ACTA AGRICULTURAE SLOVENICA

114 • 2
2019

Biotehniška fakulteta Univerze v Ljubljani
Biotechnical Faculty University of Ljubljana

Acta agriculturae Slovenica • ISSN 1581-9175 • 114—2 • Ljubljana, december 2019

Acta agriculturae Slovenica
Volume / Letnik **114** · Number / Številka **2** · **2019**

VSEBINA / CONTENTS

doi:10.14720/aas.2019.114.2.1 Original research article / izvirni znanstveni članek

Extent and financial cost of cassava postharvest loss along the cassava value chain in Kwara State, Nigeria

Samuel Temitope DANILOLA [1, 2], Raphael BABATUNDE [1], Jubril ANIMASHAUN [1]

Received May 02, 2018; accepted October 01, 2019.
Delo je prispelo 02. maja 2018, sprejeto 01. oktobra 2019.

Extent and financial cost of cassava postharvest loss along the cassava value chain in Kwara State, Nigeria

Abstract: Cassava has been identified as Africa's second most important staple food after maize, in terms of calories consumed, with Nigeria as the World leading producer. This study estimated postharvest loss of cassava along the cassava value chain in Kwara State. It estimated the size of postharvest loss of cassava; analysed the factors responsible and the financial implications of loss; and identified the strategies employed in the mitigation of loss in the study area. A three-stage random sampling technique was used to select 117 cassava farmers whom were administered structured questionnaire to elicit data analysed by the study. Descriptive statistics, Shannon's diversity index and Tobit regression model were the analytical techniques utilised. The results show that 68 % of the loss occurred at the harvesting. The loss was estimated to be about 3.8 t ha^{-1}. The financial implication was valued at \$ 300 ha^{-1}. Analysis of the factors responsible for cassava postharvest loss showed that the quantity expected at harvesting, household size and age of the farmer were significant factors affecting cassava postharvest loss. The result also revealed that farmers mitigate these losses by processing the roots and reburying unused roots into the soil. Steps needed to reduce loss have to take these factors into consideration to improve the economic status of cassava farmers-processors.

Key words: cassava; postharvest loss; financial cost; Shannon's diversity index; Tobit regression

Obseg in stroški izgube v pridelovalni verigi manioke po spravilu pridelka v državi Kwara, Nigeria

Izvleček: Manioka je postala za koruzo v Afriki druga najpomembnejša vsakodnevna hrana glede zaužitih kalorij, z Nigerijo kot vodilnim svetovnim pridelovalcem. V raziskavi je bila ocenjena velikost izgube pridelka manioke po spravilu. Analizirani so bili dejavniki, odgovorni za izgubo in njene finančne posledice. Identificirane so bile strategije, ki so bile uporabljene za blaženje izgub na preučevanem območju. Uporabljena je bila tristopenska metoda vzorčenja, v kateri je bilo 117 pridelovalcev manioke, ki so izpolnili vprašalnik za pridobitev podatkov analiziranih v tej raziskavi. Za analizo pridobljeni podatkov so bile uporabljenne metode opisne statistike, Shannonov diverzitetni indeks in Tobit regresijski model. Rezultati kažejo, da je 68 % izgube nastalo med spravilom. Izguba je bila ocenjena na okrog 3,8 t ha^{-1}, njena vrednost pa \$ 300 ha^{-1}. Analiza dejavnikov, odgovornih za izgubo pridelka manioke po spravilu je pokazala, da so bili pri tem najpomembnejši velikost pričakovanega pridelka, velikost gospodinjstev in starost pridelovalcev. Rezultati so še pokazali, da pridelovalci zmanjšujejo izgubo pridelka po spravilu s predelovanje in ponovnim zakopavanjem v tla. Koraki, potrebni za zmanjšanje izgube pridelka so torej upoštevanje teh dejavnikov in s tem izboljšati ekonomičnost predelave manioke pri pridelovalcih

Ključne besede: manioka; izguba po spravilu; finančna ocena; Shannonov diverzitetni indeks; Tobit regresija

1 University of Ilorin, Department of Agricultural Economics and Farm Management, Ilorin, Kwara State, Nigeria
2 Corresponding author, e-mail: samueldanilola@yahoo.com

1 INTRODUCTION

Cassava *(Manihot esculenta* Crantz.) is a member of the family of Euphorbiaceae, and is one of the oldest cultivated crops by human beings although the early history of cassava is still a mystery (Gulick et al., 1983). It was reported that cassava has been cultivated in northern Amazonia more than 1000 years ago (Jones, 1959) until some years back when it was postulated that cassava is likely to be originated from wild *M. esculenta* populations along the southern region of the Amazon basin (Olsen et al., 2001). Portuguese explorers introduced cassava to Africa during the 16th and 17th centuries through their trade with the African coasts and nearby islands. Africans then spread cassava further, and it is now found in almost all parts of tropical Africa. Today Nigeria and Democratic Resepublic of Congo are the largest producers of cassava in Africa alongside Brazil in the South America and Carribeans and Thailand in Asia (Department of Agriculture, Forestry and Fisheries, South Africa, 2010). Figure 1 shows the world production of cassava and the revenue generated from the sale of the commodity in dollars.

Cassava (*M. esculenta*) is Africa's second most important staple, after maize, in terms of calories consumed, with Nigeria as the world leading producer (Nweke, 2004). This is as a result of cassava's combined abilities to produce high yields under poor conditions and store its harvestable portion underground until when needed and this had made it a classic "food security crop" (Nweke, 2003). Its importance as a food crop in Africa can be clearly seen when consumption data from Africa is compared with other cassava growing regions of the world, majorly the Latin Americas. Cassava is very important in the diet of an average Nigerian. It has comparative production advantage over other staples; as a result this serves to encourage its cultivation even by the resource-poor farmers. Studies on cassava has shown that it accounts for about 70 % of the total calorie intake of more than half of Nigerians (Nweke and Enete, 1999) with its starchy roots producing more food energy per unit of land than any other staple crop in the country (de Figueroa et al., 2001).

However, despite cassava being a major constituent of the diet and also major source of income to farmers in Nigeria as a whole and specifically Kwara state, economically, it represents a wasted investment that can reduce farmers' incomes and increase consumers' expenses (Lipinski et al., 2013). Although, the level of the impact of the postharvest loss of this crop has not yet been seen from literatures, neither is the part it plays in attaining food security known. It is equally important to note that, even with our status as the leading producer of cassava in the world, we are still behind Thailand in the exportation of cassava products, and we still import some by-products of cassava processing such as industrial starch. Moreover, environmentally, postharvest loss inflict a host of impacts, including unnecessary greenhouse gas emissions and inefficiently used water and land, which in turn can lead to diminished natural ecosystems and the services they provide (Lipinski et al., 2013).

Considering the fact that, despite the prevalence of post-harvest food loss in Nigeria, there are limited studies quantifying the losses of roots and tubers, especially cassava, in Nigeria. There is therefore a need for this study on the post-harvest loss of cassava as a major constituent of Nigerian diet. Thus, the knowledge of the

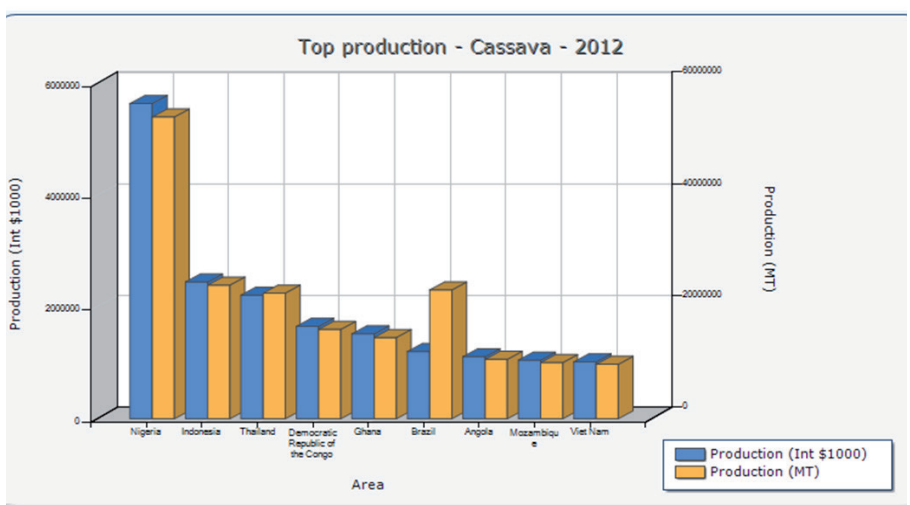


Figure 1: Graph of top cassava producers in the world as at year 2012. Source: FAOSTAT, 2013.

level and extents of postharvest losses of cassava along the cassava supply chain and their major effects would be necessary, as it would provide policy makers with the required findings to enact and implement policies needed to prevent/or mitigate these losses, and as such ensure victory in the war against food insecurity in Kwara state specifically, and global warming, in general.

In the light of the foregoing, the study was carried out to estimate the size/index of postharvest loss of cassava along the supply chain in the study area, analyse factors responsible for the postharvest losses of cassava along the supply chain, analyse the financial cost implication(s) of postharvest losses in the study area and examine the strategies employed in the mitigation of postharvest losses in the study area.

2 MATERIALS AND METHODS

The study was carried out in Kwara State whose capital is Ilorin. Kwara State of Nigeria was created on the 27th of May, 1967 along with 11 other states of the federation. The state was originally called west central state, having been carved out of the defunct northern Nigeria. At the time of creation, the state had a landmass of but this has reduced to following the boundary adjustments that accompanied excision of a segment of its eastern part to Benue State in 1976 and 6 local government areas to the present Kogi State and Niger State in 1991. However, recent survey shows that the state has a total land area of about which is about 3.5 of the total land area of the country, which is put at (KWSG, 2006).Considering the geographical location, Kwara State occupies a vantage position on the map of Nigeria. Situated between latitudes and of the equator and longitudes E and E of the equator, it lies midway between the Northern and Southern parts of Nigeria. Kwara State is divided into four zones by the Kwara State Agricultural Development Project (KWADP) in consonance with ecological characteristics, cultural practices and project's administrative convenience. These are: Zone A: Baruteen and Kaima Local Government Areas; Zone B: Edu and Patigi Local Government Areas; Zone C: Asa, Ilorin East, Ilorin South, Ilorin West and Moro Local Government Areas; and Zone D: Ekiti, Ifelodun, Irepodun, Offa, Oyun, Isin and Oke-ero Local Government Areas. The State shares boundaries with Osun, Oyo, Ondo, Kogi, Niger and Ekiti States as well as an international boundary with the Republic of Benin in the West.

The study was carried out in 2014 and a three-stage random sampling technique was used to select the sample farmers for the study. In the first stage, one (1) out of the four (4) agricultural zones in Kwara State was selected at random, while ten (10) villages were randomly selected from the list of villages in the selected zone in the second stage. The final stage involved the random selection of twelve (12) cassava farmers from each of these villages, only the data of one hundred and seventeen (117) farmers were used for further analysis.

Primary data were collected through the use of structured questionnaire in an interview schedule method. Information was collected on the socio–economic characteristics of the farmers in the study area such as age, gender, education, farm size, etc. Also, information was collected on quantities of cassava harvested and processed, losses incurred along the supply chain, amongst others. Data collected were analyzed using descriptive statistics, Shannon's diversity index and Tobit regression model based on the objectives of the study.

The Shannon's Diversity Index, H was applied to measure the spread of the postharvest loss across the cassava value chain i.e. harvesting, processing and marketing. The index of postharvest losses was obtained from the formula:

$$H = -\sum_{i}^{n}{}_{1}p_{i}\ln p_{i}$$

Where, H = Shannon's diversity index for postharvest loss,

n = number of stages in the value chain from which losses are recorded, and

$$p_i = \frac{N_i\ (loss\ at\ each\ stage\ of\ the\ value\ chain)}{total\ postharvest\ loss}$$

The tobit regression model is a statistical model which describes the relationship between a non-negative dependent variable and an independent variable and supposes that there is a latent (observable) variable. The observable variable is defined to be equal to the latent variable whenever the latent variable is above zero (but not more than 1) and zero otherwise. This model was used in the determination of the latent variable due to the presence of a maximum amount of losses that can be accommodated, beyond which the postharvest losses become unacceptable.

The econometric model for the tobit regression is implicitly stated as:

$yi^* = \beta x1 + \beta x2 + \beta x3 + \beta x4 + \beta x5 + \beta x6 + \beta x7 + e$

yi^* = percentage loss as a proportion of expected output (0 yi^* 1)

β = coefficient to be estimated

x1 = age of respondents (years)

x2 = household size
x3 = quantity expected at harvesting (kg)
x4 = use of unsold roots
x5 = causes of loss
x6 = measures to control loss
x7 = processed outputs
e = error term

Limitations

This study used recall process to elicit the losses across the different aspects of the value chain due to the poor record keeping characteristics of the farmers. Therefore, it is important to factor that in the interpretation of the result of the study.

3 RESULTS AND DISCUSSIONS

The results in table 2 show the socioeconomic characteristics of cassava farmers in the study area. It can be deduced from the table that 66.7 % of the respondents were male and 33.3 % female. The male dominance of cassava production as well as processing as against processing alone, which is female dominated (Ezedinma et al., 2007 and Muhammad-Lawal et al., 2013) could be majorly attributed to the combination of the cassava production and processing stages. The table further revealed that more than 60 % of the respondents are more than 35 years but not older than 55 years of age and majority of the farmers are married and have at least a year of formal education. Also, it can be obtained from the table that the modal years of farming experience of the farmers was between 15 and 24 years while the modal annual income falls between ₦25,001 and ₦181,000.

Estimation of the size of cassava post-harvest loss shows that the average postharvest loss of cassava per hectare of farmland under cassava cultivation in the study area was 3.80 t ha^{-1}.

$$\text{Average postharvest loss per hectare} = \frac{\text{total amount ofpostharvest loss ofcassava}}{\text{total land under cassava cultivation}}$$

$$= \frac{752.040\ \text{t}}{197.9\ \text{ha}} = 3.80\ \text{t ha}^{-1}$$

It can be deduced from table 1 that, the size of cassava postharvest loss is 752,040 kg which when expressed in percentage of total quantity expected is 33.34 %. The chain of highest loss is at harvesting which accounts for about 68 % of the total loss, followed by the loss at processing, 22 % and then loss at marketing, 10 %.The loss across the value chain is not uniformly distributed as revealed by a relatively low H-value. The intensity of the loss at harvesting stage, being the highest, is in line with the report of Bokanga (1999) where he stated that postharvest loss of cassava is the highest at harvesting in dry season due to hardness of the soil which results in heavy breakage of the roots. This further confirms the findings of Jaspreet et al. (2013) which postulates that food losses occur majorly at the field-market stage.

Tobit regression model was used to model the factors responsible for cassava postharvest loss. It was assumed that the losses beyond a threshold is regarded as postharvest loss. Ten percent was the threshold used in this study.

Table 3 shows the Tobit regression result of the factors responsible for cassava postharvest loss. The result shows that the quantity expected at harvest, household size and age of the farmers are the significant factors that determine cassava postharvest loss in the study area at 5 % level of significance. It can be deduced from the table that both the quantity expected at harvest and household size positively affects the postharvest loss of cassava. This implies the higher the quantity of cassava expected at harvesting, the more the margin of postharvest loss all things being equal. This is in conformity with the finding of Atanda et al. (2011) that postharvest loss could result when there is a bumper harvest which could overload the post-harvest handling system or exceed the consumption need and cause excessive wastage.

In a similar way, the household size was found to have a positive significant effect on cassava postharvest

Table 1: Shannon's diversity index for size/level of post-harvest loss in the study area

Stages of the cassava value chain	N_i (total loss at this stage, kg)	P_i	ln P_i	H =- ($P_i \times \ln P_i$)	Percentage loss as a proportion of total loss (%)
Harvesting	509,200	0.68	-0,39	0.26	68
Processing	166,790	0.22	-1.51	0.33	22
Marketing of roots	76,050	0.10	-2.29	0.23	10
Total	752,040			0.82	100

Source: Data analysis, 2014.

Table 2: Distribution of cassava farmers by their socioeconomic characteristics

Variables	Frequency	Percentage (%)
Sex		
Male	78	66.7
Female	39	33.3
Age (years)		
≤ 25	1	0.9
25 – 35	1	0.9
36 – 45	27	23.1
46 – 55	50	42.7
56 – 65	32	27.4
≥ 66	6	5.1
Marital status		
Single	1	0.9
Married	105	89.7
Widowed	9	7.7
Divorced	2	1.7
Years of schooling		
0	26	22.2
1 – 6	39	33.3
7 – 12	39	33.3
13 – 16	9	7.7
≥ 17	4	3.4
Farming experience (years)		
≤ 5	4	3.4
6 – 14	29	24.8
15 – 24	34	29.1
25 – 33	25	21.4
34 – 43	19	16.2
≥ 44	6	5.1
Annual income (₦)		
≤ 25,000	9	7.7
25,001 - 181,000	80	68.4
181,001 - 337,000	23	19.7
337,000 – 493,000	3	2.6
≥ 649,001	2	1.7

Source: Field survey, 2014.

loss at 5 % level of significance. This implies the larger the household size of cassava farmers, the higher the percentage of cassava postharvest loss in the study area, leaving all other factors constant. Also found to significantly affect the level of cassava postharvest loss in the study area at 5 % level was the age of cassava farmers but with a negative relationship. From Table 3, one can infer that a one year increase in the age of respondent would result in 0.33 % reduction in postharvest loss. This is true especially when the increase in age comes with more farming-processing experience and large household size. The experience, in turn, results in improved harvesting-processing practices; experience is also known to have positive effect on processors' managerial capacity, technical know-how, and adoption of extension packages (Achem and Akangbe, 2011). All these lead to a reduced level of postharvest losses. However, the remaining factors modeled were found to be insignificant to the level of cassava postharvest loss in the study area.

The analysis carried out to examine the economic cost implication of the cassava postharvest loss shows cost implication is $300/ha as shown below:

Cost of 1tonne of cassava = $78.94 t^{-1}

Average postharvest loss = 3.80 t ha^{-1}

Therefore, Cost of postharvest loss = ₦ 15,000.00 t^{-1} × 3.80 t ha^{-1} = $300 ha^{-1}

Based on the work of Abduraheem and Toluwase (2013), cassava farmers earn ₦ 68,662.50 per hectare; the postharvest loss is about 83.0 % of the income earned on a hectare.

Table 4 shows the relative percentages of the respondents that practice each postharvest control measures. The respondents utilize a combination of the postharvest loss control measures as this could imply that the use of just one of the above could be insufficient for the control of the loss. It can be deduced from the table that, the most widely used measure by cassava farmers in the study area was harvesting the roots when required while the least used measure was use of agrochemicals. This implies that majority of the cassava farmers in the study area prefer to harvest the roots as at when required to mitigate the potential postharvest loss associated with cassava. This may be attributed to the fact that harvesting of roots when required will give no room for excesses as the desired quantity will be harvested and all possible efforts will be put in place to make sure any postharvest loss is kept at barest minimum.

4 CONCLUSION AND RECOMMENDATIONS

The study has shown that the size of the cassava postharvest loss of Kwara state is very high and concrete steps have to be taken in order to reduce this loss so as to improve upon the economic status of cassava farmers-processors. The concentration of losses at the

Table 3: Tobit regression result for the factors responsible for cassava postharvest loss

Percentage loss as a proportion of output	Coefficient	Standard Error	t- value
Processed outputs (Kg)	-0.59	1.52	0.39
Measures to control loss	-0.87	1.16	-0.75
Causes of loss	2.20	1.36	1.61
Use of unprocessed roots	-0.12	1.85	-0.06
Quantity expected at harvesting (Kg)	$2.80e^{-4}$	$1.10e^{-4}$	2.54**
Household size	1.36	0.47	2.90**
Age (years)	-0.33	0.15	-2.18**
Constant	26.05	7.98	3.27
Sigma	12.94	0.89	

Source: Data analysis, 2014; ** indicate statistical significance of the coefficients at 5 % level.

Table 4: Distribution of the Respondents According to Measures Employed to Control the Post-Harvest Loss

Measures Employed	Yes		No		Total	
	Frequency	Percentage (%)	Frequency	Percentage (%)		
Use of skilled workers	12	10.3	105	89.7	117	100.0
Improved facilities	17	14.5	100	85.5	117	100.0
Delayed harvesting	20	17.1	97	82.9	117	100.0
Use of agrochemicals	6	5.1	111	94.9	117	100.0
Harvesting when required	47	40.2	70	59.8	117	100.0
Use of improved facilities	25	21.4	92	78.6	117	100.0

Source: Field survey, 2014.

harvesting stage calls for concern as there is the need for improved methods of mechanizing the harvesting process to reduce the losses at harvesting. Hence, reducing the losses at harvesting by a great percentage would imply a great reduction of the loss across the value chain. The factor responsible for postharvest loss like expected output (losses increase with increase in expected output) foretells a need to improve postharvest technology as this implies there are no adequate facilities to contain the excesses that may be harvested. From the result of the analysis of the study, the economic cost of the loss is \$300 ha^{-1}, which if mitigated and reinvested into the economy would add a required impetus to boost farmers' income and other agribusiness concern and encourage farmers for better productivity. Also, it can be deduced from the findings of the study that the methods of cassava postharvest loss mitigation adopted by farmers are not adequate enough as there are still quite a number of losses at harvesting. Hence, there should be improvements in existing methods of mitigating losses.

The study therefore recommends that should be increased study on the alternative uses of cassava roots (e.g. ethanol production) in order to reduce the already choked garri, flour and starch market and also, concerns on losses at harvesting and methods of mitigating such losses should hold central point in economic policy development and researches. Also, reasonable policies should be implemented to help the farmers reduce the losses, especially by enforcing the use of cassava flour in confectionaries across the nation. This would ensure the optimal use of cassava, hence reducing its postharvest losses.

5 REFERENCES

Abduraheem, K. A and Toluwase, S. O. (2013). Costs and returns analysis of cassava production in Ekiti State, Nigeria. *Journal of Agricultural Science, 3*(10), 454-457. Available online at http://www.scholarly journals.com/SJAS.

Achem, B.A. and Akangbe, J.A. (2011). Assessment of Constraints to Cassava Value Added Enterprises in Kwara State, Nigeria. *Journal of Agricultural Extension*, *15*(1), 124-134.

Atanda, S. A., Pessu, P. O., Agoda, S., Isong, I. U. and Ikotun, I. (2011). The concepts and problems of post–harvest food losses in perishable crops. *African Journal of Food Science, 5*(11), 603-613. Available online at http://www.academicjournals.org/AJFS.

Bokanga, M. (1999). *Cassava: Post-harvest Operations. International Institute of Tropical Agriculture* (IITA), Ibadan. Food and Agriculture Organisation of the United Nations.

de Figueroa, L., Rubenstien, L., & Gonzalez, C. (2001). Utilization of Native Cassava Starch by Yeasts. *Food Microbiology Protocols Journal. Methods in Biotechnology*, 14, 307-317. https://doi.org/10.1385/1-59259-029-2:307

Department of Agriculture, Forestry and Fisheries, South Africa (2010). *Annual Report of the department of Agriculture, Forestry and Fisheries*, The Republic of South Africa.

Ezedinma, C., Ojiako, I., Okechukwu, J., Lemehi,A., Umar, L., Sanni,M., Akoroda, F., Ogbe, E., Okoro, G., and Dixon, A. (2007): "*The cassava food commodity market and trade network in Nigeria*". IITA, Ibadan. Nigeria.

FAOSTAT, 2013. http://faostat.fao.org/DesktopModules/Faostat/Images/T20/ChartPic_d4zg47ghd0udm5xn8as3.png?eb37291b-a547-4acc-b987-31bc12fbac56.

Gulick, P., Hershey, C., and Esquinas A.J. (1983). *Genetic Resources of Cassava and Wild Relatives*. IBPGR/82/111, Rome, pp 8-45.

Jaspreet, A., Anita, R., Joan, F., and Corinne, A. (2013). Estimating Post-Harvest Food Losses: Developing a Consistent Global Estimation Framework. *Selected Poster prepared for presentation at the Agricultural & Applied Economics Association's 2013 AAEA & CAES Joint Annual Meeting, Washington,* DC, August 4-6, 2013.

Jones, W.O. (1959). *Manioc in Africa*. Stanford University Press, USA. pp 315.

Lipinski, B., Hanson, C., Lomax, J., Kitinoja, L., Waite, R., and Searchinger, T. (2013). "*Reducing Food Loss and Waste.*" Working Paper, Installment 2 of Creating a Sustainable Food Future. Washington, DC: World Resources Institute. Available online at http://www.worldresourcesreport.org.

Muhammad-Lawal, A., Omotesho, O.A., and Oyedemi, F.A. (2013). An Assessment of the Economics of Cassava Processing in Kwara State, Nigeria. *Invited paper presented at the 4th International Conference of the African Association of Agricultural Economists*, September 22-25, 2013, Hammamet, Tunisia.

Nweke, F.I. (2003). *New challenges in the cassava transformation in Nigeria and Ghana*. Environment and Production Technology Division (EPTD). Discussion Paper No. 118, International Food Policy Research Institute, Washington DC, USA.

Nweke, F.I. (2004). "*Cassava, A cash crop in Africa*. A Collaborative Study of Cassava in Africa". Working paper. No. 14.

Nweke, F.I., and Enete, A.A. (1999). Gender surprises in food production, processing and marketing emphasis on cassava in Africa: *Collaborative Study of Cassava in Africa (COSCA*). Working paper No. 19. COSCA, IITA, Ibadan, Nigeria.

Oguntade, A.E. (2013). Reducing Food Losses to Improve Food Security: *An Analysis of Cassava and Maize Value Chains in Nigeria*. Report delivered to the GIZ Gmbh, Bonn, Germany.

Olsen, K.M., and Schaal, B.A. (2001). Microsatellites variation in cassava (Manihot esculenta, Euphorbiaceae) and its wild relations: further evidence for a further evidence for a southern Amazonian origin of domestication. *American Journal of Botany, 88*, 131-142. https://doi.org/10.2307/2657133

doi:10.14720/aas.2019.114.2.2 Original research article / izvirni znanstveni članek

Comparative growth analysis, yield and quality of two cowpea (*Vigna unguiculata* L. (Walp.)) lines propagated by seed and stem cuttings

Bolaji Umar OLAYINKA [1], Mubarak Akinbode AKINWUNMI [1], Hameed ALSAMADANY[2], Abdulbaki Shehu ABDULBAKI [2, 3, 4], Yahya ALZAHRANI [2], Emmanuel Obukohwo ETEJERE [1]

Received January 19, 2019; accepted September 29, 2019.
Delo je prispelo 19. januarja 2019, sprejeto 29. septembra 2019.

Comparative growth analysis, yield and quality of two cowpea (*Vigna unguiculata* L. (Walp.)) lines propagated by seed and stem cuttings

Abstract: In the present study, a field experiment was carried out to evaluate and compare the effects of seed planting and stem cutting method of propagation on two cowpea lines (IT07K-243-1-2 and IT07K-298-15). Data assessed were subjected to student t-test to test for the significant effect of the two methods at $p \leq 0.05$. Results showed that growth characters such as plant height, number of leaves, leaf area, above-ground dry mass, leaf area index, leaf area ratio, relative growth rate, net assimilation rate and crop growth rate were significantly enhanced in seed propagation method compared to stem cutting. The yield components such as number of matured pods per plant, pod mass per plant, pod length, pod circumference, pod filling, hundred (100) seed mass, seed mass per plant, average number of seed per pod, harvest index and pod yield per metre square followed similar pattern as recorded for growth characters. Germination potential of the harvested seeds as well as vigour were better in seed planting than stem cutting. Varietal difference showed that line IT07K-298-15 had higher growth, yield and germination potential than IT07K-243-1-2. However, the protein, fat and carbohydrate contents of the latter were higher than the formal. The study concluded that the use of true seed promoted higher productivity. However, planting of stems could still be encouraged on account of a non-significant difference in some of the aforementioned attributes, thereby limiting over-reliance on seed as the main source of planting material for propagating the cowpea lines.

Key words: *Vigna unguiculata;* growth; yield; proximate composition and productivity

Primerjalna analiza rasti, pridelka in kakovosti linij kitajske vinje (*Vigna unguiculata* L. (Walp.)) razmnoženih s semeni in stebelnimi potakjenci

Izvleček: V raziskavi je bil izveden poljski poskus za ovrednotenje in primerjavo učinkov sajenja s semeni in stebelnimi potakjenci dveh linij kitajske vinje (IT07K-243-1-2 and IT07K-298-15). Podatki ocene so bili podvrženi študentskemu t- testu, da bi ugotovili značilne učinke dveh metod razmnoževanja na ravni $p \leq 0,05$. Rezultati so pokazali, da so bili rastni parametri kot so višina rastlin, število listov, listna površina, nadzemna suha masa, indeks listne površine, razmerje med listi in površino tal, relativna prirast, neto asimilacija in rast pridelka značilno večji pri razmnoževanju s semeni v primerjavi s stebelnimi potakjenci. Komponente pridelka kot so število zrelih strokov na rastlino, masa strokov na rastlino, dolžina strokov, obseg strokov, polnjenje strokov, masa 100 semen, masa semen na rastlino, poprečno število semen na strok, žetveni indeks in pridelek strokov na kvadratni meter so imele podoben vzorec kot je bil zabeležen pri rastnih parametrih. Potencial kalitve pridobljenih semen kot tudi njihova vitalnost sta bila boljša pri semenih sejanih rastlin kot pri tistih iz potaknjencev. Razlika v različicah je pokazala, da je imela linija IT07K-298-15 večjo rast, večji pridelek in potencial kalitve kot linija IT07K-243-1-2, a je bila vsebnost beljakovin, maščob in ogljikovih hidratov pri slednji večja od normale. Na osnovi raziskave lahko zaključimo, da so imele rastline vzogojene iz semen večjo produktivnost. Kljub temu pa lahko vzpodbujamo vzgojo rastlin iz stebelnih potakjencev zaradi neznačilnih razlik med nekaterimi zgoraj naštetimi parametri in s tem zmanjšamo odvisnost od semen kot glavnega vira razmnoževalnega materiala kitajske vinje.

Ključne besede: *Vigna unguiculata*; rast; pridelek; sestavine pridelka in produktivnost

1 University of Ilorin, Department of Plant Biology, Ilorin Nigeria
2 King Abdulaziz University, Faculty of Science, Department of Biological Sciences, Jeddah, Saudi Arabia
3 Federal University Dutsin-Ma, Faculty of Life Sciences, Department of Biological Sciences, Katsina State, Nigeria
4 Corresponding author, e-mail: asabdulbaki@fudutsinma.edu,ng

1 INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is of great importance both as staple and fodder crop and it is also used as cover crop to prevent soil erosion and desertification (Singh and Ntare, 1985). Cowpea constitutes a valuable source of protein as well as rich amino acid profile (Ayodele and Yalwa, 2004) and it is one of the widely cultivated leguminous crops in the savannah region of West Africa (Steele, 1996). Cowpea is a dicotyledonous species belonging to the order Fabales, family Fabaceae, sub-family Faboideae, tribe Phaseoleae, sub-tribe Phaseolinae, and genus *Vigna* (Singh, 1993; Padulosi and Ng, 2006). Like other legumes, cowpea forms a symbiotic relationship with a specific group of soil bacteria (*Rhizobium*) which makes atmospheric nitrogen available to the plant through nitrogen fixation (Tutiana et al., 2006).

Cowpea is usually propagated by seeds, however it can be propagated vegetatively using stem cuttings. An advancement in this area has been achieved through *in vitro* micropropagation through organogenesis and embryogenesis. Plant cuttings are segmented parts of plant organs that are used in vegetative propagation. Just like other vegetative propagation methods, the use of cuttings allows the production of clones. When mutations are neglected, clonally propagated plants can be considered as genetically identical to the parental plants. For crops that can be easily propagated by cuttings, this method has numerous advantages. Many new plants can be produced in a limited space from a few stock plants. It is simple and can be easily applied without special techniques such as grafting or budding. It is rapid because there is no need to produce rootstocks. It is also advantageous where a plant does not produce seeds or the seeds are sterile, or whenever seeds are not available (Bareja, 2010).

Over the last two decades, the International Institute of Tropical Agriculture (IITA) has made significant advances in improving the productivity of cowpea in sub-Saharan Africa. A number of varieties have been developed combining diverse plant types, different maturity periods, and resistance to several diseases, insect pests, and parasitic weeds, and possessing other good agronomic traits (Singh et al., 1997). These efforts could become irrelevant where there is paucity of seeds of several cowpea lines released by IITA. Another way to complement the effort of IITA is to try how these cowpea lines will perform when regenerated via vegetative propagation using stem cuttings which is an uncommon practice but can be an effective means of propagation when properly managed. It is this gap in knowledge this study aimed to address by comparing the growth, yield, proximate composition and germination potential of the harvested cowpea seeds propagated through seeds and stem cutting.

This study is therefore aimed at evaluating the regeneration and multiplication potential of cowpea using seed and stem (shoot) cuttings.

2 MATERIALS AND METHODS

2.1 STUDY AREA AND SAMPLE COLLECTION

The experiment was carried out at the Botanical Garden of University of Ilorin (Latitude 8^0, 29'N and Longitude 4^0, 35'E) between August and December, 2016**.** Seeds of the two cowpea (IT07K-243-1-2 and IT07K-298-15) lines were collected from International Institute of Tropical Agriculture, Ibadan.

2.2 SEED TREATMENT

Seeds of the two cowpea lines ('Early; IT07K-243-1-2' and 'Dual-Purpose'; IT07K-298-15) were treated using Dress Force for residual treatment.

2.3 EXPERIMENTAL DESIGN AND SEEDLING PRODUCTION

The plot layout followed a split-plot arrangement where the variety constitutes the main plot and the method of propagation was the sub-plot. The two methods of propagation within the sub-plots followed complete randomized block design with three replications. Plant to plant spacing and within row spacing were 0.3 m and 0.6 m respectively. Seeds of the two cowpea varieties obtained were group into lots A and B. Seeds of lot were planted and the emerged seedlings after four weeks were used as stock. From this stock, stem of 0.12 m in length was cut using sterile knife for planting. The seeds lot B in each variety were sown on the same day the vegetative propagation was carried out from the seedlings obtained from seeds lot A.

2.4 SOIL ANALYSIS

Soil samples were collected from different portions of the plot and subjected to standard soil tests to determine the physico-chemical properties. The organic carbon was determined using modified Walkley and Black wet oxidation method. The percent organic carbon was

multiplied by 1.72 (Van Bemmelen factor) to get percent organic matter. Soil pH was determined by the use of a pH meter. The modified Kjeldahl method was used to determine total nitrogen. Available phosphorus was determined by the Bray-1 test method with dilute acid fluoride as the extractor. The exchangeable base cations were extracted using ammonium acetate at pH 7.0. Calcium and magnesium were determined using the ethylene diamine tetra acetic acid (EDTA) titration method while potassium and sodium were determined by the flame photometer method.

2.5 MORPHOLOGICAL GROWTH CHARACTERS

Parameters such as plant height, number of leaves, stem girth, root and shoot lengths and leaf area were measured. The leaf area was measured using: Leaf area (LA) = 0.75 × (L × B) where 0.75 is the correlation factor (Abayomi and Adedoyin, 2004).

2.6 DRY MATTER PRODUCTION

After documenting the morphological growth parameters mentioned above, the plants were washed in running water after discarding the roots. Then, the above-ground plant parts in each experimental plot were packed in a polythene bags. Thereafter, these plants were oven dried at 75 ^{0}C to constant mass and the above-ground dry mass was then measured using MP1001 Electronic Balance with precision to 0.1g.

2.7 PHYSIOLOGICAL GROWTH CHARACTERS

Physiological growth parameters such as leaf area ratio (LAR), l area index (LAI), net assimilation rate (NAR), crop growth rate (CGR) and relative growth rate (RGR) were measured as secondary data from above-ground-dry mass and leaf area. The formulae of the various growth analysis components were as follows

$$LAR = \frac{\text{Leaf area } (m^2)}{\text{Above-ground dry mass (g)}}$$

$$LAI = \frac{\text{Leaf areaper plant } (m^2)}{\text{Area of canopy of plant } (m^2)}$$

of Watson (1952) as adopted by El. Naim et al. (2011) and Olayinka and Etejere (2015).

$$NAR = \frac{W2 - W1\ (Log\ eL2 - Log\ eL1)}{L2 - L1\ (t2 - t1)}$$

g m^{-2} day^{-1} described by Evans (1972) and Abayomi and Adedoyin (2004)

Crop growth rate = net assimilation rate (NAR) × leaf area index (LAI) g m^{-2} day^{-1} described by Evans (1972) and Causton and Venus, (1981).

Relative growth rate:

$$(RGR) = \frac{Log\ eW2 - Log\ eW1}{t1 - t2}\ (g\ g - 1\ day - 1)$$

Chlorophyll content was quantified according to the method described by Jnandabhiram and Sailen (2012).

2.8 PROXIMATE COMPOSITION

Proximate parameters such as moisture, ash, fibre, fat, protein and carbohydrate by difference of the air-dry harvested seeds from the two cowpea lines were determined following the methods described by AOAC (2000).

3 RESULTS AND DISCUSSION

3.1 SOIL CHARACTERISTICS

The initial pre-planting soil properties of the experimental site show that the soil is marginally fertile which implies that the soil is low in N content, organic matter, available phosphorus exchangeable bases and exchangeable cations following the soil quality ranges of ederal Ministry of Agriculture and Natural Resources (FMANR, 1990). This further implies that the soil is poor in nutrients and of low productivity. The observed 6.80 value of the pH of the soil indicates that the soil is slightly acidic and this can be attributed to the high rainfall prevalent in the area at the time of collection. Rainfall leads to leaching of the basic cations from the surface area of the soil (Agbogidi and Egho, 2012). The low organic matter content and total nitrogen could be attributed to the effects of soil erosion, leaching and bush burning predominant in the study area. Similarly, the low exchangeable cations may be due to the low clay activity and low organic content (0.48) of the soil. The CEC was low (6.76 cmol kg^{-1}) while the base saturation (67.92 %) which provides an index of soil weathering, indicates that the site has moderate fertility status. Savanna soils are inherently low in nutrients particularly nitrogen and phosphorus (Haruna et al., 2011; Haruna and Usman, 2013).

Table 1: Physical and chemical properties of the experimental surface soil (0-15 cm) before planting in 2016

Soil characteristics		
Physical properties	Particle sizes	Percent distribution (%)
	Sand	86.4
	Silt	8.9
	Clay	4.7
	Textural Class	Loamy sand
Chemical properties		
	pH (H_20)	6.80
	OC	0.28
	OM	0.48
	TKN	0.16
	AP (mg kg^{-1})	3.87
	Ca (cmol kg^{-1})	5.83
	Mg (cmol kg^{-1})	0.62
	K (cmol kg^{-1})	0.11
	Na (cmol kg^{-1})	0.15
	H + Al (cmol kg^{-1})	0.05
	ECEC (cmol kg^{-1})	6.76
	Base saturation (%)	67.92

OC = Organic carbon; OM = Organic matter; TKN = Total Kjeldah Nitrogen, AP = Available Phosphorus, Ca = Calcium; Mg = Magnesium; K = Potassium; Na = Sodium, H + Al = Exchangeable Acidity, and ECEC = Effective Cation Exchangeable Capacity.

3.2 GROWTH RESPONSE

The growth performance in terms of plant height, number of leaves, stem girth and leaf area were significantly higher in all the seed-propagated plants (Figures 1, 2, 3 and 4). The high expression of these growth attributes in all the seed propagated cowpea, compared to those propagated by stem cuttings could be due to differences in distribution of endogenous hormones. According to Li et al. 2011, morphogenesis of shoot is highly influenced by the plant hormones. In both lines, *V. unguiculata* propagated by stem cuttings followed the same growth pattern as those propagated by seed, but maintained lower magnitude from 2 weeks after planting (WAP) until final date of sampling (10 WAP). A contrasting pattern was observed only in the plant height of line IT07K-243-1-2 where the stem propagated plants showed higher magnitude. IT07K-243-1-2 recorded higher peak than IT07K-298-15 showing genotypic variation which is agreed with the findings of Agbogidi and Egho, (2012), in evaluation of cowpea varieties in agro-ecological environment. Reduced shoot development in the stem propagated *V. unguiculata* is as a result of the plants committing their carbohydrate to rooting rather than photosynthesis.

Root characteristics (root length and root-shoot ratio) significantly differed ($p < 0.05$) with respect to methods of propagation and genotype (Figures 5 and 6). In both lines, greater root length was recorded by the seed propagated *V. unguiculata* as compared to those regenerated through stem cuttings. This could be attributed to differing concentrations of the endogenous growth regulators. Li et al. (2011) in their review on regeneration in cowpea inferred that higher ratio of auxin to cytokinin concentration induces rooting of explants (stem cuttings) in a regeneration system. Root-shoot ratio which refers to the ratio of the root mass to mass of the shoot (Harris, 1992) showed significance difference between the propagation methods. In both lines, the stem propagated cowpea recorded higher ratios especially at 3 and 6 weeks after planting, an indication of acclimatization after transplanting.

This also showed that cowpea propagated by stem cutting absorbed less nutrient at the time when increase in rooting volume was predominant. According to Harris (1992), factors which improve growing conditions cause reduction in root-shoot ratio.

3.3. DRY MATTER PRODUCTION

Dry matter production of the two cowpea lines tested differed significantly with respect to methods of propagation (Figure 5). Seed propagated cowpea accumulated more dry matter and this can be an indication of the unpronounced growth observed in the stem propagated plants. However, the same pattern of increase was observed for both methods and genotypic difference was evident since line IT07K-243-1-2 recorded a greater peak for seed while IT07K-298-15 recorded greater peak for stem propagated. The similar pattern of increase in dry matter (above-ground dry mass) with time, agreed with the studies of Addo-Quaye et al. (2011) on dry matter production of three varieties of cowpea in two agro-ecological zones.

Leaf area ratio increased in magnitude followed by a decrease in line IT07K-243-1-2, a contrasting pattern was observed in IT07K-298-15 which decreased linearly until the final date of sampling, regardless of the methods of propagation (Figure 6). The seed propagated plants recorded higher peak than the stem propagated plants, an estimate of the level of reproductive strategy based on light and moisture absorbed by the plants. The lower magnitude recorded by the stem propagated of *V. unguiculata* could result from efforts

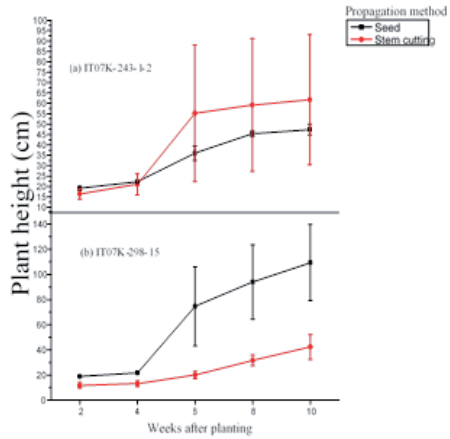


Figure 1: Plant height of *V. unguiculata* (a) IT07K-243-1-2 and (b) IT07K-298-15 as affected by different propagation methods, Vertical bars represent standard errors of mean (SEM).

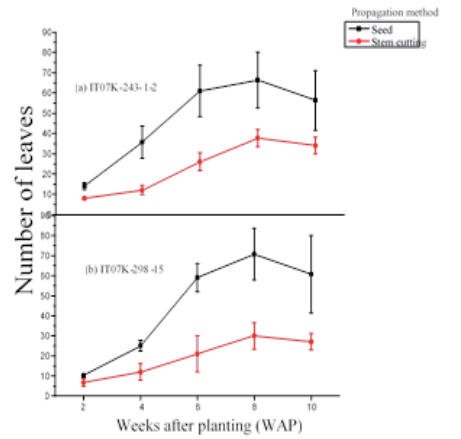


Figure 2: Number of leaves of *V. unguiculata* as affected by different propagation methods in (a) IT07K-243-1-2 and (b) IT07K-298-15. Vertical bars represent standard errors of mean (SEM).

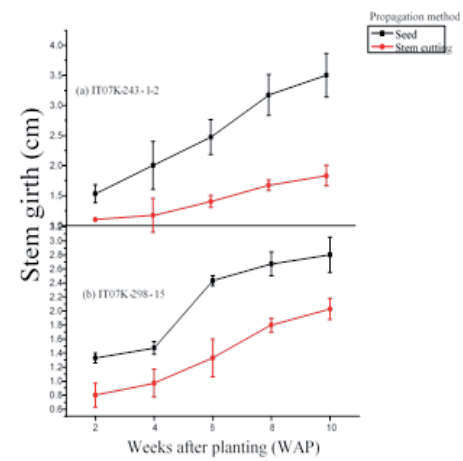


Figure 3: Stem girth of *V. unguiculata* as affected by different propagation methods in (a) IT07K-243-1-2 and (b) IT07K-298-15. Vertical bars represent standard errors of mean (SEM).

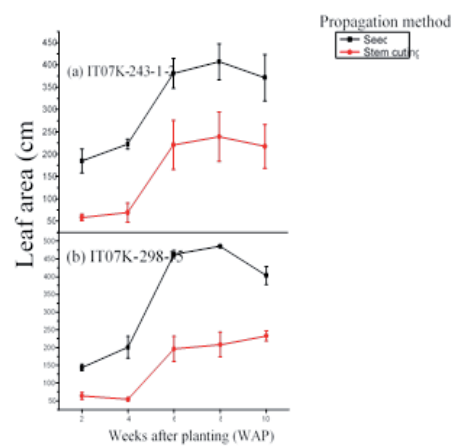


Figure 4: Leaf area of *V. unguiculata* as affected by different propagation methods in (a) IT07K-243-1-2 and (b) IT07K-298-15. Vertical bars represent standard errors of mean (SEM).

of the plant to conserve the acquired resources, according to Marron et al. (2003).

Relative growth rate, which is the rate of increase in dry mass already accumulated per unit time, was significantly affected by methods of propagation and genotype (Figure 9). There was no significant difference in RGR between the two methods of propagation in line IT07K-243-1-2 as they also followed similar pattern. Seed propagated plants recorded higher peak. RGR in IT07K-298-15 statistically differed with respect to the propagation methods with the stem propagated plants recording the higher peak. The difference in RGR further depicts the varietal differences.

Leaf area index, a descriptor of the size of the assimilatory apparatus of the plant stand, is said to be the primary factor that determines the rate of dry matter production in a stand. It also reflects differences in productive efficiency between crop varieties (Kvet et al., 1971). Leaf area index was significantly affected by the methods of propagation and genotype. Regardless of the propagation method and line, leaf area index was increasing with time until 63 days and then decreased until 84 days (Figure 10). This agreed with the pattern in leaf area index as reported by Addo-Quaye et al. (2011). For the seed propagated cowpea, IT07K-298-15 showed greater productive efficiency while IT07K-243-1-2

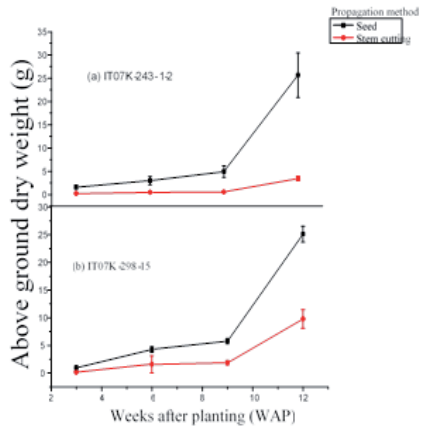


Figure 5: Above ground mass of *V. unguiculata* as affected by different propagation methods in (a) IT07K-243-1-2 (b) IT07K-298-15. Vertical bars represent standard errors of mean (SEM).

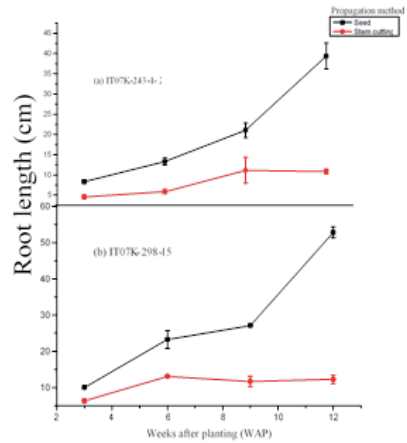


Figure 6: Root length of *V. unguiculata* as affected by different propagation methods in (a) IT07K-243-1-2 and (b) IT07K-298-15. Vertical bars represent standard errors of mean (SEM).

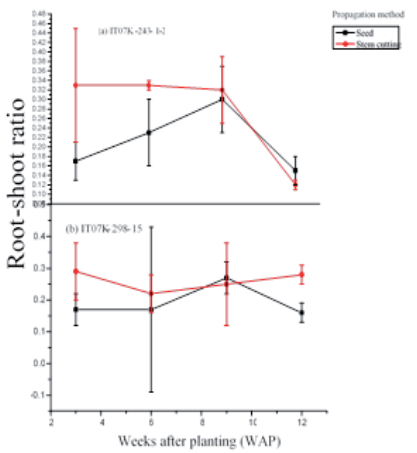


Figure 7: Root-shoot ratio of *V. unguiculata* as affected by different propagation methods in (a) IT07K-243-1-2 and (b) IT07K-298-15. Vertical bars represent standard errors of mean (SEM).

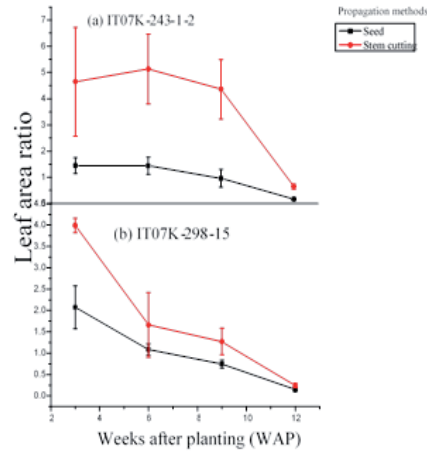


Figure 8: Leaf area ratio of *V. unguiculata* as affected by different propagation methods in (a) IT07K-243-1-2 and (b) IT07K-298-15. Vertical bars represent standard errors of mean (SEM).

showed greater efficiency for those propagated by stem cutting, another indication of varietal difference. The larger area covered by the seed propagated plants contributed to the large leaf area indices produced. Similarly, Terao et al. (1995) found that cowpea varieties with more spread collected more light than those with lesser spread and consequently produced more leaves, which resulted in larger leaf area indices. The pattern of leaf area development in this study is similar to those reported by Olayinka and Etejere (2015).

Net assimilation rate, which is the rate of dry mass increase per unit leaf area per unit time, showed significant difference between the methods at sampling interval of 63-84 days in IT07K-243-1-2 and 42-63 days in IT07K-298-15 (Figure 11). Differing pattern of increase in NAR agreed with those reported by Addo-Quaye et al. (2011). Regardless of the genotype, the seed propagated plants recorded greater values of NAR, an indication that their leaves are more efficient in producing dry matter than those regenerated by stem cutting.

Crop growth rate, an index of agricultural productivity significantly differed between the methods of propagated in the two cowpea lines tested (Figure 12). A similar pattern of slight decrease followed by rapid increase in CGR was observed in both lines.

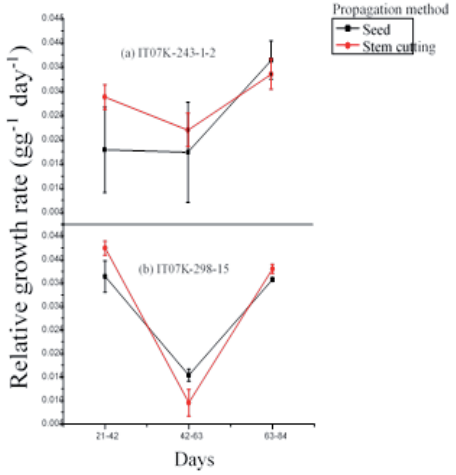


Figure 9: Relative growth rate of *V. unguiculata* as affected by different propagation methods in (a) IT07K-243-1-2 and (b) IT07K-298-15. Vertical bars represent standard errors of mean (SEM).

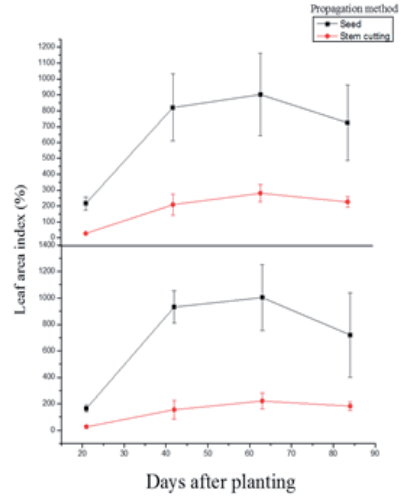


Figure 10: Leaf area index of *V. unguiculata* as affected by different propagation methods in (a) IT07K-243-1-2 and (b) IT07K-298-15. Vertical bars represent standard errors of mean (SEM).

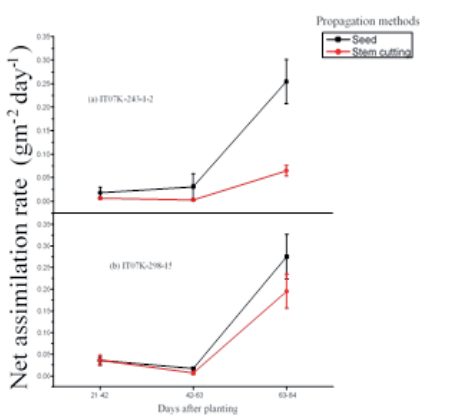


Figure 11: Net assimilation rate of *V. unguiculata* as affected by different propagation methods in (a) IT07K-243-1-2 and (b) IT07K-298-15. Vertical bars represent standard errors of mean (SEM).

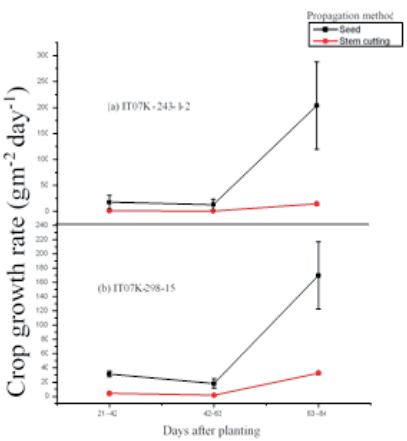


Figure 12: Crop growth rate of *V. unguiculata* as affected by different propagation methods in (a) IT07K-243-1-2 and (b) IT07K-298-15. Vertical bars represent standard errors of mean (SEM).

Cowpea propagated by seed showed greater CGR than those propagated by stem cutting. Since CGR measured the accumulation of dry matter per unit time, it was, therefore a reasonable approximation of the canopy photosynthetic rate per unit ground area (Clawson et. al., 1986). The significantly higher CGR values of IT07K-243-1-2 as against IT07K-298-15, suggested that the stands of this line produced more dry matter per unit ground area than IT07K-298-15 in spite of its lower LAI and NAR. Results of crop growth rate in this study aligned with the report of Addo-Quaye et al. (2011) and Olayinka and Etejere (2015).

3.4 CHLOROPHYLL CONTENT

The effects of methods of propagation and genotype showed significance on the chlorophyll content of *V. unguiculata* (Figures 13, 14 and 15). Similar trend in chlorophyll content was observed in the methods within each line of cowpea. Between the lines, different patterns were observed in chlorophyll a, b and total chlorophyll, an implication of the effect of genotype. Chlorophyll-*a* and Chlorophyll-*b* attributes to the accumulation of solutes in the cell sap through passive accumulation resulting from reduced cell size (Morgan, 1984) which significantly does osmotic adjustment

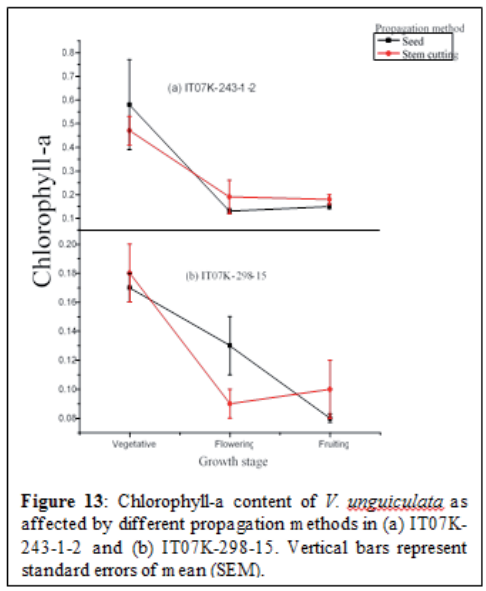


Figure 13: Chlorophyll-a content of *V. unguiculata* as affected by different propagation methods in (a) IT07K-243-1-2 and (b) IT07K-298-15. Vertical bars represent standard errors of mean (SEM).

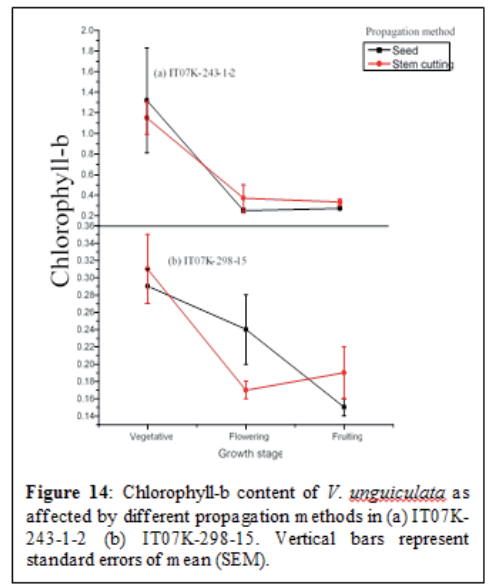


Figure 14: Chlorophyll-b content of *V. unguiculata* as affected by different propagation methods in (a) IT07K-243-1-2 (b) IT07K-298-15. Vertical bars represent standard errors of mean (SEM).

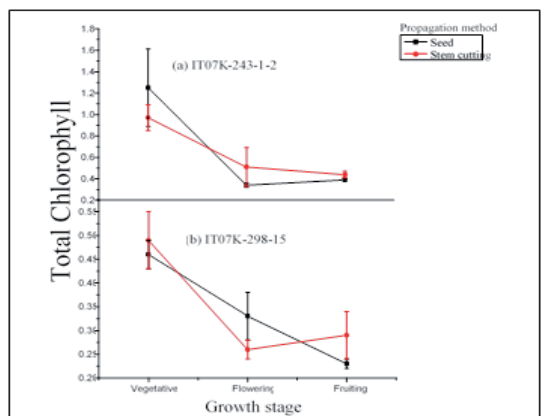


Figure 15: Total chlorophyll content of *V. unguiculata* as affected by different propagation methods in (a) IT07K-243-1-2 and (b) IT07K-298-15. Vertical bars represent standard

($\Delta\psi\pi$). Non-stomatal restrictions on CO_2 assimilation can be effectively assessed through measuring chlorophyll based parameters. Energy status of the chloroplast increases as a consequence of increased amount of total chlorophyll and Chl *a* and Chl*b* (Ranjbarfordoei et al., 2000).

3.4.1 Yield and yield components

The results of the yield components and yield such as number of matured pod per plant, pod weight per plant, pod length, pod circumference, pod filling, hundred (100) seed weight, seed weight per plant, average number of seed per pod, harvest index and pod yield per metre square followed similar pattern as recorded for growth characters.

In both lines, relatively low yield was recorded, regardless of the method of propagation. This could be as a result of low phosphorus content of the soil. Phosphorus, one of the most needed elements for crop production is critical to cowpea yield (Haruna and Usman, 2013). This is because it is reported to stimulate growth, initiate nodule formation and also influences the efficiency of rhizobium-legume symbiosis (Haruna and Aliyu, 2011). All growing plants require phosphorus for growth and development in significantly larger amount.

Table 2: Reproductive character and yield components of *Vigna unguiculata* lines as influenced by different propagation methods

Lines	Methods	No of matured pod per plant	Pod mass per plant (g)	Pod length (cm)	Pod circumference (cm)	Pod filling	Number of days to 50 % flowering
IT07K-243-1-2	Seed	18.67+1.20a	15.87+0.12a	17.62+1.00a	2.72+0.13a	0.44+0.04 a	41.33+1.86a
	Stem cutting	6.67+0.88b	7.02+0.81b	13.88+0.48b	2.21+0.16a	0.28+0.04 a	32.00+1.16 a
	P-value	0.016	0.006	0.022	0.103	0.137	0.088
IT07K-298-15	Seed	16.33+0.88 a	17.32+0.44 a	17.41+0.78 a	2.70+0.17 a	0.36+0.03 a	48.00+1.16 a
	Stem cutting	6.33+0.88b	7.33+0.48b	12.26+1.21 a	1.87+0.11 a	0.28+0.06 a	55.33+1.20b
	P-value	0.003	0.008	0.100	0.087	0.145	0.037
Mean	IT07K-243-1-2	12.00+1.53	8.84+0.71	3.74+0.56	0.51+0.18	0.15+0.06	9.33+2.96
	IT07K-298-15	10.00+0.58	9.98+0.92	5.16+1.77	0.83+0.26	0.80+0.03	7.33+1.45
	P-value	0.058	0.039	0.101	0.149	0.382	0.076

Within variety along the column means followed by the same superscript are not significantly different at $p < 0.05$

Table 3: Yield of *V. unguiculata* lines as influenced by different propagation methods and genotype

Lines	Methods of propagation	Number of seed per plant	100 seed mass (g)	Seed massper plant (g)	Average No of seed per pod	Harvest index	Pod yield (g m-2)
IT07K-243-1-2	Seed	55.33+6.98 a	17.47+0.46 a	9.02+1.02 a	7.93+0.31 a	0.24+0.05 a	40.10+4.52 a
	Stem cutting	24.67+0.88 a	11.20+0.76b	2.29+0.14b	4.31+0.68b	0.38+0.04 a	10.18+0.61b
	P-value	0.060	0.036	0.028	0.035	0.243	0.028
IT07K-298-15	Seed	49.00+3.46 a	14.84+0.29 a	8.04+0.51 a	6.44+0.87 a	0.22+0.00 a	35.73+2.28 a
	Stem cutting	18.67+1.20	12.02+0.33	3.03+0.23	3.78+0.22	0.20+0.02	13.45+1.01
	P-value	0.012b	0.010b	0.006b	0.094 a	0.462 a	0.006b
Means	IT07K-243-1-2	40.00+3.93	14.34+0.61	5.66+0.58	6.12+0.49	0.31+0.05	25.14+2.57
	IT07K-298-15	33.84+2.33	13.43+0.31	5.54+0.37	4.51+0.55	0.21+0.01	24.59+1.65
P-value		0.053	0.021	0.007	0.096	0.121	0.007

Within variety along the column means followed by the same superscript are not significantly different at $p < 0.05$.

3.5 GERMINATION POTENTIAL

The results indicated that the two methods of propagation could be said to appropriate for raising the two cowpea lines studied. However, germinability in terms of percentage germination, speed of germination seedling vigour in both cowpea lines were generally better when raised from seed planting than those obtained from stem cutting. The varietal effect showed that line IT07K-298-15 had better performance than line IT07K-243-1-2. Differential germination potential between the two lines can be attributed to the different genetic make-up. This is in agreement with results of germination potential of several cultivars of groundnut (Ahmed et al., 2017).

3.6 PROXIMATE COMPOSITION

Proximate components were significantly ($p \leq 0.05$) affected by propagation methods (Table 5) except for protein recorded in IT07K-298-15. Statistically similar values were recorded with respect to genotypes. Regardless of the propagation methods, IT07K-243-1-2 recorded higher protein, fat and carbohydrate while IT07K-298-15 recorded higher moisture ash and fibre contents. Protein content recorded in both lines was within the range of values (20-27 %) reported by Duke (1981) and Longe (1980).

4 CONCLUSION

Results of the present study show that seed planting is still better method of propagation. However, stem could still be encouraged on account of non-significant difference in some of the aforementioned attributes, thereby limiting over-reliance on seed as source of material for propagating the cowpea lines. Concerted

Table 4: Germination potential and seedling growth of the seeds harvested from two cowpea lines grown under different methods of propagation

Lines	Methods of propagation	Germination (%)	n	Seedling Vigour	Shoot length (mm)	Seminal root length (mm)
IT07K-243-1-2	Seed	20.00 ± 5.77 [a]	0.40 ±0.0.12 [a]	590.0 ±181.9 [a]	12.7±0.15 [a]	17.0±0.06 [a]
	Stem Cutting	13.33±3.33 [a]	0.27±0.07 [a]	393.33± 83.38 [a]	13.5±0.76 [a]	16.8±0.02 [a]
	P-value	0.529	0.529	0.531	0.406	0.742
IT07K-298-15	Seed	63.33±17.64 [a]	4.07±0.98 [a]	2071.70±578.62 [a]	13.4±0.03 [a]	19.3±0.33 [a]
	Stem cutting	56.67±17.64 [a]	3.67±1.71 [a]	1802.00±571.95 [a]	13.4±0.09 [a]	18.5±0.14 [a]
	P-value	0.826	0.868	0.777	0.866	0.668
Lines Mean	IT07K-243-1-2	16.67±4.50	0.34±0.041	491.67±132.65	12.9±0.46	16.9±0.04
	IT07K-298-15	60.00±17.64	3.87±1.35	1936.85±57529	13.4±0.06	18.9±0.24
p-value		<0.001	0.300	0.099	0.500	0.344

Within variety along the column means followed by the same superscript are not significantly different at $p < 0.05$..

Table 5: Proximate composition of V. unguiculata lines as influenced by different propagation methods and genotype

Lines	Propagation methods	Moisture	Ash	Fibre	Protein	Fat	Carbohydrate
IT07K-243-1-2	Seed	16.42±0.51 a	0.95±0.01 a	3.99±0.07 a	21.25±0.15 a	5.05±0.09 a	52.34±0.35 a
	Stem cutting	14.63±0.17 a	0.69±0.23 a	3.12±0.22 a	21.25±0.15 a	4.19±0.15 a	56.13±0.76 a
	P-value	0.063	0.729	0.135	0.064	0.684	0.861
IT07K-298-15	Seed	17.54±6.68 a	1.19±0.24 a	3.76±0.24 a	21.23±0.19 a	4.57±0.31 a	51.69±6.39 a
	Stem cutting	13.91±1.63 a	1.09±0.32 a	3.52±0.19 a	21.24±0.18b	4.48±0.32 a	55.76±1.98 a
	P-value	0.293	0.244	0.848	0.022	0.662	0.406
Means	IT07K-243-1-2	15.52±0.89	0.82±0.13	3.55±0.44	21.25±0.00	4.62±0.43	54.24±1.89
	IT07K-298-15	15.73±1.81	1.14±0.05	3.64±0.12	21.24±0.00	4.53±0.05	53.73±2.03
P-value		0.000	0.000	0.000	0.000	0.000	0.000

Within variety along the column means followed by the same superscript are not significantly different at $p < 0.05$.

efforts should be made to evaluate the effects of stem cutting method on the genetic variability of these cowpea lines.

5 REFERENCES

Abayomi, Y.A. and Adedoyin, G.A. (2004). Effects of planting dates and nitrogen fertilizer application on growth and yield of its contrasting genotypes of maize (*Zea mays* L.) I: leaf growth and its relationship with grain yield. *Journal of Agricultural Research & Development*, *3,* 83-97. https://doi.org/10.4314/jard.v3i1.42215

Addo-Quaye, A.A., Darkwa, A.A. and Ampiah, M.K.P. (2011). Performance of three cowpea (*Vigna unguiculata* (L.) Walp) varieties in two agro-ecological zones of Central Region of Ghana I: Dry matter production and growth analysis. *ARPN Journal of Agricultural and Biological Sciences*, *6*(2), 1-9.

Agbogidi, O.M. and Egho, E.O. (2012). Evaluation of eight varieties of cowpea (*Vigna unguiculata* (L). Walp) in Asaba agro-ecological environment, Delta State, Nigeria. *European Journal of Sustainable Development*, *1*(2), 303-314.

Ahmed, O., Olayinka, B.U., Garuba, T., Ahmed, J. and Etejere, E.O. (2017). Germination of several cultivars of groundnut in relation to incidence of fungi. *Science World Journal, 12*(1), 38-41.

Ayodele, J. T. and Yalwa, I. R. (2004). Amino Acid composition of *Vigna dekindtiana*. *Biological and Environmental Sciences Journal for the Tropics*, *1*(2), 120-126.

AOAC. (2000). *Official method of analysis* (17th Edition). Volume 1. Association of Official Analytical Chemists. Inc., Maryland, USA.

Bareja, B.G. (2010). The What, When and Why Of Using Plant Cuttings In Vegetative Propagation. *Crops Review*. Available at http://www.cropsreview.com/plant-cuttings.html.

Causton, D.R. and Venus, J.C. (1981). *The biometry of plant*

growth. London: Edward Arnold Center AgricSci (JIRCAS). Sayce, Devon, UK, pp. 1–12.

Clawson, K.L., Specht, J.E. and Blad, B.L. (1986). Growth analysis of soyabean isolines differing in pubescence density. *Agronomy Journal, 78*, 164-172. https://doi.org/10.2134/agronj1986.00021962007800010033x

Duke, J.A. (1981). *Handbook of legumes of world economic importance*. New York: Plenum Press. https://doi.org/10.1007/978-1-4684-8151-8

El-Naim, A.M., Eldouma, M.A., Ibrahim, E.A., Zaied, M.M.B. (2011). Influence of plant spacing and weeds on growth and yield of peanut (*Arachis hypogaea* L.) in rain-fed of Sudan. *Advances in Life Sciences*, *1*(2), 45–48. https://doi.org/10.5923/j.als.20110102.08

Evans, G.C. (1972). *The quantitative analysis of plant growth*. Oxford: Blackwell Scientific Publications.

Harris, R.W. (1992). Root-shoot ratio. *Journal of Arboriculture*, *18*(1), 39-42.

Haruna, I.M., Aliyu, L., Olufajo, O.O. and Odion, E.C. (2011). Growth of sesame (*Sesamum indicum* L.) as influenced by poultry manure, nitrogen and phosphorus at Samaru, Nigeria. Am. Eurasian. *Journal of Agriculture and Environmental Science, 4*, 561-568. https://doi.org/10.3923/ajcs.2013.209.215

Haruna, I.M. and Usman, A. (2013). Agronomic efficiency of cowpea varieties (*Vigna unguiculata* L. Walp.) under varying phosphorus rates in Lafia, Nassarawa State, *Nigeria. Asian Journal of Crop Science*, *5*(2), 209-215.

Jnandabhiram, C. and Sailen, P.B. (2012).Water stress effects on leaf growth and chlorophyll content but not the grain yield in traditional rice (*Oryza sativa* Linn.) genotypes of Assam, India II. Protein and proline status in seedlings under PEG induced water stress. *American Journal of Plant Sciences*, *3,* 971-980. https://doi.org/10.4236/ajps.2012.37115

Kvet J., Ondok J.P., Necas J. and Jarvis P.G. (1971). Methods of growth analysis. In: Sestak, Z., Catsky, J. and Jarvis, P.G. (Eds.), *Plant Photosynthetic Production*: Manual of Methods. Dr W. Junk N.V., The Hague. pp. 343-391.

Li, X.M., Li, J. Li, M., Tang, Y., Li, X. and Chen, L. (2011). A review on regeneration of cowpea (*Vigna unguiculata* L. Walp). *Journal of Agricultural Science and Technology*, *5*(4), 525-532.

Longe, O.G. (1980). Carbohydrate composition of different varieties of cowpea (*Vigna unguiculata*). *Food Chemistry*, *6*, 153-161. https://doi.org/10.1016/0308-8146(80)90031-X

Maroon, N., Dreyer, E., Boudouresque, E., Delay, D., petit, J.m., Delmotte, F.M. and Brignolas, F. (2003). Impact of successive drought and re-watering cycles on growth and specific leaf area of two *Populus canadensis* (Moench) clones. *Tree Physiology*, *23,* 1225-1235. https://doi.org/10.1093/treephys/23.18.1225

Morgan, J.M. (1984). Osmoregulation and water stress in higher plants. *Annual. Review of Plant Physiology, 35,* 299-319. https://doi.org/10.1146/annurev.pp.35.060184.001503

Olayinka, B.U. and Etejere, E.O. (2015). Growth analysis and yield of two varieties of groundnut (*Arachis hypogea* L.) as influenced by different weed control methods, *Indian Journal of Plant Physiology*, *20*(2), 130-136. https://doi.org/10.1007/s40502-015-0151-x

Padulosi, S., Ng, N.Q. (1997). Origin, taxonomy, and morphology of *Vigna unguiculata* (L.)Walp. In Singh B.B.,Mohan Raj, D.R., Dashiell, K.E., Jackai, L.E.N. (eds). *Advances in Cowpea Research*. Co-publication Intl Inst Tropical Agric (IITA) and Japan Intl. Res.

Ranjbarfordoei, A. Samson, R. Damne, P. V. and Lemeur, R. (2000). Effects of drought stress induced by polyethylene glycol on pigment content and photosynthetic gas exchange of *Pistacia khinjuk* and *P. mutica*. *Photosynthetic*, *38*(3), 443-447. https://doi.org/10.1023/A:1010946209484

Singh, B. B. and N'tare, (1985). Development of improved cowpea varieties in Africa. In: Singh, S.R. and Rachie, K.O. *Cowpea Research, Production and Utilization*. U.K.: John Wiley and Sons Chichester 105-115.

Steele, W. M. (1996). Cowpeas. In*:* N.W. Simmonds. *Evolution of Crop Plants*, London, UK: Longman. 183-185.

Terao, T., Watanabe, I., Matsunaga, R., Hakoyama, S. and Singh, B.B. (1995). Analysis of agro-physiological constraints in intercropped cowpea. *Cowpea Abstracts, Second World Cowpea Research Conference*, 3-7 September, Accra, Ghana.

Tutiana, K.W.O., Ibrahim, N., Cire, E.S. and Marc, N. (2006). Water condition Effects on Rhizobia competition for cowpea nodule occupancy. *African Journal of Biotechnology*, *5*(16), 1457-1463.

Watson, D. (1952). The physiological basis of variation in yield. *Advances in Agronomy*, *4,* 101-145. https://doi.org/10.1016/S0065-2113(08)60307-7

doi:10.14720/aas.2019.114.2.3 Original research article / izvirni znanstveni članek

Effects of planting dates on the growth and grain yield of two indigenous varieties of pearl millet (*Pennisetum glaucum* (L.) R.Br.) in a forest-savanna transition zone of Edo State, Nigeria

Sunday Ebonka NWAJEI [1,2], Anthony Uhunomwan OMOREGIE [1], Felix Omonkeke OGEDEGBE [1]

Received September 23, 2018; accepted October 10, 2019
Delo je prispelo 23. septembra 2018, sprejeto 10. oktobra 2019.

Effects of planting dates on the growth and grain yield of two indigenous varieties of pearl millet (*Pennisetum glaucum* (L.) R.Br.) in a forest-savanna transition zone of Edo State, Nigeria

Abstract: Effects of planting dates on the growth and grain yield of two indigenous varieties of pearl millet was carried out at the Teaching and Research Farm, Ambrose Alli University, Ekpoma. The experiment was a 2 × 5 factorial scheme fitted into a randomized complete block design with the two varieties of pearl millet ('Gero Bida' and 'Gero Badeggi') and five planting dates (April, May, June, July and August) replicated three times. The results obtained showed that delays in planting date significantly reduced growth in pearl millet examined. Similar pattern was observed on days to 50 % flowering and grain maturity. Improved growth with early sowing could have allowed increased availability of assimilates that later supported reproductive growth. These assimilates were remobilized under unfavourable climatic condition in the late cropping season to the reproductive structures. 'Gero Badeggi' sown in April, had significantly, the highest growth in the measured characters than 'Gero Bida' sown in August and other dates. 'Gero Badeggi' planted in May had the highest total grain yield (9.33 t ha^{-1}) while 'Gero Bida' planted in July had the smallest (4.27 t ha^{-1}). Therefore, 'Gero Baddegi' planted in May is a better variety for pearl millet grain production in Ekpoma.

Key words: pearl millet; *Pennisetum glaucum*; planting date; growth; yield; forest savanna; indigenous variety

Učinki datuma setve na rast in pridelek zrnja dveh avtohtonih sort biserne proso (*Pennisetum glaucum* (L.) R.Br.) v prehodnem območju gozdnate savane, država Edo, Nigeria

Izvleček: Učinki datuma setve na rast in pridelek zrnja dveh avtohtonih sort biserne proso so bili preučevani na Research Farm, Ambrose Alli University, Ekpoma. Poskus je bil narejen kot 2 × 5 faktorski naključni bločni poskus z dvema sortama biserne proso ('Gero Bida' in 'Gero Badeggi'), petimi datumi setve (april, maj, junij, julij in avgust), s tremi ponovitvami. Rezultati so pokazali, da je zakasnitev v datumu setve značilno zmanjšala rast obeh sort. Podoben učinek je bil na dneve do 50 % cvetenja in zrelost zrnja. Izboljšana rast po zgodnji setvi je povečala količino asimilatov, kar je potem povečalo reproduktivno rast. Ti asimilati so se remobilizirali kasneje v neugodnih klimatskih razmerah proti koncu rastne sezone in povečali tvorbo reproduktivnih struktur. Sorta Gero Badeggi, sejana aprila je imela značilno največjo rast med merjenimi parametri, v primerjavi s sorto Gero Bida, posejano avgusta in v drugih terminih. 'Gero Badeggi', posejana maja je imela največji celokupni pridelek zrnja (9,33 t ha^{-1}), 'Gero Bida'posejana julija pa je imela najmanjšega (4,27 t ha^{-1}). Zaradi naštetega je 'Gero Baddegi', posejana maja, primernejša sorta za pridelovanje zrnja biserne proso v Ekpomi.

Ključne besede: biserno proso; *Pennisetum glaucum*; datum setve; rast; pridelek; gozdnata savana; avtohtona sorta

1 Ambrose Alli University, Department of Crop Science, Ekpoma, Edo State, Nigeria
2 Corresponding author, e-mail: sodje4real10@gmail.com

1 INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L.) R.Br.) is a drought tolerant cereal typically grown as grain crop in semi-arid and dry areas of the world (Leila, 2008). The crop can potentially be planted as a double crop in northern areas of Nigeria. This crop produces high quality grains than any other cereals under extreme conditions like unfertile soil, intense heat and prolong drought (Amanullah et al., 2015).

The pearl millet is a cereal crop grown mostly for its grain and fodder. It is a robust, tufted, tillering annual grass, up to 300 cm high (Khan et al., 2003). Pearl millet is locally known as "Gero" and an important grain and fodder crop for savanna areas of Nigeria (Uzoma et al., 2010). Pearl millet can grow in areas with annual rainfall of 250 – 900 mm with optimum temperature of 30 – 35 °C (Raemaeker, 2001). The ideal soil pH range is 5.5 – 7.0, but it can grow in soil with pH as low as 4 and as high as 8.3 (Fahmy et al., 2010).

The world's area planted with pearl millet is about sixty five million hectares, with the major part in India and Africa (Raemaeker, 2001). World production of pearl millet stands at around 30 million tonnes annually with 97 % of this figure produced in developing countries (Gabatshele et al., 2014). In Africa, the total production of this crop is about 14 million tonnes annually, with the highest percentage produced in West Africa. In West Africa, about 13 million tonnes is produced with Nigeria, Niger, Burkina-Faso, Chad, Mali and Senegal contributing about 94 % of this annual figure. In Nigeria, the annual production record stands at about 5 million tonnes (Amodu et al., 2005).

The pearl millet is of great importance in the arid and semi-arid tropics, where it is the staple food for millions of people. Today, this crop covers the food needs of more than 500 million people in the world. It is the second most important only to sorghum, as a staple food in the savanna areas of Nigeria (Khan et al., 2003). In Nigeria, pearl millet grains are used primarily for human consumption, feeds for poultry and source of beer for brewing industries.

Planting date plays an important role on growth, development and yield of cereal crops. To pearl millet, it ensures sufficient vegetative growth and grain development for optimum harvesting (Amodu et al., 2005). In Northern Guinea Savanna zone of Nigeria, sowing dates affect pearl millet production, which must be optimal in order to successfully compete with weeds, absorb nutrient and moisture for good growth and development (Shinggu and Gani, 2012). Planting dates must be chosen within a window of opportunity defined by cropping system and available soil moisture. It also dictates to a large degree how tall the plants will get, and the potential impact of certain insects and diseases on the crop. It also determine the size of the root system, which in turn determines how much stored water the plant can utilize. It can be manipulated to counter the adverse effects of environmental stress (Uzoma et al., 2010).

Planting date can have dramatic effect on crop yield. In fact, proper planting time is important for maximizing pearl millet grain yield (Leila, 2008). In fact, Lawn et al. (1993) have argued that the differences in development of cereals sown at different times may be explained by considering an optimum temperature above which rate of development decreases. Planting time recommendation for pearl millet grain yields is commonly made based on calendar year and soil temperature (Andrews et al., 1998).

Previous study on pearl millet in Edo State focused on the evaluation of some varieties in this zone. 'Gero Bida' and 'Gero Badeggi' varieties performed best in both growth and yield characters (Omoregie & Nwajei, 2015). Hence, to obtain better growth and grain yield in this zone, it is necessary to determine the appropriate planting date since pearl millet is affected by environmental conditions, particularly photoperiod.

Besides, with dwindling revenue from petroleum and its products and the government policy to diversify the economy, there is need to revamp the agricultural sector, particularly, the cultivation of arable crops beyond their traditional agro-ecological zones to provide sufficient food and income for the ever increasing population.

The research objective was to determine the appropriate planting time on the growth and grain yield of two indigenous varieties of pearl millet in a forest-savanna transition zone of Edo

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL SITE

A field experiment was carried out during 2016 cropping season in Ekpoma. Ekpoma is located in a forest-savannah transition zone of Edo State, Nigeria. The area is located between Latitude 6° 45' North and Longitude 6° 08' East and has a mean air temperature of 29 °C, relative humidity of 70 %, sunshine of about 5 – 7 hours/day and a mean annual rainfall of 1200 – 1500 mm as established by Ighalo and Remison (2010).

Table 1: Description of the study varieties used in the experiment

NO	Name of variety	Morphological traits					
		Height (cm)	Tiller	Synchronous tillers	Head type	Seed colour	Maturity days
1	Gero Bida	75-250	2-9	3-15	Terminal-panicle	Brown	75-90
2	Gero Badeggi	80-300	4-12	2-10	Terminal-panicle	Brown	80-95

Source: Omoregie and Nwajei (2015)

2.2 MATERIALS USED

'Gero Bida' and 'Gero Badeggi' varieties of pearl millet used were obtained from National Cereals Research Institute, Badeggi, Niger State, Nigeria. The description of the pearl millet varieties are given below;

2.3 SOIL ANALYSIS

Prior to sowing, soil samples were collected at a depth of 0-15 cm with an auger. The soil samples were bulked, air dried and sieved with a 2 mm mesh sieve for the determination of the physical and chemical properties using the method described by Anderson and Ingram (1993).

2.4 PLANTING DATES AND EXPERIMENTAL DESIGN

The two varieties were planted at five dates namely: April 10th, May 10th, June 10th, July 10th and August 10th to give ten treatment combinations. The experiment was a 2 × 5 factorial scheme fitted into a randomized complete block design with three replicates to give a total of thirty plots.

2.5 LAND PREPARATION AND PLANTING

The land was manually prepared and plots demarcated according to treatment. A pinch of seeds of each of the two varieties were sown on minimally prepared beds, monthly, starting from 10th April to 10th August 2016 and later thinned to one plant per stand at one week after planting (WAP). The gross plot size was 1.25 × 3 m. The planting spacing used was 25×75 cm intra- and inter rows, equivalent to 53,333 plants/ha with a spacing of 1 m within plots and between replicates.

2.6 WEED CONTROL

Weeding was conducted manually at 3 and 7 followed by supplementary weeding at 12 WAP.

2.7 SAMPLING AND DATA COLLECTION

2.7.1 Growth parameters

A sample of four plants per plot was taken at two middle rows at two weeks intervals until crops were harvested to measure the following parameters. Plant height (cm) was taken as the height of the main culm from ground level to the tip of the plant with a measuring tape (Remison and Eifediyi, 2014) while the number of leaves per plant was determined by visual counting. The stem girth (cm) was measured by using a digital vernier caliper (150 mm × 0.01 mm IP54, BEAPO Hardware Industrial Co, LTD, Changsha, Hundan, China) at 2 cm above ground level. Total leaf area per plant (cm^2) was estimated from leaf length multiplied by the widest width, multiplied by a constant 0.75 (Remison and Eifediyi, 2014) and there after multiplied by total number of leaves/plant while number of tillers and synchronous tillers (branch) /plant (Obeng et al., 2012; Omoregie and Nwajei, 2015) were counted visually.

2.7.2 Flowering and maturity traits

Days to 50 % flowering was taken as the number of days from planting to the time when 50 % of the plants population produced flowers while days to 50 % maturity was recorded from the date of planting to the day 50 % of the plant populations of the pearl millet had matured panicles.

2.7.3 Grain yield and grain yield components

Number of terminal panicles, tiller panicles and synchronous tillers (branch) panicles/plant were visually counted. Measuring tape was used to take the length of the terminal panicles. A sensitive scale was used to take the mass of 1000 seeds while a weighing balance was used to measure the grain yield from the terminal, tiller, synchronous tiller panicles and the husk

mass. The total grain yield was determined by the addition of the grain yield from the panicles of the terminal, tillers and synchronous tillers (branch) (Obeng et al., 2012; Omoregie and Nwajei, 2015).

2.8 DATA ANALYSIS

All growth, flowering and maturity characters and grain yield data obtained were computed using the Statistical Analysis System (SAS) package version 9.0 (2002) and the means separated using Duncan's Multiple Range Test (DMRT) at 0.05 level of probability.

3 RESULTS

3.1 SOIL PROPERTIES OF THE EXPERIMENTAL SITE

The results of the soil properties show that the textural class was sandy loam, slightly acidic (5.80) with marginal organic carbon (8.40 g kg^{-1}), low nitrogen content (0.43 g kg^{-1}), moderate available phosphorus (13.05 mg kg^{-1}) and exchangeable bases (Table 2).

Table 2: Main soil properties of the experimental site prior to planting

Parameters	Values
Sand (g kg^{-1})	830
Silt (g kg^{-1})	60
Clay (g kg^{-1})	110
Textural class	Sandy loam
pH (H_20 1:1)	5.80
Organic carbon (g kg^{-1})	8.40
Total nitrogen (g kg^{-1})	0.43
Available phosphorus (mg kg^{-1})	13.05
Exchangeable calcium (cmol kg^{-1})	1.04
Exchangeable magnesium (cmol kg^{-1})	0.45
Exchangeable potassium (cmol kg^{-1})	1.03
Exchangeable sodium (cmol kg^{-1})	0.07
Total exchangeable bases (cmol kg^{-1})	
[Ca^{2+} + Mg^{2+} + K^{+} + Na^{+}]	2.59
Total exchangeable acidity (cmol kg^{-1})	
[Al^{3+} + H^{+}]	0.12
Effective cation exchange capacity (cmol kg^{-1})	2.71
Base saturation %	95.57

3.2 PLANT HEIGHT

The effects of planting date on plant height of two varieties of millet are shown in Table 3. The height increased significantly with time until 10 WAP were no further increase. At 14 WAP, plant height ranged significantly from 166.44 – 203.17 cm in 'Gero bida' and 181.00 – 233.53 cm in 'Gero Badeggi'. In all, 'Gero Badeggi' developed taller stems with an average of 208.97 cm than 'Gero Bida' (183.33 cm). April sown crops had the tallest stems while those of August had the shortest.

3.3 NUMBER OF LEAVES/PLANT

Number of leaves/plant of the two varieties of pearl millet increased from 2 WAP up to 10 WAP and thereafter decreased significantly up to 14 WAP (Table 4). At 14 WAP, the mean varied from 7.00 – 10.42 in 'Gero Bida' and 8.42 – 12.33 in 'Gero Badeggi'. On the whole, 'Gero Badeggi' had higher number of leaves/plant than 'Gero Bida'. The mean number of leaves per plant for 'Gero Badeggi' was 11.12 while that of 'Gero Bida' was 9.35. Crops planted in April produced the highest while those of August had the least number of leaves.

3.4 NUMBER OF SYNCHRONOUS TILLERS (BRANCHES)/PLANT

Results on number of branches per plant of two millet varieties revealed that branch production commenced at 6 WAP for both crops planted in April and May, while other planting dates started at 8 WAP (Table 5). With the exception of May planted crops, which peak was at 12 WAP other crops planted at other dates reached their maximum branch production at 10 WAP. At 14 WAP, the mean number of branches/plant ranged from 1.67- 5.42 and 1.67 - 4.00 in 'Gero Bida' and 'Gero Badeggi', respectively. Crops planted in May had the highest, while those of July had the least number of branches/plant. In all, both varieties gave similar number of synchronous tillers, that is, approximately 3.

Table 3: Effect of planting date on the plant height (cm) of two varieties of pearl millet at Ekpoma

Crop variety	Planting date	Weeks after planting						
		2	4	6	8	10	12	14
Gero Bida	April	4.46^{a}	17.88^{a}	81.00^{a}	174.42^{b}	203.17^{bcd}	203.17^{bcd}	203.17^{bcd}
	May	3.82^{abcd}	12.46^{b}	55.92^{b}	177.08^{b}	193.50^{cde}	193.50^{cde}	193.50^{cde}
	June	3.68^{abcd}	11.17^{b}	38.08^{cd}	157.01^{b}	177.58^{de}	177.58^{de}	177.58^{de}
	July	3.17^{cd}	10.25^{bc}	44.17b^{c}	135.92^{c}	175.99^{de}	175.99^{de}	175.99^{de}
	August	3.83^{abcd}	7.63^{c}	24.67^{de}	111.00^{d}	166.44^{e}	166.44^{e}	166.44^{e}
	Mean	3.79	11.88	48.80	151.09	183.33	183.33	183.33
Gero Badeggi	April	4.29^{ab}	18.21^{a}	91.69^{a}	211.50^{a}	233.53^{a}	233.53^{a}	233.53^{a}
	May	3.99^{abc}	12.42^{b}	42.25^{c}	164.92^{b}	226.83^{ab}	226.83^{ab}	226.83^{ab}
	June	3.47^{bcd}	13.25^{b}	36.50^{cd}	156.92^{b}	213.67^{abc}	213.67^{abc}	213.67^{abc}
	July	3.08^{d}	10.67^{bc}	27.00^{de}	131.58^{c}	189.75^{cde}	189.75^{cde}	189.75^{cde}
	August	3.46^{bcd}	7.71^{c}	17.42^{e}	117.58^{cd}	181.00^{de}	181.00^{de}	181.00^{de}
	Mean	3.66	12.45	42.92	156.50	208.97	208.97	208.97
	SL							
	Planting date	*	*	*	*	*	*	*
	Variety	ns	ns	*	*	*	*	*
	Interaction (V×D)	*	*	*	*	*	*	*

Values followed by the same superscript(s) indicated in columns are not significantly different at the 5 % probability level when using Duncan multiple range test. SL- level of significance;*significant at the 0.05 probability level; ns: values not significant; V-variety; D- planting date

3.5 TOTAL LEAF AREA/PLANT

Total leaf area per plant increased steadily with age up to 10 WAP and thereafter decreased until 14 WAP (Table 6). At 14 WAP, the mean varied from 5345.99-9952.05 cm^2 and 6187.02 – 13060.09 cm^2 in 'Gero Bida' and 'Gero Badeggi', respectively. In all, 'Gero Badeggi' significantly ($p < 0.05$) had larger total leaf area (10,431.13 cm^2) than 'Gero Bida' (8,415.20 cm^2). Total leaf area decreased with time of planting from April to August. Crops planted in April significantly gave the largest total leaf area/plant while those of August had the least.

3.6 NUMBER OF TILLERS/PLANT

Tillering in 'Gero Badeggi' was significantly higher than in 'Gero Bida' (Table 7). An average of 3 tillers/plant was obtained for 'Gero Badeggi' while that of 'Gero Bida' was 2 tillers/ plant. With the exception of crops planted in April, which tillering started at 2 WAP, May to August plants commenced tillering at 4 WAP. Number of tillers increased steadily with time until 8 WAP for both varieties and thereafter decreased till 14 WAP. At 14 WAP, the number of tillers significantly ranged from 1.08 – 3.25 in 'Gero Bida' and 2.58 – 3.83 in 'Gero Badeggi'. April planted crops significantly had the highest number of tillers/ plant while those of August had the least.

3.7 STEM GIRTH

The maximum stem girths produced by both varieties were comparable and had no significant difference (Table 8). The stem girth increased from 2WAP up to 10 and 12 WAP for both varieties. At 14 WAP, the stem girth varied from 1.68 – 2.19 cm in 'Gero Bida' and 1.86 – 2.54 in 'Gero Badeggi'. Although 'Gero Badeggi' significantly produced bigger stems than 'Gero Bida' both had similar girth sizes; approximately 2 cm. April planted crops significantly had the biggest girth than other planting dates. The smallest girth was obtained in August.

