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Vpliv pretoka zraka na sušenje krme na sušilnih napravah

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Vpliv pretoka zraka na sušenje krme na sušilnih napravah

Izvleček: V alpskih državah Evrope sušenje krme s travinja postaja vedno bolj pomembno zaradi ugotovljenih pozitivnih učinkov na maščobno kislinsko sestavo mleka. S pravilnim postopkom in tehnologijo dosuševanja krme se lahko kvaliteta dvigne na primerljiv nivo travnih silaž. Sušenje temelji na odvzemu vlage iz krme s pomočjo prevetrovanja – zraka, ki je ključen za hitrost sušenja. Poleg majhne relativne vlažnosti zraka je pomemben tudi njegov pretok skozi krmo. V literaturi se navaja spodnja mejna vrednost pretoka zraka in zgornja mejna vrednost, pri čemer je razlika 85 %. Članek opisuje izvedeni eksperiment merjenja porabe energije in učinkovitosti sušenja pri običajno v praksi uporabljenem manjšem in večjem pretoku zraka. Izkazalo se je, da pri večjem pretoku zrak porabimo za 38 % več energije na izločen kilogram vode, kot pa pri manjšem pretoku. Izvedeni preizkus dokazuje, da sušenje z velikim pretokom zraka ne prinese enako večji učinek.

Ključne besede: pretok zraka; sušenje krme; sušilna naprava; izločena voda; energija

The effect of air flow on drying fodder on drying systems

Abstract: Drying grass fodder in Europe's Alpine countries is becoming increasingly important due to positive effects on the fatty acid composition of milk. A proper approach and technology of fodder drying can raise its quality to the level of grass silage. In principle, drying fodder means extracting water from fodder by means of ventilation – the air, the key to the speed of drying. Besides low relative air humidity, its flow rate through the fodder is also important. In the literature, the lower- and upper-bound limits of air flow rates can be found, with a difference of 85 % between the two. The article describes a test, measuring energy consumption and the efficiency of drying at a low air flow, often used in practice, and a high air flow. It was found that a high air flow results in 38 % higher energy consumption per kilogramme of extracted water, compared to a low air flow. The executed test has proved that drying at a higher air flow will not have a proportionally greater effect.

Key words: air flow; drying fodder; drying system; energy; water extracted

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1 UVOD

Kmetje, sirarne in mlekarne iščejo nove priložnosti in tržne niše za prodajo pridelkov in izdelkov. Ena izmed tržnih niš, ki je bila prepoznana tudi v Sloveniji, je seneno mleko in seneno meso. Za ekonomično prirejo senenega mleka in mesa je ključno seno. To predstavlja pomemben del krmnega obroka, zato je še toliko pomembnejša njegova kakovost. V Sloveniji je sušenje krme s travinja precej razširjeno. Rezultati popisa na kmetijah v kontroli prireje mleka (5.198 kmetij) kažejo, da na približno 1/3 kmetij prevladuje siliranje, na 1/3 kmetij krmo samo sušijo ali pretežno sušijo, na 1/3 pa sta sušenje in siliranje zastopana enakovredno (Verbič in sod., 2006). Priprava sena poteka pretežno na prostem. Na sušilnih napravah posuši večji del sena le slaba tretjina kmetov (Verbič in sod., 2006). Temu primerna je tudi kakovost mrve. Rezultati analiz sena s kmetij, ki so jih v obdobju 2000-2010 opravili v slovenskih laboratorijih kažejo, da vsebuje seno v povprečju le 5,05 MJ NEL (neto energije za laktacijo) na kg sušine, kar je za 15 % manj kot pri travnih silažah (Verbič in sod, 2011). Kakovost sena je odvisna od kakovosti pokošene krme, ter od izgub in sprememb krme med sušenjem in skladiščenjem. Sušenje ali dosuševanje sena na tradicionalnih sušilih (kozolci, ostrvi), prevetrovalnih napravah s hladnim ali toplim zrakom ali na kondenzacijskih sušilnicah brez dvoma prispeva k boljši kakovosti sena. Avstrijska študija je na vzorcu, ki je vključeval prek 500 kmetij, pokazala, da suši seno na tleh manj kot ena tretjina kmetov (27,8 %), da 39,0 % kmetov seno prevetruje s hladnim zrakom, 33,2 % pa s toplim zrakom (Resch in sod., 2011). Na 6,3 % od vseh kmetij, ki seno dosušujejo na sušilnih napravah, izvajajo sušenje v valjastih balah. Rezultati analiz so pokazali, da je v Avstriji dosuševano seno precej boljše od sena, ki je sušeno na tleh. Pridelava kakovostnega sena je mogoča samo z dodatnim sušenjem na sušilnih napravah, saj pride pri sušenju na tleh do prevelikih izgub zaradi drobljenja krhkih delov travniških rastlin (lahko tudi do 30 %) in s tem do zmanjšanja hranilne vrednosti pridelanega sena. Poleg tega s sušenjem na sušilnih napravah zmanjšamo tveganja povezana z neugodnimi vremenskimi razmerami in se izognemo zmanjšanju hranilne vrednosti krme zaradi morebitnega dežja med spravilom. Sušenje s hladnim zrakom se večinoma prakticira od 30 % vlažnosti krme navzdol. Pri tem pa prihaja tudi že do povečanega drobljenja najobčutljivejših delov rastlin. Razlika med izgubami zaradi drobljenja med 50 % vlažnostjo krme in 30 % vlažnostjo krme je v povprečju 4 % do 5 %, lahko tudi 8 % (Frick in sod., 1999). Osnovni princip sušenja krme je tako pogojen z zagotavljanjem pretoka zraka skozi krmo. Tehnološki princip sušenja sena v boksu je sestavljen iz ventilatorja, ki zagotavlja pretok zraka in boksa z rešetko čez celotno površino, ki omogoča, da zrak enakomerno prehaja skozi krmo in jo suši. Pri sušenju bal ventilator zrak vpihava v kanal na katerem so položene bale. Zrak prehaja skoznje in jih suši. Osnovni princip velja za katerokoli poznano tehnologijo sušenja krme. Pretok zraka lahko rečemo, da je ključen za odvzem vlage krmi. Pri prehodu zraka ustrezne vlažnosti skozi krmo se nanj veže voda iz krme. Logično razmišljane bi bilo, da čim večja količina zraka gre skozi krmo pomeni hitrejše in s tem učinkovitejše sušenje, vendar temu ni tako, kar bo potrjeno tudi v preizkusu. Na sušilnih napravah imamo običajno na voljo omejeno količino toplote za dogrevanje, količino vpihanega zraka pa se lahko spreminja. Literatura navaja različne hitrosti/količine zraka skozi krmo. Vrednosti so različne za bale in za sušenje sena v razsutem stanju. Pri sušenju bal se navaja vrednost v količini zraka na balo. Ta je opredeljena med 1100 $m^3\ h^{-1}$ in 1500 $m^3\ h^{-1}$ (Wirleitner, 2013). Pri sušenju sena v razsutem stanju je večji razpon med spodnjo in zgornjo priporočeno mejo in sicer med 0,07 $m^3\ s^{-1}\ m^{-2}$ in 0,13 $m^3\ s^{-1}\ m^{-2}$ površine sušilnega prostora (Wirleitner, 2011).

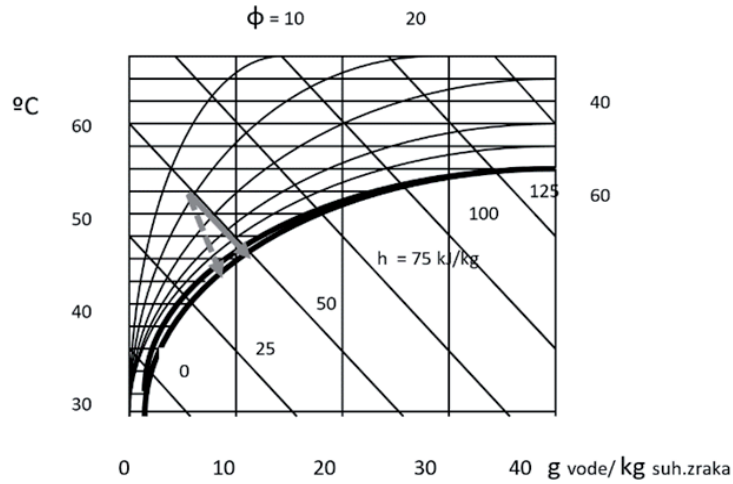
2 MATERIAL IN METODE

2.1 OSNOVNI PRINCIP SUŠENJA KRME

Bistvo procesa sušenja je, da v krmi zmanjšamo vsebnost vlage na skladiščno vrednost. Seno je dovolj suho za skladiščenje, če vsebuje najmanj 86 % SS (suhe snovi). Voda je v rastlinah prisotna v celicah in medceličnem prostoru, ki nato prehaja na površino rastline (Daszkowska-Golec in Szarejko, 2013). Pri gibanju zraka ob površini rastline se voda uparja – izhlapeva do nasičenosti plasti zraka, ki potuje ob rastlini. Zrak je nasičen z vodno paro – vlago, ko doseže relativno vlažnost 100 %. Relativna vlažnost se meri v procentih, absolutna pa se običajno podaja v g vode na kilogram zraka. Mejne vrednosti absolutne vlažnosti zraka so odvisne od temperature zraka (Tabela 1). Pri temperaturi 20 °C 1 kg zraka

Tabela 1: Absolutna in relativna vlažnost zraka
Table 1: Absolute and relative air humidity.

