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Estimation of Engel curves for household expenditure on dry bean and processed bean in Mexico

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Estimation of Engel curves for household expenditure on dry bean and processed bean in Mexico

Abstract: Dry bean is the leading source of low-cost plant-based proteins in Mexico. However, in the years following the liberalization of the economy, Mexico experienced the erosion of a self-sufficiency index for this commodity. Impending changes in the international markets for proteins compel us to reevaluate the role of dry bean for Mexico's food security. In the present paper we set out to analyze the last link of the marketing chain in Mexico's dry bean market: the consumer. Using data on household expenditure for 2018, the relationship between income and expenditure on dry bean as well as on processed bean is ascertained by means of the Working-Leser Engel Curve equations system. Due to the presence of zero-expenditure households in the sample, we followed the two-step Heckit procedure for the possible selection bias. The results suggest that the budget share for dry bean and for processed bean drops as income increases. The corrected conditional elasticity for dry bean is −0.1056. For processed bean, the elasticity is −0.2286. The negative sign indicates that both commodities are inferior goods.

Key words: plant production; plant based proteins; dry bean; economics; Engel curves; household income; food self-sufficiency; Mexico

Ocena Engelovih krivulj za izdatke gospodinjstev za suhi in predelani fižol v Mehiki

Izvleček: Suhi fižol je v Mehiki najpomembnejši cenovno ugoden vir rastlinskih beljakovin, vendar je Mehika v letih po liberalizaciji gospodarstva doživela padec indeksa samooskrbe za to proizvodno skupino. Zaradi bližajočih se sprememb v ureditvi mednarodne trgovine, ki bodo vplivale tudi na trgovanje z beljakovinsko bogatimi kmetijskimi surovinami, smo želeli ponovno oceniti pomen suhega fižola za prehransko varnost v Mehiki. V pričujočem prispevku smo analizirali zadnji člen tržne verige za suhi fižol na mehiškem trgu, potrošnika. Z uporabo podatkov o izdatkih gospodinjstev za leto 2018 smo razmerje med dohodkom in odhodki za suh in predelan fižol ugotavljali z uporabo Working-Leser-jevega sistema Engelovih krivulj. Zaradi prisotnosti gospodinjstev, ki niso imela tovrstnih stroškov, smo upoštevali dvostopenjski Heckit-ov postopek za korekcijo morebitne napake pri vzorčenju. Rezultati kažejo, da se delež proračuna za suh in predelan fižol zmanjšuje, ko se dohodek gospodinjstev povečuje. Korigirana pogojna elastičnost za suhi fižol je −0.1056. Za predelan fižol je elastičnost −0,2286. Negativni predznak potrjuje status inferiornih dobrin za obe proučevani kategoriji.

Ključne besede: poljščine; rastlinske beljakovine; suhi fižol; ekonomika; Engelove krivulje; dohodki gospodinjstev; prehranska varnost; Mehika

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1 INTRODUCTION

A notion echoed in several academic and institutional settings around the world suggests that plant-based proteins should account for a larger share of the human intake of these nutrients, replacing animal-based sources to some extent. This partial replacement is seen as an economic and environmental necessity, feasible in nutritional terms, and strategically unavoidable for the achievement of food security goals at the national and international levels. The strategic dimension is especially significant in the Developing Countries, which rely more on plant-based sources of protein (Grigg, 1995).

Although there is still uncertainty about the degree whereby climate change and the depletion of natural resources could threaten the capacity to sustain the rates of growth in agricultural output observed during the previous decades (Valin et al., 2014), the expectation is that the prices of grains and meat will increase in the long-run even if global food supply increases (Aiking, 2011).

Although average meat consumption stagnates and even declines as income increases (Vranken et al., 2014), different strategies have been suggested to adapt human diets to meet sustainability challenges (De Boer et al., 2014), one among them being the substitution of animal-based proteins for plant-based proteins (Westhoek et al., 2014).

The instrumental use of pulses in a range of areas, namely: food security, nutrition, health, sustainable agriculture and climate mitigation, is such that 2016 was declared the International Year of Pulses by the United Nations General Assembly (Calles et al., 2019). However, to fully harness these potentials, actions need to be taken in order to reverse the decline in consumption of pulses witnessed worldwide (implying a change, yet again, in consumer preferences), to encourage production (which currently takes place in marginal areas) and to further the development of their marketing chains.

The present paper is an attempt at characterizing the last link of Mexico's dry bean marketing chain, i.e., consumption, as well as other factors that ought to be considered when designing policies aimed at tackling challenges in the areas mentioned before.

Dry bean is an important source of protein, among other nutrients, in the human food supply; in fact, dry bean contains 15–25 % of protein on a dry weight basis, depending on the variety (Sathe, 2002). In Mexico, this pulse is one of the leading sources of plant-based proteins, it is the second annual crop by planted area, and it has a historical relationship with the inhabitants of the country, which is deemed as the center of origin of a number of varieties.

Four factors that could affect food security regarding the availability and prices of proteins at the national level are: a) changes in agricultural productivity levels due to climate change (Parry et al., 2004), b) the conflict between animal-based sources and plant-based sources for the natural resources required for their production, as well as the interaction between their production processes and the so-called planetary boundaries (Stehfest et al., 2009), c) changes in the consumption pattern of proteins in countries such as China and India, due to income and population growth (Gandhi & Zhou, 2014), and d) the trans-

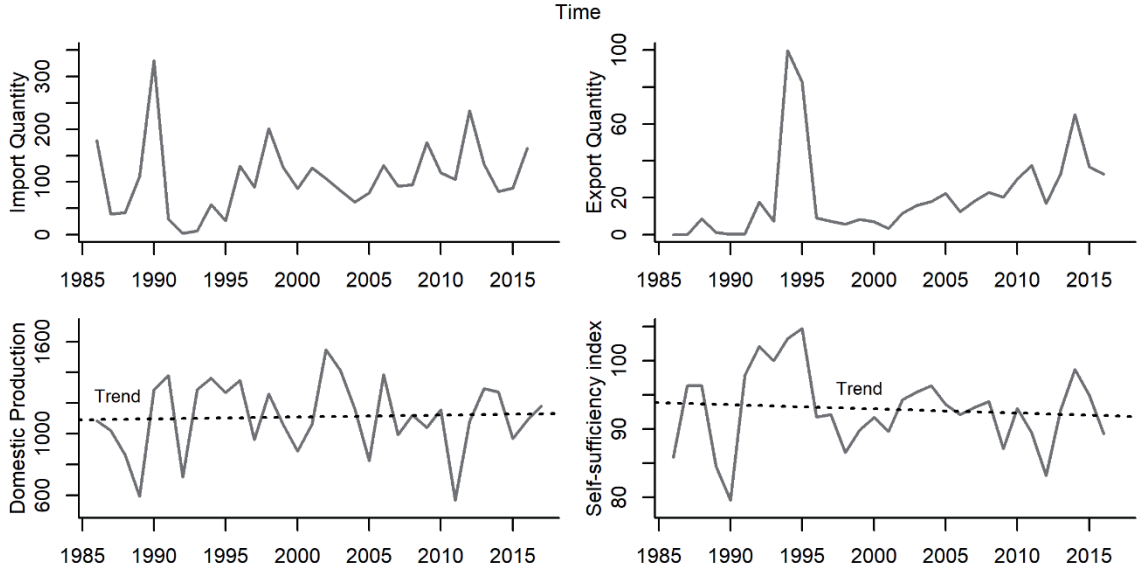

Figure 1: Components and tendency of Mexico's self-sufficiency index for dry bean (1986–2017); quantities in thousand metric tons. Source: own elaboration with data from FAO, 2019)

Table 1: Average budget share (%) for dry bean and processed bean by income decile (quarterly data), 2008–2018

	Income decile [2]										
ENIGH [1]	I [3]	II	III	IV	V	VI	VII	VIII	IX	X	Code
2008	3.590	1.507	1.023	0.854	0.616	0.517	0.376	0.277	0.174	0.084	A137
2010	3.920	1.586	1.066	0.774	0.527	0.516	0.425	0.262	0.161	0.067	Dry bean
2012	3.198	1.519	1.384	0.900	0.711	0.532	0.421	0.286	0.176	0.087	
2014	2.389	1.220	0.831	0.708	0.519	0.420	0.305	0.238	0.152	0.070	
2016	2.216	1.148	0.792	0.624	0.490	0.387	0.306	0.230	0.150	0.072	
2018	2.245	1.026	0.715	0.540	0.452	0.340	0.284	0.210	0.136	0.065	
2008	0.155	0.120	0.063	0.082	0.065	0.051	0.046	0.029	0.027	0.020	A142
2010	0.099	0.106	0.077	0.075	0.049	0.056	0.049	0.036	0.024	0.016	Proc. bean
2012	0.187	0.127	0.096	0.105	0.086	0.076	0.057	0.042	0.046	0.011	
2014	0.205	0.108	0.066	0.068	0.060	0.057	0.053	0.050	0.032	0.024	
2016	0.183	0.138	0.117	0.100	0.093	0.088	0.068	0.057	0.046	0.022	
2018	0.210	0.152	0.127	0.110	0.100	0.086	0.079	0.060	0.048	0.024	

[1] Following the procedure whereby the expenditure on other foodstuffs reported in the 'concentrado' tables were obtained. [2] The expansion factor was used when determining the income deciles. [3] Budget share for households with no income was set to 0 for decile I. Source: own elaboration with data from INEGI, 2019a.

mission of price spikes from international to domestic markets (Bekkers et al., 2017).

World production of dry beans grew steadily between 1986 and 2017, period in which it went from 17,1 to 31,4 million tons. Mexico ranked among the top ten world producers during this interval; however, its production level didn't follow this upward trend. In fact, Mexico's domestic production averaged 1,11 million tons during the same period, with significant year-over-year variations. At the same time, dry bean imports (mainly from the U.S.) showed a positive trend, but so did the relatively less significant exports (FAO, 2019). On the other hand, Mexico's population went from 81.2 million in 1990 to 119.9 million in 2015 (INEGI, 2019b). When analyzing these trends, it can be inferred that per capita consumption of dry bean among Mexicans dropped in the years after the liberalization of the economy started; yet, Mexico witnessed the slightly eroding tendency of a self-sufficiency index for

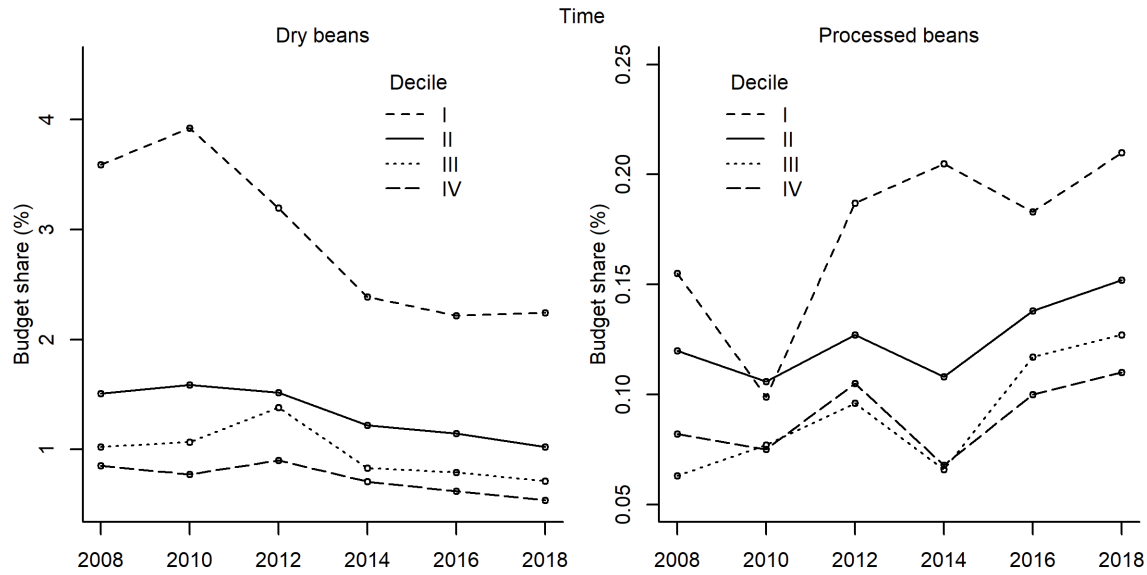

Figure 2: Average budget share for dry bean and processed bean (%) for the lower income deciles (quarterly data), 2008–2018. Source: own elaboration with data from INEGI 2019a

Table 2: Average budget share (%) for animal-based foodstuffs and non-processed pulses by income decile (quarterly data), 2008–2018

	Income decile [2]										
ENIGH [1]	I [3]	II	III	IV	V	VI	VII	VIII	IX	X	Code
2008	14.440	10.333	8.669	7.903	7.436	6.784	5.485	4.624	3.811	2.378	Animal [4]
2010	16.202	10.856	9.683	8.284	7.525	6.931	6.037	5.131	4.173	2.575	Protein
2012	15.994	10.556	10.084	8.324	8.137	7.020	6.597	5.265	4.314	2.700	
2014	14.661	10.650	10.000	9.204	8.412	7.360	6.716	6.060	4.776	3.026	
2016	13.797	10.155	8.960	8.269	7.450	6.444	6.140	5.154	4.229	2.754	
2018	13.496	9.902	8.969	8.052	7.481	6.653	6.043	5.200	4.380	2.804	
2008	0.188	0.108	0.100	0.065	0.072	0.053	0.040	0.031	0.017	0.012	Pulses
2010	0.219	0.125	0.108	0.073	0.074	0.056	0.040	0.036	0.025	0.012	Non-proc.
2012	0.160	0.113	0.106	0.078	0.048	0.075	0.040	0.034	0.020	0.012	
2014	0.121	0.147	0.109	0.079	0.088	0.074	0.040	0.034	0.030	0.012	
2016	0.198	0.120	0.102	0.081	0.061	0.055	0.041	0.031	0.022	0.012	
2018	0.172	0.116	0.098	0.072	0.068	0.063	0.048	0.038	0.027	0.015	

[1] Following the procedure whereby the expenditure on other foodstuffs reported in the 'concentrado' tables were obtained. [2] The expansion factor was used when determining the income deciles. [3] Budget share for households with no income was set to 0 for decile I. [4] Animal protein = carnes + huevo + pescado, from the 'concentrado' tables. Source: own elaboration with data from INEGI, 2019a.

this commodity (measured as the domestic production to apparent national consumption ratio) (Figure 1), since the increase in the domestic demand (driven by population growth) was met by increasing imports.

Among the factors that influence the spatial variation in the quantity and sources of protein consumed, income and cost stand out, as well as the effect of the environmental conditions on the selection of staple crops at the local level (Grigg, 1995). In the paper at hand, we set out to examine Engel's law (i.e. the principle stating that poorer households dedicate a higher share of their income to food than richer households), applied to both dry bean and processed bean. In economics, the so-called Engel curves relate expenditure on different commodities and income at the household level. The latest data available on household expenditure and income in Mexico, is provided by the 2018 national survey 'Encuesta Nacional de Ingresos y Gastos de los Hogares' (ENIGH).

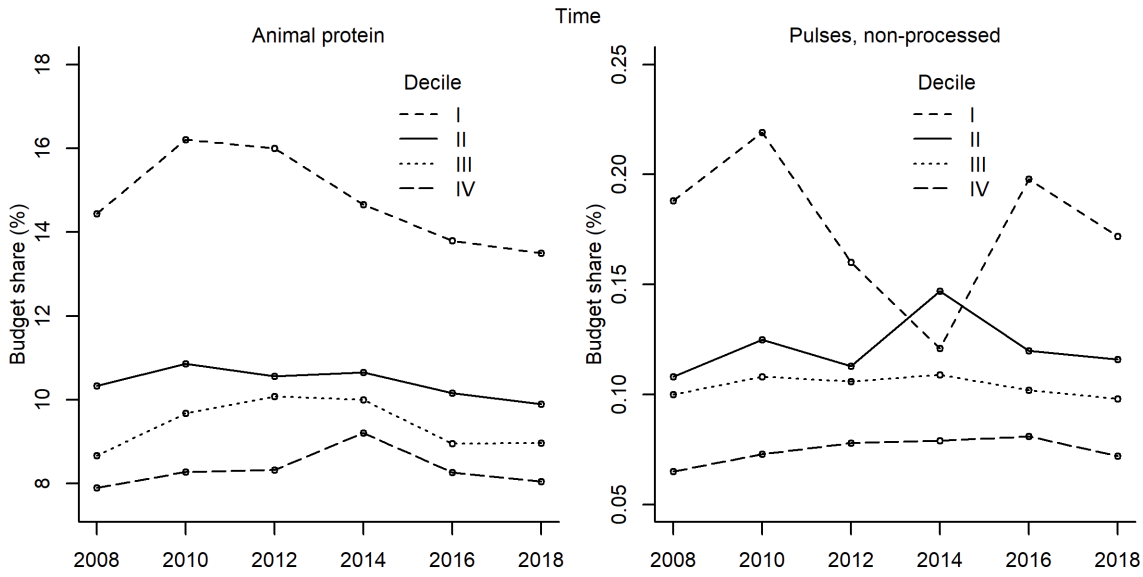

Figure 3: Average budget share for animal-based foodstuffs and non-processed pulses (%) for the lower income deciles (quarterly data), 2008–2018. Source: own elaboration with data from INEGI 2019a

Table 1 displays the budget share (i.e. the expenditure to income ratio) for both dry bean and industrialized bean across income deciles (quarterly current income). Engel's law seems to hold in the two cases. However, a closer look at the evolution of household expenditure reveals that households in the lower income deciles tend to spend far more on dry bean than on processed bean (Figure 2).

The analysis of household expenditure on dry bean is supplemented with an analysis of the expenditure on other sources of proteins. Table 2 displays the budget share for both animal-based sources of proteins (eggs, fish, and meat) and non-processed pulses (chickpeas, lentils, lima beans, and peas), during the period 2008–2018. Furthermore, the Figure 3 shows the average budget shares for animal-based foods and non-processed pulses for the lower four income deciles in the ten years' period.

2 MATERIALS AND METHODS

The 2018 ENIGH contains a set of zero-expenditure households for both dry bean and processed bean (Table 3). In single-equation representations of Engel curves, the Heckman (or Heckit) two-step procedure has been used to circumvent this censored-response problem (Saha et al., 1997).

The Heckman estimation method of Engel curves is based on the idea that censored data on household expenditure can be seen as a combination of a selection mechanism for the decision to purchase and a model for the level of the expenditure, which applies only to the sample of households with actual expenditure.

Table 3: Descriptive statistics of the expenditure variables used in the analysis

Code	Commodity	% of non-zeros	Mean [1]	Std. Dev.	Min	Max
A137	Dry bean	33.24	138.80	256.87	0.00	6,428.57
A142	Processed bean	11.81	37.28	129.47	0.00	4,049.96

[1] Mexican Pesos. Source: own elaboration with data from INEGI, 2019a.

The procedure starts off with the equation that determines the sample selection:

$$z_i = \boldsymbol{w'}_i\boldsymbol{\gamma} + u_i \quad (1)$$

Where z_i is an indicator variable equal to 1 if expenditure occurs in household i and 0 otherwise, $\boldsymbol{w}_i$ is a vector of observed socio-demographic characteristics of the household that affect the purchase decision, and $\boldsymbol{\gamma}$ is a vector of coefficients which is determined by the Maximum Likelihood (ML) estimation of a probit model where:

$$Pr[z_i = 1|\boldsymbol{w}_i] = \Phi(\boldsymbol{w'}_i\boldsymbol{\gamma}) \quad (2)$$

This step is applied on the full sample of households and its purpose is to provide with estimates of the bias correction term (also known as the inverse Mills ratio, or IMR) given by:

$$\hat{\lambda}_i = \phi\,(\boldsymbol{w'}_i\hat{\boldsymbol{\gamma}})/\Phi\,(\boldsymbol{w'}_i\hat{\boldsymbol{\gamma}}) \quad (3)$$

Where Φ is the standard normal cumulative distribution function and ϕ is the standard normal probability density function.

In the second step, Ordinary Least Squares (OLS) are applied on the model that represents the Engel curve plus the estimated bias correction term in an equation like:

$$y_i = \boldsymbol{x'}_i\boldsymbol{\beta} + \theta\hat{\lambda}_i + \varepsilon_i \quad (4)$$

Where x_i represents a vector of socio-demographic features of the household that affect the level of expenditure on a given commodity. This step uses only the truncated sample where positive expenditure is observed, i.e. where $y_i > 0$ or equivalently where $z_i = 1$.

In the paper at hand, the equation of the selection mechanism is given by:

$$z_i = \gamma_1 + \gamma_2 ln(income) + \sum_{k=3}^{k}\gamma_k w_k + u_i \quad (5)$$

Some functional forms used to examine Engel's law include: double logarithmic, quadratic, semi-logarithmic and Working-Lesser. The latter approach relates budget share y_i and the logarithm of income; it also allows a direct test of Engel's law (Holcomb et al., 1995). The Engel curve used in the second step of the Heckit follows the Working-Lesser form defined by:

$$y_i = \beta_1 + \beta_2 ln(income_i) + \sum_{k=3}^{k}\beta_k x_{k,i} + \theta\hat{\lambda}_i + \varepsilon_i \quad (6)$$

This analysis is supplemented with a corrected estimate of the income elasticity of the budget share given by (at the mean of the data):

$$e_s = 1 + \frac{1}{E(y_i)}\left[\hat{\beta}_2 + \hat{\theta}E\left(\frac{\partial}{\partial w_2}\hat{\lambda}_i\right)\right] \quad (7)$$

Where $w_2 = ln(income)$. Which is equivalent to (Saha et al., 1997):

$$e_s = 1 + \frac{1}{E(y_i)}\left[\hat{\beta}_2 - \hat{\theta}\hat{\gamma}_2\left\{E(\boldsymbol{w'}_i\hat{\boldsymbol{\gamma}})E(\hat{\lambda}_i) + E(\hat{\lambda}_i)^2\right\}\right] \quad (8)$$

The data used in this analysis is provided by Mexico's Instituto Nacional de Estadística y Geografía and are representative at the national level. The 2018 ENIGH provides with a sample of 74,647 households in a table labeled as 'concentradohogar', with records on their expenditure on selected food groups, as well as their sociodemographic features, including size and income. In this analysis, the income variable corresponds to quarterly current income.

The expenditure on dry bean was obtained following the same procedure used to get the expenditures on the food groups recorded in the 'concentradohogar' table, i.e. as the summation of the quarterly expenditure (gasto trimestral) on the code A137 from the 'gastoshogar' table plus the summation of the quarterly expenditure on the same code from the 'gastospersona' table. For the expenditure on processed bean, we replicated the procedure using the code A142.

In order for the budget shares to be confined between zero and one, households that reported having either no-income or expenditure on dry bean greater than income were removed from the sample. This rendered a subset of 74,637 households.

The sociodemographic characteristics considered in this paper are: 1) income (IngCor); 2) size of locality (TamLoc); 3) region (Region); 4) household class (ClaseHog); 5) education of the head of the household (EducaJefe); 6) sex of the head of the household (SexoJefe); 7) age of the head of the household (EdadJefe); 8) number of grown-ups (Mayores); 9) size of the household (TotInteg); and 10) socio-economic strata (EstSocio). We added dummy variables to indicate expenditure on: 11) dry bean (A137_dum); 12) processed bean (A142_dum); 13) meat products (Carnes_dum); 14) eggs (Huevo_dum); and 15) fish (Pescado_dum). The levels for size of locality (number of inhabitants) are: 100,000 and more = 1; 15,000–99,999 = 2; 2,500–14,999 = 3; 2,500 and less = 4. The region variable is a categorical one with the following levels: NW (Baja California, Baja California Sur, Chihuahua, Durango, Sinaloa, and Sonora); NE (Coahuila, Nuevo León, and Tamaulipas); W (Colima, Jalisco, Michoacán, and Nayarit); E (Hidalgo, Puebla, Tlaxcala, and Veracruz); CN (Aguascalientes, Guanajuato, Querétaro, San Luis Potosí, and Zacatecas); CS (Ciudad de México, Estado de México, and Morelos); SW (Chiapas, Guerrero, and Oaxaca); and SE (Campeche, Quintana Roo, Tabasco, and Yucatán). The education of the head of the household ranges from: Without instruction = 1, to Graduate = 11. The levels for sex of the head of the household are: Male = 1; and Female = 2. The levels for socioeconomic strata and household class are inherited from the ENIGH terminology. The socioeconomic strata are: Lower = 1; Lower middle = 2; Upper middle = 3; and Upper = 4. Finally, the household classes are: One-person = 1; Nuclear = 2; Extended = 3; Composite = 4; and Coresident = 5.

3 RESULTS

Table 4 shows the OLS Engel curve estimates for dry bean and processed bean for Mexico, using the 2018 data. It also shows the ML for the probit model used in the first stage of the Heckit procedure for dry bean. The results obtained show that the coefficient associated with the IMR is significant only for dry bean, which indicates that the correlation between the error term of the decision to purchase this commodity and the budget share of the same is different than zero.

The use of the Heckit procedure is appropriate in the case of dry bean; therefore, household expenditure on this commodity can be represented as a two-stage process. The variables included in the probit model were determined on the basis of the Akaike Information Criterion (AIC), by step-wise regression. Regarding the decision to purchase dry bean, the income variable is statistically significant. This result indicates that income affects negatively the probability of purchasing this commodity. The effect of household size is positive and statistically significant, which indicates that having a larger household increases the propensity to spend on dry bean.

In order to determine the final form of the Working-Lesser structure in the second step of the Heckit procedure, we tested for collinearity in a model with the same regressors used for the probit in the first stage, plus the IMR. Then, we removed the variable with the highest variance inflation factor (VIF) keeping the IMR, so that in the final model all VIF's fell below the cut-off value of 10. Thus, the variables in the OLS model for dry bean are a subset of the variables included in the probit model.

Both OLS models from Table 4 exhibit heteroskedasticity. It has been pointed out that the standard errors and the heteroskedasticity-robust standard errors of the OLS estimates provided by the second stage of the Heckit models, are incorrect. Although formulas to overcome this problem are available, their implementation is not always easy; however, one alternative is to use bootstrapped standard errors (Cameron & Trivedi, 2005, p. 550). We followed this approach for dry bean, whereas in the case of processed bean, we present heteroskedasticity-robust standard errors.

The Working-Lesser structure reported negative and statistically significant parameter estimates for the logarithm of current income for dry bean and processed bean.

The corrected conditional elasticity estimated at the mean of the data for dry bean is −0.1056. Whereas, the

Table 4: Heckit and OLS estimates of the Engel curve for dry bean and for processed bean

	Dependent variable:		
	1st step: $z_{i,A137}$	2nd step: $y_{i,A137}$	$y_{i,A142}$
Regressor	Probit-ML	OLS	OLS
Log(IngCor)	−0.1847*** (0.0088)	−0.0212*** (0.0005)	−0.0135*** (0.0012)
TamLoc = 2	0.0912*** (0.0176)	−0.0002 (0.0003)	
TamLoc = 3	0.1401*** (0.0181)	−0.0004 (0.0003)	
TamLoc = 4	0.1322*** (0.0164)	0.0013*** (0.0003)	
Region = NW	−0.0957*** (0.0169)	0.00004 (0.0004)	0.0014*** (0.0004)
Region = SE	0.1243*** (0.0199)	−0.0034*** (0.0004)	−0.0030*** (0.0004)
Region = SW	−0.0152 (0.0203)	0.0022*** (0.0006)	0.0002 (0.0005)
Region = CS	−0.0145 (0.0207)	−0.0004 (0.0005)	−0.0027*** (0.0005)
Region = NE	−0.1725*** (0.0200)	0.0010* (0.0006)	0.0002 (0.0007)
Region = W	0.0271 (0.0199)	−0.0006 (0.0004)	−0.0002 (0.0005)
Region = E	0.0791*** (0.0196)	−0.0013*** (0.0005)	−0.0032*** (0.0005)
ClaseHog = 2	0.0973*** (0.0253)	−0.0039*** (0.0009)	
ClaseHog = 3	0.0338 (0.0306)	−0.0040*** (0.0010)	
ClaseHog = 4	0.0240 (0.0687)	−0.0024 (0.0016)	
ClaseHog = 5	−0.3212*** (0.1169)	−0.0002 (0.0023)	
EducaJefe = 2	−0.0630 (0.1505)	−0.0028 (0.0032)	−0.0006 (0.0040)
EducaJefe = 3	−0.0249 (0.0216)	−0.0025*** (0.0007)	−0.0027** (0.0012)
EducaJefe = 4	−0.0663*** (0.0223)	−0.0034*** (0.0007)	−0.0026** (0.0013)
EducaJefe = 5	−0.1081*** (0.0336)	−0.0035*** (0.0009)	−0.0028** (0.0014)
EducaJefe = 6	−0.1385*** (0.0226)	−0.0040*** (0.0007)	−0.0025** (0.0012)
EducaJefe = 7	−0.2427*** (0.0359)	−0.0040*** (0.0008)	−0.0033*** (0.0012)
EducaJefe = 8	−0.2225*** (0.0265)	−0.0030*** (0.0007)	−0.0012 (0.0016)
EducaJefe = 9	−0.3497*** (0.0414)	−0.0005 (0.0009)	−0.0017 (0.0013)
EducaJefe = 10	−0.2934*** (0.0296)	0.0023*** (0.0008)	−0.0004 (0.0014)
EducaJefe = 11	−0.3185*** (0.0524)	0.0073*** (0.0012)	0.0038** (0.0018)
SexoJefe = 2			−0.0013*** (0.0004)
Log(EdadJefe)	0.1132*** (0.0205)	0.0028*** (0.0005)	0.0021*** (0.0004)
Log(Mayores)	0.2122*** (0.0230)	0.0033*** (0.0006)	
Log(TotInteg)	0.2248*** (0.0224)	0.0041*** (0.0006)	0.0031*** (0.0006)
EstSocio = 2	−0.2147*** (0.0147)	−0.0044*** (0.0003)	0.00002 (0.0007)
EstSocio = 3	−0.3129*** (0.0230)	−0.0028*** (0.0005)	0.0002 (0.0009)
EstSocio = 4	−0.3531*** (0.0311)	0.0007 (0.0006)	0.0022** (0.0010)
A142_dum = 1	−0.5798*** (0.0176)	−0.0025*** (0.0005)	
A137_dum = 1			−0.0030*** (0.0003)
Carnes_dum = 1	0.4133*** (0.0143)	−0.0037*** (0.0006)	−0.0019*** (0.0007)
Huevo_dum = 1	0.4305*** (0.0112)		0.0005 (0.0005)
Pescado_dum = 1	0.1379*** (0.0130)	0.0011*** (0.0003)	0.0006 (0.0005)
A137_mr		0.0019** (0.0010)	
Constant	0.1617 (0.1199)	0.2270*** (0.0047)	0.1449*** (0.0115)
Observations	74,637	24,808	8,818
R2		0.4041	0.2674
Log Likelihood	−41,773.2200		
Akaike Inf. Crit.	83,618.4300		
Residual Std. Error		0.0192 (df = 24772)	0.0148 (df = 8789)
F Statistic		479.9184*** (df = 35; 24772)	114.5816*** (df = 28; 8789)

Notes: Standard errors in parentheses; in the case of dry bean, bootstrapped estimates after 3,000 samples; in the case of processed bean, heteroskedasticity-robust. Significance levels: *$p < 0.1$; **$p < 0.05$; ***$p < 0.01$. Own elaboration with data from INEGI, 2019a.

elasticity for processed bean is −0.2286. The negative sign indicates that both commodities are inferior goods.

4 CONCLUSIONS

The use of pulses for the attainment of policy objectives in several areas demands the reversing of the downward trend in consumption that these commodities have displayed worldwide. In the present paper, the strategic importance of dry bean for Mexico is pointed out; this pulse remains as the leading source of plant-based proteins among Mexicans, since it exceeds the expenditure that households make on other pulses. However, a closer look on household expenditure revealed that the abovementioned trend manifested itself across income deciles during the last decade.

To understand the last link of the marketing chain for dry bean in Mexico, we set out to analyze household expenditure on several protein sources, including both dry bean and processed bean. For this purpose, budget share Engel curves were estimated for the two commodities using the 2018 ENIGH sample of households. Our results are in alignment with the principle stated by Engel's law, and both dry bean and processed bean turned out to be inferior goods. Therefore, policy measures aimed at the attainment of environmental, nutritional, and health goals, based on the use of dry bean and other pulses, ought to set out to change the relationship between expenditure on this commodities and income, i.e. turn them into normal goods. One alternative is the advancement of processed versions of these commodities.

Other trends worth mentioning in Mexico' market for dry bean are: the slight erosion of a self-sufficiency index in the post-liberalization era of the economy, the decline in the per-capita consumption of this commodity, and the stagnation of the production level (notwithstanding the considerable year-over-year changes). Finally, some of the expected outcomes from policies aimed at furthering dry bean production are: 1) increases in the production levels as more productive land is allocated to this end, and a decline in prices due to higher productivity.

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Pomen čebeljih pridelkov v humani prehrani

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Pomen čebeljih pridelkov v humani prehrani

Izvleček: Čebelji pridelki so naraven vir hranil in biološko aktivnih spojin, ki se uvrščajo tudi na sezname funkcionalnih sestavin. V prehrani uporabljamo predvsem med in v manjši meri cvetni prah osmukanec in matični mleček. Propolis in čebelji strup se zaradi terapevtskih lastnosti uporabljata predvsem v apiterapiji. Od osnovnih hranil je med predvsem vir sladkorjev, cvetni prah in matični mleček pa poleg teh vsebujeta še beljakovine in maščobe, cvetni prah pa tudi prehransko vlaknino. Čebelji pridelki v manjših količinah vsebujejo še bioaktivne spojine, ki imajo antioksidativno, protimikrobno, protivnetno in protivirusno delovanje. Za med so med drugim značilne fenolne spojine, proteini matičnega mlečka, oligosaharidi. Matični mleček vsebuje specifične maščobne kisline, vključno z 10-hidroksi-2-decenojsko kislino, bioaktivne peptide, proteine, v cvetnem prahu pa so različni vitamini, fenolne spojine, nenasičene maščobne kisline in druge spojine. Potrebne pa so nadaljnje raziskave in klinične študije za ovrednotenje učinkovitosti čebeljih pridelkov ter ozaveščanje potrošnikov o pomenu njihovega uživanja. Med, cvetni prah osmukanec in matični mleček so naravna živila, ki zaradi svoje sestave lahko pripomorejo k doseganju priporočenih dnevnih vnosov osnovnih hranljivih snovi, hkrati pa so lahko vir pomembnih bioaktivnih spojin, zato nedvomno sodijo v uravnoteženo prehrano človeka.

Ključne besede: živila; čebelji pridelki; med; cvetni prah osmukanec; matični mleček; prehrana ljudi; zdravje

The importance of bee products in human nutrition

Abstract: Bee products are a natural source of nutrients and biologically active compounds, which may also be found on the lists of functional ingredients. In our diets, mainly honey is used and to a lesser extent bee pollen and royal jelly. Propolis and bee venom are mainly used in apitherapy due to their therapeutic properties. Regarding the basic nutrients, honey is primarily a source of sugars, while protein and fat contents are considerable in royal jelly and pollen, which also contains dietary fiber. Bee products also contain small amounts of bioactive compounds that have antioxidant, antimicrobial, anti-inflammatory and antiviral effects. Honey is characterized by, among others, phenolic compounds, royal jelly proteins, oligosaccharides. Royal jelly contains specific fatty acids, including 10-hydroxy-2-decenoic acid, bioactive peptides, major royal jelly proteins, and pollen contains various vitamins, phenolic compounds, amino acids, unsaturated fatty acids. However, further research and clinical studies are needed to evaluate the effectiveness of bee products and to raise consumer awareness of the importance of their consumption. Honey, bee pollen and royal jelly are natural foods, which due to their composition may help to achieve the recommended daily intake of basic nutrients, and may also serve as a source of important bioactive compounds, and therefore undoubtedly belong to a balanced diet.

Key words: food; bee products; honey; bee pollen; royal jelly; human nutrition; health

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1 UVOD

Način prehranjevanja lahko pomembno vpliva na zdravje. Naravna živila in naravna prehranska dopolnila imajo v današnjem času pomembno mesto v prehrani ljudi. Del naravnih živil oz. dopolnil predstavljajo tudi čebelji pridelki, med, cvetni prah osmukanec, matični mleček in propolis. Ti pridelki imajo različno vlogo v čebelji družini, zaradi hranilne vrednosti in ugodnih funkcionalnih lastnosti pa se pogosto uporabljajo tudi v prehrani ljudi. Poleg medu, ki je v prehrani poznan že iz pradavnine, in je najbolj uporabljen čebelji pridelek, se v zadnjem času povečuje tudi uporaba cvetnega prahu in matičnega mlečka, slednjega zlasti v obliki prehranskih dopolnil. Propolis in čebelji strup se zaradi terapevtskih lastnosti uporabljata predvsem v apiterapiji (Bogdanov, 2011; Yucel in sod., 2017). Čebelji pridelki imajo visoko biološko vrednost zaradi vsebnosti hranilnih snovi in bioaktivnih spojin. Njihova zastopanost je odvisna od botaničnega in geografskega porekla, podnebnih razmer, postopkov čebelarjenja in skladiščenja čebeljih pridelkov. V vsakdanji prehrani ljudje najpogosteje posegajo po medu, ki ga uživajo samega ali kot sladilo za slajenje pijač in nekaterih drugih živil. Cvetni prah in matični mleček pa se uporabljata predvsem kot dodatek prehrani. Uporabnost čebeljih pridelkov se kaže tudi v možnosti njihovega dodajanja drugim živilom za povečanje vsebnosti bioaktivnih spojin v teh živilih in s tem večje protimikrobne in antioksidativne učinkovitosti (Viuda-Martos, 2008; Cornara in sod., 2017; Pasupuleti in sod., 2017). Prispevek povzema pridobivanje in sestavo čebeljih pridelkov ter možnost uporabe čebeljih pridelkov v vsakdanji prehrani, njihovo aplikacijo v živila in nekatere biološke lastnosti, ki lahko pozitivno delujejo na zdravje človeka.

2 PRIDOBIVANJE ČEBELJIH PRIDELKOV

2.1 PRIDOBIVANJE MEDU

Med je eno najbolj kompleksnih naravnih živil, je naravna sladka snov, ki jo izdelajo čebele *Apis mellifera* iz nektarja cvetov ali izločkov iz živih delov rastlin ali izločkov žuželk (uši, kaparjev), ki sesajo rastlinski sok na živih delih rastlin, ki jih čebele zberejo, predelajo z določenimi lastnimi snovmi, shranijo, posušijo in pustijo dozoreti v satju. Med pridobivamo iz satovja s centrifugiranjem, brez kakršnekoli obdelave, razen grobega filtriranja. Pravilnik o medu (2011) deli med glede na izvor na i) »med iz nektarja«, ki je pridobljen iz nektarja cvetov različnih rastlin, ter ii) »manin med«, ki je pridobljen predvsem iz izločkov insektov na živih delih rastlin ali izločkov živih delov rastlin. Glede na vrsto paše ločimo različne vrste medu, najpogostejše vrste slovenskega medu so podane v preglednici 1. Če so čebele nabrale nektar ali mano pretežno na eni rastlinski vrsti in med izhaja v celoti ali pretežno iz navedenega izvora, in ima njegove senzorične, fizikalno-kemijske in mikroskopske lastnosti, se ime med lahko dopolni z navedbo oznake, ki se nanaša na izvor iz cvetov ali rastlin (Pravilnik o medu, 2011).

Preglednica 1: Vrste slovenskega medu glede na pašo
Table 1: Types of Slovenian honey regarding the pasture source

Vrsta paše	Vrsta medu
Nektar	akacijev med (*Robinia pseudoacacia*)
	med oljne ogrščice (*Brassica napus*)
	ajdov med (*Fagopyrum esculentum*)
	rešeljikov med (*Prunus mahaleb*)
	regratov med (*Taraxacum officinale*)
	cvetlični med
Nektar in/ali mana	lipov med (*Tilia* sp.)
	kostanjev med (*Castanea sativa* Mill.)
	javorjev med (*Acer pseudoplatanus* L., *A. platanoides* L.)
Mana	smrekov med (*Picea abies* (L.) Karst.)
	hojev med (*Abies alba* Mill.)
	gozdni med

Po podatkih Statističnega urada RS je v Sloveniji povprečna količina proizvedenega medu 1.800 ton letno. Zaradi dolge tradicije čebelarstva v Sloveniji se večina slovenskega medu porabi doma, delež uvoženega medu predstavlja le 14 %. Po podatkih iz bilance medu se poraba medu v Sloveniji povečuje. Od leta 2000, ko je ocenjena poraba medu na prebivalca znašala nekaj več kot 1 kilogram, se je v zadnjih letih povečala na približno 1,4 kilograma. Povečuje se tudi uvoz medu, saj je domača pridelava medu manjša od skupne porabe. V zadnjih letih uvozimo največ medu iz Hrvaške, Madžarske in Nemčije, izvažamo pa ga v Italijo, Belgijo in Avstrijo (Statistični urad RS, 2019).

2.2 PRIDOBIVANJE CVETNEGA PRAHU OSMUKANCA

Cvetni prah ali pelod je značilen za vsako posamezno cvetočo rastlinsko vrsto. Je osnova spolnega razmnoževanja rastlin, saj vsebuje moške oplojevalne celice rastlin. Čebelji cvetni prah se lahko pridobiva na dva načina, kot izkopanec ali kot osmukanec. Čebele so ana-

tomsko prilagojene za nabiranje cvetnega prahu. Pri letu s cveta na cvet se jim cvetni prah oprijema telesa, pokritega z dlačicami, dodajo mu slino in nektar (ali med) iz medenega želodčka ter ga s posebnimi gibi nog zbirajo v koških na zunanji stran nog in prinašajo v panj (Kandolf, 2011). Čebelji prah izkopanec izkopljemo direktno iz čebeljega satja, v katerem je cvetni prah že fermentiran, saj v odsotnosti kisika pride do mlečnokislinskega vrenja. Vendar je to zelo zamudno, količina izkopanca je tudi zelo majhna, zato so oblikovali posebne naprave, osmukalnike, s katerimi se pridobiva cvetni prah osmukanec. Osmukalnike namestimo pred vhodom v panj, čebelam pa pri prehodu skozenj iz nožic v zbiralnik odpade nabran cvetni prah (Pucihar, 2017).

Cvetni prah osmukanec je svež čebelji pridelek, ki vsebuje veliko vode (20–30 g/100 g), v kombinaciji z visoko hranilno vrednostjo predstavlja idealen vir za razvoj plesni. Zato je potrebno cvetni prah po pobiranju ustrezno predelati in shraniti. Najpogosteje ga stabiliziramo s sušenjem, lahko ga vmešamo tudi v med, zamrznemo ali liofiliziramo (Potokar, 2010). Kakovost cvetnega prahu najbolj ohranimo, če ga svežega nemudoma shranimo v zmrzovalnik (–18 °C), na ta način uničimo tudi morebitne prisotne insekte in mikroorganizme. Po odtalitvi cvetnega prahu je pomembno, da ga takoj porabimo ali ga posušimo v električnih sušilnikih, kjer vlaga enakomerno izhlapeva in temperatura ne presega 40 °C, da preprečimo izgubo vitaminov ter hlapnih snovi, ki prispevajo k oblikovanju arome. Cvetni prah je stabilen, ko vsebuje okoli 4–8 g vode/100 g. Manjša vsebnost vode ni priporočljiva, saj postane senzorično manj sprejemljiv ter težje prebavljiv (Campos in sod., 2008; Bogdanov, 2011). Posušen cvetni prah se lahko skladišči na sobni temperaturi tudi do enega leta in pol, brez spremembe senzoričnih in mikrobioloških lastnosti. Za ohranitev bioaktivnih spojin je priporočljivo skladiščenje v temnem in suhem prostoru, pri nižjih temperaturah (Bogdanov, 2011).

2.3 PRIDOBIVANJE MATIČNEGA MLEČKA

Matični mleček je izloček krmilnih in mandibularnih žlez mladih čebel delavk (čebel dojilj). Z matičnim mlečkom hranijo vse čebelje ličinke tri dni, po tretjem dnevu pa samo ličinko, iz katere se bo razvila matica. Matica se celo življenje prehranjuje samo z matičnim mlečkom. Matični mleček pridobivajo tako, da čebelji družini brez matice dodajo umetne letvice z matičnimi nastavki, v katere cepijo en dan stare čebelje ličinke, z namenom da jih čebele dojilje preskrbijo z matičnim mlečkom. Večina proizvajalcev matičnega mlečka le-tega pobere tri dni po cepitvi ličink, ker je količina proizvedenega matičnega mlečka takrat največja (Zheng in sod., 2010; Bogdanov, 2011).

2.4 PRIDOBIVANJE PROPOLISA

Propolis sestavljajo različne rastlinske smole, ki jih čebele nabirajo na smolnatih popkih dreves, predvsem topola in breze. Dodajo še izločke svojih žlez slinavk in vosek, da postane propolis bolj lepljiv (Huang in sod., 2014; Pasupuleti in sod., 2017). V čebelji družini ima propolis pomembno zaščitno vlogo, čebele ga uporabljajo za premaz svojega bivališča, za zadelovanje notranjih špranj in razpok ter za utrjevanje satja. Čebele s propolisom zavarujejo svoje bivališče pred mikroorganizmi in mumificirajo večje tujke v čebelji družini, ki jih fizično ne morejo odstraniti iz panja. Pridobivanje tega čebeljega pridelka je zelo zahtevno, z uporabo posebnih namenskih mrežic iz živilsko neoporečnih materialov, ki jih vstavimo v panj. Te morajo biti mehansko odporne, zdržati morajo različna upogibanja in trenja, tudi po zamrznitvi. Ko mrežice odstranimo iz panjev, jih zavijemo v živilsko folijo, zamrznemo ter nato z njih postrgamo propolis. Iz njega odstranimo vosek, delce čebel in lesene delce. Iz tako pridobljenega propolisa običajno pripravimo etanolno tinkturo (Samec, 2013).

3 SESTAVA ČEBELJIH PRIDELKOV

3.1 SESTAVA MEDU

Med je kompleksna naravna mešanica ogljikovih hidratov in drugih spojin, glede na rezultate znanstvenih raziskav vsebuje preko 200 fitokemijskih spojin. Na njegovo sestavo vplivajo različni dejavniki, kot so botanično in geografsko poreklo, klimatske razmere, postopki čebelarjenja, ravnanje z medom in tudi pogoji skladiščenja. Posledica vseh teh dejavnikov je velika raznolikost vrst medu na tržišču (Bogdanov in sod., 2008; Korošec in sod., 2016; Bobiş in sod., 2017). Med je lahko tekoč ali kristaliziran, odvisno od vsebnosti vode in razmerja med glavnima sladkorjema v medu, fruktozo in glukozo. Med vsebuje tudi druge ogljikove hidrate, di- in tri-saharide. Poleg tega pa so v medu tudi beljakovine, proste aminokisline, organske kisline, fenolne spojine (fenolne kisline in flavonoidi), različni encimi, vitamini in tudi mnogi minerali. Vsebnost glavnih komponent (ogljikovih hidratov) in minornih komponent, pelodnih zrn, aktivnost encimov, vsebnost bioaktivnih spojin (flavonoidov in fenolnih kislin) in senzorične lastnosti medu vplivajo na kakovost in funkcionalne lastnosti tega čebeljega pridelka (Bogdanov, 2008; Viuda-Martos in sod., 2008; Alva-

Preglednica 2: Osnovna sestava različnih vrst slovenskega medu
Table 2: The basic composition of Slovenian honey types

		Sladkorji				
Vrsta medu	Voda (g/l00 g)	Fruktoza (g/l00 g)	Glukoza (g/l00 g)	Saharoza (g/l00 g)	Beljakovine (g/l00 g)	Prolin (mg/kg)
akacijev	13,5–17,5	33,6–45,1	21,9–31,3	2,12–8,28	0,13–0,21	197–447
lipov	14,5–17,8	33,0–43,0	29,5–39,3	0,09–3,51	0,13–0,24	225–398
kostanjev	13,7–18,2	27,7–44,9	17,4–32,7	2,02–3,29	0,31–0,40	457–776
hojev	13,8–17,7	28,1–35,0	23,6–29,6	1,00–4,89	0,18–0,36	323–506
smrekov	14,3–18,5	28,1–42,8	23,1–30,9	1,23–3,75	0,18–0,38	231–495
cvetlični	14,4–18,0	33,2–39,2	28,5–35,5	1,32–4,35	0,18–0,42	309–534
gozdni	13,5–17,0	24,9–36,4	22,9–31,6	1,72–4,61	0,20–0,49	322–461

rez-Suarez in sod., 2009; Yucel in sod., 2017; Korošec in sod., 2017; Combarros-Fuertes in sod., 2019).

Osnovna sestava različnih vrst slovenskega medu je podana v preglednici 2. Vsebnost vode je eden najpomembnejših parametrov kakovosti medu, je zakonsko omejena, in sicer je v medu lahko največ 20 g/100 g vode (Pravilnik o medu, 2011). Običajno vsebnost vode v medu ni problematična, v večini vzorcev slovenskega medu se giblje med 14 in 18 g/100 g. Med z večjim odstotkom vode je bolj tekoč in manj viskozen. Majhna vsebnost vode onemogoča rast ozmofilnih kvasovk in tako preprečuje morebitno fermentacijo medu.

Glavna sestavina medu so ogljikovi hidrati, ki zajemajo okoli 80 % delež, oziroma okrog 95 % suhe snovi v medu. Količina in razmerje med različnimi ogljikovimi hidrati v medu sta odvisna predvsem od botaničnega porekla, encimov, sestave in intenzivnosti izločanja nektarja, klimatskih razmer ter fiziološkega stanja in moči čebelje družine.

Sestava ogljikovih hidratov vpliva na fizikalnokemijske lastnosti, kot so viskoznost, kristalizacija in higroskopnost. Od sladkorjev prevladujeta glukoza in fruktoza, predstavljata od 65 do 90 % vseh ogljikovih hidratov v medu. Kot je razvidno iz preglednice 2, slovenski medovi vsebujejo od 24,9 do 45,1 g fruktoze/100 g in od 17,4 do 39,3 g glukoze/100 g (Korošec in sod., 2016). Podobne vrednosti navajajo za različne vrste medov tudi drugi avtorji (Bogdanov, 2008; Viuda-Martos in sod., 2008; Ajibola in sod., 2012). Disaharidi in oligosaharidi (trisaharidi) so v medu prisotni v precej manjših količinah, vendar je njihova vsebnost lahko kriterij za določanje botaničnega porekla in pristnosti medu. Saharoze sme biti do 5 g/100 g medu, oziroma v primeru nekaterih izjem, kot je akacijev med, do 10 g/100 g medu (Pravilnik o medu, 2011).

Glede na izvor medu, medovi iz nektarja običajno vsebujejo več monosaharidov. Pravilnik o medu (2011) za medove iz nektarja navaja skupno vsebnost fruktoze in glukoze vsaj 60 g/100 g ter za medove iz mane vsaj 45 g/100 g. Medovi iz nektarja in medovi iz mane se običajno ne razlikujejo veliko v vsebnosti disaharidov, obstajajo pa razlike v vsebnosti nekaterih trisaharidov. Erlozo, maltotriozo in panozo vsebujejo tako nektarne kot manine vrste medu, medtem ko sta rafinoza in melecitoza značilni za manin med (preglednica 3). Prisotnost melecitoze v medu iz nektarja tako nakazuje, da je v medu prisotna tudi mana (Korošec in sod., 2016).

Preglednica 3: Vsebnost ogljikovih hidratov v slovenskem medu glede na izvor medu
Table 3: Carbohydrate composition of Slovenian nectar and honeydew honey types

	Povprečje ± SD (g/100 g)	
Ogljikovi hidrati	med iz nektarja	med iz mane
Monosaharidi		
glukoza	29,38 ± 3,97	26,97 ± 2,43
fruktoza	37,27 ± 2,73	33,31 ± 3,64
Disaharidi		
saharoza	3,47 ± 1,50	3,03 ± 0,88
maltoza	2,11 ± 0,44	2,07 ± 0,67
palatinoza	0,89 ± 0,08	0,97 ± 0,23
turanoza	1,62 ± 0,25	1,83 ± 0,41
melibioza z gentiobiozo	1,86 ± 0,67	1,83 ± 0,54
Oligosaharidi (trisaharidi)		
panoza	0,59 ± 0,04	0,61 ± 0,06
erloza	1,60 ± 0,50	2,19 ± 1,03
rafinoza	< LOQ	2,21 ± 1,45
melecitoza	< LOQ	2,53 ± 1,60
maltotrioza	0,70 ± 0,14	0,92 ± 0,36

SD: standardni odklon; < LOQ: pod mejo kvantitativne določitve

Preglednica 4: Vsebnost skupnih fenolnih spojin in antioksidativna učinkovitost slovenskega medu
Table 4: Total phenolic content and antioxidant activity of Slovenian honey types

Vrsta medu	Vsebnost skupnih fenolnih spojin (mg GAE/l00 g)	Antioksidativna učinkovitost (FRAP) (μM Fe(II))
akacijev	25,7–67,9	56,8–86,0
lipov	63,4–109,0	94,6–155,1
kostanjev	146,8–272,3	238,3–469,5
hojev	163,4–285,7	320,8–582,2
smrekov	185,7–239,0	277,5–495,4
cvetlični	126,8–194,6	181,1–262,9
gozdni	192,3–270,1	371,6–494,1

GAE: ekvivalent galne kisline; FRAP: ferric reducing antioksidant power (antioksidativna moč redukcije železa)

Poleg ogljikovih hidratov vsebuje med številne organske in tudi anorganske kisline, katerih skupno vsebnost izražamo v miliekvivalentih. Prostih kislin sme med vsebovati do 50 mekv/kg (Pravilnik o medu, 2011).

Beljakovine v medu nimajo velikega prehranskega pomena, saj je njihova vsebnost majhna, običajno pod 0,5 g/100 g. V primeru slovenskega medu največ beljakovin v povprečju vsebuje kostanjev med, najmanj pa akacijev med (preglednica 2). Aminokisline v medu izvirajo iz nektarja oz. mane, cvetnega prahu in čebel. Njihova vsebnost v medu je zelo majhna, največ je prolina, ki je v povezavi z zrelostjo medu, botaničnim poreklom in pristnostjo (Korošec in sod., 2017).

Med je tudi naravni vir antioksidantov, med katerimi so najbolj pomembne fenolne kisline, flavonoidi, encimi (glukoza oksidaza, katalaza, peroksidaza) in produkti Maillardove reakcije (Bertoncelj in sod., 2007; Bogdanov, 2008; Ajibola, 2015; Bobiş in sod., 2017; Pasupuleti in sod., 2017).

Na vsebnost fenolnih spojin v medu vplivajo botanično in geografsko poreklo medu ter podnebne razmere. V medu so od flavonoidov prisotni predvsem flavoni, flavonoli in flavanoni ter različne fenolne kisline (Viuda-Martos in sod., 2008; Bertoncelj in sod., 2011). Skupna vsebnost fenolnih spojin v slovenskih medovih je podana v preglednici 4, kjer so razvidne velike razlike med posameznimi vrstami medu, najmanj jih vsebuje akacijev med, največ pa medovi iz mane, hojev, gozdni in smrekov med (Bertoncelj in sod., 2007).

Vsebnost fenolnih spojin je v tesni povezavi z antioksidativno učinkovitostjo. Medovi z večjo vsebnostjo fenolnih spojin, imajo večjo antioksidativno učinkovitost, določeno s FRAP metodo (Bertoncelj in sod., 2007; Korošec in sod., 2017).

Vsebnost skupnega pepela v medu je količina anorganskega ostanka po sežigu medu in ponazarja količino v medu prisotnih mineralnih snovi. Določanje pepela je dokaj zahtevno, zato se nadomešča z merjenjem električne prevodnosti medu, saj med tema dvema parametroma obstaja linearna zveza. Čim več je v medu prisotnih mineralnih snovi, večja je vsebnost skupnega pepela in višja je električna prevodnost (Kropf in sod., 2008). Med kot živilo ni pomemben vir elementov, skupna vsebnost pepela v medu iz nektarja običajno znaša pod 0,6 g/100 g, v medu iz mane pa do 1,0 g/100 g (preglednica 5). Raznolikost elementov v posameznem vzorcu medu je v veliki meri odvisna od sestave nektarja, mane in prsti ter prevladujočega cvetnega prahu. Iz skupine makroelementov v medu je samo kalij prisoten v količinah nad 200 mg/kg. Med vsebuje tudi različne mikroelemente (vsebnost nad 1 mg/kg) ter elemente v sledovih. Različne študije so pokazale, da ima botanično poreklo največji vpliv na vsebnost elementov v sledovih v medu (Korošec in sod., 2017).

Preglednica 5: Vsebnost pepela in nekaterih elementov v različnih vrstah slovenskega medu
Table 5: Ash and elemental content in different Slovenian honey types

Vrsta medu	Pepel (g/kg)	Povprečna vsebnost elementov ± SD (mg/kg)						
		K	Cl	Ca	S	Rb	Mn	Br
akacijev	0,4–0,9	278 ± 78	95 ± 52	17,3 ± 7,7	47 ± 19	0,72 ± 0,32	1,68 ± 1,27	0,60 ± 0,26
lipov	1,8–3,0	1800 ± 349	379 ± 139	69 ± 23	50 ± 27	5,5 ± 2,9	3,55 ± 1,56	1,02 ± 0,43
kostanjev	5,5–10,4	3590 ± 657	240 ± 217	148 ± 33	42 ± 24	17,0 ± 7,7	23,2 ± 9,0	0,55 ± 0,23
hojev	3,8–7,1	3170 ± 555	333 ± 134	35 ± 18	71 ± 26	22,0 ± 7,0	5,03 ± 1,93	0,59 ± 0,12
smrekov	4,1–6,5	2950 ± 494	322 ± 74	47 ± 17	70 ± 26	13,9 ± 6,1	7,07 ± 2,3	0,58 ± 0,22
cvetlični	1,1–2,7	1120 ± 352	264 ± 85	61 ± 25	56 ± 25	2,97 ± 1,63	3,12 ± 1,59	0,65 ± 0,25
gozdni	4,4–6,3	2940 ± 561	310 ± 79	59 ± 19	57 ± 21	13,7 ± 7,8	6,74 ± 2,51	0,59 ± 0,25

SD: standardni odklon

Preglednica 6: Osnovna hranilna sestava mešanega cvetnega prahu osmukanca slovenskega izvora
Table 6: Basic nutritional composition of Slovenian bee pollen

	Vsebnost v svežem vzorcu			Vsebnost na suho snov		
Parameter	povprečje	x_{min}	x_{max}	povprečje	x_{min}	x_{max}
voda (g/100 g)	22,73	15,70	29,20			
beljakovine (g/100 g)	17,46	13,00	22,90	22,73	16,03	32,34
maščobe (g/100 g)	7,36	4,50	12,30	9,55	6,07	15,79
pepel (g/100 g)	2,06	1,30	2,80	2,67	1,65	3,88
skupni ogljikovi hidrati (g/100 g)	50,4	39,3	60,0	65,05	54,75	73,98
energijska vrednost (kJ/100 g)	1430	1300	1540	1850	1780	1980

x_{min}: minimalna vrednost, x_{max}: maksimalna vrednost

3.2 SESTAVA CVETNEGA PRAHU

Cvetni prah osmukanec vsebuje enostavne sladkorje, vse esencialne aminokisline, nasičene in nenasičene maščobne kisline, nekatere elemente (K, Mg, Zn, Cu, Fe) in vitamine (vitamini skupine B, β-karoten, vitamin E, vitamin C), sekundarne rastlinske metabolite (flavonoidi, fitosteroli) ter prehransko vlaknino. Zaradi močne raznolikosti in prisotnih zrn cvetnega prahu različnih rastlin je v mešanem cvetnem prahu opazen velik razpon med najnižjo in najvišjo vrednostjo za vsebnost posamezne hranljive snovi (Campos in sod., 2008; Campos in sod., 2016). Cvetni prah predstavlja tudi odličen vir energije, energijska vrednost variira med 1590 in 2050 kJ/100 g (Yang in sod., 2013; Bogdanov, 2016).

Cvetni prah vsebuje veliko različnih biološko aktivnih spojin, kot so flavonoidi (katehin, kamferol, kvercetin, izoramnetin), fitosteroli in karotenoidni pigmenti (likopen in zeaksantin), ki lahko delujejo antioksidativno, protimikrobno, antikancerogeno in protivnetno (Komosinska-Vassev in sod., 2015; Denisow in Denisow-Pietrzyk, 2016; Bogdanov, 2016; Kaškonienė in sod., 2020).

Iz preglednice 6, kjer je podana hranilna vrednost slovenskega cvetnega prahu osmukanca (Lilek in sod., 2015), je razvidno, da od hranljivih snovi cvetni prah vsebuje največ ogljikovih hidratov, sledijo beljakovine in maščobe. Ker je vsebnost vode v svežem cvetnem prahu osmukancu zelo variabilna, so rezultati podani tudi na suho snov.

Podrobnejša analiza ogljikohidratne sestave cvetnega prahu osmukanca (Pucihar, 2017; Bertoncelj in sod., 2018) je pokazala, da med enostavnimi ogljikovimi hidrati v cvetnem prahu prevladujeta fruktoza (od 13,17 do 27,84 g/100 g suhe snovi) in glukoza (od 10,60 do 28,49 g/100 g suhe snovi). Cvetni prah pa je tudi dober vir prehranske vlaknine, vsebnost topne prehranske vlaknine je v območju od 0,62 do 5,21 g/100 g suhe snovi, vsebnost netopne prehranske vlaknine pa od 7,72 do 17,89 g/100 g suhe snovi (Bertoncelj in sod., 2018).

Cvetni prah vsebuje tudi različne elemente in vitamine (preglednica 7). Variabilnost v njihovi vsebnosti, predvsem pri mešanih vrstah cvetnega prahu, pripisujejo različnim vrstam cvetnega prahu rastlin. Od elementov je najbolj zastopan kalij (60 % od skupne vsebnosti)

Preglednica 7: Vsebnost elementov in vitaminov v cvetnem prahu osmukancu (Campos in sod., 2008)
Table 7: Contents of elements and vitamins in bee pollen (Campos et al., 2008)

Elementi	mg/100 g suhe snovi	Vitamini	mg/100 g suhe snovi
kalij (K)	400–2000	provitamin A (β-karoten)	1–20
magnezij (Mg)	20–300	vitamin B1 (tiamin)	0,6–1,3
kalcij (Ca)	20–300	vitamin B2 (riboflavin)	0,6–2
fosfor (P)	80–600	vitamin B3 (niacin)	4–14
železo (Fe)	1,1–17	vitamin B5 (pantotenska kislina)	0,5–2
cink (Zn)	3–25	vitamin B6 (piridoksin)	0,2–0,7
baker (Cu)	0,2–1,6	vitamin B7 (biotin)	0,05–0,07
mangan (Mn)	2–11	vitamin B9 (folna kislina)	0,3–1
		vitamin C (askorbinska kislina)	7–56
		vitamin E (tokoferol)	4–32

(Campos in sod., 2008). Cvetni prah je dober vir vitaminov, topnih v vodi, vitaminov skupine B ter vitamina C (Soares de Arruda in sod., 2013).

3.3 SESTAVA MATIČNEGA MLEČKA

Sestava matičnega mlečka je kompleksna, odvisna je od sezonskih in okoljskih dejavnikov, načina pridobivanja ter prehrane in starosti čebel. Vsebnost vode je v matičnem mlečku zelo visoka (od 60 do 70 %), suho snov pa predstavljajo ogljikovi hidrati, proteini, bioaktivni peptidi, aminokisline, maščobe ter manjša količina vitaminov in mineralov (Sabatini in sod., 2009).

Matični mleček vsebuje večinoma zelo specifične kratkoverižne mono- in dihidroksi maščobne kisline z 8–10 ogljikovimi atomi ali dikarboksilne kisline. Za matični mleček je specifična trans-10-hidroksi-2-decenojska kislina (10-HDA), ki jo je v matičnem mlečku največ (več kot 50 % vseh maščobnih kislin). 10-HDA je značilna samo za matični mleček, zato je njena vsebnost v matičnem mlečku pomemben kriterij njegove pristnosti (Ramadan in Al-Ghamdi, 2012).

Sestava sladkorjev, vsebnost vode, beljakovin in 10-HDA so najbolj pomembni kriteriji za karakterizacijo matičnega mlečka (Sabatini in sod., 2009; Bobiş in sod., 2017). Pomemben kriterij kakovosti matičnega mlečka so tudi senzorične lastnosti. Matični mleček je umazano bele do bledo rumene barve, gosto tekoč, pogosto nehomogen (zrnast, peskast) zaradi prisotnosti netopnih granul različnih velikosti. Ima vonj po kislem, rezkem, kisel okus ter ostro, pikantno aromo, lahko po vosku, po živalih. S staranjem barva matičnega mlečka temni, okus lahko postane žarek (ISO, 2016).

V preglednici 8 je podan predlog standardne sestave matičnega mlečka in rezultati analiz slovenskega matičnega mlečka.

3.4 SESTAVA PROPOLISA

Sestava propolisa je zelo raznolika, odvisna je od rastlin, na katerih so čebele nabirale surovino, od klimatskih razmer v času nabiranja pa tudi od načina pridobivanja in vrste čebel, ki imajo različne preference do posameznih rastlin. Propolis vsebuje različne smole (50 %), voske (30 %), eterična olja in druge aromatične spojine (10 %), cvetni prah (5 %) ter druge sestavine, kot so aminokisline, vitamini in minerali (Viuda-Martos in sod., 2008; Pasupuleti in sod., 2017).

V propolisu so identificirali več sto različnih spojin. Glavne so terpenoidi in fenolne spojine, kamor spadajo flavonoidi ter fenolne kisline in njihovi estri, ki so odgovorni za protivirusno in protivnetno delovanje propolisa. Naravne fenolne spojine delujejo tudi kot antioksidanti. Najbolj značilne fenolne spojine propolisa so flavonoidi pinocembrin, pinobanksin, krizin, galangin, kamferol in kvercetin, fenolne kisline cimetna, *p*-kumarna, kavna in ferulna kislina ter fenetilni ester kavne kisline (CAPE) in artepilin C (Huang in sod., 2014).

4 UPORABA ČEBELJIH PRIDELKOV V PREHRANI

Med, cvetni prah, matični mleček in propolis so čebelji pridelki, ki jih ljudje uživajo zaradi odlične hranilne vrednosti, kot tudi zaradi njihovih funkcionalnih lastnosti in biološke aktivnosti. Zaradi svojih lastnosti so tudi primeren dodatek oz. potencialna sestavina za različna živila. Potrebno pa je upoštevati nekatere previdnostne ukrepe za uporabo v prehrani v samostojni obliki ali kot dodatek živilom, da bi se izognili morebitnim alergijskim reakcijam pri osebah, občutljivih na posamezne čebelje pridelke oz. katero od njihovih sestavin. Zato je potrebno

Preglednica 8: Sestava svežega matičnega mlečka
Table 8: Composition of fresh royal jelly

Parameter	Predlog standardne sestave[1]	Zahtevana sestava po ISO 12824[2]	Slovenski matični mleček[3, 4]
vsebnost vode (g/100 g)	60–70	62,0–68,5	62,0–66,7
vsebnost maščob (g/100 g)	3–8	2–8	4,44–6,19
vsebnost 10-HDA (g/100 g)	> 1,4	≥ 1,4	2,32–3,21
vsebnost beljakovin (g/100 g)	9–18	11–18	11,6–13,6
vsebnost fruktoze (g/100 g)	3–13	2–9	2,3–4,5
vsebnost glukoze (g/100 g)	4–8	2–9	3,4–5,3
vsebnost saharoze (g/100 g)	0,5–2,0	< 3,0	0–2,0
vsebnost pepela (g/100 g)	0,8–3,0	/	0,94–1,23

[1] Sabatini in sod., 2009, [2] ISO 12824: 2016; [3] Štaudohar, 2014; [4] Kandolf Borovšak in sod., 2017; / ni podatka

z uživanjem čebeljih pridelkov začeti previdno in zaužito količino povečevati postopoma (Bogdanov, 2011).

Med ljudje v vsakdanji prehrani uživamo že od nekdaj, v zadnjem času narašča uporaba cvetnega prahu, matični mleček in propolis pa se uživata predvsem v obliki prehranskih dopolnil in uporabljata v apiterapiji. Apiterapija je veda o tem, kako si s pomočjo čebeljih pridelkov krepimo in ohranjamo zdravje. Začetki apiterapije segajo stoletja nazaj do egipčanske, grške, kitajske, babilonske in drugih civilizacij. Trditve o zdravilnih učinkih apiterapije temeljijo predvsem na dejanskih izkušnjah posameznikov in tradicionalni uporabi (Bogdanov, 2011; Fratellone in sod., 2016, Yucel in sod., 2017).

Med je eno najbolj kompleksnih naravnih živil in edino sladilo, ki ga človek uporablja brez predhodne predelave. Uživanje medu je primerno za ljudi vseh starostnih skupin, tudi za nosečnice in doječe matere, le dojenčkom in otrokom do 1. leta starosti ga zaradi možnosti prisotnih spor *Clostridium botulinum* ne smemo ponuditi. V okviru zagotavljanja uravnotežene prehrane in skrbi za zdravje je vsekakor priporočljivo, da ga vključimo v vsakodnevne obroke in z njim nadomestimo kuhinjski sladkor in druga sladila.

Ogljikovi hidrati so glavna sestavina medu in s prehranskega vidika zelo pomembni. Enostavna sladkorja, glukoza in fruktoza, predstavljata hitro izkoristljiv vir energije. Ob zamenjavi kuhinjskega sladkorja z medom pa hkrati vnesemo tudi manjše količine vitaminov, mineralov in drugih bioaktivnih spojin, ki jih sladkor ne vsebuje. Zaradi velike vsebnosti fruktoze ima med manjši vpliv na raven glukoze v krvi kot bel sladkor, kar vpliva tudi na vrednost glikemijskega indeksa. Deibert in sod. (2010) so na osnovi klinične študije z 10 udeleženci, za pet od osmih vrst nemškega medu, določili nizke vrednosti glikemijskega indeksa, pod 55. Samo gozdni med je imel vrednost nad 70, kar predstavlja visok glikemijski indeks. Vrste medu z nizkim glikemijskim indeksom, ki vsebujejo več fruktoze kot glukoze (npr. akacijev in kostanjev med), bi lahko pod ustreznim nadzorom uživali tudi diabetiki oziroma bi se potencialno lahko uporabile za obvladovanje slakorne bolezni. Vendar, ker mehanizem hipoglikemičnega učinka medu ni pojasnjen, velja v praksi zadržanost in previdnost (Meo in sod., 2017; Bobiş in sod., 2018).

Rezultati raziskav kažejo, da je za doseganje ugodnih učinkov medu na prehranski status in zdravje posameznika potrebno uživati večje količine medu, od 50 do 80 g (Bogdanov in sod., 2008) oz. od 70 do 90 g (Ajibola, 2015) dnevno, kar ni v skladu s priporočili za vnos prostih sladkorjev (WHO, 2015), h katerim prištevamo sladkorje iz medu.

Med vsebuje tudi več oligosaharidov in nekaj polisaharidov z nizko molekulsko maso in ima zato prebiotične lastnosti. Oligosaharidi so ogljikovi hidrati s 3 do 9 monomernimi enotami, ki so rezistentni na prebavo v tankem črevesu človeka, delno se razgradijo v debelem črevesu do kratkoverižnih maščobnih kislin, ki predstavljajo pomembno hranilo za mikrobioto (Mohan in sod., 2017; Cornara in sod., 2017). Oligosaharidi povečajo število in aktivnost koristnih mikroorganizmov (laktobacilov in bifidobakterij) v prebavnem traktu (Ajibola, 2015; Begum in sod., 2015; Yucel in sod., 2017; Pasupuleti, 2017). Medovi iz mane vsebujejo večjo količino (do 10 g/100 g) in več različnih oligosaharidov, zato imajo tudi večji prebiotični učinek (Bogdanov in sod., 2008).

Vsebnost posameznih vitaminov in elementov v medu je majhna, zato je njihov prispevek pri priporočenih dnevnih vnosih (Referenčne vrednosti…, 2016) zgolj neznaten. Če izpostavimo kalij, ki ga je v medu največ, bi z eno žlico medu (približno 20 g) zaužili do 5,5 % dnevnega vnosa, odvisno od vrste medu (Kropf in sod., 2009). To pomeni, da med ni pomemben vir kalija v naši prehrani, je pa v oziru zastopanosti elementov ustreznejša prehranska izbira med sladili kot kuhinjski sladkor.

Med fiziološke učinke medu spadajo poleg že omenjenega prebiotičnega učinka tudi antioksidativna in protimikrobna učinkovitost, protivnetno delovanje in zaviranje encimskega porjavenja. Medu pripisujejo tudi ugodne učinke pri zdravljenju različnih bolezni, preprečevanju pojava določenih bolezni, tudi rakavih obolenj. Po navedbah mnogih avtorjev vpliva med ugodno na delovanje srca, upočasnjuje razvoj ateroskleroze, pospešuje izločanje strupov iz telesa in pomaga pri boleznih dihal. Vse te funkcionalne lastnosti se večinoma pripisujejo fenolnim spojinam, kot so flavonoidi (Bogdanov in sod., 2008; Viuda-Martos in sod., 2008; Bobiş in sod., 2017; Pasupuleti in sod., 2017; Combarros-Fuertes in sod., 2019) in bioaktivnim peptidom, kot so defensin-1 in želeini (Cornara in sod., 2017). Med zavira rast in razvoj velikega števila mikroorganizmov zaradi velike vsebnosti sladkorjev, ki povzročijo osmotski učinek, majhne vsebnosti vode, nizke vrednosti vodne aktivnosti, nizke vrednosti pH in prisotnosti spojin z antimikrobnim delovanjem. Pod vplivom encima glukoza oksidaza, ki ga vsebuje med, nastane vodikov peroksid, ki pripomore k celjenju tkiva in deluje antibakterijsko. Zaradi teh lastnosti med že od nekdaj uporabljajo tudi za zdravljenje ran (Molan, 2006; Ajibola in sod., 2012; Yilmaz in Aygin, 2020), hkrati pa lahko pripomore tudi k zdravju ustne votline, saj preprečuje rast bakterij, ki povzročajo karies, manj vpliva na erozijo zobne sklenine (Bogdanov in sod., 2008) ter je lahko učinkovito sredstvo proti parodontozi, saj zavira delovanje parodontopatogenih bakterij in tako predstavlja cenejšo alternativno metodo zdravljenja (Podržaj, 2011).

Čebelji cvetni prah, kamor prištevamo cvetni prah

osmukanec in cvetni prah izkopanec, je pomemben vir hranil in energije in lahko predstavlja dodatek k vsakodnevni prehrani. Cvetni prah osmukanec vsebuje vse potrebne esencialne spojine, potrebne v prehrani človeka, vključno z aminokislinami in maščobnimi kislinami. Bogdanov (2011) navaja, da je dnevni vnos 10 g cvetnega prahu realen glede na ceno tega čebeljega pridelka in že omogoča preventivno delovanje. Za preventivo in izboljšanje zdravja se priporoča od 10 do 20 g cvetnega prahu dnevno, običajno 3 mesece zaporedoma, 2-krat letno. V apiterapiji pa je dnevni odmerek cvetnega prahu večji, od 20 do 50 g dnevno, zaužit 3-krat dnevno, 1 do 2 uri pred obrokom. Za večjo senzorično sprejemljivost cvetnega prahu se priporoča mešanje z medom, s sokom ali z mlečnimi izdelki, npr. jogurti ali s skuto ter sadjem. Za povečanje prebavljivosti cvetnega prahu osmukanca v organizmu in s tem razpoložljivosti posameznih hranil je ta čebelji izdelek priporočljivo pred uporabo zmleti ali namočiti zrna v topli vodi ali drugi tekočini, s čimer ovojnica zrna cvetnega prahu postane bolj prepustna (Bogdanov, 2011; Komosinska-Vassev in sod., 2015; Denisow in Denisow-Pietrzyk, 2016; Yucel in sod., 2017).

Zaradi velike variabilnosti v sestavi cvetnega prahu (preglednici 6 in 7), kot posledici različnega botaničnega izvora, je težko realno oceniti vnos posameznih makro- in mikrohranil v prehrani z uživanjem dnevno priporočene količine cvetnega prahu. Cvetni prah je dober vir beljakovin in esencialnih aminokislin ter maščobnih kislin, sladkorjev fruktoze in glukoze, kot tudi nekaterih vitaminov in mineralov. Doprinos teh komponent k priporočenemu dnevnemu vnosu je pri cvetnem prahu večji kot pri medu. Cvetni prah je tudi dober vir prehranske vlaknine (Bertoncelj in sod., 2018), komponente, za katero je ocenjeni vnos s prehrano pri prebivalcih razvitih držav prenizek glede na priporočen dnevni vnos, ki znaša najmanj 30 g na dan za odrasle (Referenčne vrednosti…, 2016). Zaužitje 20 g cvetnega prahu dnevno bi prispevalo okoli 10 % priporočenega dnevnega vnosa prehranske vlaknine. Na osnovi vsebnosti naštetih hranljivih snovi cvetni prah izboljša presnovo ter splošno telesno zmogljivost in je zelo primeren za okrevanje po boleznih ter za ljudi s premajhno telesno maso.

Kot kažejo številne raziskave v zadnjih letih ima cvetni prah poleg visoke hranilne vrednosti tudi veliko vsebnost biološko aktivnih snovi (flavonoidov, fitosterolov, različnih encimov), ki prispevajo k številnim funkcionalnim lastnostim (Komosinska-Vassev in sod., 2015; Denisow in Denisow-Pietrzyk, 2016; Yucel in sod., 2017; Cornara in sod., 2017; Kaškonienė in sod., 2020). Flavonoidi delujejo antioksidativno, protimikrobno, antikancerogeno in protivnetno, ščitijo pred pojavom ateroskleroze in drugih bolezni srca in ožilja, krepijo imunski sistem ter zavirajo prehitro staranje. Fitosteroli vplivajo na nivo holesterola v krvi in z njim povezanih bolezni srca in ožilja, zavirali naj bi tudi nastanek nekaterih vrst raka. Vse biološko aktivne spojine v cvetnem prahu imajo močno protivnetno delovanje in spodbujajo delovanje imunskega sistema. Cvetni prah tako nima le vloge prehranskega dodatka in funkcionalnega živila, ampak tudi potencialnega zdravila. Natančno stopnjo biološke učinkovitosti pa je težko določiti zaradi velike variabilnosti v sestavi tega čebeljega pridelka, ki je odvisna od botaničnega izvora. Za namen zdravljenja je nujno potrebno definirati standarde kakovosti cvetnega prahu, ki bi olajšali uporabo cvetnega prahu v medicinske namene (Denisow in Denisow-Pietrzyk, 2016).

Matični mleček in propolis se bolj kot živilo uporabljata v obliki prehranskih dopolnil ali v kombinaciji z medom. Matični mleček zaradi njegovih specifičnih senzoričnih lastnosti in visoke cene potrošniki bolj dojemajo kot domače zdravilo. Biološka aktivnost matičnega mlečka se pripisuje predvsem maščobnim kislinam (10-HDA), bioaktivnim peptidom, specifičnim proteinom matičnega mlečka (npr. rojalaktina in rojalizina) in fenolnim spojinam. Matični mleček se že od davnih časov uporablja v tradicionalni medicini, zaradi številnih pozitivnih lastnosti sodi v skupino funkcionalnih živil. Nekateri biološki in terapevtski učinki uživanja matičnega mlečka so že bili potrjeni, vendar pa kemijska sestava in biološko aktivne snovi matičnega mlečka še niso v celoti poznane. Z različnimi raziskavami so dokazali antioksidativno, protibakterijsko in protivnetno delovanje matičnega mlečka (Ramadan in Al-Ghamdi, 2012; Pasupuleti in sod., 2017; Ahmad in sod., 2020). Matični mleček antibakterijsko deluje tako na gram-pozitivne kot na gram-negativne bakterije, učinek pripisujejo specifičnim prostim maščobnim kislinam ter proteinom matičnega mlečka (Ramadan in Al-Ghamdi, 2012). Zelo razširjena je uporaba matičnega mlečka v prehranskih dopolnilih zaradi prepričanja, da ima njegovo uživanje podobne učinke na ljudi, kot jih ima na čebele. Čebela matica, ki je izključno hranjena z matičnim mlečkom, ima daljšo življenjsko dobo ter bolj razvite žleze v primerjavi s čebelo delavko (Morita in sod., 2012). Uživanje matičnega mlečka vpliva tudi na boljšo vzdržljivost pri športnikih. Športniki, ki uživajo matični mleček kot prehransko dopolnilo (1,2 g/dan), so bolj vzdržljivi v primerjavi s športniki, ki tega prehranskega dodatka ne uživajo. Med telesno dejavnostjo vzdržljivost pada zaradi povišanja lipidnih hidroperoksidov v krvi. Matični mleček s svojimi sestavinami kot antioksidant pomaga pri zniževanju hidroperoksidov v krvi in posledično pripomore k boljši vzdržljivosti (Bogdanov, 2011). Matični mleček zaradi vsebnosti specifičnih proteinov in fenolnih spojin, ki so zelo učinkovite pri odstranjevanju prostih radikalov, deluje tudi antioksidativno (Bobiş in sod., 2017: Cornara in

sod., 2017; Yucel in sod., 2017). Študije na živalih kažejo, da ima matični mleček tudi antitumorsko delovanje, ki se ga pripisuje predvsem vsebnosti 10-HDA ter nasičenim dikarboksilnim kislinam (Oršolić, 2013).

Tudi propolis je del tradicionalne medicine, vsebuje številne bioaktivne spojine, zlasti fenolne spojine, ki delujejo antioksidativno, protivirusno in protivnetno (Huang in sod., 2014; Cornara in sod., 2017; Pasupuleti in sod., 2017).

5 POMEN STANDARDIZACIJE ČEBELJIH PRIDELKOV

Na osnovi različnih bioloških lastnosti čebeljih izdelkov, dokazanih z znanstvenimi študijami, so bili izvedeni tudi poskusi aplikacij nekaterih od teh pridelkov v kliničnih okoljih, vendar je njihova farmakološka in medicinska standardizacija zaradi velike kemijske variabilnosti otežena, biološka učinkovitost čebeljih pridelkov je namreč odvisna od botaničnega in geografskega porekla, vrste medonosnih čebel, postopkov čebelarjenja in postopkov s pridelki po njihovem pridobivanju. Izolirane so bile različne spojine z dokazanim biološkim učinkom, kar kaže na pomembnost čebeljih pridelkov za odkrivanje zdravil iz naravnih virov (Cornara in sod., 2017; Pasupuleti in sod., 2017; Ahmad in sod., 2020). Potrebne pa so dodatne, ustrezne klinične študije za potrditev aktivnosti čebeljih pridelkov oz. njihovih sestavin. Zaradi nezadostnih utemeljenih znanstvenih dokazov o učinkovanju na zdravje do sedaj tudi ni bila odobrena nobena zdravstvena trditev za čebelje pridelke. Zdravstvena trditev pomeni vsako trditev, ki navaja, domneva ali namiguje, da obstaja povezava med kategorijo živil, živilom ali eno od njegovih sestavin na eni strani in zdravjem na drugi strani (Uredba 1924/2006). V postopku sprejemanja novih zdravstvenih trditev za živila je bilo na Evropsko agencijo za varnost hrane (EFSA) vloženih tudi nekaj vlog zdravstvenih trditev za matični mleček in propolis (Vujić in Pollak, 2015). Predlagane trditve so se nanašale na krepitev imunskega sistema, vitalnost organizma, povečanje antioksidativne sposobnosti organizma, ohranjanje zdravega delovanja jeter, povečanje fiziološke odpornosti organizma, krepitev zdravja zgornjih dihal, izboljšanje kakovosti življenja žensk v menopavzi, spodbujanje delovanja srca, uravnotežen nivo lipidov v krvi. EFSA je pri presojanju upravičenosti trditev za tradicionalna živila, ki se uporabljajo za domače zdravljenje, zaradi njihove naravne in sezonske variabilnosti precej zadržana. Zato je vse predlagane zdravstvene trditve zavrnila, ker živilo oziroma sestavina živila ni bila dovolj dobro opredeljena in karakterizirana ter za zatrjevani učinek ni bilo dovolj utemeljenih znanstvenih dokazov, tudi zaradi pomanjkanja ustreznih kliničnih študij. Seznam zavrnjenih zdravstvenih trditev za čebelje pridelke in utemeljitve njihove zavrnitve so dostopni na spletni strani Evropske komisije, ki vodi tako imenovani Register skupnosti v zvezi s prehranskimi in zdravstvenimi trditvami (European Commission, 2020).

Standardizacija posameznih čebeljih pridelkov v smislu standardizacije njihove sestave, ki vključuje tako vsebnost makro- in mikrohranil, kot tudi bioaktivnih spojin, je pomembna tudi za lažje vrednotenje doseganja priporočenih vrednosti posameznih hranljivih snovi glede na priporočila za vnos energije in hranil (Referenčne vrednosti…, 2016). Na ta način bi lahko v prehrano vključili čebelje pridelke z optimalnimi lastnostmi za potrebe posameznika. Hkrati pa bi standardizacija omogočila lažje preverjanje pristnosti čebeljih pridelkov, ker so le-ti zaradi visoke cene podvrženi tudi potvorbam, z definirano sestavo pa bi potvorjene čebelje pridelke lažje odkrili in s tem tudi zaščitili potrošnika.

6 DODATEK ČEBELJIH PRIDELKOV DRUGIM ŽIVILOM

V današnjem času potrošniki želijo živila, ki so bolj naravna in vsebujejo manj aditivov. Med in ostali čebelji pridelki kažejo nekatere pozitivne lastnosti, ki omogočajo njihov dodatek v različna živila. Med lahko nadomesti nekatere konvencionalne dodatke, kar omogoča tudi razvoj novih živil. Med se že od nekdaj uporablja kot sladilo v različnih pijačah in živilih, kot so brezalkoholne sadne pijače, jogurtovi napitki, športni napitki. V mleku in mlečnih izdelkih med spodbuja rast mlečnih starter kultur zaradi prisotnih oligosaharidov. Med tudi preprečuje encimsko porjavenje sadja in zelenjave in izdelkov iz njih in bi se lahko uporabljal kot alternativa sulfitom. V pekovskih izdelkih dodatek medu vpliva na zadrževanje vode, kar povzroči boljšo teksturo in izboljšane ostale senzorične lastnosti. V mesu in mesnih izdelkih med lahko preprečuje mikrobiološki kvar in oksidacijo maščob ter zmanjša nastanek heterocikličnih aromatskih aminov (Viuda-Martos in sod., 2008; Bogdanov, 2011; Yucel in sod., 2017).

Cvetni prah osmukanec se dodaja predvsem mlečnim in pekovskim izdelkom za izboljšanje njihove prehranske vrednosti. Atallah (2016) navaja, da so imeli probiotični jogurti z dodatkom cvetnega prahu (0,8 %) ali matičnega mlečka (0,6 %) boljše senzorične lastnosti (aromo, teksturo in celokupno všečnost), večjo vsebnost nekaterih elementov (Ca, P, K, Mg, Mn, Fe in Zn) ter boljše reološke lastnosti (manjša sinereza) v primerjavi z jogurtom brez dodatka prej omenjenih čebeljih pridelkov. Krystyjan in sod. (2015) so proučevali fizikalno-

kemijske in antioksidativne lastnosti keksov z dodanim cvetnim prahom. Pri pripravi keksov so del moke (od 2,5 do 10 %) nadomestili z mletim cvetnim prahom. Dodatek cvetnega prahu je vplival na povečanje vsebnosti beljakovin, sladkorjev, prehranske vlaknine in fenolnih spojin ter večjo antioksidativno učinkovitost in izboljšane senzorične lastnosti obogatenih keksov. Podobno Solgajová in sod. (2014) navajajo, da imajo keksi, obogateni s cvetnim prahom oljne ogrščice, boljšo tako hranilno vrednost kot tudi tehnološke in senzorične lastnosti.

Matični mleček in propolis se običajno dodajata medu za povečanje vsebnosti bioaktivnih učinkovin in večjo antioksidativno učinkovitost (Juszczak in sod., 2016).

7 ZAKLJUČEK

Čebelji pridelki so popolnoma naravna živila, ki jih lahko uživamo samostojno ali kot dodatek drugim živilom za izboljšanje njihove hranilne vrednosti in funkcionalnih lastnosti. Med, cvetni prah osmukanec in matični mleček imajo visoko hranilno vrednost in dokazano biološko delovanje. Z uživanjem čebeljih pridelkov doprinesemo k vnosu hranljivih snovi in dodatno zagotovimo organizmu tudi bioaktivne spojine, ki lahko ugodno vplivajo na zdravje. Ob akcijah na nacionalnem, evropskem in svetovnem nivoju in rezultatih znanstvenih raziskav o hranilni sestavi in ugodnem delovanju čebeljih pridelkov na zdravje, le-ti dodatno pridobivajo na pomenu v vsakdanji prehrani. Raziskovalni projekti z namenom karakterizacije čebeljih pridelkov, ki jih izvaja Čebelarska zveza Slovenije s sodelujočimi inštitucijami, so/bodo omogočili oblikovanje nacionalnih standardov kakovosti in posledično slovenskim potrošnikom zagotovili uživanje kakovostnih, pristnih in varnih čebeljih pridelkov.

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Influence of in ovo and pre-starter zinc and copper supplementation on growth performance and gastrointestinal tract development of broiler chickens

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Influence of in ovo and pre-starter zinc and copper supplementation on growth performance and gastrointestinal tract development of broiler chickens

Abstract: This experiment was on 350 uniform sized Cobb broiler hatching eggs (60 g) to assess the response of trace mineral supplementation (Zinc and copper) on growth performance and gastrointestinal tract development in broiler chicken. The fertile eggs were divided into groups with in ovo trace mineral solution containing zinc (80 µg) and copper (16 µg) and without in ovo administration. After hatching, the chicks were further divided into four groups: Group I served as control without in ovo and without post-hatch supplemented diet (WoINOVO-WoPHS), birds in Group II were without in ovo and with post-hatch supplemented diet (WoINOVO-WPHS) (100 % higher level of zinc 200 ppm, copper 30 ppm in diet), birds in Group III had in ovo (zinc, 80 µg; copper,16 µg) and without post-hatch supplemented diet (WINOVO-WoPHS) and birds in Group IV had in ovo and with post-hatch supplemented diet (WINOVO-WPHS). Data collected were subjected to completely randomized design. Hatchability, live weight gain, feed intake and feed conversion ratio at 0–3 wk were not affected ($p > 0.05$) by in ovo administration of the mineral. Post-hatch supplementation of zinc and copper without in ovo supplementation showed better feed conversion ratio at 3–5 wk of age. It could be recommended that for improved post-hatch performance, broiler chickens diets could be supplemented with inorganic zinc and copper.

Key words: poultry; broilers; animal nutrition; feed additives; in ovo; trace minerals; growth; gastrointestinal development; immune response

Vpliv dodatka cinka in bakra v jajce in v krmo po izvalitvi na rast in razvoj prebavil pri brojlerskih piščancih

Izvleček: Poskus je bil izveden na 350 valilnih jajcih pitovnih piščancev Cobb enotne velikosti, da bi ocenili odziv na dodatek mikromineralov (cink in baker) na rast in razvoj prebavil pri pitovnih piščancih. Oplojena jajca so bila razdeljena v dve skupini, ena je bila tretirana z raztopino cinka (80 µg) in bakra (16 µg), druga pa ne. Po izvalitvi so bili piščanci razdeljeni v štiri skupine: skupina I je služila kot kontrola brez poseganja v jajce in brez dodatka krmi po izvalitvi (WoINOVO-WoPHS), skupina II ni dobila cinka in bakra v jajce, ampak samo v krmo po izvalitvi (WoINOVO-WPHS), skupina III je dobila cink in baker v jajce, ne pa v krmo po izvalitvi (WINOVO-WoPHS), in skupina IV, ki je dobila dodatek cinka in bakra v jajce in v krmo po izvalitvi (WINOVO-WPHS). Zbrani podatki so bili uporabljeni za randomizirano zasnovo poskusa. Valilnost jajc, prirast, zauživanje in izkoriščanje krme v obdobju od izvalitve do treh tednov starosti niso kazali vpliva dodatka cinka in bakra ($p > 0,05$). Dodajanje cinka in bakra po izvalitvi brez dodajanja v jajca je bilo povezano z boljšim izkoriščanjem krme med 3. in 5. tednom starosti. Za boljše proizvodne rezultate priporočamo dodajanje anorganskega cinka in bakra v krmo za piščance.

Ključne besede: perutnina; pitovni piščanci; prehrana živali; krmni dodatki; in ovo; mikrominerali; rast; razvoj prebavil; imunski odziv

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1 INTRODUCTION

The perinatal period is a most crucial time in the development of a young chick as this is a transitional period in which the chicks undergoes metabolic and physiological shifts from the utilization of egg nutrients to exogenous feed (Ferket, 2012). However, with the current work flow of commercial hatcheries and considering time to transport and delivery of newly hatched chicks to broiler farms, the chicks are inevitably exposed to delayed feeding for 48–72 hrs (Noy et al., 2001; Panda et al., 2008; Uni and Smith, 2017). As a consequence of delayed feeding, chicks undergo starvation and allocate the limited reserves of nutrients to the upkeep of thermal regulation and metabolism which restricts growth and development (Ricklefs, 1987; Pinchasov and Noy, 1993). Delayed feeding causes poor viability and slow growth (Juul-Madsen et al., 2004), increases the weight loss (Bhanja et al., 2015), makes the hatchlings more susceptible to pathogens (Dibner, 1999), restricts the development of critical tissues (Halevy et al., 2000), influences the development of post-hatch gastrointestinal tract maturation (Geyra et al., 2001), increases mortality rate and consequently retards post-hatch growth of day old chicks (Careghi et al., 2005) as pronounced in present day stock of commercial broilers.

The development of the gut occurs throughout incubation (Romanoff, 1960), but the functional abilities of the gut only begins to develop about the time the amniotic fluid is orally consumed by the 18th day old embryo. The weight of the intestine, as a proportion of embryonic weight, increases from approximately 1 % at 17 days of incubation to 3.5 % at hatch. Rapid intestinal growth is due to great increase in cell numbers and size, due to accelerated enterocyte proliferation and differentiation and intestinal crypts formation (Uni et al., 2000, Geyra et al., 2001). Therefore, intestinal tissue growth, maturation and metabolism are of great importance in the last period on poultry embryonic development and the early post-hatch period. The sooner the intestine achieves functional capacity, quicker the chicks can utilize dietary nutrients, absorb minerals and vitamins and support the development of skeleton, immune system, breast muscle.

The current focus of broiler management needs to be shifted to the fortification of perinatal (last few days of pre-hatch to first few days post-hatch) nutrition so that the early given growth impetus results in achieving the targeted growth in less time. Accordingly, in ovo administration of nutrients in amnion prepares the opportunity for chicks to orally consume supplemented nutrients and develop their digestive and absorptive ability prior to hatch. Growth and development of the embryo and hatchling are dependent on the nutrients in the fertile egg (Richards, 1997). Residual yolk is the main source of nutrients during the transitional period between the hatch and grow-out phases (Gonzales et al., 2003; Henderson et al., 2008).

Table 1: Ingredient and nutrient composition (%) of experimental diets

	Post-hatch supplemented (0–3 days)	Starter (4–21 days)	Finisher (22–35 days)
Ingredients (%)			
Maize	57.53	58.06	62.31
Soybean meal	36.00	36.00	32.00
Sunflower oil	2.00	2.00	2.25
Limestone	1.00	1.00	1.00
Di-calcium phosphate	1.75	1.75	1.50
Salt (NaCl)	0.35	0.35	0.35
L-Lysine HCl	0.59	0.37	0.20
DL-Methionine	0.33	0.22	0.14
L-Threonine	0.20	0.00	0.00
Vitamin/mineral premix *	0.25	0.25	0.25
Analysed Nutrient composition (%)			
ME (MJ/kg) **	12.49	12.44	12.74
Crude protein	22.7	22.1	20.5
Lysine	1.68	1.34	1.11
Methionine	0.63	0.50	0.41
Threonine	0.97	0.77	0.73
Arginine	1.40	1.40	1.28
Calcium	1.04	1.04	0.98
Available phosphorus **	0.45	0.45	0.40
Zinc (ppm) ***	190.3	90.3	88.1
Copper (ppm) ***	29.3	14.3	13.8

* Trace mineral premix 0.1 %, Vit. Premix 0.1 %, B-Complex 0.02 %, Choline 0.05 %. Trace mineral premix supplied mg/kg diet: Mn = 75; Se = 0.2; Fe = 40; Zn = 70; Cu = 10. The vitamin premix supplied per kg diet: Vit. A = 8250 IU; Vit. D_3 = 1200 ICU; Vit. K = 1 mg; Vit. E = 40 IU; Vit. B_1 = 2 mg; Vit. B_2 = 4 mg; Vit. B_{12} = 10 mcg; niacin = 60 mg; pantothenic acid = 10 mg.
** calculated
*** Post hatch additional supplemental zinc @ 100ppm and copper @ 15 ppm

Micro-minerals that are important to bone formation and strength include Cu, Zn, and Mn, which are greatly reduced in concentration in the egg by the 17th day of incubation (doi) (Yair and Uni, 2011). These minerals also participate through their contribution to enzyme activity along metabolic pathways that are related to the formation of the skeletal system (Bao et al., 2007). Zinc participates in important regulatory pathways for bone and cartilage formation, such as collagen synthesis (Starcher et al., 1980), and hydroxyapatite crystallization (Sauer et al., 1997). Copper is part of the linkage between elastin and collagen, which gives the bone its tensile strength (Carlton and Henderson, 1964). Although Zn is important for collagen synthesis, Cu concentrations must be concomitantly sufficient so that fibrils are not weakened and become susceptible to breakage (Rath, 2000)

Currently, copper is added as copper sulphate to pre-mixes of blends for broiler chicken due to its antibacterial properties and to promote the effect of growth. Copper (Cu) is an essential micro element in poultry diets and is required to maintain the proper activities of metalloenzymes associated with iron metabolism. Tyrosinase, oxidase and feroxidase contain Cu, and their activities are dependent on this element, which is an integral part of the cytochrome oxidase system (Wang et al., 2013). Effects of dietary copper-loaded chitosan nanoparticle (CNP-Cu) supplementation on growth performance, haematological and immunological characteristics and the caecal microbiota in broilers were investigated. Results indicated that supplemental CNP-Cu could improve growth performance; affect the immune system (Wang et al., 2013). Varying hatchability with in ovo administration has been reported. There are reports of decreased (Ohta et al., 1999; McGruder et al., 2011) and increased (Bottje et al., 2010) and no effect (Zhai et al., 2011) of hatchability in literature. Therefore, the technique of in ovo administration, nutrient source or dose of nutrients should be perfected to the extent of reducing such loss. Hence, the present study was designed to evaluate if pre-conditioning remains as effective as in ovo supplementation of nutrients in terms of improved growth performance and feed conversion ratio.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL SITE

The animal experimental procedure was approved by ethical committee of ICAR-National Institute of Animal Nutrition and Physiology, Bangalore, India.

2.2 INCUBATION AND IN OVO TREATMENT

Three hundred and fifty uniform sized Cobb broiler eggs of 60 g average weight (55–65 g) were procured from commercial hatchery. In the meantime, three hundred and forty-four eggs (98.29 %) were sorted and incubated with the dry bulb temperature ranging from 37.22–37.78 °C and wet bulb temperature of 29.44–30.56 °C from days 1 to 18. On day 14, all the unfertile eggs (40 eggs: 88.40 % fertility) were removed after candling. On embryonic day 18, fertile eggs were divided into two groups (152 eggs per group): one without supplementation and another supplemented with in ovo enriched solution containing zinc (80 µg) and copper (16 µg) into the amnion of the embryo under a laminar flow system and then transferred into the hatching trays. The relative humidity was increased by setting the wet bulb thermometer reading of more than 32.22 °C from day 18 till hatching. At hatching, 96.9 % hatchability was from group I (147 hatches) and 87.3 % hatchability was recorded from group II to give 132 hatches. One hundred and thirty-two (132) chicks were then selected per group and further divided into four (66 chicks each) groups; Group I served as control without in ovo and without post-hatch supplemented diet (WoINOVO-WoPHS), Group II composed of hatching eggs without in ovo and with post-hatch supplemented diet (100 % higher level of zinc 200 ppm, copper 30 ppm), Group III composed of hatching eggs with in ovo and without post-hatch supplemented diet. (WINOVO-WoPHS) and Group IV consisted of hatching eggs with in ovo and with post-hatch supplemented diet (WINOVO-WPHS). The required amount of trace minerals were weighed and dissolved in the deionized water in such a concentration that 0.5 ml contained the required amount of trace minerals to be injected in one egg. Before injection, the site was suitably sterilized with 70 % ethanol and the injections were done at the broad end of the egg using 25 mm needle and the pinhole site was sealed with sterile paraffin wax immediately, and eggs were transferred to the hatching trays in the incubator. The entire in ovo procedures were completed within 20 minutes after taking out of eggs from the incubator.

2.3 BIRDS AND HOUSING

The chicks (immediately from hatchery) from the different treatment groups were randomly distributed into battery cages (6 replicates with 11 chicks in each replicate), fitted with heating arrangements, feeders, waterers and dropping trays, with 24 hours light and proper air ventilation, and reared under standard manage-

Table 2: Hatchability and chick weight

Groups	Treatments	Egg wt (g)	Chick wt (%)	Hatchability (%)
I	WoINOVO	59.3	41.3	96.9
II	WINOVO	59.4	40.1	87.3
	SEM	0.224	0.575	
	Significance	0.759	0.601	

WoINOVO: Without INOVO; WINOVO : With INOVO

ment conditions. The temperature inside the cage was maintained at 33 °C on day 1 and gradually reduced to 24–25 °C by the end of the third week and maintained. The feed and fresh drinking water were provided ad libitum during the entire experimental period.

2.4 EXPERIMENTAL DIETS

Experimental diets were prepared with maize and soybean meal as major ingredients. The dietary treatments consisted of one normal prestarter diet for group I (WoINOVO-WoPHS) and group III (WINOVO-WoPHS) and one with post-hatch supplemented diet for group II (WoINOVO–WPHS) and group IV (WINOVO-WPHS). Ingredient and nutrient composition of experimental diets are given in Table 1.

2.5 MEASUREMENTS

Body weight changes were recorded every week to ascertain the weekly and overall body weight gain. The experimental diets were given ad libitum and the residue was weighed at weekly interval in order to arrive at feed intake. Based on the data pertaining to the feed intake and body weight gain, the weekly and period wise cumulative feed conversion ratio (FCR) was calculated.

2.6 GASTROINTESTINAL TRACT DEVELOPMENT

Six birds from each treatment were sacrificed by cervical dislocation at weekly interval (0–4 weeks of age) and twelve birds from each treatment at 5 wk of age (all week data not presented). Gut development was measured by recording the weights of gizzard, proventriculus, liver as well as weight and length of duodenum, jejunum, ileum and caecum. Immune organ weight (% of live weight) and meat yield (% of live weight) were recorded at the end of the trial.

Table 3: Growth performance of broiler chicken

	Live weight gain (g/b)			Feed intake (g/b)			Feed conversion ratio		
	0–3 wk	3–5 wk	0–5 wk	0–3 wk	3–5 wk	0–5 wk	0–3 wk	3–5 wk	0–5 wk
Effect of in ovo supplementation (In ovo)									
WoINOVO	829	1072	1901	1102	1765	2866	1.33	1.65	1.51
WINOVO	843	1089	1932	1106	1807	2913	1.31	1.66	1.51
Significance	0.50	0.66	0.52	0.88	0.35	0.44	0.35	0.66	0.97
Effect of post-hatch supplemented diet (PHS)									
WoPHS	841	1077	1918	1108	1805	2912	1.32	1.68	1.52
WPHS	831	1084	1916	1100	1767	2867	1.33	1.64	1.50
Significance	0.65	0.85	0.96	0.80	0.40	0.45	0.68	0.28	0.20
Interaction effect (In ovo × PHS)									
WoINOVO-WoPHS	833	1098	1932	1095	1845[a]	2940	1.31	1.68[a]	1.52
WoINOVO-WPHS	825	1046	1870	1109	1684[c]	2793	1.35	1.62[b]	1.49
WINOVO-WoPHS	849	1055	1904	1120	1765[b]	2885	1.32	1.67[a]	1.52
WINOVO-WPHS	838	1123	1961	1092	1849[a]	2941	1.30	1.66[a]	1.50
SEM	9.93	18.95	23.43	13.22	24.95	30.46	0.01	0.02	0.01
Significance	0.98	0.13	0.23	0.47	0.01	0.10	0.21	0.01	0.61

[a, b, c] = Means in the same column bearing different superscripts differ significantly ($p < 0.05$); WoINOVO-WoPHS = without in ovo and without post-hatch supplemented diet; WoINOVO-WPHS = without in ovo and with post-hatch supplemented diet; WINOVO-WoPHS = with in ovo and without post-hatch supplemented diet; WINOVO-WPHS = with in ovo and with post-hatch supplemented diet; g/b = grams / bird

Table 4: Digestive organ weight (% of live weight) and length (cm/100g live weight) at day 0

	Duodenum		Jejunum		Ileum		Caecum		Liver	Proven-triculus	Gizzard	Yolk
Treatment	Length	Weight	Length	Weight	Length	Weight	Length	Weight	Weight	Weight	Weight	Weight
WoINOVO	19.50	1.69	42.37[b]	2.53[b]	34.43[b]	1.84[b]	8.68[b]	0.86[b]	3.03	1.09	9.24	10.05
WINOVO	19.95	1.98	46.21[a]	2.60[a]	35.47[a]	1.88[a]	10.70[a]	1.11[a]	3.24	1.26	9.59	7.13
SEM	0.59	0.08	2.05	0.16	1.49	0.09	0.62	0.07	0.08	0.12	0.35	0.95
Significance	0.18	0.17	0.04	0.04	0.04	0.01	0.01	0.01	0.65	0.37	0.13	0.09

[a, b] Means in the same column bearing different superscripts differ significantly ($p < 0.05$); WoINOVO = Without INOVO; WINOVO = With INOVO

2.7 STATISTICAL ANALYSIS

The data were subjected to one way analysis of variance (ANOVA) for completely randomized design and tested for significance between the dietary treatments means employing Tukey's HSD Post-hoc test (SAS, 2010).

3 RESULTS

3.1 HATCHABILITY AND CHICK WEIGHT

In Table 2, in ovo supplementation of trace mineral enriched solution did not show any significant difference ($p > 0.05$) in hatchability of in ovo injected group (87.3 %) compared to without in ovo supplementation group (96.9 %).

3.2 GROWTH PERFORMANCE

Live weight gain, feed intake and feed conversion ratio during 0–3 wk and overall phase was not affected ($p > 0.05$) either due to in ovo supplementation of enriched trace mineral solution, post-hatch supplemented diet or their interaction except in 0–3 wk as shown in Table 3. *In* case of feed intake, there was statistically significant differences only between groups WINOVO-WPHS, WINOVO-W0PHS and

Table 5: Digestive organ weight (% of live weight) and length (cm / 100g live weight) at 3rd wk

	Duodenum		Jejunum		Ileum		Caecum		Liver	Proven-triculus	Gizzard
	Length	Weight	Length	Weight	Length	Weight	Length	Weight	Weight	Weight	Weight
Effect of in ovo supplementation (In ovo)											
WoINOVO	2.84	2.09	6.69	3.53	6.54	3.21	1.38	1.47	2.90	1.25	4.95
WINOVO	2.47	2.40	7.15	3.91	7.06	3.57	1.47	1.74	3.41	1.49	5.32
Significance	0.07	0.18	0.29	0.12	0.17	0.07	0.46	0.18	0.03	0.12	0.39
Effect of post-hatch supplemented diet											
WoPHS	2.54	2.26	6.85	3.98	6.43	3.66	1.33	1.53	3.27	1.43	5.13
WPHS	2.77	2.22	6.98	3.46	7.17	3.12	1.52	1.68	3.04	1.32	5.13
Significance	0.25	0.75	0.72	0.04	0.15	0.01	0.13	0.68	0.30	0.39	0.90
Interaction effect (In ovo*PHS)											
WoINOVO-WoPHS	2.69	2.14	6.21	3.57	5.91	3.17	1.37	1.29	2.94	1.29	4.99
WoINOVO-WPHS	2.99	2.03	7.17	3.50	7.17	3.25	1.40	1.64	2.85	1.21	4.90
WINOVO-WoPHS	2.39	2.38	7.50	4.39	6.95	4.14	1.28	1.77	3.60	1.56	5.27
WINOVO-WPHS	2.56	2.42	6.79	3.42	7.17	3.01	1.65	1.72	3.22	1.43	5.36
SEM	0.09	0.12	0.21	0.14	0.22	0.13	0.06	0.11	0.12	0.17	0.09
Significance	0.75	0.63	0.05	0.13	0.33	0.00	0.17	0.55	0.61	0.88	0.90

WoINOVO-WoPHS = Without in ovo and without post-hatch supplemented diet; WoINOVO-WPHS = without in ovo and with post-hatch supplemented diet; WINOVO-WoPHS = With in ovo and without post-hatch supplemented diet; WINOVO-WPHS = With in ovo and with post-hatch supplemented diet

W0INOVO-WPHS but not between groups WINOVO-WPHS and W0INOVO-W0PHS. Post-hatch supplemented group without in ovo supplementation showed better feed conversion ratio at 3–5 wk of age.

3.3 DIGESTIVE ORGAN DEVELOPMENT

In ovo supplementation significantly ($p < 0.05$) increased the weight (% of live weight) of jejunum, Ileum and caecum on the day of hatch in in ovo supplemented group compared to un-injected group (Table 4). At 3rd week of age, in ovo supplementation, post-hatch supplemented diet or their interaction groups did not differ significantly ($p > 0.05$) in all digestive organs weight (% of live weight) and length (cm/100g live weight) except weight of liver, jejunum and ileum (Table 5). Digestive organs length and weight did not show any significant difference at 5th week of age in in ovo supplemented, post-hatch supplemented or their interaction group except weight of duodenum (Table 6).

4 DISCUSSION

Bakyaraj et al. (2012) reported that hatchability of 81.3 % on in ovo feeding of enriched solution containing zinc 80 µg, copper 16 µg, selenium 0.3 µg and manganese 120 mg/egg compared to sham control group (97.3 %). Dzugan et al. (2014) evaluated effects of the injection of Zn and Cd, individually and in combination and reported that in ovo injection of individual minerals negatively affected hatchability, but had no effect when injected together. Oliveira et al. (2015) studied the in ovo injection of commercial diluent containing supplemental micro-minerals (Zn, Mn and Cu) on hatchability and concluded that in ovo injection of higher mineral concentrations into the amnion interfered with embryogenesis during late incubation, due to the creation of a mineral imbalance in the residual amnion. In ovo supplementation of trace mineral enriched solution did not show any significance ($p < 0.05$) on hatch weight. Oliveira et al. (2015) observed that injection of 0.5 mg of zinc along with manganese and copper did not influence the hatch weight of chicks compared to control. Joshua et al. (2016) also reported that in ovo nano zinc injection at a graded dose (8–20 mg) had no influence on hatch weight. Favero et al. (2013) resulted in no effect on hatchability, hatchling weight and Mn and Cu content in the egg. However, the Zn content in the egg was increased by the substitution.