3.8 DAYS TO50 % FLOWERING

Plants sown in April needed higher number of days to flowering while those of May needed lower

Table 4: Effect of planting date on the plant height (cm) of two varieties of pearl millet at Ekpoma

Crop variety	Planting date	Weeks after planting						
		2	4	6	8	10	12	14
Gero Bida	April	6.42^{a}	8.08^{a}	11.53^{ab}	12.18^{c}	11.75^{bcd}	11.00^{cd}	10.25^{bc}
	May	5.65^{b}	7.83^{ab}	10.33^{bc}	11.42^{cde}	11.42^{cd}	9.67^{de}	9.58^{cd}
	June	4.82^{c}	7.25^{abcd}	9.75^{cde}	11.92^{cd}	11.42^{cd}	9.67^{de}	9.67^{cd}
	July	3.83^{d}	7.92^{ab}	9.58^{cde}	10.75^{e}	10.92^{d}	10.50^{cd}	10.42^{bc}
	August	3.67^{d}	6.46^{d}	8.33^{e}	9.42^{f}	9.25^{e}	8.08^{e}	7.00^{e}
	Mean	4.88	7.51	9.90	11.14	10.95	9.78	9.35
Gero Badeggi	April	6.67^{a}	8.08^{a}	12.33^{a}	13.58^{a}	13.58^{a}	12.90^{a}	12.17^{a}
	May	6.17^{ab}	7.75^{abc}	10.00^{cd}	13.08^{ab}	13.08^{ab}	12.42^{ab}	12.33^{a}
	June	4.83^{c}	6.75^{cd}	9.33^{cde}	12.33^{bc}	12.33^{abc}	11.58^{abc}	11.50^{ab}
	July	3.83^{d}	6.58^{d}	8.83^{de}	11.58^{cde}	11.75^{bcd}	11.25^{bc}	11.17^{ab}
	August	3.83^{d}	7.00^{bcd}	8.33^{e}	11.17^{de}	11.19^{cd}	8.50^{e}	8.42^{d}
	Mean	5.07	7.24	9.76	12.35	12.39	11.33	11.12
	SL							
	Planting date	*	*	*	*	*	*	*
	Variety	ns	ns	ns	*	*	*	*
	Interaction (V×D)	*	*	*	*	*	*	*

Values followed by the same superscript(s) indicated in columns are not significantly different at the 5 % probability level when using Duncan multiple range test. SL- level of significance;*significant at the 0.05 probability level; ns: values not significant; V-variety; D- planting date

Table 5: Effect of planting date on number of synchronous tillers (branches)/plant of two varieties of pearl millet at Ekpoma

Crop variety	Planting date	Weeks after planting						
		2	4	6	8	10	12	14
Gero Bida	April	0.00	0.00	2.00^{a}	3.17^{ns}	3.78^{ns}	3.67^{b}	2.92^{bc}
	May	0.00	0.00	1.00^{b}	3.11	3.00	5.67^{a}	5.42^{a}
	June	0.00	0.00	0.00	2.00	2.78	2.83^{b}	2.33^{bc}
	July	0.00	0.00	0.00	1.33	2.50	1.83^{b}	1.67^{c}
	August	0.00	0.00	0.00	2.50	2.67	2.67^{b}	2.08^{c}
	Mean	0.00	0.00	1.50	2.42	2.95	3.33	2.88
Gero Badeggi	April	0.00	0.00	1.33^{b}	2.08	2.94	2.83^{b}	2.33^{bc}
	May	0.00	0.00	1.00^{b}	2.00	2.74	5.53^{a}	4.00^{ab}
	June	0.00	0.00	000	2.00	2.67	1.93^{b}	1.67^{c}
	July	0.00	0.00	0.00	2.33	3.50	2.42	2.17^{c}
	August	0.00	0.00	0.00	1.83	2.58	2.50^{b}	2.17^{c}
	Mean	0.00	0.00	1.17	2.05	2.89	3.04	2.47
	SL							
	Planting date	-	-	*	ns	ns	*	*
	Variety	-	-	ns	ns	ns	ns	ns
	Interaction (V×D)	-	-	*	ns	ns	*	*

Values followed by the same superscript(s) indicated in columns are not significantly different at the 5 % probability level when using Duncan multiple range test. SL- level of significance;*significant at the 0.05 probability level; ns: values not significant; V-variety; D- planting date

Table 6: Effect of planting date on the total leaf area (cm^2/plant) of two varieties of pearl millet at Ekpoma

Crop variety	Planting date	Weeks after planting						
		2	4	6	8	10	12	14
Gero Bida	April	166.98^{b}	2095.46^{ab}	7029.44^{b}	10011.84^{bcd}	11412.57^{bc}	10682.31^{bc}	9952.05^{bc}
	May	72.91^{c}	1721.27^{bc}	5818.74^{bc}	8665.05^{cde}	9278.51^{cd}	8610.78^{c}	8533.42^{c}
	June	54.17^{cde}	1480.81^{c}	4346.88^{cd}	7764.65^{cdef}	9518.80^{cd}	9019.19^{c}	8944.79^{c}
	July	29.56^{e}	878.29^{d}	4060.59^{d}	7031.17^{ef}	9750.34^{cd}	9379.93^{c}	9299.77^{c}
	August	31.41^{e}	504.25^{d}	2281.33^{ef}	5667.85^{f}	6305.64^{e}	5411.75^{d}	5345.99^{d}
	Mean	71.01	1336.02	4707.40	7828.11	9253.17	8620.79	8415.20
Gero Badeggi	April	196.07^{a}	2416.77^{a}	9880.05^{a}	14671.47^{a}	15075.64^{a}	13878.40^{a}	13060.09^{a}
	May	60.67^{cd}	1624.15^{bc}	5800.60^{bc}	11649.47^{b}	13170.00^{ab}	12493.27^{ab}	12413.64^{a}
	June	41.56^{de}	1924.84^{bc}	3780.60^{de}	10421.17^{bc}	12705.31^{ab}	11829.98^{ab}	11752.55^{ab}
	July	30.18^{e}	904.63^{d}	3028.64^{def}	7593.74^{def}	9183.83^{cd}	8795.51^{c}	8742.33^{c}
	August	26.18^{e}	549.24^{d}	2088.09^{f}	7240.11^{ef}	8247.65^{de}	6248.85^{d}	6187.02^{d}
	Mean	70.90	1484.93	4915.60	10315.19	11676.44	10649.20	10431.13
	SL							
	Planting date	*	*	*	*	*	*	*
	Variety	ns	ns	ns	*	*	*	*
	Interaction (V×D)	*	*	*	*	*	*	*

Values followed by the same superscript(s) indicated in columns are not significantly different at the 5 % probability level when using Duncan multiple range test. SL- level of significance;*significant at the 0.05 probability level; ns: values not significant; V-variety; D- planting date

Table 7: Effect of planting date on the number of tillers/plant of two varieties of pearl millet at Ekpoma

		Weeks after planting						
Crop variety	Planting date	2	4	6	8	10	12	14
Gero Bida	April	1.00^{ns}	4.61^{a}	5.32^{a}	5.42^{a}	3.75^{a}	3.75^{ab}	3.25^{ab}
	May	0.00	2.50^{c}	3.25^{b}	3.27^{bc}	4.00^{a}	3.00^{bc}	2.58^{bcd}
	June	0.00	1.97^{cd}	2.33^{c}	2.92^{c}	3.08^{ab}	2.33^{cd}	1.83^{de}
	July	0.00	1.31^{de}	2.92^{bc}	3.08^{bc}	3.17^{ab}	2.42^{cd}	1.92^{cde}
	August	0.00	1.17^{e}	1.17^{d}	1.75^{d}	2.25^{b}	1.56^{d}	1.08^{e}
	Mean	1.00	2.32	3.01	3.29	3.25	2.61	2.13
Gero Badeggi	April	1.11	5.17^{a}	5.33^{a}	5.33^{a}	4.50^{a}	4.42^{a}	3.83^{a}
	May	0.00	3.25^{b}	3.58^{b}	3.59^{bc}	3.59^{ab}	3.50^{ab}	3.00^{ab}
	June	0.00	2.67^{bc}	3.17^{bc}	3.33^{bc}	3.33^{ab}	3.00^{bc}	2.58^{bcd}
	July	0.00	1.67^{de}	3.67^{b}	3.67^{bc}	3.33^{ab}	3.33^{bc}	2.83^{bc}
	August	0.00	1.17^{e}	2.33^{c}	3.92^{b}	4.33^{a}	3.75^{ab}	3.17^{ab}
	Mean	1.11	2.78	3.62	3.97	3.82	3.60	3.08
	SL							
	Planting date	ns	*	*	*	ns	*	*
	Variety	ns	ns	*	*	ns	*	*
	Interaction (V× D)	ns	*	*	*	*	*	*

Values followed by the same superscript(s) indicated in columns are not significantly different at the 5 % probability level when using Duncan multiple range test. SL- level of significance;*significant at the 0.05 probability level; ns: values not significant; V-variety; D- planting date.

Table 8: Effect of planting date on stem girth (cm) of two varieties of pearl millet at Ekpoma

		Weeks after planting						
Crop variety	Planting date	2	4	6	8	10	12	14
Gero Bida	April	0.57^{a}	1.65^{a}	1.77^{abc}	2.08^{bc}	2.38^{a}	2.26^{bc}	2.19^{b}
	May	0.41^{cd}	1.20^{bc}	1.83^{abc}	2.26^{ab}	2.35^{a}	2.38^{ab}	2.11^{bc}
	June	0.33^{de}	1.07^{cd}	1.63^{bc}	1.92^{cd}	2.08^{bc}	2.12^{bcde}	2.07^{bc}
	July	0.27^{e}	0.91^{c}	1.53^{bc}	1.79^{d}	1.86^{bc}	1.82^{e}	1.75^{d}
	August	0.26^{e}	0.87^{cd}	1.51^{bc}	1.73^{d}	1.74^{bc}	1.92^{de}	1.68^{d}
	Mean	0.37	1.14	1.66	1.96	2.08	2.10	1.96
Gero Badeggi	April	0.55^{ab}	1.62^{ab}	2.11^{ab}	2.36^{a}	2.56^{a}	2.61^{a}	2.54^{a}
	May	0.47^{bc}	1.53^{ab}	1.85^{ab}	2.22^{ab}	2.35^{a}	2.19^{bcd}	2.13^{bc}
	June	0.33^{de}	1.16c^{d}	1.81^{abc}	2.12^{abc}	2.15^{abc}	2.08^{bcde}	2.02^{bc}
	July	0.26^{e}	0.82^{d}	1.55^{bc}	1.90^{cd}	1.92^{bc}	1.94^{cde}	1.86^{cd}
	August	0.26^{e}	0.84^{cd}	1.48^{c}	1.94^{cd}	2.08^{abc}	2.08^{bcde}	2.02^{bc}
	Mean	0.38	1.19	1.76	2.11	2.21	2.18	2.15
	SL							
	Planting date	*	*	*	*	*	*	*
	Variety	ns	ns	ns	*	*	*	*
	Interaction (V×D)	*	*	*	*	*	*	*

Values followed by the same superscript(s) indicated in columns are not significantly different at the 5 % probability level when using Duncan multiple range test. SL- level of significance;*significant at the 0.05 probability level; ns: values not significant; V-variety; D- planting date.

number of days (Table 9). The mean number of days to flowering ranged from 54.67-68.67 days in 'Gero Bida' and 60.33-73.00 days in 'Gero Badeggi'. 'Gero Bida' flowered earlier than 'Gero Badeggi'. 'Gero Bida' needed an average of 62.93 days to flower while that of 'Gero Badeggi' was 68.40 days.

3.9 DAYS TO 50 % MATURITY

Days to 50 % maturity varied from 82.00-90.33 days in 'Gero Bida' and 86.67- 96.33 days in 'Gero Badeggi' (Table 9). 'Gero Bida' matured earlier with a mean of 83.53 days than 'Gero Badeggi' with an average of 90.33 days. June plantings needed higher number of days to maturity while those of May needed lower number of days.

3.10 LENGTH OF TERMINAL PANICLE PER PLANT

The length of terminal panicle ranged from 41.88-54.75 cm and 55.17-81.58 cm in 'Gero Bida' and 'Gero Badeggi', respectively (Table 9). 'Gero Badeggi' panicles (66.80 cm) were longer than those of 'Gero Bida' (44.46 cm). However, April plantings had the longest panicle while July had the shortest.

3.11 NUMBER OF TERMINAL PANICLES PER PLANT

The result revealed that 'Gero Bida' and 'Gero Badeggi' sown at the five dates recorded 1.00 terminal panicle/plant and did not differ significantly from each other (Table 9).

3.12 NUMBER OF TILLER PANICLES PER PLANT

Number of tiller panicles varied from 1.50 to 3.50 in 'Gero Bida' and 1.28-4.33 in 'Gero Badeggi' (Table 9). Although, 'Gero Badeggi' had higher number of tiller panicles/plant than 'Gero Bida', both were similar approximately 2 panicles per plant. April plantings had the highest number of tiller panicles/plant which differs significantly from other sown dates, while May plantings had the least.

3.13 NUMBER OF SYNCHRONOUS TILLER (BRANCH) PANICLES PER PLANT

The value for number branch panicles/plant ranged from 1.25 - 2.83 in 'Gero Bida' and 1.44 - 2.00 in

Table 9: Effect of planting date on flowering and maturity and some yield components of two varieties of pearl millet

Crop variety	Planting date	Days to 50 % flowering	Days to 50 % maturity	Length of terminal Panicle/plant (cm)	Number of terminal panicle/plant	Number of tiller panicle/plant	Number of synchronous (branch) panicle/plant	Number of seeds/ panicle
Gero Bida	April	68.67^{bc}	82.67^{d}	44.00^{e}	1.00^{ns}	3.50^{a}	2.67^{ns}	8439.00^{de}
	May	54.67^{g}	79.00^{e}	54.75^{d}	1.00	1.53^{b}	2.56	10623.33^{bc}
	June	63.33^{e}	90.33^{b}	38.33^{e}	1.00	2.25^{b}	2.83	7437.00^{e}
	July	66.33^{d}	83.67^{d}	41.88^{e}	1.00	1.75^{b}	1.25	8124.41^{de}
	August	61.67^{ef}	82.00^{d}	43.33^{e}	1.00	1.50^{b}	2.17	8406.67^{de}
	Mean	62.93	83.53	44.46	1.00	2.11	2.29	8606.08
Gero Badeggi	April	73.00^{a}	89.67^{b}	81.58^{a}	1.00	4.33^{a}	2.00	11342.33^{ab}
	May	60.33^{f}	88.67^{b}	70.67^{b}	1.00	1.28^{b}	1.44	12301.33^{a}
	June	67.33^{cd}	96.33^{a}	55.17^{d}	1.00	2.25^{b}	1.67	9571.00^{cd}
	July	72.00^{a}	90.33^{b}	59.75^{cd}	1.00	1.33^{b}	1.67	10698.50^{bc}
	August	69.33^{b}	86.67^{c}	66.83^{bc}	1.00	1.75^{b}	1.83	10441.00^{bc}
	Mean	68.40	90.33	66.80	1.00	2.19	1.72	10870.83
	SL							
	Planting date	*	*	*	ns	*	ns	*
	Variety	*	*	*	ns	ns	ns	*
	Interaction (V×D)	*	*	*	ns	*	ns	*

Values followed by the same superscript(s) indicated in columns are not significantly different at the 5% probability level when using Duncan multiple range test. SL- level of significance;*significant at the 0.05 probability level; ns: values not significant; V-variety; D- planting date

'Gero Badeggi' (Table 9). Although 'Gero Bida' had higher number of branch panicles, both varieties produced approximately 2 panicles per branch. Crops planted in April had the highest number of panicle/branch while those planted in July had the least.

3.14 NUMBER OF SEEDS PER PANICLE

Crops planted in May had the highest number of seeds per panicle while those of June had the least (Table 9). The value ranged significantly from 7437.00-10623.33 and 9571.00-12301.33 in 'Gero Bida' and 'Gero Badeggi', respectively. 'Gero Badeggi' had a higher (10,870.83) number of seeds per panicle than 'Gero Bida' (8606.08).

3.15 MASS OF 1000 SEEDS

The mean 1000 seed mass ranged from 7.43-11.40 g in 'Gero Bida' and 7.63-12.70 g in 'Gero Badeggi' (Table 10). Although, 'Gero Badeggi' produced higher (9.24 g) mass than 'Gero Bida' (8.99 g), but there was no significant difference. May planted crops had the highest while those of June had the least.

3.16 TERMINAL PANICLE MASS

April sown crops had the highest terminal panicle mass while those of July had the least (Table 10). The mean varied from 2.55-4.95 t ha^{-1}and 4.63-6.45 t ha^{-1}in 'Gero Bida' and 'Gero Badeggi', respectively. On the whole, 'GeroBbadeggi' had a higher terminal panicle weight than 'Gero Bida'. 'Gero Bida' had 3.86 t ha^{-1}terminal panicle mass while 'Gero Badeggi' had 5.53 t ha^{-1}.

3.17 HUSK MASS

The mean husk mass ranged from 0.82-2.74 t ha^{-1} in' Gero Bida' and 2.43-3.38 t ha^{-1}in 'Gero Badeggi' (Table 10). Crops sown in April produced the highest while those of June had the least. In all, 'Gero Badeggi' had a higher husk weight than 'Gero Bida' and they were

Table 10: Effect of planting date on the grain yield and yield components of two varieties of pearl millet at Ekpoma

Crop variety	Planting date	Mass of 1000 seeds (g)	Terminal panicle weight	Husk mass	Grain mass from terminal panicles	Grain mass from synchronous tiller (branch) panicles	Grain mass from tiller panicles	Total grain mass
Gero Bida	April	9.25^{c}	4.95^{b}	2.74^{ab}	2.21^{d}	1.45^{abc}	2.52^{bc}	6.18^{bcde}
	May	11.40^{b}	4.36^{bcd}	1.45^{de}	2.92^{b}	1.68^{ab}	2.57^{bc}	7.17^{bc}
	June	7.43e	3.65^{d}	1.91^{cd}	1.74^{f}	1.11^{bcd}	1.71^{c}	4.56^{ef}
	July	8.88^{cd}	2.55^{e}	0.82^{e}	1.73^{f}	0.44^{d}	2.10^{c}	4.27^{f}
	August	7.97^{cde}	3.78^{cd}	1.47^{de}	2.31^{cd}	1.51^{abc}	1.66^{c}	5.47^{cdef}
	Mean	8.99	3.86	1.68	2.18	1.24	2.11	5.53
Gero Badeggi	April	8.25^{cde}	5.97^{a}	3.38^{bc}	2.59^{c}	0.58^{d}	1.76^{c}	4.93^{def}
	May	12.70^{a}	5.82^{a}	2.45^{bc}	3.37^{a}	1.83^{a}	4.13^{a}	9.33^{a}
	June	7.63^{de}	4.63^{bc}	2.43^{bc}	2.16^{de}	1.98^{a}	3.65^{ab}	7.79^{ab}
	July	9.03^{c}	4.76^{b}	2.88^{ab}	1.87^{ef}	0.46^{d}	2.20^{c}	4.53^{ef}
	August	8.57^{cde}	6.45^{a}	3.12^{ab}	3.33^{a}	0.87^{cd}	2.24^{c}	6.44^{bcd}
	Mean	9.24	5.53	2.85	2.66	1.14	2.80	6.60
	SL							
	Planting date	*	*	*	*	*	*	*
	Variety	ns	*	*	*	*	*	*
	Interaction (V× D)	*	*	*	*	*	*	*

Values followed by the same superscript(s) indicated in columns are not significantly different at the 5 % probability level when using Duncan multiple range test. SL- level of significance;*significant at the 0.05 probability level; ns: values not significant; V-variety; D- planting date

significantly different. The average of 2.85 t ha^{-1}was for 'Gero Badeggi' while that of 'Gero Bida' was 1.68 t ha^{-1}.

3.18 GRAIN MASS FROM TERMINAL PANICLE

Crops planted in May had the highest grain mass from terminal panicles while those of July had the least (Table 10). Grain mass obtained from terminal panicles varied from 1.73- 2.92 t ha^{-1}and 1.87- 3.37 t ha^{-1}in 'Gero Bida' and 'Gero Badeggi' respectively (Table 10). On the whole, 'Gero Badeggi' had heavier (2.67 t h^{-1}) grains from terminal panicle than 'Gero Bida' (2.18 t ha^{-1}).

3.19 GRAIN YIELD FROM SYNCHRONOUS TILLER (BRANCH) PANICLES

The value ranged from 0.44-1.68 t ha^{-1}in 'Gero Bida' and 0.46-1.98 t ha^{-1}in 'Gero Badeggi' (Table 10). Although grain yield from branches was higher in 'Gero Bida' (1.24 t ha^{-1}) than 'Gero Badeggi' (1.14 t ha^{-1}), however, there was no significant difference. Crops sown in May had the highest grain yield from synchronous tiller panicles (branch) while those sown in July had the least.

3.20 GRAIN YIELD FROM TILLER PANICLES

Grain yield from tillers varied from 1.66-2.57 t ha^{-1}and 1.76-4.13 t ha^{-1}in 'Gero Bida' and 'Gero Badeggi' respectively (Table 10). In all, 'Gero Badeggi' had higher grain mass from tillers than 'Gero Bida'. The mean of 2.80 t ha^{-1}was for 'Gero Badeggi' while that of 'Gero Bida' was 2.11 t ha^{-1}. However, the highest and the least grain yield from tiller panicles were obtained from crops planted in May and August, respectively.

3.21 TOTAL GRAIN YIELD

Total grain yield ranged from 4.27-7.17 t ha^{-1}in 'Gero Bida' and 4.53-9.33 t ha^{-1}in 'Gero Badeggi' (Table 10). On the whole, 'Gero Badeggi' had significantly higher (6.51 t ha^{-1}) total grain yield than 'Gero Bida'

(5.53 t ha^{-1}). The order of performance in total grain yield was May > June > August > April > July. However, May and July sown crops significantly gave the highest and the least total grain yield, respectively.

4 DISCUSSION

Nutrient status of soil is a paramount index in crop production and food security. The soil nutrients of the site were low when compared with the critical levels of 10 g kg^{-1} organic carbon, 15 g kg^{-1} total nitrogen and 15 mg kg^{-1} phosphorus (Kparmwang et al., 2001). However, keeping in view the low nutrient status of the soil, grain yields obtained from this study commensurate with those reported by Uzoma et al. (2010) from relatively higher nutrients based soils in the guinea savannah zone of Nigeria. Fahmy et al. (2010) reported that pearl millet can grow in a wide range of environments and soils.

Plant height is an important component in the determination of growth (Khan et al., 2009). Pearl millet sown in April significantly developed the tallest stems while those planted in August developed the shortest. The possible reason is that early sown crops had availed prolonged vegetative growth, as a result, plant attained maximum height as compared to late sown crops. These results are in line with those of Maas et al. (2007) who reported that crops sown on 15th June produced significantly taller plants than those sown on 15th July.

There were significant ($p < 0.05$) differences in number of leaves/plant among planting dates of pearl millet varieties throughout the period of the study, but effect of variety was not significant from 2-6 weeks after planting. The early sown crops produced more leaves than late planted crops due to the utilization of more assimilate in producing more leaves. These results are in conformity with the findings of Andrews et al. (1998); Amanullah et al. (2015) who attributed the higher number of leaves produced by early sown crops to available moisture which enhanced utilization of more assimilate.

Millet tillering was divided into sub-terminal tillers and synchronous tillers. Sub-terminal tillers are those which arise from auxiliary bud axis while the synchronous tillers are those from the base of the leaf bud (Obeng et al., 2012). The later was referred to as branches (Omoregie & Nwajei, 2015). Number of branches/plant was significantly affected by planting date and the interaction between variety and planting date, but the effect of variety was not significant. Although 'Gero Bida' was higher than 'Gero Badeggi' with regards to number of branches, but there was no significant difference. Generally, time of planting significantly influenced the growth and development of synchronous tillers (branches) produced in both varieties. The reduction in the number of synchronous tillers after 12 WAP in both varieties was due to senescence. Obeng et al. (2012); Omoregie & Nwajei (2015) observed significant decline in the number of synchronous tillers owing to senescence which agreed with the present study.

Total plant feeding area ensures adequate photosynthesis for optimum growth and development. The leaf area of pearl millet differed significantly ($p < 0.05$) among sowing dates and planting × variety interaction, but variety was not significant from 2-6 WAP. Crops sown at early date (April/May) had the largest leaf area compared to the later dates due to moisture absorption ability and high conversion of assimilates. This is in agreement with the results of a study conducted elsewhere by Agber et al. (2012) who reported that the largest leaf area of pearl millet with early sown date was due to the availability of water during vegetative growth which increased the total leaf area of the crop.

Results in Table 6 revealed that number of tillers/ plant was significantly affected by time of planting except at 2 and 10 WAP. Number of tillers/ plant had significant difference for crops at 6, 8 12 and 14, while the interaction between plating date and variety were significant from 4 -14 WAP. This sub-terminal tillers were significantly higher when pearl millet was planted in April which reflects the crops ability to develop various vegetative and reproductive parts during early plantings with favourable environmental conditions. Uzoma et al. (2010) reported significant difference in number sub terminal tillers among sowing dates due to environmental responds.

Stem girth is a measure of plants resistance or susceptibility to lodging, but adequate stand establishment ensures good growth and stability of crops. Stem girth was significantly affected by planting date and planting date x variety interaction throughout the weeks after planting, but variety had no significant effect from 2 – 6 WAP. Early planted crops had the biggest girths and were significantly at par from late sown crops. This is consistent with the findings of Uzoma et al. (2010); Omoregie & Nwajei (2015) who report that timeliness in planting is necessary as it has great effect on the growth of millet because it places the crops growth cycle within the distribution of rains which in turn enhance good plant architecture.

Planting date, variety and planting date × variety interaction significantly affected days to 50 % flowering and maturity. However, crops sown in May needed lower number of days to flowering and maturity than other dates. This is in agreement with the earlier find-

ings by Uzoma et al. (2010); Omoregie & Nwajei (2015) who found significant difference in days to 50 % flowering and maturity of pearl millet and crops that flowered early also matured early.

Results on yield and yield components of pearl millet showed that planting date, variety and planting date x variety interaction had significant effect on length of terminal panicle/plant, number of seeds/panicle, terminal panicle mass and husk mass. There was no significant difference among the varieties in number of tiller panicles and mass of 1000 seeds while number of terminal and synchronous tiller panicles were not significantly affected by planting date, variety and planting date x variety interaction. On the whole, 'Gero Badeggi' had higher yield components than 'Gero Bida' except number of synchronous tillers/plant. Early sown crops significantly had higher yield components than late sown crops. These outstanding performances of pearl millet yield components by early sown crops over late planted crops were because early sown crops had well favourable weather conditions and gained prolonged growth period as a result more assimilates were accumulated for growth and development as well as grain production. The differences in varietal responds could have been as a result of difference in partitioning of assimilates to the grain and genotypic variation. Uzoma et al. (2010); Omoregie & Nwajei (2015) observed significant difference in yield components of millet and reported that the variety with highest yield components gave the highest grain mass which was observed in this study.

The grain yield from terminal panicles, tiller panicles, synchronous tiller (branch) panicles and total grain yield were significantly affected by planting date, variety and planting date x variety interaction. Early sown crops gave heavier grains than late sown crops due to prolonged growth period with ideal growth conditions and better vegetative characters. This result is in agreement with the results reported by Tanzubi et al. (2002); Leila (2008) who observed that pearl millet planted in May had higher grain yield due to better vegetative growth, well distributed and favourable weather conditions during grain development. This yield reduction from crops planted in June – August were due to the plants' inability to attain optimum energy reserves. This is consistent with the report of Uzoma et al. (2010) that substantial yield loss may be associated with slight delay in planting as in the case those planted from June-August or slight earliness in planting as of those planted in April which agrees with our results.

5 CONCLUSION

This study concluded that planting date of pearl millet influenced the grain yield from synchronous tiller (branch) and tiller panicles as components of total grain yield with early sown crops being more favourable than later dates. Thus, pearl millet planted in April is regarded as the option for vegetative characters and ideal for pearl millet grown for forages while May is the option for the grain production in Ekpoma, Edo state, Nigeria.

6 REFERENCES

Agber, P.I., Ter, S., & Ayuba, S.A. (2012). Assessment of pearl millet performance to early and late planting opportunities and fertilizer application models in Makurdi, Nigeria. *African Journal of Agricultural Research, 7,* 5955-5961. https://doi.org/10.5897/AJAR12.2051

Amanullah, J., Imran, K., Shahzad, A., & Amir, S. (2015). Sowing date and sowing methods influence on growth, yield and yield components of pearl millet under rainfied conditions. *Journal of Environmental and Earth Science, 5*, 2224-3216.

Amodu, J.T., Adamu, A.M., Adeyinka, I.A., Alawa, J.P., & Jegede, J.O. (2005). The nutritive value of laboratory ensiled lablab (*Lablab purpureus*) and pearl millet (*Pennisetum americanum*). *Tropical GrassLand, 39*, 117-123.

Anderson, J.M. & Ingram, J.S. (1993). *Tropical soil biology and fertility*. A hand book of soil methods (pp. 23-45). Information and press Eynsham.

Andrews, D.J., Rajewski, J.F., & Manson, S.C. (1998). Grain pearl millet: A newly developed crop. *Extension Vision* 2, 2-6.

Fahmy, A.A., Youssef, K.M., & Elshaer, H.M. (2010). Intake and nutritive value of some salt tolerant fodder grasses for sheep under saline conditions of South Sinai, Egypt. *Small Ruminant Research, 91*, 110-115. https://doi.org/10.1016/j.smallrumres.2009.11.023

Gabatshele, M.L., Thembinkosi, M., Phizana, M., Christopher, M., & Witness, M. (2014). The growth and development of two pearl millet landraces as affected by intra row spacing. *International Journal of Current Microbiology and Applied Sciences, 3*, 505-515.

Ighalo, S.O., & Remison, S.U. (2010). Effect of weeding frequency and nitrogen application on the vegetative growth of Rice. *Nigeria Journal of Agriculture and Forestry, 2*, 219 -232.

Khan, A.Z., Shah, P., Khalil, S.K., & Taj, F.H. (2003). Influence of planting date and plant density on morphological traits of determinate and indeterminate soybean cultivars under temperate environment. *Pakistan Journal of Agronomy, 2*, 146-152. https://doi.org/10.3923/ja.2003.146.152

Khan, Z.H., Khalil, S.K., Nigar, S., Khalil, I., Haq, I., Ahmad, I., Ali, A., & Khan, M.V. (2009). Phenology and yield of sweet corn landraces influenced by planting dates. *Sahad Journal of Agriculture, 25*, 153-157.

Kparmwang, T., Chude, V.O. and Abdullahi, D. (2001). The classification and genesis of benchmark soils of Bauchi State, Nigeria. *Journal of Soil Science, 14*, 30-39.

Lawn, R.J., Summerfield, R.J., Ellis, R.H., Roberts, E.H., Chay,

P.M., Brouwer, J.B., Rose, J.L. & Yeates, S.J. (1993). Towards the reliable prediction of time to flowering in six annual crops. VI. Applications in crop improvement. *Experimental Agriculture, 31*, 89-108. https://doi.org/10.1017/S0014479700025047

Leila, R. (2008). Autochthonous pearl millet ecotype (*Pennisetum glacucum* L. R. Br.) responds to different sowing dates in Tunisia. *Sjemenarstre, 25*,123-135.

Maas, A.L., & Hanna, W.W. and Mullinix, B.G. (2007). Planting date and row spacing affecting grain yield and height of pearl millet tifgrain 102 in the South Eastern Coastal plain of the United States. *Journal of Semi-Arid Tropics of Agricultural Research, 5*, 1-4.

Obeng, E., Ernest, C., Bharat, P.S., Rafina, W., Leapold, M.N., & David, M.A. (2012). Growth and grain yield of pearl millet genotypes at different levels of nitrogen fertilization in the south-eastern United State. *Journal of Agricultural Science, 4*, 155-160. https://doi.org/10.5539/jas.v4n12p155

Omoregie, A.U., & Nwajei, S.E. (2015). Evaluation of some varieties of pearl millet (*Pennisetum typhoides*) in a forest-savanna transition zone of Edo State, Nigeria. In:Omeje, S.I., Emosairue, C.C., Chukwuji, C.C., Bratte, L., Isikwenu, J.O., Iaiorhovoja, R.A. and Agbogidi, M.O. (eds). Agriculture: The Nigerian economy beyond oil. *Proceedings of the 49th Annual Conference of the Agricultural Society of Nigeria held at Delta State University*, Asaba Campus, Asaba, Nigeria 9-13th November, 2015. 2, 1346-1349.

Raemaeker, R.H. (2001). *Crop production in tropical Africa*, Brussels, Belgium. pp. 47-57.

Remison, S.U., & Eifediyi, E.K. (2014). Effects of different organic materials and NPK fertilizer on the performance of maize (*Zea mays* L.). *Poljoprivreda, 20*, 16-21.

Statitical Analysis System. (2002). *SAS user's guide: Statistics version 9.0 Cary*, NC, USA: SAS Institute Inc., 2002.

Shinggu, C.P. and Gani, M. (2012). Effects of planting methods, sowing dates and spacing on weed and the prductivity of finger millet (*Eleusine corocana* (L.) Gaertn) in the northern guinea-savanna of Nigeria. *Global Journal of Bio-Science and Biotechnology, 1*,160-162.

Tanzubil, P.B., Mensah, G.W.K. & McCaffery, A.R. (2002). Effect of millet variety and date of sowing on diapauses development in *Coniesta ignfusalis* (Hampson) in Northern Ghana. *Journal of Crop Protection, 21*, 793-798. https://doi.org/10.1016/S0261-2194(02)00039-X

Uzoma, A.O., Eze, P.C., Alabi, M., Mgbonu, F., Aboje, J.E. & Osunde, A.O. (2010). Effects of variety and planting date on the growth and yield of pearl millet in the southern guinea- savanna zone of Nigeria. *Journal of Agriculture and Veterinary Science, 2*, 122-126

doi:10.14720/aas.2019.114.2.4 Review article / pregledni znanstveni članek

Taro [*Colocasia esculenta* (L.) Schott] production in Japan: Present state, problems and prospects

Tetsuo MIKAMI [1, 2], Sakio TSUTSUI [1]

Received September 27, 2019; accepted October 10, 2019.
Delo je prispelo 27. septembra 2019, sprejeto 10. oktobra 2019.

Taro [*Colocasia esculenta* (L.) Schott] production in Japan: Present state, problems and prospects

Abstract: Taro [*Colocasia esculenta* (L.) Schott], a member of the family Araceae, is a vegetatively propagated crop with edible tubers (corms and cormels), petioles and leaf blades. Available evidence suggests that taro originated in South Central Asia, probably in the tropical region from India to Indonesia. The crop is considered to have reached Japan by the 10th century B. C.. In Japan, taro was a regional staple crop before the beginning of rice cultivation, but it is nowadays grown as a root vegetable. The corms and cormels of taro are an excellent source of carbohydrates and rich in essential minerals, vitamins and dietary fiber. Additionally, Japanese people have formed socio-cultural connections to the crop since olden times; taro has been served in traditional feasting and seasonal events. Despite having so much value, taro cultivation has shown declining trends in the past several decades. It should also be noted that little attention has been devoted to the genetic improvement of taro. In this review, an attempt is made to collect information about the commercial production and uses of Japanese taros as well as agronomic characteristics of leading cultivars, with the expectation that the synthesized information will aid in understanding the problems and prospects of taro cultivation in Japan.

Key words: agronomic characteristics; breeding; corm; cormel; cultivar; early maturing genotype; taro; traditional food

Pridelovanje tara [*Colocasia esculenta* (L.) Schott] na Japonskem: trenutno stanje, problemi in perspektive

Izvleček: Taro [*Colocasia esculenta* (L.) Schott], predstavnik družine kačnikov (Araceae), je vegetativno razmnoževana poljščina z užitnimi čebulastimi gomolji (kormi, kormiči), listnimi peclji in listnimi ploskvami. Po razpoložljivih podatkih naj bi taro izviral iz južne centralne Azije, verjetno iz tropskih predelov med Indijo in Indonezijo. Poljščina naj bi dosegla Japonsko v 10th stoletju pred Kristusom. Na nekaterih območjih Japonske je bil taro vsakodnevna hrana pred začetkom gojenja riža, danes se goji predvsem kot korenasta zelenjava. Čebulasti gomolji tara (kormi) so odličen vir ogljikovih hidratov in bogati na esencialnih mineralih, vitaminih in prehranskih vlakninah. Japonci so že od nekdaj stkali s poljščino socio-kulturne povezave, ko je bil taro postrežen na tradicionalnik praznikih in ob sezonskih dogodkih. Kljub tako veliki vrednosti, je gojenje tara v zadnjih desetletjih upadalo. Pripomniti je še potrebno, da je bilo genetskemu izboljšanju tara posvečeno premalo pozornosti. V tem pregledu je podan poskus zbiranja podatkov o komercialni pridelavi in uporabi tara na Japonskem kot tudi agronomske lastnosti vodilnih sort v pričakovanju, da bodo te informacije pomagale razumeti težave in prihodnost gojenja tara na Japonskem.

Ključne besede: agronomske lastnosti; žlahtnenje; korm; kormič; sorta; zgodaj dozorevajoči genotip; taro; tradicionalan hrana

1 Hokkaido Agricultural Laboratory for Business Development, Eniwa, Japan
2 Corresponding author, e-mail: tm-mars@fgbb.jp

1 INTRODUCTION

Taro [*Colocasia esculenta* (L.) Schott] is an ancient monocotyledonous crop belonging to the Araceae family. It is widely grown, with current worldwide production at nearly 10.2 million metric tonnes on nearly 1.7 million hectares (FAO, 2019). Nigeria produces the largest acreage (831,000 ha) of taro followed by Cameroon (227,000 ha), Ghana (184,000 ha), China (95,000 ha) and Côte d'Ivoire (67,000 ha) (FAO, 2019). According to Bown (2000), about 400 million people include taro in their diets, and in Japan it is primarily grown as a root vegetable. The plant consists of a central corm (mother tuber) from which cormels (side tubers), roots and shoots arise (Deo et al., 2009; Devi et al., 2016). While corms and cormels are economically the most important parts of the taro crop, petioles are also consumed by Japanese people (Matthews, 2004).

There is a variety of taro cultivars adapted to a range of microenvironments, from swidden fields and rainfed agriculture to paddy fields and swamps throughout the world (Chaïr et al., 2016). Among cultivated taros, two distinct botanical varieties are recognized, viz., var. *antiquorum* (Schott) Hubbard and Rehder (agronomically referred to as the "eddoe" type of taro) and var. *esculenta* (referred to as the "dasheen" type of taro) (Deo et al., 2009; Manner & Taylor, 2011; Devi, 2012). The eddoe type has a small or medium-sized central corm (unsuitable for use as food) and a number of edible side cormels, whereas the dasheen type is distinguished by the possession of a large edible central corm and very few side cormels (Deo et al., 2009; Devi, 2012). Most of the taros grown in Japan are of eddoe type (Figure 1).

Japanese people have formed socio-cultural connections to taro since olden times (Nishimoto et al., 2009; Takeuchi & Nagashima, 2012). For instance, taro has been the crop of choice for traditional feasting and seasonal events described later. It is well known that taro is an excellent source of carbohydrates (Deo et al., 2009). The crop is also emerging as a health food because of its richness in vitamins, potassium, phosphorus, calcium, magnesium, dietary fiber and so forth (Deo et al., 2009; Bhattacharjee et al., 2014). Despite having so much value, taro cultivation in Japan has shown declining trends in the past several decades (https://www.vegetable.alic.go.jp/yasaijoho/senmon/0510/senmon.html). To make taro an attractive farm crop, there is a need to put more research and funds in the cultivar development and crop management practices for improving yield, enhancing the tolerance to biotic and abiotic stresses and expanding the cultivation area. This paper begins by providing an overview of the production and uses of taros in Japan. The paper describes the agronomic characteristics of representative taro cultivars and finally, focuses on the early maturing genotypes that are tolerant to cold northern climate and considered as useful genetic resources for commercial taro cultivation under normally unfavorable growth conditions.

2 ORIGIN AND DISPERSAL INTO JAPAN

Taro most likely originated in the tropics ranging from India to Indonesia, with subsequent eastward-dispersal to China, Japan and the Pacific Islands (Devi, 2012; Helmkampf et al., 2018). From Asia, the crop also spread westward to Arabia, the Mediterranean region and Africa (Devi, 2012; Chaïr et al., 2016; Ebert & Waqainabete, 2018). It has been proposed that taro was introduced into Japan during the late Jomon period (10th century B.C.), presumably earlier than the beginning of rice cultivation (Nakao, 1966; Sasaki, 1971; Hirai et al., 1989). Several lines of evidence suggest that there were at least two different dispersal routes of taro into Japan: one route from Taiwan via the Ryukyu Islands and the other route directly from China (Kumazawa et al., 1956; Matthews er al., 1992; Matsuda & Nawata 1999). In the past, taro was one of the most important starchy crops in Japan, and at least 11 cultivars were

Figure 1: Taro plants (eddoe type) cultivated in the field (left panel) and cormels (right panel) (Photo: M. Kamei).

already recognized by the early 1600's (Kumazawa et al., 1956).

3 COMMERCIAL PRODUCTION

The annual taro production in Japan has been as much as 148,600-172,500 tonnes which was harvested from approximately 12,700 ha during the recent six years (2012-2017) (Table 1). The current production is ca. 40 % of what it was in the 1980's [for instance, taro was harvested from 29,000 ha with a total yield of 400,000 tonnes in 1982 (https://www.yasainavi.com/graph/category/ca = 23)] (Table 1). Taro farming has been on the decline throughout the country (https://www.vegetable.alic.go.jp/yasaijoho/senmon/0510/senmon.html), though the total yield of taro has remained fairly steady in the recent past (see Table 1).

One of the reasons that have direct implication in the decline is undoubtedly the demographic change taking place in the major taro growing villages. Younger populations are leaving the villages to urban areas for education, job and business opportunities. The leftover rural workforce is aging, thus there are not sufficient hands to work on the farm. Many farmers have reduced taro planting to divert more land under cash crops which give them better returns. Another reason is, as far as we can see, the change in dietary habits (Ashkenazi & Jacob, 2003; Kusaka et al., 2016). Rice, tubers (including taro) and starchy roots, vegetables, minor cereals and fish were the mainstays of the Japanese diet for some time after the World War II. The period from 1955 to 1970 was a time of diversification and westernization of eating habits. Household incomes rose during the postwar economic growth, urban areas expanded, and the consumption of milk, butter, meat, and eggs grew rapidly. On the contrary, the consumption of traditional Japanese foodstuffs such as rice, tubers and root crops decreased. In fact, taro consumption per capita per year has reduced from over 1,370 g in the late 1960's to 623 g in 2014 (https://www.vegetan.alic.go.jp/toukeiyouran.html). It is also worth mentioning that the household food budget allocated to fresh products (including fresh vegetables) has declined since 1970's because Japanese people have increased consumption of foods with greater convenience of preparation (Campo & Beghin, 2005).

4 USES

As pointed out by Matthews (2010), the continuing popularity of taro in Japan is conceivably attributed to a generally high regard for traditional foods and the continuous dissemination of cooking methods via the mass-media. Taro has been actually an important food to share with family and/or community people in gathering on special occasions. This is well illustrated by a popular dish called 'Chikuzen-ni' which is made of simmered chickin, taro cormels, lotus roots and some vegetables (http://www.japanfoodaddict.com/osechi/chikuzen-ni). The dish is often eaten when celebrating the arrival of a new year throughout the country.

Another example is a traditional soup called 'Imoni'; in the northeast region of Japan, groups of people prepare this soup around a fire near a river in celebration of the crop harvest during the late summer and early autumn. The soup contains taro, vegetables, mushrooms and thinly sliced meet (beef or pork). Either soy sauce or miso paste (fermented soybean paste) is included to flavor the soup (http://www.ajfarm.com/yamagata/12791).

Small taro cormels are steamed with their skins on and eaten after pealing their skins and slightly salting (http://www.japan-word.com/kinukatsugi). This simple dish (known as 'Kinukatsugi') is usually served for the moon viewing festival in mid-autumn. It should also be added that taro corms and cormels are frequently prepared through simmering in traditional broth and soy sauce in home-cooked dishes (Matthews, 2004; Nishimoto et al., 2009). A few cultivars bear edible petioles; petioles are commonly either peeled and cooked in soups or served after pickling in vinegar (Matthews, 2004). In addition, taro is processed to make value-added products such as chips, cakes, taro flour and local alcoholic beverages (Takeuchi & Nagashima, 2012).

5 MAJOR CULTIVARS

The Japanese archipelago spans a wide range of latitudes and offers very diverse physical environments for agriculture (Matthews, 2010). Apparently, tropical forms of taro reach their northern limit in Kyushu island at about 33° N, whereas temperate forms extend

Table 1: Production and cultivation area of taro in Japan. Source: MAFF. (2019)

	2012	2013	2014	2015	2016	2017
Cropping acreage (ha)	13,400	13,000	12,900	12,500	12,200	12,000
Total yield (t)	172,500	162,100	165,700	153,300	154,600	148,600

Table 2: List of representative taro cultivars grown in Japan

Cultivar (Ploidy)	Agronomic characteristics
Ishikawa-wase (Triploid)	An early-maturing cultivar that produces many, rather small globoid cormels and is mostly cultivated in the western area of Japan. The cormels are smooth in texture when cooked. As in 'Dotare' and 'Hasuba-imo', only cormels are edible. This cultivar most likely arose from an ancient landrace 'Kurojiku' by bud mutation (Hirai et al., 1989).
Dotare (Triploid)	A popular cultivar that forms many oval cormels with soft and sticky flesh, and exhibits moderate tolerance to cold climate. Mainly cultivated in eastern Japan.
Hasuba-imo (Triploid)	Characterized by horizontally lifted leaf laminae. The cormels are globular in shape, and have somewhat sticky texture with a sweet taste upon cooking.
Akame (Triploid)	A late-maturing cultivar characterized by reddish petioles and buds. Elliptical corms and cormels have mealy texture with a sweet taste when cooked. Both central corm and cormels are used as food.
Yatsugashira (Diploid)	A number of buds sprout from a single corm. The flesh of corm and cormels has bright creaming color and is flaky in texture with a slightly sweet flavor. The corm, cormels and petioles together constitute the edible harvest.

to about 41° N, in northern Honshu (the main island of Japan).

More than one hundred taro cultivars (mostly local landraces) have been cultivated in the country (Hirai et al., 1989). As regards cultivar identification, however, complications arise from the fact that different genotypes can be given identical or similar names and different names can be applied to the same genotypes. With this in mind, Kumazawa et al. (1956) classified the Japanese cultivars into 14 groups primarily based on the morphology of floral and vegetative organs. The classification was basically confirmed by the analyses using isozymes, tuber proteins, and ribosomal and mitochondrial DNAs (Hirai et al., 1989; Matthews et al., 1992; Isshiki et al., 1998).

Table 2 shows the agronomic characteristics of representative taro cultivars currently grown in Japan. These cultivars have been vegetatively propagated, and hence there is almost no genetic variation within the cultivars (Hirai et al., 1989; Matthews et al., 1992; Isshiki et al., 1998). Moreover, it seems likely that much of the phenotypic variations among the cultivars is ascribed to somatic mutations, selection or introduction of new genotypes from the outside of Japan (Hirai et al., 1989; Isshiki et al., 1998). Diploids (2n = 2x = 28 chromosomes) and triploids (2n = 3x = 42 chromosomes) occur in Japanese taros (Kumazawa et al., 1956). Triploids are believed to arise when unreduced gametes (1n = 2x = 28) from one parent flower meet normal gametes from another parent flower (Isshiki et al., 1999). Most of the Japanese cultivars are triploids, thus leaving one with the inference that triploids may be endowed with better adaptability and enhanced hardiness to unfavorable climates in the higher latitudes (Tahara et al., 1999; Ochiai et al., 2001; Ebert & Waqainabete, 2018).

6 A HYBRID CULTIVAR 'HIMEKAGUYA'

In Japan, little attention has been devoted to the genetic improvement of taro. The primary reason for this is the difficulty of hybrid breeding due to the inherent infertility of triploid taros predominantly grown in the country (Matthews et al., 1992). Additionally, diploid taros hardly ever flower under natural conditions in Japan, and most plants complete their field life without flowering at all (Matthews et al., 1992). The improvement programmes have been exclusively dependent upon the exploitation of the existing genetic variability among the cultivars (Banjaw, 2017). However, changes in the environment (e.g., new pests and diseases, and climatic changes), crop production technology, processing and marketing continuously require new cultivars (Ivančič & Lebot, 2000).

The discovery of gibberellic acid as an aid in promoting flowering in taro has prompted a fraction of plant breeders to look at the possibility of producing taro hybrids (Wilson & Cable, 1984). The breeding programme at the Ehime Research Institute of Agriculture, Forestry and Fisheries, released its first diploid hybrid cultivar 'Himekaguya' in 2007 (Nakagawa et al., 2016). This cultivar apparently yields more than its parents (diploid local landraces 'Takenoko-imo' and 'Touno-imo') and has good market acceptability due to its favorable eating quality (e.g., slightly mealy in texture and sweet taste) (Nakagawa et al., 2016).

7 CONCLUSIONS

Over the last few decades, insufficient quantities of taro have been grown domestically; in 2014, about 34,000 tonnes of pre-cooked frozen taro were imported

Table 3: Mean values of four characters of two early-maturing taro cultivars 'Dotare' and 'Yamato-wase'. Source: Shiga et al. (2011)

Cultivar	Plant height (cm)	Foliage mass (g)	Number of cormels per plant	Cormel yield (kg a^{-1})*	
				Marketable	Unmarketable
Dotare	106	1,677	13.2	161	121
Yamato-wase	95	1,325	11.7	138	98

Seed corms were planted in rows 100 cm apart and 40 cm distant within row on May 19, 2010. The ridges were covered with polyethylene film mulch. All plots received 0.8-1.6-2.0 ($N\text{-}P_2O_5\text{-}K_2O$) kg a^{-1} fertilizer. The four characters were recorded at harvest (October 15).
* Only cormels are edible in the two cultivars examined.

to supplement domestic production (https://www.vegetable.alic.go.jp/yasaijoho/senmon/0510/senmon.html). Nevertheless, domestically grown produce is generally preferred to imported, not only in terms of quality and taste, but also safety. Taro requires an average daily temperature above 21 °C for normal growth and does not tolerate frost (Onwneme, 1999), which restricts the increased production of the crop in Japan. Commercial taro production in the country is indeed confined to the temperate eastern, central and southwestern areas. Taro cultivation in the northernmost island, Hokkaido (latitude: 41° N - 46° N) has been considered to be impracticable due to the climate condition, especially short summer; in this prefecture, the daily average temperature generally rises above 21 °C only in July and August (http://www.data.jma.go.jp). However, it should be emphasized that Hokkaido accounts for 25 % of Japan's total cultivated acreage (http://www.pref.hokkaido.lg.jp/ns/nsi/genjyou_english_3101.pdf) and is frequently called as 'food storage' in Japan. Moreover, farm sizes (the cultivated area per farm household) in Hokkaido are on average 13 times greater than those of any other prefectures. Farmers mostly run their business on a large scale and full-time basis; in Hokkaido, business farm households, whose principal income is farming and which have at least one family member engaged in self-employed farm work for more than 60 days per year, account for 73 % in the total prefectural farm households, compared with 20 % in the other prefectures (OECD, 2009; http://www.pref.hokkaido.lg.jp/ns/nsi/genjyou_english_3101.pdf). Taro genotypes with tolerant ability to low temperature would enable this crop species to be cultivated under normally unfavorable culture conditions.

Interestingly, two early maturing genotypes have recently proven tolerant to a cooler climate (Shiga et al., 2011). The field trial for growth and yield performance of two cultivars ('Dotare' and 'Yamato-wase') was carried out during May to October of 2010 at the experimental farm (43°3′ N latitude and 141°′5 E longitude, ca. 100 m a.s.l) situated in Hokkaido. As shown in Table 3, the two genotypes produced yields comparable to or exceeding the average national yields of 1,290 kg 10 a^{-1} (MAFF, 2019). Similar results were found in a separately performed evaluation of yield parameters (M. Kamei, personal communication, May 28, 2019), implying that some early maturing cultivars would be useful genotypes for commercial taro cultivation under unfavorable growth conditions.

Nowadays, crop genomics provides plant breeders with a new set of tools and resources that allow the study of the genotype and its relationship with the phenotype (Pérez-de-Castro et al., 2012; Silva Dias, 2015). Genomic approaches are particularly useful when dealing with complex traits such as tolerance to cold climate and yield, since these traits usually have a multi-genic nature and are greatly influenced by the environment (Ivančič & Lebot, 2000). The molecular genetic researches and genetic basic data in taro are unfortunately limited in comparison with major crops (Liu et al., 2015). Nevertheless, the first taro linkage maps were generated with mainly amplified fragment length polymorphism (AFLP) and some simple sequence repeat (SSR) markers (Quero-García et al., 2006). Most recently, Soulard et al. (2017) identified a great number of single nucleotide polymorphism (SNP) loci to construct high-density genetic maps. Further efforts need to be directed towards identification and validation of a greater number of molecular markers and generating linkage maps with deeper coverage that can assist breeders in quantitative trait loci (QTL) dissection and marker assisted breeding.

8 ACKNOWLEDGEMENTS

The authors gratefully thank Mr. Makoto Nakamura (Managing Director, Hokkaido Agricultural Laboratory for Business Development) for support throughout this work and Mr. Masaru Kamei for valuable comments.

9 REFERENCES

Ashkenazi, M., Jacob, J. (2003). *Food culture in Japan*. Westport, Connecticut: Greenwood Press.

Banjaw, D. T. (2017). Review of taro (*Colocasia esculenta*) genetics and breeding. *Journal of Horticulture*, *4*, 196. https://doi.org/10.4172/2376-0354.1000196

Bhattacharjee, M., Tarafdar, J., Sadhukhan, R. (2014). Assessment of genetic diversity of some indigenous collections of upland taro [*Colocasia esculenta* var. *antiquorium* (L.) Schott] for selection of genotypes aiming at improvement in breeding programme. *IOSR Journal of Agriculture and Veterinary Science*, *7*(7), 31-43. https://doi.org/10.9790/2380-07713143

Bown, D. (2000). *Aroids. Plants of the arum family*. Portland: Timber Press.

Campo, I. S., Beghin, J. C. (2005). *Dairy food consumption, production, and policy in Japan.* CARD Working Papers. 418. https://lib.dr.iastate.edu/card_workingpapers/418

Chaïr, H., Traore, R. E., Duval, M. F., Rivallan, R., Mukherjee, A., Aboagye, L. M., …Lebot, V. (2016). Genetic diversification and dispersal of taro (*Colocasia esculenta* (L.) Schott). *PLoS ONE*, *11*(6), e0157712. https://doi.org/10.1371/journal.pone.0157712

Deo, P. C., Tyagi, A. P., Taylor, M., Becker, D. K., Harding, R. M. (2009). Improving taro (*Colocasia esculenta* var. *esculenta*) production using biotechnological approaches. *South Pacific Journal of Natural Science*, *27*, 6-13. https://doi.org/10.1071/SP09002

Devi, A. A. (2012). Genetic diversity analysis in taro using molecular markers – An overview. *Journal of Root Crops*, *38*(1), 15-25.

Devi, K. S., Singh, K. J., Devi, A. K. B., Mandal, J., Singh, H. B. (2016). Morphological evaluation of taro (*Colocasia esculenta* Linn.) genotypes collected from Manipur State of India and their yield performance. *Journal of Root Crops*, *42*(2), 18-23.

Ebert, A. W., Waqainabete, L. M. (2018). Conserving and sharing taro genetic resources for the benefit of global taro cultivation: A core contribution of the Centre for Pacific Crops and Trees. *Biopreservation and Biobanking*, *16*(5), 361-367. https://doi.org/10.1089/bio.2018.0017

FAO. (2019). *FAOSTAT database*. Retrieved from https://www.fao.org/faostat/en/#home

Helmkampf, M., Wolfgruber, T. K., Bellinger, M. R., Paudel, R., Kantar, M. B., Miyasaka, S. C., Shintaku, M. (2018). Phylogenetic relationships, breeding implications, and cultivation history of Hawaiian taro (*Colocasia esculenta*) through genome-wide SNP genotyping. *Journal of Heredity*, *109*(3), 272-282. https://doi.org/10.1093/jhered/esx070

Hirai, M., Sato, T., Takayanagi, K. (1989). Classification of Japanese cultivars of taro (*Colocasia esculenta* (L.) Schott) based on electrophoretic pattern of the tuber proteins and morphological characters. *Japanese Journal of Breeding*, *39*, 307-317. https://doi.org/10.1270/jsbbs1951.39.307

Isshiki, S., Nakamura, N., Tashiro, Y., Miyazaki, S. (1998). Classification of the cultivars of Japanese taro [*Colocasia esculenta* (L.) Schott] by isozyme analyses. *Journal of the Japanese Society for Horticultural Science*, *67*(4), 521-525. https://doi.org/10.2503/jjshs.67.521

Isshiki, S., Otsuka, K., Tashiro, Y., Miyazaki, S. (1999). A probable origin of triploids in taro [*Colocasia esculenta* (L.) Schott]. *Journal of the Japanese Society for Horticultural Science*, *68*(4), 774-779. https://doi.org/10.2503/jjshs.68.774

Ivančič, A., Lebot, V. (2000). *The genetics and breeding of taro*. Séries Repères, Montpellier: CIRAD

Kumazawa, S., Niuchi, K., Honda, F. (1956). Classification of the taro varieties in Japan. *Journal of the Japanese Society for Horticultural Science*, *25*(1), 1-10. https://doi.org/10.2503/jjshs.25.1 (in Japanese)

Kusaka, S., Ishimaru, E., Hyodo, F., Gakuhari, T., Yoneda, M., Yumoto, T., Tayasu, I. (2016). Homogeneous diet of contemporary Japanese inferred from stable isotope ratios of hair. *Scientific Reports*, *6*, 33122. https://doi.org/10.1038/srep.33122

Liu, H., You, Y., Zheng, X., Diao, Y., Huang, X., Hu, Z. (2015). Deep sequencing of the *Colocasia esculenta* transcriptome revealed candidate genes for major metabolic pathways of starch synthesis. *South African Journal of Botany*, *97*, 101-106. https://dx.doi.org/10.1016/j.sajb.2014.11.008

MAFF (Ministry of Agriculture, Forestry and Fisheries, Japan). (2019). *Statistics of taro production*. Retrieved from http://www.maff.go.jp/j/tokei/kouhyou/sakumotu/index.html (in Japanese)

Manner, H. I., Taylor, M. (2011). Farm and forestry production and marketing profile for taro (*Colocasia esculenta*). In C. R. Elevitch (Ed.), *Specialty crops for Pacific Island* agroforestry. (pp. 1-34). Holualoa, Hawaii: Permanent Agriculture Resources (PAR). http://agroforestry.net/scps

Matsuda, M., Nawata, E. (1999). Isozyme variation in taro, *Colocasia esculenta* (L.) Schott, from China, Taiwan, the Ryukyu Islands, and Japan: Its dispersal into Japan. *Tropics*, *8*(4), 397-407. https://doi.org/10.3759/tropics.8.397

Matthews, P. J. (2004). Genetic diversity in taro, and the preservation of culinary knowledge. *Ethnobotany Research and Applications*, *2*, 55-71. https://doi.org/10.17348/era.2.0.55-71

Matthews, P. J. (2010). An introduction to the history of taro as a food. In R. V. Ramanatha, P. J. Matthews, P. B. Eyzaguirre, D. Hunter (Eds.), *The global diversity of taro: Ethnobotany and conservation* (pp. 6-29). Rome: Bioversity International.

Matthews, P. J., Matsushita, Y., Hirai, M. (1992). Ribosomal and mitochondrial DNA variation in Japanese taro, *Colocasia esculenta* (L.) Schott. *Japanese Journal of Breeding*, *42*, 825-833. https://doi.org/10.1270/jsbbs1951.42.825

Nakagawa, T., Asaumi, H., Tamaki, M., Morikawa, T. (2016). 'Himekaguya', a new taro cultivar in Ehime prefecture. *Bulletin of the Ehime Research Institute of Agriculture, Forestry and Fisheries*, *8*, 19-23. (in Japanese)

Nakao, S. (1966). *The origin of cultivated plants and agriculture*. Tokyo: Iwanami-shoten. (in Japanese)

Nishimoto, T., Maegawa, H., Yamaguchi, T., Takamura, H., Matoba, T. (2009). Varietal differences in the cookability of taro corms. *Journal of Cookery Science of Japan*, *42*(2), 129-134. https://doi.org/10.11402/cookeryscience.42.129 (in Japanese)

Ochiai, T., Nguyen, V. X., Tahara, M., Yoshino, H. (2001). Geographical differentiation of Asian taro, *Colocasia esculenta* (L.) Schott, detected by RAPD and isozyme analyses. *Euphytica*, *122*, 219-234. https://doi.org/10.1023/A:1012967922502

OECD. (2009). *Evaluation of agricultural policy reforms in Japan*. Paris: OECD Publishing. https://doi.org/10.1787/9789264061545-en

Onwueme, I. (1999). *Taro cultivation in Asia and the Pacific*. Bangkok: RAP Publication.

Pérez-de-Castro, A. M., Vilanova, S., Cañizares, J., Pascual, L., Blanca, J. M., Diez, M. J., Picó, B. (2012). Application of genomic tools in plant breeding. *Current Genomics*, *13*(3), 179-195. https://doi.org/10.2174/138920212800543084

Quero-García, J., Courtois, B., Ivančič, A., Letourmy, P., Risterucci, A. M., Noyer, J. L., Lebot, V. (2006). First genetic maps and QTL studies of yield traits of taro (*Colocasia esculenta* (L.) Schott). *Euphytica*, *151*, 187-199. https://doi.org/10.1007/s10681-006-9139-y

Sasaki, K. (1971). *The agriculture before rice cultivation*. Tokyo: Nippon Hoso Shuppan Kyokai. (in Japanese)

Shiga, Y., Nakayasu, K., Fujiwara, K., Tsutsui, S. (2011). Growth and yield of early-maturing taro cultivars grown in mulched ridges. *Proceedings of the Hokkaido Society for Horticultural Research*, *44*, 40-41. (in Japanese)

Silva Dias, J. C. (2015). Plant breeding for harmony between modern agriculture production and the environment. *Agricultural Science*, *6*, 87-116. http://doi.org/10.4236/as.2015.61008

Soulard, L., Mournet, P., Guitton, B., Chaïr, H. (2017). Construction of two genetic linkage maps of taro using single nucleotide polymorphism and microsatellite markers. *Molecular Breeding*, *37*, 37. https://doi.org/10.1007/s11032-017-0646-4

Tahara, M., Suefuji, S., Ochiai, T., Yoshino, H. (1999). Phylogenetic relationships of taro, *Colocasia esculenta* (L.) Schott and related taxa by non-coding chloroplast DNA sequence analysis. *Aroideana*, *22*, 79-89.

Takeuchi, J., Nagashima, T. (2012). Low-malt beer brewing using of taro (*Colocasia esculenta* (L.) Schott) as a secondary material. *Nippon Shokuhin Kagaku Kogaku Kaishi*, *59*(3), 146-152. https://doi.org/10.3136/nskkk.59.146 (in Japanese)

Wilson, J. E., Cable, W. J. (1984). Promotion of flowering, seed production and seedling screening in minor edible aroids. In *Proceedings of the 6th Symposium of the International Society for Tropical Root Crops* (pp. 151-156). Lima: International Potato Center.

doi:10.14720/aas.2019.114.2.5 Original research article / izvirni znanstveni članek

Longevity and vigour of pigeon pea (*Cajanus cajan* (L.) Millsp.) seed stored under humid tropical ambient conditions

Moruf Ayodele ADEBISI [1], Tolulope Olaitan KEHINDE [1, 2], Tunmise Elizabeth OLADIPO [1], Isiaq Omoniyi LAWAL [3]

Received June 3, 2019; accepted October 15, 2019.
Delo je prispelo 03. junija 2019, sprejeto 15. oktobra 2019.

Longevity and vigour of pigeon pea (*Cajanus cajan* (L.) Millsp.) seed stored under humid tropical ambient conditions

Abstract: Seeds of 20 pigeon pea (*Cajanus cajan* (L.) Millsp.) genotypes were evaluated for seed longevity and vigour under humid ambient conditions. Cleaned seeds of each genotype were packed into a polyethylene bag, the packaged lot was electrically sealed and thereafter placed in a seed store under ambient conditions (30 °C, RH 75 %). Seed samples were withdrawn at 0, 60, 120, 180 and 240 days after storage for seed quality parameters evaluation. The experiment consisted of two factors which were genotype and storage duration and was a laid out in a completely randomized design with three replications. Data were collected on rate of seed germination, seed viability, seedling length, seedlings fresh mass, seedlings dry mass and seedling vigour index. Data collected were subjected to analysis of variance and significant treatment means were separated using Tukey's HSD test at 5 % probability level. PROBIT modelling was also used to predict the seed longevity of stored pigeon pea. Significant differences were observed in all seed quality attributes evaluated among the 20 pigeon pea genotypes and storage time except seedling fresh mass. Seed quality attributes decreased significantly with increasing storage periods. Genotypes NSWCC-18A, NSWCC-24, NSWC-34 and NSWCC-29A were identified to be superior for most of the seed quality attributes evaluated. PROBIT modelling result revealed that genotype NSWCC-29b had the highest storage life (16.28 months) and the highest storage potentials in terms of seed viability and other seed quality attributes of all seed lots.

Key words: PROBIT analysis; seed deterioration; seed storage; seed viability; storage period

Dolgoživost in vitalnost semen kajana (*Cajanus cajan* (L.) Millsp.) shranjenih v vlažnih tropskih razmerah

Izvleček: Dolgoživost in vitalnost semen 20 genotipov kajana (*Cajanus cajan* (L.) Millsp.) sta bili ovrednoteni v vlažnih tropskih razmerah. Očiščena semena vsakega od genotipov so bila shranjena v zaprte polietilenske vrečke, ki so bile nameščene v hrambo semen pod okoljskimi razmerami (30 °C, RH 75 %). Vzorci semen so bili odvzeti po 0, 60, 120, 180 in 240 dnevih za ovrednotenje parametrov kakovosti. Poskus je bil dvofaktorski, kjer je bil prvi dejavnik genotip, drugi pa trajanje hrambe in je bil izveden kot popolni naključni poskus s tremi ponovitvami. Zbrani so bili podatki o kalivosti semen, njihovi viabilnosti, dolžini sejank, sveži in suhi masi sejank in njihovem vitalnostnem indeksu. Podatki so bili ovrednoteni z analizo variance, poprečja značilnih obravnavanj so bila ločena s Tukeyevim HSD testom pri verjetnosti 5 %. Za predvidevanje dolgoživosti shranjenih semen kajana je bil uporaben PROBIT model. Ugotovljene so bile značilne razlike v vseh ovrednotenih kakovostnih parametrih semen med vsemi 20 genotipi kajana in med časi hrambe, z izjemo sveže mase sejank. Kakovostni parametri semen so se s časom shranjevanja značilno zmanjševali. Genotipi NSWCC-18A, NSWCC-24, NSWC-34 in NSWCC-29A so bili prepoznani kot najboljši v vseh ovrednotenih parametrih kakovosti. Na osnovi PROBIT modela je bilo odkrito, da je imel genotip NSWCC-29b med vsemi semenskimi vzorci najdaljši čas preživetja (16,28 mesecev) in največji potencial za shranjevanje glede na viabilnost semen in druge parametre kakovosti.

Ključne besede: PROBIT analiza; shranjevanje semen; propad semen; viabilnost semen; čas shranjevanja

1 Federal University of Agriculture, Department of Plant Breeding and Seed Technology, Abeokuta, Ogun State, Nigeria
2 Corresponding author, e-mail: kehindeto@funaab.edu.ng
3 Federal University of Agriculture, Department of Plant Physiology and Crop Production, Abeokuta, Ogun State, Nigeria

1 INTRODUCTION

Pigeon pea (*Cajanus cajan* (L.) Millsp.) is one of the most common tropical and subtropical legumes cultivated for its edible seeds. Pigeon pea is fast growing, hardy, widely adaptable, and drought resistant (Bekele-Tessema, 2007). Because of its drought resistance, it can be considered of utmost importance for food security in areas where rainfall is not reliable and droughts are likely to occur (Crop Trust, 2014). At the end of the dry season, pigeon pea provides green forage of outstanding value when other forages are not available (Sloan et al., 2009).

Though mainly cultivated for its edible seeds, pigeon pea can be considered a multipurpose species. Pigeon pea stems and branches are a good fuel source and basketry. Among other uses of pigeon pea, trials have shown a potential use as a raw material for paper pulp and also contribute to the environment through its use in alley cropping and as a windbreak, cover crop, shade plant and green manure (Cook et al., 2005). Despite all these numerous benefits of the crop, it is still categorized as under-utilized crop in Nigeria and little information is available on seed quality potential after harvest and during storage.

Seed is fundamental to production of crops. After maturation and harvest, seeds have to be stored until required for planting and need to maintain nearly 100 % germinations (Duffus and Slaughter, 1980). Deterioration and reduced longevity in seed is affected by enzymes activity, integrity of cell membrane, and stability of nucleic acid (Roberts, 1983). Seed quality can be influenced by environmental factors during seed production, harvesting, processing, storage and seed treatments (TeKrony et al., 1980; Copeland and McDonald, 2002; Adebisi and Ojo, 2001; Adebisi, 2004). Adetiloye (2005), Adebisi and Oyekale (2005) also reported that seed viability and seedling vigour are affected by the seed moisture level, drying temperature, seed mass, genetics constitution and length of storage. High temperature, high humidity and seed moisture content are main factors influencing seed storage behaviour (Abdul-Baki, 1980).

The longevity of seeds in storage is a good indicator of seed quantity and vigour in many crops (Ellis and Roberts, 1980; Robert, 1983; Adebisi et al., 2003, Kehinde, 2018). Ellis and Robert (1980) have developed PROBIT analysis method to quantify the initial quality of seed lot and its rate of deterioration using controlled deterioration tests. It's already well known that seed longevity is a function of storage temperature and seed moisture content (Harrington, 1972; Roberts, 1973), stresses before seed storage and initial seed quality (Ellis and Roberts, 1980), genetic make-up (Adebisi et al., 2008a; Kehinde, 2018) and pest and pathogen damages in storage (Kulik, 1995; Abdul–Rafiu, 2007). Seed deterioration in storage follows a negative cumulative normal distribution pattern (Ellis and Roberts, 1980) which make estimation of seed longevity from seed germination data using PROBIT analysis possible (Finney, 1971) and assessment of seed viability under specified conditions (Daniel, 1997; Kehinde, 2016).

Longevity of a seed is the period from seed maturation until seed death (Ellis and Roberts, 1981). Hong and Ellis (1996) reported that seed longevity varies greatly among species. This may also vary among accessions within a species. Kashyap et al. (1994) found significant differences among different cultivars and period of storage of wheat seed for all parameters of viability and vigour. There were significant differences in viability and vigour of soybean, rice, sesame and kenaf during storage (Thseng et al., 1996; Nkang and Umah 1996; Kamaswara and Jackson, 1996; Adebisi and Ajala, 2000; Adebisi et al., 2013a). Harrington (1972) reported that germination and vigour in crop decreased as storage time and relative humidity increased.

Storage of tropical seeds of different crop species is a problem under humid tropical condition due to high temperature and relative humidity. In Nigeria, pigeon pea farmers are facing problem of preservation of farm save seeds of this crop after harvest due to precarious environmental conditions of temperature and relative humidity. The seed of this crop is not commercially available in seed marketing outlets thereby reducing farmers' access to high quality seed and enabling them to contend with available stock of poor viability with poor seedling establishment and consequently reducing grain yield. However, there has been dearth of information on storage potentials of the selected 20 pigeon pea genotypes under ambient humid tropical conditions but a little information on them was their outstanding grain yield performance. Hence, there is a need to provide information to fill the knowledge gap in the focus of the research. The objectives of the study were to investigate the effect of storage duration on seed physiological quality attributes of pigeon pea genotypes stored under ambient humid tropical storage conditions, and estimate the storage life of 20 pigeon pea genotypes using PROBIT modelling techniques.

2 MATERIALS AND METHODS

2.1 SEED MATERIAL AND SOURCE

Seeds of twenty (20) genotypes of pigeon pea used

were obtained from Institute of Agriculture Research and Training (IAR&T) Ibadan, Oyo State Nigeria, and International Institute of Tropical Agriculture (I.I.T.A) Ibadan, Oyo State Nigeria. The seeds were freshly harvest in the dry season of 2017.

2.2 TREATMENT, EXPERIMENT DESIGN AND LOCATION

Two factors were investigated in the study (pigeon pea genotypes in 20 levels and storage periods in five levels). The experiment was laid out in a completely randomized design with three replications. The experiment was conducted at the seed processing and storage unit and Laboratory of Plant Breeding and Seed Technology Department, Federal University of Agriculture Abeokuta (FUNAAB), Ogun State, Nigeria.

2.3 SEED STORAGE

Cleaned seeds (300 g) of each genotype were packed into thick polyethylene bags (size 15 × 15m) and each of the packaged lot was electrically sealed and thereafter placed under ambient conditions of seed store in seed processing and storage house, FUNAAB. The average temperature and relative humidity was monitored using hygrometer for the period of storage. The packaged seeds were stored for 240 days (8 months) and samples were taken at 0, 60, 120, 180 and 240 days after storage for seed quality assessment.

2.4 SEED QUALITY EVALUATION

Standard germination tests were carried out in the laboratory of Department of Plant Breeding and Seed Technology, FUNAAB with the use of Petri dishes. Fifty seeds of each of these genotypes were placed in Petri dishes with 20 ml distilled water and then put in the incubator for maintained at 20 °C for 8 days.

From the seed germination tests above, data were collected on the following seed quality parameters:

Rate of germination: Germination counts of normal seedlings were taken on 4th day and recorded in percentage of 50 seeds sown to determine the germination rate/speed (Adebisi, 2004).

Seed germination: Germination counts of normal seedlings were taken on 8thday and expressed in percentage of 50 seeds sown according to ISTA (1995).

Seedling length (cm): Shoot lengths of 10 randomly picked seedlings per replicate were measured in centimeter.

Seedling vigour index: This was determined using the formula of Kim et al. (2002), modified by Adebisi (2004):

SVI = Germination (%) x Seedling Lenght (cm)/100

Seedling fresh mass (g): Mass of 10 randomly picked fresh seedlings per replicate from the germinated seeds under the germination test were measured using a sensitive scale. (Adebisi, 2004).

Seedling dry mass (g): Mass of 10 randomly picked seedlings per replicate were measured after oven dried for 1 hour at 130 °C temperature (ISTA, 1995).

2.5 DATA ANALYSIS

Data collected on six seed physiological quality attributes were subjected to two-way Analysis of Variance (ANOVA) with storage period and pigeon pea genotypes as the treatments. Means of significant treatments were compared using Tukey's Honest Significance Difference (HSD) test at 5 % probability level and PROBIT modelling as proposed by Ellis and Robert (1980) and reported by Adebisi et al. (2008a) was also carried on seed longevity data.

3 RESULTS

Table 1 shows the mean performance of 20 pigeon pea genotypes for seed quality parameters. In rate of germination, NSWCC-24 had the highest performance of 78 % followed by NSWC-34 while the lowest performance was recorded in TCC-6 with value of 16 %. Also, for seed viability, the highest performance was found in NSWCC-24 (77 %) but not significantly different from NSWCC-18b, NSWCC-18A, NSWCC-7D, NSWCC-34A, NSWCC-29B, NSWCC-15, NSWCC-29A (69-73 %) followed by NSWCC-18A (74 %) while the lowest performance were found in TCC-6, TCC-1, TCC-8, CITA-1, CITA-2 with range of 16 % - 24 %. NSWC-34 recorded the highest seedlings length (19.25 cm) and seedling vigour index (14. 38 %) compared to other genotypes. Seedling dry mass and fresh mass were also with highest values in NSWDD-34(1.01) and CITA-2 (6.21) respectively.

From the result in Figure 1, the highest rate of germination was attained at 0 month of storage (67 %) but statistically similar values were obtained at 2-3 months of storage with 62 and 61 %, respectively while seeds stored for 8 months recorded the lowest germination rate (39 %). For seed viability, seed stored at 2 months

Table 1: Mean performance of 20 pigeon pea genotypes for seed quality parameters over storage periods under humid tropical ambient conditions (30 °C, RH 75 %)

Genotype	Rate of Germination (%)	Seed viability (%)	Seedling length (cm)	Seedling fresh mass (g)	Seedling dry mass (g)	Seedling Vigour Index
NSWDD-34	64.00cde	66.00bc	15.35cde	3.05ab	1.01a	10.22bcd
NSWCC-18b	70.00abc	72.00ab	14.37de	2.79ab	0.87ab	10.35bcd
NSWCC-18A	69.00abc	74.00ab	18.82ab	2.82ab	0.93ab	14.44a
NSWCC-24	78.00a	77.00a	16.61a-d	2.67ab	0.82b	13.02ab
NSWCC-35A	59.00de	65.00bc	16.00a-e	3.22ab	0.84ab	10.62bc
NSWCC-29b	66.00b-e	66.00bc	16.24a-d	2.94ab	0.86ab	10.98bc
NSWCC-7D	68.00a-d	71.00ab	17.92a-d	3.07ab	0.89ab	12.88ab
NSWCC-34A	71.00abc	72.00ab	16.18a-d	2.97ab	0.79b	11.86abc
NSWCC-32	61.00cde	67.00bc	13.84de	2.87ab	0.79b	9.24cd
NSWCC-34	56.00e	58.00bc	12.57e	2.16ab	0.75b	7.30d
NSWCC-29d	69.00abc	69.00ab	14.56cde	3.18ab	0.85ab	10.39bcd
NSWCC-15	65.00b-e	68.00ab	13.27de	3.04ab	0.77b	9.38cd
NSWCC-29A	69.00abc	72.00ab	16.40a-d	3.02ab	0.89ab	12.38abc
NSWC-34	74.00ab	73.00ab	19.25a	3.17ab	0.85ab	14.38a
NSWCC-3D	64.00cde	65.00bc	15.69b-e	2.82ab	0.78b	10.40bcd
TCC-1	20.00fg	16.00d	3.57h	0.49b	0.33c	1.25e
TCC-6	16.00g	16.00d	9.03f	0.59b	0.29c	1.93e
TCC-8111	21.00fg	21.00d	5.24gh	0.46b	0.27c	1.95e
CITA-1	29.00f	24.00d	7.81fg	0.72b	0.32c	2.73e
CITA-2	27.00f	19.00d	2.66h	6.21a	0.73b	2.00e

Means followed by same alphabet along column are not different from each other at 5 % probability level according to Tukey's HSD test at 5 % probability level.

showed highest performance of 68 % above value obtained at 0 month (66 %) while the lowest value was obtained after 8 months (37 %). For seedling length, 0 month stored time had highest value (16.02 cm) followed by 2 months and 4 months with 14.89 and 14.99 cm, respectively. But seedlings fresh mass value was statistically similar during the 8 months of storage while seedling dry mass had its best performance at 0 month (0.93 g) followed by values at 2 months and 4 months while it was least at 8 months. Seeds stored for 2 months had the highest seedlings vigour index (11.59 %) above seeds stored for 0 month storage time (11.29 %) followed by 6 months of storage while least seedling vigour index was in seeds stored for 8 months (4.29 %).

Bars followed by the same alphabet along the column are not different from each other

Table 2 shows the effect of storage time on rate of seed germination in the 20 pigeon pea genotype. From the Table 2, at 0 month of storage, NSWCC-24 (93 %), NSWC-34 (92 %) and NSWCC-29A (90 %) had the highest germination rate while TCC-6 (18 %) and TCC-1 (20 %) had the least germination rate. At 2 month of storage, the highest performance was also observed in NSWCC-24 (87 %) closely followed by NSWC-34 (85 %) and NSWCC-29A (80 %) while the least performance was recorded in TCC-6 (27 %), TCC-1(25 %) and CITA-2 (23 %). Also at 4 months of storage, NSWCC-24 (83 %), NSWC-34 (80 %) were with the highest values and least performance was in TCC-6 (23 %) and TCC-1 (18 %). With increase in the storage time to 6 months, NSWCC-24 (78 %) showed the highest germination rate which was followed by NSWCC-34A (70 %). However, for seed stored for 8 months, NSWCC-18b and NSWCC-7D (57 %) had the highest germination rate and closely followed by NSWCC-34A, NSWCC-15, NSWCC-3D and NSWDD-34 with (52-53 %). The least germination rate was also recorded in TCC-8111, TCC-1 and TCC-6, with values of 8 %, 10 % and 13 %, respectively.

Effect of storage time on seed viability of 20 pigeon pea genotypes (Table 3) shows that at 0 month

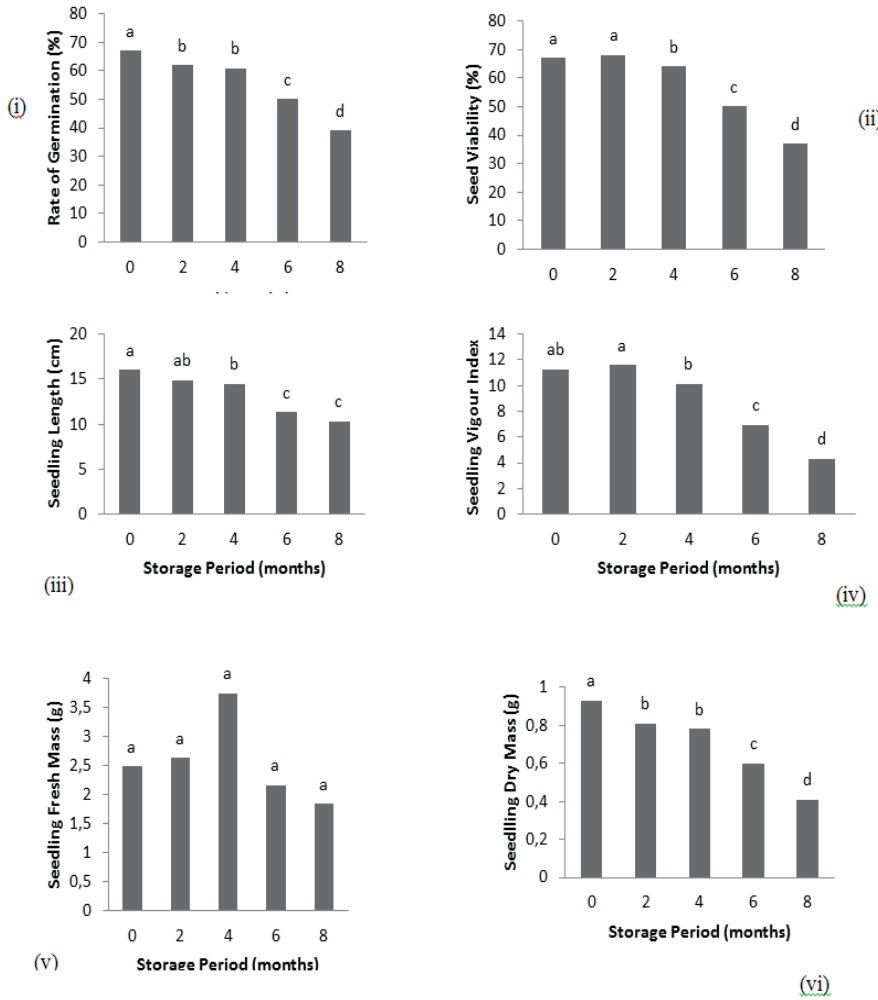


Figure 1: Changes in seed quality parameters during storage period under humid tropical ambient conditions (30 °C, RH 75 %)

of seed storage, NSWCC-24 (93 %) had the highest viability closely followed by NSWCC-18A and NSWCC-18b with values of 88 % and 87 %, respectively while TCC-6 (18 %) and TCC-1 (20 %) had the least value. At 2 months of storage, NSWCC-18A, NSWCC-24, NSWCC-29A and NSWC-34 were with highest seed viability values which ranges from 85 % - 90 % while the highest values at 4 months were obtained in NSWCC-18b (82 %), NSWCC-18A (82 %) and NSWCC-29A (82 %). Also, for seed stored for 6 months, NSWCC-24 (72 %) had the highest viability which was closely followed by NSWCC-7D and NSWCC-34 (68 %). Intrinsically, TCC-6, TCC-1, TCC-8111, CITA-2 exhibited the lowest viability of 2 - 17 % across the storage periods evaluated.

The effect of storage time on seedling length of 20 pigeon pea genotype is presented in Table 4. At 0 month of storage, NSWC-34, NSWCC-18A and NSWCC-7D were with the highest seedling lengths of 20.86 cm, 20.81 cm and 20.23 cm, respectively while least values were obtained in TCC-6 (8.96) and TCC-8111 (9.76). Similarly, NSWCC-18A (21.48) and NSWC-34 (20.79) had the highest seedling lengths for seed stored for 2 months, 4 months, 6 months and 8 months respectively. Conversely, TCC-6, TCC-1, TCC-8111, CITA-1 and CITA-2 had the lowest seedling lengths throughout the storage periods used for the research.

In Table 5, at 0 month storage period, NSWCC-29A had the highest seedling dry mass of 1.22 g followed by NSWCC-34A (1.20 g) while the lowest dry mass were found in CITA-1, TCC-8111 and TCC-1 with values of 0.48 g, 0.46 g, and 0.43 g, respectively. After 2 months storage period, NSWDD-34 (1.18 g) had the highest seedling dry mass, keenly followed by NSWCC-35A (1.09 g), NSWCC-29b (1.06 g) and NSWCC-7D (1.04 g) while the lowest dry mass was also recorded in TCC-811, CITA-1 and CITA-2, with values of (0.04 - 0.37). Similarly, the highest dry mass value was

Table 2: Effect of storage time on rate of seed germination in 20 pigeon pea stored under humid tropical ambient conditions (30 °C, RH 75 %)

Genotype	Storage Period (Months)				
	0	2	4	6	8
NSWDD-34	75cd	68c	70b	55c	52a
NSWCC-18b	87ab	67c	78a	62c	57a
NSWCC-18A	88ab	73b	70b	67b	48b
NSWCC-24	93a	87a	83a	78a	47b
NSWCC-35A	65e	60d	58d	62c	48b
NSWCC-29b	72d	75b	73b	65b	47b
NSWCC-7D	80c	75b	67c	63b	57a
NSWCC-34A	80c	73b	80a	70b	53a
NSWCC-32	75cd	70b	63c	58c	40c
NSWCC-34	63e	66c	57d	67b	37c
NSWCC-29d	83bc	77b	70b	67b	48b
NSWCC-15	67e	68c	67c	68b	53a
NSWCC-29A	90a	80a	75b	60c	40c
NSWC-34	92a	85a	80a	63b	52a
NSWCC-3D	75cd	67c	70b	57c	52a
TCC-1	18g	27f	23f	20d	13d
TCC-6	20g	25f	18f	7e	10e
TCC-8111	28f	45e	37e	4f	8e
CITA-1	31f	42e	40e	13e	13d
CITA-2	31f	23f	42e	23d	20d

Means followed by alphabet along column are not different from another according to Tukey's HSD test at 5 % probability level.

recorded for NSWDD-7D (1.09 g) after 4 months storage period, which was closely followed by NSWCC-29A (1.00 g). After 6 months storage period, NSWDD-7D (0.96 g) was the best performing genotype in terms of seed dry mass and this was followed by NSWCC-7D (0.89 g). Also, for seeds stored for 8 months, NSWDD-7D (0.83 g) was the best and it was followed by NSWCC-18b (0.66 g). Meanwhile, TCC-6, TCC-6, TCC-8111, CITA-1 and CITA-2 were the least performing genotypes throughout the storage periods used.

The effect of storage time on seedling vigour index on seeds of 20 pigeon pea genotypes is presented in Table 6. At 0 month of storage, NSWCC-18A had the highest seedling vigour index of 18.74 and was followed by NSWC-34 and NSWCC-24 with values of 17.75 and 17.03 respectively while the lowest value was in TCC-6 (1.73). After 2 months of storage, the highest seedling vigour index was also found in NSWCC-18A while NSWCC-18A showed the highest value after 4 and 8 months while NSWC-34 (11.51) was found to have the highest seedling vigour index. Conversely, the lowest values throughout the storage periods were found in TCC-6, TCC-1, TCC-8111, CITA-1 and CITA-2.

The results of PROBIT analysis of seed viability data in 20 pigeon pea genotypes over 240 days of storage (Table 7) reveal that the values in all studied varieties of genotypes of pigeon pea indicate that the seeds maintained its viability irrespective of the storage time. All genotypes recorded relatively low rate of deterioration ranging from 0.0142 in TCC-8111 to 0.0016 in TCC-1. Relatively, NSWCC-29b showed the highest time taken (493.08 days) to lose 1 PROBIT viability followed by NSWCC-35A (350.45) while TCC-811 with the value (70.56 days) recorded the lowest time taken to lose 1 PROBIT viability. NSWCC-29b recorded the highest value in days to seed half-life (244.1 days) while CITA-1 (18.6 days) which had lowest value of seed half-life. However, the longest seed storage life of (16.28 months) was obtained in NSWCC-29b followed by NSWCC-35A, NSWCC-7D, NSWCC-15 and TCC-1 with storage life of above 12 months but others exhibited storage life between 8 and 11 months except

Table 3: Effect of storage time on seed viability of 20 pigeon genotypes stored under humid tropical ambient conditions (30 °C, RH 75 %)

Genotype	Storage Period (Months)				
	0	2	4	6	8
NSWDD-34	75c	78b	70b	58b	47bc
NSWCC-18b	87a	78b	73b	65a	55a
NSWCC-18A	88a	90a	82a	62b	50a
NSWCC-24	93a	88a	82a	72a	50a
NSWCC-35A	65d	78b	73b	67a	43bc
NSWCC-29b	72c	73bc	72b	63b	52a
NSWCC-7D	80b	80ab	75a	68a	52a
NSWCC-34A	83b	82b	78a	68a	50a
NSWCC-32	82b	83a	77a	60b	33c
NSWCC-34	65d	67c	65c	58b	33c
NSWCC-29d	70c	85a	77a	60b	55a
NSWCC-15	62d	82ab	77a	67a	53a
NSWCC-29A	85b	88a	82a	63b	43bc
NSWC-34	85b	85a	80a	67a	50a
NSWCC-3D	72c	77b	73b	58b	46bc
TCC-1	18f	12f	23e	17c	10d
TCC-6	20f	23e	25e	7d	7d
TCC-8111	28e	43d	33d	2d	5e
CITA-1	32e	43d	33d	13c	1e
CITA-2	31e	18e	30d	17c	13d

Means followed by same alphabet along column are not different from another according to Tukey's HSD test at 5 % probability level.

NSWCC-29d (6.18 months), TCC-8111 (1.99 months), CITA-1 (1.24 months) and CITA-2 (2.72 months).

4 DISCUSSION

Storage period is an important stage in the seed production process, the preservation of seed during this process, that is, from harvest time to the time of its use is an essential aspect to be regarded in the production process, because the effort spent in the production phase may not be effective if the seed quality is not maintained (Oliviera et al., 1999). Also, the utilization of high quality seed lot constitutes one of the major factors responsible for successful crop production.

The analysis of variance shows that replicate effect was not significant and this could be attributed to the homogenous environment in which the research was carried out. The variations observed in all seed quality parameters among genotypes revealed that the studied 20 genotypes significantly differed in their genetic make-up, which led to a variation in their responses to different storage periods.

The study revealed that there were significant differences among the seed quality attributes in response to increased storage periods. Rate of germination, seed viability and seedling vigour index attributes decreased as storage period increased. Similarly, Khalequzzaman et al. (2012) reported that seed quality parameters were significantly influenced by the increase in storage period of French bean (*Phaseolus vulgaris* L.). Similarly, the report of the findings of Adebisi et al. (2008a) in sesame, Daniel et al., (2012) in maize and Adebisi et al., (2012 and 2013b) in water melon and kenaf respectively all corroborated the findings of this study that differences in genetic makeup of genotypes could influence storage performance in different crop species under the ambient tropical conditions.

On the storability performance, among the 20 genotypes, NSWCC-18A, NSWCC-24, NSWC-34 and NSWCC-29A were identified to have superior performance regarding most of the seed quality attributes studied, which could be due to superiority in the ge-

Table 4: Effect of storage time on seedling length of 20 pigeon genotype stored under humid tropical ambient conditions (30 °C, RH 75 %)

Genotype	Storage Period (Months)				
	0	2	4	6	8
NSWDD-34	15.77c-f	16.45d-g	15.74def	15.40abc	13.36b-e
NSWCC-18b	14.68def	14.99f-i	14.68fg	14.07bcd	13.45b-e
NSWCC-18A	20.81a	21.48a	20.17a	16.04ab	15.60ab
NSWCC-24	18.11ba	17.77cde	17.40bcd	15.36abc	14.41a-d
NSWCC-35A	16.12cde	16.72d-g	16.10c-f	15.57abc	15.46ab
NSWCC-29b	17.44bc	17.40c-f	17.18b-e	14.70abc	14.48abc
NSWCC-7D	20.23a	19.41abc	18.77ab	15.50abc	15.71ab
NSWCC-34A	17.12bcd	17.18c-f	17.17b-f	14.93abc	14.50abc
NSWCC-32	14.60de	14.57ghi	14.81efg	13.27cde	11.97def
NSWCC-34	13.04f	13.20hi	12.93gh	12.03de	11.66ef
NSWCC-29d	16.24cde	15.28e-h	15.04d-g	13.40cd	12.85cde
NSWCC-15	14.25ef	15.74efg	15.30d-g	10.90e	10.15f
NSWCC-29A	18.77ab	18.64bcd	18.50abc	14.41bcd	11.67ef
NSWC-34	20.86a	20.79ab	20.67a	17.16a	16.74a
NSWCC-3D	17.14bcd	17.01c-g	16.69b-f	13.90bcd	13.74b-e
TCC-1	8.96g	2.66k	3.20j	3.05f	9.94f
TCC-6	17.17bcd	13.11hi	12.87gh	2.00f	6.83g
TCC-8111	9.76g	9.24j	8.09i	1.17f	5.05g
CITA-1	13.28f	12.62i	11.10h	2.03f	1.37h
CITA-2	5.48g	3.47kl	3.30e	2.89f	1.00h

Means followed by same alphabet along column are not different from another according to Tukey's HSD test at 5 % probability level

netic make-up of the genotypes combined with other physical characteristics of their seeds. These genotypes, TCC-6, TCC-1, TCC-8111, CITA-1 and CITA-2 were the least storable in all the seed quality attributes which could be due to their genetic weakness and weak physical characteristics of their seeds.

The highest seed viability percentages were recorded at 0 and 2 months of storage while there was a great fall in seed viability at 6 months of storage and seeds stored at 8 months of storage had the lowest seed viability. However, seed stored at 0 month had the highest seedling vigour index while seeds store at the end of 8 months had the lowest seedling vigour. This decline in seed quality could be due to deteriorative process which occurs in all biological organisms. Ageing gradually sets in and advanced with length of storage and was further aggravated by the high temperature (30) and high relative humidity (75 %) of the ambient humid conditions which enhanced higher respiration and led to higher degradation of assimilates leading to the death of many of the stored seeds.

Seed deterioration is associated with various cellular, metabolic and chemical alterations including chromosome aberrations and damage to the DNA, impairment of RNA and protein synthesis, changes in the enzymes and food reserves and loss of membrane integrity (Kibinza et al., 2006). According to Kapoor et al. (2011), seed deterioration is the loss of seed quality (viability and vigour) due to the adverse effect of environmental factors and is a natural process which involves cytological, physiological and biochemical changes. The rate of deterioration fluctuates critically from one species to another and also among varieties of the same species (Jatoi et al., 2001; Jyoti and Malik, 2013). The deterioration is evident in the reduction in percentage germination, production of weak seedlings, loss of vigour, seed become less viable and ultimately seed death (Murthy et al., 2000; Tilebeni and Golpayegani, 2011).