T zraka [°C]	Relativna vlažnost [%]	Absolutna vlažnost [g kg-1]
20	65	11
20	100	14,7
25	65	15
25	100	20,1



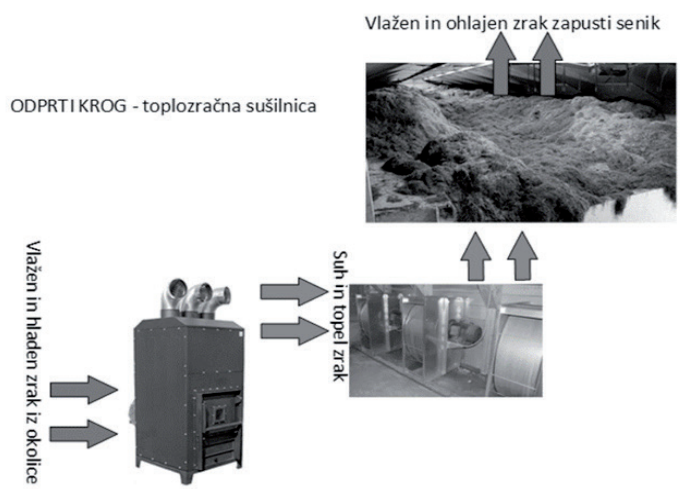
Slika 1: Prikaz adiabatnega vlaženja v h-x diagram (Benedičič in Verbič, 2013)
Figure 1: Adiabatic moisturising, shown on an h-x diagram (Benedičič and Verbič, 2013)

lahko sprejme 14,7 g vode, pri 25 °C pa 20,14 g vode, kar pomeni 37 % več (Sargent, 1980). Sušenje pri višjih temperaturah je zato hitrejše.

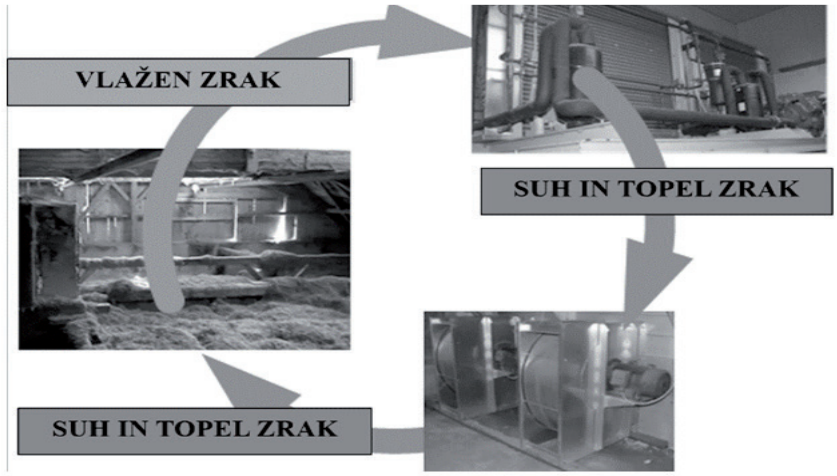
Uporaba sistemov sušenja v praksi je pokazala, da pri hladnem prevetrovanju lahko upoštevamo povprečen odvzem največ 1 g vode na m3 vpihanega zraka, pri uporabi sončne strehe 2 g vode na m3 vpihanega zraka in pri sušenju s toplotno črpalko tudi 5 g vode na m3 zraka. Pri odvzemu 0,47 g vode na m3 se zrak ohladi za 1°C (Wirleitner, 2011). To velja za idealni adiabatni proces. Sistem vlaženja zraka poteka adiabatno (Rant, 2011). Na sliki 1 je prikazan idealen adiabatni proces (polna črta), medtem ko je s prekinjeno črto prikazan dejanski proces. Razlika nastane zaradi izgube toplote.

2.2 TOPLOTA ZA SUŠENJE

Z razvojem tehnologij segrevanja zraka in poznavanjem mehanizma adiabatnega sušenja so se poleg hladnega prevetrovanja in prevetrovanja z zrakom izpod kritine razvili tudi sistemi prevetrovanja s toplim zrakom, ki jih delimo glede na vir energije: biomasa (kot vir toplote se uporabljajo drva, peleti, sekanci, žagovina); kurilno olje; plin; električna energija (toplotna črpalka).



Slika 2: Odprt zračni tok
Figure 2: Open air flow.



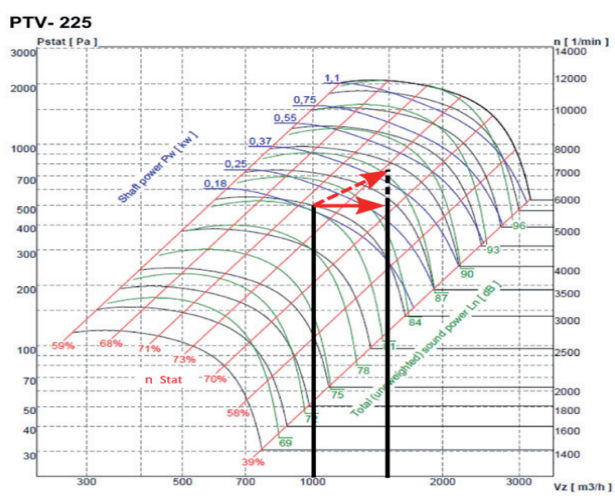
Slika 3: Zaprt zračni tok
Figure 3: Closed air flow

Biomasa, kurilno olje in plin z gorenjem proizvajajo toploto. V primeru toplozračnih peči toplota prehaja direktno na zrak, v primeru peči v katerih se segreva voda, se toplota s pomočjo toplovoda transportira do sušilne naprave kjer preko toplotnega izmenjevalca segreva zrak.

2.3 POTEK ZRAČNEGA TOKA PRI SUŠENJU

V osnovi ločimo sušenje v odprtem zračnem krogu in sušenje v zaprtem zračnem krogu. Sušenje v zaprtem zračnem krogu je sušenje s toplotno črpalko, medtem ko so vsa ostala sušenja (biomasa, plin, toplota iz bioplinarn...) v odprtem zračnem toku.

Pri odprtem zračnem toku (Slika 2) zrak sesamo iz okolice in ga preko enega izmed prej omenjenih načinov segrevamo, pri čemer se mu zmanjša relativna vlaga. Nato ga vpihavamo v sušilni boks ali bale. Zrak se pri potovanju skozi krmo navlaži in ohladi, nato ga odvajamo iz sušilne komore v okolico. Slaba stran sušenja v odprtem krogu je v tem, da vedno sesamo zrak in okolice in ga dogrevamo. To lahko predstavlja problem v mrzlih spomladanskih in jesenskih nočeh. Če je zunanja nočna temperatura 5 °C in segrevate zrak za 15 °C, temperatura vstopa zraka v boks ne bo višja kot 20 °C. Glede na zgor-



Slika 4: Karakteristika ventilatorja Oravent PTV 225 s primerom povečanja pretoka
Figure 4: Oravent PTV 225 fan characteristics with an example of increased flow.

nje ugotovitve je sposobnost zraka za odvzem vlage na 1 m^3 pri nižji temperaturi nižja kot pri višjih temperaturah. (Tabela 1).

Zaprt zračni tok (Slika 3) je značilen za kondenzacijske sušilnice. Zrak kroži od ventilatorjev preko sena kjer se navlaži do toplotne črpalke, ki ga razvlaži in segreje, ter nato ponovno do ventilatorjev. Krog potovanja zraka je tako zaprt in sklenjen. Takšen sistem ni odvisen od zunanjega vremena in zunanje temperature. Učinek sušenja je večji, saj praviloma tudi pri nizkih zunanjih temperaturah in pravilno dimenzioniranem sistemu temperatura vpihanega zraka v seno ni nižja kot 25 °C do 30 °C, kar pomeni večjo možnost odvezam vode iz krme (Tabela 1).