Many of the earlier works (Tako et al., 2005; Goel et al., 2013; Yair et al., 2013; Oliveira et al., 2015) on in ovo injection of trace minerals individually or in combination have not reported increased growth performance of post-hatch

Table 6: Digestive organ weight (% of live weight) and length (cm/100 g live weight) at 5th wk

	Duodenum		Jejunum		Ileum		Caecum		Liver	Proventriculus	Gizzard
	Length	Weight	Length	Weight	Length	Weight	Length	Weight	Weight	Weight	Weight
Effect of in ovo supplementation (In ovo)											
WoINOVO	1.52	0.99	3.38	1.92	3.22	1.79	0.94	0.82	1.72	0.41	1.93
WINOVO	1.59	1.04	3.31	2.07	3.35	1.72	0.92	0.78	1.74	0.40	2.01
Significance	0.17	0.31	0.57	0.20	0.51	0.55	0.66	0.73	0.58	0.43	0.48
Effect of post-hatch supplemented diet											
WoPHS	1.54	0.97	3.29	1.97	3.31	1.70	0.92	0.74	1.72	0.39	1.96
WPHS	1.57	1.06	3.39	2.02	3.25	1.80	0.94	0.85	1.74	0.42	1.97
Significance	0.67	0.03	0.54	0.59	0.81	0.23	0.54	0.01	0.78	0.12	0.65
Interaction effect (In ovo × PHS)											
WoINOVO-WoPHS	1.52	0.91	3.40	1.95	3.38	1.81	0.94	0.75	1.72	0.39	2.02
WoINOVO-WPHS	1.51	1.08	3.35	1.89	3.06	1.76	0.94	0.88	1.73	0.44	1.83
WINOVO-WoPHS	1.55	1.03	3.18	2.00	3.25	1.60	0.91	0.73	1.73	0.39	1.90
WINOVO-WPHS	1.63	1.05	3.43	2.15	3.45	1.84	0.93	0.83	1.76	0.41	2.11
SEM	0.03	0.02	0.11	0.05	0.09	0.05	0.02	0.02	0.03	0.01	0.05
Significance	0.93	0.04	0.46	0.34	0.40	0.33	0.86	0.36	0.91	0.59	0.22

WoINOVO-WoPHS = Without in ovo and without post-hatch supplemented diet; WoINOVO-WPHS = without in ovo and with post-hatch supplemented diet; WINOVO-WoPHS = With in ovo and without post-hatch supplemented diet; WINOVO-WPHS = With in ovo and with post-hatch supplemented diet

chicks. Joshua et al. (2016) observed a variable result on in ovo injection of graded level of nano zinc, group injected with 40 mg nano zinc showed significant increase in body weight compared to other groups at 5th week. Bakyaraj et al. (2012) reported that in ovo trace mineral supplemented group (Zinc 80 µg, selenium 0.3 µg iron 160 µg, iodine 0.7 µg per egg) showed significantly higher body weight (411.9) compared to sham control (367.8). In ovo inoculation of several nutrients (maltose, a multi-vitamin supplement, zinc-glycine, glutamine and a mixture containing all these elements and L-carnitine) to 18-day-old embryos did not influence feed intake and feed conversion ratio (dos Santos et al., 2010; Keralapurath et al., 2010; Dooley et al., 2011)

The discrepancies in various studies (Ohta and Kidd, 2001; Bhanja and Mandal, 2005; Shafey et al., 2012; Kop-Bozbay et al., 2013; Schulte-Drüggelte, 2015) could be explained by many intrinsic and extrinsic factors which affect performance of broiler birds on supplementation of in ovo nutrients. Intrinsic factor of in ovo supplementation includes the content of in ovo solution, pH of solution, osmolarity of solution, dose per egg, site of injection, day of injection, needle bore diameter, interaction effect in mixed two or more nutrients and extrinsic factor include source of hatching eggs, storage condition, weight and size of eggs, nutritive profile of hatching eggs, strain/ line/ breed of breeding birds, breeding age, feeding regimen followed by laying birds, time of hatch.

Lack of significant effects in growth performance on in ovo supplementation of trace minerals in this study may be due to ideal level of nutrient present in egg obtained from commercial hatchery. This explanation is supported by the findings of Kop-Bozbay and Ocak (2015) where they found no significant effect of in ovo supplementation of amino acids using eggs with ideal nutrients contents. Most of the researchers did not mention source of hatching eggs especially strain / breed of layer birds used. As in present trial, source of eggs is from commercial hatchery, so neutral effect on growth performance may be related to fast growing broiler strains. This explanation is in line with the findings (Sarica et al., 2009; Yamak et al., 2014; Baéza et al., 2015) that fast-growing birds were better able to perform with commercial basal diet due to the fact that nutrient requirements increase depending on growth rate and also they may be better able to digest the basal diet due to the development of the digestive tract and organs. Furthermore, Schulte-Drüggelte (2015) reported that well-nourished, healthy chicks do not respond to in ovo supplements and the degree of limiting protein synthesis of amino acids depend on the ratios and antagonistic relationship between each of these amino acids (Burnham et al., 1992; Dozier III et al., 2011) and the protein content and quality of poultry diets (Ospina-Rojas et al., 2014).

The significant difference in digestive organs weights at the 1st week of age supported by the findings of Uni et al. (2003) that *in ovo* feeding results in improved gastrointestinal tract development of hatchlings and functionally similar to that of conventional 2 day old chicks offered feed immediately after hatch. The authors also indicated that, during the last 3 days of incubation, the weight of the intestine as a proportion of embryo weight increased from approximately 1 % at 17 days of embryonic age to 3.5 % at hatch. In chicks, at 3–7 days of age, the digestive organs will grow at a faster rate as compared to other organs and the small intestine increases in weight more quickly than the body mass during the first week post-hatch (Sklan, 2001). Rapid intestinal growth is due to increase in cell number and size, accelerated enterocyte proliferation and differentiation and intestinal crypt formation (Uni et al., 2000; Geyra et al., 2001). Tako et al. (2005) observed that in ovo injection of Zn-methionine in amniotic fluid on 18th day of incubation increased the villus surface area and enhanced the expression of genes and biochemical activity of intestinal transporters and enzymes thus accelerated intestinal development.

Kop-Bozbay and Ocak (2015) observed in experiment of in ovo injection of branched chain amino acids on eggs having ideal levels of nutrient and found that in ovo injection had no effect on the hatchability, chick quality and the degree of growth promotion. Healthy chicks may not respond to in ovo supplements (Schulte-Drüggelte, 2015) and the degree of limiting protein synthesis of these amino acids depend on the ratios and antagonistic relationship between each of these amino acids in poultry diet (Burnham et al., 1992) and the protein content and quality of poultry diets (Corzo et al., 2010). The influence of in ovo supplement is greatly dependent on the maternal diet as any deficiency is overcome by extra supplementation.

4 CONCLUSIONS

In ovo supplementation of zinc and copper did not influence hatchability.

Birds on the supplementation of zinc and copper recorded better feed conversion ratio at 3–5 weeks of age.

In ovo supplementation of zinc and copper significantly increased the weight (% of live weight) of the jejunum, ileum and caecum on the day of hatch.

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Genotype and within-pod bean position microenvironment effect on seed choice for raising cocoa (*Theobroma cacao* L.) seedlings

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Abstract: The probable role of within-pod microenvironment on seed sizes, seedling vigour and biomass yield of four cocoa genotypes was investigated for two years. The respective main, sub and sub-sub plots in the split-split plot experimental design were years, genotypes and within-pod bean positions. Data were taken on cocoa bean length, width and thickness after each pod was opened. Four weekly periodic data were obtained for plant height (PH), stem girth (SG) and number of leaves (NOL); root and shoot biomass yield were also recorded. Analysis of variance revealed significant ($p \leq 0.05$) bean position, genotypes, years and some interaction effect on the studied traits. Means of the levels of the three factors differed significantly ($p \leq 0.05$). Proximal, middle and distal positions were distinct within-pod microenvironments. The pod middle cavity housed the longest, widest and heaviest beans. Trend analysis of the growing sequences of NOL, PH and SG by the four genotypes differed with bean locations. For bean length, GGE biplot respectively identified CRIN Tc1, CRIN Tc2 and CRIN Tc3 as the best genotype for middle, proximal and the distal positions. The intra-locular space within the pod enhanced differential seed development and maturation; this was evident in the seedling vigour.

Key words: bean position; cocoa; micro-environments; GGE biplot; seedling vigour

Genotip in mikrookolje glede na položaj semena znotraj ploda vplivata na izbor semen kakavovca (*Theobroma cacao* L.) za vzgojo sadik

Izvleček: Vplivi mikrookolja znotraj plodne glavice na velikost semen, vigor sadik in pridelek biomase so bili preučevani pri štirih genotipih kakavovca v dveh zaporednih rastnih sezonah. Poskus je bil zasnovan na glavnih ploskvah in treh vrstah podploskev kot poskus z deljenkami glede na leto poskusa, genotipe in položaj semen znotraj glavice. Izmerjeni so bili dolžina, širina in višina semen kakavovca potem, ko so se plodovi odprli. Na štiri tedne so bili izmerjeni višina rastlin (PH), obseg debla (SG) in število listov (NOL); izmerjeni sta bili tudi biomasa korenin in poganjkov. Analiza variance je pokazala značilno povezavo ($p \leq 0{,}05$) med položajem semen v plodu, genotipom, letom poskusa in nekatere interakcije med preučevanimi znaki. Poprečki vrednosti znakov semen glede na položaj v plodu so se razlikovali statistično značilno ($p \leq 0{,}05$). Proksimalna, srednja in distalna pozicija v plodu so imele značilna mikrookolja. Osrednja votlina ploda je imela najdaljša, najširša in najtežja semena. Analiza trendov je pokazala naraščajoče vrednosti znakov NOL, PH in SG za vse štiri genotipe in položaj v plodu. Za dolžino semen so bili na osnovi GGE biplota identificirani genotipi CRIN Tc1, CRIN Tc2 in CRIN Tc3 kot najboljši za srednji, proksimalni in distalni položaj v plodu. Intralokularni prostor v plodu je vzpodbudil diferencialni razvoj semen in njihovo zorenje, kar je bilo očitno tudi na vigorju sejank.

Ključne besede: položaj semen; kakavovec; mikrookolje; GGE biplot; vigor sejank

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1 INTRODUCTION

Cocoa (*Theobroma cacao* L.), a native crop of South America is well adapted to and flourishes productively in the rainforest ecology of West and Central Africa. The region accounts for the largest proportion of global production, especially from Cote d'Ivoire, Ghana, Cameroon and Nigeria. The economic product (i.e. the beans) whose number ranges between 20 to 60 per pod (Ortiz, 2016) are basic raw material for the production of chocolate (Motamayor et al., 2008; Amma et al., 2011).

Among the four major cocoa producing member states in the West and Central Africa, production in Nigeria is at the last place. This has grossly being attributed to low yield from most farmers' field. Mathew et al. (2012) identified the use of low quality seed for raising seedlings, low emergence and poor seedling vigour as some of the factors responsible for low productivity within the plantation. In plantation cropping, seedlings obtained from the nursery influences establishment in the field and hence the productivity in the orchard (Baiyeri, 2006). Therefore, cocoa beans meant for propagation to raise seedlings are expected to have completed their structural and functional development within the pod (the fruit) before they are plucked for use in raising seedlings (Opoku-Ameyaw et al., 2010).

The customary practice for cocoa plantation establishment in West and Central Africa has been based on use of seeds in order to generate planting material (Adewale et al., 2016). The observed norm among the farmers has been indiscriminate use of all seeds within the pod irrespective of bean size differences and the location within the pod where they are housed. Seed sizes of genotypes is not only a result of the genetic structure but also some other contributory factors. Seed growth and development is dependent on the biomass investment from the mother plant, such that seeds with high resources have bigger size. Moreover, the endosperm content determines seed sizes which differs significantly within the same fruit (Susko and Lovett-Doust, 2000; Khan et al., 2014). Why should there be significant variation in the seed sizes from the same developmental locations? Khan et al. (2014) identified: within-pod resource quantity, fertilization gradients and neighbor effect to be among some of the probable factors responsible for within-pod seed size variation. Nakamura (1988) identified proximity of the ovules to the stylar end as another important factor in *Phaseolus vulgaris* L.*;* noting that seeds closer to the style (proximal) end were significantly better in size than those nearer to the receptacle (the distal) end.

Giles (1990) strongly remarked that the within-plant variance cannot be interpreted as anything other than a random environmental variance effect; it is equally obvious that the variation in the sizes of seeds from the same pod is predominantly due to within-pod microenvironment. The fruit (pod) is a controlled environment but within it, there is environmental deviation (Singh and Pokhriyah, 2001) or intra-locular variation due to differential variation in space along the length from one end to the other. The unequal intra-ovary volume/space along the pod length affects and determines the phenotypic development of the seeds they host in various locations along the length.

The fruits provide the environmental space for the seeds and protects the seeds from unfavorable biotic and abiotic condition. However, Bennett et al. (2011) further hinted that the function of the pod to the seeds is far beyond safeguarding them to maturity as an environment, it equally regulates seed growth and maturation. The location/apartment where different seeds appears within the fruit has a role to play in the resource distribution and sharing scheme within the pods (Lee, 1988). In cocoa, Ibikunle (1967) noted that seeds which developed in the expanded (i.e. middle) part of the cocoa pod produced bigger-sized beans and hence seedlings with better vigour.

Poor seedling survival at nursery and reduced population of established seedlings on the field are among the attending problem of using all healthy beans from every portions within the cocoa pod. The knowledge of cocoa seedling performance based on positional location within the pod is a needed quest, hence the present investigation, so that beans with no promising vigour will be excluded from use in the propagation scheme. Reports from seed quality test for field establishment of maize (Cruz- Garcia et al., 1995; Moreno-Martinez et al*.,* 1998), barley (Copeland and McDonald, 2001) and rapeseed (Ghassemi-Golezani et al*.*, 2010) noted that low quality seeds produces low vigour seedlings with very poor field establishment. The present study was therefore proposed because there is rare information on the typical role of bean position in the pod on the structural development of the beans. Attempts by Iremiren et al. (2007) and Hammed et al. (2013) were on a single genotype each, hence, genotype and within-pod environment interaction on seedling vigour could not be highlighted in their investigations.

Consideration of the housing environment of the cocoa bean as a link to the expression of seedling vigour has not been well attempted in cocoa, hence the need for the present investigation. Highlighting the significance of the bean position within the pod, its relevance to determining bean size and hence, the seedling vigour is worthwhile for identification of portion(s) within the fruit where the most suitable beans capable of supporting good seedling vigour for optimum field establishment is/

are located. Therefore, the two years replicated experiment which employed four different cocoa genotypes has the following objectives: to identify the role of bean positions within the pod and its interaction with genotypes in the determination of growth and developmental traits of cocoa seedlings.

2 MATERIALS AND METHODS

With an interest to understand the relative differential performances of cocoa seeds, usually called beans in the different localized positions within the fruit capsules called pods of different cocoa genotypes and the possible link of the same to seedling vigour and development; a research was conducted for two consecutive years at the Cocoa Research Institute of Nigeria (CRIN), Idi-Ayunre, Ibadan, Nigeria. Pods for the experiment were obtained from the hybrid trial plot established in 1999 at the institute. Four physiologically matured cocoa pods were harvested per genotype during the main season (October-November) in three replications in 2014 and 2015. The four hybrids genotypes (CRIN 2011) used for the study were: CRIN Tc1 (T65/7 × N38), CRIN Tc2 (T101/15 × N38), CRIN Tc3 (P7 × PA150), and CRIN Tc4 (T56/7 × T57/22). Each fruit was longitudinally opened and beans were partitioned based on their nearness to the two ends of the pod and the middle as: the proximal (toward the stylar tip), middle (the expanded portion of the fruit) and distal (part closest to the receptacle). An image of cocoa pod delineating the three sections within the pod is shown in Plate 1.

Beans within each class were cleaned with sawdust to remove the mucilaginous pulp. Twenty beans were sampled for each of the three positions per genotype and metric measurements on length, width and thickness were taken on the cleaned beans using the venier caliper following Omokhafe and Alika (2004) and Kaushik et al. (2007). Individual mass of the sampled beans for the three positions of each genotypes were also measured and recorded.

The three classified groups of beans per genotype were pre-germinated for 72 hours before they were sown into the polythene bags in the nursery. This was repeated for two years. The experimental design employed was split-split plot design with years, genotypes and bean positions as main, sub and sub-sub plot factors respectively. The number of replications used was three.

Among the measurements taken on the seedlings after germination were: number of leaves per plant, plant height and stem girth. Data on the morphological characteristics continued from 2nd weeks after seedling emergence to the sixth month. Destructive sampling was done for the sampling unit after the termination of the experiment to obtain the fresh and dry root, shoot and total biomass yield. The data were subjected to analysis of variance (ANOVA) and means of the different main effects were separated by Tukey's honestly significant differences. The association between the bean indices and the harvested biomass after destructive sampling were tested by correlation analysis. All analysis were carried out in SAS (version 9.4, 2011). Furthermore, trend analysis was done using R Development Core Team (2013) to understand the sequence of response of the growth data taken at intervals.

Traits with significant genotype by bean position interactions from the ANOVA were further partitioned using the "which won where" option in the GGE biplot in GEA-R (Pacheco et al., 2016). From the component of the ANOVA, genetic estimates were calculated for phenotypic and genotypic coefficients of variation (PCV

Plate 1: The three cross-sections of an opened cocoa pod housing cocoa beans

and GCV) following the method of Singh and Chaudhay (1999):

$PCV = (\sigma^2_p / X)^{½}$……...........…………………. .Eq. 1

$GCV = (\sigma^2_g / X)^{½}$……......................................…Eq. 2

Where σ^2_p, σ^2_g and *X* are phenotypic and genotypic variances and grand mean respectively.

Broad sense heritability (Hbs) was expressed as the percentage of the genotypic variance to the phenotypic variance for split-split plot design as described by Bokmeyer et al. (2009), cited in Clark and Watkins (2012) and modified as follows:

$Hbs = \sigma^2_g / \sigma^2_g + \sigma^2_{gy/y} + \sigma^2_{gp/p} + \sigma^2_{gr(y)/rp} + \sigma^2_{gyp/yp} + \sigma^2_{e/ryp}$ ……
……...Eq. 3

Repeatability (r_c) was estimated following Ortiz and Ng (2000), as follows:

$r_c = \sigma^2_g/(\sigma^2_y + \sigma^2_{gy})$ …......................…………….Eq. 4

3 RESULTS

Table 1 shows the significant ($p < 0.05$) differences in the bean metric traits for years, varieties, within-pod

Table 1: Variance components for the bean metric traits, correlations among them and their mean performances based on years, varieties and bean position

Sources of Variation	DF	BL (cm)	BW (cm)	BT (cm)	BM. (g)
Replications	2	0.04	0.03	0.02	0.08
Years	1	4.86***	0.03	1.34***	5.08***
Error (a)	2	0.05	0.03	0.04	0.05
Varieties	3	3.36***	2.89***	2.24***	8.42***
Years*Varieties	3	0.98***	0.52***	0.42***	3.69***
Error (b)	12	0.04	0.03	0.03*	0.06
Bean Positions	2	1.35***	0.62***	0.56***	1.23***
Years* Bean Positions	2	0.87***	0.06	0.04	0.58***
Varieties* Bean Positions	6	0.27***	0.07**	0.36***	0.45***
Years*Varieties* Bean Positions	6	0.35***	0.04	0.15***	0.57***
Error (c)	32	0.05	0.02	0.02	0.08
	Correlations among the three bean metric traits with bean mass				
Bean mass	0.96ns	0.99*	0.99**	-	
	Mean separation of the three main effects				
Years	2014	2.11b	1.14a	0.65b	1.68a
	2015	2.31a	1.13a	0.76a	1.48b
Varieties	CRIN Tc 1	2.37a	1.27a	0.83a	1.89a
	CRIN Tc 2	2.34a	1.26a	0.81a	1.71b
	CRIN Tc 3	2.07b	0.95c	0.53c	1.33c
	CRIN Tc 4	2.06b	1.06b	0.66b	1.39c
Bean Positions	Proximal	2.13b	1.11b	0.75a	1.53b
	Middle	2.31a	1.20a	0.64b	1.68a
	Distal	2.19b	1.08b	0.73a	1.53b

† BL - Bean Length, BW - Bean Width, BT - Bean Thickness, BM. - Bean mass.
*, ** and *** - Significance at p = 0.05, 0.01 and 0.001
Mean comparison is along the column for year, varieties and bean position; means with the same alphabet are not significantly different from each other

bean positions, years by varieties, years by bean positions and varieties by bean position interactions. Bean length and its thickness differed significantly ($p \leq 0.05$) among the two years and higher significant values occurred in 2015. However, bean mass was significantly ($p \leq 0.05$) higher (1.68 g) in 2014 compared to 1.48 g in 2015 (Table 1). Beans length, width and thickness were highest for CRIN Tc1 and 2, but minimum values for the same were obtained in CRIN Tc3. The four metric traits (length, width, thickness and mass) of the beans varied significantly ($p < 0.05$) with positions where the beans were located within the pod. Beans in the middle position had significant ($p < 0.05$) longer, wider and heavier beans mass when compared to beans at the proximal and distal positions (Table 1). Furthermore in Table 1, individual bean mass had strong to very strong and significant ($p \leq 0.01$) correlation with bean width (r = 0.99) and thickness (r = 0.99).

Table 2 shows different sources of variation in the analysis of variance and the mean values of some traits at the termination of the experiment for the beans from the three within-pod environments for the four genotypes in the two years. The fresh shoot mass of the cocoa seedlings was not significantly enhanced in this study (Table

Table 2: Variance components for the seedling biomass traits, correlations among them and their mean performances based on years, varieties and bean position

Sources of Variation	DF	SFM	SDM	RFM	RDM
Replications	2	4.2	2.47	1.24	1.02***
Years	1	6.73	25.32***	0.31	1.89***
Error (a)	2	10.03	0.07	14.42	0.04
Varieties	3	31.32*	1.98*	2.85*	0.49***
Years*Varieties	3	7.47	0.23	3.68*	0.01
Error (b)	12	8.27	0.18	1.07	0.04
Bean Positions	2	11.05	1.74	4.91**	0.46***
Years* Bean Positions	2	14.72	0.45	3.68*	0.01
Varieties* Bean Positions	6	6.29	0.44	2.362	0.12
Years*Varieties* Bean Positions	6	11.34	0.69	1.35	0.06
Error (c)	32	6.02	0.64	1.04	0.06
	Correlations among the fresh and dried biomass of the shoot and root				
Shoot Dry Mass	0.99**	-	0.88ns	0.95ns	
	Mean separation of the three main effects				
Years	2014	9.90a	2.51b	4.69a	0.79b
	2015	10.51a	3.69a	4.56a	1.12a
Varieties	CRIN Tc 1	10.87ab	3.56a	5.20a	1.19a
	CRIN Tc 2	8.73b	2.77b	4.37b	0.93b
	CRIN Tc 3	9.54ab	2.98ab	4.36b	0.89b
	CRIN Tc 4	11.67a	3.09ab	4.58b	0.81b
Bean Positions	Proximal	9.91a	2.99a	4.23b	0.86b
	Middle	10.98a	3.41a	5.12a	1.11a
	Distal	9.72a	2.90a	4.53ab	0.89b

† SFM – Shoot fresh mass, SDM – Shoot dry mass, RFM – Root fresh mass, RDM – Root dry mass*, ** and *** - Significance at p = 0.05, 0.01 and 0.001

Mean comparison is along the column for year, varieties and bean position; means with the same alphabet are not significantly different from each other

Table 3: Mean performances of the vegetative growth traits across different years varieties and bean positions in the pod

	NOL 6WAS	NOL 10WAS	NOL 14WAS	NOL 18WAS	NOL 22WAS	PH 6WAS	PH 10WAS	PH 14WAS	PH 18WAS	PH 22WAS	SG 6WAS	SG 10WAS	SG 14WAS	SG 18WAS	SG 22WAS
	Year														
2014	4.75b	6.89a	8.80a	10.68a	13.22a	16.43a	19.63a	21.82a	25.67a	27.43a	0.40a	0.58a	0.63b	0.67a	0.92a
2015	5.23a	7.27a	8.53a	9.66b	11.86a	14.89b	18.36b	21.82a	23.41b	27.36a	0.22b	0.45b	0.66a	0.74a	0.82a
	Varieties														
CRIN Tc 1	5.16ab	7.23ab	8.52b	9.99ab	12.32a	16.25a	20.84a	23.77a	25.92a	28.18a	0.31a	0.45a	0.66a	0.75a	0.89a
CRIN Tc 2	4.53c	6.35c	7.81b	9.37b	12.09a	14.65b	17.40b	19.56b	22.95b	26.71b	0.28a	0.57a	0.64a	0.74a	0.81a
CRIN Tc 3	4.70bc	6.88bc	8.60b	10.29ab	12.56a	14.52b	16.88b	19.30b	22.58b	25.56b	0.33a	0.61a	0.70a	0.76a	0.90a
CRIN Tc 4	5.56a	7.87a	9.72a	11.03a	13.19a	17.21a	20.86a	23.70a	26.69a	29.11a	0.33a	0.53a	0.56a	0.68a	0.89a
	Cocoa Beans Position														
Proximal	4.86a	6.80a	8.44a	9.86ab	12.34ab	15.71ab	18.96ab	21.5b	24.22b	27.52ab	0.34a	0.59a	0.65a	0.83a	0.98a
Middle	5.13a	7.31a	9.13a	10.89a	13.42a	16.36a	19.78a	22.78a	25.83a	28.65a	0.34a	0.53a	0.59a	0.65a	0.82a
Distal	4.97a	7.14a	8.42a	9.75b	11.85b	14.91b	18.24b	20.46b	23.56b	26.00b	0.25a	0.51a	0.71a	0.81a	0.81a

NOL-Number of leaves, PH- Plant height, SG-Stem Girth, WAS- weeks after sowing
Mean comparison is along the column for years, varieties and bean positions; means with the same alphabet are not significantly different

Table 4: Some genetic estimates of some variables

Traits	PCV	GCV	GCV:PCV	Heritability (%)	Repeatability
Bean length	134.63	123.30	91.59	83.88	0.77
Bean width	167.87	159.92	95.26	90.75	0.85
Bean thickness	192.70	178.89	92.83	86.17	0.84
Bean mass	258.05	230.85	89.46	80.03	0.70
Shoot fresh mass	198.76	175.23	88.16	77.73	0.81
Shoot dry mass	88.21	79.92	90.60	82.08	0.90
Root fresh mass	112.68	78.46	69.63	48.48	0.44
Root dry mass	75.88	71.44	94.15	88.64	0.98
NOL6WAS	95.90	87.95	91.71	84.11	0.82
NOL10WAS	107.46	101.40	94.37	89.05	0.92
NOL14WAS	119.43	113.76	95.25	90.73	0.97
NOL18WAS	104.27	91.69	87.93	77.33	0.76
NOL22WAS	96.80	56.48	58.35	34.04	0.29
PH6WAS	153.71	139.67	90.87	82.57	0.73
PH10WAS	216.38	209.40	96.77	93.65	0.91
PH14WAS	241.21	227.25	94.21	88.76	0.83
PH18WAS	189.17	177.50	93.83	88.05	0.82
PH22WAS	166.93	127.03	76.10	57.91	0.50
SG6WAS	53.21	17.96	33.75	11.39	0.11
SG10WAS	94.86	58.25	61.41	37.71	0.41
SG14WAS	126.20	88.47	70.10	49.14	0.47
SG18WAS	56.98	29.07	51.01	26.02	0.23
SG22WAS	56.67	18.57	32.77	10.74	0.10

NOL-Number of leaves, PH- Plant height, SG-Stem girth taken at four weeks interval from the 6th to the 22nd weeks after planting

2), however, the dry shoot mass shown in 2014 had a lead and significant ($p \leq 0.05$) value of 3.69 g compare to 2.51 g in 2015; the trend was same for the dry root mass (Table 2). The middle positional beans produced the highest significant ($p \leq 0.05$) mean for root fresh and dry mass. Neither year nor the three beans position inside the cocoa pod affected the shoot fresh mass. However, among the varieties, the highest fresh and dry root mass value was observed in CRIN Tc1. Moreover, shoot fresh and dry mass had a strong (0.99) and significant ($p \leq 0.01$) correlation (Table 2).

From the means in Table 3, significantly ($p \leq 0.05$) higher mean was obtained for NOL6 weeks after sowing (WAS) and SG14WAS in 2015. However, higher significant ($p \leq 0.05$) values were obtained for NOL-18WAS, PH6WAS, PH10WAS, PH18WAS, SG6WAS and SG10WAS in 2014 (Table 3). Among the four genotypes, CRIN Tc1 and CRIN Tc4 had the significantly ($p \leq 0.05$) higher means for number of leaves and plant heights at 6th to the 22nd WAS (Table 3). Beans originating from the middle of the pod produced significantly ($p \leq 0.05$) the highest number of leaves (18 and 22 WAS) and plant height (6th to 22nd WAS) in Table 3.

For all the variables studied in this experiment, the phenotypic coefficient of variation were higher than the genotypic coefficient of variation (Table 4). The proportion of the genetic component in the phenotypic coefficient of variation ranged between 32.77 (SG22WAS) to 96.77 (PH10WAS). Stem girth 22 WAS which had the lowest GCV: PCV, equally had the lowest broad sense heritability (10.74) and repeatability (0.10). The highest (90.75) broad sense heritability occurred in bean width while the highest (0.98) repeatability was recorded for root dry mass (Table 4).

Table 5 unveiled the specific pattern of variability and sequence of response of each of the four genotypes to three vegetative and agronomic variables. The sources of variation from the table includes: the total treatment, each of the two factors within the treatment (i.e. intervals and bean position) and variability based

Table 5: Trend analysis of the growth traits measured at intervals in correspondence to the three bean positions within the pod for four cocoa varieties

		CRIN Tc 1			CRIN Tc 2		
Sources of Variation	DF	NOL	PH	SG	NOL	PH	SG
		Mean Squares			Mean Squares		
Treatments	14	7.27***	18.14***	0.11	6.99**	17. 49***	0.06
Interval (In)	4	24.54***	58.51***	0.24*	23.84***	55.55***	0.11
In-Linear	1	66.86***	190.71***	0.35*	67.02***	176.61***	0.18
In-Quadratic	1	1.70**	7. 45**	0.15	7.17*	0.55	0.03
In-Cubic	1	1.15*	2.08	0.05	5.7*	0.03	0.01
In-quantic	1	0.05	0.20	0.01	1.89	0.67	0.14
Bean Position(BP)	2	1.17*	8.13**	0.06	0.58	10.35***	0.03
BP-Linear	1	0. 48	0.29	0.10	0.60	18.66***	0.05
BP-Quadratic	1	1.87**	15.98***	0.03	0.57	12.02*	0.01
Error	8	0.15	0. 46	0.05	0.17	0.24	0.04
		CRIN Tc 3			CRIN Tc 4		
Sources of Variation	DF	NOL	PH	SG	NOL	PH	SG
		Mean Squares			Mean Squares		
Treatments	14	9.13**	19.17**	0.04	7.22**	20.05***	0.03***
Interval(In)	4	28.09***	62.76***	0.04	24.26**	63.12***	0.11***
In-Linear	1	83.66***	189.68***	0.03	75.19***	206. 46***	0.28***
In-Quadratic	1	0.29	2. 46	0.02	0.03	1.09	0.12***
In-Cubic	1	0.54	0.14	0.04	0.31	0.56	0.004**
In-quantic	1	0.001	0.34	0.001	0.04	0.17	0.003*
Bean Position(BP)	2	5. 49*	3.15	0.01	1.31**	10.35**	0.0003
BP-Linear	1	2.96*	0.98	0.002	7.38*	3.23	0.00007
BP-Quadratic	1	7.99**	5.32	0.02	7.56*	17. 49**	0.0006
Error	8	0.55	1.39	0.04	0.17	0.93	0.0003

Note. DF - Degree of freedom, *,**,and*** - significance at p- 0.05, 0.01 and 0.001, NOL - Number of leaves, PH -Plant height; SG - Stem girth

on different forms of trend for each of the two factors. Highly significant ($p \leq 0.01$) variabilities were noted for the fifteen treatment combinations and the five intervals of data measurements for number of leaves, plant height and stem girth of the four genotypes. However, there was significant ($p \leq 0.05$) treatments effect on stem girth for CRIN Tc4 while interval effect was equally significant ($p \leq 0.05$) for CRIN Tc1 and 4 for stem girth (Table 5). With respect to bean position as a source of variation, its effect was significantly ($p \leq 0.05$) notable for number of leaves and plant height for CRIN Tc1 and 4; CRIN Tc2 and 3 showed respective significance ($p \leq 0.05$) for plant height and number of leaves (Table 5). Number of leaves showed linear ($p \leq 0.001$), quadratic ($p \leq 0.01$) and cubic ($p \leq 0.05$) response for CRIN Tc1 and CRIN Tc2. Moreover, trend for plant height was linear ($p \leq 0.001$) and quadratic ($p \leq 0.001$) for CRIN Tc1 but only linear for CRIN Tc2 while the trend response for stem girth was only linear ($p \leq 0.05$) for CRIN Tc1 (Table 5). Within the same table, CRIN Tc3 and CRIN Tc4 showed only significant ($p \leq 0.05$) linear response for number of leaves and plant height. Specifically, the stem girth displayed significant ($p \leq 0.05$) linear to quantic responses for CRIN Tc4 (Table 5). Still from Table 5, the trend response with respect to bean position for plant height was both linear ($p \leq 0.05$) and quadratic ($p \leq 0.05$) in CRIN Tc2, number of leaves and plant height displayed quadratic ($p \leq 0.05$) response in CRIN Tc1 and CRIN Tc4. However, for CRIN Tc3 and CRIN Tc4, the number of leaves exhibited both linear ($p \leq 0.05$) and quadratic ($p \leq 0.05$) responses.

Factors 1 and 2 (Figure 1) cumulatively explained the total variance. The proximal, the middle and the distal position were three distinct within-pod environments (each appearing in different sector) for cocoa bean length

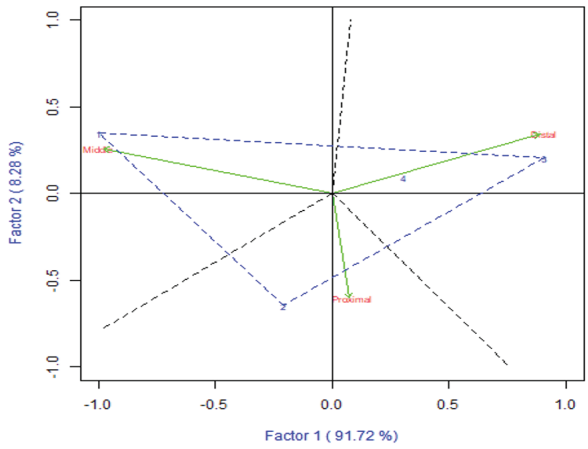

Figure 1: Bean position by varieties interaction display for cocoa bean length
1 – CRIN Tc1, 2 – CRIN Tc2, 3 – CRIN Tc3 and 4 – CRIN Tc4

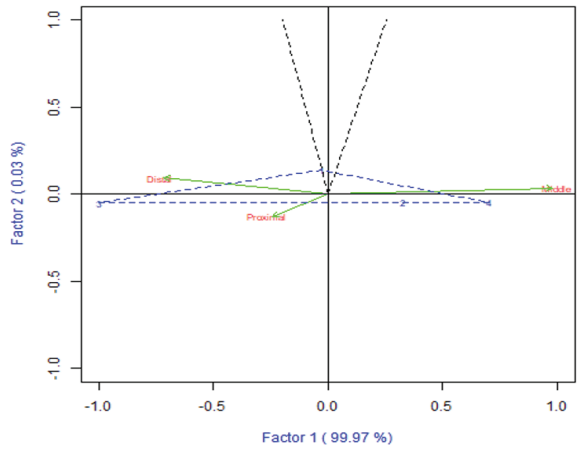

Figure 2: Bean position by varieties interaction displayed for cocoa bean thickness
1 – CRIN Tc1, 2 – CRIN Tc2, 3 – CRIN Tc3 and 4 – CRIN Tc4

determination. Each of the three distinct within-pod microenvironments identified different best performing genotype; such that CRIN Tc1, CRIN Tc2 and CRIN Tc3 respectively had the best bean length in the middle, proximal and the distal portion within the pod (Figure 1). Only two sectors were prominent in Figure 2 and the three within-pod positions differentially dispersed within the major sector. However, CRIN Tc1, CRIN Tc3 and CRIN Tc4 were respectively the vertex genotype for distal, proximal and middle within-pod microenvironment respectively for cocoa bean thickness determination.

Figure 3 displayed the bean position by varieties interaction for cocoa bean width. The three within-pod locations were distinct for bean width determination. The trapezia polygon had four sectors which identified CRIN Tc1 as the vertex genotype for beans at the middle cavity, CRIN Tc3 for the distal and CRIN Tc4 for the proximal within-pod location (Figure 3). The polygonal display of the bean position by varieties interaction for cocoa bean mass was a triangle with three sectors (Figure 4). The proximal and the distal within-pod microenvironments were accommodated in one mega environment while the middle portion of the pod environment was alone in another sector as another mega environment. The vertex genotype for both the proximal and distal within-pod environment was CRIN Tc3, but the sector which captured the middle environment had CRIN Tc4 as the vertex genotype (Figure 4).

3 DISCUSSION

The performances of the four bean metric measurements and seedling vegetative traits were affected by the

year effect. This seem to reveal that the variables are not stable but very plastic as they significantly responded to changes in the wider environment of yearly climatic variation. The four studied genotypes and the three bean positions equally distinguished themselves on the four beans metric and other vegetative traits. This further substantiate that character expression is dependent on the environment, the genotype and the interaction of both (Crossa et al., 1991; Mortazavian and Azizinia, 2014). Moreover, by this study, the two different years (2014 and 2015), the four cocoa varieties (CRIN Tc1, CRIN Tc2, CRIN Tc3 and CRIN Tc4) and the three bean positions (proximal, middle and distal) were unique treatments in the experiments.

The environment within the ovary is not consistently uniform, hence, compartments within the seed-developing space do impacts and determines the physical and physiological traits of the seed (Illipronti et al., 2000). The spacious hollow at the middle cavity of the cocoa pod may have supported the recorded bean features of longer length, wider width and heavier mass of the beans which developed in the area. It is clear from our result that the length and width of the bean rather than the thickness were noted to be more important in bean mass determination. We equally noted that beans from the middle position supported increased biomass yield of roots and shoots for the four cocoa genotypes. In *Cryptocarya alba* (Molina) Looser, large sized seeds were associated with larger shoots, roots and number of leaves (Chacon et al., 1998). The reduced value for the same traits at the proximal and distal ends could be due to some constriction of the hollow within the pods. However, beans at the two ends were significantly thicker than those in the middle of the cavity. This seems to infer that adequate space is very necessary for efficient seed development in the ovary.

The tapering structure of the pod at proximal and distal ends may be very key in the reduction on the sizes of the seeds located in and towards the two ends. So, the available space within the pod (which varies along its length) could be a determinant of the seed sizes and hence mass of beans in the cocoa pods. This result is in consonance with the report of Iremiren et al. (2007) and Hammed et al. (2013) on their work with a genotype called F3 Amazon. Following the descriptive pod and pod apex shape by Phillip-Mora et al. (2013), the pod shape of the four genotypes used in this study was more similar to 'Angoleta' with the pod apex ranging between acute to obtuse. However, for whichever shape the cocoa pod has, the middle part (which is usually raised or expanded) seem to provide wider space for bean development compare to the two tapering ends. The identified significant within-pod variation which leads to three group of beans from the same pod observed in this study may not be true for 'Calabacillo' pod shaped cocoa (Phillip-Mora et al., 2013) which are usually ball-like round. Contrary to the report of Perin et al., (2002) on melon (*Cucumis melo* L.) where stems are the organs mostly affected by seed size, our result negates their assertion, the significant differences in the metric traits on the beans did not affect cocoa seedlings stem girth as it does to other vegetative traits.

Genotypes are genetic entity with specific characteristics which makes them different from another one. Bekele et al (2006) had much earlier noted that there exist considerable genetic variation in fruit size, shape and bean size of cocoa. The four cocoa genotypes used in this study differed in sizes of their beans and the impact of each of the bean traits on vegetative and biomass yield

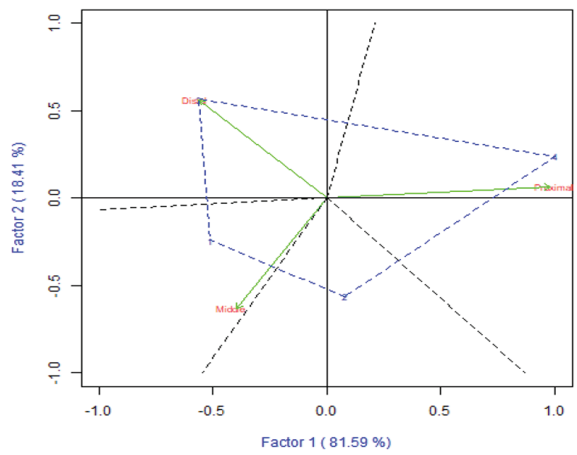

Figure 3: Bean position by varieties interaction display for cocoa bean width
1 – CRIN Tc1, 2 – CRIN Tc2, 3 – CRIN Tc3 and 4 – CRIN Tc4

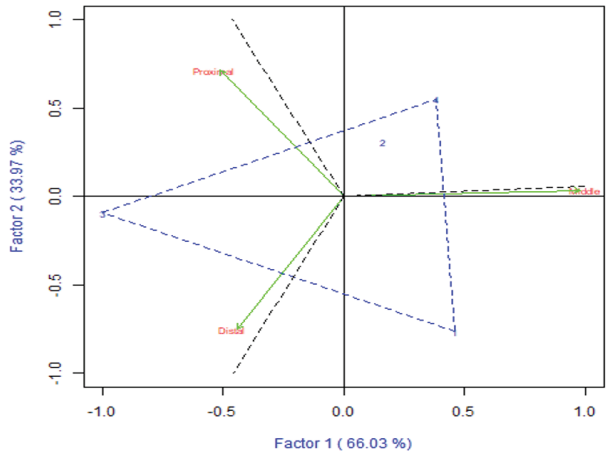

Figure 4: Bean position by varieties interaction display for cocoa bean mass
1 – CRIN Tc1, 2 – CRIN Tc2, 3 – CRIN Tc3 and 4 – CRIN Tc4

of the four cocoa genotypes; this information is a useful resource on which selection programme on wet bean sizes can thrive.

Our research outcome conforms to the expected norm that the phenotypic variance component are always higher than the genotypic component. However, variables which shows very small deviation are remarked to be reliable traits (Adewale et al., 2010), because the proportion which accrued to environmental variation is small while the quantity of variation for the genetic portion is high. The observed high genetic components in the phenotypic expression of the four bean metric traits, number of leaves and plant height measured at different interval in this study is remarkable. High and positive correlation existed between broad sense heritability and repeatability, revealing that traits with high broad sense heritability will have high repeatability. It is noteworthy that traits with high repeatability may have low response to environmental variation, hence the corresponding high broadsense heritability could be due to additive gene action.

Our research considered two notable environments: the within-pod environment (a micro environment) and the year (a macro environment), both which affected the expression of the bean metric traits and vegetative characteristics differently. GGE biplot identified the proximal, middle and distal positions within the pod environments for cocoa bean length, width, thickness and mass to be very unique. Hammed et al. (2013) who observed this three distinct divisions along the cocoa pod length, like us, did remark that the beans in the middle were longer, wider, thicker and heavier than other beans from the two extreme ends of the pod. For the three metric measurements on the bean, the GGE biplot clearly distinguish bean sizes, noting that the length, width and thickness of beans differ in respect to the positions where they are located along the inner cavity of the pod length. This therefore infers that the environment where beans are located during development primarily determines the phenotypic expression of its length, width, thickness and mass. The significant differences for preference of the four genotypes for the various within-pod location is a reflection of genotypic variation.

From this study therefore, mass of individual beans did not differ at proximal and distal positions, hence the zoning of the two microenvironments as a single mega-environment by GGE biplot for the trait. This further denotes that the mass of beans from the two ends of the cocoa pod do not differ from each other; meaning that the variation between both for mass of individual bean was not significant enough (Yan and Kang, 2003). Furthermore, the relevance of the individual bean mass of the two positions (proximal and distal) within the cocoa pod is the same. The long-standing recommendation of utilizing Cocoa beans from the middle cavity rather than the two ends (Ibikunle, 1967; Iremiren et al., 2007; Hammed et al., 2013) may have stemmed from the conspicuous variation in bean size and mass among beans from the same pod coupled with the linear correspondence between heavier seeds and high seedling vigour (Enayatgholizadeh et al., 2011).

4 CONCLUSION

Our work clearly revealed that the three inner locations (proximal, middle and distal) along cocoa pods length are prominent in distinguishing cocoa bean sizes; identifying the cocoa beans in the middle of the pod to be of the highest quality for all the metric measurements

including bean mass. We suggest consideration of the influence of pod position on the cacao tree in subsequent related work to assess its probable influence on bean sizes relative to within pod positions. Where adequate bean may not be available for seedling generation, seeds from the two ends of the pod may not be discarded as seedlings from them improved in their growth with active photosynthesis after establishment. However, selection of and usage of beans in the middle of the pod could lead to the production of good, uniform and vigorous seedlings that will support higher cocoa productivity.

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Intensification of the drying process of small seed oilseeds using microwave electromagnetic radiation

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Intensification of the drying process of small seed oilseeds using microwave electromagnetic radiation

Abstract: One of the important and crucial stages of post-harvest treatment of rapeseed is drying. The purpose of the article is to improve the drying process of seeds of small seed oil crops using electromagnetic radiation of the microwave range in order to increase its productivity and determine the optimal operating parameters. The article describes the construction of a new microwave (UHF) dryer with a capacity of 200 kg h^{-1} for drying small-seeded crops. Curves were obtained that show the dependence of the heating temperature of seeds on microwave power, the effect of initial seed moisture and heating temperature on drying kinetics. The ratio of the stages of microwave heating and cooling was determined, which allows to increase the drying efficiency.

Key words: grain drying; rapeseed; electromagnetic radiation; drying kinetics; drying device

Pospeševanje sušenja majhnih semen oljnih poljščin z mikrovalovnim elektromagnetnim sevanjem

Izvleček: Eden od pomembnejših in ključnih postopkov pri požetveni obravnavi semen oljne ogrščice je sušenje. Namen prispevka je izboljšanje procesa sušenja majhnih semen oljnih rastlin z mikrovalovnim elektromagnetnim sevanjem z namenom povečanja produktivnosti in določiti optimalne operacijske parametre. Članek opisuje zgradbo novega mikrovalovnega (UHF) sušilnika z zmogljivostjo 200 kg h^{-1} za sušenje majhnih semen oljnih poljščin. Krivulje kažejo odvisnost temperature segretih semen od moči mikrovalovnega sušilnika, učinka začetne vlažnosti semen in odvisnost sušilne temperature od kinetike sušenja. Določeno je bilo razmerje med gretjem in hlajenjem mikrovalovnega sušilnika, ki omogoča povečanje učinkovitosti sušenja.

Ključne besede: sušenje zrnja; oljna ogrščica; elektromagnetno sevanje; kinetika sušenja; sušilnik

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1 INTRODUCTION

In agricultural practice, small seed oil crops such as rapeseed, mustard, and saffron milk, which are in great demand for agriculture and industry, are becoming increasingly important (Håkansson et al., 2013; Kovalyshyn, 2015; Kovalyshyn et al., 2015). One of the most important operations of oilseed cultivation technology is post-harvest seed treatment (Håkansson et al., 2013; Soares et al., 2016; Moreno et al., 2017). After ripening, rapeseed has a moisture content that amount from 14 to 27 %, and the recommended humidity for storage should be no more than 7 to 8 %. Due to the high humidity in the seeds of oilseeds, oxidative processes can begin, leading to a decrease in their quality. Therefore, timely drying will allow to maintain high sowing and technological qualities of seeds (Ganeev et al., 2009; Ganeev & Masalimov, 2009; Masalimov et al., 2018).

Due to the lack of special technological equipment, drying of oilseed grain is performed on grain drying equipment, which are distinguished by type and method of action (Gabitov et al., 2018). Existing drying methods are mainly based on thermal effects on the material, which in turn leads to a deterioration in the quality of the finished product (Jokiniemi & Ahokas, 2014; Skakov, Rakhadilov & Sheffler, 2013).

The choice of drying equipment must be made taking into account the physical and mechanical parameters of a particular culture. It is necessary to classify them to determine the most optimal construction of dryers. Drying devices can be classified according to a number of features, the main ones being the method of supplying heat, the construction of the drying chamber, the operating mode, the state of the grain layer and the construction (GOST, 2008; Sutjagin et al., 2017). The most widespread is the direction of drying grain using the convective method of heat supply. The convective grain dryers are simple and most productive (Soares et al., 2016; Maier, 2017; Manikantan et al., 2018). However, these grain dryers are characterized by high metal consumption, high cost and high energy costs (Sutjagin et al., 2017). In shaft type grain dryers operating on gaseous and liquid fuels, when drying food and industrial grain crops, the regulated specific energy consumption should not exceed 4.56 MJ kg^{-1} of evaporated moisture, and 5.74 MJ kg^{-1} when drying seed grain (GOST, 2008). In practice, convective drying devices that operate without heat recovery consume up to 6 MJ kg^{-1} of evaporated moisture due to the fact that most of the thermal energy is carried away irrevocably by the drying agent.

On the territory of the Russian Federation, the drum and shaft grain dryers with convective heat supply are most common, which, when drying the seed, often lead to grain injury. In addition, in shaft dryers, grain is often subjected to local overheating, which in turn leads to protein denaturation in the germ (Shizhuang et al., 2017).

The main significant drawback of convective grain dryers is the high energy costs due to the occurrence of a temperature gradient in the material being dried, which leads to a decrease in the drying rate (Jokiniemi et al., 2015). One of the solutions to this drawback is the differentiation of the supply of thermal energy consisting in the alternation of heating the material with its cooling (Jokiniemi et al., 2015). With the development of technology and technics, recently drying methods such as microwave and thermos-radiation methods have begun to spread, the feature of which is the penetration of electromagnetic waves into the depth of the material being dried. This leads to heating of the inner part of the grain bypassing the outer layer (Rogov, 1988).

During infrared drying (thermal radiation), the rays are absorbed by the product, which ensures a more uniform heating of the material in depth compared to convective drying (Rogov, 1988). This, in turn, leads to a decrease in the temperature gradient and direct transfer of steam from inside to outside under the influence of the gradient of total pressure (Darvishi et al., 2013; Béttega et al., 2014; Zhao et al., 2017). It should be borne in mind that increasing the temperature of infrared heating can lead to damage to the grain germ. In turn, a forced decrease in the heating temperature leads to a decrease in the drying rate and, as a consequence, to an increase in the duration of the drying process, a decrease in the productivity of the dryer, and an increase in energy consumption (Rogov, 2015; Karimov et al., 2016; Martynov et al., 2018). The microwave drying is characterized by internal heating of the material (Li et al., 2014; Zhao et al., 2017). Therefore, thermal diffusion of moisture, directed from the center to the surface of the body, increases the speed of microwave drying. However, in the case of microwave drying of grain, in the absence of temperature and moisture control inside the grain, the probability of germ death due to possible local overheating is high (Ganeev, 2011; Fajzrahmanov et al., 2014; Fajzrahmanov, 2015).

At the moment, the problem of the dependence of grain temperature in the inner layers on the power of electromagnetic radiation from microwave is poorly studied. Therefore, the most promising direction is the development of a drying device based on microwave heating of the material and conducting experimental research to identify the operating parameters of the installation, which allows to obtain a high-quality finished product. The purpose of the study is to increase the

drying efficiency of small seed oilseeds by applying microwave electromagnetic radiation.

2 METHODS

The choice of the type of dryer and drying method for a particular material is impossible without taking into account its physical and thermophysical properties. In addition, the correct use of the laws of heat and moisture transfer is necessary to determine the most suitable drying mode. The drying process is characterized by internal and external moisture transfer. The optimal combination of technological methods used to increase internal and external moisture transfer will significantly intensify the drying process.

The kinetics of moisture transfer in capillary-porous colloidal bodies, which include seeds of agricultural crops, is generally determined by the difference in its potentials (temperature, moisture content). The intensity of internal moisture transfer is described by the well-known equation of non-isothermal moisture conductivity:

$$q_m = q_{m_U} + q_{m_T} = -a_m \cdot \rho_0 \cdot \nabla U - a_m \cdot \delta \cdot \rho_0 \cdot \nabla T, \quad (1)$$

where is the density of the moisture conduction flux, kg ($m^{-2} \cdot h^{-1}$); is the flux density of thermal moisture conductivity, kg ($m^{-2} \cdot h^{-1}$); is the moisture diffusion coefficient in the grain, $m^2 s^{-1}$; is the density of absolutely dry grain, kg m^{-3}; is the thermogradient coefficient, 1 K^{-1}; and are the gradients of concentration (moisture content) and temperature, kg_{moist} ($kg^{-1}_{dry\ matter}$ m^{-1}) and K m^{-1}.

In this equation, the first term characterizes the movement of moisture in the material under the influence of a moisture gradient, and the second - under the influence of a temperature gradient. When convectively dried, the heat from the upper layers of the material is transferred to the inside, therefore, the gradientsandhave opposite signs, i.e. thermal moisture conduction impedes the advancement of moisture from the surface of the material to its surface (Fajzrahmanov, 2015).

As can be seen from equation (1), by reducing the inhibitory effect of thermal moisture conduction or by increasing the flow of moisture conduction, the intensity of internal moisture transfer can be increased. The moisture flow can be increased by increasing the moisture gradient. This can be achieved by exposing the material to electromagnetic radiation in the microwave range (Ganeev, 2011).

During microwave heating, the humidity and temperature gradients have the same orientation, and in this case, moisture is removed not only under the influence of thermodynamic forces, but also under the influence of excess pressure arising inside the material (Budnikov, 2008):

$$q_m = -a_m \cdot \rho_0 \cdot \nabla U - a_m^T \cdot \rho_0 \cdot \nabla T - \gamma_\rho \cdot \nabla p \quad 2$$

where is the coefficient of molecular transfer of steam, kg ($m^{-1} \cdot s^{-1} \cdot Pa^{-1}$); is the steam pressure gradient, Pa m^{-1}.

During microwave heating, the overpressure gradient sharply intensifies the internal mass transfer, while the transfer occurs both by molecular diffusion and by filtration through pores and capillaries. The mechanism of heat and moisture transfer during microwave heating compared with convective heating is shown in Figure 1.

When using microwave heating, the drying process is limited by external moisture transfer. Due to intensive

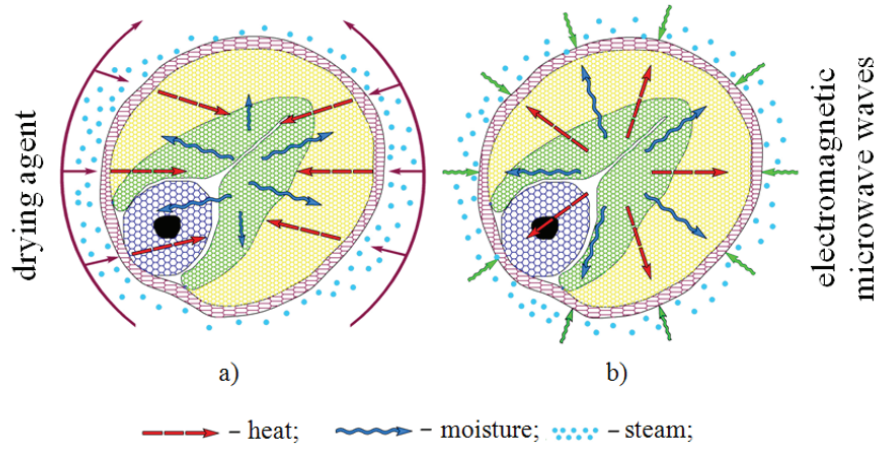

Figure 1: The mechanism of heat and moisture transfer with various heating methods
a - convective heating; b - microwave heating

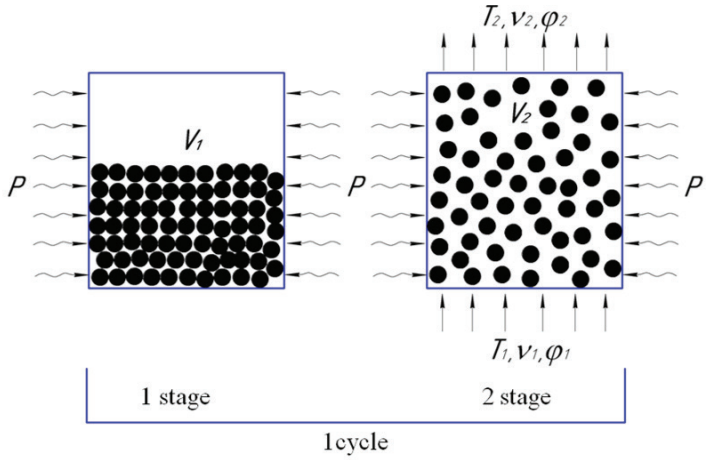

Figure 2: Technological scheme of the drying process
P - microwave E energy flows; V1 and V2 - the volume occupied by seeds at stages 1 and 2 of drying; T1, v1, φ1 - temperature, speed and humidity of the air flow at the entrance to the seed layer; T2, v2, φ2 - temperature, speed and humidity of the air flow at the exit of the seed layer.

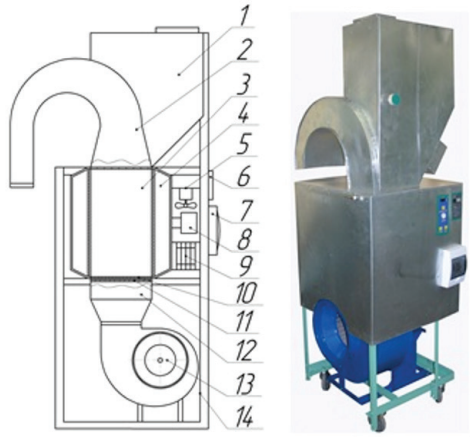

Figure 3: Microwave dryer
1 - hopper; 2 - unloading device; 3 - drying chamber; 4 - resonator chamber; 5 - magnetron cooling system; 6 – control panel; 7 - start-up protection block; 8 - magnetron with a waveguide; 9 - inverter; 10 - wireless radio-transparent mesh; 11 - shielding mesh; 12 - air distribution channel; 13 - fan; 14 - frame

movement, moisture leaving the inner layers condenses on the surface of the material and creates a barrier layer that prevents further moisture transfer. Therefore, the use of only microwave energy for drying is impractical.

By increasing the speed of the drying agent and increasing the active surface of the material, an increase in external moisture transfer can be achieved (Friesen et al., 2014). For small seed crops, drying in a fluidized bed is recommended. When drying in a fluidized bed, the active surface of the material increases to 100 %, which ensures the greatest uniformity of drying.

The technological scheme of drying small seed oilseeds has been developed, which allows to significantly intensify the drying process and reduce the risk of prolonged exposure to high temperatures on the dried material. According to the developed scheme, the drying process consists of several cycles, each of which includes two stages (Figure 2). At the first stage, the drying object is heated to the required temperature by exposure to microwave electromagnetic radiation. At this stage, there is an intensive release of moisture from the material, which accumulates on its surface. At the second stage, the material is cooled by atmospheric air in a fluidized bed. At this stage, the moisture is removed from the surface of the material and cooling is performed to the required temperature. Due to cooling in the fluidized bed, heat and moisture transfer are intensified, and uniform mixing of material particles occurs.

The drying technological scheme is implemented in a microwave drying unit developed at Bashkir State Agrarian University, the novelty of technical solutions of which is confirmed by the patent of the Russian Federation for invention. The scheme and photo of the drying unit are shown in Figure 3.

The device consists of a frame on which a resonator chamber with a built-in drying chamber is installed; a hopper and an unloading device are located above it. The drying chamber is made of radiolucent material (fluoroplast F4) in the form of a cylinder. The drying chamber is connected to a fan through an air distribution channel. A magnetron with a maximum power of P_H = 850 W and a set oscillation frequency of 2.45 GHz is connected to the resonator chamber through a rectangular waveguide.

To study the influence of the main factors on the drying process, rapeseed was selected as the drying object.

Of the many factors affecting the drying process, four main ones were chosen: W_H - initial seed moisture, P - microwave power; T - seed heating temperature; t_{exp} is the exposure time of microwave heating. The limits of factors change are given in Table 1.

The intervals of variation of factors were revealed based on the study of the researchers (Budnikov, 2008), as well as taking into account the technological requirements and construction features of the drying unit.

The studies were performed in triplicate. The reproducibility error was estimated from parallel experiments on the basis that the setting of parallel experiments, as a rule, does not give completely identical results (Ganeev, 2011; Martynov et al., 2018; Masalimov et al., 2018). According to parallel observations, the variance of reproducibility was determined. The homogeneity of the dispersions was checked using the Cochren test, the calculated value of which was compared with tabular data. The verification of individual regression coefficients for significance was carried out using Student's test at a significance level of p = 0.05. The adequacy of the obtained model was checked according to the Fisher criterion at a significance level of p = 0.05. The processing the results of the experiments, as well as calculating the calculated values of the criteria, was carried out using the software packages "Statistica" and "Mathcad". The experiments were conducted to identify the effect of the power of microwave electromagnetic radiation on changes in seed temperature. And we also studied the influence of the main mode parameters on the kinetics of drying of seeds, which allows to determine the basic laws of the drying process.

When studying the influence of microwave electromagnetic radiation power on temperature changes, the initial seed moisture was = 14.4 %; the drying was carried out at a conditional humidity W_K = 8 ... 8.5 %. The power by microwave electromagnetic radiation was regulated in the range P = 470 ... 850 W. The microwave power was a variable parameter, and the initial humidity and seed heating temperature were constant.

When studying the influence of the main operating parameters on the drying kinetics, the influence of the initial moisture and the temperature of heating the seeds on the drying kinetics was revealed. In the first case, the variable parameter was the initial humidity, which varied in the range 13 ... 25 %, which corresponds to the values of the harvesting moisture of seeds of agricultural crops in most regions of Russia. The power of the microwave electromagnetic radiation was constant and equal to P = 850 W, the maximum heating temperature of the seeds was also the same for all experiments - T = 50 °C. In the second case, the variable parameter was the heating temperature of the seeds, and the microwave power and the initial humidity were constant P = 850 W, W_H = 14.3 %. The temperature of heating the seeds during drying was in the range T = 40 ... 70 °C. The temperature was measured throughout the drying process using infrared temperature sensors. Additionally, the temperature in the seed layer was measured with kerosene thermometers, which are not affected by electromagnetic radiation.

The drying process of seeds was carried out in accordance with the developed technological scheme. At the first stage of each cycle, the seeds were heated under the influence of electromagnetic radiation in the microwave range, at the second stage, the shift was cooled in the fluidized bed. The air temperature supplied for cool-

Table 1: The limits of factors change

Terms of planning	Coded value	Factors value in the plan points			
		x_1	x_2	x_3	x_4
		W_H, %	P, W	T, °C	t_{exp}, c
Main interval	0	18	550	45	22,5
Variation interval	Δ	7	300	15	17,5
Upper level	+1	25	850	60	40
Lower level	-1	11	250	30	5

ing the seeds was 20 ... 27 °C, which is close to the average monthly air temperature during grain harvesting.

In addition, convection drying and full-cycle microwave drying were carried out. The rape seeds with an initial moisture content of W_H = 25 % were dried to a conditional moisture content of W_K = 7 ... 8 %, with a maximum heating temperature of seeds to 38 ... 40 °C. The temperature of the drying agent during convective drying did not exceed 60 ... 65 °C.

3 RESULTS AND DISCUSSION

The preliminary research results showed that the intensity of the temperature increase of rapeseed when exposed to electromagnetic radiation from microwave significantly depends on the initial humidity. At high initial humidity, the temperature of the seeds rises more intensively (Figure 4). This is due to the change in the value of the coefficient of dielectric loss, and as a consequence, a change in the amount of heat accumulated in the seeds.

At the initial stage of heating - 20 ... 25 seconds, the temperature of the seeds changes insignificantly, especially for seeds with low initial humidity (Figure 5). Further, the intensity of temperature changes increases sharply. The higher the initial seed moisture, the faster the temperature rises. The high rate of change in seed temperature is observed up to a heating temperature of 75 ... 80 °C. Further, the intensity of the temperature increase decreases.

From the graphs shown in Figures 4 and 5 it can be seen that with a microwave E power of P = 850 W, the change in seed temperature is on average 1.5 ... 2 times faster than with a power of P = 470 W. Therefore, the temperature of the seeds during microwave heating is highly dependent on the power of electromagnetic radiation. The higher the power of electromagnetic radiation, the more intensively the temperature rises.

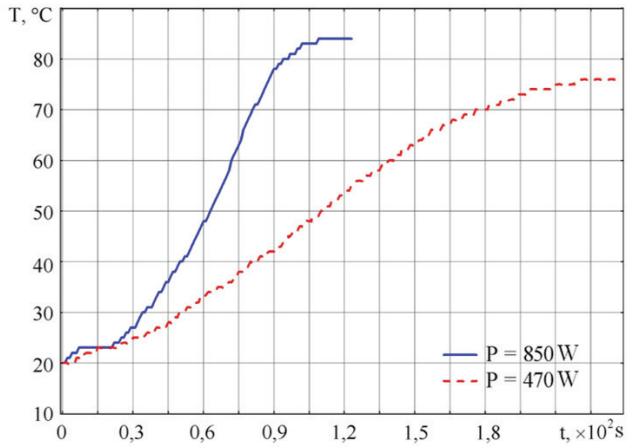

Figure 4: Graphs of the temperature change in of rapeseed when microwave heating (WH = 22%)

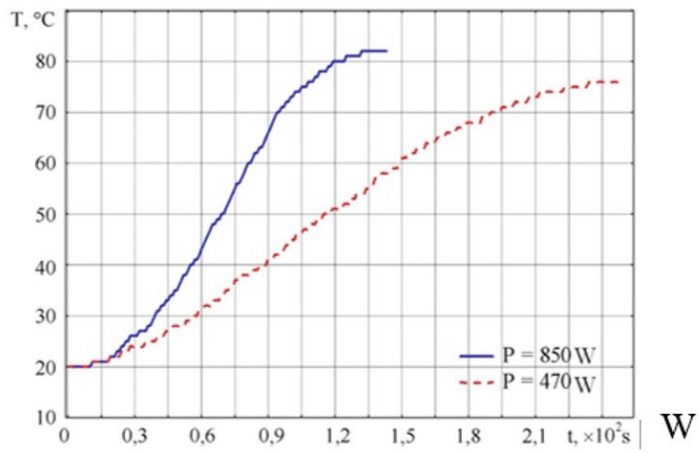

Figure 5: Graphs of the temperature changes of rapeseed at microwave heating (WH = 16%)

The study results of the effect of the initial seed moisture on the drying kinetics show that, at a high initial humidity W_H = 25 %, there is a sharp decrease in seed moisture at the initial time, due to the high speed of the first drying period. In experiments where seeds with an initial humidity of close conditional humidity W_H = 13.5 and 15 % were studied, this was not observed, the drying speed is not high even at the initial stage (Figure 6). Many researchers have noted the effect of initial seed moisture on increasing the drying rate in the first period (Gabitov et al., 2018; Martynov et al., 2018).

As can be seen from the graphs, the kinetics of drying using microwave heating significantly depends on the initial moisture content of the seeds. The critical humidity value also depends on the initial moisture content of the seeds. The higher it is, the greater the first critical humidity.

In convective drying due to insufficient supply of moisture from the internal parts of the material to the surface, the deepening of the evaporation zone occurs which leads to a continuous increase in the temperature of the material (Ganeev, 2011; Fajzrahmanov, 2015). The beginning of the temperature increase of the material indicates a transition from a period of constant drying speed to a period of decreasing drying speed. This transition is determined by the critical humidity, the value of which is influenced by the initial moisture of the material, the temperature of the drying agent, and the form of contact of moisture with the material. Unlike the temperature of the drying agent, the influence of microwave power on critical humidity is negligible. Therefore, the transition from a period of a constant speed of drying to a period of a decreasing speed of drying is determined mainly by the forms of the connection of moisture with the material.

One of the main parameters affecting the drying process is the value of the final temperature for heating the seeds. Figure 7 shows the graphs of the drying

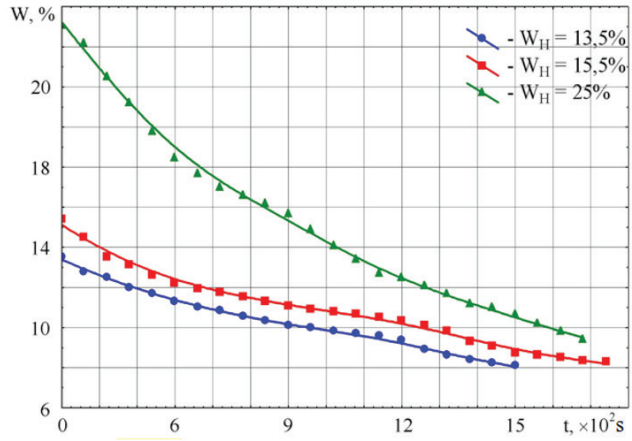

Figure 6: Curves of drying rape seeds with different initial humidity

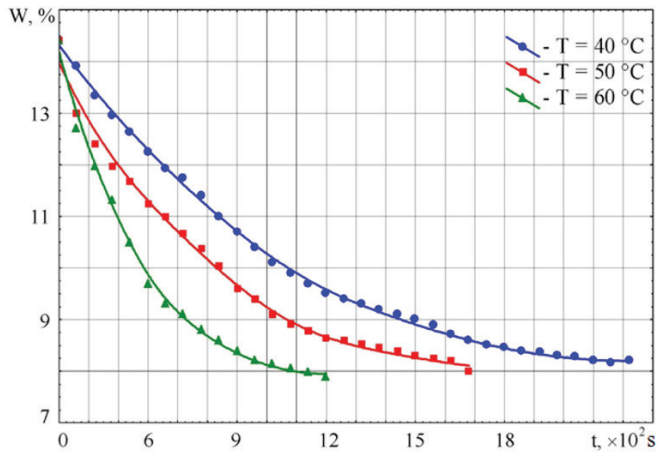

Figure 7: Curves of drying rapeseed at different temperatures of seed heating

of rape seeds with various final temperatures for heating the seeds. The analysis of the obtained curves shows that high-temperature microwave heating significantly reduces drying time.

The results of previous studies have shown that the value of the initial humidity affects the heating rate of seeds (Ganeev, 2011; Martynov et al., 2018; Masalimov et al., 2018). Therefore, for drying seeds with high initial humidity, it is necessary to select milder drying modes.

Drying rapeseed and other crops with high microwave power and temperature can lead to the destruction of the biological structure of seeds, reducing technological and sowing indicators. When choosing microwave power and drying temperature, it is necessary to take into account the final purpose of the seeds.

The results of studying the dynamics of seed heating showed that the temperature of seeds during microwave heating increases rapidly, so it is necessary to maintain the optimum temperature of the seeds during microwave drying, which does not lead to a decrease in their quality.

According to the selected technological scheme of drying at the second stage, it was planned to purge the drying object with atmospheric air in order to cool it. It was noted that cooling in the pseudo-fluidized bed facilitates the rapid and uniform removal of moisture from the surface of the drying object. It should be noted that the timely start of the cooling stage avoids heating to a critical temperature, leading to a decrease in seed quality. The researchers found that for the organization of the pseudo-fluidized bed of rapeseed should be equal to 1.5 ... 2.3 m s^{-1} (Fajzrahmanov, 2015; Kovalyshyn et al., 2015).

For the developed microwave drying device, the optimal value of the air flow rate was established experimentally, which allows uniformly mixing rape seeds in a pseudo-fluidized bed. With an air flow rate of 0.7 ... 1 m^3 s^{-1}, the velocity at the exit from the seed layer was 1.8 ... 2.1 m s^{-1}. To determine the most effective combination of drying steps, two alternatives of the drying steps were considered: 1 - heating the seeds to the required temperature and blowing in the fluidized bed until a constant temperature is reached; 2 - heating of seeds to the required temperature and cooling by 5 ... 15 °C.

Figure 8 shows the kinetics curves of microwave drying of rapeseed with periodic cooling in the fluidized bed until a constant seed temperature is reached.

The analysis of the drying kinetics graph (Figure 8), on each cycle, the nature of the temperature change is different. After each cooling cycle, the final temperature is higher than the previous one. Moreover, with a decrease in the moisture content of the material to 10 ... 9 %, the temperature of the seeds rises sharply. The explanation for this may be the onset of critical humidity, at which the transition from a period of constant to a period of decreasing drying speed begins. The period of decreasing drying rate is characterized by a continuous increase in temperature, and a continuous decrease in the drying rate (Ganeev et al., 2018). The implementation of this option of the cooling stage is difficult due to the difference in the final temperature between the cycles, which does not allow to precisely determine the time allotted to the cooling stage.

The results of the study of the kinetics of drying made it possible to establish the optimal ratio of the duration of the drying stages. At the first stage, microwave heating of the seeds to the required temperature is carried out, and at the second stage, by cooling with a decrease in the temperature of the seeds by 10 °C from the drying temperature. In the course of this research, it was

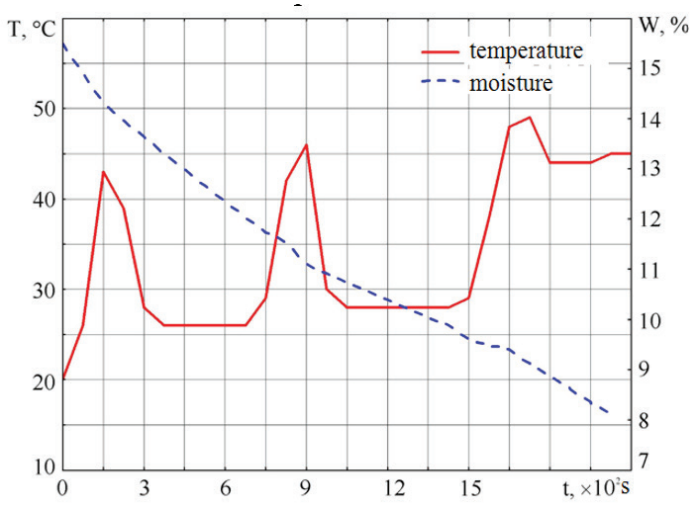

Figure 8: Kinetics of microwave drying of rapeseed with periodic cooling to a constant temperature (P = 850 W, WH = 15.5 %, Tavg = 45 °C)

found that when the seeds are cooled by 5 ... 6 °C, there is uneven cooling of the seeds along the layer thickness. The cooling at 13 ... 15 °C led to unreasonable energy consumption due to the continuous operation of the fan. Taking into account the cooling time of the seeds, uniformity of cooling across the layer thickness and energy consumption, the optimal value of the change in the temperature of the seeds at the cooling stage corresponding to 8 ... 10 °C was established. At these temperatures, the duration of the first and second stages are in the same time interval and amount to 1.5 ... 2 minutes, depending on the microwave power. Depending on the initial humidity, the conditioned moisture of the rapeseed is achieved in 8 ... 12 drying cycles.

Figure 9 shows the graphs of the drying of rapeseed with periodic cooling at 8 ... 10 °C from the drying temperature. From the graphs it is seen that the dynamics of heating between cycles in this case is also different, but no sharp jumps in temperature are observed. This scheme of alternating stages allows you to fully automate the drying process and select the operating modes of the microwave drying unit.

The results of comparative experiments of convective and microwave drying of rapeseed are shown in Figure 10.

The analysis of the drying curves shows that the use of microwave energy of electromagnetic radiation for drying rapeseed can reduce the drying time, in comparison with the convective method, by 1.5 ... 1.7 times. In addition, microwave drying does not contaminate

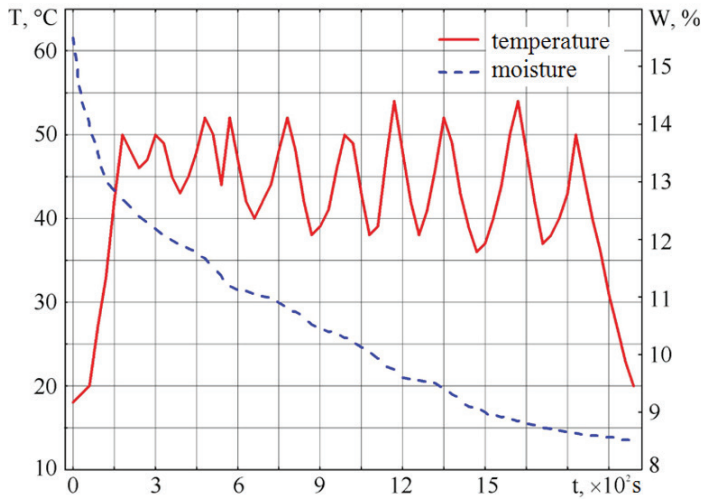

Figure 9: Kinetics of microwave drying of rapeseed with periodic cooling at 8 ... 10 °C (P = 600 W, WH = 15.5 %, Tavg = 45 °C)

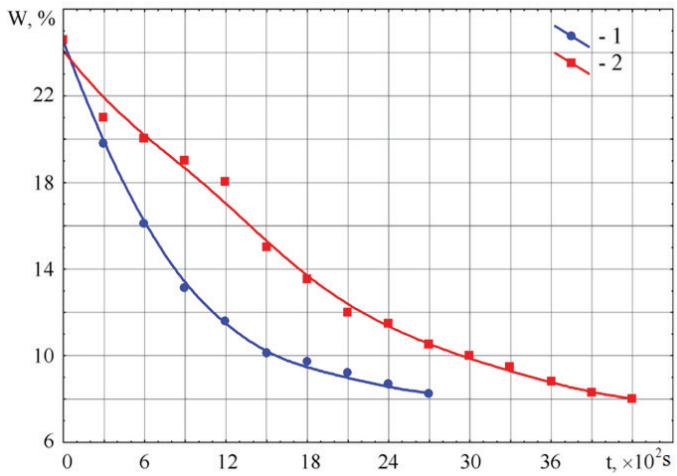

Figure 10: Curves of drying rape seeds with different ways (WH = 25%)
1 - MICROWAVE drying (P = 850 W, T = 38 ... 40 °C); 2 - convective drying (Ta.s = 60 ... 65 °C, T = 38 ... 40 °C)

the seeds and the surrounding air with fuel combustion products as in convective drying.

In the study area involved in the problem of drying small-seeded crops, works are devoted to increasing the drying efficiency by improving the convective drying method, which is traditional for most countries. Using the energy of electromagnetic radiation from the microwave range for drying small seed oilseeds is poorly studied. In other studies dealing with the problems of using microwave energy for drying crops, in most cases, microwave heating is considered as an additional source of heat to convective heating. Drying modes with short-term (2-3 sec.) exposure to high-power microwave electromagnetic radiation are proposed, which leads to overheating and loss of seed quality of grain. Furthermore, some researchers suggest using microwave heating as a preliminary before convective drying or active ventilation of the grain, which can lead to an increase in energy costs for performing these operations (Fajzrahmanov, 2015; Ganeev, 2011). Our studies have confirmed the possibility of using the energy of electromagnetic radiation from the microwave range as the main heating method for drying materials without the use of additional heating methods. The use of microwave heating can significantly intensify the drying process of small seed oilseeds, while maintaining their technological and sowing qualities.

4 CONCLUSIONS

The construction of a microwave drying device in which a two-stage technological scheme of drying is implemented is proposed. At the first stage, the seeds are heated to the required temperature, by exposing them to electromagnetic radiation in the microwave range. At the second stage, the seeds are cooled with outside air in a pseudo-fluidized bed. Based on the results of the drying kinetics study, the optimal ratios of the duration of its stages were determined, according to which, after heating to the desired temperature, cooling at 8 ... 10 °C from the set temperature follows at the first stage. At the same time, the conditional seed moisture content of 7 ... 8 %, depending on the initial importance, is achieved in 8 ... 12 cycles. The preliminary research results showed that the use of microwave energy of electromagnetic radiation for drying rapeseed can reduce the drying time, compared with the convective method, by 1.5 ... 1.7 times. The rational drying mode was determined: microwave power P = 600 W, seed heating temperature T = 50 °C, exposure time of microwave heating t_{exp} = 20 min. With these parameters of the mode, the quality of the dried seeds is at a satisfactory level and the minimum heat consumption is Q_{sat} = 4.1 MJ kg^{-1} of moisture, which is 1.4 times lower than in convection-type dryers. The proposed drying method can be used both in agricultural production and in the food and chemical industries. As studies have shown, this drying method allows to increase the productivity of the process compared to existing methods. Using microwave energy in the developed drying device makes it possible to use it as a universal device for drying, disinfecting and biostimulating seeds.

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Yield and quality of two sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll) cultivars are influenced by foliar application of salicylic acid, irrigation timing, and planting density

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Yield and quality of two sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll) cultivars are influenced by foliar application of salicylic acid, irrigation timing, and planting density

Abstract: Two field experiments were conducted to evaluate the time of foliar application of 100 ppm salicylic acid (SA), two irrigation (IR) timings, three levels of spacing (SP) hill^{-1} with different plant density on growth, yield and quality characters of two sugar beet cultivars ('Samba' and 'Farida'). The results revealed that the foliar application of 100 ppm SA at 30 days after planting (DAP) and 14 days after the first application significantly influenced top fresh mass and root biomass of sugar beet plants. Conversely, the increasing period between planting and first irrigation scheduling led to significant differences in fresh mass, sugar yield, and sucrose % as well as purity % of sugar beet. Plants density with 60 × 20 cm spacing hill^{-1} was found to be better than the other two spacings for major characters, particularly root fresh mass, and Total soluble solids and purity %. Inversely, spacing at 60 × 15 cm, between hills gave the maximum levels of top fresh mass, root yield and sugar yield in the first season. The interaction effect between spacing hill^{-1} at 60 × 20 cm and 100 ppm SA applied at 30 DAP gave the maximum levels of increment for most of the studied characters, particularly for cultivar 'Farida'.

Key words: planting density; sugar quality; salicylic acid; sugar beet; irrigation timing; yield

Vpliv foliarnega dodajanja salicilne kisline, časa namakanja in gostote setve na pridelek in kakovost dveh sort sladkorne pese (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll)

Izvleček: Izvedena sta bila dva poljska poskusa za ovrednotenje vpliva časa foliarnega dodajanja 100 ppm salicilne kisline (SA), dveh terminov namakanja (IR), treh gostot setve (SP), na pridelek in kakovostne parametre dveh sort sladkorne pese ('Samba' in 'Farida'). Rezultati so pokazali, da je foliarno dodajanje 100 ppm SA 30 dni po setvi (DAP) in 14 dni po prvi uporabi DAFA značilno vplivalo na svežo maso nadzemnih delov in biomaso korenov sladkorne pese. Naraščanje časa med setvijo in prvim zalivanjem je privedlo do značilnih razlik v sveži masi, pridelku sladkorja, v odstotku saharoze in v čistosti posevka sladkorne pese. Gostota z razmakom rastlin 60 × 20 cm se je izkazala boljša od ostalih dveh za večino merjenih lastnosti, še posebej v sveži masi korenov, v odstotku TSS in odstotku čistosti. Obratno je dal razmak 60 × 15 cm največjo svežo maso nadzemnih delov, pridelka korenov in sladkorja v prvi sezoni. Interakcija gostote setve 60 × 20 cm in dodatek 100 ppm SA 30 dni po setvi je dala največje vrednosti vseh preučevanih znakov, še posebej pri sorti 'Farida'.

Ključne besede: gostota setve; kakovost sladkorja; salicilna kislina; sladkorna pesa; režim namakanja; pridelek

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1 INTRODUCTION

Sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll) is a temperate crop also cultivated in subtropical countries. It is generally considered as a crop of temperate region, however, it is largely cultivated also in sub-tropical countries, where it can be grown successfully during the winter season (Brar et al., 2015). It is a biennial plant and one of the most essential sugar crops. The global cultivated area of sugar beet in 2014 was 4.47 million ha with a total root yield of 266.8 million tons (FAOSTAT, 2016). In Egypt, the cultivated area in the year 2016 was 254, 991 ha with the root yield of 13.3 million tons (FAOSTAT, 2016). Sugar beet is a widely adaptive crop and grows in multiple agro-ecological conditions. It takes a shorter period to maturity than the sugar cane plant and also productivity per unit time is higher and requires less water than sugarcane (Brar et al., 2015). Many environmental and agronomic factors such as biofertilization, irrigation, planting spacing sowing methods had influenced the production and quality of sugar beet (Abdelaal, 2015a; Abdelaal and Tawfik, 2015; Omar et al., 2019a,b). The main target for growers and sugar companies is to improve their quality and increase the extracted sugar (Awad et al., 2013). Therefore to get maximum benefits from sugar beet, there is a need to select the most appropriate varieties, to reduce planting time, to optimize planting methods, planting density, sowing depth as well as to provide adequate crop nutrition and irrigation schedule (Seadh et al., 2013; Brar et al., 2015).

However, among the abiotic stresses, water deficit is one of the most environmental factors threatening the agricultural production and the main reason of crop loss worldwide, reducing morphological characters and yield components of plants (Abdelaal, 2015b; EL Sabagh et al., 2019a). Due to drought stress the growth duration, growth, and development, as well as yield, are decreased (EL Sabagh et al., 2019e). Furthermore, under drought stress, the accumulation of osmoprotectants like proline is noticed (EL Sabagh et al., 2019d).

Reduced photosynthetic rates of plants have a direct effect on growth characters such as decrease dry mass and leaf area (Gong et al., 2005). Under drought stress, nitrogen metabolism, enzyme activities and protein synthesis can be impaired (Saneoka et al., 2004). However, plants have many mechanisms to adjust abiotic stress by changing the morpho-physiological characters (Todaka et al., 2012; Molla et al., 2019; Yassin et al., 2019). Neseim et al. (2014) found that under drought stress, morphological characters such as root yield and white sugar/fedden (0.42 ha) were significantly reduced, whereas, total soluble phenols and free amino acid concentrations in leaves and roots were significantly increased that ultimately led to surviving under stress condition.

It was previously reported, that root diameter, percentage of sucrose, and root yield, as well as sugar yield (t/fedden (0.42 ha)) of sugar beet, increased significantly with larger plant spacing from 20 to 30 cm (Nafei et al., 2010; Shalaby et al., 2011). While, Ramazan (2002) observed that root yield and sugar content were the highest at closer planting density of 103600 plants/ha (i.e 45 × 20 cm spacing), as compared to 555000 (45 × 40 cm), 73000 (43 × 30 cm) and 88900 (45 × 25 cm) plants ha^{-1}. Similarly, Bhullar et al. (2010) reported that the highest root and sugar yield of sugar beet were produced from the planting density at 1,00,000 plants ha^{-1} (50 x 20 cm) as compared with planting densities 83,333 plants ha^{-1} (60 x 20 cm) and 1,11,111 plants ha^{-1} (60 x 15 cm).

The previous studies have reported that the application of osmoprotectants under stressful environment (biotic and abiotic) help to maintain plant growth and yield. Moreover, osmoprotectants led to alleviate the injurious effect of stress conditions and enhance the growth characters and yield parameters of different crops moreover, it helps to survive under different biotic and abiotic stress. (El?? Sabagh et al., 2019 b,c).

Salicylic acid (SA) is recognized as a phytohormone produced after a chain of chemical reactions as benzoic acid derivative and plays a vital role in many physiological process such as photosynthesis, nutrient uptake, membrane permeability and also help to survive under different biotic and abiotic stress playing a key role in systemic acquired resistance (Noreen et al., 2009; Abdelaal, 2015b). Moosavi (2012) and Abido et al. (2015) observed that foliar spray of SA led to improve plant growth characters and enhanced the tolerance capacity of plants under abiotic stress as well as it protects the plant from oxidative stress by increasing antioxidant enzymes activity, finally increasing the fresh root and shoot mass of sugar beet and sunflower plants (Merwad, 2015; Noreen et al., 2017a,b). Furthermore, However, the foliar application of 100 mg l^{-1} SA gave the highest values for growth characters of stevia plants (reported by El-Housini et al., 2014); soybean plants (Mishra and Prakash, 2013).

There is an insufficient amount of information about the effect of SA on sugar beet growth and productivity that are linked to water deficit and density population under field conditions. The main target for the cultivation of sugar beet is to extract sugar of high yield and quality. Therefore, to get maximum benefits from sugar beet there is a need to select the most appropriate varieties, planting methods, planting density, providing adequate crop nutrition and irrigation schedule. Considering the important issues, two field experiments were conducted to evaluate the foliar application of 100 ppm, irrigation

(IR) and spacing (SP) on growth, yield and quality characters of two sugar beet cultivars ('Samba' and 'Farida').

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL SITES

Two field experiments were conducted at Kalabsho, El-Dakahlia Governorate, Egypt (30° 35′41.9" N latitude and 32° 16′ 45.8" E longitude) in consecutive two winter seasons 2016-17 and 2017-18. The area is characterized by a short warm-winter and long-hot summer. The annual average rainfall and relative humidity are about 40.4 mm and 65.4 %. The area of study exhibits certain desertification features because the surface Nile water does not adequately reach to the ends of canals. Groundwater is the major source of irrigation.

2.2 EXPERIMENTAL TREATMENTS, DESIGN AND PLANT MATERIALS

Two sugar beet cultivars namely 'Farida' and 'Samba' were used in the experiment. Treatments included:

three levels of plant density such as 44465 plants/fedden (0.42 ha), 33335 plants/fedden (0.42 ha), and 26665 plants/fedden (0.42 ha);

three types of plant spacing (SP) hill^{-1} such as at 60 x 15 cm , 60 x 20 cm and 60 x 25 cm;

foliar application of 100 ppm SA, applied at 30 DAP and 14 days after the first application,

and two irrigations (IR) times, one applied at 20 DAP and another one applied at 30 DAP.

All treatments were arranged in a split-split plot design and repeated four in four blocks to minimize the biasness.

To minimize the experimental errors, two irrigations' times (IR) were arranged in main plots, while hill spaces (spacing (SP) hill^{-1} with three levels of plant density were arranged in sub-plots and two sugar beet cultivars were located in sub-sub-sub plots.

2.3 EXPERIMENTAL PROCEDURE

Four seeds were sown in hills on 4th and 3rd October in 2016-17 and 2017-18 seasons. Each sub-plot contained 6 rows, which were 60 cm apart. Potassium at 24 kg K_2O/fedden (0.42 ha) and phosphorus at 30 kg P_2O_5/fedden (0.42 ha) were applied in the soils during final land preparation. Ammonium nitrate (33.5 % N) at 100 kg N/fedden (0.42 ha) was added at two equal doses after thinning and one month later after the first application. Foliar application of 100 ppm SA was applied 30 DAP and 14 days after the first application (DAFA). Harvest date was after 210 days from sowing.

2.4 DATA COLLECTION

2.4.1 Morphological characters

At harvesting time ten plants were randomly taken from each sub-sub-sub plot to determine morpho-physiological and yield characters. Morphological characters such as root diameter (cm), root fresh mass (kg plant^{-1}) and top fresh mass (kg plant^{-1}) were recorded.

2.4.2 Yield and quality evaluation

Total soluble solids (TSS %) were estimated in the juice of fresh roots by using Hand Refractometer. Sucrose percentage (%) was determined polarimetrically on lead acetate extract of fresh macerated roots according to the method of (Le Docte, 1927; Dutton et al., 1961). Apparent purity percentage (%) was determined as a ratio between sucrose % and TSS % of roots. Sugar beet plants from each plot were harvested topped to calculate root yield and top yield (t/fedden (0.42 ha)). Sugar yield (t/fedden (0.42 ha)) was calculated as follows: Sugar yield (t/fedden (0.42 ha)) = Root yield (t/fedden (0.42 ha)) x sucrose %.

2.5 STATISTICAL ANALYSES

Data represent the mean ± SD. The student's t-test was used to determine whether significant difference ($p < 0.05$) existed between mean values according to O'Mahony (1986).

3 RESULTS AND DISCUSSION

3.1 YIELD AND QUALITY PARAMETERS OF SUGAR BEET ARE INFLUENCED BY SA, IRRIGATION TIMES, DIFFERENT SPACINGS AND CULTIVARS

After two years of observation, the results of the study revealed that foliar application of 100 ppm SA at 30 DAP and 14 DAFA significantly influenced the top fresh mass and root biomass of sugar beet plants under both the two growing seasons. Conversely, the increasing

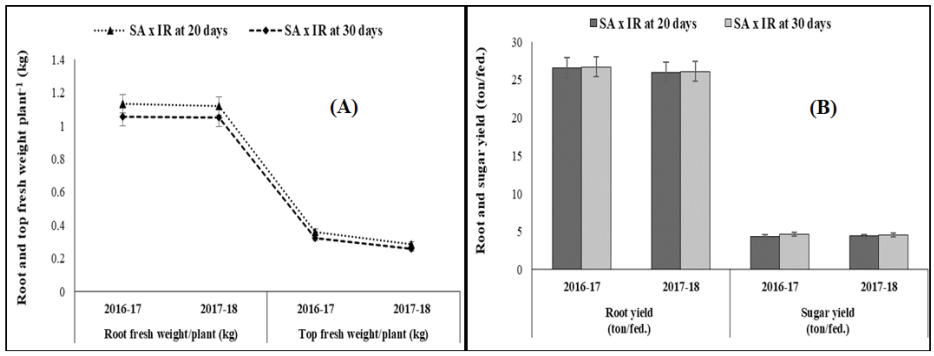

Figure 1: (A): Root and top fresh mass plant^{-1} of sugar beet are influenced by SA (applied at 30 DAP and 14 days after first application), and irrigation (IR) at 20 and 30 DAP. (B): Root and sugar yield (t/fedden (0.42 ha) of sugar beet are influenced by the application of SA (applied at 30 DAP and 14 days after first application), and irrigation (IR) at 20 and 30 DAP.

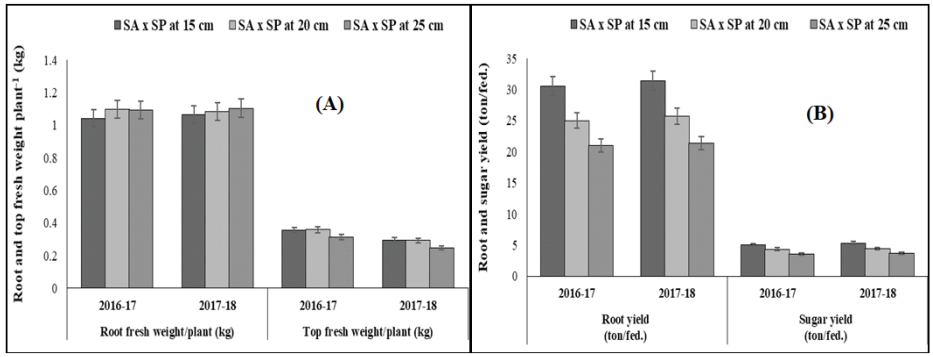

Figure 2: (A): Root and top fresh mass plant^{-1} of sugar beet are influenced by SA (applied at 30 DAP and 14 DAFA), and spacing (SP) hill^{-1} at 60 x15, 60 x 20 and 60 x 25 cm. **(B):** Root and sugar yield (t/fedden (0.42 ha) of sugar beet are influenced by SA (applied at 30 DAP and 14 DAFA), and spacing (SP) hill^{-1} at 60 x 15, 60 x 20 and 60 x 25 cm.

period of irrigation led to significant differences in fresh mass, sugar yield, and sucrose % as well as purity % in both the seasons (Figure 1 (A & B), 2 (A & B) and 3 (A & B)). Prolongation of irrigation to 30 days gave the highest values of sugar yield in the two seasons, whereas the increment of root and sugar yield was not significant in the first season (2016-17). The influence of prolonged period between last irrigation on morphological characters such as root fresh mass and top fresh mass are similar to the results which have been reported by Jain et al. (2010) and Abdelaal et al. (2017).

In the present study, planting at space 20 cm between hills with the application of SA was more promising than other spaces and gave the highest levels of root fresh mass and top fresh mass in both seasons. However, the increment of root and sugar yield was significant and obtained with SA and 15 cm space between hills. Regarding the effect of cultivars, the maximum levels of root fresh mass, top fresh mass, and root yield, as well as sugar yield, were obtained with the cultivar 'Farida' compared to 'Samba'. The results of the present study concerning cultivars are similar to the findings of Ramadan, (1999) and Awad et al (2012), who also observed significant variations between different cultivars, due to the application of SA, IR and also for SP.

Results presented in Figure 4 (A & B) & 5 (A) on the interaction effects between 100 ppm SA, water regimes before harvest and hills' spacing were signifi-

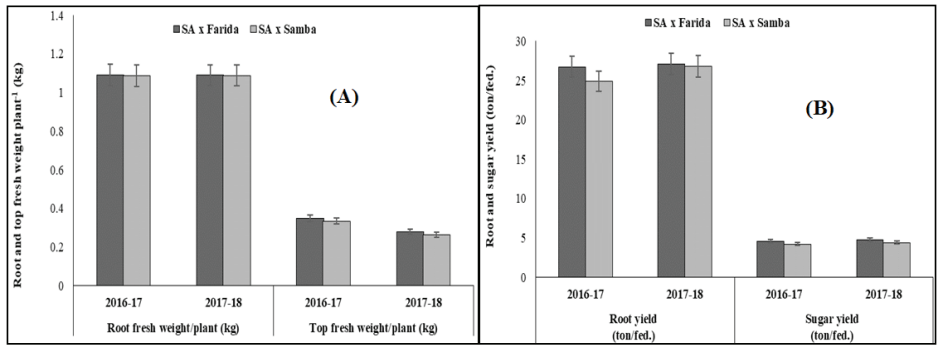

Figure 3: (A): Root and top fresh mass plant^{-1} of sugar beet are influenced by SA (applied at 30 DAP and 14 DAFA), and cultivars ('Farida' and 'Samba'). (**B):** Root and sugar yield (t/fedden (0.42 ha)) of sugar beet is influenced by SA (applied at 30 DAP and 14 DAFA), and cultivars ('Farida' and 'Samba').

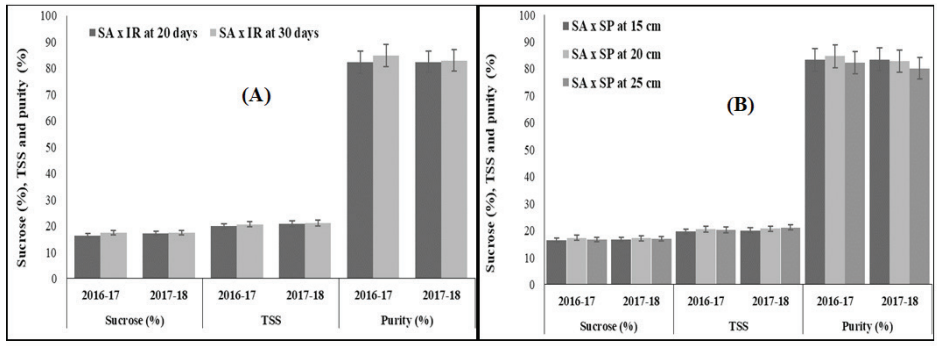

Figure 4: (A): Sucrose (%), total soluble solids (TSS %) and purity (%) of sugar beet are influenced by SA (applied at 30 DAP and 14 DAFA), and irrigation (IR) at 20 and 30 days. (**B):** Sucrose (%), TSS and purity (%) of sugar beet are influenced by SA (applied at 30 DAP and 14 DAFA), and spacing (SP) hill^{-1} at 60 x 15, 60 x 20 and 60 x 25 cm.

cant on the most of the characters of sugar beet in both seasons under the study. Nevertheless, the interaction effects between SA, regime water and spacing hills were not significant on TSS % in the second season only. The valuable effect of SA on root fresh mass, top fresh mass, and root yield may be due to its role in increasing chlorophyll concentration and enhancement photosynthetic process as well as decreasing the injurious effect of water deficit on plants Abdelaal (2015b). These effects are in agreement with those recorded by Azooz et al. (2011) and Kang et al. (2013). The authors found the relationship between SA, IR, and SP for root fresh mass, top fresh mass, root yield and also for sucrose (%), TSS and purity (%).

3.2. YIELD AND QUALITY PARAMETERS OF SUGAR BEET ARE INFLUENCED BY INTERACTION EFFECT OF SA, IRRIGATION, SPACING, AND CULTIVARS

The results of the current study presented in Figure 5 (B), and 6 (A & B) revealed that the interaction effects between SA, last irrigation and hill spacing were significant on root fresh mass, top fresh mass, root and sugar yield, sucrose %, TSS % as well as purity % in both seasons. The maximum values of root fresh mass, top fresh mass, root yield, and sugar yield were obtained with SA, 15cm between plants and period between last irrigation and harvest date at 20 days in both seasons (Figure 5(B), 6(A)). Like-

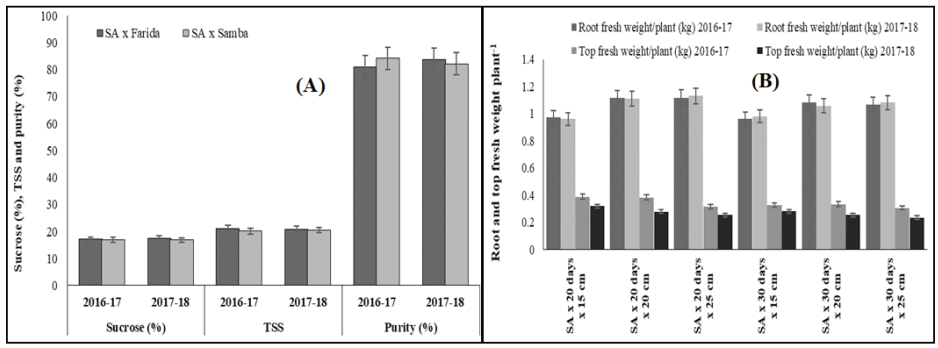

Figure 5: (**A):** Sucrose (%), TSS and purity (%) of sugar beet are influenced by SA (applied at 30 DAP and 14 DAFA), and cultivars ('Farida' and 'Samba'). **(B):** Root and top fresh mass plant^{-1} of sugar beet are influenced by interaction effect of SA (applied at 30 DAP and 14 DAFA), irrigation (IR) at 20 and 30 days, and spacing (SP) hill^{-1} at 60 x 15, 60 x 20 and 60 x 25 cm.

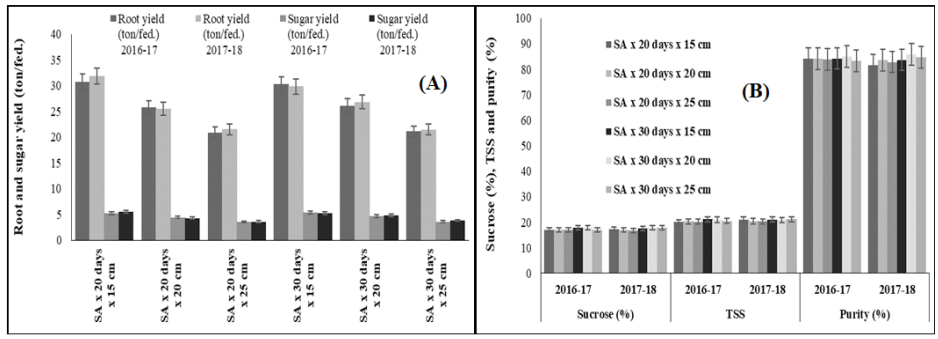

Figure 6: (**A):** Root and sugar yield (t/fedden (0.42 ha) of sugar beet are influenced by interaction effect of SA (applied at 30 DAP and 14 DAFA), irrigation (IR) at 20 and 30 days, and spacing (SP) hill^{-1} at 60 x 15, 60 x 20 and 60 x 25 cm. (**B):** Sucrose (%), TSS and purity (%) of sugar beet are influenced by interaction effect of SA (applied at 30 DAP and 14 DAFA), irrigation (IR) at 20 and 30 days, and spacing (SP) hill^{-1} at 60 x 15, 60 x 20 and 60 x 25 cm.

wise, the highest levels of sucrose %, TSS %, and purity % were obtained with the interaction between SA, the period between last irrigation and harvest date at 30 days and 20 cm between plants (Figure 6 (B)). It might be due to the reduction of competition between plants for light and nutrients, consequently improving plant growth and production (Nafei et al., 2010).

Referring to the effect of interaction between SA, the period between last irrigation and harvest date as well as cultivars on root fresh mass, root yield, sugar yield, and quality characteristics, presented data in Figures 7 (A & B) displayed a significant effect on most characteristics in the two growing seasons. The maximum values of root yield, sugar yield, sucrose %, and purity % were recorded with the interaction between SA, the period between last irrigation and harvest date at 30 days and 'Farida' cultivar compared with other treatments. Increasing the prevention period of water supply before harvesting led to increasing the concentrations of sucrose and purity %. These results are in harmony with the achieved results by Sohrabi and Heidari (2008), who also found the maximum values of plant biomass such as root, top biomass , sugar yield due to the combined effect of SA, application times of irrigation and different crop cultivars.

Regarding to interaction effects between SA, hill spacing and cultivars on fresh mass of root, root yield, sugar yield, sucrose %, TSS %, and purity % obtained

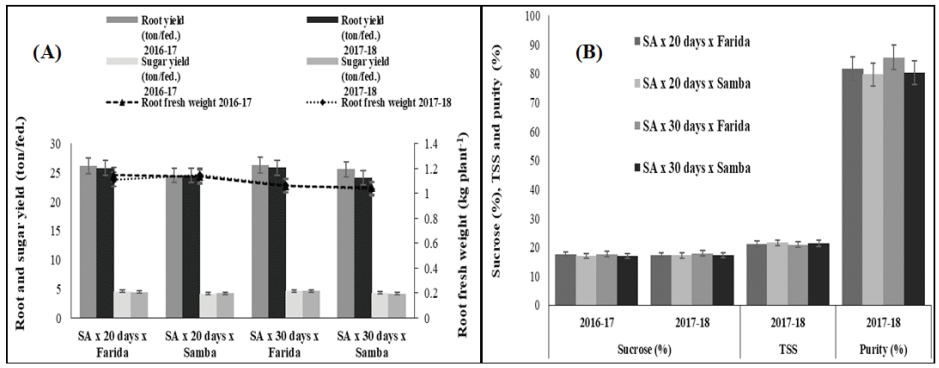

Figure 7: (A): Root fresh mass plant^{-1} (kg), root yield (t/fedden (0.42 ha) and sugar yield (t/fedden (0.42 ha)) of sugar beet is influenced by interaction effect of SA (applied at 30 DAP and 14 DAFA), irrigation (IR) at 20 and 30 days, and cultivars ('Farida' and 'Samba'). (**B):** Sucrose (%), TSS and purity (%) of sugar beet are influenced by interaction effect of SA (applied at 30 DAP and 14 DAFA), irrigation (IR) at 20 and 30 days, and cultivars ('Farida' and 'Samba').

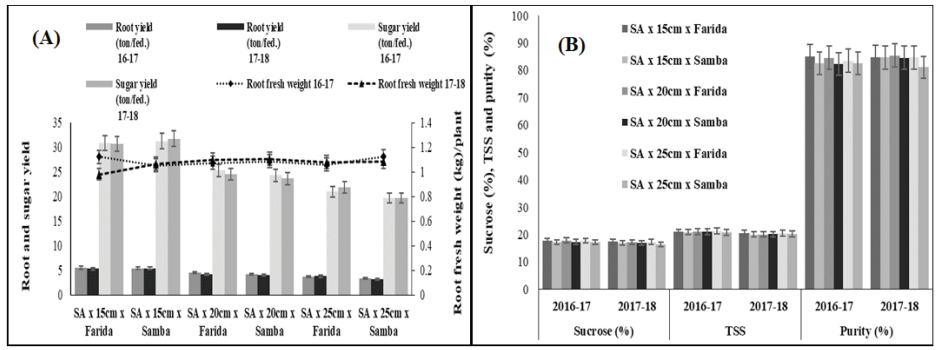

Figure 8: (A): Root fresh mass plant^{-1} (kg), root yield (t/fedden (0.42 ha)) and sugar yield (t/fedden (0.42 ha)) of sugar beet is influenced by interaction effect of SA (applied at 30 DAP and 14 DAFA), spacing (SP) hill^{-1} at 60 x 15, 60 x 20 and 60 x 25 cm, and cultivars ('Farida' and 'Samba'). (**B):** Sucrose (%), TSS and purity (%) of sugar beet are influenced by interaction effect of SA (applied at 30 DAP and 14 DAFA), spacing (SP) hill^{-1} at 60 x15, 60 x 20 and 60 x 25 cm, and cultivars ('Farida' and 'Samba').

results in Figure 8 (A) showed that the maximum levels of fresh mass of root, root yield, and sugar yield were recorded at treatment interaction between SA, 15 cm hill spacing and 'Farida' cultivar as well as interaction between SA, 15cm hill spacing and 'Farida' cultivar respectively in both season comparing with other treatments. The same trend was observed with the combined effect of SA, IR, plants' spacing and cultivars'(Ramadan, 1999; Shalaby et al., 2011). Furthermore, the maximum levels of sucrose % and purity % were obtained with the interaction between SA, 15 cm hill spacing and 'Farida' cultivar Figure 8 (B). The assumption is confirmed by several earlier findings but for different crops, who also revealed that application of SA influenced the growth, photosynthesis and carbohydrate metabolism of maize (Zhou et al., 1999; Khodary, 2004), sugar beet (Ghoulam et al., 2001) and sugarcane (Du et al., 1998) under stressed condition.

Results presented in Figure 9, clearly show that the highest levels of sucrose, root and sugar yield were recorded with the interaction between SA, the period between last irrigation and harvest date at 30 days, 15cm spacing hills and 'Farida' cultivar. These findings are in agreement with the observation of Awad et al. (2014). The results may be due to the essential role of SA in the enhancement of cellular osmolytes and improve photosynthetic pigments as well as plant production under water deficit conditions (Abdelaal, 2015b).