Furthermore, the study observed that storage period under ambient should be given due consideration when storing seeds, irrespective of genotypes available for storage. The environmental conditions in storage are very difficult to maintain and highly influenced the period of seed survival (Jyoti and Malik, 2013). Lower

Table 5: Effect of storage time on seedling dry mass of 20 pigeon pea genotypes stored under humid tropical ambient conditions (30 °C, RH 75 %)

Genotype	Storage Period (Months)				
	0	2	4	6	8
NSWDD-34	0.97de	1.18a	1.09a	0.96a	0.83a
NSWCC-18b	0.98de	0.97b-f	0.88b-f	0.86abc	0.66b
NSWCC-18A	1.18ab	1.07abc	0.97abc	0.83abc	0.61bc
NSWCC-24	0.94de	0.92def	0.87b-f	0.77bc	0.60bc
NSWCC-35A	0.92de	1.09ab	0.91b-e	0.82bc	0.46d-g
NSWCC-29b	0.88e	1.06abc	0.91b-e	0.86abc	0.58bcd
NSWCC-7D	1.02cd	1.04bcd	0.93bcd	0.89ab	0.56b-e
NSWCC-34A	1.20a	0.78g	0.77f	0.74cd	0.48cde
NSWCC-32	0.97de	0.97b-f	0.85c-f	0.76bc	0.43efg
NSWCC-34	0.96de	0.84f	0.83def	0.79bc	0.33g
NSWCC-29d	0.98de	0.95c-f	0.95def	0.74cd	0.61bc
NSWCC-15	0.94de	0.86efg	0.83bcd	0.75cd	0.48c-f
NSWCC-29A	1.22a	0.99b	1.00ab	0.81bc	0.41fg
NSWC-34	1.02cd	0.92def	0.91b-e	0.78bc	0.60bc
NSWCC-3D	1.05bcd	0.87efg	0.80ef	0.62d	0.56b-e
TCC-1	1.15abc	0.32j	0.18h	2.03^{-16}e	9.20^{-16} e
TCC-6	0.73f	0.48hi	0.54g	8.92^{-17}e	8.80^{-16} e
TCC-8111	0.46f	0.37ij	0.51g	1.33^{-16}e	1.18^{-15} f
CITA-1	0.48f	0.61h	0.49g	9.00^{-17}e	3.30^{-15} f
CITA-2	0.30f	0.04k	0.26h	2.04^{-15}e	3.08^{-14} f

Means followed by same alphabet along column are not different from another according to Tukey's HSD test at 5 % probability level.

temperature and relative humidity delayed seed deterioration process thereby leads to prolong viability period (Mohammadi et al., 2011).

Earlier reports by Adebisi et al. (2003, 2008b), Esuruoso (2010), Adebisi and Oyekale (2005) and Oni (2012) have utilized probit modelling to predict storage life of soybean, rice, kenaf, okra, and sesame, respectively under ambient humid storage conditions. In this study, the result of PROBIT modelling showed that the seeds maintained its viability, irrespective of the storage time of a period of 240 days. The pigeon pea seeds had very low rate of deterioration, in all the (20) genotypes used in this study implying that seed longevity was prolonged. NSWCC-29b had the highest value in days to seed half-life indicating high storability potential of such seeds, while CITA-1 had the lowest value in days to seed half-life indicating that this genotype was least storable under ambient humid conditions. Both genotypes also exhibited same rate for storage life. Nevertheless, the PROBIT modelling predicted that NSWCC-29b can be stored for an average of 16.28 months before it starts deteriorating, if the seeds are put under good storage conditions.

5 CONCLUSIONS

There were highly significant differences in all included seed quality parameters among 20 studied pigeon pea genotypes. These seed quality parameters were greatly influenced by storage period and all seed quality parameters declined with increase in storage duration due to the intrinsic factors in the seeds, irrespective of genotype as well as other factors such as endosperm size, seed coat, hormonal profile of the seed, the type of assimilate that is predominant in the seed among others. Storage period of pigeon pea under ambient conditions should be between 1-6 months in order to maintain high seed quality parameters. NSWCC-18A, NSWCC-24, NSWC-34 and NSWCC-29A were the best genotypes across the seed quality attributes across the 240 days of storage and should be considered in selection for high quality seeds. The highest estimated seed

Table 6: Effect of storage time on seedling vigour index on 20 pigeon pea genotypes stored under humid tropical ambient conditions (30 °C, RH 75 %)

Genotype	Storage Period (Months)				
	0	2	4	6	8
NSWDD-34	11.89e	12.99de	11.02f	9.07b-f	6.14abc
NSWCC-18b	12.70de	11.68e	10.84f	9.13b-e	7.04ab
NSWCC-18A	18.74a	19.31a	16.38a	9.25abc	7.80a
NSWCC-24	17.03ab	15.71bc	14.20abc	11.01ab	7.15ab
NSWCC-35A	10.80ef	13.18de	11.88c-f	10.52ab	6.70ab
NSWCC-29b	12.92de	12.83d	13.31c-f	9.33a-d	7.49ab
NSWCC-7D	16.11bc	15.47bc	14.10bbc	10.63ab	8.09a
NSWCC-34A	14.26cd	14.09cd	13.43b-e	10.20abc	7.30ab
NSWCC-32	11.95de	12.12d	11.36e	6.80f	3.98c
NSWCC-34	8.53d	8.75f	8.39g	6.97ef	3.88c
NSWCC-29d	12.06de	13.18de	11.59def	8.04c-f	7.08ab
NSWCC-15	9.29f	13.06de	11.83def	7.24def	5.45bc
NSWCC-29A	15.95bc	16.51b	15.13ab	9.16b-e	5.17bc
NSWC-34	17.75ab	17.17ab	16.54a	11.51a	8.37a
NSWCC-3D	12.40de	12.97e	1316c-f	8.10c-f	6.36ab
TCC-1	1.73h	3.30h	0.73i	0.49g	0.00d
TCC-6	3.47gh	3.03h	3.15h	1.76-15h	0.00d
TCC-8111	2.82gh	4.17gh	2.75hi	1.76-15h	0.00d
CITA-1	4.16g	5.43g	3.62h	0.41g	0.00d
CITA-2	4.08g	6.29g	1.00i	0.51g	0.20d

Means followed by same alphabet along column are not different from another according to Tukey's HSD test at 5 % probability level.

storage life of 16.28 months was derived for genotype NSWCC-29b. Storage of pigeon pea seeds under favourable ambient environments offer good potential for short term pigeon pea seed quality maintenance.

6 RECOMMENDATIONS

Seed quality of pigeon pea deteriorates as storage time increases therefore, storage period should not exceed 6 months NSWCC-18A, NSWCC-24, NSWC-34 and NSWCC-29A were recommended for breeding process with utmost aim of high seed quality.

7 REFERENCES

Abdul- Rafiu, A.M. (2007). *Effect of genotype and seed treatments on Sitophilus zeamais infestation and maize seed quality*. M. Agric., Dissertation. Department of Plant Breeding and Seed Technology, Federal University of Agriculture, Abeokuta, Nigeria 83 pp.

Abdul-Baki, A. A. (1980). Biochemical aspect of vigour. *Hortscience, 15*, 765-771.

Adebisi, M. A. (2004). *Variation, stability and correlation studies in seed quality and yield characters of sesame*, PhD Thesis., Federal University of Agriculture, Abeokuta, Nigeria 148 pp.

Adebisi, M. A and Ajala, M. O. (2000). Effect of seed dressing chemicals and period of storage on soybean seed vigour. *Journal of Tropical Forest Resources, 16*(1), 126-135.

Adebisi, M. A. and Ojo, D. K. (2001). Effect of genotypes on soybean seed quality development under West African rain fed conditions. *Pertanika Journal of Tropical Agriculture*, *24*(2), 139-145.

Adebisi, M. A., Daniel, I. O. and Ajala, M. O. (2003). Storage life of soybean seed after seed dressing. *Journal of Tropical Agriculture, 42*, 3-7.

Adebisi, M. A., Ola, J. A., Akintobi, D. C. A and Daniel, I. O. (2008a). Storage life of sesame (*Sesamum indicum* L.) seed under humid tropical conditions. *Seed Science and Technology, 36*, 379-387. https://doi.org/10.15258/sst.2008.36.2.11

Adebisi, M. A. Adeniyi, T. A, Akintobi, D. C. A and Ajala M. O. (2008b). Varietal differences in seed physiological quality

Table 7: PROBIT parameters of the seed viability data in 20 Pigeon pea genotypes stored under humid tropical ambient conditions (30 ºC, RH 75 %) over 240 days storage time

Genotype	*Intercept	**Slope	***Sigma	*%P_{50} (Days)	*#Seed storage life (Months)
NSWDD-34	1.06	-0.0071	141.66	149.41	9.96
NSWCC-18b	1.32	-0.0079	131.29	169.43	11.30
NSWCC-18A	1.85	--0.0122	86.75	152.94	10.20
NSWCC-24	2.02	-0.0130	86.`13	163.17	10.88
NSWCC-35A	0.87	-0.0034	350.45	207.00	13.80
NSWCC-29b	0.87	-0.0049	493.08	244.16	16.28
NSWCC-7D	1.36	-0.0081	196.25	189.00	12.60
NSWCC-34A	1.37	-0.0082	128.53	172.27	11.48
NSWCC-32	1.59	-0.0120	84.77	130.69	8.71
NSWCC-34	0.76	-0.0062	173.94	128.68	8.58
NSWCC-29d	1.34	-0.0233	190.47	92.71	6.18
NSWCC-15	0.77	-0.0032	256.42	199.35	13.29
NSWCC-29A	1.69	-0.0114	89.15	147.41	9.83
NSWC-34	1.53	-0.0096	108.86	162.48	10.83
NSWCC-3D	0.97	-0.0062	160.88	155.45	10.36
TCC-1	0.61	-0.0016	274.32	197.20	13.15
TCC-6	0.41	-0.0073	248.03	137.87	9.19
TCC-8111	0.16	-0.0142	70.56	29.60	1.98
CITA-1	0.25	-0.0115	77.87	18.63	1.24
CITA-2	0.34	-0.0048	161.90	40.80	2.72

*Intercept is PROBIT estimate of initial seed viability
**slope is the rate of seed deterioration
***Sigma is time taken for seed lot to lose 1 probit viability
*% P50 is seed half- life in days
*# Seed storage life estimated as P50 value multiplied by 2 then divided by the 30 days of a month

of West Africa rice after heat treatment. *The Nigerian Agric. Journal*, *38*(2), 159-169.

Adebisi, M. A., Abdul-Rafiu, A.M., Abdul, R. S., Daniel, I. O. and Tairu, F. M. (2012). Seed longevity and vigour of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) seeds stored under ambient humid tropical conditions. *Nigerian Journal of Horticultural Science, 17*, 169-178.

Adebisi, M.A., Kehinde, T. O., Salau, A. W., Okesola, L. A., Porbeni, J. B. O, Esuruoso, O. and Oyekale, K. O. (2013a). Influence of different seed size fractions on seed germination, seedling emergence and seed yield characters in tropic soybeans (*Glycine max* (L.) Merr.) *International Journal of Agricultural Research*, *8*, 26-33. https://doi.org/10.3923/ijar.2013.26.33

Adebisi, M.A., Esuruoso, O. A., Adetumbi, J. A., Abdul-Rafiu, A. M., Kehinde, T. O., Ajani, O. O., and Agboola, O. (2013b). Shelf life of Kenaf (*Hibiscus cannabinus* L.) seed stored under humid tropical conditions. *Plant Breeding and Seed Science Journal, 67*, 75-86. https://doi.org/10.2478/v10129-011-0071-5

Adebisi, M. A and Oyekale, K. O. (2005). Effect of seed treatment and storage containers on the maintenance of viability of okra seed. *Asset Journal Series* A, *5*(1), 81-89.

Adetiloye, P. O, (2005). Effect of seed weight, seed drying and seed soaking on germination and seedling vigour of bell pepper (*Capsicum annuum*) *Asset Journal Series* A, *5*(1), 31-34.

Bekele-Tessema, A. (2007). *Profitable agroforestry innovations for eastern Africa: experience from 10 agro climatic zones of Ethiopia, India, Kenya, Tanzania and Uganda*. World Agroforestry Centre (ICRAF), Eastern Africa Region.

Cook, B. G., Pengelly, B. C., Brown, S. D., Donnelly, J. L., Eagles, D. A., Franco, M. A., Hanson, J., Mullen, B. F., Partridge, I. J., Peters, M., Schultze-Kraft, R. (2005). *Tropical forages*. CSIRO, DPI & F (Qld), CIAT and ILRI, Brisbane, Australia.

Copeland, L. O and McDonald, M. B. (2002). *Principles of Seed Science and Technology* (3rd ed.). Chapman and Hall, New York.

Crop Trust, (2014). *Pigeon Pea: Food for Drought*. www.croptrust.org

Daniel, I. O. (1997). *Conservation of West African yam (Di-*

oscorea spp) germplasm, physiology of seed and pollen storage. Ph.D. Dissertation. University of Ibadan, Ibadan, Nigeria. 89pp.

Daniel. I. O., Oyekale, K. O., Ajala, M. O., Sanni, L. O., Okelana, M .A., Adetumbi, J. A., Akintobi, D. C. A. and Adebisi, M. A. (2012). Moisture Sorption in Commercial Hybrid Maize (*Zea mays* L) seeds during Storage at Ambient Tropical Conditions. *Research Journal of Seed Science, 5*(1), 32-37. https://doi.org/10.3923/rjss.2012.32.37

Duffus, C. M. and Slaughter, J. C. (1980). *Seed and their uses*. Chichester: John Wiley and Sons.

Ellis, R. H and Roberts, E. H. (1980). Improved equation for the prediction of seed longevity. *Annuals of Botany*, *45*, 13-30. https://doi.org/10.1093/oxfordjournals.aob.a085797

Ellis, R. H and Roberts, E. H. (1981). The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology, 9*, 373-409.

Esuruoso, O. A. (2010). *Effect of storage duration and seed invigoration treatments on quality of kenaf (Hibiscus cannabinus L.) seed stored under ambient conditions*. M. Agric. Dissertation. Department of Plant Breeding and Seed Technology, Federal University of Agriculture Abeokuta, Nigeria. 81pp.

Finney, D. J. (1971). *Probit analysis*. 3rd Edition Cambridge University Press, London. 333 pp

Harrington, J. F. (1972). Seed storage and longevity. In: *Seed Biology*. Vol 3. Ed. T.T. Kozlowski. Academic Press. NY. Pp 145-245. https://doi.org/10.1016/B978-0-12-395605-7.50009-0

Hong, T.D. and R.H. Ellis. (1996). A protocol to determine seed storage behavior. IPGRI Technical Bulletin No. 1. *International Plant Genetic Resources Institute*, Rome, Italy.

International Seed Testing Association (ISTA). (1995). "Internal rules for seed testing Rules 1995." *Seed Science and Technology,* 13, 322-326.

Jatoi, S. A., Afzal, M., Nasim, S. and Anwar, R. (2001). Seed deterioration study in pea, using accelerated ageing techniques. *Pakistan Journal of Biological Science, 4*(12), 1490-1494. https://doi.org/10.3923/pjbs.2001.1490.1494

Jyoti and Malik, C. P. (2013). Seed Deterioration: A Review. *International Journal of Life Sciences Biotechnology and Pharma Research, 2*(3), 374-386.

Kapoor, N., Arya, A., Siddiqui, M. A., Kumar, H. and Amir, A. (2011). Physiological and Biochemical Changes During Seed Deterioration in Aged Seeds of Rice (*Oryza sativa* L.). *American Journal of Plant Physiology*, *6*, 28-35. https://doi.org/10.3923/ajpp.2011.28.35

Kashyap, R. K., Chandhary, O. P and Sheorani, I. S. (1994). Effect of insecticide and seed treatment on seed viability and vigour in wheat cultivars. *Seed Science and Technology, 22*, 503-577.

Kasmaswara, R. M. and Jackson, M. T. (1996). Seed production environment and storage longevity of Japonica rice (*Oryza sativa* L.). *Seed Science Research, 6*, 17-21. https://doi.org/10.1017/S0960258500002956

Kehinde, T. O. (2016). *Digital Imaging Characterization and Potential Longevity of Kenaf (Hibiscus cannabinus L.) Seeds Using Artificial Ageing Treatments*. Doctor of Philosophy Thesis. Federal University of Agriculture, Abeokuta, Ogun State, Nigeria 102pp.

Kehinde, T. O. (2018). Potential Longevity of Kenaf (*Hibiscus cannabinus* L.) Seeds under Accelerated Ageing. *Nigerian Journal of Seed Science, 2*, 56-68.

Khalequzzaman, K. M., Rashid, M.M., Hasan, M.A. and Reza, M.A. (2012). Effect of Storage periods on the Seed quality of French Bean (*Phaseolus vulgaris*). *Bangladesh Journal of Agricultural Research, 37*(2), 195-205. https://doi.org/10.3329/bjar.v37i2.11221

Kibinza, S., Vinel, D., Côme, D., Bailly, C. and Corbineau, F. (2006). "Sunflower seed deterioration as related to moisture content during ageing, energy metabolism and active oxygen species scavenging", *Physiologia Plantarum, 128*(3), 496-506. https://doi.org/10.1111/j.1399-3054.2006.00771.x

Kim, S. H, Choe, Z. R., Kang, J. H., Copeland, L. O., and Elias, S. G. (2002). Multiple seed vigour indices to predict field emergence and performance of barley. *Seed Science and Technology, 22*, 29-38.

Kulik, M. M. (1995). Seed Quality and Microorganisms. In: *Basic Mechanisms and Agricultural Implications.* Eds. A. S Basara Food Products Inc. NY 153-171.

Mohammadi, H., Soltani, A., Sadeghipour, H. R. and Zeinali, E. (2011). Effect of seed aging on subsequent seed reserve utilization and seedling growth in soybean. *International Journal of Plant Production, 5*(1), 65-70.

Murthy, U. M., Kumar, P. P., and Sun, W. Q. (2000). Mechanisms of seed ageing under different storage conditions for *Vigna radiata* (L.) Wilczek: lipid peroxidation, sugar hydrolysis, Maillard reactions and their relationship to glass state transition. *Journal of Experimental Botany*, *54*(384), 1057-1067. https://doi.org/10.1093/jxb/erg092

Nkang, A. and E. O. Umah. (1996). Six month storability of five soybean cultivar as influence by stage of harvest, storage, temperature and relative humidity. *Seed Science and Technology, 25*, 93-99.

Oliviera, J., Carvalho, A., Viera Maac, M. L. M, and Von Pinho, E. V. R. (1999). Compartamento de semante de milho colhidas por diferented motodos, sob condicoes de amasen convencional. *Ciencia e Agrotechnologia, 23*, 289-302.

Oni, O. D. (2012). *Comparative efficacy of crude plant powders on physiological quality of sesame (Sesamum indicum L.) seeds stored under ambient conditions.* M. Agric Dissertation, Plant Breeding and Seed Technology Department, Federal University of Agriculture, Abeokuta, Nigeria. Pp 101.

Roberts, E. H. (1973). Predicting the storage life of seeds. *Seed Science and Technology*, *1*, 499-522.

Roberts, E. H. (1983). Loss of Seed Viability during Storage. In: Thompson, J.R. (ed). *Advances in Research and Technology of Seeds*, Part 8. Wageningen, The Netherlands Pp. 9-34.

Sloan, J., Heiholt, J., Iyer, H., Metz, S., Phatak, S., Rao, S., and Ware, D. (2009). *Pigeon pea: Amultipurpose, drought resistant forage, grain and vegetable crop for sustainable southern farms.* 2009 Annual Report, SARE Research and Education Project.

TeKrony, D. M., Egli, D. B and Philis A. D. (1980). Effects of field weathering and viability and vigour of soybean seed.

Agronomy Journal, 72,749-753. https://doi.org/10.2134/agronj1980.00021962007200050014x

Thseng , F. S., Hou, F. F. and Wu, S.T. (1996). Varietal differences in germination rate, storability and flooding tolerance of soybean produced from different cultural seasons and locations. *Journal of Crop Science, 65*, 525-531. https://doi.org/10.1626/jcs.65.525

Tilebeni, G. H. and Golpayegani, A. (2011). "Effect of seed ageing on physiological and biochemical changes in rice seed (*Oryza sativa* L.)". *International Journal of AgriScience, 1*(3), 138-143.

doi:10.14720/aas.2019.114.2.6 Original research article / izvirni znanstveni članek

Cadmium toxicity in African yam bean (*Sphenostylis stenocarpa*) (Hochst. ex A.Rich.) Harms genotypes

Pascal C. OKOYE[1],[2], Geoffrey O. ANOLIEFO [1], Beckley IKHAJIAGBE [1], Edokpolor O. OHANMU [1,3], Francis A. IGIEBOR [1,4], and Ephraim ALIU [1,5]

Received May 22, 2018; accepted October 24, 2019.
Delo je prispelo 22. maja 2018, sprejeto 24. oktobra 2019.

Cadmium toxicity in African yam bean (*Sphenostylis stenocarpa)* (Hochst. ex A.Rich.) Harms genotypes

Abstract: The aim of the study was to investigate the growth responses of African yam bean (*Sphenostylis sternocarpa* (Hochst. ex A. Rich.) Harms (AYB) to cadmium pollution. Top garden soil (0 – 10 cm) was obtained as pooled and polluted with cadmium (as $CdCl_2$) at the rate of 12 mg kg^{-1}, which is equivalent to 3 times the ecotoxicological screening value of Cd. The polluted soils were made ready for use 3 days later. Nine selected AYB accessions (TSs-87, TSs-89, TSs-90, TSs-91, TSs-92, TSs-93, TSs-94, TSs-95, and TSs-96) were pre-soaked for 30 minutes and then sown in the polluted and unpolluted soils. Data collected were subjected to ANOVA, and means were separated at 95 % confidence interval. Results showed that incidence of cadmium pollution significantly delayed seedling emergence in all tested AYB accessions by at least one day ($p < 0.05$). Despite exposure to Cd, TSs-96 attained 50 % emergence faster than other accessions. Although there were general reductions in yield due to exposure to Cd, TSs-92 showed the least percentage yield reduction (50 %), compared to 74 % yield reduction in TSs-93, thereby suggesting a comparatively better yield capacity compared to the other test accessions. Overall, decrease in total chlorophyll content seems to be the major reason of injury in Cd-exposed plants.

Key words: toxicity; heavy metal; *Sphenostylis stenocarpa*; cadmium; yield

Toksičnost kadmija za izbrane genotipe afriškega gomoljastega fižola(*Sphenostylis stenocarpa*) (Hochst. ex A.Rich.) Harms

Izvleček: Namen raziskave je bil preučiti rastni odziv afriškega gomoljastega fižola (*Sphenostylis sternocarpa* (Hochst. ex A. Rich.) Harms (AYB) na onesnaženje tal s kadmijem. Vzorčena je bila vrhnja plast vrtnih tal (0 – 10 cm), onesnažena s kadmijem (kot $CdCl_2$) v velikosti 12 mg kg^{-1}, kar je trikratni ekvivalent priporočene ekotoksikološke vrednosti za Cd. Onesnažena tla so bila pripravljena za poskus v treh dneh. Izbranih je bilo devet akcesij AYB (TSs-87, TSs-89, TSs-90, TSs-91, TSs-92, TSs-93, TSs-94, TSs-95, in TSs-96), katerih semena so bila predhodno namočena za 30 minut in potem posejana v onesnažena in neonesnaženea tla. Zbrani podatki so bili ovrednoteni z ANOVA, kjer so bila poprečja ločena pri 95 % intervalu zaupanja. Rezultati so pokazali, da je onesnaženje s kadmijem značilno zamaknilo vznik kalic AYB pri vseh preiskušenih akcesijah najmanj za en dan ($p < 0,05$). Kljub izpostavitvi Cd, je akcesija TSs-96 dosegla 50 % kalivost hitreje kot vse ostale. Kljub splošnemu upadu pridelka zaradi izpostavljenosti kadmiju, je akcesija TSs-92 pokazala najmanjši upad pridelka (50 %), v primerjavi s 74 % upadom pri akcesiji TSs-93, kar kaže na njeno boljšo primerjalno sposobnost prilagoditve v primerjavi z drugimi genotipi. Na splošno je bil glavni razlog poškodb zaradi Cd pri vseh izpostavljenih rastlinah upad celokupne vsebnosti klorofila.

Ključne besede: toksičnost; težka kovina; *Sphenostylis sternocarpa*; kadmij; pridelek.

1 University of Benin, Environmental Biotechnology and Sustainability Research Group, Department of Plant Biology and Biotechnology, Nigeria
2 Corresponding author, e-mail: pascal.okoye@lifesci.uniben.edu
3 Edo University, Department of Plant Biology and Biotechnology, Iyamho, Nigeria
4 Wellspring University, Department of Microbiology, College of Natural and Applied Sciences, Benin City, Nigeria
5 Iowa State University,Department of Genetics, Development, and Cell Biology, College of Agriculture and Life Sciences, United States of America

1 INTRODUCTION

Anthropogenic activities, including increased rate of industrialization, concentrated agriculture, and all-embracing mining accompanied by escalating population and rapid civilization, have not only inflicted damaging effect on the accessibility of natural endowments but also resulted in extensive and severe contamination of fundamental constituents of life worldwide (Abolghassem et al., 2015). Amidst the repercussion of human-induced disturbance of natural biogeochemical cycles, accentuated buildup of heavy metals (HMs) is of great concern due to dietetic, biological, and environmental reasons (Ali et al., 2013). Aside from having atomic mass of over 20 and density higher than 5 g cm^{-3}, HMs can trigger mutagenic, cytotoxic and genotoxic repercussions on plants, animals, and higher beings via the food chains, irrigation, aquifers, and surrounding atmosphere (Rascio & Navari-Izzo, 2011). Even so, they are not biodegradable and are extremely persistent in water and soil.

Cadmium (Cd), a powerful phytotoxic heavy metal, is frequently released into the arable soil from industrial processes and farming practices (Sarvajeet et al., 2013). It is estimated that around 30,000 tons of Cd are released into the environment annually, of which 13,000 tons resulted from human activity. Cd is easily taken up by plant roots and transported to aerial parts, thus entering into the food chain causing health problems in animals and humans (Gallego et al., 2012) . Pál et al. (2006) recounted that the uptake of Cd ions takes place in competition with that of essential elements such as K, Mg, Ca, and Fe, across the same transmembrane transporter. The main symptoms of Cd-induced toxicity in plants are stunted growth, chlorosis, leaf epinasty, altered chloroplast ultrastructure, photosynthetic inhibition, inactivation of enzymes in CO_2 fixation, induced lipid peroxidation, suppression of pollen germination and tube growth, and disturbance of the nitrogen (N) and sulphur (S) metabolism (Gill & Tuteja, 2011). The disproportionate uptake of the element from the soil creates two fold problems; first is the contamination of harvested crops, which become the entry route of heavy metal in human diet, and secondly the inhibition of metabolic processes and, in severe cases, death of plants results in heavy decline in crop yield as well as potential threat to food security (Singh & Aggarwal, 2006). Zong et al. (2007) have reported that plant species and also genotypes within the species differ greatly in their tolerance to Cd stress.

African yam bean (AYB), though an underutilized legume, contains a large amount of essential amino acids, including lysine, histidine, methionine, and isoleucine, that are vital to human metabolism. Food and Agriculture Organisation, and Wealth Health Organization have described the amino acid profile of AYB as being comparable with those of whole hen's eggs, and therefore meet the daily requirement of the organizations (Adewale & Dumet, 2010). Potter (1992) opined that AYB has the potential of fostering food security in Africa due to its resistance to pest infestation. While lack of improved varieties with dwarf erect architecture, shorter growth period and easier to cook seed coats have been identified as obstacles to large scale commercial cultivation of *S. stenocarpa stenocarpa* (Hochst. ex A.Rich.) Harms, effects of elevated environmental pollution with heavy metals pose a great threat to the potential use of the crop as source of plant protein in developing nations.

Heavy metals are known to hamper enzyme functions needed for plant metabolism through their interference with microbial activities in the soil. These deleterious effects (direct and indirect) culminate in dwindling plant growth which at times leads to domino effects of plant's death. There is need to identify possible cultivars that are resistant to toxic effects of HMs; hence the present study suffices. Cadmium (Cd) was selected for this study on the basis of its preponderance in the environment due to its presence in sewage and certain fertilizers used in agriculture (Gallego et al., 2012). Therefore, this investigation was undertaken to evaluate the growth and physiological responses of African yam bean (AYB) genotypes to cadmium pollution, as well as draw conclusion from results obtained on the ability of AYB to thrive on cadmium polluted soils without posing health risks to both animal and human consumers upon maturity. Furthermore, the question the present study intended to answer was whether cadmium pollution could elicit morphological and physiological changes in selected accessions of AYB and, if possible, which part of the plant could be adjudged the main indicator of cadmium-pollution related stress.

2 MATERIALS AND METHODS

2.1 COLLECTION AND POLLUTION OF SOIL

Top soil (0 – 10 cm) was obtained as pooled from 10 locations devoid of previous exogenous activities with heavy metals or fertilizers. Before proceeding with the experiment, a sample of the pooled soil was analyzed according to Asema et al. (2015) for selected physicochemical parameters. Twenty (20) kg of the sun-dried soil was thereafter measured into experimental bags.

2.2 PROCUREMENT OF METAL AND SOIL CONTAMINATION

The chloride form of Cd, chosen on the basis of its high solubility in water as well as availability within the locality of the present study, was used in the experiment ($CdCl_2$). The water holding capacity (1.6 l) of the soil was predetermined (Anoliefo et al., 2016) to be 816 ml kg^{-1}. Thus, 0.24 g (3 ESV of Cd) of cadmium chloride ($CdCl_2$) was dissolved in 1.6 l of water and used to saturate 20 kg of soil and allowed to attenuate naturally for a period of one week. The ecological screening value (ESV) of Cd is 4 mg kg^{-1} (Efroymson et al, 1997a) which is the limit above which Cd becomes detrimental to plants. A second set of experimental bags were presented, but these were not polluted with Cd. AYB accessions were sown in Cd-polluted and clean soils respectively, with a view to ascertaining response to Cd-induced stress.

2.3 PLANTING OF AYB SEEDS

Viable accessions of AYB (TSs-87, TSs-89, TSs-90, TSs-91, TSs-92, TSs-93, TSs-94, TSs-95, and TSs-96) were obtained from the Genetic Resource Centre (GRC) of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Five seeds (unsterilized) were sown in each experimental bag after soaking in distilled water for about 30 minutes. After two weeks of sowing of AYB, the plants were staked on 3 m-long poles.

2.4 MORPHOLOGICAL STRESS RESPONSES

The physical appearances of the plant in response to the experimental conditions were observed and recorded on periodic basis. Care was also taken to ensure that the progression of chlorosis was recorded. In this case, whenever chlorosis was noted, the leaf was immediately tagged so that chlorotic progress would be followed up till when the leaf became entirely chlorotic. Similar procedure was followed in order to designate the progression of necrosis in Cd-exposed and unexposed plants.

2.5 ASSESSMENT OF PLANT PRODUCTIVITY AND GROWTH INDICES

The assessment of plant productivity adopted in this study was based on the previous works of El-sheshneny et al. (2014) while the growth indices employed in monitoring the development of the test plant was according to the previous research findings of Ozalkan et al. (2010). Plant growth indices including leaf area index (LAI), relative growth rate (RGR), net assimilation rate (NAR), and crop growth rate (CGR) were determined according to Ozalkan et al. (2010). Other plant parameters considered were plant dry mass (Bashan & de-Bashan, 2005), catalase activity (Aebi, 1983), superoxide dismutase activity (Kumar et al*., 2*012) and total nitrogen (Raveh & Avnimelech, 1979).

2.6 STATISTICAL ANALYSIS

Data were collected and subjected to analysis of variance and differences in means were separated at 95 % confidence interval using SPSS® software version 20.

3 RESULTS AND DISCUSSION

The physicochemical composition of the soil used for the experiment has been presented on Table 1. Results showed that the pH level of the soil was 5.97 prior to contamination with cadmium (Cd); while electric conductivity and total nitrogen content were 301.21S cm^{-1} and 0.18 % respectively. There was presence of heavy metals in the garden soil; concentration of iron (Fe) was 1011.92 mg kg^{-1} whereas zinc (Zn) and manganese (Mn) were 30.12 mg $kg^{-1}$and 17.03 mg kg^{-1} correspondingly. Cd concentration in the experimental site was negligible at a concentration less than 0.001 mg kg^{-1}The description of AYB collected from IITA seed bank showed that the accessions originated in Nigeria.

3.1 EFFECT OF CADMIUM ON MORPHOLOGICAL PARAMETERS OF YOUNG EMERGENT OF AYB

It generally took between 2 - 3 days for emergence of AYB in unexposed and Cd-exposed conditions. In the present study, a plant was tagged "Cd-exposed" when exposed to Cd, the plants that were not subjected to Cd contamination were tagged "Cd-unexposed". Emergence was noticed in Cd-unexposed TSs-87 and TSs-89 at 2 days of sowing, but delayed by 1 day in their Cd-exposed counterparts ($p < 0.05$).Observably, it took an average difference of 1 day between the Cd-exposed and unexposed AYB accessions for half of the seeds to emerge irrespective of prior exposure to cadmium pollution. However, while the final emergence

was 100 % in all unexposed accessions of AYB, there was an average of 25 % reduction in final emergence of seeds exposed to cadmium contamination. Presence of metal did not in any way affect the first day of emergence of the test plant. Perhaps, this may be because water, that was readily available in both treated and untreated plants, is a major requirement for germination initiation (Raveneau et al., 2011). Nevertheless, beyond the first day of germination, the effects of cadmium became evident, for example the time taken to reach 50 % emergence was usually delayed by one day in the Cd-exposed plants. Thus, the presence of the metal warranted that for the time to 50 % emergence to be attained, it usually would delay by one day in Cd-exposed plants compared to the unexposed counterparts. Dobroviczká et al. (2013) reported that bean seed grown in Cd polluted soil did not show any apparent effect on the initial emergence parameters of the test plant. However, Thakur & Singh (2014) observed that with regard to progression of seedling emergence of soybean there was a slight decrease in the germination and emergence percentage of the Cd-exposed plants. These findings support the argument of the present study, in relation to the effects of Cd contamination on seedling emergence, that although emergence of seedlings was not inhibited during germination, there was a decrease by one day in the time to 50 % emergence in Cd-exposed plants.

In assessing plant dry mass, it was observed that there was a general reduction in plant dry mass when seeds were exposed to cadmium pollution. Dry mass of unexposed TSs-89 decreased from 0.18 g to 0.14 g after exposure to cadmium, whereas those of TSs-90 and TSs-92 showed similar outcome (0.18 - 0.15 g) and (0.17 - 0.14 g) respectively. Interestingly, the average reduction in plant dry mass occasioned by cadmium pollution in all tested AYB accessions was 0.035 g (19.44 %). This could be due to Cd interference with

Table 1: Physicochemical properties of soil and African yam bean seed description. These are background mean concentrations (n = 5).

Physicochemical properties of soil		Description of African yam bean collected from IITA				
Parameters	Mean value (n = 5)	Accession name	Country of origin	GRIN taxa	Taxonomy number	Collecting number
Ph	5.97 ± 0.67	TSs-87	Nigeria	GRIN:35250	3602	TB78-182-B
Electric conductivity (μs cm^{-1})	301.21 ± 23.01	TSs-89	Nigeria	GRIN:35250	3602	TB78-187
Total organic carbon (%)	0.49 ± 0.09	TSs-90	Nigeria	GRIN:35250	3602	TB78-187-B
Total nitrogen (%)	0.18 ± 0.06	TSs-91	Nigeria	GRIN:35250	3602	TB78-187-C
Exchangeable acidity (meq 100 g^{-1})	0.22 ± 0.08	TSs-92	Nigeria	GRIN:35250	3602	TB78-187-D
Na (meq 100 g^{-1})	10.90 ± 2.11	TSs-93	Nigeria	GRIN:35250	3602	TB78-187-E
K (meq 100 g^{-1})	1.48 ± 0.62	TSs-94	Nigeria	GRIN:35250	3602	TB78-190
Ca (meq 100 g^{-1})	14.32 ± 3.10	TSs-95	Nigeria	GRIN:35250	3602	TB78-190-B
Mg (meq 100 g^{-1})	12.01 ± 3.22	TSs-96	Nigeria	GRIN:35250	3602	TB78-190-C
NO^{-2} (mg kg^{-1})	16.43 ± 2.03	TSs-87	Nigeria	GRIN:35250	3602	TB78-182-B
NO^{-3} (mg kg^{-1})	30.01 ± 4.28	TSs-89	Nigeria	GRIN:35250	3602	TB78-187
Soil texture		TSs-90	Nigeria	GRIN:35250	3602	TB78-187-B
Clay (%)	5.43 ± 0.88	-	-	-	-	-
Silt (%)	7.36 ± 1.74	-	-	-	-	-
Sand (%)	84.81 ± 12.12	-	-	-	-	-
Heavy metals		-	-	-	-	-
Fe (mg kg^{-1})	1011.92 ± 73.38	-	-	-	-	-
Cd (mg kg^{-1})	< 0.001	-	-	-	-	-
Mn (mg kg^{-1})	17.03 ± 3.22	-	-	-	-	-
Pb (mg kg^{-1})	0.03 ± 0.01	-	-	-	-	-
Cu (mg kg^{-1})	3.93 ± 0.01	-	-	-	-	-
Zn (mg kg^{-1})	30.12 ± 3.06	-	-	-	-	-

physiological processes such as photosynthesis, translocation and partitioning of dry material in AYB. As has been shown (Neto & Bartels, 1992), water stress in cowpea decreased photosynthetic rate which in turn reduced yield, and ultimately plant dry matter, by decreasing the amount of assimilates required for plant growth and yield. An important observation made during the present study was the fact that average reduction in plant dry mass occasioned by cadmium pollution in all tested AYB accessions was 19.44 %. This remark agrees with the findings of Hassanein et al. (2013) who subjected lettuce and turnip to heavy metal stress and then obtained 19.45 % Cd-mediated reduction in shoot dry mass for turnip. Naz et al. (2015) reported similar observation for chickpea after exposure to heavy metal stress. Incidence of cadmium contamination resulted in shrinking in size of stem diameter in AYB accessions. Stem diameter ranged from 3 cm to 4.03 cm in unexposed and 2.26 cm to 3.89 in Cd-exposed plants. Aside from TSs-95 where the different in stem diameter between Cd-exposed and unexposed plants is 1 cm, other AYB accessions showed a difference less than 1 cm with slight variation. Interestingly, cadmium contamination had no effect on stem diameter of TSs-93 as reading remained 3.03 cm in both Cd-exposed and unexposed plants. Bhattarai & Subudhi (2018) revealed that varieties of plant within the same species show unique tolerance to environmental stress based on their genome. However, the scope of the present study could not pinpoint the gene that conferred the unique characteristic to TSs-93.

Results for seedling vigour of African yam bean after exposure to cadmium indicated that heavy metal contamination had notable effect on test plants (Table 2). At 28 days after sowing, estimated seedling vigour index I for unexposed TSs-87 was 47.91 as against 41.03 recorded in the Cd-exposed accession. Incidence of cadmium pollution resulted in reduction of vigour index I from 68.47 in unexposed TSs-89 to 46.38 in the Cd-exposed counterpart. Although similar observation was recorded for TSs-92 in which seedling vigour index I decreased from 45.02 (unexposed) to 40.01 (Cd-exposed), effects of cadmium pollution was not significant with respect to seedling vigour index I of TSs-92

Table 2: Effect of treatment on germination parameters of African yam bean at 7 days after sowing

Treatment	Final emergence %	50 % emergence (days)	Fresh mass of emergent (g)	Dry mass of emergent (g)	Hypocotyl length (cm)	Petiole length (cm) 14 days	Stem diameter (cm)	Seedling vigour index I	Seedling vigour index II
TSs-87_U	100.00 ^{d}	3.10 ^{a}	0.41 ^{i}	0.15 ^{efg}	3.23 ^{cdef}	1.03^{ab}	3.00^{abc}	47.91 ^{i}	0.23 ^{e}
TSs-87_Cd	62.50 ^{a}	4.03 ^{c}	0.34 ^{fg}	0.13 ^{d}	2.60 ^{abc}	0.93 ^{ab}	2.26 ^{a}	41.03 ^{f}	0.13 ^{cd}
TSs-89_U	75.00 ^{b}	3.10 ^{a}	0.41 ^{i}	0.18 ^{ij}	2.90 ^{bcde}	1.36 ^{b}	4.00 ^{d}	68.47 ^{l}	0.23 ^{e}
TSs-89_Cd	62.66 ^{a}	4.03 ^{c}	0.35 ^{g}	0.14 ^{ef}	2.13 ^{a}	1.30 ^{ab}	3.25 ^{bc}	46.38 ^{h}	0.11 ^{bcd}
TSs-90_U	100.00 ^{d}	3.16 ^{a}	0.41 ^{i}	0.18 ^{ij}	3.58 ^{e}	1.40 ^{b}	3.06 ^{abc}	82.01 ^{m}	0.15 ^{d}
TSs-90_Cd	87.50^{c}	3.46 ^{b}	0.37 ^{h}	0.15 ^{efgh}	2.11 ^{a}	1.03 ^{ab}	2.26 ^{a}	22.85 ^{c}	0.08 ^{b}
TSs-91_U	100.00 ^{d}	3.10 ^{a}	0.32 ^{d}	0.11 ^{bc}	2.94 ^{bcde}	0.93 ^{ab}	3.79 ^{d}	33.57 ^{d}	0.26 ^{e}
TSs-91_Cd	62.50 ^{a}	4.03 ^{c}	0.26 ^{c}	0.14 ^{ef}	2.10 ^{a}	0.86 ^{ab}	2.88 ^{ab}	16.61 ^{a}	0.12 ^{cd}
TSs-92_U	100.00 ^{d}	3.03 ^{a}	0.40 ^{i}	0.17 ^{ij}	2.58 ^{abc}	1.06 ^{ab}	4.01 ^{d}	45.02 ^{g}	0.23 ^{e}
TSs-92_Cd	66.67 ^{ab}	4.06 ^{c}	0.33 ^{de}	0.14 ^{e}	2.1 ^{a}	1.01 ^{ab}	3.89 ^{d}	40.00 ^{f}	0.11 ^{bcd}
TSs-93_U	100.00 ^{d}	3.06 ^{a}	0.52 ^{k}	0.18 ^{j}	3.23 ^{cdef}	1.20 ^{ab}	3.03 ^{abc}	65.75 ^{k}	0.33 ^{f}
TSs-93_Cd	62.50 ^{a}	4.03 ^{c}	0.41 ^{i}	0.16 ^{gh}	2.42 ^{ab}	1.36 ^{b}	3.03 ^{abc}	55.03 ^{j}	0.13 ^{cd}
TSs-94_U	100.00 ^{d}	3.03 ^{a}	0.17 ^{b}	0.17 ^{hi}	2.59 ^{abc}	0.93 ^{ab}	3.06 ^{abc}	86.00 ^{n}	0.10 ^{bc}
TSs-94_Cd	62.50 ^{a}	4.03 ^{c}	0.13 ^{a}	0.03 ^{a}	2.81 ^{abcd}	1.06 ^{ab}	2.85 ^{ab}	21.17 ^{b}	0.02 ^{a}
TSs-95_U	100.00 ^{d}	5.06 ^{d}	0.33 ^{ef}	0.16 ^{fgh}	0.064 ^{f}	1.13 ^{ab}	4.03 ^{d}	99.30 ^{p}	0.24 ^{e}
TSs-95_Cd	62.50 ^{a}	6.06 ^{e}	0.26 ^{c}	0.11^{ac}	3.35 ^{def}	1.03 ^{ab}	3.03 ^{abc}	37.13 ^{e}	0.14 ^{d}
TSs-96_U	100.00 ^{d}	3.13 ^{a}	0.43 ^{j}	0.16 ^{fgh}	2.60 ^{abc}	0.93 ^{ab}	3.96 ^{d}	96.28 ^{o}	0.26 ^{e}
TSs-96_Cd	75.00 ^{b}	5.30 ^{d}	0.44 ^{j}	0.10 ^{b}	2.13 ^{a}	0.63 ^{a}	3.55 ^{cd}	33.54 ^{d}	0.12 ^{bcd}
p-value	1.93 x10^{-17}	2.52 x 10^{-24}	7.74 x10^{-38}	2.22 x10^{-22}	5.52x10^{-6}	4.9 x10^{-1}	3.04 x 10^{-8}	44.34 x10^{-55}	1.18 x 10^{-19}

Means with the same alphabetic superscripts on the same column do not differ from each other ($p > 0.05$)

AYB accession. Similarly, unexposed TSs-91 and TSs-96 had identical seedling vigour index at 0.26; however, unlike the situation in TSs-87 and 89, the former had similar response to cadmium pollution with respect to seedling vigour index II at 0.12. Comparable outcomes have been reported in previous investigations (Talebi et al., 2014)

There was general reduction in shoot length of AYB at 20 weeks after sowing (WAS) as occasioned by cadmium pollution. Shoot length ranged from 104.11 cm in unexposed TSs-89 plants to 126.18 cm in the unexposed TSs-93 plant (Table 3). Cadmium pollution triggered the reduction of shoot length in TSs-87 from 112.03 cm to 93.70 cm in unexposed and Cd-exposed plants respectively. While TSs-90 showed the lowest shoot length at 84.07 cm for Cd-exposed accession, Cd-exposed TSs-92 had the longest shoot (98.71 cm). Similar outcome was observed by Pappalardo et al. (2016) who reported that high concentrations of arsenic, even in combination with Cd, stopped the seminal root emergence from the germinated seed, thus hampering the development of shoot. However, the present investigation observed that reduction in shoot length elicited by cadmium contamination was obvious in older plants than in younger ones. A notable remark from the results was the fact that cadmium pollution had significant effect on the number of leaves per plant in all tested AYB accessions. At 20 WAS, number of leaves in unexposed TSs-87 stood at 38.30 as against 29.07 in Cd-exposed TSs-87. There were over 50 % reduction in number of leaves in TSs-90 (42.13 to 15.26), TSs-91 (40.23 to 18.16), TSs-94 (33.39 to 15.10), and TSs-96 (34.40 to 15.01). Generally, reduction in number of leaves impacts on the photosynthetic rate and resultant productivity of plants exposed to cadmium pollution (Eshighi, 2010).

Effect of treatment on below ground parameters of African yam bean at 20 WAS has been presented on Table 3. Results underpinned a general decline in root length in all accessions of AYB due to exposure to cadmium contamination. Unexposed TSs-87 showed root length of 26.06 cm while the same that was subjected to cadmium stress had a reduction in root length at 20.10 cm. Similar outcome was observed in unexposed TSs-89 (21.21 cm), Ts-90 (26.23 cm), and TSs-91 (27.06 cm) where root length decreased to 12.30 cm, 20.13 cm, and 23.03 cm in Cd-exposed plant respectively. Observably, there was 50 % reduction in root length of TSs-96 accession exposed to cadmium pollution. Nitrate inhibition has been shown to have many effects, including a decrease in nodule number, nodule mass, and N_2 fixation activity, as well as the acceleration of nodule senescence or disintegration; therefore, nitrate inhibition cannot be explained simply (Ohyama et al., 2012). The mechanisms of the initiation of lateral roots have been studied previously (Fukai et al., 2002) however, the regulation of primary and lateral root growth in relation to nodule growth in African yam bean has not yet been fully evaluated. Even so, Iranpour et al. (2016) reported that the application of cadmium did significantly decrease nodule fresh mass in *Phaseolus vulgaris* L. This previous report supports the findings of the present study which opine that the number of root nodules per plant reduced in all AYB accessions upon treatment. In addition, cadmium (Cd) treat-

Figure 1: African yam bean genotypes at (a) 2 weeks and (b) 4 weeks after sowing

Figure 2: An overview of the experimental plot showing 6 week-old African yam bean plants

ment also caused a noticeable change in tuber yield of TSs-96 (12.65 g – 4.11 g). However, the most significant effect of Cd contamination with respect to tuber yield was observed in TSs-95 with 78.3 % reduction in tuber yield compared to 71.5 % and 61.4 % decline detected in TSs-93 and TSs-89 in that order.

Table 3: Effects of treatment on above and below ground parameters of African yam bean at 20 weeks after sowing

Treatment	Shoot length (cm)	No. of primary branches	No. leaves plant^{-1}	Root length (cm)	No. of primary root branches	No. of root nodules per plant	Nodules yield (g)	Tuber plant^{-1}	Tuber yield (g)
TSs-87_U	72.03^{h}	6.16^{g}	38.3^{i}	26.06^{hi}	5.02^{e}	11.21^{e}	1.48^{ab}	4.03^{e}	8.08^{g}
TSs-87_Cd	63.70^{f}	4.11^{de}	29.07^{d}	20.10^{d}	2.05^{a}	9.18^{d}	1.01^{a}	2.21^{b}	3.31^{abc}
TSs-89_U	74.11^{j}	4.21^{de}	55.20^{m}	21.21^{e}	5.02^{e}	16.81^{h}	2.03^{bcd}	6.16^{f}	12.2^{i}
TSs-89_Cd	63.24^{f}	4.04^{de}	36.11^{h}	12.30^{a}	3.4c^{d}	9.11^{d}	1.13^{a}	1.06^{a}	3.48^{abcd}
TSs-90_U	80.03^{m}	4.08^{de}	42.13^{l}	26.35^{i}	5.01^{e}	18.68^{i}	2.55d^{ef}	3.21^{cd}	3.95^{cde}
TSs-90_Cd	54.07^{c}	2.52^{ab}	15.26^{b}	20.13^{d}	3.07^{bc}	4.15^{a}	1.35^{a}	1.40^{a}	2.70^{a}
TSs-91_U	56.07^{d}	2.88^{b}	40.23^{j}	27.06j	5.67^{e}	11.34^{ef}	1.55^{abc}	4.07^{e}	9.09^{h}
TSs-91_Cd	42.96^{a}	2.66^{ab}	18.16^{c}	23.03^{g}	4.08^{d}	5.27^{b}	0.95^{a}	1.10^{a}	4.65^{e}
TSs-92_U	73.33^{i}	7.24^{h}	41.30^{k}	21.10^{e}	4.08^{d}	14.02^{g}	2.11^{cd}	3.86^{e}	9.10^{h}
TSs-92_Cd	64.71^{g}	5.17^{f}	30.15^{e}	17.25^{c}	7.01^{f}	13.67^{g}	1.00^{a}	2.06^{d}	3.70^{bcd}
TSs-93_U	76.18^{k}	3.55^{cd}	56.46^{n}	28.01^{k}	7.68^{f}	23.02j	3.05^{fg}	5.67^{f}	13.01^{j}
TSs-93_Cd	51.93^{b}	2.06^{a}	38.09^{i}	22.05^{f}	2.23^{a}	14.03g	1.22^{a}	3.00^{cd}	5.02^{f}
TSs-94_U	82.53^{n}	6.27^{g}	33.39^{j}	25.69^{f}	5.01^{e}	19.01i	2.89^{efg}	3.54^{de}	3.28^{abc}
TSs-94_Cd	65.13^{g}	6.06^{g}	15.10^{b}	21.36^{e}	2.27^{a}	14.50g	2.00^{bcd}	2.16^{b}	3.03^{ab}
TSs-95_U	79.07^{l}	4.52^{e}	40.20^{j}	21.14^{e}	5.01^{e}	16.67h	2.44^{de}	8.18^{g}	13.96^{k}
TSs-95_Cd	52.10^{b}	3.06^{bc}	12.00^{a}	17.36^{c}	3.33^{cd}	8.21c	1.40^{a}	1.00^{a}	3.03^{ab}
TSs-96_U	83.10^{n}	8.20^{i}	34.40^{g}	28.15^{k}	7.71^{f}	24.03k	3.15^{g}	6.00^{f}	12.65^{ij}
TSs-96_Cd	60.08^{e}	5.18^{f}	15.01^{b}	14.38^{b}	2.51^{ab}	12.05f	1.00^{a}	2.70^{bc}	4.11^{de}
p-value	1.46x 10^{-51}	1.17 x 10^{-21}	1.89 x 10^{-57}	2.11x 10^{-37}	4.13x 10^{-20}	4.17 x 10^{-36}	3.44 x 10^{-34}	3.53 x 10^{-24}	1.18 x 10^{-32}

Means with the same alphabetic superscripts on the same column do not differ from each other ($p > 0.05$).U = Unexposed, Cd = Cd-exposed.

3.2 EFFECT OF CADMIUM POLLUTION ON YIELD OF AYB

Considering the consequence of cadmium (Cd) treatment on reproductive capacity of African yam bean, results showed that there were observable changes between Cd-exposed and unexposed AYB accessions with regard to days to flower bud initiation. It took 35 days for development of flower bud to be initiated in the unexposed TSs-87 plant, while that of the Cd-exposed TSs-87 plant occurred on the 36th day (Table 4). Where flower bud was initiated in unexposed TSs-89 at 36th day after sowing (DAS), the same initiation occurred in Cd-exposed TSs-89 a day before (35). This has great implication on the total productivity of a plant, in that a plant yield is a function of the number of flower it produces. Anjum et al. (2008) reported that exposure to Cd results in reduced plant growth and biomass in *Brassica campestris* L. respectively. This occurs when Cd absorbed through the root system significantly inhibits plant growth by interfering with water and mineral uptake, photosynthesis and nitrogen metabolism. This confirms the results obtained in the present study as the yield of African yam bean (AYB) was significantly reduced in cadmium-Cd-exposed AYB accession, particularly with regard to biomass production. Conversely, in the work carried out by Kavvadias et al. (2012) it was observed that Cd contamination showed no significant changes in biomass of parsley. However, it is noteworthy that in Kavvadias et al. (2012), the study was conducted with 100 mg kg^{-1} of Cd as the highest concentration unlike the presence study in which the concentration of Cd in the soil was 240 mg kg^{-1}. This supports the findings of Paschalidis et al. (2013) whose studies revealed that cadmium could cause reductions in yield of plants.

Effects of cadmium pollution on pod maturation of African yam bean (AYB) was studied and it was observed that first pod was formed between 58 – 68 days in unexposed AYB accessions while first pod formation in plants under the influence of cadmium exposure occurred between 60 – 70 days. Pod formation was relatively faster in unexposed plants accessions than in Cd-exposed ones. Unexposed TSs-87 and TSs-

Table 4: Effects of treatment on reproductive capacity of African yam bean (DAS)

Treatment	Length of peduncle (cm)	Days to FBI	Days to 50 % FBI	Flower bud size (mm)	No. of flowers plant^{-1}	Flowering duration (DAS)	Days to 1st pod formation (DAS)	Days to 50 % maturity (DAS)	Maturity period (Days)
TSs-87_U	2.95^{f}	35.05^{cdef}	43.01^{b}	0.88d^{ef}	23.03^{cd}	21.01^{f}	58.04^{a}	75.07^{b}	20.00^{e}
TSs-87_Cd	1.90^{bc}	35.81^{efg}	42.28^{b}	0.49^{a}	21.11^{bc}	14.66^{e}	60.10^{ef}	82.30^{ef}	26.04^{j}
TSs-89_U	2.17^{cde}	36.67^{g}	42.37^{b}	0.82^{cde}	27.67^{e}	23.66^{h}	60.03^{def}	76.37^{c}	18.89^{cd}
TSs-89_Cd	1.13^{a}	35.05^{cdef}	41.00^{b}	0.55^{a}	20.08^{b}	12.28^{a}	61.40^{gh}	82.70^{ef}	22.38^{g}
TSs-90_U	2.11^{cde}	33.67^{b}	39.03^{b}	0.97^{efg}	33.05^{f}	20.33^{f}	59.26^{cd}	76.18^{c}	18.15^{bc}
TSs-90_Cd	1.13^{a}	34.23bcd	40.03^{b}	0.76^{bcd}	23.04^{cd}	12.67^{ab}	60.77^{fg}	83.05f	21.14^{f}
TSs-91_U	2.61^{ef}	34.68bcde	42.34^{b}	1.06^{gh}	23.07^{cd}	18.01^{e}	59.34^{cde}	75.72^{bc}	19.01^{cd}
TSs-91_Cd	1.41^{ab}	36.03fg	44.35^{b}	0.94^{efg}	20.15^{b}	12.16^{a}	62.08^{h}	82.04^{e}	23.153^{gh}
TSs-92_U	2.11^{cde}	34.01bc	39.03^{b}	0.97^{efg}	33.05^{f}	20.33^{f}	59.03^{c}	78.36^{d}	18.00^{b}
TSs-92_Cd	1.21^{a}	35.03cdef	29.10^{a}	0.75^{bcd}	20.34^{b}	13.01^{b}	62.05^{h}	84.36^{g}	23.08^{gh}
TSs-93_U	3.00^{f}	35.34def	42.04^{b}	1.01^{fgh}	19.17^{b}	22.35^{g}	58.72^{b}	68.35^{a}	16.67^{a}
TSs-93_Cd	2.10^{cde}	33.85b	40.01^{b}	0.64^{ab}	14.54^{a}	17.68^{de}	61.44^{gh}	82.45^{ef}	22.44^{g}
TSs-94_U	2.50^{de}	35.14def	44.00^{b}	0s.82^{cde}	28.01^{e}	23.66^{h}	57.71^{a}	75.07^{b}	20.34^{ef}
TSs-94_Cd	1.95^{bcd}	35.37def	43.68^{b}	0.52^{a}	24.34^{d}	17.15^{d}	61.08^{g}	84.06^{g}	26.91^{k}
TSs-95_U	2.83^{cde}	35.05cdef	43.34^{b}	0.78^{bcd}	23.03^{cd}	20.68^{f}	67.02^{i}	82.34^{ef}	19.01^{cd}
TSs-95_Cd	1.42^{ab}	36.71g	44.00^{b}	0.56^{a}	20.38^{b}	14.00^{c}	70.99^{l}	93.27^{h}	25.08^{i}
TSs-96_U	3.00^{f}	35.67efg	42.37^{b}	1.15^{h}	19.17^{b}	22.35^{g}	68.08^{k}	83.03^{f}	19.14^{d}
TSs-96_Cd	2.14^{cde}	30.55a	40.76^{b}	0.71b^{c}	19.01^{b}	18.07^{e}	70.45^{l}	93.51^{h}	23.78^{h}
p-value	2.8 x 10^{-10}	4.4 x 10^{-12}	1.76 x 10^{-1}	6.18 x 10^{-12}	7.25 x 10^{-19}	1.17 x 10^{-32}	2.68 x 10^{-32}	7.38 x 10^{-37}	2.14 x 10^{-25}

FBI = Flower bud initiation, DAS = Days after sowing. U = Unexposed, Cd = Cd-exposed.
Means with the same alphabetic superscripts on the same column do not differ from each other ($p > 0.05$).

Table 5: Effects of treatment on yield parameters of African yam bean

Treatment	Seed cavity	Locules pod^{-1}	Pod length (cm)	Single pod mass (g)	Pods peduncle^{-1}	No. of pods plant^{-1}	Seed No. pod^{-1}	Seed mass pod^{-1}	Plant yield (kg m^{-2})
TSs-87_U	Pr	7.05^{cd}	6.04^{c}	3.70^{bcd}	2.06^{ab}	12.07^{e}	6.05^{a}	2.97^{cd}	0.11^{b}
TSs-87_Cd	Pr	6.25^{ab}	5.41^{b}	3.37^{abc}	1.39^{a}	6.01^{a}	6.14^{ab}	2.41^{ab}	0.04^{a}
TSs-89_U	Pr	7.19^{cd}	6.15^{c}	4.24^{de}	3.21^{c}	12.34^{f}	8.08^{fg}	3.25^{d}	0.13^{b}
TSs-89_Cd	Pr	5.70^{a}	5.03^{b}	3.05^{ab}	2.42^{bc}	6.09^{a}	6.39^{abc}	2.09^{ab}	0.03^{a}
TSs-90_U	Pr	7.39^{de}	5.21^{b}	4.17^{de}	2.56^{bc}	13.51^{h}	7.12^{cd}	3.08^{cd}	0.14^{b}
TSs-90_Cd	Pr	7.37^{de}	4.03^{a}	4.02^{cde}	2.01^{ab}	7.45^{cd}	6.09^{a}	2.05^{a}	0.04^{a}
TSs-91_U	Pr	7.11^{cd}	6.07^{c}	3.70^{bcd}	2.09^{ab}	12.06^{ef}	6.24^{ab}	2.60^{bc}	0.11^{b}
TSs-91_Cd	Pr	5.59^{a}	5.15^{b}	2.74^{a}	1.40^{a}	6.15^{a}	6.34^{abc}	2.01^{a}	0.03^{a}
TSs-92_U	Pr	6.90^{bc}	6.31^{c}	3.91^{cde}	3.21^{c}	13.14^{gh}	7.44^{def}	3.05^{cd}	0.12^{a}
TSs-92_Cd	Pr	6.10^{a}	5.07^{b}	3.03^{ab}	2.06^{ab}	8.08^{d}	6.36^{abc}	2.05^{a}	0.06^{a}
TSs-93_U	Pr	8.14^{e}	5.93^{c}	5.04^{f}	2.86^{bc}	12.54^{fg}	8.01^{efg}	3.11^{cd}	0.19^{c}
TSs-93_Cd	Pr	7.03^{cd}	4.02^{a}	4.11^{cde}	2.11^{ab}	6.70^{ab}	6.08^{a}	2.13^{ab}	0.05^{a}
TSs-94_U	Pr	8.48^{e}	5.32^{b}	4.59^{e}	2.57^{bc}	13.74^{h}	6.94^{bcd}	3.02^{cd}	0.14^{b}
TSs-94_Cd	Pr	7.07^{cd}	4.10^{a}	4.12^{cde}	2.38^{bc}	6.15^{a}	7.19^{d}	2.15^{ab}	0.05^{a}
TSs-95_U	Pr	9.08^{f}	5.17^{b}	5.15^{e}	2.50^{bc}	11.47^{e}	8.58^{g}	3.21^{d}	0.13^{b}
TSs-95_Cd	Pr	7.36^{cd}	4.17^{a}	4.04^{cde}	2.09^{ab}	6.01^{a}	6.03^{a}	2.08^{ab}	0.03^{a}
TSs-96_U	Pr	9.04^{f}	5.08^{a}	5.05^{f}	2.84^{bc}	13.74^{h}	7.28^{de}	3.35^{d}	0.13^{b}
TSs-96_Cd	Pr	7.07^{cd}	4.04^{a}	4.02^{cde}	2.06^{ab}	6.99^{bc}	6.08^{a}	2.08^{ab}	0.05^{a}
p-value		3.68 x 10^{-12}	2.57 x 10^{-14}	4.36 x 10^{-8}	7.82 x 10^{-4}	1.11 x 10^{-10}	3.27 x 10^{-9}	6.21 x 10^{-9}	4.12 x 10^{-11}

Means with the same alphabetic superscripts on the same column do not differ from each other ($p > 0.05$).U = Unexposed, Cd = Cd-exposed. Pr = Present.

93 produced their first pod in 58 days whereas their Cd-exposed equivalence showed pod formation in 60 and 61 days respectively. Similar outcome was observed with regard to days to 50 % maturity. It took 93 days for half of the pods in Cd-exposed TSs-96 to attained maturity as against 82 days observed in Cd-exposed TSs-87, TSs-89, TSs-91, and TSs-93. Previous investigations have shown that cadmium induces significant differences in pod characteristics of legumes (Rout et al., 1999). Ghani (2010) reported that accumulation of Cd^{2+} in plant parts of mungbean (*Vigna radiata* (L.) R. Wilczek altered the number of pods per plant, average pod length, and fresh and dry mass of pods per plants. Evidence from present study proved that cadmium (Cd) contamination has no effect on seed cavity (Table 5) on ridges on pod in both Cd-exposed and unexposed plants as both showed cavity on ridges. There were 7.05 locules per pod in unexposed TSs-87 as against 6.25 in the Cd-exposed accession. Number of locules recorded for unexposed TSs-89, TSs-90, and TSs-91 was fairly similar at 7.19, 7.39, and 7.11 correspondingly whereas their Cd-exposed complements had 5.70, 7.37, and 5.59 in that order. Observably, there was no prominent difference in locules number in TSs-90 for both Cd-exposed and unexposed plants.

In terms of effects of cadmium pollution on pod production in African yam bean, results showed that number of pods per plant decreased with incidence of cadmium exposure. There was at least 50 % reduction in the number of pods in all Cd-exposed accessions when compared to their unexposed counterparts. Number of pods per plant reduced from 12 to 6 in unexposed and Cd-exposed TSs-87, TSs-89, TSs-91, and TSs-93 in that order. Cd-exposed TSs-95 was most affected by cadmium in relation to plant yield as it stood at 0.03 kg m^{-2}. Cd-exposed TSs-92, TSs-93, TSs-94, and TSs-96 were fairly tolerant to cadmium pollution as their yield was higher than other Cd-exposed counterparts in the test plant. Similar effects were observed by Ghani (2010) when mungbean was exposed to cadmium pollution.

3.3 EFFECT ON PHYSIOLOGICAL PARAMETERS

At 42 days after sowing, the effects of cadmium pollution on some pigmentation parameters of emer-

gents of African yam bean was studied and results showed that the amount of chlorophyll-a (Chl-a) decreased in Cd-exposed plants compared to the value in unexposed category (Table 6). It was noticed that in the face of cadmium pollution, Cd-exposed TSs-87, TSs-89, and TSs-96 have similar quantity of chl-a (0.13 mg g^{-1}) although their unexposed equivalence differ at 0.09 mg g^{-1}, 0.12 mg g^{-1}, and 0.11 mg g^{-1} in that order. Nevertheless, it was observed that the amount of Chl-b in both Cd-exposed and unexposed TSs-90 remained the same at 0.14 mg g^{-1} regardless of cadmium pollution. Cadmium usually attack protein by disrupting the conformation of the molecules through its affinity for sulfhydryl and carboxylic groups (Gonçalves et al., 2009). Furthermore, chlorophyll–a and chlorophyll–b contents showed significant decline at the applications of Cd and the results were in consonance with earlier reports where Cd inhibited the biosynthesis of chlorophyll and generated a kind of senescence (Qian et al., 2009). Nada et al. (2007) reported that Cd stress decreased chlorophyll content in leaf of almond (*Prunus dulcis* L.) seedling. Consequently, chlorophyll pigments seem to be one of the main reasons of heavy-metal injury in plants.

Concerning the carotenoids co ntent in test plants during the present study, there was an increase as occasioned by cadmium stress. The amount of carotenoids increased from 1123 mg g^{-1} in unexposed TSs-89 to 1173 mg g^{-1} in the Cd-exposed counterpart. The increase in carotenoids was due to the oxidative stress triggered by the presence of Cd^{2+} in the test plant as these isoprenoid compounds have been shown to act as signaling molecules during oxidative stress. Ramel et al. (2012b) reported a significant increase in b–carotene when *Arabidopsis thaliana* (L.) Heynh. was exposed to photooxidative stress. This is contrary to the findings of Paunov et al., 2018 who reported a decrease in carotenoid concentration when durum wheat was exposed to Cd stress. It was observed that in some accessions of AYB, tocopherol content increased after exposure to cadmium pollution whereas in others the reverse was the case. Tocopherol level decreased from 1.80 mg g^{-1} in unexposed TSs-87 to 1.63 mg g^{-1} in the Cd-exposed accession. Similar observation was made for TSs-89, TSs-90, TSs-93, and TSs-94 in which tocopherol level reduced from 0.82 – 0.65 mg g^{-1}, 0.14 – 0.08 mg g^{-1}, 1.72 – 1.36 mg g^{-1}, and 1.72 – 1.45 mg g^{-1} correspondingly. In TSs-95 and TSs-96 that were not subjected to cadmium stress, the concentration of tocopherol was 1.11 mg g^{-1} and 0.54 mg g^{-1} respectively, and after exposure to cadmium pollution, concentration increased to 1.74 mg $g^{-1}$and 2.00 mg g^{-1}in that order. Studies have shown that plant tissues differ a great deal in their tocopherol composition (Munne-Bosch & Alegre, 2002). This explains the discrepancy in tocopherol content as observed in the present study. In addition, based on the findings of Munne-Bosch (2005), plants with net loss of tocopherol are described as being sensitive to the prevalent stressor. This statement supports the suggestion of the present study that TSs-89, when compared across the board in all ramifications, shows high sensitivity to Cd contamination.

Interference with assimilation of nitrate nitrogen, due to cadmium pollution, was observed in Cd-exposed TSs-87 accession of African yam bean (AYB) including other accessions (Table 6). Concentration of nitrate nitrogen reduced from 453 in unexposed TSs-87 to 100.25 in the Cd-exposed plant. This showed that Cd impeded nitrogen uptake in the Cd-exposed TSs-87. Gouia et al. (2000) revealed that treating bean with 50 µM Cd for 10h reduced total nitrate uptake by 20 % compared to control plants. They further observed that the decrease was higher at 12 % of nitrate uptake in control plants with 100 µM Cd. Unlike the aforementioned accession, it was observed that nitrate nitrogen level increased from 346.33 in unexposed TSs-86 to 433.58 in the stress accession. The present study suspects that TSs-86 has a metabolic shunt that prevent excess Cd uptake, thus allowing for increased nitrogen uptake in the face of Cd pollution. Earlier studies by Zhang et al. (2014) reported that ample nitrogen uptake inhibits Cd uptake through GSH pathway. Level of nitrate nitrogen in unexposed TSs-90 stood at 963.95 while cadmium treated counterpart showed lesser value at 336.78. Reduction in nitrate nitrogen in Cd-exposed TSs-91 was alarming. It reduced from 337.11 in unexposed accession to 77.11 in Cd-exposed plant. Effects of cadmium exposure on the concentration of nitrate nitrogen were more prominent in TSs-91 compared to other AYB considered during the investigation. With regard to ability to assimilate nitrate nitrogen under cadmium stress, TSs-96 showed greater tolerance than any other accession as the Cd-exposed TSs-96 showed nitrate nitrogen of 566.56 as compared to 616.29 in the unexposed counterpart. Incidence of cadmium contamination resulted to change in ammonium nitrogen content of AYB accessions (Mokhele et al., 2012).

Table 7 shows a presentation of plant growth indices as affected by plant's exposure to treatments at 20 WAS. Results showed that leaf area index (LAI) increased in some AYB accessions while others experienced a decreased from Cd-exposed to unexposed plants and vice versa. According to the reports by Paunov et al. (2018), plants do not show general growth pattern in response to cadmium stress. They observed that shoot/root length ratio in *Pfaffia glomerata* (Spreng.)

Table 6: Effects of treatment on some biochemical parameters of developed emergent of African yam bean at 42 DAS

Treatment	Chlorophyll-a (mg g^{-1})	Chlorophyll-b (mg g^{-1})	Total chlorophyll (mg g^{-1})	Carotenoids (mg g^{-1})	Tocopherol (mg g^{-1})	Lycopene (mg g^{-1})	Catalase (mg g^{-1})	Superoxide dismutase (mg g^{-1})	Nitrate nitrogen (mg g^{-1})	Ammonium nitrogen (mg g^{-1})
TSs-87_U	0.09^{a}	0.11^{d}	0.21^{d}	1300.26^{g}	1.80^{h}	496.36^{a}	5.10^{ef}	0.80^{k}	453.00^{g}	44.04^{f}
TSs-87_Cd	0.13^{b}	0.15^{g}	0.28^{k}	1017.53^{a}	1.63^{g}	588.56^{d}	9.25^{j}	0.81^{k}	100.25^{n}	40.48^{d}
TSs-89_U	0.12^{b}	0.10^{c}	0.23^{f}	1123.80^{b}	0.82^{d}	617.25^{e}	3.30^{b}	0.38^{a}	346.33^{e}	31.62^{a}
TSs-89_Cd	0.13^{b}	0.08^{b}	0.22^{e}	1173.53^{d}	0.65^{bc}	1132.85^{q}	3.63^{bc}	0.62^{ef}	433.58^{f}	42.63^{e}
TSs-90_U	0.12^{b}	0.14^{f}	0.26^{i}	1377.20^{l}	0.14^{a}	664.18^{g}	5.07^{ef}	0.42^{b}	963.95^{m}	36.25^{b}
TSs-90_Cd	0.09^{a}	0.14^{f}	0.24^{g}	1344.06^{i}	0.08^{a}	888.40^{n}	6.58^{h}	0.70^{h}	336.78^{d}	37.87^{c}
TSs-91_U	0.17^{b}	0.15^{g}	0.29^{l}	1278.00^{f}	0.95^{d}	533.50^{b}	1.64^{a}	0.57^{d}	337.11^{d}	53.88^{h}
TSs-91_Cd	0.12^{b}	0.12^{e}	0.24^{g}	1574.13^{o}	2.52^{j}	916.76^{o}	6.06^{gh}	0.63^{e}	77.136^{a}	40.81^{d}
TSs-92_U	0.12^{b}	0.14^{f}	0.27^{j}	1300.26^{g}	0.14^{a}	954.66^{p}	3.58^{bc}	0.54^{c}	463.21^{h}	24.52^{m}
TSs-92_Cd	0.03^{ab}	0.18^{j}	0.24^{g}	1332.73^{h}	0.80^{cd}	731.33^{h}	5.52^{fg}	0.62^{e}	308.47^{c}	63.37^{i}
TSs-93_U	0.11^{ab}	0.01^{a}	0.11^{a}	1442.13^{m}	1.72^{gh}	775.62^{j}	4.15^{cd}	0.70^{h}	548.46^{i}	31.17^{a}
TSs-93_Cd	0.07^{ab}	0.66^{k}	0.18^{c}	1472.46^{n}	1.36^{f}	870.82^{m}	4.39^{de}	0.78^{j}	337.11^{d}	74.25^{k}
TSs-94_U	0.08^{ab}	0.11^{d}	0.24^{g}	1344.26^{i}	1.72^{gh}	627.04^{f}	3.59^{bc}	0.67^{g}	558.68^{j}	88.08^{l}
TSs-94_Cd	0.09^{b}	0.16^{h}	0.30^{m}	1573.80^{o}	1.45^{f}	533.17^{b}	9.11^{j}	0.59^{d}	337.11^{d}	48.67^{m}
TSs-95_U	0.07^{ab}	0.17^{i}	0.28^{k}	1148.13^{c}	1.11^{e}	552.41^{c}	4.99^{ef}	0.64^{f}	558.68^{j}	44.04^{f}
TSs-95_Cd	0.09^{ab}	0.16^{h}	0.30^{m}	1375.40^{k}	1.74^{gh}	748.26^{i}	5.10^{ef}	0.72^{h}	308.47^{c}	64.45^{k}
TSs-96_U	0.11^{ab}	0.14^{f}	0.25^{h}	1365.20^{j}	0.54^{b}	795.20^{l}	4.49^{de}	0.76^{i}	616.29^{l}	64.33^{k}
TSs-96_Cd	0.13^{b}	0.12^{e}	0.17^{b}	1274.13^{e}	2.00^{i}	786.08^{k}	7.77^{i}	0.83^{l}	566.56^{k}	70.15^{j}
p-value	3.86×10^{-1}	1.02×10^{-54}	1.09×10^{-40}	4.95×10^{-83}	2.11×10^{-29}	9.14×10^{-57}	9.87×10^{-22}	4.9×10^{-35}	1.6×10^{-84}	2.09×10^{-67}

DAS = Days after sowing, U = Unexposed, Cd = Cd-exposed. Means with the same alphabetic superscripts on the same column do not differ from each other ($p > 0.05$)

Pedersen when exposed to 20 µM Cd, increased significantly but there was only slight increment when after exposure to more than 20 µM. This explains why there was no general pattern in leaf area index in the present study. In comparing Cd-exposed and unexposed AYB accessions, LAI increased by 0.01 in TSs-89 and TSs-92, as well as decreased in TSs-87 by the same margin. LAI in unexposed and Cd-exposed TSs-95 and TSs-96 are comparable as there was increase from 0.03 – 0.07 in both plant accessions correspondingly. There was general decline in relative growth rate when cadmium Cd-exposed plants were examined alongside the unexposed plants. The highest reduction in plant dry mass production occurred in Cd-exposed TSs-96 at 752.81. Observably, plant dry mass production reduced by 23.33 between Cd-exposed and unexposed TSs-90. In addition, cadmium contamination caused a decrease in plant basal area in Cd-exposed accessions of AYB. Similar results were reported by Thakur and Singh (2014) after they exposed soybean to different concentrations of Cd.

In determining the effects of cadmium pollution on plant dry matter accumulation of African yam bean at 20 weeks after planting, results showed a general decline in overall foliar yield of AYB accessions. As recorded in Table 7, overall foliar yield range between 15.22 – 34.21 g in plants that were not exposed to cadmium, whereas foliar yield in Cd-exposed plants ranged from 10.06 – 25.32 g. There was 5.16 g reduction in overall foliar yield between Cd-exposed and unexposed TSs-87 while that of TSs-91 was 5.83 g. Overall, foliar yield reduced from 19.66 g in unexposed TSs-89 to 18.07 g in Cd-exposed TSs-87. Estimation of plant dry mass showed that incidence of cadmium stress significantly reduced plant dry mass in all accessions of AYB. Up to 50 % reduction in plant dry mass was observed in TSs-89, TSs-92, and TSs-95. Previous studies reported similar trend with respect to plant exposure to elevated cadmium concentration. Skrebsky et al. (2008) confirmed a reduction in shoot and total dry mass of *Pfaffia glomerata* (Spreng.) Pedersen [es]when exposed to 80 µM of Cd. Increase in dry mass of durum wheat was inhibited by approximately 50 % after exposure to cadmium (Paunov et al., 2018).

Figure 3 shows the level of accumulation of cadmium in leaf of developed emergent of African yam

Table 7: Presentation of plant growth indices as affected by plant's exposure to treatments at 20 weeks after sowing

Treatment	leaf area index	relative growth rate	net assimilation rate	Crop growth rate	plant dry mass production	Overall foliar yield (g)	Plant dry mass (g)	Root dry mass (g)	Shoot:Root Ratio
TSs-87_U	0.03^{a}	1.12^{ab}	6.95^{e}	0.54^{abc}	264.93^{e}	15.22^{c}	3.01^{bc}	1.09^{bc}	1.43^{ab}
TSs-87_Cd	0.02^{a}	0.66^{a}	2.47^{b}	0.46^{a}	128.04^{a}	10.06^{a}	1.37^{a}	0.26^{a}	2.30^{cd}
TSs-89_U	0.02^{a}	4.10^{ghi}	40.16^{l}	1.64^{f}	534.30^{k}	19.66^{f}	11.21^{i}	2.48^{de}	3.92^{ghi}
TSs-89_Cd	0.03^{a}	2.99^{ef}	14.65^{g}	1.33^{ef}	420.38^{j}	18.07^{e}	6.14^{g}	1.34^{c}	5.10^{j}
TSs-90_U	0.02^{a}	2.13^{cd}	21.21^{i}	0.84^{abcd}	224.72^{c}	30.25^{j}	6.41^{g}	2.01^{d}	2.01^{bc}
TSs-90_Cd	0.07^{cd}	1.62^{bc}	4.42^{cd}	0.72^{abcd}	247.95^{d}	22.63^{gh}	4.11^{de}	1.07^{bc}	2.86^{def}
TSs-91_U	0.02^{a}	2.03^{c}	6.66^{e}	0.83^{abcd}	123.34^{a}	15.83^{c}	3.01^{bc}	1.09^{bc}	1.79^{bc}
TSs-91_Cd	0.04^{b}	0.70^{a}	1.51^{a}	0.48^{ab}	109.98^{q}	10.00^{a}	1.07^{a}	0.25^{a}	3.55^{fgh}
TSs-92_U	0.02^{a}	4.45^{i}	39.03^{k}	1.61^{f}	762.78^{n}	18.67^{e}	11.04^{i}	2.10^{d}	3.28^{fg}
TSs-92_Cd	0.03^{a}	3.03^{ef}	15.16^{g}	2.46^{g}	573.10^{l}	18.06^{e}	6.55^{g}	1.40^{c}	4.50^{ij}
TSs-93_U	0.02^{a}	2.09^{cd}	21.20^{i}	1.01^{de}	405.44^{h}	30.59^{j}	6.08^{g}	2.01^{d}	2.01^{bc}
TSs-93_Cd	0.02^{a}	1.57^{bc}	9.07^{f}	0.96^{cde}	300.76^{f}	23.07^{h}	4.01^{de}	0.99^{bc}	3.03^{ef}
TSs-94_U	0.03^{a}	3.66^{gh}	7.31^{e}	0.55^{abc}	206.04^{b}	16.85^{d}	3.46^{cd}	0.95^{bc}	3.31^{fg}
TSs-94_Cd	0.06^{c}	4.02^{ghi}	2.11^{ab}	0.42^{a}	800.71^{o}	12.14^{b}	2.77^{b}	0.74^{ab}	8.22^{k}
TSs-95_U	0.03^{a}	4.13^{hi}	22.35^{j}	1.16^{de}	388.22^{g}	33.08^{k}	8.80^{h}	2.63^{ef}	2.41^{cde}
TSs-95_Cd	0.07^{d}	2.65^{de}	4.01^{c}	0.83^{abcd}	388.22^{g}	25.32^{i}	4.55^{ef}	2.00^{d}	1.10^{a}
TSs-96_U	0.03^{a}	3.01^{ef}	15.74^{h}	0.92^{bcde}	1042.55^{p}	34.21^{l}	6.66^{g}	2.98^{f}	1.45^{ab}
TSs-96_Cd	0.07^{cd}	3.50^{fg}	5.00^{d}	0.87^{abcd}	752.81^{m}	22.27^{g}	5.00^{f}	0.96^{bc}	4.11^{ij}
p-value	1.36×10^{-19}	3.64×10^{-18}	3.27×10^{-50}	3.09×10^{-22}	1.89×10^{-11}	$1.73x\ 10^{-41}$	2.21×10^{-29}	1.98×10^{-14}	2.95×10^{-21}

Means with the same alphabetic superscripts on the same column do not differ from each other ($p > 0.05$).U = Unexposed, Cd = Cd-exposed.

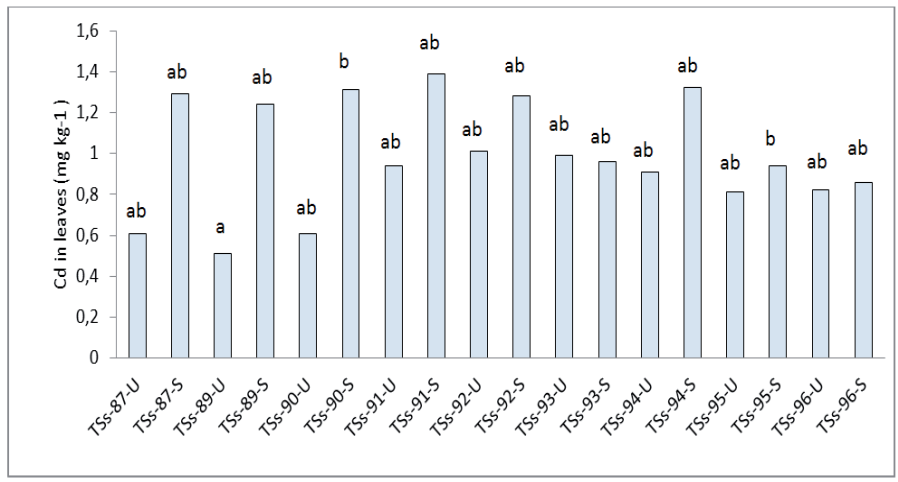


Figure 3: Accumulation of cadmium in leaves of developed emergent of African yam bean at 42 DAS. Bars with the same alphabetic superscripts do not differ from each other ($p > 0.05$). U = Unexposed, Cd = Cd-exposed.

bean at 42 DAS. Results showed that certain level of cadmium was detected in leaf of some AYB accessions exposed to cadmium pollution. Metal concentration rose from 0.61 in unexposed TSs-87 to 1.29 in Cd-exposed TSs-87. Previous experiments showed that Cd accumulate rapidly in plants even though it is not required at any concentration by the plants (Vasiliadou & Dordas, 2009). The present study confirmed that effects of Cd were measured on growth and development of AYB despite that the plant accumulated small amount in the aerial part. Previous investigation by Ding et al. (2014) showed that Cd accumulated more in aerial parts, thus agreeing with the findings of the present study. This is primarily because of the incorporation of exudates from the root which alters redox states and pH of the rhizosphere and allow for enhanced Cd mobility (Quezada-Hinojosa et al., 2015). Comparable observation was made for TSs-89 where Cd concentration increased from 0.51 in plant without cadmium pollution to 1.24 in cadmium exposed plant, whereas in TSs-91 metal concentration increased by 0.45 in Cd-exposed plant when compared to the unexposed accession. Observably, there was no significant change in the level of cadmium found in both Cd-exposed and unexposed TSs-93 at 0.96 and 0.99 respectively.

Component plot in rotated space for quantification of oxygen compounds in plant during exposure to cadmium treatment has been presented in Figure 4. Re-

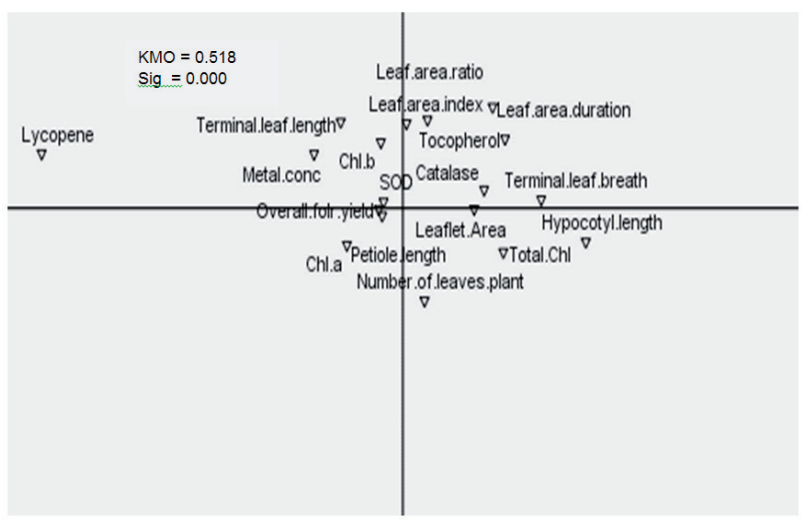


Figure 4: Component plot in rotated space for quantification of oxygen compounds in plant leaves during cadmium exposure

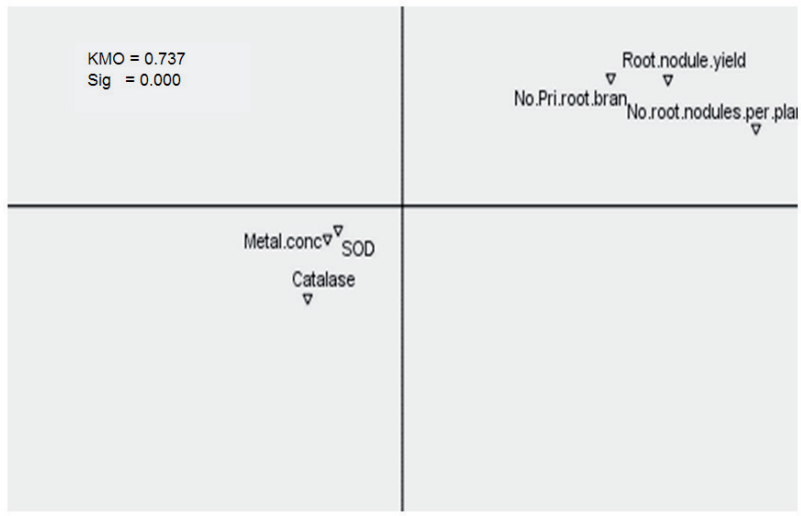


Figure 5: Component plot in rotated space for activity of superoxide dismutase and catalase in African yam bean

sults showed that most parameters including leaf area ratio, terminal leaf length, number of leaves per plant and metal concentration were clustered towards the zero point (point of interception between vertical and horizontal axes). This showed that these parameters contributed less to the variation that involved oxygen compounds in AYB after cadmium treatment. It was observed that lycopene and carotenoids contributed more than superoxide dismutase and catalase in the variation that ensued in AYB after exposure to cadmium contamination. KMO of 0.510 showed that the results of the principal component analysis were reliable.

Concerning the activity of superoxide dismutase (SOD) and catalase in AYB under the influence of cadmium treatment, results showed that parameters such as number of primary branches, root nodule yield, and metal concentration had little control on the activity of the enzymatic antioxidants — SOD and catalase. Figure 5 showed that root length and number of nodules per plant were the key parameters that controlled the activity of SOD and catalase in AYB after exposed to cadmium contamination. KMO and measure of sampling adequacy showed that the results presented in Figure 7 were reliable in identifying the principal component that had much influence on the variations observed in AYB during the study.

4 CONCLUSION

Morphological and physiological responses of nine accessions of African yam bean (*Sphenostylis sternocarpa*) to Cd pollution have been investigated. It can be inferred that Cd engendered different morphological and physiological changes in different accessions of African yam bean (AYB). Although incidence of cadmium toxicity negatively affected the period of seedling emergence in contaminated plants, some accessions showed more tolerance capability than others. This work also demonstrated that, in all AYB accessions tested, nitrogen is assimilated more in nitrate form than as ammonium nitrogen. However, cadmium reduced nitrogen assimilation more in the form of nitrate than as ammonium nitrogen. Among all accessions considered in this study, TSs-92 and TSs-89 were mostly affected by Cd in terms of yield and tolerance, whereas TSs-93and TSs-90 were the least affected. The question therefore is whether these accessions have any genetic capability to justify these characteristics. This, however, is a study for another time.

5 ACKNOWLEGEMENT

The study was funded by the authors. The authors are very grateful to Professor Michael Abberton, Head of Genetic Resources Centre of the International Institute of Tropical Agriculture, Ibadan, Nigeria, for supplying the AYB accessions. The authors also wish to thank Mr. Osazuwa Omoregie and Mrs H. O. Obayuwana for their support during the laboratory sections of the study.

6 REFERENCES

Abolghassem, E., Yulong, D., Farzad, M., & Yinfeng, X. (2015). Heavy metal stress and some mechanisms of plant de-

fense response. *The Scientific World Journal*. https://doi.org/10.1155/2015/756120

Adewale, D. & Dumet, D. (2010). African yam bean, a crop with food security potentials for Africa. *Journal of African Technological Developmental Forum,6*, 66–71.

Aebi, H. E. (1983). Catalase. In: *Methods of enzymatic analysis*. Bergmeyer, H.U. (Ed.). Verlag Chemie Weinhem 273 – 286 pp.

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals—concepts and applications. *Chemosphere, 91*(7), 869–881. https://doi.org/10.1016/j.chemosphere.2013.01.075

Anjum, N. A., Umar, S., Ahmad, A., Iqbal, M., & Khan, N. A. (2008). Ontogenic variation in response of *Brassica campestris* L. to cadmium toxicity. *Journal of Plant Interaction, 35*, 189–198. https://doi.org/10.1080/17429140701823164

Anoliefo, G. O., Ikhajiagbe, B., Okoye, P. C., & Osayi, O. (2016). Utilizing local soap-derived biosurfactant for degradation of petroleum hydrocarbon polluted soils, sustainable remediation in focus. *Annals of Science and Techonology, 1*(1), 43–51. https://doi.org/10.22366/ast.2016.01.007

Asema, S. U. K., Tanveer, S. T., & Sultan, S. (2015). Analysis of soil samples for its physico-chemical parameters from Aurangabad City. *International Journal of Innovative Research and Development, 4*(13), 85–88.

Bashan, Y & de-Bashan, L. E. (2005). Fresh-weight measurement of roots provide inaccurate estimates of the effects of plant growth-promoting bacteria on root growth: a critical examination. *Soil Biology and Biochemistry, 37,* 179 –1804. https://doi.org/10.1016/j.soilbio.2005.02.013

Bhattarai, U. & Subudhi, P. K. (2018). Identification of drought responsive QTLs during vegetative growth stage of rice using a saturated GBS-based SNP linkage map. *Euphytica, 214*(38), https://doi.org/10.1007/s10681-018-2117-3

Ding, C., Zhang, T., Wang, X., Zhou, F., Yang, Y., & Yunlong, Y. (2014). Effects of soil type and genotype on cadmium accumulation by rootstalk crops, implications for phytomanagement. *International Journal of Phytoremediation*, *16*, 1018–1030. https://doi.org/10.1080/15226514.2013.810581

Dobroviczká, T., Piršelová, B., Mészáros, P., Blehová, A., Libantová, J., Morav- ***Číková***, J. & Matušíková, I. (2013). Effects of cadmium and arsenic ions on content of photosynthetic pigments in the leaves of *Glycine max* (L.) Merrill. *Pakistan Journal of Botany, 45*(1), 105–110.

Efroymson, R. A., Will, M. E., & Suter, G. W. (1997a). *Toxicological benchmarks for screening contaminants of potential concern for effects on terrestrial plants:* 1997 revision. ES/ER/TM-85/R3. Oak Ridge National Laboratory, Oak Ridge, TN.

El-Shesheny, M. A., Hendawy, S. H., & Ahmed, K. M. (2014). Assessment of productivity, botanical composition and nutritive value of some plant communities at Sidi-Barrani in North Western Coast of Egypt. *Annals of Agricultural Sciences, 59*(2), 155–163. https://doi.org/10.1016/j.aoas.2014.11.001

Eshghi S., Mahmoodabadi, M. R., Abdi, G. R., & Jamali, B. (2010). Zeolite ameliorates the adverse effect of cadmium contamination on growth and nodulation of soybean plant (*Glycine max* L.). *Journal of Biological and Environmental Science 4*, 43–50.

Fukai, H., Tameda, S., Masuda, H., & Tasaka, M. (2002). Lateral root formation is blockedby a gain-of-function mutationin the solitary-root/IAA14 gene of Arabidopsis. *Plant Journal, 29*, 153–168. https://doi.org/10.1046/j.0960-7412.2001.01201.x

Gallego, S. M., Pena, L. B., Barcia, R. A., Azpilicueta, C. E., Iannone, M. F., Rosales, E. P., Zawoznik, S., Gropppa, M. D., & Benavides, M. P. (2012). Unravelling cadmium toxicity and tolerance in plants, insight into regulatory mechanisms. *Environmental and Experimental Botany, 83*, 33–46. https://doi.org/10.1016/j.envexpbot.2012.04.006

Ghani, A. (2010). Effect of cadmium toxicity on the growth and yield components of Mungbean [*Vigna radiate* (L.) Wilczek]. *World Applied Sciences Journal, 8*, 26–29.