Osrednji element vsake sušilne naprave je ventilator. Ta zagotavlja potreben pretok zraka, ki mora premagovati odpor pri prehodu zraka skozi krmo. Temu pravimo, da ventilator zagotavlja potreben statični tlak za premagovanje odpora pri pretoku zraka. Medsebojni vpliv je obratno sorazmeren. Na sliki 4 je prikazan vpliv povečanja pretoka na povečanje potrebne moči za pogon ventilatorja. V prvem primeru (puščica s polno črto) se pretok pri konstantnem tlaku poveča iz 1000 $m^3 h^{-1}$ na 1500 $m^3 h^{-1}$, posledično se poveča potrebna moč iz 0,19 kW na 0,30 kW. Realno se pri povečanju pretoka zraka skozi krmo poveča tudi upor in s tem potreben statični tlak. Na sliki 4 je prikazan primer (puščica s črtkano črto) istočasnega povečanj pretoka zraka in upora. Potrebna moč ventilatorja se dvigne iz 0,19 kW na 0,43 kW, kot prikazuje slika 4. Večji pretok zraka pomeni večjo potrebno moč in s tem večjo porabo energije (Wirleitner, 2013).

2.4 PARAMETRI PREIZKUSA

Kmet kot odločevalec in investitor se odloča za uvedbo določene tehnologije tudi na podlagi predvidenega povečanja produktivnosti, uvedbe novih izdelkov ali zmanjšanja stroškov. Poleg dodane vrednosti pri trženju novih izdelkov je stroškovni vidik eden izmed pomembnejših pri uvedbi tehnologije sušenja na kmetiji ali kmetijskem podjetju. Stroški sušenja so eden izmed spremljanih parametrov preizkusa. Izražen bo v obliki porabljene energije glede na izločeno vodo. Manjša je poraba energije na enoto izločene vode učinkovitejše in cenejše je sušenje. Naslednji parameter, ki smo ga spremljali je masa izločene vode. Osnovni cilj sušenja je izločiti čim več vode iz rastlin in s tem rastlino posušiti. Intenzivnost sušenja je mogoče definirati v masi izločene vode na časovno enoto. Čim večja je ta, tem hitrejše in učinkovitejše je sušenje. Pretok zraka ne bo opazovan ampak predhodno nastavljen na dve vrednosti in sicer spodnjo vrednost 0,85 $m^3 s^{-1} m^{-2}$in zgornjo vrednost 0,13 $m^3 s^{-1} m^{-2}$.

2.5 PREIZKUS

V okviru preizkusa smo sušili lucerno. Ta je vse pomembnejša krmna rastlina. Po podatkih Statističnega urada smo jo v Sloveniji v devetdesetih letih prejšnjega stoletja pridelovali na približno 10.000 ha. Nato se je njena uporaba močno zmanjšala. Predvsem zaradi finančnih podpor za beljakovinske rastline v okviru shem neposrednih plačil se zanimanje za pridelovanje lucerne spet povečuje, tako da smo jo v letu 2016 pridelovali že na približno 5600 ha. Sušenje lucerne v razsutem stanju je bilo izvedeno v posebej pripravljenem testnem zabojniku (Slika 5).

Zabojnik je bil kvadratne oblike z dimenzijo stra-

Slika 5: Testni zabojnik za izvedbo preizkusa
Figure 5: Test box for test execution.

nice 1,25 m in višino 2 m. Izdelan je bil kot sušilni boks za razsuto stanje krme. V spodnjem delu v oddaljenosti 0,3 m od dna zabojnika je bila nameščena rešetka. Pod to rešetko je bil na bočni strani zalogovnika nameščen frekvenčno krmiljeni ventilator in električni grelec stalne toplotne moči 9 kW. Višina nalaganja krme v zalogovnik je bila 1,7 m. Na zgornji strani je bil zalogovnik nihajno vpet na tehtnico, ki je beležila spremembo teže zaradi izhlapele vode iz sušeče se krme. Ventilator je zrak preko električnega grelca sesal iz okolice in ga vpihoval pod rešetko na dnu zabojnika. Sistem je deloval na principu odprto krožnega načina sušenja. Zrak za sušenje smo zajemali iz okolice, izhodni zrak iz zabojnika pa se je odvajal v okolico Med sušenjem smo spremljali maso zabojnika s krmo in tako določili maso izhlapele vode. Sušenje je potekalo intervalno med manjšim pretokom zraka (0,85 $m^3 s^{-1} m^{-2}$) in večjim pretokom zraka (0,13 $m^3 s^{-1} m^{-2}$). Ne glede na pretok zraka, smo dovedenemu zraku dovajali 9 kW toplotne energije na uro. Sušenje je potekalo 12 ur s šestimi izmeničnimi intervali (prvi interval sušenje z manjšin pretokom, drugi z večjim pretokom, naslednji ponovno z manjšim, ...).

3 REZULTATI IN RAZPRAVA

Preizkus je potekal 12 ur. V tem času smo izločili 38,5 kg vode. Ventilator je pri manjšem pretoku (0,85 $m^3 s^{-1} m^{-2}$) imel porabo električne energije 27 W h, pri večjem pretoku (0,13 $m^3 s^{-1} m^{-2}$) pa 43 W h. V povprečju smo pri manjšem pretoku na izločili 3,01 kg vode na uro sušenja, pri večjem pretoku pa 3,48 kg vode na uro sušenja. Količina izločene vode pri manjšem pretoku se med posameznimi intervali ni bistveno razlikovala (v povprečju za 1 %), med tem ko se je pri višjih intervalih v povprečju za 6 %. V Kolikor preračunamo količino izločene vode na pretok zraka ugotovimo, da se je pri manjšem pretoku izločalo 6,02 g vode na m^3 zraka, pri večjem pretoku pa 4,97 g vode na m^3 zraka. Iz dobljenih rezultatov ugotovimo, da vrednost pretoka zraka ni merilo za merjenje učinkovitosti sušenja ampak to mora bit povezano z količino izločene vode. Rezultati kažejo, da 40 % večji pretok zraka omogoči le 21 % večje izločanje vode. Glede na časovno enoto pa to pomeni le 15 % več izločene vode na uro. Zanimiva je tudi ugotovitev, da pri večjem pretoku zraka izločanje vode na enoto pretoka zraka hitreje pada kot pri manjšem pretoku zraka. Rezultati preizkusa potrjujejo, da voda v rastlinah potrebuje določen čas, za prehod iz rastlin v vodno paro zraka. Prevelika hitrost zraka skozi krmo pomeni manjše nasičenje zraka z vlago in s tem manjšo učinkovitost sistema.

Drug spremljan parameter je bila poraba energije za pogon ventilatorja. Statični tlak v zabojniku se skozi sušenje ni spreminjal, tako da je bila poraba energije v posameznih intervalih enaka (27 W h pri manjšem pretoku in 43 W h pri večjem pretoku). Glede na izločeno vodo je na kg izločene vode porabil 8,99 W h pri večjem pretoku pa 12,4 W h. Povečanje porabe energije glede na izločen kilogram vode je bil tako 38 %.

4 ZAKLJUČEK

V literaturi navedeni razpon med manjšim in večjim pretokom zraka je absolutno prevelik. Z večjim pretokom zraka ne dvigujemo učinkovitosti sistema, ampak le stroške sušenja. Pri 40 % večjem pretoku zraka je poraba energije za pogon ventilatorja za 38 % večja, kljub temu, da je sušenje le za 15 % učinkovitejše. V preizkusu smo primerjali dve vrednosti pretoka zraka, večjo in manjšo. Postavi se vprašanje ali je manjša vrednost pretoka zraka že optimalna vrednost? Ali bi z dodatnim manjšanjem pretoka zraka dosegli še boljše rezultate? Na to bi lahko odgovorili z novimi preizkusi, predvsem pa z raziskavo mehanizma prestopa vode iz rastline na zrak, ki rastlino obteka. S poznavanjem teh parametrov bi lahko nadaljevali optimiranje sušenja krme.

5 LITERATURA

Benedičič, J., & Verbič, J. (2013). Sušenje lucerne s kondenzacijsko sušilnico = Alfalfa drying by the use of condensation dryer. V: ČEH, Tatjana (ur.), KAPUN, Stanko (ur.). *Proceedings of the 22nd International Scientific Symposium on Nutrition of Farm Animals: Zadravec-Erjavec Days 2013, Radenci, 14th and 15th November 2013*. Murska Sobota: Kmetijsko gozdarska zbornica Slovenije, Kmetijsko gozdarski zavod, str. 67-71.

Daszkowska-Golec, A. and Szarejko, I. (2013). Open or Close the Gate – Stomata Action Under the Control of Phytohormones in Drought Stress Conditions. *Frontiers in Plant Science*, 4. https://doi.org/10.3389/fpls.2013.00138

Frick, R., Ammann, H., Wyss, U., Daccord, R. (1999) Einsatz von Intensivaufbereitern in der Futterwerbung. *FAT Berichte, 532*, Tänikon, Eidgenössische Forschungsanstalt für Agrarwirtschaft und Landtechnik, str. 20.