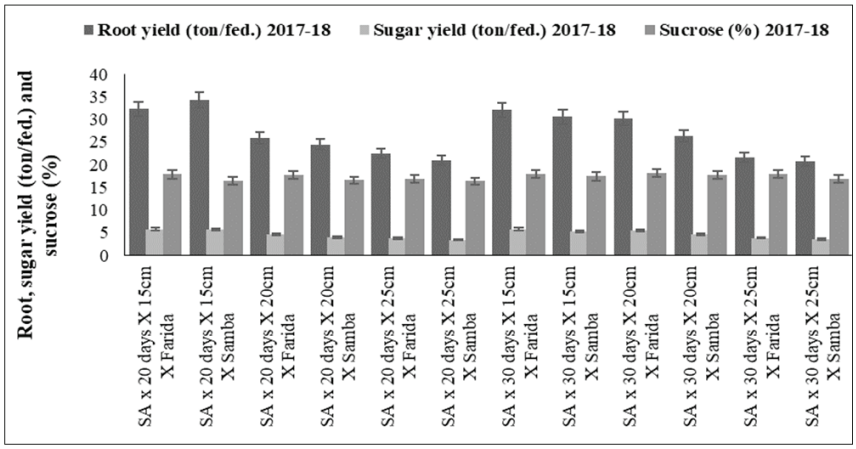

Figure 9: Root, sugar yield and sucrose (%) of sugar beet are influenced by interaction effect of SA (applied at 30 DAP and 14 DAFA), irrigation (IR) at 20 and 30 days, spacing (SP) hill^{-1} at 60 x 15, 60 x 20 and 60 x 25 cm, and cultivars ('Farida' and 'Samba').

4 CONCLUSION

Our results of the study suggest that the foliar application of 100 ppm SA at 30 DAP and 14 DAFA significantly influenced the top fresh mass and root biomass of sugar beet plants under both the two growing seasons. Conversely, the increasing period between planting and first irrigation led to significant differences in fresh mass, sugar yield, and sucrose % as well as purity % of sugar beet in both the seasons. Plants spacing hill^{-1} of 60 × 20 cm with 33335 plants/fedden (0.42 ha)) were found to be better than the other two spacings for most of the characters, particularly root fresh mass, and sucrose total soluble solids (TSS %) and purity %. Inversely, spacing at 60 × 15 cm (with 44465 plants/fedden (0.42 ha)), between hills gave the maximum levels of top fresh mass, root yield and sugar yield in the first season. The interaction effect between spacing hill^{-1} at 60 × 20 cm (33335 plants/fedden (0.42 ha)) and 100 ppm SA applied at 30 DAP gave the maximum levels of increment for most of the studied characters, mainly for the cultivar 'Farida' than 'Samba'.

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Seasonal incidence and bionomics of rose aphid, *Macrosiphum rosae* (Linnaeus, 1758), (Hemiptera: Aphididae) in Kashmir, India

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Seasonal incidence and bionomics of rose aphid, *Macrosiphum rosae* (Linnaeus, 1758), (Hemiptera: Aphididae) in Kashmir, India

Abstract: Rose is the principal flower of the world floriculture industry that is being exclusively used as cut flower, potted plant and garden plant. It plays significant part in numerous industries *viz.* food, perfumery and cosmetic industries. About 96 % of women's perfumes contain true Bulgarian rose oil. Roses are well acclimatized in Jammu & Kashmir because of its suitable agro climatic conditions which can permit its large scale production and rose products produced in the state are at par with the international standards. But the aesthetic and commercial value of roses is greatly lowered by numerous insect pests resulting in low yield. However, its major pest include aphid species most notoriously *Macrosiphum rosae* that pose many challenges and threats to rose plant cultivation. Aphid colonies on roses result in reduction of medical value of the plant and cause economic losses to growers particularly during spring and summer season. In order to reduce the economic losses inflicted by rose aphid, it is necessary to study different biological parameters of this pest species so that an effective management plan can be formulated.

Key words: floriculture; rose; *Macrosiphum rosae*; economic losses; biological parameters

Sezonsko pojavljanje in bionomika vrtnične uši (*Macrosiphum rosae* (Linnaeus, 1758), Hemiptera: Aphididae) v Kašmirju, Indija

Izvleček: Vrtnica je glavna cvetlica v svetovni florikulturni industriji, ki se uporablja za rezano cvetje, kot lončnica in zasaditev vrtov. Ima pomembno vlogo v različnih industrijskih panogah kot so živilstvo, parfumerska in kozmetična industrija. Okrog 96 % ženskih parfumov vsebuje olje prave bolgarske vrtnice. Vrtnice so dobro prilagojene razmeram v Jammu in Kašmirju zaradi primernih agroklimatskih razmer, ki omogočajo velikopovršinsko gojenje vrtnic in proizvodi iz njih so v državi pripravljeni po mednarodnih standardih. Estetsko in komercialno vrednost vrtnic v veliki meri zmanjšujejo številne škodljive žuželke, kar zmanjšuje pridelek. Največji škodljivci so listen uši in med njimi vrtnična uš (*Macrosiphum rosae* (Linnaeus, 1758)), ki predstavlja resen izziv gojenju vrtnic. Kolonije listnih uši na vrtnicah zmanjšujejo medicinsko vrednost vrtnic in povzročajo ekonomsko izgubo pridelovalcem, še posebej spomladi in poleti. Z namenom zmanjšanja ekonomskih izgub, ki jih povzročajo listne uši na vrtnicah, je potrebno preučiti različne biološke parametre tega škodljivca za pripravo plana učinkovitega upravljanja.

Ključne besede: florikultura; vrtnica; *Macrosiphum rosae*; ekonomske izgube; biološki parametri

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1 INTRODUCTION

Rose is an attractive pricky ornamental shrub belonging to genus *Rosa* of family Rosaceae. It possesses a climbing or trailing habit and is commonly called as "Queen of Flowers". Roses are exclusively used in beauty and decorations, without these gardens are not considered complete. Apart from adoration, attar extracted from roses is used in making various fragrant mixtures. Roses produce rose oil, rose water, all of which are valuable and important base material for a number of industries such as perfume, pharmaceutical and cosmetic (Ayci et al., 2005). But the main use of roses is in cut flower industry and landscaping.

Roses are also well- known for their medicinal value and are used in manufacture of large number of medicines. The rose hips which are valued for food are also the richest natural source of vitamin C (having thrice more Vitamin C content than citrus fruits) and are pressed commercially to give rose hip syrup that has been long used to prevent scurvy (Anonymous, 1982). Rose hip herbal tea helps to strengthen the immune system and can further prevent colds and flu (Ziegler et al., 1986). Medicinally, it is also an important nervine used to treat depression and anxiety. Roses possess a unique ability to firm boggy or damaged tissue, reduce inflammation and lessen bacterial proliferation while encouraging the growth of healthy tissue which makes it ideal in the treatment of many microbial infections (Jacoby & Wokes, 1944). Mahmood et al., (1996) and Basim & Basim (2003) reported anti HIV properties of rose oil and its ability to stop and kill some strains of *Xanthomonas*.

However, roses are inhabited by numerous pests which include moths from the family Tortricidae, Hymenoptera from the family Tenthredinidae, Argidae and Cynipidae as well as numerous aphid species. Out of which, the aphid species, *Macrosiphum rosae* (Linnaeus, 1785) is a key pest of roses, with worldwide distribution (Blackman and Eastop, 2000). The significant damage by rose aphid is to the inflorescences, especially at bud burst (Fig. 1). This species cause direct damage by sap sucking from young leaves and developing flower buds which in turn results in discoloration of leaves, stunted growth and gall formation, while as indirect loss is incurred by honeydew secretion on flowers and surface of leaves on which molds grow resulting in reduction in photosynthesis and finally the yield (Jalalizand, 2012). All these factors together cause significant damage to rose plants by decreasing their beauty and the value of cut flowers. Rose aphid is also involved in transmitting viral diseases such as pea mosaic, cauliflower mosaic and cabbage black ring spot (Manddahar, 1987).

Now- a- days, floriculturists round the world do not recommend use of insecticides on flowers and as such aphids are controlled by predators and parasitoids (Bari and Sardar, 1998). As a result, increasing number of studies involving biological control of aphids by natural enemies has become an important feature of pesticide free management strategies (Zehnder et al., 2007). Mehrparvar et al., 2016 reported that natural enemies of *M. rosae* include four species of Coccinellidae [*Hippodamia variegata* (Goeze, 1777), *Coccinella septempunctata* (Linnaeus, 1758), *Adalia bipunctata* (Linnaeus, 1758) and *Exochomus nigromaculatus* (Goeze, 1777)], three species of Syrphidae [*Syrphus vitripennis* Meigen 1822, *Ischiodon aegyptius* (Wiedemann, 1830) *and Scaeva albomaculata* (Macquart, 1842)], two species of Chamaemyiidae [*(Leucopis glyphinivora* Tanasijitshuk, 1958 and *Leucopis* spp. Meigen, 1830], one species of Chrysopidae [*Chrysoperla carnea* (Stephens, 1836)], a few species of Anthocoridae [*Orius niger* (Wolff, 1811) *Orius minutus*

Figure 1: Infestation of young flower bud by *M. rosae*

(Linnaeus, 1758) and *Anthocoris limbatus* Fieber, 1836] and Miridae [*Deraeocoris punctulatus* (Fallen, 1807) and *Deraeocoris* spp.(Kirschbaum, 1856)], all of these act as predators of rose aphid and four species of parasitoids i.e., Braconidae parasitic wasps [*Aphidius rosae* Haliday 1833*, Aphidius ervi* Haliday, 1834*, Praon volucre* (Haliday, 1833) and *Ephedrus plagiator* (Nees,1811)] in Iran. In addition to this, numerous researchers *viz*. Chakrabrati and Gosh, 1970; Maezler, 1977; Mohammed and Mallah, 1987; Tomiuk et al., 1990; Jaskiewicz, 1995; Dixon and Agarwala, 1999, Gadakh, 2014 etc have worked on various aspect of different rose aphid species under different climatic conditions and have obtained varied results but in Kashmir little information is available on this particular insect pest. A precise knowledge about seasonal incidence and biology of pest is essential for any effective control plan to succeed. Keeping in view the economic importance of roses, the degree of damage inflicted by *M. rosae* and in order to develop a sound pest management strategy against it, the present investigation was carried out to study its incidence, population build up and biology along with the relative susceptibility of different rose cultivars to *M. rosae.*

2 MATERIAL AND METHODS

2.1 PERCENTAGE INCIDENCE OF *M. rosae*

Random surveys were carried out fortnightly at special rose growing ornamental gardens in Srinagar district of Kashmir for collection of rose aphids from March to December 2015. These include Shalimar, Nishat, Kashmir University Botanical Garden (KUBG) and Naseem bagh campus. At all experimental sites, five plants were randomly selected and from each plant three twigs were examined for calculation of aphid infestation. The aphids were brushed off from apical tender portions i.e. shoots, buds, flowers and occasionally from underside of leaves of rose plants using camel hair brush on to a white paper and counted to determine aphid severity at different locations. For calculation of percentage of brown, green, alate and apterous forms, separate counts were done from onset of aphids on rose plants till their disappearance.

The apical portions of rose plants were considered infested even if only one aphid was observed on it whereas un- infested rose plants were devoid of any rose aphid. Percentage incidence was calculated by the formula

Incidence = n/ N × 100

Where, n = number of infested shoots

N = total number of shoots examined.

2.2 IDENTIFICATION OF ROSE APHID

Identification of the pest was done by studying their morphological characters under microscope and comparing them with literature available on their morphology. The diagnosis of the species was confirmed according to key provided by David (1975).

2.3 STOCK CULTURE OF ROSE APHID

In order to study biology, a stock culture of rose aphids was maintained on fresh, tender apical portions of rose plants in the Entomology Research Laboratory of Department of Zoology, University of Kashmir under natural conditions of temperature and humidity. Rose aphids were brushed off from infested rose plant portions using camel hair brush into collection tubes. In the case of heavy infestation, apical 10 cm tender shoots were trimmed carefully, put in sealed polythene bag, and carried to the laboratory (Reshi et al., 2008). Fresh food in the form of fresh tender apical rose plant portions were provided to them on daily basis and these were kept in large beakers which in turn were placed in large glass rearing jars having their open end covered with muslin cloth for ventilation.

2.4 STUDY OF DIFFERENT BIOLOGICAL PARAMETERS OF ROSE APHIDS

Adult apterous viviparous parthenogenetically producing female rose aphids collected from field were placed singly on potted rose plant in the laboratory and left overnight for laying young ones. After 12 hrs, all mothers except a newly laid nymph were removed from each plant and kept in 70 % alcohol. The nymph was reared and examined daily from its birth till death for recording different biological parameters. Observations were recorded on duration and number of nymphal instars, pre- reproductive, reproductive, post reproductive period and adult longevity (Ghetiya, 1992). The data was collected for apterous and alate forms separately in two seasons i.e. late spring (May- June) and autumn (October- November). Each parameter was replicated thrice.

2.5 RESPONSE OF DIFFERENT *Rosa* SPP. TO *M. rosae*

The response of different cultivars of *Rosa* spp to *M. rosae* was studied under natural field conditions at Kashmir University Botanical Garden *viz*. Grand Gala,

Golden Gate, Konfettii, Naranga and Nobless. Aphid intensity was recorded separately for each cultivar of *Rosa* spp. at fortnightly interval by examining apical 10 cm portion of rose plant. The observations were recorded from the appearance of aphid in the field and experiment for each species was repeated five times. Mean aphid intensity was calculated by transforming the observations to corresponding square root values. Further, the relative susceptibility of different cultivars was determined on the basis of relative mean population load during the study period and categorized following Malik & Deen (1998):

1. Highly resistant (HR): value between 0.0 to mean – Critical difference (CD)at 5 % level

2. Resistant (R): value between HR to mean – CD at 5 % level

3. Moderately resistant (MR): value between R to mean

4. Low resistant (LR): value between MR to mean + CD at 5 % level

5. Susceptible (S): value between LR to mean + CD at 5 % level

6. Highly susceptible (HS): value above S

3 RESULTS

3.1 SEASONAL INCIDENCE OF *M. rosae* INFESTING ROSES

The data from different localities of Srinagar, Kashmir on aphid no/ twig i.e. aphid infestation and percentage incidence (Table 1 & 2) revealed that insect pest

Table 1: Infestation of rose aphid, *M. rosae* at four different sites of Srinagar district from March to December 2015

S. No.	Month	KUBG	Nishat	Shalimar	Naseem bagh	Mean aphid infestation*
1	March	0.00	0.17	0.23	0.00	0.40
2	April	9.00	7.13	12.60	8.47	9.30
3	May	29.03	32.06	34.86	25.15	30.27
4	June	1.70	9.93	12.26	0.38	6.07
5	July	0.13	1.5	2.03	0.00	0.91
6	August	6.26	11.93	15.33	4.66	9.50
7	September	21.40	24.02	27.00	17.80	22.55
8	October	4.87	7.20	10.93	9.53	8.13
9	November	1.07	2.87	3.00	2.01	2.23
10	December	0.00	0.27	0.33	0.00	0.60

*Mean based on aphid population counted on 3 twigs/ plant/ site.

Table 2: Incidence of *M. rosae* at four experimental sites of district Srinagar, Kashmir

		Percentage incidence				
S. No.	Month	KUBG	Nishat	Shalimar	Naseem bagh	Mean*
1	March	0.00	13.66	19.42	0.00	8.27
2	April	30.00	37.66	42.00	27.33	34.25
3	May	72.02	87.00	102.00	63.13	81.04
4	June	20.12	54.22	60.00	13.66	37.00
5	July	20.00	14.00	26.66	0.00	15.16
6	August	24.13	33.33	50.00	20.66	32.03
7	September	66.00	70.33	82.66	40.00	64.75
8	October	46.33	33.66	40.00	20.00	35.00
9	November	12.00	19.00	25.33	8.00	16.08
10	December	0.00	10.66	14.00	0.00	6.16

*Mean based on aphid population counted on 3 twigs/ plant/ site.

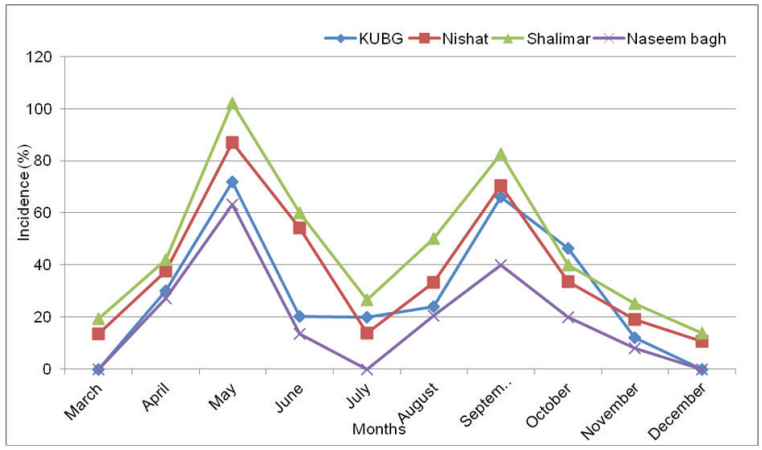

Figure 2: Monthly incidence of *M. rosae* at four sites of district Srinagar, Kashmir

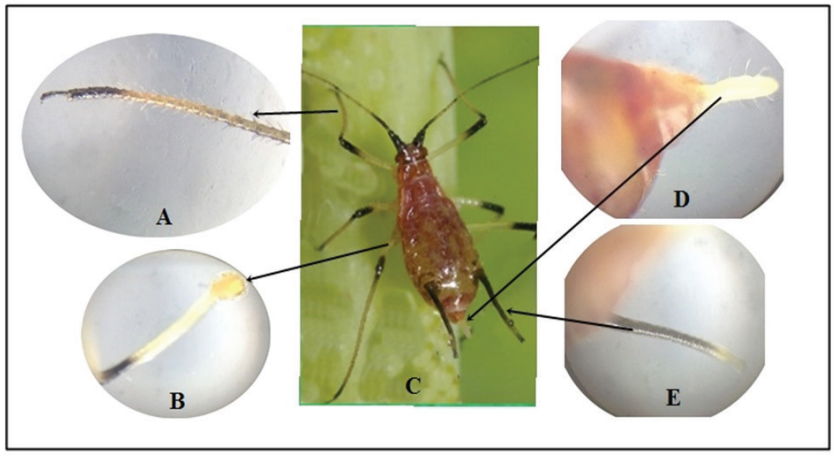

Figure 3: Identification of *M. rosae*. A. Tibia, B. Femur, C. Adult, D. Cauda and E. Siphunculi

occurred during March at Nishat and Shalimar having mean aphid no/ twig and percentage incidence of 0.17 and 0.23 and 13.66 and 19.42 at two sites respectively. The pest population reached its first peak at all sites during May with mean aphid no/ twig recorded as 29.03, 32.06, 34.86 and 25.15 while percentage incidence was 72.02, 87.00, 102.00 and 63.13 for KUBG, Nishat, Shalimar and Naseem bagh respectively. Afterwards, a rapid reduction in aphid no / twig and percentage incidence of pest population was observed at all sites till July. However, the aphid population starts building up from August and reached to its 2nd peak in September with mean aphid no. / twig 21.40, 24.02, 27.00 and 17.80 with percentage incidence of 66.00, 70.33, 82.66 and 40.00 at KUBG, Nishat, Shalimar and Naseem bagh respectively (Fig. 2). The pest population as per mean aphid no/ twig and percentage incidence showed reduction in October and November. During December the pest population was observed at Nishat and Shalimar but it completely disappeared at KUBG and Naseem bagh.

3.2 PEST IDENTIFICATION

The study on various morphological features of adult apterous viviparous specimens collected from district Srinagar revealed that this insect pest is medium sized, broadly spindle shaped with dark head, siphunculi (a pair of horn shaped tubes on abdomen) dark throughout that bent outside and reticular at distal end (Fig. 3). The average antennal length was 3.6 mm, with a cluster of rhinaria arranged all over the surface at the base of 3rd antennal segment, caudal end possessing 10- 14 hairs and leg femora and tibiae wee pale at the base and dark black in distal parts. These morphological characters were compared with taxonomic review of genus *Macrosiphum* occurring in India, provided by David (1975)

and Blackmann & Eastop (2000) and it was concluded that the insect pest is *M. rosae.*

3.3 PERCENTAGE OF BROWN AND GREEN MORPHS OF *M. rosae*

The data obtained from the fields on percentage of brown and green morphs of *M. rosae* are depicted in Table 3. From the data on overall population of both brown and green aphids, it is clear that brown morphs varied from 54.79 % to 60.00 % which is slightly higher than green morph ranging from 40.00% to 45.20%. Further, both brown and green morphs were observed on the plants at the same time throughout the infestation season i.e. colour morphs were normally distributed but slightly biased towards the brown morphs (Fig. 4).

3.4 PERCENTAGE OF ALATE AND APTEROUS FORMS OF *M. rosae*

The data on percentage of alate and apterous forms is represented in Table 4 which indicates that a significant percentage of alate form ranging from 16.00 % to 20.51% while as apterous form range from 79.48 % to 84.00 % were observed in the beginning of season. Afterwards, the percentage of alate forms declined as low as 3.19 % in the 1st week of August. Later, their percentage increased considerably and outnumbered the apterous forms towards the end of the season ranging from 39.64 % to 81.82 % of adult population in the first week of November.

3.5 BIOLOGY OF ROSE APHID, *M. rosae*

The present studies on the biological aspects conducted on stock culture of rose aphids maintained on tender apical portions of rose plant under laboratory conditions revealed that these aphids undergo both parthenogenetic and viviparous modes of reproduction producing both wingless and winged forms. In vivo observations on the biological aspects of the rose aphid were indicative of the fact that the newly born nymphs passed through four nymphal instars before molting into adult. The data on duration of nymphal instars and total developmental period for apterous as well as for alate form of the rose aphids in two seasons i.e. late spring and autumn are given in Table 5.

Table 3: Percentage of brown and green morphs of *M. rosae* at four selected sites of Srinagar, Kashmir

S. No.	Sites	Total aphid population*	Brown aphid population	Green aphid population	Percentage of brown morphs	Percentage of green morphs
1	KUBG	826	470	356	56.90	43.09
2	Nishat	1268	726	542	57.26	42.74
3	Shalimar	1635	981	654	60.00	40.00
4	Naseem bagh	584	320	264	54.79	45.20

*Data based on aphid population on 3 twigs/ host plant/ site.

Figure 4: Severe infestation of apical portions of rose plant by brown and green morphs of rose aphid, *M. rosae*

Table 4: Percentage of alate and apterous forms of *M. rosae* at KUBG, Srinagar during 2015

S. No.	Month	Total aphid population	Apterous aphid population	Alate aphid population	Percentage of apterous aphid form	Percentage of alate aphid form
1	March	50	42	8		16.00
2	April	320	230	90	71.87	28.12
3	May	848	674	174	79.48	20.51
4	June	178	167	11	93.82	6.18
5	July	94	91	3	96.80	3.19
6	August	128	121	7	94.53	5.46
7	September	386	353	8	91.45	8.55
8	October	565	341	224	60.35	39.64
9	November	98	30	68	30.61	69.39
10	December	22	4	18	18.18	81.82

*Data based on aphid population on 3 twigs/ host plant/ site.

Table 5: Duration of various developmental stages of *M. rosae* in two different seasons of Kashmir valley

Season	Phase of rose aphid	Nymphal instar	Observations			Duration of nymphal instars (Mean ± SD)*	Pre- reproductive period (days)	Total developmental period (days)
			1	2	3			
		I	2.5	3.0	2.0	2.7 (± 0.35)		
		II	2.5	2.0	1.5	2.2 (± 0.29)		
		III	2.0	3.0	2.0	2.3 (±0.58)		
	Apterous	IV	3.3	2.5	3.5	3.1 (±0.53)	1.50	11.80
		I	3.0	3.5	2.9	3.1 (±0.32)		
		II	2.7	2.4	2.1	2.4 (±0.30)		
		III	3.0	2.5	2.8	2.8 (±0.25)		
Late Spring	Alate	IV	5.2	4.5	4.8	4.8 (±0.35)	2.93	16.03
		I	2.5	3.0	2.6	2.7 (±0.26)		
		II	1.5	2.0	2.8	2.1 (±0.65)		
		III	2.8	3.2	2.0	2.7 (±0.61)		
	Apterous	IV	4.0	3.5	3.0	3.5 (±0.50)	2.25	13.25
		I	3.7	3.3	4.0	3.7 (±0.35)		
		II	2.6	2.4	3.4	2.8 (±0.53)		
		III	3.0	2.5	3.5	3.0 (±0.50)		
Autumn	Alate	IV	5.5	5.7	5.3	5.5 (±0.20)	6.00	21.00

*Mean of 3 replications/ season/ for each phase of aphid life.

In apterous form 2nd instar usually took least time in comparison to other instars while for alate forms 4th instar is the longest instar, since aphids develop wings in this very stage and as a result this instar takes more time. *M. rosae* showed polymorphism in its generations with predominant brown morphs in contrast to green morphs. Throughout the infestation season, colonies of rose aphids comprising of alate and apterous forms, were seen crowding on rose plants at the same time (Fig. 5).

The data on mean reproductive period, total fecundity (nymphs laid/ female) and rate of reproduction (nymphs laid/ day/ female) for apterous and alate forms in the late spring and autumn season are shown in Table 6. The data on mean duration of post reproductive period, adult longevity and total longevity of apterous form

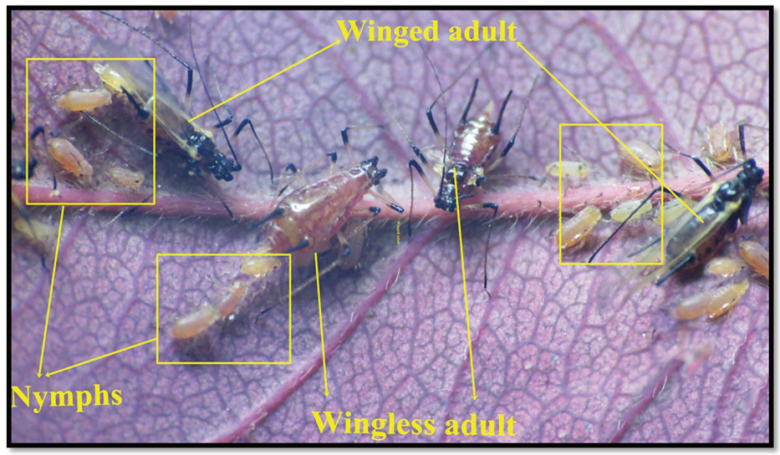

Figure 5: Colony of rose aphid depicting different phases in the life cycle of *M. rosae*

Table 6: In vitro studies on reproductive and longevity of *M. rosae* in two seasons of Kashmir

Season	Phase of aphid	Reproductive period	Total fecundity	Rate of reproduction	Post reproductive period	Adult longevity	Total longevity
	Apterous	14.63	94.62	6.46	4.25	18.44	30.24
Late spring	Alate	9.80	50.87	5.19	8.87	14.36	30.39
	Apterous	20.10	65.12	3.24	9.50	28.50	41.75
Autumn	Alate	12.35	30.74	2.49	12.75	23.00	44.00

was recorded as 4.25, 18.44, 30.24 days in late spring and 9.50, 28.50, 41.75 days in autumn respectively whereas for alate form the post reproductive period, adult longevity and total longevity were found to be 8.87, 14.36, 30.39 days in late spring and 12.75, 23.00 and 44.00 days in autumn respectively.

The pest first appeared in the 3rd week of March and remained active on the rose plants for about ten months of the year. Under laboratory conditions, the rose aphid was observed to complete its life cycle from 3rd week of March to 4th week of December, reproducing parthenogenetically, viviparously all the year round.

3.6 RESPONSE OF DIFFERENT *Rosa* SPP.. TO *M. rosae*

The results on screening of different cultivars of *Rosa* spp. *viz.* Grand Gala, Golden Gate, Konfettii, Naranga and Nobless against rose aphid, *M. rosae* are shown in Fig. 6 which clearly depicts that 'Konifitti' had least mean aphid intensity than rest of cultivars. Further, 'Grand Gala' was found to have highest mean aphid intensity during the whole growing season was designated as highly susceptible while as 'Naranga' and 'Nobless' were classified as moderately susceptible and susceptible respectively (Table 7). However, Golden Gate and Konfetti were grouped as resistant and highly resistant cultivars with mean aphid intensity of 1.91 and 1.58 respectively.

4 DISCUSSION

4.1 SEASONAL INCIDENCE OF *M. rosae*

During the survey of ornamental shrubs carried out for the purpose of collection of rose aphids, it was evident that this insect pest first appeared on the apical tender portions of rose pant in the 3rd week of March in the Srinagar district (Table 1 & 2). The findings were in close association with the prior findings of Mohammed & Mallah (1987) and Jaskiewicz (1995) who concluded that this aphid species first appeared in the spring season on the rose shoots in mid May and mid February in Poland and Iraq respectively. A large number of aphids forming huge colonies were also observed by Bhagat & Ahmad (1995) on *Rosa* spp. at Jammu during spring season. Rani & Mohan (1997) observed the *M. rosae* clusters were found around the growing shoots of rose plant during Oct.- Feb. when the weather is cool and cloudy in Bangalore. However, Atwal & Dhingra (1971) and

Table 7: Mean aphid intensity of five cultivars of *Rosa* spp. at KUBG, Srinagar

S. No.	Cultivars	Replications			Mean aphid intensity per shoot*	Response category
		1	2	3		
1	Grand Gala	8.00	5.00	7.00	20.00 (4.53)	Highly Susceptible
2	Golden Gate	0.93	0.99	1.23	3.15 (1.91)	Resistant
3	Konfettii	0.56	0.84	0.60	2.00 (1.58)	Highly Resistant
4	Naranga	2.19	2.13	3.00	7.35 (2.80)	Moderately Susceptible
5	Nobless	3.05	4.60	5.00	12.65 (3.63)	Susceptible

* Mean of observations recorded from March to December 2015; five replications for each cultivar; figures in parenthesis are √x+0.5 transformed values.

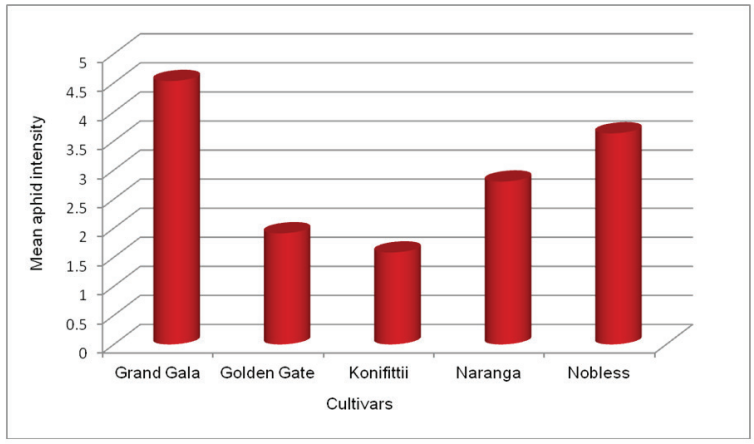

Figure 6: Relative susceptibility of different rose cultivars to *M. rosae*

Hole et al. (1998) reported that the pest first appears in November and 3rd week of January in Punjab and Pune states of India respectively. This difference in appearance of pest may be ascribed to the different agroclimatic conditions/ seasons, latitudinal clines and different growth stages of host plant in the different regions of the country.

The population of aphids increased steadily, achieving its 1stpeak in the month of May when rose plants were in flowering and bud stages. Interestingly, a 2ndpeak which was lower than the 1st one was observed in 1st week of October. This observation was credited to the drop in high temperature and to the 2ndflush of vegetative growth on rose bushes which augment its vulnerability to the pest. The population of pest starts declining sharply in the month of October and completely disappears thereafter in December. These observations coincide with the prior observations of Maezler (1977) who recorded three peaks of *M. rosae* in spring- summer with respect to the three growth flushes of rose in South Australia.

Thereafter, with the further fall in temperature and production of alate form of pest, population started declining further and completely vanishes from rose plants in the 3rdand 4thweek of December. The complete disappearance of the pest from roses has been observed in different months from different regions of the world e.g. Atwal & Dhingra (1971) reported from Punjab that the pest disappears in the month of May, at the end of October from Lublin, Poland (Jaskiewicz, 1995), by the mid-June in Mosul, Iraq (Mohammed & Mallah, 1987) and by the end of April in Pune, Maharashtra (Hole et al., 1998) which may be attributed to the agroclimatic factors, latitudinal clines and the growth stages of the rose bushes.

4.2 PERCENTAGE OF BROWN AND GREEN MORPHS OF *M. rosae*

In our study, two phenotypic morphs i.e. brown and green representing clones of *M. rosae* colonizing rose plants either separately or together were observed. These findings are in uniformity with many previous researchers *viz.* David et al. (1958), Atwal & Dhingra (1971), Tomiuk & Wohrmann (1990) and Chen De Qiao et al. (1997).

As far as percentage of brown and green morphs of *M. rosae* is considered, the observed bias towards brown form is attributed to high reproductive rates and better adaptability of this morph in comparison to its green morph as suggested by Tomiuk & Wohrmann (1990).

4.3 PERCENTAGE OF ALATE AND APTEROUS FORMS OF *M. rosae*

Throughout the study period, both apterous and alate forms were found to variable extent. In the beginning of season, percentage of alate forms increased gradually up to mid May. Atwal & Dhingra (1971) have also observed that alate population progressively increased from the beginning of season. But as the population peaked to its maximum in the 4th week of May, thereafter their population declined possibly in response to crowding and high temperature as suggested by Maezler (1977) and Atwal & Dhingra (1971). Das (1918) pointed out that possibly the alate forms may be carried to high altitude cooler regions along with wind debris. The alate percentage remained very low in the hotter months but October onwards; their percentage increased progressively and towards the end of season more than 70 % population was alate form. Atwal & Dhingra (1971) observed more than 80 % population as alate form towards the end of season. This urge was possibly in response to short photoperiod and maturity of host plants as suggested by Grewal & Bains (1975) for *Macrosiphum* (*Sitobion) avenae* Fabricius, 1775. Howard and Dixon (1992, 1995) also suggested that maturity of plants induces alate production.

The production of alatae in most of aphid species is in response to increased aphid density and in some species even small increase in population triggers wing formation (Johnson, 1965; Lees, 1967; Shaw, 1970). The immediate reason for such changes appears to be increased tactile stimulation between aphids that is mainly mediated by antennae in some species (Johnson, 1965). In addition to this, the mere occurrence of particular natural enemy was known to elicit an increase in winged morph production in pea aphid, *Acyrthosiphon pisum* Harris,1776 (Dixon and Agarwala, 1999; Kunert & Weisser, 2003). The induction of winged morphs is triggered by either predator avoidance behaviour or from the release of aphid alarm pheromone (Kunert et al., 2005). Aphid or plant pathogens (i.e., fungi or viruses) and facultative aphid endosymbionts may also affect wing development (Muller et al., 2001; Leonardo and Mondor, 2006). Furthermore, several abiotic factors like temperature may influence wing induction either directly or indirectly through host plant (Johnson and Birks, 1960; Schaefers and Judge, 1971; Liu, 1994). In clones (that do not undergo sexual reproduction), appearance of wings may be ascribed to change in photoperiod (Lees, 1966). Wing development in aphids is thus an evolutionary phenomenon by which they undergo either sexual reproduction or migrate to favourable environments.

4.4 BIOLOGY OF *M. rosae*

The study of biology revealed that pest passed through four nymphal instars. This was in consonance with the findings of Atwal & Dhingra (1971) and Mohammad & Mallah (1987). The apterous form passed through four instars with the total longevity of 30.24 and 41.75 days in spring and autumn seasons respectively. For the alate form it was 30.39 and 44.00 days in two seasons (late spring and autumn) respectively. These observations are slightly higher than those recorded by Atwal & Dhingra (1971) for *Macrosiphum rosaeformis* (Das, 1918). The variation may be due to the host/ species differences and agroclimatic conditions but in close conformity with the observations of Reshi et al., 2008 in Kashmir for *M. rosae*. Further, it was noted that the 2ndinstar of apterous form takes the least time in comparison to other instars while for the alate forms the 4thinstar took the longest time, since aphids develop wings in this very stage and as a result this instar takes more time. Similar observations were made by Mohammad & Mallah (1987) for *M. rosae* in Somul, Iraq.

The pre- reproductive period of apterous and alate forms in late spring and autumn was 1.50, 2.25 and 2.93, 6.00 days. This is higher than *M. rosaeformis* as reported by Atwal & Dhingra (1971) and the observed difference may be attributed to agroclimatic disparity followed by difference in pest and host plant used for the study.

As far as total developmental period of rose aphid was concerned, the present observations are in close agreement with that of Atwal & Dhingra (1971) and Kakar & Sood (1989) for *M. rosaeformis* and with Reshi et al., 2008 for *M. rosae*. In present study, mean reproductive period of apterous form in two seasons is 14.63 and 20.10 days whereas for alate form it is 9.80 and 12.35 days respectively. These are in association with the results of previous workers *viz.* Atwal & Dhingra (1971) for *M. rosaeformis*.

This pest was observed to reproduce parthenogenetically and viviparously all the year round. Reshi et al., 2008 reported that the pest undergo 7 complete generation from 3rd week of March to 4th week of December. The pest did not possess any sexual stage and thus appeared anholocyclic. Observations of David (1957, 1975) and Maezler (1977) in India and South Australia respec-

tively supported the anholocyclic mode of reproduction in *M. rosae* on roses. Wohrmann et al., 1991 reported that the ability of German clones to undergo sexual reproduction is stronger than the Australian clones on manipulation of environmental conditions in the laboratory. He further suggested that this disparity in the mode of reproduction in *M. rosae* may be due to the genetic and environmental factors. Sexual phase is triggered by environmental changes in the temperate regions (Hille Ris Lambers 1966 and Lees, 1966) and many clones and certain aphid species have lost the ability to undergo sexual reproduction either partially or completely (Lee, 1966 and Simon et al., 1991).

4.5 RELATIVE SUSCEPTIBILITY OF ROSE CULTIVARS TO *M. rosae*

The present study revealed that none of rose cultivar was found to be free from aphid infestation. Throughout the study period, variable aphid intensity was recorded on different cultivars. This is in conformity with the observations of David et al.(1958) who reported that both garden and wild roses are attacked by this insect pest. From the analysis of mean aphid intensity, it is clear that Grand Gala harboured maximum aphids whereas Konifittii had minimum mean aphid intensity. Furthermore, Naranga and Golden Gate demonstrated moderate mean aphid intensity. These are in uniformity with findings of Akhtar & Khaliq (2003) who ascertained that none of *Rosa* spp. was aphid free. Further, certain rose varieties were more susceptible to the attack of thrips than others. Rani & Sridhar (2003) observed that more no. of thrips attacked red and orange flowers than yellow flowers which may be explained by the fact that colour of rose may act as source of attractant for insect pests like thrips and aphids. A number of morphological characters (like colour, thorns etc) and presence of chemical compounds (like presence of catechin i.e. 1, 2 benzenediol) in sap of rose plant act as feeding deterrent for numerous pests as reported by Rani & Sridhar (2003).

5 CONCLUSIONS

In the present study, an extensive survey of rose plants was carried out to monitor aphid populations on them. The results obtained revealed that the pest remained active for ten months of the year with its first incidence in the month of March. Maximum aphid population was observed in May after which the population declined till the month of July. The aphid population reached a 2nd peak in the month of October. Thereafter, population plummeted till their complete disappearance in the month of December. Both alate and apterous forms of *M. rosae* were observed to pass through 4 nymphal instars in two different seasons (late spring and autumn). The developmental period, pre reproductive period, reproductive period, post reproductive period and adult longevity of apterous morphs in spring were recorded as 11.80, 1.50, 14.63, 4.25 and 18.44 days whereas in autumn, these phases were of 13.25, 2.25, 20.10, 9.50 and 28.50 days duration respectively. Alate morph of rose aphid had developmental period, pre reproductive period, reproductive period, post reproductive period and adult longevity of 16.03, 2.93, 9.80, 8.87 and 14.36 days in spring season. The durations of these phases in autumn season for alate morph were recorded as 21.00, 6.00, 12.35, 12.75 and 23.00 days respectively. Further, none of the rose cultivars studied during the entire growing season escaped the aphid attack. 'Konfettii' was categorized as highly resistant with lowest mean aphid intensity of just 2.00 aphids/ shoot while 'Grand Gala' was classified as highly susceptible cultivar with highest mean aphid intensity of 20.00 aphids/ shoot. In conclusion, the geographical position of Kashmir valley makes it better suited to meet huge demand of cut flowers in Middle East than Netherlands which is situated much far away from this region. As such the future research should focus on inter specific hybridization programs for development of aphid resistant cultivars of roses. This can provide much needed impetus to nascent floriculture industry of our state.

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Evaluation of production conditions of tomato grafted with different tobacco rootstocks and determining nicotine content and quality of fruit

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Evaluation of production conditions of tomato grafted with different tobacco rootstocks and determining nicotine content and quality of fruit

Abstract: This study aimed to investigate the effects of grafting tomato on different tobacco rootstocks on quality factors and nicotine content. The commercial variety (*Solanum lycopersicum* 'H2274') (BIOTECH) of the tomato was used as the scion plant, and six different tobacco (*Nicotiana tabacum* L.) rootstocks were used: Taşova, Tekel, Muş, Samsun, Dişbudak, Hasankeyf cultivars. Cleft grafting method was used in all plants. Yield of non-grafted and grafted plants grown in open-field conditions was calculated, and there was a significant increase in yield in grafted tomatoes compared to non-grafted tomatoes. There was significantly increased lycopene and β-carotene levels (mg kg^{-1}), especially in 'Tekel', 'Taşova', 'Samsun', and 'Hasankeyf' tobacco grafts. There was a statistically significant difference between grafted and non-grafted plants according to 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical retention capacities and total phenol (TP) values. Evaluation of quality determinants including pH values, titratable acidity values (citric acid %), soluble solid content (SSC) (°Brix) , fruit size ratios, showed that tomatoes grafted with 'Muş' tobacco rootstock were of higher quality. There was no significant difference between grafted and non-grafted plants according to nicotine analysis of the tobacco-grafted tomatoes, and due to acceptable ranges of nicotin level on tobacco grafted tomato plants were considered to be suitable for consumption. It could be concluded that grafting practices have significantly positive effects on tomato yield and quality.

Key words: grafting; nicotine; quality; tobacco; tomato; yield

Ovrednotenje pridelovalnih razmer paradižnika cepljenega na različne podlage tobaka in določitev vsebnosti nikotina in kakovosti plodov

Izvleček: Namen raziskave je bil preučiti vplive cepljenja paradižnika na različne podlage tobaka glede na vsebnost nikotina in kakovost plodov. Kot cepič je bila uporabljena komercialna sorta paradižnika *Solanum lycopersicum* 'H2274' (BIOTECH), kot podlaga pa šest sort tobaka (*Nicotiana tabacum* L.): Taşova, Tekel, Muş, Samsun, Dişbudak, Hasankeyf. V vseh primerih je bila uporabljena metoda cepljenja v precep. Izmerjen je bil pridelek cepljenih in necepljenih rastlin, ki so rastle na prostem. Ugotovljeno je bilo, da so imele cepljene rastline značilno večji pridelek kot necepljene. Vsebnosti likopena in β-karotena (mg kg^{-1}) so se značilno povečale, še posebej pri paradižniku cepljenem na podlage tobaka 'Tekel', 'Taşova', 'Samsun', in 'Hasankeyf'. Med cepljenimi in necepljenimi paradižniki je bila statistično značilna razlika v retencijski sposobnosti prostih radikalov z 2, 2-difenil-1-pikrilhidrazilom (DPPH) in v vsebnosti celokupnih fenolov (TP). Ovrednotenje kakovostnih parametrov, vključno s pH, vsebnostjo titrabilnih kislin (kot odstotek citronske kisline), topnih snovi (SSC) (°Brix), velikostjo plodov, je pokazalo, da so imeli paradižniki cepljeni na podlago tobaka 'Muş' večjo kakovost. Med cepljenimi in necepljenimi paradižniki ni bilo značilne razlike v vsebnosti nikotina, tudi vsebnost nikotina na tobak cepljenih paradižnikov je bila na sprejemljivi ravni in so bili primerni za uživanje. Zaključimo lahko, da ima cepljenje paradižnikov na podlage tobaka značilno pozitivne učinke na pridelek paradižnika in njegovo kakovost.

Ključne besede: cepljenje; nikotin; kakovost; tobak; paradižnik; pridelek

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1 INTRODUCTION

At the same rate required to satisfy basic human needs, rising world population also increases the demand for agricultural products. In order to supply this growing demand, research on faster and more inexpensive plant production methods to enhance quality, yield potential, and tolerance against stressful conditions have been enhanced. In this scope, grafting practices are gaining more and more relevance every day. Grafting enables the cultivation of agricultural products in different climates and soil conditions by utilizing benefits from the characteristics of different rootstocks. Besides vegetative reproduction, the yield of plants resistant to biological and environmental stress without damaging product quality positively effects crop and has become a method of producing plants with broader ecological tolerance. This method is based on placing the scion plant intended to be reproduced or improved on top of the rootstock plant by conjoining the cambium regions. The success of grafting depends on many internal and external factors. Successful grafting may be associated with the water content of tobacco, the selection of appropriate rootstock, and suitable grafting conditions. As the root system of plants effects vegetative growth, non-grafted and grafted plants may vary in growth performance (Haberal et al., 2016). Earlier studies (Moore, 1984) have stated that scion and rootstock selection is one of the most significant factors to effect yield in grafting practices. Therefore, several grafting combinations have been attempted in the past and their effects on increasing yield have been investigated (Kacjan-Maršić & Osvald 2004; Khah et al., 2006). In our institute, tobacco-tomato combinations obtained using the cleft grafting method with tobacco rootstock was previously demonstrated to affect the plant growth, positively fruit yield, and quality in greenhouse-grown tomatoes (Yasinok et al., 2009).

Antioxidants are compounds that protect cells from the damage of unstable molecules known as free radicals. Reactive free radicals, formed in metabolic reactions such as respiration and digestion, contain one or more unpaired electrons and have the potential to cause serious damage to the body (Diplock, 1998). Although the human body has its own antioxidant defense system to prevent damage, environmental factors decrease defensive resistance and render it inadequate in damage prevention. In order to limit this damage, herbal antioxidants that collaborate with the body's various defense systems are considered effective alternatives. These exogenous, natural antioxidants include compounds such as: vitamins C and E, selenium, β-carotene, lycopene, lutein and other carotenoids, flavonoids, phenolic acid, and terpenes (Hennig & Toborek, 1993; Aruoma, 1994; Burr, 1994). In addition to natural antioxidants, nowadays, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary-butylhydroquinone (TBHQ), and propylgallate (PG) are used. However, studies have shown that these synthetic antioxidants have side effects (Kehrer & Digiovanni, 1990). Therefore, there is a growing interest in natural antioxidants in the fields of food chemistry and medicine (Madhavi et al., 1995).

This study was conducted to evaluate the antioxidant capacities, quality, nicotine content, and yield of tobacco-grafted tomato plants compared to non-grafted tomato plants, considering open-area production conditions, under the ecological conditions of the Ankara region.

2 MATERIAL AND METHODS

2.1 SOWING AND GRAFTING

For grafting trials, the H2274 (Biotech) commercial variant of the tomato plant (*Lycopersicum esculentum* Mill.) was used as the scion, whereas six different commercial tobacco (*Nicotiana tabacum* L.) variants were used for rootstocks: Tekel (TE), Muş (M), Taşova (T), Samsun (*Nicotiana tabacum*) (S), Hasankeyf (*Nicotiana rustica* L.) (H), and Dişbudak (D).

Seeds were germinated in greenhouse conditionsat a humidity of 45-55 % and a temperature of 23-25 °C. Cleft grafting was made when tomato seedlings had 3-4 leaves, and tobacco seedlings had 6-7 leaves. The grafted seedlings were grown in a conditioning chamber for fusion in 16/8 day/night period at a humidity of 90-95 % and a temperature of 23-25 °C degrees for 10 day

2.2 CULTIVATION OF GRAFTED PLANTS IN OPEN-FIELD CONDITIONS AND CALCULATION OF YIELD

Successfully grafted plants of various combinations as well as control group plants consisting of H2274 seedlings were transferred to a pre-cultivated two-acre field. Seedlings were watered with the drip irrigation system with 4 l h^{-1} irrigation capacity according to weather conditions, and appropriate maintenance was carried out. Fruit yield was expressed as the mass of harvested tomato fruit per plant.

2.3 DETERMINING PIGMENT CONTENT

Lycopene and carotene extractions were performed in accordance with the low volume hexane extraction method protocol (Fish et al., 2002). In this study, 0.05 %

(w/v) butylated hydroxytoluene (BHT) in acetone, 95 % ethanol, and hexane were used.

Previously pureed non-grafted and grafted tomatoes in various combinations were weighed as 0.5 g, and afterwards, 0.05 % (M/V) (BHT) in acetone, and 95 % ethanol and hexane were added and vortexed. Distilled water was added to the samples which were shaken in the 180-rpm shaker. Supernatant of the samples brought to room temperature and spectrophotometer (HITACHI U-1800) readings were measured at 453 nm for β-carotene and 503 nm for lycopene. The results were presented as mg kg^{-1}.

2.4 DETERMINING TOTAL PHENOLIC CONTENT

The total content of phenolic compounds was determined using the Folin-Ciocalteu method (Slinkard & Singleton, 1977). The regularly harvested grafted and non-grafted tomatoes, were dried for 48 hours at 60 °C in oven and pulverized to a powder form. 10 % etanolwas added to 0.05 g of powder material which was incubated at 4 °C for overnight. After incubation, the samples were centrifuged at 5000 rpm for 5 minutes, and the supernatant was filtered through a 0.45 µm filter. Folin reagent, dH_2O and 10 % sodium carbonate were added to the samples and incubated for 30 minutes in 40 °C water bath. The samples were measured with spectrophotometer at 765 nm, and the results were expressed as standard gallic acid equivalents (GAE).

2.5 DPPH FREE RADICAL SCAVENGING ACTIVITY

Spectrophotometric evaluation of electron retention capacity and stable DPPH free radical scavenging activity of the tomato samples was performed in accordance with the protocol specified by Sharma and Bhat (2009). The powders obtained from previously prepared grafted and non-grafted tomato samples were mixed with 1 ml methanol and centrifuged at 5000 rpm. Samples prepared in different dilutions were mixed with 200 mM methanolic DPPH and left to incubate in dark for 30 minutes.

A 50 % inhibition concentration (IC50) was calculated using the concentration-dependent inhibition percentage (I % = (Ablank-Asample / A blank) × 100) curve, and these values were compared with the IC50 of standard antioxidants.

2.6 NICOTINE ANALYSIS

Pureed tomato samples were homogenized with distilled water in a glass homogenizer (Yasinok et al., 2009). After homogenization process nicotine extraction with toluene was performed. Diphenylamine was used as the internal control during the extraction. Nicotine analysis of the extracted samples was carried out with phosphorus detector gas chromatography using appropriate columns and standards at Anadolu University Plant, Drug and Scientific Research Center (EEskisehir, Turkey).

2.7 QUALITY ASSESSMENT

2.7.1 Fruit height and diameter

The diameters and lengths of the products of the grafted and non-grafted tomato plants were measured using fruit calipers and yielded average results.

2.7.2 pH and titratable acidity

pH was analyzed potentiometrically using the INOLAB brand WTW series pH meter. A homogenous mixture of freshly collected and pureed grafted and non-grafted tomatoes was prepared, and pH was measured. For the acidity analysis, the homogeneous samples were mixed with distilled water and titrated to pH 8.1 using 0.1 M NaOH. The acidity of tomatoes was calculated as citric acid according to the following formula:

acidity % (in citric acid) = S × 0.0064 × F × 100 / sample amount (ml or g)

S = Consumption, amount of 0.1 N NaOH spent (ml)

F = Factor of sodium hydroxide solution (F = 1 if the solution has a normality of 0.1) (Flores et al., 2010).

2.7.3 Soluble solids content (SSC)

Soluble solids content was determined using FUJI handheld refractometer. A sufficient amount of fluid from tomato samples were placed on the prism of the refractometer and the brix (amount of substance dissolved in 100 g) readings were taken.

2.8 STATISTICAL ANALYSIS

All data was expressed as mean ± standard error of the means (SEM), and derived from at least four replicates. IBM SPSS Statistics 25 package program was used for statistical evaluation. Descriptive statistics are expressed as mean and standard deviation for continuous data and

frequency and percentage for discrete data. In this regard, in the comparison of mean values of continuous variables between two groups, Independent Sample t-test was used for parametric tests and Mann Whitney U test for non-parametric tests. In the comparison of continuous variables when there were more than two groups, One-way ANOVA was used for parametric tests and Kruskal Wallis test for non-parametric tests. Results of the analysis of tested hypotheses was compared with a *p* value of 0.05 in which values less than 0.05 were considered statistically significant.

3 RESULTS

3.1 FRUIT YIELD

A statistically significant difference was observed between grafted and non- grafted tomato groups according to total yield means. There was a significant increase in the yield of grafted tomatoes, but there was no significant difference between the grafted groups. According to the data on fruit yield, the lowest yield of fruit mass per plant was observed in the tomatoes grafted to 'Hasankeyf' tobacco rootstock, and the highest value was observed in tomatoes grafted to 'Samsun' tobacco rootstock (Figure 1).

Fruit yield of the harvested tomatoes in the years 2014, 2015, and 2016 was compared according to tobacco types and no significant difference was observed between the types.

3.2 PIGMENT CONTENT

According to lycopene and β-carotene carotenoids, which have strong antioxidant effects, there was no significant difference in 'Muş' and 'Dişbudak' grafted tomatoes compared to the nongrafted control group, while there was a significant difference in the 'Taşova', 'Tekel', 'Samsun', and 'Hasankeyf' grafted groups. When grafts were evaluated among themselves, there were significant differences between 'Tekel', 'Muş' and 'Dişbudak'; between 'Tekel' and 'Muş'; and between 'Muş', 'Samsun', and 'Hasankeyf' tobacco-grafted tomatoes (Figure 2). While there were partial differences when all grafts were evaluated in total, no single graft type showed statistical significance.

Tomatoes of the 'Hasankeyf' grafted plants had the highest lycopene content, whereas tomatoe of the 'Muş' grafted plants had the lowest amount. Similarly, 'Hasankeyf' grafted tomatoes also had the highest β-carotene content, whereas, 'Muş' grafted tomatoes had the lowest amount.

3.3 TOTAL PHENOLIC CONTENT (TPC)

Except for the 'Muş' and 'Taşova' graft types, tobacco-grafted tomatoes showed significant differences in total phenolic compounds compared to the control group. When grafts were compared among themselves, significant differences were found between 'Taşova' and 'Tekel', 'Dişbudak', and 'Hasankeyf'; between 'Tekel' and 'Muş', 'Samsun', 'Dişbudak', and 'Hasankeyf'; between 'Muş', and 'Dişbudak' and 'Hasankeyf'; and between 'Samsun' and 'Disbudak' and 'Hasankeyf' (Figure 3). The highest total phenol content was found in tomatoes grafted on 'Muş' tobacco.

3.4 DPPH FREE RADICAL SCAVENGING ACTIVITY (FRSA)

Radical scavenging activity of tomato extracts were

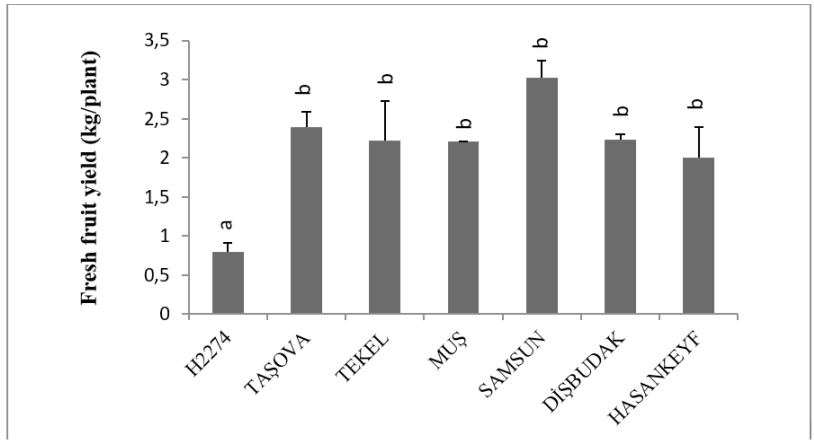

Figure 1: Fresh fruit yield of non-grafted and grafted tomatoes. Standart error of the means were derived from four biological replicates. The different letters emphasize the statistical difference ($p < 0.05$).

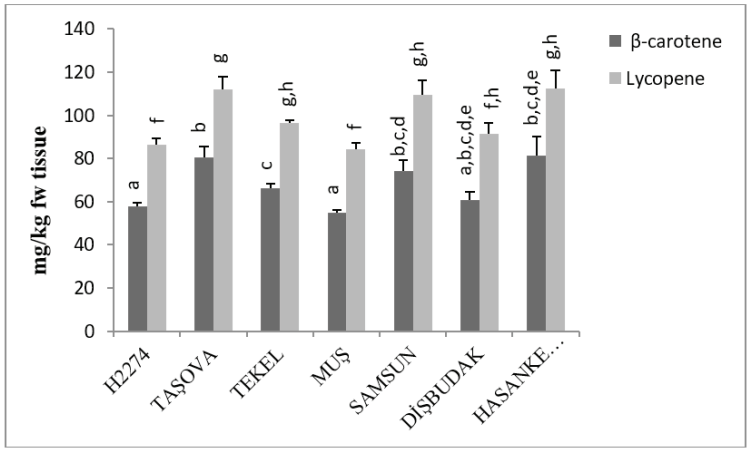

Figure 2: Distribution of lycopene and beta carotene values in grafted and non-grafted plants per fresh weight. Standart error of the means were derived from four biological replicates. The different letters, which are defined to evaluate the lycopene and β -carotene within themselves, emphasize the statistical difference ($p < 0.05$).

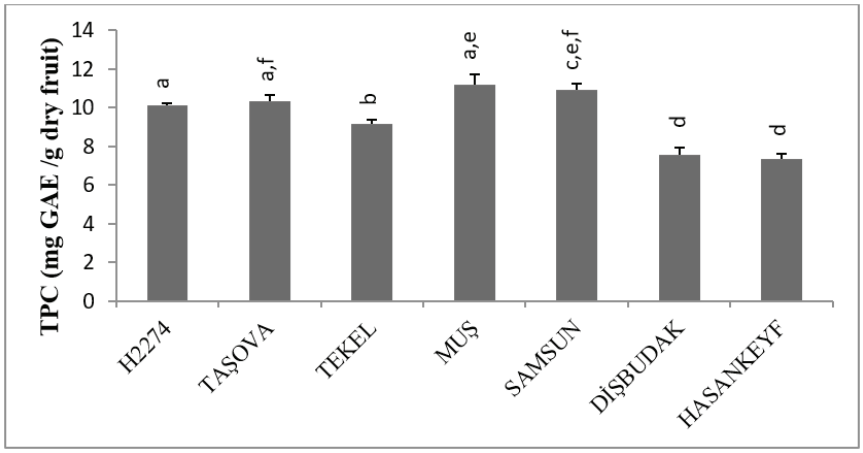

Figure 3: Total phenolic content of the fruits. Standart error of the means were derived from four biological replicates. The different letters emphasize the statistical difference ($p < 0.05$).

determined with decreased absorbance of the reduction of DPPH radicals. The comparison between tobacco-grafted tomatoes and non-grafted tomatoes showed significantly increased activity in tomatoes grafted with 'Dişbudak' and 'Hasankeyf' tobacco. There was no significant difference in tomatoes grafted with 'Taşova' tobacco and non-grafted tomatoes (Figure 4). Tomatoes grafted with 'Samsun' tobacco were determined to have the best radical scavenging activity.

According to the total evaluation of methods to determine antioxidant properties, there was a significant increase in tomatoes grafted with 'Samsun' tobacco.

3.5 NICOTINE ANALYSIS

There was no significant difference between tomatoes grafted with various tobacco variants and non-grafted tomatoes according to nicotine content. Considering the harvest time, there was no significant difference in nicotine content between newly emerged tomatoes from seedlings and tomatoes collected at the end of the harvest. There was no significant difference in nicotine content among the tobacco types of grafted tomatoes (Figure 5).

3.6 QUALITY PARAMETERS

3.6.1 Physical quality parameters

There were statistically significant differences between the groups. 'Taşova', 'Muş' and 'Samsun' tobacco grafted plants showed significant difference compared to

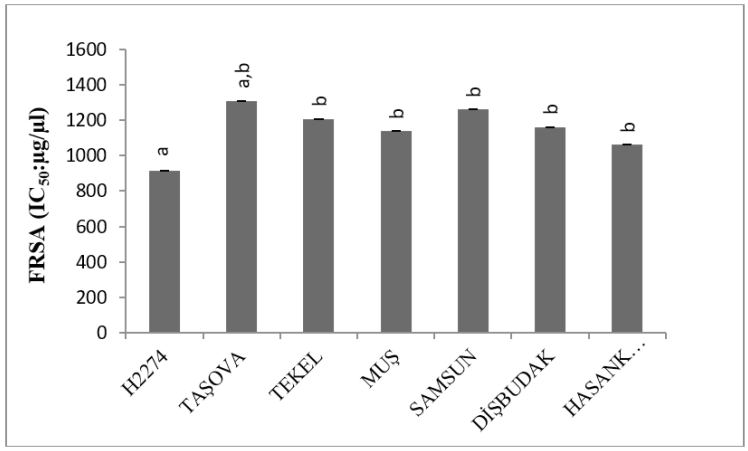

Figure 4: IC50 of free radical scavenging activity in fruits. Standart error of the means were derived from four biological replicates. The different letters emphasize the statistical difference ($p < 0.05$).

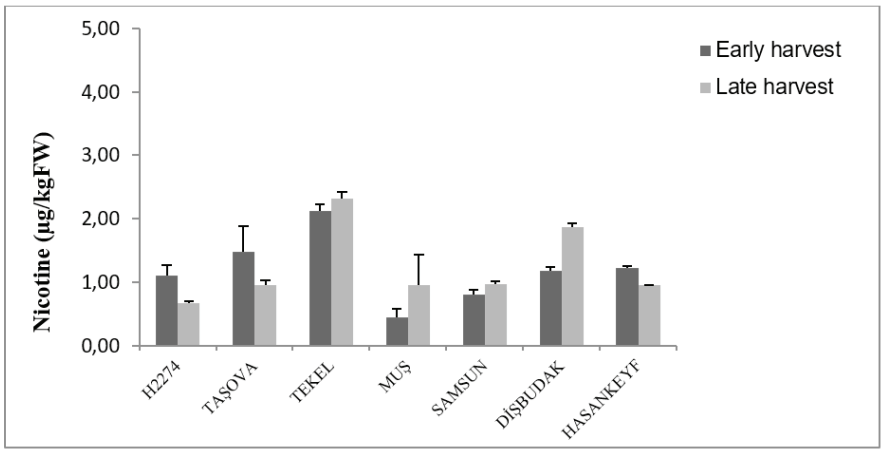

Figure 5: The distribution of nicotine content in H2274 tomatoes and tobacco grafted tomatoes. Standard error of the means was derived from three biological replicates. There was no statistical difference in the control group of the grafted tomatoes, within the grafted groups or the early and the late harvest.

the control group according to total size parameters. According to physical characteristics such as massght and diameter, 'Samsun' tobacco-grafted tomatoes had significantly increased values compared to both non-grafted tomatoes and other graft types.

3.6.2 Chemical quality parameters

There was a statistically significant difference in 'Muş' grafted tomatoes compared to control group tomatoes according to quality parameters such as soluble solids content , pH, and titration. While there was a significant difference between the graft groups in terms of soluble solids content and titration, there was no significant difference in pH levels.

According to the results, tomatoes grafted with 'Dişbudak' tobacco had the highest concentration of water-soluble dry matter. There were significant difference in 'Tekel' and 'Muş' grafts in terms of pH and titratable acidity, the important taste components and parameters that help prevent deterioration.

4 DISCUSSION

H2274 variant of tomato plants were successfully grafted with 'Taşova', 'Tekel', 'Muş', 'Samsun', 'Dişbudak', and 'Hasankeyf' tobacco rootstocks by using cleft grafting method. Previous grafting different types of tomatoes with tobacco conducted in our institution resulted in good survival (Yasinok et al., 2009). Additionally, Khah et

Table 1: Physical quality of grafted and non-grafted tomatoes

Traits	H2274	'TAŞOVA'	'TEKEL'	'MUŞ'	'SAMSUN'	'DİŞBUDAK'	'HASANKE
Fruit diameter (cm)	6.18±0.18^{a}	5.22±0.53^{b}	5.8±0.22^{b}	5.04±0.38^{b}	7.3±0.24^{c}	6.52±0.30^{d}	5.08±0.37^{b}
Fruit Height (cm)	5.44±0.27^{a}	4.12±0.40^{b}	5.62±0.15^{a}	3.92±0.35^{c}	6.22±0.19^{d}	5.4±0.36^{a}	5.66±0.54^{a}
Fruit Mass (g)	125.44±13.78^{a}	71.98±13.86^{b}	138.24±8.56^{b}	66.64±6.86^{b}	175.79±15.17^{c}	129.53±8.23^{b}	91.74±14.81^{b}

Data are expressed as mean ±standart error of the means (SEM). The different letters in the same row emphasize the statistical differences ($p < 0.05$)

Table 2: Chemical quality element values of grafted and non-grafted tomatoes

Traits	H2274	TAŞOVA	TEKEL	MUŞ	SAMSUN	DİŞBUDAK	HASANKEYF
SSC(^{0}Bx)	4.57±0.22^{a,c}	4.47±0.17^{a,c}	4.67±0.14^{a}	5.02±0.03^{b}	5.27±0.33^{b,c}	5.35±0.24^{b}	5.00±0.11^{a,b}
pH	4.56±0.03^{a}	4.61±0.01^{a,b}	4.59±0.01^{b}	4.6±0.01^{b}	4.59±0.03^{a,b}	4.53±0.02^{a,b}	4.50±0.04^{a,b}
TA(citric acid) %	0.42±0.01^{a,d}	0.37±0.01^{a,b,c}	0.39±0.01^{b}	0.36±0.01^{b}	0.42±0,01^{a,b,c}	0.43±0.01^{c,d}	0.38±0.01^{d}

Data are expressed as mean ± standart error of the means (SEM). The different letters in the same row emphasize the statistical differences ($p < 0.05$)

al. (2006) showed that grafting tomato plants with compatible rootstocks had positive effects on performance and that grafted plants in greenhouse were sturdier than non-grafted plants. Our study revealed a significant difference in yield between grafted and non-grafted plant groups. The results showed that tomatoes were grafted to compatible rootstocks. Grafting was also found to significantly increase fruit weight per plant.

The protective effects of fruits and vegetables against various diseases are believed to stem from the antioxidant compounds they contain including carotenoids, phenolic acids, and flavonoids (Abuajah et al., 2015; Kaur & Kapoor, 2001). Since the methods used to determine the amount of antioxidant activity are performed under different oxidation conditions and vary in substrate, probe, and reaction conditions to measure different oxidation products, more accurate results are obtained by implementing and comparing multiple methods (Frankel & Meyer, 2000).

It is known that antioxidant food products have the ability to prevent bitterness and taste deterioration due to oxidation. In addition to these characteristics, due to their role in preventing several diseases caused by stress or aging, antioxidants have begun to gain importance and have been studied in experimental, clinical and epidemiological research (Zavala et al., 2004). Therefore, it is crucial to measure the changes in antioxidant content of fruits and vegetables.

The natural composition of tomatoes includes antioxidant compounds such as tocopherol, ascorbic acid, lycopene and β-carotene flavonoids, and phenolic acids (Meyer et al., 2000; Maslarova, 2001; Heinonen, 2002). The antioxidative activity of tomatoes results from the synergistic effect of several phytochemicals (Heinonen, 2001; Maslarova, 2001; Heineken, 2002).

Several studies focused on the optimal conditions for maximum biosynthesis of lycopene and β-carotene and had varying results (Dumas et al., 2003). In the current study, lycopene values were between 84.5 mg kg^{-1} and 112.6 mg kg^{-1} and the highest values were obtained in tomatoes grafted with Hasankeyf tobacco. Similar to our results, Frusciante et al. (2007) reported that lycopene contents in fresh tomatoes vary between 18.6 and 146.2 mg kg^{-1} according to data gathered from different resources. Based on the results of the current study, we conclude that 'Taşova', 'Tekel', 'Samsun', and 'Hasankeyf' tobacco grafted tomatoes can be preferred for lycopene and β-carotene pigments with antioxidant characteristics.

Increased DPPH free radical scavenging activity was observed in 'Tekel', 'Muş', 'Samsun', 'Dişbudak', and 'Hasankeyf' tobacco-grafted tomatoes. The presence of phenolic compounds in 'Tekel', 'Samsun', 'Dişbudak', and 'Hasankeyf' tobacco tomatoes is important in terms of their role in scavenging radicals. The fact that these tomatoes have a strong antioxidant and anti-radical activity shows that their use in healthcare could be beneficial. There were statistically significant differences in 'Tekel', 'Samsun', 'Dişbudak', and 'Hasankeyf' tobacco grafted tomatoes according to total phenolic content. These results are valuable in determining their role in radical scavenging activity.

In tobacco-tomato grafting, nicotine content was evaluated to examine whether or not yielded tomatoes are suitable or healthy for consumption. Yasinok et al. (2009) found increased nicotine content in grafted fruit. After grafting, a very low level of nicotine was detected in

tomato fruits. Dawson (1942) reported a high quantity of alkaloid accumulation in leaves of tomato plants grown on tobacco rootstocksAndersson et al. (2003) reported that 30–40 % of orally consumed nicotine reaches the systemic circulation, a person could be exposed to almost 21.3 µg of nicotine in his/her diet and only 6.4–8.5 µg nicotine would enter the systemic circulation. Yasinok et al. (2009).In our study nicotin content of non grafted tomatoes obtained 1.10 µg/7 kg fm in early harvested plants and 0.67 µg/7 kg fm at late harvested tomatoes, also in nicotin content of grafted plants , there was no significant difference in nicotine content between non-grafted tomatoes and tobacco-grafted tomatoes. This shows that rootstock and scion selection is important, and indicates that tobacco-tomato graft is reliable and can be used.

According to the analysis of physical parameters, 'Taşova', 'Muş', and 'Samsun' tobaccos yielded larger fruit. This suggests that tomato plants with physically smaller fruit could be improved by being grafted with these tobacco variants.

Low titratable acidity is an indicator of better fruit quality and taste (Özenç et al., 2017). In this context, a significant decrease was observed in tomatoes grafted with 'Tekel' and 'Muş' tobaccos.

The content of soluble solids content is an important factor in identifying ripeness in fruits and may change depending on fruit type, ripeness phase, and storage conditions (Özbay & Ateş, 2015). High amount of dry-substance is suggested to be associated with longer-lasting fruit (Özenç et al., 2017).

It is reported that the soluble solids content in tomatoes vary between 2.9 % and 7 % (Bargefurd & Harker, 1998; Şalk et al., 2008; Ünlü & Padem, 2009; Danneh et al., 2015). Similarly, our results showed a range between 4.48 and 5.35 °Bx. Additionally, the best results were observed in tomatoes grafted with 'Muş' and 'Dişbudak' tobaccos. This suggests that durability could be achieved by grafting 'Muş' and 'Dişbudak' tobacco to tomatoes that are not as durable. The analyses showed that 'Muş' tobacco grafting enhances physical and chemical quality of tomatoes.

As tomato mostly consists of water, short spoilage time, especially in ripe products, causes commercial problems. Antioxidant compounds have positive effects on deterioration and human health, and tomatoes are a rich source of these compounds. This demonstrates the importance of innovations improving antioxidant characteristics in tomatoes.

According to the results of our study, 'Samsun' tobacco was the most compatible and applicable graft for tomato plants in terms of size, yield, and antioxidant capacity. Tomatoes grafted with tobacco can be used as a rich antioxidant source compared to non-grafted tomatoes. Because of the low nicotine content, grafted tomato plants were considered to be safe and suitable for consumption.

The grafting method described in this study may guide vegetable growers, as it increases tomato yield, performance, and quality, also allowing them to make more profit.

5 ACKNOWLEDGEMENT

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Estragole-rich essential oil of summer savory (*Satureja hortensis* L.) as an eco-friendly alternative to the synthetic insecticides in management of two stored-products insect pests

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Estragole-rich essential oil of summer savory (*Satureja hortensis* L.) as an eco-friendly alternative to the synthetic insecticides in management of two stored-products insect pests

Abstract: The lesser grain borer [*Rhyzopertha dominica* (Fabricius, 1792)] and the red flour beetle [*Tribolium castaneum* (Herbst, 1797)] are among the cosmopolitan damaging pests on several stored-products. The overuse of chemical pesticides in the control of such pests caused several side-effects including environmental contaminations, human health problems, and insect pests' resistance. In this circumstance, researchers have focused on safe and effective alternatives to chemical pesticides. In the present study, the insecticidal efficiency of essential oil extracted from the summer savory (*Satureja hortensis* L.) was assessed on the *R. dominica* and *T. castaneum* adults. The chemical profile of essential oil was evaluated through a gas chromatography-mass spectrometer, in which estragole, β-ocimene and d-limonene were the main components. The essential oil had considerable fumigant toxicity on insect pests. The mortality of insects was dependent on the essential oil concentration and exposure time. Probit analysis indicated that *R. dominica* with low LC_{50} values (Lethal Concentration to kill 50 % of tested insects) was more susceptible than *T. castaneum*. Accordingly, *S. hortensis* essential oil with a high level of phenylpropanoid and terpenic compounds can be recommended as an efficient and natural alternative to the detrimental chemicals in the management of *R. dominica* and *T. castaneum*.

Key words: essential oil; estragole; Satureja hortensis; fumigation; coleopteran pests

Na estragolu bogato eterično olje vrtnega šetraja (*Satureja hortensis* L.) kot okolju prijazna alternativa sintetičnim insekticidom pri zatiranju dveh vrst skladiščnih škodljivih žuželk

Izvleček: Žitni kutar [*Rhyzopertha dominica* (Fabricius, 1792)] in rižev mokar [*Tribolium castaneum* (Herbst, 1797) sta kozmopolitski vrsti škodljivcev, ki povzročata škodo na mnogih uskladiščenih pridelkih. Prekomerna raba insekticidov pri zatiranju takšnih škodljivcev ima številne stranske učinke, vključno z onesnaževanjem okolja, zdravstvenimi problemi ljudi in odpornostjo škodljivih žuželk. V tej raziskavi so se raziskovalci osredotočili na varno in učinkovito alternativo sintetičnim insekticidom. Insekticidna učinkovitost eteričnega olja iz vrtnega šetraja (*Satureja hortensis* L.) je bila preizkušena na odraslih osebkih obeh vrst zgoraj omenjenih škodljivcev. Kemična sestava eteričnega olja je bila ovrednotena s plinskim kromatografom in masnim spektrometrom, ugotovljeno pa je bilo, da so estragol, β-ocimen in d-limonen glavne sestavine. Zaplinjevanje z eteričnim oljem je imelo znaten toksični učinek na škodljivi žuželki. Smrtnost žuželk je bila odvisna od koncentracije eteričnega olja in časa izpostavitve. Analiza Probit je pokazala, da je vrsta *R. dominica* z manjšimi LC_{50} vrednostmi bolj občutljiva kot vrsta *T. castaneum*. Glede na to bi lahko eterično olje iz vrtnega šetraja z veliko vsebnostjo fenilpropanoidov in terpenov priporočili kot učinkovito in naravno alternativo škodljivim kemikalijam pri zatiranju omenjenih škodljivcev.

Ključne besede: eterično olje; estragol; *Satureja hortensis*; zaplinjevanje; škodljivi hrošči

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1 INTRODUCTION

Secondary metabolites announce the evolution of chemical defenses in plants which are often formed as by-products throughout the production of primary metabolites. Secondary metabolites have several essential roles especially in the protection against herbivores and in the attraction of pollinators' (Dinan, 1995; Bohinc et al., 2012). Plant-derived essential oils as well-known secondary metabolites can be produced in several aerial parts including leaves, flowers, seeds, stems and the roots of aromatic plants. Essential oils are generally composed of isoprene units as terpenes and phenylpropane (Bakkali et al., 2008). Although terpenes such as monoterpenes (2 units of isoprene, C_{10}), sesquiterpenes (3 units of isoprene, C_{15}), and diterpenes (4 units of isoprene, C_{20}) have a high quantity, the monoterpenoids (oxygenated monoterpenes) are often the most components of the many essential oils (Breitmaier, 2006; Abdel-Tawab, 2016). Along with the application of essential oils in the perfumery and pharmaceutical industries, their lethal and sub-lethal effects especially fumigant toxicity of essential oils have been approved toward different class and orders of main insect and acari herbivores (Regnault-Roger et al., 2012; Rojht et al., 2012; Ebadollahi & Jalali-Sendi, 2015).

Summer savory [*Satureja hortensis* L. (Lamiaceae)], as an aromatic spice and food preservative, widely distributed and/or cultivated in many countries. It used in Iranian traditional medicine to treat intestinal and stomach disorders such as indigestion and diarrhea, muscle pain, thrombosis, and cardiovascular diseases (Hajhashemi et al., 2000; Yazdanparast et al., 2008). Moreover, along with antibacterial, antifungal, antioxidant, and cytotoxic activities of *S. hortensis*, its potential on the insect pest management have also been documented (Mahboubi & Kazempour, 2011; Miladi et al., 2013; Gombač & Trdan, 2014; Farzaneh et al., 2015; Ghorbanpour et al., 2016).

R. dominica (lesser grain borer) and *T. castaneum* (red flour beetle) are among the cosmopolitan serious pests of stored-products such as cereal and legume grains, dried fruits, spices, flours, leather, and even packaging materials made from wood and paper. Further, the quality of infested products strongly reduces due to the residues of insect bodies and their unpleasant smell (Villaverde et al., 2007; Edde, 2012).

As part of a program aimed at studying the insecticidal activity and chemical composition of plant essential oils, we have assessed the fumigant toxicity and chemical profile of *S. hortensis* essential oil against *R. dominica* and *T. castaneum*. Hope the range of introduced active bioagents derived from aromatic plants has extended by the results of the present study.

2 MATERIALS AND METHODS

2.1 ESSENTIAL OIL EXTRACTION AND ANALYSIS

Fresh 10 cm aerial parts from the shoots of *S. hortensis* were sampled for essential oil extraction. The specimens were collected during April and May 2019 from Parsabad region (Latitude: 39°38' N, Longitude: 47°52' E, and height: 52 m), Ardebil province, Iran. The samples were dried at room temperature within a week and then ground using an electric grinder. Fifty grams ground plant material was poured into a Clevenger apparatus equipped with a 1000 ml balloon. The essential oil was extracted within 3 h and the obtained oil was stored in a refrigerator at 4 °C.

Chemical profile of the *S. hortensis* essential oil was assessed using a gas chromatographic system (Agilent model 7890B) equipped with the mass spectrometer detector (Agilent model 5977A) according to Ebadollahi et al. (2017): chromatographic separation was performed on the HP-5MS (5 % phenyl-methyl-polysiloxane) capillary column (30 m length, 0.25 mm internal diameter, and 0.25 µm film thickness) with 70 eV ionization energy. The injected volume was 1.0 µl with 280 °C temperature. The temperature program of the column was set from 50 to 350 °C. Helium (99.999 %) was used as a carrier gas at 1 ml minute^{-1}. The component was identified by comparison of their mass spectra with those from Wiley's MS library (7th edition) and NIST (National Institute of Standards Technology) in the library.

2.2 TESTED INSECTS

The adult insects of *R. dominica* were obtained from the colonies at the Department of plant protection, University of Mohaghegh Ardebili, Ardabil, Iran. The adult insects of *T. castaneum* were collected from contaminated wheat grains in the warehouses of Parsabad city (Latitude: 39°38' N, Longitude: 47°52' E, and height: 52 m), Ardabil province, Iran. Adult insects were separately released on wheat grains in the breeding container. Adult insects were removed 48 h later and grains with insects' eggs were kept in an incubator at 25 ± 2°C and 65 ± 5 % relative humidity in dark (Arnaud et al., 2005). Synchronized adult insects with 1 - 7 old-days were selected.

2.3 BIOASSAY

The fumigation bioassay was done according to the study of Ebadollahi (2018): twenty adults of both insects

were separately located in 340 ml fumigant chambers. The tested concentrations of essential oil, based on the preliminary experiments, were from 11.76 to 47.06 µl l^{-1} and from 21.00 to 55.15 µl l^{-1} for *R. dominica* and *T. castaneum*, respectively. The essential oil concentrations were poured on the 2 × 3 cm piece of filter papers which were sealed to the inside of the container lids and the lids were closed using parafilm. Experiments were conducted for control groups without adding essential oil concentration. Each treatment was repeated 4 times and the insects' mortality was documented after 24, 48 and 72 h intervals.

2.4 STATISTICAL ANALYSIS

Variance analysis was used to assess the significant effects of essential oils' concentrations and the exposure times. To compare the effects of independent factors concentration and exposure time on the insects' mortality, the ω^2 comparison was used. Calculation of lethal concentrations (LC), lethal times (LT) and linear regression analysis along with heterogeneity of the data by a Chi-squared test were done using SPSS software version 24 (IBM, Chicago, USA).

3 RESULTS

3.1 CHEMICAL COMPOSITION OF ESSENTIAL OIL

Chemical analysis of *S. hortensis* essential oil identified 17 components at 99.21 %, in which 83.02 % are phenylpropanoid constituents. Five different groups of terpenes were also recognized in the essential oil, in which the monoterpene hydrocarbons (15.38 %) had the highest amount followed by sesquiterpenoids (0.43 %), monoterpenoids (0.26 %), a sesquiterpene hydrocarbon (0.08 %), and a diterpene (0.04 %). Estragole (82.10 %) as

Table 1: Chemical composition of the essential oil isolated from Iranian *Satureja hortensis*

Compound	Retention Time (minute)	Formula and Classification	Percentage
α-Pinene	5.30	$C_{10}H_{16}$ MH	0.91
Camphene	5.57	$C_{10}H_{16}$ MH	0.04
Sabinene	6.03	$C_{10}H_{16}$ MH	0.06
β-Pinene	6.09	$C_{10}H_{16}$ MH	0.09
β-Myrcene	6.33	$C_{10}H_{16}$ MH	0.12
d-Limonene	7.08	$C_{10}H_{16}$ MH	2.25
β-Ocimene	7.46	$C_{10}H_{16}$ MH	11.86
α-Terpinene	8.27	$C_{10}H_{16}$ MH	0.05
Rosefuran	8.43	$C_{10}H_{14}O$ M	0.08
Estragole	11.51	$C_{10}H_{12}O$ Ph	82.10
E,E-2,6-Dimethyl-3,5,7-octatriene-2-ol	11.54	$C_{10}H_{16}O$ M	0.07
Bornyl acetate	14.23	$C_{12}H_{20}O_2$ M	0.11
Methyl Eugenol	18.71	$C_{11}H_{14}O_2$ Ph	0.92
Germacrene-D	21.05	$C_{15}H_{24}$ SH	0.08
Spathulenol	23.76	$C_{15}H_{24}O$ S	0.31
Caryophyllene oxide	23.89	$C_{15}H_{24}O$ S	0.12
Eicosane	32.78	$C_{20}H_{42}$ DH	0.04
MH: Monoterpene Hydrocarbon			15.38
M: Monoterpenoid			0.26
SH: Sesquiterpene Hydrocarbon			0.08
S: Sesquiterpenoid			0.43
DH: Diterpene Hydrocarbon			0.04
Ph: Phenylpropanoid			83.02
Total			99.21

Table 2: Results of the variance analysis of *S. hortensis* essential oil fumigation on the adults of *R. dominica* and *T. castaneum* after 24, 48 and 72-h exposure times

Insect	Source of Variation	df	*F*	*p*-value	ω^2
R. dominica	Concentration	4	467.987 *	<0001	22.516
	Time	2	155.009 *	<0001	3.713
	Time × Concentration	8	1.594	0.154	0.057
T. castaneum	Concentration	4	324.572 *	<0001	17.793
	Time	2	142.271 *	<0001	3.884
	Time × Concentration	8	1.106	0.377	0.012

* Significant at α = 1%

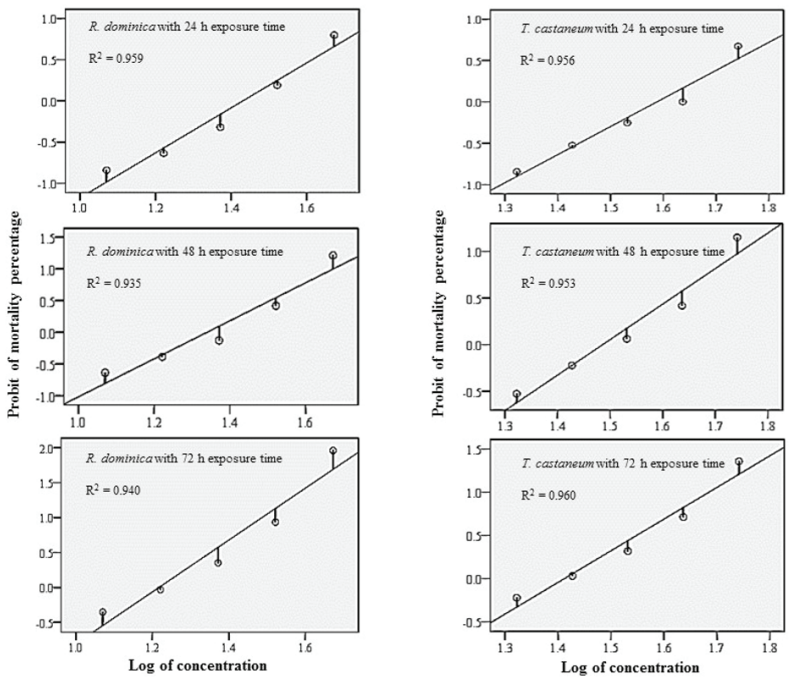

Figure 1: Concentration – mortality lines for fumigant toxicity of *S. hortensis* essential oil against the adults of *R. dominica* and *T. castaneum* after 24, 48 and 72-h exposure times

a phenylpropanoid constituent had the highest amount and monoterpene hydrocarbons β-ocimene (11.86 %), and dl-limonene (2.25 %) were in the next points (Table 1).

3.2 FUMIGANT TOXICITY

Results of the fumigant toxicity indicated that essential oil of Iranian *S. hortensis* had considerable toxicity on the *R. dominica* and *T. castaneum* adults. The results of variance analysis were summarized in Table 2. Concentrations of essential oil and exposure times had statistically significant effects on the insects' mortality but their interaction wasn't significant. Furthermore, based on the ω^2 values, among these factors, the effect of essential oil concentration was more effective.

The calculated R^2 values for concentrations-mortality correlation were 0.959, 0.935 and 0.940 for *R. dominica* and 0.956, 0.953 and 0.960 for *T. castaneum* after 24, 48 and 72-h exposure times, respectively. So, there is a direct correlation between the concentrations of essential oil and mortality of both insects (Figure 1).

Probit analysis indicated the calculated LC_{50} values (lethal concentration to kill 50 % of tested insects) of essential oil were significantly decreased from 24 h to 72 h for both insects (Table 3). For example, the 24 h-LC_{50} value of essential oil with 95 % confidence limits was 27.212

Table 3: Results of Probit analysis for fumigant toxicity of *S. hortensis* against the adults of *R. dominica* and *T. castaneum*

Insect	Time (h)	LC_{50} (95 % confidence limits) (μl l^{-1})	χ^2 (df = 3)	Slope ± SE	Significance *
R. dominica	24	27.212 (24.657 - 30.361)	3.893	2.740 ± 0.294	0.273
	48	22.193 (20.140 - 24.385)	7.062	2.897 ± 0.298	0.070
	72	16.466 (12.128 - 20.013)	5.830	3.321 ± 0.329	0.120
T. castaneum	24	38.908 (35.951 - 42.688)	3.425	3.386 ± 0.412	0.331
	48	30.757 (28.377 - 33.070)	3.810	3.691 ± 0.419	0.283
	72	25.747 (23.020 - 28.021)	2.745	3.506 ± 0.429	0.433
Insect	Concentration (μl l^{-1})	LT_{50} (95 % confidence limits) (h)	χ^2 (df = 1)	Slope ± SE	Significance *
R. dominica	47.06	10.301 (2.944 - 16.210)	1.765	2.060 ± 0.515	0.184
T. castaneum	55.15	12.682 (5.479 - 18.103)	2.023	2.282 ± 0.503	0.155

* Since the significance level is greater than 0.05, no heterogeneity factor is used in the calculation of confidence limits. The number of insects for calculation of LC_{50} values is 400 for each time. The number of insects for calculation of LT_{50} values is 240 for each concentration.

(24.657 - 30.361) μl l^{-1} which was decreased to 16.466 (12.128 - 20.013) μl l^{-1} after 72 h. Further, according to Table 3, adults of *R. dominica* with low LC_{50} values were significantly susceptible than *T. castaneum* adults to the *S. hortensis* essential oil at all exposure times.

The lethal times to kill 50 % of tested insects (LT_{50} values) are also shown in Table 3. At a high tested concentration of *S. hortensis* essential oil (47.06 μl l^{-1}), the LT_{50} value was 10.301 (2.944 - 16.210) h against *R. dominica* adults. This value for *T. castaneum* adults with a concentration of 55.15 μl l^{-1} was calculated as 12.682 (5.479 - 18.103) h.

4 DISCUSSION

The composition of *S. hortensis* essential oil have been investigated in the previous studies; carvacrol (11.0 %), ρ-cymene (19.6 %), sabinene (4.4 %), γ-terpinene (16.0 %), and thymol (28.2 %) were found as major compounds by Mahboubi and Kazempour (2011). Thymol, ρ-cymene, γ-terpinene, and carvacrol were not detected in the present study but a trace of sabinene (0.06 %) was determined. In contrast, estragole and β-ocimene as major components of present work were not detected in the study of Mahboubi and Kazempour (2011). Farzaneh et al. (2015) showed carvacrol (48.0 %), ρ-cymene (11.7 %), myrcene (2.5 %), α-pinene (2.5 %), γ-terpinene (24.2 %) were the main components. From these constituents, myrcene (0.12 %) and α-pinene (0.91 %) with different amounts were recognized in the essential oil of present study. In the other study, Miladi et al. (2013) also revealed that monoterpenoids (59.11 %) were the main chemical class of *S. hortensis* essential oil which is parallel with our results but they announced other components such as carvacrol, β-caryophyllene, *p*-cymene, and γ-terpinene. In contrary, Mohammadhosseini and Beiranvand (2013) showed that the monoterpene hydrocarbons such as myrcene, α-pinene, β-pinene, α-terpinene, and α-thujene had the highest amount in the *S. hortensis* essential oil. These differences in the chemical profile of *S. hortensis* essential oil in the present and above-mentioned studies can be due to the variations in some of the influential factors, such as geographical and growing conditions, drying and extraction methods, ontogenetic stages, and season (Sefidkon et al., 2006; Pfefferkorn et al., 2008; Rezvanpanah et al., 2011; Ghorbanpour et al., 2016).

Insecticidal properties of *S. hortensis* essential oil were acknowledged in some recent studies; appropriate fumigant toxicity of this oil was proved against Mediterranean flour moth [*Ephestia kuehniella* (Zeller, 1879)], Indianmeal moth [*Plodia interpunctella* (Hubner, 1813)], and *T. castaneum* (Mollaei et al., 2011). The calculated 48 h-LC_{50} value for *T. castaneum* in this work (192.350 μl l^{-1}) is much higher than the corresponding LC_{50} in the present study (30.757 μl l^{-1}). In the study of Tozlu et al. (2011), the *S. hortensis* essential oil with a high amount of carvacrol, β-caryophyllene, p-cymene, δ-terpinene, and α-terpinene was very toxic against the broad bean weevil [*Bruchus dentipes* (Baudi, 1886)]. They concluded that the *S. hortensis* essential oil toxicity is directly related to its components. Along with the fumigant toxicity of *S. hortensis* essential oil, the contact toxicity, repellency, and disruption in the enzymes' activity were also described (Mollaei et al., 2011; Heydarzade & Moravvej, 2012; Magierowicz et al., 2019). The results of these studies indicated that *S. hortensis* essential oil has considerable

insecticidal activities against stored-product insect pests which are in accordance with our findings.

Estragole or methyl chavicol, as two major compounds identified in the present study, is a GRAS (Generally Recognized As Safe) nominated material and approved for food procedure (De Vincenzi et al., 2000). Its name originates from "estragon" which is a French word of tarragon (*Artemisia dracunculus* L.) (Misztal et al., 2010). Along with cytotoxic and antimicrobial properties of estragole (Bagamboula et al., 2004; Andrade et al., 2015), toxicity of this compound has also been approved against some of damaging stored-product insect pests including *T. castaneum*, the rice weevil [*Sitophilus oryza* (Linnaeus, 1763)], the maize weevil [*Sitophilus zeamais* (Motschulsky, 1855)], the booklice [*Liposcelis bostrychophila*, Badonnel, 1931], the cigarette beetle [*Lasioderma serricorne* (Fabricius, 1792)], and the adzuki bean beetle [*Callosobruchus chinensis* (Linnaeus, 1758)] (Kim & Ahn, 2011; Wang et al., 2011; Kim & Lee, 2014; Guo et al., 2015). Furthermore, the insecticidal properties of other main components identified in the present study including d-limonene and β-ocimene were also documented (Tripathi et al., 2003; Guo et al., 2015; Kang et al., 2018). Accordingly, the fumigant toxicity of *S. hortensis* essential oil may be attributed to such constituents. However, the existence of synergistic effects between other compounds is also possible.

5 CONCLUSION

Synthetic pesticide residues can be found in different parts of our surrounding environment from water and soil to everybody's foods and even human breast milk samples (Damgaard et al., 2006; Nicolopoulou-Stamati et al., 2016; Trdan, 2016). Regarding the pests' management, due to the overusing of synthetic chemicals, the other side-effects such as resurgence and outbreak of new pests, several pest-resistant reports on the different classes of synthetic pesticides, and detrimental effects on valuable noun-target organisms including parasitoids and predators have also been documented (Köhler et al., 2013; Cruz et al., 2017; Sudo et al., 2018). Therefore, urgent efficacious tools for the reduction of synthetic chemical utilization and for announcing eco-friendly agents with fewer public health risks are required. Because of the low toxicity to the mammals and pose a minimum risk, the plant essential oils considered safe (Viciolle et al., 2012). The prospective pesticidal activity of several plants essential oils have been stated in recent years (Isman & Grieneisen, 2014), and the range of these eco-friendly bio-agents was extended in the present study through the introduction of Iranian phenylpropanoid-rich summer savory as a toxic agent against two damaging coleopteran insect pests *R. dominica* and *T. castaneum*. However, based on the short residual lifetime (Isman, 2006), it is recommended that such essential oils be tested in the better applicable form such as "controlled release technique" through micro- and nano-encapsulation. Furthermore, the pesticidal ability of this plant essential oil on the other pests and its adverse effects on beneficial biocontrol agents should be more investigated.

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Improvement of yield and yield stability in safflower using multivariate, parametric and non-parametric methods under different irrigation treatments and planting date

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Improvement of yield and yield stability in safflower using multivariate, parametric and non-parametric methods under different irrigation treatments and planting date

Abstract: Development of superior genotypes with high adaptability to different environments is considered as one of the most important goals in safflower breeding programs. In this study, ten parametric and six non-parametric measures along with the additive main effects and the relevant multiplicative interaction (AMMI) model were used to evaluate genotype by environment interaction (GE) in 15 safflower genotypes across 12 test environments) combination of year, planting date and moisture conditions) during growing seasons in 2016 and 2017. AMMI analysis revealed significant differences among the genotypes and their GE interactions. The different stability statistics were substantiated by rank correlation coefficient. Rank-correlation coefficients revealed positive and significant correlations between mean seed yield and superiority index ($r = 0.99^{**}$), and significant and negative correlation with bi, R^2, D_{ij} and non- parametric measures ($NPi^{(2)}$, $NPi^{(3)}$ and $NPi^{(4)}$). Based on most stability parameters, the Mex.295 genotype (G_{10}) was found to be the most stable for seed yield. IL.111 genotype (G_9) recorded the highest mean yielding genotype regarded as the most favorable safflower genotype. In conclusion, both stability and seed yield should be simultaneously considered to exploit useful effects of G × E interactions in safflower breeding programs.

Key words: safflower; parametric and non-parametric measures; yield, rank correlation

Izboljšanje pridelka žafranike in njegove stabilnosti z multivariatnimi parametričnimi in neparametričnimi metodami pri različnem namakanju in datumih setve

Izvleček: Razvoj superiornih genotipov z veliko prilagodljivostjo različnim okoljem je eden izmed najvažnejših ciljev v žlahniteljskih programih žafranike. V raziskavi je bilo uporabljenih deset parametričnih in šest neparametričnih meril vključno z glavnimi aditivnimi učinki in modelom pomembnih multiplikativnih interakcij (AMMI) za ovrednotenje interakcije genotipa z okoljem (GE) pri 15 genotipih žafranike, preiskušenih v 12 okoljih)kombinacija leta poskusa, datuma setve in vlažnostnih razmer) v rastnih sezonah 2016 in 2017. AMMI analiza je odkrila značilne razlike v interakcijah genotipov z okoljem. Različne statistične metode za ovrednotenje različnih vidikov stabilnosti pridelka so bile uspešno nadomeščene s koeficientom gradualne korelacije. Ti koeficienti so odkrili pozitivne in značilne korelacije med poprečnim pridelkom semena in indeksom superiornosti ($r = 0.99^{**}$), in značilne negativne korelacije z bi, R^2, D_{ij} in neparametričnimi merili ($NPi^{(2)}$, $NPi^{(3)}$ in $NPi^{(4)}$). Na osnovi večine parametrov stabilnosti je bil genotip Mex.295 ,(G_{10}) prepoznan kot najbolj stabilen za pridelek semena. Genotip IL.111 (G_9) je bil prepoznan kot najboljši genotip žafranike z največjim poprečnim pridelkom. Zaključimo lahko, da je v žlahtniteljskih programih žafranike potrebno hkrati upoštevati velikost in stabilnost pridelka, če hočemo izkoristiti koristne interakcije okolja in genotipa (G × E).

Ključne besede: žafranika; parametrična in neparametrična merila; pridelek; korelacija rangov

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1 INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is mainly grown in dryland conditions of the world as an oilseed crop with diverse genetic backgrounds and the pharmaceutical industry uses (Kumar et al., 2016). Safflower have tremendous potential for cosmetic industry and organic food and other usages as biofuel, soap, varnish making, food coloring, flavoring, dyes, medicines and bird feed (Golkar, 2014; Kumar et al., 2016). With the development of global changes, researchers from all over the world increasingly pay attention to drought as a major abiotic stress limiting growth and productivity of crops. Iran is known as one of the highest genetic diversity for safflower in the world (Knowles, 1969). It hosts a large number of native landraces with improved yields in seed and oil (Golkar, 2014).

Drought can be regarded, as a major fundamental abiotic stress factor limiting and restricting the crop plants growth and production (Farooq et al., 2012 Hussain et al., 2016). So, drought stress has recently attracted increasing attention in breeding programs due to its exacerbating impact of it by climate change (Hussain et al., 2016). In drought affected regions and semi- arid agro-ecosystems, safflower is considered as a promising alternate crop due to its high adaptability to drought conditions (Omidi Tabrizi, 2006; Kar et al., 2007; Hussain et al., 2016). The yield of safflower is influenced by such different factors as location and date of planting, soil available water, air temperature, and light intensity (Dajue & Mundel, 1996), especially during its seedling and flowering stages (Hussain et al., 2016). Different environments usually have significant fluctuation on seed yield of different genotypes due to the different responses of the genotypes to environmental features including environmental stresses (biotic and abiotic).

Hence, seed yield is influenced by genotype (G), environment (E) and genotype × environment interactions (G×E) in a number of genotypes that are grown in a wide range of environments (Gauch, 2006). The seed yield of safflower genotypes varies a lot due to the high dependence of their yield on both genotypic and environmental conditions (Omidi Tabrizi, 2006; Ebrahimi et al., 2016). In safflower breeding programs, interpretation of G × E interactions plays a major role to identify the superior genotypes across various environments (Pourdad & Mohammadi, 2008). Also, the obtained results from G × E analysis determine the phenotypic stability of genotypes in each tested environment (Abdulahi et al., 2009). In such situations, the breeder is often faced with the choice either to develop some special genotypes for a specific adaptations and/or to choose the genotypes with a high general adaptations that can perform well in a wide range of environments (Pourdad & Mohammadi, 2008) Thus, it is necessary to study the adaptability and stability of new genotypes with diverse origins for cultivation in different planting dates and moisture regimes in its cultivation regions as Iran.

Different methods have been commonly used to determine the extent of G × E interaction effects under different growing conditions (Becker & Leon, 1988). These methods include multivariate analysis (Gauch, 2006), parametric methods (Eberhart & Russell, 1966), and non-parametric ones (Thennarasu, 1995). Parametric methods, as the most common approach, depend on assumptions made regarding the distributional patterns of about genotypic, environmental, and G × E interaction effects (Huehn, 1996). The most common ones include regression coefficient (b_i) (Eberhart & Russell, 1966), regression coefficient (B_i) (Perkins & Jinks, 1968), variance of deviations from regression (s^2_{di}) (Eberhart & Russell, 1966), Wricke' s ecovalance (W^2_i) (Wricke, 1962), coefficient of variability (CV_i) (Francis & Kannenberg, 1978), and stability variance () (Shukla, 1972). Most breeding programs exploit combinations of some parametric and some non- parametric approaches (Becker & Leon, 1988). Non-parametric approaches are based on no assumption about the distribution of model residuals and homogeneity of variances (Nassar & Huehn, 1987; Farshadfar et al., 2012). Multivariate techniques have been commonly employed in stability analysis in order to provide more information regarding the real multivariate response of genotypes to different environments (Purchase et al., 2000). Multivariate analysis serves three purposes: (i) to remove noise from the data pattern, (ii) to make a summary of the data, and (iii) to show the structure existing in the data. Additive main effects and multiplicative interactions (AMMI) model combines the main effects and interactions of genotype by environment. This method have its own capacities as identification of the ideal test conditions, choice of genitors, and formulation of recommendations for regionally adapted cultivars (Gauch & Zobel, 1996; Ebdon & Gauch, 2002). The AMMI stability value (ASV) was developed by Purchase et al. (2000) based on the AMMI model scores ($IPCA_1$ and $IPCA_2$) for each genotype. The ability of safflower varieties to function appropriately in different environmental conditions has been well confirmed by plant breeders and agronomists. The present study is intended to identify the potential of native and exotic safflower genotypes for cultivation in arid and semi-arid regions based on the best sowing dates. So, the main objective of this study was to investigate the genotype by environment interactions for the seed yield of safflower genotypes, as evaluated under different environmental conditions (year, sowing date, and moisture regimes) and 2) to find stable safflower

genotypes having high seed yields in a wide range of environments.

2 MATERIALS AND METHODS

This experiment was conducted in 2016 and 2017 at the Research Farm located at Isfahan University of Technology, in Lavark, Isfahan (32° 32′N, 51° 23′ E, 1630 m asl), Iran. The soil at the site is silty clay loam with the pH value of 7.8. In each of the study years, fifteen safflower accessions from various topographical regions (both native and exotic accessions) were planted (Table 1) with three replications at each of the two dates designated as early sowing (15 March) and late sowing (15 April). Plants were irrigated uniformly at the budding stage. The non-stress treatment involved irrigation when 40 % of the total available water was depleted from the root zone. In the medium and high drought stress treatments, irrigation was applied when depletion of 60 % and 85 of the total available water from the root zone occurred, respectively. Irrigation depth was determined using the formulae: $I = [(\theta_{FC} - \theta_i)/100]\ D \times B$), where, I is the irrigation depth (cm) and θ_{FC} (–0.03 MPa) denotes the soil

Table 1: Safflower genotype origins and the environmental characteristics of the environments used to analyze genotype × environment interaction on safflower seed yield using parametric and nonparametric measures

Genotype characteristics				
Genotype	Name	Origin	Genotype type	Mean seed yield (g/plant)
G_1	AC Sunset	Canada	-	13.72
G_2	KMP30	Karaj, Iran	Selected from mutation	15.94
G_3	GE_{62918}	Germany	-	11.45
G_4	Mex.7-37	Mexico	-	9.30
G_5	KMP51	Karaj, Iran	Selected from mutation	12.20
G_6	C_{111}	Isfahan, Iran	Selected from landrace	13.43
G_7	K_{21}	Kordestan, Iran	Selected from landrace	9.91
G_8	Padideh	Isfahan, Iran	Selected from landrace	12.51
G_9	IL.111	Auromieh, Iran	Selected from landrace	14.46
G_{10}	Mex.295	Mexico	Pedigree method	17.22
G_{11}	Mex.117	Mexico	-	11.56
G_{12}	A_2	Azerbayejan, Iran	-	13.24
G_{13}	Gol Sefid	Isfahan, Iran	Selected from landrace	14.77
G_{14}	PI-25090	Turkey	-	8.91
G_{15}	Golmehr	Isfahan, Iran	Zarghan 279 × IL.111	18.42
Environment characteristics				
Environment	Year- Location- Sowing date-Irrigation treatment			Mean seed yield /plant (g/plant)
E_1	2016- Lavark- 15 March- Non-drought stress			18.56
E_2	2016- Lavark- 15 March - Medium drought stress (60 % FC)			13.63
E_3	2016- Lavark- 15 March- High drought stress (85 % FC)			13.93
E_4	2016- Lavark- 15 April- Non-drought stress			12.51
E_5	2016- Lavark- 15 April- Medium drought stress (60 % FC)			9.13
E_6	2016- Lavark- 15 April- High drought stress (85 % FC)			8.24
E_7	2017- Lavark- 15 March- Non-drought stress			21.78
E_8	2017- Lavark- 15 March - Medium drought stress (60 % FC)			14.51
E_9	2017- Lavark- 15 March High - drought stress (85 % FC)			13.76
E_{10}	2017- Lavark- 15 April- Non-drought stress			13
E_{11}	2017- Lavark- 15 April- Medium drought stress (60 % FC)			9.83
E_{12}	2017- Lavark- 15 Apri- High drought stress (85 % FC)			8.75

gravimetric moisture percentage at field capacity (22 %); on the other hand, θ_i (–1.5 MPa) indicates the soil gravimetric moisture percentage at the irrigation time (10 %), D refers to the root-zone depth (50 cm), and B relates to the soil bulk density at the root zone (1.3 g cm^{-3}) (Clarke et al., 2008). The characteristics of the different genotypes and environments used in this study are reported in Table 2.

2.1 STATISTICAL ANALYSIS

2.1.1 Variance analysis

For detection the magnitude effects of genotype, environment and genotype × environment, a combined analysis of variance carried out, based in three replication in each environment. The soft ware GEA-R (v.4.1) (Pacheco et al., 2015) were used for all of the calculations.

2.1.2 AMMI analysis

The additive main effects as well as multiplicative interaction (AMMI) model were employed according to the following formula (Gauch & Zobel, 1996):

$$y_{ijk} = \mu + g_i + e_i + \sum_{n=1}^{N} \delta_n \xi_{in} \eta_{jn} + \theta_{ij} + \varepsilon_{ijk}$$

where, μ represents the grand mean, g_i refers to the main effect of the i^{th} genotype, and e_j denotes the main effect of the j^{th} environment. GEI is captured by:

$$\sum_{n=1}^{N} \delta_n \xi_{in} \eta_{jn}$$

In this equation, represents the Eigen value of the n^{th} interaction principal component analysis (IPCA) which is retained in the AMMI model, refers to the eigen vector taken for the i^{th} genotype from the n^{th} IPCA, indicates the Eigenvector considered for the j^{th} environment from the n^{th} IPCA, indicates the GEI residual, n shows the number of IPCA kept in the model and finally, e_{ijK} stands for the random error term.

2.1.3 Parametric statistics

1) Coefficient of variation (C.V)

Coefficient of variability (CVi) and mean yield (Francis & Kannenberg, 1978) were used to measure the stability of each genotype. Genotypes with low CVs and high average yields were considered as the most desirable ones.

$$CVi = (\sqrt{\delta^2}/\bar{X}i.)\times 100$$

2) Regression approaches

Eberhart and Russell (1966) used the linear regression coefficient (bi) (a part) and pooled deviation mean squares ((b section) to study the G×E interaction.

$$(a)\, b = 1 + \frac{\sum_i (Xij - \bar{X}i. - \bar{X}.j + \bar{X}..)(\bar{X}.j - \bar{X}..)}{\sum_j (\bar{X}.j - \bar{X}..)^2}$$

$$(b)\, \delta^2_{di} = \frac{\sum [Xij - \bar{X}i. - \bar{X}.j + \bar{X}..] - b_i{}^2 \sum_{j=1}^{q} (X_{.j} - X_{i.})^2}{e-2} - \frac{\delta^2_e}{r}$$

According to Perkins and Jinks (1968), the stable variety in each genotype is defined by small values of D_{ij} and non-significance of $B_i = 1$.

$$Bi = \frac{\sum_j (X_{ij} - \bar{X}i. - \bar{X}.j + \bar{X}..)(\bar{X}.j - \bar{X}..)}{\sum_j (\bar{X}.j - \bar{X}..)^2}$$

D_{ij}

3) Coefficient of determination (R^2)

The most stable genotype is characterized by the minimum value of R^2 (Pinthus, 1973).

$$R_i^2 = \frac{b_i^2 \sum_i (\bar{X}ij - \bar{X}..)^2}{\sum_j (\bar{X}ij - \bar{X}i.)^2}$$

Here, X_{ij} represents the safflower yield of the genotype i in the environment j, $X_{i.}$ denotes the mean safflower yield of the genotype i, $X_{.j}$ stands for the mean safflower yield in the environment j, $X..$ is the grand mean, b_i denotes the regression coefficient, e is taken as the number of environments and finally, g indicates the genotypes number.

Wricke covalence (W_i^2)

$$W_i^2 = \sum_j^e (X_{ij} - \frac{X_{i.}}{e} - \frac{X_{.j}}{g} + \frac{X_{..}}{ge})^2$$

4) Shukla's stability variance parameter (δ_i^2).

Then, estimation of the unbiased stability for each genotype was determined using Shukla's stability variance (Shukla, 1972):

$$\delta_i^2 = \left(\frac{p}{(p-2)(q-1)}\right) W_i^2 - \frac{SSGE}{(p-1)(q-1)(p-2)}$$

With this statistics, the most stable genotype is the one that minimizes (δ_i^2).

5) Superiority index (*Pi*) (Lin & Binns, 1988)

$$P_i = \frac{\sum_1^n (Xij - Mj)^2}{2e}$$

where, X_{ij} is the safflower yield of genotype *i* in environment *j*, M_j is the safflower yield of the reference genotype in environment *j*, and *e* is the number of environments.