Gill, S. S. & Tuteja, N. (2011). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry, 48*, 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Gonçalves, J. F., Antes, F. G., Maldaner, J., Pereira, L. B., Tabaldi, L. A., Rauber, R., Rossato, L. V., Bisognin, D. A., Dressler, V. L., Flores, M. E., & Nicoloso, F. T. (2009). Cadmium and mineral nutrient accumulation in potato plantlets grown under cadmium stress in two different experimental culture conditions. *Plant Physiology and Biochemistry, 47*, 814–821. https://doi.org/10.1016/j.plaphy.2009.04.002

Gouia, H., Ghorbala, M. H., & Meyer, C. (2000). Effects of cadmium on activity of nitrate reductase and on other enzymes of the nitrate assimilation pathway in bean. *Plant Physiology and Biochemistry, 38*, 629–638. https://doi.org/10.1016/S0981-9428(00)00775-0

Hassanein, R. A., Hashem, H. A., El-Deep, M. H., & Shouman, A. (2013). Soil contamination with heavy metals and its effect on growth, yield and physiological responses of vegetable crop plants (Turnip and Lettuce). *Journal of Stress Physiology and Biochemistry, 9*(4), 145–162.

Iranpoura, M., Lakzian, A., & Zarenia, M. (2016). The effects of cadmium and cow manure on nodulation and growth attributes of common bean (*Phaseolus vulgaris* L.*). International Journal of Environmental and Agricultural Research, 2*(10), 69–74.

Kavvadias, V., Paschalidis, C., Vavoulidou, E., Petropoulis, D., & Koroki, A. (2012). Effects of soil amended with cadmium and lead on growth, yield, and metal accumulation and distribution in parsley. *Communications in Soil Science and Plant Analysis, 43*(1 – 2), 161–175. https://doi.org/10.1080/00103624.2012.634708

Kumar, A., Dutt, S., Bagler, G., Ahuja, P. S., & Kumar, S. (2012). Engineering a thermo-stable superoxide dismutase functional at subzero to > 50°C, which also tolerates autoclaving. *Scientific Reports, 387*, https://doi.org/10.1038/srep00387

Mokhele, B., Zhan, X., Yang, G., & Zhang, X. (2012). Review, Nitrogen assimilation in crop plants and its affecting factors. *Canadian Journal of Plant Science*, *92*, 399–405. https://doi.org/10.4141/cjps2011-135

Munne-Bosch, S. & Alegre, L. (2002). The function of tocopherols and tocotrienols in plants. *Critical Review in*

Plant Science, 21, 31–57. https://doi.org/10.1080/0735-260291044179

Munne-Bosch, S. (2005). The role of α-tocopherol in plant stress tolerance. *Journal of Plant Physiology, 162*, 743–748. https://doi.org/10.1016/j.jplph.2005.04.022

Nada, E., Ferjani, B. A., Ali, R., Bechir, B. R., Imed, M., & Makki, B. (2007). Cadmium induced growth inhibition and alteration of biochemical parameters in almond seedlings grown in solution culture. *Acta Physiologiae Plantarum, 29*, 57–62. https://doi.org/10.1007/s11738-006-0009-y

Naz, H., Naz, A., & Ashraf, S. (2015). Impact of heavy metal toxicity to plant growth and nodulation in chickpea grown under heavy metal stress. *International Journal for Research in Emerging Science and Technology, 2*(5), 248–260.

Neto, M. C. & Bartels, P. G. (1992). Dry matter partitioning of cowpea [*Vigna unguiculata* (L.) Walp.] under water deficit conditions. *Vegetable Report, 93*, 167–170.

Ohyama, T., Fujikake, H., Yashima, H., Tanabata, S., Ishikawa, S., Sato, T., Nishiwaki, T., Ohtake, N., Sueyoshi, K., & Ishii, S. (2012). Effect of nitrate onnodulation and nitrogen fixation of soybean. In El-Shemy, H. A., (Ed.) *Soybean Physiology and Biochemistry*. Intech, Rijeka, Croatia, pp 333–364. https://doi.org/10.5772/17992

Ozalkan, C., Sepetoglu, H. T., Daur, I., & Sen, O. F. (2010). Relationship between some plant growth parameters and grain yield of chickenpea (*Cicer arietinum* L.) during different growth stages. *Turkish Journal of Field Crops, 15*(1), 79–83.

Pál, M., Horvá, E., Janda, T., Páldi, E., & Szalai, C. (2006). Physiological changes and defense mechanisms induced by cadmium stress in maize. *Journal of Plant Nutrition and Soil Science, 169*, 239–246. https://doi.org/10.1002/jpln.200520573

Pappalardo, H., Leonardi, C., Genovese, C., Toscano, V., Melilli, M. G., & Raccuia, S. A. (2016). Effects of heavy metals on seedlings germination and growth in different cardoon genotypes. *Acta Horticulturae,1147*, 281 – 288. https://doi.org/10.17660/ActaHortic.2016.1147.39

Paschalidis, C., Kavvadias, V., Dimitrakopoulou, S., & Koriki, A. (2013) Effects of cadmium and lead on growth, yield, and metal accumulation in cabbage. *Communications in Soil Science and Plant Analysis*, *44*(1-4), 632 – 644. https://doi.org/10.1080/00103624.2013.745373

Paunov, M., Koleva, L., Vassilev, A., Vangronsveld, J., & Goltsev, V. (2018) .Effects of different metals on photosynthesis, Cadmium and zinc affect chlorophyll fluorescence in durum wheat. *International Journal of Molecular Sciences*, *19*, https://doi.org/10.3390/ijms19030787

Potter, D. (1992). Economic Botany of *Sphenostylis* (Leguminosae). *Economic Botany, 46*, 262–275. https://doi.org/10.1007/BF02866625

Qian, H., Li, J., Sun, L., Chen, W., Sheng, G. D., & Liu, W. (2009). Combined effects of copper and cadmium on *Chlorella vulgaris growth and photosynthesis-related gene transcription. Aquatic Toxicology, 94*, 56–61. https://doi.org/10.1016/j.aquatox.2009.05.014

Quezada-Hinojosa R., Föllmi, K. B., Gillet, F., & Matera, V. (2015). Cadmium accumulation in six common plant species associated with soils containing high geogenic cadmium concentrations at Le Gurnigel, Swiss Jura Mountains. *Catena, 124*, 85 – 89 https://doi.org/10.1016/j.catena.2014.09.007

Ramel, F., Birtric, S., Soubigou-Taconnat, L., Triantaphylides, C., & Havaux, M. (2012b). Carotenoids oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. *Proceedings of the National Academy of Sciences of the United States of America, 109*, 5535–5540. https://doi.org/10.1073/pnas.1115982109

Rascio, N. & Navari-Izzo, F. (2011). Heavy metal hyperaccumulating plants, how and why do they do it? And what makes them so interesting? *Plant Science, 180*(2), 169–181. https://doi.org/10.1016/j.plantsci.2010.08.016

Raveh, A. & Avnimelech, Y. (1979). Total nitrogen in water, soil and plant material with persulphate oxidation. *Water Research, 13*(9), 911–912. https://doi.org/10.1016/0043-1354(79)90227-6

Raveneau, M. P., Coste, F., Moreau-Valancogne, P., Lejeune-He´naut I., & Durr, C. (2011). Pea and bean germination and seedling responses to temperature and water potential. *Seed Science Research* https://doi.org/10.1017/S0960258511000067

Rout, G. R., Samantaray, S., & Das, P. (1999). Differential cadmium tolerance of Mungbean and rice genotypes in hydroponic cultures. *Acta Agriculturae Scandinavica, 49*, 234–241. https://doi.org/10.1080/090647100750001604

Sarvajeet, S. G., Mirza, H., Kamrun, N., Anca, M., & Tuteja, N. (2013). Importance of nitric oxide in cadmium stress tolerance in crop plants. *Plant Physiology and Biochemistry, 63*, 254–261. https://doi.org/10.1016/j.plaphy.2012.12.001

Singh, S. & Aggarwal, P. K. (2006). Effect of heavy metals on biomass and yield of different crop species. Indian *Journal of Agricultural Science*, *76*, 688–691.

Skrzyska-Polit, E., Dr_kiewicz, M., & Krupa, Z. (2010). Lipid peroxidation and antioxidative response in Arabidopsis thaliana exposed to cadmium and copper. *Acta Physiologiae Plantarum, 32*, 169–175. https://doi.org/10.1007/s11738-009-0393-1

Thakur, A. K. & Singh, K. J. (2014). Seed emergence is more appropriate criterion for testing cadmium toxicity in soybean. *International Journal of Food, Agriculture and Veterinary Sciences, 4*(2), 129–134.

Vasiliadou, S. & Dordas, C. (2009). Increased concentration of soil cadmium affects plant growth, dry matter accumulation, Cd, and Zn uptake of different tobacco cultivars (*Nicotiana tabacum* L.). *International Journal of Phytoremediation, 11*(2), 115–130. https://doi.org/10.1080/15226510802378400

Yang, H. Y., Shi, G. X, Xu, Q. S., & Wang, H. X. (2011). Cadmium effects on mineral nutrition and stress-related induces in *Potamogeton criprus*. *Russian Journal of Plant Physiology, 58*, 253–260. https://doi.org/10.1134/S1021443711020245

Zhang, F., Wan, X. & Zhong, Y. (2014). Nitrogen as an important detoxification factor to cadmium stress in poplar plants. *Journal of Plant Interactions, 9*(1), 249–258. https://doi.org/10.1080/17429145.2013.819944

Zong, L. G., Sun, J. K., Shen, Q. Y., & Zhang, X. P. (2007). Impacts of cadmium and lead pollution in soil on shoot vegetables growth and toxic-symptoms. *Asian Journal of Ecotoxicology, 2*(1), 63–68.

doi:10.14720/aas.2019.114.2.7 Original research article / izvirni znanstveni članek

The effect of biorational insecticides on the citrus aphids and their predator, *Coccinella septempunctata* L.

Behnam AMIRI BESHELI [1, 2], Amir HosseinTOORANI [3], Habib ABBASIPOUR [3]

Received November 11, 2018; accepted October 15, 2019.
Delo je prispelo 11. novembra 2018, sprejeto 15. oktobra 2019.

The effect of biorational insecticides on the citrus aphids and their predator, ***Coccinella septempunctata*** **L.**

Abstract: To determine selective effectiveness for specific pesticides on biological control species, we evaluated the contact toxicity of different treatments including 10 ml l^{-1} dishwashing liquid, Dayabon 5, 6, 7, 8, 9 and 10 ml l^{-1}, Palizin 1.5, 2.5 and 2.5 ml l^{-1}, Palizin 1.5, 2 and 2.5+Citrol oil 5 ml l^{-1}, Tondoxir 2 and 3+Bartar soap 1 ml l^{-1}, Malathion 2 ml l^{-1} and control (water) on the adult aphids of the most important citrus gardens and their predator, *Coccinella septempunctata* L. in the laboratory conditions. The results revealed that the Palizin treatment 2.5+Citrol oil 5 ml l^{-1}, caused the highest rate of the mortality of the citrus green aphid, *Aphis spiraecola* Patch, 1914 in 36 hours. Concentrations of 7 to 10 ml l^{-1}, Dayabon and Palizin 2.5+Citrol oil 5 ml l^{-1}, as well as 3 ml^{-1} Tondexir + Bartar soap 1 ml^{-1} had the highest mortality of the citrus brown aphid, *Aphis citricidus* (Kirkaldy, 1907), 36 hours after treatment (100 %). In addition, the treatments of Palizin 2 ml^{-1}+ Citrol oil of 5 ml^{-1}, as well as 2 ml l^{-1} Tondexir+Bartar soap 1 ml l^{-1} and concentrations of 5 ml l^{-1} and 6 ml^{-1} of Dayabon produced the same amount of the mortality of the citrus black aphid, *Toxoptera aurantii* (Boyer de Fonscolombe, 1841). Tondexir 3 ml l^{-1}+Bartar soap 1 ml l^{-1} in 24 hours after treatment caused the highest rate of the mortality of the cotton aphids, *Aphis gossypii* Glover, 1877 (83.88 %). Malathion treatment caused a 100 % mortality of the predator ladybird 36 hours after treatment, while the lowest amount was observed in the Dayabon at 10 ml l^{-1} with 33.34 % mortality.

Key words: citrus aphids; *Coccinella septempunctata*; mortality; botanical pesticides

Učinek biološko primernih insekticidov na uši citrusov in njihovega predatorja (*Coccinella septempunctata* **L.**)

Izvleček: Za ovrednotenje selektivne učinkovitosti posebnih pesticidov pri biološkem uravnavanju vrst je bila ovrednotena kontaktna toksičnost različnih snovi, ki so obsegale obravnavanje z 10 ml l^{-1} pomivalne tekočine, obravnavanje z Dayabonom v koncentracijah 5, 6, 7, 8, 9 in 10 ml l^{-1}, Palizinom v koncentracijah 1,5, 2,5 in 2,5 ml l^{-1}, s Palizinom 1,5, 2 in 2,5 + citrolnim oljem 5 ml l^{-1}, Tondoxirjem 2 in 3+milom Bartar 1 ml l^{-1}, Malathionom 2 ml l^{-1} in kontrolo (voda) na odrasle uši v najpomembenjših nasadih citrusov in na njihovega predatorja, *Coccinella septempunctata* L. v laboratorijskih razmerah. Rezultati so odkrili, da je povzročilo obravnavanje s Palizinom 2,5 + citrolnim oljem 5 ml l^{-1} največjo smrtnost zelene uši citrusov (*Aphis spiraecola* Patch, 1914) po 36 urah. Koncentracije 7 do 10 ml l^{-1} Dayabona in Palizina 2,5 + citrolnega olja 5 ml l^{-1}, kot tudi 3 ml l^{-1} Tondexirja + Bartar mila 1 ml l^{-1} so imele največjo smrtnost rjavih citrusovih uši, *Aphis citricidus* (Kirkaldy, 1907), 36 ur po obravnavanju (100 %). Dodatno so obravnavanja s Palizinom 2 ml l^{-1}+ citrolnim oljem 5 ml l^{-1}, 2 ml l^{-1} Tondexirja + Bartar mila 1 ml l^{-1} in Dayabona v koncentracija 5 ml l^{-1} in 6 ml l^{-1} povzročile enako smrtnost črne citrusove uši, *Toxoptera aurantii* (Boyer de Fonscolombe, 1841). Tondexir, 3 ml l^{-1} + Bartar milo, 1 ml l^{-1} sta 24 ur po ubravnavanju povzročila največjo smrtnost bombaževe uši, *Aphis gossypii* Glover, 1877 (83,88 %). Obravnavanje z Malathionom je povzročilo 100 % smrtnost polonice 36 ur po obravnavanju, medtem, ko je bila njena najmanjša smrtnost, 33,4 % , pri obravnavanju z Dayabonom pri 10 ml l^{-1}.

Ključne besede: uši citrusov; *Coccinella septempunctata*; smrtnost; botanični pesticidi

1 Sari Agricultural and Natural Sciences University, Faculty of Crop Sciences, Department of Plant Protection, Sari, Iran
2 Corresponding author, e-mail: behnamamiri39@yahoo.com
3 Shahed University, Faculty of Agricultural Sciences, Department of Plant Protection, Tehran, Iran

1 INTRODUCTION

Aphids are important pests of citrus growth because of direct damage, mainly on young plants, or because of virus transmission. These species are widespread and four of them, green citrus aphid, *A. spiraecola* Patch, cotton aphid, *A. gossypii* Glover, 1877, brown citrus aphid, *Toxoptera citricidus* (Kirkaldy, 1907) and black citrus aphid, *T. aurantii* (Boyer de Fonscolombe, 1841), are especially abundant (Lapchin et al., 1994). These are the most important pests of citrus trees in northern Iran (Abbasipour & Basij, 2016). The citrus aphids are responsible for direct and indirect damages. The former is highly correlated to the aphid species and to the density of its population, as well as to the species and the age of infested citrus trees. Damage consists of leaves' deformation and a reduced development of the infested shoots; such injuries have a negative effect mostly on the growth of young trees. In addition, the aphid infestation to the flowers and the very young fruits may cause their drop. Indirect damages are caused by the excretion of the honeydew, on which, sooty mould fungi raise later. But the greatest threat of the aphid infestation on citrus is undoubtedly represented by their efficiencies as the vector of the citrus virus diseases and particularly of the "Tristeza" (Cavalloro & Martino, 1985).

Nowadays, several groups of insecticides are being used against the main citrus pests (Tena and García-Marí, 2011; Urbaneja et al., 2012). Furthermore, especially in the case of clementines (*Citrus clementina* Hort. ex Tan.) because of their tender flushes, citrus aphids and the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) are considered important pests (Jacas et al., 2010; Urbaneja et al., 2012).

Ladybirds are one of the largest groups of predator insects found in many habitats, including crops, gardens, forests and other places (Ali & Rizvi, 2010). The seven-spotted ladybird, *Coccinella septempunctata* L. (Col.: Coccinellidae) is one of the most common predator species of aphids. The origin of this insect is Asia and Europe and is now widely considered one of the most widely known species in the regions Nearctic, Palearctic, and Oriental (Honek & Martinkova, 2005; Barjadze et al., 2009). This species has been reported from all regions of Iran and in various ecosystems (Ansaripour et al., 2012). Regarding the spread of aphids, limiting chemical control is necessary to reduce the amount of used pesticides and to detect and apply non-chemical methods.This can be done by introducing low-risk biological agents and pesticides with specific dosage and time.

In contrast to the destructive impacts of chemical insecticides, the use of biorational pesticides seems a safe and convenient solution to control these pests. Palizin is a water-soluble wetting agent having an herbal origin and formulated as a 65 % water-soluble concentrated liquid. Sirinol, peppermint, and eucalyptus extracts are used for making the compound. Dayabon, another tested compound has been formulated based on thyme essential oil (EC 10 %). Tondexir, the green, the concentrated liquid contains red pepper extract (EC 85 %). This combination, like the previous composition, acts by contact and causes the death of the pest through the respiratory system disorder (Anonymous, 2015; Imani & Toorani, 2016).

Unfortunately, and despite the importance of predator ladybeetles, *C. septempunctata* in the citrus agroecosystem, there is scarce and disperse literature on the side-effects of the pesticides used in citrus on it (Suma et al., 2009; Zappalà, 2010). Thus, the aims of this study were to evaluate and compare the biorational insecticides with conventional pesticides for control of some important aphids of citrus orchards and to evaluate their impact on its main predator, *C. septempunctata*. This information will allow to assess the acute toxicity of biorational pesticides on citrus aphids and their predators.

2 MATERIALS AND METHODS

2.1 PLANT AND APHID CULTURES

The 'Thomson Navel' variety of orange *Citrus sinensis* L. seedlings were planted in plastic pots (20 cm diameter × 30 cm height) containing a 1:1 mixture of perlite: vermiculite in a greenhouse at 25 ± 2 °C and 16L: 8D h photoperiods. Stock laboratory cultures of the studied aphids, including the citrus green aphid, *A. spiraecola*, the cotton aphid, *A. gossypii*, the brown citrus aphid, *T. citricidus* and the citrus black aphid, *T. aurantii* were established on its respective host plants from individuals that were field-collected from the same host plant in Sari city of Mazandran province, north of Iran in 2017 and were reared under the same physical conditions as the plant.

Early colonies of aphids were collected from infested citrus orchards in Sari, Iran, and transferred to the laboratory. Adult aphids were placed on citrus leaves to obtain a similar population of adults after a few days of nymphs were appeared. Then, adult aphids were separated from the leaves and the aphids were placed into the germinator. After several days and emergence

of same-aged adult aphids, they were used for bioassay experiments.

2.2 PREDATOR LADYBIRD REARING

A number of the seven-spotted ladybird, *C. septempunctata* were collected during the winter that were fed and matured in citrus orchards. They were then transferred to the germinator at 25 ± 2 °C, 65 ± 5 % RH and 16L : 8D h photoperiods. Adults and nymphs of citrus aphids were daily used to feed the ladybirds.

2.3 BIOASSAY TESTS

For each treatment, ten replicates were considered, and in each replicate, 30 adult aphids were placed on leaves of the host plant in Petri dishes with a diameter of 8 and a height of 1.5 centimeters. The treatments included: 1) Aveh dishwashing liquid (10 ml l^{-1}), 2) Dayabon (5 ml l^{-1}), 3) Dayabon (6 ml l^{-1}), 4) Dayabon (7 ml l^{-1}), 5) Dayabon (8 ml l^{-1}), 6) Dayabon (9 ml l^{-1}), 7) Dayabon (10 ml l^{-1}), 8) Palizin (1.5 ml l^{-1}), 9) Palizin (2 ml l^{-1}), 10) Palizin (2.5 ml l^{-1}), 11) Palizin (1.5 ml l^{-1}) + Citrol oil (5 ml l^{-1}), 12) Palizin (2 ml l^{-1}) + Citrol oil (5 ml l^{-1}), 13) Palizin (2.5 ml l^{-1}) + Citrol oil (5 ml l^{-1}), 14) Tondexir 2 ml l^{-1} + Bartar Soap 1 ml l^{-1}, 15) Tondexir 3 ml l^{-1}+ Bartar soap 1 ml l^{-1}, 16) Malathion 2 ml l^{-1} and 17) control (water). Then, a certain amount of each treatment was carried out with a sampler and poured into a liter of water and sprayed with a 10-milliliter (Potter precision spray tower) spray bottle (Potter Precision Spray Tower, 2000) on each leaf. Due to the fact that the capacity of the device is from a half to one milliliter, at each stage, one milliliter was sprayed on the leaf containing aphids, and this operation was repeated for 10 times, for each leaf at 10 ml spray. All experiments were replicated three times. Petri-dish containing aphids were place in the germinator at 25 ± 2 °C, 65 ± 5% RH and 16L : 8D h photoperiods. Live and dead aphids were counted and their mortality rate was estimated at 12, 24 and 36 hours after treatment. Insects that were unable to move their legs and tentacles against hot needle excitation were considered dead. In case of death in the control, the percent mortality of the treatments was corrected by Abbott's formula (Finny, 1971). These experiments were performed on each of the four-aphid species, separately in 10 replicates and 30 aphids per replicate.

Table 1: Percentage mean (±SE) mortality of the green citrus aphid, *Aphis spiraecola* treated with different chemical compounds in different times

	Mean (±SE) mortality at different intervals (h)		
Treatment	12 h	24 h	36 h
dishwashing liquid (Ave) 10 ml l^{-1}	46.78 ± 0.81^{ef}	50.62 ± 0.57^{fg}	28.28 ± 0.82^{k}
Dayabon 5 ml l^{-1}	26.81 ± 0.61^{h}	36.71 ± 0.78^{i}	45.37 ± 0.62^{i}
Dayabon 6 ml l^{-1}	26.41 ± 0.46^{h}	37.55 ± 0.43^{hi}	41.73 ± 0.55^{j}
Dayabon 7 ml l^{-1}	28.40 ± 0.65^{h}	37.15 ± 0.66^{i}	49.69 ± 1.61^{h}
Dayabon 8 ml l^{-1}	38.04 ± 0.51^{g}	40.24 ± 0.79^{h}	52.06 ± 0.84^{h}
Dayabon 9 ml l^{-1}	43.53 ± 0.69^{f}	53.18 ± 0.60^{f}	62.61 ± 0.56^{f}
Dayabon 10 ml l^{-1}	49.68 ± 0.61^{d}	58.21 ± 0.61^{f}	71.76 ± 0.55^{e}
Palizin 1.5 ml l^{-1}	28.64 ± 0.61^{h}	32.88 ± 0.85^{j}	51.94 ± 0.51^{h}
Palizin 2 ml l^{-1}	38.08 ± 0.50^{g}	49.05 ± 0.66^{g}	58.40 ± 0.49^{g}
Palizin 2.5 ml l^{-1}	58.22 ± 0.73^{c}	61.76 ± 0.67^{g}	72.32 ± 0.68^{e}
Palizin 1.5 ml l l^{-1} + Citrol oil 5 ml l^{-1}	45.80 ± 0.78^{ef}	50.34 ± 0.45^{fg}	63.34 ± 0.65^{f}
Palizin 2 ml l^{-1} + Citrol oil 5 ml l^{-1}	59.49 ± 0.55^{c}	67.86 ± 0.55^{c}	82.18 ± 0.47^{c}
Palizin 2.5 ml l^{-1} + Citrol oil 5 ml l^{-1}	78.89 ± 0.49^{a}	84.37 ± 0.38^{a}	99.37 ± 0.30^{a}
Tondexir 2 ml l^{-1} + Bar-Tar wetting agent 1 ml l^{-1}	68.42 ± 0.47^{b}	72.30 ± 0.55^{b}	77.11 ± 0.46^{d}
Tondexir 3 ml l^{-1} + Bar-Tar wetting agent 1 ml l^{-1}	76.65 ± 0.76^{b}	83.49 ± 0.48^{a}	95.21 ± 0.65^{b}
Malathion 2 ml l^{-1}	58.79 ± 0.51^{c}	63.44 ± 0.60^{d}	39.49 ± 1.04^{j}
Control (water)	7.57 ± 0.38^{i}	7.66 ± 0.38^{k}	12.21 ± 0.43^{l}

* Means were compared by Tukey's range test at 0.05 level. The similar letters indicate no significant difference.

The adult ladybirds, *C. septempunctata* were used for bioassay experiments. The method of bioassay tests for ladybird was similar to that of aphids, with the exception that 10 adult insects were used in each replication and, the number of live and dead insects were estimated at 12, 24, 36, 48 and 60 hours after treatment. The experiments were bio-assayed with 16 treatments and control with 10 replicates for each of the compounds.

2.4 STATISTICAL ANALYSIS.

In the case of mortality in the control, the mortality percentage of other 16 treatments was modified using the Abbott formula (Finney, 1971). The data were subjected to a two-way analysis of variance (ANOVA) and the means were compared by Tukey's range test at 0.05 level, using SPSS program (SPSS, 2006).

3 RESULTS

The results of bioassay experiments on the effects of different treatments of various compounds on the black citrus aphid, *T. aurantii*, the brown aphid, *T. citricidus*, the cotton aphid *A. gossypii* and the green citrus aphid, *A. spiraecola*, as well as the seven-spotted ladybird insects, are listed in Tables 1 to 5.

The results of analysis of bioassay data for the green citrus aphid, *A. spiraecola* showed that there is a significant difference between various treatments at the test times. For a period of 12 hours (df = 16, F = 1000.804, $p < 0.001$), 24 hours (df = 16, F = 1048.339, $p < 0.001$) and for 36 hours (df = 16, F = 988.059, $p < 0.001$), based on these results, the treatment Palizin 2.5+ Citroloil 5 ml l^{-1} had the highest mortality at 12, 24 and 36 h, which was 76.89, 84.37 and 99.37 %, respectively.

Among Dayabon treatments, treatment of 10 ml l^{-1} had the highest mortality rate at different times, so that at 36 hours about 71.76 % of aphids died. For the dishwashing liquid 10 ml l^{-1} treatment, the mortality rate increased from 12 hours to 24 hours and 36 hours. Indicating that the time of 36 hours after treatment had the highest mortality rate (58.28 %) (Table 1).

According to the variance analysis results of bioassay testing of the black citrus aphid, *T. aurantii*, a significant difference was observed between all treatments at test times (for 12 hours, df = 16, F = 680.645, $p < 0.001$; for 24 hours, df = 16, F = 2605.078, $p < 0.001$ and for 36 hours, df = 16, F = 146.352, $p < 0.001$). The results

Table 2: Percentage mean (±SE) mortality of the black citrus aphid, *Toxoptera aurantii* treated with different chemical compounds in different times

	Mean (±SE) mortality at different intervals (h)		
Treatment	12 h	24 h	36 h
dishwashing liquid (Ave) 10 ml l^{-1}	55.57 ± 1.0^{g}	95.31 ± 0.80^{b}	1.08 ± 0.37^{d}
Dayabon 5 ml l^{-1}	66.72 ± 0.68^{e}	100 ± 0.0^{a}	100 ± 0.0^{a}
Dayabon 6 ml l^{-1}	75.04 ± 0.70^{d}	100 ± 0.0^{a}	100 ± 0.0^{a}
Dayabon 7 ml l^{-1}	92.09 ± 0.72^{bc}	100 ± 0.0^{a}	100 ± 0.0^{a}
Dayabon 8 ml l^{-1}	96.68 ± 0.99^{ab}	100 ± 0.0^{a}	100 ± 0.0^{a}
Dayabon 9 ml l^{-1}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
Dayabon 10 ml l^{-1}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
Palizin 1.5 ml l^{-1}	31.70 ± 0.70^{i}	39.15 ± 0.61^{g}	48.32 ± 0.51^{c}
Palizin 2 ml l^{-1}	41.44 ± 0.50^{h}	52.39 ± 0.72^{f}	76.38 ± 0.65^{b}
Palizin 2.5 ml l^{-1}	61.52 ± 0.62^{f}	73.35 ± 0.69^{d}	84.19 ± 0.93^{b}
Palizin 1.5 ml l^{-1} + Citrol oil 5 ml l^{-1}	55.61 ± 0.92^{g}	64.88 ± 0.69^{e}	77.50 ± 0.81^{b}
Palizin 2 ml l^{-1} + Citrol oil 5 ml l^{-1}	90.80 ± 0.81^{c}	98.29 ± 0.54^{c}	100 ± 0.0^{a}
Palizin 2.5 ml l^{-1} + Citrol oil 5 ml l^{-1}	98.38 ± 0.62^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
Tondexir 2 ml l^{-1} + Bar-Tar wetting agent 1 ml l^{-1}	90.13 ± 0.78^{c}	88.16 ± 0.89^{c}	100 ± 0.0^{a}
Tondexir 3 ml l^{-1} + Bar-Tar wetting agent 1 ml l^{-1}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
Malathion 2 ml l^{-1}	73.64 ± 3.28^{d}	98.11 ± 0.70^{d}	39.49 ± 1.64^{b}
Control (water)	11.14 ± 0.62^{j}	10.64 ± 0.78^{h}	11.02 ± 0.79^{d}

* Means were compared by Tukey's range test at 0.05 level. The similar letters indicate no significant difference.

Table 3: Percentage mean (±SE) mortality of the brown citrus aphid, *Aphis citricidus* treated with different chemical compounds in different times

	Mean (±SE) mortality at different intervals (h)		
Treatment	12 h	24 h	36 h
dishwashing liquid (Ave) 10 ml l^{-1}	41.84 ± 1.04^{h}	82.65 ± 0.90^{c}	32.05 ± 0.98^{h}
Dayabon 5 ml l^{-1}	47.0 ± 0.85^{g}	67.61 ± 0.84^{de}	72.38 ± 0.55^{d}
Dayabon 6 ml l^{-1}	54.61 ± 0.87^{f}	70.53 ± 0.93^{d}	81.34 ± 0.72^{c}
Dayabon 7 ml l^{-1}	84.20 ± 0.59^{d}	96.89 ± 1.08^{ab}	100 ± 0.0^{a}
Dayabon 8 ml l^{-1}	91.96 ± 0.48^{c}	96.55 ± 1.28^{ab}	100 ± 0.0^{a}
Dayabon 9 ml l^{-1}	96.26 ± 0.53^{b}	100 ± 0.0^{a}	100 ± 0.0^{a}
Dayabon 10 ml l^{-1}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
Palizin 1.5 ml l^{-1}	25.83 ± 0.68^{i}	37.66 ± 0.58^{h}	41.95 ± 1.08^{g}
Palizin 2 ml l^{-1}	34.35 ± 0.66^{i}	40.73 ± 0.71^{h}	42.83 ± 0.72^{g}
Palizin 2.5 ml l^{-1}	44.29 ± 0.76^{gh}	48.22 ± 0.77^{g}	52.60 ± 0.70^{f}
Palizin 1.5 ml l^{-1} + Citrol oil 5 ml l^{-1}	40.90 ± 0.70^{h}	54.75 ± 0.72^{f}	63.91 ± 0.67^{e}
Palizin 2 ml l^{-1} + Citrol oil 5 ml l^{-1}	55.67 ± 0.75^{f}	65.56 ± 0.82^{e}	85.48 ± 0.81^{b}
Palizin 2.5 ml l^{-1} + Citrol oil 5 ml l^{-1}	73.91 ± 0.65^{j}	95.84 ± 0.86^{b}	100 ± 0.0^{a}
Tondexir 2 ml l^{-1} + Bar-Tar wetting agent 1 ml l^{-1}	70.99 ± 0.87^{e}	82.56 ± 0.64^{c}	85.69 ± 0.88^{b}
Tondexir 3 ml l^{-1} + Bar-Tar wetting agent 1 ml l^{-1}	85.13 ± 1.24^{d}	98.54 ± 0.81^{ab}	100 ± 0.0^{a}
Malathion 2 ml l^{-1}	54.73 ± 0.75^{f}	83.67 ± 0.77^{c}	62.78 ± 0.77^{e}
Control (water)	7.95 ± 0.47^{k}	8.73 ± 0.57^{i}	9.24 ± 0.56^{i}

* Means were compared by Tukey's range test at 0.05 level. The similar letters indicate no significant difference.

of the mean comparison indicated that the treatments of Dayabon 9 and 10 ml l^{-1}, as well as Tondexir + Bartar soap 3 + 1ml l^{-1} had the highest effect in 12 hours after the treatment and resulted in the mortality of 100 % of the black citrus aphids. In 36 hours after treatment, all concentrations of Dayabon, Tondexir + Bartar soap, Dishwashing liquid (Ave) 10 ml l^{-1} and Palizin 2, 2.5 + citrol oil 5 ml l^{-1} treatments caused 100 % of aphid mortality. While the lowest mortality rate was recorded for control (11.92 %) (Table 2).

The results of the variance analysis of bioassay data from the brown citrus aphid, *T. citricidus* showed a significant difference between the treatments at different time of testing. At 12 hours (df = 16, F = 1255.853, $p < 0.001$), 24 hours (df = 16, F = 1160.900, < 0.001) and 36 hours (df = 16, F = 2046.868, $p < 0.001$) post treatments. The results of the mean comparison showed that when Palizin insecticide was combined with citrol oil, its efficiency was increased. At 36 hours after treatment, the concentrations of Palizin 0.5, 2 and 2.5 ml l^{-1}, caused 41.95, 42.83 and 52.60 % mortality, respectively, but when there were combined with citrol oil, mortality increased to 63.91, 85.48 and 100 %, respectively. In the treatment of Malathion, over a period of 12 hours after treatment to 24 and 36 hours, the mortality rate increased from 54.73 to 83.67 % and 92.78 % mortality, respectively (Table 3).

The results of the variance analysis of bioassay tests in different treatments on the cotton aphid, *A. gossypii* showed that there was a significant difference between treatments at 12, 24 and 36 hours. At 12 h (df = 16, F = 871.371, $p < 0.001$), 24 hours (df = 16, F = 844.016, $p < 0.001$) and 36 hours (df = 16, F = 322.346, $p < 0.001$). Regarding the results of the mean comparison at 12 hours after the treatment, the highest mortality rates occurred in Tondexir 3 + Bartar soap 1ml l^{-1} and Malathion 2 ml l^{-1} treatments, which was 75.40 and 74.32 %, respectively. In the 24 hours after treatment, as in 12 hours, the two treatments with 83.89 and 82.73 % mortality. But 36 hours after treatment, Tondexir 3ml l^{-1} + soap Bartar 1ml l^{-1} treatment alone was found in the first group with 88.73 % of mortality. Meanwhile, Malathion 2ml l^{-1} was placed in the second group (b) (Table 4).

Based on results of the variance analysis of data from bioassay tests of different treatments on the seven-spotted ladybird, *C. septempunctata* at different times after treatment showed a significant difference among treatments at 12 to 60 hours after treatment. For 12 hours (df = 16, F = 47.631, $p < 0.001$), for 24 hours

Table 4: Percentage mean (±SE) mortality of the citrus aphid, *Aphis gossypii* treated with different chemical compounds in different times

	Mean (±SE) mortality at different intervals (h)		
Treatment	12 h	24 h	36 h
dishwashing liquid (Ave) 10 ml l^{-1}	50.34 ± 0.68^{ef}	53.47 ± 0.79^{f}	62.44 ± 0.91^{d}
Dayabon 5 ml l^{-1}	29.56 ± 0.66^{h}	34.28 ± 0.81^{gh}	34.94 ± 0.48^{f}
Dayabon 6 ml l^{-1}	35.81 ± 0.75^{h}	36.02 ± 0.57^{g}	42.33 ± 0.65^{e}
Dayabon 7 ml l^{-1}	44.22 ± 0.72^{h}	57.93 ± 0.56^{e}	63.45 ± 1.75^{d}
Dayabon 8 ml l^{-1}	55.53 ± 0.73^{g}	62.68 ± 0.61^{d}	70.27 ± 0.55^{bc}
Dayabon 9 ml l^{-1}	54.98 ± 0.78^{f}	62.78 ± 0.87^{cd}	70.53 ± 0.98^{bc}
Dayabon 10 ml l^{-1}	58.34 ± 0.82^{d}	68.65 ± 0.49^{b}	74.84 ± 0.79^{ab}
Palizin 1.5 ml l^{-1}	9.57 ± 0.58^{h}	13.02 ± 0.65^{j}	25.81 ± 0.92^{g}
Palizin 2 ml l^{-1}	12.40 ± 0.54^{g}	22.30 ± 0.76^{i}	26.70 ± 2.38^{g}
Palizin 2.5 ml l^{-1}	21.56 ± 1.03^{c}	31.37 ± 0.65^{h}	36.48 ± 0.61^{ef}
Palizin 1.5 ml l^{-1} + Citrol oil 5 ml l^{-1}	42.89 ± 0.79^{ef}	55.0 ± 0.93^{ef}	66.37 ± 2.24^{cd}
Palizin 2 ml l^{-1} + Citrol oil 5 ml l^{-1}	54.52 ± 0.81^{c}	65.15 ± 0.91^{bcd}	75.03 ± 1.0^{ab}
Palizin 2.5 ml l^{-1} + Citrol oil 5 ml l^{-1}	55.45 ± 0.80^{a}	66.63 ± 1.0^{bc}	75.23 ± 0.72^{ab}
Tondexir 2 ml l^{-1} + Bar-Tar wetting agent 1 ml l^{-1}	61.95 ± 0.68^{b}	62.07 ± 0.83^{d}	63.37 ± 0.73^{b}
Tondexir 3 ml l^{-1} + Bar-Tar wetting agent 1 ml l^{-1}	75.40 ± 0.80^{b}	83.89 ± 0.89^{a}	78.73 ± 0.24^{a}
Malathion 2 ml l^{-1}	74.32 ± 0.73^{c}	82.73 ± 0.88^{a}	62.78 ± 0.55^{bc}
Control (water)	8.36 ± 0.85^{i}	8.60 ± 0.90^{k}	11.81 ± 0.72^{h}

* Means were compared by Tukey's range test at 0.05 level. The similar letters indicate no significant difference

(df = 16, F = 84.149, $p < 0.001$), for 36 hours (df = 16, F = 124.517, <0.001), for 48 hours (df = 16, F = 102.704, $p < 0.001$) and for 60 hours (df = 16, F = 188.976, $p < 0.001$). According to the results presented in Table 5, the highest mortality rate of *C. septempunctata* ladybird were occurred at 12, 24, 36, 48 and 60 hours after treatment, by Malathion 2ml l^{-1}, which was equal to 69, 92, 100, 100 and 100 %, respectively. While among other treatments at different times after treatment, the highest mortality rate was related to the Dayabon 10 ml l^{-1} treatment, 36 hours after treatment 35.20 % (Table 5).

4 DISCUSSION

Knowledge of the use of selective insecticides for the pests and their natural enemies is very important in the integrated pest management. Especially when these compounds are used as pest control tools (Sterk et al., 2003). Previous studies using the above treatments showed that the highest mortality rate of the green citrus aphid, *A. spiraecola* was observed for Tondexir with a concentration of 3 ml l^{-1} + Bartar soaps 1 ml l^{-1} (58 %) and the lowest rate after control treatment (6 %), was produced by Palizin 1.5 and 2 ml l^{-1} (16 and 18 %) treatments, respectively (Imani &Toorani, 2016). In this experiment, the treatments of Tondexir 3+ Bartar soap 1 ml l^{-1} and Palizin with 1.5 ml and 2 ml l^{-1} of after 24 hours of the treatments, had the mortality of 83.49, 32.88 and 49.05 %, respectively on the green citrus aphid. In addition, the treatment Palizin 2.5 ml l^{-1} + citrol oil 5 ml l^{-1} had the highest mortality rate in 24 hours after treatment (84.37 %), which indicates that with increasing concentration, the efficiency of this compound has increased, as well as citrol oil 5 ml l^{-1} has a synergistic role and increases the palizin function.

Moreover,the experiments carried out on the black citrus aphid, *T. aurantii* in our case revealed that all Dayabon treatments, Palizin 2.5+ citrol oil 5 ml l^{-1} and Tondexir 3 + Bartar soap 1 ml l^{-1} treatments caused 100 % mortality, 24 hours after treatment (Table 2). Similar to our research, in the experiment on the black citrus aphid, *T. aurantii*, all concentrations of Dayabon had the highest mortality rates (100 %) (Toorani et al., 2016).

Similarly, the highest mortality rate of the Australian mealybug, *Icerya purchasi* Maskell, 1878 was observed by Heydariet al. (2016c) for Dayabon treatments with concentrations of 9 and 10 ml l^{-1} and Tondexir

Table 5: Percentage mean (± SE) mortality of the seven-spot ladybird, *Coccinella septempunctata*, treated with different chemical compounds, in different times

	Percentage mean (±SE) mortality at different intervals (h)				
Treatment	12 h	24 h	36 h	48 h	60 h
dishwashing liquid (Ave) 10 ml l^{-1}	6.0 ± 1.63^{def}	17.60 ± 2.44^{defg}	21.0 ± 1.34^{cde}	9.17 ± 2.13^{bcdef}	0.0 ± 0.0^{c}
Dayabon 5 ml l^{-1}	4.0 ± 1.63^{f}	10.11 ± 2.11^{fghi}	8.10 ± 1.77^{gh}	7.45 ± 1.87^{bcdef}	5.0 ± 2.7^{ab}
Dayabon 6 ml l^{-1}	13.0 ± 1.53^{bcdef}	17.40 ± 1.37^{defg}	10.0 ± 1.51^{fgh}	3.30 ± 1.55^{f}	1.30 ± 0.0^{c}
Dayabon 7 ml l^{-1}	20.0 ± 2.98^{b}	15.20 ± 1.02^{efgh}	15.80 ± 2.14^{cde}	7.10 ± 1.86^{cdef}	5.70 ± 2.7^{ab}
Dayabon 8 ml l^{-1}	17.0 ± 1.53^{bc}	27.49 ± 2.48^{bcd}	21.20 ± 2.09^{bcd}	16.0 ± 3.20^{bcd}	3.80 ± 2.3^{ab}
Dayabon 9 ml l^{-1}	18.0 ± 2.0^{bc}	23.70 ± 1.98^{bcde}	26.0 ± 1.59^{bcd}	16.90 ± 1.54^{bc}	2.70 ± 1.0^{c}
Dayabon 10 ml l^{-1}	18.0 ± 2.94^{bc}	30.80 ± 1.06^{b}	33.40 ± 1.52^{b}	14.50 ± 2.79^{bcde}	5.20 ± 1.0^{ab}
Palizin 1.5 ml l^{-1}	4.0 ± 1.63^{f}	7.50 ± 1.61^{ghi}	6.30 ± 1.78^{h}	3.60 ± 1.38^{ef}	1.0 ± 1.0^{c}
Palizin 2 ml l^{-1}	6.0 ± 1.63^{def}	6.20 ± 1.81^{hi}	7.20 ± 1.32^{h}	5.20 ± 1.90^{def}	4.30 ± 1.0^{ab}
Palizin 2.5 ml l^{-1}	5.0 ± 2.24^{ef}	8.80 ± 1.23^{fghi}	8.60 ± 2.43^{gh}	4.10 ± 1.73^{ef}	2.60 ± 1.0^{c}
Palizin 1.5 ml l^{-1} + Citrol oil 5 ml l^{-1}	9.0 ± 1.79^{cdef}	13.20 ± 2.12^{efghi}	9.90 ± 2.04^{gh}	13.20 ± 2.33^{bcdef}	7.90 ± 2.7^{ab}
Palizin 2 ml l^{-1} + Citrol oil 5 ml l^{-1}	15.0 ± 1.67^{bcde}	15.70 ± 1.38^{efgh}	19.70 ± 2.34^{def}	6.60 ± 2.42^{cdef}	2.0 ± 1.0^{c}
Palizin 2.5 ml l^{-1} + Citrol oil 5 ml l^{-1}	22.0 ± 2.91^{b}	11.50 ± 2.88^{fghi}	17.80 ± 2.52^{defg}	11.40 ± 2.75^{bcdef}	4.60 ± 1.3^{ab}
Tondexir 2 ml l^{-1} + Bar-Tar wetting agent 1 ml l^{-1}	16.0 ± 2.21^{bcd}	19.30 ± 3.28^{cdef}	25.0 ± 2.07^{bcde}	2.90 ± 1.97^{f}	3.30 ± 2.7^{ab}
Tondexir 3 ml l^{-1} + Bar-Tar wetting agent 1 ml l^{-1}	20.0 ± 2.98^{b}	28.60 ± 2.98^{bc}	29.70 ± 3.25^{bcd}	18.70 ± 3.98^{b}	11.60 ± 2.0^{b}
Malathion 2 ml l^{-1}	69.0 ± 3.48^{a}	92.0 ± 3.51^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
Control (water)	4.0 ± 1.63^{f}	4.20 ± 1.72^{i}	6.50 ± 1.78^{h}	5.50 ± 1.84^{def}	40.80 ± 1.0^{ab}

* Means were compared by Tukey's range test at 0.05 level. The similar letters indicate no significant difference..

with concentrations of 2 and 3+ Bartar Soap of 1 ml l^{-1} with 93, 99.33, 95 and 100 % mortality, respectively.

Based on studies conducted in laboratory conditions, the LC_{50} values of the two extracts of red pepper, *Capsicum annum* L., and garlic extract on *A. gossypii* were 135.74 and 140.69 ppm, respectively. The result has shown the higher efficiency of pepper extracts (Kazem & El-shereif, 2010). The use of coconut oil soap (Palizin) with concentrations of 1500 and 2500 ppm in Iranian cucumber greenhouses reduced the population of *A. gossypii* aphid by 75.9 and 90.6 % mortality (Baniameri, 2008). In this research, Palizin 2.5 ml l^{-1}iter + Citrol oil 5 ml $l^{-1}$and Tondexir 3 ml l^{-1}+ Bartar soap 1 ml l^{-1}have high efficacy in reducing the population of aphids and cause a high mortality rate, as the impaction of the pest to these compounds causes gas exchange and metabolism problems, as well as the destruction of the cuticle, and finally causes the death of insects (Baniameri, 2008).

In the present study, Tondexir 2+Bartar soap 1 ml l^{-1} and Palizin 2 + citrol oil 5 ml l^{-1}, causing 72.30 and 67.86 % mortality of the green citrus aphid, *A. spiraecola* (Table 1); 98.16 and 98.29 % of the black citrus aphid, *T. aurantii* (Table 2); 82.56 and 65.56 % of the brown citrus aphid, *A. citricidus* (Table 3) and 62.07 and 65.15 % of the cotton aphid, *A. gossypii*, (Table 4) respectively. Whereas, the results of the mortality of larvae of citrus leaf miner, *P. citrella* showed that Tondexir, Sirinol, Palizin, 2000 ppm + mineral oil 5000 ppm and Spinosad 750 ppm + mineral oil 5000 ppm, produced 31.75, 45, 35.75 and 76 % mortality, respectively after 24 hours (Amiri-Besheli, 2009). It seems that the aphids tested in the present research are much less resistant than citrus leaf miner larvae. The reason for that is due to Palizin and Tondexir agents which had a contact effect. For the larvae of the citrus leaf miner, they were inside leaf tissue and were relatively protected by leaf tissue, so that the tested insecticides had less contact with the larvae, and lower mortality rates were observed. Also, the highest mortality rate of the oleander yellow aphid, *A. nerii* Fonscolombe, 1841, was occurred in the treatments of Tondxir 3 + Bartar soap 1 ml l^{-1} (94.46 %), Dayabon 10 ml l^{-1} (89.70 %) and acetamipride (90.17 %), and no significant difference was observed among the three treatments (Heydari et al., 2016a). The difference in the mortality rate of the treatments used in the present study with the mentioned research can be attributed to the difference in aphid species, because the resistance of each pest is different to the insecticides comparatively to other species.

Our results showed that almost in most tests, Palizin 1.5 and 2 ml l^{-1} treatments had a significantly lower effect than other treatments (Table 2, 3 and 4). But when these treatments were combined with citrol oil of 5 ml l^{-1}, their mortality increased dramatically. But, it can be concluded that the mortality rate of the similar treatments in the present study was much higher than Imani & Toorani (2016) research. The reason for this difference is due to the difference in the test conditions in the two studies. For example, tested aphids in the previous research were directly collected from citrus gardens and treatments were applied, whereas, in the present study, the aphids were reared for a generation in the laboratory and then were tested. This suggests that the method of insect rearing and the surrounding environmental conditions and the feeding type of an insect can effect on its resistance to insecticides.

In the present study, new botanical pesticides such as Dayabon, Palizin and Tondexir had a very little effect on the seven-spotted ladybird (Table 5). In previous studies, garlic extract (Sirinol) with a concentration of 2500 ppm has a lesser impact on natural enemies such as predator ladybirds and pistachio parasitoid in comparison with the Mospilan and Consult insecticides (Kabiri et al., 2012). In the present study, similar to the above-mentioned research, Tondexir 3 +Bartar soap 1 ml l^{-1}, containing garlic and pepper extract, had a lower effect on the seven-spotted ladybird than the chemical pesticides, malathion, caused 29.70 and 100 % mortality on ladybird at 36 hours after treatment, respectively (Table 5).

Previous researches, as in our case, showed that the botanical pesticides used in the present study had a great impact on pest infestation, but had little effect on the natural enemy. For example, the results of Heydari et al. (2016b) showed that the highest percentage of mortality in the nymphs of citrus cushion, *Pulvinaria aurantii* Cockerell, 1896 (100 %) was occurred in the Dayabon treatment of 9 and 10 ml l^{-1}, and the lowest (except for control) (34 %) was observed in Palizin 1.5 ml l^{-1}. In the case of the predator ladybird, *Cryptolaemus montrouzieri* Mulsant, 1850 the highest and lowest mortality rates were observed in Dorsban 2 ml l^{-1} (76.66 %) and Dayabon 5 ml l^{-1}, Palizin 2 ml l^{-1} (3.33) treatments. In addition, in a research carried out in field conditions with the same treatments, it showed that Dayabon 9 and 10 ml l^{-1} treatments, in addition to controlling the citrus cushion, *P. aurantii* and not having an adverse effect on its predator, can be a suitable substitute for other high risk chemical insecticides (Tooranie et al., 2017). Furthermore, Kabiri et al. (2012) showed that three pesticides, Sirinol, Palizin and Tondexir had very low toxicity on the ladybird, *Oenopia conglobate* (Linaeus, 1758), one of the most important predators of the pistachio psylla, *Agonoscena pistaciae* Burckhardt & Lauterer, 1989, and were classified into a group of harmless insecticides for this predator. Due to the fact that the botanical pesticides used in this study have a direct contact effect and, by closing the respiratory tract of the skin, cause mortality of the pests, therefore, there is less effect on the mortality of *C. septempunctata* ladybird because the elytra of insect prevent insecticide to contact with the cuticle of insect.

5 CONCLUSION

The results of this study indicate that the botanical pesticides used to control the important aphids of citrus orchards, had a desirable level. Also, according to the results, Palizin 2 and 2.5 + Citrol oil 5 ml l^{-1} and Tondexir concentrations with Bartar soap and also Dayabon 10 ml l^{-1} treatment, in addition to effective control on the citrus aphids, had a negative effect on the seven-spotted ladybird which is the most important natural enemy in citrus orchards. So, they could be a good alternative to other high-risk chemical insecticides.

6 ACKNOWLEDGEMENTS

This work was supported by the Entomology section of the Department of Plant Protection of Shahed University, Tehran, and Sari Agricultural Sciences and Natural Resources University, Iran.

7 REFERENCES

Alam, M. Z., Alam, M. S. & Abbas M. (1972). Status of different stem borers as pests of rice in Bangladesh. *IRRN, 21*(2), 15-17.

Chen, R. z., Li, X. y., Liu, M., Yi, B. r., Sheng, C. f. & Ma, J. y. (2007). A preliminary study on generation of *Chilo suppressalis* (Walker, 1863) in Changchun City using sex pheromone technique. *Journal of Jilin Agricultural Sciences, 32*, 37-39.

Abbasipour, H. & Basij, M. (2016). *Identification and control of citrus pests*. Applied Higher Education Institution of Agricultural Jihad, pp. 89-94.

Ali, A. & Rizvi, P. Q. (2010). Age and stage specific life table of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) at varying temperatures. *World Journal of Agricultural Sciences*, *6*(3), 268-273.

Amiri-Beheshti, B. (2009). Toxicity evaluation of Tracer, Palizin, Sirinol, Runner and Tondexir with and without min-

eral oils on *Phyllocnistis citrella* Stainaton. *African Journal of Biotechnology*, *8*, 3382-3386.

Anonymous. (2015). *Environmentally friendly pesticides*. Available from: http://www.kimiasabzavar.com

Ansaripour, A., Aghasi, K. & Bedoreh, M. (2012). Density and sex ratio of seven spotted ladybird (*Coccinella septempunctata*) in three altitudes of Khorramabad district. *Life Science Journal*, *9*, 830-834.

Baniameri, V. (2008). Study of efficacy of different concentrations of insecticidal soap, in comparison oxydemeton-methyl to control *Aphis gossypii* in greenhouse cucumber. *IOBC/wprs Bulletin*, *32*, 13-16.

Barjadze, S., Kvavadze, E., Kvavadze, E. & Tsertsvadze, R. (2009). Food spectrum of *Coccinella septempunctata* L. (Coleoptera, Coccinellidae) in the urban habitats of Tbilisi (Georgia). *Revue Francaise de Entomologie (N.S.)*, *31*(1), 30-32.

Campbell, C. D., Walgenbach, J. F. & Kennedy, G.G. (1991). Effect of parasitoids on lepidopterous pests in insecticide-treated and untreated tomatoes in Western North Carolina. *Journal of Economic Entomology*, *84*(6), 1662-1667. https://doi.org/10.1093/jee/84.6.1662

Cavalloro, R. & Martino, E.D.I. (1985). Integrated Pest Control in Citrus Groves. *Proceedings of the Expert's Meeting*, Acireale, 26-29 March 1985, published for the European communities, 599 p.

Heydari, S., Toorani, A.H. & Abbasipour, H. (2016a). Comparison of Plant and Chemical Pesticide Effects on *Aphis nerii* Boyer de Fonscolombe (Hem.: Aphididae). *The 5th Congress of Agriculture and Sustainable Natural Resources, Tehran, Iran*, https:// www.civilica.com/Paper-NACONF05-NACONF05_084.html

Heydari, S., Toorani, A.H., Dostarkalkenari, L. & Abbasipour, H. (2016b). Comparison effects of botanical pesticides on first instar nymphs of the citrus cushion, *Pulvinaria aurantii* Cockerell and its ladybird predator, *Cryptolaemus montrouzieri* Mulsant. *22nd Iranian Plant Protection Congress, Tehran, Iran*.

Heydari, S., Toorani, A.H., Dostarkalkenari, L. & Abbasipour, H. (2016c). Comparison effect of botanical pesticides on first instar nymphs of the Australian mealybug, *Icerya purchasi* Maskell and its ladybird predator, *Novius cardinalis* Mulsant. *22nd Iranian Plant Protection Congress, Tehran, Iran*.

Honek, A. & Martinkova, Z. (2005). Long term changes in abundance of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) in the Czech Republic. *European Journal of Entomology*, *102*(3), 443- 448. https://doi.org/10.14411/eje.2005.063

Imani, A. & Toorani, A. H. (2016). Study of the Effect of Plant and Chemical Pesticides on Citrus *Aphis citrus*, *Aphis citricola* V. d. G (Hem.: Aphididae). *3rd International Conference on Sustainable Development, Solutions and Challenges Focusing on Agriculture, Natural Resources, Environment and Tourism, Tabriz, Iran*, https://www. civilica.com/Paper-ICSDA03-ICSDA03_002.html

Jacas, J.A., Karamaouna, F., Vercher, R. & Zappala`, L. (2010). Citrus pest management in the northern Mediterranean basin (Spain, Italy and Greece). In: Ciancio A, Mukerji KG (eds.) *Integrated Management of arthropods pests and insect borne diseases*. Springer, NL. Dordrecht, the Netherlands, pp 3-27. https://doi.org/10.1007/978-90-481-8606-8_1

Kabiri, M., Amiri-Beheshti, B. & Basirat, M.A. (2012). Comparison of toxicity of the botanical insecticide, Sirinol and two chemical insectides, Mospilan and Consult, in two natural enemies of the pistachio psyllid, coccinellid predator (*Oenopia conglobate*) and parasitic wasp (*Psyllaephaus pistaciae*). *African Journal of Biotechnology*, *11*, 13888-95. https://doi.org/10.5897/AJB11.4159

Kazem, M.G. T. & El-Shereifi, S.A.E.H.N. (2010). Toxic Effect of Capsicum and Garlic Xylene Extracts in Toxicity of Boiled Linseed Oil Formulation against some Piercing Sucking Cotton Pests. *American- Eurasian Journal of Agricultural & Environmental Science*, *8*, 390-396.

Lapchin, L., Gtrvot, H. & Brun, P. (1994). Spatial and temporal heterogeneity in population dynamics of citrus aphids at a regional scale. *Ecological Research*, *9*, 57-66. https://doi.org/10.1007/BF02347242

Matthews, G.A. (1999). Pesticides, IPM and training. *Phytoparasitica*, *27*, 253-256. https://doi.org/10.1007/BF02981480

Potter Precision Spray Tower (2000). Burkard Manufacturing Co. Ltd. http://pollenuk.worc.ac.uk/Burkard/ Default.html.

SPSS Institute.(2006). *SPSS Base 15.0 for Windows*, User's Guide Computer Program Version by SPSS Inc., Chicago, IL.

Sterk, G., Heuts, F., Merck, N. & Bock, J. (2003). Sensitivity of non-target arthropods and beneficial fungal species to chemical and biological plant production products results of laboratory and semi-field trials. p. 306–313. In: Proc. *1st International Symposium on Biological Control of Arthropods, Honolulu, Hawaii, USA*, 14–18 January 2002, 532 pp.

Suma, P., Zappala`, L., Mazzeo, G. & Siscaro, G. (2009). Lethal and sublethal effects of insecticides on natural enemies of citrus scale pests. *Biocontrol*, *54*, 651-661. https://doi.org/10.1007/s10526-009-9215-z

Tena, A. & García-Marí, F. (2011). Current situation of citrus pests and diseases in the Mediterranean Basin. *IOBC/WPRS Bulletin*, *60*, 349-361.

Toorani, A.H. & Abbasipour, H. (2016). Study of the Effect of Plant and Chemical Pesticides on Citrus Aphids, *Toxoptera aurantii* B.d.F (Hem.:Aphididae). *3rd Conference on New Findings in the Environment and Agricultural Ecosystems, Tehran, Iran*, https://www.civilica.com/Paper-AGRO-CONGRESS03- 459.html

Toorani, A. H., Abbasipour, H. & Doostdar Kalkenari, L. (2017). Toxicity of selected biorational insecticides to *Pulvinaria aurantii* Cockerell and its predator, *Cryptolaemus montrouzieri* Mulsant in citrus field, *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science*, *67*(8), 723-729. https://doi.org/10.1080/09064710.2017.1338745

Urbaneja, A., Catalán, J., Tena, A. & Jacas, J.A. (2012). *Gestiónintegrada de plagas de cítricos*. http://www. gipcitricos. ivia.es.

Zappala`, L. (2010). Citrus integrated pest management in Italy. In: Ciancio A, Mukerji KG (eds.) *Integrated management of arthropods pests and insect borne diseases*. Springer, Dordrecht, pp 3-27. https://doi.org/10.1007/978-90-481-8606-8_4

doi:10.14720/aas.2019.114.2.8 Original research article / izvirni znanstveni članek

Salicylic acid and potassium nitrate promote flowering through modulating the hormonal levels and protein pattern of date palm *Phoenix dactylifera* 'Sayer' offshoot

Hussein J. SHAREEF [1, 2]

Received April 21, 2019; accepted October 14, 2019.
Delo je prispelo 21. aprila 2019, sprejeto 14. oktobra 2019.

Salicylic acid and potassium nitrate promote flowering through modulating the hormonal levels and protein pattern of date palm *Phoenix dactylifera* 'Sayer' offshoot

Abstract: Salicylic acid enhances the flowering process in the plant by creating new proteins under salinity stress. The study was to determine the role of salicylic acid (500 ppm) and potassium nitrate (1500 ppm), on flowering of date palm 'Sayer' offshoots under salinity effect. Application of salicylic acid increased the number of clusters, the number of new leaves, the content of carbohydrates, ascorbic acid, indoleacetic acid, zeatin, gibberellin, and abscisic acid significantly under salinity compared with control. Although the measured parameters were the highest in plants treated with salicylic acid, there was no distinction among potassium nitrate treatment under saltwater, and salicylic acid treatment with saltwater. Salicylic acid and potassium nitrate treatment demonstrated some amazing contrasts in protein patterns in light of gel electrophoresis. Plants treated with salicylic acid with fresh water and with saltwater showed five and six protein bands, respectively, that differed in the molecular mass of one polypeptide compared to control with freshwater. However, there was a difference in the molecular mass of two polypeptides compared to control with salt water, which showed six bands. In contrast, potassium nitrate application showed five protein bands, whether with freshwater or with saltwater. The findings could facilitate to elucidate the flowering mechanisms in date palm.

Key words: abscisic acid; clusters number; zeatin; electrophoresis; gibberellin; indoleacetic acid

Salicilna kislina in kalijev nitrat pospešujeta cvetenje stranskih poganjkov dateljeve palme (*Phoenix dactylifera* 'Sayer') z moduliranjem ravni hormonov in vzorca proteinov

Izvleček: Salicilna kislina pospešuje cvetenje preko tvorbe novih proteinov v razmerah slanostnega stresa. Namen raziskave je bil določiti vlogo salicilne kisline (500 ppm) in kalijevega nitrata (1500 ppm) na cvetenje stranskih poganjkov dateljeve palme ('Sayer') v razmerah slanosti. Uporaba salicilne kisline je značilno povečala število stranskih poganjkov, število novih listov, vsebnost ogljikovih hidratov, askorbinske kisline, indolocetne kisline, zeatina, giberelina in abscizinske kisline v razmerah slanosti v primerjavi s kontrolo. Čeprav so imeli vsi merjeni parametri največje vrednosti pri obravnavanju s salicilno kislino, ni bilo razlike v obravnavanjih s kalijevim nitratom in salicilno kislino v istih razmerah. Obravnavanja s salicilno kislino in kalijevim nitratom so imela velike razlike v vzorcu proteinov, določenem z gelsko elektroforezo. Rastline, ki so bile tretirane s salicilno kislino, sladko in slano vodo so imele pet oziroma šest proteinskih trakov, ki so se razlikovali v molekulski masi enega izmed polipeptidov v primerjavi s kontrolo, kjer je bilo samo obravnavanje s sladko vodo. Kakorkoli, v primerjavi s kontrolo, kjer je bilo obravnavanje samo s slano vodo in je bilo šest proteinskih trakov, je bila razlika v molekulski masi dveh polipeptidov. Obravnavanje samo s kalijevim nitratom je pokazalo samo pet proteinov, ne glede na obravnavanje s slano ali sladko vodo. Ti izsledki bi lahko pomagali razjasniti mehanizem cvetenja pri dateljevi palmi.

Ključne besede: abscizinska kislina; število poganjkov; zeatin; elektroforeza; giberelin; indolocetna kislina

1 University of Basrah, Dept. of Date Palm Research Center, Basrah, Iraq
2 Corresponding author, e-mail: husseinshareef@live.com

1 INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is usually exposed to abiotic stresses in an arid and semi-arid region. Notably, high temperatures, the shortage of irrigation water, and soil salinity are the real constraining variables to productivity (Allbed et al., 2017). The production of fruits is restricted in the extreme conditions. In any case, their development and yield profitability are reduced (Moustafa et al., 2018), where the yield reduced to half at 18 dS m^{-1} and 100 % at 32 dS m^{-1} (Elsadig et al., 2017). The production of date palm fruits relies upon the number of clusters and the success of the pollination process. Flowering in date palm is delayed when presented to unforgiving natural conditions. Date palm flowering is a complex process in which numerous natural factors and the physiological material of the plant interact. However, the transformation of the axillary buds to floral buds in the date palm relies upon a high sugar level and a low nitrogen C/N ratio. For this situation, the role of the ecological conditions includes a substantial impact on the regulation of this quantitative relation, particularly the impact of salinity, whether the salinity of the soil or water system conditions (Shareef, 2016). We have observed earlier that foliar application of salicylic acid on offshoots induced flowering in date palm under salt stress, especially at the early stage (Shareef et al., 2017).

Salicylic acid (2-hydroxybenzoic acid) (SA), is thought to activate general plant resistance ordinarily related to activation of defense genes (Tamaoki et al., 2013). Moreover, SA has a specific role in plant growth, induction of flowering, and uptake of ions. Thus, it has an essential role in the regulation of flowering (Wada & Takeno, 2013). The formation of the buds as a result of the SA application is unusual as SA changes the synthesis and signaling pathways of plant hormones, like jasmonic acid, ethylene, and auxin (IAA) (Vlot et al., 2009). Auxin, abscisic acid, and zeatin gradually decrease during flowering in date palm leaves in early varieties and then rise after flowering while the gibberellin (GA3) increased (Cheruth et al., 2015). Gibberellins accordingly are hormones responsible for dynamics in the survival and persistence of plants (Lymperopoulos et al., 2018). The effects on the phase of plant development and blossoming at the suitable season increased plant production. The interaction of environmental signals with endogenous biological process signals the main mechanisms which control flowering time in plant and are stimulated by rising SA (Zhang et al., 2018). Khayyat et al. (2018) found that the stimulation of the flowering of the saffron plant was successful using 2 mM SA or 1000 ppm potassium.

Under the water shortage conditions, leaf stomata do not function actively in plants with potassium deficiency and, therefore, cause excessive water loss (Farooq et al., 2015). Potassium (K) plays a vitally role in the physiological processes of photosynthesis, in the formation of carbohydrates and proteins, the transfer of water and nutrients, in use of nitrogen (N), and the stimulation of early plant growth (Lakudzala, 2013). In plant tissues, the transport of water, nutrients, and carbohydrates is enhanced by potassium application (Safar-Noori et al., 2018). Potassium ions induce gene expression in light of environmental changing (Zhang et al., 2018a). Gene expression drives the biological process and stress properties. It is, therefore responsible for subsequent translation modifications of the pure nucleus proteins and sometimes methylation of deoxyribonucleic acid (Zhang et al., 2018b). The process of flowering is controlled by a group of genes made up of gene expression (Yan et al., 2019).

This study aimed to check whether the exogenous use of salicylic acid (SA) or potassium nitrate (KNO_3) will positively influence the growth and formation of flower buds in the late flowering date palm.

2 MATERIALS AND METHODS

2.1 EXPERIMENT FIELD

The experiment was equipped at the General Authority of Palm station, Burjysia, Basrah, Iraq (latitude 30 ° 22 ‘ 6.294 “N and longitude 47 ° 36 ‘ 39.639 “) at 26 km of Basrah center, in 2017 and 2018 growing season. In the experiment, 30 plants used, Sayer date palm cultivar was five years old, planted on 5 x 5 m in sandy loam. The drip irrigation system was used. The normal EC value for soil was 12 dS m^{-1}. The average temperature of the experiment months was in October 26.2 °C, November 20.4 °C, December 14.4 °C, January12.2 °C, February 14.1 °C, March 18.1 °C, and April 23.8 °C. Plants were treated with saltwater and freshwater on April 1st in season 2017, according to the block design during the six months before the treatments, and continued until the samples were taken on April 1st in the season of 2018 after the flowers were completed in this variety. The plants were treated with foliar treatment once, with one plant for each replicate on October 1st in the season of 2017 as follows: only spray water (0 dS m^{-1}) + irrigation with freshwater (EC water 1.5 dS m^{-1}) (control); spray only with water + irrigate with salt water (EC 8 dS m^{-1}) (control); foliar spray of 500 ppm salicylic acid (SA) + irrigation with fresh water; foliar spray of SA at

500 ppm + irrigated with saltwater; foliar spray with potassium nitrate (KNO_3) 1500 ppm + irrigated with freshwater; foliar spray with KNO_3 1500 ppm + irrigated with saltwater. On the 1st of April in the season of 2018, the data on plant parameters described in the following subchapters were recorded.

2.2 NUMBER OF CLUSTERS

The date palm starts to flower usually in February until April. The flowering is completed at the Hillawi cultivar, usually in April. The number of clusters was determined by calculating the number of clusters per plant to different treatments in April one time.

2.3 THE NUMBER OF NEW LEAVES

At the beginning of October, the number of leaves was assessed before the treatment time for each plant. The number of new leaves is completed in date palm in the spring. Therefore, at the beginning of April, the number of newly formed leaves was determined according to the following formula:

New leaves = Total of leaves on April – Total of leaves before treatment.

2.4 CARBOHYDRATE ANALYSIS

Soluble carbohydrates were determined after Yemm & Willis (1954). Samples of fresh pinnae were weighed (0.2 g) and homogenized using 70 % ethanol. Then they were filtered, and the use of benzene removed pigments. An aliquot of 0.2 ml of leaf extract was added to 1.0 ml of 5 % phenol + 5 ml H_2SO_4 95 % to react in a water bath for 10 min at 100 °C. Soon after, the test tube was cooled in an ice bath, and then the absorbance was read at 620 nm.

2.5 ASCORBIC ACID (ASA) CONTENT

AsA content was determined by employing a slightly changed methodology of Luwe et al. (1993) in which 0.5 g of green leaf pieces were ground in liquid nitrogen and afterward homogenized in 1 % cold trichloroacetic acid. The homogenate was then centrifuged at 12,000 x g for 20 minutes at 4 °C, and the supernatant (50 µl) blended with100 mM potassium phosphate buffer, and ascorbate estimated at 265 nm.

2.6 HORMONES ANALYSIS

Five g of a fresh leaf tissue sample, which was homogenized in 70 % methanol was stirred overnight at 4 °C. The extract was filtered through Whatman filter paper (No. 1) and evaporated under vacuum. The pH of the aqueous phase was adjusted to 8.5 using 0.1 M phosphate buffer. Later the aqueous phase was partitioned using methanol twice. The methanol phase was removed by a rotary evaporator. The aqueous phase pH was adjusted to 2.5, using 1 N hydrochloric acid (HCI). Phytohormones were determined by the injection of the concentrate into a reversed-phase HPLC, C18 column in an isocratic elution mode utilizing a portable stage comprising of acetone: water (26:74) with 30 mM phosphoric acid as per Tang et al. (2011). The pH was kept up at 4, utilizing 1 N sodium hydroxide. A temperature was kept upt at 25 °C. The flux rate was 0.8 ml min^{-1}, and the elution of the phytohormones was observed at 208, 265, 270, and 280 nm for indoleacetic acid, abscisic acid, gibberellins, and zeatin, respectively.

2.7 EXTRACTION OF PROTEINS AND GEL ELECTROPHORESIS

Proteins were extracted by homogenizing the 333 mg of solidified dried leaf to 1 ml of extraction cradle [0.2 M, tris-hydroxymethyl aminomethane (Tris) + 0.001 M ethylene diamine tetra acetic acid + (Na_2 + EDTA) + 12 % glycerol + 0.01 M dithio threitol (DTT) + 0.05 mM phenyl methyl sulfonyl fluoride (PMSF)] by utilizing the mortar and pestle. At that point the samples were centrifuged at 15,000 × g for 15 min. Splitting buffer consisted of 0.125 M Tris HCl (pH 6.8) + 4 % SDS + 20 %, glycerol + 10 % b-mercapto ethanol + 0.01 % bromo phenol blue. Protein samples were denaturized by bubbling in the water bath at 90 °C for 3 min. Protein electrophoresis was performed in an irregular SDS polyacrylamide gel, as indicated by a strategy depicted by Laemmli (1970).

2.8 STATISTICAL ANALYSIS

Randomized completely block design of six treatments of salicylic acid and potassium nitrate replicated five times were utilized. Experimental data on all factors were analyzed by ANOVA. SPSS variant 19.0 (SPSS, Chicago, IL), and Duncan test were used for different correlations treatments considered at the $p \leq 0.05$ levels.

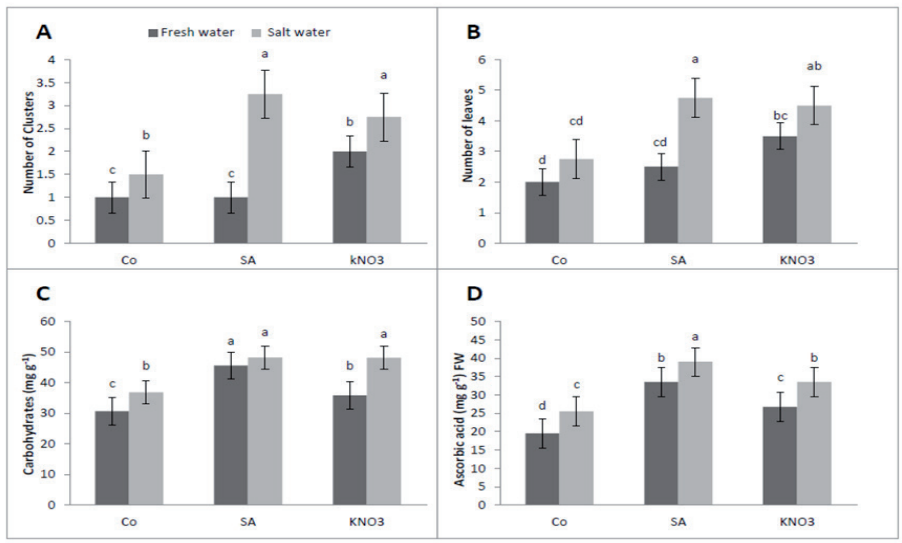


Figure 1: Salicylic acid and potassium nitrate effects on the number of clusters (A), number of new leaves (B), carbohydrates (C), and ascorbic acid (D)content in leaves of date palm offshoots after irrigated with salt water or with fresh water. The means of five replicates ± SE are presented. Bars with different letters are significantly different at $p \leq 0.05$ after a Duncan correction.

3 RESULTS

3.1 EXOGENOUS UTILIZATION OF SA AND KNO_3 PROMOTES FLOWERING, NUMBER OF NEW LEAVES, CARBOHYDRATES AND ASCORBIC ACID

The highest flowering rate was observed through SA treatment in salinity conditions followed by KNO_3 under the same conditions, whereas the lowest clusters number was found in control irrigated with fresh water, compared with saltwater (Fig. 1 A). The number of new leaves was fundamentally influenced by treatments; however, there was no difference between control, freshwater, and saltwater. Although the highest value of this variable was seen in plants treated with SA, there was no distinction among KNO_3 treatment under saltwater and SA treatment with salt water (Fig. 1 B). Application of both KNO_3 and SA treatments significantly increased carbohydrates in leaves under salt stress; there was no difference among treatments in this trait (Fig. 1 C). Ascorbic acid content under salt stress was higher than with freshwater in control; however, SA increased significantly ascorbic acid content under salt stress compared with other treatments (Fig. 1 C).

3.2 SA AND KNO_3 MODULATE IAA, CK, ABA, AND GA3 LEVELS

The application of treatments significantly increased IAA, CK, and GA3 levels, either under saltwater or freshwater, compared with controls (Fig. 2). Whereas the content of ABA increased under salt stress only, and no significant effect of treatment was observed under normal conditions. SA increased ABA and GA3 content significantly under salt stress (Fig. 2 C, D).

3.3 SA AND KNO_3 MODULATE THE PROTEIN PATTERN OF LEAVES

The protein pattern of date palm leaves was determined according to the molecular mass (KD) of protein bands affected by SA and KNO_3 under freshwater and saltwater conditions by SDS-PAGE gel electrophoresis (Fig. 3). The leaf proteins in both controls were separated into five different polypeptides through acrylamide gels (Fig. 3). The control treatment with freshwater has a molecular mass of polypeptides 236.000, 132.072, 69.333, 40.642, and 34.389 KD, whereas control with salt water, had different molecular mass to three polypeptides 177.500, 44.179, and 30.090 KD. The application of SA showed five and six-band of proteins with fresh water and with saltwater, respectively, that differed in the molecular mass of one polypeptide compared to control with fresh water 28.232 KD. Whereas, SA with saltwater changed the molecular mass of two polypeptides compared to control with salt water 17.090 and 13.980 KD and showed six bands (Fig. 3). However, potassium application showed five bands of protein, whether with freshwater or with saltwater. One of the polypeptides

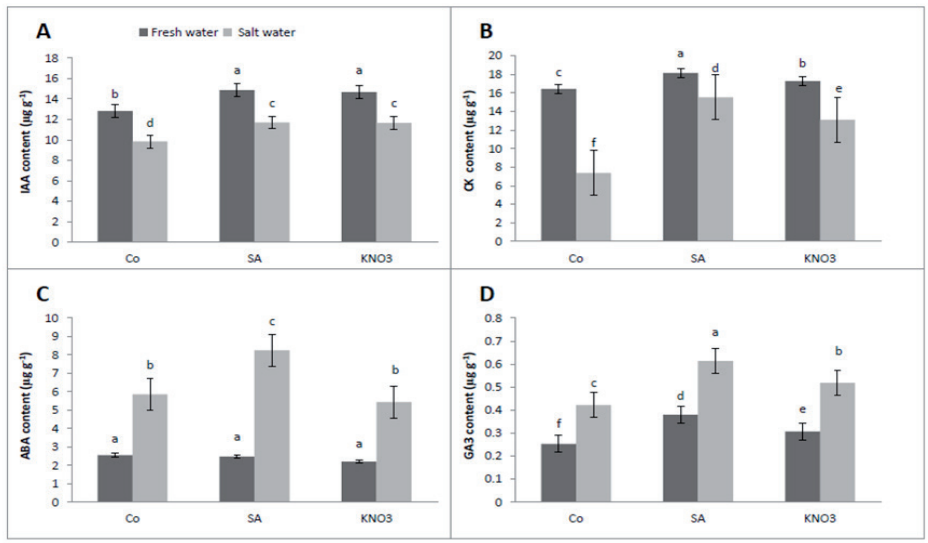


Figure 2: Salicylic acid and potassium nitrate effects on IAA (A), CK (B), ABA (C), and GA3 content (D) in leaves of date palm offshoots under irrigation with salt water or with fresh water. The means of five replicates ± SE are presented. Bars with different letters are significantly different at $p \leq 0.05$ after a Duncan correction.

differed in the molecular mass compared to the treatment of potassium with fresh water 33.188 KD. In contrast, two polypeptides differed in the molecular mass compared to potassium with saltwater treatment 33.600 and 14.646 KD (Fig. 3).

4 DISCUSSION

Date palm offshoots give a few numbers of floral buds that turn into a cluster at the beginning of their transformation from the vegetative to the flowering phase under extreme conditions. The salicylic acid-treated plant accelerated flowering initiation in plants under the salinity conditions (Wada & Takeno, 2013). An increase in the number of clusters observed after the application of SA (Fig. 1 A) was due to its effect on increase of carbohydrates to higher levels than the impact of salinity alone (Fig. 1 C). SA acts as an internal signal to regulate the physiological processes (Appu & Muthukrishnan, 2014).

Desoky and Merwad (2015) reported that spraying the leaves of wheat plants with SA increased the soluble carbohydrate content. Carbohydrate content in the plant increased the chances of the axillary buds transformation into a floral bud (Dierck, 2016). The number of increased leaves reflected the increase in the number of clusters (Fig. 1B, A). Inducing flowering, when connected to salicylic acid, might be the consequence of the positive impact on plant development. It can be indirect as SA adjusts the synthesis and signaling pathways of other plant hormones, including gibberellin, auxin, and abscisic acid (Fig. 2). SA and K^+ ion have a role in cell division and stimulate secondary metabolism to produce antioxidants such as ascorbic acid. However, SA with saltwater increased AsA content (Fig. D). AsA is a primary co-factor in the synthesis of ethylene, gibberellic acid, and abscisic acid. Therefore, the endogenous level of AsA can affect the signaling of synthesis of those molecules (Anwar et al., 2018). Also, AsA is considered as an organic acid that stimulates the process of flowering (Akram et al., 2017)ascorbic acid (AsA. The high AsA content in plants supports a particular balance in preventing pigment damage and membrane injury (Costa et al., 2018). The antioxidant activity of ascorbate peroxidase and superoxide dismutase, and synthesis of special protein groups were increased by SA and AsA, together with salt stress (Al-Mayahi, 2016).

SA increased ABA and GA3 significantly under salt stress (Fig. 2 C, D). Salicylic acid rose IAA, CK and ABA levels, enhanced cell division in the apical meristem, and improved flowering of the plant (Sytar et al., 2019). In contrast, Alonso-Ramírez et al. (2009) reported that there was a cross-talk between SA and GA3 in *Arabidopsis thaliana* during abiotic stress conditions. GA3 induced flowering in early varieties of date palm (Cheruth et al., 2015). ABA has molecular effects on the downstream states of the autonomous biological pathway and, as such, improves the plant's capacity to encounter the change to flowering (Duncan et al., 2018).

The proteins are the final product of genetic pathways inside the plant cells that are created in light of cell

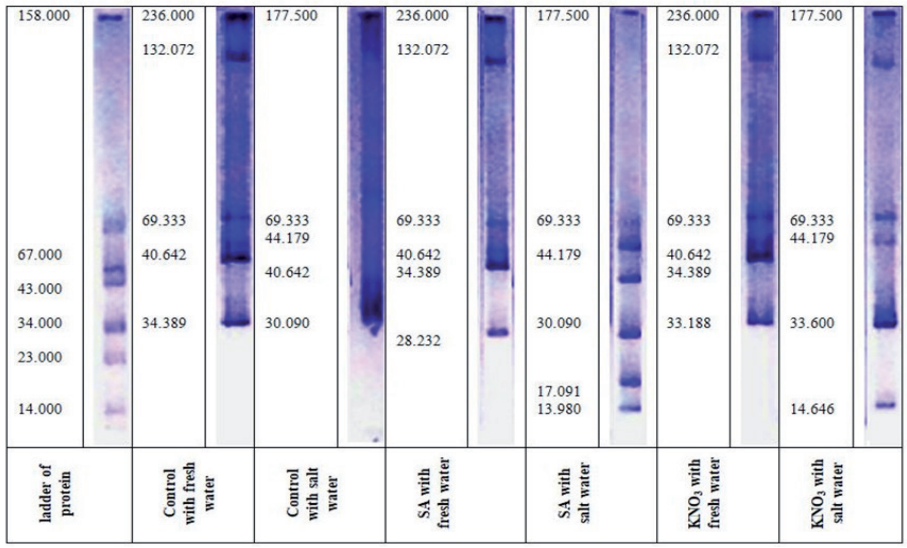


Figure 3: hematic diagrams of the electrophoretic protein pattern of date palm leaves with the molecular mass (KD) of protein bands affected by salicylic acid and potassium nitrate under freshwater and saltwater conditions.

needs and moved imbalances in some areas throughout entirely different stages of life and stress conditions (Razavizadeh, 2015)Brassica napus L.. Applications of SA and K^+ showed new protein bands with low molecular mass and the disappearance of other groups (Fig. 3). Salinity and SA induced *de novo* caused compilation of particular polypeptides and regulated the expression of salt-stress-tolerant proteins (Amirbakhtiar et al., 2019)which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. Salt stress is one of the major adverse environmental factors limiting crop productivity. Considering Iran as one of the bread wheat origins, we sequenced root transcriptome of an Iranian salt tolerant cultivar, Arg, under salt stress to extend our knowledge of the molecular basis of salinity tolerance in Triticum aestivum. RNA sequencing resulted in more than 113 million reads and about 104013 genes were obtained, among which 26171 novel transcripts were identified. A comparison of abundances showed that 5128 genes were differentially expressed due to salt stress. The differentially expressed genes (DEGs. In *Arabidopsis thaliana* and different other flowering plants, it was suggested that molecular mechanisms which include gibberellic acid regulate the LFY promoter (Blazquez, 1998). However, ABA-induced RNA-binding proteins SNF5 and FCA control flowering time and stress responses (Fahraji et al., 2014) kinetin and salicylic acid may increase yield of different crops due to reduction in stress induced inhibition of plant growth. Salicylic acid (SA. In this respect, SA and salinity conditions together induced high levels of GA3 and ABA which caused synthesis of new isozymes of low molecular mass to form the new floral buds. These findings could facilitate understanding the mechanisms of flowering and salt-tolerance of the date palm.

5 CONCLUSION

Exogenous application of SA and KNO_3 enhanced the biochemical mechanisms of flowering in the date palm through conferring adaptation to salinity stress and creating new isozyme. IAA, ABA, CK, and GA3 successfully increased the number of the cluster. Furthermore, these hormones in the plant under salinity conditions contribute to the process of flowering by the formation of specific proteins through the epigenetic pathway to promote the transformation of the offshoots into an adult plant. This study is the first, which highlighted the stimulative effect of SA and KNO_3 on the flowering of the date palm.

6 REFERENCES

Akram, N.A., Shafiq, F. & Ashraf, M. (2017). Ascorbic Acid-A Potential Oxidant Scavenger and Its Role in Plant Development and Abiotic Stress Tolerance. *Frontiers in Plant Science, 8*, 1-17. https://doi.org/10.3389/fpls.2017.00613

Al-Mayahi, Ahmed M. W. (2016). Influence of salicylic acid (SA) and ascorbic acid (ASA) on in vitro propagation and salt tolerance of date palm (*Phoenix dactylifera* 'Nersy'). *Australian Journal of Crop Science*, *10*(7), 969–976. https://doi.org/10.21475/ajcs.2016.10.07.p7640

Allbed, A., Kumar, L., & Shabani, F. (2017). Climate change impacts on date palm cultivation in Saudi Arabia. *Journal of Agricultural Science*, *155*(8), 1203–1218. https://doi.org/10.1017/S0021859617000260

Alonso-Ramírez, A., Rodríguez, D., Reyes, D., Jiménez, J. A., Nicolás, G., López-Climent, M., & Nicolás, C. (2009). Cross-talk between gibberellins and salicylic acid in early stress responses in Arabidopsis thaliana seeds. *Plant Signaling and Behavior*, *4*(8), 750–751. https://doi.org/10.4161/psb.4.8.9175

Amirbakhtiar, N., Ismaili, A., Ghaffari, M.R., Firouzabadi, F.N. & Shobbar, Z.S. (2019). Transcriptome response of roots to salt stress in a salinity-tolerant bread wheat cultivar. *Plant Physiology, 177*, 475-489. https://doi.org/10.1371/journal.pone.0213305

Anwar, A., Liu, Y., Dong, R., Bai, L., Yu, X. & Li, Y. (2018). The physiological and molecular mechanism of brassinosteroid in response to stress: A review. *Biological Research, 51*, 1–15. https://doi.org/10.1186/s40659-018-0195-2

Appu, M., & Muthukrishnan, S. (2014). Foliar Application of Salicylic Acid Stimulates Flowering and Induce Defense Related Proteins in Finger Millet Plants. *Universal Journal of Plant Science*, *2*(1), 14–18. https://doi.org/10.13189/ujps.2014.020102

Blazquez, M. A. (1998). Gibberellins Promote Flowering of Arabidopsis by Activating the LEAFY Promoter. *The Plant Cell Online*, *10*(5), 791–800. https://doi.org/10.1105/tpc.10.5.791

Cheruth, A. J., Kurup, S. S., & Subramaniam, S. (2015). Variations in Hormones and Antioxidant Status in Relation to Flowering in Early, Mid, and Late Varieties of Date Palm (*Phoenix dactylifera*) of United Arab Emirates. *The Scientific World Journal*, *1*, 1–8. https://doi.org/10.1155/2015/846104

Costa, S.F., Martins, D., Agacka-mo, M., Czubacka, A. & Araújo, S.D.S. (2018). Strategies to Alleviate Salinity Stress in Plants. In V. Kumar et al. (eds.) *Salinity Responses and Tolerance in Plants*. pp. 307–337. https://doi.org/10.1007/978-3-319-75671-4

Desoky, E.S.M. & Merwad, A.R.M. (2015). Improving the Salinity Tolerance in Wheat Plants Using Salicylic and Ascorbic Acids. *Journal of Agricultural Science, 7*, 203–217. https://doi.org/10.5539/jas.v7n10p203

Dierck, R. (2016). *Shoot branching: analysis of axillary bud outgrowth in Chrysanthemum morifolium*. Ph.D. Thesis, Ghent University, Gent, Belgium pp,165.

Duncan, D.R., Phillips, G.C., Thorpe, T.A. & Dai, W. (2018). *Cellular & Developmental Biology*. Springer, pp. 135.

Elsadig, E.H., Aljuburi, H.J., Elamin, A.H.B. & Gafar, M.O. (2017). Impact of organic manure and combination of N P K S, on yield, fruit quality and fruit mineral content of Khenazi date palm (*Phoenix dactylifera* L.) cultivar. *Journal of Scientific Agriculture, 1*, 335. https://doi.org/10.25081/jsa.2017.v1.848

Fahraji, S.S., Kheradmand, M.A. & Mahdi, M. (2014). Effect of Salicylic acid on germination, leaf area, Shoot and root growth in crop plants. *International Research Journal of Applied and Basic Sciences, 8*, 1454–1458. https://doi.org/10.1007/978-3-319-14714-7_10

Farooq, M., Hussain, M., Wakeel, A. & Siddique, K.H.M. (2015). Salt stress in maize: effects, resistance mechanisms, and management. A review. *Agronomy for Sustainable Development, 35*, 461–481. https://doi.org/10.1007/s13593-015-0287-0

Khayyat, M., Jabbari, M., Fallahi, H.R. & Samadzadeh, A. (2018). Effects of corm dipping in salicylic acid or potassium nitrate on growth, flowering, and quality of saffron. *Journal of Horticultural Research, 26*, 13–21. https://doi.org/10.2478/johr-2018-0002

Laemmli UK. (1970). Cleavage of structural proteins during the assembly of the head bacteriophage T4. *Nature*, *227*, 680–685. https://doi.org/10.1038/227680a0

Lakudzala, D. D. (2013). Potassium Response in some Malawi Soils. *International Letters of Chemistry, Physics and Astronomy*, *13*(2), 175–181. https://doi.org/10.18052/www.scipress.com/ILCPA.13.175

Luwe, M.W.F., Takahama, U. & Heber, U. (1993). Role of ascorbate in detoxifying ozone in the apoplast of spinach (*Spinacia oleracea* L.) leaves. *Plant Physiology*, *101*, 969–976. https://doi.org/10.1104/pp.101.3.969

Lymperopoulos, P., Msanne, J. & Rabara, R. (2018). Phytochrome and Phytohormones: Working in Tandem for Plant Growth and Development. *Frontiers in Plant Science, 9*, 1–14. https://doi.org/10.3389/fpls.2018.01037

Moustafa A.R.A., Abdel-Hamid N.A., Abd El-Hamid A.A., El-Sonbaty, S.M.R. & Abd El-Naby, S.K.M. (2018). Improving fruit set, yield and fruit quality of khadrawi date palm cultivar. *Arab Universities Journal of Agricultural Sciences, 26*, 1461–1469. https://doi.org/10.21608/ajs.2018.34126

Razavizadeh, R. (2015). Protein pattern of canola (*Brassica napus* L.) changes in response to salt and salicylic acid in vitro. *Biological Letters*, *52*(1–2), 19–36. https://doi.org/10.1515/biolet-2015-0012

Safar-Noori, M., Assaha, D. V. M. & Saneoka, H. (2018). Effect of salicylic acid and potassium application on yield and grain nutritional quality of wheat under drought stress condition. *Cereal Research Communications*, *46*(3), 558568. https://doi.org/10.1556/0806.46.2018.026

Shareef H. J., Jasim, A. M. & Abass, M. F. (2017). Molecular Analysis of Anti-salinity Compounds on Date Palm offshoots (*Phoenix dactylifera* L.) cultivars using RAPD. *Journal of Environmental Science*, *6*(February), 061–071.

Shareef, H. J. (2016). Enhancing Fruit Set and Productivity in Date Palm (*Phoenix Dactylifera* L.) Berhi Cultivar Using Boron and Potassium. *Journal of Environmental Science, 5*, 108–114.

Sytar, O., Kumari, P., Yadav, S., Brestic, M. & Rastogi, A. (2019). Phytohormone Priming: Regulator for Heavy Metal Stress in Plants. *Journal of Plant Growth Regulation, 38*, 739–752. https://doi.org/10.1007/s00344-018-9886-8

Tamaoki, D., Seo, S., Yamada, S., Kano, A., Miyamoto, A., Shishido, H., Miyoshi, S., Taniguchi, S., Akimitsu, K. & Gomi, K. (2013). Jasmonic acid and salicylic acid activate a common defense system in rice. *Plant Signaling & Behavior, 8*, 8–10. https://doi.org/10.4161/psb.24260

Waadt, R., Hsu, P.K. & Schroeder, J.I. (2015). Abscisic acid and other plant hormones: Methods to visualize distribu-

tion and signaling. *BioEssays, 37*, 1338–1349. https://doi.org/10.1002/bies.201500115

Wada K. C. & Takeno K. (2013). Salicylic Acid-Mediated Stress-Induced Flowering. In Hayat, S., Ahmad, A., & Alyemeni, M.N. (eds.), *Salicylic Acid* (pp. 163-182) Springer Science+Business Media Dordrecht. https://doi.org/10.1007/978-94-007-6428-6_9

Yan, F., Zhou, H., Yue, M., Yang, G., Li, H., Zhang, S. & Zhao, P. (2019). Genome-wide identification and transcriptional expression profiles of the f-box gene family in common walnut (*Juglans regia* L.). *Forests, 10*, 1–20. https://doi.org/10.3390/f10030275

Yemm, E.W., & Willis, A.J. (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal*, *57*, 508–514. https://doi.org/10.1042/bj0570508

Zhang, Y., Lv, Y., Jahan, N., Chen, G., Ren, D. & Guo, L. (2018a). Sensing of abiotic stress and ionic stress responses in plants. *International Journal of Molecular Sciences, 19*, 1–16. https://doi.org/10.3390/ijms19113298

Zhang, Z., Zhuo, X., Zhao, K., Zheng, T., Han, Y., Yuan, C. & Zhang, Q. (2018b). Transcriptome Profiles Reveal the Crucial Roles of Hormone and Sugar in the Bud Dormancy of Prunus mume. *Scientific Reports, 8*, 1–15. https://doi.org/10.1038/s41598-018-23108-9

doi:10.14720/aas.2019.114.2.9 Original research article / izvirni znanstveni članek

Changes of antioxidant enzymes in 'Thomson-Navel' orange during induction of resistance to green mold (*Penicillium digitatum* (Pers.) Sacc.) as provoked by jasmonic acid, epibrassinolide, chitosan and cinnamon essential oil

Behrooz ALIJOO [1], Vahid ABDOSSI [1, 2], Vahid ZARRINNIA [3], Sepideh Kalateh JARI [1], Mohammad CHAMANI [4]

Received May 16, 2019; accepted September 30, 2019.
Delo je prispelo 16. maja 2019, sprejeto 30. septembra 2019.