Rant, Z. (2011). Termodinamika knjiga za uk in prakso, Fakulteta za strojništvo, str. 609

Resch, R., Buchgraber, K., Wiedner, G., Frank, P., Meusburger, C., Pichler, M., Stromberger, W., Tiefenthaller, F., Wurm, K. Praxisheu. (2011). Raufutterqualität auf österreichischen Betrieben. *Irdning, Lehr- und Forschungszentrum für Landwirtschaft Raumberg-Gumpenstein*, str. 56.

Sargent, G. P., 1980: Computation of vapour pressure, dewpoint and relative humidity from dry- and wet-bulb temperatures. *Meteorological Magazine*, *109*, 238-246.

Verbič J., Babnik D., Jeretina J., Perpar T. (2006). Navade rejcev pri krmljenju krav v Sloveniji in njihov vpliv na mlečnost,

sestavo mleka in zdravstveno stanje. *V: Zbornik predavanj 15. posvetovanja o prehrani domačih živali „Zadravčevi-Erjavčevi dnevi"*. Kapun, S. (ur.), Čeh, T. (ur.). Murska Sobota, Kmetijsko gozdarska zbornica Slovenije in Kmetijsko gozdarski zavod Murska Sobota, 119-135.

Wirleitner, G. (2011). Energieefficienter Heutrocknungstechnik 2011, *Heugala 2011,* Bundesanstalt für alpenländische Landwirtschaft Gumpenstein.

Wirleitner, G. (2013). Neue Dimensionen in der Rundballentrocknung, *Bautagung Raumberg – Gumpenstein 2013*, Raumberg Gumpenstein.

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Population fluctuations of the pistachio twig borer, *Kermania pistaciella* Amsel, 1964 (Lep.: Oinophylidae) using delta pheromone trap

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Population fluctuations of the pistachio twig borer, *Kermania pistaciella* Amsel, 1964 (Lep.: Oinophylidae) using delta pheromone trap

Abstract: Population fluctuations of adult insects of pistachio twig borer, *Kermania pistaciella* Amsel were studied using delta pheromone traps and sampling from 2016-2017 in Kashan pistachio orchards. Delta type traps baited with sex pheromones were hung in pistachio orchards ('Akbari') at 20 lures per one hectare and the numbers of captured male insects were counted every three days. *Kermania pistaciella* males have begun to attract the traps from 18th March and 8th April in the first and second year, respectively and this trend continued to 31st April and 4th May and flight period of about 42 days in the first year and the second year that lasted 27 days. First, peak and end of each catch insects occurred on 18 March, 8-21 April and 31 April in the first year and on 8 March, 23-26 April and 4 May in the second year, respectively. There was no association in the first year and a weak positive association in the second year of study between the average daily temperature and the number of *K. pistaciella* moths captured in pheromone traps. It seems that moths capture was different because of the weather conditions of these orchards was different in two consecutive years.

Key words: pistachio; *Kermania pistaciella*; population fluctuations; pheromone trap; parasitoid

Sledenje fluktuacij populacije molja, zavrtača pistacijevih vejic, *Kermania pistaciella* Amsel, 1964 (Lep.: Oinophylidae), s fermonskimi pastmi

Izvleček: V raziskavi je bilo preučevano nihanje populacije odraslih zavrtačev pistacijevih vejic, *Kermania pistaciella* Amsel, s fermonskimi pastmi v rastnih sezonah 2016 in 2017 v nasadih pistacije v Kashanu, Iran. Pasti s spolnimi fermoni so bile obešene na pistacije ('Akbari'), po 20 na hektar, število ujetih samcev je bilo prešteto vsake tri dni. Samci vrste *Kermania pistaciella* so se začeli pojavljati v pasteh od 18. marca in 8. aprila v prvem in drugem letu in so se pojavljali do 31. aprila, oziroma 4. maja. Obdobje njihovega izleta je trajalo v prvem letu 42 dni, v drugem letu pa 27 dni. Prvo pojavljanje, višek in konec izleta je bilo v prvem letu 18. marca, od 8 do 21 aprila, in 31. aprila in 8. marca, od 23 do 26 aprila in 4. maja v drugem letu. Med številom ujetih moljev v fermonskih pasteh in povprečno dnevno temperaturo v prvem letu opazovanja ni bilo povezave, v drugem letu opazovanja pa je bila ta povezava rahlo pozitivna. Izgleda, da je bil ulov moljev v obeh letih različen zaradi različnih vremenskih razmer v sadovnjaku.

Ključne besede: pistacija; *Kermania pistaciella*; nihanja populacij; fermonske pasti; parazitoidi

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1 INTRODUCTION

Pistachio trees (*Pistacia vera* L.) have a lot of economic value in Iran. Pistachios are mainly planted in the eastern and central parts of Iran. In 2009, about 225,000 million tons of pistachios, about 50 % of world pistachio production, were produced in Iran (FAO, 2013). As a strategic product, pistachio has a special place in agricultural production. This product forms a major part of non-oil exports (about 40 %) (Panahi et al., 2003), which accounts for more than $ 500 million annually (Hokmabadi, 2011). Pistachio is a rich source of some important and vital nutrients, including linoleic fatty acids (Garcia et al., 1992).

The pistachio twig borer, *Kermania pistaciella* Amsel, 1964 (Lepidoptera: Oinophylidae), is an important pest of pistachio trees throughout the main pistachio-producing areas of Iran and Southeast Anatolia regions of Turkey (Mart et al., 1995; Mehrnejad, 2001; Yanik & Yücel, 2001; Abbaszadeh et al., 2006; Avand-Faghih et al., 2016). The insect has one generation per year and females lay eggs on pistachio flowers and fruit clusters in early spring. The larvae penetrate the plant tissue and bore tunnels in the twigs, feeding on xylem and pith tissues. The canals in the young wood destroy the core of branches and prevent the growth of young branches, dehydrating them (Samih et al., 2005). Larval development takes almost 10 months and the last (4^{th}) instar overwinters inside twig. Larvae leave their tunnels the following year during early March and find suitable places on twigs to form cocoons in which they pupate and, after approximately 3 weeks, emerge as adults (Küçükaslan, 1966; Mehrnejad, 2001; Achterberg & Mehrnejad, 2002; Abbaszadeh et al., 2006).

Larval feeding inside the twig causes severe economic damage by fruit drop, twig weakening, and death. Most pistachio plantations in Iran and Turkey are treated every year with insecticides to suppress *K. pistaciella* populations. Insecticides, however, pose a serious threat to the environment and are harmful to natural enemies. Therefore, insecticide applications should be used during the main periods of parasitoids' activity (Mehrnejad, 2002; Özgen et al., 2012). Toward this aim, the sex pheromone of the pistachio twig borer was identified, and it is now used in the field for monitoring activity of males and timing of the insecticidal sprays (Gries et al., 2006). The situation calls for the development of new pest control methods that would be based on more environment-friendly practices, which in turn, require better knowledge of *K. pistacialla* biology and ecology.

Awareness of the distribution of insects and their range in a region in biological control, assessment of the potential distribution of species in the field of ecology, the development and preservation of biological resources, paleontology, invasive species of pests and diseases, assessment of the impact of environmental changes in spatial distribution has been used (Tognelli et al., 2009; Barber-mussin et al., 2012). Determining the distribution of species helps us to understand the geographical distribution and the appropriate habitat selection in the form of animal geography in order to better manage pests (Pearson et al., 2007; Tognelli et al., 2009).

Today, the study of pest population changes is one of the most important parameters that have a significant role in controlling it in each specific region with its study of the biology of pests. This plays an important role in determining the time of non-chemical control, the natural enemies releasing time, the appropriate time for using mineral compounds, the time of installation of pheromone traps etc., as well as determining the exact time of using chemical pesticides (Bassirat, 2008). Awareness of insect dispersion and its range in a region is important in biological control, evaluation of the distribution potential of species and selection of crops for cultivation (Gressitt, 1958).

Detection of pest populations, pest biology and the development of mass-trapping method for direct control of pests was used by pheromones and other attractants, practically all over the world against a wide range of pest infestations, and these methods are an essential part of pest control programs (Carde, 1990; Cronin et al., 2000; Devetak et al., 2014; Trdan et al., 2019). They can use many of the chemicals used by pest insects to communicate with each other as a tool. It was valuable to manage them (Crade, 1990). In order to increase the efficiency of pheromone traps and turn them into more accessible tools in pest control programs, factors such as shape, size, the location of the correct installation of traps and other things have been considered (Zamani et al., 2012).

Because the population of hibernating larvae of the pistachio twig borer depends on the continuity of extreme cold during the cold season (Mollaei et al., 2016), the appearance and the flight peak of the pest in different years depends on the different conditions of the region weather. Many experiments have been carried out in recent years to use the natural pheromone of *K. pistaciella* in pistachio orchards. Use of pheromone traps to estimate the population of the pest, studying the biology and behavior, the time of emergence and the flight peak, and the end of the period of insect flight in nature, investigate on the effective rate of insecticides, pest dispersion, mating disruption method and, most importantly, the mass trapping to reduce population and prevent damage in pistachio orchards (Fakhri et al., 2016). Different types of traps such as delta, funnel, colorful sticky cards, indoor tubs, cylinders, and trays of water are used. The experi-

ments show that the pistachio twig borer is not sensitive to specific colors, but delta traps, cylinders, and trays have the highest rate of capturing.