6) AMMI stability value (ASV)

For each genotype and each environment, the AMMI stability value (ASV) is estimated on the basis of the relative contribution of $IPCA_1$ to $IPCA_2$ scores (the interaction principle component axes 1 and 2, respectively) and can be applied to the interaction sum of squares (ss), which is as follows (Purchase et al., 2000)

$$ASVi = \sqrt{[\frac{SS_{IPCA1}}{SS_{IPCA2}}(IPCA1\ score)^2] + (IPCA2\ score)^2}$$

where, $\frac{SS_{IPCA1}}{SS_{IPCA2}}$ is the weight given to the IPCA-1 value. Smaller IPCA scores represent a more stable genotype in different environments.

2.1.4 Non-parametric measures

In this study, the following two non-parametric stability statistics are derived on the basis of genotypes yield rank in each environment (m = number of environments) (Nassar & Huehn, 1987; Huehn, 1996):

$$S_i^{(1)} = 2\sum_j^{m-1}\sum_{i=j+1}^{n}|r_{ij} - r_{ij}|/[m(m-1)]$$

$$S_i^{(2)} = \sum_{j=1}^{m}(r_{ij} - \bar{r}_{i.})^2/(m-1)$$

Non-parametric stability measures were calculates as follows (Thennarasu (1995):

$$NP_i^{(1)} = \frac{1}{m}\sum_{j=1}^{n}|r_{ij}^* - M_{di}^*|$$

$$NP_i^{(2)} = \frac{1}{m}(\sum_{j=1}^{n}|r_{ij}^* - M_{di}^*|/M_{di})$$

$$NP_i^{(3)} = \frac{\sqrt{\sum(r_{ij}^* - \bar{r}_i^*)^2}}{\bar{r}_{i.}}$$

$$NP_i^{(4)} = \frac{2}{m(m-1)}\left[\sum_j^{m-1}\sum_{(j=j+1)}^{m}|r_{ij}^* - r_{ij}^*|/\bar{r}_{i.}\right]$$

The different stability parameters were statistically compared in this study by employing Spearman's coefficient of rank correlation (r_s) (Steel & Torrie, 1980). Furthermore, the significance of ranks was tested for the studied genotypes using Kruskal–Wallis *H* test (Kruskal & Walis, 1952).For a test of genotypic differences, the test statistic (H) is almost X^2- distributed, with degrees of freedom being g–1.

3 RESULTS

3.1 ANALYSIS OF GENOTYPE × ENVIRONMENT INTERACTION BY AMMI MODEL

The AMMI model revealed that the seed yield was considerably influenced through genotype, environment, and genotype × environment interaction (Table 2). AMMI analysis of variance partitioned the GE interaction into three interaction principal component axes (IPCA), all of which were significant for seed yield, while the three first principal components explained 92.61 % of the GE interaction. Based on this analysis, of the total sum of squares, 52.65 % could be attributed to the environmental effects; these included 25.34 %, which could be attributed to genotypic effects and 22 %, which could be related to GEI effects for seed yield.

The biplot showed that genotypes G_6 and G_{10} had the lowest IPCA1 scores (Figure 1). Given the angle that is estimated between the genotype *i* vectors and the environment *j*, the interaction effect (G×E) could be assumed to be positive for acute angles, while it is expected to be negligible for right angles; also, it can be postulated to be negative for obtuse angles. So, G_9 and G_4 showed to be specifically adaptable to the best environments including E_7 (i.e., non-drought stress condition with the early sowing date in 2017) and E_1 (i.e., non- drought stress condition with the early sowing date in 2016) (Figure 1). The genotypes G_8 and G_{15} showed to be specifically adaptable to environment E5 (i.e., medium drought stress with the early sowing date in 20165) and E_{11} (i.e., medium drought stress with the early sowing date in 2016) (Figure 1). In the worst environments for seed yield (i.e., E_6 and E_{12}), G_{14} showed the highest specific adaptation (Figure

Table 2: AMMI analysis of variance (a) and the first two AMMI scores for 15 safflower genotypes across 12 environments

Source of variation	DF	MS Seed yield (g/ plant)			
Environment (E)	11	727.75**			
Genotype (G)	14	275.22**			
G×E	154	21.73**			
IPCA1	24	71.89**			
IPCA2	22	38.48**			
IPCA3	20	26.44**			
Noise	88	2.8			
Error	360	17.52			
	Genotypic scores			Environmental scores	
Genotype	IPCA1	IPCA2	Environment	IPCA1	IPCA2
G1	-0.28	-0.04	E1	-0.74	-0.65
G2	0.20	0.091	E2	-0.161	0.69
G3	-0.40	-0.33	E3	-0.38	0.22
G4	-0.30	0.061	E4	0.34	0.13
G5	0.41	0.07	E5	0.98	-0.32
G6	-0.04	0.427	E6	-0.10	-0.17
G7	-0.50	0.707	E7	-0.58	-0.71
G8	0.66	-0.0097	E8	-0.18	0.65
G9	-1	-0.587	E9	-0.29	0.40
G10	0.10	-0.077	E10	0.26	0.20
G11	0.46	-0.66	E11	1	-0.29
G12	0.20	0.35	E12	-0.13	-0.13
G13	-0.15	0.64			
G14	-0.15	0.64			
G15	0.90	-0.17			

1). Genotypes and environments away from the center of the biplot showed large G × E interactions, displaying some specific kind of adaptation. Genotypes which were near the origin, including G_6 and $G_{10,}$ were found to have large stability statistics (Figure 2). Considering its high seed yield, G_{10} had the most specific adaptation to environments E_1 and E_7 (Figure 1).

The Heat Map graph in Figure (2) was drawn to gain a better understanding of the genotypic clustering based on their seed yield performance (g/plant) in different environments. Clearly, the environments may be divided into three different groups including favorable (E_7 followed by E_1), medium (E_2, E_3, E_4, E_8, E_9 and E_{10}), and unfavorable (E_{12} followed by E_5, E_6 and E_{11}) environments (Figure 2). Also, the genotypes can be categorized into high (G_4, G_9 and G_{10}), intermediate (G_1, G_3, G_6, G_7, G_{13} and G_{14}), and low yield (G_8 and G_{12}) ones. In the heat map legend, six different color represented different ranges of seed yield (g/plant) from E_1 to E_{12}, demonstrating the relative seed yield of each genotype in different environments.

3.2 PARAMETRIC MEASUREMENTS

Seed yield (SY) was used as the first parameter to evaluate the genotypes; thus, the genotypes G_4, G_9 and G_{10} were identified as the one with the highest but G_8 and G_{12} as those with the lowest mean yields across the 12 environments (Table 3). Moreover, the genotypes G_1, G_3, G_4, G_7 and G_9 recording regression coefficients (b_i) higher than unity exhibited yield performances greater than the average and were found adaptable to favorable environments, whereas the remaining ones with b_i values less than unity recorded the least average yields, which could be hardly adapted to all environments, and could only have specific adaptation to low yielding environments. Regarding stability parameters, the least values for W^2i,

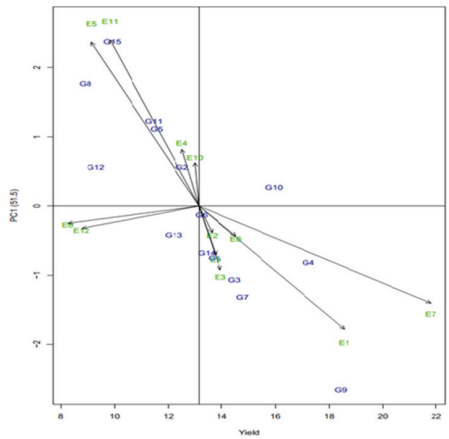

Figure 1: Biplot of mean seed yield (g/plant) for safflower and first IPCA axis (AMMI1) of the safflower genotypes grown in different environments

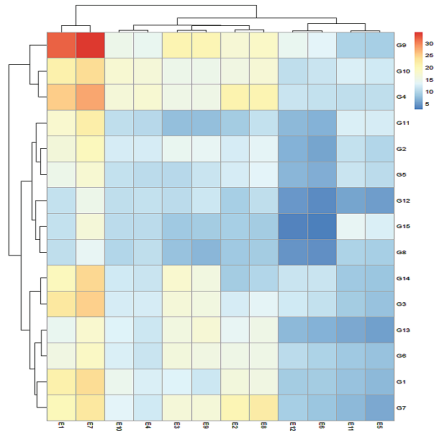

Figure 2: Heat map graph showing the clustering of the 15 safflower genotypes studied across 12 environments based on their yield performance (g/plant)

CV, D_{ij}, and ASV were denoted to G_{10} (Table 3). Comparison of ranks of yield means identified G_{10} and G_9 as the one with the least (4.09) and highest (11.36) mean yields, indicating the highest and least yield stability measures, respectively (Table 3).

3.3 NON-PARAMETRIC MEASUREMENTS

Table 4 reports the results of non-parametric stability statistics ($Si^{(1)}$ and $Si^{(2)}$) due to Thennarasu (1995) calculated based on the ranks of adjusted yield means. The non-parametric methods identified G12 with $Si^{(1)}$, $Si^{(2)}$, $NPi^{(2)}$, $NPi^{(3)}$, and $NPi^{(4)}$ ranks as the most stable genotype for seed yield among the studied genotypes, whereas G_9 with $Si^{(1)}$, $NPi^{(2)}$, $NPi^{(3)}$, and $NPi^{(4)}$ ranks was found to be the most unstable one but ranking the best for seed yield . With respect to $NPi^{(1)}$, the most stable and non-stable genotypes were G_2 and G_{14}, respectively. The highest (12.42) and the least (3) means of ranks belonged to G_{14} and G_{12} genotypes, respectively (Table 4). The significance tests for $Si^{(1)}$ and $Si^{(2)}$ were conducted by calculating the Z_i values (Nassar and Huehn, 1987) on the basis of the ranks of the adjusted data; then they were summed up over the genotypes (Table 4). No significant differences were found among the 15 genotypes grown in the 12 environments in regard to rank stability; this was because as both these statistics recorded values less than the critical value of $X^2_{0.05}$, df = 15 = 24.99. Among the individual Z values, none of the genotypes was shown to be considerably unstable, in comparison to others, with the exception of G_{15} with a $Zi^{(2)}$ greater than the critical value of $X^2_{0.05}$, 1 = 3.84).

To gain a better understanding of the rank means

Table 3: Mean safflower yield values, and ranks (numbers in parentheses) of 10 parametric stability measures for the 15 safflower genotypes tested across the 12 test environments to analyze G × E interactions for safflower seed yield

GEN	SY	CV (%)	bi	S^2di	Bi	Dij	R^2	W^2_i	σ^2i	Pi	ASV	Mean of ranks
G1	13.72 (6)	38.61 (11)	1.26(12)	-3.23 (4)	0.26(12)	2.35(4)	0.92 (14)	36.20(4)	3.24 (4)	23.8(6)	0.58(5)	7.45
G2	12.51 (9)	31.07 (5)	0.91(7)	-3.97(2)	-0.08(7)	1.61(2)	0.90(13)	17.30(2)	1.25 (2)	37.09 (9)	0.43(3)	5.54
G3	14.46 (5)	35.86 (7)	1.19(11)	-1.18(9)	0.19(11)	4.40(9)	0.85(10)	50.50(7)	4.74(7)	19.82(4)	0.88(10)	8.18
G4	17.22 (2)	33.73 (6)	1.36(14)	-1.54(8)	0.36(14)	4.04(8)	0.89(12)	64.02(8)	6.15(8)	7.34(2)	0.63(6)	8
G5	11.56 (11)	26.13 (2)	0.67(3)	-3.75(3)	-0.32(3)	1.83(3)	0.81(9)	36.58(5)	3.28 (5)	47.58(11)	0.85(9)	5.81
G6	13.24 (8)	26.34(3)	0.76(5)	-2.67(6)	-0.23(5)	2.92(6)	0.78(7)	38.67(6)	3.50 (6)	33.18(8)	0.43(2)	5.63
G7	14.77 (4)	41.16 (14)	1.3(13)	4.74(14)	0.30(13)	10.33(14)	0.74(6)	119.97(13)	12.02 (13)	20.93(5)	1.23(12)	11
G8	8.913 (15)	30.76 (4)	0.51(1)	-1.95(7)	-0.48(1)	3.63(7)	0.56(2)	78.95(10)	7.72(10)	78.62(15)	1.35(13)	7.72
G9	18.42 (1)	43.12 (15)	1.84(15)	3.19(11)	0.84(15)	8.79(11)	0.87(11)	215.40(15)	22.03(15)	3.38(1)	2.11(15)	11.36
G10	15.94 (3)	25.48 (1)	0.97(9)	-4.37(1)	-0.02(9)	1.21(1)	0.93(15)	12.24(1)	0.72 (1)	15.03(3)	0.22(1)	4.09
G11	11.45 (12)	39.92 (12)	0.86(6)	4.18(13)	-0.13(6)	9.77(13)	0.57(3)	101.08(12)	10.04(12)	48.49(12)	1.14(11)	10.18
G12	9.29 (14)	37.08 (9)	0.76(4)	-2.98(5)	-0.23(4)	2.60(5)	0.80(8)	35.80(3)	3.19(3)	70.22(13)	0.55(4)	6.54
G13	12.20 (10)	36.82 (8)	0.93(8)	1.23(10)	-0.06(8)	6.82(10)	0.69(5)	69.14(9)	6.69(9)	41.69(10)	0.72(8)	8.63
G14	13.43 (7)	37.40(10)	1.02(10)	3.43(12)	0.02(10)	9.0(12)	0.67(4)	90.40(11)	8.92(11)	30.33(7)	0.70(7)	9.81
G15	9.91 (13)	40.80 (13)	0.58(2)	6.30(15)	-0.41(2)	11.9(15)	0.33(1)	149.6(14)	15.13(14)	70.87(14)	1.82(14)	10.63

SY: Mean seed yield; CVi : Coefficient of variation (Francis & Kannenberg, 1978), bi: linear regression coefficient (Eberhart & Russell, 1966); S^2_{di}: deviation from regression (Eberhart & Russell, 1966); Bi: Regression coefficient (Perkins & Jinks, 1968); D_{ij}: Deviation from regression (Perkins & Jinks, 1968); R^2: Coefficient of determination; W^2i: Wricke's Ecovalance; σ^2i: Shukla (1972) stability variance; Pi : superiority index (Lin and Binns,1988), and ASV: AMMI stability value.

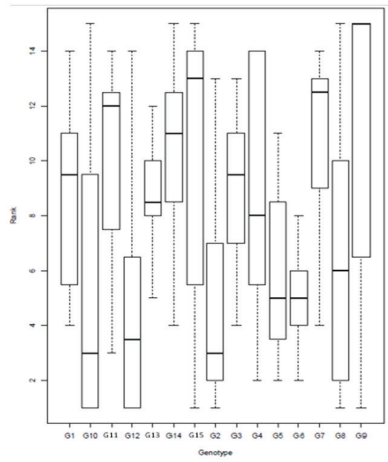

Figure 3: Box and whisker plot of rank means for the genotypes evaluated at the twelve locations in this study. In the case of each genotype, a box is the interquartile range, a heavy horizontal line indicates the median; on the other hand, fine horizontal lines show the minimum and maximum values with the exclusion of the outliers and extreme values.

Table 4: Mean yield values and ranks (numbers in parentheses) of the non-parametric stability parameters for the 15 genotypes over the 12 environments investigated.

Genotype	SY (g/plant)	Si (1)	Zi (1)	Si(2)	Zi (2)	$NPi^{(1)}$	$NPi^{(2)}$	$Npi^{(3)}$	$NPi^{(4)}$	Ranks mean
G1	13.72 (6)	0.76 (11)	0.39	10.18(10)	0	2.66 (10)	0.41(9)	0.41(7)	0.11(10)	9
G2	12.51 (9)	0.39(2)	0.97	4.82(3)	0.67	1.41(1)	0.15(3)	0.23(3)	0.04(3)	3.42
G3	14.46 (5)	0.76(12)	0.39	8.82(9)	0.04	2.41(9)	0.48(13)	0.48(10)	0.12(12)	10
G4	17.22 (2)	0.47(6)	0.40	5(5)	0.63	1.75(4)	0.7(14)	0.61(14)	0.15(14)	8.42
G5	11.56 (11)	0.62(8)	0	8.18(8)	0.09	1.83(7)	0.16(4)	0.27(5)	0.06(4)	6.71
G6	13.24 (8)	0.42(4)	0.73	4.27(2)	0.82	1.75(5)	0.26(6)	0.25(4)	0.07(5)	4.85
G7	14.77 (4)	0.68(9)	0.07	14.55(12)	0.46	2.83(13)	0.43(11)	0.57(13)	0.11(9)	10.14
G8	8.913 (15)	0.45(5)	0.52	8 (7)	0.11	1.83(8)	0.12(2)	0.2 (2)	0.03(2)	5.85
G9	18.42 (1)	0.52(7)	0.17	5.91(6)	0.42	1.75(6)	0.87(15)	0.84(15)	0.18 (15)	9.28
G10	15.94 (3)	0.40(3)	0.97	4.91(4)	0.65	1.66(3)	0.41(10)	0.50(11)	0.11(11)	6.42
G11	11.45 (12)	0.85(13)	1.04	19.64(14)	2.15	3.33(14)	0.31(7)	0.42(9)	0.08(8)	11
G12	9.29 (14)	0.24(1)	2.68	3(1)	1.22	1.41(2)	0.10(1)	0.12(1)	0.02(1)	3
G13	12.20 (10)	0.7(10)	0.13	12.64(11)	0.14	2.75(12)	0.32(8)	0.38(6)	0.08(7)	9.14
G14	13.43 (7)	1.02(15)	3.10	17.18(13)	1.18	3.58(15)	0.44(12)	0.52(12)	0.13(13)	12.42
G15	9.91 (13)	0.97(14)	2.38	25.09(15)	5.34	2.66(11)	0.20(5)	0.41(8)	0.08(6)	10.28
Test statistics		$E(Si^{(1)}) = 0.616$		$E(Si^{(2)}) = 10.14$						
		$Var(Si^{(1)}) = 0.052$		$Var(Si^{(2)}) = 41.75$						

for the genotypes evaluated, the box and whisker plot shown in Figure 3 was used. The median, upper, and lower quartiles and interquartile range for the mean of ranking (both parametric and non-parametric methods) are depicted for each genotype in this graph. The least rank mean (4.62) was observed for G_2 and G_{12} genotypes, showing no significant differences from G_6 and G_{10} (Figure 2). The highest rank mean (11.12) was denoted to G_9. The genotypes G_1, G_3, G_4, G_7, G_{11}, G_{13}, G_{14}, and G_{15} showed no significant differences in their rank means (Figure 2). The test of significant differences among the ranks of genotypes for all the stability parameters revealed an H value) 63.37) greater than the critical value of $X^2_{(005, 14)} = 23.68$, revealing the significant differences between the genotypes studied in regards to rank stability.

3.4 ASSOCIATION AMONG THE PARAMETRIC AND NON-PARAMETRIC MEASURES

Each of the parameters mentioned above produced a genotype order (Tables 3 and 4). Correlations between the ranks were then calculated and a PC analysis was performed based on this rank correlation (Table 5 and Figure 4). The Spearman's rank correlations between parametric and non-parametric measures are reported in Table 5. Clearly, the mean yields were significantly and negatively correlated with the stability measures of bi, R^2, D_{ij}, NPi $^{2)}$,$NPi^{(3)}$, and $NPi^{(4)}$ (Table 5), but significantly and positively correlated with Pi (r = 0.99**). The C.V parameter showed positive and significant correlations with s^2_{di}, σ^2_i, W_i, $Si^{(1)}$, $Si^{(2)}$, $NPi^{(1)}$, and ASVI (Table 5). The regression coefficient (bi) established a negative and significant correlation with Pi, but it conversely, positive and significant correlations with $NPi^{(2)}$, $NPi^{(3)}$, and $NPi^{(4)}$. The s^2_{di} established significant and positive correlations with σ^2_i, W_i, ASVI, Si $^{(1)}$, $Si^{(2)}$, $NPi^{(1)}$, and $NPi^{(4)}$. The superiority index (Pi) was negatively and significantly correlated with R^2, D_{ij}, $NPi^{(2)}$, $NPi^{(3)}$, and $NPi^{(4)}$ (Table 5). W_i

Table 5: Spearman rank correlations between mean of seed yield (S_Y), stability parameters and non-parametric measures for the safflower genotypes across different environments

	SY	CV	bi	s^2_{di}	R^2	$\sigma^2 i$	D_{ij}	$W^2 i$	Pi	Si(1)	Si(2)	$NPi^{(1)}$	$NPi^{(2)}$	$NPi^{(3)}$	$NPi^{(4)}$
CV	-0.12	1													
bi	-0.91**	0.38	1												
s^2_{di}	0.01	0.77**	0.15	1											
R^2	-0.65**	-0.31	0.54*	-0.70**	1										
$\sigma^2 i$	-0.03	0.72**	0.15	0.93**	-0.66**	1									
D_{ij}	-0.91**	0.38	1.00	0.15	0.54*	0.15	1								
W^2_i	-0.03	0.72**	0.15	0.93**	-0.66**	1.00	0.15	1							
Pi	0.99**	-0.08	-0.91**	0.07	-0.69**	0.03	-0.91**	0.03							
$Si^{(1)}$	0.00	0.54*	0.14	0.70**	-0.47*	0.60**	0.14	0.60**	0.04						
$Si^{(2)}$	0.13	0.57*	0.03	0.74**	-0.58*	0.66**	0.03	0.66**	0.19	0.93**					
$NPi^{(1)}$	0.11	0.55*	0.08	0.75**	-0.63**	0.66**	0.08	0.66**	0.15	0.90**	0.93**				
$NPi^{(2)}$	-0.89**	0.34	0.89**	0.35	0.31	0.36	0.89**	0.36	-0.88**	0.38	0.23	0.26			
$NPi^{(3)}$	-0.81**	0.44	0.80**	0.48*	0.18	0.50	0.80**	0.50	-0.77**	0.42	0.36	0.32	0.93**		
$NPi^{(4)}$	-0.85**	0.34	0.85**	0.32	0.33	0.34	0.85**	0.34	-0.84**	0.43	0.26	0.27	0.98**	0.93**	
ASVI	0.12	0.61**	0.00	0.73**	-0.57*	0.86**	0.00	0.86**	0.16	0.52*	0.61*	0.54	0.17	0.31	0.15

* and ** significantly correlated at 0.05 and 0.01, respectively.

Figure 4: Biplot of the first two principal components for the studied genotypes and their ranks in terms of different parametric and non-parametric parameters.

had positive and significant correlations with Pi, Si $^{(1)}$, Si $^{(2)}$, NPi$^{(1)}$, and ASVI, indicating that these measures led to similar results. Figure 4 illustrates a biplot of the first two principal components of stability ranks (PCA_1 vs. PCA_2), which accounted for 80.95 % of the variance in the original variables. Simultaneous examination of both axes discloses the presence of the following three groups: Group 1 consisting of NPi$^{(2)}$, NPi$^{(3)}$, NPi$^{(4)}$, and Bi (Figure 4); Group 2 composed of CVi, b_i, S^2_{di}, D_{ij}, W^2_i, σ^2_i, ASV, Si$^{(1)}$, Si$^{(2)}$, and NPi$^{(1)}$; and Group 3 comprising seed yield and Pi (Figure 4). R^2 is located in a separate section of the biplot.

4 DISCUSSION

The interaction of genotype and environment complicates the identification of superior genotypes containing better stability across a wide range of environments (Annicchiarico, 2002). Hence, GE interactions need to be modelled and adequately interpreted for different plant species (Abdulahi et al., 2009). Stability and wide adaptation are of vital importance for semi-arid regions of highly varying characteristics. Selection of suitable planting date for safflower is a first management decision aimed at seed yield stability in safflower under arid conditions (Dajue & Mundel, 1996; Caliskan & Caliskan, 2018). Evaluation of safflower seed yield under different moisture conditions, multi-sowing dates, and over different years might revealed hidden trends in genotype × environment interactions, thereby complicating the decisions related to selecting or recommending the more appropriate lines of the highest stability (Pourdad & Mohammadi, 2008).

In this study, the study of genotype × environment interaction revealed the differential response of safflower genotypes to environmental conditions and showed the possibility of selecting for stable genotypes. A large proportion of the square sums for environment in our GE analysis indicated diversity among the environments studied. This finding is in agreement with those previously reported on safflower (Abdulahi et al., 2009; Moghaddam & Pourdad, 2009; Ebrahimi et al., 2016). The biplot constructed with seed yield and $IPCA_1$ for genotype and environment on the X and Y axes, respectively, can be interpreted by comparing the *i* scores of $IPCA_1$ for each genotype and environment (Gauch & Zobel, 1996). The lowest $IPCA_1$ scores of the genotypes G_6 and G_{10} indicate that they had the lowest interaction with environment (Figure 1). By definition, stable genotypes are the ones with the minimal variance for yield in a wide range of environments (Lin & Binns, 1988). So, this biological concept of stability cannot be helpful to the vast majority of plant breeders as they are commonly looking genotypes having high mean yields and a good potential in order to ensure r better environmental conditions (i.e., the concept of dynamic stability) (Becker & Leon, 1988). According to Eberhart and Russell (1966), a stable variety will be one with $b_i = 1$ and = 0. Genotypes with bi > 1 could be better adapted to favorable growing conditions; on the other hand, it could be argued that those with $b_i < 1$ would be adaptable to the environmental conditions that are not favorable; finally, those who regression coefficients equals unity would show an average adaptation to all environmental conditions. With this regression coefficient (b_i), G_9 followed by G_4, G_7 and G_3 would adapt to favorable environments (adequate moisture and early sowing date) (Figure 2 and Table 3). Thus, genotypes G_{10} and G_2 were considered as the most stable because of their least values of S^2_{di} and their b regression coefficients close to unity (Table 3). In agreement with Pinthus (1973), the genotypes G_9 and G_{15} with higher coefficients of determination were considered to be unstable while G_{10} followed by G_5 and G_6 that recorded the lowest coefficients of determination were categorized as stable (Table 3).

Regarding other parametric stability parameters, G_{10} recorded the least values for W^2_i, CV, D_{ij}, and ASV (Table 3). Hence, this genotype could be considered as the one with the highest stability for seed yield while G_9 and G_{15} were instable due to their higher values of W^2i, a parameter that had the greatest contribution to the GE interaction. Regarding the superiority index, the highest P_i value was recorded by the genotypes with the largest yield difference from that of the reference genotype. It must be noted that a low value of P_i indicates the relatively high stability of a cultivar. The genotype G_9 followed by G_4 and G_{10} were, therefore, considered as stable ones when judged on the basis of this index (Table 3). In the present research, there was a highly positive and significant correlation between P_i and mean yield (Table 5), revealing that the P_i parameter could be helpful to identify stable genotypes having high yields. NP $(i)^1$, NP $(i)^2$, and NP $(i)^3$ were highly correlated, indicating that these four parameters could be used interchangeably in the GE interaction study of safflower. Genotypes with fewer changes in ranks are also considered to be more stable (Becker & Leon, 1988). In non-parametric methods, Si$^{(1)}$ estimates are based on all the possible pairwise rank differences across environments for each genotype, whereas Si$^{(2)}$ ones are based on variances of ranks for each genotype across environments (Nassar & Huehn, 1987). The two statistics of Si$^{(1)}$ and Si$^{(2)}$ showed only slight similarities in ranking the genotypes (Table 4). Some stability statistics including Si$^{(1)}$, Si$^{(2)}$, and NPi$^{(1)}$ indicate the static concepts of stability; so, they cannot be correlated with mean yield (Huehn, 1996). This find-

ing agrees well with those reported in Mohebodini et al. (2006). The positive and significant correlation between P_i and SY (Table 5) demonstrated that lower values of Pi could be used for selection of high yield genotypes in safflower, as also reported for lentils (Mohebodini et al., 2006). While different stability statistics indicate a considerable, average, or minimal stability performance, the stability values have been found not to yield direct information contributing to making firm conclusions (Mohebodini et al., 2006). This is the reason why both parametric and non-parametric methods have been simultaneously used to analyze yield stability in different plant species such as durum wheat (Mohammadi et al., 2007), tallfescue (Dehghani et al,. 2016), lentil (Sabaghnia et al., 2006), barley (Khalili and Pour-Aboughadareh, 2016), and *Cicer arietinum* L. (Farshadfar et al., 2012). In the AMMI method used for safflower (Ebrahimi et al., 2016) and the parametric methods developed (Omidi Tabrizi, 2006; Pourdad & Mohammadi, 2008), use has been made of seed yield stability analysis. Also, oil yield stability has been evaluated in safflower genotypes across different geographical regions (Ebrahimi et al., 2016). However, simultaneous analyses of these two has not yet been reported. Given the importance of proper sowing date under drought stress conditions, the present study was conducted to show how early sowing dates (15 April) could lead to the highest yield stability under non-drought conditions.

5 CONCLUSION

The present study showed that useful exploitation of GE interaction effects toward more precise genotype selection with respect to yield, and performance stability. Based on the results obtained in this study, G_6 as a stable genotype recording an average seed yield may be recommended for regions where growing conditions are unfavorable or undergo high fluctuations. The AMMII biplot and parametric measures indicated that, based on a dynamic definition of stability, G_9 (from Mexico) followed by G_4 (from Iran) are favorable selections for yield. The results obtained from six non-parametric measures identified G_{12} followed by G_2 as the most stable genotypes. Hence, these genotypes can be used for improved safflower adaptation to the environments under study. Finally, based on a static concept of stability, the genotype G_{10} was recognized as the superior one offering a good combination of yield and stability for cultivation in both drought and non-drought environments. It is, therefore, suggested that both seed yield and stability methods should be exploited simultaneously to define the useful effects of GE interaction for selecting the best safflower genotypic selection. Further studies are, however, required to evaluate seed yield stability with different seed densities and in different geographical regions with diverse climates.

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Correlation and path coefficient analysis among agro-morphological and biochemical traits of okra [*Abelmoschus esculentus* (L.) Moench] genotypes in Ethiopia

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Correlation and path coefficient analysis among agro-morphological and biochemical traits of okra [*Abelmoschus esculentus* (L.) Moench] genotypes in Ethiopia

Abstract: Thirty six okra genotypes were evaluated for different agro-morphological and biochemical traits at Melkassa Agricultural Research Center, Ethiopia during 2018 main season using 6 x 6 simple lattice design. The objectives were to assess the correlation of agro-morphological and biochemical traits with fruit yield and to partition the correlation in to direct and indirect effects through path analysis. The genotypic correlation was positive and significant for fruit yield per hectare with stem diameter, plant height, leaf length, leaf width, peduncle length, fruit length, fresh fruit mass, hundred seed mass, seed yield per hectare and ash content. The phenotypic correlation was positive and significant for fruit yield per hectare with stem diameter, plant height, number of branches, leaf length, leaf width, peduncle length, fruit length, fresh fruit mass, number of fruits per plant, hundred seed mass, seed yield per hectare, ash, fat and protein content. Path coefficient analysis indicated fresh fruit mass and seed yield per hectare had positive direct effects on fruit yield per hectare at phenotypic and genotypic levels. These traits also exerted high to low positive indirect effects through other traits on fruit yield at genotypic and phenotypic level. In conclusion, this study showed the presence of association of traits with fruit yield indicating that the prime importance of these traits while selecting higher yield okra genotypes.

Key words: correlation; genotypic correlation; path analysis; phenotypic correlation

Korelacija in multipla regresijska analiza med agro-morfološkimi in biokemijskimi lastnostmi genotipov okre [*Abelmoschus esculentus* (L.) Moench] v Etiopiji

Izvleček: Šestintrideset genotipov okre je bilo ovrednoteno na osnovi agro-morfoloških in biokemijskih lastnosti v Melkassa Agricultural Research Center, Etiopija, v glavni rastni sezoni 2018 v nepopolnem bločnem poskusu. Predmet raziskave je bil oceniti korelacijo med agro-morfološkimi in biokemijskimi lastnostmi okre s pridelkom plodov in razdeliti korelacijo na neposredne in posredne učinke z multiplo regresijsko analizo. Genotipska korelacija je bila pozitivna in značilna za pridelek plodov na hektar, s premerom stebla, višino rastlin, dolžino in širino lista, dolžino plodnega peclja, dolžino plodu, svežo maso plodu, maso stotih semen, pridelkom semen na hektar in vsebnostjo pepela. Fenotipska korelacija je bila pozitivna in značilna za pridelek plodov na hektar, s premerom stebla, višino rastlin, številom stranskih poganjkov, dolžino in širino listov, dolžino plodnega peclja, dolžino plodu, svežo maso plodu, številom plodov na rastlino, maso stotih semen, pridelkom semen na hektar, vsebnostjo pepela, maščob in beljakovin. Multipla regresijska analiza je pokazala, da imata sveža masa plodov in pridelek semen na hektar pozitivni neposredni učinek na pridelek plodov na fenotipski in genotipski ravni. Te lastnosti sta izkazali tudi velike do majhne pozitivne posredne učinke skozi druge lastnosti na pridelek plodov na genotipski in fenotipski ravni. Na osnovi te raziskave lahko zaključimo, da obstaja povezava med lastnostmi in pridelkom plodov, ki so najvažnejše pri odbiranju genotipov okre z velikim pridelkom.

Ključne besede: korelacija; genotipska korelacija; multipla regresijska analiza; fenotipska korelacija

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1 INTRODUCTION

Okra [*Abelmoschus esculentus* (L.) Moench] is a member of the family Malvaceae. It originated somewhere around Ethiopia and was cultivated by the ancient Egyptians by the 12th century BC. Its cultivation spread throughout the Middle East and North Africa (Lamont, 1999). The Nile Basin seems to have been the route by which this crop spread through North Africa, the Eastern Mediterranean, Asia, and to India. Okra reached the new world by the way of Brazil and Dutch Guinea. African slaves brought okra to North America by way of New Orleans (Bisht et al., 1995). The crop is grown in many parts of the world, especially in tropical and subtropical countries (Kumar et al., 2010). India, Nigeria, Sudan and Mali lead the production of Okra in the world (FAOSTAT, 2017).

Correlation coefficient is numerical measure which is used to find out the degree of relationship between two or more traits. The intensity of correlation between different variable is represented by r. The correlation coefficient, r ranges from -1 to 1. If r is -1, there is 100 % correlation between two variables, but both vary in opposite direction (negative correlation). On the other hand, if r is +1, it indicates perfect correlation (100 %) where both traits vary in the same direction (positive correlation). If r = 0 there is no correlation at all between two variables, that is the two variables are independent of each other or no correlation indicates that genes concerned are located far apart on the same chromosome or they are located on different chromosomes. Also, in plant genetics and breeding studies, correlated traits are key importance because of genetic causes of correlations through pleiotropic action or developmental interactions of genes and changes brought about by a natural or artificial selection (Falconer & Mackay, 1996; Sharma, 1998).

Path coefficient analysis partitions the correlation in to direct and indirect effects and thus may be useful in choosing the characters that have direct and indirect effects on yield (Balai et al., 2014) and also simultaneously captures the effects of intricate relationship among various traits under investigation. Therefore, this study was aimed to analyse and determine the traits having greater association with fruit yield of okra and path analysis for different agro morphological and biochemical traits in okra.

2 MATERIAL AND METHODS

2.1 DESCRIPTION OF THE STUDY SITE

The field study was conducted at Melkassa Agriculture Research Center, Ethiopia in 2018 main cropping season (rainy season). Melkassa is located $8^0$24'59.20" N latitude and $39^0$19'15.19" E longitude with an altitude of 1548 meter above sea level (MARC, 2008). The biochemical contents of the seeds were determined at Ethiopian Biodiversity Institute food and nutrition laboratory (total ash and crude fat), Debrezeyit Agriculture Research Center (crude fibre) and Melkassa Agriculture Research Center (total protein).

2.2 EXPERIMENTAL MATERIALS AND DESIGN

Total of 36 okra genotypes was used from six geographic regions (South Western, Western, North Western and Northern Ethiopia (24 landrace accession collected by Ethiopian Biodiversity Institute) one released variety and two genotypes from Humera Agriculture Research Center), nine commercial varieties (eight from India and one from USA) were included. The genotypes were planted 6 × 6 simple lattice design with two replications.

2.3 DATA COLLECTION

Data were collected for phenology traits (days to 50 % emergence, days to 50 % flowering and days to 90 % maturity), growth and yield related traits (plant height, stem diameter, number of primary branches per stem, number of internodes, internodes length, leaf length, leaf width, peduncle length, fruit length, average fruit mass, number of tender fruits per plant, number of ridges on fruit, fruit yield per hectare, number of seeds per capsule, hundred seed mass and seed yield per hectare) and biochemical content of the seed determined (total ash, total fat, crude fibre and total protein) following the procedure of Association of Official Analytical Chemists (AOAC, 2000).

2.4 DATA ANALYSIS

2.4.1 Phenotypic and genotypic correlation coefficient analysis

Phenotypic (rp) and genotypic (rg) correlations between two traits were estimated using the formula suggested by Johnson et al. (1955) and Singh & Chaudhury (1985).

$$rp = \frac{\sigma^2 Pxy}{\sqrt{(\sigma^2 px)\,(\sigma^2 py)}}$$

$$rg = \frac{\sigma^2 G\ xy}{\sqrt{(\sigma^2 gx)\ (\sigma^2 gy)}}$$

Where,

rp = Phenotypic correlation coefficient

rg = Genotypic correlation coefficient

$\sigma^2 Pxy$ = Phenotypic covariance between variables x and y

$\sigma^2 G\ xy$ = Genotypic covariance between variables x and y

$\sigma^2 px$ = Phenotypic variance of variable x

$\sigma^2 gx$ = Genotypic variance of variable x

$\sigma^2 py$ = Phenotypic variance of variable y

$\sigma^2 gy$= Genotypic variance of variable y

The coefficient of correlation at phenotypic level was tested for significance by comparing the values of correlation coefficient with tabulated r value at g-2 degree of freedom, where 'g' is number of genotypes. However, the coefficient of correlations at genotypic level was tested for the significance using the formula described by Robertson (1959).

$$t = \frac{(rgxy)}{SErgxy}$$

The calculated "t" value was compared with the tabulated ''t" value at g-2 degree of freedom at 5 % level of significance. Where, g = number of genotypes, rgxy = genotypic correlation coefficient and SErgxy = standard error of genotypic correlation coefficient between character x and y which will be calculated as:

$$SErgxy = \sqrt{\frac{(1 - r^2)^2}{2H^2x\ H^2y}}$$

Where: SE_{rgxy} = standard error of genotypic correlation coefficient between character x and y, H^2x = Heritability value of character x and H^2y = heritability value of character y.

2.4.2 Path Coefficient Analysis

Based on genotypic and phenotypic correlations, path coefficient analysis which refers to the estimation of direct and indirect effects of the fruit yield attributing characters (independent character) on fruit yield (dependent character) was calculated based on the method used by Dewey & Lu (1959) as follows:

$$rij = pij + \Sigma rikpkj$$

Where, rij = mutual association between the independent character (i) and dependent character (j) as measured by the genotypic and phenotypic correlation coefficients. Pij = direct effect of the independent character (i) on the dependent variable (j) as measured by the genotypic path coefficients, and Σrikpkj = Summation of components of indirect effect of a given independent character (i) on a given dependent character (j) via all other independent characters (k).

The residual effect, which determines how the best the causal factors account for the variability of the dependent factor yield, were computed using the formula:

$$1 = P^2R + \Sigma p\ ij\ rij$$

Where, p^2R is the residual effect and p ijrij is the product of direct effect of any variable and its correlation coefficient with yield. SAS 9.0 (SAS, 2004) was used for both genotypic and phenotypic correlation and path analysis.

3 RESULT AND DISCUSSIONS

3.1 PHENOTYPIC AND GENOTYPIC CORRELATION COEFFICIENT

3.1.1 Phenotypic and genotypic correlations of fruit yield with other traits

Fruit yield per hectare showed positive and significant correlations with stem diameter, plant height, leaf length, leaf width, peduncle length, fruit length, fresh fruit mass, hundred seed mass, seed yield per hectare and total ash both at the genotypic and phenotypic levels. Fruit yield per hectare had positive and significant phenotypic correlations with number of branches, number of internodes, number of fruit ridge, number of fruit per plant, total fat and protein content and these traits had positive but nonsignificant genotypic correlations with fruit yield per hectare (Table 1).The presence of significant correlation of these traits with fruit yield per hectare both at genotypic and phenotypic levels indicated prime importance of these traits in selection program to identify okra genotypes with high fruit and seed yield. This result is in agreement with Muluken et al. (2015), Mihretu et al. (2014), Saitwal et al. (2011) and Dhankhar & Dhankhar (2002).

3.1.2 Correlation coefficients among other traits

Day to 50 % flowering with days to maturity had significant correlation at phenotypic and genotypic levels. Days to 50 % emergence had a significant association with days to 50 % flowering at genotypic levels, but days to 50 % emergence had positive and nonsignificant correlations with day to 50 % flowering at phenotypic level and with days to maturity both at phenotypic and genotypic levels. Days to 50 % flowering and days to maturity showed positive and significant associations with stem diameter and number of internodes at phenotypic and genotypic levels. Days to 50 % emergence and days to maturity had positive and significant correlations with peduncle length both at phenotypic and genotypic levels (Table 1). This suggested that the selection of genotypes for phenology traits (days to seedlings emergence, flowering and maturity) may not affect the selection of genotypes for plant growth performances. Muluken et al. (2015) also indicated that a positive correlation between traits allows the selection of genotypes simultaneously for the correlated traits.

Fozia (2018) reported that the phenology traits (days to emergence, days to 50 % flowering and days to maturity) showed significant association among them both at genotypic and phenotypic levels except the genotypic correlation between days to emergence and days to maturity was positive but nonsignificant. She also observed genotypic correlation of phenology traits with growth traits of okra was nonsignificant except the genotypic correlation of days to emergence with number of internodes per plant and days to maturity with stem diameter showed negative and positive significant genotypic association, respectively.

The genotypic and phenotypic correlations among stem diameter, plant height and number of branches were positive and significant. These traits also had positive and significant correlations at genotypic and phenotypic levels with other growth traits viz., number of internodes, internode length, leaf length, leaf width and peduncle length except stem diameter with internode length at genotypic and phenotypic levels and plant height with peduncle length at genotypic and phenotypic level showed nonsignificant associations. Leaf length with leaf width; leaf length and leaf width with peduncle length had positive and significant correlations at genotypic and phenotypic levels. Number of internodes with leaf length, leaf width and peduncle length; internode length with leaf length showed positive and significant phenotypic correlations.

Stem diameter and plant height with hundred seed mass and seed yield per hectare; stem diameter with fresh fruit mass; plant height with number of fruits per plant had positive and significant genotypic and phenotypic correlations. In addition, plant height and fresh fruit mass showed positive and significant phenotypic correlation. Leaf width and peduncle length with fruit length, fresh fruit mass, number of fruits per plant, hundred seed mass and seed yield per hectare had positive and significant genotypic and phenotypic correlations. Number of internodes and internode length with seed yield per hectare showed positive and significant associations at genotypic and phenotypic levels. The results indicated that the selection of genotypes for one of or more of growth traits particularly stem diameter and plant height is a simultaneous selection for other growth traits and most of the fruit yield components. Ehab et al. (2013) also, indicated that the selection on the basis of any of the significantly positive inter-related characters was expected to give a desired correlated response in other characters.

Fruit length with fresh fruit mass and hundred seed mass had positive and significant correlation at genotypic and phenotypic level. However, fruit length had positive and nonsignificant correlation with seed per capsule at genotypic and phenotypic level. Fresh fruit mass had positive and significant correlation with number of fruit ridge at genotypic and phenotypic level. Number of fruit ridge with number of fruits per plant had negative and significant correlation at phenotypic level. Therefore, it is important to give attention to number of ridges in the process of the selection of okra genotypes for high number of fruits per plant. Akinyele & Osekita, (2006); Nwangburuka et al. (2012) and Ahiakpa et al. (2012) also suggested that negative association of traits was difficult or practically impossible to improve through simultaneous selection of those traits. The sign of genetic correlations between two characters can either facilitate or impede selection progress and $r = 0$ or nonsignificant carries the implication of no correlation between the two characters (Singh & Chaudhary, 1977; Falconer & Mackay, 1996; Sharma, 1998).

The other important yield of okra is its seed yield which can be used for different purpose. The seed can be used as a substitute for coffee and the oil content of the seed is quite high at about 40 % (Anwar et al., 2011). The correlation of seed yield had positive and significant correlation coefficient with stem diameter, plant height, number of internods, internod length, leaf length, leaf width, peduncle length, number of fruits per plant and fruit yield per hectare at genotypic and phenotypic level. It had also positive and nonsignificant correlation with fruit length both at genotypic and phenotypic level (Table 1). Fozia (2018) reported that seed yield per plant had positive and significant correlation with plant height, stem diameter, branch, number of internodes, internod

Table 1: Genotypic (above diagonal) and phenotypic (below diagonal) correlation coefficient among fruit and fruit related traits of 36 Okra genotypes evaluated at Melkassa 2018

Traits	EM	FPF	DM	SD	PH	BR	NOI	IL	LL	LW	PL
EM		0.340*	0.3221	0.105	-0.103	0.1316	-0.013	-0.006	0.198	0.220	0.360*
FPF	0.227		0.965**	0.341*	0.262	0.2417	0.359*	0.124	-0.031	-0.119	0.183
DM	0.171	0.943**		0.418*	0.286	0.2349	0.395*	0.206	0.016	-0.033	0.341*
SD	0.065	0.239*	0.248*		0.611**	0.5580**	0.652**	0.248	0.494**	0.551**	0.659**
PH	-0.062	0.189	0.175	0.506**		0.577**	0.528**	0.467**	0.369*	0.308	0.271
BR	0.092	0.223	0.209	0.409**	0.466**		0.478**	0.152	0.525**	0.366*	0.254
NOI	0.037	0.259*	0.224	0.636**	0.494**	0.4008**		0.002	0.255	0.183	0.322
IL	-0.021	0.089	0.144	0.155	0.435**	0.1473	-0.034		0.328	0.293	0.311
LL	0.153	-0.032	-0.025	0.452**	0.374**	0.4041**	0.338**	0.298*		0.811**	0.462**
LW	0.229	-0.081	-0.052	0.527**	0.291*	0.2463*	0.233*	0.221	0.761**		0.619**
PL	0.236*	0.128	0.261*	0.526**	0.227	0.2400*	0.294*	0.226	0.372**	0.466**	
FL	0.227	-0.123	-0.072	0.187	0.009	-0.1322	-0.152	0.190	0.286*	0.389**	0.377**
NFR	0.212	0.213	0.174	0.191	0.015	-0.1104	0.005	-0.136	0.036	0.182	0.132
FFM	0.200	0.132	0.099	0.298*	0.284*	0.2114	0.171	0.022	0.436**	0.469**	0.325**
NFPP	0.135	-0.049	0.037	0.229	0.354**	0.2038	0.232	0.495**	0.497**	0.387**	0.347**
FYPH	0.209	0.171	0.168	0.389**	0.343**	0.2364*	0.308**	0.144	0.548**	0.493**	0.358**
SPC	0.160	-0.036	-0.046	0.239*	0.223	0.0692	0.046	0.103	0.199	0.349**	0.264*
HSM	0.071	0.158	0.186	0.414**	0.396**	0.0850	0.116	0.249*	0.343**	0.467**	0.391**
SYPH	0.116	-0.074	0.009	0.472**	0.402**	0.1378	0.377**	0.396**	0.525**	0.599**	0.472**
ASH	-0.024	-0.096	-0.109	0.272*	0.176	0.1638	-0.081	0.173	0.259*	0.294*	0.293*
FAT	0.223	-0.061	-0.031	0.107	0.099	0.0934	-0.061	0.187	0.329**	0.333**	0.224
FIBER	0.083	-0.001	-0.081	-0.099	-0.080	0.0814	0.035	-0.119	0.028	0.066	-0.204
PROTEIN	0.097	0.126	0.099	0.087	-0.024	-0.0192	0.063	-0.055	0.139	0.212*	0.127

**, *, significant at $p < 0.01$, $p < 0.05$ respectively. EM = days to 50 % emergence, FPF = days to 50 % flowering, DM = days to maturity,SD = stem diameter, PH = plant height, BR = number of branches, NOI = number of internodes, IL = internode length, LL = leaf length, LW = leaf width, PL = peduncle length, FL = fruit length, NFR = number of fruit ridge, FFM = fresh fruit mass, NFPP = number of fruit per plant, FYPH = fruit yield per hectare, SPC = seed per capsule, HSM = hundred seed mass, SYPH = seed yield per hectare, total ash, total fat, crude fibre, protein content

Table 1: Continue

Traits	FL	NFR	FFM	NFPP	FYPH	SPC	HSM	SYPH	ASH	FAT	FIBER	PROTEIN
EM	0.225	0.269	0.195	0.135	0.176	0.172	0.086	0.092	0.014	0.141	0.091	0.119
FPF	-0.118	0.270	0.196	-0.084	0.273	-0.066	0.197	-0.098	-0.087	-0.041	-0.004	0.136
DM	-0.032	0.233	0.159	0.056	0.287	-0.085	0.247	0.029	-0.103	0.064	-0.084	0.112
SD	0.185	0.172	0.465**	0.306	0.548**	0.183	0.525**	0.483**	0.287	0.108	-0.129	0.104
PH	-0.119	-0.029	0.278	0.403*	0.346*	0.176	0.377*	0.401*	0.195	-0.006	-0.100	-0.026
BR	-0.167	-0.084	0.238	0.209	0.279	0.092	0.111	0.169	0.164	0.131	0.086	-0.021
NOI	-0.265	-0.016	0.201	0.259	0.327^{ns}	-0.056	0.103	0.376*	-0.116	-0.163	0.066	0.076
IL	0.229	-0.149	0.017	0.534**	0.154	0.132	0.259	0.455**	0.176	0.209	-0.154	-0.059
LL	0.324	-0.006	0.514**	0.589**	0.631**	0.067	0.314	0.514**	0.295	0.359*	0.037	0.173
LW	0.424**	0.119	0.567**	0.478**	0.616**	0.287	0.482**	0.616**	0.337*	0.412*	0.065	0.260
PL	0.406*	0.121	0.379*	0.444**	0.457**	0.272	0.496**	0.560**	0.316	0.258	-0.204	0.138
FL		0.102	0.368*	0.228	0.384*	0.131	0.383*	0.176	0.412*	0.287	0.005	0.109
NFR	0.131		0.389*	-0.294	0.283	0.311	-0.030	-0.202	0.224	0.018	-0.131	0.097
FFM	0.349**	0.377**		0.045	0.932**	0.241	0.446**	0.263	0.553**	0.199	0.172	0.298
NFPP	0.160	-0.278*	0.035		0.299	-0.085	0.102	0.676**	0.011	0.256	-0.156	0.079
FYPH	0.329**	0.266*	0.876**	0.312**		0.123	0.401*	0.434**	0.424**	0.274	0.147	0.298
SPC	0.184	0.314**	0.249*	-0.072	0.179		0.155	0.327	0.127	0.167	-0.041	0.060
HSM	0.392**	0.011	0.442**	0.099	0.399**	0.218		0.299	0.408*	0.152	-0.112	0.025
SYPH	0.194	-0.093	0.262*	0.623**	0.458**	0.390**	0.335**		-0.011	0.322	-0.066	0.222
ASH	0.367**	0.224	0.502**	0.002	0.355**	0.121	0.347**	-0.007		-0.007	-0.145	-0.123
FAT	0.344**	0.079	0.196	0.224	0.252*	0.190	0.156	0.275*	0.015		0.1465	0.140
FIBER	0.009	-0.118	0.155	-0.127	0.134	-0.046	-0.088	-0.051	-0.139	0.118		0.331*
PROTEIN	0.099	0.087	0.276	0.074	0.254*	0.053	0.026	0.202	-0.119	0.117	0.327**	

**, *, significant at $p < 0.01$ and $p < 0.05$, respectively. EM = days to 50 % emergence, FPF = days to 50 % flowering, DM = days to maturity SD = stem diameter PH = plant height, BR = number of branches, NOI = number of internode, IL = internode length, LL = leaf length, LW = leaf width, PL = peduncle length, FL = fruit length, NFR = number of fruit ridge, FFM = fresh fruit mass , NFPP = number of fruit per plant, FYPH = fruit yield per hectare, SPC = seed per capsule, HSM = hundred seed mass, SYPH = seed yield per hectare, total ash, total fat, crude fiber, protein content

length, number of fruit ridge, number of seed per capsule and hundred seed mass.

The genotypic and phenotypic correlation coefficient showed crude fibre with total protein was the only positive and significant correlation among biochemical traits at genotypic and phenotypic level. However, ash with leaf width, fruit length, fresh fruit mass and hundred seed mass; crude fat with leaf length and width had positive and significant correlation at genotypic and phenotypic level. Similar result had reported by Prasath et al. (2017) crude fibre content exhibited positive and significant correlation with protein content.

3.2 PATH ANALYSIS

3.2.1 Genotypic path coefficient analysis of fruit yield with other traits

Stem diameter, leaf length, leaf width, peduncle length, fruit fresh mass, seed yield per hectare, and ash content had positive and highly significant ($p < 0.01$) correlation, and plant height, fruit length and hundred seed mass had positive and significant ($p < 0.05$) correlation with fruit yield per hectare. Majority of these traits also exerted positive direct effect on fruit yield per hectare. Lenka & Mishra (1973) rated the direct and indirect effects into negligible (0.00-0.09), low (0.10-0.19), moderate (0.20-0.29), high (0.30-1.00) and very high (> 1.00). Accordingly, fresh fruit mass (0.901) had positive and high direct effect on yield while leaf length (0.219) and seed yield per hectare (0.146) exerted positive moderate and low direct effect on fruit yield per hectare, respectively.

In addition, fresh fruit mass had exerted high positive indirect effect on fruit yield via stem diameter, leaf length, leaf width, peduncle length, fruit length, hundred seed mass and ash content. Fresh fruit mass also had moderate positive indirect effect on fruit yield via seed yield per hectare and plant height. This indicated that the positive and significant genotypic correlations of traits with fruit yield were due to the direct effects of the traits on yield. Therefore, it is possible to suggest that the traits could be used for indirect selection of genotypes for high fruit yield. Prasath et al. (2017), Rambabu et al. (2019) the direct effect of traits on fruit yield per hectare favours yield improvement through selection. These suggested that indirect selection based on these traits will be effective in yield improvement.

Fruit length (0.088), stem diameter (0.078), peduncle length (0.032), plant height (0.019) had positive and negligible direct effects on fruit yield. Whereas, leaf width (-0.197) and ash content (-0.116) exerted low negative direct effects on fruit yield. Hundred seeds mass (-0.069) negative but negligible direct effects on fruit yield. If the trait has positive correlation and the direct effect of the trait is negative or negligible, the positive correlation of the trait is because of the indirect effects through other traits. In such situation, the indirect causal factors/traits are to be considered simultaneously for selection (Singh & Chaudhary, 1977).

The residual effect in the present genotypic path study was 0.058 (Table 2) showing that 94.19 % of the variability in the fruit yield per hectare was explained by the component factors. The remaining 5.8 % is explained by other traits not considered in this study.

3.2.2 Phenotypic path coefficient analysis of fruit yield with other traits

The phenotypic correlation coefficient computed between fruit yield per hectare and other traits showed the presence of significant association with stem diameter, plant height, namber of branches, number of internods, leaf length, peduncle length, fruit length, fresh fruit mass, number of fruit per plant, hundred seed mass, seed yield per hectare, ash, fat and protein content. This implies the importance of partitioning the correlation coefficients into direct and indirect effects on fruit yield per hectare, so as to determine the selection criteria for fruit yield improvement in okra.

Fresh fruit mass had high (0.911) positive direct effect on fruit yield followed by number of fruits per plant (0.257) which exerted a moderate direct effect on fruit yield per hectare. Stem diameter (0.134), leaf length (0.124) and seed yield per hectare (0.106) had low direct effect on fruit yield per hectare. Number of internods, fruit length, number of fruit ridge, hundred seed mass and fat content had positive and negligible direct effect on fruit yield per hectare. On the other hand, plant height, number of branches, peduncle length, ash and protein content had exerted negative and negligible direct effect on fruit yield per hectare (Table 3). Leaf width had negative and low direct effect on fruit yield per hectare.

On other hand, fresh fruit mass had exerted high and positive indirect effect on fruit yield via leaf length, leaf width, fruit length, number of fruit ridge, hundred seed mass and ash content. It had also exerted moderate and positive indirect effect on fruit yield through stem diameter, plant height, peduncle length, seed yield per hectare and protein content. In this study, the results of phenotypic path coefficient analysis showed that fresh fruit mass followed by number of fruits per plant, seed yield per hectare, stem diameter and leaf width could be used as selection criteria for high fruit yield per hectare in okra genotypes. Besides positive and highly significant correlation of these traits with fruit yield per hectare, the traits had high, mod-

Table 2: Partitioning of genotypic into direct (diagonal) and indirect (off diagonal) effects 36 okra genotype by path coefficient analysis at Melkassa in 2018
Residual factor = 0.05

Traits	SD	PH	LL	LW	PL	FL	FFM	HSM	SYPH	ASH	rg
SD	**0.079**	0.012	0.108	-0.109	0.021	0.016	0.419	-0.036	0.070	-0.033	0.548**
PH	0.048	**0.019**	0.081	-0.061	0.009	-0.010	0.250	-0.026	0.058	-0.023	0.345*
LL	0.039	0.007	**0.219**	-0.160	0.015	0.028	0.463	-0.022	0.075	-0.034	0.631**
LW	0.043	0.006	0.178	**-0.197**	0.020	0.037	0.511	-0.033	0.090	-0.039	0.615**
PL	0.052	0.005	0.101	-0.122	**0.032**	0.036	0.341	-0.034	0.082	-0.037	0.456**
FL	0.015	-0.002	0.071	-0.084	0.013	**0.088**	0.332	-0.026	0.026	-0.048	0.384*
FFM	0.037	0.005	0.113	-0.112	0.012	0.032	**0.901**	-0.031	0.038	-0.064	0.932**
HSM	0.041	0.007	0.069	-0.095	0.016	0.034	0.402	**-0.069**	0.043	-0.047	0.401*
SYPH	0.038	0.008	0.113	-0.121	0.018	0.015	0.237	-0.020	**0.146**	0.001	0.433**
ASH	0.023	0.004	0.065	-0.066	0.010	0.036	0.498	-0.028	-0.002	**-0.116**	0.424**

* = significant, ** = highly significant, SD = stem diameter PH = plant height, LL = leaf length, LW = leaf width, PL =peduncle length, FL = fruit length, FFM = fresh fruit mass, HSM = hundred seed mass, SYPH = seed yield per hectare, ash content, rg = genotypic correlation coefficient.

Table 3: Partitioning of phenotypic into direct (diagonal) and indirect (off diagonal) effects of 36 okra genotype by path coefficient analysis at Melkassa in 2018. Residual factor = 0.12

Traits	SD	PH	BR	NOI	LL	LW	PL	FL	NFR	FFM	NFPP	HSM	SYPH	ASH	FAT	PRO	rp
SD	**0.134**	-0.049	-0.006	0.029	0.056	-0.104	-0.043	0.004	0.008	0.272	0.059	0.007	0.050	-0.026	0.002	-0.003	0.390**
PH	0.068	**-0.097**	-0.007	0.023	0.046	-0.058	-0.018	0.000	0.001	0.258	0.091	0.007	0.042	-0.017	0.002	0.001	0.343**
BR	0.055	-0.045	**-0.015**	0.018	0.050	-0.049	-0.020	-0.003	-0.004	0.193	0.052	0.001	0.015	-0.015	0.002	0.001	0.236*
NOI	0.085	-0.048	-0.006	**0.046**	0.042	-0.046	-0.024	-0.003	0.000	0.156	0.060	0.002	0.040	0.008	-0.001	-0.003	0.308**
LL	0.061	-0.036	-0.006	0.016	**0.124**	-0.151	-0.030	0.006	0.001	0.397	0.128	0.006	0.055	-0.024	0.007	-0.006	0.548**
LW	0.071	-0.028	-0.004	0.011	0.094	**-0.198**	-0.038	0.008	0.007	0.427	0.100	0.008	0.063	-0.028	0.007	-0.008	0.493**
PL	0.070	-0.022	-0.004	0.014	0.046	-0.093	**-0.081**	0.008	0.005	0.296	0.089	0.007	0.050	-0.027	0.005	-0.005	0.358**
FL	0.025	-0.001	0.002	-0.007	0.035	-0.077	-0.031	**0.020**	0.005	0.319	0.041	0.007	0.020	-0.034	0.008	-0.004	0.329**
NFR	0.026	-0.001	0.002	0.000	0.004	-0.036	-0.011	0.003	**0.040**	0.344	-0.072	0.000	-0.010	-0.021	0.002	-0.003	0.266**
FFM	0.040	-0.027	-0.003	0.008	0.054	-0.093	-0.026	0.007	0.015	**0.911**	0.009	0.008	0.028	-0.047	0.004	-0.011	0.876**
NFPP	0.031	-0.034	-0.003	0.011	0.062	-0.077	-0.028	0.003	-0.011	0.032	**0.257**	0.002	0.066	0.000	0.005	-0.003	0.312**
HSM	0.055	-0.038	-0.001	0.005	0.042	-0.092	-0.032	0.008	0.000	0.404	0.026	**0.017**	0.035	-0.033	0.003	-0.001	0.400**
SYPH	0.063	-0.039	-0.002	0.017	0.065	-0.119	-0.038	0.004	-0.004	0.239	0.160	0.006	**0.106**	0.001	0.006	-0.008	0.458**
ASH	0.036	-0.017	-0.002	-0.004	0.032	-0.058	-0.024	0.007	0.009	0.458	0.001	0.006	-0.001	**-0.094**	0.000	0.005	0.355**
FAT	0.014	-0.010	-0.001	-0.003	0.041	-0.066	-0.018	0.007	0.003	0.178	0.058	0.003	0.029	-0.001	**0.022**	-0.005	0.252*
PRO	0.012	0.002	0.000	0.003	0.017	-0.042	-0.010	0.002	0.003	0.252	0.019	0.000	0.021	0.011	0.003	**-0.040**	0.254*

* = significant, ** = highly significant, SD = stem diameter PH = plant height, BR= number of branches, NOI = number of internode, LL = leaf length, LW = leaf width, PL = peduncle length, FL = fruit length , NFR = number of fruit ridges, FFM = fresh fruit mass, NFPP = number of fruits per plant, HSM= hundred seed mass, SYPH = seed yield per hectare, ash content, fat content, PRO = protein content, rp = phenotype correlation coefficient

erate and low positive direct effect. If the correlation coefficient between causal factor and the effect is almost equal to its direct effect, the correlation explains the true relationship and the direct selection through these traits will be effective (Singh & Chaudhary, 1977). Kumar & Reddy (2016) reported similar result with the present result that, fruit length had positive and negligible direct effect on fruit yield.

The residual effect from phenotypic path analysis was 0.12 (Table 3), indicating that all the traits included in the study explained high percentage of variation (88.27 %) in fruit yield per hectare in okra and other factors not included in the study can explain 11.73 %. The residual effect determines how much best the causal factors or dependent variables account for the variability of dependent variable (Singh & Chaudhary, 1977; Dabholkar, 1992).

4 CONCLUSION

The genotypic correlation coefficient between fruit yield per hectare and most of the other traits was positive and significant except days to 50 % emergence, days to 50 % flowering, days to maturity, number of branches, number of internods, number of fruit ridge, number of fruits per plant, seed per capsule, fat, fibre and protein content. The phenotypic correlation coefficient between fruit yield per hectare and all traits was positive and significant except days to 50 % emergence, days to 50 % flowering, days to maturity, internods length, seed per capsule and fibre content. Among the traits; fresh fruit mass and seed yield per hectare had positive direct effects on fruit yield per hectare at phenotypic and genotypic levels. Number of fruit per plant had also a moderate direct effect on fruit yield per plant at phenotype level. These traits also exerted high to low positive indirect effects through other traits on fruit yield at genotypic and phenotypic level. Therefore, these traits will have practical importance in selection of okra genotypes for high fruit yield per hectare.

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Introducing of some species of genus *Allium* subgenus *Melanocrommyum* from Iran as new sources of allicin

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Introducing of some species of genus *Allium* subgenus *Melanocrommyum* from Iran as new sources of allicin

Abstract: Allicin is a sulfur compound found in genus *Allium* characterized by numerous biological and pharmacological properties. *Melanocrommyum,* the second largest subgenus of *Allium*, has about 10 sections and 82 species in Iran. In this study, allicin content of aerial part, aerial part fresh mass and allicin yield belonging to 17 wild populations of six species of *Allium* sect. *Acanthoprason* and *Asteroprason* growing in different region of Iran, were analyzed. Allicin content evaluation using HPLC method showed its variation between populations from 26.98 to 58.11 mg g^{-1} FW, also showing that all the tested populations of *Allium* are rich in allicin. The average of aerial part fresh mass and allicin yield varied between populations from 0.49 g to 1.66 g and from 14 mg to 78 mg, respectively. The populations were classified in four major groups using dendrogram generated by UPGMA method of cluster analysis. However, grouping of populations was not completely related to species and geographical regions. This study is the first evaluation of allicin content in wild populations of *Allium* sect. *Acanthoprason* and *Asteroprason* in Iran. High amount of allicin in these populations make them a new sources of allicin.

Key words: *Acanthoprason*; *Asteroprason*; medicinal plant; population; variation; allicin content

Uvajanje nekaterih vrst iz rodu *Allium*, podrodu *Melanocrommyum* iz Irana kot novih virov alicina

Izvleček: Alicin je žveplo vsebujoča snov v rodu *Allium* s številnimi biološkimi in farmakološkimi lastnostmi. Podrod *Melanocrommyum* je drugi največji podrod v rodu *Allium*, v Iranu z okoli 10 sekcijami in 82 vrstami. V raziskavi so bili analizirani nadzemni deli na vsebnost alicina in svežo maso pri 17 divjih populacijah šestih vrst iz rodu *Allium* ,sekcij. *Acanthoprason* in *Asteroprason*, ki rastejo na različnih območjih Irana. Vsebnost alicina, ovrednotena s HPLC metodo je pokazala razlike med populacijami v razponu od 26,98 do 58,11 mg g^{-1} na svežo maso, kar kaže, da so vse populacije preiskušenih vrst bogate na alicinu. Poprečna sveža masa in vsebnost alicina nadzemnih delov je med populacijami variirala od 0,49 g do 1,66 g in od 14 mg do 78 mg. Z generiranjem dendrograma po UPGMA metodi in klasterski analizi so bile populacije združene v 4 glavne skupne. Grupiranje populacij se ni popolnoma ujemalo z vrstami in geografskimi regijami izvora. Ta raziskava je prva v Iranu, ki je ovrednotila vsebnost alicina v divjih populacijah vrst iz rodu *Allium, Acanthoprason* in *Asteroprason*. Zaradi velike vsebnosti alicina so vrste iz teh popuacij lahko njegov nov naravni vir.

Ključne besede: *Acanthoprason*; *Asteroprason*; zdravilne rastline; populacija; variabilnost; vsebnost alicina

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1 INTRODUCTION

Plants of the genus *Allium* belonging to monocotyledonous flowering plants comprise more than 900 accepted species, with main center of diversity in the eastern Mediterranean area, Southwest and Central Asia (Fritsch & Abbasi, 2013). *Allium* species have been *used* for centuries as *vegetables,* as well as *medicinal plants. Several studies have been conducted showing Allium's therapeutic properties as well as numerous reports referring to their* antioxidant, antibacterial, antifungal, antiparasitic, antiseptic, anti-inflammatory, anticancer, antidiabetic, cardioprotective, antiatherosclerosis, hepatoprotective and immunomodulatory properties (Benkeblia, 2004; Galeone et al., 2006; Rizwani & Shareef, 2011; Feng et al., 2012; Lu et al., 2012; Nicastro et al., 2015; Sobolewska et al., 2015; Huang et al., 2016; Rad et al., 2017; Zeng et al., 2017). Most of medicinal properties of *Alliums* are mainly attributed to phenolic (like flavonoids: kaempferol, myricetin and quercetin derivatives) and sulfur-containing compounds and beneficial elements such as selenium (Omar & Al-Wabel, 2010; Nwachukwu & Slusarenko, 2014; Soto et al., 2016). Organic sulphur compounds like alliin, allicin, allyl sulfide, (E)-ajoene, (Z)-ajoene and 1,2-vinyldithiin are responsible for odor, flavor and most of biological activities of *Alliums* (Block, 1992; Benkeblia & Lanzotti, 2007). Among these, allicin (diallylthiosulfinate) has received more attention due to its significant human health benefits (Oommen et al., 2004; Rahman, 2007; Borlinghaus et al., 2014; Ye et al., 2016).

Allicin's structure and activities were described by Cavallito and Bailey in 1944 for the first time (Cavallito & Bailey, 1944). This unstable sulfur compound is composed from alliin by the action of alliinase released from vacuoles upon crushing or damaging *Allium* tissues (Jones et al., 2007). Allicin is now clearly accepted as a biologically active compound, and several documents have been published in this field (Ali et al., 2000; Li et al., 2010; Wallock-Richards et al., 2014; Gruhlke et al., 2017). Garlic (*Allium sativum* L.) is a main source of allicin among cultivated *Alliums*. Nevertheless, there are many wild *Allium* species that may have potentially some levels of allicin which needs investigation.

Melanocrommyum, the second largest subgenus of *Allium*, comprises about 10 sections and 82 species in Iran (Fritsch & Abbasi, 2013). Some species in *Acanthoprason* and *Asteroprason,* two sections in this subgenus, are used by folk peoples as wild leafy vegetable and medicinal herbs. These plants have specific smell like garlic. In spite of long local traditional usages, there is no research on their beneficial compounds like allicin. These species are threatened with *extinction* because of wild-harvesting as the only way to reach them. Awareness and knowledge about their potent in production of healthy metabolites is needed for domestication and breeding of these species.

In this study allicin content of 17 wild populations belonging to six species of *Allium* sect. *Acanthoprason* and *Asteroprason* which were collected from different regions of Iran, as a central part of diversity of this genus, were investigated.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

Seventeen populations of *Allium* including six species of sect. *Acanthoprason* and *Asteroprason* from diverse geographical origin which were described by Fritsch and Abbasi (2013) were collected during the flowering stage. Table 1 gives the geographical location of populations. All plants were cut 1cm above ground, weighted and kept in a freezer at -80 °C.

2.2 SAMPLE PREPARATION

Allicin was extracted from randomly ten plants of each population in two replications according to Baghalian et al. (2005). In brief, each 800 mg powder sample was placed in an ultrasonic bath at 4 °C for 5 min with 20 ml of distilled water. Tubes were incubated for 30 min at room temperature. The supernatant were then separated by centrifuging at 6000 g for 30 min. The supernatant (10 ml) were added to 15 ml of solution which contains 1 % (v/v) solution of anhydrous formic acid and methanol (4:6) and centrifuged at 6000 g for 5 min. The extracts were analyzed as quickly as possible.

20 mg butyl parahydroxybenzoate in 100 ml of methanol–water (50:50) was used as internal standard. 0.5 ml of internal standard was added to supernatant and make up the volume to 10 ml and 20 µl of it was injected into the HPLC.

2.3 DETERMINATION OF ALLICIN

The allicin were determined according to the method of Baghalian et al. (2005). The HPLC analysis was carried out on a Knauer HPLC system (Berlin, Germany) equipped with a Knauer C18 column (25 cm × 4.6 mm) and a PDA detector. The mobile phase was methanol–water (50 : 50) at a flow rate of 0.7 ml min^{-1}. Elution was monitored at 254 nm. The percentage of allicin was calculated by using the following equation:

Allicin (%) = "s_1 m_2 * 22.75" /"s_2 m_1"

Where s_1 and s_2 are the area of the peak corresponding to allicin and internal standard and m_1 and m_2 are the mass of the *Allium* powder and butyl parahydroxybenzoate in internal standard solution, respectively. The allicin content was expressed as mg g^{-1} FM.

Mean allicin yield of each population (per plant) were calculated by using percentage of allicin and aerial part fresh mass.

2.4 DATA ANALYSIS

Pearson correlation and cluster analyses (UPGMA) were carried out on the data of allicin content and aerial part fresh weight using the statistical software SPSS (SPSS Inc., Chicago, USA).

3 RESULTS AND DISCUSSION

The aerial part allicin content of 17 populations of *Allium* belonging to *Acanthoprason* and *Asteroprason* sections collected from different regions of Iran are shown in Figure 1. The percentage of allicin content varied from 26.98 to 58.11 mg g^{-1} FM, where the highest content was found for Shen Jari population of *A. pseudobodeanum*, followed by Dehdasht of *A. minutiflorum* (57.95 mg g^{-1} FM), Pir Baba Ali of *A. subakaka* (56.87 mg g^{-1} FM) and Shirpala, another population of *A. pseudobodeanum* (55.94 mg g^{-1} FM), while the lowest content belonged to Taze Abad Oryeh population of *A. kurdistanicum*, followed by Vali Abad of *A. derderianum* (27.42 mg g^{-1} FM).

Variation in allicin content of different ecotypes of garlic as a main source of this valuable metabolite is reported in previous studies (Baghalian et al., 2005; Wang et al., 2014; Mostafa et al., 2015; Panahandeh et al., 2016). Allicin content of 212 accessions of garlic from different

Table 1: *Allium populations* including six species of sect. *Acanthoprason* and *Asteroprason* collected from various locations of Iran

Pop. no.	Section	Species	Location (Province)	Latitude (N)	Longitude (E)	Altitude (m)
1	*Acanthoprason*	*A. derderianum* Regel.	Dareh Oson (Tehran)	35°51′248"	51°25′786"	2645
2	*Acanthoprason*	*A. derderianum* Regel.	Vali Abad (Mazandaran)	36°18′856"	51°11′1"	2421
3	*Acanthoprason*	*A. derderianum* Regel.	Kochka (Mazandaran)	36°18′232"	51°04′53"	2248
4	*Acanthoprason*	*A. derderianum* Regel.	Vandarin (Mazandaran)	36°22′55"	51°1′41"	2926
5	*Acanthoprason*	*A. kurdistanicum* Maroofi & R.M. Fritsch	Taze Abad Oryeh (Kurdistan)	35°7′42"	47°40′309"	2332
6	*Acanthoprason*	*A. minutiflorum* Regel.	Dehdasht (Kohgiluyeh and Boyer-Ahmad)	30°50′315"	50°33′067"	1920
7	*Acanthoprason*	*A. subakaka* Razyfard & Zarre	Pir Baba Ali (Kurdistan)	35°6′17"	47°39′26"	2351
8	*Acanthoprason*	*A. subakaka* Razyfard & Zarre	Jame Shoran (Kurdistan)	35°5′733"	47°39′175"	2318
9	*Acanthoprason*	*A. subakaka* Razyfard & Zarre	Ghalelan (Kurdistan)	35°4′965"	47°39′245"	2618
10	*Asteroprason*	*A. elburzense* W.	Band e Yakhchal (Tehran)	35°50′648"	51°25′775"	2277
11	*Asteroprason*	*A. elburzense* W.	Emamzadeh Ebrahim (Tehran)	35°50′5"	51°25′10"	2120
12	*Asteroprason*	*A. elburzense* W.	Kamelat (Tehran)	35°44′514"	52°04′594"	2372
13	*Asteroprason*	*A. elburzense* W.	Abnik (Tehran)	35°51′353"	51°25′414"	2567
14	*Asteroprason*	*A. elburzense* W.	Ghabre Oros (Tehran)	35°51′618"	51°25′25"	2821
15	*Asteroprason*	*A. elburzense* W.	Kandovan Tunnel (Mazandaran)	36°9′56"	51°19′16"	2672
16	*Asteroprason*	*A. pseudobodeanum* R.M. Fritsch & Matin	Shen Jari (Tehran)	35°4′50"	52°50′372"	2290
17	*Asteroprason*	*A. pseudobodeanum* R.M. Fritsch & Matin	Shirpala (Tehran)	35°51′171"	51°25′458"	2515

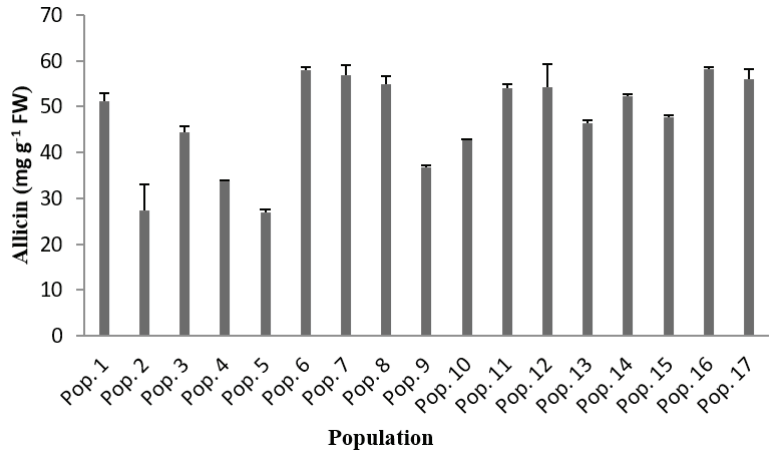

Figure 1: Schematic diagram representing the percentage of aerial part allicin in different populations of *Allium* belonging to *Acanthoprason* and *Asteroprason* sections from Iran

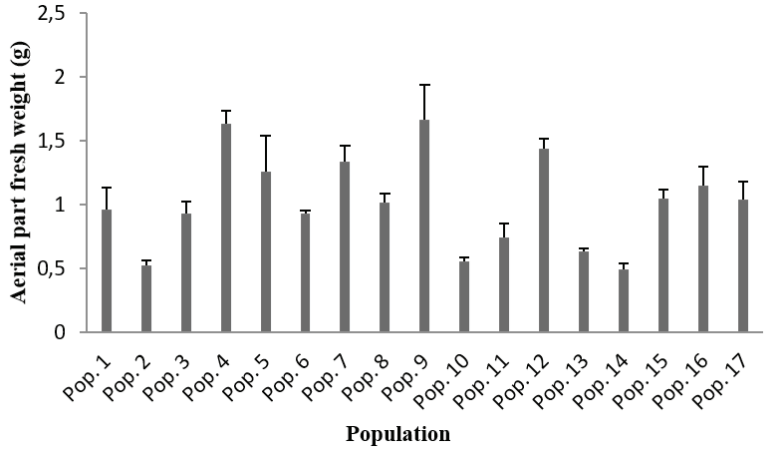

Figure 2: The average of aerial part fresh mass of different populations of *Allium* belonging to *Acanthoprason* and *Asteroprason* sections from Iran

provinces and areas of China ranged from 0.81 to 3.01 % (Wang et al., 2014). In an investigation of 24 Iranian garlic ecotypes from different areas, allicin was the highest in a local selected clone from northeast of Iran (13 % DW) (Baghalian et al., 2005). In the present work, high amount of allicin (2.69-5.81 % FM) was found in all the tested populations of *Allium*. So it was indicated that all studied populations are suitable for allicin production and pharmaceutical usage.

The average of aerial part fresh mass per plant in these populations ranged from 0.49 g in Ghabre Oros (*A. elburzense*) to 1.66 g in Ghalelan population (*A. subakaka*) (Figure 2). Variation in morphological parameters between species, populations and genotypes of *Alliums* is supported by previous literatures (Panthee et al., 2006; Karpaviciene, 2012; Khosa et al., 2014; Wang et al., 2014; Shiga et al., 2015; Silva et al., 2015; Hirata et al., 2016; Jafari et al., 2017).

Based on the obtained results, there was no correlation between percentage of allicin and aerial part fresh masst and these two characters were affected by species and environmental conditions.

Calculation of allicin yield of aerial part for each population shown in Figure 3 indicated that the average of allicin yield was the highest in Kamelat population (*A. elburzense*) (78 mg) and the lowest in Vali Abad (*A. derderianum*) (14 mg).

Due to variation of aerial part fresh mass among population, calculation of allicin yield appears to be a good parameter for evaluation of populations and finding the promising populations which can be selected for domesticating and breeding programs. Based on the results, Kamelat followed by Pir Baba Ali population have

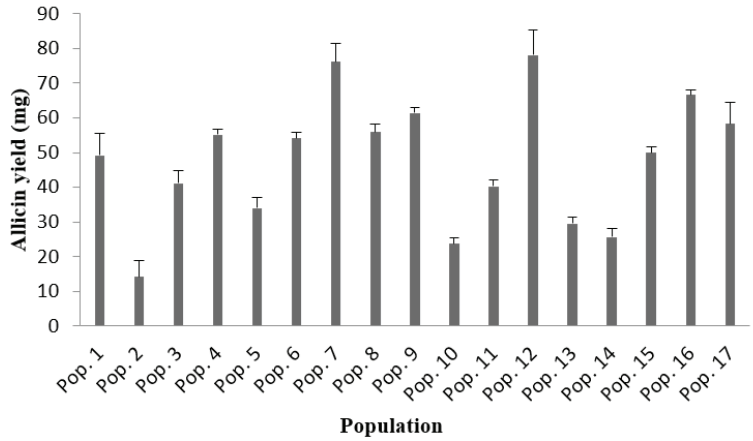

Figure 3: The average of aerial part allicin yield of different populations of *Allium* belonging to *Acanthoprason* and *Asteroprason* sections from Iran

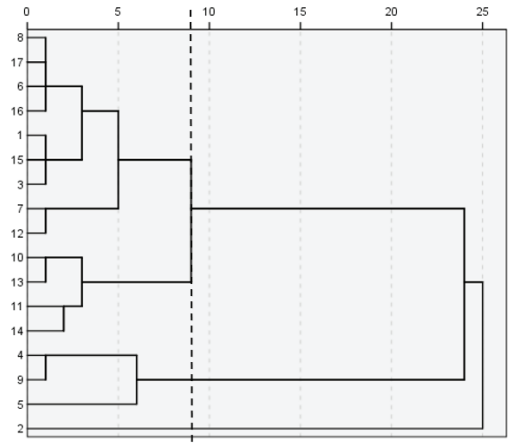

Figure 4: Cluster analysis of different populations of *Allium* belonging to *Acanthoprason* and *Asteroprason* sections from Iran using UPGMA method

the highest allicin yield and could be suitable candidates for breeding.

Dendrogram generated by UPGMA method of cluster analysis is presented in Figure 4. By applying cluster analysis, four main groups and some subgroups were evident. Jame Shoran (*A. subakaka*), Shirpala (*A. pseudobodeanum*), Dehdasht (*A. minutiflorum*), Shen Jari (*A. pseudobodeanum*), Dareh Oson (*A. derderianum*), Kandovan Tunnel (*A. elburzense*), Kochka (*A. derderianum*), Pir Baba Ali (*A. subakaka*), Kamelat (*A. elburzense*) populations were placed in cluster I. Four populations of *A. elburzense* from Tehran province (Band e Yakhchal, Abnik, Emamzadeh Ebrahim and Ghabre Oros) were assigned to cluster II. Cluster III was composed of Vandarin (*A. derderianum*), Ghalelan (*A. subakaka*) and Taze Abad Oryeh (*A. kurdistanicum*) populations. Finally, Vali Abad (*A. derderianum*) population formed cluster IV. Grouping of the populations were not completely related to species and geographical regions.

4 CONCLUSIONS

This study is the first evaluation of allicin content in wild populations of *Allium* sect. *Acanthoprason* and *Asteroprason* in Iran. Our results showed that these wild populations present considerable variation in percentage of aerial part allicin, aerial part fresh mass and allicin yield. High amount of allicin in these populations make them new sources of allicin. Conservation, domestication and breeding of studied populations are critical to exploitation and prevention of danger of their extinction. Allicin rich plants are desirable for medical industry and

Kamelat and Pir Baba Ali populations are good candidates for these purposes.

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Combinative breeding for large seeds in soybean

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Combinative breeding for large seeds in soybean

Abstract: Technological qualities of the seeds, including their mass, play an important role in the purposeful use of soybean for food production. The purpose of this study is to determine the potential of specific crosses and recombinant lines in the combinative breeding of high yielding large-seeded soybean varieties. During the period of 2018-2019 the F_3 and F_4 hybride generations of crosses with participation of the ultra-early mature and large-seeded cultivar Romantica were studied. Data were used to evaluate: presence and extent of positive transgressive forms by absolute seed mass in F_3 family crosses; genotypic diversity and additive variance at specific crosses; the effectiveness of selection of the trait large seed in F_3. According to the results, transgressive selection can be successfully used to reach the goal large seeds in soybean. The efficiency of selection of transgressive forms in F_3 generation is high. The genetic potential to combine a high specific mass of seeds with a high yield of seeds per plant has been established for the Romantica cross with the Bulgarian standard variety Srebrina. Recombinant lines suitable for intensive selection for the trait lage seed were obtained from the 'Romantica' x 'Oria' combination. The 'Saikai 20' x 'Romantica' cross possess a very high degree of transgressive segregations.

Key words: soybean; combinative breeding; absolute seed mass

Kombinacijsko žlahtnjenje soje za večja semena

Izvleček: Tehnološke lastnosti semen, vključno z njihovo maso imajo pomembno vlogo pri namenski rabi soje v pridelavi hrane. Namen raziskave je bil določiti potencial specifičnih križanj in rekombinantnih linij v kombinirani vzgoji visokodonosnih sort soje z velikimi semeni. V obdobju 2018-2019 so bile preučevane F_3 in F_4 hibridne generacije iz križanj zelo zgodaj dozorevajočih sort soje in sorte Romantica z velikimi semeni. Pridobljeni podatki so bili ovrednoteni glede na naslednje parametre: prisotnost in obseg pozitivnih transgresivnih oblik z veliko absolutno maso semen v družini F_3 križancev; genotipsko raznolikost in aditivno spremenljivost pri specifičnih križanjih; učinkovitost izbora lastnosti velikih semen v F_3 generaciji. Glede na rezultate je transgresivna selekcija lahko uspešno uporabljena za vzgojo sort soje v velikimi semeni. Učinkovitost izbora transgresivnih oblik v F_3 generaciji je bila velika. Genetski potencial za kombinacijo lastnosti velika specifična masa semen z velikim pridelkom semen na rastlino je bil dosežen pri križanju sorte Romantica s standarno bolgarsko sorto Srebrina. Rekombinantne linije, primerne za intenzivno selekcijo za lastnost velika semena so bile dobljene s kombinacijo 'Romantica' x 'Oria'.Tudi križanje 'Saikai 20' x 'Romantica' je imelo veliko stopnjo transgresivnih segregacij.

Ključne besede: soja; kombinacijsko žlahtnjenje; absolutna masa semen

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1 INTRODUCTION

Legumes are widespread around the world, providing more than 69 % of the protein, and 30 % of the fats/oils needed for human diet. In the grain legumes - pea, soybean and common bean the protein content ranged from 20 % to 40 % depending on genotype and environment. Important is the fact that legumes require minimal amounts of soil fertilizers because they possess the ability to absorb nitrogen from the air through symbiotic interactions with nitrogen fixing bacteria. This ability affirm them as the world's plant protein sources, providing sustainable agriculture. Climate specificity of Bulgaria could be described as a heavy continental. The Bulgarian soybean varieties are created for the specific soil and climatic conditions of the country along with the developed agro-technologies. In Bulgaria the Experimantal Soybean Station in Pavlikeny is the research organization where the Bulgarian soybean varieties are created and its main scientific activity is to develop varieties adapted to the country-specific agro-climatic environment and with high nutritional and technological quality of the grain. Technological qualities of the grain, including its specific absolute mass, play an important role in the purposeful use of soybean for food production. The mass of 100 seeds is an important component in seed yield, which is monitored in breeding productivity programs. The mass of 100 seeds possess complex genetic control. According to the study of Assefa et al. (2019), 14 quantitative trait loci (QTL) related to this trait were identified. Independent selection by absolute seed mass is considered easy and effective because of medium to high inheritance estimates (Cober et al., 1997; Krisnawati and Adie, 2015), but it is also important to associate this trait with productivity and adaptive potential. In their study (Mian et al., 1996) comment significant positive genotypic correlations between seed mass and seed yield per plant. On the other hand, the trait large seed is negatively correlated with the number of pods formed by the plant and the number of seeds per pod (Miladinović et al., 2011). Because these are the main criteria for selection in the direction of productivity, often high-yielding varieties are not large-seeded. It is important to note that seed mass is also strongly influenced by environmental factors. According to our research, genotypically defined trait large seed have a phenotypic realization when the reproductive phases of grain filling R7-R8 take place outside the period 1-15 August, when the drought period in Bulgaria is most frequent and most severe. In this regard, high and stable trait expression was observed in varieties of very early (MG 000-00) and medium to late (MG I) maturity groups (Naydenova and Georgieva, 2019).

The nature of inheritance of the absolute mass of the seeds, established by the dialle crosses of the varieties grown in Bulgaria, is defined as additive (Kien, 1989). This is a reason transgressive selection to be considered as a potentially effective method to improve the trait. Transgressive variability in hybrid F_2 and F_3 families due to the recombination of additive alleles has been observed and further used in our selection program for all quantitative traits structuring seed yield in soybean (Aleksieva, 2001). According to Jambormias et al. (2015) the finding of multiple transgressive segregates at the F_3 generation will enables an early selection to be carried out simultaneously for several multiple traits on self pollinated crops.

The purpose of this study is to identify positive transgressive forms by absolute mass of seeds, as well as to determine the potential of specific crosses and recombinant lines in the combinative breeding of high yielding large-seeded soybean varieties.

2 MATERIAL AND METHODS

2.1 CHARACTERISTICS OF PARENTAL GENOTYPES

Romantica variety is an ultra-early mature Ukrainian variety (MG00), with a vegetation period of about 90 days for the condition of Northern Bulgaria. In three years comparative variety trial, the value of the trait mass of 100 seeds for this genotype is 15.22 g (Naydenova and Georgieva, 2019). Unfavorable characteristics of the variety are low growth habitus and low positioning of the first pods.

Oria variety is an early mature Canadian variety (MG0) with a corresponding average mass of 100 seeds 14.14 g. Filling of the seeds in this genotype varies greatly in the years.

Felix variety is an early mature Romanian variety (MG0) with a mass of 100 seeds 13.27 g. It is characterized by a stable phenotypic expression of the trait, as well as high grain productivity.

Srebrina is a Bulgarian medium-early variety (MGI) with high adaptive and productive potential. The mass of 100 seeds for this genotype identified in the above mentioned comparative variety trail is 12.10 g, with trait values varying slightly over the years.

'Saikai 20' is a Japanese variety, midle-late maturity group (MGII), characterised with high-protein content. The genotype is small-seeded (mass of 100 seeds

– 11.15 g), with cracked pods under Bulgarian environmental conditions.

2.2 FIELD EXPERIMENTS

The crosses performed with these varieties were conducted in 2015 in the region of Pavlikeni (43°24'N, 25°32'E, 144 m), which is in the temperate continental climate zone with a well-established continental rainfall regime - with a maximum in May-June and a minimum in August-September. In 2017 the selection in F_2 was made according to the growth habitus, by early maturity, height of positioning of the first pods, and not crackble pods. Generations F_3 and F_4 were tracked down in a breeding nursery compared to the parental genotypes over two consecutive years (2018-2019). The offspring of each elite genotype harvested in F_2 generation was sown in one separate row 4 m in length, with a row spacing of 70 cm and 10 cm in row distance, 10 plants per linear meter.

2.3 PHENOTYPIC MEASUREMENTS AND DATA ANALYSIS

On the basis of visual assessment, 4 plants of each row were selected (10 % intensity of selection was applied), and the values of the trait mass of 100 seeds (g) and the yield of seeds per plant (g) were monitored for 20 plants of each family/studied cross. The mass of 100 seeds was evaluated in duplicate for each genotype. From the biometric measurements carried out, the mean values, limit values and variance by absolute mass of the seeds in F_3 generation were determined. The positive transgressive forms of the studied trait were established in crosses, respectively families. Transgression is represented by the relative difference of the trait value of the individual hybrid combinations against that of the large seeded parent. On the base of the phenotypic data of the trait large seed (mass of seeds) in combination with the production of seed per plant, 46 lines were selected, and traced down in the fourth generation for stabilization of the trait large seed. The lines were sown in rows of 2 m in length, at 70 cm between rows and 5 cm in row distance, 20 plants per linear meter, in two randomized repeats. In the F_4 generation, from each line, eight genotypes were selected, which were also subjected to biometric analysis.

Data from both generations (F_3 and F_4) were used to evaluate genotypic diversity and additive variance by absolute seed mass by family, respectively at specific crosses. Genotype diversity is represented by the inheritance coefficient H_{bs}^2 calculated as the ratio of genotype to phenotype variance in F_3 generation. The additive variance is represented by the inheritance coefficient h_{ns}^2, which is calculated by the parent-offspring covariance (F_4) using the formula $h_{ns}^2 = b/2r_{xy}$, where b is the regression coefficient between the trait values in the parental genotypes (x) and those in the offspring (y); r - is the correlation coefficient (Yankulov et al., 1993).

In order to evaluate the effectiveness of selection of the trait absolute seed mass in F_3, a rank analysis was performed. Spearman's rank correlation coefficient (r_S) was used to estimate the relationship between seed mass of the elites selected in the F_3 generation and that of their offspring in the F_4 generation. The coefficient value is calculated using the formula given, where: d is the difference in genotype rank numbers by generation; n is the volume of the samples extract.

$$r_s = 1 - \frac{6\sum d^2}{n(n^2-1)}$$

Statistical and graphical data processing was done using Microsoft Excel 2010.

3 RESULTS AND DISCUSSION

According to the results (Table 1), the highest transgression rate by mass of 100 seeds is the hybrid combination in which both parental components are large-seeded - Romantica X Oria. For the family of this cross, a very high mean value of the trait was observed, as well as the highest degree of segregation with respect to its threshold values. Krisnawati and Adie (2015) had successfully obtained soybean large seed size lines established after crosses of large seeded parental genotypes (> 14 g/100 seeds). For the cross of the same maternal genotype with the Bulgarian variety Srebrina, high positive transgressions were also achieved, as well as a high range of phenotypic variability.

Using the Romantica variety as a pollen paternal component, lower mean values of the trait and significantly lower levels of positive transgressions were obtained. In these crosses, depending on the maternal component, contrasting results were also observed for variance of the trait. The highest hybrid variability was observed in the F_3 population of the crossing breed where the Japanese variety Saikai 20 was the mother genotype. The results could be explained both by the large difference in trait values in parental components and that transgressive segregations are more likely in genetically distant genotypes (Stelkens et al., 2014). The 'Felix' x 'Romantica' cross family has the lowest variance in terms of the tracking trait.

Table 1: Mean values, limit values, variance and degree of positive transgression for families by trait mass of 100 seeds (g) in F_3 generation

	Hybrid combinations			
	'Romantica' x 'Srebrina'	'Romantica' x 'Oria'	'Felix' x'Romantica'	'Saikai 20' x'Romantica'
Mass of 100 seeds (m_{100}), g	19.3	21.9	18.5	17.6
Min	15.4	17.6	17.4	16.8
Max	23.2	26.4	19.5	20.4
σ^2, g	5.3	10.9	0.8	13.0
Values of positive transgression	0.5 - 14.3%	1.4 - 36.1%	1.3 - 6.5%	0.2 - 10.8 %
Significance of genotypic variance in F_3 generation of family	$p < 0.01$	$p < 0.01$	$p = 0.10$	$p < 0.001$
H^2	0.18	0.24	0.23	0.90

Investigation of heterogeneous F_2 and F_3 families, followed by testing only the best of the lines, is considered to be an effective method in early generation of soybean selection (St Martin and Geraldi, 2002). In this case, in addition to the mean and limit values for the families studied, estimates of genotypic effect and genotypic diversity within them are also important. According to the variance analysis, the genotypic control in the variation of seed mass between the lines was significant ($p < 0.01$-0.001) for three of four families/crosses studied - Table 1. This implies the efficiency of the selection by trait still in F_3 generation. On the other hand, according to the values of the inheritance coefficient, the genotypic diversity in F_3 generation of the crosses 'Romantica' x 'Srebrina', 'Romantica' x 'Oria', and 'Felix' x 'Romantica' is relatively low, where additional breeding criteria were used for the selection of elites (seed yield per plant, harvest index, growth habitus). The genetic differences in the studied generation of the 'Saikai 20' x 'Romantica' cross are significant ($H^2 = 0.90$), which could be accepted as an indicator of both gene recombination leading to a significant increase in seed mass and the likely presence of spontaneous mutational variability of the trait.

It is important to consider the effect of environmental factors in determining the genotypic significance of the breeding trait in the individuals (Rosenzweig et al., 2016). The highest phenotypic variance established in families derived from parental components differing in maturity – 'Romantica' (MG00) x 'Srebrina' (MGI) and 'Saikai 20' (MGII) x 'Romantica' (MG00) – is possibly due to environmental impact. In the segregating hybrid populations, the individuals have different timing of grain-filling phenophases, which is a source of environmental variance by the traced trait (the differences in precipitation during the phenophases of grain filling R_7-R_8 in very early and middle-early offspring amounts to almost 50 mm). This requires the seed mass to be evaluated in relation to early maturity in these crosses. At the same time, high phenotypic variability by trait, coupled with a slightly segregation in maturity, is observed for the cross of early maturity 'Romantica' (MG00) x 'Oria' (MG0) varieties. In this case, some of the variability may be associated with a specific genotypic response to the larger feeding area in which the F_3 offspring were grown – row spacing 70 cm and 10 cm in row distance, 10 plants per linear meter. In our previous studies based on standard Bulgarian soybean varieties we found a difference in genotypic response of the trait seed mass related to sowing density (Georgiev et al., 2019). In this regard, an additional evaluation of the expression of the trait in crops with higher density is required, which was done in F_4 generation of crosses.

According to the results of the determing of phenotype by seed mass in combination with the seeds production per plant (Figures 1) higher frequency of offspring, significantly exceeding the parental forms is observed for the fourth hybrid generation of the cross 'Romantica' x 'Srebrina' - Fig. 1A, Fig. 2A. In relation to the results for the previous generation of this cross, the large seeded is connected to the maturity period, and the highest seed mass was observed for the offspring of the late mature F_3 genotypes. For genotype R5/13/2 an extremely high mass value of 100 seeds was reported - 28.9 g.

In F_4, the offspring of the cross 'Romantica' x 'Oria', under conditions of genotypic and environmental competition, were observed only two elites, superior in terms of both productivity and seed mass of the parent components (Fig. 1B, Fig. 2B). It is important to note that there are also a large number of offsprings with very high matter of the breeding trait (>20 g), which exhibit higher in-

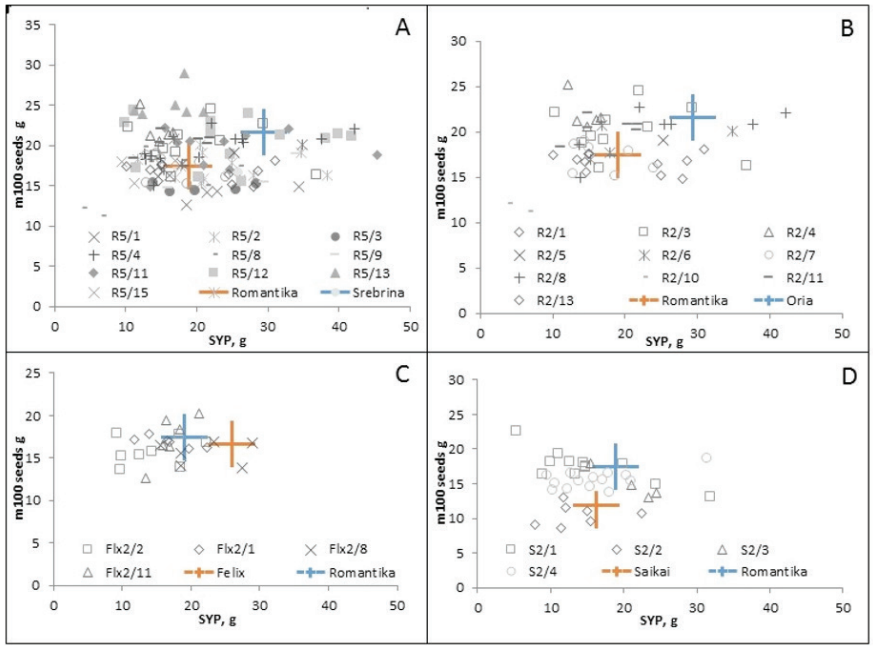

Figure 1: Scattering of seed yield per plant (SYP, g) by mass of 100 seeds ($m_{100,}$ g) in F4 generation of crosses (A-D)

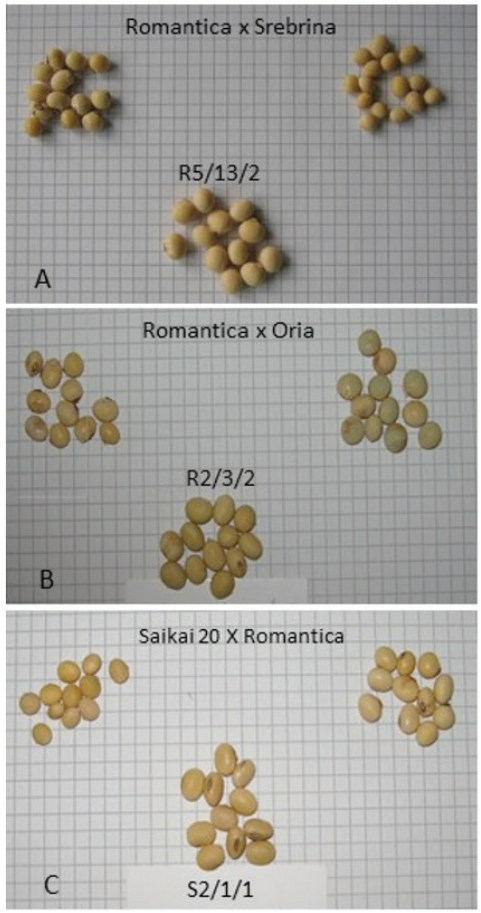

Figure 2: Representative elite lines from F4 generation (A-C)

Table 2: Correlation coefficient between mass of 100 seeds (m_{100}), seed yield per plant (SYP) and number of seeds per plant (NSP) in F_4 generation, rank correlation coefficient by trait m_{100} values in F_3 and F_4 generations (r_S), inheritance coefficient of m_{100} (h_{ns}^2)

Hybrid combinations	'Romantica' x 'Srebrina'	'Romantica' x 'Oria'	'Felix' x 'Romantica'	'Saikai 20' x 'Romantica'
$r_{m100/SYP}$	0.13	0.07	0.12	-0.22
$r_{m100/NSP}$	-0.28	-0.10	-0.15	-0.62
r_S	0.60	0.69	0.54	0.80
h_{ns}^2	0.47	0.56	0.39	0.54

dividual productivity than that of the mother variety. This allows a high intensity of selection by the mass of seeds in the family.

Due to the low hybrid variability presented above, the number of 'Felix' x 'Romantica' progeny monitored in F_4 is small. According to the results, the cross has no potential for the desired combination of high seed mass with high plant seed yield - Fig. 1 C.

In a large number of F_4 offsprings from the 'Saikai 20' x 'Romantica' cross, a significant increase in the selected trait values was observed against the maternal genotype. In the offspring of one of the selected line in the previous generation, genotypes with very high seed mass values (> 20 g) and with high yield of seeds per plant (> 30 g) were observed (Fig.1 D, Fig.2 C). Genotypes have been found to exceed the Romantica variety for both traits, which is the parent component with higher meaning of these traits.

The efficiency of identifying offspring with high genotypic meaning by mass of 100 seeds in F_3 estimated by the rank correlation coefficient (r_s) is very high for the 'Romantica' x 'Oria' and 'Saikai 20' x 'Romantica' crosses - Table 2. Also relevant are the results of the assessment of additive inheritance by the trait in the commented crosses - the inheritance coefficient h_{ns}^2 possess corresponding values of 0.56 and 0.54, the additive variance is about half of the observed phenotypic variance.

According to the values of the correlation coefficient ($r_{m100/SYP}$), the mass of the seeds was not related to the yield of seeds per plant (Table 2). A significant compensating dependency between seed mass and number of seeds per plant ($r_{m100/NSP}$ = -0.62) was observed for recombinant lines from the 'Saikai 20' x 'Romantica' cross. According to Fujii et al. (2018) some genetic factors for large seeds reduce the number of pods and fertility by decreasing either the number of pods per plant or the number of ovules per pod. Definitely further studies are needed to find out whether the regulation of these components is due to a common genetic factor or closely related ones.

4 CONCLUSION

According to the results presented in this study, transgressive selection can be successfully used to reach the goal large seeds in soybean. The efficiency of selection of transgressive forms in F_3 generation is high. The valuable combinative variability in seed mass obtained in all the crosses studied, identifies the Romantica variety as an important genetic source in the selection for the trait large seed. The genetic potential to combine a high specific mass of seeds with a high yield of seeds per plant has been established for the 'Romantica' cross with the Bulgarian standard variety Srebrina.

Recombinant lines suitable for intensive selection for the trait lage seed were obtained from the 'Romantica' x 'Oria' combination. The 'Saikai 20' x 'Romantica' cross possess a very high degree of transgressive segregations. The hybrid material derived from it could be successfully used to study the genetic factors affecting both traits - the number of seeds per plant and their specific mass.

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Effect of foliar or soil application of selenium on some morphological and physiological traits of garden pansy (*Viola x wittrockiana* Gams) grown under salinity stress

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Effect of foliar or soil application of selenium on some morphological and physiological traits of garden pansy (*Viola x wittrockiana* Gams) grown under salinity stress

Abstract: Salinity stress is one of the most important plant stresses in Iran. In this regard, a factorial experiment was conducted to investigate the effects of salinity stress on the garden pansy. The investigated factors were containing sodium selenate (0, 2, 4 and 8 mg l^{-1}), its method of application (foliar and soil applications) and salinity stress (0, 3 and 6 dS m^{-1}). The obtained results indicated that salinity leads to the significant reduction in morphological traits, chlorophyll a and b contents. Under the salinity of 6 dS m^{-1}, when sodium selenate was used in the soil, the fresh and dry mass of flower increased by 11.34 and 10.39 %, respectively, compared to the control. However, the use of sodium selenate by foliar application led to the increasing fresh and dry mass of garden pansy's flower by 25.10 and 25.41 %, respectively. Also, the content of chlorophyll a increased by 12.93 % under the salinity of 6 dS m-1 with applying 8 mg l^{-1}sodium selenate compared to the case of non-application. The superoxide dismutase activity decreased by 26.13 % compared to the non-sodium selenate usage treatment. In conclusion the foliar application of sodium selenate at the concentraion of 8 mg l^{-1} resulted in the garden pansy's growth improvement.

Key words: garden pansy; superoxide dismutase; number of flowers; salinity stress; chlorophyll content

Učinek foliarnega dodajanja selena na nekatere morfološke in fiziološke lastnosti vrtne mačehe (*Viola x wittrockiana* Gams) v razmerah slanostnega stresa

Izvleček: Slanostni stres je eden najpomembnejših stresov za rastline v Iranu. V tem pogledu je bil izveden faktorski poskus za preučevanje vpliva slanostnega stresa na vrtno mačeho. Preučevani so bili naslednji parametri: koncentracija natrijevega selenata (0, 2, 4 in 8 mg l^{-1}), način njegove uporabe (foliarno in talno dodajanje) in velikost slanostnega stresa (0, 3 in 6 dS m^{-1}). Rezultati so pokazali, da je slanostni stres vodil k značilnemu zmanjšanju morfoloških latnosti in vsebnosti klorofila a in b. V razmerah slanostnega stresa 6 dS m^{-1} in ob talni uporabi natrijevega selenata sta se sveža in suha masa cvetov povečali za 1,34 in 10,39 % v primerjavi s kontrolo. Foliarno dodajanje natrijevega selenata pa je povečalo svežo in suho maso cvetov vrtne mačehe za 25,10 in 25,41 %. Tudi vsebnost klorofila a se je v razmerah slanosti 6 dS m^{-1}in uporabi natrijevega selenata 8 mg l^{-1} povečala za 12,93 % v primerjavi z razmerami brez dodatkov selenata. Aktivnost superoksid dizmutaze se je pri dodatku selena zmajšala za 26,13 % v primerjavi z obravnavanjem brez selenata. Zaključimo lahko, da je foliarno dodajanje natrijevega selenata v koncentraciji 8 mg l^{-1} izboljšalo rast vrtne mačehe.

Ključne besede: vrtna mačeha; superoksid dismutaza; število cvetov; slanostni stres; vsebnost klorofila

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1 INTRODUCTION

Viola x wittrockiana Gams, a garden pansy, (from Violaceae family) is of great economic importance. It contains salicylic acid, phenolic acids such as caffeic acid and their derivatives (Vukics et al., 2008). Garden pansy is used to decorate urban green spaces and to promote the mental well-being of citizens, but it experiences all kinds of environmental stresses such as drought, salinity, high temperature and cold.

Among the various stresses, salinity is one of the most important ones which severely restrict the productivity, especially in the arid and semi-arid regions (Ashraf & Harris, 2004). According to the conducted studies, 30 and 50 % of the agricultural ground will be destroyed by salinity within the next 25 years and by the middle of the 21st century, respectively and this will have negative effects on the agricultural production (Shahid et al., 2018). Salinity stress is a serious environmental threat to the agricultural fields, which causes green fields to become arid and non-cultivable lands and reduces the plant growth and crop yield (Khan et al., 2015). The salinity stress is mostly obtained by high concentrations of sodium (Na^+) and chloride (Cl^-) ions within the soil solution (Hasegawa et al., 2000). High salinity results into the ionic and osmotic stresses which lead to the plant death as a consequence (Hu & Schmidhalter, 2005; Mahajan et al., 2005). Furthermore, salinity stress yields yellow and brown flowers and therefore the ornamental value of the plants is reduced (Cassaniti et al., 2012; Matraszek et al., 2015). This stress causes premature aging of the leaves, chloroplast damage and chlorophyll content reduction. Chlorophyll decrement results in reduced photosynthesis and plants which maintain more chlorophyll content during the stress, have higher photosynthetic efficiency and are tolerant to the stress (Sharma & Dubey, 2005). In salt tolerance, numerous compounds such as sugars, organic acids and nitrogen-containing ones such as amino acids, amides, imides and proteins act as osmotic adjusters. These compounds help maintain turgor pressure, cell volume and reduce the stress effects (Ashraf & Harris, 2004). Various methods are available for reducing the salinity effects. Many researchers have examined the organic and inorganic substances in order to reduce the effects of salt toxicity (Liang et al., 2006; Ashraf et al., 2010; Hasanuzzaman et al., 2013; Diao et al., 2014). In a study by Satyendra et al. (1999) a positive correlation was observed between the peroxidase enzymes activities and soil salinity.