Changes of antioxidant enzymes in 'Thomson-Navel' orange during induction of resistance to green mold (*Penicillium digitatum* (Pers.) Sacc.) as provoked by jasmonic acid, epibrassinolide, chitosan and cinnamon essential oil

Abstract: Pathogenic agents are one of the causes of post-harvest citrus fruit loss. Therefore, the aim of this study was to evaluate the effect of post-harvest treatments with jasmonic acid (ja), epibrassinolide (epiBL), chitosan (chi) and cinnamon essential oil (cin) on induction of resistance to the citrus green mold (*Penicillium digitatum* (Pers.) Sacc.) and reduction of fungal growth by improving the activity of some antioxidant enzymes in 'Thomson-Navel'orange. For this purpose, a factorial experiment was conducted in a completely randomized design. Treatments included positive and negative control, jasmonic acid (5, 10, 20 and 40 µl l^{-1}), epibrassinolide (1, 4, 7 and 10 µmol l^{-1}), chitosan (2.5, 5, 7.5 and 10 g l^{-1}) and cinnamon essential oil (250, 500, 750 and 1000 ppm). Characteristics such as lesion diameter and activities of antioxidant enzymes including SOD, APX, CAT and POD were evaluated for a period of 96 hours with 24 hour intervals. The results indicated that all treatments significantly decreased the lesion diameter of fruits. Consequently, chitosan treatments (7.5 and 10 g l^{-1}) and cinnamon essential oil (750 and 1000 ppm) inhibited the spread of fungal infection better than other treatments, and therefore reduced the growth of green mold. Also, different concentrations of (ja) and (chi) increased the activity of SOD and APX enzymes, while different concentrations of (epiBR) and (cin) stimulated the activity of POD and CAT enzymes. Finally, the present study proposes using natural products as an appropriate alternative to fungicides in order to reduce the citrus green mold rot.

Key words: peroxidase; catalase; superoxide dismutase; ascorbate peroxidase; citrus green mold; inoculation; lesion diameter

Spremembe v antioksidacijskih encimih pri pomaranči 'Thomson-Navel' med indukcijo odpornosti na zeleno plesen (*Penicillium digitatum* (Pers.) Sacc.), vzpodbujene z jasmonsko kislino, epibrasinolidom, hitozanom in cimetovim eteričnim oljem

Izvleček: Patogeni so agensi, ki povzročajo izgubo pridelka citrusov po obiranju. Namen raziskave je bil ovrednoti učinek tretiranja plodov citrusov po obiranju z jasmonsko kislino (ja), epibrasinolidom (epiBL), hitozanom (chi) in cimetovim eteričnim oljem (cin) na indukcijo odpornosti proti zeleni plesni citrusov (*Penicillium digitatum* (Pers.) Sacc.) in zmanjšanje rasti glive z izboljšanjem aktivnosti nekaterih antioksidacijskih encimov v 'Thomson-Navel' pomarančah. V ta namen je bil izveden popolni naključni faktorski poskus. Obravnavanja so obsegala kontrolo in tretiranje pomaranč z jasmonsko kislino (5, 10, 20 in 40 µl l^{-1}), epibrasinolidom (1, 4, 7 and 10 µmol l^{-1}), hitozanom (2,5; 5; 7,5 and 10 g l^{-1}) in cimetnim eteričnim oljem (250, 500, 750 in 1000 ppm). Znaki kot so premer lezije s plesnijo in aktivnost antioksidacijskih encimov kot so SOD, APX, CAT in POD so bili ovrednoteni po obdobju 96 ur s 24 urnimi intervali. Rezultati so pokazali, da so vsa obravnavanja značilno zmanjšala premer lezij s plesnijo na plodovih. Obravnavanja s hitozanom (7,5 in 10 g l^{-1}) in eteričnim oljem cimeta (750 in 1000 ppm) so bolj zavrla širjenje glive kot ostala in so tako zmanjšala rast zelene plesni. Različne koncentracije jasmonske kisline in hitozana so povečale aktivnosti SOD in APX encimov, medtem, ko so različne koncentracije epibrasinolida in cimetovega eteričnega olja stimulirale aktivnost POD in CAT enzimov. Na osnovi te raziskave lahko priporočamo uporabo naravnih snovi kot primerno alternativo fungicidom pri zmanjševanju gnitja plodov citrusov zaradi zelene plesni.

Ključne besede: peroksidaza; katalaza; superoksid dismutaza; askorbat peroksidaza; zelena plesen citrusov; inokulacija; premer lezij

1 Islamic Azad University, Department of Horticultural Sciences, Science and Research Branch, Tehran, Iran
2 Corresponding author, e-mail: abdossi@srbiau.ac.ir
3 Islamic Azad University, Department of Plant Protection, Science and Research Branch, Tehran, Iran
4 Islamic Azad University, Department of Animal Sciences, Science and Research Branch, Tehran, Iran

1 INTRODUCTION

Citrus is considered as an important fruit in all over the world, and is cultivating in all parts of the world and have a 4,000 years history in China's agricultural system (Ladanyia and Ladaniya, 2010). Iran has the eighth rank in global citrus production with a production of 1,946169 tons in 2016 (FAO, 2019). Post-harvest diseases cause significant damage to harvested fruits and vegetables during transporting and storing (Sharma et al., 2009).There are two major factors that make plant products more susceptible to damage: The more water content in the fruit provides the pathogen attack conditions, and after that, wound is generated at the plant's organs during harvest and transportation (Sharma et al., 2009). Following skin damage, many species of pathogenic fungi attack it. More than 20 species of citrus diseases have been reported up to date, during the post-harvest period. Among them, the green and blue molds rot that are produced by *P. digitatum* (Pers.) Sacc. and *P. italicum* Wehmer fungi, respectively, are the worst due to they can easily result in fruit decay and deterioration. Generally, the rate of fruit rot remains approximately between 10 % and 30 %, however it increases up to 50 % in worse conditions and causes widespread economic losses, especially in developing countries (Ladanyia and Ladaniya, 2010). Nowadays, post-harvest citrus diseases are mainly controlled by chemical fungicides such as imazalil, benzimidazole, thiabendazole, prochloraz and pyrmethanil, which long-term and persistent using of them causes resistance to pathogens along with environmental pollution. Since fungicides remain on the surface of citrus fruits and have been reported to be harmful for human health, using alternative methods are expanding as a global trend that are more profitable and efficient, less toxic to the environment and human and also are inexpensive (Jimenez-Reyes et al., 2018). Over the past thirty years, extensive studies have been performed to develop new methods based on the microbial antagonists for biological control of postharvest pathogens (Droby et al., 2009).

Enzymes are key factors in the management of fresh products quality. Each enzyme plays an important role in the plant's physiological process. Previous reports demonstrated that the major enzymes in the plant (Polyphenol Oxidase, Phenylalanine Ammonia Lyase, Catalase and Superoxide Dismutase) are regulating the defense mechanism against fungal pathogens in fruits (Wang et al., 2009).

Methyl jasmonate is considered as an important plant hormone that can modulate plant defense responses, including antioxidant systems (Wasternack and Hause, 2013). This material has an important influence on the content of secondary metabolites in different types of fruit and is also important for the development of natural defense systems against abiotic stresses and post-harvest decay (González et al., 2003). During a study, the preventive action of methyl jasmonate (MeJA) was evaluated alone and in combination with the antagonistic yeast (*Cryptococcus laurentii* (Kuff.) C.E. Skinner) in suppressing the green mold in citrus fruits.The results showed that at the concentration level 100 mol $l^{-1}$of methyl jasmonate, the incidence of disease and decay diameters decreased compared to the control. Also, the use of methyl jasmonate increased the activity of polyphenol oxidase, peroxidase and catalase compared to the control (Guo et al., 2014).

Brassinoestroides are considered as the sixth group of plants hormones (Luan et al., 2013).It is also proposed that brassinoestroides are natural, non-toxic, and environmental friendly phytohormones, and consequently can be used in agriculture to improve growth, yield and post-harvest quality (Coll et al., 2015). In a study, the effects of brassinosteroids (BRs) on the blue mold caused by *Penicillium expansum* Link and jujube fruit senescence were investigated. Brassinosteroids at 5 µm concentration, effectively inhibited the growth of blue mold and increased the activity of defense-related enzymes such as phenylalanine ammonia lyase, polyphenol oxidase, catalase and superoxide dismutase (Zhu et al., 2010).

Chitosan is a natural compound that has good potential in agriculture for controlling plant diseases. This molecule shows toxicity, and inhibits the growth and development of fungi (Zhang et al., 2011). In addition, chitosan is a polysaccharide and produced by chitin deacetylation and can also be used to form a fully edible semipermeable layer on the outer surface of the fruit to extend its post-harvest life and reduce different types of fungus-induced decay during storage process (Bautista-Banos et al., 2006). During a research, the effects of chitosan on green mold and quality characteristics of citrus fruit were investigated. The results of an *in vivo* study showed that the green mold was significantly reduced by the chitosan treatment. In the same way, the activity of chitinase and glucanase enzymes in coated fruits increased. Evidence has shown that the effect of chitosan coating on green mold of citrus fruits may be attributed to its antifungal properties to the pathogen or to the development of biochemical defense responses in coated fruits (El Guilli et al., 2016).

Herbal essences indicated antimicrobial activity against a variety of pests and plant diseases. Several studies have investigated the potential of herbal essences as antifungal agents, including cinnamon essence (Abdolahi et al., 2010). Cinnamon (*Cinnamomum verum* J. Presl) is from Lauraceae family, *Cinnamomum* genus and *Cinnamomum verum* (*C. zeylandicum* Blume). It has been

determined that the essential oil of cinnamon contains 50-80 % cinnamaldehyde and about 2 % eugenol and the essential oil of its leaves is rich in eugenol (70-75 %)(Shan et al., 2007). Antifungal activity of cinnamon, pepper and garlic extracts combined with chitosan as a covering on fungal rot of banana cluster (*Colletotrichum musae* (Berk. & M. A. Curtis) Ar., *Fusarium* spp and *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.*)* were evaluated under laboratory conditions. The results showed that cinnamon essential oil with a concentration of 5 mg l^{-1}, completely prevented germination of conidia and mycelium growth in all fungi (Win et al., 2007).

The purpose of this study was to evaluate the effects of treatments to control post-harvest disease of citrus green mold on orange fruit during storage process and also to protect the environment from the harmful effects of fungicides. In this study, we investigate the antifungal activity of jasmonic acid, epibrassinolide, chitosan and cinnamon essential oil against citrus green mold.

2 MATERIALS AND METHODS

2.1 PREPARATION OF PLANT MATERIALS

Thomson-Navel oranges (*C. sinensis* 'Thomson Navel') were harvested from a commercial orchard located in Tonekabon, Iran. In a physiological maturation state and after full dyeing in early 2016, were immediately transferred to the Plant Pathology Laboratory of Islamic Azad University, Science and Research branch in Tehran. Then, uniform fruits in terms of size and color and without physical damage were selected for treatment application.

2.2 PREPARING OF PATHOGEN INOCULUM

In order to prepare the pathogenic inoculum, the *Penicillium digitatum* (Per.) Sacc. fungus was isolated from the infected 'Thomson-Navel' oranges and cultured on potato dextrose agar medium (Yao and Tian, 2005).After 2 weeks, spores were removed from the culture medium to prepare spore suspension using sterile distilled water (containing 0.5 ml Tween 80). Then, spore suspension of the green mold pathogen was prepared using the Hemocytometer with a concentration of 1×10^5 spore ml^{-1} (Qin et al., 2003).

2.3 APPLYING TREATMENTS AND INOCULATING FRUITS WITH PATHOGEN

For surface sterilization, the fruits were immersed in sodium hypochlorite 0.1 % for 30 seconds and then washed twice with the sterilized distilled water. After drying, fruits were dipped for 3 minutes in pre-prepared solutions of jasmonic acid (5, 10, 20 and 40 µl l^{-1}), epibrassinolide (1, 4,7 and 10 µmol l^{-1}), chitosan (2. 5, 5, 7.5 and 10 g l^{-1}) and cinnamon essential oil (250, 500, 750 and 1000 ppm). Control treatments included infected control(healthy fruits inoculated with the pathogen spore and dipped in sterile distilled water containing 0.05 % tween 80) and healthy control (healthy fruits dipped in sterile distilled water containing 0.05 % tween 80) (Reddy et al., 1997). After 24 hours of fruit treatment, they were then wounded with a sterilized nail at 2 points (4 mm deep and 3 mm in diameter) in the equatorial zone and inoculated with 20 µl spore suspension of *P. digitatum*. After that, the inoculated fruits were placed in special plastic bags to maintain the relative humidity of about 95 % and stored at 20 °C for 96 hours. All traits were evaluated over a period of 96 hours (every 24 hours) (Peng et al., 2009). Each treatment was replicated three times, and the experiment was conducted twice.

Chemicals: all compounds for the treatments were purchased from Aldrich Chemicals (Milwaukee) and to extract the essential oil of cinnamon, the dried cinnamon pieces were crushed by milling.

2.4 EVALUATION OF LESION DIAMETER AND ANTIOXIDANT ENZYMES ACTIVITY MEASUREMENTS

In order to evaluate the lesion diameter of the fruit, from each fruit at specific times (immediately, and 24, 48, 72 and 96 hours after inoculation) two diameters of infection were obtained, and their average was considered as the lesion diameter for that fruit at that specific time. Also, a portion of the healthy mesocarp tissue was removed for enzymatic studies at immediately, 48, 72 and 96 hours after inoculation (enzymatic evaluation was not performed at 24 hours after inoculation due to no infection). Samples were then mixed and frozen immediately in liquid nitrogen, then stored at -80 °C (Peng et al., 2009).

2.4.1 Extraction of enzyme

The enzyme extract was prepared by homogenizing 1 g of tissue sample with 3 ml sodium phosphate buffer, at (pH 6.7). The homogenate was centrifuged at 18,000 × g for 15 min at 4 °C. After complete sedimen-

tation, the supernatant served as the enzyme source (Mac-Adam et al., 1992).

2.4.2 Superoxide dismutase activity (SOD)

To measure the superoxide dismutase enzyme, 1500 µl phosphate buffer 50 mmol, 300 µl sodium carbonate 50 mmol, 300 µl methionine 12 mmol, 300 µl nitro blue tetrazolium chloride 75 µmol, 300 µl riboflavin 1 µmol and 300 µl enzyme extract were used. After stirring the mixture, the glass test tubes were placed at a distance of 35 cm from each other under a 15-watt fluorescent lamp for 10 minutes. The reaction was stopped by turning off the lamp and the absorption of the reaction mixture was read at 560 nm with a spectrophotometer. Also, a test tube containing the reaction mixture with the exception of the enzyme extract was used as control. A unit of the superoxide dismutase activity was considered as an enzyme level, resulting in a 50 % reduction of nitro blue tetrazolium light recovery (Giannopolitis, 1977).The SOD activity was expressed as U g^{-1} protein.

2.4.3 Catalase activity (CAT)

The reaction complex consists of 1.5 ml potassium phosphate buffer at concentration of 100 mmol (pH = 7), 0.5 ml of hydrogen peroxide 7.5 mmol and 50 µl of enzyme solution. The volume of samples was adjusted to 3 ml by adding distilled water. The reaction begins with the addition of hydrogen peroxide and a decrease was submitted in absorbance of the samples at 240 nm in one minute. The change in absorbance obtained in one minute, was subjected to a molar offset of this reaction, which is 36.6 mM cm^{-1}, and the enzyme activity was determined by the calculation of the amount of hydrogen peroxide degraded by the enzyme (Aebi, 1984). The CAT activity was expressed as U g^{-1} protein.

2.4.4 Peroxidase activity (POD)

For this purpose, 3 ml of sodium phosphate buffer at concentration of 0.1 molar and 50 µl of pure guaiacol liquid ($C_7H_8O_2$) and then 50 µl of hydrogen peroxide (H_2O_2) 3 % were added to the enzyme extract and immediately the changes in the light absorption at 436 nm were recorded using a spectrophotometer at intervals of 15 seconds for 3 minutes. After adding oxygenated water and guaiacol, the solution was reddish-brown. To calculate the activity of the peroxidase enzyme, the last absorption number was reduced from the first absorbed number and divided into 3 (Mac-Adam et al., 1992). The POD activity was expressed as U g^{-1} protein.

2.4.5 Ascorbate peroxidase (APX)

The amount of ascorbate peroxidase enzyme activity was measured by Ranieri et al. (2003) method. As a result of the reaction between ascorbate peroxidase and ascorbic acid and H_2O_2, dehydroascorbate is produced and recorded at a wavelength of 290 nm. The reaction medium contained 600 µl of 0.1 mmol EDTA, 1500 µl phosphate buffer 50 mmol (pH = 7), 400 µl ascorbic acid 0.5 mmol, 400 µl H_2O_2 30 % and 100 µl of enzyme extract. Enzyme activity measurements were recorded within 4 minutes. Over time, the amount of absorption increased and the APX activity was expressed as U g^{-1} protein.

2.5 STATISTICAL ANALYSIS

This experiment was a factorial based on completely randomized design with 18 treatments and each treatment including 3 replications. Statistical analysis of the data and comparison of the meanings were performed using SAS 9.1 and MSTAT-C and comparing the meanings was performed with Duncan's multiple range test.

3 RESULTS AND DISCUSSIONS

According to Table 1, the main effect of time (storage period) and the main effect of treatments on the lesion diameter and activity of all antioxidant enzymes of fruit was significant at 1 % level. Also, the interaction effects of treatment in time on fruit lesion diameter, as well as the activity of catalase and peroxidase enzymes were significant at a probability level of 1 %, but did not have any significant effect on other enzymes.

3.1 LESION DIAMETER

The lesion diameter on the surface of 'Thomson-Navel' orange was initially zero. However, it gradually increased over time, at 48 hours after inoculation, and every 24 hours, there was a significant difference with other courses, and on the fourth day (96 hours after inoculation) it reached its highest level (Table 2). Regarding the process of controlling the fungal rot of the fruit under the influence of treatments in 96 hours after inoculation, all treatments except the treatment of 10 µl l^{-1} jasmonic acid prevented the growth of green mold on the surface of the

Table 1: Analysis of variance of different treatments on the amount of lesion diameter and activity of CAT, POD, SOD and APX enzymes in orange fruit of Thomson-Navel variety within 96 hours after inoculation.

		Sum of squares				
Sources of changes	Degrees of freedom	Amount of lesion diameter	CAT	SOD	POD	APX
Time (storage period)	3	109/38**	21668325**	4/13**	3984/2**	33385/5**
treatment	17	0/78**	399316**	0/352**	58/46**	892/17**
Treatment × Time	51	0/08**	85421**	0/142ns	24/66**	40/97ns
Test error	144	0/015	30038	0/129	9/6	37/65
Coefficient of variation		4/69	3/77	15/43	7/54	8/48

** show significant effect at 1 statistically level; ns: statistically non-significant

Table 2: Interaction effects of the treatment in time on the mean lesion diameter (cm) on the surface of 'Thomson-Navel' orange fruit during 96 hours after inoculation

	Time (Hours)									
Treatments	0		24		48		72		96	
Control (+)	0	t	0	t	0	t	0	t	0	T
Control (-)	0	t	0	t	1.4	o	3.22	f	4.75	A
JA 5 µl l^{-1}	0	t	0	t	1.05	pqr	2.65	ijk	3.89	De
JA 10 µl l^{-1}	0	t	0	t	1.13	opq	2.54	jkl	4.47	Ab
JA 20 µl l^{-1}	0	t	0	t	1.06	pqr	2.56	jkl	3.97	Cde
JA 40 µl l^{-1}	0	t	0	t	1.11	opq	2.48	klm	4.03	Cd
EP 1 µmol l^{-1}	0	t	0	t	1.36	op	2.9	ghi	3.81	De
EP 4 µmol l^{-1}	0	t	0	t	0.39	s	2.22	mn	3.7	E
EP 7 µmol l^{-1}	0	t	0	t	1.25	opq	3.01	fgh	4.24	Bc
EP 10 µmol l^{-1}	0	t	0	t	1.02	pqr	2.68	ijk	3.9	De
Cin 250 ppm	0	t	0	t	1.3	op	2.91	ghi	3.8	De
Cin 500 ppm	0	t	0	t	1.25	opq	3.17	fg	4.07	Cd
Cin 750 ppm	0	t	0	t	0.81	r	2.34	lmn	2.78	Hij
Cin 1000 ppm	0	t	0	t	1.03	pqr	2.39	k-n	2.95	f-i
Chi 2.5 g l^{-1}	0	t	0	t	1.36	op	3.04	fgh	4.08	Cd
Chi 5 g l^{-1}	0	t	0	t	1.2	opq	2.22	mn	3.2	Fg
Chi 7.5 g l^{-1}	0	t	0	t	1.12	opq	2.15	n	3.03	Fgh
Chi 10 g l^{-1}	0	t	0	t	0.96	qr	2.11	n	2.9	Ghi

*In each column, means with the similar letters are not significant different ($p < 0.05$) using LSD test.

fruit and also caused a significant difference to the negative control. So negative control treatment (untreated and infected with green mold) with a 4.75 cm diameter resulted in the highest lesion diameter on the surface of the fruit, which approximately all of the surface of fruit covered with green mold mycelium. On the other hand, positive control treatment, showed the lowest lesion diameter in 96 hours after inoculation, which had no infection, followed by, the essential oil of cinnamon at concentrations of 750 and 1000 ppm, and chitosan at concentrations of 7.5 and 10 g l^{-1}, without significant differences, could prevent from expansion of fungal infection on the fruit surface. The results indicated that 750 ppm cinnamon treatment reduced fungal infection by 53 % compared with negative control.

3.2 EVALUATION OF SUPEROXIDE DISMUTASE ENZYME ACTIVITY

According to Table 3 and 4, the highest amount of

Table 3: Mean comparison of the effect of the jasmonic acid (ja) and epibrassinolide (epiBL) on the superoxide dismutase (SOD) and ascorbate peroxidase (APX) activity in 'Thomson-Navel' orange fruit during 96 hours after inoculation

		After 96 hours of inoculation						
	Day 0	Control^{+}	Control^{-}	1 µmol.l^{-1}	4 µmol.l^{-1}	7 µmol.l^{-1}	10 µmol.l^{-1}	
SOD (Ug^{-1} protein)	2.54	2.42±0.18abc	2.08±0.25c	2.19±0.3bc	2.23±0.2bc	2.36±0.35abc	2.42±0.22abc	
APX (U g^{-1} protein)	52.1	76.15±2.97bcdef	70.15±2.97i	75.63±2.61defgh	77.08±2.66cdef	76.26±2.67bcdef	74.2±2.23gh	epiBL
	Day 0	Control^{+}	Control^{-}	5 µl.l^{-1}	10 µl.l^{-1}	20 µl.l^{-1}	40 µl.l^{-1}	
SOD (U g^{-1} protein)	2.54	2.42±0.18abc	2.08±0.25c	2.33±0.2bc	2.29±0.14bc	2.37±0.25abc	2.694±0.24a	
APX (U g^{-1} protein)	52.1	76.15±2.97bcdef	70.15±2.97i	80.07±2.76a	78.79±2.59b	77.34±2.58bcde	74.46±2.2fgh	JA

*Means are average values of three replications with the standard error. Values followed by the same letter in the rows are not significantly different at (p = 0.05) according to Duncan's multiple range test.

Table 4: Mean comparison between the effect of the chitosan (chi) and cinnamon essential oil (cin) on the superoxide dismutase (SOD) and ascorbate peroxidase (APX) activity in 'Thomson-Navel' orange fruit during 96 hours after inoculation

		After 96 hours of inoculation						
	Day 0	Control^{+}	Control^{-}	2.5 g.l^{-1}	5 g.l^{-1}	7.5 g.l^{-1}	10 g.l^{-1}	
SOD (Ug^{-1} protein)	2.54	2.42±0.18 abc	2.08±0.25 c	2.11±0.22 c	2.25±0.27 bc	2.5±0.26 ab	2.44±0.28 abc	
APX (U g^{-1} protein)	52.1	76.15±2.97 bcdef	70.15±2.97 i	75.99±2.6 cdefgh	74.7±2.53 abcd	76.71±2.61 bcdef	77.3±2.36 bcd	Chi
	Day 0	Control^{+}	Control^{-}	250 ppm	500 ppm	750 ppm	1000 ppm	
SOD (U g^{-1} protein)	2.54	2.42±0.18 abc	2.08±0.25 c	2.25±0.13 bc	2.16±0.32 bc	2.34±0.14 bc	2.69±0.21 a	
APX (U g^{-1} protein)	52.1	76.15±2.97 bcdef	70.15±2.97 i	76.41±2.6 bcdef	76.47±2.54 bcdef	76.07±2.75 cdefgh	73.39±2.44 h	Cin

*Means are average values of three replications with the standard error. Values followed by the same letter in the rows are not significantly different at (p = 0.05) according to Duncan's multiple range test.

SOD activity with value of 2.694 (U g $^{-1}$ protein) was observed in jasmonic acid treatment at a concentration of 40 µl l^{-1}.The lowest activity of the enzyme was obtained by negative control treatment with the value of 2.089 (U g $^{-1}$ protein) which except for 40 µl l^{-1} concentration of jasmonic acid, cinnamon 1000 ppm and chitosan 7.5 g l^{-1}, had not significant difference to other treatments.

3.3 EVALUATION OF ASCORBATE PEROXIDASE ENZYME ACTIVITY

According to Table 3 and 4, all treatments increased enzyme activity and caused a significant difference with negative control. So, the lowest ascorbate peroxidase activity was observed in negative control treatment with a value of 70.157 (U g $^{-1}$ protein). On the contrary, Apx enzyme activity was the highest in the treatment of jasmonic acid at a concentration of 5 µl l^{-1} with a value of 80.076 (U g $^{-1}$ protein) which except for 5 g l^{-1} chitosan, had a significant difference to other treatments.

3.4 EVALUATION OF CATALASE ENZYME ACTIVITY

The activity level of the catalase enzyme, was initially 3648 (U g $^{-1}$ protein) and increased over time. However, the effect of the treatments on the activity of the enzyme during the measurement period did not follow a steady pattern and at different times, it gave different responses to the concentrations in treatments (Table 5). Regarding the process of enzyme changes under the influence of treatments, all treatments, except 5 µl l^{-1} jasmonic acid

Table 5: Interaction effects of the treatment in time on changes in the catalase (CAT) activity level (U g $^{-1}$ protein) of the 'Thomson-Navel' orange fruit in 96 hours after inoculation

	Time (Hours)							
Treatments	0		48		72		96	
Control (+)	3648	y	4261	w	4425	uv	4575.67	Stu
Control (-)	3648	y	4094	x	4190.33	wx	4412	V
JA 5 µl l^{-1}	3648	y	4744.67	o-r	4622.33	rst	4472	Tuv
JA 10 µl l^{-1}	3648	y	4839	l-q	4833.67	l-q	4830.67	l-q
JA 20 µl l^{-1}	3648	y	5077	e-i	4957	h-n	5020.33	f-k
JA 40 µl l^{-1}	3648	y	5078.33	e-i	4899	j-o	4936	i-n
EP 1 µmol l^{-1}	3648	y	4879.33	k-p	4881.33	k-p	5271.67	Abc
EP 4 µmol l^{-1}	3648	y	4994.33	f-m	5052	f-j	5347.67	A
EP 7 µmol l^{-1}	3648	y	5133	c-g	5114.33	c-h	5159.33	b-f
EP 10 µmol l^{-1}	3648	y	5054.67	f-j	5230.33	a-e	5246.33	a-d
Cin 250 ppm	3648	y	4710.33	qrs	4963	g-n	5102	d-i
Cin 500 ppm	3648	y	4825.33	m-q	5100.67	d-i	5297	Ab
Cin 750 ppm	3648	y	4999.33	f-l	4985	g-m	5307	Ab
Cin 1000 ppm	3648	y	4723.67	p-s	4978.33	g-n	5023	f-k
Chi 2.5 g l^{-1}	3648	y	4626.33	rst	4813.33	n-q	4730	p-s
Chi 5 g l^{-1}	3648	y	4268.33	w	4432.33	uv	4640.67	Rs
Chi 7.5 g l^{-1}	3648	y	4570	s-v	4705.67	qrs	4703	Qrs
Chi 10 g l^{-1}	3648	y	4458	uv	4475.33	tuv	4694.33	Qrs

*Means are average values of three replications with the standard error. Values followed by the same letter in the rows are not significantly different at (p = 0.05) according to Duncan's multiple range test.

treatment, increased enzyme activity and showed a significant difference compared to negative control. 96 hours after inoculation, the negative control had the lowest activity of the catalase enzyme with a value of 4412 (U g $^{-1}$ protein) and the highest activity was 5347 (U g $^{-1}$ protein) associated with epibrassinolide treatment at a concentration of 4 µmol l^{-1}.This process was observed by passing about 48 and 72 hours from inoculation. Therefore, the effect of the treatments increased with an increase in the length of the period from 48 hours to 96 hours, resulting in more enzyme changes.

3.5 EVALUATION OF PEROXIDASE ENZYME ACTIVITY

In general, the amount of peroxidase activity after infection with green mold was initially 28.75 (U g $^{-1}$ protein) and increased over time. However, similar to the changes in the catalase enzyme activity, the effect of the treatments on the activity of the peroxidase during the measurement period did not follow a constant pattern and at different times, it gave different responses to the treatment's concentrations (Table 6). Regarding the changes in the activity of the enzyme under the influence of treatments, except for treatment of 10 µl l^{-1} jasmonic acid, other treatments indicated a significant difference compared to the negative control treatment. 96 hours after inoculation, the negative control had the lowest activity of the peroxidase enzyme with a value of 41.73 (U g $^{-1}$protein) and the highest activity was 60.63 (U g $^{-1}$protein) associated with epibrassinolide treatment at a concentration of 10 µmol l^{-1} which had a significant difference to all treatments.

3.6 CORRELATIONS BETWEEN ANTIOXIDANT ENZYMES AND LESION DIAMETER

According to table 7, the enzymes activity of catalase, superoxide dismutase, ascorbate peroxidase at 1% probability level and peroxidase at 5 % probability level were negatively correlated with lesion diameter. Therefore, along with increase of the enzyme activity, the incidence and spread of the infection decreased (marked gray color).

Table 6: Interaction effects of the treatment in time on changes in the catalase (CAT) activity level (U g $^{-1}$ protein) of the 'Thomson-Navel' orange fruit in 96 hours after inoculation

	Time (Hours)							
Treatments	0		48		72		96	
Control (+)	28.75	w	36.88	uv	42.51	k-t	44.29	h-s
Control (-)	28.75	w	35.88	v	41.85	l-u	41.73	m-u
JA 5 µl l^{-1}	28.75	w	40.14	q-v	43.68	i-s	42.78	k-t
JA 10 µl l^{-1}	28.75	w	41.81	l-u	45.49	e-p	41.74	m-u
JA 20 µl l^{-1}	28.75	w	39.44	r-v	45.83	e-o	46.88	d-k
JA 40 µl l^{-1}	28.75	w	46.25	e-n	49.72	c-f	42.85	k-t
EP 1 µmol l^{-1}	28.75	w	39.38	s-v	44.44	g-r	51.64	Bcd
EP 4 µmol l^{-1}	28.75	w	40.69	p-v	46.22	e-n	46.17	e-n
EP 7 µmol l^{-1}	28.75	w	46.74	d-m	46.76	d-l	51.32	Bcd
EP 10 µmol l^{-1}	28.75	w	41.6	n-u	54.51	bc	60.63	A
Cin 250 ppm	28.75	w	38.33	tuv	43.63	i-s	44.63	g-q
Cin 500 ppm	28.75	w	40.9	o-u	48.17	d-j	47.08	d-k
Cin 750 ppm	28.75	w	45.9	e-o	44.93	f-q	55.35	Bcd
Cin 1000 ppm	28.75	w	42.29	k-t	50.36	b-e	49.14	d-h
Chi 2.5 g l^{-1}	28.75	w	40.21	q-v	45.69	e-p	45.76	e-o
Chi 5 g l^{-1}	28.75	w	43.4	j-s	49.31	d-g	45.97	e-n
Chi 7.5 g l^{-1}	28.75	w	46.67	d-m	44.31	g-s	48.96	d-h
Chi 10 g l^{-1}	28.75	w	43.68	is	48.54	d-i	45.35	f-p

*Means are average values of three replications with the standard error. Values followed by the same letter in the rows are not significantly different at (p = 0.05) according to Duncan's multiple range test.

Table 7: Correlation coefficient between enzymes and lesion diameter.

	CAT	SOD	POD	APX	Lesion Diameter
CAT	1.000				
SOD	0.637**	1.000			
POD	0.894**	0.739**	1.000		
APX	0.601**	0.68**	0.65**	1.000	
Lesion Diameter	-0.559**	-0.461**	-0.397*	-0.57**	1.000

**and *show significant effect at 1 and 5 statistically levels, respectively.

Generally, with respect to the results of this experiment, treatments result in induction of fruit resistance to green mold rot at different concentrations by increasing antioxidant enzymes activity. It was observed that using treatments such as jasmonic acid at concentrations of 5 and 40 µl l^{-1}, epibrassinolide at concentrations of 4 and 10 µmol l^{-1}, chitosan at concentrations of 5 and 7.5 g l^{-1}, and cinnamon essential oil at concentrations of 750 and 1000 ppm, improved the activity of the antioxidant enzymes of the fruit. Among these, cinnamon essential oil treatments with a concentration of 750 and 1000 ppm and chitosan at concentrations of 7.5 and 10 g l^{-1}, more effectively prevented the spread of fungal infection on the fruit surface (Table 2).

It appears that induction of fruit resistance during the post-harvest period is an applicable strategy for reducing the incidence of diseases, due to the activation of defense mechanisms within the plant itself, which has broad-spectrum antibacterial activity (Walters et al., 2005). Increasing the activity of the antioxidant enzymes is one of the strategies of plants to deal with a variety of stresses. One of these enzymes is catalase, which converts hydrogen peroxide into water, thereby preventing damage of these free radicals. Therefore, high levels of antioxidant

enzymes play an important role in inhibiting free radicals and reducing oxidative damage (Dinget al., 2002; Wang et al., 2014).

Studies have shown that methyl jasmonate is involved in the defensive mechanisms against post-harvest diseases through a complex signaling network of fruit-based monitoring interactions, which indicates an increase in the expression of proteins associated with the disease (PR) (such as chitinase, glucanase or heat shock proteins) and the accumulation of phytoalexins in the host or other antifungal compounds (Ding et al., 2002; Wang et al., 2014). Studies of Asghari and Hasanlooe (2015) have also shown that the use of methyl-jasmonate in post-harvest time has increased the activity of catalase enzyme in strawberries, which is consistent with the results of this study. Studies of Yao and Tian (2005) on peaches showed that between concentrations of 1, 10, 100 and 500 μmol l^{-1} of methyl jasmonate, the best and the most economical concentration for eliminating microbial growth is 1 μmol l^{-1}. Methyl jasmonate, also causes accumulation of plant defensive proteins against pathogens in some cases (Wang et al., 2003).

Ge et al. (2015) reported that Peach treatments with brassinolide, due to its effect on increasing resistance proteins such as superoxide dismutase and peroxidase enzymes, reduced the decay caused by the *P. expansum* Link which is consistent with the findings of this study. In jujube, EBR stimulated the activity of defense-related enzymes (CAT, SOD etc.) which consequently increases the resistance to the fungus pathogen of blue mold (Zhu et al., 2010). Liu et al. (2016) found that epibrassinolides-treated grapes had better post-harvest quality and a lower incidence of *Botrytis* rot. Zhu et al. (2015) proposed that EBR could reduce the incidence of post-harvest disease in 'Satsuma' mandarin, possibly attributable to the accumulation of H_2O_2, stress-related metabolites, and also the induction of stress-related genes. It has been suggested that BRs may help reducing decay by inducing disease resistance in fruits and also delaying their senescence (Champa et al., 2015).

Chitosan has a double effect on host-pathogen interactions as followings: It has both a direct antifungal effect and the ability of inducing defense mechanisms in plant (Romanazzi et al., 2013). First reason for the antimicrobial activity of chitosan is the positive charge amino group. Second, it interferes with negatively charged microbial cell membranes, which resulting in leakage of proteins and other intracellular constituents of pathogens (Jayaraj et al., 2009). In addition, chitosan may enter fungal cells, which inhibit the absorption of essential nutrients, and as a result inhibit or slow down the synthesis of mRNA and protein (Zhang et al., 2011).

The result of Chien et al. (2007) indicated that, by covering the 'Murcott Tangor' citrus fruit with different concentrations of chitosan, the highest concentration of chitosan had the greatest effect on fruit quality maintenance and control of fungal growth which is consistent with results obtained from this research. Jongsri et al. (2016) found that treatment of mangoes with high molecular mass chitosans resulted in the highest activity of catalase and ascorbate peroxidase enzymes; also, treatment with high molecular mass chitosan delays the decomposition of these two antioxidant enzymes. According to findings from Zeng et al. (2010) peroxidase enzyme activity significantly increased in 'Navel' orange fruits covered by chitosan, which protects plant tissues from damages caused by high levels of activated oxygen. Therefore, chitosan coatings increase the activity of antioxidants, which its main task is to protect the fruit against oxidative stresses, including senescence and ripening, and thus increasing the storage period of fruit. Results of Badawy and Rabea (2009) demonstrate that chitosan treatment reduces fungal rot, so the accumulation of mycotoxin in apples is reduced at ambient temperature.

The production and consumption of medicinal herbs in the pharmaceutical and food industries are expanding due to the active biological compounds. In addition, extensive research has begun to show that secondary metabolites of certain medicinal plants are effective in preventing the growth of pathogenic fungi and are an appropriate alternative to chemical pesticides (Pretorius et al., 2002). Negative effects of essences on fungal spore sporulation may be caused by the effect of volatiles released by the essences on mycelium surface growth or the reception / transmission of signals that were involved in the transition from vegetative to reproductive phases in the fungus (Tzortzakis and Economakis, 2007). However, suppression of spore production by plant essence can play an important role in limiting the spread of the pathogen by reducing the release of spores in the storage space and on the fruit surface (Tzortzakis and Economakis, 2007). In this study, cinnamon essential oil showed significant antifungal activity in controlling decay caused by green mold in 'Thomson-Navel' orange. The effect of essential oil in preventing fungal growth is caused by its active ingredients. Essential oil analysis has shown that the main ingredient of cinnamon essential oil is cinnamaldehyde, of which its antifungal and antimicrobial properties have been proven (Bendahou et al., 2008; Tajkarimi et al., 2010).

Wu et al. (2017) stated that citrus treatment with the main ingredient of cinnamon essential oil (cinnamaldehyde) induced superoxide dismutase activity; also, treatment with cinnamaldehyde increased the activity of catalase and peroxidase enzymes at the end of storage compared to the control samples. It was also stated that the increase of the superoxide dismutase enzyme resulted

in effective conversion of O_2 to H_2O_2 and then increased H_2O_2 decomposition by catalase enzyme under the influence of cinnamaldehyde treatment.

Han et al. (2006) and Xu et al. (2009) reported that POD activity in control treatment of the pepper plant and fruit coated with chitosan and cinnamon essential oil increased after harvest and the amount of its activity in the treated fruit at the end of storage is increasingly more than the control treatment which shows the effect of chitosan and cinnamon essential oil on increasing POD activity during storage.

4 CONCLUSIONS

In conclusion, the present study confirmed that treatments of 750 and 1000 ppm of cinnamon essential oil and 7.5 and 10 g l^{-1} of chitosan, as natural substances improved the enzyme activity compared to other treatments, and thus inhibited the growth of green mold on 'Thomson-Navel' oranges in post-harvest conditions. After completing investigation of their effects on the qualitative characteristics of the fruit and achieving favorable results, it is suggested to commercially produce and apply them at their lower concentration (because of its economic costs) as a substitute for high-consumption of chemical fungicides for the purpose of reducing postharvest waste of oranges.

5 REFRENCES

Abdolahi, A., Hassani, A., Ghosta, Y., Javadi, T.and Meshkatalsadat, M. (2010). Essential oils as control agents of postaharvest Alternaria and Penicillium rots on tomato fruits, Journal of Food Safety, 30, 341-352. https://doi.org/10.1111/j.1745-4565.2009.00211.x

Aebi, H. E. (1984). Catalase in vitro. Methods in Enzymology, 105, 121-126. https://doi.org/10.1016/S0076-6879(84)05016-3

Asghari, M., Hasanlooe, A.R. (2015). Interaction effects of salicylic acid and methyl jasmonate on total antioxidant content, catalase and peroxidase enzymes activity in 'Sabrosa' strawberry fruit during storage. ScientiaHorticulturae, 197, 490-495. https://doi.org/10.1016/j.scienta.2015.10.009

Asghari, M., Hasanlooe, A.R. (2016). Methyl jasmonate effectively enhanced some defense enzymes activity and Total Antioxidant content in harvested "Sabrosa" strawberry fruit. Food science & nutrition, 4(3), 377-383. https://doi.org/10.1002/fsn3.300

Badawy, M.E.I., Rabea, E.I. (2009). Potential of the biopolymer chitosan with different molecular weights to control postharvest gray mold of tomato fruit. Postharvest Biology and Technology, 51(1), 110-117. https://doi.org/10.1016/j.postharvbio.2008.05.018

Bautista-Banos, S., Hernandez-Lauzardo, A.N., Velazquez-del Valle, M. G., Hernandez-Lopez, M., AitBarka, E., Bosquez-Molina, E. and Wilson, C. L. (2006). Chitosan as Potential Natural Compounds to Control Pre and Postharvest Diseases of Horticultural Commodities. Crop Protection, 25: 108-118. https://doi.org/10.1016/j.cropro.2005.03.010

Bendahou, M., Muselli, A., Grignon-Dubois, M., Benyoucef, M., Desjobert, J.M., Bernardini, A.F. (2008). Antimicrobial activity and chemical composition of Origanum glandulosum Desf. essential oil and extract obtained by microwave extraction: Comparison with hydro distillation. Food Chemistry, 106(1), 132-139. https://doi.org/10.1016/j.foodchem.2007.05.050

Champa, W.A.H., Gill1, MIS., Mahajan, BVC., Aror, NK., BediS. (2015). Brassinosteroids improve quality of table grapes (Vitis vinifera 'Flame Seedless'. Tropical Agricultrucal Research, 26(2):368 - 379. https://doi.org/10.4038/tar.v26i2.8099

Chien, P.J., Sheu, F., Lin, H.R. (2007). Coating citrus ('Murcott Tangor') fruit with low molecular weight chitosan increases postharvest quality and shelf life. Food chemistry, 100(3), 1160-1164. https://doi.org/10.1016/j.foodchem.2005.10.068

Coll, Y., Coll, F., Amorós, A., Pujol, M. (2015). Brassinosteroids roles and applications: an up-date.Biologia, 70(6):726-732 doi: 10.1515/biolog-2015-0085. https://doi.org/10.1515/biolog-2015-0085

Ding, C.K., Wang, C.Y., Gross, K.C., Kenneth, C.S., David, L. (2002). Jasmonate and salicylate induce the expression of pathogenesis-related-protein genes and increase resistance to chilling injury in tomato fruit. Planta 214, 895-901. https://doi.org/10.1007/s00425-001-0698-9

Droby, S., Wisniewski, M., Macarisin, D. and Wilson, C. (2009). Twenty years of postharvest biocontrol research: is it time for a new paradigm. Postharvest Biology and Technology, 52,137e145. https://doi.org/10.1016/j.postharvbio.2008.11.009

El guilli, M., Hamza, A., Clement, C., Ibriz, M., Aitbarak, E. (2016). Effectiveness of postharvest treatment with chitosan to control citrus green mold. Agriculture, 6(2), 12. https://doi.org/10.3390/agriculture6020012

FAO/WHO (2019). http://www.fao.org/faostat.

Ge, Y.H., Li, C.Y., Tang, R.X., Sun, R.H., Li, J.R. (2015). Effects of postharvest brassinolide dipping on quality parameters and antioxidant activity in peach fruit. In III International Symposium on Postharvest Pathology, Using Science to Increase Food Availability, 1144.377-384. https://doi.org/10.17660/ActaHortic.2016.1144.56

Giannopolitis, S. (1977). Superoxide dismutase. I. Occurrence in higher plants. Plant Physiology, 59, 309-314. https://doi.org/10.1104/pp.59.2.309

González-Aguilar, G., Buta, J., Wang, C. (2003). Methyl jasmonate and modified atmosphere packaging (MAP) reduce decay and maintain postharvest quality of papaya 'Sunrise'. Postharvest Biology and Technology, 28, 361-370. https://doi.org/10.1016/S0925-5214(02)00200-4

Guo, J., Fang, W., Lu, H., Zhu, R., Lu, L., Zheng, X., Yu, T. (2014). Inhibition of green mold disease in mandarins by preventive applications of methyl jasmonate and antagonis-

tic yeast Cryptococcus laurentii. Postharvest biology and technology, 88, 72-78. https://doi.org/10.1016/j.postharvbio.2013.09.008

Han, J. H., Tao, W.Y., Hao, H.K., Zhang, B.L., Jiang, W.B., Niu, T.G., et al. (2006). Physiology and quality responses of fresh-cut broccoli florets pretreated with ethanol vapor. Journal of Food Science, 71, 385-389. https://doi.org/10.1111/j.1750-3841.2006.00042.x

Jayaraj, J., Rahman, M., Wan, A., Punja, Z.K. (2009). Enhanced resistance to foliar fungal pathogens in carrot by application of elicitors. Annals of Applied Biology, 155, 71-80. https://doi.org/10.1111/j.1744-7348.2009.00321.x

Jiménez-Reyes, M.F., Carrasco, H., Olea, A., Silva-Moreno, E. (2018). Natural compounds: A sustainable alternative for controlling phytopathogens. PeerJ Reprints, 6, e26664v1. https://doi.org/10.7287/peerj.preprints.26664v1

Jongsri, P., Wangsomboondee, T., Rojsitthisak, P., Seraypheap, K. (2016). Effect of molecular weights of chitosan coating on postharvest quality and physicochemical characteristics of mango fruit. LWT-Food Science and Technology, 73, 28-36. https://doi.org/10.1016/j.lwt.2016.05.038

Ladanyia, M., Ladaniya, M. (2010). Citrus Fruit: Biology, Technology and Evaluation. Academic Press: San Diego, CA, USA.

Liu, Q., Xi, Z., Gao, J., Meng, Y., Lin, S., Zhang, Z. (2016). Effects of exogenous 24 epibrassinolide to control grey mould and maintain postharvest quality of table grapes. International Journal of Food Science and Technology, 51(5), 1236-1243. https://doi.org/10.1111/ijfs.13066

Luan, L. Y, Zhang. Z. W, Xi, Z. M, Huo, S. S, Ma, L. N. (2013). Brassinosteroids regulate anthocyanin biosynthesis in the ripening of grape berries. South African Journal of Enology and Viticulture, 34(2):196-203. https://doi.org/10.21548/34-2-1094

Mac-Adam, J.W., Nelson, C.J., Sharp, R.E. (1992). Peroxidase activity in the leaf elongation zone of tall fescue I. Spatial distribution of ionically bound peroxidase activity in genotypes differing in length of the elongation zone. Plant Physiology, 99(3), 872-878. https://doi.org/10.1104/pp.99.3.872

N. G. Tzortzakis, C. M. Economakis. (2007). Innovative Food Science and Emerging Technologies, 8, 253. https://doi.org/10.1016/j.ifset.2007.01.002

Peng, J., Zheng, Y., Tang, S., Rui, H., Wang, C. (2009). Enhancing disease resistance in peach fruit with methyl jasmonate. Journal of the Science of Food and Agriculture, 89(5), 802-808. https://doi.org/10.1002/jsfa.3516

Pretorius, J.C., Zietsman, P.C., Eksteen, D. (2002). Fungitoxic properties of selected South African plant species against plant pathogens of economic importance in agriculture. Annals of Applied Biology, 141(2), 117-124. https://doi.org/10.1111/j.1744-7348.2002.tb00203.x

Qin, G.Z., Tian, S.P., Xu, Y., Wan, Y.K. (2003). Enhancement of biocontrol efficacy of antagonistic yeasts by salicylic acid in sweet cherry fruit. Physiological and Molecular Plant Pathology, 62(3), 147-154. https://doi.org/10.1016/S0885-5765(03)00046-8

Ranieri, A., Castagna, J., Pacini, B., Baldan, A., Mensuali. Sodi, G.F. (2003). Soldatini Early production and scavenging of hydrogen peroxide in the apoplast of sunflowers plants exposed to ozone. Journal of Experimental Botany, 54, 2529-2540. https://doi.org/10.1093/jxb/erg270

Reddy, M.V.B., Angers, P., Gosselin, A., Arul, J. (1997). Characterization and use of essential oil from Thymus vulgaris against Botrytis cinerea and Rhizopus stolonifer in strawberry fruits. Phytochemistry, 47, 1515-1520. https://doi.org/10.1016/S0031-9422(97)00795-4

Romanazzi, G.; Murolo, S.; Feliziani, E. (2013). Effects of an innovative strategy to contain grapevine Bois noir: Field treatment with resistance inducers. Phytopathology, 103, 785-791. https://doi.org/10.1094/PHYTO-01-13-0031-R

Shan, B., Cai, Y.Z., Brooks, J.D., Corke, H. (2007). The in vitro antibacterial activity of dietary spice and medicinal herb extracts. International Journal of food microbiology, 117(1), 112-119. https://doi.org/10.1016/j.ijfoodmicro.2007.03.003

Sharma, R.R., Singh, D., Singh, R. (2009). Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. Biological control, 50, 205-221. https://doi.org/10.1016/j.biocontrol.2009.05.001

T, M., Ibrahim, S., Cliver, D. (2010).Antimicrobial herb and spice compounds in food. Food Control, 21(9), 1199-1218. https://doi.org/10.1016/j.foodcont.2010.02.003

Walters, D., Wash, D., Newton, A., Lyon, G. (2005). Induced resistance for plant disease control: Maximizing the efficacy of resistance elicitors. Phytopathology, 95, 1368-1373. https://doi.org/10.1094/PHYTO-95-1368

Wang Q, Lai TF, Qin GZ, Tian SP. (2009). Response of jujube fruits to exogenous oxalic acid treatment based on proteomic analysis. Plant Cell Physiol, 50:230-242. https://doi.org/10.1093/pcp/pcn191

Wang, C.Y., Fung, R., Ding, C. (2003). Reducing chilling injury and enhancing transcript levels of heat Shock proteins, pr-proteins and alternative oxidase by methyl jasmonate and methyl salcylate in tomatoes and peppers. Meeting Abstract, 38, 860.

Wang, K.T., Jin, P., Han, L., Shang, H.T., Tang, S.S., Rui, H.J., et al. (2014). Methyl jasmonate induces resistance against Penicillium citrinum in Chinese bayberry by priming of defense responses. Postharvest Biology and Technology, 98, 90-97. https://doi.org/10.1016/j.postharvbio.2014.07.009

Wasternack, C., Hause, B. (2013). Jasmonates-Biosynthesis and Role in Stress Responses and Developmental Processes. Annals of Botany, 111, 1021-1058. https://doi.org/10.1093/aob/mct067

Win, N.K.K., Jitareerat, P., Kanlayanarat, S., Sangchote, S. (2007). Effects of cinnamon extract, chitosan coating, hot water treatment and their combinations on crown rot disease and quality of banana fruit. Postharvest biology and technology, 45(3), 333-340. https://doi.org/10.1016/j.postharvbio.2007.01.020

Wu, Y., Duan, X., Jing, G., Ouyang, Q., Tao, N. (2017). Cinnamaldehyde inhibits the mycelial growth of Geotrichum citri-aurantii and induces defense responses against sour rot in citrus fruit. Postharvest Biology and Technology, 129, 23-28. https://doi.org/10.1016/j.postharvbio.2017.03.004

Xu, W.T., Peng, X.L., Luo, Y.B., Wang, J., Guo, X., Huang,

K.L. (2009). Physiological and biochemical responses of grape fruit seed extract dip on 'Redglobe' grape. LWT-Food Science and Technology, 42, 471-476. https://doi.org/10.1016/j.lwt.2008.09.002

Yao, H.J., Tian, S.P. (2005). Effects of a biocontrol agent and methyl jasmonate on postharvest diseases of peach fruit and the possible mechanisms involved. Journal of Applied Microbiology, 98(4), 941-950. https://doi.org/10.1111/j.1365-2672.2004.02531.x

Zeng, K., Deng, Y., Ming, J., Deng, L. (2010). Induction of disease resistance and ROS metabolism in navel oranges by chitosan. Scientia Horticulturae, 126, 223-228. https://doi.org/10.1016/j.scienta.2010.07.017

Zhang, H., Li, R. and Liu, W. (2011). Effects of Chitin and Its Derivative Chitosan on Postharvest Decay of Fruits: A Review. International Journal of Molecular Sciences, 12(2), 917-934. https://doi.org/10.3390/ijms12020917

Zhu, F., Yun, Z., Ma, Q. (2015). Effects of exogenous 24-epibrassinolide treatment on postharvest quality and resistance of Satsuma mandarin (Citrus unshiu). Postharvest Biology and Technology, 100, 8-15. https://doi.org/10.1016/j.postharvbio.2014.09.014

Zhu, Z., Zhang. Z.Q., Qin, G.Z., Tian, S.P. (2010). Effects of brassinosteroids on postharvest disease and senescence of jujube fruit in storage. Postharvest Biology and Technology, 56, 50-55. https://doi.org/10.1016/j.postharvbio.2009.11.014

doi:10.14720/aas.2019.114.2.10 Original research article / izvirni znanstveni članek

Does ozone treatment of maize seeds influence their germination and growth energy?

Dmitry NORMOV [1], Evgenii CHESNIUK [1], Andrey SHEVCHENKO [1], Tatiana NORMOVA [1], Raisa GOLDMAN [1], Denis POZHIDAEV [1], Tanja BOHINC [2], Stanislav TRDAN [2, 3]

Received August 07, 2019; accepted November 05, 2019.
Delo je prispelo 07. avgusta 2019, sprejeto 05. novembra 2019.

Does ozone treatment of maize seeds influence their germination and growth energy?

Abstract: This article evaluated the pre-sowing treatment of seeds with electroozonation. A full factor experiment on the influence of the parameters of the technological process of electroozone treatment on the sowing qualities of maize seeds was carried out. Based on the experimental data, the effect of ozone concentration, treatment time and the time before sowing and after treatment on the germination ability, germination energy and growing energy of maize seeds was determined. According to the results of the study, the corresponding regression equations were calculated, graphical dependencies were constructed and method parameters for the pre-sowing treatment of maize seeds with ozone were determined.

Key words: electroozonation; ozone; treatment time; ozone concentration; time before sowing; maize seeds; germination ability; germination energy; growing energy.

Ali tretiranje semena koruze z ozonom vpliva na njegovo kalitev in energijo kalivosti?

Izvleček: Prispevek obravnava tretiranje semena koruze pred setvijo z elektroozoniranjem. V njem so predstavljeni rezultati faktorskega poskusa vpliva različnih dejavnikov tehnološkega procesa elektroozoniranja na dejavnike kakovosti semena koruze. V tej zvezi so predstavljeni vplivi koncentracije ozona, časa tretiranja z ozonom in časa pred setvijo (po tretiranju z ozonom) na odstotek kalitve, energijo kalivosti in energijo rasti semena koruze. Ob upoštevanju rezultatov raziskave so izračunane regresijske enačbe, izdelani grafični prikazi in predstavljeni ključni parametri semena koruze, tretiranega z ozonom pred setvijo.

Ključne besede: elektroozoniranje; ozon: čas tretiranja; koncentracija ozona; čas pred setvijo; seme koruze; odstotek kalitve; energija kalivosti; energija rasti

1 Kuban State Agrarian University, Ulitsa Kalinina 13, Krasnodar, Krasnodarskiy kray, 350004, Russia
2 University of Ljubljana, Biotechnical Faculty, Dept. of Agronomy, Jamnikarjeva 101, SI-1000 Ljubljana
3 Corresponding author, e-mail: stane.trdan@bf.uni-lj.si

1 INTRODUCTION

To increase production and improve the quality of crop production, it is necessary to reduce crop losses from diseases, various pests, and weeds. Pre-sowing treatment of planting material is of great importance in the intensive system of crop production. Today, the main process of pre-sowing treatment of seeds is sterilization aimed at destroying external and internal infections. Despite the external effectiveness of the use of chemical processing methods, they have a number of serious drawbacks. The use of chemicals is associated with danger to humans and environmental pollution (Yeoh, 2014). The development of new alternative seed treatment methods is therefore an important scientific task. During the 1930s to 1940s, professor Chizhevsky systematized and substantiated the positive effect of the use of charged particles of oxygen and ozone when exposed to living organisms and plants (Chizhevsky, 2015).

Ozone (O_3) is a chemically highly reactive compound that is an allotropic form of oxygen with powerful oxidizing properties. Ozone ranks highly in reactivity, second only to fluorine (Normov, 2003). Moreover, unlike other oxidizing agents, in the process of chemical reactions, ozone decomposes into molecular and atomic oxygen and limiting oxides that do not pollute the environment and do not lead to the formation of carcinogenic substances, such as oxidation products of chlorine or fluorine (Booker, 2009).

Despite the relatively large number of publications devoted to the use of ozone, its antibacterial and stimulating properties have not been fully studied; therefore, the purpose of our study was to determine the effect of ozone on the sowing qualities of seeds.

2 MATERIALS AND METHODS

Under laboratory conditions, germination energy, germination ability and growing energy were determined. The standard method stipulates that in seed control laboratories, the germination ability of maize is determined at a constant temperature of +20 °C. One hundred seeds were germinated in a germinative bed with sand moistened to full capacity. The experiment was conducted with four replicates. Germination energy was determined on days 3-4, and the germination ability (the number of seeds that gave normal seedlings in % of 100 planted) was determined between days 7 and 10 (Dospehov, 1985).

Seedlings with a well-developed root, equal to or more than the length of a seed, and a stem with a size of half a seed were considered normal and were used in the experiment. Seeds that had only a shoot or root were considered to not have successfully germinated, as were those that were sick, misshapen, rotten, or split. There were many "hard" seeds among the freshly harvested maize seeds. These seeds with a hard pericarp did not swell and did not germinate; by spring, their number decreased. Therefore, the seed germination ability of the maize was determined two months before sowing.

We proposed a new accelerated method for determining the sowing qualities of seeds in rolls. This method makes it possible to visualize and examine the influence of the physical method of ozone exposure (ozone) on the development of seedlings to accelerate the experimental results.

Dry seeds (100 pieces in each batch) were laid out on a film covered with a layer of filter paper. The size of the strip was 25 × 100 cm. The paper was moistened with water. Seeds were placed at a distance of 5 cm from the top edge and 1 cm from each other. It was covered with the same roll of filter paper and formed into a roll.

The seed roll was placed vertically in a 250 cm^3 tank, which was then filled completely with water. The seeds germinated in a ventilated chamber at a constant temperature of 28-30 °C. Germination energy was determined on the second day, and germination ability was determined on the sixth day.

The seeds were inspected on the sixth day. Rotten seeds and seeds that gave abnormal shoots were removed. The upper layer of the filter paper was replaced with a new layer. During the day, the room was aired. The growing energy was determined on the 10th day: length of the seminal root and the shoot, the total length of the seedling, and the mass of 100 seedlings were measured. The growing energy of seedlings was evaluated visually by the length and thickness of the seminal root, the intensity of development and the mass of the seedling.

The seeds were treated according to the following scheme: four levels of ozone concentration X2 (10, 24, 48, 72 mg m^{-3}) with four levels of treatment time X1 (3, 5, 7, 9 minutes.). Then, the seeds were planted after four equal intervals of time X3 (1, 10, 20, 30 days).

For the measurement of the ozone concentration, we chose the iodometric method. This method has been used to measure the concentration of ozone in the air at a content of 4-10 %.

The iodometric method for measuring the concentration of ozone in a gas is as follows: ozone-containing gas is passed through a solution of potassium iodide and sulfuric acid. As a result of the chemical interaction of ozone with potassium iodide, an equivalent amount

of free iodine is released according to the following equation:

$$O_3+2KJ+H_2SO_4=J_2+K_2\ SO_4+O_2+H_2O, \quad (1)$$

The iodine released in this reaction is titrated with sodium sulfate (sodium thiosulfate) according to the equation below:

$$2Na_2\ S_2\ O_3+J_2=Na_2\ S_4\ O_6\ 2NaJ, \quad (2)$$

Titration was carried out in the presence of an indicator - starch - and was carried out until the solution was discoloured, that is until complete fixation of the free iodine was achieved.

Two molecules of thiosulfate are consumed for each molecule of ozone in the reaction during the titration. Thus, the amount of thiosulfate used during titration is proportional to the amount of ozone reacting, and if the reaction is complete, then the amount of ozone in a gas (C) can be determined by the amount of thiosulfate in accordance with the expression below:

$$C=E_0\frac{V_m M_m}{V}, \quad (3)$$

where E_0 is the molar mass equivalent of ozone;

$$M_m\left(\frac{1}{2}\cdot O_3\right)=24 \quad g/mol, \quad (4)$$

where M_m is the molar concentration of the thiosulfate solution; V_m is the volume of the thiosulfate solution used during titration, ml; and V is the volume of a gas that has passed through the solution of thiosulfate, l.

To normalize the ozone concentration to ambient conditions, it is necessary to measure the atmospheric pressure and the ambient temperature and then calculate the reduced concentration of using the following formula:

$$C_o=C\frac{P_0\cdot T}{P\cdot T_0}, \quad (5)$$

where P is the atmospheric pressure, mm Hg;
P_0 - 760 mmHg, normal pressure;
T - ambient air temperature, K;
T_0 - 293 K - normal temperature.

The ozone concentration was determined as follows: first, we prepared a solution (13.7 g K_2HPO_4 + 14.1 g NaH_2PO_4) in 1000 ml of H_2O. Then, in contrast to the standard technique, which suggests using a 5 % solution, we prepared a 0.1 N potassium iodide buffer solution (KI). The ozone-air mixture produced by the installation under study was passed through 40-50 ml of a one-molar solution of potassium iodide (KI). After passing the ozonised gas, the resulting solution was poured into a flask and acidified with 5 ml of a 2 N solution of HCl. The released iodine was titrated with a 0.01 N solution of sodium hyposulfite, also prepared from the standard titrimetric substance, to a slightly yellow colour, after which 1 ml of a 1 % starch solution was added, and the titrant was added until the blue colour disappeared. The ozone content was calculated by the following formula:

$$C = 24 \times V \times N/V, \quad (6)$$

where C is the ozone content, mg l^{-1};

N is the normality of the hyposulfate solution used for titration, N;

24 is the conversion factor for the moles of sodium hyposulfite to ozone; and

V is the volume of ozonised gas passed through a solution of potassium iodide, l.

3 RESULTS AND DISCUSSION

In the laboratory of electrical technologies of KubSAU, an experiment was set up to identify the effect of various concentrations and exposures of an ozone-air mixture on the germinating energy of maize seeds. For this experiment, we treated 8 batches of 400 seeds with ozone at a concentration of 32 mg m^{-3} for different exposure times. Seeds were planted and germinated in accordance with the requirements for determining the sowing qualities of seeds. In addition to the treated seeds, control seeds were planted. With to the results of the experiment, a dependence curve was generated.

After analysing Figure 1, we can conclude that the area of positive effect of ozone on maize seeds lies within 3-9 minutes of exposure time.

To more accurately determine the effect of ozone on the seeds, a full-factor experiment was established.

According to the results of the experiment, the following dependence curve was constructed:

An increase in seedling germination energy was observed in all treatment modes. The seedling germination energy increase at the lowest level of exposure to ozone (X1 - 3 minutes; X2 - 24 mgm^{-3}; X3 - 1 day) was approximately 2 %. This suggests an insufficient amount of the ozone-air mixture for full activation of the supply of nutrients inside the seed. However, at the same time, it should be noted that the positive effect of

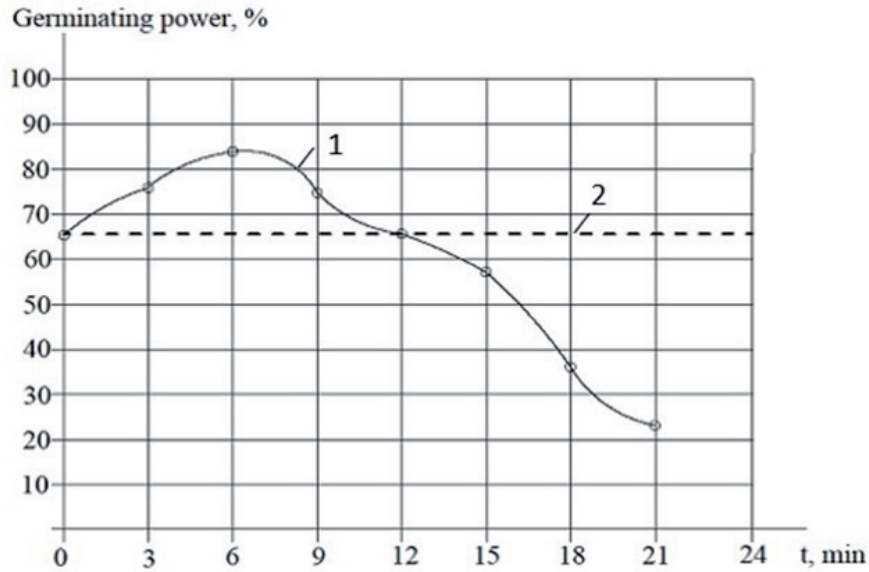


Figure 1: Preliminary experimental data. line 1 - treated seeds; 2 - untreated seeds

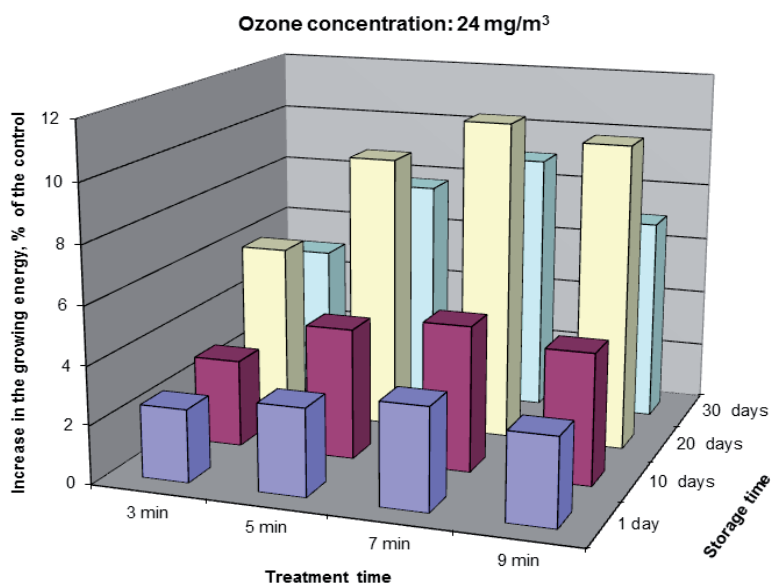


Figure 2: The effect of different ozone treatment modes on maize seeds

the ozone-air mixture on the germination energy began to decline at X1 = 9 minutes.

This histogram shows that the most appropriate mode for treating maize seeds was the one in which the treatment time (X1) was 7 minutes and the time before sowing (X3) was 20 days. At this level of treatment, the increase in germination energy compared with that of the control was more than 10 %, which indicates an increase in the sowing qualities of seeds.

As the histogram shows, the time before sowing is also an important factor. Thus, at X3 = 1 day and a treatment time of 7 minutes at a concentration of 24 mg m^{-3}, the germination energy increased by approximately 2 %; after 10 days, it was 4.5 %; 10 days later, its level rose to 10.5 %, and after another 10 days, it began to decline and was 9 %. Based on this, we can say that the ozone was completely absorbed by the seed and activated the processes inside the seed in 20 days. This effect was observed in all modes considered in the work.

With the help of the computer program "STATIS-

TICA 6.0", the data were processed, and a multiple regression equation was obtained, as follows:

$$Y1 = 3.66 + 0.46 \cdot X1X3 + 0.40 \cdot X2X3 - 5.96 \cdot X33 + 7.86 \cdot X32 - 1.99 \cdot X3 - 0.26 \cdot X1X2X3 - 1.23 \cdot X1 - 3.7 \cdot X13 + 4.9 \cdot X12 - 1.88 \cdot X23 + 1.89 \cdot X22.$$

where *X1* - Ozone treatment time of seeds, min;
X2 - Concentration, mg m^{-3};
X3 - Time before sowing, days; and
Y1 - Dependent variable germination energy, %.

As a result, a close (r = 0.96) correlation between the germination energy and the studied factors was established. It was also found that 92 % of the *Y1* variation (germination energy) is "explained" by all *X* variables.

The shift determines the predicted value of *Y* when all variables *X* are equal to 0. In our case, this *Y* value was 3.66 % and interpreted as follows: the typical germination energy of an untreated maize seed that was 3.66 %.

Regression coefficients were interpreted as the influence of each variable on the value of germination energy if all other independent ("explanatory") variables remain unchanged.

The regression coefficient for the interaction between the grain ozone treatment time and the time before sowing (b X1X3 = 0.46) indicates that, when all other conditions are held constant, the germination energy increases by 0.46 % when the grain treatment time and the time before sowing interact. In addition, when the grain ozone treatment time changes by one unit, germination energy decreases by 1.23 %. Thus, an interpretation of all coefficients was made for the variables *X* in the event that these values are significant (significance level $p < 0.05$). If the coefficient was insignificant, i.e., $p > 0.05$, it was not subjected to interpretation and was removed from the equation because it did not carry additional information to predict the value of germination energy.

Germination ability is one of the most significant indicators of crop yield. An increase in germination ability directly indicates an increase in the final crop yield. Ozone treatment also increases the germination ability due to the activation of the internal energy of seeds without changes at the gene level and due to the disinfection of seeds.

Based on the data obtained in the course of the experiment, a figure was constructed that reflects the effect of the ozone-air mixture on the object under study. Thus, the following figure shows the germination variability depending on the concentration and time before sowing maize seeds of the T22MB variety.

The relations shown in Figure 3 were approximated by the program, so there are negative concentrations and times before sowing shown. It should also be noted that at a concentration equal to zero and a change in the values of time before sowing, an increase in germination ability occurred; this change is associated with the approximation conditions incorporated in the software.

Considering the relations presented in Figure 3, we can say that the most acceptable methods for increasing the germination ability of maize seeds are those in which the ozone concentration ranges from 20 to

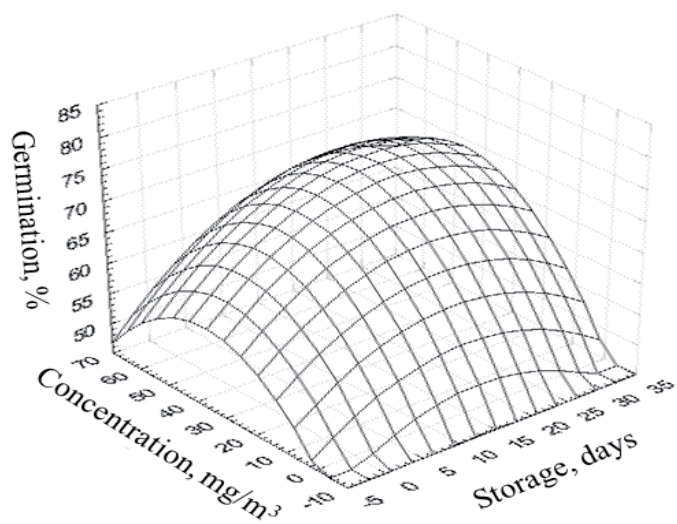


Figure 3: Increased germination ability of maize seeds under the influence of ozone concentration and time before sowing after treatment

40 mg m^{-3} and in which the time before sowing after treatment is 15-25 days. At such conditions, the germination ability was approximately 80 %, while the initial germination of the grain was approximately 65 %.

It should also be noted that with increasing ozone concentration *X2*, the germination *Y2* begins to decrease. During the experiment, it was noted that seedlings at high doses of treatment had black (burnt) ends. This suggests that the oxidative properties of ozone began to degrade the structure of cells and partially destroyed them at high concentrations. Consequently, with a further increase in concentration, the grain will receive a lethal dose of ozone and will die due to cell destruction.

When *X2* is approximately 70 mg m^{-3} and the treatment time *X1* = 5 minutes, the germination ability was approximately 50 %, with a control grain germination rate of 65 %. With a further increase in ozone concentration, the germination continued to decrease, and eventually, the maize kernels were killed.

After conducting regression analysis, we obtained the following regression equation:

$$Y2 = 52.9 + 0.35\ X2X3 - 5.66\ X13 - 2.49\ X1 - 0.096\ X23 - 2.25\ X22 - 1.79\ X33 + 1.5\ X32 + 0.39\ X1X3 + 7.68\ X12 + 2.66\ X2 - 0.28\ X1X2.$$

where *Y2* - dependent variable, the germination of maize seeds.

The data obtained indicate a sufficient correlation (r = 0.94) between germination ability and the studied factors. At the same time, in 88 % of cases, the factors included in the equation influence germination ability, and 12 % are controlled by other factors that were not taken into account when building the mathematical model.

It should be noted that the coefficients for variable interaction of the grain treatment time with ozone with ozone concentration (*X1X2*) and the squared (*X22*) and cubed (*X23*) concentrations were not significant and could not only be interpreted but could also be removed from the equation due to the absence of effects on grain germination ability. Thus, the equation took the following form:

$$Y2 = 52.9 + 0.35\ X2X3 - 5.66\ X13 - 2.49\ X1 - 1.79\ X33 + 1.5\ X32 + 0.39\ X1X3 + 7.68\ X12 + 2.66\ X2.$$

Considering the equation, we can say that the combination of factors *X1X3* (treatment time and time before sowing) had the greatest influence on *Y2*. Their influence was 0.39 % when all other conditions are held constant. This was despite the fact that the treatment time alone (*X1*) reduced the treatment efficiency by 2.49 %. This suggests that the use of ozone seed treatment without a storage period before sowing is impractical. The combination of factors *X2X3*, similarly to *X1X3,* had an effect on seed germination ability, but the effect in percentage was somewhat smaller (0.35 %).

Of the single factors, the factors *X12*, *X2* and *X32* had the greatest positive influence, with coefficients of 7.68, 2.66, and 1.5, respectively.

The remaining factors had a negative effect on the germination ability of maize seeds since the coefficients of the indicators had negative signs.

The growing energy of seedlings determines the degree of their development. Thus, if the plant is underdeveloped, i.e., has a weak root and shoot, it may not achieve maturity of the cob or will do so much later

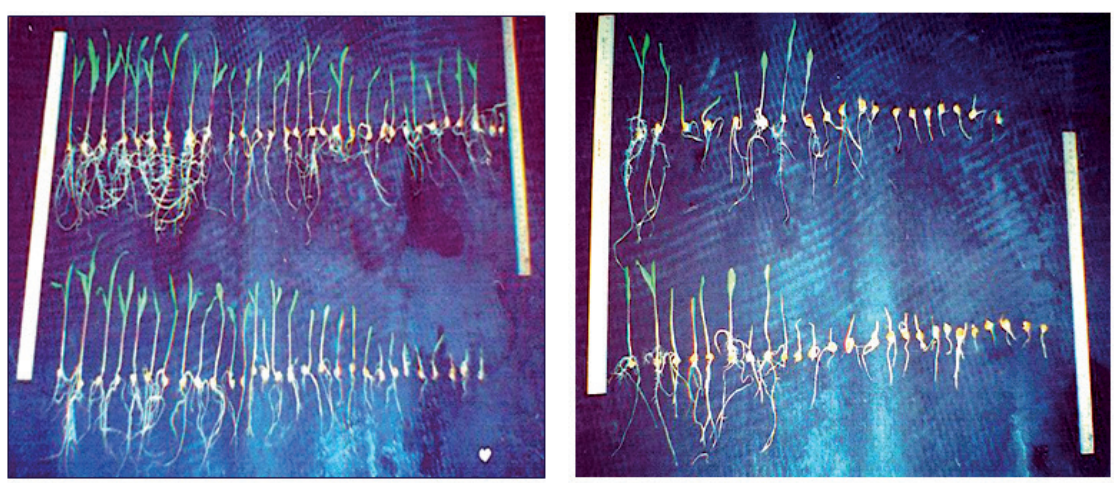


Figure 4: Samples of germinated maize seeds (treated - left; untreated - right)

than normally developed plants, which is unacceptable because harvesting time is limited by agrotechnical requirements.

Growing energy can also influence the number of cobs on a plant. Therefore, usually one stalk of maize has one cob or two that are underdeveloped. If the growing energy of the plant is high, then the plant will have a decreased vegetative period, and it will be able to gain a larger supply of nutrients before the summer drought, and as a result, it will exhibit improved growth and produce more healthy and developed cobs.

The experiment showed that the treated seeds had a higher growing energy, and, visually, they had a more saturated colour. The root system of the treated seeds was strong and had one long, strong main root (seminal root, approximately 10 cm) with small sprouts. The untreated seeds had a root system of approximately 5 cm in length. Photographs of the germinated seeds are presented in Figure 4.

It is necessary to resort to statistical processing of experimental data to determine the degree of influence of the studied factors on the growing energy of seedlings.

After statistical processing of experimental data, we obtained a multiple regression equation that is expressed below:

$$Y3 = 23.8 + 1.77\ X3 - 1.29\ X33 - 2.49\ X13 + 2.37\ X12 - 4.05\ X23 + 5.75\ X22 - 1.0\ X2.$$

where *Y3* is the dependent variable, growing energy, %.

A close correlation has been established between the variable factors used and the growing energy of seedlings - R = 0.91. In addition, in 84 % of cases, the factors studied had an effect on the growing energy of the seedlings, and in 16 %, they were controlled by other factors that were not taken into account during the experiment. Such factors may be the ambient temperature during seed germination, air humidity, composition of water used for watering plants, etc.

A large level of significance was observed for the growing energy indicator *X22* a = 5.75. This indicates that when all other indicators are held constant, the growing energy is 5.75 % than that of the control higher due to the performance of seeds after treatment.

According to the data obtained during the experiment, the histogram shown in Figure 5 was constructed.

It can be seen from the graph that the maximum value of the growing energy was reached at an ozone concentration (*X2*) of 48 mg m^{-3}, a treatment time (*X1*) of 5 minutes and a time after treatment and before sowing (*X3*) of 20 days. With these method parameters, ten typical plants reached 61 mm. This is two times more than with the same treatment mode but no wait time after treatment and before sowing (28 mm). To construct the graph, we used the data on the length of a maize seed kernel.

With an increase in processing time of more than 5 minutes, there was a decrease in the length of seedlings. For example, when the time before sowing was 10 days, the concentration of ozone was 24 mg m^{-3}, and the treatment time was 5 minutes, the growing energy of the seedlings was 45 mm (for this experiment we took this indicator in relation to untreated grain). With an increase in treatment time up to 9 minutes, the growing energy decreased and was measured as 37 mm. The same effect was observed at other time levels before

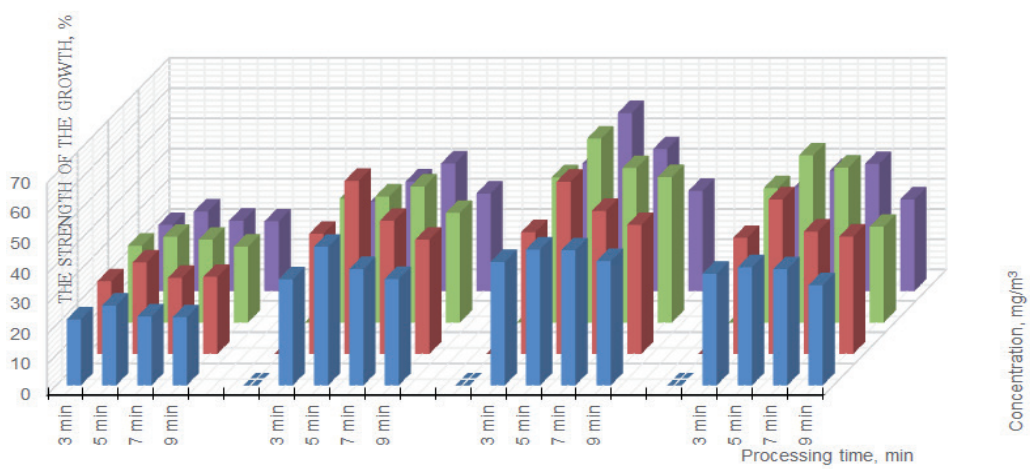


Figure 5: Changes in the growing energy of maize seedlings caused by ozone-air treatment and time before sowing after treatment

sowing. This suggests an overdose of the ozone-air mixture during treatment.

4 REFRENCES

Booker, F.L. (2009). The ozone component of global change: Potential effects on agricultural and horticultural plant yield, product quality and interactions with invasive species, *Journal of Integrative Plant Biology, 51*, 337-351.

Chizhevsky, A.L. (2015). *Earth and space*. Earth echo of cosmic storms. Academic project: 272 p. https://doi.org/10.1111/j.1744-7909.2008.00805.x

Dospehov, B.A. (1985). *Methods of field experience: the textbook*. Agropromizdat: 351 p.

Gerlach K.A. (1995). *Effects of ozone on soybean seed vigor*. Sustaing Planet Faith. Charleston: 44.

Mendes F., Maier D.E., Mason L.J. and Woloshuk C.P. (2003). Penetration of ozone into columns of stored grains and effects on chemical composition and processing performance. *Journal of Stored Products Research*, 39, 1, p. 3344. https://doi.org/10.1016/S0022-474X(02)00015-2

Normov, D.A. (2003). Drying and bactericidal properties of ozone. *Physical and technical problems of creating new technologies in the agro-industrial complex*: Collection of scientific papers. SSAU, Stavropol: 219-220.

Pat. 2248111 Russian Federation, IPC A01 S1 / 00 Method of presowing treatment of agricultural crops / Normov, D.A., Oskin S.V., Shevchenko A.A., Saprunova E.A./ applicant and patentee of KubSAU. - № 2003123158/13; declare 07/22/03, publ. 03/20/05, Bull. № 23: 3 p.

Rezchikov V.G., Churmasov, A.V., Gavrilova, A.A. (1998). The effect of ozone on the germination of pea and sea-buckthorn seeds. *Technics in agriculture*. Chelyabinsk: 14-17.

Sun J, Feng Z, Ort DR (2014) Impacts of rising tropospheric ozone on photosynthesis and metabolite levels on field grown soybean. Plant Science, *226*, 147–161. https://doi.org/10.1016/j.plantsci.2014.06.012

Tzortzakis, N. G., Taybi, T., Antony, E., Singleton, I., Borland, A., & Barnes, J. (2013). Profiling shifts in protein complement in tomato fruit induced by atmospheric ozone-enrichment and/or wound-inoculation with *Botrytis cinerea. Postharvest Biology and Technology, 78*, 67–75. https://doi.org/10.1016/j.postharvbio.2012.12.005

Voznesensky V.A. 1981. *Statistical Methods for Experimental Planning in Techno-Economic Research.* Finance and Statistics: 259 p.

Yeoh, W. K., Ali, A., & Forney, C. F. (2014). Effects of ozone on major antioxidants and microbial populations of fresh-cut papaya. *Postharvest Biology and Technology, 89*, 56–58. https://doi.org/10.1016/j.postharvbio.2013.11.006

doi:10.14720/aas.2019.114.2.11 Original research article / izvirni znanstveni članek

Izračun vodne bilance tehtalnega lizimetra za oceno napajanja vodonosnika

Špela ŠERJAK[1], Vesna ZUPANC[2], Barbara ČENČUR CURK [3]

Received September 04, 2019; accepted October 28, 2019.
Delo je prispelo 04. septembra 2019, sprejeto 28. oktobra 2019.

Izračun vodne bilance tehtalnega lizimetra za oceno napajanja vodonosnika

Izvleček: Ljubljansko polje je pomemben vodonosnik, ki je vir pitne vode za širšo okolico Ljubljane, zato je poznavanje vodne bilance zelo pomembno. Pri tem je ključno poznavanje dinamike toka vode skozi nezasičeno cono ter načina napajanja vodonosnika. Z oceno vodne bilance dobimo vpogled, kako se napaja vodonosnik in obnavlja podzemna voda. S pomočjo vgrajenega monolitnega tehtalnega lizimetra v Kležah v Ljubljani smo ocenili vodno bilanco za hidrološko leto od marca 2014 do februarja 2015. Pri izračunu smo uporabili meritve mase lizimetra in iztoka ter padavine in dejansko evapotranspiracijo, izračunane iz spremembe mase lizimetra. Večje padavinske dogodke smo razvrstili po času trajanja, intenziteti ter količini padavin. Meritve padavin kažejo, da je bilo obravnavano obdobje izjemno mokro. Padavine so v izbranem hidrološkem letu prispevale k pozitivni vodni bilanci zgornjega sloja nezasičene cone in k napajanju Ljubljanskega vodonosnika na območju vodarne Kleče.

Ključne besede: vodna bilanca; napajanje vodonosnika; monolitni tehtalni lizimeter; Ljubljansko polje; nezasičena cona

Calculation of water balance of the weighing lysimeter for assessment of aquifer recharge

Abstract: Ljubljana field aquifer is an important source of drinking water for the Ljubljana city and surrounding areas. Knowledge of the water balance and of the water flow dynamics through the unsaturated zone and recharge of Ljubljana field aquifer is crucial. The water balance assessment of the upper unsaturated zone provides an insight into groundwater recharge and renewal. With the help of build-in monolith weighing lysimeter in Kleče in Ljubljana we have assessed the water balance for hydrological year from March 2014 to February 2015. Water balance parameters, precipitation and evapotranspiration were determined from the changes in the mass of lysimeter and outflow tank. Precipitation events were evaluated based on their duration, intensity and the amount of precipitation. Evapotranspiration and the duration of precipitation were estimated based on the changes of the lysimeter mass. Results show that the chosen period was extremely wet. In the selected hydrological year, precipitation contributed to positive water balance of the upper unsaturated zone, as well as to the recharge of the aquifer.

Key words: water balance; groundwater recharge; monolithic weighing lysimeter; Ljubljansko polje; unsaturated zone

1 spela.serjak@gmail.com
2 Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo, Jamnikarjeva 101, 1000 Ljubljana, vesna.zupanc@bf.uni-lj.si
3 Univerza v Ljubljani, Naravoslovnotehniška fakulteta, Oddelek za geologijo, Aškerčeva cesta 12, 1000 Ljubljana, barbara.cencur@ntf.uni-lj.si
Prispevek je del magistrske naloge Špele Šerjak pod mentorstvom doc. dr. Barbare Čenčur Curk in doc. dr. Vesne Zupanc.

1 UVOD

V zadnjih letih so vse bolj aktualne raziskave vpliva urbanega okolja na količino in kakovost podzemne vode (Meissner in sod., 2007). Raziskave so usmerjene v določanje količinskega stanja podzemne vode, način napajanja ter obnavljanja vodonosnikov. Poznavanje mehanizmov napajanja vodonosnika je zelo pomembno za upravljanje in varovanje podzemnih vodnih virov. Izračuni členov vodne bilance omogočajo vpogled v mehanizem napajanja vodonosnika. Poleg tega je določitev količine vode, ki se infiltrira skozi talni profil, nujna za napoved prenosa onesnaževal znotraj nezasičene cone (Meissner in sod., 2007).

Na območju vodonosnika Ljubljanskega polja so narejene številne študije o kakovosti in količinskemu stanju ter dinamiki podzemne vode. Peščeno-prodni vodonosnik se v glavnem napaja iz dveh komponent, in sicer iz reke Save, ki se infiltrira skozi zelo prepustne sedimente ter iz padavin, ki se infiltrirajo do vodonosnih plasti na Ljubljanskemu polju (Breznik, 1969; Urbanc in Jamnik, 1998, Vrzel in sod., 2018). Deleža omenjenih komponent sta v različnih delih vodonosnika dokaj različna (Jamnik in Urbanc, 2000). Kakovost podzemne vode Ljubljanskega polja so obravnavali Urbanc in Jamnik (2007) ter Bračič Železnik in sod., (2011). Čeprav so bile opravljene številne raziskave, ki so se ukvarjale z ugotavljanjem hidrogeoloških lastnosti vodonosnika Ljubljanskega polja (Šram in sod., 2012), ostaja napajanje vodonosnika še vedno relativno neraziskano. Predvsem je težko opredeliti infiltracijo padavin in tok vode skozi heterogeno nezasičeno cono vodonosnika zaradi spremenljivih fizikalnih in kemijskih lastnosti nezasičene cone (Vižintin in sod., 2009; McGrath in sod., 2015). V študiji Zupanc in sod., 2012, je bila narejena ocena vodne bilance zgornje nezasičene cone, a je bilo to narejeno za sušno obdobje, zato smo procese v nezasičeni coni in vodno bilanco ovrednotili za hidrološko leto z več padavinami.

2 METODE DELA

Lizimeterska postaja v Klečah (Slika 1 in 2) je bila zgrajena v osemdesetih letih prejšnjega stoletja z namenom ocenjevanje vodne bilance lizimetra (Brilly in Gorišek, 1985). Takrat sta bila narejena dva lizimetra v betonskem jašku z možnostjo spremljanja iztoka v kontrolnem jašku. Leta 2010 je bil na mestu starega lizimetra vgrajen monolitni tehtalni lizimeter (von Unold in Fank, 2008), katerega površina je 1 m^2, globina pa 2 m. Lizimetri so naprave, ki se uporabljajo za določitev posameznih členov vodne bilance (dejanska evapotranspiracija, napajanje vodonosnika) in raziskave dinamike toka vode in prenosa snovi v nezasičeni coni na številnih področjih, tako v agronomiji, hidrologiji, meteorologiji in v okoljskih znanostih (Pintar, 2003; Bračič Železnik in sod., 2011; Meissner in sod., 2010; Kohfahl in sod., 2019; Klammler in Fank, 2014; Schrader in sod. 2013). Znotraj monolitnega lizimetra v Klečah so na globinah 50 cm, 100 cm in 150 cm nameščeni senzorji za meritve vodnega potenciala (tenziometri) in količine vode ter vzorčevalniki vode. Na globini 190 cm sta nameščena dva tenziometra (en znotraj lizimetra in en zunaj) za uravnavanje robnih pogojev na dnu lizimetra. Meritve omogočajo izračun vodne bilance lizimetra (Zupanc in sod., 2012).

Tehtalni sistem ima natančnost do 30 g in omogoča neprekinjeno spremljanje količine vode znotraj lizimetra. Zaradi velike občutljivosti lizimeter zazna že zelo majhne spremembe v teži, zato je treba pri vrednotenju niza podatkov upoštevati vse možne napake zaradi zunanjih vplivov: košnja trave, hoja živali, rast trave itd. Rast vegetacije nismo ocenjevali, ker smo predvidevali konstantno višino trave. za natančnejše ovrednotenje bi potrebovali dnevnik košenj. Zaradi tega je zelo pomembno, da se vse aktivnosti in morebitne napake na lizimetru skrbno beležijo (čas košnje, odvzema vzorcev in/ali podatkov, odbobje brez baterije, obdobje nedelovanja posamezne sonde ipd.).

Členi osnovne vodne bilance lizimetra so padavine (P), evapotranspiracija (ET), iztok (O) in sprememba zaloge vode v tleh (ΔS), ki so vsi podani v mm (enačba (1)). Iztok (O) je masa vode, ki izteče iz lizimetra med dvema časovnima korakoma, t.j. med dvema meritvama mase vode, odtekle iz lizimetra. Na zalogo vode v lizimetru (S) vpliva razmerje med posameznimi členi osnovne vodne bilance. V članku obravnavamo spremembo zaloge vode v tleh, ker celokupne zaloge vode nismo ocenjevali.

$$P-ET-O-\Delta S=0 \quad (1)$$

K zmanjševanju mase lizimetra prispevata iztok in evapotranspiracija. Slednja je bila izračunana kot razlika spremembe mase lizimetra in iztoka v dveh časovnih korakih:

$$(W_i-W_{i+1})-(O_{i+1}-O_i)=ET_a \quad (2)$$

kjer so masa lizimetra (kg), masa posode za iztok (kg), dejanska evapotranspiracija (mm) ter časovni korak. Vsi parametri morajo biti merjeni v enakem časovnem koraku. Na lizimeterski postaji Kleče sta iztok in masa lizimetra merjena v 1-minutnem intervalu. Spremembo mase lizimetra W smo izračunali kot

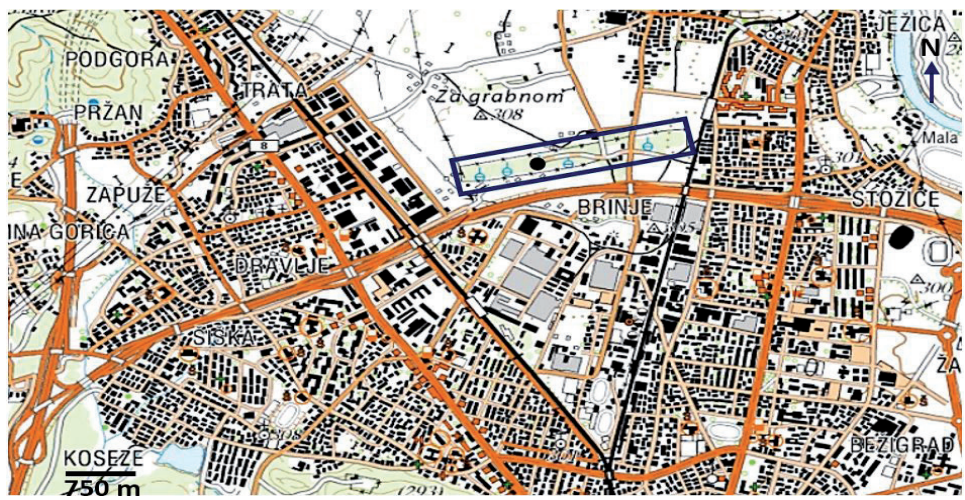
razliko med začetno vrednostjo, preden ta začne strmo upadati, in končno vrednostjo, ko se proces evapotranspiracije prekine.