Considering the importance of this pest in pistachio orchards in Kashan region of Iran and the necessity of adopting the best method of control, it is necessary to study the population fluctuations and factors that cause these changes. Despite the importance of *K. pistaciella* as an important pest of pistachio, any information has been published on its population fluctuation using delta traps. The aim of this study was to investigate the population fluctuations of adult moths of the pistachio twig borer, *K. pistaciella* using delta traps in Kashan pistachio orchards, in order to determine the time of occurrence of flight peak and to know about the most optimal time for chemical control.

2 MATERIALS AND METHODS

2.1 STUDY SITE

This research was carried out in Isfahan province, Aran and Bidgol city, and in the Kavirat region in the pistachio orchards of Hossein Abad and Abouzid Abad towns. The city of Aran and Bidgol with an area of 6051 square kilometers has a warm and dry climate, the average rainfall of the city is 120 ml, and the depth of evapo-

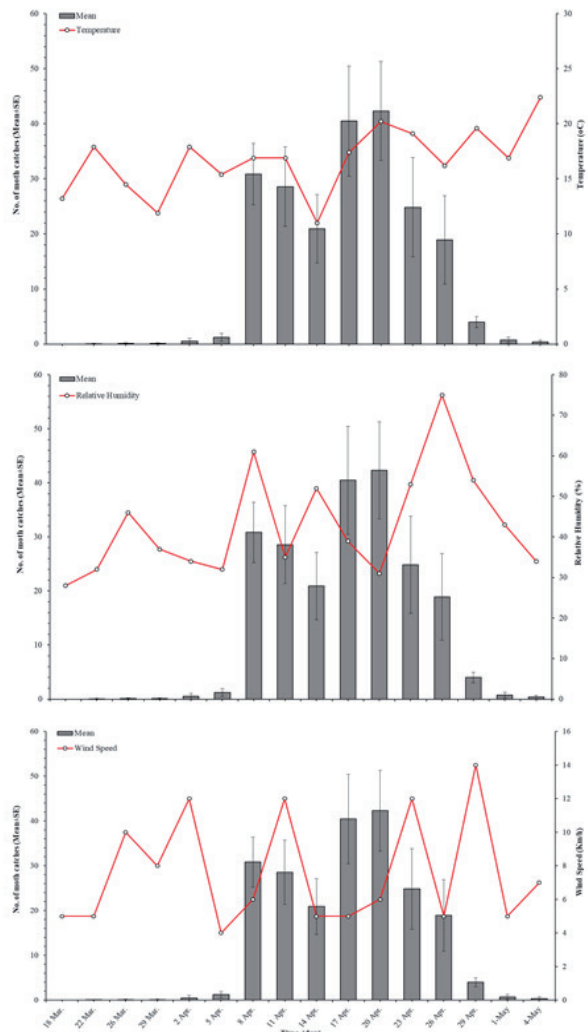

Figure 1: Population fluctuations of the pistachio twig borer, *K. pistaciella* in the pheromone traps in studied orchards in 2015-2016. The sampling dates represent the end dates of each trapping period. The solid line represents the average temperatures (°C), the relative humidity (%) and the wind speed (km h^{-1}) in each trapping interval.

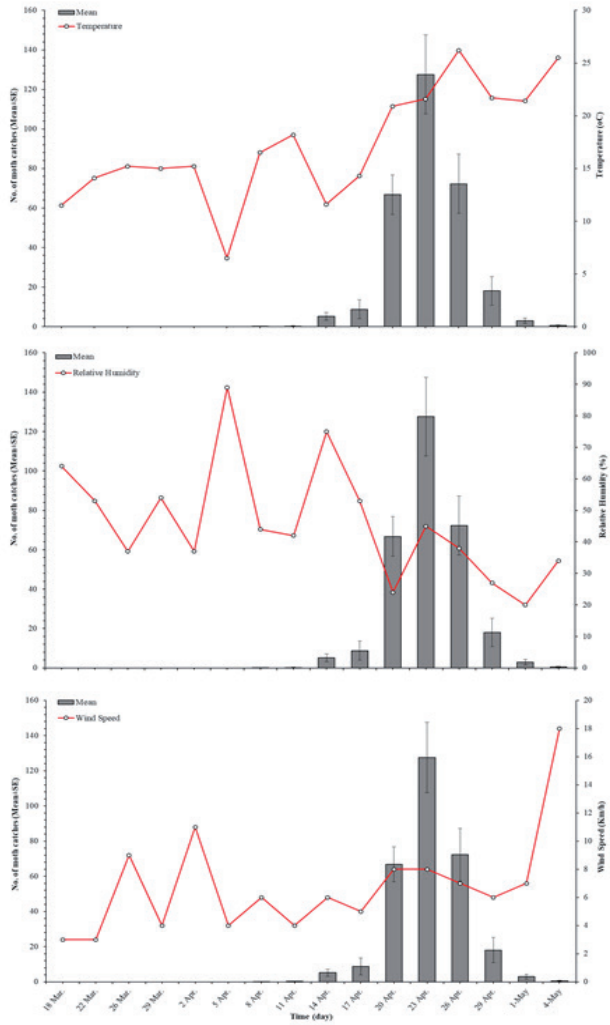

Figure 2: Population fluctuations of the pistachio twig borer, *K. pistaciella* in the pheromone traps in studied orchards in 2016-2017. The sampling dates represent the end dates of each trapping period. The solid line represents the average temperatures (°C), the relative humidity (%) and the wind speed (km h^{-1}) in each trapping interval..

ration is 2626 ml, which has also been faced with drought crisis in recent years. The geographical characteristics of pistachio orchards were with latitude 51° 38' N and longitude 33° 55' E. The age of pistachio trees was almost the same and the average age was 17 years.

For this purpose, five pistachio gardens were selected with Akbari cultivar and their geographical characteristics are as follows:

site 1: N (33° 50' 02") and E (51° 57' 09")
site 2: N (33° 53' 50") and E (51° 44' 23")
site 3: N (33° 50' 55") and E (51° 47' 04")
site 4: N (33° 55' 32") and E (51° 41' 41")
site 5: N (33° 53' 47") and E (51° 46' 48")

Weather data including average, the highest, and the lowest daily temperatures, relative humidity and wind speed were obtained from a nearby meteorological station.

2.2 PHEROMONE TRAP CATCHES

First, according to the reports of previous years, for the purpose of studying the population fluctuations, delta pheromone traps were installed on March 5, 2016, and the sampling was repeated the following year. Sex pheromone used in the experiment was provided by the Pherobank Company, Netherlands, with a concentration of one milligram of pheromone per capsule. According to previous researches, the pistachio twig borer, *K. pistaciella* is not susceptible to certain colours. Therefore, delta traps were selected from two yellow and white colours.

Twenty delta-type traps per hectare (approximately 20 meters apart) were installed in five pistachio gardens before leaf and fruit buds were opened and every three days, number of captured male insects were counted. Traps were re-baited at one-month intervals. Traps were located on the highest part of the plant at a height of one meter, approximately, and at a detected distance in the field. The captured male moths were collected and counted every three days. Traps were re-baited at one-month intervals. Pheromone traps were installed at an altitude of one meter, approximately, and on the northern side of the trees, in the exterior of the twigs to be less exposed to the wind and direct sunlight. The captured male moths were collected and counted every three days in the early hours of the day.

3 RESULTS

3.1 POPULATION FLUCTUATIONS OF ADULTS

The results of the sampling of adult insects using the pheromone trap and by counting the captured adult insects in the traps in the two years are presented in Figs 1 and 2. On the basis of population fluctuations, in the first year, the emergence period of adult insects of *K. pistaciella*, was observed for 42 days from March 29 to May 4, a false peak was occurred on the 8th of April and the true peak was observed on April 20th. In the second year, a 27 days period was observed from April 8 to May 4, and a true peak was observed on April 23rd and there was no false peak.

3.2 THE RELATIONSHIP BETWEEN TEMPERATURES, HUMIDITY AND WIND SPEED WITH POPULATION FLUCTUATIONS OF ADULTS

The difference in population changes over two consecutive years is due to instability and the difference in climate conditions and environmental factors, especially the temperature, relative humidity and wind speed of the studied gardens, as shown in Figs 1 and 2.

The correlation coefficient between number of moth's catches of *K. pistaciella* and weather parameters revealed that mean temperature (R^2 = 0.031 for 2015-16) and (R^2 = 0.143 for 2016-17) exhibited no association in the first year and a weak positive association in the second year of study.

4 DISCUSSION

Pistachio twig borer, *K. pistaciella,* male insects started to be attracted by pheromone traps in on March 22 in the first year and in the second year from April 8th.