Selenium is an essential micronutrient for humans and animals (Matos et al., 2017; Supriatin et al., 2015). Although sodium selenate is unevenly distributed around the globe, its concentration ranges from 0.1-1 mg kg^{-1} soil (Bocchini et al., 2018). This element can be useful or harmful to the plant depending on its concentration and type of the plant species (Draho novský et al., 2016). Germ et al. (2007) indicated that sodium selenate is dangerous to the plants at high concentrations but can have beneficial effects at lower ones. Recent studies have shown that sodium selenate plays an important role in the plant tolerance to the environmental stresses including salinity (Feng et al., 2013; Bocchini et al., 2018; Munshower et al., 2018; Shahid et al., 2018; Tan et al., 2018). Selenium is not an essential ingredient in the plants but acts as an antioxidant protecting plants against UV radiation regulates plant growth and protects them against pathogens (Kaur et al., 2014). It protects the cell membrane against the salinity stress conditions (Hawrylak-Nowak, 2009). There are some evidence of the positive effects of selenium on the growth and performance of tomato (*Solanum lycopersicum* L.) (Diao et al., 2014; Zhu et al., 2016), lemon balm (*Melissa officinalis* L.) (Habibi & Sarvary, 2015) and canola (*Brassica napus* L.) (Hashem et al., 2013; Bybordi, 2016) at low concentrations. Sodium selenate has beneficial effects on the plants' growth and tolerance to the stresses through increasing their antioxidant capacity (Hasanuzzaman et al., 2010; Djanaguiraman et al., 2005; Rios et al., 2009). Further to these applications, selenium increases the antioxidant acids such as salicylic acid, jasmonic acid and hormones such as ethylene (Hasanuzzaman et al., 2013).

Sodium selenate is considered as an effective micronutrient in reducing the non-biological stresses such as salinity. Selenium fertilizer is used in four ways including the seed soaking, seed dressing, foliar and soil applications. Nowadays, selenium application technology is used as foliar or base fertilizer to increase the selenium content within the crops (Pezzarossa et al., 2012). The aim of this study is to investigate the effect of sodium selenate and its application method on the garden pansy plant under the salinity stress conditions in Iran.

2 MATERIALS AND METHODS

2.1 CULTIVATION AND TREATMENT OF PLANTS

The present research aims to investigate the effect of sodium selenate in both foliar and soil applications on the ornamental garden pansy's flowers (*Viola x wittrockiana* 'Queen Yellow Bee') under salinity stress using a factorial experiment in a completely randomized design with three replications conducted in the greenhouse of horticulture department of Science and Research Branch of Tehran, Islamic Azad University in 2019. The 4-leaf

ornamental garden pansy transplants were prepared from the Flower and Plant Center of Mahalat city. The transplants were then transferred to the 15 cm-diameter pots containing culture medium (a mixture of perlite and cocopeat at 70 : 30 ratios) and kept in the greenhouse for two weeks for adaptability. During this time period, they were fed with Hogland's nutrient solution to the amount of half of the recommended concentration with irrigation water once a week (Hoagland & Arnon, 1950). EC of nutrient solution was 1-1.4 ds m^{-1}. Plants were treated with sodium chloride (NaCl) at three levels of 0 (control), 3 and 6 dS m^{-1}. Salinity treatments in volume of 50 ml was irrigated regularly as required (every two weeks) and it was applied until complete flowering of the plant. Salt used in this experiment was purchased from Elgomhouria Company, Amiria, Cairo. The selenium concentrations of 0 (control), 2, 4 and 8 mg l^{-1} as sodium selenate (Na_2SeO_4) have been applied in two ways of leaf foliar and soil applications. Leaf application was applied immediately after transplanting and continued every two weeks until the end of the experiment. The solution pH was initially adjusted between 5.8 and 6.5 with minute additions of HCI or NaOH as needed. Foliar application of sodium selenate was done in the evenings and 10 ml volume was consumed for each pot. Two weeks after the last application of the treatments, leaf and root samples were collected in order to perform experiments. The average day and night temperatures were 15–25 and 12–15 °C, respectively, relative humidity was about 60 % and light to darkness estimated as 14 to 10 h with light intensity of 160 µmol m^{-2} s^{-1}. Eight replicates (individual plants) were used for each treatment.

2.2 MORPHOLOGICAL TRAITS MEASUREMENT

The shoot height was measured by a ruler. Thus, from the plant's collar to the shoot apex was considered as the height of the shoot. The cultivated plants in each pot were cut from the collar section by scissors and shoot and flower were weighed. The fresh mass were measured using a digital scale with accuracy of 0.01 g. After drying the different parts of plant in the oven at 72 °C for 24 h, their dry mass were measured by digital scale.

2.3 CHLOROPHYLL CONTENT MEASUREMENT

The chlorophyll content measurement was carried out according to the method of Lichtenthaler and Wellburn (1983). At first, 0.1 g of the plant leaf sample was thoroughly grinded in Chinese mortar together with 3 ml of 80 % acetone and the extract's final volume reached 15 ml. The extract was then filtered at the speed of 5000 g for 10 min using a centrifuge. The spectrophotometer device (Shimadzu UV-160) was utilized to measure the absorption rate of the samples. First, the apparatus was set to zero with 80 % acetone and then the absorption rates of the extract were read by spectrophotometer at the wavelengths of 663 and 645 nm for chlorophyll a and b, respectively.

Chlorophyll a = (19.3A663 - 0.86A645) V/100W
Chlorophyll b = (19.3A645 - 3.6A663) V/100W

2.4 ASSESSMENT OF THE ENZYMES ACTICITY

The catalase enzyme's activity was measured with spectrophotometry method and based on the absorption reduction of hydrogen peroxide for 30 s at a wavelength of 240 nm. The reaction mixture contained 50 mM K phosphate buffer (pH = 7), 15 mM hydrogen peroxide and 100 µl of enzyme extract. The reaction was started by adding hydrogen peroxide and the absorption reduction measured for 30 s. The degraded amount of hydrogen peroxide was calculated using the extinction coefficient equal to 40 $mM^{-1}cm^{-1}$ (Velikova et al., 2001). The measurement of superoxide dismutase was conducted using the method presented in Giannopolitis and Ries (1977). To measure the activity of this enzyme, the reaction mixture was prepared in a final volume of 1 ml including 50 mM phosphate buffer (pH = 7.8), 0.013 M methionine, 0.01 µM EDTA and 2 µM riboflavin and maintained in the complete darkness. Immediately after adding riboflavin, 3 ml of it was poured into the test tube and 100 µl of protein sample added to each tube. The test tubes were placed in a distance of 30 cm from the light source and the samples' absorption values were read at the corresponding wavelength after 16 minutes. The device was calibrated at the wavelength of 560 nm. The enzyme activity was expressed in enzyme unit per mg protein in each sample. The total protein content in the enzyme extracts was determined according to Bradford (1976) procedure, using bovine serum albumin as a standard.

2.5 MEASURING CONCENTRATION OF ELEMENTS

To measure Cl^-, 100 mg of powdered plant tissue was poured into the Falcone tube and extracting was performed after adding 10 ml of 0.5 M nitric acid and drying for 1 h at 80 °C. The amount of 1 ml of the extract was used for Cl^- reading according to the colorimetry method

at the wavelength of 480 nm using Epoch setup (Munns & Tester, 2008). In order to measure the Na^+ and K^+ contents, the garden pansy's leaves were completely dried in open air after harvesting. The samples were then powdered using a mortar. 0.3 g of the powdered samples were weighed and converted into ash in the furnace at 500 °C for 6 h and then dissolved in 5 ml of 2 M nitric acid solution. The solution's volume was finally reached 25 ml with double distillation water and filtered with Whatman No.1 filter paper. Then, measurement was performed using flame photometry device (PFP7 model manufactured by JENWAY Company, UK) (Chapman & Pratt, 1962). The Unico spectrophotometer made in USA was used to measure the P concentration of the root and shoot. For this purpose, the plant samples were first converted into ash within the furnace (550 °C). Then, 1 ml of Barton reagent and 70 ml of 70 % perchloric acid were added to the ash samples. After that, their volume reached 10 ml with double distillation water. The absorbance of each solution was measured by spectrophotometer at wavelength of 450 nm (Ryan et al., 2007). For measuring Se concentration, 5 g dried powder samples were digested with 25 ml of a 4:1 mixture of HNO_3 and $HClO_4$ at 130 °C for 60 min. After cooling, 5 ml of concentrated HCl was added to the sample for reduction of Se^{+6} to Se^{+4} and continued for 20 min at 115 °C until the sample was completely mineralized. The Se concentration of test solution was analyzed by atomic absorption (Liu & Gu, 2009).

2.6 DATA ANALYSES

The experiment was repeated twice under the same conditions and data were statistically analyzed using the SAS statistical software (version 9.3, SAS Institute, Cary, N.C.). Comparison of the mean data at significance level of 5 % was performed by Least Significant Difference Test.

3 RESULTS

3.1 MORPHOLOGICAL TRAITS

Comparison of the garden pansy's average height indicated that salinity significantly reduce the plant height. The highest shoot height (5.75 cm) was observed in the zero salinity and 8 mg l^{-1} sodium selenate treatment which was not significantly different from other levels of sodium selenate at this salinity level (Table 1). The highest shoot diameter (5.87 mm) was reported in zero (control) salinity treatments (Table 1). The salinity stress led to a significant decrease in the shoot diameter (Table 2). However, this decrement was lower in the treatments containing sodium selenate and this element moderated the effect of salinity on the shoot diameter (Table 1). Sodium selenate significantly increased the shoot fresh mass. The highest fresh mass (79.33 g) was observed in

Table 1: Interaction effects of different levels of salinity and sodium selenate concentration on shoot height, shoot diameter, fresh weight of the shoot and flower diameter

		Mean			
Salinity level (dS m^{-1})	Sodium selenate concentration mg l^{-1}	Shoot height (cm)	Shoot diameter (mm)	Fresh mass of the shoot (g)	Flower diameter (cm)
0	0	5.75±0.21	5.38±0.21	78.00±2.31	5.40±0.41
	2	5.52±0.21	5.42±0.31	78.67±2.47	5.41±0.31
	4	5.57±0.16	5.72±0.22	82.83±2.14	5.72±0.24
	8	5.75±0.17	5.87±0.17	85.67±2.19	5.87±0.34
3	0	5.28±0.20	4.65±0.14	70.50±1.99	4.65±0.24
	2	4.87±0.31	4.32±0.15	71.00±2.09	4.32±0.25
	4	5.43±0.32	4.90±0.19	74.50±2.01	4.90±0.24
	8	5.28±0.17	4.93±0.20	76.83±2.47	4.93±0.33
6	0	3.82±0.18	3.38±0.21	56.33±2.33	3.38±0.37
	2	3.95±0.22	4.02±0.23	57.50±2.17	4.02±0.21
	4	4.45±0.19	4.37±0.31	61.00±2.17	4.37±0.14
	8	4.50±0.19	4.55±.31	63.67±2.39	4.55±0.17
LSD* ($p \leq 0.05$)		0.30	1.84	2.35	0.26

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (± SD).

Table 2: Interaction effects of different types of application and salinity level on shoot diameter and number of flowers

Trait	Salinity level (dS m^{-1})	Type of application	
		Soil	Foliar
Shoot diameter	0	5.46±0.27	5.73±0.41
	3	4.53±0.36	4.87±0.28
	6	3.90±0.37	4.26±0.63
LSD* ($p \leq 0.05$)		1.30	
Number of flowers	0	13.75±0.98	15.17±1.41
	3	8.42±1.02	11.17±1.03
	6	4.33±0.97	5.83±0.95
LSD* ($p \leq 0.05$)		0.82	

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (± SD).

Table 3: Interaction effects of different types of application and sodium selenate concentration on fresh mass of the shoot, number of flowers per plant and flower diameter

Type of application	Sodium selenate concentration mg l^{-1}	Mean		
		Fresh mass of the shoot (g)	Number of flowers per plant	Flower diameter (cm)
Soil	0	68.11±2.22	9.33±0.98	4.47±0.54
	2	67.22±3.14	8.00±0.24	4.41±0.50
	4	71.11±3.01	9.22±0.87	4.79±0.47
	8	71.44±3.04	8.78±0.65	4.86±0.63
Foliar	0	68.44±2.55	9.11±0.87	4.48±0.34
	2	70.89±3.78	9.89±0.69	4.76±0.33
	4	74.44±2.98	11.11±0.67	5.20±0.41
	8	79.33±2.65	12.78±0.66	5.38±0.39
LSD* ($p \leq 0.05$)		1.91	0.95	0.21

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (± SD).

8 mg l^{-1} sodium selenate foliar treatment which was significantly different from other treatments (Table 1). Also, the highest fresh mass (85.67 g) was achieved in zero salinity (control) treatment with applying 8 mg l^{-1} sodium selenate (Table 3). The highest shoot dry weight (39.01 g) was observed in the sodium selenate foliar treatment at the concentration of 8 mg $l^{-1}$and zero salinity (Table 4).

By applying 8 mg l^{-1} sodium selenate in soil, the number of flowers per plant decreased by 5.89 % compared to the treatment without its usage, while the same concentration with foliar application led to an increment of 40.27 % in the mentioned number (Table 3). Salinity stress significantly reduced the flower diameter but this decrease was lower in treatments containing sodium selenate compared to the control one (Table 1). The biggest flower (5.38 cm) was observed in the foliar treatment of sodium selenate at 8 mg l^{-1}, (Table 3). Under the salinity of 6 dS m^{-1}, when using sodium selenate in the soil, the flower's fresh and dry mass increased by 11.34 and 10.39 % compared to the control (no sodium selenate usage).

Respectively, while using sodium selenate in terms of foliar application under these conditions, led to the increments of 25.10 and 25.41 % in the fresh and dry mass of the garden pansy's flowers (Table 4).

3.2 CHLOROPHYLL CONTENT AND ENZYME ACTIVITIES

Salinity of 3 dS m^{-1} resulted in the decreased chlorophyll a and b contents (Table 5). However, under a salinity of 6 dS m^{-1} with 8 mg l^{-1}sodium selenate application, the chlorophyll a content increased by 12.93 % rather than not using it (Table 6). Sodium selenate usage in both soil and foliar applications reduced the negative

Table 4: Interaction effects of different levels of salinity, types of application and sodium selenate concentration on dry massof the shoot, fresh and dry massof the flower

Salinity level (dS m^{-1})	Sodium selenate concentration mg l^{-1}	Mean					
		Dry mass of the shoot (g)		Fresh mass of the flower (g)		Dry mass of the flower (g)	
		Soil	Foliar	Soil	Foliar	Soil	Foliar
0	0	33.05±1.02	33.33±1.24	8.97±0.97	9.30±0.64	3.80±0.4	3.65±0.3
	2	32.21±1.1	34.32±1.64	9.60±0.64	9.70±0.64	3.81±0.4	3.80±0.8
	4	33.33±0.99	36.88±1.34	9.77±0.94	9.90±0.64	3.37±0.5	3.87±0.4
	8	33.61±0.97	39.01±1.33	9.80±0.63	10.10±0.74	3.11±0.6	3.96±0.6
3	0	30.07±0.98	29.93±1.47	8.63±0.87	8.53±0.85	3.28±0.1	3.33±0.7
	2	29.68±1.01	30.5±1.06	8.00±0.74	8.53±0.64	3.23±0.4	3.34±0.7
	4	32.07±1.30	31.06±1.10	8.43±0.68	8.70±0.67	2.27±0.3	3.41±0.6
	8	32.21±1.21	32.91±1.07	8.30±0.74	9.10±0.74	2.22±0.5	3.57±0.3
6	0	24.14±1.24	24.43±1.22	5.73±0.65	5.77±0.63	2.59±0.3	2.28±0.4
	2	23.31±1.50	25.86±1.32	5.67±0.32	5.90±0.72	2.53±0.4	2.34±0.5
	4	24.72±1.37	27.45±1.64	6.60±0.54	6.87±0.63	3.80±0.3	2.72±0.5
	8	24.66±1.68	29.74±1.17	6.47±0.90	7.70±0.60	3.81±0.2	3.06±0.6
LSD* ($p \leq 0.05$)		1.40		0.37		0.12	

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (± SD).

Table 5: Interaction effects of salinity level and different types of application on chlorophyll a and b contents

Trait	Salinity level (dS m^{-1})	Type of application	
		Soil	Foliar
Chlorophyll a (mg g^{-1})	0	0.76±0.05	0.80±0.02
	3	0.62±0.09	0.70±0.03
	6	0.55±0.08	0.61±0.04
(LSD* ($p \leq 0.05$)		0.05	
Chlorophyll b (mg g^{-1})	0	0.26±0.04	0.27±0.04
	3	0.21±0.03	0.24±0.03
	6	0.16±0.01	0.17±0.03
LSD* ($p \leq 0.05$)		0.01	

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (± SD).

effect of salinity on the chlorophyll a and b contents (Table 7). Salinity stress resulted in the significant increase of enzyme's activity (Table 5) and the minimum activity of catalase was observed in the 8 mg l^{-1} sodium selenate in foliar treatment (Table 7). The highest activity of superoxide dismutase was achieved in 6 dS m^{-1} salinity treatment and no sodium selenate application (Table 6). This treatment had no significant difference with that of 2 mg l^{-1} sodium selenate application. Under the salinity stress of 6 dS m^{-1} with 8 mg l^{-1}sodium selenate application, the enzyme's activity reduced by 26.13 % rather than not applying it (Table 6).

3.3 CONCENTRATION OF ELEMENTS

Salinity stress increased Cl^- and Na^+ concentrations of the shoot (Table 8). However, sodium selenate had a moderating effect on them and Cl^- concentration of root and shoot were lower in treatments containing this substance (Tables 8). Cl^- and Na^+ concentrations of the shoot under the salinity of 6 dS m^{-1} with 8 mg l^{-1} application of sodium selenate decreased by 18.65 and 23.92 %, respectively compared to the case of not using sodium selenate (Table 8). K^+ concentration in the shoot significantly decreased with increasing salinity stress (Table

Table 6: Interaction effects of different levels of salinity and sodium selenate concentration on chlorophyll b, catalase and superoxide dismutase enzyme activities

Salinity level (dS m^{-1})	Sodium selenate concentration mg l^{-1}	Mean		
		Chlorophyll a (mg g^{-1})	Catalase activity (μg H_2O_2 $^{-1}$ min^{-1}mg)	Superoxide dismutase (unit mg $protein^{-1}$)
0	0	0.74±0.04	0.55±0.04	12.70±0.17
	2	0.78±0.09	0.55±0.04	13.00±0.32
	4	0.79±0.10	0.54±0.08	13.03±0.14
	8	0.82±0.06	0.54±0.07	13.10±0.17
3	0	0.65±0.04	0.80±0.09	21.60±0.21
	2	0.63±0.11	0.79±0.06	20.27±0.32
	4	0.68±0.09	0.77±0.07	17.65±0.40
	8	0.69±0.08	0.76±0.06	16.28±0.33
6	0	0.54±0.06	0.92±0.07	25.22±0.28
	2	0.55±0.07	0.89±0.06	24.80±0.50
	4	0.62±0.06	0.87±0.08	20.53±0.34
	8	0.62±0.07	0.86±0.06	18.63±0.37
LSD* ($p \leq 0.05$)		0.05	0.01	1.26

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (± SD).

Table 7: Interaction effects of different types of application and sodium selenate concentration on chlorophyll a and b and catalase enzyme activity

Type of application	Sodium selenate concentration mg l^{-1}	Mean		
		chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Catalase activity (μg H_2O_2 min^{-1} mg^{-1})
Soil	0	0.64±0.07	0.21±0.02	0.75±0.05
	2	0.63±0.09	0.20±0.01	0.76±0.04
	4	0.66±0.10	0.21±0.3	0.74±0.03
	8	0.65±0.09	0.21±0.03	0.74±0.05
Foliar	0	0.64±0.08	0.20±0.04	0.75±0.04
	2	0.67±0.12	0.21±0.02	0.72±0.03
	4	0.73±0.11	0.24±0.02	0.71±0.03
	8	0.77±0.06	0.25±0.03	0.70±0.02
LSD* ($p \leq 0.05$)		0.059	0.01	0.01

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (± SD).

9), but sodium selenate application increased this element's concentration (Table 10). Soil and foliar applications with 8 mg l^{-1}sodium selenate led to the increment in the K^+ concentration of shoot to the amounts of 4.95 % and 22.62 %, respectively (Table 10). It was observed the salinity stress decrease the concentration of P^{+3} in the shoot (Table 9). Applying selenium sodium in the soil and its foliar application at the concentration of 8 mg l^{-1}, increased the concentration of Se in the shoot compared to the control by 63.72 and 68.10 %, respectively (Table 10).

4 DISCUSSION AND CONCLUSION

It was observed that the salinity stress significantly decreases the plant height of garden pansy but this decrement is lower in sodium selenate containing treatments and its higher levels improved the height and reduced the

Table 8: Interaction effects of different levels of salinity and sodium selenate concentration on Cl^- and Na^+ concentration in shoot

		Mean	
		Shoot	
Salinity level (dS m^{-1})	Sodium selenate concentration mg l^{-1}	Cl^- (mg g^{-1})	Na^+ (mg g^{-1})
0	0	13.17±0.33	1.58±0.16
	2	12.67±0.24	1.67±0.10
	4	13.17±0.22	1.63±0.09
	8	13.17±0.19	1.70±0.2
3	0	20.83±0.34	1.98±0.12
	2	21.17±0.42	1.90±0.10
	4	21.17±0.32	1.47±0.13
	8	19.17±0.23	1.43±0.08
6	0	32.17±0.23	2.72±0.08
	2	31.17±0.15	2.40±0.12
	4	30.00±0.17	2.12±0.13
	8	26.17±0.19	2.07±0.11
LSD* ($p \leq 0.05$)		2.15	0.20

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (± SD).

Table 9: Interaction effects of different types of application and salinity level on Cl^- and K^+ concentration in shoot and Cl^- and P^{+3} concentration in root

		Means			
		Shoot		Root	
Type of application	Salinity level (dS m^{-1})	Cl^- (mg g^{-1})	K^+ (mg g^{-1})	Cl^- (mg g^{-1})	P^{+3} (mg g^{-1})
Soil	0	13.00±0.43	24.55±1.1	33.25±0.33	2.63±0.09
	3	21.58±0.32	15.38±0.9	43.83±0.18	2.58±0.08
	6	31.58±0.32	18.96±0.8	79.33±0.32	2.02±0.10
Foliar	0	13.08±0.15	27.69±1.2	32.67±0.43	3.10±0.08
	3	19.58±0.21	16.02±1.0	44.33±0.39	2.56±0.06
	6	28.17±0.27	21.72±0.8	76.58±0.51	1.90±0.06
LSD* ($p \leq 0.05$)		1.52	1.01	1.38	0.23

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (± SD).

influence of the salinity stress. The negative effects of salinity on the plant growth are due to the low osmotic potential of soil solution (osmotic stress), special ionic effects (salinity stress), nutrients imbalance or combination of these factors. Hence, as the plant grows under salinity conditions, its photosynthetic activity decreases and results in a decrement in the shoot height. As the minerals concentration increases, the osmotic pressure of the soil solution increases, thus increasing the amount of energy the plant requires in order to absorb water from the soil, which reduces water absorption, increases respiration and decreases plant height and yield (Malash et al., 2008; Hawrylak et al., 2019). Decrease in the height of other ornamental plants under the salinity stress has also been reported (Mirlotfi et al., 2015; Nofal et al., 2015; Kozminska et al., 2017). Salinity stress significantly decreased the shoot fresh and dry mass, but in the treatments containing sodium selenate, the fresh mass decrement was lower. Under the salinity stress, as the salt concentration increases, the osmotic potential of the solution increases, water absorption and cells' turgor pressure decrease consequently. Water withdrawal from the cells prevents

Table 10: Interaction effects of different levels of type of application and sodium selenate concentration on K and Se concentration in shoot and Cl^- and K concentration in root

		Mean			
		Shoot		Root	
Type of application	Sodium selenate concentration mg l^{-1}	K^+ (mg g^{-1})	Se^+ (mg g^{-1})	Cl^- (mg g^{-1})	K^+ (mg g^{-1})
Soil	0	19.39±0.7	10.44±0.91	53.56±1.05	14.44±1.1
	2	18.73±0.9	12.44±0.82	54.22±1.03	17.56±0.9
	4	19.99±0.8	19.56±0.87	51.33±1.00	18.78±1.0
	8	20.40±0.8	28.78±0.94	49.44±1.07	21.89±1.2
Foliar	0	19.42±0.9	10.56±0.76	53.67±1.10	14.33±0.9
	2	20.08±1.0	13.44±0.86	51.22±1.70	14.33±0.8
	4	22.64±0.8	20.78±0.69	50.44±1.98	16.67±1.02
	8	25.10±1.1	33.11±0.29	49.44±1.76	17.89±1.03
LSD* ($p \leq 0.05$)		1.17	1.30	1.60	1.38

*Least Significant Difference. Data presented are mean values obtained from 8 independent replications (± SD).

them from growing. On the other hand, with shrinking and falling leaves, the source of assimilates production in the plant decreases. Therefore, the amount of material reaching the cells is significantly reduced, which eventually causes both reducing number and size of the cells and consequently reduce the fresh and dry mass of the organs (Rawson et al., 1998). In general, the increasing soil salinity causes a significant reduction in the growth and crop yield. Salinity affects all major processes such as growth, photosynthesis, protein synthesis, lipid metabolism and energy. Further to these, it affects all stages of plant life from germination to biomass and seed productions (Pardia et al., 2004). In this study sodium selenate application improved the growth of garden pansy. Consistent with these results, Turakainen (2007) in a greenhouse experiment showed that selenium-treated potato (*Solanum tuberosums* L.) had higher yield rather than the control, which might be due to the antioxidant effects in delaying the plant aging.

Salinity stress led to the significant decrease in the chlorophyll content of the garden pansy. Also, a significant decrease in the chlorophyll volume with increasing NaCl concentration in other ornamental plants has been previously reported (Bayat et al., 2012; Al Hassan et al., 2015, 2016a, b; Kumar et al., 2017). The reducing photosynthetic pigments appear to be a general response to the salinity stress (Parihar et al., 2015). In addition, salinity stress causes premature leaf aging, chloroplast breakage and reduced chlorophyll content. Chlorophyll content declination results in reduced photosynthesis and plants which maintain more chlorophyll content during the stress, have higher photosynthetic efficiency and are resistant to it (Sharma & Dubey, 2005). Application of selenium as foliar solution increased the chlorophyll a and b content in the garden pansy. Confirming these results, Shahzadi et al. (2017) reported that foliar application of selenium leads to an increment in the chlorophyll content of barely (*Hordeum vulgare* L.). Application of appropriate levels of selenium reduces damage to chloroplasts and thus increases the leaves' chlorophyll content (Chu et al., 2010; Yao et al., 2011; Malik et al., 2012; Wang, 2011). Significant increase has been observed in the catalase enzyme's activity and superoxide dismutase enzyme's activity of garden pansy under salinity stress. Decrease in the active oxygen species within the plants exposed to the drought and salinity stresses has been observed by using selenium in canola (Hasanuzzaman et al., 2011; Hasanuzzaman & Fujita, 2011) and white clover (*Trifolium repens* L.) seedlings (Wang, 2011).

Salinity led to the increment in Na^+ and Cl^- concentration in shoots and root of the garden pansy. Na^+ accumulation in the plants is usually associated with inhibition of enzymatic activities, physiological processes and K^+ concentration decrease as these two elements compete for passing across the membrane's width by carriers (Rodríguez-Navarro, 2000). In addition, K^+ decrement has negative effects on the photosynthesis, osmotic regulation, protein biosynthesis and trigger pressure (Gierth & Mäser, 2007). However, compared to the control, the application of 8 mg l^{-1} selenium with foliar and soil application, led to the K^+ increments of 22.62 and 4.95 % in the shoot, 34.03 and 19.89 % in the root, respectively. Similarly, Pazurkiewicz et al. (2008) reported that the selenium application causes an increment in the K^+ content of maize. The adjustment of the absorption and distribution of some essential elements by selenium is an im-

portant mechanism in the reaction to the antioxidants involved in reducing the levels of reactive oxygen species (Feng et al., 2013). Application of selenium in terms of both soil and foliar applications increased this element's concentration in the garden pansy's shoots but this increment was higher for foliar application. The efficiency decrement of the increasing selenium in the plant by its soil application might be due to less plant access to this element in the soil. Confirming the results, Wanga et al. (2013) reported that both foliar and soil applications of selenium have positive effect on increasing selenium concentration in some plants without any negative on other nutrients. Furthermore, they reported that foliar application of selenium is more effective than the soil counterpart.

Salinity stress significantly reduced the plant's height, number and diameter of the flower, dry and fresh mass of the shoot, and flower, chlorophyll content, P^{+3} and K^{+} concentrations of the garden pansy but the activity of antioxidant enzymes (catalase and superoxide dismutase) increased under these conditions. The sodium selenate application was observed to reduce the influences of salinity stress on the investigated traits of the garden pansy. The sodium selenate foliar application at the concentration of 8 mg l^{-1}, was the best treatment for increasing the shoot growth as well as flower growth under the salinity stress.

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Effects of spermine and putrescine polyamines on capsaicin accumulation in *Capsicum annuum* L. cell suspension cultures

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Effects of spermine and putrescine polyamines on capsaicin accumulation in *Capsicum annuum* L. cell suspension cultures

Abstract: This study examined the effects of different concentrations of spermine (Spm) and putrescine (Put) elicitors on capsaicin production at different times in cell suspension culture of peper (*Capsicum annuum* L'Kahramanmaraş Hat-187'.), raised from pepper seeds. Callus was obtained from hypocotyl explants of pepper seedlings germinated *in vitro* conditions, and cell suspensions were prepared from calluses. Spm (0.1, 0.2 and 0.4 mg l^{-1}) and Put (0.1, 0.2 and 0.4 mg l^{-1}) elicitors were applied on cell suspensions, and control groups free from elicitor treatment were created. The amount of capsaicin in cells was found to be higher in the control groups and samples treated with Spm elicitors when compared to filtrates. The highest increase in the capsaicin amount in cells was determined on day 12 of elicitation with 0.2 mg l^{-1} Spm application. The highest capsaicin amount passing into the filtrate was determined as 0.1 mg l^{-1} Spm on day 8. The most effective Put concentration and time on capsaicin amount were found as 0.2 mg l^{-1} Put on day 12 in both cells and filtrates. The highest total capsaicin was also determined in the 0.2 mg l^{-1} Spm application on day 12 with 312.747 ± 8.70 µg g^{-1} of culture. Exogenous treatment of Spm and Put elicitors affected capsaicin accumulation.

Key words: capsaicin; *Capsicum annuum* L.; cell filtrate; pepper; polyamines

Učinki poliaminov spermina in putrescina na akumulacijo kapsaicina v suspenzijski kulturi celic paprike *Capsicum annuum* L.

Izvleček: V raziskavi so bili preučevani učinki različnih koncentracij spermina (Spm) in putrescina (Put) kot elicitorjev na tvorbo kapsaicina v različnih časovnih intervalih v suspenzijski celični kulturi paprike (*Capsicum annuum* 'Kahramanmaraş Hat-187'. Kalus je bil pridobljen iz izsečkov hipokotila kalic paprike, ki je vzkalila v *in vitro* razmerah, celične suspenzije so bile pripravljene iz kalusov. Spm (0,1; 0,2 in 0,4 mg l^{-1}) in Put (0,1; 0,2 in 0,4 mg l^{-1}) sta bila dodajana kor elicitorja v celične suspenzije, hkrati so bile vzpostavljene kontrolne celične kulture brez elicitorjev. Količina kapsaicina v celicah je bila večja v kontrolnih skupinah in vzorcih tretiranih celic z elicitorjem Spm kot pa v filtratu. Največje povečanje kapsaicina v celicah je bilo določeno po 12 dneh elicitacije z dodatkom 0,2 mg l^{-1} Spm. Največja količina kapsaicina v filtratu je bila določena osmi dan pri dodajanju 0,1 mg l^{-1} Spm. Najbolj učinkovita koncentracija Put, 0,2 mg l^{-1}, za tvorbo kapsaicina je bila 12 dni po dodajanju, v celicah kot v filtratu. Največja celokupna vsebnost kapsaicina, 312,747 ± 8.70 µg g^{-1}kulture, je bila določena pri dodajanju 0,2 mg l^{-1} Spm na dvanajsti dan. Zaključimo lahko, da je dodajanje Spm in Put kot elicitorjev vplivalo na akumulacijo kapsaicina.

Ključne besede: kapsaicin; *Capsicum annuum* L.; celični filtrat; paprika; poliamini

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1 INTRODUCTION

Capsaicin is one of the most important alkaloids obtained from pepper plant, and it is used as a food additive due to its bitter aroma. It is used in the preparation of pharmacological compounds and in the treatment of rheumatic diseases as well. At the same time, the tonic and carmine form of *Capsicum* preparations are used for indigestion (Ramachandra Rao & Ravishankar, 2002; Barbero et al., 2008; Hayman & Kam, 2008). It takes 4-5 months to grow the pepper plant to be used for the production of capsaicin under normal conditions and therefore a continuous production process of capsaicin can be carried out over a long period of time and for a limited period of time. Continuous growth of pepper free cell cultures under *in vitro* conditions is a way to provide continuity for capsaicin production. Application of stabilized culturing systems and changing the environmental factors in order to increase the secondary metabolite compounds is accepted as a more efficient and newer method than suspension culturing system. When externally applied chemical elicitors are removed from callus and suspension culture cells, the amount of secondary metabolite compounds synthesized by the cells is also reduced. Therefore, methods have been developed to increase the synthesis of secondary metabolite compounds. Generally, the variety and amount of hormones applied to the culture medium are determinative on the amount of product synthesized (Lindsey, 1985). Two different compounds; salicylic acid and methyl jasmonate were applied separately and together to the suspension cultures of *Capsicum frutescens* L. cells (Sudha & Ravishankar, 2003b) and both compounds were found to increase capsaicin synthesis and accumulation in culture cells. But, only salicylic acid increased the activity of capsaicin synthase enzyme, while methyl jasmonate compound had no effect on enzyme activity. According to these results, it can be concluded that increasing effect of methyl jasmonate on capsaicin concentration is due to its activity preventing capsaicin degradation or conjugation with other molecules. In addition, polyamine production of the cells increased with salicylic acid application and decreased with the application of methyl jasmonate (Sudha & Ravishankar, 2003b).

Putrescine (Put), spermidine (Spd) and spermine (Spm) polyamines are low molecular mass substances present in all living organisms (Vuosku et al., 2018). Polyamines and their biosynthetic enzymes are involved in a wide range of metabolic events ranging from protecting plants against stress to cell organogenesis (Kaur-Sawhney et al., 2003; Puyang et al., 2015). When applied to plants alone, polyamines can be effective in many physiological events such as aging, embryogenesis, root growth, flowering, cell division, nucleic acid and protein synthesis and germination (Chen et al., 2018). Elicitation can be an important strategy to improve *in vitro* production of plant secondary metabolites. It has been previously demonstrated that in cell and organ cultures, biotic and abiotic elicitors have effectively stimulated production of almost all chemical classes of plant secondary metabolites (Brooks et al., 1986; Ramirez et al., 2016). The studies on secondary metabolite production by exogenous polyamine application are insufficient in tissue culture studies. Thus, the aim of this study was to prepare cell suspension cultures from hypocotyl explants of 'Kahramanmaraş-Hat 187' (*Capsicum annuum* L.) pepper seedlings and add different concentrations of Spm and Put into them and the amount of capsaicin accumulating in the samples received from cell suspension cultures (cell+filtrate) were determined. Also, an attempt was made to determine the most effective elicitor concentration and time of application on capsaicin accumulation.

2 MATERIAL AND METHOD

2.1 PLANT MATERIAL

Pepper (*Capsicum annuum* 'Kahramanmaraş-Hat 187') seeds used in the study were provided from Kahramanmaraş Agricultural Research Institute (Turkey).

2.2 STERILIZATION OF SEEDS AND GERMINATION

Pepper (*Capsicum annuum* L.) seeds used in the study were subjected to surface sterilization before sowing to sterile medium in sterile magenta box. They were dipped into 70 % ethyl alcohol for three minutes and then sterilized with commercial 6 % sodium hypochlorite for 30 minutes followed by washing with sterile distilled water in the sterile cabin. Sterilized pepper seeds were placed into each one of Magenta box containing about 50 ml hormone-free Murashige & Skoog (1962) (MS) sterile basic nutrient medium, and the caps of flasks where seeds were germinated were closed and covered by stretch film (Ellialtıoğlu et al., 1998).

2.3 CULTURE CONDITIONS

Pepper seedlings were germinated in the Murashige & Skoog's (MS) medium (Murashige & Skoog, 1962) without hormone and were used as explant source after

completing the four week incubation period under sterile conditions in magenta containers in the growth chamber which was adjusted to 16 hours light (27 µmol m^{-2} s^{-1}) and 8 hours dark photoperiodic order (Figure 1). MS medium containing 1.0 mg l^{-1} *2,4-dichlorophenoxyacetic acid* (2,4-D) 0.1 mg l^{-1} kinetin, 3 % sucrose and 0.7 % agar was used in order to obtain callus from hypocotyl explants. After adjusting the pH of the MS medium to 5.7 and sterilizing that at autoclave, hypocotyl explants were placed horizontally on the medium. The developing callus tissues were taken as subculture into MS nutrient medium containing 1.0 mg l^{-1} 2,4-D, 0.1 mg l^{-1} kinetin, 3 % sucrose and 0.7 % agar. Callus tissues in magenta boxes were developed at the same photoperiodic order in the growth chamber (Figure 1).

2.4 CELL SUSPENSION CULTURE

MS nutrient medium including 1.0 mg l^{-1} 2,4-D with 0.1 mg l^{-1} kinetin and 3 % saccharose that was used for callus development were used also in the suspension culture, only agar was not added to the medium. 2 g callus tissues was added to each 100 ml erlenmeyer flasks containing 40 ml liquid nutrient medium. Erlenmeyer flasks containing the cell suspensions were incubated on a shaker at 100 rpm and 25 °C (Ellialtıoğlu et al., 1998).

2.5 ELICITOR APPLICATIONS

Cell suspension cultures developed for 30 days were transferred to the new nutrient medium, after which elicitor applications were performed and putrescine and spermine were used as elicitor. Trials were repeated in triplicate. Put (0.1, 0.2, 0.4 mg l^{-1}) and Spm (0.1, 0.2, 0.4 mg l^{-1}) were added as elicitors into 100 ml erlenmayers containing cells and 40 ml suspension culture in the sterile flask by means of a sterile micropipette. Sterile distilled water was used for control samples. Following the elicitor treatments, suspension cultures placed on a shakers at 25 °C were subjected to the vacuum filtration process by means of a Buchner funnel vacuum pump over Whatman no.2 filter paper to separate the cell and its liquid phase after 8, 10 and 12th day, and cells and liquid phase samples parts were separated. Samples were stored at -70 °C until further processing.

2.6 CAPSAICIN EXTRACTION

For capsaicin extraction, 0.1 g cells were received and grinded using a mortar in 8 ml ethyl acetate three times. They were then centrifuged at 3000 rpm for 10 minutes and the supernatants on the surface were collected. The collected supernatants were evaporated at 55 °C temperature at 180 rpm under low pressure by using rotary evaporator by ensuring that the ethyl acetate part remained almost dry. The residue was dissolved in 1 ml ethyl acetate and taken into labelled sample bottles. Samples were stored in the deep freezer at -70 °C until they were analysed (Johnson et al., 1990).

2.7 CAPSAICIN ANALYSIS FROM EXTRACTS

Determination of capsaicin was conducted in accordance with Palacio method (1977, 1979). Accordingly, 1 ml ethyl acetate was added to 200 µl sample and 0.1 ml of 5 % $VOCl_3$ were added before measuring on the spectrophotometer. Measurement was carried out quickly after addition of $VOCl_3$ to prevent color loss.

2.8 STATISTICAL ANALYSIS

The trials were organized to create an experimental design with 3 repetitions in randomized blocks. Data presented are mean values ± standard deviation of measurement for 3 replicates. The datas were evaluated with

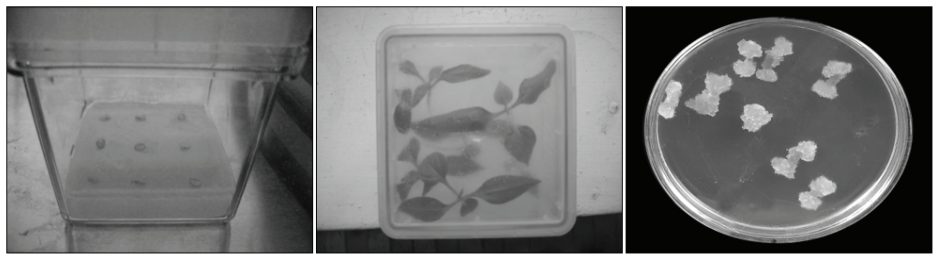

Figure 1: Seeding of pepper seeds in hormone-free MS basic nutrient medium and pepper seedlings in MS basic nutrient medium and callus tissues developing from hypocotyl explant

repeated measures variance analysis technique in factorial order using SPSS 24.0 package program. The alpha level was set at 5 %.

3 RESULTS AND DISCUSSION

3.1 THE EFFECT OF SPM ON ACCUMULATION OF CAPSAICIN

In cells, it was determined that the amount of capsaicin increased in all other applications and days when compared with the control groups except for the application of 0.1 mg l^{-1} Spm on day 8. When compared with the control group, the maximum increase was observed on day 12 in the 0.1 and 0.2 mg l^{-1} Spm applications: the increases in their values were approximately 200 % and 295 %, respectively (Table 1.). When all applications and days were compared, the highest capsaicin amount was determined on day 12 in the 0.2 mg l^{-1} Spm application in cells ($p < 0.05$). This capsaicin amount composed about 90 % of total capsaicin (Table 1.).

The amount of capsaicin passing from cells to filtrate except for 0.4 mg l^{-1} Spm application on day 12 increased when compared to the control group in all Spm applications while the highest capsaicin amount was determined in 0.1 mg l^{-1} Spm application on day 8. Compared to control, the increase in its values was approximately 150 % ($p < 0.05$). The lowest capsaicin amount was determined as 15.359 ± *1.52* µg 40 ml^{-1} culture medium in 0.4 mg l^{-1} Spm application on day 12 ($p < 0.05$) (Table 1).

The highest total capsaicin was determined in the 0.2 mg l^{-1} Spm application on day12 with approximately 312.747 ± 8.70 µg g^{-1} of culture (Table 1). Except 0.4 mg l^{-1} Spm application on day 12, compared to control, all applications increased the total capsaicin amount ($p < 0.05$). This result shows that the application of high concentrations of Spm for a long time may have a stress effect on the cells. The findings showed that the Spm increased the accumulation of capsaicin (Table 1).

3.2 THE EFFECT OF PUT ON CAPSAICIN ACCUMULATION

In cells, the highest capsaicin amount on days 8,10, 12 was determined in control groups. Compared to the control group, all of the elicitor concentrations caused a decrease in the amount of capsaicin ($p < 0.05$) (Table 2.). When all Put applications and days were compared, the maximum capsaicin amount was determined as 37.84 ± 2.53 µg g^{-1} fm in the 0.2 mg l^{-1} Put application on day 8 and as 36.49 ± 3.11 µg g^{-1} fm on day 12. The lowest amount of capsaicin was found in the 0.1 mg l^{-1} Put concentration on days 8 and 10 ($p < 0.05$) (Table 2).

Compared to the control group, all of the Put concentrations caused an increase in the amount of capsaicin in filtrate of cells ($p < 0.05$) (Table 2). The highest amount of capsaicin in the filtrate was determined as

Table 1: Effect of Spm application on capsaicin accumulation in cells and filtrate of *C. annuum* 'KM-187' at different concentrations and days. The data represent the mean of three replications and error bars indicate SD

		Capsaicin		
Days	Applications	Cell (µg g^{-1} fm)	Filtrate (µg 40 ml^{-1} culture medium)	Total (culture) (µg g^{-1})
8	Control	70.204 ± 3.590	22.361 ± 1.925	92.563 ± 5.015
	0.1 mg l^{-1} Spm	40.079 ± 2.102	**50.951 ± 7.411**	91.030 ± 5.588
	0.2 mg l^{-1} Spm	89.709 ± 5.457	28.535 ± 3.309	118.242 ± 6.758
	0.4 mg l^{-1} Spm	97.991 ± 4.880	31.703 ± 3.022	129.695 ± 7.757
10	Control	63.767 ± 1.748	29.406 ± 2.595	93.171 ± 1.017
	0.1 mg l^{-1} Spm	89.943 ± 1.403	29.024 ± 1.996	118.966 ± 0.334
	0.2 mg l^{-1} Spm	67.668 ± 2.026	33.970 ± 4.158	101.637 ± 6.081
	0.4 mg l^{-1} Spm	145.898 ± 6.336	37.667 ± 6.124	183.695 ± 7.696
12	Control	71.966 ± 2.428	25.883 ± 1.683	97.849 ± 2.959
	0.1 mg l^{-1} Spm	**214.306 ± 3.423**	32.810 ± 2.541	**247.116 ± 5.951**
	0.2 mg l^{-1} Spm	**281.824 ± 7.397**	30.930 ± 2.934	**312.747 ± 8.70***
	0.4 mg l^{-1} Spm	73.082 ± 2.676	15.359 ± 1.524	88.442 ± 4.191

Table 2: Effect of Put application on capsaicin amount in cells and filtrate of *C. annuum* 'KM-187' at different concentrations and days. The data represent the mean of three replications and error bars indicate SD (n = 3)

		Capsaicin		
Days	Applications	Cell (μg g^{-1} fm)	Filtrate (μg 40 ml^{-1} culture medium)	Total (culture) (μg g^{-1})
8	Control	40.004 ± 1.313	15.094 ± 1.824	54.098 ± 0.963
	0.1 mg l^{-1} Put	12.636 ± 2.171	15.545 ± 2.490	28.181 ± 5.048
	0.2 mg l^{-1} Put	**37.845 ± 2.538**	32.147 ± 3.309	69.992 ± 8.092
	0.4 mg l^{-1} Put	20.820 ± 2.935	23.928 ± 3.863	44.495 ± 6.217
10	Control	43.492 ± 0.660	16.950 ± 3.635	60.442 ± 3.750
	0.1 mg l^{-1} Put	11.720 ± 2.163	26.479 ± 4.863	38.199 ± 5.829
	0.2 mg l^{-1} Put	31.163 ± 5.230	37.263 ± 4.420	68.426 ± 8.850
	0.4 mg l^{-1} Put	18.136 ± 0.181	31.883 ± 4.450	50.020 ± 4.435
12	Control	44.740 ± 1.094	16.540 ± 0.487	61.280 ± 0.655
	0.1 mg l^{-1} Put	15.586 ± 0.583	20.536 ± 5.334	36.122 ± 5.916
	0.2 mg l^{-1} Put	**36.491 ± 3.110**	**44.287 ± 1.972**	**80.784 ± 4.97***
	0.4 mg l^{-1} Put	23.244 ± 0.520	29.178 ± 3.308	52.422 ± 3.024

44.29 ± 1.97 μg g^{-1} fm in the 0.2 mg l^{-1} Put concentration on day 12; compared to control, the increase in value was 173 % ($p < 0.05$). This capsaicin amount composed about 54.8 % of total capsaicin (80. 784 ± 4.97 μg g^{-1} of culture).The lowest amount of capsaicin was found in control groups ($p < 0.05$) (Table 2). In filtrate of cells, the most effective elicitation concentration and time were 0.2 mg l^{-1}. Put and on day 12, respectively. The highest total capsaicin was determined in the 0.2 mg l^{-1} Put application on day 12 with 80.784 ± 4.97 μg g^{-1} of culture (Table 2).

4 DISCUSSION

Capsaicin biosynthesis takes place through two metabolic pathways as phenylpropanoid pathway and valine metabolic pathway. The first part of the aromatic biosynthesis pathway is commonly shared with the phenylpropanoid metabolism in all plants. This suggests that the exogenous elicitor treatment might forward the metabolic pathway in this direction. The elicitation of secondary metabolites in plant cell cultures has been reported in the majority of plant types (Ramachandra Rao & Ravishankar 2002). Increasing secondary plant metabolites enhances survival, permanence and competitiveness of a plant (Cheong & Choi, 2003; Wasternack & Hause et al., 2013). It is known that various biotic and abiotic elicitors increase the production of phytochemicals in culture cells several times (Zhao et al., 2005; Savitha et al., 2006; Namdeo, 2007; Baenas et al., 2014). The use of elicitors, which can induce the synthesis of these substances in tissues of different plant species under *in vitro* conditions, is considered as an alternative method. Elicitors are molecules that activate the signal transduction cascade and result in activation and expression of genes related to the biosynthesis of secondary metabolites (Zhao et al., 2005). Thus, various elicitors that might stimulate the synthesis of these substances in tissues of different plant types under *in vitro conditions* were addressed, and effective concentration and durations that need to pass after the treatment were determined. Elicitor concentration and induction time are accepted as key factors affecting cell growth and product yield for plant cell suspension culture (Wang et al., 2015). Studies have reported that different elicitors have different effects on capsaicin synthesis. The effect of polyamine compounds on capsaicin accumulation is another subject under investigation. In the study carried out in callus cultures of *Pinus virginiana* Mill. plant, high peroxidase (POX) activity and increase in callus development were determined in brown callus cultures with the addition of exogenous polyamine (Tang et al., 2004). An increase in the growth rate and synthesis of capsaicin was observed as a result of the application of 0.1 mmol l^{-1} Put to the suspension culture medium of *Capsicum frutescens* L. cells (Sudha & Ravishankar, 2003a). In addition, it was found that capsaicin synthase activity increased in Put applied *Capsicum frutescens* cultures. Capsaicin synthase, a terminal enzyme of the capsaicin biosynthetic pathway, catalyses the condensation reaction between vanilyalamine and nonanoic acid

to form capsaicin. It has been suggested that this increase in the amount of capsaicin results from an increase in the activity of capsaicin synthase. Also, Ahern et al. (2006) reported that Spm, Spd and Put are potential ligands for the capsaicin receptor TRPV1 and that they can regulate the activity of TRPV1 through the cationic charges of polyamines. They found that spermine at low concentrations could potentially increase activity and responses. In this study, we studied the production of capsaicin from the callus of *Capsicum annuum*. The findings obtained in our study show that Put elicitor was an increasing effect on capsaicin accumulation. The most effective Put concentration and application time on total capsaicin amount in *C. annuum* 'KM-187' cell suspension culture were determined as 0.2 mg l^{-1} and on day 12. Taking into account all application concentrations and days, the highest increase in total capsaicin amount was determined at 0.2 mg l^{-1} Spm concentration on day 12 in the cell suspension culture (cell + filtrate). The amount of capsaicin in cells was found to be higher in the control groups and samples treated with Spm elicitors when compared to filtrates. The differences in the amount of capsaicin in the control groups for both elicitors may be due to cell suspensions initiated at different times. These results show that elicitor concentration and incubation time play a key role in the increase observed in the capsaicin amount. Therefore, both concentrations and incubation periods of elicitors should be optimized for a maximum elicitation of the capsaicin synthesis.

5 CONCLUSION

As a result of this study, it was found out that the capsaicin amount in cells in the Spm applications was more than in filtrates. The most effective concentration and application time for both elicitors was found to be 0.2 mg l^{-1} and on day 12. These increases in the amount of capsaicin may be due to an increase in capsaicin synthase activity. More detailed studies are needed to put forward the mechanism behind the changes in capsaicin amount. This study showed that Spm and Put can be used to induce capsaicin accumulation in cultured plant cells. There is a need for screening a number of possible various elicitors for their effect on capsaicin synthesis before applying them in industrial scale.

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Polifenoli – med zaščito nevronov in potencialno toksičnostjo

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Polifenoli – med zaščito nevronov in potencialno toksičnostjo

Izvleček: Polifenoli predstavljajo skupino sekundarnih metabolitov, ki jih najdemo v živilih, kot so: sadje, zelenjava, vino, čaj, oljčno olje in čokolada. Te spojine imajo poleg močnih antioksidantivnih lastnosti tudi protivnetne. Številne študije so potrdile njihovo potencialno vlogo pri preprečevanju in zdravljenju različnih patoloških stanj, povezanih z oksidativnim stresom in vnetjem. Mednje sodijo rak, srčno-žilne in nevrodegenerativne bolezni. Slednje globalno predstavljajo enega od glavnih vzrokov smrtnosti in so zato veliko socialno in finančno breme. Številne raziskave so pojasnile nekatere mehanizme delovanja polifenolov kot antioksidativnih in protivnetnih spojin in pojasnile njihovo vlogo pri zdravljenju/preprečevanju določenih bolezenskih stanj. Ugotavljajo, da polifenole lahko uporabljamo kot zaščitne/profilaktične spojine kot tudi terapevtske spojine. Zadostno količino lahko dosežemo z uživanjem prehrane, bogate s polifenoli, v obliki prehranskih dopolnil ali s formulacijami, kot so nutracevtiki. Zdravstveni učinki polifenolov so odvisni tako od zaužite količine kot od njihove biološke razpoložljivosti. Vendar pa lahko njihova čezmerna uporaba povzroči pomisleke glede varnosti zaradi kopičenja teh molekul v organizmu, zlasti če upoštevamo, da so predpisi na področju prehranskih dopolnil zelo ohlapni. Zato se pričujoči pregledni članek osredotoča na poglavitne pozitivne učinke polifenolov, ki izvirajo iz naravnih virov, z vidika zaščite nevronov, obravnava pa tudi možne varnostne pomisleke z vidika nevrotoksičnosti.

Ključne besede: polifenoli; zaščita nevronov; nevrotoksičnost; biološka razpoložljivost; modeli *in vitro*

Poyphenols – between neuroprotection and neurotoxicity

Abstract: Polyphenols are a group of secondary metabolites found in a wide variety of foods, such as fruits, vegetables, wine, tea, olive oil and chocolate. These compounds, in addition to their antioxidant activity, also possess strong anti-inflammatory properties. Numerous studies have therefore confirmed their potential role in preventing and treating various pathological conditions associated with oxidative stress and inflammation. Among these, the most prevalent ones include cancer, cardiovascular and neurodegenerative diseases, which globally represent one of the main causes of death and are therefore a major social and financial burden, Numerous studies have clarified some of the mechanisms of action of polyphenols as antioxidant and anti-inflammatory compounds and have clarified their role in treatment/prevention of certain conditions. It was shown that polyphenols could be used both as protective/prophylactic compounds and as therapeutic compounds. A sufficient amount can be achieved either by consuming a diet, rich in polyphenols, or in the form of dietary supplements and nevertheless with formulations such as nutraceuticals. The health effects of polyphenols depend not only on the amount consumed but also on their bioavailability. However, their overconsumption can cause safety concerns due to the accumulation of these molecules in the body, especially considering that the regulatory legislation in the field of dietary supplements is rather loose. Therefore, this review focuses on the major positive effects of natural-derived polyphenols, and addresses potential safety concerns, with a focus on neuroprotection and neurotoxicity.

Kew word: polyphenols; neuroprotection; nevrotoxicity; bioavailability; models *in vitro*

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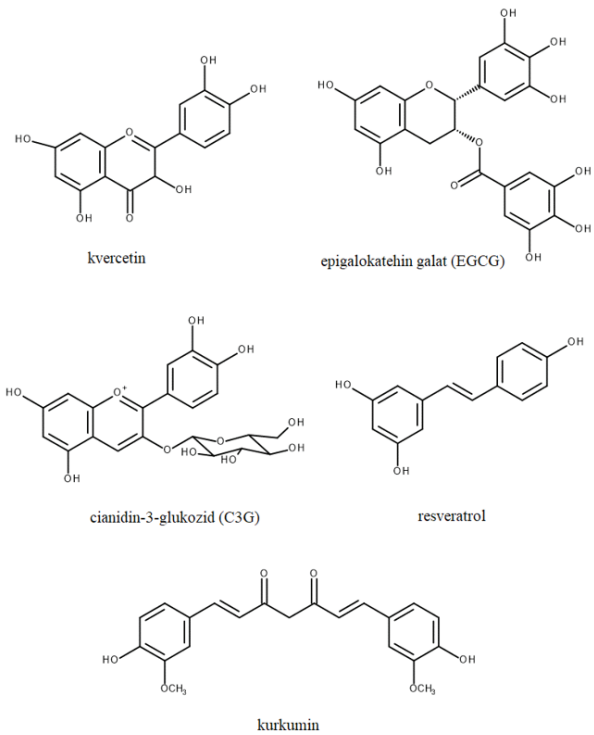
1 UVOD

Polifenoli (polifenolne spojine, fenolne spojine) so naravno prisotna mikrohranila, ki jih rastline nujno potrebujejo za svoj obstoj (Bravo, 1998). Mednje sodijo spojine, zgrajene iz enega ali več fenolnih obročev (Slika 1), najdemo pa jih v številnih živilih, kot so vino, zeleni čaj, grozdje, zelenjava, rdeče sadje in kava (D'Archivio et al., 2007; Manach et al., 2004). Večina polifenolov sodi med močne antioksidante (Noda et al., 2002; Zafra-Stone et al., 2007), imajo pa lahko tudi protivnetne učinke (Fernandes et al., 2006; Yu et al., 2016). Ravno to je pritegnilo pozornost številnih raziskovalcev, ki so dokazali njihovo potencialno vlogo pri preprečevanju in zdravljenju različnih patoloških stanj, povezanih z oksidativnim stresom in vnetnimi procesi. Mednje sodijo močno razširjene bolezni, kot so: rak, srčno-žilne in nenazadnje tudi nevrodegenerativne bolezni, med katere sodita tudi Alzheimerjeva in Parkinsonova bolezen (Abib et al., 2011; Afshari et al., 2019; Hartman et al., 2006; Liu et al., 2016; Poti et al., 2019). Za slednji kronični stanji je značilna predvsem izguba nevronov in nevrodegeneracija. Čeprav so klinične manifestacije nevrodegenerativnih bolezni povezane predvsem s staranjem, velja, da se začetek bolezni in izguba nevronov začneta pojavljati postopoma skozi celotno življenje, precej preden se pojavijo prvi simptomi. Ena glavnih težav pri izbiri terapij pri nevrodegenerativnih boleznih je, da z zdravljenjem pogosto začnemo šele, ko opazimo prve simptome in ko je veliko število nevronov že odmrlo. Ravno zato je zelo pomembno, da poznamo načine, kako ohraniti nevrone zdrave in kako zmanjšati tveganje za nevrodegeneracijo. Novi pristopi za preprečevanje in/ali premagovanje nevrodegeneracije bi dolgoročno lahko povečali zdravje možganov in drugih delov živčevja ter zmanjšali tveganje za nevrodegeneracijo, zato bi s tem imeli velik vpliv ne samo na družbo kot tako, temveč tudi na svetovno ekonomijo.

Nekatere raziskave so v preteklosti pokazale, da ima prehrana lahko ključno vlogo pri ohranjanju zdravja, kar je sprožilo razvoj cele vrste prehranskih dopolnil (Virmani et al., 2013). Kot ena izmed možnih rešitev se tako kaže naravno povečanje notranje obrambe možganov in preprečevanje ali vsaj zmanjšanje začetnih poškodb nevronov, ki vodijo v nevrodegenerativne procese. Ravno zato se je več raziskav osredotočilo na pomen uživanja naravnih proizvodov ali ustreznih prehranskih dopolnil, ki bi lahko zaščitila nevrone pred poškodbami. Običajna človeška prehrana je sicer bogata s polifenoli. Po podatkih Phenol-Explorer (Perez-Jimenez et al., 2010) jih povprečen Evropejec zaužije okrog 1 g na dan. Še posebej dobre vire polifenolov predstavljajo oreščki, čaj, kava, češnje, citrusi, zelenjava, čokolada in rdeče vino, ki so del tako imenovane mediteranske prehrane, ki jo UNESCO



Slika 1: Predstavniki nekaterih polifenolov, vključenih v raziskave zaščite nevronov
Figure 1: Representatives of polyphenols compounds that showed neuroprotection properties

uvršča na seznam nesnovne kulturne dediščine. V različnih državah pa se glavni prehranski viri polifenolov lahko razlikujejo glede na tradicionalne prehranjevalne navade. Tako so v severno in vzhodnoevropskih državah glavni prehranski viri polifenolov predvsem napitki, kot sta kava in čaj (Grosso et al., 2014; Zamora-Ros et al., 2016), medtem ko so v južnoevropskih in sredozemskih državah pomembni prehranski viri oreški, oljčno olje, sadje in zelenjava (Godos et al., 2017; Tresserra-Rimbau et al., 2013). Živilska industrija se je v zadnjem času začela zanimati tudi za stranske produkte, ki nastanejo pri predelavi sadja in zelenjave (npr. oljčne pogače, čebulni listi ipd.), saj so lahko bogat vir polifenolov in jih zato lahko uporabimo kot dodatke v funkcionalnih živilih ali kot prehranska dopolnila (Marranzano et al., 2018). Zanimivo je, da so nekateri polifenoli značilni samo za določeno sadje ali zelenjavo, medtem ko večino lahko najdemo v več virih (D'Archivio et al., 2010). Večina sadja in zelenjave vsebuje več kot eno značilno vrsto polifenolov (Lorenzo et al., 2019). Zavedati pa se moramo, da je količina polifenolov v isti rastlini lahko tudi različna in je odvisna od posameznega dela rastline korenine, stebla, listi, cvet, plod), pa tudi od klimatskih in rastnih razmer (Lorenzo et al., 2019; Marranzano et al., 2018). Ravno zato je zelo težko oceniti dejansko količino naravno zaužitih polifenolov. Vse to je potrebno upoštevati, ko govorimo o pozitivnih učinkih polifenolov na naše zdravje v primerjavi z možnimi negativnimi posledicami, povezanimi s potencialnimi toksičnimi učinki kot posledice njihovega kopičenja v telesu.

2 POZITIVNI UČINKI POLIFENOLOV NA MOŽGANE

Nevrodegenerativne bolezni so patološka stanja, pri katerih se določene skupine nevronov poškodujejo ali odmrejo, kar vpliva na normalno delovanje centralnega živčnega sistema in se izraža s slabšimi kognitivnimi in/ali motoričnimi funkcijami. Mnoge od teh bolezni so običajno povezane s staranjem, pri čemer velja, da se nevrodegeneracija skozi leta pojavlja v subklinični obliki, pri čemer prihaja do odmiranja nevronov postopoma skozi celo življenje, še preden so opazni prvi klinični znaki. Trenutne napovedi kažejo, da se bo število dementnih prebivalcev stalno povečevalo in lahko do leta 2030 prizadene kar polovico starostnikov (Noble & Burns, 2010). Številne raziskave (Glass et al., 2010; Lee et al., 2003; Ono et al., 2003; Weinreb et al., 2004) so proučevale celične mehanizme nevrodegeneracije pri več patoloških stanjih, kot so Alzheimerjeva, Parkinsonova in Huntingtonova bolezen, pa tudi amiotrofična lateralna skleroza. Vendar zaenkrat še ni na voljo učinkovitih terapij za zdravljenje teh bolezni, z določenimi zdravili lahko le zmanjšamo ali začasno zavremo njihove simptome.

Kljub specifičnim kliničnim slikam pa ima veliko teh bolezni nekatere skupne mehanizme, med katerimi prevladujeta nevronsko vnetje (Amor et al., 2010) in oksidativni stres (Glass et al., 2010; Tarawneh & Galvin, 2010). V preglednem članku (Calabrese et al., 2007) je bil zato natančno predstavljen pomen zmanjšanja ekspresije oksidativno-stresnih regulatornih genov pri staranju in nevrodegeneraciji, pa tudi možna zaščita z antioksidanti. Ravno zato so pristopi, ki lahko upočasnijo ali preprečijo nastanek bolezni in preprečujejo nevrodegeneracijo, enako pomembni kot tisti, ki so namenjeni zdravljenju teh bolezni. Pri tem je bila prehrana prepoznana kot pomembna, kar je vodilo v celo vrsto raziskav, osredotočenih na pomen uživanja izdelkov, tako v obliki živil kot v obliki prehranskih dopolnil, ki lahko zaščitijo nevrone (Costa et al., 2016; Solanki et al., 2015).

Biološka aktivnost molekul, ki izvirajo iz živil, je bila prvič prepoznana v poznih 40. letih prejšnjega stoletja, ko sta Schraufstatter in Bernt (Schraufstatter & Bernt, 1949) dokazala antibakterijsko delovanje kurkumina. Kasneje so pritegnili pozornost tudi drugi prehranski polifenoli, zlasti resveratrol, ki je bil prepoznan kot možni razlog za povezavo med uživanjem rdečega vina v Franciji in nizko stopnjo srčno-žilnih bolezni (Sun et al., 2002). Zasluge za pozitivne učinke lahko pripišemo antioksidativnim lastnostim prehranskih polifenolov, v tem primeru resveratrola.

V literaturi pa je je opisanih tudi več primerov polifenolov, ki kažejo obetavne sposobnosti preprečevanja in zdravljenja nevrodegenerativnih bolezni (Gray et al., 2018; Lorenzo et al., 2019; Mani et al., 2018; Palazzi et al., 2018; Ullah & Khan, 2018; Ulusoy & Sanlier, 2019). Veliko raziskav je bilo ravno tako narejenih na resveratrolu, za katerega je bilo ugotovljeno, da lahko zaščiti nevrone v študijah *in vitro* (Granzotto & Zatta, 2014; Sun et al., 2002; Zhang et al., 2013). Zanj je bilo tudi dokazano, da sodeluje pri zmanjšanju nevronskega vnetja, ki ga povzroča mikroglija, pri varovanju možganov pred hipoksično-ishemičnimi poškodbami in pri izboljšanju kognitivnih sposobnosti v modelu Alzheimerjeve bolezni (Granzotto & Zatta, 2014; Sun et al., 2002; West et al., 2007; Zhang et al., 2013). Zdi se, da zmanjšuje tudi s staranjem povezano zmanjšanje kognitivnih zmožnosti in poveča kognitivno funkcijo z modulacijo SIRT1 (Cao et al., 2018). Opisani pa so tudi še drugi možni mehanizmi zaščite nevronov (Menard et al., 2013; Pan et al., 2019).

Obstaja še več drugih študij, ki so vključevale polifenole, zlasti tiste iz rdečega vina ali zelenega čaja (Mandel et al., 2006), in so se osredotočile na njihovo vlogo pri zaščiti nevronov pri različnih nevrodegenerativnih boleznih. Med njimi je tudi študija, ki je dokazala zaščito

nevronov z epigalokatehin galatom (EGCG) pred nevrotoksičnimi učinki β-amiloidnih proteinov (Zhang et al., 2014). Ista molekula je kazala tudi sposobnost, da s povečanjem LC3-II (Lee et al., 2015) zavira translokacijo Baxa in citokroma c, lahko pa tudi modulira mitohondrijsko funkcijo (Oliveira et al., 2016). Dokazano je bilo tudi, da lahko EGCG z lahkoto prestopa model človeške krvno-možganske membrane (BBB) in zaščiti nevrone pred celično smrtjo kot posledico oksidativnega stresa *in vitro* (Pogačnik et al., 2016). Podobna študija na živalskem modelu BBB je prav tako pokazala, da bi lahko nekateri ostali flavonoidi lahko dosegli možgane (Faria et al., 2014), ni pa še povsem jasno, kakšni so mehanizmi zaščite nevronov.

Potrebno pa je poudariti, da se pozitivna vloga polifenolov po svoji intenzivnosti razlikuje tudi glede na izvor in vrsto živila. Na primer, pri modelu podgan z inducirano Alzheimerjevo boleznijo je bilo ugotovljeno, da je bila boljša zaščita nevronov dosežena z dodatkom zelenega čaja kot s črnim čajem ali rdečim vinom (Schimidt et al., 2017). V podobnem mišjem modelu je sok granatnega jabolka ?vplival na zmanjšanje odlaganja amiloidov in istočasno izboljšal rezultate testov vedenja (sposobnost učenja in pomnjenja v Morrisovem vodnem labirintu in hitrost plavanja) živali (Hartman et al., 2006). Zanimiva je nedavna objava, v kateri je zapisano, da je z dodatkom borovnic v hrano podgan mogoče zmanjšati vnetno reakcijo (Willis et al., 2010), še zlasti pri starejših osebkih. Opazili pa so tudi zaščito nevronskih celic pred oksidativnim stresom in zmanjšano aktivacijo mikroglije (Garcia et al., 2017). Na modelu Parkinsonove bolezni pa so bili doseženi podobno dobri rezultati s kurkuminom (Mythri & Bharath, 2012), pa tudi z nekaterimi zdravilnimi rastlinami, ki se uporabljajo v tradicionalni medicini, kot je npr. vrsta *Centella asiatica* (L.) Urban, s katero so dosegli tako zmanjšanje mitohondrijske disfunkcije kot tudi oksidativnega stresa. Z uporabo ekstraktov iste rastline je prišlo tudi do izboljšanja kognitivne zmožnosti pri Alzheimerjevi bolezni *in vivo* (Gray et al., 2018).

Poleg opisanih primerov naraščajoče število raziskav kaže na možnost epigenetske modulacije preko uživanja polifenolov, in sicer le-ti lahko vplivajo na modulacijo pro- in protivnetnih mikroRNK (Lee et al., 2015; Tili & Michaille, 2016). Nedavni pregledni članek (Boyanapalli & Tony Kong, 2015) je izpostavil epigenetsko modulacijo kurkumina, vključno z inhibicijo DNK metiltransferaz, regulacijo sprememb histona z regulacijo histon acetiltransferaze in histonske deacetilaze (HDAC), pa tudi z regulacijo mikroRNA.

Na podlagi opisanih izsledkov raziskav lahko zaključimo, da je vključitev polifenolov v prehrano ali njihova uporaba v obliki prehranskih dopolnil ali nutracevtikov obetavna pri preprečevanju več različnih patologij, med katere sodijo tudi nevrodegenerativne bolezni. Obsežen seznam vseh raziskanih bolezenskih stanj (depresija, ekscitotoksičnost glutamata, epilepsija, motnja sluha in vida ter nevrodegenerativne bolezni), pa tudi študije *in vitro*, *ex vivo* in *in vivo*, pri katerih so bili ovrednoteni mehanizmi delovanja polifenolov, je podan v preglednem članku Szwajgier et al. (2017). Do podobnih zaključkov so prišle tudi zanimive epidemiološke študije na splošni populaciji, na kateri so ugotavljali povezavo med prehranskimi polifenoli in depresivnimi simptomi (Chang et al., 2016; Godos et al., 2018).

Opisane potencialne koristne lastnosti polifenolov, bodisi kot zaščitnih/profilaktičnih spojin bodisi kot terapevtskih molekul, lahko dosežemo tako z uživanjem naravne prehrane, bogate s polifenoli, kot tudi z uporabo prehranskih dopolnil ali v obliki nutracevtikov. Dokazano je bilo tudi, da so učinki polifenolov na zdravje odvisni tudi od zaužite količine in njihove biološke razpoložljivosti (Tresserra-Rimbau et al., 2018). Po drugi strani pa prekomerno uživanje polifenolov lahko povzroči pomisleke glede varnosti zaradi kopičenja velikih količin teh molekul v organizmu, zlasti če upoštevamo ohlapno zakonodajo na področju prehranskih dopolnil.

3 MODELI ZA UGOTAVLJANJE VLOGE POLIFENOLOV V TELESU

3.1 PREBAVNI MODEL

Biološka razpoložljivost polifenolov je ključna za njihove pozitivne/negativne učinke na človeško telo. Nekatere raziskave kažejo, da je le-ta majhna (Pandareesh, Mythri, & Srinivas Bharath, 2015), opisani pa so bili že tudi potencialno toksični učinki, kadar so bile učinkovine uporabljene v velikih koncentracijah. Prebavni modeli *in vitro* so zato zelo pomembni del raziskav za preučevanje strukturnih sprememb, prebavljivosti in sproščanja sestavin hrane v simuliranih pogojih prebave. Najpogosteje uporabljene biološke molekule, vključene v prebavne modele, so prebavni encimi (pankreatin, pepsin, tripsin, kimotripsin, peptidaza, α-amilaza in lipaza), žolčne soli in mucin, temperatura prebave pa je 37 °C. Najpogosteje uporabljeni modeli simulirajo prebavo v želodcu in v tankem črevesu. Želodčna faza se začne z dodatkom raztopine pepsina in uravnavanjem pH na 2,0 po inkubaciji pri 37 °C v pokriti stresni vodni kopeli 1 uro. Faza tankega črevesja se začne s uravnavanjem pH na 5,3, sledi dodajanje encimske raztopine tankega črevesa (lipaze, pankreatina in žolčnih soli). Končni pH vzorca je uravnan na 7,5, čemur sledi 2-urna inkubacija pri 37 °C. Vzorce med prebavo lahko večkrat analiziramo s sistemom HPLC/DAD, da ugotovimo pretvorbe bioak-

tivnih spojin (polifenolov) (Hur et al., 2011; Minekus et al., 2014).

3.2 MODEL KRVNO-MOŽGANSKE PREGRADE (BBB)

Glede na to, da je bila na celičnih modelih dokazana zmožnost polifenolov, da zaščitijo nevrone, je ključno vedeti, ali izbrane spojine dosežejo ciljni organ (možgane), saj so lahko samo na ta način učinkovite. Večina modelov *in vitro* uporablja poenostavljen *in vitro* model, ki posnema lastnosti človeške BBB, in je sestavljen iz monoplastnih mikrovaskularnih endotelijskih celic človeških možganov (HBMEC) (Bernas et al., 2010). Te celice je mogoče gojiti na porozni membrani, pri čemer dobimo model z dvema predeloma, zgornji predstavlja krvni obtok, spodnji pa možgansko tkivo. Prisotnost molekul v spodnjem predelu kaže na to, da so le-te zmožne preiti BBB in so tako razpoložljive za možgane (Deli et al., 2005; Faria et al., 2012; Faria et al., 2010). Pri tem pa je seveda treba biti pozoren na to, da ne pride do poškodovanja HBMEC, kar se zagotovi z meritvami električne prevodnosti in prehajanja fluorescentno označenega barvila.

3.3 CELIČNE KULTURE

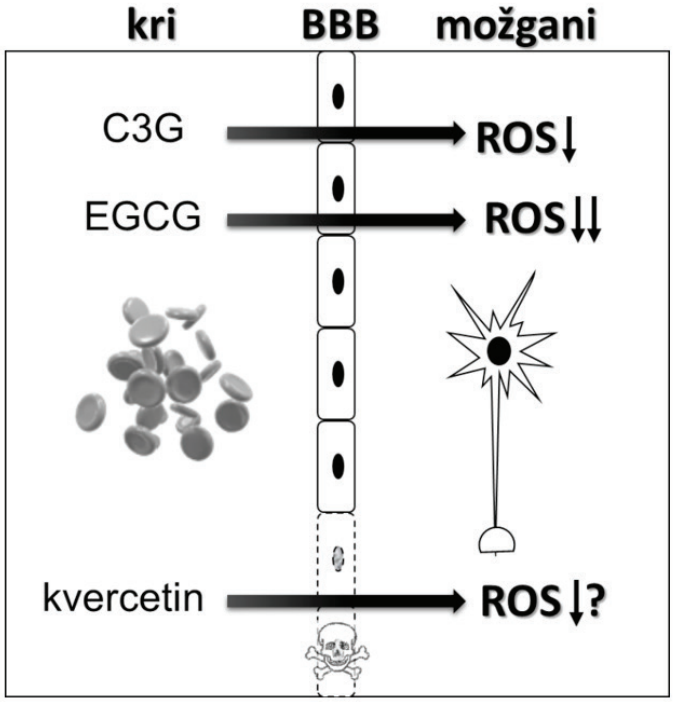
Zmožnost polifenolov, da zaščitijo nevrone (ali njihovo nevrotoksičnost), lahko ugotavljamo z različnimi modeli *in vitro*. Kot preprost model lahko uporabimo primarne nevronske celične kulture iz možganov podgan, ki veljajo za najbolj dovzetne za nevrodegenerativne spremembe (Lee et al., 2003; Pogačnik et al., 2016; Zhang et al., 2013). Večinoma se pri teh raziskavah uporablja astrocite in mikroglije, ki so glavni protagonisti možganskega imunskega odziva (Capiralla et al., 2012; Garcia et al., 2017; Nones et al., 2010; Willis et al., 2010; Yamamoto et al., 2017). Celični stres za posnemanje patoloških stanj v kontrolnih celicah in celicah, ki jih tretiramo s polifenoli, je potrebno izbrati glede na bolezen, ki jo želimo inducirati. V ta namen se uporabljajo: izpostavljenost oksidativnemu okolju (Aquilano et al., 2008), MPP + (široko uporabljani Parkinsonov induktor) (Anandhan et al., 2013; Mani et al., 2018), Aβ-peptidi za AD (Zhang et al., 2013) ali bakterijski lipopolisaharid (LPS) kot induktor nevronskega vnetja (Capiralla et al., 2012). Z uporabo teh modelov lahko ocenimo celično smrt in njene specifične mehanizme (apoptoza, nekroza, nekroptoza, avtofagija) ter parametre in poti oksidativnih poškodb, vnetnega odziva in epigenetskih sprememb.

Najobetavnejše rezultate, pridobljene na modelih *in vitro*, je potrebno preveriti tudi z modeli *in vivo*, na primer na toksikološko tretiranih glodavcih (Pan et al., 2019; Rasheed et al., 2018) ali glodavcih s specifičnimi boleznimi (Gray et al., 2018; Schimidt et al., 2017), ki jih lahko hranimo z običajno hrano, s polifenolno bogatimi rastlinskimi ekstrakti ali pa izolirano bioaktivno molekulo polifenola. Te raziskave so pomembne tudi za oceno stranskih učinkov *in vivo* in za ugotavljanje varne uporabe kateregakoli preiskovanega polifenola (Charradi et al., 2018; Hu et al., 2018; Liu et al., 2017; Shen et al., 2017). Rezultate študij *in vitro* in rezultate na živalskih modelih pa je potrebno potrditi tudi s človeško pilotno študijo, v kateri je mogoče spremljati koristne in škodljive učinke (Molino et al., 2016).

4 VARNOST V SVETU POLIFENOLOV

Zaenkrat se je le malo raziskav osredotočilo na varno uporabo polifenolov za preprečevanje in zdravljenje bolezni, čeprav je to ključnega pomena za nadaljnje spodbujanje njihove uporabe za zagotavljanje zdravja ljudi. V naši študiji (Pogačnik et al., 2016) smo ocenili tri strukturno različne flavonoidne polifenole: kvercetin, (eden izmed najbolj zastopanih flavonoidov v živilih), monomerni flavanol epigalokatehin galat EGCG (najdemo ga v nekaterih semenih stročnic, v grozdju in v zelenem čaju (Afzal et al., 2015; Rezai-Zadeh et al., 2008)) in antocianin cianidin-O-3-glukozid (C3G), ki ga najdemo kot pigment v številnih rdečih jagodah, zlasti v borovnicah (Crozier et al., 2009; Kelly et al., 2017). Ti polifenoli so pokazali zmerno do veliko antioksidativno učinkovitost z antioksidativnimi testi *in vitro*, več avtorjev pa je ugotovilo, da jih je mogoče po peroralni uporabi določiti v plazmi v relativno velikih koncentracijah (Egert et al., 2008; Kay et al., 2005; Mereles & Hunstein, 2011). Vsi trije polifenoli so v naši študiji (Pogačnik et al., 2016) pokazali raznolik profil glede na preučevane parametre. Medtem ko je EGCG pokazal zmerno nevrotoksičnost, je bila to molekula, ki je najmanj poškodovala model BBB in je najbolj učinkovito zaščitila primarne podganje nevrone pred nekrozo in apoptozo, povzročeno z oksidativnim stresom. Po drugi strani je C3G sicer kazal zelo majhno nevrotoksičnost, a je primarne podganje nevrone zaščitil le pred nekrozo, ne pa tudi pred apoptozo; poleg tega je že pokazal rahlo destabilizacijo modela BBB. In končno, kljub številnim obetavnim raziskavam (Carrasco-Pozo et al., 2019; Dajas et al., 2015; Zhu et al., 2013), je kvercetin pokazal najslabši profil, saj je močno poškodoval model BBB, bil je zmerno do zelo nevrotoksičen, medtem ko nevronov praktično ni zaščitil pred oksidativnim stresom (Slika 2).

Kot že omenjeno, je le malo raziskav osredotočenih



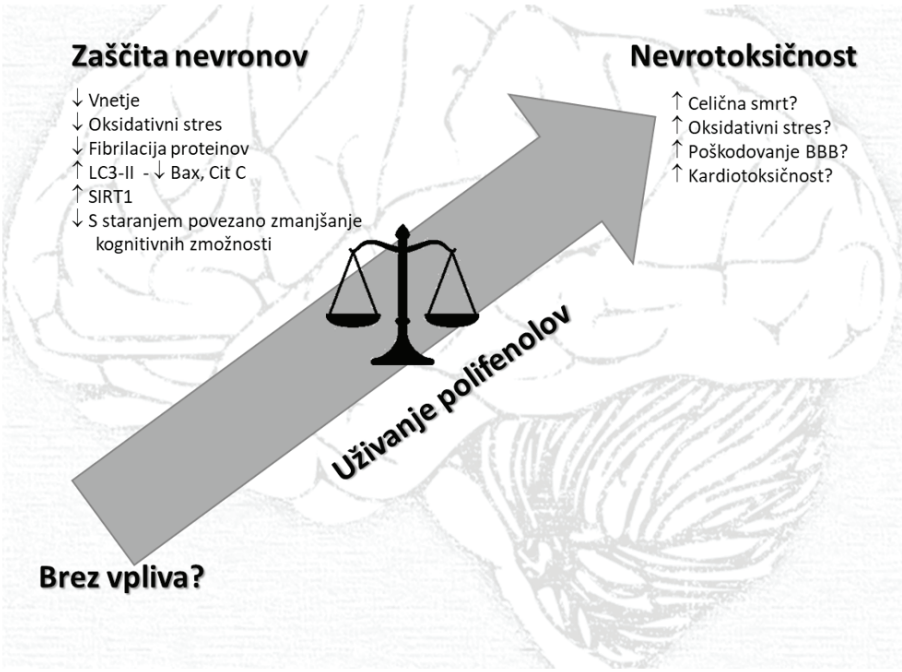
Slika 2: Shema prehajanja izbranih polifenolov (cianidin-3-glukozid (C3G), epigalokatehin galat (EGCG) in kvercetin) preko modela krvno-možganske pregrade (BBB) in njihov vpliv na zaščito primarnih podganjih nevronov pred reaktivnimi kisikovimi spojinami (ROS).
Figure 2: Schematic presentation of blood-brain barrier (BBB) model, crossed by selected polyphenols compounds (cyanidine-O-3-glucoside (C3G), epigallocatechin gallate (EGCG) and quercetin) and their neuroprotection against reactive oxygen species (ROS).

na toksičnost oziroma varnost uživanja polifenolov. Nekatere študije navajajo, da je redno uživanje zelenega čaja in ekstraktov zelenega čaja varno (Shen et al., 2017) zlasti v obliki tradicionalne infuzije (Liu et al., 2017; Wang et al., 2012), vendar svarijo pred uporabo koncentriranih ekstraktov z velikim odmerki posameznih sestavin v trdni obliki. Zato bodo potrebne dodatne študije, da se zagotovi njihova varna uporaba (Hu et al., 2018). Rezultati raziskav kažejo, da je neposredna uporaba zmernih odmerkov resveratrola varna in lahko zaščitijo srce (Johnson et al., 2011). Uporaba dodatka resveratrola (Longevinex) v študiji na živalih ni povzročila škodljivih učinkov, kar kaže na koristne učinke in varno uporabo (Sangeetha et al., 2013). Uživanje ekstrakta grozdnih pečk je bilo varno za zdrave podgane tudi v relativno velikih ponavljajočih se odmerkih. Pokazali so se tudi antioksidativni in protivnetni učinki (Charradi et al., 2018). Nasprotno pa so rezultati raziskave, kjer so diabetičnim mišim intraperitonealno vbrizgavali velike odmerke EGCG, pokazali na kardiotoksičnost (Rasheed et al., 2018).

Učinkovitost polifenolov je mogoče izboljšati z njihovo vključitvijo v nove farmacevtske formulacije, s katerimi bi jih tarčno dostavljali. S tem bi se izognili potencialnim škodljivim učinkom, kot je pokazala nedavna študija z uporabo kurkumina v obliki nanomicelijske trdne disperzije (Parikh et al., 2018). Vendar pa se moramo zavedati, da imamo na trgu slabo regulirana komercialna prehranska dopolnila in nutracevtike, kar je lahko problematično z vidika njihove varne uporabe, kar je poudarjeno tudi v preglednem članku, v katerem so avtorji sistematično pregledali medicinske preparate na osnovi granatnega jabolka in njihovo uporabo pri zdravljenju rakavih obolenj (Vlachojannis et al., 2015).

5 ZAKLJUČEK

Polifenoli so obetavne spojine za preprečevanje in potencialno zdravljenje številnih človeških patologij, med katere sodijo tudi nevrodegenerativne bolezni. Potrebno je poudariti, da je študij vpliva naravnih spojin na človeško telo zelo kompleksen, saj je pri tem potrebno upoštevati številne faktorje. Obstajajo namreč bistvene razlike med delovanjem čistih učinkovin in učinkom naravnih izvlečkov, ki vsebujejo mnoge spojine, ki lahko med seboj vplivajo tudi sinergistično. Pomembni pa so



Slika 3: Prikaz glavnih mehanizmov zaščite nevronov s polifenoli z možnimi varnostnimi pomisleki, ki bi bili lahko posledica prevelikih zaužitih količin
Figure 3: Presentation of main polyphenol neuroprotection mechanisms with potential toxic effects caused by overconsumption.

tudi učinki njihovih modificiranih ali razgradnih produktov, ki nastajajo v procesu prebave in/ali prenosa po telesu. Poleg že omenjenega prehoda preko BBB ne smemo spregledati prehoda spojin iz prebavil v krvni obtok, pomemben pa je tudi njihov vpliv na spolne celice ali zarodek. Zavedati se je tudi potrebno, da vpliv posameznih spojin ni vedno neposreden, saj v nekaterih primerih niso potrebne velike količine učinkovine na tarčnem mestu.

Vse to bo potrebno upoštevati v nadaljnjih raziskavah, ki bodo usmerjene v ugotavljanje morebitnih škodljivih stranskih učinkov, zlasti zaradi njihovega potencialnega kopičenja v organizmu (Slika 3). Potrebnih bo še več študij, da bi ugotovili, kakšna je povezava med količino zaužitih polifenolov in njihovimi varnimi plazemskimi koncentracijami. Dokler ne bodo opravljene te raziskave, je priporočljivo uživanje živil bogatih s polifenoli, kot so npr. sadje, zelenjava, čaj in kava. Prekomerna uporaba prehranskih dopolnil, ki vsebujejo velike količine polifenolov, pa je še vedno slabo regulirana s strani zakonodaje, kar lahko povzroči previsoke vsebnosti teh spojin v organizmu in večje tveganje za škodljive učinke. Kljub temu so takšna dopolnila lahko koristen vir, kadar je normalno prehranjevanje onemogočeno ob določenih bolezenskih stanjih ali dietah.

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Možnosti zatiranja izbranih plevelnih vrst v Evropi z žuželkami

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Možnosti zatiranja izbranih plevelnih vrst v Evropi z žuželkami

Izvleček: Zatiranje plevelov z žuželkami je vse bolj pomembno, saj ima kemično zatiranje plevelov (uporaba herbicidov) velik vpliv na okolje in posledično tudi na organizme, ki v njem živijo. Uporaba žuželk za zatiranje plevelov tako predstavlja alternativo herbicidom. V članku so predstavljene možnosti zatiranja nekaterih razširjenih in trdovratnih plevelov v Evropi z njihovimi naravnimi sovražniki – žuželkami. Predstavljene so naslednje kombinacije najpogostejših plevelov in njihovih naravnih sovražnikov: topolistna kislica - ščavje (*Rumex obtusifolius* L.) – *Gastrophysa viridula* (De Geer, 1775), kodrastolistna kislica (*Rumex crispus* L.) – *Apion violaceum* (Kirby, 1808), pelinolistna ambrozija (*Ambrosia artemisiifolia* L.) – *Ophraella communa* (LeSage, 1986) in *Zygogramma suturalis* (Fabricius, 1775), njivski osat (*Cirsium arvense* (L.) Scop.) – *Cassida rubiginosa* (Müller, 1776), plezajoča lakota (*Galium aparine* L.) – *Halidamia affinis* (Fallen, 1807) in *Sermylassa halensis* (Linnaeus, 1767), ptičja dresen (*Polygonum aviculare* L.) in navadni slakovec (*Fallopia convolvulus* L.) – *Gastrophysa polygoni* (Linnaeus, 1758) ter na koncu še njivski slak (*Convolvulus arvensis* L.) – *Galeruca rufa* (Germar, 1824) in *Tyta luctuosa* (Denis in Schiffmuller, 1775).

Ključne besede: pleveli; biotično zatiranje; žuželke; rastlinojede žuželke; povezava žuželka – gostiteljska rastlina; odziv gostitelja; ovipozicija

Potential of controlling selected weeds in Europe with insects

Abstract: Weed control by insects is increasingly important, as chemical weed control (the use of herbicides) has an important impact on the environment and, consequently, on all organisms living there. The use of insects to control weeds thus represents an alternative to herbicides. The article presents the suppression of some widespread and persistent weeds in Europe with their natural enemies - insects. The following combinations presented below are: broad-leaved dock (*Rumex obtusifolius* L.) – *Gastrophysa viridula* (De Geer, 1775), curly dock (*Rumex crispus* L.) – *Apion violaceum* (Kirby, 1808), common ragweed (*Ambrosia artemisiifolia* L.) – *Ophraella communa* (LeSage, 1986) and *Zygogramma suturalis* (Fabricius, 1775), creeping thistle (*Cirsium arvense* (L.) Scop.) – *Cassida rubiginosa* (Müller, 1776), cleavers (*Galium aparine* L.) – *Halidamia affinis* (Fallen, 1807) and *Sermylassa halensis* (Linnaeus, 1767), common knotgrass (*Polygonum aviculare* L.) and black-bindweed (*Fallopia convolvulus* L.) – *Gastrophysa polygoni* (Linnaeus, 1758) and as the last one field bindweed (*Convolvulus arvensis* L.) – *Galeruca rufa* (Germar, 1824) and *Tyta luctuosa* (Denis in Schiffmuller, 1775).

Key words: weeds;, biological control; insects; phytophagous insects; relation insect - host plant; host plant response; oviposition

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1 UVOD

Bistvo biotičnega zatiranja plevela z žuželkami je, da skušamo zatreti ciljno plevelno vrsto z vnosom žuželke, primerne glede na gostiteljsko rastlino. Klasično biotično zatiranje plevela je omejeno predvsem na monofagne vrste žuželk (to so tiste, ki se hranijo in preživijo samo na eni vrsti rastlin). Vendar pa pri zatiranju plevelov z žuželkami obstajata dve tveganji. Prvo je, da vnesene žuželke predstavljajo grožnjo za neciljne rastline, medtem, ko je drugo, da žuželka ne bo sposobna učinkovito zatreti ciljnega plevela (Kluge, 2000). Biotično zatiranje plevelov z žuželkami je ena izmed najbolj znanih alternativ kemičnemu zatiranju (Sankaran, 1990). Pri tujerodnih plevelih je potreben dodaten nadzor njihovega širjenja, saj zaenkrat še ni poznanih učinkovitih naravnih sovražnikov za njihovo zatiranje. Veliko samoniklih rastlinskih vrst, ki rastejo v 'domačem' območju razširjenosti, niso obravnavane kot pleveli, ker se pojavljajo v manjši gostoti in v razpršenih sestojih, ki jih napadajo različne žuželke. Težave pa se pojavijo, ko se takšne rastline naselijo na novo območje brez naravnih sovražnikov, kjer se začnejo zelo hitro širiti in povečevati gostoto, še posebno, če so za to ugodni tudi drugi okoljski dejavniki (Sankaran, 1990). Zgled takšnega 'divjega' plevela je v Indiji *Parthenium hysterophorus* L. (Asteraceae). Žuželke, s katerimi zatiramo plevel, le-tega tako poškodujejo, da propade ali pa le prispevajo k splošnemu zmanjšanju njegove rasti, vigorja in razmnoževalne sposobnosti. Za zatiranje te agresivne plevelne vrste so uporabili različne pristope (mehansko, kemični, biološko), vendar je večina teh pristopov neučinkovitih, ker se ta plevel izjemno hitro širi in raste. Ugotovili so, da je učinkovito zatiranje s hroščem *Zygogramma bicolorata* (Pallister, 1953), vendar vseeno metoda ni dovolj učinkovita, saj hrošči le-tega tako poškodujejo tako, da prispevajo le k splošnemu zmanjšanju njegove rasti, vigorja in razmnoževalne sposobnosti (Kumar, 2014). Če ena vrsta žuželke ne more zatirati plevela, se lahko za potrebe povečanja učinkovitosti zatiranja uporabi druge (Sankaran, 1990).

Namen pričujočega članka je širjenje informacij o možnostih biotičnega zatiranja plevelov v Evropi z njihovimi naravnimi sovražniki, ki so na Stari celini domorodni ali splošno razširjeni. Za razliko od nekaterih drugih območij sveta, kjer je uporaba rastlinojedih organizmov za zatiranje plevelov bolj razširjena (zlasti v ZDA) (Bürki in sod., 1997; Pitcairn, 2018), je to področje v Evropi strokovno podhranjeno in ga bo potrebno okrepiti, da bo v prihodnosti mogoča tudi praktična uporaba rastlinojedih organizmov pri zatiranju plevelov. Pleveli, omenjeni v članku, so bili izbrani naključno med vsemi najbolj razširjenimi in najbolj trdovratnimi v Evropi (npr. osat in njivski slak sta še posebej trdovratna zaradi svojih dolgih korenin). Tudi žuželke, naravni sovražniki izbranih plevelov, so bile izbrane na podlagi njihove razširjenosti na območju Evrope in pozitivnih izkušenj z njimi pri zatiranju plevela. Raziskovalci so namreč pri vsaki žuželčji vrsti ugotovili sposobnost zatiranja plevelov.

2 PREGLED DOSEDANJIH OBJAV O USPEŠNOSTI ZATIRANJA IZBRANIH PLEVELOV Z ŽUŽELKAMI

2.1 TOPOLISTNA KISLICA - ŠČAVJE (*Rumex obtusifolius* L.) IN HROŠČ G*astrophysa viridula* (De Geer, 1775)

2.1.1 Splošno o hrošču *Gastrophysa viridula*

Gastrophysa viridula je vrsta hrošča, ki se v Evropi pojavlja v vseh državah, z izjemo Slovenije, Hrvaške, Luksemburga, Ukrajine, Romunije, Litve, Moldavije, Grčije, Makedonije in Iberskega polotoka. Dolžina teh hroščev se razlikuje med spoloma – samci so dolgi 4 mm in samice 7 mm. Med parjenjem imajo samice povečan abdomen. Tako samci kot samice so zelene barve s kovinskim sijajem. Za zeleno obarvanost je odgovoren večplastni hitinski sloj, ki se izmenjuje s plastmi, ki vsebujejo melanin. Tudi noge so zelene barve s kovinskim sijajem in močno grajene. Tipalke so nazobčane in srednje dolge. Hrošči so oligofagi, kar pomeni, da se prehranjujejo le z nekaj vrstami rastlin, ki so v tem primeru različne kislice. Razmnoževalna doba traja od marca do oktobra. Na leto se pojavijo do 4 rodovi, s tem, da zadnji rod prezimi v obliki odraslih hroščev. Samice odložijo več kot 1000 jajčec, ki jihodlagajo v skupine po 20 do 45 skupaj, na spodnjo stran listov gostiteljske rastline. Jajčeca so ovalna, kremne do rumene barve, pred izleganjem pa oranžna. Po 3 do 6 dneh se iz jajčec izležejo ličinke, ki so lahko različnih odtenkov, vse od zelenkasto sive pa do temno rjave. Ličinke dosežejo dolžino do 8 mm. V starejših razvojnih stadijih izločajo snovi, ki odvračajo kompetitorje od hranjenja na listih kislice. Ličinka se zabubi 2 cm globoko v tleh in po 6 do 9 dneh se pojavijo odrasli hrošči (Martinková in Honěk, 2004; UK Beetle …, 2019).

2.1.2 Biotično zatiranje topolistne kislice z vrsto *G. viridula*

Zaradi specializiranega načina hranjenja – ličinke in hrošči se hranijo na listih kislice – ta hrošč velja za potencialno 'sredstvo' za zatiranje vrst iz rodu *Rumex*. Pojavljanje hrošča *G. viridula* na rastlinah kislice vpliva na zmanjšanje vigorja, ne vpliva pa na propad rastlin. Vpliv tega hrošča na rastline je podkrepljen še z enim biotičnim agensom, rjo *Uromyces rumicis* (Schumach.) G. Winter (Pucciniaceae).

Kombinirani vplivi hrošča *G. viridula* in ostalih biotičnih agensov povečajo propadanje rastlin in s tem prispevajo k njihovem zatiranju (Martinková in Honěk, 2004). Učinkovitost tega hrošča kot biotičnega agensa, je odvisna od intenzivnosti hranjenja in številčnosti hroščev na rastlini. Intenzivnost hranjenja je sorazmerna s kakovostjo listov kislice, ki se proti koncu rastne dobe zmanjšuje. Hranilna vrednost gostiteljskih rastlin se poveča z rezanjem in ponovno rastjo. Kakovost listov se spreminja tudi zaradi gnojenja z dušikom in zaradi okužbe z rjo *Uromyces rumicis*. Tako gnojenje z dušikom kot rja *Uromyces rumicis* zmanjšujeta vsebnost dušika v listih, povečata pa vsebnost ogljika in vode v listih, kar vodi v povečano hranjenje osebkov *G. viridula* z listi. Okužba z rjo vpliva na manjše število odloženih jajčec in manjši odstotek izleganja ličink, medtem ko staranje listov vpliva na manjšo vitalnost samic in posledično tudi na manjšo velikost jajčec in sposobnost preživetja ličink. Intraspecifično tekmovanje vpliva tudi na gostoto populacije hroščev. Samice prenehajo z odlaganjem jajčec in zapustijo liste, ki so gosto poseljeni z ličinkami teh hroščev, zaradi izločanja odvračalnih snovi, ki jih tvorijo ličinke. Populacija hrošča *G. viridula* pogosto s hranjenjem odstrani večji del listov, vendar ponavadi to ne zadostuje za propad celotne rastline. Hatcher (1996) je ugotovil, da tudi majhne rastline kislice ne propadejo ob odstranitvi tudi do 90 % listov. Negativni učinki napada vrste *G. viridula* se bolj opazni pri rastlinah, izpostavljenih interspecifičnemu tekmovanju. Na številčnost populacije pa vpliva tudi kompleks naravnih sovražnikov, katerih učinkovitost spodbuja raznolikost vegetacije, ki obdaja rastline kislice. Hann in Kromp (2001) sta uporabila tega naravnega sovražnika za biotično zatiranje kislic na travnikih in pašnikih v sistemih ekološkega kmetijstva. Najbolj učinkovit je bil na nekošenih območjih, kjer kompeticija s travami zmanjša uspešnost rasti kislice. Ta hrošč vpliva na zmanjšano konkurenčno sposobnost in uspeh razmnoževanja rastlin iz rodu *Rumex*, čeprav je manj učinkovit kot metode rezanja korenin (Martinková in Honěk, 2004).

2.2 KODRASTOLISTNA KISLICA (R*umex crispus* L.) IN HROŠČ *Apion violaceum* (Kirby, 1808)

2.2.1 Splošno o hrošču *Apion violaceum*

Apion violaceum je vrsta hrošča, ki se hrani na semenih. V Evropi je bil najden v vseh državah, z izjemo Slovenije, Luksemburga, Estonije, Belorusije, Moldavije, Romunije, Srbije, Makedonije, Črne gore ter Bosne in Hercegovine. V dolžino zraste od 2,6 do 3,5 mm (Schmidt, 2005). Glavne značilnosti, po katerih prepoznamo hrošča, so podolgovato, hruškasto oblikovano telo z modrimi bleščečimi pokrovkami, debel rilček in razmeroma veliko telo (Mifsudi in Colonelli, 2010). Samice odlagajo jajčeca na rastline iz rodu *Rumex*, na navadno ajdo (*Fagopyrum esculentum* Moench) in na rabarbaro (*Rheum rhabarbarum* L.). Večina hroščev iz rodu *Apion*, najdenih v izvrtanih rovih v cvetnem steblu kislice kot ličinke v maju in juniju, se julija ali avgusta preobrazijo v odrasle hrošče (Scott, 1985). Samice odlagajo jajčeca skozi plast pri razvijajočih se cvetovih, stebelne liste in stebla. Ličinke se od mesta izleganja premeščajo po steblu navzdol in gredo skozi tri razvojne stopnje znotraj iste gostiteljske rastline. Prostor za zabubljenje se nahaja na zunanjem delu stebla in se oblikuje tik pred zabubljenjem. Enako kot druge vrste iz rodu *Apion*, je tudi vrsta *A. violaceum* univoltilna in prezimuje od septembra ali oktobra naprej na tleh v ostankih listov, pod debli,… (Hopkins in Whittaker, 1980). Hopkins in Whittaker (1980) sta v štiriletni raziskavi ugotovila, da se je na dveh opazovanih mestih zmanjšalo število in povprečna višina stebel kodrastolistne kislice , na katerih so bili naseljeni hrošči *A. violaceum*. Sočasno je prišlo tudi do upada števila hroščev, kar sta pripisala zmanjšani višini stebel, kar je vplivalo na privlačnost stebel za samice, ki na njih odlagajo jajčeca. Predvidevala sta, da višina stebel vpliva tako na samice, ki odlagajo jajčeca, kot tudi na preživetje ličink (Grossrieder in Keary, 2004).

2.2.2 Biotično zatiranje kodrastolistne kislice z vrsto *A. violaceum*

Hrošči iz rodu *Apion* predstavljajo daleč najbolj obetavno metodo biotičnega zatiranja kodrastolistne kislice. S hranjenjem/vrtanjem povzročajo rove na steblih in koreninah (Davies in Turner, 2012). Freese (1995) je preučeval pojavljanje hrošča *A. violaceum* na kodrastolistni kislici in ugotovil, da ta vrsta vrta rove po celotni dolžini stebel. Zgodnost pojavljanja je primerjal z vrsto *Apion miniatum* Germar, 1833 in ugotovil, da se slednja pojavi dva tedna pred hroščem *A. violaceum*, to je v začetku maja. Glede gostote hroščev (povprečno število hroščev na steblu) je ugotovil, da je bila ta na istem območju pri vrsti *A. violaceum* kar štirikrat večja kot pri hrošču *A. miniatum*, poleg tega je bilo s strani hroščev vrste *A. violaceum* napadenih kar 86 % stebel, medtem ko jih je bilo pri vrsti *A. miniatum* napadenih samo 50 %.

2.3 PELINOLISTNA AMBROZIJA (A*mbrosia artemisifolia* L.) TER HROŠČ AMBROZIJEV LEPENEC *Ophraella communa* (LeSage, 1986) IN *Zygogramma suturalis* (Fabricius, 1775)

2.3.1 Splošno o ambrozijevem lepencu

Pojav ambrozijevega lepenca so leta 2013 prvič ugo-

tovili v južni Švici (Ticino) in Severni Italiji (Lombardija) (Boriani in sod., 2013; Müller-Schärer in sod., 2014). Ta vrsta hroščev tam ni domorodna, zato sklepajo, da vzrok tiči v namernem vnosu. Hrošč se je hitro širil po Italiji in se od tam razširil tudi v Slovenijo, na Goriško, kjer so njegov pojav prvič našli v začetku avgusta 2017. Seljak in sod. (2017) so na podlagi obsega napadenega območja, gostote populacije in stopnje poškodovanosti pelinolistne ambrozije sklepali, da se je ambrozijev lepenec na to območje razširil že kakšno leto prej. To so potrdili tudi z izjavami nekaterih ljudi, ki so govorili o močnejših poškodbah pelinolistne ambrozije že leta 2016. Seljak in sod. (2017) dejanski obseg razširjenosti pri nas še ugotavljajo, z veliko gotovostjo pa lahko trdijo, da se bo zagotovo stalno povečeval, saj je hrošč dober letalec. Za zdaj so njegovo zastopanost potrdili v spodnji Vipavski dolini in v dolini Raše na Krasu.

Odrasli hrošči dosežejo velikost od 3,5 do 4,3 mm, pri tem moramo upoštevati, da so samci nekoliko manjši kot samice. Glava, oprsje in pokrovke so rumenkaste do bledo rjavkaste barve. Na temenu glave je podolžna temno rjava lisa. Na predprsju so tri podolžne rjave lise. Pokrovke imajo vdrte pike in podolžne temno rjave proge, ki so izrazitejše pri samicah, medtem ko so lahko samci tudi brez teh prog. Ličinka je vretenasto valjasta, segmentirana, rumeno sivkaste do rumenkaste barve, po bokih po vsaki strani poteka prekinjena rjava proga, noge so črne. Telo je pokrito s številnimi dlačicami, ki so na vrhu betičaste. Razvoj poteka prek treh razvojnih stopenj ličinke v 9 do 12 dneh. Buba je široko jajčasta, zaprta v značilen zapredek (kokon) iz rjavih vlaken. Jajčece je oranžnorumeno, jajčaste oblike, s kratko zoženim zgornjim delom. Navadno samica jajčeca odloži v skupine po več deset skupaj in na zgornjo stran lista. Ličinka se iz jajčeca razvije v petih dneh (Seljak in sod., 2017). Vrsta prezimi kot odrasel hrošč. Na leto se pojavijo do trije rodovi, na Kitajskem lahko tudi šest (Chen in sod., 2013).

2.3.2 Splošno o hrošču *Zygogramma suturalis*

Zygogramma suturalis izvira iz Severne Amerike, vendar je sedaj naravno zastopan tudi v Evropi, in sicer v Ukrajini, kjer se uporablja za biotično zatiranje ambrozije. Hrošč je majhen in ima rjavo glavo, sredoprsje in pokrovke. Zunanji rob pokrovk je obrobljen s široko umazano rumeno črto, ta rumena črta pa poteka tudi po sredini pokrovk. Na leto se pojavijo do 3 rodovi (prvi na sredini ali konec junija, drugi konec julija ali na začetku avgusta in tretji v začetku ali na sredi septembra), prezimijo pa kot odrasli osebki (Wan in Wang, 1989). Hrošči spet postanejo aktivni pozno v aprilu ali v začetku maja in se naselijo na mlade rastlinice ambrozije, ko so te visoke le od 2 do 5 cm. Samica v povprečju odloži 394 jajčec (Vinogradova in Bogdanova, 1989).

2.3.3 Biotično zatiranje pelinolistne ambrozije z ambrozijevim lepencem in hroščem *Z. suturalis*

V Avstraliji in na Kitajskem se je kot dolgoročni način zatiranja pelinolistne ambrozije najbolj obneslo klasično biotično zatiranje z vnosom tujerodnih vrst žuželk z območij naravnega areala rasti pelinolistne ambrozije. Kot najbolj učinkovito vrsto za zatiranje Kiss (2007) smatra hrošča ambrozijevega lepenca, kateri naj bi bil med najbolj znanimi biotičnimi agensi za zatiranje te vrste ambrozije, poleg hrošča *Z. suturalis*.

Glavni gostitelj ambrozijevega lepenca je pelinolistna ambrozija, občasno pa lahko napade tudi sončnice (*Helianthus annuus* L.), topinambur (*Helianthus tuberosus* L.), bodiče (*Xanthium* spp.) in kanadsko hudoletnico (*Erigeron canadensis* (L.) Cronquist). Ličinke, pa tudi odrasli hrošči, se prehranjujejo z listi ambrozije in jih pri tem obžrejo vse do debelejših listnih žil, ki se nato navadno posušijo. Posledice namnožitve ambrozijevega lepenca so ponekod zelo očitne. Manj izrazite so neposredne poškodbe še razvijajočih se socvetij, ki pa se navadno ne razvijejo ali se zelo slabo oblikujejo. Pri zelo močnem napadu cela rastlina odmre. Učinek napada je tem večji, čim zgodnejši je napad (se pravi čim mlajše so rastline). Že povsem razvite rastline lahko še vedno oblikujejo okrnjena socvetja iz zaloge hranil v steblu, vendar večinoma ne uspejo oblikovati semena. Biotično zatiranje pelinolistne ambrozije z ambrozijevim lepencem je dolgoročno najustreznejši in tudi najbolj učinkovit način za omejevanje njenih populacij. Velik razmnoževalni potencial in veliko število rodov zagotavljata zadostno populacijo skozi celotno rastno dobo ambrozije. Na podlagi prvih opazovanj se zdi, da je to daleč najbolj učinkovit način za zatiranje pelinolistne ambrozije (Seljak in sod., 2017).

Odrasli hrošči in ličinke hrošča *Z. suturalis* se hranijo na vrstah *Ambrosia artemisiifolia*, *Ambrosia psilostachya* in *Ambrosia trifida*. Ličinke prvega rodu se začnejo na listih hraniti v sredini maja ali na začetku junija. Ličinke drugega in tretjega rodu pa se začnejo hraniti v prvih dveh tednih avgusta. Reznik in sod. (2008) so škodo na ambroziji v njihovem poskusu opazili le na nekaj parcelah, in sicer v višini do 5 %. V okolju brez motenj, ki je stabilno, lahko hrošč *Z. suturalis* povzroči veliko škodo na rastlinah ambrozije v obdobju dveh do treh let, odvisno od začetne gostote rastlin. Večje škode se pojavijo na območjih z veliko gostoto rastlin ambrozije. Tudi samice raje odlagajo jajčeca na velike in nepoškodovane rastline. Analize Reznika in sod. (1994) so pokazale, da je absolutna gostota hrošča *Z. suturalis* določena z gosto-

to rastlin ambrozije. Poljske in laboratorijske raziskave so pokazale, da samice na zelo poškodovanih rastlinah odložijo manjše število jajčec, poleg tega pa je bolj verjetno tudi, da vstopijo v diapavzo, za razliko od tistih na zdravih rastlinah (Vinogradova in Bogdanova, 1989; Reznik, 1989). Reznik (1996) raziskavo zaključi s sklepom, da je bil načrtni vnos hrošča *Z. suturalis* v Rusiji delni in ne popolni uspeh, saj je bilo hranjenje in posledično poškodovanje rastlin pelinolistne ambrozije, z izjemo nekaj majhnih zaplat rastlin z veliko gostoto hroščev, premajhno, da bi se na ta način zmanjšala gostota te, že 'toksične' vrste plevela.

2.4 NJIVSKI OSAT (*Cirsium arvense* (L.) Scop.) IN HROŠČ *Casssida rubiginosa* (Müller, 1776)

2.4.1 Splošno o hrošču *Cassida rubiginosa*

Cassida rubiginosa je vrsta listnega hrošča, ki je bil v Evropi najden v vseh državah, razen na Irskem, v Belorusiji, Moldaviji, Andori in Estoniji. V dolžino meri od 6 do 8 mm, kot vsi hrošči iz rodu *Cassida* pa ima tudi ta pronotum (sklerenhimski del hrbta na predprsju). Telo hrošča je okroglo, z zelenimi ali rumenozelenimi pokrovkami, pri nekaterih vrstah pa se lahko na hrbtni strani pojavi značilen vzorec v obliki trikotnika. Ličinke so ovalne oblike, rjavkaste ali zelene barve, po robovih na obeh straneh pa so lahko vidne črne dlačice (McLeod in sod., 2015). Na leto se pojavi en rod, prezimijo v obliki odraslih osebkov. Samice v ciklih dolgih 6 tednov odložijo do 1000 jajčec na spodnjo stran listov. Odrasli hrošči živijo do 80 tednov, razvoj od jajčeca do odraslega osebka pa traja 6 tednov (Majka in Lesage, 2008).