Količino iztoka *O* smo izračunali kot razliko med končno in začetno vrednostjo časovnega koraka. Začetna vrednost je definirana kot začetek naraščanja, medtem ko je končna vrednost takrat, ko spremembe iztoka ni moč zaznati. Količino iztoka v enem dnevu (od 0:00 do 24:00) smo izračunali po naslednji enačbi:

$$(O_{i+1}-O_i)+(O_i-O_{i-1})+\ldots=O_{0h-24} \quad (3)$$

Pozitivne vrednosti iztoka iz lizimetra prispevajo k napajanju podzemne vode. Negativen iztok pa predstavlja pritok vode nazaj v lizimeter, ki se sproži v sušnem obdobju na podlagi meritev dveh tenziometrov, ki na globini 190 cm (dno lizimetra) znotraj in zunaj lizimetra uravnavata robne pogoje (Zupanc in sod., 2012).

S pomočjo meritev mase lizimetra smo določili tudi količino padavin (P_{lys}). Padavinski dogodek smo določili iz grafa mase lizimetra *W* v odvisnosti od časa ter nato izračunali iz spremembe mase lizimetra ΔW med zadnjo vrednostjo *W*, preden se je začela dvigati in najvišjo vrednostjo *W*, preden je ta začela upadati (posledica *ET* ali *O*). Razlika v masi predstavlja količino padavin v Klečah, izračunano po enačbi:



Slika 1: Geografska umestitev lizimetra (črn krog) z vodarno Kleče (pravokotnik; GURS, 2017)
Figure 1: Geographical position of the lysimeter (black circle) within the well field Kleče (rectangle; GURS, 2017)

Slika 2: Lizimeterska postaja v Klečah
Figure 2: Kleče Lysimeter station

$$P_{lys}=(P_{i+1}-P_i) \quad (4)$$

Izračunane vrednosti ET_a, O in P_{lys} omogočajo izračun spremembo zaloge vode ΔS za časovni niz 24 ur (od 0:00 do 24:00) po enačbi:

$$\Delta S=P-ET-O \quad (5)$$

Dejansko evapotranspiracijo, ki smo jo določili iz meritev na lizimetru Kleče, smo primerjali z referenčno evapotranspiracijo (ET_0), ki je bila izračunana po Penman-Monteithovi metodi na osnovi meritev na meteorološki postaji Ljubljana-Bežigrad (ARSO, 2018; Allen in sod., 1998).

V prispevku podrobno obravnavamo razmere na lizimetrski postaji v Klečah za mesec junij 2014, ko je bilo suho, vroče obdobje, in mesec oktober 2014, ko je bilo mokro obdobje. Prav tako smo izračunali mesečno vodno bilanco za eno hidrološko leto (obdobje marec 2014 – februar 2015).

3 REZULTATI Z DISKUSIJO

Za obravnavano hidrološko leto predstavljamo dve ekstremni vremenski obdobji (slika 3), v času katerih je bila izmerjena masa lizimetra najmanjša (junij) in največja (oktober) ter mesečno vodno bilanco.

V juniju je bilo suho in vroče obdobje z visoko temperaturo zraka (T_{max} = 35°C) in majhno količino padavin, zato so bile razmere v tleh sušne. V mesecu oktobru je bil ekstremni padavinski dogodek z obilno količino padavin, temperature pa so bile nizke, kar je ustvarilo pogoje za doseg največje mase lizimetra (mokro obdobje).

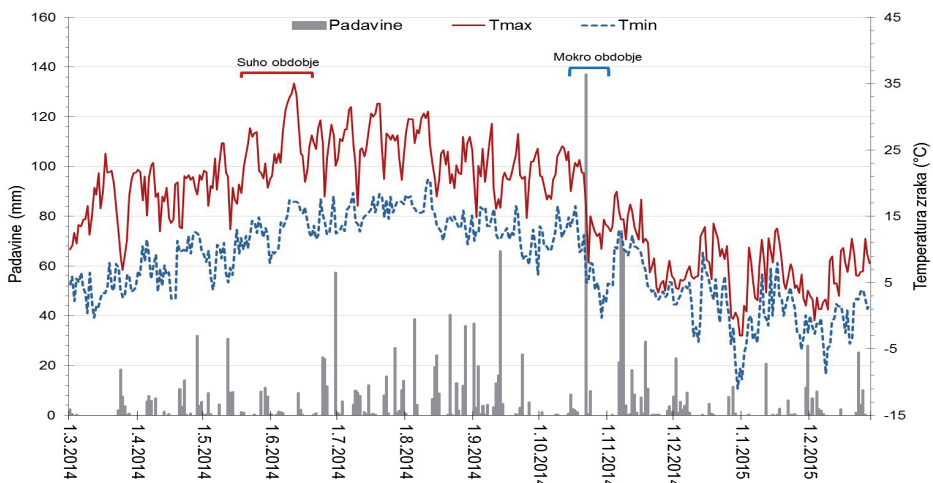
3.1 VODNA BILANCA ZA MESEC JUNIJ 2014

Na osnovi spremembe mase lizimetra W (slika 4) smo določili 15 padavinskih dogodkov, od tega dva znatna (24. 6. in 25. 6.) in tri manjše (12. 6., 13. 6. in 29. 6.). Skupna količina padavin za mesec junij 2014 je bila 87,7 mm (preglednica 1).

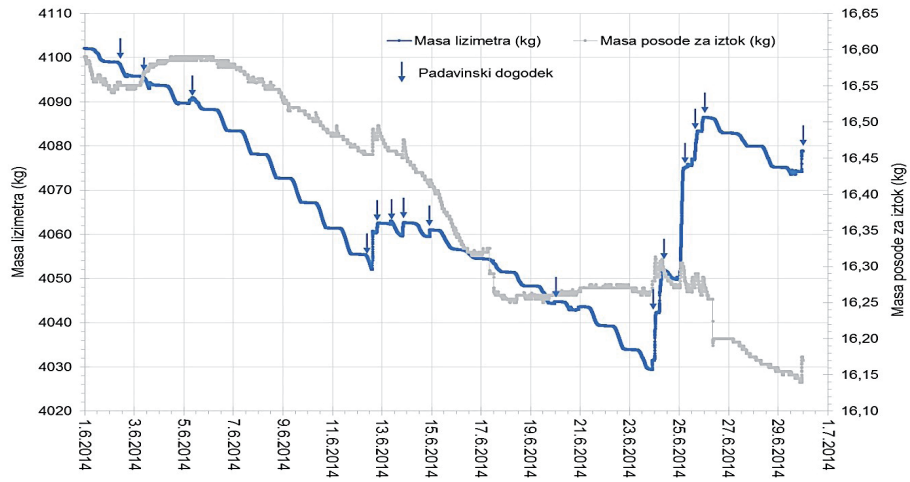
Masa lizimetra W (Slika 4) je v začetku meseca junija padla z največje vrednosti 4102,1 kg (1. 6. ob 0:00) na 4052,0 kg (12. 6. ob 14:30), kar pomeni, da se je zaloga vode v lizimetru S v tem obdobju zmanjšala (ΔS = 50,1 mm). Količina vode, ki je iztekla (O), je bila 0,1 mm.

Padavinski dogodek 12. 6. se je začel ob 14:30 (W = 4052,0 kg) in trajal do 15:22 (W = 4060,7 kg) ter se nato po krajšemu premoru nadaljeval do 20:31 (W = 4062,6 kg), kar je povečalo zalogo vode v tleh (ΔS = 10,6 mm) in količino iztoka O (0,1 mm). Po padavinskemu dogodku 12. 6. se je masa lizimetra W zmanjšala in je 23. 6. ob 20:54 dosegla najmanjšo vrednost 4029,4 kg v mesecu juniju (Slika 4). Zmanjšali sta se zaloga vode v tleh S (33,2 mm) in iztok O (0,2 mm).

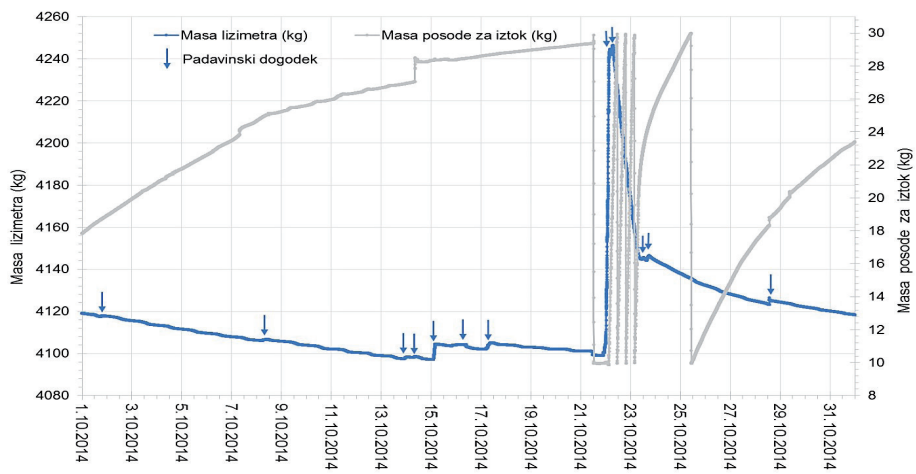
Med 23. 6. in 25. 6. sta bila zabeležena dva padavinska dogodka, ki sta doprinesla glavnino padavin v juniju (57,1 mm). Prvi se je začel 23. 6. ob 21:40 (W = 4029,4 kg) in se nadaljeval naslednjega dne do 8:03 (W = 4052,2 kg). Padavine so se po krajši preki-



Slika 3: Dnevna količina padavin (mm) ter dnevna maksimalna in minimalna temperatura zraka (°C) za meteorološko postajo Ljubljana-Bežigrad v obdobju med marcem 2014 in februarjem 2015 z označenim suhim in mokrim obdobjem (ARSO, 2018)
Figure 3: Daily rainfall (mm) and daily maximum and minimum air temperature (°C) for the meteorological station Ljubljana-Bežigrad in the period from March 2014 to February 2015 with marked dry and wet season (ARSO, 2018)



Slika 4: Masa lizimetra in posode za iztok (kg) ter zaznani padavinski dogodki v juniju 2014 (Šerjak, 2019)
Figure 4: Mass of lysimeter and outflow container (kg) and detected rainfall events in June 2014 (Šerjak, 2019)



Slika 5: Masa lizimetra in posode za iztok (v kg) ter zaznani padavinski dogodki v oktobru 2014 (Šerjak, 2019)
Figure 5: Mass of lysimeter and outflow container (kg) and detected rainfall events in October 2014 (Šerjak, 2019)

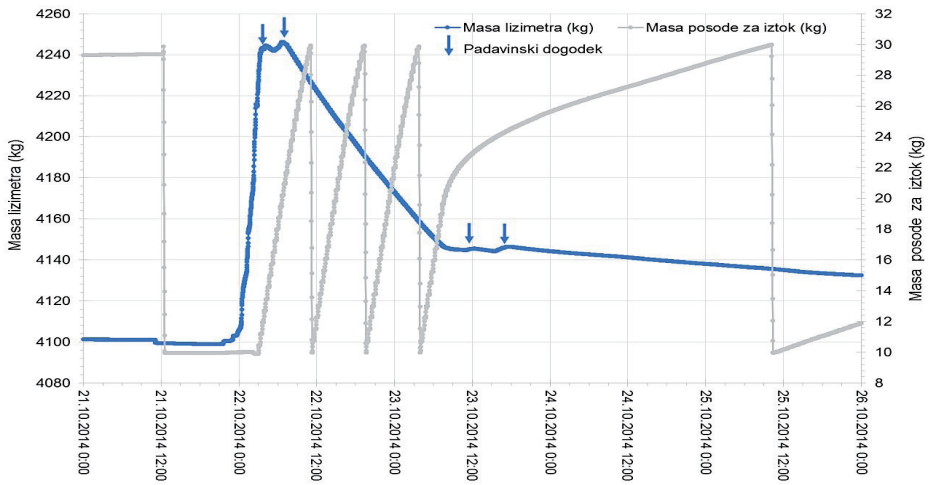
nitvi nadaljevale v drugi padavinski dogodek, ki je se je začel 24. 6. ob 21:35 (W = 4050,1 kg) in končal 25. 6. ob 23:41 (W = 4086,5 kg). Le-te so povzročile, da sta se povečala zaloga vode v tleh (ΔS = 57,1 mm) in iztok iz lizimetra O (0,1 mm).

Na sliki lahko opazimo nagel upad mase iztoka 26. 6. ob 8:20, ki se je zgodil zaradi pobiranja vzorcev, zato se ta podatek ni upošteval v izračunu vodne bilance.

3.2 VODNA BILANCA ZA OKTOBER 2014

Na osnovi spremembe mase lizimetra W (Slika 5) smo določili 12 padavinskih dogodkov, od tega enega večjega (22. 10.) in tri manjše (15. 10., 17. 10. in 21. 10.). Skupna količina padavin za mesec oktober je bila 169,7 mm (preglednica 1).

V obdobju od 1. 10. do 21. 10. ni bilo izrazitih padavinskih dogodkov, ki bi opazno vplivali na porast mase lizimetra. Zabeleženi so bili kratkotrajni padavinski dogodki 1. 10., 8. 10. ter med 13. 10. in 17. 10. Masa lizimetra W je bila med 4119,1 kg (1. 10. ob 0:00) in 4099,0 kg (21. 10. ob 20:52), kar pomeni, da se je zaloga vode v lizimetru zmanjšala (ΔS = 20,1 mm). Masa lizimetra W je 15. 10. ob 0:46 dosegla najmanjšo vrednost (4097,2 kg) za mesec oktober (slika 5). V obdobju od 1. 10. do 21. 10. je količina iztoka narastla (12,0 mm), čeprav v tem obdobju ni bilo zabeleženih večjih padavinskih dogodkov, vendar je prihajalo do izcejanja vode



Slika 6: Masa lizimetra in posode za iztok (v kg) ter zaznani padavinski dogodki v obdobju med 21. 10. in 26. 10. 2014 (Šerjak, 2019)
Figure 6: Figure 6: Mass of lysimeter and outflow container (kg) and detected rainfall events in the period from 21. 10. to 26. 10. 2014 (Šerjak, 2019)

iz lizimetra, saj so bila tla blizu zasičenja zaradi padavin, ki so bile v mesecu septembru.

V mesecu oktobru se je zgodil ekstremni padavinski dogodek (143,5 mm; slika 5 in 6). Ekstremni kratkotrajni padavinski dogodek z veliko intenziteto padavin je trajal od 21. do 22. 10. (slika 6). Padavine so se začele ob 20:52 (W = 4099,0 kg) ter trajale do 6:52 naslednjega dne (W = 4246,4 kg). Največja intenziteta naliva (34,7 mm·h^{-1}) je bila 22. 10., saj je od 0:00 do 4:05 padlo 139,0 mm padavin. Zaradi obilnih padavin sta se povečali zaloga vode v tleh ΔS (147,4 mm) in iztok vode iz lizimetra O (10,4 mm). Posoda za iztok ima omejitev 30 kg, zato se je v tem obdobju večkrat spraznila, kar se odraža kot žagasta krivulja iztoka na slikah 5 in 6.

3.3 MESEČNA VODNA BILANCA V OBDOBJU MED MARCEM 2014 IN FEBRUARJEM 2015

Izbrano hidrološko leto je bilo bogato s padavinami, ki so pozitivno vplivale na vodno bilanco lizimetra (Preglednica 1) in na napajanje vodonosnika. Na območju Kleč padavinska postaja ne deluje več, zato smo rezultate izračuna padavin za lizimeter Kleče primerjali s padavinami na meteorološki postaji Ljubljana-Bežigrad (P_{ARSO}; ARSO, 2018), za katero so podane vsote dnevnih padavin od 7 h do 7 h. V posameznih mesecih je količina padavin presegla dolgoletno povprečje ARSO (2019b). Izjemno vodnati mesic so bili avgust (178,7 mm; Preglednica 1), september (172,8 mm), oktober (169,7 mm) in november (192,3 mm) . V oktobru (22. 10.; Slika 6) je bil ekstremni padavinski dogodek (143,5 mm), ki je presegel 100-letno povratno dobo (ARSO, 2019b). Količina padavin, izračunanih iz meritev v lizimetru, odstopa od meritev na meteorološki postaji Ljubljana-Bežigrad (ARSO, 2018). Odstopanje je najmanjše v oktobru in od decembra do marca; medtem ko je največje odstopanje v mesecu novembru. Na količino padavin vplivajo številni dejavniki (npr. veter in vegetacija - gozd), zato so lahko razlike tudi na manjšem območju. Zaradi tega je potrebno vzpostaviti padavinsko postajo na lizimetru Kleče za primerjavo izračunih padavin iz podatkov lizimetra . Skupna količina padavin v obravnavanem obdobju je bila 1369,4 mm (Preglednica 1).

V obravnavanem obdobju je bila največja evapotranspiracija (Preglednica 1) v mesecu maju (103,9 mm), juniju (106,9 mm), juliju (94,7 mm) in avgustu (106,2 mm). Iz lizimetra je v izbranem hidrološkem letu izhlapelo 646,5 mm vode. V Preglednici 1 so podani rezultati izračunane mesečne dejanske evapotranspiracije (ET_a) na podlagi meritev v lizimetru Kleče ter izračunane referenčne evapotranspiracije za postajo Ljubljana-Bežigrad (ET_0; ARSO, 2018). Vrednosti ET_a in ET_0 se razlikujejo, kar so ugotovili že Zupanc s sod. (2012). ET_0 je izračunana po Penman-Monteithvi enačbi za aktivno rastočo travo, ki popolnoma prekriva tla in je zadostno preskrbljena z vodo (ARSO, 2018; Allen et al., 2006, 2011) in gre za potencialno ET. Travnata površina na lizimetru ni namakana, zato ni vedno na voljo optimalna količina vode in je dejanska evapotranspiracija lahko manjša od referenčne. Poleg tega se meteorološke razmere delno razlikujejo zaradi različnih lokacij (Kleče / Bežigrad). Na vrednosti izračunane eva-

potranspiracije lahko vplivajo tudi intenzivni nalivi po daljšemu sušnemu obdobju, ko je možen tok vode čez lizimeter (hipni preliv preko silikonske zaščite vmesnega prostora med lizimetrom in okoliškimi tlemi), kar je na grafu vidno kot nenadni strmi upad mase lizimetra in so zato posledično večje vrednosti ETa. Nekatere vrednosti dnevne ET_a pa so bile premajhne zaradi padavin, ki so se pojavile tekom dneva in so prekrile evapotranspiracijo na grafu mase lizimetra.

V prvi polovici obravnavanega obdobja (od marca do julija) se je količina iztoka iz lizimetra manjšala (pomanjkanje padavin in/ali povečana evapotranspiracija) in dosegla negativne vrednosti v maju (−0,5 mm), juniju (−0,4 mm) in juliju (−1,1 mm) (*O*; Preglednica 1). Negativne vrednosti se pojavljajo v izredno sušnih razmerah, ko se v lizimetru zaradi uravnavanja robnih pogojev črpa voda iz posode za iztok nazaj v lizimeter. V tem sušnem obdobju se vodonosnik ni napajal. Prihajalo je do kapilarnega dviga iz nižjih plasti v višje. Kapilarni dvig vode je v primerjavi s tokom vode iz vrhnjih plasti (iztok iz lizimetra) navzdol zelo majhen.

V drugi polovici izjemno namočenega hidrološkega leta se je količina iztoka iz lizimetra povečala in dosegla največje vrednosti v mesecu oktobru (105,3 mm) in novembru (159,7 mm) (Preglednica 1). Pozitivna sprememba zaloge vode v lizimetru pomeni, da so bili v obravnavanem času pritoki preko padavin večji kot izgube (ET in iztok) iz lizimetra (Bračič Železnik in sod., 2011; Zupanc in sod., 2012). V tem obdobju je bila količina padavin velika, bilo je tudi nekaj intenzivnih nalivov ter ekstremni padavinski dogodek (22. 10.). V tem obdobju je količina padavin presegla dolgoletno povprečje za meteorološko postajo Ljubljana-Bežigrad (v avgustu, septembru, oktobru, novembru in januarju). Nadpovprečne količine padavin so se infiltrirale skozi nezasičeno cono in napajale vodonosnik. Ekstremni padavinski dogodek in intenzivni nalivi so v jesenskemu obdobju (september, oktober in november) skupno doprinesli 534,8 mm padavin (Preglednica 1). V mesecu septembru, oktobru in novembru je bilo skupno 350,0 mm iztoka, kar pomeni, da je v teh treh mesecih 65 % mesečnih padavin izteklo iz lizimetra oz. napajalo podzemno vodo. V jesenskemu obdobju se je vodonosnik znatno napajal z infiltracijo padavin, kar je ugodno vplivalo na obnavljanje podzemne vode. To je skladno z ugotovitvami Zupanc s sod. (2012), ki so potrdili, da večji padavinski dogodki ali daljša padavinska obdobja doprinesejo k bogatenju podzemne vode tudi v manj mokrem obdobju. Skupna količina iztoka iz lizi-

Preglednica 1: Parametri mesečne vodne bilance za obdobje marec 2014 - februar 2015: količina iztoka iz lizimetra O(0 h–24h), sprememba zaloge vode v lizimetru ΔS, padavine na postaji Ljubljana-Bežigrad PARSO (7 h–7 h) (ARSO, 2018), padavine (meritve v lizimetru; Plys (0 h–24 h), ET0 (Ljubljana-Bežigrad; ARSO, 2018) in ETa (meritve v lizimetru) (Šerjak, 2019)
Table 1: Monthly water balance parameters for the period March 2014 - February 2015: lysimeter outflow amount O(0 h–24 h), change in soil water storage in lysimeter ΔS, rainfall for the meteorological station Ljubljana-Bežigrad PARSO (7 h–7 h) (ARSO, 2018), rainfall (lysimeter measurements; Plys (0 h–24 h), ET0 (Ljubljana-Bežigrad; ARSO, 2018) and ETa (lysimeter measurements) (Šerjak, 2019)

Mesec	$O_{(0\ h-24\ h)}$ (mm)	ΔS (mm)	$P_{ARSO\ (7\ h-7\ h)}$ (mm)	$P_{lys\ (0\ h-24\ h)}$ (mm)	$ET_{0\ (ARSO)}$ (mm)	ET_a (mm)
Marec	15,4	−17,0	35,4	36,3	64,6	37,9
April	4,1	24,9	97,5	83,1	77,5	54,0
Maj	−0,5	5,8	94,0	109,1	117,2	103,9
Junij	−0,4	−18,8	74,0	87,7	128,8	106,9
Julij	−1,1	21,5	130,3	115,1	119,6	94,7
Avgust*	6,6	65,9	205,0	178,7	99,1	106,2
September	85,0	32,6	203,6	172,8	58,6	55,1
Oktober	105,3	25,5	163,4	169,7	43,4	38,9
November	159,7	18,3	248,6	192,3	15,2	14,3
December	47,8	21,6	77,9	77,4	11,9	8,0
Januar	28,9	35,2	69,7	76,0	13,4	11,9
Februar	49,8	6,8	64,3	71,2	15,9	14,7
SKUPAJ	500,6	222,3	1463,7	1369,4	765,2	646,5

* izračuni so podvrženi tehničnim napakam, ki so se pojavljala pri beleženju podatkov (npr. nedelovanje sond zaradi pomanjkanja energije). V mesecu avgustu sta iz izračunov izključena parametra O in ΔS za 26. 8., 27. 8. in 28. 8. Iz izračunov so izključeni tudi 30. 6., 31. 8. in 28. 2.

metra v hidrološkem letu je bila pozitivna in je znašala 500,6 mm (Preglednica 1).

Spremembo zaloge vode v lizimetru predstavlja razlika med količino infiltrirane vode (padavine ali črpanje vode iz posode za iztok v lizimeter) ter ET_a in iztokom vode iz lizimetra (izguba vode; Preglednica 1). Sprememba zaloge vode v lizimetru (ΔS; preglednica 1) je bila pozitivna v mesecu aprilu (24,9 mm), maju (5,8 mm), juliju (21,5 mm), avgustu (65,9 mm), septembru (32,6 mm), oktobru (25,5 mm), novembru (18,3 mm), decembru (21,6 mm), januarju (35,2 mm) in februarju (6,8 mm). Navedeni meseci so bili bogati s padavinskimi dogodki, ki so ugodno vplivali na zaloge vode v lizimetru. Negativne vrednosti so bile v mesecu marcu (−17,0 mm) in juniju (−18,8 mm), kar sovpada s padavinskim primanjkljajem (ARSO, 2019a). Skupna vrednost spremembe zaloge vode v lizimetru v obravnavanemu obdobju je bila pozitivna (222,3 mm), kar pomeni, da so bili pritoki vode večji kakor izgube iz lizimetra.

4 ZAKLJUČKI

V prispevku smo obravnavali vodno bilanco lizimetra na območju vodarne Kleče v Ljubljani z vidika napajanja vodonosnika Ljubljanskega polja. Izračuni so bili narejeni le za eno hidrološko leto, zato rezultati ne predstavljajo dolgoletnega povprečja, temveč dinamiko procesov v izbranem hidrološkem letu (marec 2014 – februar 2015).

V obravnavanem obdobju so bile izrazite padavine ter ekstremni padavinski dogodek, ki se je zgodil 22. oktobra 2014. V celotnem obravnavanem obdobju je padlo 1369,4 mm padavin.

Z lizimetrom v Klečah smo določili dejansko evapotranspiracijo, ki je znašala 646,5 mm (oz. 47 % padavin). Sprememba zaloge vode v lizimetru v izbranem hidrološkem letu je bila pozitivna (222,3 mm oz. 16 % padavin).

Iztok iz lizimetra je znašal 500,6 mm (oz. 37 % padavin). Iztok iz lizimetra pove, kolikšno je napajanje vodonosnika iz padavin v izbranem hidrološkem letu. V prvi polovici obravnavanega obdobja (od marca do avgusta 2014) so bile razmere za napajanje vodonosnika z infiltracijo padavin manj ugodne kakor v drugi polovici hidrološkega leta (od septembra 2014 do februarja 2015). Napajanje vodonosnika z infiltracijo padavin je bilo največje v septembru, oktobru in novembru.

Padavine so prispevale tako k uskladiščenju infiltrirane vode v nezasičeni coni, kakor tudi k napajanju vodonosnika. Zaradi velike dinamike vseh členov vodne bilance je zelo pomemben izračun mesečne vodne bilance.

Lizimeter omogoča izračun parametrov vodne bilance za nezasičeno cono, ki jih sicer težko ovrednotimo, a so pomembni za celostne študije napajanja vodonosnika in modeliranja toka podzemne vode.

5 ZAHVALA

Zahvaljujemo se mag. Branki Bračič Železnik za posredovanje in uporabo podatkov. Raziskave na lizimetru Kleče so bile opravljene s finančno podporo Evropskega sklada za regionalni razvoj (European Regional Development Fund) v okviru projektov CC-WaterS (No. SEE/D/0143/2.1/X) in CC-WARE (No. SEE/A/022/2.1/X), oba programu transnacionalnega sodelovanja Jugovzhodna Evropa.

6 LITERATURA

Allen R. G., Pereira L. S., Raes D., Smith M. (1998). *Crop evapotranspiration – Guidelines for Computing Crop Water Requirements.* Rome: Food and Agriculture Organization of the United Nations.

Allen, R.G., Pruitt, W.O., Wright, J.L., Howell, T.A., Ventura, F., Snyder R. (2006). A recommendation on standardized surface resisdence for hourly calculation of reference ET_0 by the FAO56 Penman–Monteith method. *Agricultural Water Management*, *81*, 1–22. https://doi.org/10.1016/j.agwat.2005.03.007

Allen R.G., Pereira L.S., Howell T.A., Jensen M.E. (2011). Evapotranspiration information reporting: I. Factors governing measurement Accuracy: review. *Agricultural Water Management*, *98*, 899–920. https://doi.org/10.1016/j.agwat.2010.12.015

ARSO. (2018). Pregled agrometeoroloških spremenljivk: *Tabela (dnevni podatki v mesecu) za meteorološko* postajo Ljubljana Bežigrad. Pridobljeno s http://meteo.arso.gov.si/met/sl/agromet/data/month/

ARSO. (2019a). Meteorološka postaja Ljubljana Bežigrad: *Povratne dobe za ekstremne padavine (novejša različica).* Pridobljeno s http://meteo.arso.gov.si/uploads/probase/www/climate/table/sl/by_variable/return-periods/Ljubljana%20Bezigrad.pdf

ARSO. (2019b). *Mesečna povprečja višine padavin*. Pridobljeno s http://meteo.arso.gov.si/met/sl/climate/maps/monthly-mean-precipitation-maps/

Bračič Železnik B., Zupanc V., Pintar M. (2011). Naravno ozadje nitratov – meritve na lizimetru Kleče na Ljubljanskem polju. V *22. Mišičev vodarski dan: zbornik referatov* (str. 120–125). Maribor : Vodnogospodarski biro Maribor.

Breznik M. (1969). Groundwater of the Ljubljana polje and possibilities of increasing its exploitation. *Geologija, 12*, 165–184.

Brilly M., Gorišek M. (1985). *Matematični model podtalnice*

Ljubljanskega polja : II. Faza raziskave podtalne vode na Ljubljanskem Barju, Ljubljana : FAGG, Laboratorij za mehaniko tekočin.

GURS. (2017). Državna pregledna karta merila 1 : 250 000 [online]. Pridobljeno s https://www.e-prostor.gov.si/zbirke-prostorskih-podatkov/topografski-in-kartografski-podatki/topografski-podatki-in-karte/drzavne-pregledne-karte/#tab2-1044%3E.

Jamnik B., Urbanc J. (2000). Izvor in kakovost podzemne vode Ljubljanskega polja = Origin and guality of groundwater from Ljubljansko polje. *RMZ Materiali in geookolje*, *47*(2), 168–178.

Klammler G., Fank, J. (2014). Determining water and nitrogen balances for beneficial management practices using lysimeters at Wagna test site (Austria). *Science of The Total Environment*, *499*, 448–462. https://doi.org/10.1016/j.scitotenv.2014.06.009

Kohfahl, C., Molano-Leno, L., Martinez, G., Vanderlinden, K., Guardiola-Albert, C., Moreno, L. (2019). Determining groundwater recharge and vapor flow in dune sediments using a weighable precision meteo lysimeter. *Science of the Total Environment, 656*, 550–557. https://doi.org/10.1016/j.scitotenv.2018.11.415

McGrath S., Ratej J., Jovičić V., Čenčur Curk B. (2015). Hydraulic characteristics of alluvial gravels for different particle sizes across a wide range of pressure heads. *Vadose zone Journal*, *14*(3), 1539–1663. https://doi.org/10.2136/vzj2014.08.0112

Meissner R., Seeger J., Rupp H., Seyfarth M., Borg H. (2007). Measurement of dew, fog, and rime with a high precision gravitation Lysimeter. *Journal of Plant Nutrition and Soil Science*, *170*(1), 335–344. https://doi.org/10.1002/jpln.200625002

Meissner R., Prasad M. N., Laing G., Rinklebe J. (2010). Lysimeter application for measuring the water and solute fluxes with high precision. *Current Science*, *99*(5), 601–607.

Pintar M. (2003). Lizimetri v Sloveniji. V *14. Mišičev vodarski dan: zbornik referatov* (str. 104–110). Maribor : Vodnogospodarski biro Maribor.

Schrader, F., Durner, W., Fank, J., Gebler, S., Pütz, T., Hannes, M., Wollschläger, U. (2013). Estimating precipitation and actual evapotranspiration from precision lysimeter measurements. *Procedia Environmental Sciences, 19*, 543–552. https://doi.org/10.1016/j.proenv.2013.06.061

Šerjak, Š. (2019). *Določitev napajanja vodonosnika s pomočjo lizimetra v Klečah v Ljubljani*. Magistrska naloga. Naravoslovnotehniška fakulteta. https://repozitorij.uni-lj.si/IzpisGradiva.php?lang=slv&id=108920

Šram D., Brenčič M., Lapanje A., Janža M. (2012). Prostorski model visečih vodonosnikov na Ljubljanskem polju = Perched aquifers spatial model: a case study for Ljubljansko polje (central Slovenia). *Geologija*, *55*(1), 107–116. https://doi.org/10.5474/geologija.2012.008

Vižintin G., Souvent P., Veselič M., Čencur Curk B. (2009). Determination of urban groundwater pollution in alluvial aquifer using linked process models considering urban water cycle. *Journal of Hydrology, 377*(3), 261–273. https://doi.org/10.1016/j.jhydrol.2009.08.025

von Unold, G., Fank, J. (2008). Modular Design of Field Lysimeters for Specific Application Needs. Water, Air, & Soil Pollution, *8*(2), 233–242. https://doi.org/10.1007/s11267-007-9172-4

Vrzel J., Solomon K.D., Blažeka Ž., Ogrinc N. (2018). The study of the interactions between groundwater and Sava River water in the Ljubljansko polje aquifer system (Slovenia). *Journal of Hydrolgy, 556*, 384–396. https://doi.org/10.3390/w11091753

Urbanc J., Jamnik B. (1998). Izotopske raziskave podzemne vode Ljubljanskega polja = Isotope investigations of groundwater from Ljubljansko polje (Slovenia). *Geologija*, *41*(1), 355–364. https://doi.org/10.5474/geologija.1998.018

Urbanc J., Jamnik B. (2007). Porazdelitev in izvor nitratov v podzemni vodi Ljubljanskega polja. *Geologija*, *50*(2), 468–475. https://doi.org/10.5474/geologija.2007.032

Zupanc V., Nolz R., Cepuder P., Bračič-Železnik B., Pintar M. (2012). Determination of water balance components with high precision weighing lysimeter Kleče. *Acta agriculturae Slovenica*, *99*, 165–173. https://doi.org/10.2478/v10014-012-0016-1

doi:10.14720/aas.2019.114.2.12 Review article / pregledni znanstveni članek

Vezane fenolne spojine polnozrnatih žitnih pripravkov kot sestavina funkcionalnih živil: prvi del

Petra TERPINC [1]

Received September 9, 2019; accepted December 8, 2019.
Delo je prispelo 09. septembra 2019, sprejeto 08. decembra 2019.

Vezane fenolne spojine polnozrnatih žitnih pripravkov kot sestavina funkcionalnih živil: prvi del

Izvleček: Številne presnovne bolezni sodobnega časa so povezane z neuravnoteženo energijsko bogato prehrano, ki je osiromašena s prehransko vlaknino in drugimi zaščitnimi bioaktivnimi snovmi. Glede na omejen uspeh terapevtskih posegov za zdravljenje debelosti in presnovnega sindroma se je povečalo zanimanje za druge možnosti. V prvem delu se osredotočamo na pomen polnozrnatih žit v prehrani, spoznamo ključne bioaktivne komponente žit in njihovo razporeditev znotraj zrna. Izvemo, da otrobi, stranski proizvod žitno predelovalne industrije, predstavljajo neizkoriščen vir fenolnih spojin. Biosinteza slednjih poteka na endoplazmatskem retikulumu in v plastidih od koder se prenesejo do drugih organelov znotraj celice. Deaminiranje, hidroksiliranje in metiliranje so osrednje reakcije nastanka hidroksibenzojskih in hidroksicimetnih kislin. Poseben poudarek namenjamo fenolnim spojinam, ki ostanejo v trdnem ostanku po solventni ekstrakciji z vodnimi raztopinami organskih topil. Te neekstraktibilne fenolne spojine, ki so kovalentno vezane na celično steno, so v tovrstnih raziskavah pogosto prezrte in posledično je vsebnost bioaktivnih snovi v žitih nemalokrat podcenjena. Ferulna kislina, kot najpomembnejši predstavnik, je *in vitro* poznana po zaviranju bolezni, ki so posledica oksidativnega stresa – preprečuje razna rakava obolenja, srčno-žilne in nevrodegenerativne bolezni. Vezane fenolne spojine se ne razgradijo v prebavnem traktu, kot različni metaboliti se absorbirajo v krvni obtok šele po fermentaciji s pomočjo črevesne mikroflore. Zadostnemu uživanju vezanih fenolnih spojin pripisujemo izboljšano antioksidativno in protivnetno delovanje, številni dokazi pa kažejo na njihovo preventivno vlogo pri razvoju črevesnih bolezni.

Ključne besede: polnozrnata žita; bioaktivne spojine; ferulna kislina; biosinteza; metabolizem; proste, konjugirane in vezane fenolne spojine

Bound phenolic compounds of whole cereals grain as a functional food component: part one

Abstract: Numerous metabolic diseases are nowadays associated with an unbalanced energy-rich diet, depleted from dietary fibers and other protective bioactive compounds. Given the limited success of therapeutic interventions to treat obesity and the metabolic syndrome, there has been an increased interest in other strategies. In part one, the focus is made on a role of whole cereals grain in diet, the most important bioactive components and their distribution in grains. We find out, that bran, a by-product of the grain processing industry, represents an unexploited source of phenolic compounds. Their biosynthesis takes place on the endoplasmic reticulum and other plant organelles from which they are transported to other cellular compartments. Deamination, hydroxylation and methylation are the main reactions involved in the formation of hydroxybenzoic and hydroxycinnamic acids. Special emphasis is made on phenolic compounds which remain in the solid residue after aqueous-organic solvent extraction. Non-extractable phenolic compounds are covalently bound to the cell wall materials, consequently the amount of bioactive compounds in cereals are often underestimated. Ferulic acid, as the most important representative, may acts against disorders related to oxidative stress, including cancer, diabetes and neurodegenerative diseases. Insoluble bound phenolic compounds are not degraded in the digestive tract, they are absorbed into the bloodstream as different metabolites after they are subjected to fermentation by the intestinal microflora. Adequate consumption of bound phenolic compounds can lead to improve antioxidant and anti-inflammatory properties, and there are many evidences suggesting their role in intestinal diseases prevention.

Key words: whole grain cereals; bioactive compounds; ferulic acid; biosynthesis; metabolism; free, conjugated and bound phenolic compounds

1 Univerza v Ljubljani, Biotehniška fakulteta, Jamnikarjeva 101, SI-1000 Ljubljana, e-mail: petra.terpinc@bf.uni-lj.si

1 UVOD

Prehranske navade potrošnikov se spreminjajo. Hrana danes ni več samo vir energije in potrebnih hranil za normalno delovanje organizma, ampak tudi način, kako preprečiti razvoj določenih bolezni ter izboljšati mentalno in fizično počutje posameznika (Menrad, 2003). Funkcionalno živilo pomeni živilski izdelek, ki je obogaten s specifičnimi sestavinami in doprinese k zdravju potrošnika bolj kot v primeru enakega živila brez njihovega dodatka. Izdelek z oznako 'funkcionalno' živilo mora biti v obliki običajnega živila v okviru vsakodnevnega prehranskega režima (Siró in sod., 2008). Potrošniki vse več posegajo po živilih, ki so pridobljena iz naravnih surovin, vsebujejo potencialno koristne snovi in imajo posebne fiziološke koristi (Kaur in Singh, 2017). Žitni otrobi, stranski proizvodi pri predelavi riža, pšenice, ovsa, ječmena, sirka, prosa, rži in koruze, so pridobili ključno vlogo pri razvoju tovrstnih izdelkov (Patel, 2015).

2 POMEN ŽIT V PREHRANI

Žita so pomemben del vsakodnevne prehrane, saj bistveno prispevajo k vnosu ključnih hranil v človeško telo, zlasti kadar zaužijemo celotno zrno. V svetovnem merilu žitni izdelki predstavljajo glavni vir energije, pomemben vir proteinov, mineralov in vitaminov (Koistinen in Hanhineva, 2017; Oghbaei in Prakash, 2016). Večino zrn za potrebe industrije izpostavimo raznim tehnološkim postopkom kot so luščenje, brušenje, mletje in/ali sejanje, ki osiromašijo hranilno vrednost nastalega končnega produkta (Oghbaei in Prakash, 2016; Seetharaman in Abdel-Aal, 2014). Pšenica, riž in koruza so najpomembnejše rastline, katerih plodovi (semena) predstavljajo največji delež v prehrani človeka. Manj zastopana, a še vedno pomembna so oves, ječmen, rž, tritikala, sirek, proso (McKevith, 2004) in psevdožita ajda, amarant ter kvinoja (Alvarez-Jubete in sod., 2010).

Žitnim otrobom pripisujemo antiaterogene, antihipertenzivne in hipoglikemične lastnosti. Organizem ščitijo pred oksidativnim stresom, dajejo občutek sitosti in vplivajo na urejeno prebavo (Patel, 2015). Številne študije potrjujejo, da zadostno uživanje polnozrnatih živil pripomore k zmanjšani pojavnosti presnovnega sindroma (sladkorne bolezni tipa 2, debelosti, povišanega tlaka, povečane vsebnosti trigliceridov, itd.). Redno poseganje po tovrstnih izdelkih pomaga pri preprečevanju razvoja srčno-žilnih bolezni in vnetnih procesov (Belobrajdic in Bird, 2013; Borneo in León, 2012; Fardet, 2010).

3 BIOAKTIVNE KOMPONENTE ŽIT

Bioaktivne komponente so tiste spojine v živilih, ki so sposobne vplivati na metabolične procese v človeškem organizmu in tako prispevati k izboljšanemu zdravju posameznika. Med zaščitnimi fiziološkimi mehanizmi bioaktivnih komponent omenimo mehanski učinek (npr. predstavniki netopne vlaknine povečajo občutek sitosti, čas prehoda hrane skozi prebavni trakt in količino blata) in učinek na hormone (npr. Zn, Se in nikotinska kislina sodelujejo pri aktivaciji hormonov). Med njihove najkoristnejše lastnosti nadalje prištevamo antioksidativno (večina bioaktivnih komponent), protivnetno (npr. n-3 α-linolenska kislina; Cu in ferulna kislina) in antikarcinogeno delovanje (večina bioaktivnih komponent). Nekatere bioaktivne komponente so vključene v regulacijo genov (npr. flavonoidi), sporazumevanje celic (npr. nekatere fenolne spojine), spet druge inhibirajo ali inducirajo delovanje encimov (npr. nekateri minerali in elementi v sledovih). Omenjene mehanizme delovanja je v svojem obsežnem pregledu literature podrobneje predstavil Fardet (2010). Zavedati se je potrebno, da biološka razpoložljivost spojine lahko močno odstopa od njene biološke dostopnosti – prevladujoča bioaktivna komponenta v zaužitem živilu ni nujno tudi najbolj zastopan aktivni metabolit v tarčnem tkivu (Liu, 2007). Kljub številnim študijam, ki opisujejo biološke učinke bioaktivnih spojin *in vitro*, je poznavanje njihove absorpcije v človeškem organizmu še vedno precej okrnjeno (Heleno in sod., 2015).

Žitna zrna so vir številnih sekundarnih metabolitov, ki preprečujejo kronične bolezni oz. izboljšujejo zdravstveno stanje ljudi. Mednje prištevamo fenolne kisline, flavonoide, karotenoide, alkilresorcinole, avenantramide, tokoferole, fitosterole, lignane, organožveplove spojine in druge (Koistinen in Hanhineva, 2017). Gre za spojine z različno kemijsko zgradbo (razlikujemo hidrofilne ali lipofilne) ter razširjenostjo v naravi (bodisi so spojine specifične za skupino rastlin ali splošno razširjene). Spojina avenantramid je kemijsko fenolni alkaloid, ki je zelo razširjena in biološko aktivna, najbolj pa so poznani avenantramidi iz ovsa. Topne spojine z majhno molsko maso, se praviloma nahajajo v ovojnici zrna in predstavljajo glavni fenolni antioksidant omenjene vrste žita. Poleg dokazanega močnega antioksidativnega delovanja *in vitro* in *in vivo*, jim pripisujejo še protivnetno, anti-proliferativno in anti-iritativno delovanje. Zadostno uživanje avenantramidov zagotavlja dodatno zaščito pred srčno-žilnimi obolenji, rakom debelega črevesa in draženjem kože (Arendt in Zannini, 2013). Med spojine, ki so biološko učinkovite spadajo tudi antinutrienti kot so fitinska kislina, tanini, inhibitorji tripsina in drugi (Oghbaei in Prakash, 2016).

Poleg naštetih, velja opozoriti tudi na spojine, ki imajo v našem telesu koristno funkcijo, ne da bi se v telesu absorbirale. Primer so fitosteroli in β-glukani, ki v črevesju preprečujejo absorpcijo holesterola (Jesch in Carr, 2017; Ostlund in sod., 2003; Zhu in sod., 2016). Spet druge, primer so nekatere fenolne spojine, so podvržene razgradnji s strani mikrobiote, pri čemer nastali razgradnji produkti vplivajo tako na sestavo mikrobiote kot na biološko funkcijo, ki jo bodo ti imeli (Aura, 2008; Espín in sod., 2017; Selma in sod., 2009; Williamson in Clifford, 2017).

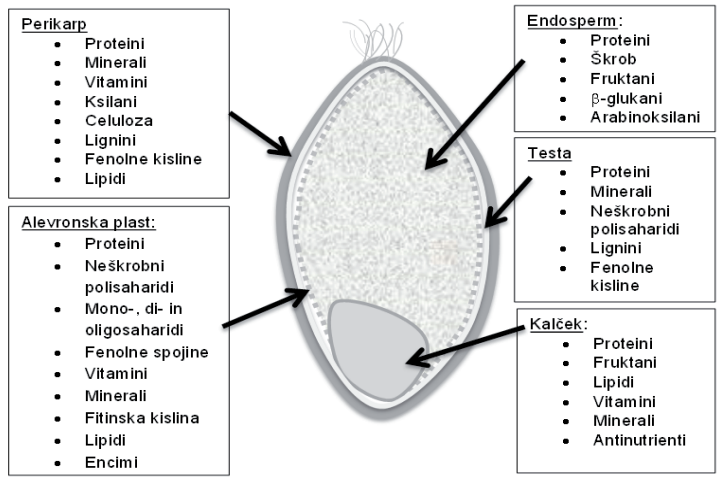
Preden lahko določeno spojino povežemo s pozitivnim delovanjem, je potrebno vedeti, koliko neke spojine je biološko dostopne in/ali razpoložljive v organizmu. Koncentracija bioaktivne spojine v prebavnem traktu je določena z njeno koncentracijo v živilu, z načinom priprave živila, količino zaužitega živila in biološko dostopnostjo spojine (Ribas-Agustí in sod., 2018). Kot primer vzemimo lignane. Njihov bogat vir sta laneno in sezamovo seme, ki ju povprečen človek dnevno zaužije relativno malo. Čeprav pšenica in rž na drugi strani vsebujeta precej manj lignanov, imata omenjeni žiti ključno vlogo pri njihovem vnosu v telo, saj teh semen zaužijemo precej več (Koistinen in Hanhineva, 2017).

4 ZGRADBA ŽITNEGA ZRNA IN RAZPOREDITEV BIOAKTIVNIH KOMPONENT V NJEM

Žitno zrno je posebno zgrajen plod iz družine trav (*Poaceae*), imenovan kariopsa. Sestoji iz semena, ki ga sestavljata endosperm in kalček ter večplastne ovojnice, ki ju gradita zrasli plodna (perikarp) in semenska ovojnica (testa) (Slika 1). Pri določenih vrstah, npr. pri ovsu, ječmenu, rižu, prosu itd. jo obdaja še dodatna plast, ki jo tvori predpleva. Pretežno škrobnati endosperm običajno prispeva k celotni masi zrna približno 75 – 85 %, medtem ko delež kalčka in zunanjih plasti variira glede na vrsto žita in sorto (Liu, 2007). Otrobi so zmes več zmletih plasti perikarpa, teste in alevronske plasti. V njih prevladuje prehranska vlaknina, ki jo glede na topnost v vodi razdelimo na topno (v stiku z vodo nastane raztopina) in netopno (v stiku z vodo se ne raztopijo). Hkrati otrobi predstavljajo pomemben vir vitaminov, mineralov in bioaktivnih spojin kot so alkilresorcinoli, ferulna kislina, flavonoidi, karotenoidi, lignani in steroli. Perikarp, ki se razdeli na zunanji in notranji del, je znan po veliki vsebnosti netopne vlaknine in vezanih fenolnih kislinah. Testa je bogata z alkilresorcinoli in steroli, pod njo se nahaja hialinska plast (ni prikazana na sliki). Alevronska plast, ki je sicer anatomsko že del endosperma, se smatra kot najbolj notranja plast ovojnice zrna, ki se pri običajni meljavi odstrani skupaj z ostalimi plastmi. Vsebuje velik delež lignanov in proteinov z uravnoteženo aminokislinsko sestavo, topno in netopno prehransko vlaknino, fitinsko kislino, fenolne kisline, karotenoide, lignane, antocianine, izoflavonoide, lipide, vitamine E in vitamine skupine B ter minerale. Poudariti velja, da se v alevronski plasti nahaja tudi precej hidrolitičnih encimov (amilaz, proteinaz, citolitičnih encimov itd.), ki imajo ključno vlogo med kaljenjem zrna. Kalček je bogat s proteini (vir žveplo vsebujočih aminokislin in glutationa), ogljikovimi hidrati (zlasti saharozo in rafinozo) in minerali. Vsebuje velik delež maščob (je pomemben vir α-linolenske kisline) in posledično v maščobah topnega vitamina E, ki je znan antioksidant. Glede na ostale dele zrna ima kalček največjo vsebnost vode, vendar koncentracija posameznih vodotopnih vitaminov ni nujno največja v kalčku. Uporabo kalčka za proizvodnjo pekovskih izdelkov omejuje njegova nestabilnost in prisotnost antinutrientov kot so rafinoza, fitinska kislina in aglutinin. Zadnji, največji del zrna predstavlja endosperm, čigar glavnino tvorijo škrobna zrnca, ki so obdana s proteini, predvsem glutenom. V celičnih stenah endosperma prevladujejo arabinoksilani, precej manj je β-glukanov, β-glukomanov in celuloze. Kljub temu, da slednje predstavljajo prehransko pomembne spojine, velja opozoriti, da jih napram otrobom, endosperm vsebuje relativno malo (Arendt in Zannini, 2013; Fardet, 2010; Brouns in sod., 2013; Onipe in sod., 2015).

Po kemijski sestavi v žitnih zrnih prevladujejo ogljikovi hidrati (cca. 60 - 75 %), sledijo proteini (cca. 10 - 15 %), voda (12 - 15 %) in lipidi (cca. 2 %). Glavnino mineralnih snovi predstavljajo kalijevi, magnezijevi in kalcijevi fosfati oz. sulfati. V manjših koncentracijah so prisotne še spojine železa, cinka, natrija. Med vitamini najdemo največ predstavnikov skupine B (tiamin, riboflavin, niacin) (Călinoiu in Vodnar, 2018; Fraś in sod., 2016; Ragaee in sod., 2006). Strukturni polisaharidi žit kot so rezistentni škrob, β-glukani, arabinoksilani, celuloza in fruktani se v prebavnem traktu ne razgradijo, zaradi česar jih uvrščamo med prehransko vlaknino (Gong in sod., 2018). Med prehransko vlaknino uvrščamo tudi ne-polisaharidno spojino lignin, ki prav tako predstavlja pomemben strukturni del celičnih sten.

Pšenično zrno vsebuje približno13 % prehranske vlaknine in najmanj 2 % drugih bioaktivnih komponent, kar skupaj predstavlja 15 % delež. Slednje je precej manj kot v otrobih, kjer skupni delež vlaknin in bioaktivnih komponent znaša več kot polovico celotne frakcije, s čimer zlahka pojasnimo njihov upad v prečiščenih žitih (Fardet, 2010). Večina pšenice se zmelje v moko in porabi za izdelavo raznoraznih pekovskih



Slika 1: Bioaktivne komponente znotraj pšeničnega zrna niso enakomerno razporejene, koncentrirane so v ovojnici zrna (skupek različnih plasti perikarpa, teste in alevronske plasti), zato prečiščena žita izgubijo glavnino zaščitnih spojin (povzeto po Arendt in Zannini (2013); Fardet (2010); Brouns in sod. (2013); Onipe in sod. (2015))
Figure 1: Bioactive compounds in wheat grain are unevenly distributed within its different parts, they are concentrated in outer layers of the kernel (pericarp, testa, aleurone layer), therefore the products that lack the bran and germ fraction have lost most of their protective compounds (summarized by Arendt and Zannini (2013); Fardet (2010); Brouns et al. (2013); Onipe et al. (2015))

proizvodov. Med mletjem se zaradi izboljšanja tehnoloških lastnosti in podaljšanja trajnosti končnega izdelka (moke) pšeničnemu zrnu odstrani otrobe in kalček. Posledično zmleto zrno praviloma vsebuje več škroba, a manj proteinov, mineralov in vitaminov. Prečiščena moka je nadalje osiromašena s topno (β-glukani, fruktani, rafinoza, stahioza itd.) in netopno vlaknino (ksilani, celuloza, lignini, hemiceluloza itd.). V zunanjih plasteh zrna in v kalčku se nahaja tudi večina fenolnih kislin in flavonoidov, medtem ko jih celične stene endosperma vsebujejo neprimerljivo manj (Fardet, 2010; Oghbaei in Prakash, 2016; Slavin in sod., 2000). Na spremenjeno hranilno vrednost lahko znatno vpliva že tako preprost postopek kot je frakcioniranje delcev s siti (Oghbaei in Prakash, 2016).

Uporaba prečiščene moke je tako razširjena predvsem na račun bistveno boljših tehnoloških lastnosti kot jo ima polnozrnata moka. Glavni stranski proizvod žitno predelovalne industrije, otrobi, tako predstavljajo neizkoriščen vir mikrohranil, prehranske vlaknine in raznih fitokemikalij. Številni raziskovalci se ukvarjajo z iskanjem ustrezne rešitve, kako otrobe kar najpogosteje vključiti v prehrano ljudi. Glavni izziv ostaja razvoj izdelka z izboljšano prehransko vrednostjo, ki hkrati ohranja tudi sprejemljivo senzorično in tehnološko kakovost (Le Bleis in sod., 2015; Patel, 2015).

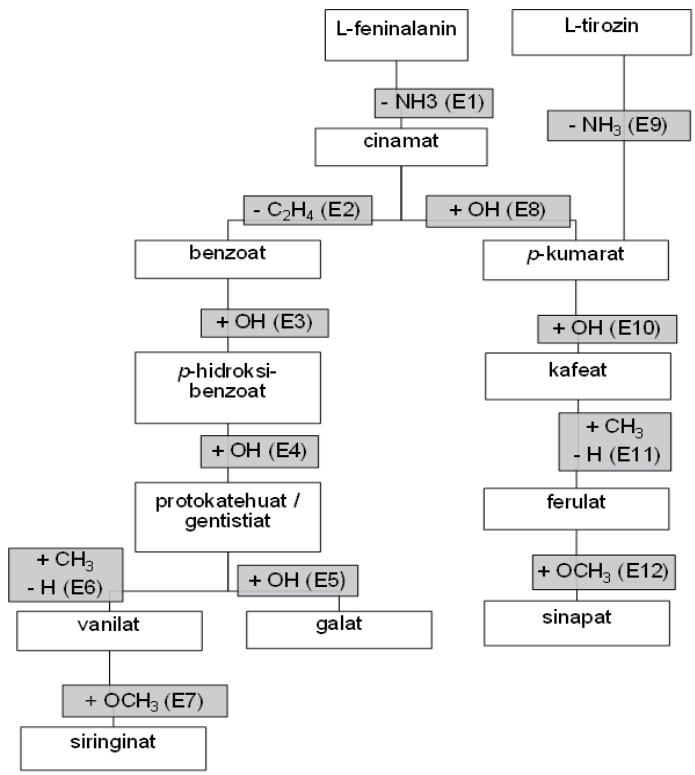
5 BIOSINTEZA FENOLNIH SPOJIN

Fenolne spojine so spojine z vsaj enim aromatskim obročem, na katerega je vezana (ena ali več) hidroksilna skupina. Mednje spadajo fenolne kisline (C6-C1 in C6-C3), flavonoidi (C6-C3-C6), stilbeni (C6-C2-C6), kumarini (C6-C3), tanini (hidrolizabilni in kondenzirani), lignani $(C6\text{-}C3)_2$ in lignini $(C6\text{-}C3)_n$. Fenolne spojine so sekundarni metaboliti rastlin s ključno vlogo pri razmnoževanju in rasti rastlin, delujejo kot obramba pred patogenimi organizmi, paraziti in plenilci, ponekod so odgovorni tudi za barvo rastlin (Liu, 2007). Fenolne spojine dajejo rastlinam grenak, oster, trpek okus in posledično zmanjšujejo sprejemljivost živil, ki so z njimi obogatena v večji meri. Nekatere fenolne spojine, predvsem tiste z veliko molsko maso, smatramo lahko celo za antinutriente; zaradi tvorbe kompleksov vplivajo na manjšo prebavljivost škroba, motijo absorpcijo proteinov ter zmanjšujejo dostopnost železa (Drewnowski in Gomez-Carneros, 2000). Naštete lastnosti vsekakor precej omejujejo njihovo uporabo pri razvoju funkcionalnih izdelkov.

Biosinteza fenolnih spojin v večji meri poteka na citoplazemski površini endoplazmatskega retikuluma. V pentoza-fosfatni poti nastane eritroza-4-fosfat, ki vstopa v reakcijo s fosfoenolpiruvatom. V zaporedju reakcij, imenovanih šikimatna pot, se šikimska kislina pretvori v dve aromatski aminokislini, fenilalanin in tirozin (Shahidi in Yeo, 2016). Glavni prekurzor fenolnih kislin, ki nastajajo po fenilpropanoidni poti je tako fenilalanin in v manjšem obsegu tirozin (Slika 2). Preklop aminokislin iz primarnega v sekundarni metabolizem je možen zaradi delovanja encima fenilalanin amonia liaze/tirozin amonia liaze (PAL / TAL). Pri tem se še

nič ne ve, zakaj in kdaj pride do take preusmeritve fenilalanina v biosintezo fenolnih spojin. Odcep amino skupine iz molekule fenilalanina in/ali tirozina privede do nastanka cimetne kisline. Osrednjo reakcijo deaminiranja katalizira PAL. V nadaljevanju sledi vezava hidroksilne (-OH) skupine na položaj 4 v aromatskem obroču, reakcijo katalizira encim cinamat-4-hidroksilaza (CAH, C4H). Slednje omogoča nastanek *p*-kumarne kisline (Petersen in sod., 2010). Njuna derivata, kavna in ferulna kislina, nastaneta z vezavo hidroksilne in metilne skupine na aromatski obroč *p*-kumarne kisline. Deaminiranje, hidroksiliranje in metiliranje so tako tri osrednje reakcije nastanka fenolnih kislin (Heleno in sod., 2015), vendar si raziskovalci niso povsem enotni o podrobnem poteku fenilpropanoidne poti. Čeprav v splošnem velja, da ferulna kislina nastane neposredno iz *p*-kumarne kisline, nekateri raziskovalci encimu *p*-kumarat-3-hidroksilaza pri delovanju *in vivo* pripisujejo večjo afiniteto do derivatov *p*-kumaroil estrov kot do *p*-kumarne kisline, s čimer hidroksiliranje slednje in njeno direktno pretvorbo v kavno kislino postavljajo pod vprašanje. Podobno naj bi imel encim kafeat-3-metil transferaza večjo preferenco do 5-hidroksiferulne kisline kot do kavne kisline (de Oliveira in sod., 2015).

Metabolično so hidroksicimetne kisline same po sebi bolj ali manj nereaktivne, za nastanek njihovih derivatov je potrebna predhodna aktivacija karboksilne skupine, ki jo katalizira encim *p*-kumarat-CoA ligaza (4CL). Omenjen encim lahko deluje na različno substituirane hidroksicimetne kisline, pri čemer se *p*-kumarna, kavna in ferulna smatrajo za dobre substrate, medtem ko sta cimetna in sinapinska kislina slabi ali celo zelo slaba substrata. Aktivacija hidroksicimetne kisline v tioester koencim A predstavlja izhodišče za številne



Slika 2: Biosintezna pot hidroksibenzojskih in hidroksicimetnih fenolnih kislin. Encimi: (E1) fenilalanin amonia liaza, (E2) oksidaza, (E3) benzoat- 4-hidroksilaza, (E4) *p*-hidroksibenzoat-3-hidroksilaza, (E5) protokatehuat- 5-hidroksilaza, (E6) protokatehuat-3-O-metiltransferaza, (E7) vanilat-5-hidroksilaza in vanilat-5-O-metiltransferaza, (E8) cinamat-4-hidroksilaza, (E9) tirozin amonia liaza, (E10) *p*-kumarat-3-hidroksilaza, (E11) kafeat-3-O-metiltransferaza, (E12) ferulat-5-hidroksilaza in kafeat/5-hidroksiferulat-O-metiltransferaza (povzeto po Heleno in sod. (2015))

Figure 2: Biosynthesis of hydroxybenzoic and hydroxycinnamic phenolic acids. Enzymes: (E1) Phenylalanine ammonia lyase, (E2) Oxidase, (E3) Benzoate-4-hydroxylase, (E4) *p*-hydroxybenzoate-3-hydroxylase, (E5) protocatechuate-5-hydroxylase, (E6) protocatechuate-3-O-methyltransferase, (E7) vanillate-5-hydroxylase and vanillate-5-O-methyltransferase, (E8) cinnamate-4-hydroxylase, (E9) tyrosine ammonia lyase, (E10) *p*-coumarate-3-hydroxylase, (E11) caffeate-3-O-methyltransferase, (E12) ferulate-5-hydroxylase and caffeate / 5-hydroxyferulate-O-methyltransferase (summarized by Heleno et al. (2015)).

druge spojine: flavonoide, izoflavonoide, stilbene itd. (Petersen in sod., 2010). Prekurzor druge skupine fenolnih kislin (ti. hidroksibenzojskih kislin) je benzojska kislina, ki nastane tako, da se stranska veriga cimetne kisline skrajša za dva ogljikova atoma (Slika 2). Podobno kot v primeru cimetne in *p*-kumarne kisline, lahko reakciji hidroksiliranja in metiliranja potečeta tudi na molekuli benzojske kisline, rezultat česar je nastanek ustreznih derivatov, tj. protokatehujske in *p*-hidroksibenzojske kisline (Heleno in sod., 2015). Transport nastalih fenolnih kislin z endoplazmatskega retikuluma do ostalih organelov v rastlinski celici poteka ali s pomočjo veziklov ali transmembranskih prenašalnih proteinov (Shahidi in Yeo, 2016).

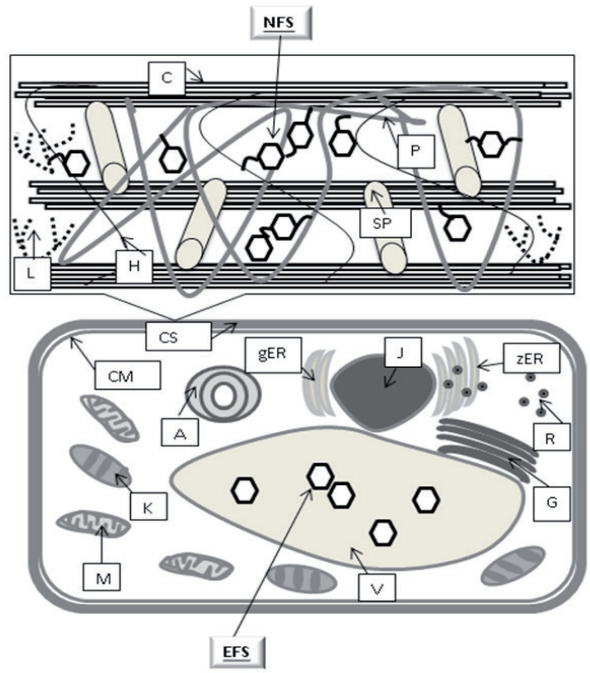
6 PROSTE, KONJUGIRANE IN VEZANE FENOLNE SPOJINE

Do nedavnega biološko aktivne spojine polnozrnatih pripravkov niso bile deležne tolikšne pozornosti kot tiste v sadju in zelenjavi, čeprav zadostno uživanje polnozrnatih proizvodov strokovnjaki že dlje časa povezujejo z zmanjšano obolelostjo za številnimi kroničnimi boleznimi sodobnega časa. Raziskave zadnjih let (Liu, 2007) so pokazale, da je vsebnost skupnih bioaktivnih komponent in antioksidativna aktivnost polnozrnatih izdelkov/živil pogosto podcenjena, še zlasti če upoštevamo, kolikokrat dnevno se izdelki iz žit znajdejo na naših jedilnikih. Eden glavnih vzrokov je ta, da so v preteklosti raziskave vključevale zgolj proste in le šibko vezane spojine, ki so med ekstrakcijo bolj ali manj uspešno prešle v vodno raztopino metanola, etanola ali acetona, medtem ko večina vezanih fenolnih spojin ni bila niti določena v trdnem preostanku po ekstrakciji. Izkazalo se je, da te postanejo dostopne šele po bolj specifični obdelavi rastlinskega materiala (Adom in Liu, 2002; Naczk in Shahidi, 2006).

Fenolne spojine se v rastlinski celici torej ne nahajajo zgolj v prosti obliki, ampak lahko tvorijo tudi topne konjugate in netopno vezane fenolne spojine. Kot pove že samo ime, se proste fenolne spojine nahajajo v prosti, tj. nevezani obliki; konjugirane topne fenolne spojine so vezane na topne molekule z majhno molsko maso (ogljikove hidrate, proteine, lipide) in so ujete v vakuoli (Acosta-Estrada in sod., 2014; Shahidi in Yeo, 2016). Pri nastanku topnih konjugatov v žitih pride običajno do tvorbe kovalentnih vezi (estrske in etrske vezi), lahko pa so ti posledica tudi nastalih vodikovih vezi in hidrofobnih interakcij med različnimi molekulami (Xu in sod., 2019). V rižu, koruzi, pšenici in prosu so v frakciji topnih konjugatov določili bistveno več ferulne kot *p*-kumarne kisline; med monosaharidi pa so v spremenjenih deležih prevladovale arabinoza, ksiloza in glukoza (Rao in Muralikrishna, 2004). Proste fenolne spojine in topne konjugate tako uvrščamo med ekstraktibilne fenolne spojine, vezane ali netopne fenolne spojine pa med neekstraktibilne (Pérez-Jiménez in sod., 2013; Xu in sod., 2019). Tvorba topnih konjugatov vpliva na topnost fenolnih spojin ter še na nekatere druge pomembne lastnosti. Topni ogljikovi hidrati so spojine z več hidroksilnimi skupinami, ki posledično povečajo hidrofilnost pripetih fenolnih spojin, zlasti flavonoidov. Slednje vodi v njihovo izboljšano biorazpoložljivost. Paucar-Menacho in sod. (2017) so v koruzi določili flavonoide konjugirane predvsem z monosaharidi (primeri: kvercetin-*O*-glukozid, kamferol-O-glukozid) in disaharidi (primeri: kvercetin-3-*O*-rutinozid, izoramnetin-3-*O*-rutinozid). Nadalje je možna vzpostavitev estrskih, vodikovih ali elektrostatskih vezi tudi med fenolno spojino in oligosaharidi oz. polisaharidi. Polisaharidi iz vrst topne prehranske vlaknine ujamejo in zaščitijo fenolne spojine. Tako nastali konjugat zaščiti fenolno spojino pred drugimi oksidanti in svetlobo. Poleg tega so topni polisaharidi donorji vodika in tako pripomorejo k boljšemu antioksidativnemu delovanju konjugiranih fenolnih spojin. Fenolne spojine v obliki topnih konjugatov so torej dokazano učinkovitejše kot so njihove pripadajoče hidrolizirane oblike (Xu in sod., 2019).

Zadnjo, količinsko najbolj zastopano obliko, predstavljajo netopno vezane fenolne spojine, ki so na različne načine vključene v celično steno (Slika 3). Lahko so s kovalentno vezjo pritrjene na gradnike celične stene kot so pektin, celuloza, hemiceluloza ali strukturni proteini (Shahidi in Yeo, 2016). Funkcionalna skupina fenolne kisline, ki sodeluje pri nastanku etrske vezi z ligninom je hidroksilna skupina, pripeta na aromatski obroč. Kadar pa kisline vstopajo v reakcijo s strukturnimi proteini ali neškrobnimi polisaharidi preko karboksilne skupine, govorimo o nastanku estrske vezi (Acosta-Estrada in sod., 2014). Pri sintezi netopne fenolne spojine je možen tudi nastanek C-C vezi (C-glikozidi). Netopne fenolne spojine imajo pomembno vlogo pri vzdrževanju integritete celične stene, zagotavljajo tako fizično kot kemijsko bariero, ščitijo pred vdorom patogenih organizmov, s svojo značilno trpkostjo pa odvračajo insekte in živali pred zaužitjem (Shahidi in Yeo, 2016).

Številni literaturni viri potrjujejo dejstvo, da prosta oblika predstavlja zgolj manjši delež vseh prisotnih fenolnih kislin v žitih, te se tako pretežno nahajajo v vezani obliki (kot topne ali netopne): 85 % v koruzi, 76 % v pšenici, 75 % v ovsu in 62 % v rižu. Rjavi riž vsebuje približno 88 % fenolnih spojin vezanih, ječmen pa med 55 % in 89 %, odvisno od sorte (Acosta-Estrada



Slika 3: Razporeditev ekstraktibilnih (EFS) in neekstraktibilnih (NFS) fenolnih spojin v rastlinski celici. Zgradba celice: (V) vakuola, (M) mitohondrij, (K) kloroplast, (A) amiloplast, (J) jedro, (R) ribosom, (gER) gladki endoplazmatski retikulum, (zER) zrnati endoplazmatski retikulum, (G) golgijev aparat, (CM) celična membrana, (CS) celična stena. Strukturni elementi celične stene: (C) celuloza, (H) hemiceluloza, (SP) strukturni protein, (L) lignin, (P) pektin

Figure 3: Distribution of extractable (EFS) and non-extractable (NFS) phenolic compounds in a plant cell. Cell structure: (V) vacuole, (M) mitochondria, (K) chloroplast, (A) amyloplast, (J) nucleus, (R) ribosome, (gER) smooth endoplasmic reticulum, (zER) granular endoplasmic reticulum, (G) Golgi apparatus, (CM) cell membrane, (CS) cell wall. Cell wall structural elements: (C) cellulose, (H) hemicellulose, (SP) structural protein, (L) lignin, (P) pectin.

in sod., 2014). Uživanje žitnih proizvodov tako pripomore k vnosu vezanih fenolnih spojin le pri uporabi celega zrna ali otrobov. V raziskavi, ki so jo pred leti izvedli Mattila in sod. (2005) so primerjali vsebnost fenolnih spojin v različnih žitih in njihovih izdelkih. Največjo vsebnost skupnih fenolnih spojin so po hidrolizi vzorca kvantitativno določili s HPLC v otrobih pšenice (4527 mg kg^{-1}) in rži (4190 mg kg^{-1}) ter v polnozrnati moki iz teh semen (1342 oz. 1366 mg kg^{-1}). V prečiščeni pšenični moki so določili le še zanemarljiv delež fenolnih spojin (167 mg kg^{-1}).

Ferulna kislina je prevladujoča fenolna kislina v žitnih zrnih. V 100 g suhe snovi pšeničnega zrna najdemo približno 8 – 20 mg ferulne kisline; ta lahko predstavlja vse do 90 % skupnih fenolnih spojin. Ferulna kislina se nahaja predvsem v zunanjih plasteh zrna. Alevronska plast in perikarp zajemata 98 % celotne količine ferulne kisline, zato ne preseneča, da je njena vsebnost v moki v tesni korelaciji z mletjem in sejanjem (Oghbaei in Prakash, 2016). V pšenici se ferulna kislina skorajda vsa nahaja v vezani obliki, podobno je tudi pri koruzi, ovsu in rižu (Adom in Liu, 2002). Običajno je pritrjena na hemicelulozo, kjer prevladuje tvorba estrske vezi med ferulno kislino in arabinoksilanom. Ferulna kislina, ki tvori estrsko vez z arabinoznimi ostanki arabinoksilana (s hidroksilno skupino na mestu C-5), se lahko hkrati poveže tudi z molekulo lignina preko etrske vezi. Nadalje je možen nastanek dimera ferulnih kislin dveh sosednjih arabinoksilanov; takšna prečna zamreženost blokira delovanje hidrolaz, kar zmanjša učinkovitost encimske razgradnje in s tem prebavljivost polisaharidov celične stene. Prepletenost celične stene z estri ferulne kisline (oz. njenimi dimeri) in arabinoksilanom ter ligninom vpliva na številne lastnosti celične stene kot so adherenca, raztegljivost, dostopnost in biorazgradljivost (Oliveira in sod., 2019). Ferulna kislina je poznana po zaviranju bolezni, ki so posledica oksidativnega stresa. Boz (2015) v svojem pregledu literature navaja, da je spojina sposobna nevtralizirati različne proste radikale, ki bi sicer povzročili oksidativne poškodbe celične membrane in DNA. Po antioksidativni učinkovitosti je ferulna kislina primerljiva z ostalimi fenolnimi kislinami, njen potencial je odvisen od izbire *in vitro* testa, zlasti učinkovito se je izkazala pri lovl-

jenju alkilperoksilnih radikalov v emulziji (Terpinc in Abramovič, 2010). Med njene koristne učinke prištevamo tudi uravnavanje strjevanja krvi, zniževanje nivoja holesterola in trigliceridov ter posledično manjše tveganje za razvoj srčno-žilnih bolezni (Boz, 2015). Nadalje de Oliveira Silva in Batista (2017) v svojemu članku bralcu predstavita protimikrobno in protivnetno delovanje ferulne kisline, njeno zaščitno vlogo pri razvoju sladkorne bolezni in različnih rakavih obolenj, vse več raziskav pa zadostno uživanje ferulne kisline povezuje s preprečevanjem in lajšanjem nevrodegenerativnih bolezni, predvsem Parkinsonove in Alzheimerjeve bolezni ter depresije.

7 METABOLIZEM VEZANIH FENOLNIH SPOJIN

Bioaktivne lastnosti fenolnih spojin so precej dobro raziskane predvsem *in vitro*. Številne študije, ki se ukvarjajo z njihovo biološko učinkovitostjo, ignorirajo vprašanje njihove dostopne koncentracije kot tudi presnovno obliko, v kateri fenolne spojine dejansko krožijo znotraj krvnega obtoka. Ko pride do zaužitja enostavnih fenolnih spojin, ki so v prosti obliki, se te v tankem črevesju hitro absorbirajo. V tankem črevesu in jetrih so podvržene konjugiranju, proces povzroči številne spremembe v njihovi prvotni strukturi. Reakcije glukoronidiranja, metiliranja ali sulfatiranja tako privedejo do nastanka ustreznih derivatov fenolnih kislin. Strukturne spremembe bodisi izboljšajo bodisi zmanjšajo biološko aktivnost zaužitih fenolnih spojin (Heleno in sod., 2015).

Omenjen proces ni pomemben samo z vidika detoksifikacije, nastanek ustreznih derivatov hkrati poveča hidrofilnost izhodnih molekul, ki se tako z žolčem ali urinom lažje odstranijo iz telesa. Zaradi učinkovitih mehanizmov konjugacije, so aglikoni prisotni v krvi v zelo majhnih koncentracijah. Transport konjugiranih derivatov fenolnih spojin po krvi poteka s pomočjo albuminov. V dvanajstniku so izpostavljeni delovanju bakterijskih encimov, zlasti β-glukoronidaz. Po tem koraku lahko pride do njihove ponovne absorpcije, kar vodi do daljše prisotnosti fenolnih spojin v telesu (Heleno in sod., 2015).

Ferulna kislina in ostale hidroksicimetne kisline, ki so vezane na celično steno, niso dostopne človeškim encimom prebavnega trakta, ampak njihovo razgradnjo katalizirajo encimi črevesne mikroflore, zlasti ksilanaze in esteraze. Tako so vezane fenolne spojine po zaužitju v prebavnem traktu človeka podvržene številnim encimskim reakcijam, ki spremenijo njihove fizikalne in kemijske lastnosti. Fenolne spojine se sprostijo iz živila s pomočjo encimsko katalizirane hidrolize v želodcu kot tudi kasneje med fermentacijo vlakninskega matriksa v debelem črevesu. Močno na sam proces vplivajo pH razmere v prebavnem traktu. Človeško telo lahko absorbira zgolj 5 – 10 % vseh fenolnih spojin, ki so po večini monomeri ali dimeri. Zanemarljiv del sproščenih fenolnih spojin iz vezanih oblik se podobno, kot to velja za proste fenolne spojine, tako absorbira že v tankem črevesu, čemur sledi tvorba konjugatov z drugimi spojinami, da lahko preidejo v krvni obtok. Preostalih 90 – 95 % fenolnih spojin (oligomeri, polimeri) je rezistentnih na encimsko razgradnjo v zgornjem delu prebavil in dosežejo debelo črevo v intaktni obliki. Tu so podvrženi fermentaciji s strani bifidobakterij in mlečnokislinskih bakterij, ki so del naravne črevesne mikrobiote. Omenjeni mikroorganizmi izločajo najrazličnejše ekstracelularne encime, ki so sposobni razgraditi celično steno in/ali hidrolizirati kovalentne vezi vezanih fenolnih spojin. Tako sproščene fenolne spojine naj bi posredno preko fermentacije pomembno prispevale k znižanju pH in posledično k zaviranju rasti mikroorganizmov, ki inducirajo raka. Tiste fenolne spojine, ki niso fermentabilne oz. se ne morejo absorbirati, naj bi kazale protimikrobno in antioksidativno delovanje in tako zavirale rast patogenih bakterij. Po drugi strani pa so prisotni mikroorganizmi sposobni pretvoriti nekatere fenolne spojine v aktivnejše spojine, ki se lažje absorbirajo (Călinoiu in Vodnar, 2018; Gong in sod., 2018). Metabolizem fenolnih kislin s strani mlečnokislinskih bakterij je povezan z delovanjem dekarbosilaz in reduktaz. Prva skupina encimov omogoča dekarboksiliranje hidroksibenzojske ali hidroksicimetne kisline in s tem nastanek njihovih ustreznih 4-vinil derivatov, reduktaze pa so odgovorne za hidrogeniranje dvojne vezi. Opisane transformacije so specifična lastnost posameznega seva; sevi, ki so sposobni pretvoriti ferulno kislino v 4-vinilgvajakol, niso nujno zmožni pretvarjati tudi drugih hidroksicimetnih kislin. Lahko pa različni sevi omogočajo nastanek drugačnih metabolitov iz istega substrata (Gänzle, 2014).

8 ZAKLJUČEK

Pretežni del zdravju koristnih spojin je v žitnem zrnu koncentriran v zunanjih plasteh zrna, ki se jih med proizvodnjo prečiščene (bele) moke odstrani. Številne raziskave preteklih let pričajo o pomembnosti uživanja polnozrnatih žitnih izdelkov oz. otrobov. Slednji so pomembni z vidika vnosa številnih bioaktivnih komponent, tudi fenolnih spojin. Med najpomembnejšimi predstavniki velja omeniti ferulno kislino, ki se v žitih skoraj vsa nahaja v vezani obliki. Absorpcija vezanih

fenolnih kislin je v primerjavi z njihovo prosto obliko precej otežena. Pretežni del vezanih fenolnih spojin se ne more absorbirati v tankem črevesu, ampak preidejo v debelo črevo, kjer jih prisotni mikroorganizmi fermentirajo in tako delno sprostijo iz celičnih sten. Številni raziskovalci podpirajo teorijo, da je pozitiven učinek uživanja polnozrnatih žit omogočen preko dveh mehanizmov: (1) otrobi kot vir prebiotikov (fruktanov, rafinoze, stahioze) uravnavajo sestavo in aktivnost črevesne mikrobiote ter odpravljajo ekološko neravnotežje (2) črevesna mikrobiota je sposobna pretvoriti določene neškrobne polisaharide in fenolne spojine iz otrobov v metabolite, ki imajo lahko tudi boljšo biološko aktivnost kot zaužite spojine. Vsekakor so za lažje razumevanje ključne vloge vezanih fenolnih spojin, ki jih ti imajo pri uravnavanju našega zdravja, potrebne nadaljnje raziskave in sodelovanje strokovnjakov različnih ved.

9 VIRI

Acosta-Estrada, B. A., Gutiérrez-Uribe, J. A., Serna-Saldívar, S. O. (2014). Bound phenolics in foods, a review. *Food Chemistry, 152*, 46-55. https://doi.org/10.1016/j.foodchem.2013.11.093

Adom, K. K. & Liu, R. H. (2002). Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry, 50*(21), 6182-6187. https://doi.org/10.1021/jf0205099

Alvarez-Jubete, L., Arendt, E. K., Gallagher, E. (2010). Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients. *Trends in Food Science & Technology, 21*(2), 106-113. https://doi.org/10.1016/j.tifs.2009.10.014

Arendt, E. K. & Zannini, E. (2013). *Cereals grains for the food and beverage industries*. Cambridge, Woodhead Publishing Ltd.

Aura, A.-M. 2008. Microbial metabolism of dietary phenolic compounds in the colon. *Phytochemistry Reviews, 7*(3), 407-429. https://doi.org/10.1007/s11101-008-9095-3

Belobrajdic, D. P. & Bird, A. R. (2013). The potential role of phytochemicals in wholegrain cereals for the prevention of type-2 diabetes. *Nutrition Journal, 12*(1), 62. https://doi.org/10.1186/1475-2891-12-62

Borneo, R., León, A. E. (2012). Whole grain cereals: functional components and health benefits. *Food & Function, 3*(2), 110-119. https://doi.org/10.1039/c1fo10165j

Boz, H. (2015). Ferulic acid in cereals -- a Review. *Czech Journal of Food Science, 33*(1), 1-7. https://doi.org/10.17221/401/2014-cjfs

Brouns, F. J. P. H., van Buul, V. J., Shewry, P. R. (2013). Does wheat make us fat and sick? *Journal of Cereal Science, 58*(2), 209-215. https://doi.org/10.1016/j.jcs.2013.06.002

Călinoiu, F. L., Vodnar, C. D. (2018). Whole grains and phenolic acids: A review on bioactivity, functionality, health benefits and bioavailability. *Nutrients, 10*(11). https://doi.org/10.3390/nu10111615

de Oliveira, D. M., Finger-Teixeira, A., Rodrigues Mota, T., Salvado,r V. H., Moreira-Vilar, F. C., Correa Molinari, H. B., Craig Mitchell, R. A., Marchiosi, R., Ferrarese-Filho, O., Dantas dos Santos, W. (2015). Ferulic acid: a key component in grass lignocellulose recalcitrance to hydrolysis. *Plant Biotechnology Journal, 13*(9), 1224-1232. https://doi.org/10.1111/pbi.12292

de Oliveira Silva, E. & Batista, R. (2017). Ferulic acid and naturally occurring compounds bearing a feruloyl moiety: A review on their structures, occurrence, and potential health benefits. *Comprehensive Reviews in Food Science and Food Safety, 16*(4), 580-616. https://doi.org/10.1111/1541-4337.12266

Drewnowski A., Gomez-Carneros C. (2000). Bitter taste, phytonutrients, and the consumer: a review. *The American Journal of Clinical Nutrition, 72*(6), 1424-1435. https://doi.org/10.1093/ajcn/72.6.1424

Espín, J. C., González-Sarrías, A., Tomás-Barberán, F. A. (2017). The gut microbiota: A key factor in the therapeutic effects of (poly)phenols. *Biochemical Pharmacology, 139*, 82-93. https://doi.org/10.1016/j.bcp.2017.04.033

Fardet, A. (2010). New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? *Nutrition Research Reviews, 23*(1), 65-134. https://doi.org/10.1017/s0954422410000041

Fraś A., Gołębiewska K., Gołębiewski D., Mańkowski D. R., Boros D., Szecówka P. 2016. Variability in the chemical composition of triticale grain, flour and bread. *Journal of Cereal Science, 71*, 66-72. https://doi.org/10.1016/j.jcs.2016.06.016

Gänzle, M. G. (2014). Enzymatic and bacterial conversions during sourdough fermentation. *Food Microbiology, 37*, 2-10. https://doi.org/10.1016/j.fm.2013.04.007

Gong, L., Cao, W., Chi, H., Wang, J., Zhang, H., Liu, J., Sun, B. (2018). Whole cereal grains and potential health effects: Involvement of the gut microbiota. *Food Research International, 103*, 84-102. https://doi.org/10.1016/j.foodres.2017.10.025

Heleno, S. A., Martins, A., Queiroz, M. J. R. P., Ferreira, I. C. F. R. (2015). Bioactivity of phenolic acids: Metabolites versus parent compounds: A review. *Food Chemistry, 173*, 501-513. https://doi.org/10.1016/j.foodchem.2014.10.057

Jesch, E. D., Carr, T. P. (2017). Food ingredients that inhibit cholesterol absorption. *Preventive nutrition and food science, 22*(2), 67-80. https://doi.org/10.3746/pnf.2017.22.2.67

Kaur, N. & Singh, D. P. (2017). Deciphering the consumer behaviour facets of functional foods: A literature review. *Appetite, 112*, 167-187. https://doi.org/10.1016/j.appet.2017.01.033

Koistinen, V. M. & Hanhineva, K. (2017). Mass spectrometry-based analysis of whole-grain phytochemicals. *Critical Reviews in Food Science and Nutrition, 57*(8), 1688-1709. https://doi.org/10.1080/10408398.2015.1016477

Le Bleis, F., Chaunier, L., Chiron, H., Della Valle, G., Saulnier, L. (2015). Rheological properties of wheat flour dough and French bread enriched with wheat bran. *Journal of Cereal Science, 65*, 167-174. https://doi.org/10.1016/j.jcs.2015.06.014

Liu, R. H.(2007). Whole grain phytochemicals and health.

Journal of Cereal Science, 46(3), 207-219. https://doi.org/10.1016/j.jcs.2007.06.010

Mattila, P., Pihlava, J.-M., Hellström, J. (2005). Contents of phenolic acids, alkyl- and alkenylresorcinols, and avenanthramides in commercial grain products. *Journal of Agricultural and Food Chemistry, 53*(21), 8290-8295. https://doi.org/10.1021/jf051437z

McKevith, B. (2004). Nutritional aspects of cereals. *Nutrition Bulletin, 29*(2), 111-142. https://doi.org/10.1111/j.1467-3010.2004.00418.x

Menrad, K. (2003). Market and marketing of functional food in Europe. *Journal of Food Engineering, 56*(2), 181-188. https://doi.org/10.1016/S0260-8774(02)00247-9

Naczk, M. & Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis, 41*(5), 1523-1542. https://doi.org/10.1016/j.jpba.2006.04.002

Oghbaei, M., Prakash, J. (2016). Effect of primary processing of cereals and legumes on its nutritional quality: A comprehensive review. *Cogent Food & Agriculture, 2*(1), 1136015. https://doi.org/10.1080/23311932.2015.1136015

Oliveira, D. M., Mota, T. R., Oliva, B., Segato, F., Marchiosi, R., Ferrarese-Filho, O., Faulds, C. B., dos Santos, W. D. (2019). Feruloyl esterases: Biocatalysts to overcome biomass recalcitrance and for the production of bioactive compounds. *Bioresource Technology, 278*, 408-423. https://doi.org/10.1016/j.biortech.2019.01.064

Onipe, O. O., Jideani, A. I. O., Beswa, D. (2015). Composition and functionality of wheat bran and its application in some cereal food products. *International Journal of Food Science & Technology, 50*(12), 2509-2518. https://doi.org/10.1111/ijfs.12935

Ostlund, J. R. E., Racette, S. B., Stenson, W. F. (2003). Inhibition of cholesterol absorption by phytosterol-replete wheat germ compared with phytosterol-depleted wheat germ. *The American Journal of Clinical Nutrition, 77*(6), 1385-1389. https://doi.org/10.1093/ajcn/77.6.1385

Patel, S. (2015). Cereal bran fortified-functional foods for obesity and diabetes management: Triumphs, hurdles and possibilities. *Journal of Functional Foods, 14*, 255-269. https://doi.org/10.1016/j.jff.2015.02.010

Paucar-Menacho, L. M., Martínez-Villaluenga, C., Dueñas, M., Frias, J., Peñas, E. (2017). Optimization of germination time and temperature to maximize the content of bioactive compounds and the antioxidant activity of purple corn (*Zea mays* L.) by response surface methodology. *LWT - Food Science and Technology, 76*, 236-244. https://doi.org/10.1016/j.lwt.2016.07.064

Pérez-Jiménez, J., Díaz-Rubio, M. E., Saura-Calixto, F. (2013). Non-extractable polyphenols, a major dietary antioxidant: occurrence, metabolic fate and health effects. *Nutrition Research Reviews, 26*(2), 118-129. https://doi.org/10.1017/s0954422413000097

Petersen, M., Hans, J., Matern, U. (2010). Biosynthesis of phenylpropanoids and related compounds. V M. Wink (Ur.), *Annual Plant review,s Biochemistry of Plant Secondary Metabolism, Second edition* (str. 182-257).Singapore: Blackwell Publishing, Ltd. https://doi.org/10.1002/9781444320503.ch4

Ragaee, S., Abdel-Aal, E.-S. M., Noaman, M. (2006). Antioxidant activity and nutrient composition of selected cereals for food use. *Food Chemistry, 98*(1), 32-38. https://doi.org/10.1016/j.foodchem.2005.04.039

Rao, R. S. P. & Muralikrishna, G. (2004). Non-starch polysaccharide–phenolic acid complexes from native and germinated cereals and millet. *Food Chemistry, 84*(4), 527-531. https://doi.org/10.1016/S0308-8146(03)00274-7

Ribas-Agustí, A., Martín-Belloso, O., Soliva-Fortuny, R., Elez-Martínez, P. (2018). Food processing strategies to enhance phenolic compounds bioaccessibility and bioavailability in plant-based foods. *Critical Reviews in Food Science and Nutrition, 58*(15), 2531-2548. https://doi.org/10.1080/10408398.2017.1331200

Seetharaman, K. & Abdel-Aal, E.-S. M. (2014). The impact of milling and thermal processing on phenolic compounds in cereal grains AU - Ragaee, Sanaa. *Critical Reviews in Food Science and Nutrition, 54*(7), 837-849. https://doi.org/10.1080/10408398.2011.610906

Selma, M. V., Espín, J. C., Tomás-Barberán, F. A. (2009). Interaction between phenolics and gut microbiota: Role in human health. *Journal of Agricultural and Food Chemistry, 57*(15), 6485-6501. https://doi.org/10.1021/jf902107d

Shahidi F. & Yeo J. 2016. Insoluble-bound phenolics in food. *Molecules, 21*(9), 1216. https://doi: 10.3390/molecules21091216

Siró, I., Kápolna, E., Kápolna, B., Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance—A review. *Appetite, 51*(3), 456-467. https://doi.org/10.1016/j.appet.2008.05.060

Slavin, J. L., Jacobs, D., Marquart, L. 2000. Grain processing and nutrition. *Critical Reviews in Food Science and Nutrition, 40*(4), 309-326. https://doi.org/10.1080/10408690091189176

Terpinc, P. & Abramovič, H. (2010). A kinetic approach for evaluation of the antioxidant activity of selected phenolic acids. *Food Chemistry,* 121(2), 366-371. https://doi.org/10.1016/j.foodchem.2009.12.037

Williamson, G. & Clifford, M. N. (2017). Role of the small intestine, colon and microbiota in determining the metabolic fate of polyphenols. *Biochemical Pharmacology, 139*, 24-39. https://doi.org/10.1016/j.bcp.2017.03.012

Xu, M., Rao, J., Chen, B. (2019). Phenolic compounds in germinated cereal and pulse seeds: Classification, transformation, and metabolic process. *Critical Reviews in Food Science and Nutrition*, 1-20. https://doi.org/10.1080/10408398.2018.1550051

Zhu, F., Du, B., Xu, B. (2016). A critical review on production and industrial applications of beta-glucans. *Food Hydrocolloids, 52*, 275-288. https://doi.org/10.1016/j.foodhyd.2015.07.003

doi:10.14720/aas.2019.114.2.13 Review article / pregledni znanstveni članek

Vezane fenolne spojine polnozrnatih žitnih pripravkov kot sestavina funkcionalnih živil: drugi del

Petra TERPINC [1]

Received September 9, 2019; accepted December 2, 2019.
Delo je prispelo 09. septembra 2019, sprejeto 02. decembra 2019.

Vezane fenolne spojine polnozrnatih žitnih pripravkov kot sestavina funkcionalnih živil: drugi del

Izvleček: Hrana na bazi žit bistveno prispeva k vnosu energije v človeško telo, saj se pogosto znajde na naših jedilnikih, medtem ko polnozrnata živila iz žit pripomorejo k večjemu vnosu mikronutrientov kot, če ta uživamo v prečiščeni obliki. Vezane fenolne spojine, ki so nakopičeni v otrobih žit, se transformirajo v dvanajstniku, v debelem črevesu pa jih prisotna mikrobiota v procesu fermentacije pretvori v metabolite, ki se lahko absorbirajo. V drugem delu je predstavljena analiza fenolnih spojin, s poudarkom na hidrolizi in ekstrakciji vezanih fenolnih spojin, njihovi kvantifikaciji ter identifikaciji. Ker slaba biorazpoložljivost neekstraktibilnih fenolnih spojin kritično omejuje izkoriščanje njihovega širokega potenciala, se v članku dotaknemo tudi tehnik in novih strategij, s katerimi je možno sprostiti fenolne spojine iz netopno vezanih oblik z obdelavo živil. Pobližje se spoznamo z dvema trenutno zelo aktualnima pristopoma, kaljenjem in fermentacijo. S transformacijo vezanih fenolnih spojin v lažje dostopne proste fenolne spojine, lahko poleg ugodnega antikancerogenega učinka na debelo črevo, izkoristimo tudi njihovo antioksidativno in protimikrobno učinkovitost. Zavedanje potrošnikov in njihovo povpraševanje po zdravi hrani je privedlo do zahtev po vključevanju naravnih sestavin v proizvodnjo izdelkov z dodano vrednostjo. Pridobivanje ferulne kisline iz polnozrnatih pripravkov žit in njihova vključitev v funkcionalne prehrambene proizvode je vsekakor pomembno raziskovalno področje v prihodnosti.