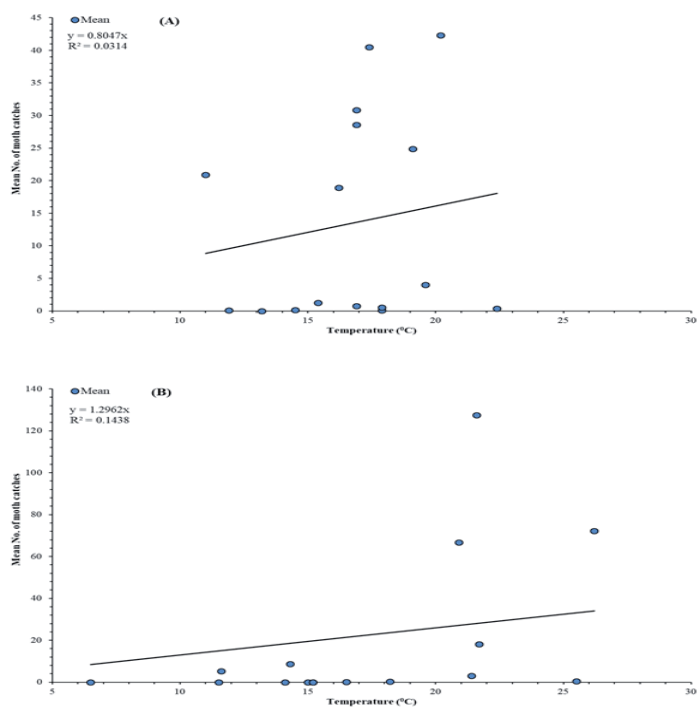

Figure 3: Scatter plot showing a positive correlation between average daily temperature (°C) and mean number of the pistachio twig borer, *K. pistaciella* adults captured in pheromone traps in in studied orchards in A) 2015-2016 and B) 2016-2017

This trend was continued in the first and second year to the fourth of May. The flight period lasted for 42 days in the first year and 24 days in the second year. It seems that these differences in population fluctuations over the two years are due to different temperature and humidity conditions. In the first year, relative humidity varied from 31-75 % and in the second year from 20-89 % and the mean temperature in the first year varied from 11-22 °C, and in the second year it varied from 11-26 °C.

Based on the results of the first capture of adult insects, the flight peaks and the end of the capturing in the first year were recorded on March 22, 8-20 April, and May 4, and in the second year of experiment they were recorded on April 8, April 23, and May 4, respectively. The results showed that the incidence of the peak of flight period of male insects in the trap varied in five different orchards. It seems that the different weather conditions of these gardens have caused this in one year and different weather conditions differently for two consecutive years.

Based on the results of Zamani et al. (2012), the first capture of adult insects, the peaks of the flight period and the end of the capturing in 2012 were recorded on April 20, May 9, and May 30, respectively. Funnel traps in comparison with Delta trap had a greater number of insects, and there was no significant difference in trapping and geographic location in attracting insects. According to Yaniki & Yildirim (2016), *K. pistaciella* males first emerged in early to mid- April, and they had a four - five week flight period in orchards of Bozova and Hilvan Counties of the Province of Sanliurfa, Turkey.

The number of insects captured in the flight peak varied in different orchards, with an average of between 4 and 153 in the first year and 27 to 330 individuals in the second year. Studies in Rafsanjan pistachio orchards (Bassirat, 2006) showed that adult emergence occurred from early April to early May and flight peak from 25 to 30 April. There is a difference in the biology of the pest in the two regions of Kashan and Rafsanjan due to differences in weather conditions of the two regions. The presence of atmospheric unstable conditions, especially in the spring, such as the sudden fall of temperature, thunder shower and sporadic rainfall, monsoon rains and even hail and no precipitation, in some years, cause the fluctuations in the population curve of adult insects and false pixels.

In another study in order to control the population of pests, the effect of shape and direction of the installation of pheromone trap in the crown of the tree and the geographical location in attracting the adult pest insects were studied. The experiment was conducted as a factorial experiment in a completely randomized design with two funnel and delta traps, two direction of north and south axis of the tree crown and three geographical regions of the pistachio orchards of Shahin-e-Shahr and Meymeh of Isfahan province. Traps were installed at 50 meters intervals with two replications. The number of captured insects per traps were counted from late March to early June on a weekly basis. Based on the results of the first adult insect capturing, the flight peaks and the end of flight period in 2012 were recorded on April 20, May 9, and May 30, respectively. In addition, funnel trap in compare to the delta trap captured more insects, and there was no significant difference in the attraction of insects to trap installation direction and trap and geographic position (Zamani et al., 2012).

Based on the previous studies conducted by Bassirat during the years of 2002-2008, the peaks numbers were observed in some years during the flight period of adults of *K. pistaciella*, so the results of the research also confirmed previous findings in this context (Bassirat, 2016). Also, based on previous research (Bassirat, 2008), if the percentage of emergence was considered as the basis for the time of the control, the peak of the appearance of insects in four years and in two regions, on average, approximately was coincided with 65 % of the emergence of adult insects. Research has shown that the use of 500 units of pheromones per hectare to disturb mating of *K. pistaciella* adult moths is more effective than chemical control. The research showed that the development of the mating disruption method to control the pistachio twig borer is more preferable in the production of healthy food and environmental protection (Avand-Faghih et al., 2016).

The current studies revealed that the weather parameters such as temperature and relative humidity has great influence on the mean numbers of Pistachio twig borer adults. The peaks of adult moths was occurred with the mean air temperature of 20 to 25°C. According to previous studies (Bassirat, 2008), the total daily effective temperature for the peak of the appearance of insects with respect to the minimum thermal threshold is 12 °C. Therefore, due to the effective daily temperature in the area, the time of emergence of insects can be determined and planted to control the pest. The influence of the mean daily temperature on the capture of *K. pistaciella* moths, as revealed by correlation analysis, can be explained by the fact that the temperature was close to 25 °C, which is the ideal temperature for the moths to mate (Fakhri, 2018; Abbaszadeh et al., 2006). As can be seen in Figs. 1 and 2, the mean number of *K. pistaciella* moths captured in the present study was the highest when the temperature was near 25 °C, as it was between April 17 and 23.

5 CONCLUSION

In the present study, we found positive correlations among the number of *K. pistaciella* males captured, temperature, and relative humidity. The Delta pheromone trap was efficient in capturing a large numbers of *K. pistaciella* male adults. The flight peaks and the end of the capturing in the first year were recorded on March 22, 8-20 April, and May 4, and the second year was recorded on April 8, April 23, and May 4, respectively. Also for effective management of the pest, local hanging of pheromone traps is suggested.

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7 REFERENCES

Abbaszadeh, G., Seyedoleslami, H., Samih, M.A. & Hatami, B. (2006). Bioecology of pistachio twig borer moth *Kermania pistaciella* Amsel, in Rafsanjan and Isfahan-Iran. *Communications in Agricultural and Applied Biological Sciences*, *71*(2 Pt B), 563-569.

Achterberg, C.Van. & Mehrnejad, M.R. (2002). The braconid parasitoids (Hymenoptera: Braconidae) of *Kermania pistaciella* (Lepidoptera: Tineidae: Hieroxestinae) in Iran. *Zoologische Mededelingen*, *76*, 27-40.

Avand-Faghih, A., Naseri, M., Mohammadipour, A. & Farid, S. (2016). Evaluation of mating disruption for controlling the pistachio twig borer, *Kermania pistaciella* Amsel (Lep.: Oinophylidae). *Proceedings of 22nd Iranian Plant Protection Congress*, 27-30 August 2016 - College of Agriculture and Natural Resources, University of Tehran, Karaj, IRAN.

Barber-mussin, M., Jiiguet, F., Albert, C.H. & Thuiller, W. (2012). Selecting pseudo-absences for species distribution models: how, where and how many? *Methods in Ecology and Evolution, 3*(2), 327-338. https://doi.org/10.1111/j.2041-210X.2011.00172.x

Bassirat, M. (2006). Determination of heat requirements for pistachio twig borer moth, *Kermania pistaciella*. *Acta Horticulturae*, *726*, 519-524. https://doi.org/10.17660/ActaHortic.2006.726.86

Bassirat, M. (2008). Estimating the Heat Requirements for Pistachio Twig Moth, *Kermania pistaciella* Amsel in Field Condition. *Journal of Science and Technology* of *Agriculture and Natural Resources*, *12*(45), 339-349.

Bassirat, M. (2016). *The pistachio twig borer, Kermania pistaciella and its control methods*. Journal of Pistachio Research Institute, Horticultural Research Institute, Agricultural Research and Development Research Organization, 17 p.

Carde, R.T. (1990). Principles of mating disruption. In Ridgway, R.L., Silverstein, R.M. and Inscoe, M.N. (Eds). *Behavior-modifying chemicals for insect management, applications of pheromones and other attractants*. Marcel Dekker, INC, New York. pp. 47-71.