2.4.2 Biotično zatiranje njivskega osata s hroščem *C. rubiginosa*

Hrošč se hrani na različnih vrstah iz družine Asteraceae, vključno z osati (*Cirsium*, *Carduus*, *Onopordum*) in glavinci (*Centaurea*) (McLeod in sod., 2015). Odrasle hrošče navadno najdemo na spodnji strani listov, medtem ko se ličinke hranijo na zgornji strani listov. Hrošči se lahko hranijo tudi s cvetnim prahom. Njivski osat je agresivna, invazivna plevelna vrsta in ga je težko zatreti z mehanskimi in kemičnimi pristopi (Liu in sod., 2000). Na Novi Zelandiji je bil leta 2007 hrošč vnesen za potrebe zatiranja njivskega osata in predstavlja enega izmed najbolj obetavnih biotičnih agensov za zatiranje osata (Hettiarachchi in sod., 2018). Cripps in sod. (2019) so ugotovili, da hrošči *C. rubiginosa* z objedanjem listov povzročijo precejšnje zmanjšanje gostote populacije in razširjanje njivskega osata v rastni dobi. Naredili so tudi primerjavo vpliva poškodb hroščev na rastline osata med dvema letoma. Ugotovili so, da se je v obravnavanju, kjer je bilo nanesenih od 10 ali 20 ličink na poganjek, gostota rastlin hitro zmanjšala, kar je v nasprotju z drugim opazovanim letom, ko se je gostota poganjkov zmanjšala ne glede na obravnavanje. Oktobra in novembra so opazili najmanj poškodb zaradi hranjenja, vendar se gostota poganjkov in višina rastlin nista zmanjšali. Večje objedanje listov je bilo povezano z zmanjšanim deležem poganjkov, ki so bili sposobni reprodukcije (Cripps in sod., 2019). V kar nekaj raziskavah so potrdili vpliv hranjenja hroščev in ličink v rastni dobi na rastline osata, poleg tega so tudi vse pokazale določen učinek hranjenja na rastlino (npr. zmanjšana višina poganjkov, preživetje in biomasa).

2.5 PLEZAJOČA LAKOTA (*Galium aparine* L.) TER GRIZLICA *Halidamia afffinis* (Fallén, 1807) IN HROŠČ *Sermylassa halensis* (Linnaeus, 1767)

2.5.1 Splošno o grizlici *Halidamia affinis*

Grizlica *Halidamia affinis*, predstavnik reda kožekrilcev, se v Evropi pojavlja povsod, z izjemo Slovenije, Norveške, Portugalske, Ukrajine, Estonije, Litve, Belorusije, Bosne in Hercegovine, Črne gore, Makedonije, Grčije in Albanije. Odrasle grizlice imajo glavo, sredoprsje in krila črno obarvane, z vmesnimi oranžnimi deli na mezopleuronu (lateralno površje sredoprsja) in izločalnih žlezah. Zadek je oranžen, z izjemo prvega, drugega in zadnjega segmenta zadka, ki so v večji meri obarvani črno. Noge so oranžne s temno rjavkastim odtenkom. Gosenice so mlečno bele do prozorne. Glava je oranžna s temnimi očmi. Na oprsju so trije pari nog, na zadkovih členih pa je še 8 parov mesnatih izrastkov, ki podobni nogam, imenovani panožice. Grizlice v dolžino dosežejo od 5 do 6,5 mm, najbolj aktivno pa letajo od aprila do junija.

2.5.2 Splošno o hrošču *Sermylassa halensis*

Hrošč *Sermylassa halensis* je v Evropi zastopan v večini držav, z izjemo Slovenije, Hrvaške, Makedonije, Črne gore, Bosne in Hercegovine, Grčije, Albanije, Portugalske, Irske, Norveške, Finske, Romunije, Moldavije, Latvije, Estonije in Belorusije (Gruev, 2005). V dolžino doseže od 5 do 7 mm. Pokrovke so kovinsko zelene ali modre, redkeje tudi bakrene. Na vrhu glave imajo veliko črno liso. Vratni ščit in noge so rumenorjave barve, tudi oranžne. Prezimijo v razvojnem stadiju jajčec, na območjih z milejšimi zimami tudi kot odrasli osebki. Tipalke so nazobčane in temneje obarvane (UK Beetle …, 2019).

2.5.3 Biotično zatiranje plezajoče lakote z grizlico *H. affinis* in hroščem *S. halensis*

Odrasle grizlice in pagosenice so oligofagi in se hranijo na rastlinah iz rodu *Galium* (Pavlinec, 1992). Največjo škodo povzročajo predvsem pagosenice, ki se intenzivno hranijo na listih različnih rastlin iz tega rodu. Ob močnem napadu pagosenic se začnejo rastline sušiti, še pred tem pa se zmanjša fotosintetska sposobnost rastlin (Batra, 1984).

Gostiteljske rastline hrošča *S. halensis* so rastline iz rodov *Galium* in *Clinopodium*. Odrasli hrošči se hranijo na listih, medtem ko ličinke najdemo tako na listih kot na steblih. Hrošč velja za potencialni biotični agens za zatiranje plevela zaradi svojih množičnih napadov na rastline iz rodu *Galium*, pa tudi zaradi povzročitve popolne defoliacije (Pavlinec, 1992).

2.6 PTIČJA DRESEN (*Polygonum aviculare* L.) IN NAVADNI SLAKOVEC (*Fallopia convolvulus* L.) IN HROŠČ *Gastrophysa polygoni* (Linnaeus, 1758)

2.6.1 Splošno o hrošču *Gastrophysa polygoni*

Ti hrošči so v Evropi naravno zastopani po celotni Evropi, z izjemo Estonije, Moldavije in Albanije. Čeprav so relativno majhni (4-5 mm), odrasle hrošče na njivah z lahkoto prepoznamo po njihovem oranžnordečem predprsju, nogah in zadku, medtem ko so glava in pokrovke kovinsko zelene ali modre barve. Najpogosteje tega 'listnega' hrošča najdemo na njivah z žiti, kjer se prehranjuje na rastlinah iz družine dresnovk (*Polygonum* spp., *Fallopia* spp.) ali na kislicah (*Rumex* spp.). Prezimijo odrasli hrošči, ki svoja zimska zavetišča zapustijo konec aprila in v začetku maja. Na leto se pojavita dva rodova (lahko pa jih je tudi več). Samica odlaga jajčeca v skupinah, obložena z lepljivo snovjo, na spodnjo stran listov. Število jajčec se razlikuje med rodovi. Bube so dolge od 5 do 5,5 mm, rumene barve z rjavimi dlačicami, rastočimi v vzporednih črtah. Celoten razvoj prvega rodu traja od 35 do 69 dni, pri drugemu rodu pa 31 do 53 dni (Simpkins, 2012).

2.6.2 Biotično zatiranje ptičje dresni in navadnega slakovca s hroščem *G. polygoni*

Prave gostiteljske rastline hrošča *G. polygoni* so predstavniki iz družine Polygonaceae, predvsem iz rodov *Polygonum*, *Fallopia* in *Rumex*, Clark in sod. (2004) pa so poročali tudi o drugih, naključnih gostiteljskih rastlinah. V Evropi je najpomembnejši gostitelj ptičja dresen, občasno pa se pojavi tudi na navadnemu slakovcu. MacNay (1955) meni, da je hrošč *G. polygoni* koristen, saj s prehranjevanjem na navadnemu slakovcu povzroča njegovo defoliacijo. Kmetje so celo pobirali hrošče in jih načrtno spuščali na njive, kjer je bila velika gostota tega plevela (McDonald, 1956). Popolno defoliacijo so opazili na kar nekaj območjih pokrajine Saskatchewan v Kanadi, največ v njenem zahodno-osrednjem delu (McDonald in sod., 1956).

2.7 NJIVSKI SLAK (*Convolvulus arvensis* L.) TER SLAKOV LEPENEC (*Galeruca rufa* [Germar, 1824]) IN SLAKOV MOLJ (*Tyta luctuosa* [Denis in Schiffermüller, 1775])

2.7.1 Splošno o slakovemu lepencu

Slakov lepenec je v Evropi zastopan v Italiji. Na leto se pojavijo od 2 do 3 rodovi, in sicer od marca do septembra (Rosenthal in Hostettler, 1980). Prezimijo odrasli hrošči. V dolžino zrastejo 4-8 mm. Pokrovke, glava in vratni ščit so svetlo do temno rjave barve, medtem ko je telo sivorjave barve. Noge so črne barve, tipalke so nitaste in členaste (Rosenthal in Hostettler, 1980).

2.7.2 Splošno o slakovemu molju

Slakov molj je v Evropi zastopan v vseh državah, z izjemo Irske, Norveške, Finske in Estonije. Odrasel molj doseže v dolžino 11 mm, razpon kril pa meri od 22 do 25 mm. Sprednji par kril je temno rjave barve, s svetlejšimi črtami, medtem ko je zadnji par kril enakomerne temno rjave barve. Na vseh štirih krilih se pojavlja po ena bela lisa. Rob kril je resast in belo obarvan (Gaoter in sod., 2003). Na leto se pojavita do dva rodova (pozno spomladi in poleti), v toplejših območjih tudi trije. Samica izleže od 400 do 500 jajčec, ki jih najdemo v suhih, peščenih in apnenčastih tleh. Gosenice so rjave, s tremi pari nog na oprsju in štirimi pari panožic. Na vsaki strani njenega telesa poteka po ena svetlejša črta (Doremi, 2019).

2.7.3 Biotično zatiranje njivskega slaka s slakovim lepencem in slakovim moljem

Hrošč se hrani samo na rastlinah iz rodov *Convolvulus* in *Calystegia*. Zmerna populacija hroščev lahko povzroči defoliacijo, ki je dovolj močna za zmanjšanje števila cvetov na njivskem slaku in posledično manjše število novo zraslih rastlin zaradi manjšega števila semen (Rosenthal in Hostettler, 1980). Pri naravno zasto-

panih populacijah hroščev se le-ti po pričakovanjih na gostiteljskih rastlinah pojavljajo skozi celo rastno dobo (Rosenthal, 1981). Na njivskem slaku se hranijo tako ličinke kot odrasli hrošči (Rosenthal, 1981), ko pa se ne hranijo jih najdemo na rastlinskih ostankih na površju tal (Rosenthal, 1995).

V ZDA so za biotično zatiranje molja, katerega glavni gostitelj je njivski slak, odločili leta 1980, vendar o njegovem obstanku v okolju niso poročali (Julien, 1992). Tudi rezultati raziskave žuželk v Južni Evropi, povezanih z njivskim slakom, so razkrili, da je slakov molj eden najbolj razširjenih defoliatorjev, ki ima največji vpliv na zmanjšanje populacije tega plevela (Rosenthal in Buckingham, 1982). Najbolj 'učinkovit' razvojni stadij slakovega molja so gosenice, ki se, v glavnem samo ponoči (Tóth in sod., 2004), hranijo z listi in cvetovi slaka, pri čemer povzročijo zelo veliko škodo (Tóth in Cagáň, 2005; Doremi, 2019).

3 SKLEPI

Biotično zatiranje plevela z žuželkami, pa naj bodo domorodne ali tujerodne, vključuje kompleks medsebojnih vplivov morfoloških, biokemičnih, fenoloških in drugih dejavnikov, tako v gostiteljski rastlini kot v žuželki. Specifičnost gostitelja je najpomembnejši pogoj, da se žuželka uporablja kot sredstvo za zatiranje plevela. Medtem ko žuželka na začetku išče ustrezno gostiteljsko rastlino, se odzove na različne dražljaje, sčasoma izbere pravega in ostane na rastlini, ki je najbolj ustrezna za rast in hranjenje. Nekateri taksoni plevelov so odporni na napade določenih žuželk ali pa jih prenesejo, brez, da bi dobili kakšne poškodbe. Lahko pa jih poškodujejo drugi povzročitelji. Več vrst žuželk, ki se pojavljajo na istem plevelu hkrati, vendar kažejo komplementarne in ne tekmovalne navade hranjenja in odlaganja jajčec, ima lahko sinergijski učinek pri zatiranju plevela. Poleg podnebja so biotični dejavniki, kot so paraziti, plenilci in patogeni organizmi, ter biokemične in fenološke spremembe ranljivosti gostiteljskih rastlin med glavnimi vzroki, ki vplivajo na učinkovitost naravnih sovražnikov plevelov (Sankaran, 1990). V Evropi zatiranje plevelov z naravnimi sovražniki, za razliko od nekaterih drugih območij sveta, še ni razširjeno, saj so tovrstne aktivnosti organizacije EPPO usmerjene zlasti v zatiranje gospodarsko škodljivih žuželk in pršic, v prihodnje pa pričakujemo razmah raziskovalnih aktivnosti na področju biotičnega zatiranja plevelov in njihovo postopno vpeljavo v sisteme rastlinske pridelave. Na podlagi pisanja tega preglednega članka smo ugotovili, da bi bile lahko žuželke kot naravni sovražniki plevelov ena od ustreznih alternativ herbicidom.

4 VIRI

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Influence of edaphoclimatic conditions on stem production and stem morphological characteristics of 10 European hemp (*Cannabis sativa* L.) varieties

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Influence of edaphoclimatic conditions on stem production and stem morphological characteristics of 10 European hemp (*Cannabis sativa* L.) varieties

Abstract: Six dioecious (Antal, KC Dóra, Kompolti hibrid TC, Monoica, Tiborszallasi and Tisza) and four monoecious (Fedora 17, Futura 75, Santhica 27 and USO 31) European hemp varieties were sown at a density of 300 viable seeds per m^2 and a row spacing of 12.5 cm in a three-year field trial (2017-2019) to evaluate the yield of the stems (fresh and dry) and some other biometric characteristics of the stems. No pesticides were used during plant growth to suppress weeds, diseases and pests. The highest yield of fresh and dry stems was achieved by the variety Antal with 12.3 t ha^{-1} and 5.3 t ha^{-1}, respectively, followed by the varieties Futura 75 and Tiborszallasi. The lowest yields of fresh and dry stems were recorded for the monecious variety USO 31 (6.0 and 2.6 t ha^{-1}). In general, dioecious varieties had higher and thicker stems than monoecious varieties. The year of production had a highly significant impact on all variables, in particular on the proportion of weed biomass, which was the highest in 2019 (77.2 %), when weather conditions were most unfavourable for hemp cultivation. The correlation analysis between fresh/dry stem yields and weed biomass was highly negative (-0.85 and -0.83) and strongly statistically significant ($p < 0.001$), indicating the issue related to weed management.

Key words: hemp; *Cannabis sativa* L.; yield of stem; weather conditions; weeds

Vpliv pedo-klimatskih razmer na pridelek in morfološke lastnosti stebel 10 evropskih sort navadne konoplje (*Cannabis sativa* L.)

Izvleček: Šest dvodomnih (Antal, KC Dóra, Kompolti hibrid TC, Monoica, Tiborszallasi in Tisza) in štiri enodomne (Fedora 17, Futura 75, Santhica 27 in USO 31) evropske sorte navadne konoplje smo sejali pri gostoti 300 kalivih semen na m^2 na medvrstno razdaljo 12,5 cm v letih 2017 do 2019 z namenom ovrednotiti pridelek svežih in suhih stebel ter določiti nekatere druge morfološke lastnosti stebel. Fitofarmacevtska sredstva za zatiranje plevelov, bolezni ali škodljivcev med rastjo rastlin niso bila uporabljena. Največji pridelek svežih in suhih stebel je dosegla sorta Antal, in sicer 12,3 t ha^{-1} in 5,3 t ha^{-1}, sledili sta sorti Futura 75 in Tiborszallasi. Najmanjši pridelek svežih in suhih stebel je dosegla enodomna sorta USO 31 (6,0 and 2,6 t ha^{-1}). Dvodomne sorte so imele v povprečju višja in debelejša stebla od enodomnih sort. Leto pridelave je imelo velik vpliv na vse spremenljivke, še posebno na odstotek plevela v skupni biomasi, ki je bil največji v letu 2019 (77,2 %), ko so bile vremenske razmere najmanj ugodne za pridelavo navadne konoplje. Korelacijska analiza med pridelkom svežih/suhih stebel in odstotkom biomase plevela je bila negativna (-0,85 in -0,83) ter močno statistično značilna ($p < 0,001$), kar je pokazalo na problem plevelov pri pridelavi navadne konoplje.

Ključne besede: navadna konoplja; *Cannabis sativa* L.; pridelek stebel; vremenske razmere; pleveli

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1 INTRODUCTION

From the 16th to the 18th century, hemp (*Cannabis sativa* L.) was the most important fibre crop in Europe, together with flax (Struik et al., 2000). The hemp stems have been produced because of the long and strong bast fibres traditionally used in the textile and paper industry (Mandolino & Carboni, 2004). Due to the high tensile strength and excellent fineness of hemp fibres, new applications are emerging today, e.g. in geotextiles and biocomposites (Schäfer & Honermeier, 2006; Salentijn et al., 2015). The woody part of the hemp stem is called hurd, also shive, and is used for animal bedding, pulp production and the manufacture of building materials, e.g. hemp concrete (Karus & Vogt, 2004; Elfordy et al., 2008). This versatile use of hemp stems makes hemp one of the oldest non-food crops used worldwide (Schultes, 1970).

The cultivation of hemp is less demanding compared to some other crops (e.g. maize and wheat). Hemp can be grown with minimal or even no pesticides. Due to its rapid juvenile growth, the plant itself successfully suppresses weeds. It could be grown in a relatively narrow crop rotation and has a low fertilization requirement (van der Werf, 1994). However, hemp is a crop whose cultivation is very much influenced by soil and weather conditions.

As far as climatic conditions are concerned, a humid atmosphere is most suitable for hemp cultivation when growing for stem and fibres (Ranalli, 1999). For the production of one kg dry matter hemp needs 300-500 l water. During the vegetative growth phase 250-300 mm of rainfall is required and a total of 500-700 mm of water for the whole season. Precipitation in June and July has a very large influence on plant growth when the seeds are sown in late spring (Bócsa & Karus, 1998). Excess or lack of water in the early stages of development is crucial for yield formation (Struik et al., 2000).

Edaphically, hemp grows well in deep or medium deep soils with a sandy loam structure. Clay loam, heavy clay and sandy soils are less suitable for hemp cultivation (Amaducci et al., 2015). Highly fertile soil for stem production is soil on which a potential yield of 10 000 kg dry matter per ha can be achieved (Bócsa & Karus, 1998). This type of soil is well drained with a pH around 6.0 (Barron et al., 2003).

The main goal of hemp stem production is usually the extraction of bast fibres. The fibre yield is a product of fibre content and stem yield (Berenji et al., 2013). Moreover, fibre yield is also directly related to stem yield (Hennik, 1994). The fibre content in the stem is primarily influenced by the genotype, e.g. it is advisable to use varieties with a longer vegetative cycle for stem production (Struik et al., 2000). On the other hand, stem yield could be influenced by the use of different agricultural techniques (time of sowing, sowing density, fertilisation, time of harvest, etc.) (Amaducci et al., 2008). However, the edaphoclimatic and seasonal growing conditions could not be influenced by labour and therefore have a decisive influence on the agronomic performance of hemp.

The aim of the study was to evaluate the agronomic performance of 10 different European hemp varieties in terms of stem production during the three-year growing period. Secondly, the influence of growing conditions on stem characteristics and proportion of weed biomass was also evaluated. Finally, a correlation analysis was performed to gain insight into the relationships between the variables studied.

2 MATERIAL AND METHODS

2.1 EXPERIMENTAL SITE AND SOIL CHARACTERISTICS

The field experiments were carried out at Ljubljana (Biotechnical Faculty), Slovenia (46° 3' N, 14° 30' E, altitude 295 m) during the 2017-2019 growing seasons. On the test site the soil was medium deep and hydromeliorated with a texture of 31.9 % clay, 43.2 % silt and 24.9 % sand. The soil fertility characteristics for each year of the field trials are shown in the Table 1.

2.2 VARIETIES USED AND THE DESIGN OF THE FIELD TRIAL

10 commercial hemp varieties used in this study are

Table 1: Chemical properties of the soil at the test site

Year	pH in KCl	P_2O_5 (mg / 100 g soil)	K_2O (mg / 100 g soil)	Organic matter (%)
2017	6.6	15.2	16.3	3.6
2018	6.8	19.5	15.9	3.9
2019	6.9	11.9	9.3	4.5

Table 2: Some characteristics of hemp varieties used in field trials

Variety	Origin	Sexual type	Vegetative cycle*	Applications
Antal	Hungary	Dioecious	Late	Flowers/CBD
Fedora 17	France	Monoecious	Early	Seed/CBD/fibre
Futura 75	France	Monoecious	Late	Seed/CBD/fibre
KC Dóra	Hungary	Dioecious	Late	Seed/CBD/fibre
Kompolti hibrid TC	Hungary	Dioecious	Medium	Fibre
Monoica	Hungary	Dioecious	Medium	Seed/CBD/fibre
Santhica 27	France	Monoecious	Medium	Seed/fibre
Tiborszallasi	Hungary	Dioecious	Late	Seed/fibre
Tisza	Hungary	Dioecious	Late	Seed/fibre
USO 31	Ukraine	Monoecious	Early	Seed/fibre

* Early < 125 days; Medium < 135 days; Late < 145 days

listed in the Table 2. Data on variety characteristics were obtained from Flajšman et al. (2018) and Ihempfarms (2019).

The previous crops for the first, second and third year of the field trial were soybeans, maize and lupines, respectively. Every year, the residues of the previous crops were ploughed in autumn and the field was left fallow over the winter. Before sowing the field was fertilized with 500 kg ha^{-1} of NPK 0:14:28 and 260 kg ha^{-1} of calcium ammonium nitrate (27 % N). No additional fertilizers were used during the growth of the plants. No pesticides were used to suppress weeds, diseases and pests. Sowing was carried out in May (4 May 2017, 29 May 2018 and 8 May 2019) with a Wintersteiger plot seeder. The experiment was designed as a randomized, complete block experiment with three replications. The experimental plot size was 18 m^2 (6 x 3 m), with a plant density of 300 viable seeds per m^2 and a row spacing of 12.5 cm. The harvests were preformed manually in August (17 August 2017, 28 August 2018 and 22 August 2019). For each plot, only the inner 4 m^2 were used to determine the variables. First, the total biomass (hemp plants + weeds) was cut at the ground and weighed . Secondly, the biomass of the first m^2 was separated and weighed on the hemp plants and weeds. The weight data were used to determine the proportion (percentage) of weed biomass in the total biomass and the yield of fresh stems. Thirdly, the hemp plants were separated from first m^2 by sexual type (only for dioecious varieties) and counted to determine the sex ratio and number of plants per m^2 at harvest. Fourthly, 25 plants per sexual type (male, female and/or monoecious) of the same m^2 were randomly selected for the determination of mass, height and stem diameter. Finally, the same 25 plants per sexual type were dried at 55 °C until

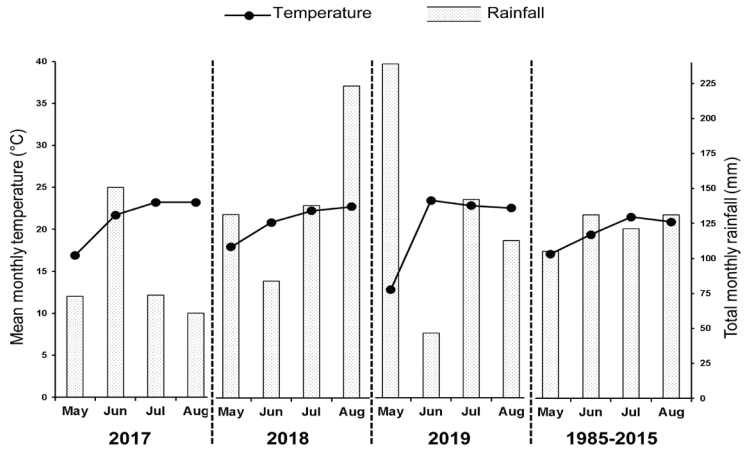

Figure 1: Mean monthly temperature and total monthly precipitation from May to August for the field experiments from 2017-2019 and the long-term average (1985-2015). The association between precipitation and temperature on the y-axes is 6 mm : 1 °C, which is adapted to the Slovenian climate conditions. If the temperature curve is higher than the columns, a slight drought is expected.

constant mass was achieved for the determination of the yield of dry stems.

2.3 WEATHER CONDITIONS

Temperatures were above the long-term average in almost all years and months. Notable exceptions were May 2019, when the average temperature was only 12.9 °C, compering to 17.2 °C of the long-term average, and June 2019, when the average temperature was 4 °C above the long-term average. The arrangement of the precipitation showed more variation regarding the month and year of the field trials. The lack of precipitation was the highest in June 2019 and August 2017, when only the amount 35 % and 46 % of the long-term average occurred. On the other hand, the surplus of precipitation was the highest in May 2019 and August 2018, with the amount of 227 % and 170 % of the long-term average precipitation for these months, respectively (Figure 1).

2.4 STATISTICAL ANALYSES

The data of the variables studied over three years were first subjected to a combined analysis of variance (ANOVA). Year taken as a factor, variety and year × variety interaction were considered fixed effects and determined to be significant if $p < 0.05$. Replications were considered random effects. Significant differences in the mean values given by ANOVA were evaluated using the Duncan test ($\alpha = 0.05$). The data were analysed with the package 'agricolae' in the R-Software version 3.2.5 (R Core Team, 2016). Furthermore, the analysis of the correlation between the response variables was also calculated using the same R-package. The graphics were drawn by the Microsoft Excel programme.

3 RESULTS AND DISCUSSION

3.1 YIELD OF STEMS

The yields of fresh and dry stems were significantly influenced by the year ($p < 0.001$), but the variety had no statistically significant effect ($p = 0.0889$ for the yield of fresh stems and $p = 0.0729$ for the yield of dry stems). There was no interaction between the two factors. The highest average yields of fresh and dry stems were achieved by the Antal variety with 12.3 t ha^{-1} and 5.3 t ha^{-1}, respectively, and Futura 75 with 11.4 t ha^{-1} of fresh and 4.7 t ha^{-1} of dry stems. The lowest yields of fresh and dry stems were measured for the varieties Santhica 27 (6.7 t ha^{-1} and 2.6 t ha^{-1}) and USO 31 (6.0 t ha^{-1} and 2.6 t ha^{-1}) (Figure 2). The influence of the growing season and environmental characteristics can be summarised with the Futura 75 variety, which has been used in many field trials in Europe. In Latvia (Tang et al., 2016), for example, the yield of dry steam of this variety reached 20.3 t ha^{-1}, whereas in France the stem yield was only 2.8 t ha^{-1} (Tang et al., 2017). In two-year field trials by Amaducci et al. (2008), the variety Futura 75 (10.7 t ha^{-1} - 13.1 t ha^{-1}) has outyielded the variety Tiborszallasi (8.4 t ha^{-1} - 10.6 t ha^{-1}) on dry stems. Cosentino et al. (2012) also tested these varieties in a two-year field trial, in which the average yield of the dry stems of Futura 75 was around 4 t ha^{-1} and 6 t ha^{-1} for Tiborszallasi. In our field trial, the yields of the two mentioned varieties were comparable (average 4.7 t ha^{-1}) with only 2.5 % yield difference to the benefit of the Tiborszallasi (Figure 2).

Baldini et al. (2018) tested many different monoecious varieties. The two-year averages (2016 and 2017) of the dry stem yields were 6.0 (3.8), 4.0 (2.6), 7.7 (4.2), 7.8 (4.6) and 8.3 (4.7) t ha^{-1} for the varieties Fedora 17, USO 31, KC Dora, Monoica and Futura 75, respectively. In brackets are shown the average values of the dry stem yield from our study and lower yields are observed. Intriguingly, when comparing only the common year 2017, the yields of Fedora 17 (8.4 t ha^{-1}), USO 31 (5.0 t ha^{-1}), KC Dora (7.6 t ha^{-1}), Monoica (8.6 t ha^{-1}) and Futura 75 (8.4 t ha^{-1}) from our study differ only slightly from those of Baldini et al. (2018). However, poor weather conditions in the subsequent years 2018 and 2019 led to a decrease in average stem yields in this study (see below).

Dioecious varieties are more suitable for stem production because of the quantity and quality of the fibres (Bosca, 1999). In this trial, the average yield of dry stems of six dioecious varieties (4.6 t ha^{-1}) was 35 % higher than the average yield of four monoecious varieties (3.4 t ha^{-1}). In addition, varieties that start flowering later and have a longer vegetative cycle are more suitable for stem production in terms of yield, since the accumulation of dry matter in the stems decreases very rapidly after the start of flowering (Struik et al., 2000). In our three-year field trial, five early or medium varieties (3.5 t ha^{-1}) achieved only 74 % of the dry stem yield of five late varieties (4.7 t ha^{-1}). Similarly, the dioecious varieties yielded a higher yield dry stems than the monoecious varieties in the study by Cosentino et al. (2012).

The growing season had a very important influence on the stem yields. The highest average yield of fresh (17.1 t ha^{-1}) and dry (7.6 t ha^{-1}) stems was achieved in 2017, followed by 2018, and the lowest yield of fresh (2.7 t ha^{-1}) and dry (1.1 t ha^{-1}) stems happened in 2019. Tang et al. (2016) conducted an extensive study with 14 commercial hemp varieties in four different European

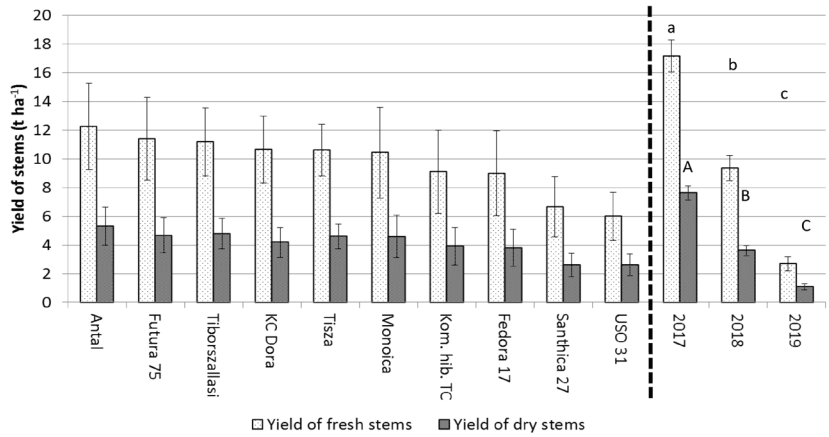

Figure 2: The yield of fresh and dry stems is given as an average by factor variety and as an average by factor year. The lower case letters indicate statistically significant differences in yield of fresh stems and the upper case letters indicate statistically significant differences in yield of dry stems. The main factors (variety and year) are separated by the dotted line and different letters (small or capital) indicate the differences within factor year. Error bars indicate standard errors of the mean values

countries (Latvia, Czech Republic, France and Italy). The yield of dry stems varied considerably among varieties and also among locations, e.g. the yield of dry stems increased from 8.3 t ha^{-1} in France to 19.5 t ha^{-1} in Latvia for the variety Tiborszallasi and from 7.3 t ha^{-1} (Czech Republic) to 22.1 t ha^{-1} (Latvia) for the variety KC Dora. Although their research was not multiyear trial, the differences in growing environments had a huge impact on the performance of the hemp varieties.

3.2 BIOMETRIC CHARACTERISTICS OF PLANTS AND OTHER MEASUREMENTS

Although the same sowing density was used for all varieties in all years, both factors (the year and the variety) but not their interaction influenced the number of plants at harvest. The effect of variety was not statistically significant ($p = 0.0557$) and 107 plants m^{-2} (Kompolti hibrid TC) up to 240 plants m^{-2} (Tisza) were observed (Table 3). The proportion of male plants in the dioecious varieties ranged from 32.0 % to 48.8 % (data not shown). Amaducci et al. (2008) reported that plant density together with harvest time is the most important parameter determining fibre production. Mediavilla et al. (2001) found that 170 plants m^{-2} is typical density for fibre production for Central Europe. On the other hand, Amaducci et al. (2002) stated that 90 to 100 plants m^{-2} represents an optimal density for stem production. In our three-year field trial, the year of production had a statistically significant influence ($p < 0.001$) on the number of plants at harvest, with the first year showing the highest number (304 plants m^{-2}) and the second year the lowest (108 plants m^{-2}). The seasonal effect on the number of plants at harvest was also recorded elsewhere (Cromack, 1998; Struik et al., 2006).

The proportion of weed biomass was significantly influenced by both factors, but not by their interaction. The highest average percentage of weed biomass was found for the variety Santhica 27 (51.3 %) and the lowest for the variety Tisza (27.9 %). The year had a very strong influence on the proportion of weed biomass ($p < 0.001$); the highest percentage was found in 2019 (77.2 %). On the contrary, the average percentage of weed biomass was very low in 2017 (7.6 %). Hemp is known for its ability to suppress weeds independently due to its rapid growth and canopy closure (Kraenzel et al., 1998). This assertion was only confirmed in the first year of our trials. In the second year, and especially in the third year, the hemp plants could not overcome the weeds and its biomass increased significantly.

The plant height differed significantly between varieties ($p < 0.01$) where the stem diameter did not. On the other hand, both variables were statistically significantly influenced by the year of production. For neither of these two variables, no interaction between the factors was found. The highest variety was Antal with an average height of 137.7 cm, followed by Tiborszallasi (130.3 cm) and Tisza (128.9 cm). The lowest varieties were Fedora 17 (101.6 cm), USO 31 (90.1 cm) and Santhica 27 (86.5 cm). In 2017 the plants were the highest with an average height of 150.0 cm. The plants were more than twice as small in 2019, averaging 74.3 cm. The average stem diameter did not vary from variety to variety (average 3.96 mm), but the influence of the year of production was statistically significant ($p < 0.01$), when in 2018 the stems were thickest (4.73 mm) and in 2019 the stems were thinnest (3.15 mm) (Table 3). Plant height and stem diameter are primar-

Table 3: Influence of variety and year on the average number of plants per m^2 at harvest, the proportion of weed biomass, the average plant height and the average stem diameter

Variety	Number of plants per m^2 at harvest		Proportion of weed biomass (%)		Plant height (cm)		Stem diameter (mm)	
Tisza	240 ± 32		27.9 ± 07.5	c	128.9 ± 08.7	ab	4.03 ± 0.20	
Fedora 17	216 ± 39		33.7 ± 11.2	abc	101.6 ± 15.1	bc	3.81 ± 0.32	
Tiborszallasi	211 ± 29		33.2 ± 10.4	bc	130.3 ± 12.2	ab	4.02 ± 0.30	
Antal	194 ± 43		36.5 ± 11.0	abc	137.7 ± 16.1	a	4.19 ± 0.36	
Monoica	188 ± 27		38.2 ± 38.2	abc	125.1 ± 17.9	ab	4.02 ± 0.42	
USO 31	181 ± 28		50.4 ± 11.9	a	090.1 ± 10.4	c	3.30 ± 0.32	
KC Dora	175 ± 32		31.1 ± 10.4	bc	124.1 ± 13.3	ab	4.28 ± 0.32	
Futura 75	175 ± 31		34.0 ± 11.6	bc	121.7 ± 17.8	ab	4.06 ± 0.46	
Santhica 27	174 ± 40		51.3 ± 12.1	a	086.5 ± 10.8	c	3.37 ± 0.30	
Kom. hib. TC	107 ± 28		46.5 ± 12.3	ab	122.8 ± 17.8	ab	4.53 ± 0.49	
p	*ns*		*		**		*ns*	
Year								
2017	304 ± 10	a	07.6 ± 0.9	c	150.0 ± 5.3	a	4.00 ± 0.13	b
2018	108 ± 13	c	32.0 ± 3.4	b	126.3 ± 5.8	b	4.73 ± 0.16	a
2019	147 ± 06	b	77.2 ± 3.5	a	074.3±5.9	c	3.15 ± 0.18	c
p	***		***		***		**	

Mean values followed by same letters are not significantly different at the 5% level of probability; ****p* < 0.001; **p* < 0.01; **p* < 0.05; *ns* - not significant

ily determined by genotype, and dioecious varieties are typically characterized by both a higher stem height and a thicker basal stem diameter than monoecious varieties (Bennett et al., 2006; Amaducci et al., 2008; Cosentino et al., 2013). Indeed, the average stem height of six dioecious varieties in this study was 128.2 cm compared to 100.0 cm of four monoecious varieties and the average stem diameter of the dioecious varieties was 4.18 mm compared to 3.63 mm of the monoecious varieties. Furthermore, in the study by Baldini et al. (2018), USO 31 and Fedora 17 had a lower plant height than KC Dora, Futura 75 and Monoica. Amaducci et al. (2008) used the varieties Futura 75 and Tiborszallasi in their two-year study and reported an average stem height of 174 and 192 cm, respectively, and an average stem diameter of 5.6 mm without statistically significant difference between the varieties. The values for both variables and both varieties are higher compared to our study, indicating that the growing conditions in our experiment were not optimal for hemp growth throughout the experimental period.

3.3 THE CORRELATION ANALYSIS

All six variables were used for the correlation analysis and all correlations were statistically significant, only the number of plants had no influence on the stem diameter (Figure 3). The number of plants at harvest had a high correlation with the yield of dry stems (0.61). Tang et al. (2017) found that increasing plant density up to 240 plants m^{-2} also increases the stem yield. On the contrary, Amaducci et al. (2002) noticed that an increase in plant density meant only a small increase in the fresh and dry mass of the stems. Population density usually has a negative effect on the height of plants, as they compete for light and sources of nutrients (Legros et al., 2013). However, we found a positive but not very high correlation (0.40), probably due to the low number of plants at harvest in 2018 and 2019. The correlation analysis for the year 2017 alone showed a negative correlation (-0.27), but this was not statistically significant (data not shown). Tang et al. (2017) reported that stem height ($R2 \approx 0.9$) and diameter ($R2 \approx 0.8$) are strongly correlated with the yield of dry stems, which was also confirmed in our study. A high and negative correlation between weed biomass and stem yield (fresh or dry) was also observed, indicating the massive negative effect weeds have had on hemp production (see further discussion below).

3.4 THE MEASURED VARIABLES WERE SIGNIFICANTLY INFLUENCED BY THE GROWING SEASON

The year of production had a statistically significant

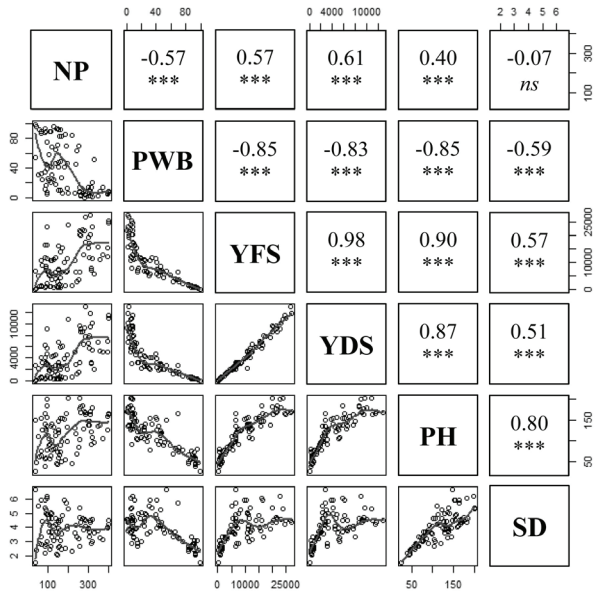

Figure 3: Correlation plots among 6 traits. NP - number of plants per m^2 at harvest, PWB - proportion of weed biomass, YFS - yield of fresh stems, YDS - yield of dry stems, PH - plant height and SD - stem diameter; *** indicates significance at 0.001 level of probability; ns - not significant

influence on all measured variables, which was already shown in Figure 2 and Table 3. The Figure 4 shows a detailed comparison of the impact of the production year expressed on an index scale. The year 2017 was the most favourable for hemp cultivation, where only the stem dimeter did not perform best compared to the other two years. In addition, the proportion of weed biomass was also the lowest. The year 2019 had the most adverse growing conditions, mainly due to the unfavourable arrangement of temperature and precipitation in May and June. The year 2018 was also less favourable due to high rainfall in mid-May, which postponed sowing until the end of May, followed by low precipitation in June and high temperatures in July (Figure 1). The shorter growing season in 2018 due to late sowing had a negative effect on stem growth and thus on stem yield. The most pronounced negative effect of the hostile growing conditions of 2018 and 2019 is shown by the proportion of weed biomass, which was 4.2 and 10.2 times higher, respectively, compared to 2017. The correlation analysis also pointed to the significant negative impact of the biomass fraction of weeds on all measured variables, especially on stem yields. Since the test site and the agricultural technique used in the trials were the same in all three years, only the weather conditions could be the reason for the increase in the biomass proportion of weeds from 2017 to 2019. Jankauskiene et al. (2014) came to the same conclusions, finding a significant difference in the average density of weeds at full hemp emergence between two experiments carried out in two different growing periods (2006-2007 and 2010-1012), but at the same testing location.

In the literature, hemp is described as a crop that requires little or no weed control during growth (Amaducci et al., 2015; Fike, 2016). However, there are very few scientific facts supporting this claim, as verified by Sandler & Gibson (2018). These authors indicated that the reason for the lack of weed management in the majority of published, peer-reviewed studies could be the fact that hemp truly does not need active weed control. Furthermore, almost no mechanical or manual weeding was used in these studies. In the second scenario, which is more likely, the non-use of herbicides in hemp production could be related to the sensitivity of plants to many commonly used active compounds in herbicides. The authors concluded that hemp production should not be determined by weed manifestation and that detailed studies on weed intervention are needed to prevent yield losses. Our study confirmed that weeds can have a very strong negative impact on hemp performance, especially on the stem yield, which is the main concern when growing hemp for fibre production. Unfavourable weather conditions in 2018 and 2019 resulted in a low coverage of hemp plants per area (108 and 147 plants m^2 at harvest for 2018 and 2019, respectively). This means that more space was available for weeds to develop and compete with hemp. Due to low number, the hemp plants could not close canopy area and were therefore overgrown by various weeds. The results of this study thus showed that weeds significantly reduced hemp stem yield

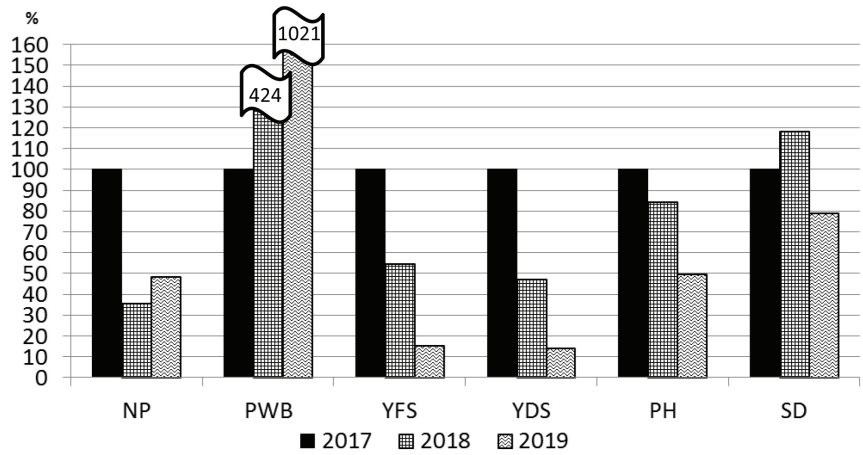

Figure 4: The comparison of the measured variables in relation to the year of the experiment, expressed on an index scale, with the year 2017 set at 100. NP - number of plants per m^2 at harvest, PWB - proportion of weed biomass, YFS - yield of fresh stems, YDS - yield of dry stems, PH - plant height and SD - stem diameter

and other variables under unfavourable weather conditions, most likely due to a lower number of hemp plants. Since no herbicide has yet been approved for the use of hemp in Europe (Legros et al., 2013; Sandler & Gibson, 2018), active weed management for hemp cultivation under field conditions would be necessary in the future.

4 CONCLUSIONS

A three-year trial with 10 European hemp varieties showed that the dioecious varieties outperformed the monoecious varieties in terms of stem yields, plant height and stem diameter. The most productive variety in terms of fresh and dry stem yield was the dioecious variety Antal with 12.3 t ha^{-1} and 5.3 t ha^{-1}, respectively. The year had a very strong influence on all measured variables; unfavourable weather conditions in 2019 (cold and wet May, hot and dry June) led to a sharp decline in all measured variables, including the lowest stem yields. In this year, the proportion of weed biomass was the highest. A highly negative and strongly statistically significant correlation between the biomass of weeds and the yield of the stems showed that weeds can strongly affect the production of hemp. Under the unfavourable weather conditions, when the hemp plants do not cover canopy due to low number of plants and are therefore not able to compete with fast growing weeds, effective weed management would be even more necessary.

5 ACKNOWLEGEMENT

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Monitoring and population changes of *Tuta absoluta* (Meyrick, 1917) on tomato under greenhouse conditions in an arid expanse of south-eastern Algeria

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Monitoring and population changes of *Tuta absoluta* (Meyrick, 1917) on tomato under greenhouse conditions in an arid expanse of south-eastern Algeria

Abstract: The population changes of *Tuta absoluta* was surveyed during three growing seasons in greenhouse tomatoes in Biskra. Introduced in 2009 for the first time, it seems to be well established on tomato crops in Biskra; while their natural enemies remained lacking, due possibly to pesticides overuse. All pest stages were present on tomato plants during the three cropping seasons. Important numbers of males were captured during the first growing season and the least during the third growing season. The first adults' flight spread out between October and December. Adults' flight significantly rose at the end of the plant cycle due to increased temperatures in all cultivation seasons. This can provide information on the infestation levels for the following cultivation years. The numbers of immature were low during the three cultivation seasons. March, April and May seem more favorable to the different leaf miner instars development for the three cropping seasons. This was due probably to temperature rising.

Key words: population changes; *Tuta absoluta;* tomato crop; Biskra; Algeria

Spremljanje sprememb v populacijah paradižnikovega molja, *Tuta absoluta* (Meyrick, 1917), na paradižniku, gojenem v rastlinjakih v sušnih območjih jugo-vzhodne Alžirije

Izvleček: Spremembe v populaciji paradižnikovega molja (*Tuta absoluta*) so bile spremljane v treh rastnih sezonah na paradižniku v rastlinjakih v Biskri. Paradižnikov molj se je prvič pojavil leta 2009 in se je v nasadih paradižnika v Biskri dobro udomačil medtem, ko so njegovi naravni sovražniki še vedno odsotni, verjetno zaradi prevelike uporabe pesticidov. Vsi razvojni štadiji škodljivca so bili najdeni na paradižniku v vseh treh rastnih sezonah. Največje število samcev je bilo ujeto med prvo rastno sezono in najmanjše med tretjo rastno sezono. Prvi izlet odraslih žužel je bil med oktobrom in decembrom. Izlet odraslih se je značilno povečal proti koncu rastne sezone paradižnika zaradi dviga temperature v vseh rastnih sezonah, kar lahko kaže na stopnjo okužbe v naslednji rastni sezoni. Število nedoraslih osebkov je bilo majhno v vseh treh preučevanih sezonah. Marec, april in maj so bili najprimernejši za različne vmesne razvojne štadije minerjev v vseh treh rastnih sezonah, verjetno zaradi dviga temperature.

Ključne besede: spremembe populacij; *Tuta absoluta*; paradižnik; Biskra; Alžirija

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1 INTRODUCTION

Tomato crops, *Lycopersicon esculentum* Miller, are currently cultivated worldwide in greenhouses and in open fields (Lange & Bronson, 1981). It is the most cultivated plant and largely consumed vegetable in Algeria after potatoes. The greenhouse tomato cultivation underwent a major expansion in the Sahara region and mainly in Ziban (Biskra) due to availability of water and good soil quality in certain localities. Biskra was ranked as the first producer of early vegetables nationally. Greenhouse crops are usually more exposed to fungal, viral and pest attacks due to elevated ambient moisture and temperature. The infestation can occur on plant aerial parts (stems, leaves, flowers, fruits) and/ or roots. The main pests of tomatoes are nematodes, insects or other arthropods (Lange & Bronson, 1981). *Tuta absoluta* (Meyrick, 1917) is devastating pest of economic importance on tomato crops and other solanaceous crops (Medeiros et al., 2005; Bawin et al., 2017).The tomato borer leaf miner originates from South America. It is considered the most damaging pest of tomato crops in South America (Torres et al., 2001) since 1960s (Guedes & Picanço, 2012); and is currently impacting crops in the Mediterranean countries of Europe and North Africa (Desneux et al., 2010; Caparros-Megido et al., 2012, 2013). Recently, it was found in Senegal (Pfeiffer et al., 2013). The larvae dig galleries in tomato leaves, fruit and stems (Picanço et al., 1998) and consequently open pathogen penetration pathways. Crops severely damaged can reach up to 100 % of yield losses (Desneux et al., 2010). It harms several cultivated and wild plants (Vargas, 1970; Garcia & Espul, 1982). It is new pest of tomatoes in Algeria, detected for the first time in 2008 in Mostaganem coastal region (Guenaoui, 2008) and in 2009 in Biskra (Allache & Demnati, 2012; Allache et al., 2012). It became the most important pest of tomato crops in Algeria since 2008 (Gacemi & Guenaoui, 2012) due to its severe damage observed in greenhouses and open fields (Badaoui & Berkani, 2010). Chemical control was the main method used in South American countries to manage this pest. Unfortunately, these chemicals, have caused insects resistance, leaving residue on food products and in the environment; threatening human safety and have eradicated beneficial insects (Siqueira et al., 2000, 2001; Lietti et al., 2005). In South America, the pest is faced with several insects parasitoids and predators including, the egg parasitoid *Trichogramma* spp. (Medeiros et al., 2011); and many others antagonist of different stages (Sanchez & Redolfi de Huiza, 1985; Desneux et al., 2010). Some have been tested and used in biological control against *T. absoluta* with promising results. The mirid bugs *Nesidiocoris tenuis* (Reuter, 1895) and *Macrolophus pygmaeus* (Rambur, 1839) were the most tested bugs. Larvae and adults are known to be consumers of eggs and larvae of *T. absoluta* (Urbaneja et al., 2009). The knowledge of biological processes and development of this pest in the arid agricultural systems of Biskra is important to establish an efficient management program. The objective of this research was to monitor population dynamics of *Tuta absoluta* in tomato crop during three growing seasons in Biskra.

2 MATERIALS AND METHODS

2.1 STUDY SITE

The study was carried out in the south east of Algeria in the province of Biskra (34°51′01" N, 5°43′40" E). Currently, *Tuta absoluta* was found exclusively on tomato crops which are cultivated essentially under greenhouse conditions in Biskra. The infestation of tomato plants in the studied greenhouses occurred naturally. Tomato production has significant economic value at local and national level. The losses produced by this pest can be considerable. The crops were set up in late September and early October. During the study, tomato diseases and pests were recorded, including mainly botrytis, alternaria, mites, whiteflies, aphids, moth, agromyzid leaf miner and thrips. These pests were subjected to chemical treatments used by the farmers. Usually, tomato crop dries towards May due to high temperatures characterizing the region.

2.2 INSECT ADULTS MONITORING

Population development of *Tuta absoluta* was surveyed over three seasons. Adult monitoring started from the transplanting of plants until its desiccation. The survey was conducted in tomato greenhouses with a surface area of 400 m² (50 m in length and 8 m wide) containing about 800 plants. The distance between plants line was 1 m and between plants was 40 cm. Two traps Delta type (Russell IPM) equipped with pheromone capsules were used, at 1 m 20 cm from the ground surface, for capturing adults; they were placed in each greenhouse entrance separated by 30 m from each other. The pheromone capsules were renewed each month. The number of adults was recorded every week. The objective of traps was, first to detect the beginning of adult flight and secondly to study population changes.

2.3 SAMPLING METHOD OF IMMATURE STAGES

To investigate the immature stages (eggs, larvae

and pupae), sampling of leaves was important (Allache & Demnati, 2012; Allache et al., 2012). Immature stages can be encountered on all plant parts; but in this work, sampling concerned only leaves. Twenty leaves were collected randomly every week, and they were put individually in paper bags. Sampling of leaves started three weeks after plant transplantation depending on plant vigor. The leaves were brought back to laboratory and observed under a binocular magnifying glass. Eggs, larvae and pupae were then counted on each tomato leaf.

2.4 STATISTICAL ANALYSIS

Data obtained were normalized using a square root transformation (x + 0.5) before analysis. An ANOVA test was performed to differentiate between means and a LSD test for means comparison using a 5 % level of significance was applied.

3 RESULTS

3.1 TOTAL INDIVIDUALS COLLECTED

The total and average numbers of the different developmental stages of *T. absoluta* on tomato were monitored for three cropping seasons. Over the three cropping seasons, the rates of adults caught during the 2009/2010 season was high (43.96 %) compared to the two subsequent seasons. The captures were low in 2010/2011 and 2011/2012, amounting to respectively 28 and 27 %. The lowest number of males was caught in 2011/2012 growing season. The high number of catches was recorded particularly towards the end of the crop cycle. ANOVA analysis showed that adults caught were not significantly different between the three growing seasons ($p > 0.05$) (Table 1).

In October, a large number of adults were trapped during the 2011/2012 growing season. The least adults' captured was recorded in November. During the 2011/2012 growing season, March appears to be the most prolific concerning the number of adult caught. The number of caught individuals was most important in April for the 2009/2010 growing season. May was the most prolific month for the cropping season 2010/2011, the number of adults captured was important compared to the two other cropping years (Table 2).

Concerning total eggs laid on leaves, it was found that during the first crop season (2009/2010), a large numbers were recorded compared to the second and third cultivation growing seasons (Table 1). ANOVA analysis showed a significant difference between these three crop seasons ($F = 8.96$, $p = 0.000$). LSD test performed between the second and the third year revealed no difference ($p = 0.116$).

The number of eggs deposited was more pronounced from March. Majority of eggs were deposited on leaves during the 2009/2010 cropping season (March-April); and in May for the 2010/2011 growing season (Table 2).

Larval activity on leaves varied during and between the crop campaigns (Table 1). The most important activity was registered in the 2009/2010 growing season and the lowest during the 2011/2011 season. ANOVA analysis showed significant differences between larval numbers during these growing seasons ($F = 17.90$, $p = 0.000$). From December to April, during the growing season 2009/2010, the number of larvae recorded was most important compared to the two next crop years. However, a significant number was registered in May during the 2010/2011 cultural season (Table 2).

The pupae number recorded was very low and variable between all crop seasons. During the third season, only two pupae were counted on tomato leaves. The most important number of pupae collected was during the 2009/2010 crop season. ANOVA tests demonstrated significant differences between growing seasons ($F = 24.18$; $p = 0.000$); no difference was noted between the second and the third year ($p = 0.131$). An absence of pupae was observed during the growing season 2011/2012. Even so, the most important pupae numbers were noted during the 2009/2010 growing season. March, April and May appear most favorable to pupation of *T. absoluta* (Table 2).

We noted a lack of presence and activity of most

Table 1: Comparison of means of the different stages of *Tuta absoluta* between crop seasons

	Insect stage (Mean ± SD)			
Crop season	Adults	Eggs	Larvae	Pupae
2009/2010	71.57±102.21^{a} (2004)	20.07±18.83^{a} (562)	33.89±19.50^{a} (949)	5.82±4.62^{a} (163)
2010/2011	46.25±131.89^{a} (1295)	10.00±14.33^{b} (280)	13.11±28.96^{b} (367)	1.39±3.18^{b} (39)
2011/2012	45.00±47.18^{a} (1260)	3.86±8.27^{b} (108)	1.93±4.01^{c} (54)	0.07±0.26^{b} (2)

LSD test was used to differentiate between means. The values in the columns with the same lowercase letter are not statistically different (confidence level $p < 0.05$). Values in parentheses represent the total of detected individuals at each different stage of *Tuta absoluta*.

Table 2: Comparison between means per month of the different stages of *T. absoluta* during three cropping season

	2009/2010							
	Oct.*	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May*
Adults	0.00±0.00	1.25±2.50^{a}	30.80±23.64^{a}	23.25±5.34^{a}	15.50±4.20^{a}	76.00±39.12^{a}	227.00±64.61^{a}	402.00±0.00
Eggs	0.00±0.00	2.00±4.00^{a}	10.60±3.05^{a}	10.50±2.52^{a}	11.50±1.73^{a}	32.60±14.47^{a}	47.75±12.12^{a}	59.0±0.00
Larvae	0.00±0.00	6.75±13.50^{a}	38.20±4.03^{a}	24.75±4.27^{a}	25.75±7.50^{a}	44.60±2.79^{a}	58.50±7.33^{a}	72.00±0.00
Pupae	0.00±0.00	0.00±0.00^{a}	4.00±1.58^{a}	2.50±2.08^{a}	5.50±1.00^{a}	9.60±2.07^{a}	12.50±0.58^{a}	13.00±0.00
	2010/2011							
Adults	0.00±0.00	0.00±0.00^{a}	0.80±1.30^{b}	1.00±0.82^{b}	3.25±2.63^{b}	30.00±27.10^{b}	109.75±39.36^{b}	685.00±0.00
Eggs	0.00±0.00	0.00±0.00^{a}	0.20±0.45^{b}	2.75±0.50^{b}	7.25±1.26^{b}	14.60±3.44^{b}	24.75±3.77^{b}	67.00±0.00
Larvae	0.00±0.00	0.00±0.00^{a}	0.00±0.00^{b}	0.00±0.00^{b}	2.25±3.86^{b}	11.80±3.77^{b}	39.00±5.89^{b}	143±0.00
Pupae	0.00±0.00	0.00±0.00^{a}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}	1.00±0.71^{b}	4.75±1.25^{b}	15.00±0.00
	2011/2012							
Adults	82.00±0.00	11.50±4.51^{b}	23.60±13.83^{a}	13.50±4.44^{c}	34.25±44.55^{a}	99.40±9.24^{a}	126.25±25.51^{b}	164.00±0.00
Eggs	0.00±0.00	0.25±0.50^{a}	0.40±0.55^{b}	0.50±0.58^{b}	0.50±1.00^{c}	2.00±2.35^{c}	18.00±13.59^{b}	19.00±0.00
Larvae	0.00±0.00	0.00±0.00^{a}	0.00±0.00^{b}	0.25±0.50^{b}	0.00±0.00^{b}	1.00±1.23^{c}	8.50±3.70^{c}	14.00±0.00
Pupae	0.00±0.00	0.25±0.50^{a}	0.00±0.00^{b}	0.25±0.50^{b}	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{c}	0.00±0.00

* It was not possible to make statistical analysis for all stages due to lack of data. One sampling was done in October due to newly transplanted tomato plants. Likewise for May, this was due mainly to desiccation and uprooting of tomato seedlings by the farmer. The values followed by the same letters in lowercase in the same column for the same stage were not significant at $p < 0.05$.

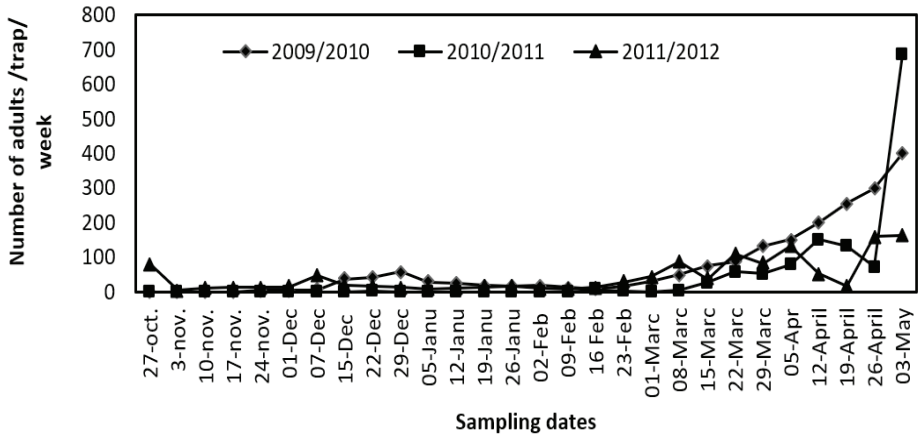

Figure 1: Flight activity and population changes in pheromone traps of *Tuta absoluta* males in Biskra greenhouses during three cropping campaigns (2009/2010; 2010/2011; 2011/2012)

common natural enemies of the leaf miner tomato borer during the three cropping seasons.

3.2 FLIGHT ACTIVITY AND POPULATION CHANGE OF ADULTS

The first flight of adults of *T. absoluta* was recorded in pheromone traps on November 24th, 2009 for the first growing season, December 15th, 2010 for the second and earlier for the third year (October 27th, 2011) (Figure 1).

Adults occurred during all tomato phenological cycles. At the beginning of the tomato cultivation season, the numbers of male caught in pheromone traps was low. Thereafter, this number became very important towards the end of the tomato cycle. The observations revealed intense adult activity with increasing temperatures from the end of February until crop harvest for the three growing seasons. Development changes of *T. absoluta* adults for the different growing seasons was highly significant (2009-2010: $F = 37.93$, $p = 0.000$ / 2010-2011: $F = 182.86$, $p = 0.000$ / 2011-2012: $F = 5.65$, $p = 0.000$). The number of individuals caught in traps ranged from 5 to 402 adults in the first growing season (2009/2010); four peaks were registered. During the 2010/2011 crop season, between 1 and 685 adults were captured, with three peaks observed. In the third cropping season (2011/2012), there were between 5 and 164 adults, with six peaks observed. The population of tomato leaf miner borers increased with time in the three cropping seasons; most adults number were trapped in spring.

4 DISCUSSION

The survey was conducted throughout all phenological plant cycles. Tomato crops seem to be the only host plant used by *Tuta absoluta* in Biskra. This was also reported by Allache et al. (2012), while Vargas (1970), Garcia & Espul (1982) and Guenaoui et al. (2011), reported their development on several plants belonging to cultivated and wild solanacous species. *Tuta absoluta* seems to be well established in this location given the continual presence throughout the plant cycle and during the three cropping seasons. Currently it is among the major pests that cause important threats on tomato crop in Algeria. There were very few captured males early in the growing season due to low temperatures. This number gradually increased during the end of the cropping season. Similar observations were stated by Cocco et al. (2013, 2015), Cherif & Lebdi-Grissa (2014) and Harbi et al. (2015). Balzan & Moonen (2012) underlining exponential growth of *T. absoluta* captured during warmer periods and high numbers at crop harvest. These may be due to increased temperatures (Lacordaire & Feuvrier, 2010; Allache et al., 2015). Whereas El-Aassar et al. (2015) highlighted a decrease in population of *T. absoluta* caught.

In October, during the third cultivation season (2011/2012), an important number of adults were trapped. These high captures can be due to population built-up during the previous cultivation season as suggested by Cocco et al. (2013).

The adult flight activity takes place during the same period in autumn; which means when the summer-autumn temperatures become favorable (decline in high temperatures in late September), the adults' flight is activated. Martins et al. (2016) reported an upper and lower limit of development temperatures estimated between 14 °C and 35 °C. Unlike the present study, Mamay & Yanik (2012) reported an adult flight activity much later, in early May in Şanliurfa

(Turkey); and in January in Takelsa, Tunisia (Cherif et al., 2013).

In absence of cultivated tomatoes during intercropping periods, alternative host species can serve as infestation reservoirs for the tomato leaf miner borers (Cocco et al., 2015). Given the extreme temperatures in Biskra during the summer, all plants potentially favorable for its development will dry out at that time. A study of aestivation form of *T. absoluta* is needed in this location to build a pest control plan before adults' flight activity.

Knowing the first adults' flight and major flight activity periods are important for farmers to take appropriate pest control decisions. According to Cherif et al. (2013), information about the population structure combined with adults' flight activity are essential to control this pest and to determine the best intervention time according to larvae sensitivity and extent of damage.

All *T. absoluta* instars were present on tomato leaves in the greenhouses during all cropping seasons. This is confirmed by the results obtained by Lebdi-Grissa et al. (2010) and Cocco et al. (2015). The tomato leaves were more attractive to the females' egg laying (Galdino et al., 2015; Salama et al., 2015). In this study, the amounts of eggs deposited on leaves were low; even so, 2009/2010 cropping season was the year which more eggs were laid.

Important numbers of eggs were noticed in Marsh, April and May. These numbers were low in 2010/2011 and 2011/2012 cropping years. This was most likely due to low temperatures early in the season during the egg hatching. Likewise, Cherif & Lebdi-Grissa (2014) registered low egg numbers during the autumn-winter period; this number increased in spring. After the same authors, the leaf miner *T. absoluta* development was related to temperature. Remating of *Tuta absoluta* increased number of eggs laid, fertility and female longevity (Lee et al., 2014). Sampling methodology can also explain low egg numbers. Lebdi-Grissa et al. (2010) indicated that eggs were laid on young leaves; however, in the present study, sample of leaves were taken on the middle part of the plants.

Tuta absoluta larvae were present throughout all stages of the phenological plant cycle during the 2009/2010 cropping season; these numbers were more important compared to the two subsequent seasons. March, April and May appeared to be very favorable for larval development, perhaps because the 2009/2010 crop season was warmer than the following years. It may also be due to larvae movement; it should be noted that leaves were sampled essentially in the middle canopy. Galdino et al. (2015) reported that older larvae can move around the plant, to young leaves, with more nutrition, increasing their performance. The same authors reported that larvae were able to identify sites on plants where their performances are high.

Larvae of leaf miners reaching pupae stage in leaf tissue appeared very low, principally for the second and the third cropping seasons. This may be due to larval pupation, which was performed generally in the ground; however, some old larvae complete their developmental stage in leaf galleries (Lebdi-Grissa et al., 2010). The abiotic factor like temperature (Lebdi-Grissa et al., 2010); and biotic factors like, larval natural mortality, pupae viability and nutritional quality of the plants could have an effect on leaf miner development (Torres et al., 2001). Furthermore, to the sampling procedure which don't take in consideration pupae fallen on the ground or to larval chemical control (Allache et al., 2012). According to Coelho and França (1987), very little larval pupation takes place on leaves, stems and fruit; this could be the cause for the low numbers found in this study.

Our study showed that at the end of the third year, the pests were well installed and continually present on the tomato crop, while predators were not found. On the contrary, Cocco et al. (2015) observed parasitoid activity on potato and tomato crops during a 2-years study. In Jordan, Al-Jboory et al. (2012) noted three hemipteran insects and one parasitic wasp on *T. absoluta*. Unlike the present study, Mahdi et al. (2011) recorded two predatory mirid bugs in Mitidja (Algiers) (*Nesidiocoris tenuis* (Reuter, 1895) and *Macrolophus caliginosus* (Wagner 1951)) and a parasitic eulophid wasp *Diglyphus* spp. Several natural enemies could develop on *T. absoluta* depending on its dispersion area of origin. Some of these species are used successfully in biological control with appreciable levels of parasitism (Desneux et al., 2010).

The main causes for the lack of tomato leaf miner borer antagonists in the study area can be explained by their sensitivity and the overuse of pesticides by the farmers in pest control programs.

5 CONCLUSION

The rising temperatures influenced the development of *Tuta absoluta*. This pest seems well established in tomato crops in Biskra. Thus their natural enemies were not found during this study. Increased knowledge about adult flight dates and population changes is important to develop an efficient control strategy.

Changing farmer behavior and control method (which was mainly chemical) and developing other strategies based on safer environmental techniques are fundamental for providing antagonists possibility, time and space to develop.

In Italy, Balzan & Moonen (2012) suggested changes

of the current strategies used. They stated that frequent use of pesticides not only disrupts the biological control but also makes the agroecosystems more susceptible to pest invasions; which leads to a dependence on external inputs of antagonists. Harbi et al. (2012) considered that insect-proof screens combined with sex pheromone mass trapping can be very efficient to decrease *T. absoluta* populations. However Cherif et al. (2013) discussed the unsuitability for egg laying of certain plant varieties, which can provide effective prophylactic techniques for reducing *T. absoluta* infestation.

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Fertilizer application enhances establishment of cacao seedlings in plant-parasitic nematodes infected soil

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Fertilizer application enhances establishment of cacao seedlings in plant-parasitic nematodes infected soil

Abstract: Low soil fertility, pests and diseases are major problems of growth and establishment of cacao seedlings on the field. Cocoa production increases by new plantings and rehabilitation of moribund farms, but a build-up of plant-parasitic nematodes (PPN) causing dieback and declining soil fertility has discouraged many farmers, leading to a reduction in crop productivity. In this study, the potentials of some organic wastes as fertilizers and their effects on establishment of cacao seedlings in PPN infected soils was investigated at Ibadan and Owena of Southwestern Nigeria. Goat dung (GD), organic fertilizer (OF), organo-mineral fertilizers (OMF) and NPK 15:15:15 were applied at 200, 400 and 600 kg ha-1, respectively, to cacao seedlings one month after transplanting, while unfertilized served as control. Results from the experiments showed a significant increase in percentage survival of cacao seedlings under organic fertilizers at Ibadan and Owena compared to NPK and control even at the lowest rate of 200 kg ha^{-1} 3 years after transplanting. The incorporation of GD, OF and OMF significantly reduced the population densities of PPN compared to control. Therefore, GD, OF and OMF at 200 kg ha^{-1} are recommended for soil application to enhance the field establishment of cacao seedlings in the soil infected with PPN.

Key words: fertilizers; plant-parasitic nematodes; cacao seedlings; establishment; organic wastes

Uporaba gnojil pospešuje rast sadik kakavovca v tleh okuženih s parazitskimi ogorčicami

Izvleček: Slaba rodovitnost tal, škodljivci in bolezni so glavni problem pri vzgoji sadik kakavovca na prostem. Pridelava kakava se povečuje z novimi nasadi in obnovo zanemarjenih kmetijskih zemljišč, a pojav parazitskih ogorčič (PPN), ki povzročajo propad sadik in zmanjšana rodovitnost tal jemljeta pri tem mnogim kmetom pogum, kar vodi v zmanjšanje v pridelavi te kulture. V tej raziskavi je bil preučevan potencial nekaterih organskih ostankov kot gnojil in njihov vpliv na rast sadik kakavovca v z ogorčicami (PPN) okuženih tleh v Ibadanu in Oweni, v jugovzhodni Nigeriji. Uporabljeni so bili kozji gnoj (GD), organska gnojila (OF), organsko-mineralna gnojila (OMF) in NPK 15 : 15 : 15 v odmerkih 200, 400 in 600 kg ha^{-1}, v nasadu kakavovca en mesec po presaditvi in kot kontrola nepognojen nasad. Rezultati poskusa so pokazali značilno povečanje preživetja sadik kakavovca pri gnojenju z organskimi gnojili v Ibadanu in Oweni v primerjavi z gnojenjem s NPK in kontrolo, celo pri najmanjšem gnojenju z organskimi gnojili, 200 kg ha^{-1}, 3 leta po presaditvi. Vnašanje GD, OF in OMF v tla je značilno zmanjšalo gostoto populacij ogorčic v primerjavi s kontrolo. Zaradi tega priporočamo gnojenje z GD, OF in OMF v odmerku 200 kg ha^{-1} za uspešno rast sadik kakavovca v tleh okuženih s parazitskimi ogorčicami.

Ključne besede: gnojila; rastlinske parazitske ogorčice; sadike kakavovca; uspešna vzgoja; organski odpadki

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1 INTRODUCTION

Cocoa (*Theobroma cacao* L.) is cultivated in the humid tropics of the world (Yanelis et al., 2012) with more than 70 % production coming from Africa as a source of income for producing countries (Simo et al., 2018). The crop production is dominated by small-scale farmers who live and work in the cocoa belt providing them employment and income (Minimol et al., 2015; Ngoh Dooh et al., 2015). However, cocoa production has witnessed a downward trend due to declining soil fertility, pests and diseases, aging trees and low yields from smallholder farms. Low farm gate prices paid to farmers make it difficult for them to afford expensive inputs to increase soil fertility and yield, such as mineral fertilizers, and pesticides to control pests and diseases adverse effects. There are also concerns that the projected global temperature rise and subsequent increase in potential evapotranspiration and demand for plant water may lead to further drought stress during the dry season and deterioration of cocoa climate conditions (Läderach et al., 2013; Schroth et al., 2016). Cocoa production increases through new plantings and rehabilitation of moribund farms, but the build-up of plant-parasitic nematodes causing die-back of cacao seedlings in nurseries and young plantations and declining soil fertility caused many farmers to be discouraged leading to a reduction in crop productivity (Orisajo et al., 2012; Orisajo, 2018). The need to pay attention to soil fertilization is now almost as important as the control of pests and diseases in cocoa. Tropical soils are inherently low in soil organic matter and fertility status; hence external fertilizer supply is a key factor in raising crop production.

Fertilization is an indispensable agricultural practice in which organic and inorganic fertilizers are used primarily to improve plant nutrition and hence crop productivity (Tian et al., 2015; Francioli et al., 2016). Inorganic fertilizers which perform a decisive role in improving crop productivity are wildly applied. The production and application of these fertilizers cause serious environmental damage like greenhouse gas emissions, eutrophication (Copetti et al., 2016), pollution (De Notaris et al., 2018), leaching and contamination of groundwater thereby posing risk to human health (Huang et al., 2018; Jalali & Latifi, 2018). The continuous application of NPK leads to increase in the soil compactness, decrease in the soil pH (Adamtey et al., 2016), soil porosity, and organic carbon level (Chaudhary et al., 2017) as well as decrease in soil beneficial microorganism populations (Wei et al., 2017). Continuous excessive applications of inorganic fertilizer can also lead to nutrient accumulation in soil, and eventual P and N loss from soil to aquatic ecosystems (Qiao et al., 2012; Yan et al., 2013). Excessive N and P applications will also deteriorate the soil quality and reduce the soil's production levels (Zhang et al., 2015). With rising costs of chemical fertilizer and the aforementioned growing concerns over the environmental impact of excessive fertilizer application, there has been an increasing scrutiny on how nutrients are managed on farms (Chen et al., 2014).

Organic fertilizers (manures) are gaining attention as the alternative to inorganic fertilizers. Organic manure produced from biomass and animal conventionally plays an important role in recycling of nutrients (Hasler et al., 2015). When added to soils, organic manure enhances soil fertility by increasing nutrient availability (Cavagnaro, 2014), soil organic carbons (Xie et al., 2014), available N and P, micronutrients, soil aggregation, and water holding capacity, as well as leading to a high soil buffering capacity against external disturbances (Yu et al., 2012; Liang et al., 2012; Chaudhary et al., 2012; Sogn et al., 2018). Though, the benefits associated with organic amendments majorly depend upon the type and application rate of organic fertilizers (Jones & Healey, 2010).

The application of organic material, though a traditional practice to improve soil fertility and structure, is also known as a control method for soil- borne diseases, including plant-parasitic nematodes (Hassan et al., 2010; Houx et al., 2014). In recent years, a variety of organic materials, such as animal and green manures, compost, and proteinaceous wastes, are used for this purpose (Summers, 2011; Stirling et al., 2011; Renco & Kovacik, 2012; Olabiyi & Oladeji, 2014; Abolusoro et al., 2015; Rudolph & DeVetter, 2015; Tiyagi et al., 2015; Briar et al., 2016; Forge et al., 2016; Atandi et al., 2017; Shiferaw et al., 2017). Incorporation of organic amendments has been shown to be detrimental to plant parasitic nematodes (Wang et al., 2004) due to release of NH_4, formaldehyde, phenol, volatile fatty acids and toxic compounds (Oka, 2010; McSorley, 2011; Briar et al., 2016). It was generally postulated that the adverse influence of organic amendment on plant-parasitic nematode is referred to increasing host resistance to nematode infection and enhancement of growth performance (Country & Millon, 2008).

This work aims to examine the effects of organic and organo-mineral fertilizers on plant-parasitic nematodes, cacao seedlings growth and establishment on the field. This will possibly ameliorate the current frustration faced by small-scale farmers on poor establishment of cacao seedlings and thereby increasing the crop production and income.

2 MATERIALS AND METHODS

2.1 STUDY AREA

Field experiments were carried out at the Cocoa Re-

search Institute of Nigeria (CRIN) experimental farms in Ibadan, Oyo State and Owena, a CRIN Substation in Ondo State, Nigeria. Ibadan lies between the latitude 7^0 30' N and longitude 3^0 54' E at an altitude of 1222 m above sea level. It is located in the tropical rain forest ecosystem with mean solar radiation of 18MJ m^{-2} day^{-1} and an annual average rainfall of 2000 mm with a bimodal pattern. Owena lies between the latitude 7^0 15' N and longitude 5^0 12' E at an altitude of 367 m above sea level. It is located in the tropical rain forest ecosystem with mean solar radiation of 30MJ m^{-2} day^{-1} and an annual average rainfall of 1500 mm with a bimodal pattern.

The experiment was conducted over three years on the False horn plantain (*Musa* spp. L., AAB – group 'Agbagba') as shade crop planted with cacao (*Theobroma cacao* 'F3 Amazon') in Ibadan and Owena. The experiment was set as a randomized complete block design involving four fertilizer types: goat dung (GD), organic (OF), organo-mineral fertilizer (OMF) and NPK 15:15:15, which were separately applied at 200, 400, 600kg ha^{-1} and unfertilized served as control. Each treatment had 3 replications. Healthy sword suckers of plantain of approximately uniform size (50-60 cm tall, 30-40 cm pseudostem girth) pared to remove lesions were planted at a spacing of 3 x 3 m. Cocoa seedlings of 5 months old were planted four weeks later at the same spacing.

2.2 PROCUREMENT OF FERTILIZERS AND PROXIMATE ANALYSIS

Organic (OF) and organo-minerals fertilizers (OMF) used for the experiments were obtained from the Sunshine Fertilizers, Ministry of Agriculture, Ondo State. They were manufactured in 2016 with batch numbers 30172, 30110, respectively. Goat dung (GD) was collected from Goat farms in Ilesha Garage, Akure, Ondo State. The GD was collected from pens with good farm sanitation, air-dried, carefully sorted to remove foreign materials and packed in 50 kg bags. The analysis was conducted to determine the nutrient content of the fertilizers using the wet digestion method (Odu et al., 1986). After drying, the fertilizer sample was finely ground in a mortar at approximately 80 °C for 12 hours. The 0.5 g sample was then weighed into a 100-ml Berzelius beaker. Five millilitres (5 ml) of nitric acid (HNO_3) and 2 ml of perchloric acid ($HClO_4$) were added, covered with a watch glass and digested by heating to a final volume of 5 ml. Ten millilitres (10 ml) of water was then added and the digested solution was filtered through an acid-washed filter paper into a 50 ml volumetric flask. The filter paper was washed with water and the filtrate diluted to volume with deionized water. The filtrate was read under atomic adsorption spectrometer, flame photometer and colorimeter for macro and micronutrients in the sample.

2.3 SOIL SAMPLES COLLECTION AND ANALYSIS

Soil samples were collected randomly from each of the experimental sites at both locations (Ibadan and Owena) with the aid of soil auger at 0 - 30 cm depth. For the pre-cropping analysis, the samples were bulked together and mixed thoroughly, air dried at room temperature and analysed for various elements. Particle analysis was determined using the hydrometer method (Kettler et al., 2001). Organic carbon determination was by the potassium dichromate oxidation method (Zhang et al., 2001). The total nitrogen (N) was determined by Kjeldahl method; available P by ammonium-vanadomolybdate colorimetric method; exchangeable K and Na by flame photometer; and exchangeable Mg, Ca and Mn were determined using atomic absorption spectrophotometer (Ryan et al., 2001). Soil pH was read on pH meter (1:1 water). Soil was assayed to confirm the presence and the initial population density of the plant-parasitic nematodes (Coyne et al., 2007). Aliquots of 100 ml soil was put into a set up that has two plastic sieves with extractor tissue sandwiched in between. The plastic sieves with the soil were thereafter placed in a plastic bowl, and water was added to the extraction bowl just enough to wet the soil. The set-up was left undisturbed for 48 hours. Thereafter, the plastic sieve containing the soil was removed briskly, and the nematode suspension in the bowl was poured into a nalgene wash bottle and allowed to settle. The supernatant was siphoned out, and the suspension containing nematodes was then poured into a labelled beaker, and adjusted to 10 ml by adding water. This was homogenized and 1ml of the suspension was taken with the use of pipette, dispensed into the nematode counting dish and examined under a high power stereomicroscope. Nematodes were transferred with a picker to a slide with a drop of water, covered (with a cover slip) and examined under an Olympus compound microscope for identification using taxonomic keys (UNL, 2019) and counted. The identification and counting was repeated three times and mean population of nematodes per sample calculated. Two grams (2 g) each of the organic fertilizers used were also analysed for nutrient composition.

2.4 FERTILIZER APPLICATION AND DATA COLLECTION

The fertilizers were applied to treatment plots one month after transplanting using ring method of appli-

cation at 5 cm away from the base of cacao. Monthly Data collection on growth parameters (plant height, stem girth, number of leaf, and leaf area and number of branches) commenced 3 months after transplanting. Leaf samples (4th leaf) were collected from 4 tagged cocoa seedlings at 12 months after transplanting and were analysed in the laboratory for chemical composition. The experiments were monitored for 36 months (144 weeks after planting). Survival count was carried out 12 months after transplanting. At 15 months after transplanting, soil samples were collected from treatment plots and were processed and analysed for physical properties (sand silt, loam, clay, soil moisture content and soil bulk density), chemical properties (soil organic matter, soil pH, N, P, K, Mg, Ca, and Na), and plant-parasitic nematodes population densities using aforementioned standard procedures.

2.5 DATA ANALYSIS

Nematode population densities were $\log_{10}(x + 1)$ transformed and percentage data were square-root-transformed prior to analysis to stabilize variances (Gomez & Gomez, 1984), while the other data collected were not transformed. Only the predominant plant-parasitic nematode species were included in the data analysis. Analyses of variance (ANOVA) were carried out to test for main effects and interactions. Pre-planned comparisons between treatment combinations were tested with linear contrasts. All analyses were performed using GENSTAT.

3 RESULTS AND DISCUSSIONS

3.1 NUTRIENT COMPOSITION OF THE ORGANIC MATERIALS

The nutrient composition of the organic materials applied to the soil is presented in Table 1. The C : N ratio of the organic fertilizers used are 8.2, 9.4, 9.8 for goat dung, organo-mineral fertilizer and organic fertilizer, respectively. Changes in the C : N ratio of aggregates may reflect the degree of organic materials decomposition within aggregate fractions (Baldock et al., 1992). Higher C : N ratios of aggregates suggest that soil organic C is relatively fresh or little altered, whereas, soil organic C is more decomposed and relative aged when the C : N ratio of aggregates is low (Chen et al., 2010). Difference in soil organic matter quality within aggregate fractions will result in difference in the types of nutritional substrates available, which may directly affect the natural microbial communities (Bending et al., 2002). In general, amending the soil with organic materials having low C : N ratio (less than 20) resulted in rapid mineralization of N in the form of NH_{4+} or NO_3 – for absorption and uptake by plant roots (Powers & McSorley, 2000). The fertilizers used in these experiments have low C : N and this appeared to have positive effects on the survival of the cacao seedlings.

3.2 SURVIVAL AND GROWTH OF CACAO SEEDLINGS AS AFFECTED BY FERTILIZER APPLICATION

Results indicated that fertilizers applied significantly ($p < 0.05$) increased the survival of cocoa seedlings 12 months after planting in the field. The percentage survival of cacao seedlings under organic fertilizers at Ibadan and Owena increased significantly compared to NPK and control even at the lowest rate of 200 kg ha^{-1} used in the experiment (Table 2). However, application of 600 and 400 kg ha^{-1} of NPK enhanced the survival of the cacao seedlings compared to the control. In the same vein, growth of cacao seedlings was consistently improved by the fertilizer application compared with the control at both locations (Table 3). Application of goat dung, organo-mineral fertilizer and organic

Table 1: The nutrient composition of the organic materials

Properties	Goat dung (GD)	Organo-mineral fertilizer (OMF)	Organic fertilizer (OF)
pH (water)	8.17 ± 0.04	7.00 ± 0.03	7.30 ± 0.02
Organic carbon (%)	40.1 ± 0.13	40.5 ± 0.12	36.4 ± 0.13
Organic matter (%)	69.1 ± 0.15	69.8 ± 0.14	62.8 ± 0.15
Total nitrogen (%)	4.9 ± 0.01	4.3 ± 0.01	3.7 ± 0.01
Available P (cmol kg^{-1})	113.24 ± 0.17	138.06 ± 0.17	7.08 ± 0.17
K^+ (cmol kg^{-1})	0.41 ± 0.01	0.19 ± 0.01	5.56 ± 0.01
Mg^{++} (cmol kg^{-1})	1.20 ± 0.01	1.00 ± 0.01	6.00 ± 0.01
Ca^{++} (cmol kg^{-1})	2.60 ± 0.12	2.00 ± 0.12	13.10 ± 0.15
Na^+ (cmol kg^{-1})	0.38 ± 0.01	0.18 ± 0.01	2.30 ± 0.02
C : N	8.2 ± 0.03	9.4 ± 0.04	9.8 ± 0.03

fertilizer at 200, 400 and 600 kg ha^{-1} led to a significant increase in the height of cacao compared with NPK and control (Table 3). Similar pattern was observed for other growth parameters measured. In contrast, there was a significant reduction in plant height, stem girth, number of leaves, leaf area and number of branches of cacao in unfertilized plots. The increase in growth parameters could be attributed to the enhanced nitrogen and phosphorus uptake by the plant using organic amendments (Pandit et al., 2018). Organic manures have been shown to supply required plant nutrients, improve soil structure and promote plant growth (Agbede et al., 2014, 2017). The addition of organic manure in soil may encourage the immobilization of bioavailable nitrogen and phosphorus, which may otherwise be lost through leaching or emissions in the environment (Sun et al., 2018). The inclusion of organic manure may also generate higher transpiration rates leading to higher water retention in the soil. Hence, more availability of water soluble nutrients may cause the crop yield improvement (Doan et al., 2015).

Application of inorganic fertilizer, NPK, even at the lowest rate 200 kg ha-1 also improved cacao growth significantly compared with the control (Table 3). This is in agreement with the earlier study that the use of appropriate levels of NPK fertilizers have good effects on plant growth factors (Irshad et al., 2006). NPK application enriched the availability of macro nutrients, nitrogen, phosphate, and potassium in the soil. These nutrients therefore, were readily absorbed by the crops. In crop metabolism, these nutrients are utilized in carbohydrate synthesis, cellulose, proteins, hormones, and enzymes. All these processes triggered the growth of plant organs such as plant height, stem diameter, number of leaves, leaf area and number of branches as reported in this present study. This result was in line with the previous studies conducted by Mandal et al. (2009) and Bandyopadhyay et al. (2010). In their studies, applications of NPK also triggered the growth of vegetative crops.

3.3 RELATIONSHIPS BETWEEN PLANT-PARASITIC NEMATODES AND CACAO GROWTH

Relationships between the predominant plant-parasitic nematode population densities recovered and vegetative growth of young cacao revealed various statistically significant interactions (Table 4). *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, *Pratylenchus coffeae* Goodey, 1951 and *Radopholus similis* (Cobb, 1893) Thorne, 1949 population densities were negatively correlated with the survival percentage of the cacao seedlings ($r = -0.69$, $p < 0.01$; $r = -0.58$, $p < 0.05$ and $r = -0.46$, $p < 0.05$, respectively). Furthermore, *M. incognita* was negatively correlated with the plant height ($r = 0.91$, $p < 0.01$), leaf area ($r = -0.61$, $p < 0.01$) and number of branches ($r = -0.51$, $p < 0.05$). This confirmed the previous reports that root-knot nematodes, *M. incognita*, damage on cacao seedlings led to stunted growth of the plants (Afolami & Caveness, 1983; Afolami & Ojo, 1984) Similarly, *Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956, *P. coffeae* and *R. similis* population densities were negatively correlated with plant height ($r = -0.46$, $p < 0.05$; $r = -0.51$, $p < 0.05$; $r = -0.43$, $p < 0.05$, respectively), while they have no significant correlation with leaf area and number of

Table 2: Survival rate (%) of cacao seedlings as affected by fertilizer application at Ibadan and Owena (12 months after transplanting)

Treatments		Ibadan experiments	Owena experiments
Fertilizers	Rates (kg ha^{-1})		
Goat dung	600	94.44a	94.44ab
	400	94.44a	94.44ab
	200	94.44a	88.33abc
Organo-mineral fertilizer	600	90.44a	83.33abc
	400	88.88a	83.33abc
	200	77.77ab	83.33abc
Organic fertilizer	600	94.44a	100.00a
	400	90.44a	83.33abc
	200	83.33ab	83.33abc
NPK 15 : 15 : 15	600	66.66b	72.22bc
	400	77.77ab	72.21bc
	200	72.21ab	66.88cd
Control		66.66b	49.89d

Treatment means within each column followed by the same letters are not significantly different from each other using Tukey's HSD at 5 % level.

Table 3: Effects of fertilizer types and rates on cocoa growth parameters at 12 months after transplanting in Ibadan and Owena

		Ibadan					Owena				
Treatments (kg ha^{-1})		Plant height (cm)	Stem girth (cm)	Number of leaves	Leaf area (cm^2)	Branche (no)	Plant height (cm)	Stem girth (cm)	Number of leaves	Leaf area (cm^2)	Branches (no)
Goat dung	600	92.58ab	2.46abc	52.31a	48.17ab	8.83a	99.55a	2.26ab	51.35a	41.17a	5.47ab
	400	88.33ab	2.47abc	36.05b	40.58abc	6.92ab	79.00ab	2.42ab	36.24b	33.08ab	5.42ab
	200	80.33abc	2.27abc	34.12b	44.92abc	8.08ab	101.17a	2.38a	35.23b	37.42ab	5.42ab
Organo- mineral fertilizer	600	90.75ab	1.99abc	50.18a	54.08ab	7.83ab	86.17ab	2.60a	50.15a	44.11a	5.50ab
	400	90.50ab	2.09abc	50.13a	46.00abc	5.57abc	78.17ab	1.93ab	50.10a	39.75ab	5.57ab
	200	81.50abc	1.97abc	36.13b	29.83bc	5.50bc	91.69ab	2.19ab	37.11b	36.36ab	5.07ab
Organic fertilizer	600	109.83a	2.59ab	49.74a	55.92ab	9.33a	90.92ab	2.07ab	50.13a	40.50ab	6.93a
	400	95.42ab	2.79a	34.67b	61.33a	7.00ab	89.00ab	1.99ab	34.54b	33.00b	5.05ab
	200	82.42abc	2.23abc	33.63b	41.42abc	6.50ab	73.50ab	1.86ab	33.87b	20.50c	4.43ab
NPK 15 : 15 : 15	600	68.89bc	1.97abc	50.23a	31.45bc	6.47ab	83.33ab	2.17ab	50.13a	33.50b	5.52ab
	400	64.08bc	1.65bc	39.10b	30.58bc	5.52abc	81.70ab	1.89ab	38.27b	30.42b	5.42ab
	200	68.33bc	2.23abc	37.15b	36.06abc	4.56bc	78.92ab	1.81ab	38.13b	23.72c	3.83ab
Control		50.42c	1.56c	20.33c	19.50c	2.53c	64.51b	1.39b	24.33c	17.56c	1.75b

Treatment means within each column followed by the same letters are not significantly different from each other using Tukey's HSD at 5 % level

Table 4: Linear correlation matrix (half) of mean values for plant-parasitic nematode population densities / 100 g soil, percentage survival, plant height, leaf area and branches of young cacao

	Hm	Pc	Rs	Survival (%)	Plant height (cm)	Leaf area (cm^2)	Branches (no)
M. incognita (J2)	0.96**	0.41*	0.67**	-0.69**	-0.91**	-0.61**	-0.51*
H. multicinctus	-	0.46*	0.72**	0.24	-0.46*	-0.12	-0.24
P. coffeae		-	0.84**	-0.58*	-0.51*	-0.15	-0.18
R. similis			-	-0.46*	-0.43*	-0.15	-0.17
Survival (%)				-	0.89**	0.63**	0.51*
Plant height (cm)					-	0.71**	0.53*
Leaf area (cm^2)						-	0.28

Mi: Meloidogyne incognita; Hm: Helicotylenchus multicinctus; Pc: Pratylenchus coffeae; Rs: Radopholus similis.
Correlation coefficient significant at *$p < 0.05$, **$p < 0.01$.

branches (Table 4). However, plant height was positively correlated with survival percentage ($r = 0.89$, $p < 0.01$), leaf area ($r = 0.71$, $p < 0.01$) and number of branches ($r = 0.53$, $p < 0.05$).

3.4 EFFECTS OF ORGANIC FERTILIZERS ON POPULATION DENSITIES OF PLANT-PARASITIC NEMATODES

The incorporation of goat dung, organo-mineral fertilizer and organic fertilizer at 200, 400 and 600 kg ha^{-1} led to a significant reduction in the population densities of these plant-parasitic nematodes compared with NPK fertilizer and control (Table 5). This is in agreement with earlier studies that soil amendments with different types of organic manures are effective in reducing the population densities of many soil-borne plant pathogens including plant-parasitic nematodes (Hassan et al., 2010; Shiferaw et al., 2017). Organic manure has been reported to be rich in several compounds especially nitrogen and phenolics (Hassan et al., 2010; Renco & Kovacik, 2012). Nitrogen in the organic manure after conversion into ammonia (Thoden et al., 2011) has been reported to kill several plant parasitic nematodes (Lazarovits et al., 2001). Phenols and other nematostatic chemicals released from organic matters into amended soil significantly decreased the nematodes population (Oka 2010; Briar et al., 2016). Several researchers using organic soil amendments have reported satisfactory results on the plant growth and yield in a variety of crops with marked reduction in the population of plant-parasitic nematodes (Orisajo et al., 2008; Pakeerathan et al., 2009; Iqbal et al., 2012; Chaudhary & Kaul, 2013; Abolusoro et al., 2015; Adepoju et al., 2017). All the treated plants showed significant and satisfactory results when compared to untreated control. Our findings in this study are similar with the aforementioned earlier reports. In the same vein, application of NPK at 200, 400 and 600 kg ha^{-1} 600 also had a significant lower population densities of *M. incognita*, *H. multicinctus*, *P. coffeae* and *R. similis*. Our findings were consistent with earlier studies that the use of appropriate levels of NPK fertilizers have good effects on plant growth factors with resultant reductions in plant-parasitic nematode populations (Irshad et al., 2006; Ameen et al., 2013; Osman et al., 2015; Kolawole et al., 2018). Contrarily, nematode populations were reported to have increased due to NPK and manure combined with chemical fertilizer (Hu et al., 2018). Other studies also reported an increase in the total number of nematodes due to the use of chemical fertilizers (Li et al., 2016; Hu et al., 2017).

4 CONCLUSION

Improving the agronomic conditions for plant growth is an important factor for increasing the plant tolerance to plant-parasitic nematodes (Charegani et al., 2010). Results from this study have shown that the addition of fertilizers to the soil will improve the survival and growth of cacao seedlings. With rising costs of chemical fertilizer and the growing concerns over the environmental impact of excessive fertilizer application, goat dung, organo-mineral fertilizer and organic fertilizer at 200 kg ha^{-1} are recommended for soil application. These have been shown to enhance the field establishment of cacao seedlings in the soil infected with plant-parasitic nematodes.

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Table 5: Effects of fertilizer types and rates on mean plant-parasitic nematode population densities / 100 g soil in Ibadan and Owena

Treatments	Ibadan				Owena			
	Meloidogyne incognita (000)	*Helicotylenchus multicinctus* (000)	*Pratylenchus coffeae* (000)	*Radopholus similis* (000)	*Meloidogyne incognita* (000)	*Helicotylenchus multicinctus* (000)	*Pratylenchus coffeae* (000)	*Radopholus similis* (000)
GD 600	0.28e	0.01c	0.36c	0.01c	0.33e	0.01c	0.37c	0.01c
GD 400	0.28e	0.02c	0.37c	0.01c	0.33e	0.01c	0.37c	0.01c
GD 200	0.27e	0.02c	0.35c	0.02c	0.33e	0.01c	0.38c	0.01c
OMF 600	0.35d	0.01c	0.33c	0.02c	0.44d	0.01c	0.37c	0.01c
OMF 400	0.34d	0.02c	0.33c	0.02c	0.44d	0.01c	0.38c	0.01c
OMF 200	0.34d	0.01c	0.36c	0.02c	0.43d	0.01c	0.38c	0.01c
OF 600	0.16f	0.01c	0.33c	0.03c	0.19f	0.01c	0.40c	0.01c
OF 400	0.16f	0.01c	0.35c	0.02c	0.19f	0.01c	0.40c	0.01c
OF 200	0.17f	0.01c	0.37c	0.02c	0.19f	0.01c	0.41c	0.02c
NPK 600	1.67c	0.22b	2.02b	0.14b	1.81c	0.21b	3.01b	0.14b
NPK 400	1.63b	0.23b	1.97b	0.14b	1.77b	0.21b	3.01b	0.14b
NPK 200	1.61b	0.23b	2.01b	0.14b	1.76b	0.22b	3.02b	0.15b
Control	7.63a	2.12a	8.36a	3.53a	7.01a	1.25a	7.84a	3.41a

Treatment means within each column followed by the same letters are not significantly different from each other using Tukey's HSD at 5 % level.

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Seasonal incidence of apple leaf miner (*Lyonetia clerkella* (L., 1758), Lepidoptera, Lyonetiidae) in Kashmir, India

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Seasonal incidence of apple leaf miner (*Lyonetia clerkella* (L., 1758), Lepidoptera, Lyonetiidae) in Kashmir, India

Abstract: The seasonal incidence of apple leaf miner (*Lyonetia clerkella* [L., 1758], Lepidoptera, Lyonetiidae) was investigated in four districts of Kashmir valley from March 2015 to April 2016. The prevalence of infestation was found higher in Srinagar (70.6 %) and Bandipora (65.3 %) as compared to Pulwama (9.3 %) and Baramulla (6.6 %). Infestation intensity was found at its peak during the month of May (2015) in all the four districts. In Srinagar, the percent infestation intensity during May (2015) was found to be 58.69 % (± 11.46 SD), while as in Bandipora, Pulwama and Baramulla, it was found as 55.71 % (± 12.59 SD), 6.04 % (± 1.97 SD) and 4.27 % (± 1.12 SD) respectively. Infestation of intensity was observed to decline linearly from the first week of June (2015) and disappeared completely with the beginning of winter season. Further, seven generations of *L. clerkella* were found under laboratory conditions. The1st, 2nd, 3rd, 4th, 5th, and 6th generations completed in 39, 40, 37, 39, 45 and 48 days respectively. However, final generation (7th) was found relatively longer in duration, extended from the 4th week of September (2015) till April of the following year (2016) for about 204 days. In general, the life cycle of *L. clerkella* is similar to those described for other leaf miner species.

Key words: *Lyonetia clerkella*; prevalence; infestation intensity; seasonal history

Sezonsko pojavljanje sadnega listnega zavrtača (*Lyonetia clerkella* (L., 1758), Lepidoptera, Lyonetiidae) v Kašmirju, Indija

Izvleček: Sezonsko pojavljanje sadnega listnega zavrtača (*Lyonetia clerkella* (L., 1758), Lepidoptera, Lyonetiidae) je bilo preučevano v štirih območjih doline Kašmirja od marca 2015 do aprila 2016. Razširjenost okužbe je bila večja v Srinagarju (70,6 %) in Bandipori (65,3 %) v primerjavi s Pulwamo (9,3 %) in Baramullo (6.6 %). Velikost okužbe je dosegla višek v maju (2015) v vseh štirih območjih. V Srinagarju je bil odstotek okužbe, v maju 2015 58,69 % (± 11,46 SD), medtem, ko je bil v Bandipori, Pulwami in Baramulli 55,71 % (± 12,59 SD), 6,04 % (± 1,97 SD) in 4,27 % (± 1,12 SD). Velikost okužbe je linearno upadala od prvega tedna v juniju (2015) in je popolnoma izginila z začetkom zimske sezone. V laboratorijskih razmerah je bilo ugotovljenih sedem generacij sadnega listnega zavrtača. Prva, druga, tretja, četrta, peta in šesta generacija so zaključile svoj razvoj po 39, 40, 37, 39, 45 in 48 dneh. Zadnja, sedma generacija, je imela daljši razvoj, ki je trajal od četrtega tedna v septembru 2015 do aprila 2016, skupno okrog 204 dni. V splošnem je življenski krog sadnega listnega zavrtača podoben tistim, ki so opisani za ostale vrste listnih zavrtačev.

Ključne besede: *Lyonetia clerkella*; raširjenost; velikost okužbe; sezonsko pojavljanje

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1 INTRODUCTION

Kashmir is famous for the deliciousness of its temperate fruits in every part of India (Rather & Buhroo, 2015). Thousands of fruit orchards consisting mainly of apple (*Malus domestica* Borkh) can be seen in many parts of the valley. However, a wide variety of insect pests also occur on apple trees from the onset of foliage till the end of autumn period. Among these pests, apple leaf miner (*Lyonetia clerkella* (L., 1758) is regarded as one of the most common, widespread and destructive in many parts of the valley (Ahmed & Bhat1987; Rather & Buhroo, 2015).

The female moths of *L. clerkella* deposit their eggs singly into the leaf parenchyma (Adachi, 1998). The larvae feed on mesophyll immediately after being hatched. Thereafter, the hatched caterpillars form serpentine mines in the upper half of leaves that are visible to the unaided eyes. However, leaf epidermis is not damaged during the mesophyll devouring and larvae occupy mines till the next phase of their life cycle. The mines with variable lengths that range from narrow linear galleries to wide chambers appear as whitish or grey areas on the leaves. Mines excavated by the larvae of *L. clerkella* were found to be one of the major causes that reduce the photosynthetic capacity of leaves and cause their premature abscission (Spencer, 1973; Parrella, 1987; Parrella & Jones, 1987). The larval tunneling has also been reported to provide a way for the pathogens to enter into the tissues of the plants (Zitter & Tsai, 1977) and decline the annual yield of fruits (Wolfenbarger, 1954; Ledieu & Heyler, 1985; Minkenberg & Van Lenteren, 1986).

The re-emergence of hibernated moths of *L. clerkella* occurs with the flowering of apple trees. As the day temperature rises in spring, male and female population of these moths emerge in about the equal numbers (Faeth, 1985). After arrival, these miner insect pests target the leaves of the host plants. The female moths discriminate and select the suitable leaves for oviposition. Leaf selection is therefore an important aspect as far as insect-plant interactions are concerned (Faeth et al., 1981).

There is no information available on the prevalence and intensity of infestation of this miner pest in the Indian sub-continent. Therefore, the aim of the present study was to investigate the various aspects of *L. clerkella* including prevalence, infestation intensity and the number of generations.

2 MATERIAL AND METHODS

In order to evaluate the infestation prevalence of *L. clerkella* in different districts of Kashmir such as Srinagar (34° 51'.161" N, 74° 47' 50.535" E, Elev. 1,585 m asl) Bandipora (34° 41' 670" N, 74° 68' 69. 425" E, Elev. 2,183 m asl) Pulwama (33° 52' 18.627" N and 74° 53' 57.753" E, Elev. 1,740 m asl) and Baramulla (34° 12' 72.732" N, 74° 20' 53.732" E, Elev. 1,615 m asl) (Fig. 1), three sites in each of these districts were selected.

Fifty host trees (*Malus domestica* 'Red Delicious') at each site were surveyed during the peak season (May 2015) of this pest. Number of infested trees at each site was counted. Percent prevalence of infestation for every district was calculated by the following formula:

$$\frac{\textit{Total No. of infested trees in three sites of a district}}{\textit{Total No. of trees at these sites}} \times 100$$

For assessing the infestation intensity in the various months of a year (2015), three sites in every district (Srinagar, Bandipora, Pulwama and Baramulla) were

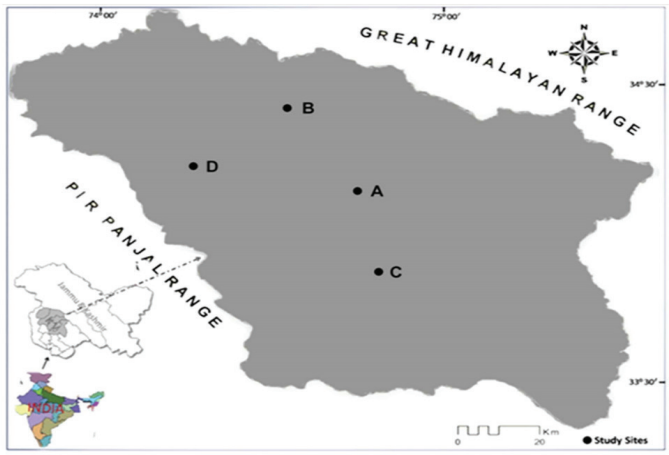

Figure 1: Map showing sampling sites (A = Srinagar, B = Bandipora, C = Pulwama and D = Baramullah)

selected. The sampling was done as per the method adopted by Adachi (2002). Five infested trees at each site were surveyed randomly at every survey during the final week of every month, from April to December (2015). One branch from each of these host trees at every site was chosen and the total number of leaves (fresh and mined) and mines of this branch were counted. Infestation intensity for each district was thereafter calculated by the following formula:

$$\frac{Total\ number\ of\ infested\ leaves\ in\ three\ sites}{Total\ number\ of\ mines\ they\ bear} \times 100$$

In order to determine the number of generations *L. clerkella* produces under laboratory conditions, methodology adopted by Rather and Buhroo (2015) was followed. Leaves carrying cocoons of this pest were collected from the field and brought in the laboratory. Small sections of these leaves with cocoons were incised and placed inside the 7 litre transparent plastic bottles mounted upon the 2.5 feet apple plants (Figs. 2–3). These bottles had cross ventilations with the dimensions of 10 x 10 cm covered with one layer of nylon mesh for the free circulation of air. About 25–30 cocoons were introduced in each of these bottles. Dead moths of the first generation were collected for preservation and leaves were constantly being observed with the help of torch light (being placed on the underside of the leaves) and high power magnifying glass (Fig. 4). After the appearance of mines, bottles were again mounted on these experimental plants until the formation of cocoons. This process was repeated till the end of final generation. The duration of each generation was thereafter calculated from the time of oviposition till the death of adult moths.

Images were taken by Canon EOS 1200-D camera attached with Reynox super macro 250-D lens on a 75–250 mm zoom lens. Analysis of variance (ANOVA)

Figure 2 & 3: 2 Leaf sections with cocoons 3. Bottles mounted on apple plants

Figure 4: Mine observation by placing a torch at the inner side of a leaf

followed by Tukey's multiple comparison test was done in SPSS (Version 20). The significance of differences between means was determined at $p < 0.05$.

3. RESULTS

3.1 PREVALENCE

One hundred and six (70.6 %) out of 150 host trees in Srinagar were found infested with *L. clerkella* and 98 (65.3 %) trees were found infested in Bandipora (Fig. 5). The situation was quite different in the other two districts. Fourteen (9.3 %) trees in Pulwama and merely 10 (6.6 %) trees in Baramulla were found infested. No significant difference ($p > 0.05$) in infestation prevalence was found between Srinagar and Bandipora, and between Pulwama and Baramulla. However, this difference was found statistically significant ($p < 0.05$) between Srinagar–Bandipora and Pulwama–Baramulla districts (Fig. 5).

3.2 INFESTATION INTENSITY

The seasonal variation in infestation intensity (%) in different districts of Kashmir is given in Table 1. The intensity of infestation was found relatively high in May (2015) than the other months (April and June – December 2015) in all the study areas of the valley, Srinagar, Bandipora, Pulwama and Baramulla. However, the rate of infestation was found much more in intensity in Srinagar and Bandipora districts as compared to other two areas of the Valley (Pulwama and Baramulla). Although the signs of infestation appeared from the third week of April in all these districts, infestation was however found with varying intensities among them throughout the year. In the month of April, the intensity of infestation in Srinagar, Bandipora, Pulwama and Baramulla were calculated to be 7.61 % (± 2.87 SD), 6.74 % (± 2.36 SD), 2.19 % (± 0.61SD) and 1.01 % (± 0.31SD) respectively (Figs. 6–9). The percent infestation intensity of district Srinagar and Bandipora were found statistically similar but different than Pulwama and Baramulla (Table 1).

Infestation intensity was found at its peak during the month of May (2015) in all the four districts. In Srinagar, the percent infestation intensity was found to be 58.69 % (± 11.46 SD) while as in Bandipora, Pulwama and Baramulla, it was calculated as 55.71 % (± 12.59 SD), 6.04 % (± 1.97 SD) and 4.27 % (± 1.12 SD) respectively. The percent infestation intensity of district Srinagar and Bandipora are statistically similar but statistically different from Pulwama and Baramulla (Table 1).