Ključne besede: polnozrnati žitni izdelki; hidroliza vezanih fenolnih spojin; biorazpoložljivost fenolnih spojin; kaljenje; fermentacija; funkcionalna živila

Bound phenolic compounds of whole cereal grains as a functional food component: part two

Abstract: Since they are eaten regularly, cereals based food make a significant contribution to the daily energy intake, meanwhile in whole-grain form they contribute to higher micronutrients intake that refined cereal products. The bound phenolic compounds, which are accumulated in cereal bran, play a key role in the duodenum, where they are transformed to the absorbable metabolites by microbial fermentation. In part two, an analysis of phenolic compounds is presented, with emphasis on the hydrolysis and extraction procedure for bound phenolic compounds, their quantification and identification. Due to poor bioavailability of non-extractable phenolic compounds, which critically limits the exploitation of their wide potential, the article also discusses techniques and new strategies that enable the release of phenolic compounds from insoluble bound forms during food processing. Two current approaches, germination and fermentation, are presented in more details. With transformation of bound phenolic compounds to more easily accessible free phenolic compounds, we also benefit from their antioxidant and antimicrobial efficacy in addition to a favorable anti-cancer effect on the colon. The awareness of consumers and their demand for healthier foods led to the exploration and incorporation of natural ingredients in the production of value added products. The extraction of ferulic acid from whole grain cereal products and its incorporation in functional food products is definitely an important area of future research.

Key words: whole-grain cereal products; hydrolysis of bound phenolic compounds; bioavailability of phenolic compounds; germination; fermentation; functional foods

1 Univerza v Ljubljani, Biotehniška fakulteta, Jamnikarjeva 101, SI-1000 Ljubljana, e-mail: petra.terpinc@bf.uni-lj.si

1 ANALIZA FENOLNIH KISLIN

Glede na različno naravo fenolnih kislin, ki jih najdemo v žitih, je pomembno izbrati metodo, ki v največji možni meri zajame vse tarčne molekule. Analiza fenolnih kislin sestoji iz več korakov, postopek običajno vključuje ekstrakcijo, hidrolizo, čiščenje oz. izolacijo, identifikacijo in kvantifikacijo. V Preglednici 1 so navedene posamezne fenolne kisline in njihova razširjenost med žiti in psevdožiti.

Ne glede na vrsto žita, je eden ključnih korakov homogenizacija vzorca. Ta zagotavlja reprezentativen vzorec (ustrezen delež endosperma, kalčka in zunanjih plasti), po drugi strani pa le zadostna homogenizacija omogoča dobro ekstraktibilnost in analitično ponovljivost. Zmanjšanje velikosti delcev vpliva na prenos mase. Z namenom zagotavljanja čim večje kontaktne površine med tarčnimi molekulami in ekstrakcijskim topilom, se v literaturi največkrat omenja mletje, redkeje pa drobljenje v možnarju ali uporaba stiskalnice (Domínguez-Rodríguez in sod., 2017). Pred homogenizacijo je vzorec priporočljivo stabilizirati s tekočim dušikom (inaktivacija encimov, ohranjanje fenolnih spojin), pa tudi sicer je pomembno, da se analiza izvede čim hitreje po zaključeni homogenizaciji. Obstajajo številni dokazi, da na izplen fenolnih spojin v veliki meri vplivajo razmere ekstrakcije, med drugim izbira ekstrakcijskega topila (Gunenc in sod., 2015), način ekstrakcije (Kumar in sod., 2016), čas in temperatura ekstrakcije (Wang in sod., 2008). Domínguez-Rodríguez in sod. (2017) opozarjajo, da je potrebno v vzorcu upoštevati tudi vsebnost vode. Sušenje vzorca lahko povzroči kontrakcijo celic, kar potencialno ovira ekstrakcijo analita iz notranjosti celice. Zato je vpijanje vode in nabrekanje celic do določene mere zaželeno, vendar je prisotnost vode sočasno v tesni korelaciji z zmanjšano stabilnostjo vzorca (nastale spremembe so posledica kemijskih in encimskih reakcij). Sušenje na zraku je dolgotrajen postopek, ki zahteva relativno visoke temperature, kar lahko vodi v pretvorbe fenolnih spojin. Dobro alternativo, ki zagotavlja maksimalno dobit in ohranitev fenolnih spojin, predstavljata sušenje z zamrzovanjem (liofilizacija) in vakuumsko sušenje.

Ekstrakcijske tehnike delimo na konvencionalne in napredne. Prvi sklop je osnovan na principu solventne ekstrakcije, tj. uporabi organskih topil (primeri: metanol, etanol, aceton) ali mešanici topil (metanol/voda, nakisan metanol/voda, aceton/voda, …) (Domínguez-Rodríguez in sod., 2017). Zaradi več hidroksilnih skupin (ki so polarne) in aromatskega obroča (ki je nepolaren), je večina fenolnih spojin, ki na svoj osnovni skelet nimajo pripete dodatne molekule, dobro topna v polarnih in srednje nepolarnih topilih (voda/aceton/metanol in dietileter/etilacetat). Iz tega razloga jih lahko opredelimo kot topne proste fenolne spojine. Topni konjugati sestojijo iz fenolne spojine in polarnega dela (v žitih najpogosteje sladkorja), ki poveča topnost fenolnih spojin v polarnih topilih. Posledično lahko tudi topne konjugate ekstrahiramo s polarnimi topili kot so voda, metanol, aceton oz. jih očistimo prostih

Preglednica 1: Razdelitev fenolnih kislin in njihova prisotnost v zrnih žit in psevdožit (povzeto po Irakli in sod. (2012a); Wang in sod. (2014); Călinoiu in Vodnar (2018); Van Hung (2016); Gawlik-Dziki in sod. (2012); Tang in sod. (2016); Dykes in Rooney (2007); Mir in sod. (2018)).

Table 1: Classification of phenolic acids and their presence in cereal and pseudocereal grains (summarized by Irakli et al. (2012a); Wang et al. (2014); Călinoiu and Vodnar (2018); Van Hung (2016); Gawlik-Dziki et al. (2012); Tang et al. (2016); Dykes and Rooney (2007); Mir et al. (2018)).

	Fenolna kislina	Žito	Psevdožito
Hidroksibenzojske	Galna	Riž, proso, oves, pšenica, pira, ječmen, rž, koruza, tritikala	Amarant, ajda, kvinoja
	Gentistična	Proso, pšenica	
	p-Hidroksibenzojska	Ječmen, oves, riž, proso, pšenica, pira, koruza, rž, tritikala	Amarant, ajda, kvinoja
	Protokatehuična	Riž, proso, rž, ječmen, oves, koruza, pšenica, pira, tritikala	Amarant, kvinoja
	Salicilna	Pšenica, pira, ječmen, oves, tritikala	
	Siringična	Pšenica, pira, koruza, oves, proso, ječmen, rž, riž, tritikala	Amarant, kvinoja
	Vanilinska	Ječmen, oves, proso, pšenica, pira, rž, koruza, riž, tritikala	Amarant, ajda, kvinoja
Hidroksicimetne	Cimetna	Proso, pšenica, oves	Kvinoja
	Ferulna	Koruza, pšenica, pira, ječmen, rž, proso, riž, oves, tritikala	Ajda, kvinoja
	Kavna	Oves, riž, ječmen, pšenica, pira, koruza, proso, rž, tritikala	Ajda, kvinoja
	p-Kumarna	Koruza, pšenica, pira, ječmen, rž, proso, riž, oves, tritikala	Amarant, ajda, kvinoja
	Sinapinska	Rž, riž, pšenica, pira, koruza, ječmen, proso, oves, tritikala	Ajda, kvinoja

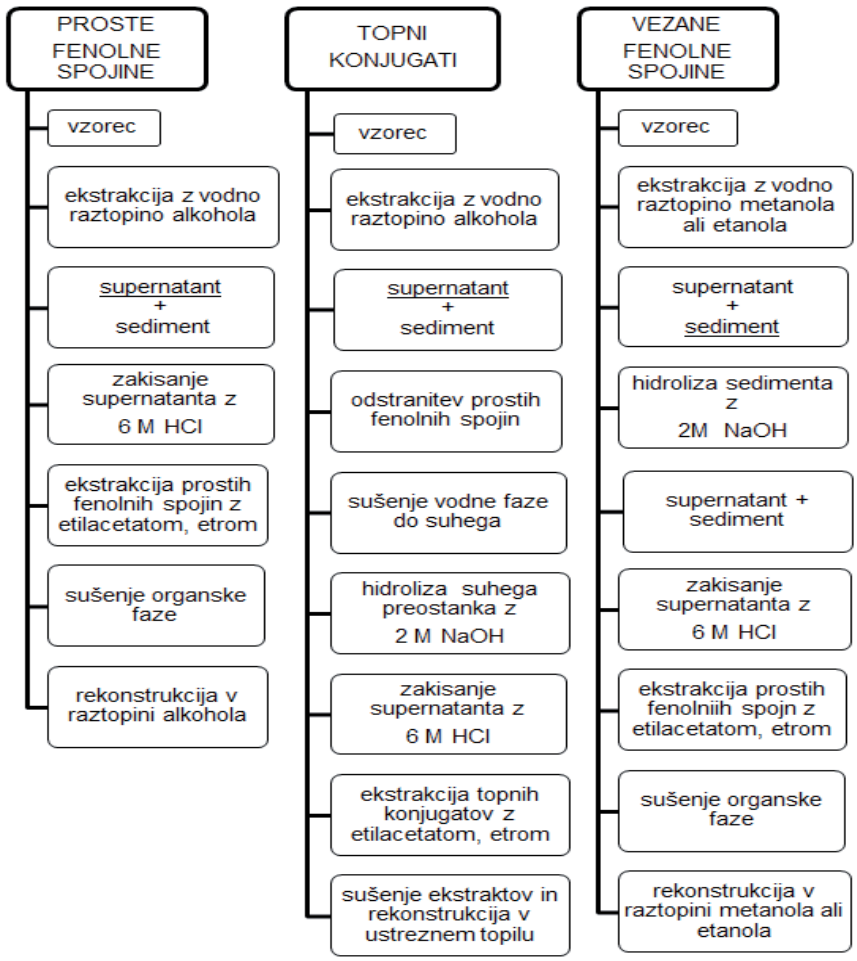
fenolnih spojin s pomočjo dietiletra/etilacetata (Xu in sod., 2019). Ekstrakcijo trdno-tekoče (kadar je matriks v trdni obliki) ali tekoče-tekoče (kadar je vzorec tekoč) lahko uporabimo tudi kot fazo čiščenja za odstranitev ekstraktibilnih fenolnih spojin. Če želimo v vzorcu določiti netopno vezane fenolne spojine, ga moramo najprej z opisanim postopkom očistiti prostih fenolnih spojin in topnih konjugatov (Domínguez-Rodríguez in sod., 2017).

V zadnjem desetletju konvencionalne ekstrakcijske metode vse pogosteje nadomeščajo sodobne tehnike, ki imajo številne prednosti: so hitrejše, avtomatizirane, bolj ponovljive in selektivne, zagotavljajo boljši izkoristek ekstrakcije, zahtevajo manjšo poraba toksičnih topil oz. vključujejo topila, ki so za okolje manj škodljiva. Mednje uvrščamo uporabo ultrazvoka, mikrovalov, pulzirajočega elektromagnetnega polja, encimov, visokega hidrostatičnega tlaka, tekočin pod pritiskom in superkritičnih tekočin (Dahmoune in sod., 2014; Das in sod., 2017; Kumar in sod., 2016). Po uporabi omenjenih tehnik, netopne fenolne spojine praviloma še vedno ostajajo vezane na celični matriks (Domínguez-Rodríguez in sod., 2017). Slabo topni polimeri kot so celuloza, arabinoksilan, hemiceluloza ali kompleks polisaharid-protein, onemogočajo topnost vezanih fenolnih spojin v vodi in organskih topilih, zato jih pojmujemo tudi kot netopne ali neekstraktibilne fenolne spojine. Fenolne kisline bolj ali manj uspešno sprostimo iz vezane oblike, če vzorec obdelamo z bazo, kislino ali encimi, pri čemer je izplen odvisen od razmer hidrolize (Acosta-Estrada in sod., 2014; Arranz in Saura Calixto, 2010; Kim in sod., 2006; Verma in sod., 2009). V povezavi s prekinitvijo glikozidnih in estrskih vezi, ki jih fenolne spojine najpogosteje tvorijo v žitnih zrnih, se v dosedanjih raziskavah največkrat omenjata alkalna in kislinska hidroliza. Eden izmed možnih pristopov za analizo prostih, konjugiranih in netopno vezanih fenolnih spojin je prikazan na Sliki 1 in podrobneje predstavljen v eni naših prejšnjih raziskav (Terpinc in sod., 2011a). Hidrolizi (alkalni, kislinski, kombinirani), ki sprosti topne in netopno vezane fenolne spojine, običajno sledi ekstrakcija hidroliziranih molekul s pomočjo etilacetata, dietiletra ali zmesi etilacetata in etra (v razmerju 1:1).

Kislinska hidroliza povzroči razpad glikozidnih vezi in sprosti se sladkorni del, medtem ko običajno na estrske vezi nima vpliva (Domínguez-Rodríguez in sod., 2017). Različni raziskovalci (Chen in sod., 2014; Kim in sod., 2006) poročajo, da vodi kislinska hidroliza pri povišanih temperaturah do izgub določenih fenolnih spojin, tvorbe furfurala in njegovih derivatov. Na drugi strani lahko z alkalno hidrolizo sprostimo fenolne spojine iz polisaharidov tako, da razcepimo estrske vezi. Obdelava vzorca z različnimi koncentracijami natrijevega hidroksida se je izkazala kot učinkovit način sproščanja omenjenih tarčnih molekul iz riža (Irakli in sod., 2018), pšenice (Zhang in sod., 2018), ovsa in ječmena (Gangopadhyay in sod., 2015). Postopek naj ne bi vodil v kemijsko modifikacijo fenolnih spojin (Domínguez-Rodríguez in sod., 2017). Tako kislinska kot alkalna hidroliza sta nespecifični in lahko privedeta do strukturnih sprememb na fenolnih spojinah, zaradi česar ne moremo sklepati o strukturi izhodnih spojin v netretiranem vzorcu. Bolj specifična, netoksična in učinkovita metoda vključuje uporabo encimov: glukoronidaz, pektinaz, celulaz, hemicelulaz, glukanaz, tanaz in amilaz. Kljub številnim prednostim, je uporaba encimov za razcep glikozidnih vezi in razgradnjo celične stene slabo razširjena v praksi, predvsem na račun njihove omejene uporabe. Encimi za svoje delovanje običajno potrebujejo mile pogoje delovanja (pH, temperatura in tlak). K manjšemu izplenu netopne frakcije pripomore tudi velik delež celuloze in lignina v celičnih stenah rastlin. Eden učinkovitejših pristopov je kombinacija različnih načinov hidrolize. Alves in sod. (2016) so ugotovili, da predhodno tretiranje vzorca z α-amilazami (15 min obdelava pri 37 °C) zmanjša viskoznost hidrolizata (alkalna hidroliza je prav tako potekala pri 37 °C) in tako za več kot trikrat poveča ekstraktibilnost vezanih fenolnih spojin v primerjavi s klasično alkalno hidrolizo pri sobni temperaturi, oz. je izplen primerljiv z alkalno hidrolizo pri 70 °C. Čeprav velja kemijska hidroliza za učinkovit pristop študije vezanih fenolnih spojin, te metode običajno vključujejo visoke temperature in ostre alkalne ali kisle razmere. Ti kot taki ne odražajo realnega stanja v človeškem telesu (Acosta-Estrada in sod., 2014), zato je relevantnost dobljenih rezultatov vprašljiva.

Določitev prostih in vezanih fenolnih spojin v žitih običajno zahteva dodaten korak, ki je namenjen odstranitvi interferenc in koncentriranju vzorcev. Z ekstrakcijo na trdnem nosilcu (SPE) lahko iz žitnih zrn učinkovito odstranimo sladkorje in druge relativno polarne spojine: organske kisline, aminokisline, proteine. Številni poskusi optimizacije omogočajo velike izkoristke in dobro ponovljivost (Irakli in sod., 2012a, 2012b).

Najbolj razširjen način za ločitev in karakterizacijo posameznih fenolnih spojin je HPLC. Čeprav je znotraj omenjene tehnike možna uporaba številnih kolon, se večina analitikov odloči za uporabo kolone z reverzno fazo C16 ali C18, ki izboljša separacijo analitov. Izbira mobilne faze lahko variira, vendar ta običajno vključuje gradientno spreminjanje razmerja med organsko in vodno fazo. Slednji se z namenom učinkovitejše ločbe in resolucije kromatografskih vrhov pogosto doda ocetno ali mravljično kislino. Še nedolgo tega so za prvo izbiro



Slika 1: Protokol za analizo različnih oblik fenolnih spojin:. v prvi stopnji (stolpec levo) iz vzorca z ekstrakcijskim topilom (običajno z vodno raztopino alkohola) pridobimo ekstraktibilne fenolne spojine. Za uspešen prehod prostih fenolnih spojin iz bolj v manj polarno topilo (običajno dietileter in/ali etilacetat) je potrebno zagotoviti dovolj nizek pH (ker je topnost tarčnih fenolnih kislin v posamezni fazi odvisna od njihove pKa vrednosti), čemur sledi rekonstrukcija prostih fenolnih spojin v ustreznem topilu za nadaljnje analize. Po odstranitvi prostih fenolnih spojin se v vodni raztopini alkohola (supernatantu) nahajajo topni konjugati, ki jih podvržemo hidrolizi (stolpec na sredini), čemur sledi zakisanje hidrolizata in ekstrakcija sproščenih topno vezanih fenolnih spojin z organskim topilom. Prav tako je hidroliza potrebna v primeru netopno vezanih fenolnih spojin (stolpec desno). Slednjo izvedemo tako, da trdemu preostanku po ekstrakciji dodamo NaOH, in nadaljujemo s protokolom za ekstrakcijo prostih fenolnih spojin.

Figure 1: Protocol for the analysis of different forms of phenolic compounds: in the first step (left column), extractable phenolic compounds are obtained from the sample with an extraction solvent (usually an aqueous alcoholic solution). A successful transition of free phenolic compounds from more to less polar solvent (usually diethyl ether and/or ethyl acetate) requires a sufficiently low pH (since the solubility of the target phenolic acids depends on their pKa value), followed by the reconstruction of free phenolic compounds in appropriate solvent for further analysis. After removal of free phenolic compounds, soluble conjugates are present in the aqueous alcoholic solution (supernatant) and subjected to hydrolysis (middle column), followed by acidification of the hydrolyzate and extraction of the released soluble phenolic compounds with an organic solvent. Hydrolysis is also required in the case of insoluble phenolic compounds (right column). The latter is carried out by the addition of NaOH to the solid residue after extraction, followed by the protocol for the extraction of free phenolic compounds.

organske faze veljala topila kot so propanol, butanol ali etilacetat, ki pa so jih v zadnjem času v veliki meri nadomestila predvsem metanol ali acetonitril (Koistinen in Hanhineva, 2017).

HPLC instrumenti so pogosto sklopljeni z UV/Vis ali DAD detektorjem. Fenolne kisline v žitnih zrnih absorbirajo svetlobo različnih valovnih dolžin, odvisno od strukture molekule. Za derivate benzojske kisline je

to območje med 200 – 290 nm, za spojine, ki izhajajo iz cimetne kisline pa 270 – 360 nm. Ta tip detekcije ne pove ničesar o strukturi posameznih spojin. Zaradi boljše občutljivosti, širšega območja detekcije in večje natančnosti, je UV-VIS detektor v veliki meri nadomestila masna spektroskopija (MS) (Koistinen in Hanhineva, 2017). Med najpogostejšimi načini ionizacije se omenja elektrosprej ionizacijo (ESI), ki pride v poštev za detekcijo toplotno občutljivih analitov z majhno in srednjo molsko maso. Ta pristop omogoča razlikovanje med različnimi fenolnimi spojinami in daje informacijo o položaju glikozidne vezi, glavno pomanjkljivost pa predstavlja pomanjkanje avtentičnih standardov (Domínguez-Rodríguez in sod., 2017). Strukturo vezanih fenolnih spojin lahko določimo tudi z uporabo jedrske magnetne resonance (NMR) (Anokwuru in sod., 2018; Wang in sod., 2015), ki ima številne prednosti pred MS, vendar se NMR v praksi zaradi visokih stroškov opreme in omejenega števila študij teh spojin, v ta namen uporablja relativno redko.

2 SPROSTITEV VEZANIH FENOLNIH SPOJIN Z OBDELAVO ŽIVIL

Medtem, ko nekatera semena stročnic in riž uživamo cela, večino žitnih zrn predelamo v moko. S tem vplivamo na matriks, tj. na okolico, v katero so hranila vgrajena znotraj zrna, posledično pa na samo razpoložljivost teh komponent (Oghbaei in Prakash, 2016). Nadalje je potrebno upoštevati način, kako je zrno vključeno v živilo, kot intaktno ali v zmleti obliki. Musa-Veloso in sod. (2018) so ugotovili, da je imelo uživanje kruha narejenega iz polnozrnate moke na dvig glukoze v krvi podobne posledice kot kruh iz bele, tj. prečiščene moke, medtem ko je dodatek celih zrn bistveno zmanjšal glikemični indeks.

Obdelava živil je eden ključnih korakov, ki določa kakšna bo biološka razpoložljivost in dostopnost bioaktivnih komponent. Na eni strani lahko obdelava živil vodi v zmanjšanje vsebnosti fenolnih spojin, hkrati pa obstajajo številni načini s katerimi olajšamo cepitev vezi, povečamo količino osvobojenih vezanih fenolnih spojin in izboljšamo njihovo absorpcijo v prebavilih (Călinoiu in Vodnar, 2018). Različni pristopi temeljijo na zmanjšanju velikosti delcev, razgradnji rastlinskega materiala in/ali razgradnji prehranske vlaknine. Struktura celičnega matriksa in pa način, kako je fenolna spojina vezana v ta matriks, pomembno vplivata na njeno fiziološko funkcijo (Ribas-Agustí in sod., 2018; Wang in sod., 2015). V sklopu mehanske obdelave omenimo mletje, drobljenje in brušenje. Pristop je osnovan na povečanju specifične površine delcev, ki ekstrakcijskemu topilu omogoča lažji dostop do fenolnih spojin. V to skupino spada tudi mikrofluidizacija, ki temelji na homogenizaciji matriksa pod visokim tlakom brez uporabe organskih topil. Tudi tu pride do znatnega zmanjšanja redukcije velikosti delcev, pri čemer ti ekspandirajo, kar razrahlja njihovo trdno strukturo in tako olajša ekstrakcijo fenolnih spojin. Naslednjo kategorijo predstavlja toplotna obdelava, ki vključuje tehnike kot so parjenje, avtoklaviranje, sušenje in praženje. Predpostavlja se, da avtoklaviranje, zahvaljujoč visokim temperaturam in tlaku ter hitremu zmanjševanju tlaka ob zaključku procesa, omogoči sprostitev vezanih fenolnih spojin na račun porušene strukture celične stene in delne hidrolize prehranske vlaknine. Toplotno obdelavo običajno spremlja tvorba novih spojin (npr. produktov Maillardove reakcije) z znatno antioksidativno učinkovitostjo. Eno novejših tehnik predstavlja tudi kuhanje z ekstrudiranjem, ki lahko, prav tako kot neustrezna toplotna obdelava, povzroči razpad termolabilnih molekul. Omenjeni proces prekine integriteto celične stene in kovalentne vezi v fenolnih kompleksih z veliko molsko maso se razcepijo. Med različnimi načini transformacije vezanih fenolnih spojin, velja izpostaviti biološko transformacijo. Fermentacija in kaljenje trenutno predstavlja enega najbolj aktualnih področij v sklopu tovrstnih raziskav.

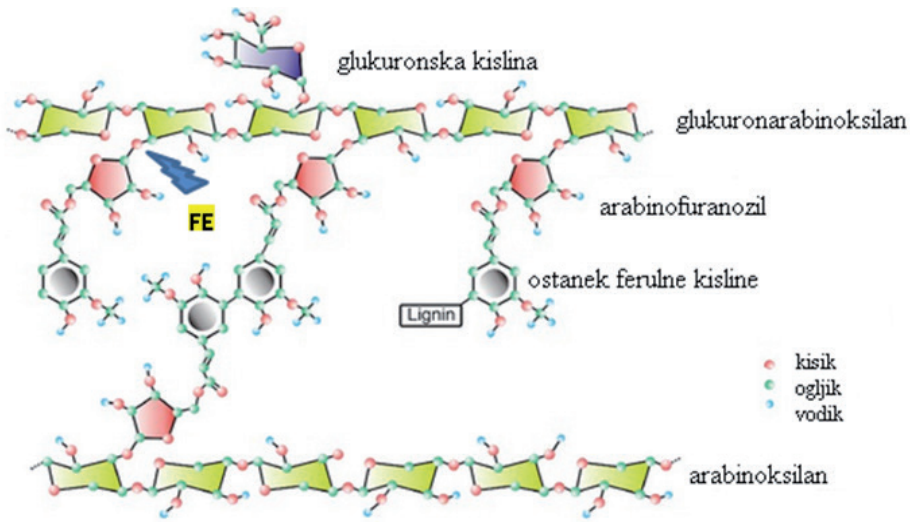
Kaljenje se že stoletja uporablja z namenom mehčanja strukture zrna, izboljšanja hranilne vrednosti in zmanjševanja antinutritivnih komponent. Proces kaljenja vpliva na okus, aromo, stabilnost in prebavljivost zrn (Singh in sod., 2015). Primarni cilj je spodbuditi razvoj hidrolitičnih encimov, sintezo novih in aktivacijo tistih, ki so v nekaljenem zrnu v neaktivni obliki. Biosintezne poti v kalečem zrnu vodijo do strukturnih sprememb obstoječih spojin kot tudi do sinteze novih, bioaktivno pomembnih molekul. Xu (2019) povzema, da se med kaljenjem z vidika metabolizma fenolnih snovi odvijajo trije procesi. Prvič, v celici se vrši sinteza fenolnih spojin iz glukoze ali aromatskih aminokislin. Pentozno-fosfatna pot, glikolitična in šikimatna pot zagotovijo prekurzorje, ki je največkrat fenilalanin. Ta se v citosolu pretvori v fenolne kisline, te pa v endoplazmatskem retikulumu naprej v flavonoide, stilbene, kumarine. Nastale molekule lahko v nadaljevanju polimerizirajo ali se vežejo z drugimi makromolekulami (polisaharidi, proteini, lipidi), ki jih celica tako vezane skladišči v celični steni ali vakuoli. Drugič, hidrolitični encimi med kaljenjem razgrajujejo makrohranila in posledično sproščajo fenolne spojine iz vezanih oblik. Tretjič, celica fenolne spojine potroši, ko ti lovijo proste radikale ali služijo kot intermediati signalnih molekul.

Znano je, da razmere namakanja (Xu in sod., 2009; Yang, 2001), temperatura kaljenja (Chavarín-Martínez

in sod., 2019; Paucar-Menacho in sod., 2017), čas kaljenja (Kim in sod., 2018; Paucar-Menacho in sod., 2017) ter način osvetljevanja (Xiang in sod., 2017) značilno vplivajo na fenolni profil kaljenih žitnih zrn. V eni naših prejšnjih raziskav (Terpinc in sod., 2016) smo med drugim ugotavljali vpliv razmer kaljenja na fenolni profil dveh sort ajde. Daljše kaljenje je vodilo v večjo vsebnost skupnih fenolnih spojin. Presenetljivo smo največji relativni prirast opazili pri izoorientinu, orientinu in izoviteksinu, medtem ko se je vsebnost najbolj zastopane fenolne spojine, rutina, relativno malo spremenila. Biološka aktivacija zrn vpliva na vsebnost skupnih fenolnih spojin tako v kalčkih kot v preostalemu delu kalečega zrna, pri čemer je vpliv na ekstraktibilne fenolne spojine drugačen kot na vezane fenolne spojine (Gan in sod., 2017). Žal se številne raziskave zadnjih let (Boubakri in sod., 2017; Falcinelli in sod., 2017; Niroula in sod., 2019) še vedno osredotočajo na ekstraktibilne fenolne komponente, čeprav imajo tudi v kaljenih žitnih zrnih glavno vlogo vezane fenolne spojine (Chen in sod., 2017; Hung in sod., 2011; Ti in sod., 2014). V začetnih fazah kalitve poteka razgradnja makromolekul, tj. proteinov, ogljikovih hidratov, lipidov, kar vodi v porast njihovih presnovnih produktov, tj. prostih aminokislin, enostavnih sladkorjev in organskih kislin. Aktivnost hidrolitičnih encimov omogoča sprostitev fenolnih spojin iz celične stene, kar v začetni fazi pomeni upad vezane frakcije in porast proste (Hübner in Arendt, 2013). Alvarez-Jubete in sod. (2010) so primerjali vsebnost ekstraktibilnih fenolnih spojin v kaljenih zrnih ajde, amaranta, kvinoje in pšenice in zabeležili dvakratno povečanje proste frakcije v ajdi, pšenici in kvinoji, kar za štirikrat pa se je njihova vsebnost povečala v amarantu. Kot že omenjeno, v kalčku poleg primarnih metabolitov med kaljenjem nastajajo tudi sekundarni metaboliti, vključno s fenolnimi spojinami. Ti se nato vgrajujejo v celične stene novo nastalih rastlinskih celic, kar pri starejših kalčkih vodi v ponoven porast vezanih fenolnih spojin in upad proste oblike (Gan in sod., 2017; Singh in Sharma, 2017). Da različne razmere kaljenja značilno vplivajo na razmerje med prostimi in vezanimi fenolnimi spojinami smo potrdili tudi z lastnimi raziskavami (Krek, 2018; Golob, 2018). Biosinteza in transformacija fenolnih spojin lahko nadalje zavisi tudi od stresnih razmer med kaljenjem (Chen in sod., 2019; Ma in sod., 2019), niso pa vse spojine enako dovzetne za tovrstne spremembe. Hung in sod. (2011) v svoji raziskavi navajajo, da je kaljenje pšenice vodilo v porast derivatov hidroksibenzojske kisline, medtem ko se je vsebnost derivatov hidroksicimetne kisline zmanjšala. H kopičenju vezanih fenolnih snovi je najbolj prispevala ferulna kislina, med prostimi fenolnimi spojinami pa siringična kislina. Med kaljenjem v zrnih žit poleg fenolnih kislin nastajajo tudi druge fenolne spojine z biološko učinkovitostjo. Ti in sod. (2014) poročajo o spremenjeni vsebnosti tako prostih kot vezanih flavonoidov v primerjavi z nekaljenim rižem. Nenazadnje, kot povzema Oghbaei in Prakash (2016), številne študije povezujejo kaljenje in slajenje žit z izboljšano prehransko vrednostjo, prebavljivostjo in lažjo dostopnostjo hranil ter manjšo vsebnostjo nekaterih antinutrientov.

Podobno kot kaljenje, tudi fermentacija spremlja človeka že zelo dolgo časa. Mehanizem, s katerim povečamo biorazpoložljivost in dostopnost nevezanih fenolnih spojin s pomočjo fermentacije vključuje tako encime iz žitnih zrn kot mikroorganizmov. Predpostavlja se, da encimi lahko katalizirajo razgradnjo celične stene, poleg tega pa naj bi proces fermentacije zajemal tako sintezo novih spojin kot tudi encimsko transformacijo različnih že prisotnih bioaktivnih komponent. Učinkovitost te tehnike (podobno kot pri ostalih) zavisi od vrste žita, vrste mikroorganizma in razmer same fermentacije (zlasti temperature, časa, pH) (Wang in sod., 2014). Kot že omenjeno, je ferulna kislina v glavnem vezana na arabinoksilane. Nadalje lahko pride tudi do kovalentnih povezav med dvema ferulnima kislinama, ki sta pritrjeni na sosednja arabinoksilana (Slika 2), ali do tvorbe med ostankom ferulne kisline in tirozinom. Encim, ki katalizira cepitev te vezi (kot tudi sprostitev ostalih hidroksicimetnih kislin iz vezanih oblik), se imenuje feruloil esteraza (tudi ferulna kislina esteraza). Uspešno so ga izolirali iz številnih plesni in bakterij, njeno aktivnost pa so zaznali celo v ječmenu in prosu; tako raznolik izvor daje feruloil esterazam zelo širok spekter optimalnih razmer delovanja ((pH 3.0 – 10.0) in (T 20 – 75 °C)). Viri navajajo, da encim postane aktiven med kaljenjem, njegova aktivnost med kaljenjem postopoma upada, njegovo delovanje pa upočasni nizek pH (3,5) in povišana temperatura. Številne raziskave v zadnjih letih potrjujejo smiselnost uporabe feruloil esteraz za ekstrakcijo bioaktivnih komponent (Gänzle, 2014; Faulds, 2010; Oliveira in sod., 2019).

Prisotnost omenjenega encima, ki katalizira razpad estrske vezi med ferulno kislino in sladkorjem so potrdili tudi v laktobacilih, ki so naravno prisotni v našem črevesju. O znatnem porastu skupnih prostih fenolnih spojin poročajo v primeru fermentacije pšeničnih otrobov (Moore in sod., 2007). Avtorji so neodvisno od seva kvasovk določili največji relativni prirast siringinske, manjši *p*-kumarne in najmanjši ferulne kisline. Hkrati pa je bil vpliv omenjenih kvasovk na vsebnost vanilinske kisline izrazito negativen, kar nakazuje na različno zmožnost presnavljanja različnih fenolnih kislin v vezani obliki. Zaporedna uporaba kaljenja in fermentacije lahko biorazpoložljivost vezanih fenolnih spojin še dodatno izboljša. V eni izmed takšnih razi-



Slika 2: Celična stena je bogata z arabinoksilani. Osnovno ogrodje predstavlja veriga ksilana, ki je razvejana z arabinozo ali glukoronsko kislino. Arabinozni ostanki so pogosto zaestreni s s ferulno kislino, feruloilni estri polisaharidov lahko oksidativno polimerizirajo, kar vodi v nastanek dimerov in oligomerov, ki prečno povežejo bližnje glukoarabinoksilane; sprostitev ferulne kisline omogoča encim feruloil esteraza (FE) (prirejeno po de Oliveira in sod. (2015)).
Figure 2: Arabinoxylans are abundant in the cell wall. They are composed by a core chain of xylan branched with arabinose and glucuronic acid. The arabinose residues are often esterified with feruloyl residues, feruloyl esterified to polysaccharides can further oxidatively polymerize to produce dehydrodimer and oligomers that cross–link vicinal glucuronoarabinoxylan; ferulic acid can be released by the action of an enzyme feruloyl esterase (FE) (adapted by de Oliveira in sod. (2015)).

skav (Katina in sod., 2007) so z uporabo kvasovk (*Saccharomyces cerevisiae* Meyen ex E.C. Hansen) znatno pripomogli k porastu vsebnosti skupnih fenolnih kislin in sicer za kar 110 % (zgolj s kaljenjem je bilo povečanje 87 % v primerjavi z nekaljenimi zrni). Fermentacija je bistveno pripomogla k dvigu prostih fenolnih kislin, zabeležili pa so tudi dvig zaestrenih, glikoziliranih in vezanih fenolnih kislin. Kot zanimivost povejmo, da so tako ob uporabi posamezne vrste mlečnokislinskih bakterij (*Lactobacillus plantarum* ((Orla-Jensen, 1919) Bergey in sod., 1923) ali *Lactobacillus brevis*) kot tudi mešane kulture (*Saccharomyces cerevisiae*, *Lactobacillus plantarum* in *Lactobacillus brevis*) ali celo spontane fermentacije, dobili primerljive rezultate za zaestrene, glikozilirane in vezane fenolne kisline, je pa v vseh naštetih primerih prišlo do izrazito negativnega vpliva na prosto frakcijo fenolnih spojin. Slednje ni v skladu z raziskavo, ki so jo Konopka in sod. (2014) opravili na pšenici in rži. Fermentacija s kvasovkami je povečala vsebnost proste ferulne kisline 10-krat, uporaba kislega testa pa 11-krat. Še večje razlike so se pokazale pri fermentaciji iz polnozrnate moke rži, kvasovke so vsebnost proste ferulne kisline povečala 13-krat, starter kultura mlečnokislinskih bakterij pa kar 25-krat. Tudi sicer lahko v nedavnem pregledu literature, ki jo je pripravil Gobbetti (2019), najdemo številne dokaze o smotrnosti uporabe kislega testa z namenom sprostitve vezanih fenolnih spojin. Antognoni in sod. (2019) poročajo o precejšnjemu doprinosu fermentacije k vsebnosti ferulne kisline. Trije izmed osmih preizkušenih sevov *Lactobacillus plantarum* so testo uspešno obogatili s prosto ferulno kislino, vendar kljub relativno zelo velikemu prirastu, je bila vsebnost prostih fenolnih spojin še vedno precej manjša od vsebnosti topnih konjugatov ter predvsem netopno vezane ferulne kisline. Avtorji so razlike pojasnili s tem, da imajo preizkušeni sevi različno sposobnost za prekinitev estrske vezi, s katero je ferulna kislina vezana na celično steno (torej preko aktivnosti esteraz) kot tudi na račun razlik v aktivnosti dekarboksilaz fenolnih kislin. Specifično sposobnost razgradnje/ hidrolize/ presnove fenolnih spojin s strani posameznih sevov mlečnokislinskih bakterij potrjuje tudi naslednja raziskava. Hole in sod. (2012) poročajo, da je fermentacija polnozrnatega ječmena in oluščenih zrn ovsa z izbranimi probiotičnimi bakterijami (*Lactobacillus johnsonii*, *Lactobacillus reuteri* (Kandler in sod., 1982), *Lactobacillus acidophilus* ((Moro, 1900) Hansen in Mocquot, 1970)) znatno povečala vsebnost prostih fenolnih kislin in tako posledično tudi njihovo biorazpoložljivosti. Raziskava je dala še en pomemben zaključek. Na primeru *Bifidobacterium animalis* ((Mitsuoka, 1969) Scardovi in Trovatelli, 1974) so pokazali, da velika aktivnost encima feruoil esteraze (ki je značilna tudi za zgoraj omenjene probiotike) še ne pomeni nujno velike

učinkovitosti pri sproščanju vezane ferulne kisline. Fermentacija z mlečnokislinskimi bakterijami je vplivala tudi na spremembo skupnih vezanih fenolnih spojin, njihova vsebnost se je v ječmenu povečala, pri ovsu pa zmanjšala, prav tako je bil različen vpliv različnih bakterij na posamezne vezane fenolne kisline znotraj istega žita kot tudi med žiti. Porast vezane frakcije v ječmenu so raziskovalci povezali s povečano vsebnostjo topne vlaknine in posledično lažjo dostopnostjo (ekstraktibilnostjo) vezane frakcije.

3 VEZANE FENOLNE SPOJINE V VLOGI BIOAKTIVNIH KOMPONENT

V žitnih zrnih se nahaja veliko različnih komponent, ki po zaužitju vplivajo na zdravstveno stanje ljudi. Poleg zaviranja delitve rakavih celic v debelem črevesu (Madhujith in Shahidi, 2007; Shi in sod., 2015), uživanje vezanih fenolnih spojin povezujemo tudi z drugimi koristnimi posledicami. Katehinom in fenolnim kislinam pridobljenim iz golega ječmena Zhu in sod. (2015) pripisujejo inhibicijo rasti rakavih celic v jetrih. Raziskave opravljene na kvinoji (Hemalatha in sod., 2016; Tang in sod., 2016), so pokazale inhibitorni učinek na delovanje α-glukozidaze (ključni encim pri prebavi kompleksnih ogljikovih hidratov) in pankreasne lipaze (vloga pri absorpciji trigliceridov). Podobne rezultate je dala tudi študija fenolnih spojin, pridobljenih iz ovsa (Cai in sod., 2012) in prosa (Pradeep in Sreerama, 2017). Nadalje so raziskovalci (Boue in sod., 2016) na primeru barvnega riža ugotovili, da otrobi odstranjujejo glukozo iz krvnega obtoka v maščobne celice ter istočasno upočasnjujejo razgradnjo škroba do glukoze v tankem črevesu. Inhibicija α-glukozidaze in pankreasne lipaze lahko predstavlja učinkovito strategijo uravnavanja nivoja glukoze v krvi in debelosti; dveh ključnih dejavnikov za razvoj sladkorne bolezni tipa 2.

Nič manj ni pomembno njihovo antioksidativno, protivnetno in antimikrobno delovanje (Slavin, 2003). Vse fenolne kisline zahvaljujoč svoji strukturi izražajo določen antioksidativni potencial. Prostim radikalom oddajo elektron ali vodikov atom in jih tako stabilizirajo (Terpinc in Abramovič, 2010). Poleg tega ne smemo pozabiti tudi na njihove presnovne produkte. Eden takšnih, ki smo ga podrobneje že predstavili, 4-vinilgvajakol, se je izkazal kot uspešnejši antioksidant pri lovljenju alkilperoksilnega radikala v emulziji od svojega izvornega substrata, ferulne kisline (Terpinc in sod., 2011). Vezane fenolne spojine so v številnih *in vitro* antioksidativnih testih pokazali znatno večjo antioksidativno učinkovitost v primerjavi s prosto in konjugirano obliko (Chandrasekara in Shahidi, 2011; Chen in sod., 2017; Liyana-Pathirana in Shahidi, 2006; Pang in sod., 2018). Literaturni viri navajajo, da so fenolni antioksidanti pšeničnih otrobov (Liyana-Pathirana in Shahidi, 2007; Yu in sod., 2005) sposobni inhibirati LDL oksidacijo, kot možen mehanizem se omenja vezavo apolipoproteinov B. Pred leti so z *in vitro* testi dokazali (Serpen in sod., 2007), da je alkalna hidroliza znatno zmanjšala antioksidativno učinkovitost spojin pridobljenih iz pšeničnih in ječmenovih otrobov, kar govori v prid teoriji, da so fenolne spojine bolj učinkovite pri lovljenju prostih radikalov, ko so fenolne spojine vezane na netopne komponente. Tudi v študiji, ki so jo Price in sod. (2012) opravili na zdravih, prekomerno hranjenih udeležencih, starih 45 – 65 let, se je na analizi krvne plazme pokazalo, da je vnos izdelkov obogatenih z alevronsko plastjo pšeničnih zrn znatno pripomogel k povečani koncentraciji mikrohranil in spojin, s potencialno oksidativnim delovanjem. Do podobnih zaključkov so prišli tudi Costabile in sod. (2008), ki so poudarili, da uživanje polnozrnate pšenice pripomore k povečani koncentraciji ferulne kisline v krvi (analizirali na tešče). Slednje je bil po njihovem mnenju dokaz konstantnega sproščanja antioksidantov v krvni obtok. Istočasno pa so v svoji študiji potrdili vlogo polnozrnatih žitnih zrn kot prebiotikov in ugotovili, da so pripravki za zajtrk iz polnozrnate pšenice bolj zaznamovali črevesno mikrobioto (povečala populacijo bifido- in mlečnokislinskih bakterij) kot pšenični otrobi.

4 VEZANE FENOLNE SPOJINE KOT SESTAVINA FUNKCIONALNIH ŽIVIL

Dodatek vezanih fenolnih spojin v živilo je lahko idealno nadomestilo za aditive (antioksidativno delovanje) in konzervanse (protimikrobno delovanje). Načini vključevanja v živilo so različni. Priprava ekstraktov zahteva dodatno delo, uporabo topil, ustrezno opremo. Po drugi strani lahko prevelika količina v živilo dodanih vlaknin z vezanimi fenolnimi spojinami predstavlja tehnološko in senzorično omejitev, zato je ekstrakt pogosto priročnejša rešitev. V študiji, kjer so mesu dodali ekstrakte rženih in pšeničnih otrobov (Šulniūtė in sod., 2016) je to vodilo v izboljšano oksidativno stabilnost mesnega izdelka in pripomoglo k večji vsebnosti bioaktivnih komponent s potencialno pozitivnim učinkom na zdravje. Tudi v primeru ajde se je izkazalo, da bi se ekstrakt fenolnih spojin pripravljen iz otrobov lahko uporabil kot sestavina z antioksidativnim delovanjem, z *in vitro* testom pa je bila potrjena tudi sposobnost zaviranja tvorbe in razvoja rakastih celic na jetrih (Li in sod., 2016). Dodatek riževih otrobov (30 %) je 5-krat povečal antioksidativno učinkovitost pšeničnega kru-

ha, pri čemer bistveno ni vplival na splošno senzorično sprejemljivost (Irakli in sod., 2015). Vključitev ekstrakta riževih otrobov v piškote je pripomogla k manjši oksidaciji prisotnih maščob (Bhanger in sod. 2008). Nadalje, najnovejše raziskave kažejo na to, da kombinacija dveh ali več fitokemikalij različnega izvora pomembno vpliva na biološko učinkovitost ter razpoložljivost posamezne bioaktivne komponente (Gawlik-Dziki in sod., 2013; Phan in sod., 2018; Wang in Zhu, 2017). Podobne rezultate je dala tudi sočasna uporaba različnih žit. Raziskovalci (Serpen in sod., 2012) so poročali o sinergističnem in antagonističnem delovanju polnozrnatih pripravkov, ki so jih v različnih kombinacijah dodajali v kruh. Poleg izboljšane antioksidativne učinkovitosti, je uporaba ustrezne kombinacije semen vodila v manjšo vsebnost akrilamida, učinkovito omejevanje tvorbe 5-hidroksimetilfurfurala in večjo koncentracijo furozina (Serpen in sod., 2012). Opozorimo še na izsledke nedavne raziskave, ki so jo izvedli Montemurro in sod. (2019). Ta je pokazala, da fermentacija kaljenih zrn pšenice, ječmena in kvinoje s kislim testom ne vpliva zgolj na fenolne spojine, ampak sočasno poveča vsebnost peptidov, prostih aminokislin, γ-aminomaslene kisline ter zmanjša koncentracijo fitinske kisline, kondenziranih taninov, rafinoze in inhibitorjev tripsina. Še več, s tako pripravljenimi zrni so obogatili kruhe iz pšenične moke in uspeli razviti senzorično sprejemljive izdelke z veliko prebavljivostjo proteinov in majhno dostopnostjo škroba.

5 ZAKLJUČEK

Potrebna so nova dognanja na področju sproščanja in ekstrakcije vezanih fenolnih spojin, da bodo ta lahko še učinkoviteje uporabljena kot funkcionalna sestavina živil. Stranski proizvodi žitno predelovalna industrije, zlasti otrobi, predstavljajo bogat in poceni vir bioaktivnih komponent in izkazujejo velik potencial za tovrstno uporabo v prihodnosti. Dodatek vezanih fenolnih spojin lahko izboljša kakovost živila in mu podaljša rok uporabe. Poleg izboljšane antioksidativne stabilnosti mesa in piškotov, je uporaba ustrezne kombinacije semen vodila v manjšo vsebnost akrilamida, učinkovito omejevanje tvorbe 5-hidroksimetilfurfurala in večjo koncentracijo furozina v kruhu. Fermentacija kaljenih polnozrnatih semen omogoča izboljšanje prehranske vrednosti izdelkov, poveča vsebnost peptidov, prostih aminokislin, γ-aminomaslene kisline ter zmanjša koncentracijo nekaterih antinutrientov. Omenjen pristop velja za varen, poceni in tradicionalen postopek in kot tak bo gotovo dobro sprejet tudi s strani potrošnika, saj ponuja izdelke sprejemljive senzorične in tehnološke kakovosti. Razvijanje okusnejših polnozrnatih živil z dodano vrednostjo na eni strani ter sodelovanje industrije, strokovnjakov in vladnih predstavnikov na drugi, bo potrošniku gotovo pomagalo prepoznati, kupiti in zaužiti več tovrstnih izdelkov.

6 VIRI

Acosta-Estrada, B. A., Gutiérrez-Uribe, J. A., Serna-Saldívar, S. O. (2014). Bound phenolics in foods, a review. *Food Chemistry, 152*, 46-55. https://doi.org/10.1016/j.foodchem.2013.11.093

Alvarez-Jubete, L., Arendt, E. K., Gallagher, E. (2010). Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients. *Trends in Food Science & Technology, 21*(2), 106-113. https://doi.org/10.1016/j.tifs.2009.10.014

Alves, G. H., Ferreira, C. D., Vivian, P. G., Monks, J. L. F., Elias, M. C., Vanier, N. L., de Oliveira, M. (2016). The revisited levels of free and bound phenolics in rice: Effects of the extraction procedure. *Food Chemistry, 208*, 116-123. https://doi.org/10.1016/j.foodchem.2016.03.107

Anokwuru, C., Sigidi, M., Boukandou, M., Tshisikhawe, P., Traore, A., Potgieter, N. (2018). Antioxidant activity and spectroscopic characteristics of extractable and non-extractable phenolics from *Terminalia sericea* Burch. ex DC. *Molecules, 23*(6), 1303. https://doi.org/10.3390/molecules23061303

Antognoni, F., Mandrioli, R., Potente, G., Taneyo Saa, D. L., Gianotti, A. (2019). Changes in carotenoids, phenolic acids and antioxidant capacity in bread wheat doughs fermented with different lactic acid bacteria strains. *Food Chemistry, 292*, 211-216. https://doi.org/10.1016/j.foodchem.2019.04.061

Arranz, S. & Saura Calixto, F. (2010). Analysis of polyphenols in cereals may be improved performing acidic hydrolysis: A study in wheat flour and wheat bran and cereals of the diet. *Journal of Cereal Science, 51*(3), 313-318. https://doi.org/10.1016/j.jcs.2010.01.006

Bhanger, M. I., Iqbal, S., Anwar, F., Imran, M., Akhtar, M., Zia-ul-Haq, M. (2008). Antioxidant potential of rice bran extracts and its effects on stabilisation of cookies under ambient storage. *International Journal of Food Science & Technology, 43*(5), 779-786. https://doi.org/10.1111/j.1365-2621.2007.01515.x

Boubakri, H., Jdey, A., Taamalli, A., Taamalli, W., Jebara, M., Brini, F., Riciputi, Y., Pasini, F., Christian, M., Verardo, V. (2017). Phenolic composition as measured by liquid chromatography/mass spectrometry and biological properties of Tunisian barley. *International Journal of Food Properties, 20*(2), 1783-1797. https://doi.org/10.1080/10942912.2017.1359186

Boue, S. M., Daigle, K. W., Chen, M.-H., Cao, H., Heiman, M. L. (2016). Antidiabetic potential of purple and red rice (*Oryza sativa* L.) bran extracts. *Journal of Agricultural and Food Chemistry, 64*(26), 5345-5353. https://doi.org/10.1021/acs.jafc.6b01909

Cai, S., Wang, O., Wang, M., He, J., Wang, Y., Zhang, D., Zhou, F., Ji, B. (2012). In *vitro* inhibitory effect on pancreatic lipase activity of subfractions from ethanol extracts of fermented oats (*Avena sativa* L.) and synergistic effect of three phenolic acids. *Journal of Agricultural and Food Chemistry, 60*(29), 7245-7251. https://doi.org/10.1021/jf3009958

Călinoiu, L. F. & Vodnar, D. C. (2018). Whole grains and phenolic acids: A review on bioactivity, functionality, health benefits and bioavailability. *Nutrients, 10*(11), 1615. https://doi.org/ 10.3390/nu10111615

Chandrasekara, A. & Shahidi F. (2011). Determination of antioxidant activity in free and hydrolyzed fractions of millet grains and characterization of their phenolic profiles by HPLC-DAD-ESI-MSn. *Journal of Functional Foods, 3*(3), 144-158. https://doi.org/10.1016/j.jff.2011.03.007

Chen, P. X., Tang, Y., Zhang, B., Liu, R., Marcone, M. F., Li, X., Tsao, R. (2014). 5-Hydroxymethyl-2-furfural and derivatives formed during acid hydrolysis of conjugated and bound phenolics in plant foods and the effects on phenolic content and antioxidant capacity. *Journal of Agricultural and Food Chemistry, 62*(20), 4754-4761. https://doi.org/10.1021/jf500518r

Chen, Z., Ma, Y., Yang, R., Gu, Z., Wang, P. (2019). Effects of exogenous Ca^{2+} on phenolic accumulation and physiological changes in germinated wheat (*Triticum aestivum* L.) under UV-B radiation. Food Chemistry, *288*, 368-376. https://doi.org/10.1016/j.foodchem.2019.02.131

Chen, Z., Wang, P., Weng, Y., Ma, Y., Gu, Z., Yang, R. (2017). Comparison of phenolic profiles, antioxidant capacity and relevant enzyme activity of different Chinese wheat varieties during germination. *Food Bioscience, 20*, 159-167. https://doi.org/10.1016/j.fbio.2017.10.004

Costabile, A., Klinder, A., Fava, F., Napolitano, A., Fogliano, V., Leonard, C., Gibson, G. R., Tuohy, K. M. (2008). Wholegrain wheat breakfast cereal has a prebiotic effect on the human gut microbiota: a double-blind, placebo-controlled, crossover study. *British Journal of Nutrition, 99*(1), 110-120. https://doi.org/ 10.1017/s0007114507793923

Dahmoune, F., Spigno, G., Moussi, K., Remini, H., Cherbal, A., Madani, K. (2014). *Pistacia lentiscus* leaves as a source of phenolic compounds: Microwave-assisted extraction optimized and compared with ultrasound-assisted and conventional solvent extraction. *Industrial Crops and Products, 61*, 31-40. https://doi.org/10.1016/j.indcrop.2014.06.035

Das, A.K., Adsare, S.R., Das, M., Kulthe, P.S., Ganesan, P. (2017). Advanced techniques in extraction of phenolics from cereals, pulses, fruits, and vegetables. V M.W. Siddiqui, V. Bansal, K. Prasad (Ur), *Plant Secondary Metabolites, vol 2: Stimulation, Extraction and Utilization* (str. 27-76).: New York, NY: Apple Academic Press, Inc.

de Oliveira, D. M., Finger-Teixeira, A., Rodrigues Mota, T., Salvador, V. H., Moreira-Vilar, F. C., Correa Molinari, H. B., Craig Mitchell, R. A., Marchiosi, R., Ferrarese-Filho, O., Dantas dos Santos, W. (2015). Ferulic acid: a key component in grass lignocellulose recalcitrance to hydrolysis. *Plant Biotechnology Journal, 13*(9), 1224-1232. https://doi.org/ 10.1111/pbi.12292

Dykes, L. & Rooney, W. (2007). Phenolic compounds in cereal grains and their health benefits. *Cereal Foods World*, *52*, 105-111.

Domínguez-Rodríguez, G., Marina, M. L., Plaza, M. (2017). Strategies for the extraction and analysis of non-extractable polyphenols from plants. *Journal of Chromatography A, 1514*, 1-15. https://doi.org/10.1016/j.chroma.2017.07.066

Falcinelli, B., Benincasa, P., Calzuola, I., Gigliarelli, L., Lutts, S., Marsili, V. (2017). Phenolic content and antioxidant activity in raw and denatured aqueous extracts from sprouts and wheatgrass of einkorn and emmer obtained under salinity. *Molecules, 22*(12), 2132. https://doi: 10.3390/molecules22122132

Faulds, C. B. (2010). What can feruloyl esterases do for us? *Phytochemistry Reviews, 9*(1), 121-132. https://doi.org/10.1007/s11101-009-9156-2

Gan, R.-Y., Lui, W.-Y., Wu, K., Chan, C.-L., Dai, S.-H., Sui, Z.-Q., Corke, H. (2017). Bioactive compounds and bioactivities of germinated edible seeds and sprouts: An updated review. *Trends in Food Science & Technology, 59*, 1-14. https://doi.org/10.1016/j.tifs.2016.11.010

Gangopadhyay, N., Hossain, M. B., Rai, D. K., Brunton, N. P. (2015). A review of extraction and analysis of bioactives in oat and barley and scope for use of novel food processing technologies. *Molecules, 20*(6), 10884-10909. https://doi: 10.3390/molecules200610884

Gänzle, M. G. (2014). Enzymatic and bacterial conversions during sourdough fermentation. *Food Microbiology, 37*, 2-10. https://doi.org/10.1016/j.fm.2013.04.007

Gawlik-Dziki, U., Swieca, M., Dziki, D. (2012). Comparison of phenolic acids profile and antioxidant potential of six varieties of spelt (*Triticum spelta* L.). *Journal of Agricultural and Food Chemistry, 60*(18), 4603-4612. https://doi: 10.1021/jf3011239

Gawlik-Dziki, U., Świeca, M., Dziki, D., Baraniak, B., Tomiło, J., Czyż, J. (2013). Quality and antioxidant properties of breads enriched with dry onion (*Allium cepa* L.) skin. *Food Chemistry, 138*(2), 1621-1628. https://doi.org/10.1016/j.foodchem.2012.09.151

Gobbetti, M., De Angelis, M., Di Cagno, R., Calasso, M., Archetti, G., Rizzello, C. G. (2019). Novel insights on the functional/nutritional features of the sourdough fermentation. *International Journal of Food Microbiology, 302*, 103-113. https://doi.org/10.1016/j.ijfoodmicro.2018.05.018

Golob, K. (2018). *Vpliv osvetljevanja s svetlobo različnih valovnih dolžin na antioksidativni potencial kaljene pšenice : magistrsko delo.Ljubljana:* Biotehniška fakulteta, Oddelek za živilstvo.

Gunenc, A., HadiNezhad, M., Farah, I., Hashem, A., Hosseinian, F. (2015). Impact of supercritical CO_2 and traditional solvent extraction systems on the extractability of alkylresorcinols, phenolic profile and their antioxidant activity in wheat bran. *Journal of Functional Foods, 12*, 109-119. https://doi.org/10.1016/j.jff.2014.10.024

Hemalatha, P., Bomzan, D. P., Sathyendra, Rao B. V., Sreerama, Y. N. (2016). Distribution of phenolic antioxidants in whole and milled fractions of quinoa and their inhibitory effects on α-amylase and α-glucosidase activities. *Food Chemistry, 199*, 330-338. https://doi.org/10.1016/j.foodchem.2015.12.025

Hole, A. S., Rud, I., Grimmer, S., Sigl, S., Narvhus, J., Sahlstrøm, S. (2012). Improved bioavailability of dietary phenolic acids in whole grain barley and oat groat following fermentation with probiotic *Lactobacillus acidophilus*, *Lactobacillus johnsonii*, and *Lactobacillus reuteri. Journal of Agricultural and Food Chemistry, 60*(25), 6369-6375. https://doi.org/10.1021/jf300410h

Hübner, F. & Arendt, E. K. (2013). Germination of cereal grains as a way to improve the nutritional value: A Review. *Critical Reviews in Food Science and Nutrition, 53*(8), 853-861. https://doi.org/10.1080/10408398.2011.562060

Hung, P. V., Hatcher, D. W., Barker, W. (2011). Phenolic acid composition of sprouted wheats by ultra-performance liquid chromatography (UPLC) and their antioxidant activities. *Food Chemistry, 126*(4), 1896-1901. https://doi.org/10.1016/j.foodchem.2010.12.015

Irakli, M., Katsantonis, D., Kleisiaris, F. (2015). Evaluation of quality attributes, nutraceutical components and antioxidant potential of wheat bread substituted with rice bran. *Journal of Cereal Science, 65*, 74-80. https://doi.org/10.1016/j.jcs.2015.06.010

Irakli, M., Kleisiaris, F., Kadoglidou, K., Katsantonis, D. (2018). Optimizing extraction conditions of free and bound phenolic compounds from rice by-products and their antioxidant effects. *Foods, 7*(6), 93. https://doi.org/10.3390/foods7060093

Irakli, M. N., Samanidou, V. F., Biliaderis, C. G., Papadoyannis, I. N. (2012a). Development and validation of an HPLC-method for determination of free and bound phenolic acids in cereals after solid-phase extraction. *Food Chemistry, 134*(3), 1624-1632. https://doi.org/10.1016/j.foodchem.2012.03.046

Irakli, M. N., Samanidou, V. F., Biliaderis, C. G., Papadoyannis, I. N. (2012b). Simultaneous determination of phenolic acids and flavonoids in rice using solid-phase extraction and RP-HPLC with photodiode array detection. *Journal of Separation Science, 35*(13), 1603-1611. https://doi.org/10.1002/jssc.201200140

Katina, K., Liukkonen, K. H., Kaukovirta-Norja, A., Adlercreutz, H., Heinonen, S. M., Lampi, A. M., Pihlava, J. M., Poutanen, K. (2007). Fermentation-induced changes in the nutritional value of native or germinated rye. *Journal of Cereal Science, 46*(3), 348-355. https://doi.org/10.1016/j.jcs.2007.07.006

Kim, K.-H., Tsao, R., Yang, R., Cui, S. W. (2006). Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chemistry, 95*(3), 466-473. https://doi.org/10.1016/j.foodchem.2005.01.032

Kim, M. J., Kwak, H. S., Kim, S. S. (2018). Effects of germination on protein, γ-aminobutyric acid, phenolic acids, and antioxidant capacity in wheat. *Molecules„23*(9), 2244. https://doi.org/10.3390/molecules23092244

Koistinen, V. M. & Hanhineva, K. (2017). Mass spectrometry-based analysis of whole-grain phytochemicals. *Critical Reviews in Food Science and Nutrition, 57*(8), 1688-1709. https://doi.org/10.1080/10408398.2015.1016477

Konopka, I., Tańska, M., Faron, A., Czaplicki, S. (2014). Release of free ferulic acid and changes in antioxidant properties during the wheat and rye bread making process. *Food Science and Biotechnology, 23*(3), 831-840. https://doi.org/10.1007/s10068-014-0112-6

Krek, M. (2018). *Vpliv osvetljevanja s svetlobo različnih valovnih dolžin na antioksidativni potencial kaljene pire : magistrsko delo*. Ljubljana: Biotehniška fakulteta, Oddelek za živilstvo.

Kumar, P., Yadav, D., Kumar, P., Panesar, P. S., Bunkar, D. S., Mishra, D., Chopra, H. K. (2016). Comparative study on conventional, ultrasonication and microwave assisted extraction of γ-oryzanol from rice bran. *Journal of food science and technology, 53*(4), 2047-2053. https://doi.org/10.1007/s13197-016-2175-2

Li, F., Zhang, X., Zheng, S., Lu, K., Zhao, G., Ming, J. (2016). The composition, antioxidant and antiproliferative capacities of phenolic compounds extracted from tartary buckwheat bran (*Fagopyrum tartaricum* (L.) Gaerth). *Journal of Functional Foods, 22*, 145-155. https://doi.org/10.1016/j.jff.2016.01.027

Liyana-Pathirana, C. M. & Shahidi, F. (2006). Importance of insoluble-bound phenolics to antioxidant properties of wheat. *Journal of Agricultural and Food Chemistry, 54*(4), 1256-1264. https://doi.org/10.1021/jf052556h

Liyana-Pathirana, C. M. & Shahidi, F. (2007). The antioxidant potential of milling fractions from breadwheat and durum. *Journal of Cereal Science, 45*(3), 238-247. https://doi.org/10.1016/j.jcs.2006.08.007

Ma, Y., Wang, P., Wang, M., Sun, M., Gu, Z., Yang, R. (2019). GABA mediates phenolic compounds accumulation and the antioxidant system enhancement in germinated hulless barley under NaCl stress. *Food Chemistry, 270*, 593-601. https://doi.org/10.1016/j.foodchem.2018.07.092

Madhujith, T. & Shahidi, F. (2007). Antioxidative and antiproliferative properties of selected barley (*Hordeum vulgarae* L.) cultivars and their potential for inhibition of low-density lipoprotein (LDL) cholesterol oxidation. *Journal of Agricultural and Food Chemistry, 55*(13), 5018-5024. . https://doi.org/10.1021/jf070072a

Mir, N. A., Riar, C. S., Singh, S. (2018). Nutritional constituents of pseudo cereals and their potential use in food systems: A review. *Trends in Food Science & Technology, 75*, 170-180. https://doi.org/10.1016/j.tifs.2018.03.016

Montemurro, M., Pontonio, E., Gobbetti, M., Rizzello, C. G. (2019). Investigation of the nutritional, functional and technological effects of the sourdough fermentation of sprouted flours. *International Journal of Food Microbiology, 302*, 47-58. https://doi.org/10.1016/j.ijfoodmicro.2018.08.005

Moore, J., Cheng, Z., Hao, J., Guo, G., Liu, J.-G., Lin, C., Yu, L. (2007). Effects of solid-state yeast treatment on the antioxidant properties and protein and fiber compositions of common hard wheat bran. *Journal of Agricultural and Food Chemistry, 55*(25), 10173-10182. https://doi.org/10.1021/jf071590o

Musa-Veloso, K., Poon, T., Harkness, L. S., O'Shea, M., Chu, Y. (2018). The effects of whole-grain compared with refined wheat, rice, and rye on the postprandial blood glucose response: a systematic review and meta-analysis of randomized controlled trials. *The American Journal of Clinical*

Nutrition, 108(4), 759-774. https://doi.org/10.1093/ajcn/nqy112

Niroula, A., Khatri, S., Khadka, D., Timilsina, R. (2019). Total phenolic contents and antioxidant activity profile of selected cereal sprouts and grasses. *International Journal of Food Properties, 22*(1), 427-437. https://doi.org/10.1080/10942912.2019.1588297

Oghbaei, M.& Prakash, J. (2016). Effect of primary processing of cereals and legumes on its nutritional quality: A comprehensive review. *Cogent Food & Agriculture, 2*(1), 1136015. https://doi.org/10.1080/23311932.2015.1136015

Oliveira, D. M., Mota, T. R., Oliva, B., Segato, F., Marchiosi, R., Ferrarese-Filho, O., Faulds, C. B., dos Santos, W. D. (2019). Feruloyl esterases: Biocatalysts to overcome biomass recalcitrance and for the production of bioactive compounds. *Bioresource Technology, 278*, 408-423. https://doi.org/10.1016/j.biortech.2019.01.064

Pang, Y., Ahmed, S., Xu, Y., Beta, T., Zhu, Z., Shao, Y., Bao, J. (2018). Bound phenolic compounds and antioxidant properties of whole grain and bran of white, red and black rice. *Food Chemistry, 240*, 212-221. https://doi.org/10.1016/j.foodchem.2017.07.095

Paucar-Menacho, L. M., Martínez-Villaluenga, C., Dueñas, M., Frias, J., Peñas, E. (2017). Optimization of germination time and temperature to maximize the content of bioactive compounds and the antioxidant activity of purple corn (*Zea mays* L.) by response surface methodology. *LWT - Food Science and Technology, 76*, 236-244. https://doi.org/10.1016/j.lwt.2016.07.064

Phan, M. A. T., Paterson, J., Bucknall, M., Arcot, J. (2018). Interactions between phytochemicals from fruits and vegetables: Effects on bioactivities and bioavailability. *Critical Reviews in Food Science and Nutrition, 58*(8), 1310-1329. https://doi.org/10.1080/10408398.2016.1254595

Pradeep, P. M., Sreerama, Y. N. (2017). Soluble and bound phenolics of two different millet genera and their milled fractions: Comparative evaluation of antioxidant properties and inhibitory effects on starch hydrolysing enzyme activities. *Journal of Functional Foods, 35*, 682-693. https://doi.org/10.1016/j.jff.2017.06.033

Price, R. K., Wallace, J. M. W., Hamill, L. L., Keaveney, E. M., Strain, J. J., Parker, M. J., Welch, R. W. (2012). Evaluation of the effect of wheat aleurone-rich foods on markers of antioxidant status, inflammation and endothelial function in apparently healthy men and women. *British Journal of Nutrition, 108*(9), 1644-1651. https://doi.org/10.1017/s0007114511007070

Ribas-Agustí, A., Martín-Belloso, O., Soliva-Fortuny, R., Elez-Martínez, P. (2018). Food processing strategies to enhance phenolic compounds bioaccessibility and bioavailability in plant-based foods. *Critical Reviews in Food Science and Nutrition, 58*(15), 2531-2548. https://doi.org/10.1080/10408398.2017.1331200

Serpen, A., Capuano, E., Fogliano, V., Gökmen, V. (2007). A new procedure to measure the antioxidant activity of insoluble food components. *Journal of Agricultural and Food Chemistry, 55*(19), 7676-7681. https://doi.org/10.1021/jf071291z

Serpen, A., Gökmen, V., Mogol, B. A. (2012). Effects of different grain mixtures on Maillard reaction products and total antioxidant capacities of breads. *Journal of Food Composition and Analysis, 26*(1), 160-168. https://doi.org/10.1016/j.jfca.2012.02.001

Shi, J., Shan, S., Li, Z., Li, H., Li, X., Li, Z. (2015). Bound polyphenol from foxtail millet bran induces apoptosis in HCT-116 cell through ROS generation. *Journal of Functional Foods, 17*, 958-968. https://doi.org/10.1016/j.jff.2015.06.049

Singh, A. & Sharma, S. (2017). Bioactive components and functional properties of biologically activated cereal grains: A bibliographic review. *Critical Reviews in Food Science and Nutrition, 57*(14), 3051-3071. https://doi.org/10.1080/10408398.2015.1085828

Singh, A. K., Rehal, J., Kaur, A., Jyot, G. (2015). Enhancement of attributes of cereals by germination and fermentation: A review. *Critical Reviews in Food Science and Nutrition, 55*(11), 1575-1589. https://doi.org/10.1080/10408398.2012.706661

Slavin, J. (2003). Why whole grains are protective: biological mechanisms. *Proceedings of the Nutrition Society, 62*(1), 129-134. https://doi.org/10.1079/pns2002221

Šulniūtė, V., Jaime, I., Rovira, J., Venskutonis, P. R. (2016). Rye and wheat bran extracts isolated with pressurized solvents increase oxidative stability and antioxidant potential of beef meat hamburgers. *Journal of Food Science, 81*(2), H519-H527. https://doi.org/10.1111/1750-3841.13197

Tang, Y., Zhang, B., Li, X., Chen, P. X., Zhang, H., Liu, R., Tsao, R. (2016). Bound phenolics of quinoa seeds released by acid, alkaline, and enzymatic treatments and their antioxidant and α-glucosidase and pancreatic lipase inhibitory effects. *Journal of Agricultural and Food Chemistry, 64*(8), 1712-1719. https://doi.org/10.1021/acs.jafc.5b05761

Terpinc, P., Abramovič, H. (2010). A kinetic approach for evaluation of the antioxidant activity of selected phenolic acids. *Food Chemistry, 121*(2), 366-371. https://doi.org/10.1016/j.foodchem.2009.12.037

Terpinc, P., Cigić, B., Polak, T., Hribar, J., Požrl, T. (2016). LC–MS analysis of phenolic compounds and antioxidant activity of buckwheat at different stages of malting. *Food Chemistry, 210*, 9-17. https://doi.org/10.1016/j.foodchem.2016.04.030

Terpinc, P., Polak, T., Poklar Ulrih, N., Abramovič, H. (2011). Effect of heat treatment of camelina (*Camelina sativa*) seeds on the antioxidant potential of their extracts. *Journal of Agricultural and Food Chemistry, 59*(16), 8639-8645. https://doi.org/10.1021/jf2016072

Terpinc, P., Polak, T., Šegatin, N., Hanzlowsky, A., Ulrih, N. P., Abramovič, H. (2011). Antioxidant properties of 4-vinyl derivatives of hydroxycinnamic acids. *Food Chemistry, 128*(1), 62-69. https://doi.org/10.1016/j.foodchem.2011.02.077

Ti, H., Zhang, R., Zhang, M., Li, Q., Wei, Z., Zhang, Y., Tang, X., Deng, Y., Liu, L., Ma, Y. (2014). Dynamic changes in the free and bound phenolic compounds and antioxidant activity of brown rice at different germination stages. *Food Chemistry, 161*, 337-344. https://doi.org/10.1016/j.foodchem.2014.04.024

Van Hung, P. (2016). Phenolic Compounds of cere-

als and their antioxidant capacity. *Critical Reviews in Food Science and Nutrition,* 56(1), 25-35. https:// doi: 10.1080/10408398.2012.708909

Verma, B., Hucl, P., Chibbar, R. N. (2009). Phenolic acid composition and antioxidant capacity of acid and alkali hydrolysed wheat bran fractions. *Food Chemistry, 116*(4), 947-954. https://doi.org/10.1016/j.foodchem.2009.03.060

Wang, J., Sun, B., Cao, Y., Tian, Y., Li, X. (2008). Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran. *Food Chemistry, 106*(2), 804-810. https://doi.org/10.1016/j.foodchem.2007.06.062

Wang, S., Zhu, F. (2017). Dietary antioxidant synergy in chemical and biological systems. *Critical Reviews in Food Science and Nutrition, 57*(11), 2343-2357. https://doi.org/10.1080/10408398.2015.1046546

Wang, T., He, F., Chen, G. (2014). Improving bioaccessibility and bioavailability of phenolic compounds in cereal grains through processing technologies: A concise review. *Journal of Functional Foods, 7*, 101-111. https://doi.org/10.1016/j.jff.2014.01.033

Wang, W., Guo, J., Zhang, J., Peng, J., Liu, T., Xin, Z. (2015). Isolation, identification and antioxidant activity of bound phenolic compounds present in rice bran. *Food Chemistry, 171*, 40-49. https://doi.org/10.1016/j.foodchem.2014.08.095

Xiang, N., Guo, X., Liu, F., Li, Q., Hu, J., Brennan, C. S. (2017). Effect of light- and dark-germination on the phenolic biosynthesis, phytochemical profiles, and antioxidant activities in sweet corn (*Zea mays* L.) Sprouts. *International journal of molecular sciences, 18*(6), 1246. https://doi.org/10.3390/ijms18061246

Xu, J. G., Tian, C. R., Hu, Q. P., Luo, J. Y., Wang, X. D., Tian, X. D. (2009). Dynamic changes in phenolic compounds and antioxidant activity in oats (*Avena nuda* L.) during steeping and germination. *Journal of Agricultural and Food Chemistry, 57*(21), 10392-10398. https://doi.org/10.1021/jf902778j

Xu, M., Rao, J., Chen, B. (2019). Phenolic compounds in germinated cereal and pulse seeds: Classification, transformation, and metabolic process. *Critical Reviews in Food Science and Nutrition*, 1-20. https://doi.org/10.1080/10408398.2018.1550051

Yang, F., Basu, T. K, Ooraikul, B. (2001). Studies on germination conditions and antioxidant contents of wheat grain. *International Journal of Food Sciences and Nutrition, 52*(4), 319-330. https://doi.org/10.1080/09637480120057567

Yu, L., Zhou, K., & Parry, W. J. (2005). Inhibitory effects of wheat bran extracts on human LDL oxidation and free radicals. *LWT - Food Science and Technology, 38*(5), 463-470. https://doi.org/10.1016/j.lwt.2004.07.005

Zhang, J., Ding, Y., Dong, H., Hou, H., Zhang, X. (2018). Distribution of phenolic acids and antioxidant activities of different bran fractions from three pigmented wheat varieties. *Journal of Chemistry, 2018*, 1-9. https://doi.org/10.1155/2018/6459243

Zhu, Y., Li, T., Fu, X., Abbasi, A. M., Zheng, B., Liu, R. H. (2015). Phenolics content, antioxidant and antiproliferative activities of dehulled highland barley (*Hordeum vulgare* L.). *Journal of Functional Foods, 19*, 439-450. https://doi.org/10.1016/j.jff.2015.09.053

doi:10.14720/aas.2019.114.2.14 Original research article / izvirni znanstveni članek

Analiza vpliva vgrajenega sanacijskega materiala na rekultivacijo opuščenega peskokopa

Sonja CERAR [1, 2], Špela BAVEC [1]

Received November 4, 2019; accepted December 7, 2019.
Delo je prispelo 04. novembra 2019, sprejeto 07. decembra 2019.

Analiza vpliva vgrajenega sanacijskega materiala na rekultivacijo opuščenega peskokopa

Izvleček: V okviru sanacije opuščenega peskokopa Drtija (Moravče) se kot sanacijski material uporabljajo gradbeni kompoziti, ki se proizvajajo z mešanjem nenevarnih in inertnih odpadkov ter naravnega avtohtonega materiala (kremenov pesek, glina). V prispevku smo ugotavljali kemično sestavo že vgrajenih sanacijskih materialov ter vpliv le-teh na rekultivacijo površinskih vrhnjih plasti. V ta namen smo z bagrskimi izkopi vzorčili sanacijski material na 3 lokacijah do globine 3 m, okoliška površinska tla (0-10 cm) na dveh lokacijah ter izcedno vodo na 1 lokaciji. Rezultati kažejo, da so vgrajeni sanacijski materiali obremenjeni s kovinami (Cd, Cr, Cu, Ni, Pb, Zn), ki pa so v danih razmerah slabo topne. Slednje nakazujejo tudi koncentracije kovin v izcedni vodi, ki se pojavljajo znotraj območja spodnje meje določanja (LOQ). Od organskih snovi so v izcedni vodi prisotne povečane vsebnosti fenola in formaldehida, ki pa v sanacijskem materialu nista zaznavna, z izjemo fenola v dveh vzorcih. Vgrajeni sanacijski materiali bodo v sklopu končne sanacije prekriti s plastjo gline in humusa ter zatravljeni ali pogozdeni, zato ugotavljamo, da vpliv sanacijskih materialov na rekultivacijo vrhnje plasti ni pričakovan. Glede na rezultate je najbolj smiselna rekultivacije vrhnje plasti s pogozditvijo.

Ključne besede: peskokop kremenovega peska; rekultivacija; sanacija; sanacijski material; Moravče

Impact analysis of inbuilt rehabilitation material on the recultivation of abandoned sandpit

Abstract: For the rehabilitation and reclamation of abandoned sandpit in Drtija (Moravče), construction composites are used as rehabilitation materials. Construction composites are produced by mixing of recycled non-hazardous waste and natural materials. In the presented study, the chemical composition of the rehabilitation materials, which are already built-in on abandoned surfaces and impact analysis on recultivation of the upper layer, was evaluated. For this purpose, the rehabilitation materials were sampled at 3 locations with test pit excavations up to a depth of 3 m, surrounding topsoils (0-10 cm) at 2 locations and leachate at 1 location. The results show that the in-built rehabilitation materials are burdened with metals (Cd, Cr, Cu, Ni, Pb, Zn), which are, however, poorly soluble under the given conditions. The latter is also indicated by the concentrations of metals in the leachate, which are within the lower limit of quantification (LOQ) area. Regarding organic substances, the presence of phenol and formaldehyde was found in the leachate. In rehabilitation materials only phenol was found in two samples. Considering that the rehabilitation materials will be covered with a layer of clay and humus and will be tilled or afforested as part of the final rehabilitation step, the impact of rehabilitation materials on the reclaimed soil is not expected. According to results of this study recultivaton of the upper layer with planting of woods is recommended.

Key words: silica sandpit; recultivation; rehabilitation; rehabilitation material; Moravče

1 Geološki zavod Slovenije, Ljubljana, Slovenija
2 Korespondenčni avtor, e-naslov: sonja.cerar@geo-zs.si

1 UVOD

Destruktivno spreminjanje okolja temelji na prepričanju, da so bistvene sestavine naravnega okolja praktično neizčrpne, a je za kmetijska zemljišča v zadnjem obdobju mogoče zaslediti opozorila, da je že presežena regeneracijska zmogljivost (Vrščaj, 2010; Pintar in sod., 2010). Obseg kmetijskih zemljišč se zmanjšuje, okoljske zahteve pa se zaostrujejo, zato marsikje v Sloveniji ni več mogoče racionalno kmetovati, še manj pa kmetovati ekološko, saj za to ni dovolj površin. Slovenija je po obsegu kmetijskih obdelovalnih površin (njive in vrtovi) na repu držav članic Evropske unije (Pintar in sod., 2010). S problematiko zmanjšanja kmetijskih zemljišč zaradi tesnjenja tal oziroma odkopi, ki predstavlja globalno grožnjo varnosti preskrbe s hrano in socialni stabilnosti ter biotski raznovrstnosti in ekosistemom pa se srečujejo tudi po svetu (Montanarella, 2017).

Dinamično ravnovesje se lahko poruši ob človekovih posegih, kot npr. površinskih kopih (gramoznice, peskokopi, kamnolomi...), kjer zaradi odvzema mineralnih surovin prihaja do spreminjanja topografije (večje depresije, strma pobočja itd.) (Sharma in sod., 2004). S spremembo režima v poznih osemdesetih in kasnejšo prilagoditvijo zakonodaje za vstop v Evropsko skupnost je prišlo tudi do sprememb okoljske zakonodaje (Elliot in Udovč, 2005). Velik korak k izboljšanju neravnovesja, povzročenega v okolju s površinskimi kopi, je bil narejen s sprejetjem Zakona o rudarstvu (2010), ki je nosilce rudarske pravice zavezal k sanaciji rudarskih prostorov. Na izkoriščenih površinskih kopih, ki predstavljajo degradirano pokrajino, morajo nosilci rudarske pravice po zaključku izkoriščanja izvesti sanacijska rudarska dela, da se odpravijo posledice, ki so nastale pri izvajanju rudarskih del in izvede dokončna sanacija okolja (96. člen Zakona o rudarstvu (2010)). Površja, ki je bilo izpostavljeno izkopavanju mineralnih surovin, ni mogoče v celoti vrniti v prvotno stanje (Grčman in Zupanc, 2018). Degradirano območje se lahko preuredi v sekundarne habitate (Urbanc in Berg, 2005), v športno-rekreacijske površine, deponije komunalnih in drugih odpadkov (Knez in Regent, 1993), izgradnjo rastlinskih čistilnih naprav (Griessler Bulc in Šajn Slak, 2009) ali v kmetijske površine (Krümmelbein in sod., 2010). Pri rekultivaciji degradiranega območja za kmetijsko rabo želimo čas, ki ga naravna sukcesija potrebuje, bistveno skrajšati. Z ukrepi nekatere procese pospešimo, npr. vnos hranil, predvsem dušika (Čop in sod., 2009); nekatere, kot je spiranje nitrata in drugih onesnažil, ublažimo (Laner in sod., 2011), zmanjšamo toksičnost tal (Grčman in sod., 2001), z ozelenitvijo izboljšamo strukturo tal in zmanjšamo nevarnost erozije (Zupanc in Grčman, 2016). Pri izvedbi rekultivacije površinskega kopa v kmetijsko površino je zelo pomembno, da nosilec rudarske pravice že v naprej načrtuje, v kakšno kmetijsko površino bo površinski kop rekultiviran, ali bo to njiva, travnik, pašnik, gozd itd., in se temu primerno loti načrtovanja ter izvajanja rekultivacije. V nasprotnem primeru prihaja do slabega izvajanja in s tem posledično do otežene uporabe zemljišča (Škornik Grdina, 2016).

V Sloveniji se je v letu 2018 izkoriščalo 25 mineralnih surovin na skupno 180 nahajališčih, ki zajemajo skupno 206 pridobivalnih prostorov s koncesijsko pogodbo (rudarsko pravico za izkoriščanje) (Senegačnik in sod., 2019). Med slednjimi je tudi družba Termit d. d., ki je pričela z izkoriščanjem kremenovega peska že leta 1960 in se še danes ukvarja s proizvodnjo in predelavo kremenovih peskov ter izdelavo pomožnih livarskih sredstev za livarne in železarne. Ocena družbe Termit d. d. iz leta 2004 je pokazala, da je potrebno sanirati 2 milijona m^3 opuščenih kopov (Pavlin in sod., 2018) oz. približno 20 ha površin (Vajović in sod., 2016). Sanacija z naravnimi materiali ni prišla v poštev, saj bi s tem ustvarili nova degradirana območja drugje. Zaradi slednjega so v podjetju pričeli z reciklažo industrijskih odpadkov v gradbene kompozite, ki so skladni s Slovenskim tehničnim soglasjem (STS). Gradbena industrija spada med največje porabnike naravnih materialov, zato igra pomembno vlogo pri reciklaži odpadkov v gradbene surovine za doseganje trajnostnega razvoja (John in Tinker, 1998; Barbuta in sod., 2015). Mauko Pranjič in sod. (2014) poročajo, da med najbolj perspektivne industrijske odpadke za uporabo v gradbeništvu spadajo žlindra ter elektrofiltrski pepel iz sežiga premoga, biomase, komunalnih odpadkov in papirniškega mulja. Poleg tega je v primerih, kadar odpadki niso okolijsko inertni, možno nevarne komponente trajno imobilizirati z različnimi vezivi ali postopki (Colliviginarelli in Sorlini, 2002; Mauko Pranjič in sod., 2014; Ramesh in sod., 2014; Chuang in sod., 2018). Imobilizacija potencialno nevarnih snovi (PNS) v odpadkih poteka s pravim razmerjem materialov, ustrezno vlago in zgoščevanjem med vgradnjo in tako PNS ne ogrožajo okolja (Pavlin in sod., 2018). Kljub temu pa obstaja dvom o učinkovitosti vgrajevanja odpadkov, ki temelji na interakciji med gradbenimi materiali in okoljem, v katerem so vgrajeni (Podlipnik, 2019). Ugotavljanje stanja v okolju, kjer so gradbeni kompoziti že vgrajeni, je torej ključno za boljše razumevanje te problematike in predstavlja pomemben izziv za strokovnjake iz področja varstva okolja.

Ta prispevek vključuje rezultate in ugotovitve prve raziskave (Cerar in Bavec, 2017) že vgrajenih gradbenih kompozitov, ki se uporabljajo kot sanacijski material na opuščenem kopu Drtija (Moravče). Glavni cilj tega pri-

spevka je z uporabo geokemičnih preiskav opredeliti: (1) kemično sestavo sanacijskega materiala, okoliških površinskih tal in izcednih vod obravnavanega območja ter njihovo medsebojno povezavo ter (2) potencialni vpliv sanacijskega materiala na bodočo rekultivacijo. Na podlagi ugotovitev je predlagana tudi vrsta prostorske rabe po končni sanaciji.

2 MATERIAL IN METODE

2.1 OBRAVNAVANO OBMOČJE

Obravnavano območje se nahaja približno 1 – 1,5 km vzhodno od Moravč, približno 30 km vzhodno od Ljubljane. Razprostira se med dolino potoka Drtijščice, na zahodu, naseljem Zgornja Dobrava na vzhodu, naseljem Straža pri Moravčah na severu ter z obrati in poslovno stavbo Termit na jugu (Slika 1).

Obravnavano območje predstavlja umetni zasip nekdanjega odkopa kremenovega peska Drtija s sanacijskim materialom. V okolici se nahajajo večinoma gozdne in kmetijske površine. Vzhodno od obravnavanega območja se nahaja usedalni bazen (Slika 1), ki je urejen z varnostnim nasipom za preprečevanje izliva vode v okolje. Usedalni bazen je nastal kot posledica pridobivanja kremenovega peska, zaradi česar je nastala depresija, v kateri so se zadrževale oziroma izlivale meteorne vode z območja peskokopa ter iz potoka Stražca.

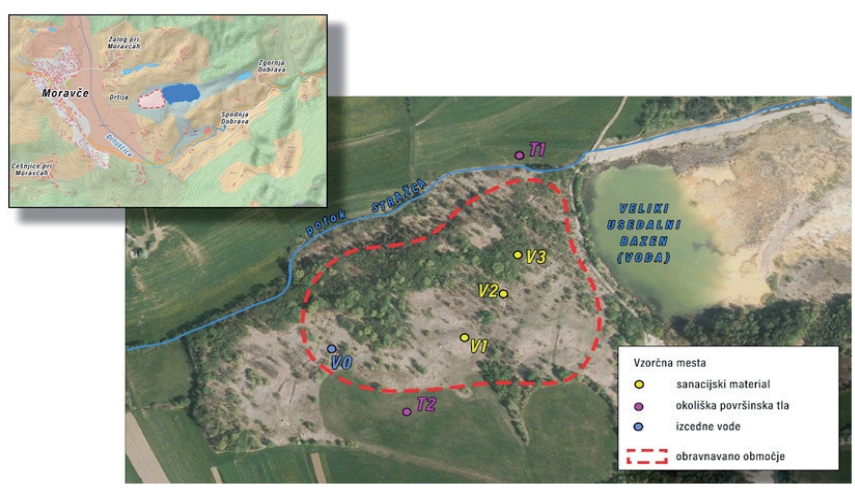
V morfološkem pogledu gre za podolgovato območje površine približno 5 ha, ki se razprostira v smeri vzhod – zahod. Nadmorska višina območja je približno 380 m.

Najpomembnejši vodotok predstavlja potok Drtijščica, ki teče približno 400 m južno od obravnavanega območja. Drtijščica izvira na treh lokacijah pod Sv. Lenartom na nadmorski višini približno 550 m. Njena struga poteka v smeri vzhod – zahod, pri vasi Drtija pa povije proti severu. Ob naselju Trnava se izliva v reko Radomljo na približno 340 m n. v. (Atlas okolja, 2019).

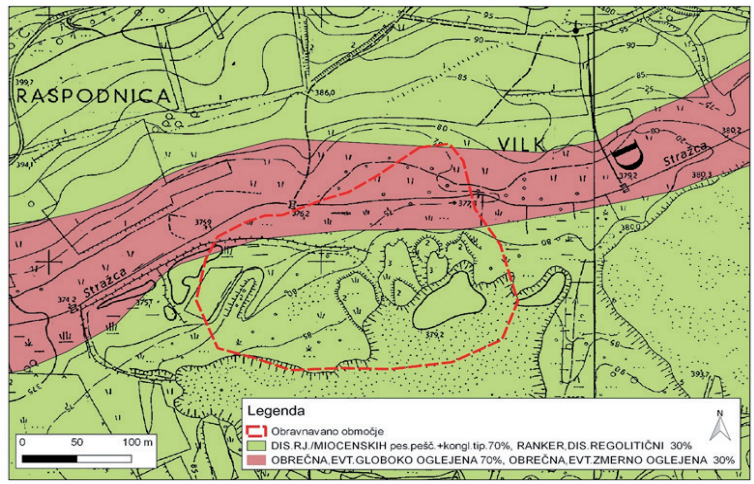
Pomemben vodotok predstavlja tudi potok Stražca, ki teče na severnem obrobju obravnavanega območja v smeri proti zahodu, kjer se pri naselju Zalog pri Moravčah izliva v Drtijščico. Potok Stražca izvira na treh lokacijah na severovzhodni strani območja, na nadmorski višini približno 420 m.

Geološke razmere na obravnavanem območju opredeljuje sinklinalna struktura, zapolnjena s plastmi neogenskih sedimentov (lapornata glina (sivica), kremenovi peski, prod in peščena glina), imenovana Moravška sinklinala. Podlago neogenskim sedimentom predstavljajo v glavnem zgornjetriasni plastoviti in masivni apnenci ter dolomiti, ki izdanjajo v severnem in južnem krilu sinklinale, mestoma pa še karnijski laporovec in laporast apnenec. Iz teh kamnin je zgrajeno tudi južno in severno obrobje Moravške doline. Južno sinklinalno krilo ima strmejši vpad kot severno sinklinalno krilo. Skupna debelina terciarnih plasti v Moravški sinklinali znaša okrog 250 m, medtem ko je na obravnavanem območju debelina ocenjena na približno 90 m (Lapajne, 1993).

Terciarni sedimenti so pričakovano srednje prepustni ($K \sim 4{,}6 \times 10^{-6}$ m s^{-1}), prav tako je izdatnost vodnjakov v teh sedimentih ocenjena na 3-4 l min^{-1} (Marinko in sod., 1975). Prepustnost apnencev je ocenjena na $3{,}88 \times 10^{-3}$ m s^{-1} (Rogelj in Karahodžič, 2004). Gladina podzemne vode se lokalno lahko nahaja na kontaktu med krovnino in talnino različnih slojev terciarnih se-



Slika 1: Obravnavano območje z lokacijami vzorčenja na letalskem posnetku med leti 2009 in 2011
Figure 1: Study area with sampling locations on an aerial photo map between 2009 and 2011



Slika 2: Pedološka karta obravnavanega območja v merilu 1:25000 (Vir: MKGP, 2016)
Figure 2: Pedological map of study area at scale 1:25.000 (Source: MAFF, 2016)

dimentov (kremenov pesek, glina, lapor, itd.). Smer toka podzemne vode je od vzhoda proti zahodu (Lapajne, 1993).

Glede na Pedološko karto Slovenije 1:25.000 (MKGP, 2016) se na miocenskih peskih, peščenjakih in konglomeratih nahajajo distrična rjava tla (70 %) in distrični regolitični ranker (30 %), na aluviju pa obrečna, evtrična globoko oglejena tla (70 %) in obrečna, evtrična zmerno oglejena tla (30 %) (Slika 2).

Večji del obravnavanega območja predstavlja rudarski prostor z odkopi kremenovega peska in saniranimi območji. Posledično so bila v večji meri avtohtona tla odstranjena. Le ta pa se predvidoma nahajajo izven rudarskega prostora.

2.2 IZVEDBA TEHNIČNE SANACIJE IN REKULTIVACIJE TAL

Po podatkih podjetja Termit d. d. je ob svojem nastanku, leta 1960, podjetje pričelo izkoriščati pesek v zahodnem delu, ki predstavlja obravnavano območje. Na tem območju se je izkoriščanje zaključilo leta 1978. Odkopani del se je najprej uporabljal kot bazen za vodo iz separacije. Bazen se je polnil z vodo približno šest let (Slika 3a). Nato so s krovninskimi materiali in glinami zapolnili odkop (Slika 3b). Med tem časom se je teren počasi sušil in utrjeval, dokler ni postal toliko trden, da so ga lahko spomladi leta 1993 poravnali z buldožerjem. Poravnan teren so nato še zasejali s travo (Slika 3c). Na površino 7 ha so navozili rodovitni material, ga pognojili in zatravili (Slike 3d, e in f). To območje takrat še ni bilo dokončno sanirano, saj je bilo zaraščeno z močvirskim drevjem in močvirsko travo in kot takšno neuporabno za kmetijsko obdelavo. Zato so leta 2015 na obravnavanem območju v skladu z rudarskim projektom (Vajović in sod., 2016) pričeli z izvajanjem končne tehnične sanacije opuščene odkopne jame peskokopa. Kot sanacijski material se od takrat uporabljajo različni gradbeni kompoziti (Pavlin in sod., 2018). Gradbene kompozite proizvaja Termit, in sicer s predelavo nenevarnih odpadkov in dodajanjem naravnega materiala, t. j. kremenovega peska. Delež in sestava sta skladna s predhodno pridobljenim Slovenskim tehničnim soglasjem (STS), ki ga na podlagi Zakona o gradbenih proizvodih (ZGPro) podeljuje Zavod za gradbeništvo Slovenije (ZAG).