Cronin, J.T., Hayes, J.L. & Turchin, P. (2000). Evaluation of traps used to monitor southern pine beetle aerial populations and sex ratios. *Agricultural and Forest Entomology*, *2*, 69-76. https://doi.org/10.1046/j.1461-9563.2000.00053.x

Devetak, M., Bohinc, T., Kač, M. & Trdan, S. (2014). Seasonal dynamics of the cabbage armyworm (*Mamestra brassicae* [L.]) and the bright-line brown-eyes moth (*Mamestra oleracea* [L.]) in Slovenia. *Horticultural Science*, *41*(2), 80-88. https://doi.org/10.17221/209/2013-HORTSCI

Fakhri, N. (2018). *Study of population fluctuations of the pistachio twig borer, Kermania pistaciella Amsl (Lep.: Oinophilidae) and identification of its parasitoids in Kashan, Iran pistachio orchards*. MSc thesis in Agricultural Entomology, Faculty of Agriculture, Shahed University, Tehran, Iran, 97 p.

Fakhri, N., Abbasipour, H. & Askarianzadeh, H. (2016). Study of population fluctuations of the pistachio twig borer, *Kermania pistaciella* Amsl (Lep.: Tineidae) using delta pheromone trap in Kashan pistachio orchards. *Proceedings of 22nd Iranian Plant Protection Congress*, 27-30 August 2016 - College of Agriculture and Natural Resources, University of Tehran, Karaj, IRAN, p. 631.

Garcia, J.M., Agar, I.T. & Streif J. (1992). Fat content and fatty acid composition in individual seeds of pistachio varieties grown in Turkey. *Gartenbauwissenschaft*, *57*, 130-133.

Gressitt, J.L. (1958). Zoogeography of insect. *Annual Review of Entomology*, *99*, 822-827. https://doi.org/10.1146/annurev.en.03.010158.001231

Gries, R., Khaskin, G., Daroogheh, H., Mart, C., Karadag, S., KubilayEr, M., Britton, R. & Gries, G. (2006). (2S, 12Z)-2-Acetoxy-12-heptadecene: major sex pheromone component of pistachio twig borer, *Kermania pistaciella*. *Journal of Chemical Ecology*, *32*, 2667–2677. https://doi.org/10.1007/s10886-006-9190-0

Hokmabadi, H. (2011). *Diagnostic of Environmental and Non-environmental Damaging Factors Incoming to Pistachio Product*. Agricultural Research and Education Organization, Tehran, Iran. (in Persian)

Küçükaslan, N. (1966). *Pistachio of Harmful branch Moth (Kermania pistaciella Amsel) (Lepidoptera: Oinophilidae) of Biology and Chemical Control*. Sabri Aş. Basımevi. İstanbul. 64 s.

Mehrnejad, M.R. (2001). The current status of pistachio pests in Iran. In: Ak B.E. (ed.). *XI GREMPA Seminar on Pistachios and Almonds*. Zaragoza: CIHEAM, 2001. p. 315-322. (Cahiers Options Méditerranéennes; n. 56). 11. GREMPA Seminar on Pistachios and Almonds, 1999/09/01-04, Sanliurfa (Turkey).

Mehrnejad, M.R. (2002). The natural parasitism ratio of the pistachio twig borer moth, *Kermania pistaciella*, in Iran. *Acta Horticultrae*, *591*, 541-544. https://doi.org/10.17660/ActaHortic.2002.591.83

Mart, C., Celik, M.Y. & Yigit, A. (1995). Biological observations and chemical control of pistachio twig borer, *Kermania pistaciella* Ams. (Lep.: Oinophilidae), injurious in pista-

chio orchards in Turkey. *Acta Horticulturae, 419*, 373–378. https://doi.org/10.17660/ActaHortic.1995.419.62

Mollaei, M., Izadi, H., Simek, P. & Kostal, V. (2016). Overwinterring biology and limits of cold tolerance in larva of pistachio twig borer, *Kermania pistaciella*. *Bulletin of Entomological Research*, *106*, 538-545. https://doi.org/10.1017/S0007485316000237

Özgen, İ., Bolu, H. & Beyarslan, A. (2012). *Chelonus flavipalpis* Szépligeti, 1896 and *Mirax rufilabris* Haliday, 1833 (Hymenoptera, Braconidae): two new larva-pupa parasitoids of Pistachio twig borer *Kermania pistaciella* Amsel, 1964 (Lepidoptera: Oinophilidae) with the parasitization ratios from Turkey. *Munis Entomology & Zoology*, *7*(1), 238–242.

Panahi, B., Ismaeil Pour, A., Farbood, F., Moazen Pour Kermani, M. & Farivar Mahin, H. (2003). *Pistachio guide*. Office of Educational Technology of the Ministry of Agriculture.

Pearson, R.G., Raxworthy, C.J., Nakamura, M. & Peterson, A.T. (2007). Predicting species distribution, from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *Journal of Biogeography*, *34*, 102-117. https://doi.org/10.1111/j.1365-2699.2006.01594.x

Samih, M., Alizadeh, A. & Saberi Riseh, R. (2005). *Pistachio Pests and Diseases in Iran and their IPM*. Organization of Jihad-eUniversity, Tehran.

Tognelli, M.L., Roig-Junet, S.A., Marvaldi, A.E., Flores, G.E. & Lobo, J.M. (2009). An evalution of methods for modelling distribiution of Patagonian insects. *Revista Chilena de Historia Natural*, *82*, 347-360. https://doi.org/10.4067/S0716-078X2009000300003

Trdan, S., Čuk, J., Poženel, A., Kralj, M.B., Rot, M., Carlevaris, B., Žežlina, I., Vidrih, M., Laznik, Z. & Bohinc, T. (2019). Field testing of different synthetic attractants for mass trapping of common European cockchafer (*Melolontha melolontha* [L.], Coleoptera, Scarabaeidae) adults. *Acta agriculturæ Scandinavica. Section B, Soil and plant science*, *69*(2), 174-180. https://doi.org/10.1080/09064710.2018.1524020

Yaniki, E. & Yildirim, Y. (2016). Effectiveness of mass trapping for control of the pistachio twig borer, *Kermania pistaciella*. *Bulletin of Insectology, 69*(1), 35-40.

Yanik, E. & Yücel, A. (2001). The pistachio (*P. vera* L.) pests, their population development and damage state in Sanliurfa province. pp. 301–309 in Ak, B.E. (Ed) XI. GREMPA Seminar on Pistachios and Almonds.

Zaragoza, CIHEAM, *Cahiers Options Méditerranéennes*, 56. Zamani, Z., Khajehali, J. & Sabzalian, M.R. (2012). Influence of trap type, trapping location and cardinal direction on the capture of the Pistachio twig borer moth, *Kermania pistaciella* Amsel. (Lepidoptera: Tineidae) in Isfahan. *Plant Pests Research*, *2*(2), 53-62.

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Feasibility of different crop rotations for cultivation in salt affected soils

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Feasibility of different crop rotations for cultivation in salt affected soils

Abstract: Crop rotation can be used as an effective technique for managing salt-affected soils, however selection of suitable crop rotation at farmer field is very intricate decision which depends on expected net revenue, available resources and preserving the soil quality. In this perspective a study was conducted to evaluate a suitable crop rotation scheme for salt affected soils in term of economic value and improving the soil health. Seven crop rotation used were; T_1 = Wheat-Rice, T_2 = Wheat-Sesamum, T_3 = Ispagol-Rice, T_4 = Ispagol-Qulfa, T_5 = Tukhum-e-blangoo-Qulfa, T_6 = Ajwain-Niazboo, T_7 = Saunf-Podina. A moderately salt affected field {pH_s = 8.65, EC_e 5.20 dS m^{-1}, SAR = 27.73 (mmol $l^{-1})^{1/2}$} was selected. The experimental design was randomized complete block design (RCBD) with three replications having plot size of 4 m x 6 m. Results of two years study showed that maximum grain yield was recorded by rice wheat rotation and maximum net income (208352 Rs. ha^{-1}) and BCR (4.72) was also observed in rice-wheat crop rotation over all other crop rotations. With respect to ameliorative affect, rice- wheat rotation also showed a significant positive impact on chemical properties of salt affected soil. Therefore, it is suggested that rice wheat crop rotation is the most suitable and economically attractive cropping scheme in salt affected soil which has potential to provide better long-term income to farmers, improve soil health and combat soil deterioration caused by salinity.