The level of infestation intensity was observed to decline linearly from the first week of June till the last week

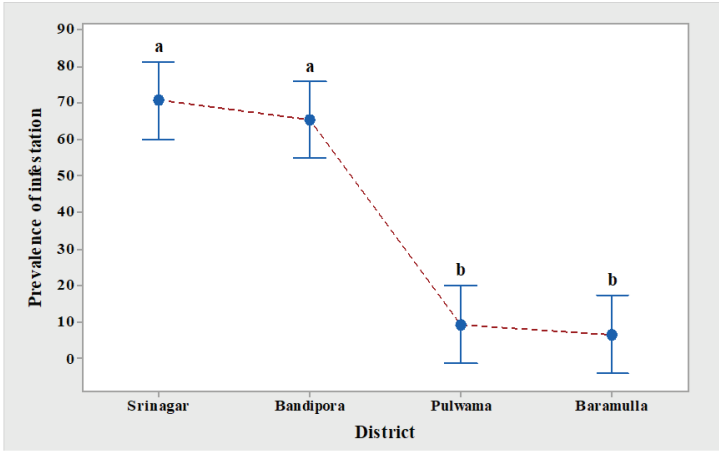

Figure 5: Prevalence of infestation in different districts of Kashmir

Table 1: One way ANOVA showing seasonal variation in infestation intensity (%) in different districts of Kashmir (Mean ± SD)

District	March	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
Srinagar	$0.00^{a} \pm 0.00$	$7.61^{a} \pm 2.87$	$58.69^{a} \pm 11.46$	$37.29^{a} \pm 16.19$	$32.61^{a} \pm 9.58$	$27.07^{a} \pm 5.07$	$13.43^{a} \pm 6.28$	$7.92^{a} \pm 4.92$	$3.73^{a} \pm 1.36$	$0.00^{a} \pm 0.00$
Bandipora	$0.00^{a} \pm 0.00$	$6.74^{a} \pm 2.36$	$55.71^{a} \pm 12.59$	$47.07^{a} \pm 17.08$	$23.01^{a} \pm 9.86$	$8.17^{b} \pm 2.15$	$6.55^{b} \pm 1.10$	$2.75^{b} \pm 0.99$	$1.22^{b} \pm 0.65$	$0.00^{a} \pm 0.00$
Pulwama	$0.00^{a} \pm 0.00$	$2.19^{b} \pm 0.61$	$6.04^{b} \pm 1.97$	$4.85^{b} \pm 0.49$	$4.15^{b} \pm 1.09$	$4.16^{c} \pm 0.86$	$4.49^{c} \pm 1.76$	$1.80^{b} \pm 0.81$	$1.63^{b} \pm 1.30$	$0.00^{a} \pm 0.00$
Baramulla	$0.00^{a} \pm 0.00$	$1.01^{b} \pm 0.31$	$4.27^{b} \pm 1.12$	$4.40^{b} \pm 0.61$	$3.41^{b} \pm 1.09$	$2.45^{c} \pm 0.3$	$2.36^{c} \pm 1.66$	$2.58^{b} \pm 1.65$	$1.23^{b} \pm 0.65$	$0.00^{a} \pm 0.00$

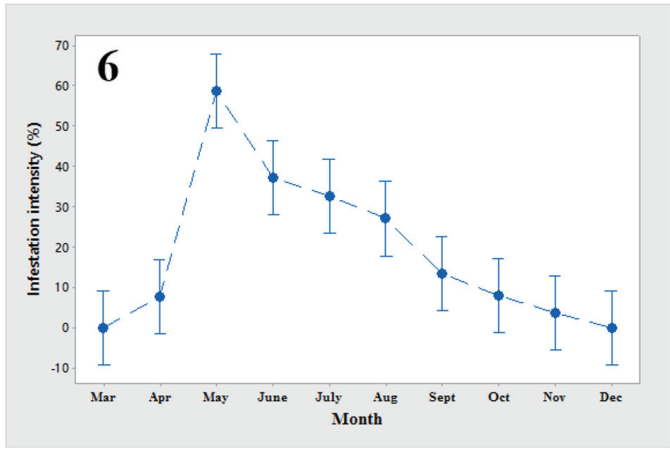

Figure 6: Infestation intensity in various months of a year; Srinagar

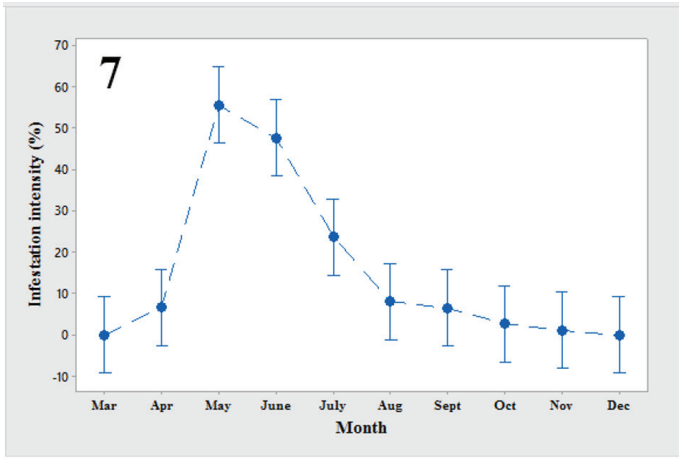

Figure 7: Infestation intensity in various months of a year; Bandipora

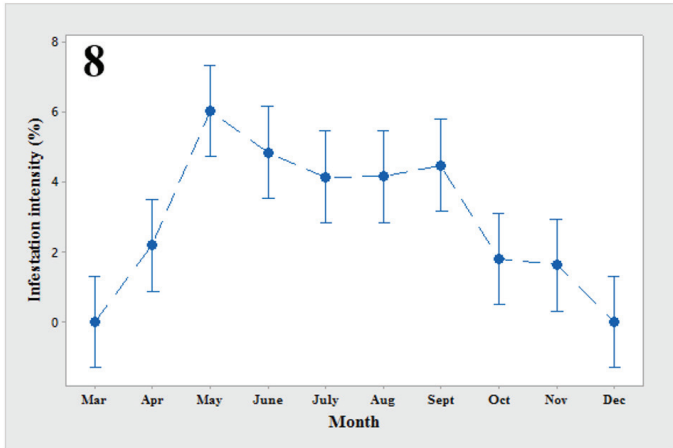

Figure 8: Infestation intensity in various months of a year; Pulwama

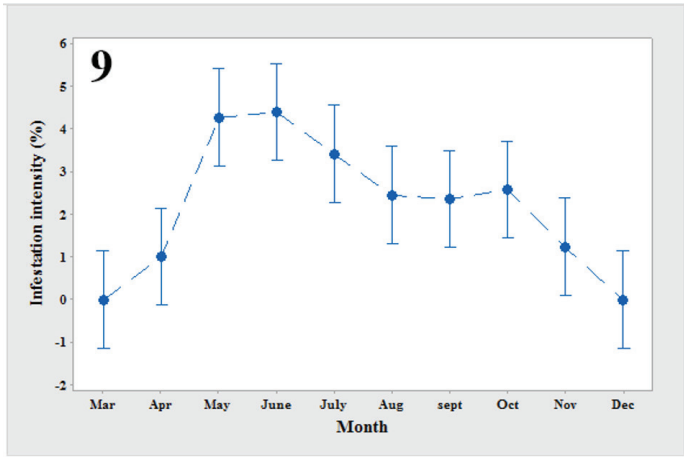

Figure 9: Infestation intensity in various months of a year; Baramulla

of November (2015). The intensity of infestation was found least in the month of November (2015). In Srinagar, infestation intensity in this month was calculated as 3.73 % (± 1.36 SD) while as in the other three areas of the valley, Bandipora, Pulwama and Baramulla, it was found to be 1.22 % (± 0.65 SD), 1.63 % (± 1.30 SD) and 1.23 % (± 0.65 SD) respectively. The percent infestation intensity of district Srinagar was found statistically different from all the other three areas.

3.3 SEASONAL HISTORY

Seven generations of *L. clerkella* were found in the laboratory (Fig. 10). First generation started from 12th of April (2015) and ended on 20th of May (2015). Second generation begin from 8th of May (2015) and completed on 16th of June (2015). Third generation commenced from 5th June (2015) and ended on 11th July (2015). Fourth generation begin from 27th of June (2015) and completed on 4th of August (2015). Fifth generation started from 21st of July (2015) and ended on 3rd September (2015). Sixth generation commenced on 21st of August (2015) and completed on 7th of October (2015). Similarly, 7th generation extended from 24th of September (2015) to mid spring (15th April) of the following year (2016). Moths of the final generation (7th) hibernated during the winter months, from 2nd week of November (2015) till March of the following year ((2016) (Fig. 10).

Thus, the first, second, third, fourth, fifth, and sixth generations of *L. clerkella* completed in 39, 40, 37, 39, 45 and 48 days respectively. However, final generation (7th) was found relatively longer in duration, extended from the 4th week of September (2015) till April of the following year (2016) for about 204 days.

The swarms of hibernated moths were first seen from 11th to 20th of April in the year 2015 and the average day temperature of this period was recorded to be 21 °C (Table 2). However in 2016, the swarms of these moths

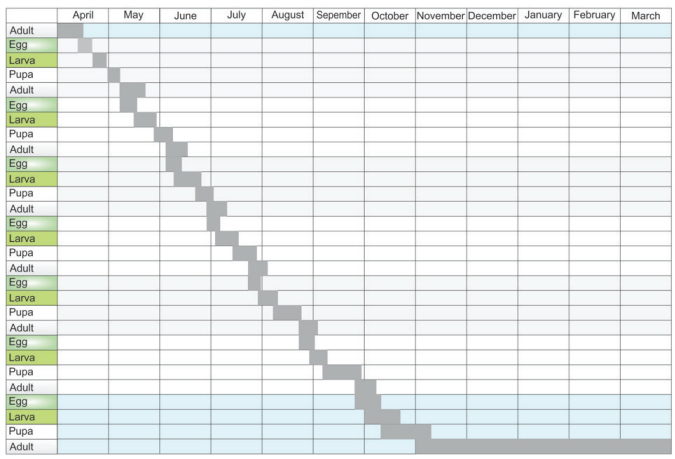

Figure 10: Seasonal history of *L. clerkella* as seen in laboratory

were observed from 17th to 25th of April with an average day temperature of 21.4 °C.

4. DISCUSSION

In the present study, the prevalence and infestation intensity was found to fluctuate seasonally in different study areas (Srinagar, Bandipora, Pulwama and Baramulla) of the valley with relatively high level of prevalence and intensity being observed in the month of May. The highest prevalence was found in Srinagar and Bandipora districts (70.6 % and 65.3 %). Similarly, the highest intensity of infestation was found in Srinagar, 58.69 % (± 11.46 SD) followed by Bandipora, 55.71 % (± 12.59 SD) whereas, least infestation intensity was found in Pulwama 6.04 % (± 1.97 SD) and Baramulla 4.27 % (± 1.12 SD). A significant difference in infestation intensity was also observed among these four districts (Table 1). The variations in prevalence and infestation intensity among different districts of the valley (April to November 2015) could be attributed to the factors such as insecticides, parasitism, temperature and food quality as reported by Dixon (1987) and Feeny (1970).

These insect pests resume their activities in spring under varying temperatures at different places of the world (Kholchenkov, 1974). In Azerbaijan, Kholchenkov (1974) reported that *L. clerkella* appear in spring at the temperature of 12 °C. However, in Kashmir, the swarms of hibernated moths of this pest were found to appear in early spring, between 11th to 25th of April at an average day temperature of 21.2 °C (Table 2).

Since *L. clerkella* is widely distributed in many parts of the world, the number of generations subjected to specific temperatures and elevations were reported varying from place to place. Agata et al. (2007) reported three generations of *L. clerkella* at the altitude of 200–300 m and 1–2 generations at an elevation of 750–860 m. This pest was found to develop 3–4 generations per year in Ukraine (Kholchenkov, 1974). Furthermore, Shoji & Ueno (1981) reported that the apple leaf miner moths grow for 5 to 6 generations per year in Yamagata Prefecture while as Miyaji (1991) found *L. clerkella* produced 9 generations per year in Kagoshima prefecture, Japan. Apple leaf miner was also found to produce 5 generations in a year under natural conditions in Kashmir (Rather & Buhroo, 2015). The 7 generations in experimental room as observed in the present study fell within this expected range.

Relatively shorter duration of the first two generations (April to June) could be attributed to the availability of better food quality (Dixon, 1987). In the months of spring and early summer (April to June), the mesophyll content remains soft which enables the caterpillars to feed voraciously as compared to subsequent months when the available foliage dries and becomes relatively tougher due to the deposition of cellulose, hemicelluloses, pectins, and other materials (Feeny, 1970). Seasonal changes in the leaf tannin composition can also have an adverse impact on the generation time as increased tannin concentration negatively affects the growth of lepidopteron caterpillars (Varley & Gradwell, 1962). The ability of tannins to form complexes with proteins enhances defense mechanism of plants and thereby affecting the growth of insects (Feeny, 1970).

The work of many authors indicated that the duration of puparial stage of apple leaf miner insects extends relatively from the onset of autumn period because of reduction in daily temperatures (Kholchenkov, 1974; Kuznetsov & Seksyaeva, 1994; Savkovskii, 1976). In the present study, the puparial stage was also found comparatively longer in duration from the beginning of autumn season because of linear reduction in daily temperatures. The longevity of adult moths of this pest was also found to decrease with the decrease in temperature from the month of September.

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Pattern of variation and grouping of qualitative morphological characters of bambara groundnut (*Vigna subterranea* (L.) Verdc.)

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Pattern of variation and grouping of qualitative morphological characters of bambara groundnut (*Vigna subterranea* (L.) Verdc.)

Abstract: Morphological field evaluation to characterize the phenotypical features of 33 Bambara groundnut accessions was carried out at the Teaching and Research Farm of Department of Crop Science and Technology, Federal University of Technology, Owerri, Imo State; Nigeria. Qualitative morphological descriptors showed a varying degree of variation across the Bambara groundnut collections evaluated. In relation to other descriptors, ground colour of eye displayed the highest range of variation, while eye pattern of the accessions recorded the least discriminating feature. The accessions were resolved into five groups based on similarities on morphological characters and not on geographical place of origin.

Key words: Bambara groundnut; grouping; morphological variability; qualitative characters

Vzorec spreminjanja in združevanja morfološki znakov bambare (*Vigna subterranea* (L.) Verdc.)

Izvleček: V raziskavi je bilo na terenu ovrednoteno 33 akcesij bambare (voandzeje) za opredelitev njihovih fenotipskih lastnosti na Teaching and Research Farm of Department of Crop Science and Technology, Federal University of Technology, Owerri, Imo State, Nigerija. Kakovostni morfološki deskriptorji so med akcesijami pokazali različno spremenljivost. Glede na ostale deskriptorje je osnovna barva hiluma pokazala največjo spremenljivost, medtem, ko je vzorec hiluma pakazal najmanjšo spremenljivost med akcesijami. Akcesije so se združevale v pet skupin na osnovi podobnosti v morfoloških lastnostih in ne na osnovi njihovega geografskega izvora.

Ključne besede: bambara; združevanje; mofološka variabilnost; kakovostni znaki

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1 INTRODUCTION

In conventional and modern breeding programme for crop improvement, the major factor that determines its success is the availability of variation within and between species (intra and interspecific variations). Natural variations exist in both wild and cultivated plants. Several scholars in their different studies have reported the importance of screening lines for the purpose of identification and classification of natural occurring variations in crop species (Ariyo and Odulaja, 1991; Aliero and Morakinyo, 2001; Ntundu et al., 2004; Santos et al., 2012; Afolayan et al., 2014).

In detection of variation, crop species are characterised in which the differences that exist in species are used to describe germplasm of crop species. Traditionally, standard characterization of accessions involves the use of descriptor list of morphological characters. In fact, most workers have solely relied on phenotypic morphological descriptors for the characterization of local varieties of crops (Caradus and Forde, 1996; El eSAWI, 2012), despite that the use of morphological descriptors presents some limitations. The environment sometimes influences the phenotype and there may be some ambiguity in information capture and interpretation. However, the use of other methods of diversity analysis (as it is currently being practiced) like biochemical, cytological, and molecular markers have not substituted phenotypic morphological characterization of collections (William et al.,1990; Afolayan et al., 2014), rather they play a complimentary role. This is because phenotypic morphological markers easily expose heritable characters to the eye in the natural crop environment and are highly discriminating. In addition, under a wide range of environmental conditions, some morphological characters are stable. Consequently, assessment and characterization of morphological characters is used for the identification of species, families and genera. It has been reported that studies in morphological characterization of plants have immensely enhanced derivation of economic and breeding gains from germplasm collections and families of related accessions (Iwaro et al., 2003; Bekele et al., 2006; Maharaj et al., 2011). Further, phenotypic morphological characterization of collections can provide reliable information on level of genetic diversity, structure and distribution of diversity.

Morphological characterization using qualitative characters typically involve the use of leaf, flower, fruit/pod and seed descriptors (Engels et al., 1980; Bekele and Bekele 1996). In Bambara groundnut, the use of seed morphological features in the identification and classification of a wide range of germplasm has been reported (Mohammed, 2014), apparently demonstrating the usefulness of morphological descriptors in the assessment of diversity that exit in Bambara groundnut. Also to maximise efforts made in the conservation of germplasm, identification of useful and valuable characters through morphological diversity analysis is essential. Therefore, this study was set up to identify and classify morphological variation that exist in Bambara groundnut with a view to establish the pattern of variation and to cluster the collections in groups.

2 MATERIALS AND METHOD

A total of 33 accessions of Bambara groundnut (*Vigna subterranea* (L.) Verdc.) obtained from the gene bank of International Institute of Tropical Agriculture (IITA) Ibadan was used for the study. These accessions were sourced from seven major African Bambara groundnut growing countries. Nigeria and Togo had twelve accessions each. Three accessions were from Malawi, while Mali and Cameroon had two accessions each. Ghana and Zambia had one accession each.

Field experiment was conducted in the late raining season of 2014, 2015 and 2016 at the Teaching and Research Farm of Department of Crop Science and Technology, Federal University of Technology, Owerri. The experimental design was randomized complete block design (RCBD) with 3 replications. A portion of land measuring 40 m x 20 m was used. It was ploughed, harrowed and marked out into 3 blocks with a space of 1 meter between blocks. Two seeds were sown which was later thinned down to one. Planting spacing was maintained at 100 cm between rows and 20 cm within rows. Weeding, suppling and earthing up was carried out for optimum crop production. Data on morphological characters were collected as outlined in Bambara groundnut descriptor list developed by the International Bambara Groundnut Network (IPGRI, IITA, BAMNET, 2000).

Data collected from morphological qualitative characters were analysed visually and grouped based on colours, shapes, and texture using standard shape and colour charts of Royal Horticultural Society Colour Chart. In addition, statistical analysis to determine mean, and frequency distribution was carried out using GENSTAT 5.0 Release 4.23DE, Discovery Edition 3.

3 RESULT AND DISCUSSION.

The qualitative morphological traits used to characterize the Bambara groundnut accessions evaluated are shown in Tables 1 and 2. A varying degree of variation was revealed by these qualitative morphological

descriptors across the collections. Most of the qualitative characters displayed broad variability among the accessions. Comparatively, ground colour of eye displayed the highest range of variation than other qualitative morphological characters. On the other hand, eye pattern of the accessions recorded the least distinguishable qualitative morphological variability. The details of the result on the qualitative descriptors used to discriminate among the accessions is discussed below.

Growth habit: The pattern of growth habit exhibited by the evaluated Bambara groundnut accessions (Table 1) were bunch (63.6 %), semi-bunch (18.2 %), and spreading (18.2 %). A detailed breakdown of this result showed that the predominant growth habit pattern among the accessions was bunch. A greater percentage (63.6) of the entire population had it. This result on growth habit may have some implication on evolution status of the crop. Doku and Karikari (1971) reported that Bambara groundnut grew from spreading growth habit to bunch. While bunch growth habit is characterized by short internode, spreading growth habit had long internode. The leaves of bunch growth habit were clustered, unlike the leaves of spreading growth habit. Literature report revealed that farmers prefer growing Bambara groundnut accessions with short internode length; they are easy to harvest than the long internode types. Again in selection, short internode types are considered as a desirable character for crop improvement (Goli et al.,1995; Doku, 1995). The internodes of the semi-bunch fall between that of bunch and spreading growth habit. They were moderately long. Apparently, the length of the internode determined the type of growth habit the accessions exhibited. This result is in agreement with the report of Goli et al. (1995).

Terminal leaflet shape: Four types of terminal leaflet shapes were observed among the accessions as shown in Table 3. They were lanceolate, oval, elliptic, and round. The distribution of the accessions among the observed four patterns of terminal leaflet shape were 30.3 % for lanceolate, 39.4 % oval, 39.4 % elliptic and 6 % round. This qualitative morphological character displayed a reasonable level of discrimination among the accessions. Again, a careful investigation on this result also showed that none of the observed four types of terminal leaflet shapes dominated the distribution. This result has some implication on evolution status of Bambara groundnut based on terminal leaflet shape. Further, the round type of terminal leaflet shape was not popular among the collections, only two accessions (or 6 %) of the collections had it. Apparently, this type of terminal leaflet is threatened to go into extinction, hence efforts should be made to salvage it. Again, terminal leaflets were observed to be slightly larger in size than the laterals leaflets. Terminal leaflets were subtended by two stipules while lateral leaflets were subtended by one. Similar reports on the arrangement of terminal leaflet of Bambara groundnut have been observed by other workers (Goli et al., 1995; Mohammed, 2014).

Eye (hilum) pattern: There were two types; eye as thin and no eye. Accessions that had eye as thin constituted 72.7 % of the distribution, while 27.3 % had eye pattern described as no eye (Table 1). The distribution was skewed towards eye pattern described as eye as thin. More than half of the accessions had this eye pattern. This qualitative character (eye pattern of the accessions) had the least variation in discriminating among the accessions. Invariably eye pattern of the accessions did not exhibit a reasonable distinguishable identity among the collections of Bambara groundnut evaluated. This result suggests the level of relatedness among these accessions, which is an expected natural occurrence. Again, it may imply that this crop is evolving from no eye type of eye pattern towards eye as thin.

Pod shape: Investigation on pod shape of the accessions showed that 69.7 % of the accessions had pod shape described as ended in a point round on the other side, 15.2 % had the type classified as ended without point, and 12.1 % had pod shape that ended in a point with hook on the other side. Furthermore, 3 % had pod shape described as ending in 2 points on each side (Table 1). Pod shape of the accessions like terminal leaflet shape was among the traits that displayed a reasonable variation among the collections evaluated. The pod shape that dominated the distribution was the type described as ended in a point, round on the other side. Over half (69.7 %) of the population had it. This result may have some evolutionary implication on the status of pod shape in Bambara groundnut. On the other hand, the pod shape classified as ending in 2 points on each side was seriously threatened to go into extinction, only 3 % of the accession had it. Hence the need to commence breeding programme to salvage it.

Pod texture: The result on pod texture displayed by the accessions showed that 51.5 %, 30.3 %, and 9.1 % had much grove, little grove, and smooth pod texture in that order (Table 1). The variability displayed by the accessions on pod texture was comparatively narrow. Invariably this trait cannot be used in discriminating among the accessions. Hence, there is the need to use more descriptors in the characterization of the accessions to delimit them into distinct groups (Kok et al.,1989). However, the distribution was skewed towards much grove pod texture. Slightly above half (51.5 %) of the accessions evaluated had this type of pod texture.

Pod colour: The frequency and percentage distribution of pod colour of the accessions (Table 1) showed that each of these two pod colours (purple and reddish)

constituted 3 % of the distribution. 12.1 % of the accessions had pods that were black in colour, while 57.6 % had brown coloured pods. The remaining 24.3 % had yellowish-brown pods (Table 1). Pod colour displayed a reasonable level of discrimination among the accessions. The result on pod colour also showed that brown coloured pod was the most popular among the Bambara groundnut accessions evaluated, more than half (57.6 %) of the collections had it. This result is similar to that observed for much grove type of pod texture described above. Contrarily, the endangered pod colours were purple and reddish. Each of these pod colours constituted only 3 % of the distribution. Hence the need to conserve them otherwise they may go into extinction. At harvest, the colour of the matured pod varies from yellow to reddish dark brown. This result is in agreement with the findings of Goli et al (1995). A detailed investigation on pod descriptors (pod shape, pod texture and pod colour) used to discriminate among the accessions showed that pod colour displayed the highest level of variability among the Bambara groundnut accessions. This result agrees with the report of a previous study on classification of Bambara groundnut morphotypes based on seed morphological features (Mohammed, 2014). In addition, previous studies have reported variations in seed and pod morphotypes as being very useful in discriminating among Bambara groundnut collections especially for genetic improvement programme (Padulosi et al., 2002; Mohammed, 2014).

Seed shape: 39.4 % of the accessions had oval shaped seeds, 48.5 % round seeds and 12.1 % had seeds whose shape was described as others (Table 1). A further break down of this result showed that the level of discrimination of the accessions based on seed shape was relatively low, like the result on pod shape. Among the observed three types of seed shapes, none dominated the distribution, which is similar to the result on terminal leaflet shape. The result on seed shape of the accessions may have some implication on evolutionary status of the crop. However, there are several reports on the importance of seed characters of Bambara groundnut in the identification and classification of this crop (Massawe et al., 2005; Ntundu et al., 2006; Abu and Buah, 2011)

Ground colour of testa: Four types of ground colour of testa were observed among the collections evaluated as presented in Table 1. Cream colour constituted 39.4 % of the distribution while 15.2 % had dark brown colour. Furthermore, 33.3 % had dark purple, and 9.1 % light brownish red colour of testa. This qualitative morphological character displayed a reasonable level of variation among the accessions. Further, none of the observed four colours of the ground colour of testa of the accessions dominated the distribution which is similar to the results on terminal leaflet shape and seed shape.

Ground colour of eye (hilum): the result on ground colour of eye showed that brown circular and light brownish red were constituted by one accession (3 %) each. Similarly, 6.1 % of the accessions had black triangular eye and brown triangular eye each. Grey triangular ground colour of eye constituted 9.1 % of the distribution, while 18.2% of the accessions had grey butterfly-like eye. In addition, 54.5 % had the type described as others (Table 1). Ground colour of eye described as others dominated the distribution, over half of the accessions had it. On the other hand, two types of ground colour of eye; brown circular and light brownish red have almost gone into extinction. Only 3 % of the distribution had each of them. Urgent measures should be taken to salvage brown circular and light brownish red types of ground colour of eye. Comparatively, ground colour of eye displayed the highest range of variation than other traits amongst the Bambara groundnut accessions evaluated. This result offers opportunity for selection for crop improvement. It has been reported that selection is effective only when significant genetic variability exits in high frequency among the genotypes (Hahn,1997; Adebisi et al., 2001). Previous study on characterization of Bambara groundnut lines has reported a similar result (Mohammed, 2014).

Morphological traits cluster analysis or dendrogram of the 33 Bambara groundnut accession is displayed in Figure 1. Five main clusters were revealed by the dendrogram. The first group which also was the smallest had only one accession; TVSU 1688 from Togo. This accession had some outstanding morphological characters like high vigour index (9), smooth pod texture and yellowish-brown pod colour. The second group had two accessions; TVSU 1788 and TVSU 1638, and both were from Mali. Some common morphological or agronomic features of this group were two seeds per pod, bunch growth habit pattern, and earliness to number of days to 50 % emergence (8 days). Group three clustered three accessions which comprised of TVSU 1713 from Zambia, TVSU 1605 from Togo, and TVSU 1510 from Nigeria. Accessions in this group were characterized mostly by earliness; 8 days to emergence and 37 days to flowering. In addition, many accessions in this group contained only one seed per pod. Another outstanding feature among accessions in this group was their growth habit pattern was mostly semi-spreading. The fourth and the largest group had fourteen accessions. Cameroon and Malawi had one accession each; TVSU 1819 and TVSU 1769 respectively. Togo had three accession; TVSU 1697, TVSU 1614 and TVSU 1702. The remaining nine accessions were from Nigeria; TVSU 1584, TVSU 1504, TVSU 1554,

Table 1: Qualitative characterization of Bambara groundnut accessions evaluated

Trait	Growth habit	Percentage distribution	Accessions
Growth habit	Bunch	63.6	TVSU 1483, TVSU 1503, TVSU 1504, TVSU 1509, TVSU 1512, TVSU 1563, TVSU 1584, TVSU 1591, TVSU 1604, TVSU 1605, TVSU 1614, TVSU 1620, TVSU 1625, TVSU 1627, TVSU 1631, TVSU 1638, TVSU 1639, TVSU 1688, TVSU 1702, TVSU 1788 and TVSU 1917.
	Semi-spreading	18.2	TVSU 1510, TVSU 1697, TVSU 1713, TVSU 1769, and TVSU 1610
	Spreading	18.2	TVSU 1552, TVSU 1554, TVSU 1555, TVSU 1559, TVSU 1766 and TVSU 1819.
Terminal leaflet shape	Lanceolate	30.3	TVSU 1483, TVSU 1503, TVSU 1504, TVSU 1510, TVSU 1513, TVSU 1688, TVSU 1697, TVSU 1713, TVSU 1769 and TVSU 1788
	Oval	30.4	TVSU 1554, TVSU 1559, TVSU 1563, TVSU 1614, TVSU 1620, TVSU 1625, TVSU 1627, TVSU 1631, TVSU 1638, TVSU 1639, TVSU 1766, TVSU 1819 and TVSU 1917.
	Elliptic	24.3	TVSU 1509, TVSU 1512, TVSU 1584, TVSU 1591, TVSU 1604, TVSU 1605, TVSU 1610, and TVSU 1702,
	Round	6	TVSU 1552 and TVSU 1555.
Eye pattern	Eye as thin	72.7	TVSU 1483, TVSU 1503, TVSU 1509, TVSU 1510, TVSU 1512, TVSU 1513, TVSU 1584, TVSU 1591, TVSU 1604, TVSU 1605, TVSU 1610, TVSU 1614, TVSU 1620, TVSU 1625, TVSU 1627, TVSU 1631, TVSU 1638, TVSU 1639, TVSU 1688, TVSU 1702, TVSU 1766, TVSU 1769, TVSU 1788, and TVSU 1917.
	No eye	27.3	TVSU 1504, TVSU 1552, TVSU 1554, TVSU 1555, TVSU 1559, TVSU 1563, TVSU 1697, TVSU 1713, and TVSU 1819
Pod shape	Ending in a point, round on the other side	69.7	TVSU 1483, TVSU 1503, TVSU 1504, TVSU 1510, TVSU 1512, TVSU 1513, TVSU 1559, TVSU 1563, TVSU 1591, TVSU 1605, TVSU 1610, TVSU 1614, TVSU 1620, TVSU 1627, TVSU 1631, TVSU 1638, TVSU 1639, TVSU 1688, TVSU 1697, TVSU 1702, TVSU 1713, TVSU 1769 and TVSU 1917.
	Ending without point	15.2	TVSU 1766, TVSU 1625, TVSU 1604, TVSU 1584 and TVSU 1509
	Ending in a point, with hook on the other side	12.1	TVSU 1788, TVSU 1819, TVSU 1552, and TVSU 1554
	Ending in 2 points on each side	3	TVSU 1555

Table 1: continued

Pod texture	Much grove	51.5	TVSU 1503, TVSU 1509, TVSU 1512, TVSU 1563, TVSU 1605, TVSU 1614, TVSU 1620, TVSU 1625, TVSU 1627, TVSU 1631, TVSU 1638, TVSU 1639, TVSU 1697, TVSU 1702, TVSU 1766, TVSU 1819, and TVSU 1917.
	Little grove	39.4	TVSU 1483, TVSU 1510, TVSU 1552, TVSU 1554, TVSU 1555, TVSU 1559, TVSU 1584, TVSU 1591, TVSU 1604, TVSU 1610, TVSU 1713, TVSU 1769, and TVSU 1788
	Smooth	9.1	TVSU 1504, TVSU 1688 and TVSU 1513.
Pod colour	Purple	3	TVSU 1819
	Black	12.1	TVSU 1591, TVSU1625, TVSU1639 and TVSU1697
	Brown	57.6	TVSU 1483, TVSU 1503, TVSU 1504, TVSU 1509, TVSU 1510, TVSU 1512, TVSU 1513, TVSU 1554, TVSU 1555, TVSU 1559, TVSU 1563, TVSU 1584, TVSU 1604, TVSU 1605, TVSU 1610, TVSU 1627, TVSU 1631, TVSU 1638, and TVSU 1917
Seed shape	Oval	39.4	TVSU 1503, TVSU 1552, TVSU 1554, TVSU 1555, TVSU 1559, TVSU 1563, TVSU 1591, TVSU 1605, TVSU 1614, TVSU 1697, TVSU 1713, TVSU 1788 and TVSU 1917.
	Round	48.5	TVSU 1483, TVSU 1504, TVSU 1509, TVSU 1510, TVSU 1512, TVSU 1513, TVSU 1584, TVSU 1604, TVSU 1610, TVSU 1620, TVSU 1625, TVSU 1631, TVSU 1638, TVSU 1639, TVSU 1766, and TVSU 1769
	Others	12.1	TVSU 1627, TVSU 1688, TVSU 1702 and TVSU 1819.
Ground colour of testa	Cream	39.4	TVSU 1503, TVSU 1509, TVSU 1510, TVSU 1512, TVSU 1513, TVSU 1584, TVSU 1605, TVSU 1639, TVSU 1688, TVSU 1766, TVSU 1769, TVSU 1788, and TVSU 1917.
	Dark brown	18.2	TVSU 1483, TVSU 1559, TVSU 1625, TVSU 1702, TVSU 1713, and TVSU 1819
	Dark purple	33.3	TVSU 1552, TVSU 1554, TVSU 1555, TVSU 1591, TVSU 1604, TVSU 1610, TVSU 1614, TVSU 1620, TVSU 1631, TVSU 1638, and TVSU 1697
	Light brownish red	9.1	TVSU 1504, TVSU 1563, and TVSU 1627.
Ground colour of eye	Brown circular	3	TVSU 1483
	Light brownish red	3	TVSU 1627
	Black triangular	6	TVSU 1766 and TVSU 1769
	Brown triangular	6	TVSU 1503 and 1509
	Grey triangular	9.1	TVSU 1512, TVSU 1513, and TVSU 1788
	Grey butterfly-like	18.2	TVSU 1917, TVSU 1639, TVSU 1685, TVSU 1605, TVSU 1584, and TVSU 1510
	Others	54,5	TVSU 1819, TVSU 1702, TVSU 1697, TVSU 1713, TVSU 1631, TVSU 1638, TVSU 1625, TVSU 1620, TVSU 1610, TVSU 1614, TVSU 1591, TVSU 1604, TVSU 1559, TVSU 1555, TVSU 1554, TVSU 1552, TVSU 1563, and TVSU 1504

Table 2: Geographical origin and morphological (qualitative) features of the Bambara groundnut accessions evaluated

Accessions	Country of origin	Growth habit	Terminal leaflet shape	Eye pattern	Pod shape
TVSU 1483	Ghana	Bunch	Oval	Eye as thin lines	Ending in a point, round on the other side
TVSU 1503	Nigeria	Bunch	Oval	no eye	Ending in a point, round on the other side
TVSU 1504	Nigeria	Bunch	Lanceolate	Eye as thin lines	Ending in a point, round on the other side
TVSU 1509	Nigeria	Bunch	Lanceolate	Eye as thin lines	Ending in a point, round on the other side
TVSU 1510	Nigeria	Bunch	Lanceolate	Eye as thin lines	without point
TVSU 1512	Nigeria	Bunch	Lanceolate	Eye as thin lines	Ending in a point, round on the other side
TVSU 1513	Nigeria	Bunch	Lanceolate	no eye	Ending in a point, round on the other side
TVSU 1552	Nigeria	Spreading	Oval	no eye	Ending in a point, with nook on the other side
TVSU 1554	Nigeria	Spreading	Round	no eye	Ending in 2 points, on each side
TVSU 1555	Nigeria	Spreading	Oval	no eye	Ending in a point, round on the other side
TVSU 1559	Nigeria	Bunch	Oval	no eye	Ending in a point, round on the other side
TVSU 1563	Nigeria	Bunch	Elliptic	Eye as thin lines	without point
TVSU 1584	Nigeria	Bunch	Elliptic	Eye as thin lines	Ending in a point, round on the other side
TVSU 1591	Togo	Bunch	Elliptic	Eye as thin lines	without point
TVSU 1604	Togo	Bunch	Elliptic	Eye as thin lines	Ending in a point, round on the other side
TVSU 1605	Togo	Semi-bun	Elliptic	Eye as thin lines	Ending in a point, round on the other side
TVSU 1610	Togo	Bunch	Oval	Eye as thin lines	Ending in a point, round on the other side
TVSU 1614	Togo	Bunch	Oval	Eye as thin lines	Ending in a point, round on the other side
TVSU 1620	Togo	Bunch	Elliptic	Eye as thin lines	Ending in a point, with nook on the other side
TVSU 1625	Togo	Bunch	Lanceolate	Eye as thin lines	without point
TVSU 1627	Togo	Bunch	Oval	Eye as thin lines	without point
TVSU 1631	Togo	Bunch	Oval	Eye as thin lines	Ending in a point, round on the other side
TVSU 1638	Mali	Bunch	Oval	Eye as thin lines	Ending in a point, round on the other side
TVSU 1639	Mali	Bunch	Oval	Eye as thin lines	Ending in a point, round on the other side
TVSU 1688	Togo	Bunch	Oval	Eye as thin lines	Ending in a point, round on the other side
TVSU 1697	Togo	Bunch	Lanceolate	Eye as thin lines	Ending in a point, round on the other side
TVSU 1702	Togo	Semi-bun	Lanceolate	no eye	Ending in a point, round on the other side
TVSU 1713	Zambia	Bunch	Elliptic	Eye as thin lines	Ending in a point, round on the other side
TVSU 1766	Malawi	Semi-bun	Lanceolate	no eye	Ending in a point, round on the other side
TVSU 1769	Malawi	Spreading	Oval	Eye as thin lines	without point
TVSU 1788	Malawi	Bunch	Lanceolate	Eye as thin lines	Ending in a point, with nook on the other side
TVSU 1819	Cameroon	Spreading	Oval	no eye	Ending in a point, with nook on the other side
TVSU 1917	Cameroon	Bunch	Oval	Eye as thin lines	Ending in a point, round on the other side

Table 2: continued

Pod texture	Pod colour	Seed shape	Ground colour of testa	Ground colour of eye
much grooved	Brown	Round	Dark brown	Cream testa with brown circular eye
much grooved	Brown	Oval	Cream	Cream testa with brown triangular eye
much grooved	Brown	Round	Light brownish red	Others
much grooved	Brown	Round	Cream	Cream testa with brown triangular eye
little grooves	Brown	Round	Cream	Cream testa with grey butterfly-like eye
much grooved	Brown	Round	Cream	Cream testa with grey triangular eye
Smooth	Brown	round	Cream	Cream testa with grey triangular eye
little grooves	Reddish	oval	Dark purple	Others
little grooves	Brown	oval	Dark purple	Others
little grooves	Brown	oval	Dark purple	Others
much grooved	Brown	oval	Dark brown	Others
little grooves	Brown	oval	Light brownish red	Others
little grooves	Brown	round	Cream	Cream testa with grey butterfly-like eye
little grooves	Black	oval	Dark purple	Others
much grooved	Brown	round	Dark purple	Others
little grooves	Brown	oval	Cream	Cream testa with grey butterfly-like eye
much grooved	Brown	round	Dark purple	Others
much grooved	Yellowish-brown	oval	Dark purple	Others
much grooved	Yellowish-brown	round	Dark purple	Others
much grooved	Black	round	Dark brown	Others
much grooved	Brown	others	Light brownish red	Light brownish red testa with dark brown triangular eye
much grooved	Brown	round	Dark purple	Others
much grooved	Brown	round	Dark purple	Others
much grooved	Black	round	Cream	Cream testa with grey butterfly-like eye
much grooved	Yellowish-brown	others	Cream	Cream testa with grey butterfly-like eye
Smooth	Black	oval	Dark purple	Others
much grooved	Yellowish-brown	others	Dark brown	Others
much grooved	Yellowish-brown	oval	Dark brown	Others
little grooves	Yellowish-brown	round	Cream	Cream testa with black triangular eye
much grooved	Yellowish-brown	round	Cream	Cream testa with black triangular eye
little grooves	Yellowish-brown	oval	Cream	Cream testa with grey triangular eye
much grooved	Purple	others	Dark brown	Others
Much grooved	Brown	oval	Cream	Cream testa with grey butterfly-like eye

TVSU 1513, TVSU 1555, TVSU 1552, TVSU 1559, TVSU 1509 and TVSU 1503. The accessions in this cluster were similar in growth habit pattern (bunch), and their eye pattern was no eye. Again, majority of the accessions in this group had two seeds per pod. The fifth group was made up of the remaining thirteen accessions. They were mainly from Togo which had seven accessions; TVSU 1631, TVSU 1610, TVSU 1627, TVSU 1604, TVSU 1620, TVSU 1591, and TVSU 1625. Other accessions in this group were two (TVSU 1563 and TVSU 1512) from Nigeria, and one accession each from Malawi (TVSU 1766), Mali (TVSU 1639), Cameroon (TVSU1917), and Ghana (TVSU 1483). These accessions had two common distinguishing agronomic features; their pod shape was mostly the type described as ending in a point, round on the other side and they had much grooved type of pod texture.

Summarily, the result on the grouping of the accessions did not cluster the evaluated Bambara groundnut accessions into groups based on geographical origin (place of collection), rather similarity on qualitative morphological features. Hence, this situation of mixture of geographically divergent Bambara groundnut accessions in the same cluster may have arisen due to the exchange of Bambara groundnut seeds between farmers over wide geographic-ethnic region. A similar trend of association between Bambara groundnut accessions from different geographical areas has been reported by a previous genetic diversity study, and it was concluded that these accessions may be related, similar or the same lines (Ntundu et al. 2006).

4 CONCLUSION

The qualitative morphological descriptors used in

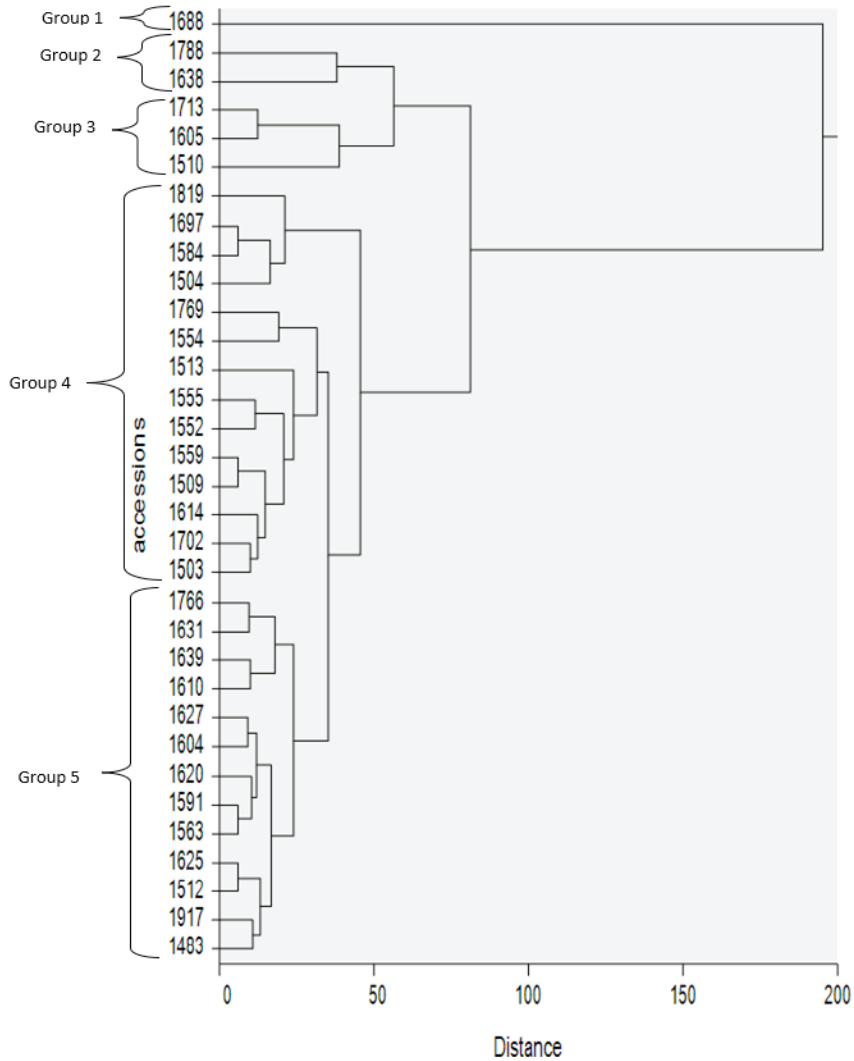

Figure 1: Dendrogram of 33 accessions of Bambara groundnut based on morphological characters

this study had clearly shown varying levels of discrimination among the accessions of Bambara groundnut evaluated. At the extremes were ground colour of eye and eye pattern that had the highest and the least level of variation respectively. Other qualitative characters like leaflet shape, pod shape, ground colour of testa and pod colour showed reasonable range of variations among the collections. In addition, it was also observed that certain features in some qualitative traits like round type of terminal leaflet shape, brown circular and light brownish red types of ground colour of eye and pod shape described as ending in 2 points on each side, may soon go into extinction. Only few accessions had each of these features. There is urgent need to adopt appropriate measures to conserve these endangered qualitative features, because of the importance of variation in crop improvement programmes. Further, the grouping pattern clustered the accessions into five groups based on similarities on morphological characters and not on their geographical origin.

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Relationship between *Aphis spiraecola* Patch, 1914 (Hemiptera: Aphididae) and citrus foliar minerals

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Relationship between *Aphis spiraecola* Patch, 1914 (Hemiptera: Aphididae) and citrus foliar minerals

Abstract: Spring and autumn flushes are generally the most infested periods by citrus aphids. Nevertheless, the role of citrus foliar minerals on aphids is not clear. Thus, this paper aims to study the correlation between certain minerals and the infestation degree of citrus varieties by *Aphis spiraecola*. Aphid counting was carried out on 12 leaves for each of the six species retained (clementine, lemon, grapefruit and three varieties of mandarin), during autumn (October 2014) and spring (April 2015) flushes. In addition, mineral contents of the leaves in P, K, Na, Ca and Li were measured for the same periods. The results showed that the infestation levels of the studied varieties were higher in the spring flush than in the autumn one. Moreover, analyzes of young leaves showed an important intraspecific (mandarin varieties) and interspecific differences in the mineral composition between the examined citrus trees. The study of the relationship between infestation levels by *A. spiraecola* and mineral content of the six examined species showed no significant correlation, suggesting a marginal role of the five analyzed minerals in the relation citrus – *A. spiraecola*.

Key words: citrus aphid; clementine; lemon; grapefruit; mandarin; flushes

Razmerje med pojavljanjem jabolčne uši *Aphis spiraecola* Patch, 1914 (Hemiptera: Aphididae) in mineralno sestavo listov citrusov

Izvleček: Spomladanski in jesenski viški rasti citrusov so navadno obdobja njihove največje okužbe z listnimi ušmi, vendar je znano zelo malo o pomenu mineralne sestave listov na njihovo pojavljanje. Namen prispevka je bil preučiti korelacijo med nekaterimi minerali v listih različnih citrusov in stopnjo okužbe z listno ušjo *Aphis spiraecola*. Štetje listnih uši je bilo izvedeno na 12 listih vsake od preučevanih vrst (klementine, limone, grenivke in treh sort mandarine), v jesenski (oktober 2014) in spomladanski (april 2015) rasti. Dodatno so bile v istem obdobju v listih izmerjene vsebnosti P, K, Na, Ca in Li. Rezultati so pokazali, da je bila stopnja okužbe pri vseh sadnih vrstah večja v obdobju spomladanske kot jesenske rasti. Analize mladih listov so še pokazale pomembne znotrajvrstne razlike (med sortami mandarin) in medvrstne razlike v mineralni sestavi pregledanih citrusov. Raziskava odvisnosti med velikostjo okužbe z vrsto *A. spiraecola* in mineralno sestavo analiziranih vrst citrusov ni pokazala značilne korelacije, kar kaže na marginalno vlogo petih analiziranih mineralov v razmerju citrusov in preučevane listne uši.

Ključne besede: listne uši citrusov; klementina; limona; grenivka, mandarina; viški rasti

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1 INTRODUCTION

Herbivorous insects identify their host plants through the morphological aspect, chemical signals and sometimes by the combined action of all these factors (Städler & Reifenrath, 2009). Upon herbivore attack, plants produce and emit volatile organic compounds, and some of them may be used in defensive strategy namely the attraction of the herbivores natural enemies (Laznik & Trdan, 2018). In addition, the performance of insects is determined directly by the quality of host plants (Sun & Ge, 2011). The main nutritional needs of insects are amino acids, vitamins, minerals, carbohydrates, lipids and sterols (Silva et al., 2005).

There is much evidence in the literature about the importance of minerals in plant resistance (Hedin et al., 1977). For instance, Khattab (2007) reported that potassium may play a role in the defense mechanism of aphid-infested plants. Several authors have mentioned that low K levels have a positive effect on aphids (Myers et al., 2005; Myers & Gratton, 2006; Hayes et al., 2009), because a lack of potassium in plants favors the accumulation of amino acids in tissues (Amtmann & Armengaud, 2009; Soetan et al., 2010). Mineral ions are important to insect's physiology in at least three major processes: enzyme activation (K, Mg, Fe, Co, Mn), trigger and control mechanisms (Na, Ca, K), and structure formation (Mg) (Silva et al., 2005). Moreover, pests need adequate quantities of several minerals to grow and reproduce (Wigglesworth, 1966). Components such as carbon and nitrogen act directly on the fertility of the pest (Awmack & Leather, 2002).

Shoot growth occurs in most types of citrus in well-defined waves (flushes). The spring flush is the most important one, containing both vegetative and reproductive shoots (Spiegel-Roy & Goldschmidt, 1996). According to Lotmani et al. (2008), the chemical composition of the leaves formed during the different flushes is generally different.

Previous studies (Lebbal & Laamari, 2015; Lebbal & Laamari, 2016) have shown that spring and autumn flushes are the most infested by aphids. Nevertheless, research on the effect of the mineral composition of citrus leaves on aphids is almost absent. Therefore, this paper aims to study the correlation between certain leaf minerals of some citrus varieties and their infestation level by *Aphis spiraecola* Patch, 1914.

2 MATERIALS AND METHODS

In order to show the effect of the leaf chemical composition on the infestation of six citrus species (clementine clone 63, lemon 'Eureka', grapefruit 'Shambar' and three varieties of mandarin: 'Ortanique', 'Carvalhal' and 'Commune'), aphid counting was carried out on 12 randomly chosen young leaves belonging to 4 trees for each of the retained varieties, at the rate of 3 leaves / tree, distributed over the different cardinal directions. These leaves were collected during autumn (October 2014) and spring (April 2015) flushes. Moreover, a foliar analysis of healthy young leaves of these same periods was realized. In total, five minerals were quantified: phosphorus (P) using colorimetry, and sodium (Na), potassium (K), calcium (Ca) and lithium (Li) using flame photometry (Jenway, model PFP7). This latter offers interesting possibilities for the study of the mineral composition of plants (Gueguen & Rombauts, 1961). The location of the young leaves taken was at the periphery of the foliage of the analyzed varieties (Martin-Prével et al., 1965), at about the height of a person. These leaves were dried and then they were crushed for later use in the determination of mineral elements.

The studied orchard (36° 42' N ; 6° 47' E ; 200 m above sea level) is situated in Skikda province (northeast of Algeria) characterized by a sub-humid climate. Its trees were planted in 2001. They were subject to almost the same technical itinerary. The used stock for graft is Troyer citrange (*Citrus sinensis* L. × *Poncirus trifoliata* Raf.) except for lemon which is grafted on volkamer lemon (*Citrus volkameriana* Pasquale). The studied orchard has been managed with limited spraying of pesticides. Weeding was performed mechanically and irrigation was applied during the dry season. Whereas, the fertilization was carried out using 46 % urea.

A correlation analysis was carried out between the level of citrus infestation and mineral content of leaves during the autumn and spring flushes. These analyzes were performed using SPSS software for Windows 10.0.5 (SPSS, Inc.).

3 RESULTS AND DISCUSSION

It was noticed that the infestation levels of these varieties were higher in the spring flush than in the autumn one (Table 1). In addition, analyzes of young leaves taken during the two flushes showed remarkable intraspecific and interspecific differences in the mineral composition. Plants do not have the same mineral requirements. Their contents in these elements affect their physiology and consequently the herbivorous insects feeding on them (Silva et al., 2005). Several authors, among others, Marchal et al. (1974), Roversi et al. (2008) and Pasković et al. (2013), indicated differences in leaf composition in nutrients for different fruit trees.

In general, concentrations of lithium and phosphorus are higher during the spring flush than in the autumn one.

The study of the relationship between degrees of aphid infestation and foliar content of the six examined cultivars showed no significant correlation (Table 2). Similarly, Harrewijn (1970) found that difference in longevity and reproduction rate of *Myzus persicae* (Sulzer, 1776) was not correlated with the total N or soluble N-content of the potato leaves.

Silva et al. (2005) revealed that variation in aphid abundance along different sampling times is correlated to C : N ratio, N, Mg, P and S, but correlations vary with cultivar and aphid species. For instance, they found no significant correlations between aphid population variation and minerals for an alfalfa resistant cultivar, except for C : N ratio. Likewise, Myers et al. (2005) observed no significant difference in mean generation time between soybean aphids feeding on the K-deficient and non-deficient soybean leaves. Nevertheless, they indicated that aphids in the K-deficient treatment exhibited significantly greater intrinsic rate of increase, finite rate of increase, and net reproductive rate relative to aphids feeding on non-deficient leaves. The yellowing associated with potassium deficient soybean leaves may preferentially attract migrating soybean aphids, placing potassium deficient fields at a further disadvantage (Hogg & Gratton, 2010).

Many correlations have been reported between some minerals and biotic parameters of aphids in subsequent studies (Douglas & van Emdeen, 2007; Djazouli, 2010; Agarwala & Das, 2012; Helfenstein et al., 2015). Miyasaka et al. (2007) mentioned that increased reproduction by *Sipha flava* (Forbes, S.A., 1885) aphids on kikuyu (*Pennisetum clandestinum* Hochst) was accompanied by high foliar N. Moreover, short development times of *Macrosiphum euphorbiae* Thomas, 1878 were associated with high P and K content in *Petunia* leaves (Jansson & Ekbom, 2002).

In the present study, aphid colonies may be affected much more by other factors (climate, primary and secondary metabolites) than by the leaf composition in these mineral elements. According to Jansson and Ekbom (2002), the complexity of plant nutrient content on aphid performance suggests that not only nutrient levels but also ratios of nutrients should be considered.

In addition, interactions between nutrients and allochemicals may be key factors in plant susceptibility to

Table 1: Variation in infestation levels (mean number ± standard error of *A. spiraecola* aphids/leaf) and mineral content (in μg g^{-1} of dry matter) of citrus leaves during the autumn and spring flushes

Flush	Parameters	Clementine Clone 63	Lemon Eureka	Grapefruit Shambar	Mandarin Ortanique	Mandarin Carvalhal	Mandarin Commune
Autumn	Infestation	13.75 ± 10.10	5.92 ± 4.91	1.58 ± 0.92	5 ± 1.51	12.83 ± 3.36	8.67 ± 4.23
	Na	0.5	0.58	0.54	0.55	0.56	0.66
	P	3.4	5.7	5.7	4.7	5.2	61.8
	Li	38	38	43.1	32.2	32.2	26.5
	K	29.4	63.4	41.8	56.4	71.8	199.9
	Ca	23071.4	9137.1	18507.9	8785.7	3428.5	1642.8
Spring	Infestation	71.08 ± 14.84	33 ± 18.98	50.17 ± 25.80	48 ± 9.90	20.83 ± 11.45	12.08 ± 5.83
	Na	0.56	0.55	0.57	0.61	0.56	0.57
	P	11.2	13.3	14.1	14.9	5.7	5.5
	Li	43.8	38	49.5	95.6	112.9	107.2
	K	52.4	60.2	62.7	92.9	71.4	73.9
	Ca	15928.5	8785.7	7000	17714.2	17714.2	12357.1

Table 2: Coefficients of correlation between the level of infestation of six citrus varieties by *A. spiraecola* and the mineral contents of their leaves

Analyzed elements Infestation degree	Na	P	Li	K	Ca
Correlation of Pearson	0.042	- 0.011	0.153	- 0.129	0.181
P	0.896	0.974	0.635	0.690	0.573

insect attack (Reese, 1983). Some allochemicals may even make certain nutrients not assimilable (Reese, 1977).

4 CONCLUSION

This study revealed that, compared with the autumn flush, the six citrus species tested had a higher aphid's infestation rate during the spring period. Furthermore, clear differences were shown in mineral composition between the examined varieties. However, statistical analysis showed no significant correlation between aphid infestation levels during these two periods and young leaf content in mineral elements (P, K, Na, Ca and Li). Further studies are desirable in this field, in order to clarify the direct and indirect contribution of each mineral element in the resistance or sensitivity of citrus to aphid attacks.

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Comparison study of flaxseed, cinnamon and lemon seed essential oils additives on quality and fermentation characteristics of lucerne silage

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Comparison study of flaxseed, cinnamon and lemon seed essential oils additives on quality and fermentation characteristics of lucerne silage

Abstract: This experiment was performed to investigate the effects of some essential oils on chemical properties and aerobic stability of lucerne silage. Treatments included lucerne silage without additives (control), lucerne silage with 60 mg cinnamon essential oil/kg, lucerne silage with 60 mg flaxseed essential oil/kg, lucerne silage with 60 mg lemon seed essential oil/kg, lucerne silage with 180 mg blend of essential oils (60 mg cinnamon + 60 mg flaxseed + 60 mg lemon seed essential oils/kg). Adding essential oils to lucerne silage reduced silage pH ($p < 0.001$) compared to control. The highest level of total volatile fatty acids (tVFA) was found when lemon seed essential oil and the lowest level when flaxseed essential oil was used. The lucerne silages treated with essential oils had the highest crude protein contents ($p < 0.01$). Untreated lucerne silage had the highest level of gas production compared to lucerne silage treated with lemon seed and flaxseed essential oils ($p < 0.01$). The essential oil additives increased the aerobic stability of the silage. It can be concluded that the use of essential oil additive in the preparation of high quality lucerne silage, can improves the quality and nutritive value of silages.

Key words: lucerne silage; essential oils; medicinal plants; nutritive value

Primerjava učinkov eteričnih olj lanenih semen, cimeta in limoninega semena na kemično sestavo in fermentacijske značilnosti silaže lucerne

Izvleček: Namen poskusa je bil preučiti učinke nekaterih eteričnih olj na kemijske lastnosti in aerobno stabilnost silaže lucerne. Obravnavanja so obsegala silažo lucerne brez dodatkov (kontrola), silažo lucerne z dodatkom cimetovega eteričnega olja, silažo lucerne z dodatkom eteričnega olja iz lanenega semena in silažo lucerne z dodatkom eteričnega olja iz semen limone (60 mg kg^{-1}) in silažo lucerne z dodatkom 180 mg mešanice eteričnih olj na kg silaže (60 mg cimetovega + 60 mg lanenega + 60 mg limoninega eteričnega olja na kg silaže). Dodajanje eteričnihj olj je znižalo pH silaže ($p < 0,001$) v primerjavi s kontrolo. Največja vsebnost celokupnih hlapnih maščobnih kislin (tVFA) je bila izmerjena pri dodatku eteričnega olja semen limone in najmanjša pri dodatku eteričnega olja iz lanenega semena. Silaža lucerne, ki je bila obdelana z eteričnimi olji je imela največjo vsebnost surovih beljakovin ($p < 0,01$). Neobdelana silaža lucerne je imela največjo proizvodnjo plina v primejavo s silažo obdelano z eteričnimi olji iz semen limone in lana ($p < 0,01$). Dodatek eteričnih olj je povečal aerobno stabilnost silaže. Zaključimo lahko, da dodatek eteričnih olj pri pripravi kvalitetne silaže lucerne izboljša njeno kakovost in hranilno vrednost.

Ključne besede: silaža iz lucerne; eterična olja; zdravilne rastline; hranilna vrednostnutritive value

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1 INTRODUCTION

Lucerne is one of the most nutritious forage crops for ruminants. In areas with adverse climates, and in areas where there is not enough time to dry the late harvested lucerne the lucerne is usually ensiled. Lucerne is often difficult to ensile because of its high protein, high buffering capacity and low water-soluble carbohydrate contents (WSC) (Khadem et al., 2009). The use of silage additives could improve the silage quality and its nutritive value. As an option for silage additives the essential oils can be used. Essential oils are natural secondary metabolites that are responsible for providing plants and spices with their characteristic essence and color (Beauchemin, 2008). These non-nutritive and biologically active compounds accumulate in various plant tissues and are extracted by steam-based distillation. Essential oils and their compounds are known to be active against a wide variety of micro-organisms, including Gram-negative bacteria, Gram-positive bacteria and fungi. Although the microbial effect of plant essential oils is recognized, there is limited research about these substances to be used as silage additives. For example, Fraser et al. (2007) observed a reduction in ammonia nitrogen (NH_3-N) concentration in culture medium by using cinnamon essential oil. McIntosh et al. (2003) also used a commercial blend of essential oils and observed inhibition of hyper-ammonia producing bacteria. Moreover, some of former studies demonstrated the potential of essential oils to alter rumen fermentation by reducing the proportion of acetate to propionate and also by inhibition of methanogenesis (Benchear et al., 2007). The aim of this study was to determine the potential use of flaxseed, cinnamon and lemon seed essential oils as silage additives on chemical composition and nutritive value of lucerne silage.

2 MATERIALS AND METHODS

2.1 ESSENTIAL OILS PREPARATION

Cinnamon, flaxseed and lemon seeds, used in this study, were purchased from local markets in Ahar and Tabriz cities. The samples obtained were cut or crushed into small pieces according to Palangi et al. (2012) procedure, oven-dried at 39 °C for 48 h and ground to pass a 1 mm-screen. Essential oils content of each plant was obtained with hydro-distillation of grinded samples using clevenger apparatus (Jahani-Azizabadi et al., 2014). The obtained essential oils were stored in refrigerator (4 °C) until they were used in the experiment.

2.2 EXPERIMENTAL TREATMENTS AND SILAGE PREPARATION

The chemical composition of ensiling material is presented in Table 1. The fourth cut of lucerne was dehydrated for 24 hours. Then it was chopped at 3-5 cm length and ensiled in laboratory scale mini pvc silos (3 ± 0.25 kg) for 60 days. Treatments included lucerne silage without additives (control), lucerne silage with 60 mg cinnamon essential oil/kg (C60), lucerne silage with 60 mg flaxseed essential oil/kg (F60), lucerne silage with 60 mg lemon seed essential oil/kg (L60), lucerne silage with 180 mg blend of essential oils (60 mg cinnamon + 60 mg flaxseed + 60 mg lemon seed essential oils/kg; M60). All additives were dissolved in 120 mg kg^{-1} aqueous ethanol solution (Chaves et al., 2012) and sprayed onto the chopped forages. The same amount of the aqueous ethanol solution was also added to the control. Three silos for each treatment were made and stored at ambient temperature (28 °C to 33 °C). All silos were opened after 60 days of ensiling and the contents were used for the determination of the silage chemical composition, nutritive value and aerobic stability. Analyses of silage composition and nutritive value were done on the contents of individual silo and averaged for use in the statistical analyses.

2.3 CHEMICAL COMPOSITION

After the opening of silages, the pH, dry matter (DM) and soluble carbohydrate (WSC) of the samples were determined. DM content of the silages was determined by oven drying of lucerne samples (65 °C for 48 h). DM, ash (CA), ether extract (EE) and crude protein (CP) contents were determined by the procedures given by AOAC (2002). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) concentrations were deter-

Table 1: Chemical composition of lucerne before ensiling (% DM)

Item	Chemical composition						
	ADF	NDF	WSC	CA	CP	pH	DM
Lucerne	17 ± 1.40	24.8 ± 1.058	3.74 ± 0.087	11.6 ± 0.028	19.6 ± 0.427	6.14 ± 0.011	22.2 ± 0.975

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; WCS: water soluble carbohydrates, CA: Crude Ash.

mined according to Van Soest et al. (1991) procedures without the use of sodium sulphite. NDF was analyzed without amylase and contains the ash. Aqueous extract was prepared from ensiled samples by mixing 20 g of forage with 180 ml of deionized water and homogenizing this mix for 1 min. Then, silage pH was determined using a portable pH meter. Ammonia-N (NH_3-N) concentration of acidified silage extracts were determined using Kjeldahl method. Phenol sulfuric acid method was used to measure WSC contents (Dubios et al., 1956). The distillation method described by Markham (1942) was used to measure total volatile fatty acids (tVFA) in silages. One ml of 25 % meta-phosphoric acid (v/w) was added to 5 ml of filtered extract to calculate the volatile fatty acids. For the determination of lactic acid (LA) contents, the method of Borshchevskaya et al. (2016) was used.

2.4 *In vitro* GAS PRODUCTION

Ruminal fluid was collected approximately 2 h after morning feeding from two fistulated sheep. Gas production was measured by Fedorak and Hrudy (1983) method. Approximately 300 mg of dried and ground (2 mm) samples were weighed and placed into serum bottles. Rumen fluid buffered with McDougall (1948)'s buffer (20 ml) was pipetted into each serum bottle. The metabolizable energy (ME; MJ kg^{-1} DM) content of samples was calculated using equation of Getachew et al. (2004) equation. The short chain fatty acid (SCFA) and organic matter digestibility (OMD) for feeds were calculated using equations of Menke et al. (1979) equations. Gas production parameters were calculated using the following mathematical model in the SAS package program according to the model reported by Ørskov and McDonald (1979).

$$P = a + b\ (1\text{-}e^{-c(t)})$$

where 'P' is the disappearance at time 't', 'a' quickly degradable fraction (or washing loss), 'b' denotes slowly degradable fraction and 'c' is constant rate of degradation of 'b' (Palangi and Macit, 2019).

2.5 AEROBIC STABILITY

Aerobic stability of silages represents the time (hours) during which the temperature of silage do not increase more than 2 °C above ambient temperature (Moran et al., 1996). Aerobic stability was determined on all treatments. About 400 g of each silage was transferred into separate 1l containers. The containers were implanted with thermocouples to monitor temperature. A double layer of sterile cheesecloth was placed over each container to prevent drying and contamination but allow penetration of air. Silage and ambient temperature were recorded manually every two hours until heating occurred.

2.6 STATISTICAL ANALYSIS

Obtained data from this study were subjected to analysis as a completely randomized design by the GLM procedure of SAS (SAS, 2002) and Duncan's multiple range test was used for the comparison of means. Significance was declared at $p < 0.01$.

3 RESULTS AND DISCUSSION

3.1 CHEMICAL COMPOSITION

Effect of essential oil additives on chemical composition of lucerne silage are shown in Table 2. The highest DM contents were observed for F60 and C60 silages which were significantly higher than other silages ($p < 0.05$). This effect could be due the consequence of limited development of specific microorganisms and therefore smaller loss of nutrients (Selwet, 2009). The CP concentrations of M60 and C60 silages were significantly higher than in other silages ($p < 0.01$). Degradation of protein in silage is a consequence of proteolytic microorganisms, such as *Clostridia* and/or enterobacteria (McDonald et al., 1991). Inhibitory effects of essential oils on growth of some microorganisms such as *Clostridia* reported in previous studies (Ismaiel and Pierson, 1990). Addition of essential oils to lucerne increased the protein content significantly after 60 d ensiling. These results were in agreement with findings of Soycan-Önenç et al. (2017) and Chaves et al. (2012). The results of current study show that the addition of M60 did not affect DM contents of silages, what was not in agreement with the findings of Soycan-Önenç et al. (2017).

The pH of silages supplemented with essential oils was significantly lower than control group ($p < 0.01$). The obtained results of this study were in agreement with the findings of Kung et al. (2000). In addition, the pH values of all silages were lower than those obtained by Bolsen et al. (1996). In the study by Soycan-Önenç et al. (2017), the pH values of 4.40 and 4.47 were determined in the silages prepared by addition of oregano and cinnamon essential oil to field peas. Higher pH values in majority of the treated silages could be the result of the reduced activity of *Lactobacillus* bacteria, as LA could decrease the pH of the silage (Kung and Ranjit, 2001). However, in some of

Table 2: Effect of essential oils on chemical properties of lucerne silage after 60 d of silage (% DM)

Treatments[1]	Chemical composition[2]										
	DM	NDF	ADF	WSC	tVFA	NH_3-N	CA	CP	LA	pH	EE
Control	24.44^{c}	49.07^{a}	22.67^{b}	4.09^{b}	12.63^{b}	84.93^{a}	11.40^{c}	11.62^{d}	69.38^{d}	4.65^{a}	4.27^{c}
C60	25.68^{b}	42.43^{bc}	25.67^{a}	4.78^{a}	11.65^{c}	79.80^{b}	11.66^{b}	12.29^{b}	80.29^{a}	3.66^{b}	3.94^{c}
F60	26.82^{a}	43.17^{b}	15.34^{d}	4.05^{b}	10.36^{d}	83.53^{a}	10.80^{d}	12.22^{c}	71.22^{c}	3.73^{b}	4.07^{c}
L60	24.50^{c}	48.03^{a}	19.34^{c}	4.63^{a}	14.90^{a}	85.16^{a}	12.31^{a}	12.37^{b}	76.23^{b}	3.61^{b}	4.73^{a}
M60	24.23^{c}	39.60^{c}	18.67^{c}	4.24^{b}	12.77^{b}	83.53^{a}	11.70^{b}	12.54^{a}	70.82^{c}	3.79^{b}	4.53^{ab}
SEM	0.308	1.039	0.615	0.066	0.049	0.660	0.518	0.043	0.244	0.055	0.123
p-value	0.0005	0.0003	<.0001	<.0001	<.0001	0.0013	<.0001	<.0001	<.0001	<.0001	0.0055

Treatment[1]-control: Lucerne silage without additives, C60: lucerne silage with 60 mg cinnamon essential oil kg^{-1}, F60: lucerne silage with 60 mg flaxseed essential oil kg^{-1}, L60: lucerne silage with 60 mg lemon seed essential oil kg^{-1}, M60: lucerne silage with 180 mg blend of essential oils (60 mg cinnamon essential oil + 60 mg flaxseed essential oil + 60 mg lemon seed essential oil kg^{-1}).
Chemical composition[2]: DM, dry matter; CP, crude protein; EE, ether extract; CA, crude ash; NDF, neutral detergent fiber; ADF, acid detergent fiber; NH_3-N: ammonia nitrogen (% of total nitrogen), tVFA: total volatile fatty acid (mmol), LA: lactic acid. WSC: water soluble carbohydrate.
Means within same column with different superscripts differ ($p < 0.05$).

the previous studies, essential oils had no inhibitory effect on lactic acid producing bacteria (Kung et al., 2008).

NH_3-N content significantly decreased in C60 silage ($p < 0.01$; Table 2). The treatments F60, L60 and M60 had no effect on NH_3-N concentration, which is in agreement with the findings of Kung et al. (2008). In the study of Hodjatpanah et al. (2016), essential oils of cinnamon added to ensiling material in amounts of 120 and 240 mg kg^{-1}, had no effect on NH_3-N in corn silage, whereas essential oils of oregano and thyme in same amounts and peppermint essential oil in amount of 120 mg kg^{-1} level decreased NH_3-N content.

The amounts of tVFAs determined in silages in this experiment was different by the addition of essential oil (Table 2). The highest amount of tVFA was found in L60 and the lowest in F60. Essential oils obtained from cinnamon and flaxseed decreased tVFA concentration in comparison to control ($p < 0.01$).

It is possible that the decrease of NDF and ADF contents is affected by pH. Soycan-Önenç et al. (2017) determined that LA content decreased in field pea silages prepared with the addition of oregano, cinnamon, and oregano+cinnamon essential oils. However, in this study it was determined that there was an increase in LA contents. While F60 and M60 reveal a decreasing effect on LA amount through inhibiting beneficial microorganisms, C60 caused increase in lactic acid amount by promoting beneficial microorganisms activity.

3.2 GAS PRODUCTION

Relative to the control, total produced gas from L60 and F60 decreased ($p < 0.01$) and M60 produced more gas ($p < 0.01$) after 120 h of incubation (Table 3). The chemical composition of silage can influence on the rumen microbial fermentation patterns (Navarro-Villa et al., 2013). Furthermore, some of essential oils have a good potential to alter rumen microbial fermentation and specially reducing rumen methanogenesis (Jahani-Azizabadi et al., 2014) and ammonia producing bacteria in the rumen (McIntosh et al., 2003).

Effects of essential oils on rumen microbial populations are dose-dependent (Macheboeuf et al., 2008). Effects of essential oils on NH3-N concentrations were only noticed during the first 120 h of incubation. At 120 h, treatments M60, F60 and L60 had higher ($p < 0.01$) ammonia concentration than the control (Table 4). The cumulative curve of gas production parameters in different lucerne silage treatments is shown in Figure 1.

At the end of incubation, the highest volume of gas produced was obtained with M60 with 148.54 ml g^{-1} DM and the lowest was for treatment F60 with 123.43 ml g^{-1} DM. The results of this experiment are in agreement with the findings of Hodjatpanah et al. (2016) and Chavez et al. (2012). Aminipour et al. (2017) used thyme essential oils as an additive to alter the fermentation characteristics of lucerne silage in ruminants and showed that thyme essential oil reduced the amount of gas produced in comparison with control silage. Fraser et al. (2007) used cinnamon essential oil which reduced the amount of gas produced after 24 h of incubation. Thestudy of Busquet et al. (2005) showed that the use of garlic essential oil reduced the amount of gas production after 17 hours of incubation and that the increasing levels of garlic essential oil levels decreased the *in vitro* gas production.

The mean gas produced from the potential degradable (*b*) was significantly different between the experi-

Table 3: The effect of different essential oils on gas production of lucerne silage (ml g^{-1} DM)

Treatments[1]	Incubation times (h)											
	2	4	6	8	12	16	24	36	48	72	96	120
Control	17.61^{b}	32.40^{b}	40.06^{b}	51.72^{b}	65.18^{b}	82.28^{b}	103.98^{b}	111.98^{b}	123.10^{b}	130.90^{b}	134.42^{b}	135.52^{b}
C60	15.51^{bc}	30.83^{bc}	38.50^{bc}	45.83^{c}	55.94^{c}	70.79^{c}	89.02^{c}	104.75^{c}	115.54^{c}	122.13^{c}	126.47^{c}	128.70^{c}
F60	14.18^{c}	27.50^{c}	35.03^{c}	45.22^{c}	56.01^{c}	69.19^{c}	88.70^{c}	99.56^{c}	110.29^{c}	118.94^{c}	121.80^{c}	123.43^{c}
L60	13.59^{c}	27.03^{c}	37.50^{bc}	50.50^{b}	60.74^{bc}	73.11^{c}	89.16^{c}	102.22^{c}	112.14^{c}	117.74^{c}	122.87^{c}	124.97^{c}
M60	20.24^{a}	40.02^{a}	50.01^{a}	62.48^{a}	76.33^{a}	91.63^{a}	110.93^{a}	126.12^{a}	137.12^{a}	143.79^{a}	147.74^{a}	148.54^{a}
SEM	0.802	1.392	1.344	1.490	1.561	1.845	2.101	1.981	2.445	2.434	2.397	2.411

Treatment[1]-control: Lucerne silage without additives, C60: lucerne silage with 60 mg cinnamon essential oil/kg, F60: lucerne silage with 60 mg flaxseed essential oil kg^{-1}, L60: lucerne silage with 60 mg lemon seed essential oil kg^{-1}, M60: lucerne silage with 180 mg blend of essential oils (60 mg cinnamon essential oil + 60 mg flaxseed essential oil + 60 mg lemon seed essential oil kg^{-1}).
Means within same column with different superscripts differ ($p < 0.05$).

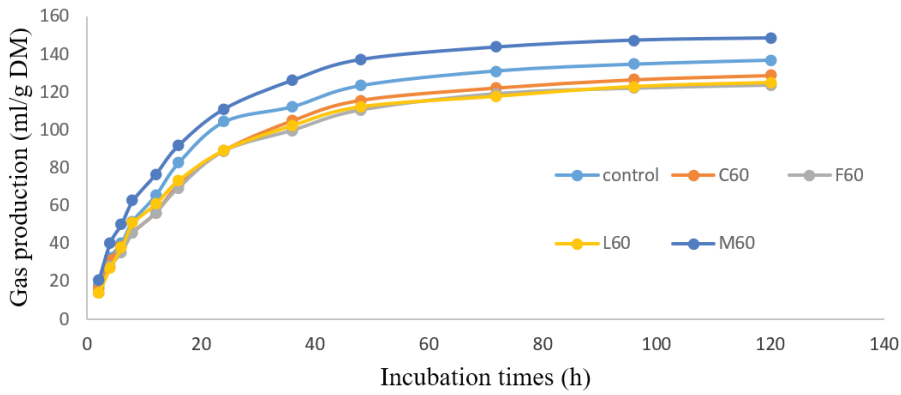

Figure 1: The effect of essential oils on gas production at different incubation times of lucerne silage

mental treatments. The treatment M60 had the highest *b* and *c* amounts among the treatments ($p < 0.01$).

Lucerne silage prepared with addition of essential oils lucerne had a significant effect on tVFA and NH_3-N. Treatments M60 and L60 had the highest tVFA and NH_3-N among treatments, respectively. Treatments F60 and C60 increased the amounts of tVFA and NH_3-N in comparison with control, respectively. Hart et al. (2008) using medium containing a set of rumen microorganisms showed that limonene, thymol, vanillin, guaiacol and oregano extract reduced rumen NH_3-N concentration. Brochers (1965) showed that the addition of thymol to rumen fluid resulted in the accumulation of amino acids and a decrease in NH_3-N concentration. He suggested that the thymol prevents the deamination of amino acids by rumen bacteria. It seems that since the plant essential oils have inhibitory effects on proteolysis and deamination, their inhibitory effects on proteolytic activities may reduce the degradation of the silage protein and consequently decrease the ammonia nitrogen content. The reduction in NH_3-N concentration has been attributed to the antimicrobial activity of essential oils. This property limits the fermentation process and reduces the breakdown of protein into ammonia. It has also been suggested that effective compounds in essential oils are able to bind to proteins, which reduces nitrogen loss.

3.3 AEROBIC STABILITY

All silages treated with essential oils improved the aerobic stability compared with control, of which control obtained 77 h, while M60, C60, L60, and F60 obtained 112, > 99.33, > 96.66, >92 h, respectively. Higher aerobic stability of treated silages was in agreement with the findings of Chaves et al. (2012). In their experiment, silages treated with oregano or cinnamon leaf essential oils at 120 mg kg^{-1} remained stable for two weeks. Exposure to air in silos may result in silage deterioration. The increase in temperature is the result of the metabolism of organic

Table 4: The effect of experimental treatments on gas production parameters of lucerne silage

Treatments[1]	Items[2]									
	pH	NE_L	SCFA	ME	OMD	DOMD	tVFA	NH_3-N	*b*	*c*
Control	6.60^{ab}	1.26^{a}	0.152^{a}	3.22^{a}	27.23^{a}	24.01^{ab}	8.62^{b}	47.22^{c}	133.74^{b}	0.0592^{b}
C60	6.63^{ab}	1.17^{bc}	0.128^{bc}	3.08^{bc}	26.55^{ab}	23.46^{bc}	7.14^{c}	29.36^{d}	126.78^{bc}	0.0526^{d}
F60	6.60^{ab}	1.20^{ab}	0.137^{ab}	3.14^{ab}	26.90^{a}	24.26^{a}	3.00^{d}	47.91^{c}	121.62^{c}	0.0539^{dc}
L60	6.50^{b}	1.10^{c}	0.1142^{c}	3.00^{c}	26.05^{b}	23.01^{c}	3.26^{d}	51.10^{a}	121.92^{c}	0.0579^{bc}
M60	6.65^{a}	1.20^{ab}	0.1360^{ab}	3.13^{ab}	26.92^{a}	23.74^{ab}	12.92^{a}	49.71^{b}	145.34^{a}	0.0645^{a}
SEM	0.042	0.026	0.006	0.036	0.240	0.202	0.438	0.467	2.488	0.002
p-value	0.1641	0.0041	0.0041	0.0047	0.0244	0.0016	<.0001	<.0001	<.0001	0.0006

Treatment[1]-control: Lucerne silage without additives, C60: lucerne silage with 60 mg cinnamon essential oil kg^{-1}, F60: lucerne silage with 60 mg flaxseed essential oil kg^{-1}, L60: lucerne silage with 60 mg lemon seed essential oil kg^{-1}, M60: lucerne silage with 180 mg blend of essential oils (60 mg cinnamon essential oil + 60 mg flaxseed essential oil + 60 mg lemon seed essential oil kg^{-1}).
ME: metabolizable energy (MJ/Kg DM); SCFA: short chain fatty acid (mmol 0.2 g^{-1} DM); DOMD: digestible organic matter in dry matter (%); NE_L: net energy lactation (MJ kg^{-1} DM); tVFA: total volatile fatty acids (mmol l^{-1}); NH_3-N: ammonia nitrogen (mg l^{-1}); OMD: organic matter digestibility (%); b: gas production of the potentially degradable (insoluble); c: fraction after 24 h incubation (ml 200 mg^{-1} DM).
Means within same column with different superscripts differ ($p < 0.05$).

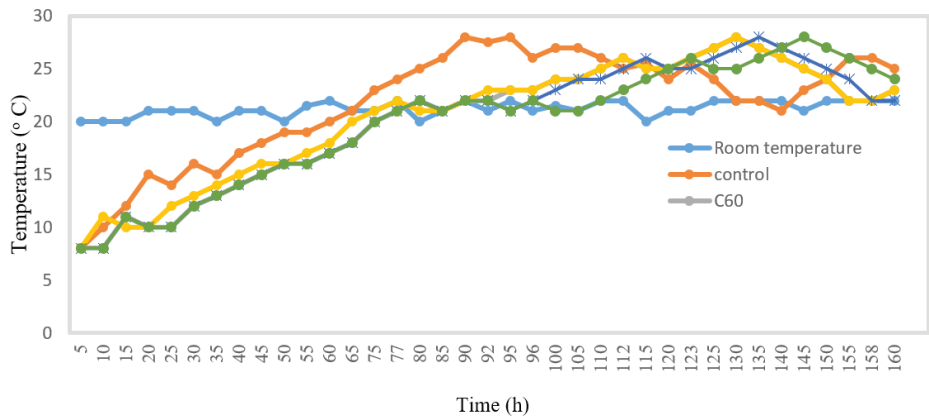

Figure 2: The effect of adding essential oils on aerobic stability of alfalfa silage

acids and nutrients by aerobic microorganisms. Changes in temperature can be an indicator of the development of aerobic deterioration of silages. In study of Chaves et al. (2012) the silages supplemented with 120 mg of pineapple or thyme essential oil kg^{-1} DM, remained stable for two weeks. Some secondary plant metabolites have been shown to inhibit the growth of some yeast species associated with aerobic degradation (Soycan-Önenç et al., 2017). In the study of Chaves et al. (2012), addition of 3 different herbal essential oils (cinnamon, oregano and sweet orange at 120 mg kg^{-1} DM) to barley silage increased aerobic stability compared to the control treatment. Hodjatpanah et al. (2016) added different herbal essential oils (oregano, thyme, cumin and cinnamon) to corn silage which improved aerobic stability of silages. The improved stability was attributed to inhibitory effects of essential oils on the growth and activities of yeast species that initiate deterioration of silages. Kung et al. (2000) reported that propionic acid, suberic acid, benzoic acid, acetic acid and ammonia are among the substances that increase the aerobic stability of silage.

4 CONCLUSION

In conclusion, some essential oils, which were used as silage additives in this study, had positive effects on quality of lucerne silage. The M60 protected silage protein against deleterious deamination by decreasing pH of the silage which increased the aerobic stability of the silage. Moreover, results regarding gas production demonstrated the potential of lemon seed and flaxseed essen-

tial oils to promote fermentation efficiency through reduction of gas production. Based on the obtained results, it can be concluded that the use of essential oil as silage additives Lucerne have a potential in improving its nutritional value as well as silage aerobic stability.

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Insecticidal activity and sublethal effects of *Beauveria bassiana* (Bals.-Criv.) Vuill. isolates and essential oils against *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae)

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Insecticidal activity and sublethal effects of *Beauveria bassiana* (Bals.-Criv.) Vuill. isolates and essential oils against *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae)

Abstract: The cotton aphid, *Aphis gossypii* Glover, 1877, is a polyphagous species and one of the most important pests of cucumber crops in Iran. In this study, virulence of three *Beauveria bassiana* (Bals.-Criv.) Vuill isolates, IRAN 108, IRAN 429C and LRC 137, as well as insecticidal activity of two essential oils extracted from *Matricaria chamomilla* L. and *Cuminum cyminum* L. were evaluated against adult stage of *A. gossypii* under laboratory conditions. The data for life table were analyzed using the age-stage, two-sex life table theory. Results showed that all isolates were pathogenic on aphid, but their virulence was varied in different isolates. The lowest calculated LC_{50} was belonged to IRAN 429C (3.9×10^4conidia ml^{-1}). The lowest LT_{50} was obtained at concentration of 10^8 and 10^7 conidia ml^{-1} for IRAN 429C (2.9 and 3.55 days, respectively). *M. chamomilla* essential oil had the lowest LC_{50} and LT_{50} values (19 µl l^{-1} air and 11.4 h), respectively. Longevity and population growth parameters, including the intrinsic rate of increase (r_m), gross reproduction rate (*GRR*), net reproductive rate (R_0), generation time (T) and finite rate of population increase (λ), were affected negatively by both agents. According to the results obtained in this study, both entomopathogenic fungi and essential oils could be used as an alternative to chemical insecticides in aphid IPM programs.

Key word: entomopathogenic fungi; biological control; integrated pest management; essential oil; sublethal dose

Insekticidna aktivnost in subletalni učinki izolatov entomopatogene glive *Beauveria bassiana* (Bals.-Criv.) Vuill. in eteričnih olj na bombaževo uš (*Aphis gossypii* Glover, 1877, Hemiptera: Aphididae)

Izvleček: Bombaževa uš (*Aphis gossypii* Glover, 1877) je polifagna vrsta in je eden izmed najpomembnejših škodljivcev kumar v Iranu. V raziskavi so bili preučevani virulenca izolatov entomopatogene glive (*Beauveria bassiana* (Bals.-Criv-) Vuill.) IRAN 108, IRAN 429C and LRC 137 in insekticidna aktivnost dveh eteričnih olj ekstrahiranih iz vrst *Matricaria chamomilla* L. in *Cuminum cyminum* L. na odrasle osebke bombaževe uši v laboratorijskih razmerah. Podatki preživetja so bili analizirali glede na starost, spol in razvojne faze škodljivca. Podatki so pokazali, da so bili vsi izolati patogeni za uši, vendar se je virulenca med izolati razlikovala. Najmanjša izračunana vrednost LC_{50} je pripadala izolatu IRAN 429C ($3,9 \times 10_4$ konidijev ml^{-1}). Najmanjša vrednost LT_{50} je bila dosežena pri koncentracijah 10^8 in 10^7 konidijev ml^{-1} za izolat IRAN 429C (2,9 in 3,55 dni). Eterično olje prave kamilice je imelo najmanjše vrednosti LC_{50} in LT_{50} (19 µl l^{-1} zraka in 11,4 h). Preživetje in parametri rasti populacije kot so potencialna rast populacije (r_m), bruto reprodukcija (*GRR*), neto reprodukcija (R_0), čas med dvema zaporednima generacijama (T) in končna velikost povečanja populacije (λ) so bili negativno prizadeti pri obeh obravnavanjih. Glede na rezultate pridobljene v tej raziskavi, bi kot alternativo kemičnim insekticidom v programih intergriranega upravljanja z listnimi ušmi lahko uporabili oboje, entomopatogene glive in eterična olja.

Ključne besede: entomopatogene glive; biološki nadzor; integrirano upravljanje s škodljivci; eterična olja; subletalna doza

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1 INTRODUCTION

Aphids are considered as an important pest of agricultural products all around the world and due to their specific biological characteristics, including the multiplicity and interference of generations and type of nutrition, as well as their resistance to some common chemical pesticides. So, different control strategies have been used against them (Abramson et al., 2006). *Aphis gossypii*, which is commonly known as cotton or melon aphid, is one of the most important pests of the plants in the families Rutaceae, Malvaceae and Cucurbitaceae all around the world and is recorded from more than 100 plant families (Van emden & Harrington, 2017). It is a cosmopolitan and polyphagous species distributed in tropical, subtropical and temperate regions, which causes direct damage by feeding on phloem sap and disrupting the plant growth, and indirectly through virus transmission and honeydew production (Martin et al., 2003). More than 50 plant viruses, both non-persistent and persistent, are transmitted by *A. gossypii* (Martin et al., 2003; Van emden & Harrington, 2017). To control this pest, various methods such as host-plant resistance, cultural practices, biological control, chemical control and integrated management have been used (Lowery & Smirle, 2003; Van emden & Harrington, 2017).

The occurrence of resistance in aphid populations to commonly used insecticides makes many of the pesticides inefficient and ineffective in aphid control programs, which is contributed to overall increases in the application of pesticides, continue to affect food and resource productivity, increase environmental exposure to chemical pesticides and etc. So, the use of anti-resistance strategies and/or using alternative methods in integrated pest management (IPM) programs are an urgent necessity (Van emden & Harrington, 2017; Wakil et al., 2017). *Beauveria bassiana* (Bals.-Criv.) Vuill. (Cordycipitaceae, Hypocreales) is a well-known entomopathogenic fungus with a broad host range infecting many arthropods including Coleoptera, Lepidoptera, Hemiptera, Formicidae and Acarina on diverse crops and it is one of the most widely used entomopathogenic fungi in biological control programs (Sowjanya Sree & Varma, 2015; Lacey, 2016). Its cosmopolitan existence, rich diversity, growing naturally in soils throughout of the world, as an endophyte inside different plants and as a pathogen acting against different insect species make it a suitable mycoinsecticde in IPM programs (Vega et al., 2008; Ragavendran et al., 2017).

Essential oils or volatile oils as commonly defined are aromatic oily liquids consisting of mixtures of volatiles mono- and sesquiterpenoids and phenyl propanoids, characterized by a strong odor and lower density than that of water (Bakkali et al., 2008; Norris et al., 2015). They have well proven antibacterial, antifungal, antiviral, antiparasitic, antioxigenic, anti-inflamatory, acaricidal and insecticidal properties (Shahriari et al., 2019; Srivastava et al., 2015). Because of their low persistence in the environment, low mammalian toxicity, non-phytotoxicity and diverse mode of actions, activities and applications, they are a very good and promise candidates in IPM programs (Liao et al., 2016; Sapindal et al., 2018). Over 3000 essential oils have been identified of which about 300 have commercial importance in the pharmaceutical, agricultural, food, health, cosmetics and perfume industries (Bakkali et al., 2008). Good insecticidal and acaricidal potential have been demonstrated in essential oils and during the different studies, their contact, fumigant, antifeedant, repellent, ability to delay development and fertility and oviposition inhibition activities has been identified (Marimuthu et al., 1997; Isman, 2000; Koul et al., 2008; Tripathi et al., 2009; Marcic, 2012; de Oliveira Cruz et al., 2013; Germinara et al., 2017). In addition, their effects in preventing the development of resistance in insect pests has been documented (Liao et al., 2016).

The aim of the present study was to assay and compare toxicity and sublethal effects of three indigenous isolates of *Beauveria bassiana*: IRAN 429C, IRAN 108 and LRC 137 and two essential oils, *Matricaria chamomilla* and *Cuminum cyminum* against *A. gossypii*.

2 MATERIALS AND METHODS

2.1 APHID COLONY

The initial colony of cotton aphid was collected from cucumber gardens in research fields of Agricultural Faculty, Urmia University, Iran and identified at species level based on morphological characteristics. It was reared and maintained on cucumber plants under laboratory conditions at 27 ± 2 °C, 65 ± 5% RH and a 16:8 (L:D) h photoperiod.

2.2 ESSENTIAL OILS

Dried parts of *M. chamomilla* plants except wooden stem and *C. cyminum* seeds were grounded into powder. 50 g of herbal powder was extracted with 600 ml of distilled water using a clevenger-type apparatus and hydro-distillation for 3 hours (Hassanpouraghdam et al., 2009). Extracted oil was dried with anhydrous sodium sulfate and stored in dark capped tubes at 4 °C until analysis.

2.3 FUNGAL ISOLATES

Three isolates of *Beauveria bassiana* were purchased from the Iranian Plant Protection Research Institute, Tehran, Iran (Table 1). All the isolates were grown on Sabouraud Dextrose Agar (SDA) medium and incubated at 25 ± 2 °C, 16:8 (L:D) h for 14 days to complete the sporulation. The conidia were directly harvested by scrapping off the colony surfaces with sterile scalpel and suspended in 15 ml of distilled water containing Tween 80. The suspension was filtered through a three-layered cheesecloth to remove fungal mycelia and substrate materials. Five concentrations form 10^4 to 10^8 conidia ml^{-1} were prepared from stock suspensions after which conidial concentration were determined based on haemocytometer (improved Neubauer) counts. Distilled water containing 0.05 % Tween 80 was used as control. The viability of spores for each isolate were determined prior to bioassays by spreading the dilute suspension (1×10^5 spore ml^{-1}) over the surface of potato dextrose agar (PDA, 39 $g\ l^{-1}$, Merck, Germany) medium. The plates were incubated at 25 ± 1 °C for 24 h and were viewed under a 400 x microscope magnification. While the length of germ tube was longer than the spore width, the spore was considered germinated. The germination rate was > 90 % in all bioassays.

2.4 INSECTICIDAL ACTIVITY

The bioassay was conducted to assess LC_{50} values. Filter paper (2 cm diameter) was soaked in different concentrations of essential oils and placed on the top of a glass bottles (305 ml), each containing 20 adult insects (< 24 h old). In order to prevent direct contact between insects and the essential oil, a cloth mesh was used to isolate filter paper. The main concentrations of essential oils tested on *A. gossypii* were 9.8, 14.8, 21.7, 32.1, and 47.4 $\mu l\ l^{-1}$ air for *M. chamomilla* and 19.7, 27.3, 37.6, 51.9, and 71.7 $\mu l\ l^{-1}$ air for *C. cyminum* for 24 h, respectively. In the control, the filter papers were soaked with distilled water. The mortality was evaluated 24 h after exposure. The experiment had three replications for each treatment. Insects that did not show any movement when touched with the brush are considered dead.

Table 1: Details of Beauveria bassiana isolates used in bioassay experiments

Accession Number	Substrate	Location
IRAN 429C	*Chilo suppressalis*	Iran
IRAN 108	Soil	Iran
LRC 137	*Leptinotarsa decemlineata*	Canada

For the test of fungi, twenty adult aphids (< 24 h old) were immersed for 30 seconds into five different spore concentrations (1×10^4 to 1×10^8 conidia ml^{-1}) of the fungal isolates. In control treatments, aphids were immersed into a 0.05 % Tween 80 solution. Each treatment had three replications and mortality data was assessed daily up to 10 days. Again, insects that did not show any movement when touched with the brush are considered dead. Dead aphids were transferred into new petri dishes containing a moist filter paper and incubated at 25 °C to observe the possible development of mycelium/conidia of the treated fungi (Kassa, 2002).

Bioassays was done to determine the median effective time to cause mortality of 50 % of the test insects (LT_{50} value), that for this study the aphids immersed for 30 seconds into five different spore concentrations (1×10^4 to 1×10^8 conidia ml^{-1}) of the fungal isolates. Also, for this test in essential oils, LC_{50} value of both oils were used. Ten adult aphids (< 24 h old) were introduced to each glass bottles (305 ml). Each treatment had three replications and the mortality was evaluated every 2, 7, 12, 18 and 24 h to obtain the end point of mortality.

2.5 SUBLETHAL EFFECTS

The sublethal effects test was carried out in a similar manner except that 50 adult aphids (< 24 h old) were immersed for 30 seconds in conidial suspensions containing 2.2×10^3 and 3.9×10^4 conidia ml^{-1} for IRAN 429C and IRAN 108 fungal isolates, respectively. This two fungal isolates were selected for sublethal studies, because of their higher efficacy to infect the aphids in previous tests. In control treatments, aphids were immersed in 0.05 % Tween 80 solution. Mortality was recorded daily from the time of emergence of the insects until last insect's life.

To determine the sublethal effects of the *M. chamomilla* and *C. cyminum* essential oils, 50 adult aphids (< 24 h old) were exposed to an LC_{30} of each essential oil (13.52 and 27.87 $\mu l\ l^{-1}$ air, respectively) for a period of 24 h. The live insect were transferred individually after 24 h to a plastic Petri plates (6 cm in diameter). Treated aphids were examined daily and the nymphs were counted and removed until the death of the last adult aphid. Aphids were considered dead if they didn't move when contacted with a needle.

3 DATA ANALYSIS

The experiments were conducted under completely

randomized design (CRD) with three replicates of each treatment. Mortality data were corrected by Abbott's formula (Abbott, 1925). The data obtained from lethal concentration assays were subjected to probit analysis for calculation of LC_{50}, LC_{30} and LT_{50} values. All statistical analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, USA). The life history raw data of *A. gossypii* were analyzed according to the age-stage, two-sex life table theory and the method described by Chi (1988) using TWOSEX-MSChart software (Chi & Liu, 1985; Chi, 2016). The intrinsic Rate of Increase (r) estimated by using iterative bisection method from the Euler-Lotka formula:

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$$

with age indexed from 0 (Goodman, 1982). The life table parameters (the age-specific survival rate (l_x); the age specific fecundity (m_x)) and the population parameters (the net reproductive rate (R_0), finite rate of increase (λ), and mean generation time (T)) were calculated according to Chi (1988) method:

The net reproductive rate (R_0):

$$R_0 = \sum_{x=0}^{\infty} l_x m_x$$

mean generation time (T):

$$T = \frac{\ln R_0}{r}$$

and finite rate of increase (λ):

$$\lambda = e^r$$

The means and standard errors of the population parameters were estimated by using the bootstrap method (Efron & Tibshirani, 1993; Huang & Chi, 2012) embedded in the TWOSEX-MSChart (Chi, 2016). The paired bootstrap test was used to compare differences (Efron & Tibshirani, 1993). Survival, fecundity and reproductive value curves were constructed using SigmaPlot (12.3).

4 RESULTS

4.1 FUMIGANT TOXICITY OF ESSENTIAL OILS

Estimated values of LC_{50} of the essential oils are summarized in Table 2. The results showed that the essential oils of *M. chamomilla* and *C. cyminum*, had good aphicidal activity against the adults of cotton aphids after 24 h at very low concentrations. LC_{50} values for *M. chamomilla* and *C. cyminum* were 19 and 37.36 µl l^{-1} air, respectively. The results obtained from LT_{50} showed that *M. chamomilla* oil affected the insects faster than *C. cyminum* oil (Table 3).

4.2 SUBLETHAL EFFECTS OF ESSENTIAL OIL

In the studies dealing with sublethal effects of essential oils on adult aphids, there were significant differences in the life table parameters such as intrinsic rate of increase (r), net reproductive rate (R_0), gross reproduction rate (*GRR*), finite rate of increase (λ) and female adult longevity among essential oils treatments and the controls. The survival rate of female adults was significantly lower in the *M. chamomilla* and *C. cyminum* than control group ($p < 0.001$) (Table 4).

Gross reproduction rate (*GRR*) in control group was higher than both of the essential oil treatments ($p < 0.001$). Intrinsic rate of increase (r) was 0.44, 0.26, and 0.26 in control and *M. chamomilla* and *C. cyminum* es-

Table 2: LC_{30} and LC_{50} values with confidence intervals of *Matricaria chamomilla* and *Cuminum cyminum* essential oils on *Aphis gossypii* adults after 24 hours

Plant species	LC_{30} (95 % C.I.) (µl l^{-1} air)	LC_{50} (95 % C.I.) (µl l^{-1} air)	Slope±S.E.	χ^2 (df)
M. chamomilla	13.52 (11.54-15.26)	19 (17.01-21.1)	3.54 ± 0.38	1.39 (3)
C. cyminum	27.87 (24.54-30.75)	37.36 (34.11-40.91)	4.11 ± 0.45	1.03 (3)

Table 3: LT_{50} and LT_{90} values of *Matricaria chamomilla* and *Cuminum cyminum* essential oils on *Aphis gossypii* adults

Plant species	LT_{50} (95 % C.I.) (hours)	LT_{90} (95 % C.I.) (hours)	Slope ± SE	χ^2 (df)
M. chamomilla	11.4 (9.82-13.19)	35.17 (27.70-50.13)	2.62±0.3	5.8 (3)
C. cyminum	13.88 (12.2-15.85)	36.38 (29.04-51.58)	3.06±0.37	3.05 (3)

sential oils, respectively. Intrinsic rate of increase significantly decreased between control and both essential oils ($p < 0.001$). The finite rate of increase in population (λ) for control and essential oils was 1.7, 1.3, and 1.3, respectively. This parameter significantly decreased in both essential oils compared with the control ($p < 0.001$). The age-specific survival rate (l_x), fecundity (m_x), and age-specific net maternity ($l_x m_x$) of *A. gossypii* are presented in Fig. 1. The beginning of oviposition in both treatments was delayed in compared with control. Also, these result showed that *C. cyminum* caused more declined faster decline in age-specific fecundities in compared with *M. chamomilla*. The mean oviposition days of *A. gossypii* were reduced significantly in *M. chamomilla* and *C. cyminum* essential oil treatments than the controls. There were no significant differences in the intrinsic rate of increase (r), gross reproduction rate (*GRR*) and finite rate of increase (λ) between essential oil treatments (Table 4).

4.3 LETHAL EFFECTS OF FUNGAL ISOLATES

All the tested fungal isolates were infective to adult aphids at the used conidial concentrations and the mortality rate of the aphids was correlated with conidium concentration. Based on the results, there was a linear relationship between the conidial concentrations of each fungal isolate and mortality of the aphids. The values of LC_{30} and LC_{50}, confidence intervals and slope of different isolates of *B. bassiana* against adult aphids are presented in Table 5. IRAN 429C and LRC 137 isolates caused the highest and lowest mortality rates in the adult aphids, re-

Table 4: Life table parameters (mean ± SE) of *Aphis gossypii* adults treated with two essential oils and in the control treatment

Parameter	*M. chamomilla*	*C. cyminum*	Control
r (day^{-1})	0.2685 ± 0.003 a	0.2691 ± 0.006 a	0.4487 ± 0.011 b
R_0(offspring/individual)	10.24 ± 0.34 a	13.22 ± 0.34 b	58.68 ± 1.29 c
GRR	16.96 ± 1.18 a	16.33 ± 0.46 a	62.68 ± 1.43 b
T (day)	8.66 ± 0.16 a	9.59 ± 0.17 b	9.07 ± 0.21 b
λ (day^{-1})	1.3080 ± 0.008 a	1.3088 ± 0.008 a	1.56± 0.018 b
Oviposition days	5.48 ± 0.22 a	7.44 ± 0.21 b	17.48 ± 0.37 c
Female adult longevity (day)	8.52 ± 0.28 a	11.72 ± 0.21 b	23.66 ± 0.25 c

r: intrinsic rate of increase; R_0: net reproductive rate; *GRR*: gross reproduction rate; T: mean generation time; λ: finite rate of increase. Standard errors were estimated by using the bootstrap technique with 100,000 resampling. Difference was compared with paired bootstrap test ($P < 0.05$). The mean followed by different lower case letters indicate significant differences between three varieties.

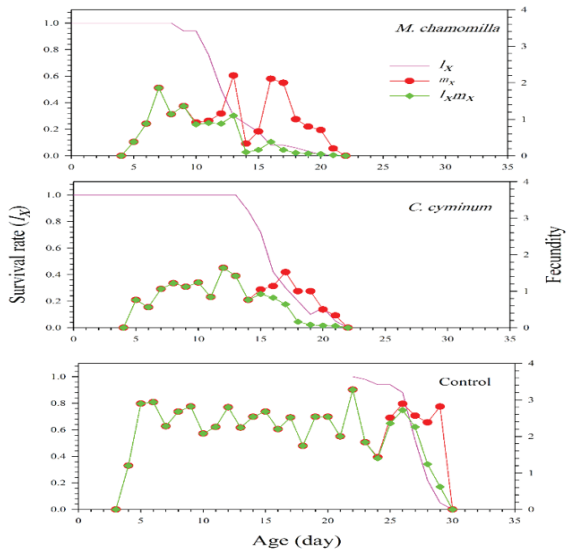

Figure 1: Age-specific survival rate (l_x), age-specific fecundity (m_x) and age-specific maternity ($l_x m_x$) of *Aphis gossypii* treated with the essential oils

spectively. The lowest LC_{50} (3.9×10^4 conidia ml^{-1}) was calculated for IRAN 429C. Based on LC_{50} values, IRAN 429C was the most virulent isolate, followed by IRAN 108 and LRC 137 (Table 5). Low mortality was observed in control treatments and no signs of fungal growth were seen on dead insects. The results of LT_{50} indicated that IRAN 429C, had the lowest LT_{50} values followed by IRAN 108 and LRC 137, respectively (Table 6). For concentrations of 10^4, 10^5 and 10^6 conidia ml^{-1}, LT_{50} wasn't calculated because there weren't reached 50 % mortality rate at the end of the experiment.

4.4 SUBLETHAL EFFECTS OF FUNGAL ISOLATES

The fungal isolates, IRAN 429C and IRAN 108 were selected to evaluate their sublethal effects because of their higher virulence against adult aphids. The life table parameters of *A. gossypii* affected by fungal isolates are summarized in Table 7. The intrinsic rate of increase (r) in the control, IRAN 429C and IRAN 108 treatments was 0.44, 0.31, 0.31, respectively (Table 8). There wasn't a significant difference between the used fungal isolates ($p > 0.05$), but significant differences were found between fungal isolates and the control treatment ($p < 0.001$).

Gross reproduction rate (*GRR*) showed significant decrease in fungal isolates in compare with control ($p < 0.001$). Net reproductive rate (R_0) was significantly higher in control than that of fungal isolates ($p < 0.001$). The finite rate of population increase (λ) for control, IRAN 429C and IRAN 108 were 1.56, 1.36 and 1.36, respectively. This parameter significantly decreased in both fungal isolates compared with control ($p < 0.001$). The oviposition period for untreated aphids was significantly higher than that treated aphids ($p < 0.001$). There wasn't significant differences in the intrinsic rate of increase (r) and finite rate of increase (λ) among the two fungal isolates ($p > 0.05$). The age-specific survival rate (l_x), fecundity (m_x), and age-specific net maternity ($l_x m_x$) of *A. gossypii* are presented in Fig. 2. The beginning oviposition in Iran 429C was delayed in compared with control. Also, these result showed that IRAN 429C caused more declined in age-specific fecundities in compared with IRAN 108. At the end-point, the results showed that sublethal doses of the essential oils had a better performance against aphids than that of fungal isolates (Table 9).

5 DISCUSSION

Organophosphates, carbamates and pyrethroids are three main groups of chemical insecticides, which

Table 5: LC_{30} and LC_{50} values with confidence intervals of *Beauveria bassiana* isolates on *Aphis gossypii* adults

Isolates	LC_{30} (95% C.I.) (conidia/ml)	LC_{50} (95% C.I.) (conidia/ml)	Slope ± S.E.	χ^2 (df)
IRAN 429C	2.2×10^3 (7.2×10^2-5.1×10^3)	3.9×10^4 (1.9×10^4-7.4×10^4)	0.41 ± 0.03	5.85 (4)
IRAN 108	3.9×10^4 (9.5×10^3-1×10^5)	4.3×10^5 (1.5×10^5-1.1×10^6)	0.50 ± 0.03	6.93 (4)
LRC 137	7.4×10^4 (6.2×10^3-3.5×10^5)	1.4×10^6 (2.9×10^5-1×10^7)	0.41 ± 0.03	12.6 (4)

Table 6: LT_{50} and LT_{90} values of *B. bassiana* isolates at 10^8 conidia mL^{-1} on *Aphis gossypii* adults

Isolates	LT_{50} (95 % C.I.) (days)	LT_{90} (95 % C.I.) (days)	Slope±S.E.	χ^2 (df)
IRAN 429C	2.90 (2.62-3.18)	9.51 (8.43-11.02)	2.48 ± 0.16	4.75 (8)
IRAN 108	3.84 (3.47-4.22)	15.71 (13.17-19.75)	2.09 ± 0.15	2.44 (8)
LRC 137	4.64 (4.17-5.14)	23.34 (18.32-32.44)	1.82 ± 0.15	0.80 (8)

Table 7: LT_{50} and LT_{90} values of *B. bassiana* isolates at 10^7 conidia mL^{-1} on *Aphis gossypii* adults

Isolates	LT_{50} (95 % C.I.) (days)	LT_{90} (95 % C.I.) (days)	Slope ± SE	χ^2 (df)
IRAN 429C	3.55 (3.19-3.91)	14.90 (12.52-18.68)	2.05 ± 0.15	1.04 (8)
IRAN 108	6.21 (5.60-6.97)	30.64 (23.17-45.16)	1.85 ± 0.16	2.51 (8)
LRC 137	4.78 (4.34-5.25)	20.76 (16.79-27.57)	2.0 ± 0.15	2.57 (8)

Table 8: Life table parameters (mean ± SE) of *Aphis gossypii* adults treated with two *B. bassiana* isolates and the control treatment

Parameter	IRAN 108	IRAN 429C	Control
r (day^{-1})	0.3142 ± 0.007 a	0.3103 ± 0.006 a	0.4487 ± 0.011 b
R_0(offspring/individual)	26.52 ± 0.74 a	19.7 ± 0.90 b	58.68 ± 1.29 c
GRR	32.23 ± 1.18 a	28.76 ± 0.98 b	62.68 ± 1.43 c
T (day)	10.43 ± 0.22 a	9.60 ± 0.18 b	9.07 ± 0.21 b
λ (day^{-1})	1.36 ± 0.010 a	1.36 ± 0.008 a	1.56 ± 0.018 b
Oviposition days	11.08 ± 0.23 a	8.88 ± 0.4 b	17.48 ± 0.37 c
Female adult longevity (day)	17.28 ± 0.2 a	13.18 ± 0.36 b	23.66 ± 0.25 c

r: intrinsic rate of increase; R_0: net reproductive rate; *GRR*: gross reproduction rate; T: mean generation time; λ: finite rate of increase. Standard errors were estimated by using the bootstrap technique with 100,000 resampling. Difference was compared with paired bootstrap test ($p < 0.05$). The mean followed by different lower case letters indicate significant differences between three varieties.

Table 9: Comparison of life table parameters (mean ± SE) of *Aphis gossypii* treated with fungal isolates and essential oils

Parameter	Treatment			
	Fungi		Essential oil	
	IRAN 108	IRAN 429C	*M. chamomilla*	*C. cyminum*
r (day^{-1})	0.3142 ± 0.007 a	0.3103 ± 0.006 a	0.2685 ± 0.003 b	0.2691 ± 0.006b
R_0(offspring/individual)	26.52 ± 0.74 a	19.7 ± 0.90 b	10.24 ± 0.34 c	13.22 ± 0.34 d
GRR	32.23 ± 1.18 a	28.76 ± 0.98 b	16.96 ± 1.18 c	16.33 ± 0.46 c
T (day)	10.43 ± 0.22 a	9.60 ± 0.18 b	8.66 ± 0.16 c	9.59 ± 0.17 b
λ (day^{-1})	1.36 ± 0.010 a	1.36 ± 0.008 a	1.3080 ± 0.008 b	1.3088 ± 0.008b
Oviposition days	11.08 ± 0.23 a	8.88 ± 0.4 b	5.48 ± 0.22 c	7.44 ± 0.21 d
Female adult longevity (day)	17.28 ± 0.2 a	13.18 ± 0.36 b	8.52 ± 0.28 c	11.72 ± 0.21 d

r: intrinsic rate of increase; R_0: net reproductive rate; *GRR*: gross reproduction rate; T: mean generation time; λ: finite rate of increase. Standard errors were estimated by using the bootstrap technique with 100,000 resampling. Difference was compared with paired bootstrap test ($p < 0.05$). The mean followed by different lower case letters indicate significant differences between three varieties.