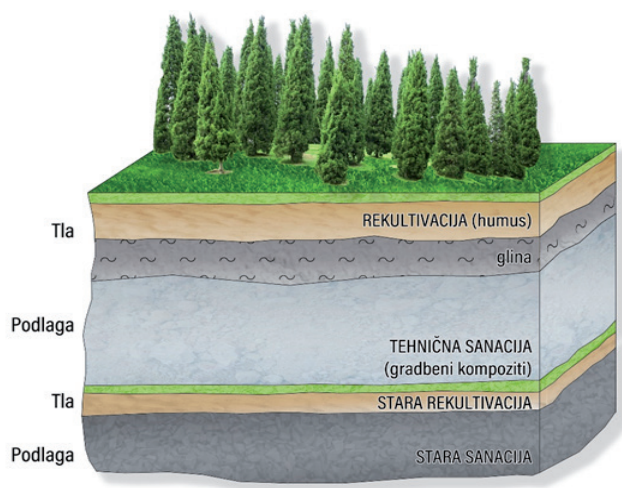
Tehnična sanacija v času preiskav še ni bila dokončno izvedena. Glede na rudarski načrt (Vajović in sod., 2016) je predvideno, da bo po končani tehnični sanaciji terena, ki predstavlja zasutje odkopanega prostora in izravnavo brežin do končnega naklona, izvedena rekultivacija vrhnjega sloja površin (Slika 4). Pri tem je navedeno, da se lahko v primeru resnih interesov in ustrezne podpore s strani občine ali lokalnih skupnosti, območje uredi tudi v druge namene (npr. turizem, rekreacija, raziskovalno območje ali kakršnokoli druga raba).

2.3 VZORČENJE

2.3.1. Sanacijski material

Vzorčenje sanacijskega materiala je bilo izvedeno z bagrskimi izkopi dne 15.3.2017 do globine 3 m na 3 vzorčnih mestih V1, V2 in V3 (Preglednica 1, Slika 1). Odvzeli smo dva tipa vzorcev: porušene in neporušene

Slika 3: Prikazi stanja okolja na območju peskokopa Drtija od leta 1981 do 1996: (a) leto 1981– zasipavanje s separacijskim muljem, (b) leto 1992 – teren zapolnjen s krovnino in glino, (c) leto 1993 – poravnan teren (približno 7 ha), (d) leto 1993 – nasutje rodovitnega materiala (mulj iz bližnjega ribnika), (e) letnica neznana – stanje po sanaciji in rekultivaciji, (f) 1995 – stanje po sanaciji in rekultivaciji (vir fotografij: Termit d. d.)
Figure 3: Indications of the environment in the area of the Drtija sand pit from 1981 to 1996: (a) year 1981 - backfill with separation sludge, (b) year 1992 - terrain filled with roof and clay, (c) year 1993 - leveled terrain (ca. 7 ha), (d) 1993 - Spillage of fertile material (sludge from a nearby pond), (e) Year unknown - Condition after rehabilitation and recultivation, (f) 1995 - Condition after rehabilitation and recultivation (Photo source: Termit d. d.)



Slika 4: Shema tehnične sanacije in rekultivacije vrhnjega sloja (prirejeno po Vajović in sod., 2016).
Figure 4: Scheme of technical rehabilitation and recultivation of the upper layer (adapted after Vajović et al., 2016)

vzorce. Slednji opredeljujejo dejansko stanje v naravi. Porušene vzorce sanacijskega materiala smo odvzeli iz izraženih plasti, ki so se pojavljale na različnih globinah. Odvzeli smo približno 3 kg vzorca. Približno 2 kg porušenega vzorca smo na terenu spravili v plastične vrečke za anorganske analize in v stekleno embalažo za organske analize ter 1 kg vzorca za arhiv v steklene embalaže. Za vsak vzorec smo na terenu zabeležili tudi njegove lastnosti (tekstura, vlažnost, barva in podobno).

Neporušene vzorce, ki v primerjavi s porušenimi vzorci predstavljajo dejanske razmere na terenu, smo na vzorčnih mestih V1, V2 in V3 odvzeli s cilindrom velikosti 14 cm x 35 cm. Globine, na katerih smo vtisnili cilinder, so podane v Preglednici 1.

2.3.2. Okoliška površinska tla

Ker smo tekom vzorčenja sanacijskega materiala opazili, da pri vgrajevanju sanacijskih materialov prihaja do prašenja delcev v bližnjo okolico, smo odvzeli tudi vzorce okoliških površinskih travniških tal z namenom ugotavljanja vpliva dejavnosti vgrajevanja sanacijskih materialov preko emisij iz zraka. Lokacije odvzema so bile pogojene z rabo tal. Travniška tla, za katera smo predvideli, da že dlje časa niso bila orana, so bila na voljo v ozkem pasu severno in jugovzhodno od obravnavanega območja. Drugod so bile obdelovalne kmetijske površine, gosto grmičevje ali gozd. Tako smo na dveh vzorčnih mestih (T1 in T2) (Preglednica 2) določili nulto stanje tal, kjer je možno dolgoročno spremljanje

Preglednica 1: Pregled podatkov o vzorčnih mestih sanacijskih materialov in njihovih lastnosti
Table 1: Review of data on sample sites of rehabilitation materials and their properties

Oznaka vzorčnega mesta	GKX	GKY	Oznaka vzorca	Globina (m)	Lastnosti materialov	Metoda vzorčenja
V1	109953	481789	V1G1	0 – 0,95	Material je temno sive barve, peščene teksture, suh, smrdi po prežganem	Bagrski izkop, vzorčenje iz žlice
			V1G2	0,95 – 1,35	Material je svetlo sive barve, peščene teksture, suh	
			V1G3	1,35 – 1,55	Material je črne barve, glinaste teksture z vidnimi primesmi peska, suh, gnetljiv	
			V1G4	1,55 – 1,75	Material je rumenorjave barve, glinaste teksture z vidnimi primesmi peska, suh/svež, enak material se ponovno pojavi na globini med 2,2- 2,6 m	
			V1G5	1,75 – 2,20	Material je temno rjave barve, glinaste teksture z vidnimi primesmi peska, moker	
			V1	0,95 – 1,30	Material je enak V1G2	Bagrski izkop, vtis cilindra (14 cm x 35 cm) z bagrsko žlico
V2	109999	481826	V2G1	0 – 0,80	Material je temno sive barve, peščene teksture, suh, mestoma se pojavljajo leče materiala svetlo sive barve	Bagrski izkop, vzorčenje iz žlice
			V2G2	0,85 – 1,85	Material je enak V2G1	
			V2G3	1,85 – 2,40	Material je sive barve, glinaste teksture, vlažen, gnetljiv, mestoma se pojavljajo leče materiala enakega kot pri V1G4	
			V2	1 – 1,35	Material je enak V2G1	Bagrski izkop, vtis cilindra (14 cm x 35 cm) z bagrsko žlico
V3	110042	481841	V3G1	0 – 0,85	Material je enak V2G3	Bagrski izkop, vzorčenje iz žlice
			V3G2	0,85 – 2,15	Material je enak V1G3	
			V3G3	2,15 – 2,85	Material je kremenov pesek, neprijetnih vonjav, suh/svež	
			V3	1,00 – 1,35	Material je enak V3G2	Bagrski izkop, vtis cilindra (14 cm x 35 cm) z bagrsko žlico

Preglednica 2: Pregled podatkov o vzorčnih mestih tal in lastnosti tal
Table 2: Review of soil sample site data and soil properties

Oznaka vzorčnega mesta	GKX	GKY	Metoda vzorčenja	Oznaka vzorca	Globina (cm)	Lastnosti tal
T1	110147	481844	Kompozitno vzorčenje	T1	0 – 10	Tla so bila ob vzorčenju sveža/vlažna, humozna, gosto prekoreninjena, drobljiva z dobro izraženo mrvičasto do grudičasto strukturo, terenska ocena teksturo je peščena ilovica, barva 10YR 4/4 (Munsell Soil), vsebnost skeleta ocenjena na 5 % s prevladujočo velikostjo ostrorobih delcev 0,5 cm.
T2	109873	481733	Kompozitno vzorčenje	T2	0 – 10	Tla so bila ob vzorčenju vlažna, srednje humozna, gosto prekoreninjena, zbita, mazava in gnetljiva z dobro izraženo mrvičasto do grudičasto strukture, terenska ocena teksture je peščena glinasta ilovica, barva 10YR 3/3 (Munsell Soil), vsebnost skeleta ocenjena na 30 % s prevladujočo velikostjo peščenih delcev (kremenov pesek), pojavljajo se tudi zaobljeni delci z velikostjo do 2 cm.

kvalitete tal v bližnji okolici, glede na to, da se sanacijski materiali trenutno še vgrajujejo. Poleg tega smo lahko primerjali vrednosti kemijskih snovi v tleh in sanacijskih materialih ter ugotavljali njuno povezavo. Vzorčenje tal je bilo izvedeno dne 21.4.2017. Izbrali smo sistem kompozitnega vzorčenja tako, da je vsak odvzeti vzorec sestavljen iz petih podvzorcev, ki smo jih homogenizirali na terenu. Prvi podvzorec je bil odvzet na GK koordinatah obravnavanega vzorčnega mesta, preostali podvzorci so bili odvzeti na krožnici, s centrom na koordinati prvega podvzorca in polmerom 5 m; drugi podvzorec v smeri V, tretji podvzorec v smeri S, četrti podvzorec v smeri Z in peti podvzorec v smeri J. Odvzeli smo po en združen vzorec na vsakem vzorčnem mestu, na globini 0 – 10 cm. Skupna teža posameznega vzorca je znašala približno 3 kg. Homogenizirani vzorec smo razdelili na posamezne dele za nadaljnje analize in jih spravili v stekleno embalažo (približno 2 kg za določitev anorganskih in organskih parametrov ter približno 1 kg za arhiv).

2.3.3. Izcedne vode

Da bi preverili možnost prehajanja kemijskih snovi iz sanacijskega materiala v okolje preko vode, smo poleg uporabe izluževalnih testov odvzeli tudi vzorec izcedne vode (VO). Izcedna voda na obravnavanem območju predstavlja infiltrirano padavinsko vodo, ki se preceja skozi sanacijski material. Glede na to, da je obravnavano območje zgrajeno v obliki nasipa, je bilo možno izcedno vodo odvzeti le na dnu brežine tega nasipa, saj odvodnja izcedne vode na dan vzorčenja ni bila urejena. Tako je bil vzorec izcedne vode odvzet dne 20.4.2017 na zahodnem delu obravnavanega območja z GKX 109941 in GKY 481661 (Slika 1), kjer je bil odvzem vzorca možen (na območju preostalih brežin izcedna voda ni bila prisotna).

Pred vzorčenjem vode smo izvedli tudi meritve terenskih parametrov (temperatura vode, pH in električna prevodnost vode) z uporabo instrumenta WTW pH/Cond 340i SET™ in meritve oksidacijsko-redukcijskega potenciala ter nasičenosti s kisikom v vodi, z instrumentom WTW Multi 3410/set C. Ob vzorčenju je bil ocenjen tudi pretok vode. Vzorci vode za organske parametre so bili odvzeti v rjavih steklenih embalažah oziroma steklenih vialah, medtem ko so bili za osnovne kemijske parametre ter kovine vzorci odvzeti v plastičnih embalažah različne kapacitete. Vsi vzorci izcedne vode so bili na terenu ustrezno obdelani s filtriranjem oziroma stabiliziranjem z dodajanjem različnih kislin.

2.4 KEMIJSKE ANALIZE

Predpriprava porušenih vzorcev sanacijskih materialov, vodnih izlužkov porušenih vzorcev sanacijskih materialov po standardu 2003/33/EC, tal in vod za fizikalno-kemijske in kemijske analize z uveljavljenimi standardnimi metodami, je bila opravljena v zunanjem akreditiranem (CSN EN ISO/IEC 17025:2005) laboratoriju ALS Czech Republic, s. r. o. Predpriprava neporušenih vzorcev sanacijskih materialov za fizikalno-kemijske analize je bila opravljena na Zavodu

za gradbeništvo (ZAG). Pripravili so vodne izlužke po standardu SIST EN 1744-1:2010+A1:2013, katerih meritev analiznih parametrov so prav tako opravili v zunanjem akreditiranem laboratoriju ALS Czech Republic, s. r. o.. Izmerjene vrednosti parametrov podane v mg/l, so bile za interpretacijo preračunane v mg kg^{-1} s. s. v skladu z razmerjem med maso vzorca in izluževalnega sredstva (destilirana voda) 1:10, računano na suho snov vzorca, zato da smo jih lahko ovrednotili v skladu s predpisanimi vrednostmi (mg kg^{-1} s. s.) za inertne odpadke.

Da bi preverili možnost prehajanja kemijskih snovi v okolje smo v porušenih in neporušenih vzorcih sanacijskega materiala določili parametre vodnega izlužka inertnih odpadkov (kovine, kloridi, fluoridi, sulfati, fenolni indeks, raztopljeni organski ogljik (DOC) in celotne raztopljene snovi), medtem ko smo v vodnih izlužkih neporušenih vzorcev dodatno določili kalcij, magnezij, natrij in kalij. Za karakterizacijo kemične sestave materialov smo v porušenih vzorcih sanacijskih materialov in tal določili celotne vsebnosti kovinskih anorganskih snovi in organskih snovi (celotni organski ogljik (TOC), ogljikovodiki (C10-C40), lahkohlapni aromatski ogljikovodiki (BTEX), policiklični aromatski ogljikovodiki (PAH), poliklorirani bifenili (PCB), formaldehid, fenol, naftol in krezol. V porušenih vzorcih tal smo določili še vsebnosti nekovinskih anorganskih parametrov (bromid, klorid, fluorid, nitrat, sulfat). Pri tem je potrebno omeniti, da smo analizirali 7 (od skupno 11) vzorcev porušenih sanacijskih materialov (V1G1, V1G5, V2G1, V2G2, V2G3, V3G2, V3G3), ki so glede na terensko oceno teksture bolje prepustni (v materialu prevladujejo peščeni delci).

V vzorcu izcedne vode smo analizirali iste parametre kot so bili analizirani v sanacijskem materialu in vodnih izlužkih, in sicer: osnovne kemijske parametre, raztopljene kovine ter nekatere organske parametre (adsorbljivi organski halogeni (AOX), fenolni indeks in fenolni derivati, celotni organski ogljik (TOC), celotni ogljikovodiki – mineralna olja (C10-C40), lahkohlapni aromatski ogljikovodiki (BTEX), policiklični aromatski ogljikovodiki (PAH) in formaldehid).

Merilna negotovost analitske metode je podana v poglavju 3. skupaj z rezultati kemijskih analiz vseh preiskovanih medijev.

2.5 VREDNOTENJE REZULTATOV

Za vrednotenje rezultatov kemijskih analiz sanacijskih materialov smo uporabili veljavne predpise. Za vrednotenje rezultatov vodnih izlužkov smo uporabili mejne vrednosti vodnega izlužka inertnih odpadkov, ki so predpisane v Uredbi o odlagališčih odpadkov (2014). Za vrednotenje rezultatov kovinskih anorganskih snovi smo uporabili največje dovoljene vrednosti anorganskih parametrov v umetno pripravljeni zemljini, ki je namenjena nasipavanju stavbnih zemljišč in nasipavanju območij mineralnih surovin za zapolnitev tal po izkopu, ki so predpisane v Uredbi o obremenjevanju tal z vnašanjem odpadkov (2008 in 2011). Za vrednotenje organskih snovi smo uporabili mejne vrednosti parametrov onesnaženosti inertnih odpadkov, ki so predpisane v Uredbi o odlagališčih odpadkov (2014).

Izmerjene vrednosti analiznih parametrov v tleh smo primerjali z mejnimi, opozorilnimi in kritičnimi vrednostmi, ki jih predpisuje Uredba o mejnih, opozorilnih in kritičnih imisijskih vrednostih nevarnih snovi v tleh (1996) ter povprečnimi vrednostmi elementov v slovenskih tleh (Gosar in sod., 2019). Izračunali smo faktorje obogatitve za posamezen element v odvzetih vzorcih tal tako, da smo izmerjene vrednosti delili s povprečnimi vrednostmi elementov v slovenskih tleh (Gosar in sod., 2019).

Za vrednotenje izcedne vode na obravnavanem območju so pomemben kriterij mejne vrednosti določene z Uredbo o emisiji snovi pri odvajanju izcedne vode iz odlagališč odpadkov (2008). Ker z omenjeno uredbo niso določene mejne vrednosti za nekatere anorganske in organske snovi smo za oceno vrednotenja uporabili še mejne vrednosti določene z Uredbo o emisiji snovi in toplote pri odvajanju odpadnih voda v vode in javno kanalizacijo (2012) ter kot najstrožji kriterij vrednotenja vod, Pravilnik o pitni vodi (2004). Uporaba teh mejnih vrednosti predstavlja konzervativen pristop vrednotenja rezultatov kemijskih analiz izcedne vode saj oba predpisa obravnavata drug tip voda in jih tako lahko uporabimo le kot primerjavo.

3 REZULTATI IN DISKUSIJA

3.1 SANACIJSKI MATERIAL

3.1.1 Celotne vsebnosti anorganskih snovi

Kemične analize so pokazale, da so celotne vsebnosti antimona in selena pod spodnjo mejo določanja (LOQ) v vseh obravnavanih vzorcih. Pri ostalih kovinah smo za izračun osnovnih statistik za vrednosti manjše od LOQ upoštevali polovične vrednosti LOQ. Preiskovani materiali vsebujejo arzen (As), kadmij (Cd), krom (Cr), baker (Cu), živo srebro (Hg), nikelj (Ni) in cink (Zn) (Preglednica 3), katerih vsebnosti smo za splošno oceno kakovosti materialov, ovrednotili glede na smernice anorganskih parametrov (As, Cd, Cr, Cu, Hg, Ni, Pn

Preglednica 3: Celokupne vrednosti kovin in As (mg kg^{-1} s. s.) v porušenih vzorcih sanacijskega materiala skupaj z mejnimi vrednostmi parametra za umetno pripravljeno zemljino (MV) ter osnovnimi statistikami
Table 3: The total values of metals and As (mg kg^{-1} d. m) in demolished samples of rehabilitation material, together with the limit values of the parameter for artificially prepared soil (MV) and basic statistics

Oznaka vzorca	As	Ba	Cd	Cr	Cu	Hg	Mo	Ni	Pb	Zn
V1G1	5,0	128	0,2	17,9	17,5	0,099	0,60	14,7	51,1	50,2
V1G5	4,44	86,7	0,2	16,5	13,2	0,073	0,98	14	41,4	41,3
V2G1	<2,5	286	*2,82*	*2.260*	*176*	0,046	122	*813*	*149*	*1.060*
V2G2	<2,5	285	*2,82*	*1.740*	*220*	0,044	111	*659*	*153*	*1.080*
V2G3	<2,5	266	*2,64*	*1.520*	*156*	0,041	95	*557*	*136*	*1.020*
V3G2	<2,5	276	<0,4	*226*	20	<0,01	2,08	48,8	9,1	41,9
V3G3	5,62	88,5	0,52	30,9	25,2	0,22	1	21	*108*	67,7
N[1]	7	7	7	7	7	7	7	7	7	7
Min.	<2,5	86,7	<0,4	16,5	13,2	0,041	0,6	14	9,1	41,3
Max.	5,0	286	2,82	2.260	220	0,22	122	813	153	1.080
Povprečje	2,86	202	1,3	830	89,7	0,1	47,5	304	92,5	480,2
Mediana	<2,5	266	0,52	226	25,2	0,05	2,08	48,8	108	67,7
MN[2]	± 20%	± 20%	± 20%	± 20%	± 20%	± 20%	± 20%	± 20%	± 20%	± 20%
MV[3]	30	/	1,1	90	90	0,7	/	55	100	450
X[4]>MV	0	/	3	4	3	0	/	3	4	3

[1]Število analiziranih vzorcev; [2]Merilna negotovost analitske metode; [3]Mejna vrednost za anorganske parametre v umetno pripravljeni zemljini (Uredba o obremenjevanju tal z vnašanjem odpadkov (2008)); [4]Število vzorcev nad mejno vrednostjo

in Zn) v umetno pripravljeni zemljini, ki je med drugim namenjena nasipavanju območij mineralnih surovin za zapolnitev tal po izkopu (Uredba o obremenjevanju tal z vnašanjem odpadkov (2008)). Mejne vrednosti Cd, Cr, Cu, Ni, Pb in Zn so presežene v vseh treh vzorcih sanacijskega materiala vertikalnega profila V2, in sicer V2G1, V2G2 ter V2G3, kar kaže na to, da so povečane vsebnosti podobno prostorsko porazdeljene. Podobna prostorska porazdelitev omenjenih kovin nakazuje na podoben izvor, ki so lahko livarski peski (Božym, 2017), pepel in žlindra (Basu in sod., 2009). V vzorcih sanacijskega materiala vertikalnega profila V3 pa sta presežena tudi krom (V3G2) in svinec (V3G3). Mejne vrednosti As in Hg niso presežene. Glede na izbrane smernice, vgrajen sanacijski material ni primeren za zapolnitev tal po izkopu mineralnih surovin, saj so presežene mejne vrednosti celotnih vsebnosti posameznih kovin. Vendar pa je potrebno opozoriti, da uradno te smernice za tovrsten material (t. j. gradbeni kompozit) na obravnavanem območju ne veljajo, zato je napačno trditi, da ti materiali niso ustrezni. V okoljevarstvenih dovoljenjih družbe Termit d .d. je namreč opredeljeno, da se gradbene materiale lahko uporablja, če so izdelani v skladu s pridobljenim STS in če kemične lastnosti predelanih materialov ustrezajo zahtevam za inertne odpadke (Uredba o odlagališčih odpadkov (2014)). Slednje pa ne predpisujejo mejnih vrednosti za celotne vsebnosti parametrov, temveč za parametre vodnega izlužka (1:10). To je na splošno gledano smiselno, saj celotne vsebnosti kovin še ne povedo veliko o njihovi mobilnosti oz. možnosti prehajanja v okolje – vodo in bioto, temveč so za to potrebne podrobnejše raziskave, in sicer fizikalno-kemijskih lastnosti, speciacije, vodotopnosti, biodostopnosti, itd. (De Matos in sod., 2001; Ogundiran in Osibanjo, 2009; Bavec in Gosar, 2016). V tej študiji smo ugotavljali vodotopnost, oz. možnost prehajanja kemičnih snovi iz sanacijskega materiala v vodo, kar so pokazale vrednosti, ki so bile izmerjene v vodnih izlužkih in izcedni vodi, ki so predstavljene v nadaljevanju.

Povprečna vsebnost barija je 202 mg kg^{-1} s. s. Značilne vsebnosti barija v naravnih – neobremenjenih, geoloških materialih so do 1.600 mg/kg, v glinenih materialih do 3.000 mg kg^{-1} (Turekian in sod., 1961). Glede na sestavo materialov barij praviloma izvira iz karbonatnih materialov. Povprečna vsebnost molibdena je 47,5 mg kg^{-1} s. s.. Od povprečnih vrednosti odstopajo vzorci vertikalnega profila V2 in sicer vzorca V2G1 in V2G2 na globini do 2 m, kjer izkopan material predstavlja temno siv pesek (material v večji meri povsem verjetno sestavlja livarski pesek) in vzorec V2G3, kjer izkopan material predstavlja sivo glino. Značilne vseb-

Preglednica 4: Vrednosti organskih snovi (v mg kg^{-1} s. s., razen za TOC v % s. s.), žarilne izgube (% s. s.) in suhe snovi (%) porušenih vzorcev sanacijskega materiala skupaj z mejnimi vrednostmi parametrov onesnaženosti za inertne odpadke ter osnovnimi statistikami

Table 4: Organic matter values (in mg kg^{-1} d. m., except for TOC in % d. m.), incineration losses (% d. m.) and dry matter (%) of demolished rehabilitation material samples, together with limit values for contamination parameters for inert waste and basic statistics

Parameter	TOC	C10-C40	Vsota PAH	Vsota BTEX	Fenol	Žarilna izguba 550°C	Suha snov 105°C
V1G1	*3,23*	137	0,50	<0,17	/	7,3	76,9
V1G5	2,29	<20	<0,16	<0,17	/	15,1	53,0
V2G1	1,75	22	0,64	<0,17	/	10,6	67,1
V2G2	*4,92*	140	0,72	0,483	1,08	6,5	86,5
V2G3	0,45	<20	<0,16	<0,17	/	5,5	77,2
V3G2	*7,09*	163	0,80	1,90	/	3,6	85,3
V3G3	1,42	42	0,31	<0,17	0,30	0,9	94,9
N[1]	7	7	7	7	2	7	7
Min.	0,45	<20	<0,16	<0,17	0,30	0,9	53,0
Max.	7,09	163	0,80	1,90	1,08	15,1	94,9
Povprečje	3,02	75	0,45	0,40	/	7,0	77,3
Mediana	2,29	42	0,50	<0,17	/	6,5	77,2
MN[2]	± 20%	± 30%	± 30%	± 40%	± 40%	± 5,0%	± 6,0%
MV[3]	3	500	6	6	/	/	/
X[4]>MV	3	0	0	0	/	/	/

[1]Število analiziranih vzorcev; [2]Merilna negotovost analitske metode; [3]Mejna vrednost parametrov onesnaženosti za inertne odpadke (Uredba o odlagališčih odpadkov (2014)); [4]Število vzorcev nad mejno vrednostjo

nosti molibdena v naravnih – neobremenjenih, geoloških materialih so do 3 mg kg^{-1} oz. v glinenih materialih do 27 mg kg^{-1} (Turekian in sod., 1961).

3.1.2. Celotne vsebnosti organskih snovi

V preglednici 4 navajamo vsebnosti TOC-a, mineralnih olj, vsote BTEX, vsote PAH-ov in fenola skupaj z žarilno izgubo in suho snovjo. Vrednosti spojin iz sklopa PCB-jev – kongenerji 2,4,4 triklorobifenil (PCB 28), 2,2',5,5 tetraklorobifenil (PCB 52), 2,2',4,5,5' pentaklorobifenil (PCB 101), 2,3',4,4',5 pentaklorobifenil (PCB 118), 2,2',4,4',5',5 heksaklorobifenil (PCB 153) in 2,2',3,4,4',5',5 heptaklorobifenil (PCB 180) ter formaldehidov, naftolov in krezolov so manjši od LOQ v vseh obravnavanih vzorcih, zato jih v preglednici 4 ne navajamo.

Preiskovani vzorci sanacijskega materiala vsebujejo v povprečju 80 % suhe snovi (s. s.) oz. 20 % vlage. Odstopa vzorec V1G5 s 53 % s. s. oz. 47 % vlage (Preglednica 4). Količina vlage je bila pričakovana, ker je bil vzorec v primerjavi z ostalimi vzorci, ki so bili pretežno suhi, že na terenu moker. Slednje pripisujemo teksturi vzorca (glina), ki predstavlja manj prepustno plast.

Ob upoštevanju vseh obravnavanih vzorcev je delež TOC-a skromen, v povprečju 3 % s. s. Glede na povprečno vrednost žarilne izgube na 550 °C, ki znaša 7 % in izmerjene vsebnosti TOC (3,02 % s. s.) je delež termično stabilnih materialov v preiskovanih zemljinah več kot 90 %. Glede na smernice za inertne odpadke (Uredba o odlagališčih odpadkov (2014)), je v manjši meri presežena mejna vrednost za TOC in sicer v treh vzorcih V1G1, V2G2 in V3G2. Glede na vrste odpadkov, iz katerih so gradbeni materiali sestavljeni, so vir povečanega TOC lahko odpadki iz predelave papirniškega mulja, predelave mulja iz vodnih vrtin, predelave vrtin in predelave fosfatov. Vsota PAH-ov, BTEX-ov ter vrednosti mineralnih olj ne presegajo mejnih vrednosti organskih parametrov za inertne odpadke. Glede na vrste odpadkov, iz katerih so sanacijski materiali sestavljeni, so vir BTEX lahko odpadki iz predelave livarskih peskov, predelave lepila in predelave mulja.

Fenol vsebujeta dva vzorca V2G2 in V3G3 sanacijskih materialov, ki sta pretežno sestavljena iz kremenovega oziroma livarskega peska. Odpadni materiali iz

Preglednica 5: Vrednosti parametrov (mg kg^{-1} s. s.) v vodnih izlužkih porušenih vzorcev sanacijskega materiala skupaj z mejnimi vrednostmi parametrov izlužka za inertne odpadke ter osnovnimi statistikami
Table 5: Values of parameters (mg kg^{-1} d. m.) in water leachates of demolished samples of rehabilitation material together with limit values of leachate parameters for inert waste and basic statistics

Oznaka vzorca	Ba	Cr	Cu	Hg	Mo	Zn	Cl^-	F^-	SO_4^{2-}	DOC	CRS[1]	Alkaliteta pH 4,5
V1G1	18,70	<0,05	<0,1	<0,0001	<0,2	0,18	90	5,86	89	67	*9.740*	17,90
V1G5	5,18	<0,05	<0,1	<0,0001	<0,2	<0,1	448	3,30	442	50	*4.340*	4,76
V2G1	2,75	0,20	<0,1	0,00011	<0,2	<0,1	152	6,42	560	33	*8.760*	10,90
V2G2	1,50	*4,46*	<0,1	0,00014	*0,72*	0,19	110	6,48	192	18	*5.460*	9,64
V2G3	0,57	<0,05	<0,1	0,0004	<0,2	<0,1	24	3,42	744	<5	1.390	0,44
V3G2	0,21	*0,87*	<0,1	<0,0001	0,26	<0,1	127	*10,60*	381	21	3.540	4,38
V3G3	2,63	0,07	0,48	0,00043	<0,2	2,58	26	5,00	70	*690*	1.050	11,00
N[2]	7	7	7	7	7	7	7	7	7	7	7	7
Min.	0,21	<0,05	<0,1	<0,0001	<0,2	<0,1	24	3,30	70	<5	1.050	0,44
Max.	18,70	4,46	0,48	0,00043	0,72	2,58	448	10,60	744	690	9.740	17,90
Povprečje	4,51	0,81	0,11	0,00	0,21	0,45	140	5,87	354	126	4.897	8,43
Mediana	2,63	0,07	<0,1	0,00011	<0,2	<0,1	110	5,86	381	33	4.340	9,64
MN[3]	**± 10%**	**± 10%**	± 10%	± 10%	± 10%	± 10%	± 10%	± 10%	± 10%	± 10%	± 10%	± 10%
MV[4]	20	0,5	2	0,01	0,5	4	800	10	1.000	500	4.000	/
X[5]>MV	0	2	0	0	1	0	0	1	0	0	4	/

[1]Celotne raztopljene snovi; [2]Število analiziranih vzorcev; [3]Merilna negotovost analitska metode; [4]Mejna vrednost parametrov izlužka za inertne odpadke (Uredba o odlagališčih odpadkov (2014)); [5]Število vzorcev nad mejno vrednostjo

predelave kremenovega peska in livarskega peska so lahko vir fenolov (Mcnaughtan in Hoyt, 1958).

3.1.3. Vrednosti parametrov v vodnih izlužkih

Vrednosti antimona, arzena, kadmija, niklja, svinca in selena so manjše od LOQ v vseh obravnavanih vodnih izlužkov porušenih vzorcev sanacijskega materiala. Za ostale parametre izmerjene vrednosti navajamo v Preglednici 5, skupaj z mejnimi vrednostmi parametrov izlužka za inertne odpadke (Uredba o odlagališčih odpadkov (2014)). Izmerjene vrednosti za alkaliteto kažejo na prisotnost karbonatnih (HCO_3^-, CO_3^{2-}) zvrsti v vzorcih materialov s peščeno teksturo.

Iz Preglednice 5 je razvidno, da vrednosti parametrov kot so barij, baker, živo srebro, cink, kloridi, fluoridi in sulfati v vodnih izlužkih porušenih vzorcev, ne presegajo mejnih vrednosti parametrov izlužka za inertne odpadke. Mejne vrednosti pa so bile presežene v posameznih vzorcih izlužkov za 5 parametrov: krom v vzorcih V2G2 in V3G2, molibden v V2G2, fluorid v vzorcu V3G2, raztopljen organski ogljik v vzorcu V3G3 ter celotne raztopljene snovi v vzorcih V1G1, V1G5, V2G1 in V2G5.

Glede na vrste odpadkov, iz katerih so sanacijski materiali sestavljeni, so vir Cr, Cu in Mo lahko odpadki iz predelave odpadne žlindre, predelave pepela in filtrskega prahu. Ob upoštevanju merilne negotovosti (± 10 %) izmed potencialno nevarnih kovin znatno izstopa samo Cr v enem vzorcu (V2G2).

Fluoridi predstavljajo reaktivno kemijsko zvrst, ki s sestavinami zemljine oz. vode, kot so kalcij in magnezij, tvorijo težko topne – kemijsko inertne spojine in tako ne prehajajo z vodo v širše okolje. Fluoridi pa z aluminijem in železom oziroma natrijem in kalijem tvorijo stabilne komplekse (Spletni vir, 2019d), ki so vodotopni. Možni izvor fluorida so lahko antropogeni – odpadni keramični materiali in vsi tisti odpadni materiali, ki nastanejo po uporabi naravnih mineralnih surovin (Ponsot in sod., 2013) lahko so pa tudi geogeni, saj glineni minerali med drugim vsebujejo tudi fluorid. Značilne vsebnosti fluorida v naravnih – neobremenjenih, geoloških materialih so do 100 mg kg^{-1} v karbonatnih kamninah oz. do 600 mg kg^{-1} v glinah (Turekian in sod., 1961).

Izmerjene vrednosti parametrov v vodnih izlužkih neporušenih sanacijskih materialov so prikazane v Preglednici 6, skupaj z mejnimi vrednostmi parametrov izlužka za inertne odpadke. Iz preglednice 6 je razvidno,

Preglednica 6: Vrednosti parametrov (mg kg^{-1} s. s.) v vodnih izlužkih neporušenih vzorcev sanacijskega materiala skupaj z mejnimi vrednostmi parametrov izlužka za inertne odpadke ter osnovnimi statistikami
Table 6: Values of parameters (mg kg^{-1} d. m.) in water leachates of undisturbed samples of rehabilitation material together with limit values of leachate parameters for inert waste and basic statistics

Oznaka vzorca	V1	V2	V3	N[2]	Min.	Max.	MN[3]	MV[4]	X[5]>MV
Ba	*25,2*	0,123	0,143	3	0,123	25,2	**± 10%**	20	1
Cd	<0,008	<0,004	0,011	3	<0,004	0,011	**± 10%**	0,04	0
Cr	<0,02	<0,01	<0,01	3	<0,01	<0,02	**± 10%**	0,50	0
Cu	0,03	0,03	0,14	3	0,03	0,14	**± 10%**	2	0
Hg	<0,00001	0,00061	0,00081	3	<0,00001	0,00081	**± 10%**	0,01	0
Mo	<0,04	0,126	0,496	3	<0,04	0,496	**± 10%**	0,50	0
Pb	2,08	<0,05	0,24	3	<0,05	2,08	**± 10%**	0,50	0
Sb	<0,2	<0,1	<0,1	3	<0,1	0,01	**± 10%**	0,06	0
Zn	0,47	2,02	3,08	3	0,47	3,08	**± 10%**	4	0
Cl^-	26	175	144	3	26	175	**± 15%**	800	0
F^-	<2	*18,2*	*13,6*	3	<2	18,2	**± 15%**	10	2
SO_4^{2-}	50	267	251	3	50	267	**± 15%**	1.000	0
DOC	292	285	330	3	285	330	**± 20%**	500	0
CRS[1]	*11.600*	1.940	2.990	3	1.940	11.600	**± 10%**	4.000	1
Alkaliteta pH 4,5	18,8	1,97	4,23	3	1,97	18,8	**± 12%**	/	/
Ca	3.680	27	93	3	27	3.680	**± 10%**	/	/
Mg	0,13	2,67	22,9	3	0,13	22,9	**± 10%**	/	/
K	1.260	32,5	34,6	3	32,5	1260	**± 10%**	/	/
P	<0,2	<0,1	1	3	<0,1	1	**± 10%**	/	/
Na	238	675	923	3	238	923	**± 10%**	/	/
B	<0,2	0,73	1,53	3	<0,2	1,53	**± 10%**	/	/

[1]Celotne raztopljene snovi; [2]Število analiziranih vzorcev; [3]Merilna negotovost analitska metode; [4]Mejna vrednost parametrov izlužka za inertne odpadke (Uredba o odlagališčih odpadkov (2014)); [5]Število vzorcev nad mejno vrednostjo

da vrednosti parametrov, kot so kadmij, krom, baker, živo srebro, molibden, svinec, antimon, cink, kloridi, sulfati in raztopljene organske snovi v vodnih izlužkih neporušenih vzorcev, ne presegajo mejnih vrednosti parametrov izlužka za inertne odpadke. Mejne vrednosti pa so presežene v posameznih vzorcih izlužkov za 2 parametra: barij v vzorcu V1 in fluorid v vzorcih V2 in V3. Znatno so povečane celotne raztopljene snovi, in sicer v vzorcu V1 (11.600 mg kg^{-1} s. s.). Med raztopljenimi snovmi pa je pomemben tudi delež snovi – kloridov in sulfatov natrija in kalija. Na osnovi izmerjenih vrednosti kemijsko sestavo raztopljenih snovi v vodnem izlužku, ni možno neposredno povezati s kemijsko sestavo preiskovanih materialov. Možnost vpliva vgrajenih sanacijskih materialov na drugih lokacijah ni izključena. Vsebnosti neraztopljenih snovi, praviloma so to netopne snovi kombinacij Ca, Mg, Ba in Sr na kationski strani ter karbonatov, sulfatov, tudi fluoridov, na anionski strani, so skladne s povečanimi vsebnostmi Ca, K in vrednosti alkalitete v vzorcu V1.

Rezultati kažejo, da so že v porušenih vzorcih sanacijskega materiala, ki predstavljajo stanje materiala pred vgradnjo, kovine slabo vodotopne. Podobne rezultate kažejo tudi izmerjene vrednosti kovin v neporušenih vzorcih, ki pa predstavljajo dejansko stanje v naravi. Zato ocenjujemo, da so iz vidika možnega prehajanja kovin iz sanacijskega materiala v vodo, materiali inertni. Da so kovine slabo vodotopne v materialih, ki jih sestavljata livarski pesek ali elektrofiltrski pepel, za katere sklepamo, da so izvor kovin v sanacijskih materialih, ugotavljata tudi Kočevar (1991a) in Božym (2017). Kočevar (1991a) tudi ugotavlja, da razen povečanih vrednosti pH v izlužkih, ni kratkoročnega vpliva na vodo.

Vodotopne frakcije kovin so najšibkejše vezane frakcije kovin in kot take zelo mobilne in potencialno dostopne za bioto. Z vodotopnimi frakcijami lahko oce-

nimo delež celotnih kovin, ki se izlužuje v talno porno vodo in je tako na voljo za privzem v rastline ali pa potuje s tokom v podzemno vodo (Pirrone and Mahaffey, 2005; Ogundiran in Osibanjo, 2009; Nwoko in sod., 2018). Raziskave namreč kažejo, da je na s kovinami obremenjenem območju potrebno za rekultivacijo vrhnje plasti v kmetijske namene pazljivo izbrati vrsto gojenih rastlin, pri čemer naj bi bile najbolj primerne rastline, katerih plodovi so namenjeni za prehrano, veliko manj pa listnate rastline in rastline z gomolji. V primeru, gojenja rastlin, ki kopičijo večje količine kovin, pa je nujno potrebno rastline dolgoročno

Preglednica 7: Primerjava izmerjenih celokupnih vrednosti (mg kg^{-1} s. s.) anorganskih snovi v vzorcih okoliških površinskih tal s povprečnimi vrednostmi v slovenskih tleh ter mejno, opozorilno in kritično vrednostjo
Table 7: Comparison of measured total values (mg kg^{-1} d. m.) of inorganic substances in surrounding surface soil samples to average values in Slovenian soil and limit, warning and critical values

Parameter	T1	T2	MN[1]	Povprečne vrednosti v slovenskih tleh (0-10 cm)[2]	Faktor obogatitve T1	Faktor obogatitve T2	Mejna vrednost[3]	Opozorilna vrednost[3]	Kritična vrednost[3]
Al	16.400	17.100	± 20%	19.000	0,9	0,9	/	/	/
As	4,86	3,41	± 20%	13	0,4	0,3	20	30	50
B	5,6	8	± 20%	2,8	2,0	2,9	/	/	/
Ba	60,8	55,3	± 20%	83	0,7	0,7	/	/	/
Be	0,725	0,795	± 20%	1	0,7	0,8	/	/	/
Ca	3.410	14.100	± 20%	20.000	0,2	0,7	/	/	/
Cr	22,4	24,7	± 20%	38	0,6	0,7	100	150	380
Co	10,1	8,30	± 20%	15	0,7	0,6	20	50	240
Cu	12,6	8,8	± 20%	25	0,5	0,4	60	100	300
Fe	21.000	18.300	± 20%	28.000	0,8	0,7	/	/	/
Li	19,2	21,9	± 20%	20	1,0	1,1	/	/	/
Mg	2.750	3.330	± 20%	9.800	0,3	0,3	/	/	/
Mn	652	115	± 20%	960	0,7	0,1	/	/	/
Hg	0,064	0,051	± 20%	0,17	0,4	0,3	0,8	2	10
Mo	0,57	< 0,4	± 20%	1,4	0,4	/	10	40	200
Ni	18,2	21,4	± 20%	34	0,5	0,6	50	70	210
P	676	354	± 20%	630	1,1	0,6	/	/	/
K	1.410	1.520	± 20%	1.300	1,1	1,2	/	/	/
Si	229	223	± 20%	/	/	/	/	/	/
Na	40	54	± 20%	79	0,5	0,7	/	/	/
Sr	10,9	21,7	± 20%	30	0,4	0,7	/	/	/
S	434	540	± 20%	430	1,0	1,3	/	/	/
Ti	109	100	± 20%	120	0,9	0,8	/	/	/
V	25,6	25,2	± 20%	49	0,5	0,5	/	/	/
Pb	24,9	16,5	± 20%	40	0,6	0,4	85	100	530
Zn	50,7	38,9	± 20%	83	0,6	0,5	200	300	720
NO_2	6,02	< 5	/	/	/	/	/	/	/
NO_2-N	1,36	< 1,5	/	/	/	/	/	/	/
SO_4	26,7	90,3	/	/	/	/	/	/	/

[1]Merilna negotovost analitska metode; [2]Gosar in sod. (2019); [3]Uredba o mejnih, opozorilnih in kritičnih imisijskih vrednostih nevarnih snovi v tleh (1996)

nadzorovati (Kočevar, 1991b v Pogačnik, 2007)). Različne vrste dreves pa so sposobne shraniti enako ali večjo količino težkih kovin kot zelnate rastline. Za dekontaminacijo onesnaženih tal so posebej primerna hitro rastoča drevesa z globokim koreninskim sistemom (topol in vrba), saj ne potrebujejo pogoste obdelave (Di Lonardo in sod., 2011, Zacchini in sod., 2011).

3.2. OKOLIŠKA POVRŠINSKA TLA

3.2.1. Celokupne vrednosti anorganskih snovi

V Preglednici 7 so navedene izmerjene celokupne vsebnosti anorganskih snovi v vzorcih okoliških površinskih tal skupaj z mejnimi, opozorilnimi in kritičnimi vrednostmi nevarnih snovi v tleh (Uredba o mejnih, opozorilnih in kritičnih imisijskih vrednostih nevarnih snovi v tleh (1996)) ter povprečnimi vrednostmi elementov v slovenskih tleh (Gosar in sod., 2019).

Vsebnosti kovin so pod mejnimi, opozorilnimi in kritičnimi vrednostmi. Glede na primerjavo s povprečnimi vsebnostmi elementov v slovenskih tleh so izmerjene vrednosti elementov v vzorcih površinskih tal T1 in T2 manjše ali istega reda velikosti. Faktor obogatitve kaže, da je samo B zanemarljivo povečan (2 × v T1 oz. 2,9 × v T2). Br, Cl, F in NO_3 oz. NO_3-N so manjši od LOQ v obeh obravnavanih vzorcih tal. Vsebnost rastlinam dostopnega oz. mobilnega nitratnega dušika NO_2-N je bila zaznana v vzorcu T1 in je majhna (1,36 mg kg^{-1}). Sulfat v tleh, ki predstavlja rastlinam dostopno oz. mobilno obliko, je bil zaznan v obeh obravnavanih vzorcih (26,7 mg kg^{-1} v T1 in 90,3 mg kg^{-1} v T2).

3.2.2. Celokupne vrednosti organskih snovi

Celotni organski ogljik (TOC) v vzorcu T1 znaša 2,8 % s. s. in v vzorcu T2 2,73 % s. s., kar pomeni da so tla srednje humozna (Zupan in sod., 2008). Vrednosti mineralnih olj v vzorcu T1 (41 mg kg^{-1} s. s.) in v vzorcu T2 (43 mg kg^{-1} s. s), so pod mejno vrednostjo (50 mg kg^{-1} s. s.) (Uredba o mejnih, opozorilnih in kritičnih imisijskih vrednostih nevarnih snovi v tleh 1996). Vrednosti BTEX-ov, PCB-jev, PAH-ov, formaldehidov, fenolov, krezolov, naftolov in fenolnega indeksa so manjši od LOQ v obeh obravnavanih vzorcih tal in s tem pod mejnimi, opozorilnimi in kritičnimi vrednostmi.

Rezultati analiz kažejo, da so okoliška površinska tla v dobrem stanju, saj se vsebnosti vseh izmerjenih parametrov nahajajo pod mejnimi, opozorilnimi in kritičnimi vrednostmi. Prav tako je ugotovljeno, da vpliv emisij iz zraka zaradi vgrajevanja sanacijskega materiala na okoliška površinska tla ni zaznan.

Odkop kremenovega peska na območju Drtije je glede na rudarski načrt (Vajović in sod., 2016) segal tudi južno od obravnavanega območja, ki pa je sedaj že saniran in rekultiviran ter zatravljen. Sklepamo, da vzorčno mesto T2 predstavlja vrhnjo plast že rekultiviranega dela odkopa Drtija. Okoliška površinska tla, ki se nahajajo v neposredni bližini jugovzhodno od obravnavanega območja in vzorca T2, je preiskovala tudi Škornik Grdina (2016). Omenjena avtorica je ugotavljala osnovne lastnosti tal že rekultivirane površine kopa Drtije, ki je bil saniran z umetno pripravljeno zemljino. Slednja se po njenih raziskavah pojavlja na 40 cm globine. Ugotovila je, da sicer tla niso zadovoljivo preskrbljena s fosforjem in kalijem, vendar pa so razmere za mikrobno aktivnost in dostopnost hranil optimalne in vsebnost organske snovi je dobra. Škornik Grdina (2006) sklepa, da je obravnavano rekultivirano zemljišče primerno za kmetijsko rabo, vendar pa bi kmetijsko zemljišče lahko izboljšali s sajenjem primerne travne ruše in ustreznim gnojilnim načrtom, ki bi izboljšal preskrbljenost tal s fosforjem, kalijem in organsko snovjo. S tem bi se izboljšale razmere za rast in razvoj rastlin.

3.3. IZCEDNE VODE

3.3.1. Terenski parametri

Iz preglednice 8 je razvidno, da je za vzorec izcedne vode VO značilen majhen pretok vode (0,05 l s^{-1}), zaradi česar je reprezentativnost rezultatov meritev za vrsto parametrov, kot na primer neraztopljene snovi, biološka in kemijska poraba kisika, povsem neprimerna.

Izcedna voda iz sanacijskega materiala ima pH vrednost 7,37 in je v okviru sprejemljivih vrednosti za vode naravnega okolja (6,5 – 8,5). Električna prevodnost (EC), kot merilo raztopljenih ionsko aktivnih snovi, je poveča-

Preglednica 8: Merjeni terenski parametri pri vzorčenju izcedne vode
Table 8: Measured field parameters of leachate sampling

Oznaka vzorca	Pretok	T	EC	pH	ORP	Razt. kisik	Razt. kisik
	L s-1	°C	µS cm^{-1}	/	mV	mg O_2 l^{-1}	% O_2
VO	0,05	11,2	3.910	7,37	-106	0,84	7,6

na (3910 µS cm^{-1}). Za primerjavo znaša mejna vrednost za pitno vodo, določena z 2500 µS cm^{-1} (Pravilnik o pitni vodi (2004)). Izmerjena vsebnost raztopljenega kisika je 0,84 mg O_2 l^{-1} ter oksidacijsko-redukcijskega potenciala -106 mV, kar pomeni, da so razmere redukcijske.

3.3.2. Anorganske snovi

V preglednici 9 so predstavljeni rezultati meritev anorganskih parametrov v izcedni vodi. Iz preglednice je razvidno, da je izcedna voda obremenjena z anorganskimi snovmi, ki so v vodi dobro topne. Od osnovnih komponent vode, ki določajo mineralizacijo vode [Na, K, Ca, Mg]// [HCO_3, Cl, SO_4], so izmerjene povečane vsebnosti kalija in natrija, ki sta v ionskem ravnotežju s povečanimi vsebnostmi klorida in sulfata. Navedene sestavine vode tudi določajo povečane vrednosti električne prevodnosti. Glede na izmerjene vsebnosti kalcija (270 mg l^{-1}) ter magnezija (35 mg l^{-1}), izmerjene vsebnosti Na, K ter Cl in SO_4 presegajo pričakovana naravna razmerja v podzemni in površinski vodi navedenih elementov (Mezga, 2014). Zato so izmerjene vsebnosti Na, K ter Cl in SO_4 ocenjene za dodatno obremenitev izcedne vode. Enake ugotovitve nakazujejo tudi rezultati analiz izlužka neporušenega vzorca V1 (Preglednica 6) in rezultati izvedenih preiskav izlužkov porušenih vzorcev V1G5, V2G1 in V2G3 (Pregledni-

Preglednica 9: Vsebnosti anorganskih in nekaterih organskih parametrov (mg l^{-1}) v izcedni vodi na obravnavanem območju
Table 9: Content of inorganic and some organic parameters (mg l^{-1}) in the leachate in the study area

Parameter	VO	MN[1]	Mejna vrednost[2]	Mejna vrednost[3]	Mejna vrednost[4]
AOX	0,03	± 32%	0,5	/	/
TOC	*712*	± 20%	/	30	/
NH_4	*44*	± 15%	50	/	0,5
BPK5	*1300*	± 15%	30	/	/
KPK	*2220*	± 15%	300	/	/
Cl	*273*	± 15%	/	/	250
F	*1,35*	± 15%	/	10	1,5
P (P_2O_5)	1,15	± 20%	/	2,87	/
SO_4^{2-}	*914*	± 15%	/	/	250
$N_{CELOK.}$	48	± 30%	/	/	/
$P_{CELOK.}$	0,50	± 20%	2	2	/
$PO_4^{3-}{}_{CELOK.}$	1,54	± 20%	/	/	/
Ca	*270*	± 10%	/	/	/
Mg	*35*	± 10%	/	/	/
K	*93*	± 10%	/	/	/
Na	*1050*	± 10%	/	/	200
As	<0,01	± 10%	/	0,1	0,01
B	0,565	± 10%	/	1,0	1,0
Cr - $_{SKUPNI}$	<0,002	± 10%	0,5	0,5	0,05
Cu	<0,002	± 10%	0,5	0,5	2,0
Ni	0,0154	± 10%	0,5	0,5	0,02
Pb	<0,01	± 10%	0,5	0,5	0,01
Hg	<0,01	± 10%	0,01	0,005	0,001
Cd	<0,0008	± 10%	0,1	0,025	0,005
Zn	0,0054	± 10%	2	2	/
Fenolni indeks	*1,26*	± 20%	/	0,1	/
Formaldehid	6,11	± 20%	/	13	/

[1]Merilna negotovost analitska metode; [2]Uredba o emisiji snovi pri odvajanju izcedne vode iz odlagališč odpadkov (2008); [3]Uredba o emisiji snovi in toplote pri odvajanju odpadnih voda v vode in javno kanalizacijo (2012); [4]Pravilnik o pitni vodi (2004).

ca 5), kjer je ugotovljeno, da so izstopajoče vsebnosti sulfatov prisotne tudi v izlužkih vzorcev sanacijskega materiala, vendar pa zaradi heterogenosti območja le-te ni možno neposredno povezati z lokacijami, kjer je bil odvzet vzorec izcedne vode. Izvor sulfatov so lahko odpadki iz predelave papirniškega mulja in pepela (Goñi in sod., 2014).

Izmerjeni sta tudi povečani vrednosti za biološko porabo kisika (BPK5) 1300 mg l^{-1} O_2 in kemijsko porabo kisika (KPK) 2220 mg l^{-1} O_2. Posledica obremenitev so majhne vsebnosti kisika, kar pomeni, da so razmere ocenjene kot »pomanjkanje kisika«. Izmerjene vsebnosti amonija v vodi so povečane (44 mg l^{-1} NH_4).

Izcedna voda vsebuje fluorid (1,35 mg l^{-1}), ki pa ne presega mejne vrednosti določene z Uredbo o emisiji snovi in toplote pri odvajanju odpadnih voda v vode in javno kanalizacijo (2012). Povečane vsebnosti fluoridov so bile ugotovljene tudi v vodnih izlužkih vzorcev sanacijskih materialov (Preglednici 5 in 6). Možni izvor fluorida so odpadni keramični materiali in vsi tisti odpadni materiali, ki nastanejo po uporabi naravnih mineralnih surovin (Ponsot in sod., 2013). Ne glede na mejno vrednost, se izmerjene vsebnosti fluorida ne ocenjujejo za pomembne, saj je fluorid reaktivna snov, ki se v naravnem okolju s kalcijem, magnezijem praviloma veže v težko topne fluoride in tako ne prehaja naprej v okolje. S sestavinami kot so železo in aluminij oziroma kalij in natrij pa tvori kemijsko stabilne komplekse, ki pa so vodotopni.

Izmerjene vsebnosti težkih kovin in arzena so v izcedni vodi na koncentracijskem nivoju spodnje meje določanja (LOQ) (Preglednica 9), kar je pričakovano, saj so že analize vodnih izlužkov pokazale, da so te kovine slabo topne v vodi in s tem tudi slabo mobilne.

Obremenitve izcedne vode z nenevarnimi anorganskimi snovmi ne predstavljajo pomembnega tveganja za širjenje morebitnega onesnaženja v okolje. Na obravnavanem območju prevladuje površinski odtok padavin, le majhen delež se jih infiltrira v tla, kar nakazuje tudi majhen pretok izcedne vode in redukcijske razmere v času vzorčenja. Zaradi tega se morebitno onesnaženje, že na majhnih razdaljah od območja sanacije razredči, ko pride v stik z neobremenjeno talno vodo iz okolice ali s padavinami. Ob tem pa se lahko spremenijo tudi redoksne razmere, ki narekujejo usodo posameznih onesnaževal v vodi. Velik delež k možnosti širjenja onesnaženja v okolje pa narekujejo tudi procesi naravnih zadrževalnih sposobnosti.

3.3.3. Organske snovi

Iz preglednice 9 je razvidno, da izcedna voda vsebuje formaldehid (6,11 mg l^{-1}), ki pa ne presega mejne vrednosti določene z Uredbo o emisiji snovi in toplote pri odvajanju odpadnih voda v vode in javno kanalizacijo (2012), ki znaša 13 mg l^{-1}. Ne glede na mejno vrednost, predstavlja formaldehid zelo mobilno snov (porazdelitveni koeficient na organski C (K_{OC}) znaša 8), za katero se ne pričakuje, da se bo vezala na suspendirane snovi v vodi ali na sediment. Nedvomno pa je pričakovana biorazgradnja, v obsegu do 90 % v dveh tednih (spletni vir, 2019a). V razmerah brezvetrja obstaja možnost, da se prisotnost formaldehida v vodi zazna tudi z vonjem, prag zaznavanja je okrog 0,83 mg l^{-1} (spletni vir, 2019b). Izmerjena vsebnost fenola merjenega in izraženega kot fenolni indeks, kar pomeni skupino fenolnih snovi, znaša 1,26 mg l^{-1}. Praviloma se fenol oz. fenolne snovi v vodnih tokovih vežejo na neraztopljene, predvsem organske delce in na delce sedimenta, biološka razgradnja tako adsorbiranih fenolnih snovi poteče v nekaj dneh, odvisno od dinamike vodnega toka. Formaldehid in fenol sta tudi naravno prisotna v rastlinah (spletni vir, 2019c). Ocenjujemo, da kljub temu, da gre pri obeh snoveh za mobilno snov, zaradi relativno hitre biološke razgradljivosti nista škodljiva za okolje.

Izmerjene vsebnosti organskih snovi iz sklopov BTEX, PAH, kloriranih derivatov fenola in mineralnih olj (C10-C40) so v izcedni vodi na koncentracijskem nivoju spodnje meje določanja (LOQ).

4 SKLEPI

V prispevku smo opredelili kemično sestavo že vgrajenega sanacijskega materiala, ki je namenjen za tehnično sanacijo opuščenega peskokopa, okoliških površinskih tal in izcedne vode. Na osnovi njihove medsebojne povezave smo opredelili potencialni vpliv sanacijskega materiala na bodočo rekultivacijo.

Rezultati študije kažejo, da sanacijski materiali v obliki gradbenih kompozitov, vsebujejo znatne količine kovin (Cd, Cr, Cu, Ni, Pb, Zn), ki pa so v danih razmerah slabo vodotopne, kar nakazujejo tudi izmerjene vrednosti le-teh v vodnih izlužkih in izcedni vodi. V izcedni vodi se kovine pojavljajo na koncentracijskem nivoju spodnje meje določanja (LOQ). Zato ocenjujemo, da kovine niso na voljo za privzem v rastline ali za prenos s tokom v podzemno vodo oz. v okolje.

Obremenitve z nenevarnimi anorganskimi snovmi (Ca, Mg, Na, K, SO_4^-, F) so bile ugotovljene v vodnih izlužkih sanacijskih materialov in izcedni vodi, ki pa ne predstavljajo pomembnega tveganja za prenos v rastline v primerjavi s kovinami. Od organskih snovi pa je določena prisotnost fenola in formaldehida. Ocenjujemo, da zaradi njune relativno velike mobilnosti in hitre

biološke razgradljivosti nista škodljiva za okolje, prav tako pa se pojavljata tudi že v rastlinah kot naravni sestavini.

S preiskavami tal je ugotovljeno, da emisije iz zraka zaradi vgrajevanja sanacijskega materiala ne vplivajo na okoliška površinska tla. Slednja so glede na zakonodajne smernice v dobrem stanju.

Glede na smernice za kovine, ki jih predpisuje Uredba o mejnih, opozorilnih in kritičnih imisijskih vrednostih nevarnih snovi v tleh (1996) sanacijski materiali (gradbeni kompoiti) zaradi potencialnih škodljivih učinkov ali vplivov na človeka in okolje niso primerni za pridelavo rastlin, namenjenih prehrani ljudi ali živali ter za zadrževanje ali filtriranje vode. Ob upoštevanju, da bo po končni tehnični sanaciji površina zapolnjena s plastmi gline in humusa ter zatravljena ali pogozdena (rekultivacija vrhnje plasti), oziroma da bo odtekanje padavin iz saniranega območja pretežno površinsko, vpliv že vgrajenih sanacijskih materialov na rekultivirana tla ni pričakovan. Iz previdnostnega načela je najbolj smiselna rekultivacija z gozdom, predvsem topoli in vrbami, saj imajo globok koreninski sistem in so sposobni črpati težke kovine. V primeru kmetijske rabe bi bilo sprejemljivo pašništvo ali pridelava krme z vzpostavljenim dolgoročnim spremljanjem kakovosti tal in krme. Uporaba rekultiviranih površin za njivsko pridelavo hrane ni priporočljiva, saj bi z gnojenjem, oranjem in namakanjem lahko porušili obstoječe stanje sanacije, ki je izvedena na način, da stabilizira in imobilizira potencialne nevarne snovi (predvsem kovine) v materialih v podlagi.

5 ZAHVALA

Ta študija je bila financirana s strani Občine Moravče. Avtorici se zahvaljujeta podjetju Termit d. d. za vse podatke, ki so bili potrebni za izvedbo študije ter Civilni iniciativi Moravče za vse informacije v zvezi z okoliščinami na obravnavanem območju. Prav tako se avtorici zahvaljujeva trem recenzentom za vse konstruktivne pripombe, ki so pomagale k izboljšavi tega prispevka.

6 VIRI

Atlas okolja, (2019). Agencija RS za okolje. Spletni portal: »http://gis.arso.gov.si/atlasokolja/profile.aspx?id=Atlas_Okolja_AXL@Arso«.

Barbuta, M., Bucur, R. D., Cimpeanu, S. M., Paraschiv, G., & Bucur, D. (2015). Wastes in Building Materials Industry. *Agroecology*, *81*.

Basu, M., Pande, M., Bhadoria, P. B. S., & Mahapatra, S. C. (2009). Potential fly-ash utilization in agriculture: a global review. *Progress in Natural Science*, *19*(10), 1173-1186. https://doi.org/10.1016/j.pnsc.2008.12.006

Bavec, Š., & Gosar, M. (2016). Speciation, mobility and bioaccessibility of Hg in the polluted urban soil of Idrija (Slovenia). *Geoderma*, *273*, 115-130. https://doi.org/10.1016/j.geoderma.2016.03.015

Bożym, M. (2017). The study of heavy metals leaching from waste foundry sands using a one-step extraction. In *E3S Web of Conferences* (Vol. 19, p. 02018). EDP Sciences.

Cerar, S., & Bavec, Š. (2017). *Analiza možnih vplivov sanacijskega materiala na območju nekdanjega odkopa Drtija v lasti družbe Termit d.d. na podzemne vode in površinske vode*. Geološki zavod Slovenije, Ljubljana.

Collivignarelli, C., & Sorlini, S. (2001). Optimisation of industrial wastes reuse as construction materials. *Waste management & research*, *19*(6), 539-544. https://doi.org/10.1177/0734242X0101900610

Chuang, K. H., Lu, C. H., Chen, J. C., & Wey, M. Y. (2018). Reuse of bottom ash and fly ash from mechanical-bed and fluidized-bed municipal incinerators in manufacturing lightweight aggregates. *Ceramics International*, *44*(11), 12691-12696. https://doi.org/10.1016/j.ceramint.2018.04.070

Čop, J., Vidrih, M., & Hacin, J. (2009). Influence of cutting regime and fertilizer application on the botanical composition, yield and nutritive value of herbage of wet grasslands in Central Europe. *Grass and Forage Science*, *64*(4), 454-465. https://doi.org/10.1111/j.1365-2494.2009.00713.x

De Matos, A. T., Fontes, M. P. F., Da Costa, L. M., & Martinez, M. A. (2001). Mobility of heavy metals as related to soil chemical and mineralogical characteristics of Brazilian soils. *Environmental pollution*, *111*(3), 429-435. https://doi.org/10.1016/S0269-7491(00)00088-9

Di Lonardo, S., Capuana, M., Arnetoli, M., Gabbrielli, R., & Gonnelli, C. (2011). Exploring the metal phytoremediation potential of three Populus alba L. clones using an in vitro screening. *Environmental Science and Pollution Research*, *18*(1), 82-90. https://doi.org/10.1007/s11356-010-0354-7

Elliott, C., & Udovč, A. (2005). Nature conservation and spatial planning in Slovenia: continuity in transition. *Land use policy*, *22*(3), 265-276. https://doi.org/10.1016/j.landusepol.2004.02.002

Goñi, S., Frías, M., Vegas, I., & García, R. (2014). Sodium sulphate effect on the mineralogy of ternary blended cements elaborated with activated paper sludge and fly ash. *Construction and Building Materials*, *54*, 313-319. https://doi.org/10.1016/j.conbuildmat.2013.12.052

Gosar, M., Šajn, R., Bavec, Š., Gaberšek, M., Pezdir, V., & Miler, M. (2019). Geochemical background and threshold for 47 chemical elements in Slovenian topsoil = Geokemično ozadje in zgornja meja naravne variabilnosti 47 kemičnih elementov v zgornji plasti tal Slovenije. *Geologija, 62*(1), 5-57. https://doi.org/ 10.5474/geologija.2019.001

Grčman, H., Velikonja-Bolta, Š., Vodnik, D., Kos, B., & Leštan, D. (2001). EDTA enhanced heavy metal phytoextraction: metal accumulation, leaching and toxicity. *Plant and Soil*, *235*(1),105-114.https://doi.org/10.1023/A:1011857303823

Grčman, H., & Zupanc, V. (2018). Compensation for soil deg-

radation after easement of agricultural land for a fixed period. *Geodetski vestnik, 62*(2), 235-248.

Griessler Bulc, T., & Šajn Slak, A. (2009). Ecoremediations–a new concept in multifunctional ecosystem technologies for environmental protection. *Desalination*, *246*(1-3), 2-10. https://doi.org/10.1016/j.desal.2008.03.039

John, V. M., & Tinker, J. (1998). Recycling waste as building materials: an Internet database. *Proceedings of the CIB World Building Congress* (pp. 611-616). https://www.irbnet.de/daten/iconda/CIB8640.pdf

Knez, M., & Regent, T. (1993). Sanacija opuščenih gramoznic na Dravskem polju. *Mišičev vodarski dan '93*, 152-160.

Kočevar, H. 1991a. *Vpliv elektrofiltrskega pepela na okolje* : magistrska naloga, Ljubljana, Fakulteta za naravoslovje in tehnologijo, 124 str.

Kočevar, H., 1991b. *Ugotavljanje vsebnosti biosencialnih in toksičnih prvin v pepelu in tleh v okolici deponije elektrofiltrskega pepela, ter ocena vpliva na rastline* : raziskovalna naloga. Ljubljana, Fakulteta za naravoslovje in tehnologijo, Oddelek za montanistiko, 35 str.

Krümmelbein, J., Horn, R., Raab, T., Bens, O., & Hüttl, R. F. (2010). Soil physical parameters of a recently established agricultural recultivation site after brown coal mining in Eastern Germany. *Soil and Tillage Research*, *111*(1), 19-25. https://doi.org/10.1016/j.still.2010.08.006

Laner, D., Fellner, J., & Brunner, P. H. (2011). Future landfill emissions and the effect of final cover installation –A case study. *Waste management*, *31*(7), 1522-1531. https://doi.org/10.1016/j.wasman.2011.02.022

Lapajne, V. (1993). *Elaborat o kategorizaciji, klasifikaciji in izračunu rezerv kremenovega peska v ležiščih Moravške terciarne kadunje (Stanje 31. dec. 1992)*. Geološki zavod Ljubljana.

Marinko, M., Lapajne, T., Radelović, G., & Ruglić, Z. (1975). *Hidrogeološko poročilo o vodonosnosti peskov površinskega kopa »Drtja« Moravče«*. Geološki zavod Slovenije, Ljubljana. Mauko Pranjić, A., Mladenović, A., Sever, K., Leban, J., Božič Cerar, A., Cotič, Z., Jurjavčič, P., Šprinzer, M. & Leben, J. (2014). *Promocija uporabe recikliranih gradbenih in industrijskih odpadkov v gradbeništvu.* Poročilo za širšo javnost. Zavod za gradbeništvo Slovenije.

Mcnaughtan, T. J., & Hoyt, H. E. (1958). *U.S. Patent No. 2,856,381*. Washington, DC: U.S. Patent and Trademark Office.

Mezga, K. (2014). *Natural hydrochemical background and dynamics of groundwater in Slovenia*: PhD. thesis. Nova Gorica, Slovenia, 226 p.

MKGP. (2016). Ministrstvo za kmetijstvo, gozdarstvo in prehrano. Spletni portal: »http://rkg.gov.si/GERK/«.

Montanarella, L. (2017). Soil sealing and land take as global soil threat: the policy perspective. In: Gardi, C. (Ed.). *Urban expansion, land cover and soil ecosystem services*. Taylor & Francis, 291-295.

Nwoko, T. p., Oluwasina, O. O., Abata, E., & Ajai, O. O. (2108). Analysis of Heavy Metals in Soil from Residential and Mechanic Dumpsites. *Journal of Environment Protection and Sustainable Development, 4*(1), 7-15.

Ogundiran, M. B., & Osibanjo, O. (2009). Mobility and speciation of heavy metals in soils impacted by hazardous waste. *Chemical Speciation & Bioavailability*, *21*(2), 59-69. https://doi.org/10.3184/095422909X449481

Pavlin, A., Horvat, B., & Ducman, V. (2018). TERMIT`s Circular Economy. In: Bogataj, M., Kravanja, Z., Novak Pintarič, Z. (eds.). *1st International conference on technologies & business models for circular economy : conference proceedings,* 5th – 7th September 2018, Portorož, 89-100.

Pintar, M., Lobnik, F., & Bohanec, B. (2010). Apel proti pozidavi kmetijskih zemljišč. *Delo 52*. Ljubljana.

Podlipnik, B. (2019). Uvodnik. *Mineralne surovine*, *15*(1), 12-23.

Pogačnik. M., 2007. *Vplivi odlagališča elektrofiltrskega pepela Termoelektrarne Trbovlje na okolje* : diplomsko delo. Univerza v Ljubljani, Filozofska fakulteta, Oddelek za geografijo. 85 str.

Ponsot, I., Falcone, R., & Bernardo, E. (2013). Stabilization of fluorine-containing industrial waste by production of sintered glass-ceramics. *Ceramics International*, *39*(6), 6907-6915. https://doi.org/10.1016/j.ceramint.2013.02.025

Pravilnik o pitni vodi (2004). *Uradni list RS,* št. 19/04, 35/04, 26/06, 92/06, 25/09, 74/15 in 51/17

Pirrone, N., & Mahaffey, K. R. (2005). Where we stand on mercury pollution and its health effects on regional and global scales. In *Dynamics of Mercury Pollution on Regional and Global Scales:* (pp. 1-21). Springer, Boston, MA. https://doi.org/10.1007/0-387-24494-8_1

Rogelj, J., & Karahodžič, M. (2004). *Strokovne hidrogeološke podlage za pridobitev vodnega dovoljenja Vrtina V-1 Ples.* Geološki zavod Slovenije, Ljubljana.

Ramesh, M., Kathic, K. S., Kathikeyan, T., & Kumaravel., A (2014). Construction materials from industrial wastes – a review of current practices. *International Journal of Environmental Reseacrh and Development, 4*(4), 317-324.

Senegačnik, A., Burger, A. & Štih, J. (2019). Stanje na področju mineralnih surovin v Sloveniji v letu 2018. *Mineralne surovine*, 15(1), 9-10.

Sharma, K.D., Kumar, P., Gough, L.P., & Sanfilipo, J.R. (2004). Rehabilitation of a lignite mine-disturbed area in the Indian Desert. *Land Degradation & Development, 15*(2), 163-176. https://doi.org/10.1002/ldr.601

Spletni vir, 2019a: https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~j5fN2q:3 (citirano: 11.9.2019).

Spletni vir, 2019b: https://www.epa.gov/sites/production/files/2016-09/documents/formaldehyde.pdf; https://www.cdc.gov/niosh/docs/81-123/pdfs/0293.pdf (citirano: 11.9.2019)

Spletni vir, 2019c: https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~41z0OK:3 (citirano: 11.9.2019)

Spletni vir, 2019d: https://pubchem.ncbi.nlm.nih.gov/compound/159692#section=Computed-Properties (citirano: 11.11.2019)

Škornik Grdina, K. 2016. *Lastnosti tal rekultiviranih zemljišč površinskih kopov: diplomsko delo = Soil characteristics of recultivated mine areas : graduation thesis*, Biotehniška fakulteta, Oddelek za agronomijo, 2428), Ljubljana, 51 pp.

Turekian, K. K., & Wedepohl, K. H. (1961). Distribution of the elements in some major units of the earth's crust. *Geological Society of America Bulletin*, *72*(2), 175-192. https://doi.org/10.1130/0016-7606(1961)72[175:DOTEIS]2.0.CO;2

Uredba o emisiji snovi pri odvajanju izcedne vode iz odlagališč odpadkov (2008). *Uradni list RS*, št. 62/08

Uredba o emisiji snovi in toplote pri odvajanju odpadnih voda v vode in javno kanalizacijo (2012). *Uradni list RS*, št. 64/12, 64/14 in 98/15

Uredba o mejnih, opozorilnih in kritičnih imisijskih vrednostih nevarnih snovi v tleh (1996). *Uradni list RS,* št. 68/96 in 41/04 – ZVO-1.

Uredba o obremenjevanju tal z vnašanjem odpadkov (2008). *Uradni list RS*, št. 34/08 in 61/11.

Uredba o odlagališčih odpadkov (2014). *Uradni list RS*, št. 10/14, 54/15, 36/16 in 37/18.

Urbanc, M., & Breg, M. (2005). Gravel plains in urban areas: gravel pits as an element of degraded landscapes. *Acta geographica Slovenica*, *45*(2), 35-61. https://doi.org/10.3986/AGS45202

Vajović, S., Hribar, T., Tori, M., Rojšek, M., Galuf, S., Čadež, F., Goleš, N., & Filipič, M. (2016). *Rudarski projekt za izvedbo pri končni sanaciji zahodnega dela odkopa Drtija z zaključnim izvajanjem del pri izkoriščanju kremenovega peska za področje Drtija – zahodni del*. GR Investicije d.o.o., št. projekta: i-19/16-VS. Ljubljana.

Vrščaj, B. (2010). Varovanje kmetijskih zemljišč in racionalna raba prostora sta osnova trajnostnega razvoja. *Kmečki glas, 67*(4). Ljubljana.

Zacchini, M., Iori, V., Mugnozza, G. S., Pietrini, F., & Massacci, A. (2011). Cadmium accumulation and tolerance in Populus nigra and Salix alba. *Biologia Plantarum*, *55*(2), 383-386. https://doi.org/10.1007/s10535-011-0060-4

Zakon o gradbenih proizvodih (2013). ZGPro-1, *Uradni list RS*, št. 82/13.

Zakon o rudarstvu (2010). ZRud-1, *Uradni list RS*, št. 14/14 – uradno prečiščeno besedilo in 61/17 – GZ.

Zupan, M., Grčman, H., Hodnik, A., Lobnik, F., Kralj, T., Rupreht, J., Šporar, M., Lapajne, S., Tič, I., Šijanec, V., Gogič, S., Mohorovičič, B., Ilc, R., & Kobal, M. (2008). *Raziskave onesnaženosti tal Slovenije*. Agencija RS za okolje, Ljubljana, 63 pp.

Zupanc, V., & Grčman, H. (2016). Hydraulic characteristics of recultivated surface mines. In: Čelkova, A. (ed.). *Transport of water, chemicals and energy in the soil-plant-atmosphere system : Proceediings of peer-reviiewed contriibutiions*, 23rd International Poster Day and Institute of Hydrology Open Day, 10th November 2016, Institute of Hydrology SAS, Dúbravská cesta 9, Bratislava, Slovak Republic. Bratislava: Ústav hydrológie Slovenskej akadémie vied., 285-289.

Recenzija knjige Zdravju in okolju prijazne metode varstva rastlin

Jaka RAZINGER [1, 2]

Enaindvajsetega novembra 2019 je izšla znanstvena monografija Zdravju in okolju prijazne metode varstva rastlin v samozaložbi Kmetijskega inštituta Slovenije. Obsežna publikacija (235 strani) obravnava metode varstva rastlin z nizkim tveganjem (MNT) s poudarkom na biotičnem varstvu rastlin. Knjiga je nastala v sodelovanju več kot tridesetih soavtorjev ob finančni podpori Ministrstva za kmetijstvo, gozdarstvo in prehrano (MKGP) – Uprave RS za varno hrano, veterinarstvo in varstvo rastlin.

Glede na naraščajoče število ekoloških pridelovalcev in problemi, ki nastajajo zaradi poznih aplikacij kemičnih fitofarmacevtskih sredstev (FFS), ter naraščanja okoljskega in zdravstvenega zavedanja potrošnikov se povečuje tudi zanimanje za uporabo MNT. Uporaba MNT, zlasti biotičnega varstva rastlin, zahteva dodatna znanja ter izkušnje ob ustrezni strokovno-raziskovalni podpori in izobraževanju končnih uporabnikov – pridelovalcev. Prav to pa je poslanstvo knjige Zdravju in okolju prijazne metode varstva rastlin, s katero avtorji želijo predstaviti znanje tujih in slovenskih raziskovalnih dosežkov domačim raziskovalcem, MKGP, kmetijskim svetovalcem, študentom agronomije, biologije in sorodnih ved ter nenazadnje pridelovalcem samim, s ciljem zmanjšati uporabo kemičnih FFS v slovenskem kmetijstvu in narediti kmetijstvo bolj trajnostno.

Knjiga je razdeljena na štiri vsebinske sklope. V prvem sklopu so obravnavana FFS, katerih aktivna učinkovina so živi mikroorganizmi (bakterije, glive in virusi [3]). Drugi sklop govori o t. i. makrobiotičnih agensih, kamor sodijo agronomsko koristne žuželke, pršice in ogorčice. V tretjem sklopu so zajeta FFS na osnovi biokemikalij, rastlinskih izvlečkov, feromonov in osnovnih snovi. V četrtem sklopu pa so predstavljeni tehnološki ukrepi za varstvo rastlin, ki vključujejo tudi nekemično zatiranje plevelov. Posamezen vsebinski sklop se začne s preglednim člankom, kateremu sledi en ali več (kratkih) znanstvenih prispevkov. Pregledni članki obsežneje povzamejo neko področje (npr. Glive kot biotični agensi; Entomopatogene ogorčice kot biotični agensi ipd.) in so navadno sestavljeni iz štirih poglavij: uvod; pregled dotičnih MNT, ki jih uporabljajo v tujini; uporaba dotičnih MNT, ki so na voljo v Sloveniji; izkušnje s področja varstva rastlin z dotičnimi MNT oz. biotičnimi agensi v Sloveniji. Znanstveni članki, ki sledijo, pa podrobneje obravnavajo primere praktičnih raziskav (t. i. case study). Avtorji so pripravili tudi terminološki slovar, ki pojasnjuje strokovno terminologijo, ki je pogosto uporabljena v knjigi.

Zaradi izjemno široke snovi, ki jo knjiga obravnava, so določene tematike zgolj na kratko predstavljene. Za primer si oglejmo tretji vsebinski sklop, Sredstva za varstvo rastlin na osnovi biokemikalij, rastlinskih izvlečkov, feromonov in osnovnih snovi. V tem, sicer obširnem poglavju, avtorji najprej tabelarično navedejo številna sredstva, nato pa predstavijo in na kratko opišejo zgolj glavne kategorije oz. razrede sredstev glede na izvor aktivne snovi. Izjema so le nekatera izbrana

1 Kmetijski inštitut Slovenije, Oddelek za varstvo rastlin, Slovenija.
2 Korespondenčni avtor, e-naslov: jaka.razinger@kis.si
3 Mnenja o tem, ali so virusi mikroorganizmi ali ne, so deljena. Po klasični biološki definiciji virusi niso organizmi, saj niso sposobni samostojnega razmnoževanja in življenja, ker za svoj obstoj nujno potrebujejo gostiteljsko celico. Vseeno pa jih npr. pri Evropskem mikrobiološkem društvu vključujejo med mikroorganizme (https://microbiologyonline.org/about-microbiology/introducing-microbes/viruses).

sredstva (npr. spinosad, piretrin), ki so podrobneje opisana. Seveda pa bi si vsako sredstvo zaslužilo svoj sestavek in podrobnejšo obravnavo. Naslednji primer je četrti vsebinski sklop, kjer avtorji obravnavajo tehnološke ukrepe za varstvo rastlin. V njem avtorji natančno obravnavajo tehnološke ukrepe v določenih kulturah kot sta na primer jagodičevje in koruza, na pa v drugih kulturah. A to ne gre pripisati površnosti avtorjev, ampak prej omenjeni obsežnosti snovi, saj če bi avtorji hoteli natančno obdelati vse MNT, bi morala biti knjiga precej bolj obsežna.

Druga pomanjkljivost knjige se tiče pregleda slovenskih raziskovalnih izkušenj s področja MNT. Tu so avtorji v glavnem povzemali objave dostopne v Zbornikih Društva za varstvo rastlin Slovenije ter periodičnih publikacijah kot je npr. Acta Agriculturae Slovenica. Na osnovi pregleda literature sklepajo, da je raziskovalno poskusništvo uporabe MNT v Sloveniji sorazmeroma nerazvito, saj veliko prispevkov zgolj teoretično obravnava možnost uporabe MNT, manj pa je prispevkov o dejanskih preizkusih učinkovitosti koristnih (makro in mikro) organizmov oz. drugih MNT. Očitno pa se pisci knjige zavedajo, da obravnavani pregled objav ne predstavlja celostnega vpogleda v stanje raziskav s področja MNT v Sloveniji in dopuščajo možnost, da niso zajeli vseh relevantnih objav.

Ne glede na te (in pa morebitne druge) pomanjkljivosti, pa si knjiga zasluži, da večkrat posežemo po njej, saj bralcu nazorno prikaže izjemno širino tematike zdravju in okolju prijaznih metod varstva rastlin. Nedvomno bo svoje bralce našla med akademiki, raziskovalci, študenti in strokovnjaki s področja varstva rastlin.

E-knjiga je brezplačno na voljo tule: https://www.ivr.si/wp-content/uploads/2018/12/Zdravju-in-okolju-prijazne-metode-varstva-rastlin_KONCNA.pdf

Ob 90-letnici akademika zaslužnega profesorja dddr. Jožeta Mačka

28. oktobra 2019 je dopolnil 90 let akademik, zaslužni profesor dddr. Jože Maček. Profesor, kot smo ga v obdobju njegovega delovanja na Biotehniški fakulteti klicali in ga še vedno kličemo njegovi nekdanji sodelavci, je bil dolgoletni glavni in odgovorni urednik ter sodelavec Zbornika Biotehniške fakultete, danes revije Acta Agriculturae Slovenica.

Akademik Maček je doktor agronomskih, zgodovinskih in ekonomskih znanosti, zaslužni profesor ljubljanske univerze, upokojeni redni profesor za fitopatologijo, gozdno fitopatologijo in fitofarmakologijo, dopisni član Hrvaške akademije znanosti in umetnosti, član Evropske akademije znanosti in umetnosti v Salzburgu, dobitnik Zoisove nagrade za življenjsko delo na področju fitomedicine, častni član Društva za varstvo rastlin Slovenije in dobitnik številnih drugih priznanj, vseh za njegovo dolgoletno kakovostno delo na področju fitomedicine in slovenske znanosti nasploh. Njegovo 38-letno pedagoško obdobje je profesor, ki je eden od najprepoznavnejših slovenskih fitomedicinskih strokovnjakov, preživel na današnjem Oddelku za agronomijo Biotehniške fakultete.

Kot raziskovalec v fitomedicini je akademik Maček deloval na več področjih. Tako je v okviru fitopatologije raziskoval patološko fiziologijo, primarni in sekundarni parazitizem, razvojne kroge parazitskih gliv in njihovo odpornost na sistemične fungicide. Ugotovil je precejšnje število za Slovenijo in prejšnjo državo novih vrst parazitskih gliv in njihovih gostiteljskih rastlin. Pri preučevanju hiponomološke favne v Sloveniji je ugotovil

več sto za Slovenijo, prejšnjo državo in jugovzhodno Evropo novih vrst in precejšnje število doslej neznanih gostiteljskih rastlin. Intenzivno je preučeval vpliv raznih skupin herbicidov na talne (tudi parazitske) mikroorganizme. Dolgo je preučeval kontaminacijo rastlin in tal z ostanki fitofarmacevtskih sredstev v Sloveniji in ugotovil sorazmerno skromno obremenitev tako rastlin (pridelkov) kot tal v Sloveniji. Preučeval je tudi ekonomiko varstva rastlin.

Profesor Maček velja za našega najbolj plodovitega pisca strokovne literature s področja fitomedicine. Razprav in člankov je v tujih in domačih revijah objavil nad 350, raznih krajših strokovnih in poljudno-strokovnih prispevkov pa nad 3000. Akademik Maček je najuspešnejši prevajalec strokovne literature iz biotehniških strok pri nas, izjemnega pomena pa je tudi njegov prispevek k obogatitvi fitomedicinske in biotehniške terminologije nasploh. V obdobju 1984-1994 je objavil 4 univerzitetne učbenike s področja posebne fitopatologije za študente agronomije. Med izvrstnimi knjigami izpod peresa profesorja Mačka je leta 2008 izdani učbenik Gozdna fitopatologija. V okviru njegovega bogatega pedagoškega dela, tako na dodiplomskem kot tudi na podiplomskem študiju, je bil mentor okrog sto diplomantom, desetim magistrandom in sedmim doktorandom.

Akademik Maček je eden od najzaslužnejših za ustanovitev Društva za varstvo rastlin Slovenije, osrednje domače stanovske organizacije raziskovalcev, svetovalcev in drugih strokovnjakov, ki delajo na področju varstva rastlin in ki danes šteje okrog 200 članov. Njegova vloga pri delovanju omenjenega društva in organizaciji prvih trinajstih posvetovanj (1993-2013) je neprecenljiva, saj je bil urednik vseh Zbornikov predavanj in referatov iz posvetovanj, ki so izjemno pomemben vir informacij o varstvu rastlin v Sloveniji v navedenem obdobju, s katerimi se je tudi dobesedno pisala zgodovina slovenskega varstva rastlin.

Nekaj dni pred njegovim 90. rojstnim dnevom je izšla knjiga Zgodovina spremljanja pojava bolezni in škodljivcev na gojenih rastlinah v Sloveniji od 15. do sredine 20. stoletja, v soavtorstvu profesorja Mačka in pokojnega prof. Franceta Adamiča. V knjigi, ki bo tudi koristen učni pripomoček študentom agronomije in zgodovine, je predstavljenih 38 domačih strokovnjakov, ki so v navedenem obdobju delovali na področju varstva rastlin. Druga za domačo strokovno javnost izjemno pomembna knjiga, ki je prav tako izšla le nekaj dni pred profesorjevim osebnim jubilejem, je njegova popolna fitomedicinska bibliografija, kjer so na 235 straneh navedeni prav vsi bibliografski zapisi o znanstvenem, strokovnem, in pedagoškem delovanju profesorja Mačka na področju fitomedicine. Tako bodo imeli prispevek profesorja Mačka slovenski fitomedicini in znanosti nasploh možnost spoznati tudi mlajši domači fitomedicinski in kmetijski strokovnjaki.

Spoštovani Profesor, hvala za vse in vse najboljše ob vašem visokem osebnem jubileju!

prof. dr. Stanislav Trdan in sodelavci
iz fitomedicinskega dela Katedre za
fitomedicino, kmetijsko tehniko,
poljedelstvo, pašništvo in travništvo

V spomin prof. dr. Franzu Pirchnerju
(1927–2019)

Franz Pirchner je bil rojen v Imstu na Tirolskem, kjer se je na domači kmetiji prvič srečal z živinorejo. Po končani osnovni šoli je obiskoval trgovsko šolo in trgovsko akademijo v Innsbrucku, po vojni pa je leta 1945 začel s študijem medicine na Univerzi v Innsbrucku, leto kasneje pa se je vpisal na Veterinarsko fakulteto na Dunaju, leta 1950 pa še na Kmetijsko univerzo (BoKu) na Dunaju. Po diplomah na Veterinarski fakulteti (1950) in Kmetijski univerzi (1952), je dve leti deloval na Tirolski kmetijski zbornici v Innsbrucku, nato pa je odšel na doktorski študij v Združene države Amerike, kjer je pod mentorstvom J.L. Lush-a leta 1957 doktoriral na Iowa State University. Po nekajletnem delu na živinorejskem inštitutu Rottenhaus pri Wieselburgu in kot genetik v perutninarski industriji v ZDA in v Nemčiji, se je leta 1964 odzval vabilu Veterinarske fakultete na Dunaju in postal redni profesor na Katedri za živinorejo, leta 1971 pa je sprejel povabilo Tehniške univerze v Münchnu, Weihenstephan, kjer je kot redni profesor za živinorejo in genetiko deloval do upokojitve leta 1995.

Njegovo raziskovalno in pedagoško delo je bilo usmerjeno v populacijsko in kvantitativno genetiko domačih živali s posebnim poudarkom na ohranjanju lokalnih pasem. Njegov učbenik Populationsgenetik in der Tierzucht (Population Genetics in Animal Breeding) je njegov najpomembnejši prispevek k razvoju genetike v živinoreji in še danes predstavlja eno temeljnih študijskih gradiv za študente živinoreje v številnih državah. Raziskovalni opus prof. Pirchnerja obsega več kot 200 znanstvenih publikacij, tekom svojega pedago-

škega delovanja pa je bil mentor 60 doktorandom, ki so k njemu prihajali iz celega sveta. Številni med njimi so danes univerzitetni profesorji na področju genetike in živinoreje. Njegovo izredno široko poznavanje strokovne literature je spodbujalo poglobljene in plodne diskusije, ki so pogosto pomenile začetek novih trendov v živinoreji. Prof. Pirchner je bil član odbora za genetsko-statistične metode pri Nemškem genetskem društvu, bil je odgovorni urednik revije *Journal of Animal Breeding and Genetics* (1975–1998), član uredniškega odbora revije *Archiv für Tierzucht*, predsednik komisije za genetiko pri EAAP (1966–1972), predsednik mednarodnega stalnega odbora WCGALP (1988–1992) in nemškega društva za znanost v živinoreji (1984–1988). Za njegov znanstveni doprinos je prejel častna doktorata Univerze v Ghentu, Belgija in ETH v Zürichu, Švica. Nemško živinorejsko združenje mu je leta 1998 podelilo medaljo Hermanna Nathusiusa. Prof. Pirchner je bil 25 let član uredniškega odbora revije *Acta Agriculturae Slovenica* (AAS), kjer je s svojimi nasveti in kakovostnimi recenzijami pomembno prispeval k rasti naše revije.

In Memoriam Prof. Franz Pirchner, PhD (1927–2019)

Born in Imst, Tyrol, Franz Pirchner was first introduced to livestock farming on a domestic farm. After graduating from elementary school, he attended the Commercial School and the College of Commerce in Innsbruck. After the world war II he entered study of medicine at the University of Innsbruck in 1945, and a year later he moved to the Veterinary Faculty in Vienna in 1946. In 1950 he started also the study of agriculture at the Agricultural University. (BoKu) in Vienna. After graduating from the Faculty of Veterinary Medicine (1950) and the Agricultural University (1952), he worked for two years at the Tyrol Chamber of Agriculture in Innsbruck. After that he moved to United States of America, where he prepared his doctorate under the mentorship of J.L. Lush. In 1957, he received his doctorate from the Iowa State University. After working for several years at the Rottenhaus Livestock Research Institute at Wieselburg and as a geneticist in the poultry industry in the US and in Germany, he accepted the invitation of the Faculty of Veterinary Medicine in Vienna in 1964 and became a full professor at the Department of Animal Production. In 1971 he accepted the invitation of the Technical University of Munich, Weihenstephan, where he kept the position of full professor of Animal breeding and genetics until his retirement in 1995.

His research and teaching work has focused on population and quantitative genetics in domestic animals with a particular focus on the conservation genetics in local breeds. His textbook Population Genetics in Animal Breeding (Populationsgenetik in der Tierzucht) is his most important contribution to the development of genetics in animal breeding and is still one of the core study materials for animal science students in many countries. The research opus of prof. Pirchner counts more than 200 scientific publications, and during his teaching career he has been a mentor to 60 doctoral students coming from all over the world. Many of them are university professors in genetics and animal husbandry today. His extraordinarily broad knowledge of the scientific literature was a motivation for numerous in-depth and fruitful discussions that often marked the beginning of new trends in livestock production. Prof. dr. Pirchner was a member of the Board of genetic-statistical methods at the German Genetic Society, editor-in-chief of the *Journal of Animal Breeding and Genetics* (1975–1998), a member of the editorial board of *Archiv für Tierzucht*, chairman of the genetics committee at EAAP (1966–1972), chairman of the WCGALP International Standing Committee (1988–1992) and the German Society for Animal Science (1984–1988). For his scientific contributions he received honorary doctorates from the University of Ghent, Belgium and the ETH in Zurich, Switzerland. In 1998, the German Association for animal production awarded him the Hermann Nathusius Medal. Prof. dr. Pirchner has been a member of the editorial board of *Acta Agriculturae Slovenica* (AAS) for 25 years, where he has made a significant contribution to the growth of our magazine through his advice and high quality reviews.

Prof. dr. Peter Dovč

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 naslednje 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. Navedbe morajo biti v slovenskem in angleškem jeziku, kadar so prispevki v slovenščini. Uredništvo revije zagotovi prevode izbranih bibliografskih elementov (naslova, izvlečka, opomb in ključnih besed) v primeru tujih avtorjev. Prispevke sprejemamo skozi celo leto.

POSTOPEK ODDAJE PRISPEVKOV

Avtorji lektorirane prispevke oddajo v elektronski obliki na spletni strani OJS Acta agriculturae Slovenica. Pred oddajo prispevka se mora avtor na spletni strani najprej prijaviti oziroma registrirati, če prvič vstopa v sistem (potrebno je klikniti na Registracija in izpolniti obrazec za registracijo). Bodite pozorni, da na dnu regi-

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 Slovenian or English language. Review articles are published upon agreement with the editor. Reports presented on 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 % of new original content, but the editorial board must be notified beforehand. If the paper is part of 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 foot note. Slovenian-language translation of selected bibliographic elements, for example the title, abstract, notes and keywords, will be provided by the editorial board. Manuscripts are accepted throughout the year.

SUBMISSION PROCESS

Manuscripts should be submitted to the Acta agriculturae Slovenica OJS site. The submitting author should be registered to the site. Click Register and fill in the registration form. Be sure to check in the Author

stracijskega 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
- Grafično gradivo naj bo naloženo v eni ZIP datoteki. Grafične slike imenujte Slika1.jpg, Slika2.eps in podobno.
- Za vsako dodatno naloženo datoteko je treba zagotoviti predvidene metapodatke.

Korak 5: Potrditev
- Potrebna je končna potrditev.

check box on the form. We advise you to check in also the Reader check box.

Submission process consists of 5 steps. Before submission, authors should go through the checklist and prepare for submission:

Step 1: Starting the submission
- Choose one of the journal sections.
- Confirm all the requirements of the Submission Preparation Checklist.
- Additional plain text comments for the editor can be provided in the relevant text field.

Step 2: Upload submission
- Upload full manuscript in the form of the Microsoft Word document file format (.doc or .docx).

Step 3: Enter metadata
- First name, last name, contact e-mail and affiliation for all authors, in relevant order, must be provided. Corresponding author has to be selected.
- Title and abstract must be provided in plain text.
- Key words must be provided (max. 8, separated by semicolons) and enter the language of the text.
- Data about contributors and supporting agencies may be entered.
- References in plain text must be provided in the relevant text filed (between each reference should be a blank line).

Step 4: Upload supplementary files
- All graphic have to be uploaded in a single ZIP file. Graphics should be named Figure1.jpg, Figure2.eps, etc.
- For each uploaded file the author is asked for additional metadata, which may be provided.

Step 5: Confirmation
- Final confirmation is required.

PODROBNEJŠA NAVODILA / DETAILED INSTRUCTIONS

http://ojs.aas.bf.uni-lj.si/index.php/AAS/about/submissions#authorGuidelines