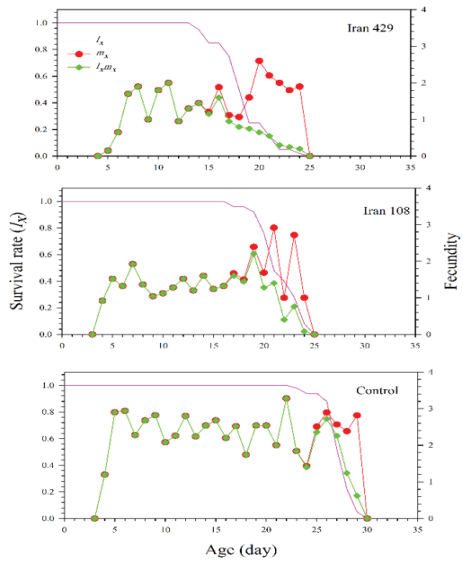

Figure 2: Age-specific survival rate (l_x), age-specific fecundity (m_x) and age-specific maternity ($l_x m_x$) of *Aphis gossypii* treated with the fungal isolates

are commonly used in aphid control. The long-term use of these insecticides caused resistance development in aphid populations which make them difficult to control as well as frequent environmental and health risks (Sadeghi et al., 2009; Asadi et al., 2018). In recent years, the insecticidal properties of essential oils and their main compounds have been investigated on various pests, some of which had promising results (Al-Jabr, 2006). The results of the present study indicates that this biological agents had a significant lethal effect on the tested pests. Also, given the LC_{50} values of these two essential oils on adult insects, it was recognized that chamomile essential oil performed better compared to other essential oil in this pest. Meanwhile, IRAN 429C isolate cause the highest mortality in adult insects compared to the other two isolates. The toxicity of plant essential oils may be due to the fact that the seeds or leaves of these plants include compounds that have anti-nutrition or toxic activity or disrupt the molting which is often fatal for insects (Champagne et al., 1989). High level of insecticidal activity of *Eucalyptus globules* L. essential oil against *Aphis gossypii* has been reported (Mareggiani et al., 2008). In another study, the insecticidal activities of *Azadirachta indica* Adr. Juss., *Eucalyptus camaldulensis* Dehn. and *Laurus nobilis* L. essential oils were evaluated against *A. gossypii* (Ebrahimi et al., 2013). According to the results, *A. indica* and *E. camaldulensis* have a greater insecticidal activity compared to *L. nobilis*. Also, fertility and life span of the treated aphids were significantly decreased. Besides, it was reported that the essential oils of *Origanum syriacum* var. *bevanii* L., *C. cyminum* L., *Pimpinella anisium* L. and *E. camaldulensis* Dehn. were effective in fumigant assays against melon aphids and green peach aphids (Isman, 2000). Therefore, the results of this study are consistent with the findings of this researcher on the control of melon aphids by essential oils such as *C. cyminum*. In an experiment, Al-Jabr (2006) proved the toxicity and repelling effect of chamomile essential oil on *Oryzaephilus surinamensis* (Linnaeus, 1758) (Coleoptera: Silvanidae) and *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrioidae). Also, El-Khyat et al. (2017) studied the insecticide activity of three essential oils, including chamomile on *Ephestia cautella* (Walker, 1863) and concluded that all of the three tested essential oils had a significant insecticidal effect on the pest. Among these, the most repelling essential oil was chamomile. The study results of AL-Jabr (2006) and El-Khyat et al. (2017) have been compatible with the results of the present study on the control potential of chamomile essential oil on insects. These reports are consistent with the present study in terms of the insecticidal activity of essential oils on the Aphididae family.

Entomopathogenic fungi are considered as one of the most promising alternatives in biological control of insect pests (Kaaya & Hassan, 2000). Gurulingappa et al. (2011) were studied the effects of endophytic *B. bassiana* and *Lecanicillium lecanii* isolates on mortality, survival and reproduction of *A. gossypii*. Results showed that the tested fungi significantly reduced the rate and period of reproduction and increased mortality of *A. gossypii*. Feng et al. (1990) compared pathogenicity of *B. bassiana* and *Verticillium lecanii* R. Zare & W. Gams, isolates against six species of cereal aphids. Although both fungal species were pathogenic on aphids, but *B. bassiana* was more virulent than that of *V. lecanii*. In a study, virulence of six *B. bassiana* isolates was studied on Russian wheat aphid based on LC_{50} and LD_{50} indices (Feng & Johnson, 1990). Although all isolates infect the aphids, but their virulences were very different and only one isolate showed significant virulence on aphids with the lowest LC_{50}. Similar results were obtained in pathogenicity assessment of indigenous isolates of *B. bassiana* on adult insects of Russian wheat aphids. In an experiment, fertility of *Aphis craccivora* Koch, 1854 were studied under the influence of *B. bassiana* isolate (Zaki, 1998). Result showed that by increasing in concentration of fungal conidia, the fertility rate was decreased which is in accordance with our finding. Our results were in agreement with those obtained by Kim (2007) who examined the effect of *Lecanicillium attenuatum* Zare & W. Gams CS625 on the reproduction of the cotton aphid. They found net reproduction rate of aphid nymphs was reduced and the reduction was corresponded well with spore concentration.

Based on the results obtained in this and previous studies, both entomopathogenic fungi and essential oils are good alternative candidates to chemical pesticides in aphid control programs. Further studies are needed to evaluate the insecticidal activities of these promise biological control agents directly under greenhouse and field conditions.

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Modeling the chemical properties of sesame oil under the influence of pulsed electric field using the artificial neural networks

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Modeling the chemical properties of sesame oil under the influence of pulsed electric field using the artificial neural networks

Abstract: In this study, PEF pretreatment was used to improve the efficiency of screw press method on the properties of extracted sesame seeds oil. Sesame seeds were treated at different PEF intensities (250, 3250 and 6250 Vcm^{-1}) and pulse numbers (10, 30 and 50). Then, the oil was extracted using a screw press at 33 rpm. Some physicochemical properties of the obtained oil including oil extraction efficiency, acidity index, determination of total phenolic compounds and activity of the inhibition of the DPPH free radical were evaluated. The results showed that the oil extraction efficiency initially increased at first but it showed reduction during PEF pretreatment at higher intensities. Increase in the applied PEF intensity and pulse number lead to an increase in the acidity and total phenolic compounds. While the oxidative stability of the oil reduced at the more intensive PEF conditions. However, the antioxidant activity was firstly increased and then decreased during PEF pretreatment. In addition, artificial neural network model was used to predict the effect of different PEF pretreatment conditions on the physicochemical properties of the extracted oil. The best model was the feed forward neural network with sigmoid hyperbolic tangent conduction function, Levenberg – Marquardt training function with 5-6-2 topology.

Key words: sesame seeds oil extraction; pulsed electric field; artificial neural network model; physicochemical properties

Modeliranje kemijskih lastnosti sezamovega olja pod vplivom pulzirajočega električnega polja z uporabo umetnih nevronskih mrež

Izvleček: V raziskavi je bilo uporabljeno predhodno obravnavanje semen sezama s pulzirajočim električnim poljem (PEF) za izboljšanje učinkovitosti stiskanja in izboljšanje kakovosti olja. Semena sezama so bila izpostavljena različnim jakostim PEF (250, 3250 in 6250 V cm^{-1}) in različnim številom pulzov (10, 30 in 50). Olje je bilo potem iztisnjeno pri 33 rpm. Ocenjene so bile nekatere fizikalno-kemične lastnosti dobljenega olja kot so učinkovitost ekstrakcije, indeks kislosti, vsebnost celokupnih fenolov in velikost inhibicije DPPH prostega radikala. Rezultati so pokazali, da se je učinkovitost ekstrakcije v začetku obravnavanja povečala, a se je potem zmanjševala z večanjem moči PEF. Povečanje moči PEF in števila pulzov je vodilo k povečanju kislosti in vsebnosti celokupnih fenolov. Pri tem se je oksidativna stabilnost olja zmanjšala v razmerah večje moči PEF. Antioksidativna aktivnost olja se je v začetku obravnavanja s PEF povečala in nato zmanjšala. Dodatno je bil za predvidevanje učinkov obravnavanja s PEF na fizikalno-kemijske lastnosti iztisnjenega olja uporabljen model umetne nevronske mreže. Najboljši model je bil dosežen z nevronsko mrežo s sigmoidno-hiperbolično funkcijo, in Levenberg – Marquardtovo funkcijo učenja s 5-6-2 topologijo.

Ključne besede: ekstrakcija olja iz semen sezama; pulzirajoče električno polje; model umetne nevronske mreže; fizikalno-kemijske lastnosti olja

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1 INTRODUCTION

Sesame seeds (*Sesamum indicum* L.) is cultivated in Asia and some parts of Africa particularly in Sudan, Nigeria and Ethiopia. The land under cultivation and the yield of sesame seeds were respectively 42.95 thousand hectare and 40.44 thousand tons in Iran during 2015 to 2016 (Abdollahi et al., 2018).The oil content of these seeds is ranged between 28 % to 59 % (Biabani & Pakniyat, 2008). Sesame oil has pleasant flavor and aroma causing make it possible to be used as salad dressing and cooking oil. This oil is also used in preparing the shortening, margarine, cosmetics, perfume and drugs (Boselli et al., 2009). The sesame oil causes a decrease in human's blood pressure and cholesterol level due to containing phytosterols, tocopherols and lignans (Ogawa et al., 1995). This oil contains about 85 % unsaturated fatty acids but it shows good stability against oxidative rancidity (Rostami et al., 2014). Sesame oil has been recognized for its health-promoting properties, and more recently, studies have revealed that polyphenolic compounds found in sesame oil are responsible for its therapeutic effects (Jacklin et al., 2003). Among the compounds in sesame oil, sesamin is among the phenolic compound at the highest concentration. Ferulic acid, vanillic acid, syringic acid and gallic acid were of high content among phenolic compounds in sesame oil (Wu et al., 2016)

Cold press method is applied to produce the oil and press cake without using chemical materials; hence, the obtained oil and press cake are suitable for human consumption and herbivorous animals. Application of the cold press method shows some advantages such as low cost initial and operative investments, simplicity and wide range of production capacity (from low scale up to about 100 tons per hour). However, there is a high amount of oil residue (10-20 %) in the press cake. In this method, different factors such as press pressure, rotational speed of screw press, seed moisture content and process temperature influence the oil extraction efficiency. In addition, the control of seed moisture content and temperature are critical to prevent from the denaturation of press cake proteins (Azadmard-Damirchi et al., 2010) (Anderson et al., 1996).

Pulsed electric field (PEF) can been used as a pretreatment to enhance the efficiency of the oil extraction during screw pressing. In this method, the placed seeds (submerged in aqueous medium) between two electrodes are exposed to high voltage pulses (Asavasanti et al., 2011). The PEF process time is very short (about microsecond) and it can be performed at room temperature, which resulted to PEF known as a non-thermal method. The PEF technology prevents undesirable changes in physicochemical properties of the food so it shows advantages in comparison to the heating treatments (Pourzaki & Mirzaee, 2009; Schroederetal., 2009).During PEF treatment, the microorganisms inactivation is achieved via electroporation phenomena (Quass, 1997). Electroporation is related to the effect of PEF on pore formation in the cell membrane. In fact, an external electric field creates a potential of additional membrane transfer that is more than natural potential of cell and when total potential of membrane reaches to the critical limit of about 1 V, then tearing will occur. This membrane tearing can be irreversible or reversible. The reversible tearing is used in cell incitation, gene transfer and increase in metabolic activities of cell(Hamid Bakhshabadi et al., 2018). Low energy PEF treatment was successfully used to produce fat from micro-algae to lowering(La Choi et al., 2016). Researchers mentioned that PEF process conditions including applied intensity, frequency, pulse width and treatment time influenced the efficiency of oil extraction of sunflower seeds. They introduced the PEF treatment as effective method in the seeds oil extraction in large scales (Shorstkii et al., 2017).

The artificial neural network (ANN) is a simple simulated modeling method that is inspired by studying the living beings' mind and neural network system. High performance of biologic systems arising from nature is parallel to their neurons programming. An ANN performs this structure through distributing simulation in attached small and simple processor units (Fausett, 1994). ANN with input layer of three neurons (pressure, temperature and process time)was used to predict the efficiency of oil extraction from black caraway (output layer) using supercritical solvent method (Full ana et al., 2000). Nazari et al. (2015) used feed forward ANN to predict the oxidative stability of olive oil during storage time. Therefore, the aim of this study was to investigate the effect of PEF pretreatment condition (intensity and pulse numbers) on different physicochemical properties of the extracted sesame seeds oil. In addition, ANN modeling was used to predict efficiency of oil extraction from sesame seeds using PEF pretreatment.

2 MATERIALS AND METHODS

2.1 MATERIALS AND EQUIPMENT

The sesame seeds, containing 46.7 % oil, was provided from local market of Ferdous town (Iran). At first, the external materials were separated and removed from the sesame seeds and then it was kept in resistant plastic bags against air and moisture penetration. The used equipment were laboratory sieve, grinding machine (Huddinge 14105, Sweden), Desiccator, laboratory oven

(Memert, Germany), digital balance (Gec Avery, made in England), pulsed electric field machine (made in Food Industry Institute of Iran), Kjedahl device (Auto Analayser 130 Tecator CO) and oil extracting screw press (Kern Kraft, Germany).

2.2 OIL EXTRACTION PROCESS

The sesame seeds were pretreated at different PEF intensities (250, 3250, 6250 V cm^{-1}) using various pulse numbers (10, 30 and 50) according to Bakhshabadi et al.(2017) method. Then, the oil of PEF pretreated seeds was extracted using screw press with speed of 34 rpm.

2.3 THE OIL EXTRACTION EFFICIENCY

The percentage of oil extraction efficiency was calculated using Eq. 1. A digital balance with the precision of 0.01 was applied to weight the samples.

$$\text{Oil extraction efficiency}\,(\%) = \frac{\text{mass of extracted oil}\,(g)}{\text{mass of initial seeds}\,(g)} \times 100$$

2.4 MEASUREMENT THE OIL ACIDITY

The acidity of the oil was measured according to AOCS Cd 3-63 method (AOCS, 1993a).

2.5 DETERMINATION OF OIL OXIDATIVE STABILITY

According to AOCS Cd 12b-92 method, the oil stability against oxidation was determined using a Rancimat (AOCS, 1993b).This device works based on change in electric conduction of water existing in Rancimat container through compounds resulted from oxidative reaction of oil existed from device cell. In this study, the input airflow speed was set on 20 liter per hour.

2.6 DETERMINATION OF THE CONTENT OFTOTAL PHENOLIC COMPOUNDS

The content of total phenolic compounds was determined using spectroscopy method. At first, 1 g of oil was mixed thoroughly with 3 ml of methanol solution: water (90:10) for 4 minutes. Then, the solution was centrifuged at the 3000 rpm for 5 min. 20 micro liter of the obtained supernatant was mixed with water (8.2 ml) and Folin Ciocalteu reagent (0.5 ml). After 5 min, 1 ml of sodium carbonate 10 % was added to the mixture and it was left in a dark place at the room temperature for 1 hour. Finally, the absorbance of the samples was recorded using a spectrophotometer at the wavelength equals to 765 nm. Gallic Acid solution (0 to 1000 micro gram per ml) was used to prepare the standard curve and the content of total phenolic compounds was reported as mg of gallic acid per kg of sample (Bail et al., 2008).

2.7 THE ACTIVITY OF THE DPPH FREE RADICAL INHIBITION

2-2-di-phenyl-1-picryl Hydrazine (DPPH), is a lipophilic radical showing the maximum absorption in 517 nm wavelength. The DPPH radicals react with antioxidants or other radicals resulting in decrease in their contents and absorption. Decrease in DPPH molecules has direct relation with available hydroxyl groups; and, the hydroxyl groups give hydrogen to the DPPH radicals and changed their color from dark violet to light yellow.

In this method, 1 ml of 0.1 mM methanol solution of DPPH was mixed completely with1 ml of extracted oil and was then placed in a dark place at the room temperature for 15 min. The absorption of the mixture was read at 517 nm wavelength and percentage of DPPH free radical inhibition was calculated using Eq. 2 (Long et al., 2011).

$$\text{Activity of the inhibition of the DPPH free radical}\,(\%) = \frac{AS - AC}{AC} \times 100$$

Where AS and AC are optical absorption of sample and control, respectively.

2.8 STATISTICAL ANALYSIS

The response surface methodology using the Design-Expert version 6.0.2 was used to represent the graph of conditions of the PEF pretreatments (intensity and pulse number) on different physicochemical properties of the extracted oil. Finally, the neural network tool of MATLAB software was used to determine the optimum neural network. For the purpose of designing this network, two intakes of PEF intensities and pulse numbers were defined in a two-line matrix and the amount of oil extraction efficiency, acidity, oxidative stability, content of phenolic compounds and activity of the inhibition of the DPPH free radical were defined in a five-line matrix as outputs. Different neural networks contains activation functions and different learning and also the amount of different neurons in the hidden layer were designed.

Then, their efficiency become distinguished using two criteria of evaluating the correlation coefficient (R^2) and mean squared error (MSE) using Eq. 3 and 4, respectively. At first, through examining the various neural networks, the feed forward neural network, with the highest efficiency was chosen. The amount of learning cycles were regarded 1000. Regarding these cases, different neural networks were designed in a manner containing a hidden layer with different number of neurons (1 to 10). To connect the input layer to the hidden layer, the hyperbolic, logarithm and linear sigmoid tangent activation functions were used in various test and error stages of networks. The linear activation function in the fixed form was also used to connect the hidden layer to the output layer. In addition to the mentioned cases two different learning patterns include Levenberg -Marquardt learning algorithm and resilient back propagation (trainrp) were used in different networks and their influence on the networks were evaluated.

$$R^2 = 1 - \frac{\sum_{i=1}^{N}\left(Y_{pi} - Y_{ei}\right)^2}{\sum_{i=1}^{N}\left(Y_{pi} - \overline{Y}\right)^2}$$

$$MSE = \frac{1}{N}\sum_{i=1}^{N}\left(Y_{pi} - Y_{ei}\right)^2$$

Where Y_{pi} is the ratio of predicted features by the network Y_{ei}, is the ratio of features resulted from performing experiments and tests and $\bar{Y}$ is the mean of laboratory characteristics ratios and N is the total number of observations.

Entering the raw data will lead to a decrease in the speed and precision of network. Hence, to obtained acceptable and reliable results, it is necessary to normalize the entered data. In this study, the input and outputs were normalized between 0 and 1 using Eq. 5:

$$V_N = \frac{V_R - V_{min}}{V_{max} - V_{min}}$$

Where V_N is the normalized data. V_R represents the initial raw data. V_{max} and V_{min} are the maximum and minimum amounts of initial data, respectively.

3 RESULTS AND DISCUSSION

3.1 EFFECT OF PEF PRETREATMENTCONDITIONS ON OIL EXTRACTION EFFICIENCY

Fig. 1 represents the effect of PEF pretreatment conditions on the oil extraction efficiency. It was found that the applied pulse had no significant effect on this parameter ($p > 0.05$). However, the oil extraction efficiency was considerably affected by the applied PEF intensity ($p < 0.05$). During PEF pretreatment at higher intensities, the oil extraction efficiency initially increased due to the electrical degradation and permeability of the cells (Schroeder et al., 2009), but it then decreased as a result of further damage to the internal structure of the grains and closure of the oil outlets. The results are in agreement with Guderjan et al. (2005) findings.

3.2 EFFECT OF PEF PRETREATMENT CONDITIONS ON OIL ACIDITY

The PEF pretreatment significantly affected the oil acidity ($p < 0.05$) as shown in Fig. 2. The increase in the acidity could be related to the rising in the lipase activity during

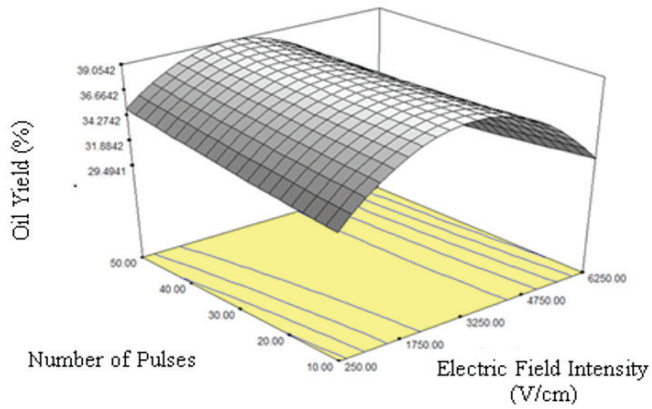

Figure 1: Effect of PEF intensity and pulse number on the oil extraction efficiency

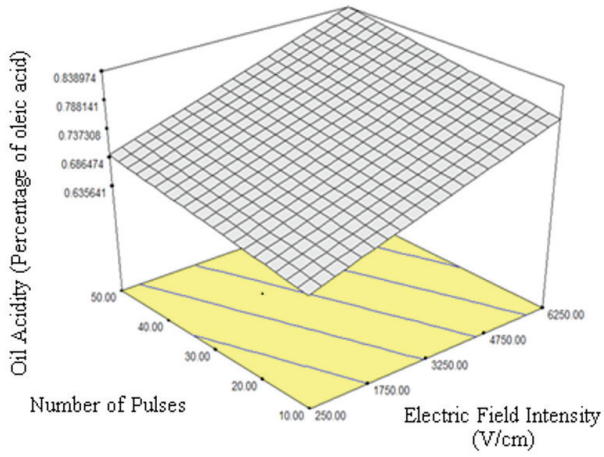

Figure 2: Changes in oil acidity influence by the PEF pretreatment conditions

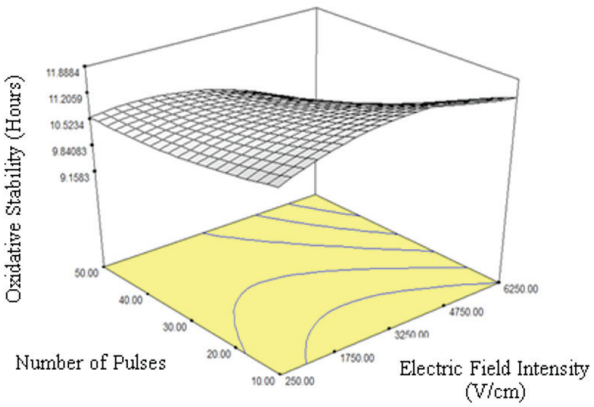

Figure 3: Influence of electric field intensity and pulse number on oil oxidative stability

PEF treatment; as, the activity of this enzyme resulted in production of free fatty acids which are undesirable constituents in edible oils (Guderjan et al., 2007). The same results were reported by Puértolas and de Marañón (2015) during studying the effect of PEF process on the olive oil properties.

3.3 EFFECTOF PEF PRETREATMENT CONDITIONS ON OXIDATIVE STABILITY OF OIL

Fig. 3 represents that the oxidative stability of oil decreased with increasing in the PEF intensity and number of pulses ($p < 0.05$). During PEF pretreatment, increase in free fatty acids content caused decrease in oxidative stability of the oil. However, the oil oxidative stability showed partial increase at the beginning of PEF process. The reason for the decrease in oxidative stability can be attributed to the increase in free fatty acids.

3.4 EFFECT OF PEF PRETREATMENT CONDITIONS ON CONTENT OF TOTAL PHENOLIC COMPOUNDS

The obtained results showed that the content of the total phenolic compounds influenced by the PEF pretreatment conditions ($p < 0.05$). The content of total phenolic compounds increased as the intensity of the electric field and the number of pulses increased (Fig. 4) as Sarkis et al. (2015) reported. This result could be attributed to the effect of PEF electroporation on release phenolic compounds into the oil (Boussetta et al., 2014).

3.5 EFFECT OF PEF PRETREATMENT CONDITIONS ONANTIOXIDANTACTIVITY

The PEF intensity and the pulse number had considerable effect on activity of the inhibition of the DPPH free

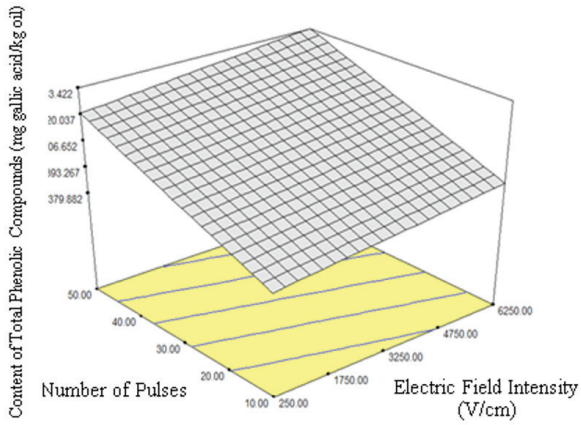

Figure 4: The alteration in the content of total phenolic compounds during PEF pretreatment

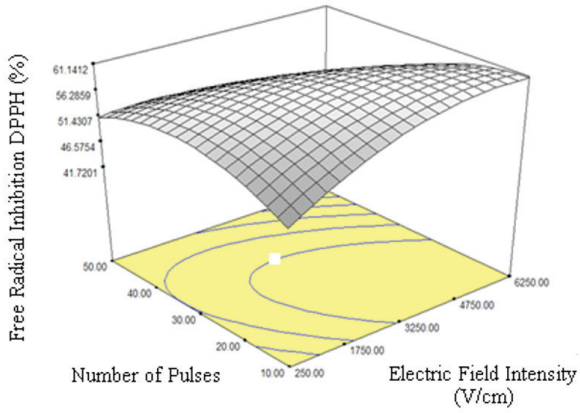

Figure 5: Effect of PEF intensity and pulse number on activity of the inhibition of the DPPH free radical

radical ($p < 0.05$). At low pulse numbers, the antioxidant activity enhanced by rise in the applied PEF intensity, but this trend reversed during pretreatment at high pulse numbers (Fig. 5). Electroporation phenomena causes the release of antioxidants such as tocopherols that result in higher activity of the inhibition of the DPPH free radical. In addition, the reduction in antioxidant activity can be also attributed to the destructive effect of higher PEF intensities on the chemical structures of antioxidants. These results were in agreement with the reports results by Guderjan et al. (2005).

3.6 ARTIFICIAL NEURAL NETWORKS MODELING

Tables 1 to 3 represent comparison the effect of hidden layer neurons number and type of learning pattern on the prediction accuracy of the feed forward back propagation neural networks for using PEF pretreatment in sesame oil extraction with sigmoid hyperbolic tangent conduction function, logarithm and linear functions and learning cycle of 1000, respectively.

The feed forward neural network with sigmoid hyperbolic tangent conduction function, Levenberg – Marquardt training function with topology of 2-6-5 (input layer with 2 neurons, a hidden layer with 6 neurons, and output layer with 5 neurons) was selected as the optimized neural network ($R^2 = 0.998$ and MSE = 0.00013). In addition, the high correlation coefficient of this optimized network against laboratory data for 5 regarded output variables shows high precision and accuracy of this model (Fig.6). However, the neural network has the least accuracy to measure the amount of oil oxidative stability in comparison to the studied parameters.

Fa and Okunola (2015) used methods of response surface and ANN to optimize the oil extraction from sesame seeds. The highest oil extraction efficiency (85.70 %)

Table 1: Comparison of the effect of hidden layer neurons number and type of learning function and sigmoid hyperbolic tangent activation function on accuracy and precision of predicting different characteristics of sesame oil extracted using PEF pretreatment

Neurons number	R^2	MSE	Trainlm	trainrp
			R^2	MSE
2	0.384	0.34809	0.899	0.00187
3	0.984	0.00037	0.799	0.05649
4	0.993	0.00191	0.962	0.00621
5	0.924	0.00360	0.995	0.00477
6	0.998	0.00013	0.996	0.00446
7	0.997	0.00680	0.998	0.00399
8	0.995	0.00891	0.997	0.00388
9	0.997	0.00758	0.992	0.00551
10	0.989	0.00689	0.997	0.00368

Table 2: Comparison of the effect of hidden layer neurons number and type of learning function and sigmoid logarithm activation function on accuracy and precision of predicting different characteristics of sesame oil extracted using pulsed electric field pretreatment

Neurons number	R^2	MSE	Trainlm	trainrp
			R^2	MSE
2	0.630	0.05789	0.799	0.00888
3	0.734	0.01112	0.876	0.00658
4	0.798	0.00999	0.877	0.00655
5	0.810	0.00871	0.988	0.00498
6	0.819	0.00863	0.983	0.00782
7	0.774	0.00998	0.991	0.00098
8	0.899	0.00769	0.992	0.00099
9	0.995	0.00177	0.992	0.00099
10	0.887	0.00122	0.994	0.00089

Table 3: The effect of hidden layer neurons number and type of learning function and linear activation function on accuracy and precision of predicting different characteristics of sesame oil extracted using pulsed electric field pretreatment

Neurons number	R^2	MSE	Trainlm	trainrp
			R^2	MSE
2	0.677	0.054	0.698	0.04567
3	0.783	0.041	0.881	0.04746
4	0.794	0.0399	0.771	0.03671
5	0.765	0.0296	0.698	0.02314
6	0.766	0.0370	0.713	0.03670
7	0.777	0.0429	0.715	0.03133
8	0.775	0.0307	0.751	0.02415
9	0.795	0.023	0.729	0.02648
10	0.791	0.047	0.733	0.02354

was obtained using neural networks; as, the mass of sesame samples, extraction time and solvent amount were 54.71 g, 44.88 min and 165.8 ml, respectively. Therefore, ANN was more effective in predicting the oil extraction process rather than the response surface method. They reported that regarding the complexity and multiplicity of effective factors in oil extraction particularly in industrial scale, ANN method can be introduced as an acceptable model for modeling these processes in industrial scales, too. ANN methods showed proper accuracy and precision in predicting the oxidation stability of canola oil with fatty acids in input layer and induction time in output layer (Dehghani et al., 2012). Przybylski and Zambiazi (2000)showed the high accuracy of applying ANN model in predicting the oxidative stability of vegetable oil when partial oil composition is known. A good predictability was obtained when composition of the major fatty acids and the amounts of tocopherols and tocotrienols were used with accelerated conditions, respectively.

As shown in Fig. 7, regarding the topology of selected ANN model (2-6-5), the weight matrix for the input layer to the hidden layer was a 2 × 6 referring to the connection of 2 input layer neurons to 6 hidden layer neurons (A matrix). The weight matrix for hidden layer to the output layer was a 6 × 5 (connection of 6 hidden layer neurons to 5 output layer neurons) representing as B matrix.

$$A=\begin{pmatrix} -7.73 & -3.14 \\ -5.42 & 5.57 \\ -6.96 & -2.97 \\ 1.67 & -0.17 \\ -0.69 & 0.66 \\ -5.09 & 8.76 \end{pmatrix}$$

$$B=\begin{pmatrix} 5.11 & 5.77 & -2.83 & -0.06 & 2.99 & -5.02 \\ -4.13 & -16.56 & 2.62 & 5.73 & 14.29 & 6.53 \\ -37.05 & 40.27 & 3.05 & 6.15 & 16.35 & -25.67 \\ 2.63 & -8.38 & 1.10 & 1.59 & 0.25 & 5.28 \\ 2.66 & 2.39 & 0.57 & 0.68 & 0.09 & -3.27 \end{pmatrix}$$

Meanwhile, bias matrixes for hidden layer (matrix C) and output layer (matrix D) were matrixes of 1 × 6 and 1 × 5.

It was determined that the efficiency of oil extraction and oxidative stability increased with an increase in the applied PEF intensity. However, as higher pulse numbers were applied, the higher oil extraction efficiency and lower oxidative stability were observed. In addition, the oil acidity and the content of total phenolic compounds increased by rise in the applied PEF intensity and pulse numbers. However, it was found activity of the inhibition of the DPPH free radical was increased at first and decreased then during PEF pretreatment at higher intensity and pulse numbers.

$$C=\begin{pmatrix} 8.08 \\ 5.56 \\ 0.24 \\ 1.28 \\ 0.68 \\ 5.01 \end{pmatrix} \qquad D=\begin{pmatrix} -5.88 \\ 0.24 \\ 7.04 \\ 0.02 \\ -2.01 \end{pmatrix}$$

4 CONCLUSION

In this study, PEF pretreatment was used to improve the effect of screw press method on the extracted sesame seeds oil properties. The obtained results revealed that the oil acidity and the content of the phenolic compounds increased during PEF pretreatment at higher intensity and pulse number. While the oxidative stability of the oil reduced at the more intensive PEF conditions. The oil extraction efficiency initially increased at first but it showed reduction during PEF pretreatment at higher intensities. At low pulse numbers, the antioxidant activity enhanced by rise in the applied PEF intensity, but this trend reversed during pretreatment at high pulse numbers. ANN model was used to predict the effect of different PEF pretreatment conditions on the physicochemical properties of the extracted oil. Considering the R^2 and MSE, the feed forward neural network with sigmoid hyperbolic tangent conduction function, Levenberg – Marquardt training function with topology of 5-6-2 (input layer with 2 neurons, a hidden layer with 5 neurons, and output layer with 5 neurons) was selected as the most effective model. The high accuracy and precision of this model shows its effectiveness in optimizing and controlling the process conditions in order to produce the high quality oil as well as save time and energy.

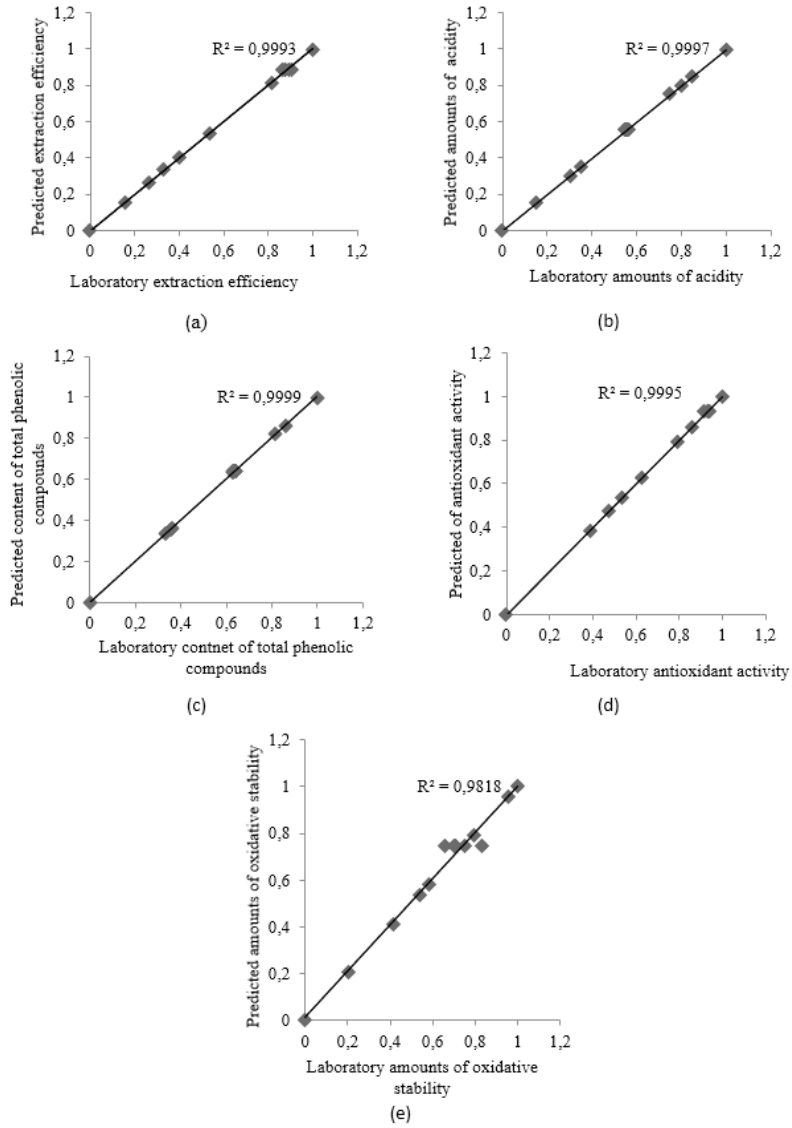

Figure 6: The predicted amounts of oil extraction efficiency (a), acidity (b), content of total phenolic compounds (c), antioxidant activity (d) and oxidative stability (e) using ANN model for optimized topology of (2-6-5) vs. laboratory amounts

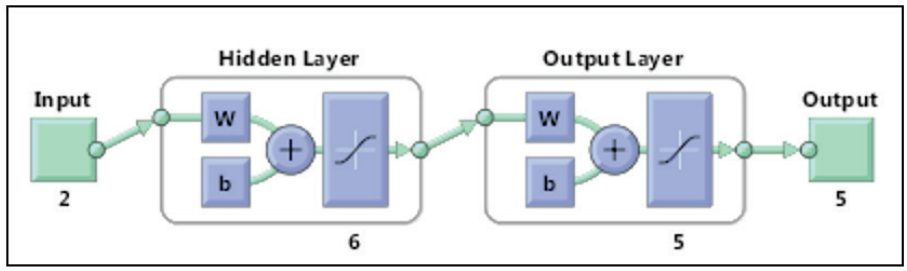

Figure 7: Schematic of different layers of the optimized ANN model with sigmoid hyperbolic tangent function using in predicting the effect of PEF pretreatment on oil properties

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Delovanje dunajskega Terezianuma in Theodorja Kravine na področju ekonomije in agronomije

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Delovanje dunajskega Terezianuma in Theodorja Kravine na področju ekonomije in agronomije

Izvleček: Theodor Kravina von Kronstein (1720-1789) se je rodil v Slovenski Bistrici. Kot jezuit je postal prefekt in kasneje rektor dunajske vojaške, pozneje splošne akademije Theresianum. Pričujoči sestavek obravnava njegovo delo *Entwurf der oekonomischen Kenntnisse*, ki je izšlo leta 1773 in predstavlja sistematičen oris ekonomskih znanj, ki so jih poučevali na Theresianumu. Šlo je predvsem za praktična znanja v smislu poznavanja zemlje, rastlin, mineralov in surovin ter tehnik njihove predelave v končne izdelke. V delu Kravina opisuje tudi "ekonomski vrt", šolsko kmetijsko gospostvo in mineraloške zbirke, ki so pod njegovim vodstvom močno izboljšale kakovost pouka.

Ključne besede: agrarna ekonomika; Slovenija; zgodovinski pregled

Activity of Vienna Terezianum and Theodor Kravina on the field of economics and agronomy

Abstract: Theodor Kravina von Kronstein (1720-1789) was born in Slovenska Bistrica. As jesuit he became prefect and later rector of Vienna Military Academy, later general Academy Theresianum. The contribution deals with his work entitled *Entwurf der oekonomischen Kenntnisse*, published in 1773 representing systematic outline of economic sciences, tought at Theresianumu. It was predominantly about practical expertises in knowing the soil, plants, minerals and raw materials and techniques of their processing into final products. In this published monography Kravina described also the »Economic garden«, school agricultural enterprise and mineral collections, which all improved significantly under his leadership the quality of schooling process.

Key words: agricultural economics; Slovenia; historical outline

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1 UVOD

V slovenskem agronomskem slovstvu doslej še nikjer ni bila omenjena dunajska prvotno vojaška, pozneje splošna akademija Theresianum, kjer so poučevali večinoma sinove zemljiških gospodov za častnike in za službe v državni upravi in diplomaciji ter na področju kmetijstva in siceršnjega gospodarstva. Predstavljeno tudi še ni bilo dokaj programatsko delo direktorja omenjenega zavoda, slovenjebistriškega rojaka, Theodorja Kravine. Lani pa je v Zgodovinskem časopisu 73/2019/3-4/160/346-365, bilo objavljeno delo: Andrej Sušjan - Stanislav Južnič: Theodor Kravina von Kronstein in njegov *Oris ekonomskih znanj*. Da bi to tehtno pomanjkljivost odpravili, smo se z uredništvom *Acta agriculturae Slovenica* dogovorili, da v tej publikaciji objavimo nekoliko skrajšano delo obeh avtorjev Andreja Sušjana in Stanislava Južniča, kar je za tisk pripravil Jože Maček. Kravinovo delo *Entwurf der oekonomischen Kenntnisse* je izšlo na Dunaju 1773. Na to delo je opozoril že prof. Vladimir Murko, ki pa mu kljub intenzivnemu iskanju in poizvedovanju ni uspelo najti nobenega ohranjenega izvoda, tako da je natančnejša vsebina dela ostala neznana. Knjigo je šele pred nekaj leti odkril dr. Dragan Božič in tako se je naposled ponudila možnost, da se podrobneje seznanimo z njeno vsebino.

Namen prispevka je predstaviti in ovrednotiti delo Theodorja Kravine von Kronsteina *Entwurf der oekonomischen Kenntnise.*

V uvodnem delu prispevka je orisana Kravinova življenjska pot, kot izhaja iz razmeroma skopih ohranjenih podatkov. Osrednji del prispevka je namenjen predstavitvi posameznih poglavij omenjenega ekonomskega dela, ki je nastalo v obdobju razsvetljenstva, ko so na področju ekonomske misli merkantilistično ekonomsko doktrino postopno nadomestile liberalne ideje in se je oblikovala klasična ekonomska teorija. V sklepnem delu nas bo zato zanimalo, ali Kravinov *Oris ekonomskih znanj* odraža tedanje trende na področju razvoja ekonomske misli, ali pa gre za specifično delo, ki z liberalno klasično politično ekonomijo ni povezano.

2. THEODOR KRAVINA VON KRONSTEIN

Theodor Kravina (Cravina, Gravina) se je rodil leta 1720 v Slovenski Bistrici (Windisch Feistritz). Njegov oče Johann Georg Cravina je bil srebrar (Salzversilbrerer) in je delal predvsem za tamkajšnje grofe Wildenstein in Attems. Kot proizvajalec posrebrenega okrasja je dobro zaslužil in si kupil posest v Slivnici pri Mariboru. Leta 1714 je dobil tudi plemiški naziv von Cronstein (Cronstain, Kronstein). Theodor se je leta 1730 v Gradcu vpisal na nižje študije, ki pa jih je nato končal v Varaždinu. Preselitev v Varaždin je bila verjetno povezana z njegovo odločitvijo za vstop v tamkajšnji jezuitski red, v katerega je bil sprejet leta 1736, takoj po zaključku študija retorike. S tem je očitno dokončno pokopal upe svojih domačih, da bo nadaljeval družinsko srebrarsko obrt. Vstopil je v noviciat v zavodu sv. Ane na Dunaju in hkrati nadaljeval študij filozofije. V letih 1741/42 ga že najdemo kot predavatelja gramatike v Varaždinu. Od leta 1749 do leta 1773 je bila njegova življenjska pot tesno povezana z ugledno šolo Theresianum (Terezijanišče) na Dunaju, na kateri je predaval matematiko in fiziko ter mehaniko, opravljal visoke vodstvene funkcije ter skrbel za urejanje in vzdrževanje cesarskih muzejskih zbirk in posesti, ki so bile v


Entwurf
der
oekonomischen Kenntnisse,
welche
in dem kaiserlichen königlichen Theresianum
der adelichen Jugend beygebracht
werden.
WIEN,
gedruckt bey Joseph Kurzböck, k. k. illirisch. und orient.
Hofbuchdruckern, und Buchhändlern.
1773.

Slika 1: Naslovna stran Kravinovega Orisa ekonomskih znanj (Vir: https://www.digital.wienbibliotek.at/wbrobv/content/titleinfo/1453214)
Figure 1: Front page of Kravina's »Outline of economic knowledges (Source: https://www.digital.wienbibliotek.at/wbrobv/content/titleinfo/1453214)

sklopu šole. Po razpustitvi jezuitskega reda je leta 1773 dobil proštijo v dokaj velikem mestu Zwettl v Spodnji Avstriji, kjer je leta 1789 tudi umrl.

2.1 KRAVINOVO DELO *ENTWURF DER OEKONOMISCHEN KENNTNISSE*

2.1.1 Okoliščine v času nastanka dela in avtorjeve uvodne opredelitve

Kravinov *Oris ekonomskih znanj* je nastal v neposredni povezavi z njegovim delovanjem na dunajskem Terezianumu, ugledni izobraževalni inštituciji, ki so jo 1746 ustanovili jezuiti s podporo in pod pokroviteljstvom Marije Terezije. Kravina je leta 1749 na Terezianumu postal prefekt višjih študijev, pozneje je bil vicerektor, leta 1770 pa je prevzel funkcijo rektorja, ki jo je opravljal vse do razpustitve jezuitskega reda 1773, ko je ta izobraževalna ustanova za nekaj časa tudi prenehala delovati. Terezianum je bil zamišljen kot "Ritterakademie", torej vojaška šola, pozneje splošna akademija, namenjena predvsem sinovom iz starih plemiških družin, ki bi tu od uglednih profesorjev pridobivali vrhunska znanja, potrebna za delo v armadi, državni upravi in diplomaciji pa tudi v gospodarstvu. S tega vidika je razumljiv (relativno dolg) naslov Kravinovega dela, ki pravi, da gre za oris oz. načrt ekonomskih znanj, ki jih na cesarsko-kraljevem Terezianumu posredujejo plemiški mladini. Knjiga oz. bolje rečeno knjižica ni ekonomski učbenik, kot bi morda kdo pričakoval, temveč načrt oz. sistematičen pregled ekonomskih znanj oz. področij, s katerimi je po mnenju Kravine treba seznaniti mlade plemiče. Hkrati je bil namen dela očitno tudi predstaviti in promovirati naravoslovni muzej in ekonomsko-botanični vrt ter gospostvo, ki so delovali v okviru Terezianuma. Kravina je namreč, kot pravi Murko, v sklopu šole ustanovil mineraloške in zoološke muzejske zbirke, kemijski laboratorij in kmetijsko posestvo, vse s ciljem čim bolj nazornega in učinkovitega poučevanja oz. prenašanja ekonomskih znanj na mlade generacije plemstva. Kot bomo videli v nadaljevanju, je Kravina ekonomska znanja razumel zelo pragmatično in sicer predvsem kot dobro poznavanje naravnih resursov, zemlje, rastlin, mineralov, surovin ter tehnik njihove obdelave in predelave v končne izdelke. Že na uvodnih straneh knjige postavi trditev, da je bistvo ekonomije v znanju, kako iz določene količine zemlje pridobiti čim več. To znanje pa so po njegovem potrebovali ravno pripadniki plemiškega stanu, saj so bili praviloma zemljiški lastniki in je bilo od njihovega umnega gospodarjenja z zemljo in s proizvodi zemlje odvisno tudi izobilje oz. blagostanje države kot celote.

S tega vidika lahko rečemo, da je bilo Kravinovo razumevanje ekonomije do neke mere blizu stališčem francoskih fiziokratov. Fiziokratska šola ekonomske misli, ki je izvor bogastva videla izključno v zemlji (kmetijstvo se je štelo za edino gospodarsko dejavnost, ki ustvarja presežek), je bila namreč v drugi polovici 18. stoletja zelo priljubljena zlasti v Franciji, kjer je tudi nastala. Njene ideje o potrebi po modernizaciji kmetijskih posestev z vpeljavo sodobnih tehnik obdelave in kmetijskih strojev so se širile tudi v druge evropske države, kjer so našle prostor predvsem v okviru t. i. agrarnih oz. kmetijskih družb, ki pa se niso ukvarjale le z izboljšavami poljedelstva in živinoreje, ampak so promovirale tudi merkantilistične politike, vezane na povečanje nacionalnih konkurenčnosti skozi napredek manufakturne proizvodnje, vpeljevanje novih tehnoloških postopkov, širjenje znanj o inovacijah v kmetijski in industrijski proizvodnji, izboljšanje kvalitete proizvodov in večanje produktivnosti v vseh segmentih gospodarstva. V Avstriji je bilo delovanje kmetijskih družb, ki so delovale po posameznih pokrajinah (med drugim tudi na Kranjskem, oz. v vseh tedanjih upravnih slovenskih deželah, opomba J. Maček) vpeto v širši okvir širokopoteznega procesa terezijanskih reform, katerih končni cilj je bilo doseganje maksimalnega izkoristka domačih resursov in splošno povečanje družbene učinkovitosti, tako v gospodarstvu kot tudi v državni upravi. Tudi sama ustanovitev akademije Terezianum je bila posledica tega obsežnega reformnega procesa, in sicer v tistem njegovem delu, ki se je nanašal na modernizacijo izobraževalnega sistema v monarhiji. G. van Swieten, osebni zdravnik in svetovalec Marije Terezije, je bil zadolžen za reformo univerzitetnega študija. On je bil tisti, ki je po zgledu rodnega Leidna predlagal, da se v visokošolski sistem vpelje nizozemski model "vrta" kot učinkovitega izobraževalnega sredstva. Na podlagi tega predloga je posledično nastal tudi t. i. ekonomski vrt Terezianum, ki ga je ustanovil prav Kravina. Izmed vseh treh "učnih vrtov", ki so sredi 18. stoletja nastali na Dunaju, je bil, kot ugotavlja M. Klemun, ravno ekonomski vrt najbolj praktično orientiran, usmerjen v sistematičen prikaz uspešne vzgoje rastlin in njihove uporabe ter njihovega družbenega pomena.

Prevladujoča družbenopolitična klima v habsburški monarhiji je bila torej v času, ko je Kravina zasnoval in napisal svoje ekonomsko delo, pod vplivom terezijanskih reform, usmerjenih v splošni družbeni napredek na osnovi povečevanja znanja, uvajanja tehničnih novosti in posledičnega večanja gospodarske učinkovitosti. Zato ne preseneča, da je v knjigi ves čas prisotna ideja o največjem možnem izkoristku domače zemlje (in domačih virov nasploh) ter o vzdrževanju in krepitvi gospodarske moči kot prioritetah ekonomskega izobraževanja. Kravina sprva razdeli ekonomijo na dve področji. Prvo področje se ukvarja s proučevanjem obdelovanja zemelj-

skih površin (die Bearbeitung der Oberfläche der Erde), drugo pa z izkoriščanjem zemeljske notranjosti oz. s tako imenovanimi podzemnimi proizvodi (die unterirdischen Erzeugnissse). Nato pa opredeli še tretje področje ekonomije, ki ga imenuje javna splošna oz. skupna ekonomija (die oeffentliche und (all)gemeine Oekonomie) in se nanaša na menjavo proizvodov zemlje (pridobljenih tako iz površja kot tudi iz notranjosti zemlje) z drugimi ljudmi in deželami. Kravina pravi, da se tretje področje ekonomije pri tistih, ki nanj gledajo bolj s političnega vidika, imenuje tudi komercij oz. trgovina (das Commerz oder die Handlung). Pri tem je zanimivo, da se od tovrstnega "političnega" razumevanja ekonomije takoj distancira in poudari, da se njegov načrt poučevanja ekonomije ne nanaša na napotke za menjavo proizvodov, temveč predvsem na njihove značilnosti, fizikalne sestavine, načine njihove uporabe, predelave itd. Tej trojni klasifikaciji sledi tudi struktura Kravinove knjige, ki ima štiri poglavja. Prvo poglavje obravnava ekonomijo zemeljskega površja, drugo poglavje ekonomijo, vezano na notranjost zemlje in zadnje poglavje splošno ekonomijo, pod katero Kravina razume predvsem seznanjanje s fizikalnimi lastnostmi različnih proizvodov, pridobljenih bodisi na površju, bodisi iz notranjosti zemlje in namenjenih menjavi. Vmes je vključeno še obsežno tretje poglavje, ki govori vse tisto, čemur bi danes rekli človeški in materialni viri ekonomije (delovna sila), delovna živina, zemlja, orodja in stroji itd.. Ravno v tem poglavju Kravina razvije še nekatere druge implikacije vzdrževanja in povečevanja tako imenovanih moči (Kraefte) v ožjem in širšem pomenu besede ter opozori na pomen povezanosti med posameznimi panogami gospodarstva. Poglejmo si v nadaljevanju nekoliko pobliže Kravinove poglede po posameznih poglavjih dela.

2.2 EKONOMIJA PROIZVODOV ZEMELJSKEGA POVRŠJA

V prvem poglavju, ki nosi naslov "Von der Oekonomie der Oberfläche der Erde", Kravina uvodoma pravi, da mora pouk na tem področju mladega plemiča naučiti, da bo v osnovi razumel gospodarstvo svojega posestva oz. da bo sposoben posestvo voditi in izboljševati, nadzorovati svoje uradnike in vse druge podrejene, pa tudi pravilno presojati vsebino ekonomskih knjig. V nadaljevanju se izkaže, da Kravina v okviru tega dela ekonomije predvsem poudarja pomen dobrega poznavanja proizvodov, ki jih dajejo različni deli posestev (njive, vrtovi, pašniki, gozdovi, hribi, vode), in tudi uporabe ter predelave teh proizvodov. Mlademu človeku je po Kravinovem mnenju treba posredovati obstoječe znanje o teh proizvodih, ki ga bo s svojim razmišljanjem lahko razvijal dalje. Ob tem Kravina nakaže tudi pedagoški pomen oblikovanja razstavnih oz. muzejskih zbirk (s katerimi se je, kot že omenjeno, v Terezianumu sam veliko ukvarjal), saj pravi, da je zelo pomembno, da se mlademu človeku predmeti poučevanja predstavijo v njihovi naravni obliki.

Temu je namenjen tudi t. i. "ekonomski vrt", ki je sistematično razdeljen na več prostorov (Plätze), ki jih Kravina v nadaljevanju poglavja sistematično predstavi. Imenuje jih tudi "oddelki predmetov ekonomije" (die Abteilungen der Gegenstände dieser Oekonomie) in jih učitelj med svojim predavanjem razkaže plemiškim učencem. Na prvem prostoru se učenci seznanijo z različnimi vrstami rodovitnih in nerodovitnih tal. Za nerodovitne vrste tal, kamor se uvrščajo pesek, mivka, lapor, kreda, grušč in skala, Kravina pravi, da z vidika človeške prehrane sicer ne dajejo sadov, so pa pomembne za prehrano določenih rastlin, ki v takem okolju dobro uspevajo. Na drugem prostoru so na eni strani plodovi (Ackerfrüchte), ki jih dajejo s plugom obdelane njive, kot npr. različne vrste pšenice, koruze, ovsa, itd. (alles was einen mit dem Pfluge bearbeiteten Boden fordert), in na drugi strani vrtne rastline, ki jih ljudje bodisi delno (samo njihove liste in/ali korenine) bodisi v celoti uporabljajo za prehrano. Tretji prostor je predviden za pašnike in travnike kot tudi za tiste rastline, ki sicer uspevajo le v vlažnih in močvirnih krajih ali celo povsem pod vodo. Četrti prostor je namenjen za vse rastline, ki se uporabljajo za barvanje. Na petem prostoru rastejo raznovrstna sadna drevesa in na šestem prostoru divja in domača drevesa, katerih les je namenjen kurjavi ali ga uporabljajo različni obrtniki, kot npr. mizarji, kolarji, tesarji itd. Kot pravi Kravina, pouk poteka tako, da učitelj pojasni tako splošne značilnosti rastlin kot tudi posebnosti posameznih vrst rastlin. Razloži, kako posamezni deli rastlin (korenine, skorja, listi) prispevajo k rasti in dajanju plodov, kako tečejo hranilni sokovi po rastlinah, kako rastline dihajo, počivajo, rastejo, kakšne so bolezni rastlin in tudi kako rastline odmirajo. Predstavi optimalne pogoje v smislu temperature in vlage, pri katerih določene rastline uspevajo, ter tudi načine njihovega presajanja.

V nadaljevanju poglavja se Kravina še enkrat vrne k opisanim prostorom in kratko oriše, o čem poteka pouk na vsakem posameznem prostoru. V okviru prvega prostora se mladina seznani s povečevanjem rodovitnosti tal z mešanjem različnih vrst zemlje, uporabo gnoja in pluga, pa tudi z izkoriščanjem nerodovitnih tal, kjer uspevajo nekatere rastline, ki jih je možno uporabiti v živinoreji ali barvarstvu. Drugi prostor obsega rastline, ki človeka "hranijo in oblačijo", zato mora pouk tukaj pojasniti izbiro pravilnega položaja njiv in polj, obdelavo zemlje s pomočjo pluga, brane in drugih orodij, zasnovo kuhinjskega in okrasnega vrta s pravilno izbiro rastlin glede na značilnosti zemlje, na pridobivanje in pravilno shranje-

vanje semen, glavne značilnosti posameznih rastlin ter kateri njihovi deli so za človeka lahko koristni ali škodljivi. Na tretjem prostoru učitelj obravnava glavne značilnosti polj, pašnikov in voda, različne vrste zelišč, trav in detelj, ki se uporabljajo za rejo živine in perutnine (pri čemer so nekatere trave za živali zdravilne, druge pa lahko strupene), razlike med prehrano konj, rogate živine, ovac in perutnine, lego in možne izboljšave pašnikov ter tudi čebelarstvo. V okviru tega prostora lahko učitelj po Kravinovem mnenju obravnavo razširi še na t.i. gospodarsko živino (das sogenannte Wirtschaftsvieh), njene značilnosti, uporabo in predelavo njenih izločkov, volne, rogov, kopit, kože, pa tudi samo vzrejo te živine, njeno vrednost in ceno. Kravina pravi, da je predstavljanje značilnosti gospodarske živine mladini možno tudi, če živina ni prisotna, medtem ko so večje težave v tem pogledu v zvezi s perutnino. Na koncu se učenci seznanijo še z obsežno zbirko različnih rastlinskih sovražnikov, kot so hrošči, gosenice, kobilice in mravlje, ter z doslej znanimi sredstvi za njihovo uničevanje. Na četrtem prostoru je pouk omejen na barvilne rastline, ki uspevajo v naših krajih, vendar je teh malo oz. so, kot pravi Kravina, še nepoznane in morda čakajo, da jih bodo odkrili ravno sedanji plemiški učenci. Gre za poznavanje priprave in uporabe teh rastlin, pa tudi za primerjavo koristi in škod, ki jih prinašajo drugi tovrstni proizvodi, tudi tisti iz tujine. Na petem prostoru, ki obsega divja in domača drevesa, se obravnava vrste in značilnosti različnih dreves, čas njihove rasti, najboljšo lego in najprimernejšo zemljo za posamezna drevesa ter primeren čas za sečnjo. Govora pa je tudi o uporabi lesa za zgradbe in orodja ter o lesni trgovini in o cenah lesa. Na šestem prostoru se plemiški učenci seznanijo z značilnostmi sadnega drevja, z različnimi vrstami jabolk, hrušk, koščičastega sadja, s sredstvi zoper številne bolezni sadnega drevja, pa tudi z izdelavo sadnih sokov.

2.3 EKONOMIJA MINERALOV, KAMNIN IN DRUGIH PODZEMNIH PROIZVODOV

Drugo poglavje z naslovom "Die Oekonomie der Erzeugnisse in den unterirdischen Lagen der Erde" kratko oriše vse, kar spada v ekonomijo podzemnih proizvodov, pri čemer Kravina uvodoma poudari, da uporaba tega znanja omogoča sprejemanje sklepov, ki niso relevantni le za plemiškega posestnika, ampak še bolj za celotno državo. Za ta namen vzpostavljena zbirka zato vsebuje različne vrste zemlje, peska in kamnin, ki jih Kravina v nadaljevanju poglavja sistematično našteva. Glede zemlje mora biti zbirka sestavljena iz vzorcev črne in rdeče zemlje kot tudi močvirne zemlje in šote. Poleg tega mora vsebovati tudi primere mešanic zemlje ter vzorce krede in laporja. Pri vsakem predmetu zbirke je, kot pravi Kravina, treba pokazati njegove prednosti oz. možne načine uporabe, kot npr. v proizvodnji posodja in porcelana, v barvarstvu in pleskarstvu, gospodinjstvu, kmetijstvu, zgradbah. Enako velja za različne vrste peska (zrnati pesek, mivka itd), kjer so ravno tako možne različne uporabe v kmetijstvu in gospodinjstvu, tovarnah, za glajenje itd. Veliko prostora Kravina nameni različnim vrstam kamnin (apnenec, marmor itd) in z njimi povezanih kristalov. Pravi, da je treba predstaviti lastnosti kamnin, načine njihove obdelave oz. pridobivanja leska, seznaniti pa se je treba tudi z delom v kamnolomih. Prav tako je treba učiti o načinih uporabe kamnin v zgradbah, tovarnah in topilnicah, kako se kamnine žgejo oz. nasploh naredijo primerne za uporabo. Pokazati je treba zlasti koristnost marmorja in alabastra z vidika njune uporabe pri krašenju cerkva, mestnih trgov, palač, v kiparstvu itd. Kravina poudari, da je v zbirki že zelo veliko tujih in še več domačih vrst marmornih kamnin z obrazložitvami, ki se nanašajo tako na njihovo vrednost kot tudi na načine njihove obdelave. Sem sodijo različne vrsta skrilavcev, peščenjakov, brusnih kamnov, prodnikov, opalov, oniksov itd. Kravina pravi, da je uporaba vseh teh kamnin pomembna za gospodarstvo, mnoge od njih so v različnih ozirih za človeka nepogrešljive tudi v vsakodnevni uporabi. Nekatere pa služijo človeku samo za okras in ga, kot je kritičen Kravina, s svojo bolj namišljeno kot dejansko vrednostjo vse prepogosto zavedejo v nezmerno trošenje.

Ob koncu poglavja Kravina opozori, da bi podrobnejši opis vseh zvrsti tega dela narave presegel omejitve kratkega orisa. Zato zaključi le z navedbo še preostalih glavnih področij te, v veliki meri že ustvarjene zbirke, pri čemer spomni, da pri obravnavi posameznih področij za popoln pouk mladine ne zadošča le predstavitev njihovih značilnosti, ampak je treba omeniti tudi okoliščine njihovega nastanka, uporabe, obdelave, cene ter vse z njimi povezane prednosti. Področja, ki jih tu navaja, se nanašajo na različne vrste soli, žvepla polkovin (Halbmetale), kovin, okamnin, odtisov, koral, školjčnih usedlin, kovinskih zmesi ter pripravkov iz zemlje, kamnin, soli itd.

2.4 MATERIALNA IN NEMATERIALNA SREDSTVA EKONOMIJE

Najobsežnejše, najbolj raznovrstno in z vidika ekonomije tudi najbolj zanimivo je tretje poglavje z naslovom »Die oekonomischen Hilfsmittel«, ki pravzaprav obravnava materialna in nematerialna sredstva oz. fizične in človeške resurse ekonomije. V njem Kravina vpelje koncept (ekonomskih) moči (die Kräfte), opredeljenih v ožjem in širšem smislu, nato pa razpravlja o načinih vzdrževanja in povečevanja teh moči pa tudi o »morali«,

ki je potrebna za učinkovito delo oz. upravljanje posestva.

Kravina najprej poudari pomen človekovega truda (die Bemühungen des Menschen), s katerim se proizvodi zemlje (lahko bi rekli ponudba) uskladijo z željami ljudi (torej z povpraševanjem). Za večjo učinkovitost tega truda pa so pomembni tudi ekonomski »pripomočki«, ki na podlagi (načel) fizike in mehanike povečujejo tako vložene moči (die Kräfte selbst) kot tudi učinkovitost njihove uporabe (die Anwendung der Kräfte), pri čemer je za slednje potrebna tudi zdrava »morala« (gesunde Moral) oz. zavedanje, da se za koristno uporabo moči zahteva njihovo poznavanje, znanje o možnostih uporabe in seveda tudi pripravljenost za uporabo. Kravina pravi, da je pomoč pri obdelavi zemlje človek vedno našel v vlečni in tovorni živini. Vendar pa sama moč živine pogosto ne zadošča, zato človek uporablja tudi orodja in stroje, s katerimi poveča tako svojo moč kot tudi moč živine. Iz tega Kravina izpelje sklep, da so poleg znanja o lastni moči, o moči živine in njeni vzdržljivosti še kako pomembna tudi znanja o mehaniki (die mechanischen Kenntnisse). Kljub vzdržljivosti živine namreč uporaba njene moči v vseh primerih ni možna, zlasti ko gre za potrebo po nepretrganem delovanju nekega stroja, po veliki začetni sili ali za nemožnost živino ali človeka pripeljati na določeno lokacijo. V takih okoliščinah je človek, na podlagi izkušenj in razmisleka, začel uporabljati sredstva, kot so zračni pritisk ter gibanje in moč vode, ki so, kot pravi Kravina, povečala ali pa v celoti nadomestila živo silo. Iz tega razloga so postala zelo pomembna področja aerometrije (Aerometrie), hidravlike (Hydraulik) in hidrostatike (Hydrostatik).

Po kratki digresiji, v kateri opozori na potencialne nevarnosti, ki jih prinaša voda, Kravina v nadaljevanju izpostavi različne postopke, kot sta npr. metodi izparevanja (Ausdünstung) in taljenja (Schmelzkunst), ki so pomembni tako za predelavo proizvodov zemeljskega površja kot podzemnih proizvodov in ki lastnikom omogočajo realizirati vse prednosti njihovih posestev. Sem se uvrščajo tudi umetnost varjenja piva (Bräukunst), pridobivanj sokov iz rastlin in vinarstvo, pa tudi predelava žita, hmelja in drevesneskorje, pri čemer je po mnenju Kravine potrebno tudi določeno znanje kemije in mineralogije. Ti postopki koristijo celotnemu kmetijstvu, njihova uporaba pa, kot se slikovito izrazi Kravina, ne le povečuje človeške moči, ampa v določenih primerih lahko tudi povsem nadomesti človeško nemoč.

Kot smo že poudarili, Kravina v svojem delu v ospredje postavlja plemiškega zemljiškega lastnika, ki mu gre večina donosa zemlje, hkrati pa njegova uspešnost povečuje blagostanje celotne države. Toda istočasno opozarja, da je moč plemiškega lastnika predvsem v moči njegovih podložnikov, ki jo je lastnik dolžan povečevati. K temu ga zavezuje trojna obveznost, in sicer največja možna skrb za podrejene, skrb za lastno korist oz. ohranjanje svojega položaja in skrb za povečevanje blagostanja države. To trojno obveznost lastnika mora učitelj ekonomije po Kravinovem mnenju svojim učencem ob vseh priložnostih vcepljati v glavo (eintragen), če želi da bo njegovo poučevanje res uspešno. Ko mladenič prepozna resničnost teh obveznosti, ga bo vnema po učinkovitem delu spodbudila, da bo našel načine za njihovo izpolnitev.

Kravina učinkovito ekonomsko delovanje, kot smo že omenili, pogojuje tudi z ustrezno moralo (zur Oekonomie erfo(r)derliche Moral), kar po njegovem mnenju zajema tri stvari, in sicer, (1) da mora lastnik poznati vse dele ekonomije in njim pripadajoče pripomočke ter imeti resnično željo, da jih dejavno uporabi, (2) zavedanje, da lastnikova znanja lahko dokončno realizirajo le njegovi podrejeni in (3) da mu pri izpeljavi načrtov pomagata dve skupini ljudi, to so gospodarski uradniki (die Wirtschaftsbeamten) in duhovniki (die Seelsorger). Vlogo slednjih Kravina vidi predvsem v motivaciji podložnikov. Pravi namreč, da ni dovolj, da podložnik samo zna uporabljati svoje moči, ampak mora biti tudi njegova volja usmerjena k temu, da svoje moči uporablja z vnemo in veseljem. Za tovrstno usmerjanje volje pa je po njegovem mnenju najpomembnejše ravno prizadevno delo duhovnika. Glede uradnikov Kravina priporoča, da naj imajo poleg drugih talentov tudi nekaj naravoslovnega znanja, ki ga lahko nato z branjem knjig s tega področja še povečujejo in so tako bolj kos svojemu delu.

Iz tako definiranih moralnih obveznosti Kravina izpelje tezo, da mora biti glavna skrb lastnika namenjena ohranjanju moči, in sicer tako v dobesednem pomenu telesne moči njega in njegovih podložnikov kot tudi v posrednem smislu moči njegovega premoženja oz. posesti. Za telesne moči meni, da se vzdržujejo z zdravo prehrano, telesno higieno in urejenim bivanjskim okoljem oz. nasploh z urejenim (čeprav delovnim) življenjem. Lastnik mora biti pozoren tudi na morebitne bolezni podložnikov in jim priskrbeti potrebna zdravila, kar bo v končni fazi koristno tudi zanj. Glede vzdrževanja moči in izboljševanja posestva pa Kravina ponovno izpostavi vlogo široko zastavljenega sistema poučevanja, ki mora pokrivati vse - od uporabe proizvodov zemlje in vzrokov neuspevanja do uporabe živine, izkoriščanja naravnih prednosti vode in zraka, skrbi za zdravje in celo primerne vzgoje otrok.

Sledi zadnja tretjina tretjega poglavja, ki je vsebinsko in tudi stilsko nekoliko specifična. Prinaša namreč strnjeno in mestoma tudi nekoliko nejasno razpravo, ki bi jo na kratko lahko označili kot avtorjevo zahtevo po strukturni usklajenosti in uravnoteženosti najprej posameznega posestva in nato gospodarstva kot celote.

Za razliko od preostalih delov knjige, ki večinoma samo sistematično naštevajo glavna področja znanj, ki jih je treba posredovati plemiškim mladeničem kot bodočim gospodarjem posestev, se tukaj pojavi tudi razmislek o določenih makroekonomskih implikacijah, povezanih z gospodarskim napredkom. Kravina pravi, da se uspešen lastnik posestva lahko loti tudi manufakture oz. tovarniške dejavnosti (kar se je marsikje po Evropi in tudi v Avstriji tedaj dejansko že dogajalo), vendar je pomembno, da takšen razvoj negativno ne vpliva na poljedelstvo. Poudarja namreč, da je gospodarstvo treba gledati celovito in vselej tehtati koristi in škode, tako poljedelstva na eni strani kot tovarn in manufaktur na drugi. Vsi deli ekonomije zahtevajo enako pazljivost (eine gleiche Vorsicht) in posamezne veje gospodarstva morajo druga drugo podpirati. V nasprotnem primeru se skupna prednost (to lahko razlagamo kot konkurenčno prednost oz. moč strukturno usklajenega gospodarstva) vse prelahko izgubi. Kadar lastnik pretirano težo daje eni dejavnosti, lahko to povzroči, kot pravi Kravina, da bosta trud in delo v drugih dejavnostih izgubljena. Na agregatni ravni takšen razvoj lahko pripelje do pomanjkanja živil in delovne sile, kar bi povratno negativno vplivalo na kmetijstvo in živinorejo. Kravina zato poudarja potrebo po ravnotežju (Gleichgewicht) vseh delov ekonomije skupaj njihovimi resursi, ki pa se lahko doseže in vzdržuje le tako, da se vsakemu delu gospodarstva posveti primerna skrb. Ob tem je treba izpostaviti, da Kravina tukaj zelo smiselno uporabi koncept ravnotežja, in sicer v smislu strukturne uravnoteženosti ekonomije. Ta koncept je seveda dobro poznal iz svojih predavanj o statiki. V ekonomski misli 18. stoletja pojem ravnotežja še ni bil prevladujoč teoretični koncept, so ga pa že uporabljali Italijan Ferdinando Galiani (1728-1787) in pa zlasti francoski fiziokrati, na primer Francois Quesnay (1694-1774) in Anne-Robert-Jaques Turgot (1727-1781).

Kravina po drugi strani tudi opozarja, da ni dobro, če se ekonomski resursi (nekega posestva) preveč razpršijo v različne uporabe, saj se s tem izgubi iz vida prvotno jasen cilj koristnosti. V nasprotju s prejšnjimi trditvami Kravina tu očitno nakazuje pomen specializacije in hkrati pravi, da se vrednost pod rokami delavcev povečuje in da končna vrednost proizvodov posledično presega vrednost surovin (npr. lan¸ ki ga pridelamo na polju, ali kovine, pridobljene iz rud, izkopanih iz zemlje). Vendar pa po Kravinovem mnenju le redki, tako med lastniki kot na ravni države, dejansko dobro poznajo te postopke in procese plemenitenja oz. povečevanja vrednosti (diesen immer fruchtenden Abwechslungen und Verwandlungen), zaradi česar so lastniki pogosto prehitro zadovoljni s svojimi ekonomskimi dosežki.

V nadaljevanju v tem kontekstu zasledimo tudi določeno kritiko fiziokratizma, ko Kravina oceni, da pretirano poveličevanje poljedelstva kot najpomembnejše panoge pravzaprav pomeni apriorno zanemarjanje drugih delov ekonomije. Hkrati pa bo poljedelstvo po njegovem mnenju ob takem razmišljanju postalo nerodovitno. Kravina tu očitno že opozarja na problem padajočih donosov v kmetijstvu, saj v nadaljevanju eksplicitno zapiše, da moči zemlje kljub vsem pripomočkom ostajajo omejene in jih ni mogoče v nedogled povečevati. Vedno več dela se kaže v vse manjšem presežku proizvodov. Glede pustih in strmih krajev, kjer si s poljedelsko proizvodnjo »ni mogoče povrniti niti vrednosti pluga«. Kravina predlaga, da jih poskusijo izkoristiti za rudarstvo (ki ga je po njegovem mnenju tudi možno imeti za neko vrsto poljedelstva) in morda bodo bogato poplačali vloženi trud. Poleg tega bi se država tako otresla odvisnosti od uvoza nujno potrebnih rudarskih proizvodov, za katere tujci praviloma zahtevajo zelo visoko ceno. Prebivalstvo po mnenju Kravine narašča glede na to, koliko ima na voljo dobrin tam, kjer prebiva. Presežek (kmetijstva) naj bi se razdelil sam po sebi do stanja blaginje (bis zu dem ansehnlichen Stand), ki državi omogoča mir in varnost.

Proti koncu poglavja Kravina še enkrat opozori na vlogo duhovnika, ki vpliva na voljo ljudi in poskrbi, da se znanje udejanja (macht die ausgebreiteten Kenntnisse endlich tätig). Poleg pouka o veri naj v pridigah, pa tudi v prijateljskih pogovorih in zaupnih srečanjih z ljudmi, govori o vzrokih, zaradi katerih so ljudje organizirani v družbe, kako potrebne so države in kako potrebna je raznolikost stanov in njihova hierarhija. Od tega, kako je organizirana država, je odvisna varnost ljudi, pa tudi njihovo zdravje in celotna sreča. Kravina pravi, da so božja navodila (göttliche Anordnungen), da naj se človek podredi svojemu predpostavljenemu (torej gospodarju) ter živi v skladu s svojim poklicem in ne varčuje svojih moči. Stanovi se morajo med sabo podpirati. Poljedelec oz. kmet hrani sebe, meščane in vojake, vojak pa brani meščane in kmete. Duhovnik mora torej farane vedno opominjati na to, da kar posameznik daje državi, pravzaprav porabi sam.

To pa je tudi moralni nauk, ki naj ga po mnenju Kravine učitelj ekonomije posreduje svojim plemiškim učencem - bodočim lastnikom posestev. Kajti največ je odvisno od lastnikov. Ti morajo spodbujati vnemo svojih podložnikov in svoje znanje uporabljati v prid domovine. Ekonomski pogledi lastnika, ki zna motivirati svoje podrejene, bodo delovali tudi v njegovi odsotnosti. Posestvo pa bo prispevalo k delovanju vseh drugih delov ekonomije, ki so medsebojno odvisni.

2.5 SPLOŠNA EKONOMIJA MENJAVE

Zadnje poglavje je razmeroma kratko, v njem Kra-

vina samo na hitro oriše zbirko, ki naj bi poučno prikazovala različne vrste trgovskega blaga. Poglavje ima naslov »Von der gemeinen oeffentlichen Oekonomie«, pod čemer Kravina pravzaprav razume menjavo dobrin. Predmet tega dela ekonomije so, kot pravi, vse snovi, surove ali predelane, proizvodi in umetnine, ki izhajajo bodisi iz zemeljskega površja bodisi iz podzemnih leg in se kupujejo ali menjujejo med pokrajinami in različnimi narodi ter se nato v skladiščih in na stojnicah ponudijo v nakup. Toda kot je nakazal že v uvodu, Kravine ne zanimajo načela delovanja same trgovine, mehanizem trga, logika ponudbe in povpraševanja, gibanje cen ipd., ampak se osredotoča na pomen dobrega poznavanja blaga, ki je predmet menjave. Sprašuje se, kako naj bo plemiški mladenič sposoben pravilno ocenjevati različne vrste trgovskega blaga, njegove materiale, poreklo, načine njegove predelave, če jih ni nikoli opazoval ali sploh videl; kako naj ima o njih jasno predstavo; kako naj pozna njihove različne uporabe, kraje njihovega izvora, najboljše načine predelave, njihovo pomembnost v tujih deželah, možnost njihovega nadomeščanja z domačimi proizvodi. Zato mora tovrstna znanja pridobiti, in sicer ne le znanja o značilnostih materialov in proizvodov, ampak tudi o samih tovarnah in manufakturah, zgodovini narave in različnih mehaničnih uporabah, da bo tako ne le znal koristno izbirati med dobrimi in slabimi materiali in med različnimi neenakimi predelavami, ampak bo lahko neki material in njegovo predelavo tudi izboljšal. Tu se po Kravinovem mnenju kaže potreba po novi zbirki, ki je popolnoma drugačna od prejšnjih zbirk. V njej morajo biti vzorci različnih materialov (Stoffe) in umetnin (Kunstwerke), pa tudi primeri najbolj pomembnih strojev in orodij (Maschinen und Werkzeuge). Zaradi velike raznovrstnosti trgovskega blaga je to zelo obsežna zbirka, saj se npr. samo iz kože, volne ali dlake živali izdelujejo številne vrste usnja in sukna, ki se razlikujejo po načinu obdelave, trpežnosti in barvi, kar vse so pomembne okoliščine za trgovino in menjavo. Podobno velja za svilo in les ter za tako rekoč neomejene možnosti predelave stekla in kovin. Kravina predlaga razdelitev materialov in proizvodov v zbirki na tri področja, in sicer (1) za prebivanje, oblačila in okras, (2) za prehrano in razvedrilo, (3) za namene zdravljenja. To so kot pravi Kravina, trije koristni deli ekonomije, za katere je zelo pomembno, da jih plemiški mladenič pozna.

V zaključku poglavja Kravina še enkrat poudari pomen posredovanja ekonomskih znanj mladim. Pravi, da so vsi, ki se sprašujejo o pomenu zbirk ter o tem kaj lahko lastniki in država pričakujejo od ekonomske znanosti, mirni, saj se s tovrstnim izobraževanjem mlademu plemiču daje priložnost, da se nauči, kako iz svojega posestva lahko potegne vse možne prednosti in tako v največji meri prispeva k izobilju v državi, pa tudi kako s svojim trgovskim znanjem in delovanjem lahko podpira trgovino celotne države.

3 ZAKLJUČEK

Kravinov *Oris ekonomskih znanj* je specifično delo, ki je nastalo v kontekstu njegovega angažiranega delovanja na dunajskem Terezianumu. Ne gre za ekonomsko teoretično delo, kakršna so v tedanjem času publicirali Kravinovi sodobniki, angleški in francoski politični ekonomisti, kot npr. Adam Smith (1723-1790), Etienne de Condillac (1714-1780) ali že omenjeni Anne-Robert-Jaques Turgot, ter nemški oz. avstrijski merkantilisti, kot npr. že omenjena Joseph von Sonnenfels in Johann H. von Justi (oba sta bila nekaj časa Kravinova sodelavca na Terezianumu). Zlasti angleška klasična politična ekonomija, ki je nastala v tem obdobju, se je namreč že zelo poglobljeno ukvarjala s teoretičnimi vprašanji, z vprašanjem večanja bogastva narodov oz. z gospodarsko rastjo, s preučevanjem problematike vrednosti in razdelitve ter z analizo mehanizma trga in cen, hkrati pa tudi promovirala idejo liberalizma in svobodne konkurence. Francoski ekonomisti tega obdobja so sicer večinoma prisegali na fiziokratizem, ki je favoriziral izključno kmetijsko proizvodnjo, toda bili so tudi zagovorniki svobodnega trga ('laissez faire') ter začetniki ekonomskega modeliranja *(Tableau Economique)*. Nemški in avstrijski merkantilizem pa se je po drugi strani veliko ukvarjal z ekonomsko politiko države in učinkovitostjo državne administracije. V Kravinovem delu najdemo bolj malo od naštetega. Izmed teoretičnih ekonomskih konceptov se v delu, kot smo pokazali, pojavita le koncept ravnotežja oz. strukturne usklajenosti ekonomije in zakon padajočega donosa, razen tega pa Kravina uporablja še koncept blagostanja oz. izobilja v državi, kar je primerljivo s Smithovim konceptom bogastva naroda, razmišlja pa tudi o procesih povečevanja vrednosti, kar je pravzaprav skladno s kasnejšim konceptom dodane vrednosti. Tudi skrb za vzdrževanje in povečevanje moči lastnikov, podložnikov in naslednjih generacij (v smislu ohranjanja zdravja ter povečevanja znanj in spretnosti) o čemer Kravina na široko piše v tretjem poglavju, je blizu modernim razmišljanjem o pomenu človeškega kapitala. Drugače pa Kravina ne načenja nobenih ekonomsko teoretičnih vprašanj (vezanih na delovanje trga, definicijo vrednosti, oblikovanje cene, gibanje razdelitvenih deležev ipd.) ne promovira neke širše ekonomske filozofije (liberalizma ali intervencionizma) niti se ne dotika vprašanj ekonomske politike. Res pa je, da je glede angažiranja države očitno velik pomen pripisoval izobraževalni politiki, torej povečevanju obstoječega znanja, kreiranju novih znanj

in prenašanju znanj (vključno z ekonomskim) na mlade generacije.

Oris ekonomskih znanj je pravzaprav nekakšen učni načrt, ki sistematično predstavlja ekonomska področja oz. ekonomska znanja, ki jih je Kravina videl kot pomembna za učinkovito vodenje zemljiških posestev in ki jih je šola Terezianum zato posredovala mladim plemičem kot bodočim zemljiškim lastnikom. Skozi opis posameznih poglavij knjige smo videli, da pod »ekonomskimi znanji« Kravina ni razumel ekonomske teorije ali politike, temveč predvsem dobro poznavanje raznovrstnosti sveta narave, zemlje in njenih plodov, surovin, načinov in možnosti njihove uporabe, pa tudi praktično seznanjenost s tehnološkimi postopki in orodji za obdelavo proizvodov in surovin¸ pridobljenih iz zemlje, in njihovo predelavo v končne izdelke. V tem smislu je, kot smo že omenili, v delu čutiti duh fiziokratizma, torej gre za poudarjanje pomena narave in zemlje ter proizvodov, ki jih pridelamo na zemlji ali pridobimo iz zemlje, in predvsem za zahtevo po optimalnem izkoristku naravnih virov, s katerimi država razpolaga. Toda medtem ko so francoski fiziokrati v okviru t. i. produktivnega razreda v ospredje pogosto že postavljali velike kmetijske zakupnike oz. podjetnike (to je kapitaliste), ki so od plemiških zemljiških lastnikov najemali velike površine zemlje in jih obdelovali z najeto delovno silo in modernimi stroji. Kravina izhaja iz obstoječega podložniškega sistema in odgovornost za gospodarsko blagostanje države vidi predvsem v plemstvu oz. v njegovi sposobnosti učinkovito voditi posestva, spremljati tehnološki razvoj, uvajati novosti itd. ter seveda v ustrezni motiviranosti podložnikov, za kar naj bi skrbela tudi duhovščina. Relevantna »ekonomska znanja« za to pa so plemiški sinovi imeli možnost pridobiti na Terezianumu, kjer so se ob bogatih naravoslovnih muzejskih zbirkah in v ekonomsko-botaničnem vrtu - oboje je ustanovil Kravina s ciljem izboljšanja kakovosti pouka - lahko dodobra seznanili z zvrstmi zemlje oz. tal, rastlinami in živalmi, kmetijskimi in industrijskimi proizvodi ter obdelovalnimi in proizvodnimi tehnikami.

Theodor Kravina je kot rektor Terezianuma nedvomno dosegel visok ugleden in tudi odgovoren položaj v izobraževalnem sistemu avstrijske monarhije. S tega vidika je treba presojati tudi njegovo knjigo. Z njo je želel promovirati učni program te elitne izobraževalne institucije, pri oblikovanju katerega je tudi sam sodeloval ter hkrati poudariti pomen ekonomskih znanj za učinkovito vodenje kmetijskih posestev in industrijskih manufaktur, kar je videl kot enega glavnih dejavnikov gospodarskega in splošnega družbenega napredka. Kravinova knjiga sicer ni primerljiva s klasičnimi političnoekonomskimi deli, ki so v drugi polovici 18. stoletja nastajali po Evropi. Lahko pa rečemo, da je bila s poudarjanjem pomena izobraževanja skladna z jezuitsko tradicijo vsestranskega izobraževalnega dela, hkrati pa je odražala tudi napredni duh terezijanskih reform in razsvetljenstva nasploh.

4 SUMMARY

The article presents the volume Entwurf der oekonomischen Kenntnisse by Theodor Kravina von Kronstein which was published in Vienna in 1773. Theodor Kravina (1720-1789) was son of a silversmith from Slovenska Bistrica. Having finished the basic studies in Graz und Varaždin, he entered the Jesuit order in 1736 and went on to study philosophy in Vienna. Between 1749 and 1773 his life path was closely tied to the renowned Viennese school Theresianum, where he taught mathematics, physics, and mechanic, held leading positions and organized and maintained its museum collections and estates. Following the dissolution of the Jesuit order in 1773, he received a provostry in Zwettl, Lower Austria, where he worked and stayed until his death.

Kravina's *Outline of Economics* Knowledge stems directly from his work at Theresianum, where he was a prefect and later on, a rector. Theresianum was an elite school, intended first and foremost for sons from old noble families. Here they obtained knowledge required for working in civil service and diplomacy, as well as economy. His volume is a systematic overview of economic knowledge skills that young noblemen were tought at Theresianum. Concurrently the volume aimed to promote the natural science museum along with the economic and botanical garden operating at Theresianum in order to allow for demonstrative teaching and consequently imparting knowledge to nobility's young generations. In the period when Kravina wrote the book, the prevailing social and political climate in Habsburg Monarchy was impacted by Theresian reforms, which were directed at a general social advance resting on improved knowledge, the introduction on new techniques, and, consequently, enhanced economic efficiency. The idea about the best possible use of ones« land (and sources in general) is present throughout the book, as is that maintenance and growth of economic power as priorities of economic education. Kravina understood economic knowledge in a very pragmatic manner, namely mostly as knowledge and produce finished goods. It was his belief that members of the nobility required these skills because they were, as a rule, landowners and the state's welfare depended on their ingenious management of land and produce.

Kravina divided economy into two categories, which he described in the first and the second chapter respetively. The first category includes the examination of cultivating the earth's surface (*Die Oekonomie der Oberfläche der Erde)*, while the second one addresses the

exploitation of the interior of the earth (Die Oekonomie der Erzeugniss in den unterirdischen Lagen der Erde). As to the first category, Kravina stressed the importance of knowledge of produce provided by various parts of estates (fields, gardens, pastures, forests, waters), its use and processing. He maintained that young people should be tought about produce and processing techniques, which will enable him to expand and develop their knowledge of the subject in matter. Theresianum's economic gardens was particurably suitable for teaching econonomy; its sections (grain crops, garden plants, fruit trees, etc.) are presented systemically in the first chapter. The second category is intended for teaching types of soils, minerals, rocks, and their use in different economic activities. Theresianum's mineralogical collections, which are described in detail in the second chapter, were used to this end. The book's third chapter (gemeinen offentlichen Oekonomie Oekonomieter) is the longest; it discusses economic means (Die oekonomischen Hilfsmittel) or what we nowadays refer to as humans and material resources, workforce, working cattle, soil, tools, machinery, etc.). In this chapter Kravina developed also a somewhat broader view of economy and drew attention to maintenance and enhancement of production power (including human capital) as well as connectedness or structural harmony among specific economic activities (the concept of equlibrium). The last chapter contains the definition of the third sphere of economics, which Kravina referred to as public common economy (Von der *gemeinen offentlichen Oekonomie*) and is associated with the exchange of produce with other people and lands. However Kravin was not interested in the principles of trade, market mechanism, movement of prices and suchlike; he argued that teaching economics ought to be directed first and foremost at knowledge of produce that is subject to exchange, its characteristics, ingredients, usage, processing, etc.

As Theresianum rector, Theodor Kravina obtained a high, esteemed but also responsible position in the Austrian monarchy's educational system and his book is to be assessed from this perspective. By means of his book he wanted to promote this reputable educational instution«s learning programme, which he had co-created, and at the same time emphasize the importance of economic skills as practical knowledge of natural sciences, techniques and production for efficient management of farms and industrial mills, which he regarded as one of main factors of economic and social progress. Having established the »economic garden« and collections, Kravina improved the quality of education at Theresianum significantly. His book is not comparable with the classical political economy written in Europe in the second half of the 18. century (Smith, Turgot, etc.). However, we can state that by accentuating the importance of education Kravina's book was in line with the Jesuit tradition of universal education and at the same time reflected the progressive spirit of Theresian reform and the Age of Enlightenment in general.

5 VIRI

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Senecio vulgaris L. recorded as a new host plant for the root-knot nematode *Meloidogyne luci*

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Senecio vulgaris L. recorded as a new host plant for the root-knot nematode _Meloidogyne luci_

Abstract: *Meloidogyne luci* is a polyphagous plant parasitic nematode species with a potential to cause great losses in agricultural production. *M. luci* can parasitize over thirty important crop species as well as ornamentals, herbs and weeds. In this report we documented a weed plant common groundsel (*Senecio vulgaris* L.) as a new naturally-infected host species which could act as a reservoir for this pest.

Key words: root-knot nematode; *Meloidogyne luci;* weed; host plant; *Senecio vulgaris*; common groundsel; reservoir

Dokumentiranje nove gostiteljske rastline _Senecio vulgaris_ L. za rastlinsko parazitsko ogorčico _Meloidogyne luci_

Izvleček: *Meloidogyne luci* je polifagna rastlinsko parazitska ogorčica, ki je zmožna povzročiti velike izgube v kmetijski proizvodnji. Ogorčica *M. luci* lahko namreč parazitira prek trideset pomembnih kmetijskih rastlinskih vrst kot tudi okrasne rastline, zelišča in plevele. V tem prispevku smo dokumentirali plevelno rastlino navadni grint (*Senecio vulgaris* L.) kot novo, naravno okuženo gostiteljsko rastlino, ki lahko služi kot rezervoar za omenjenega škodljivca.

Ključne besede: ogorčice koreninskih šišk; *Meloidogyne luci;* plevel; gostiteljska rastlina; *Senecio vulgaris*; rezervoar

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1 SHORT COMMUNICATION

Meloidogyne luci Carneiro et al. 2014 is a recently described root-knot nematode species with a potential to cause great losses in agricultural production (Carneiro et al., 2014). This plant parasitic nematode is prevalent in South America, Europe and Asia as its presence was documented in Argentina, Bolivia, Brazil, Chile, Ecuador, Greece, Guatemala, Iran, Italy, Portugal, Slovenia and Turkey (reviewed in Carneiro et al., 2014; Janssen et al., 2016; Maleita et al., 2018; reviewed in Gerič Stare et al., 2017). Its distribution may be even broader than reported as species identification is challenging and was misidentified as *Meloidogyne ethiopica* Whitehead,1968, in the past (Gerič Stare et al., 2017, 2019). Important crop plants such as potato (*Solanum tuberosum* L.), maize (*Zea mays* L.) and tomato (*Solanum lycopersicum* L.) are host plants for *M. luci* (Strajnar et al., 2011; Conceição et al., 2012; Santos et al., 2019; Maleita et al., 2018), which could have serious impact for agricultural production in Europe and world-wide. This polyphagous pest can further parasitize numerous crop species such as okra (*Abelmoschus esculentus* (L.) Moench), kiwifruit (*Actinidia deliciosa* (A.Chev.) C.F.Liang & A.R.Ferguson), onion (*Allium cepa* L.), celery (*Apium graveolens* L.), chard (*Beta vulgaris* var. cicla (L.) Schuebl. & G.Martens), beet (*Beta vulgaris* var. conditiva L.), cabbage (*Brassica oleracea* var. capitate L.), cauliflower (*Brassica oleracea* var. botrytis L.), kohlrabi (*Brassica oleracea* var. gongylodes L.), broccoli (*Brassica oleracea* var. *italica* Plenck), pepper (*Capsicum annuum* L.), endive (*Cichorium endivia* L.), chicory (*Cichorium intybus* var. foliosum L.), melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai); cucumber (*Cucumis sativus* L.), carrot (*Daucus carota* L.), buckwheat (*Fagopyrum esculentum* Moench), florence fennel (*Foeniculum vulgare* Mill. ssp. *vulgare* var. azoricum (Mill.) Thell.), soybean (*Glycine max* (L.) Merr.), sunflower (*Helianthus annuus* L.), barley (*Hordeum vulgare* L.), lettuce (*Lactuca sativa* L.), lucerne (*Medicago sativa* L.), tobacco (*Nicotiana tabacum* L.), rice (*Oryza sativa* L.), common bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L.), yakon (*Smallanthus sonchifolius* (Poepp.) H.Rob.), peach (*Prunus persica* (L.) Batsch), radish (*Raphanus sativus* L. var. radicula), aubergine (*Solanum melongena* L.), spinach (*Spinacia oleracea* L.), grapevine (*Vitis vinifera* L.) and sweet corn (*Zea mays* L. var. saccharata) (Strajnar et al., 2009; Carneiro et al., 2014; Bellé et al., 2016). *M. luci* can also thrive on ornamentals such as snapdragon (*Antirrhinum majus* L.), cabbage-palm (*Cordyline australis* (G.Forst.) Endl.), sedum (*Hylotelephium spectabile* (Boreau) H.Ohba), lavender (*Lavandula angustifolia* Mill.) and rose (*Rosa* sp. L.), herb curled dock (*Rumex patientia* L.) and weed creeping woodsorrel (*Oxalis corniculata* L.) (Strajnar et al., 2009; Carneiro et al., 2014; Santos et al., 2019). As there is a great concern that this pest could spread to new areas with suitable hosts and climate conditions, the species was included in the EPPO Alert list of harmful organisms (EPPO, 2017).

We have tested efficiency of bionematicide VOTiVO™ (Bayer CropScience AG) based on bacteria *Bacillus firmus* Bredemann and Werner, 1933, for the protection of tomato plants against infestation with *M. luci* (Susič et al., 2020; Širca et al., 2019). The test was conducted in the microplots at the site of Agricultural Institute of Slovenia from April to September in 2018 and 2019. At the end of a growing season 2019 (114 days after nematode infestation) when tomato plants were uprooted and evaluated

Figure 1: *Senecio vulgaris* recorded as a new host plant for the root-knot nematode *Meloidogyne luci*. Left: Roots of *S. vulgaris* infested with the root-knot nematode *M. luci*; arrows point to nematode infestation in the host roots. Right: Above ground part of the plant shows no symptoms of nematode infestation.

for nematode infestation several weed plants were found growing in the microplots as well. The weed was identified as common groundsel (*Senecio vulgaris* L.) based on morphological characters. Typical root-knot nematode infestation symptoms were observed on *S. vulgaris* roots (Figure 1). Identity of nematode females dissected from galls was confirmed as *M. luci* using isoenzyme electrophoresis as described previously by Strajnar et al. (2009).

While *S. vulgaris* L. is a documented host plant for several *Meloidogyne* species such as *Meloidogyne microtyla* Mulvey et al., 1975 (Townshend at al., 1984), *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood 1949 (Amin, 1994), *Meloidogyne hapla* Chitwood, 1949 (Bélair & Benoit, 1996) and *Meloidogyne chitwoodi* Golden, O'Bannon, Santo & Finley, 1980 (Kutywayo & Been, 2006), this is a first report of *S. vulgaris* L. being a host plant for *M. luci* as well. *S. vulgaris* is a widely distributed weed and ruderal species. The significance of description of a new naturally-infected host of *M. luci* is a fact that the weed species *S. vulgaris* L. could act as a reservoir of this plant pathogenic nematode in agricultural production settings. The producers using resistant crop varieties like tomato bearing the *Mi* resistance gene to control *M. luci* infestation in greenhouses or fields should therefore be aware that this widely spread weed could maintain nematode population.

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Ensuring food security amid novel coronavirus (COVID-19) pandemic: Global food supplies and Pakistan's perspectives

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Recently, novel coronavirus (COVID-19) has reached to the level of pandemic (WHO, 2020) turning the world upside down (Nkengasong, 2020). Its fear has caused more panic than virus itself while making economic downturns of the unabated outbreak substantial. Globally, economic crunch has started to be witnessed in developed nations with trickle down impact on developing economies (Beltrami, 2020). Across the world except sanitizers and other medical supplies (face masks, protection suits, ventilators etc.), overall food supplies have remained stable despite the spread of dreadful virus in over 195 countries and territories. There are sufficient food stockpiles in worst hit countries like China, USA, Italy, Spain, Iran, France, Germany etc. However, extent of deterioration in nutritional security of already unsecured and vulnerable populace in developing countries of South Asia will be determined by a bunch of factors including time span taken to flatten the curve of infected persons and especially policies adopted by respective governments to curtail the pandemic. Among South Asian countries, Pakistan is a developing country having over 210 million populace with agriculture as economic backbone (shares 18% to national GDP along with provides employment to 38% labor force) (Iqbal et al., 2019).

Regarding staple crops productivity, Pakistan produced just over 25 million tons of wheat, while rice and maize production remained 7.5 and 5.9 million tons respectively during 2018-19 (Government of Pakistan, 2019). As per preliminary estimates of Asian Development Bank (ADB), Pakistan may incur losses amounting to US$ 5 billion caused by decline in GDP growth due to decline in services sector (airline business, FBR tax collections), exports, foreign remittances etc. due to pandemic (Haider, 2020). This outbreak is feared to seriously exacerbate prevalent food and nutritional insecurity owing to substantial reduction in net incomes and jobs rationlization. It is writing on the wall that reduced purchasing power of masses is bound to alter eating patterns leading to poor nutrition. On other side, panic purchase of staple food as witnessed in many developed countries of world, is feared to result in localized price hikes owing to breakage of supply chains (Askew, 2020). As malnutrition multiplies vulnerability to infectious diseases thus there are growing fears that spread of disease among poorer segments of the society can inflict unthinkable mortality rates. In fragile economies as that of Pakistan, economic crunch can inflict more harm to general masses than disease itself. The production and consumption paradigm (Table 1) indicates that except pulses and vegetable oil, Pakistan is self-sufficient in other staple foods. Despite the fact that Pakistan is 8th largest wheat producing country, the need of hour is to ensure its regulated supplies as it fulfills 72 % caloric needs of common Pakistanis.

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1 COVID-19 TRIGGERED THREATS TO AGRICULTURAL ECONOMY

One of the serious threats posed to agricultural economy in Pakistan amid novel coronavirus pandemic is labor shortages owing to illness and fears of pandemic (Daily times, 2020). Similarly, lockdown triggered transport interruptions have resulted in reduced supplies of essential farm inputs. In addition, limited access of growers to markets due to quarantine measures and disruption of farm supply chains may lead to substantial loss of food caused by reduced productivity and increased wastages. Moreover, farmers also need to adhere to social distancing which is bound to reduce agricultural productivity, while lack of farm mechanization is bound to turn the situation from bad to worse. Closing of restaurants may inflict serious economic cost to farmers who supply fresh farm produces. Similarly, high-value, organically grown fruits and vegetables as well as cash crops growers are likely to suffer more losses compared to staple crops growers owing to lack of marketing and export facilities. Like other developing countries, agricultural services sector (governmental agricultural extension departments and privately owned pesticide, seed and fertilizer industries) in Pakistan is not digitized and resultantly rely on face-to-face communications. Likewise, harvesting season of wheat and potatoes in many parts of Pakistan is just around the corner, while lack of farm mechanization has made it impossible to avoid face-to-face contact of farm labors which may result in higher labor cost caused by shortage. Maintenance of orchards and picking of fresh fruits may also be hit the hardest leading to disrupting supply chain of fresh fruits. Closure of farm machinery workshops in the wake of anticipated strict lockdown and cordoning off many intensively infected areas may result in grounding of numerous threshers, reapers, combine harvesters etc. which can adversely affect wheat harvesting (March to May).

2 REQUIRED POLICY LEVEL ASSERTIONS

It is worth mentioning that Kazakhstan which is world's biggest wheat flour shipper has effectively banned its exports along with other agricultural produces like carrots, sugar and potatoes. Similarly, Serbia stopped exporting sunflower just at the emergence of COVID-19, while Russia is also planning to withhold wheat exports. Algeria and Turkey have issued tenders to initiate large scale procurement of wheat and rice from domestic and international markets in order to off-set the drastic impacts of pandemic on local panic buying of food commodities (Martin, 2020). Keeping in view the emerging food nationalism and to alleviate the drastic impacts of novel coronavirus (COVID-19) on food and nutritional security of people in Pakistan, some serious policy level initiatives are needed to be taken before it becomes the matter of too little too late.

First and foremost thing is to temporarily follow protectionism for staple food commodities with tax exemptions. There is a dire need to bolster economically fragile consumers' purchasing power which may go a long way in sustaining the nutritional status of populace.

There is need to realize that all awareness pertaining to adopting health hygiene against COVID-19 can go down the drain if socially isolated and food insecure population cannot make both ends meet. Strict and vigilant monitoring of staple foods supplies through federal and provincial agencies along with establishing virtual control rooms having direct communication with general populace can assist to regulate price control mechanism.

Table 1: Production and consumption paradigm of staple crops and food commodities in Pakistan during 2018-19 (Saleem, 2020; Government of Pakistan, 2019)

Crops	Area (million hectares)	Production (millions/ million tons)	Consumption (million tons)	Per capita consumption (Kg/liter per year)	Storage (million tons)
Wheat	8.79	25.6	25.4	115	5.0
Rice	2.81	7.5	3.5	13	-
Maize	1.3	6.3	4.9	-	-
Sugar	-	6.5	5.4	25	-
Pulses	1.18	0.40	1.4	-	-
Poultry	-	1.51	1.50	7	-
Beef + mutton	-	4.4	3.9	-	-
Milk	-	59	59.0	119	-
Vegetable oil	0.748	0.5	1.6	12	-
Eggs	-	18	17.1	80	-

Amid COVID-19 pandemic, China which is the biggest producer and consumer of rice has pledged to buy locally grown rice in higher than ever quantities for adding it to national strategic stockpiles (Martin, 2020). The fact remains even before incidence of this pandemic; China maintains strategic reserves of wheat and rice for one year. Following the foot-steps of China, provincial level stocks of staples (wheat and rice) in Pakistan need to be increased by temporarily acquiring storage facilities which may be used to bridge supply and demand gaps. Moreover, as the wheat harvesting season is about to begin, so storage capacity of Pakistan Agricultural Storage and Services Corporation (PASSCO) needs to be substantially enhanced which currently stands at 0.9 million tons for wheat. Overall, wheat procurement by federal and provincial government's stands below 5 million tons, however keeping in view dreadful pandemic, government procurement must be between 6-10 million tons. Likewise, rice consumption in Pakistan is 3.5 million tons against annual production of 7.5 million tons. However unlikely to wheat, federal and provincial governments have zero control over rice procurement and storage (Iqbal, 2015), while this policy needs to be amended for initiating rice procurement as that of wheat. Moreover, maize (6.5 million tons production during 2018-19) must also be procured as an emergency supplement to wheat flour. Otherwise natural disasters like prevalent pandemic are bound to inflict heavy losses along with jeopardizing food related governance.

Provision of effective support (monetary and commodities) to most vulnerable segments of the society especially daily wagers enabling them to sustain periods of lockdown and thereafter scenarios of projected economic crunch.

Government need to increase targeted spending in the form of subsidies on farm implements leading to promotion of small scale farm mechanization can go a long way in boosting crops productivity along with enabling farmers to cope with emerging any pandemic in future.

Digitization of agriculture services need to be initiated and media campaigns can be utilized to create awareness among farming community in order to minimize net losses on crops production caused by quarantine and lockdown situations.

Given the extent of problem we are facing now, provision of subsidies on farm inputs (seeds, fertilizers, pesticides) has the potential to trigger agricultural economic activities leading to increasing area under staple crops and reducing food wastage.

If history is any guide, inflated bread costs have always triggered political unrest leading to national instability. Thus deeming wheat flour as strategic commodity, there is dire need to formulate regulatory amendments for amending the functioning of private milling industry (over 1000 flour mills) which cater the needs of over 40 % populace in the country. Moreover, government owned utility stores outlets must be utilized to make sure the availability of wheat flour, pulses and vegetable oil on subsidized rates which is bound to have positive psychological impacts on people by looking ample wheat flour in shelves.

Provincial livestock departments in coordination with domestic dairy industries can be used to maintain the supplies of milk in metropolitan and urban areas. It has the potential to provide sufficient income to dairy farmers along with ensuring sustainable milk supplies.

Since social distancing requirement cannot allow establishing cumulative food banks or group meals owing to risk of spreading the infection, bolstering the network of welfare organizations (Saylani, Edhi, Chippa, Akhuwat etc.) with infrastructure and financially along with establishing volunteer organizations for delivering food in respective localities can assist to reach the most vulnerable segments of the society for fulfilling their immediate daily dietary needs.

Last but not least, calm and calculated decision making even in the wake of emerging supply chain hiccups instead of adopting beggar-thy-neighbor policies will ultimately determine the effectiveness of policy-making and disaster responsiveness.

The silver lining of this grim scenario is that warmer climate for most part of year in Pakistan, younger population, less dense rural areas where most of Pakistani populace resides and limited travelling networks within and outside country, may assist to flatten the curve of infected persons sooner than expected. Pakistan produces sufficient quantities of staple crops (wheat and rice), thus if it is ensured to procure additional quantities of wheat, rice, maize etc. on government level then emerging supply gaps owing to panic buying can be effectively dealt with. Most importantly tax exemptions and rigorous vigilance of food supply chain can sooth the drastic effects of panic buying. Lastly, prudent policies at government level formulated in line with indigenous socio-economic paradigms can ensure sustainable supply of food commodities to vulnerable segments of the country leading to overcoming of the pandemic. Last but not least, it must be recognized that scientific education is the most beneficial defense equipment for humanity, while the discipline of medicine constitutes the most demanding expertise currently and in times to come.

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Navodila avtorjem

UVOD

Acta agriculturae Slovenica je četrtletna odprtodostopna znanstvena revija z recenzentskim sistemom, ki jo izdaja Biotehniška fakulteta Univerze v Ljubljani. Revija sprejema izvirne in še neobjavljene znanstvene članke v slovenskem ali angleškem jeziku, ki se vsebinsko nanašajo na širše področje rastlinske pridelave in živalske prireje in predelave. Zajema sledeče teme: agronomija, hortikultura, biotehnologija, fiziologija rastlin in živali, pedologija, ekologija in okoljske študije, agrarna ekonomika in politika, razvoj podeželja, sociologija podeželja, genetika, mikrobiologija, imunologija, etologija, mlekarstvo, živilska tehnologija, prehrana, bioinformatika, informacijske znanosti in ostala področja, povezana s kmetijstvom. Pregledne znanstvene članke sprejemamo v objavo samo po poprejšnjem dogovoru z uredniškim odborom. Objavljamo tudi izbrane razširjene znanstvene prispevke s posvetovanj, vendar morajo taki prispevki zajeti najmanj 30 % dodatnih izvirnih vsebin, ki še niso bile objavljene. O tovrstni predhodni objavi mora avtor obvestiti uredniški odbor. Če je prispevek del diplomske naloge, magistrskega ali doktorskega dela, navedemo to in tudi mentorja na dnu prve strani. Avtorji omenjenih del morajo biti tudi soavtorji članka, ki podaja izsledke dela. Navedbe morajo biti v slovenskem in angleškem jeziku, kadar so prispevki v slovenščini. V primeru člankov v slovenščini so dolžni avtorji priskrbeti angleški prevod naslova, izvlečka s ključnimi besedami in naslovov slik in tabel. Uredništvo revije zagotovi prevode izbranih bibliografskih elementov (naslova, izvlečka, opomb in ključnih besed) v primeru tujih avtorjev. Prispevke sprejemamo skozi celo leto samo preko spleta v OJS sistemu.

POSTOPEK ODDAJE PRISPEVKOV

Avtorji lektorirane prispevke oddajo v elektronski obliki na spletni strani OJS Acta agriculturae Slovenica.

Author guidelines

INTRODUCTION

Acta agriculturae Slovenica is an open access peer-reviewed scientific journal published quarterly by the Biotechnical Faculty of the University of Ljubljana, Slovenia. The journal accepts original scientific articles from the fields of plant production (agronomy, horticulture, plant biotechnology, plant-related food-and-nutrition research, agricultural economics, information-science, ecology, environmental studies, plant physiology & ecology, rural development & sociology, soil sciences, genetics, microbiology, food processing) and animal production(genetics, microbiology, immunology, nutrition, physiology, ecology, ethology, dairy science, economics, bioinformatics, animal production and food processing, technology and information science) in the Slovenian or English languages. Review articles are published upon agreement with the editor. Reports presented at conferences that were not published entirely in the conference reports can be published. Extended versions of selected proceedings-papers can also be considered for acceptance, provided they include at least 30 % new original content, but the editorial board must be notified beforehand. If the paper is part of a BSc, MSc or PhD thesis, this should be indicated together with the name of the mentor at the bottom of the front page and will appear as footnote. Authors of mentioned thesises should be also coauthors of manuscript. Slovenian-language translation of selected bibliographic elements, for example the title, abstract, and key words, will be provided by the editorial board. Manuscripts are accepted throughout the year. Only online submissions are accepted..

SUBMISSION PROCESS

Manuscripts should be submitted to the Acta agriculturae Slovenica OJS site. Complete manuscripts in-

Oddajo kompleten članek (naslov, avtorji z njihovimi naslovi, označen mora biti korespondenčni avtor in naveden njegov elektronski naslov, besedilo z vsemi poglavji in vključenimi ilustracijami (slike, tabele, enačbe). Pred oddajo prispevka se mora avtor na spletni strani najprej prijaviti oziroma registrirati (priporočamo, da je to korespondenčni avtor), če prvič vstopa v sistem (potrebno je klikniti na Registracija in izpolniti obrazec za registracijo). Bodite pozorni, da na dnu registracijskega obrazca ne pozabite odkljukati potrditvenega polja »Avtor«, sicer oddaja prispevka ne bo mogoča.

Postopek oddaje prispevka poteka v petih korakih. Priporočamo, da se avtor pred oddajo najprej seznani s postopkom in se na oddajo prispevka pripravi:

Korak 1: Začetek oddaje prispevka
- Izbrati je treba eno od sekcij,
- pri rubriki »Pogoji za oddajo prispevka« morate potrditi vsa potrditvena polja,
- dodatna pojasnila uredniku je mogoče vpisati v ustrezno polje.

Korak 2: Oddaja prispevka
- Naložite prispevek v formatu Microsoft Word (.doc ali .docx).

Korak 3: Vpis metapodatkov
- Podatki o avtorjih: ime, priimek, elektronski naslovi in ustanove vseh avtorjev v ustreznem vrstnem redu. Korespondenčni avtor mora biti posebej označen.
- Vpišite naslov in izvleček prispevka.
- Vpišite ključne besede (največ 8, ločeno s podpičjem) in označite jezik besedila.
- Vnesete lahko tudi podatke o financerjih.
- V ustrezno besedilno polje vnesite reference (med posameznimi referencami naj bo prazna vrstica).

Korak 4: Dodajanje morebitnih dodatnih datotek
- Za vsako dodatno naloženo datoteko je treba zagotoviti predvidene metapodatke.

Korak 5: Potrditev
- Potrebna je končna potrditev.

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- Final confirmation is required.

PODROBNEJŠA NAVODILA / DETAILED INSTRUCTIONS

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