Key words: crop rotation; rice; wheat; salinity; cost benefit

Primernost različnih kolobarjev za pridelavo na slanih tleh

Izvleček: Kolobarjenje lahko uporabimo kot učinkovito tehniko za obvladovanje učinka slanosti tal, vendar je izbira ustreznega kolobarjenja za kmeta zelo zapletena odločitev, ki zavisi od pričakovanega neto dohodka, razpoložljivih sredstev in ohranjanja kakovosti tal. V povezavi s to tematiko je bila izvedena študija, katere namen je bil ovrednotiti primernost različnih načinov kolobarjenja na slanih tleh z ekonomskega vidika in vidika izboljšanja uporabnosti tal. Uporabljenih je bilo naslednjih sedem načinov kolobarjenja: T1 = pšenica (*Triticum aestivum* L.) – riž (*Oryza sativa* L.), T2 = pšenica – sezam (*Sesamum indicum* L.), T3 = indijski trpotec (*Plantago ovata* Forssk.) - riž, T4 = indijski trpotec - navadni tolščak (*Portulaca oleracea* L.), T5 = lalemancija (*Lallemantia royleana* Benth. in Wall.) - navadni tolščak, T6 = iranska kumina (*Carum copticum* L.) - navadna bazilika (*Ocimum basilicum* L.), T7 = navadni komarček (*Foeniculum vulgare* Mill.) - poprova meta (*Mentha piperita* L.). Izbrana so bila zmerno slana tla (pHs = 8.65, ECe = 5.20 dS m^{-1}, SAR = 27.73 (mmol $l^{-1})^{1/2}$). Poskus je bil zasnovan v naključnem bločnem razporedu (RCBD) s tremi ponovitvami in velikostjo parcelic 4 m × 6 m. Rezultati dvoletnega poskusa so pokazali, da je bil v kolobarju riža s pšenico dosežen največji pridelek zrnja in največji neto dohodek (208352 Rs. ha^{-1}) kot tudi največji količnik med stroški in prihodkom (BCR; 4.72). Kolobar riža s pšenico je pokazal tudi značilne pozitivne učinke na kemijske lastnosti slanih tal. Zaradi naštetega se za načrtovanje rotacije poljščin na slanih tleh priporoča kolobar riža s pšenico kot najbolj primeren in ekonomnsko najbolj obetaven, saj ima večji potencial dolgoročnega zagotavljanja prihodkov kmetov, izboljšuje lastnosti slanih tal in zmanjšuje verjetnost njihovega slabšanja.

Ključne besede: kolobar; riž; pšenica; slanost; stroški/prihodki

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1 INTRODUCTION

A key challenge of 21st century agriculture is to provide the food, fiber and fuel for an expanding population while preserving the soil fertility and providing adequate farm profitability to farmers (Robertson and Swinton, 2005). Furthermore, agricultural land loss due to salinity is one of the main problems to sustainable agriculture as approximately 20 % of the world's cultivated land are salt affected (Sumner, 2000). In general, continued irrigation with poor quality ground water, poor infield management and a deficient drainage system are main causes of expansion of land salinization (Gehad, 2003). So in current scenario salt-affected soils are of growing importance to meet food demand of growing population and this situation necessitates some forward planning and need to find out several management and agronomic practices that work satisfactory for utilizing and preserving this natural resource (Gehad, 2003).

Rice (*Oryza sativa* L.), cultivation started nearly 11,500 years ago (Gnanamanickam, 2009) and approximately half of the world's population consumed the rice as staple food (Ma et al., 2007). To ensure food security for the expanding population, rice production should be increased by 50 % in rice consuming countries (Jinni and Joseph, 2017). Wheat (*Triticum aestivum* L.), is also a main vegetable protein source and is cultivated all around the world due to its adaptation to a various range of climates. Globally it is a major food crop, which is cultivated on approximately 200 million hectares with an average production of 600 million tons (Rajarm & Braun, 2006). Medicinal plants are also becoming increasingly popular in modern society and are used all over the world as natural alternatives to synthetic chemicals (Wyk & Wink, 2004). It may be worthwhile to explore potential of medicinal plants for salt affected soil, which may be beneficial for mankind. Previously many researchers have described the economical and medicinal importance of several halophytes (Dagar, 1995). Consequently, concerted research efforts are required for their immense potential to be planted on salt affected soils as valuable resource and cash crop on an urgent basis.

Crop rotation is an agricultural practice, which implicates cultivation of different crops on same field. Selection of suitable crop rotation at farmer field is very intricate decision. Economists assume that farmers tend to pursue activities that improve their utility, or well-being, generate revenue, lessen the monetary and physical risk, decrease labor demands, and are comfortable or pleasurable (Bowman and Zilberman, 2013). One of the main issues influencing crop production choices by farmers is the expected market price of selected commodity and the resulting estimated net revenue, in addition to the relative economic risk associated with production of potential commodities (Huirne et al., 2000). A farmer's earnings and capacity to achieve credit, farming systems, skill and technologies and willingness to invest in new crops will also influence the choice of crops (Knowler and Bradshaw, 2007; Salassi et al., 2013). In a study consisting of different crop rotation Dogan et al. (2008) reported that wheat and sunflower using as main crop were more profitable rotation system under rain fed conditions having net profit of \$474 and \$482 ha^{-1} $year^{-1}$, respectively. Likewise, Nel and Loubser (2004) stated that crop rotation consisting of sunflower, soyabean were most effective rotations with maximum net while mitigating financial risk. Similarly, a field trail showed that inclusion of oilseeds crop in cereals generate the maximum net return, reducing the financial risk through improved production stability (Dhuyvetter et al., 1996). To evaluate the economic assessment of different crop rotation consisting of: corn–soybean–corn (CSC), alfalfa–alfalfa–corn (AAC), continuous corn (CCC), soybean–wheat–corn (SWC), soybean–corn– corn (SCC) and soybean–alfalfa–corn (SAC). Goplen et al. (2018) reported that alfalfa–alfalfa–corn (AAC) was most dominant crop rotation with the highest net return of \$919 ha^{-1} yr^{-1}, mostly due to more stable prices of alfalfa. Jat et al. (2012) studied the economic performance of ten rice-based cropping sequences. Results revealed that rice - fenugreek - okra was most productive (25.73 t ha^{-1}) cropping system with maximum return of (96,286 Rs ha^{-1}).

However, in salt affected soils there is need to combine profitability in combination with other production factors like soil remediation, soil fertility and soil physical and chemical properties (Popp et al., 2005; Yao et al. 2013). Furthermore, crop rotation could be used as an alternative approach to improve soil health and combat soil deterioration. It improves soil structure (Yazar, 2008), increases soil organic matter (Bremer et al., 2008; Bhatti and Khan, 2012) and water use efficiency (Tanaka et al., 2005), improves crop nutrient use efficiency (Karlen et al., 1994), reduces grain yield variability (Varvel, 2000) and improves grain quality (Kaye et al., 2007).

Furthermore, when good quality water supplies are limited a suitable crop rotation is the only means for managing salt-affected soils and maintaining crop yields (Kaur et al., 2007). Crop rotation resulted in several improvements, in soil physical and chemical properties and is also suggested for salt affected soil, especially when crops with varying degrees of salinity tolerance are used (Lacerda et al., 2011). For suitable crop rotation in salt affected soils, selected crop should be either salt tolerant or tolerant cultivars must be selected from sensitive or medium tolerant crops with high economic value (Ouda et al., 2016; Kishk, 2000). Likewise, appropriate crop ro-

tation in salt-affected soils can accelerate the reclamation process by reducing evaporation and upward transport of salt in the soil (Brady and Well, 2008).

Abro and Mahar (2007) reported that in rice-wheat cropping system, salinity indicators like soil EC_e, pH and SAR were significantly lowered after the rice harvest, however, a minor increase in EC_e and pH were recorded whereas, the SAR levels dwindled further after wheat harvest. Similarly in a study Liu et al. (2013) reported that the rice-barley crop rotation lowered soil EC_e after a reclamation time of more than 10 years. Zhang and He (2004) found that rice plantation resulted the addition of soil organic matter. The paddy soil management for 50 years favored the enhancement of soil organic carbon and decreased the concentrations of Ca, Mg, and Na (Chen et al., 2011). Fu et al. (2014) found that rice-barley crop rotation had more ameliorative effect on soil properties and significantly decreased the pH value than cotton-barley crop rotation system at the same year. Similarly, in a study Zou et al. (2011) concluded that the management of rice crop increased the accumulation of organic matter, which tends to converge soil pH to neutral. In addition, leaching effects of irrigation reduces the soil salinity (Iost et al., 2007; Fu et al., 2012).

So, keeping the all above facts in consideration the work presented in this paper was designed to evaluate a suitable crop rotation scheme for salt affected soils which will not only benefit the overall productivity and profitably of the farm but also improve the soil health.

2 MATERIAL AND METHODS

This field trial was conducted for two successive seasons from 2011-12 to 2012-13 at Soil Salinity Research Institute Pindi Bhattian. A moderately salt affected field {pH of soil saturated past (pH_s) = 8.65, electrical conductivity of soil extract (EC_e) = 5.20 dS m^{-1}, sodium absorption ratio (SAR) = 27.73 $(mmol\ l^{-1})^{1/2}$} was selected. The experimental design was randomized complete block design (RCBD) with three replications having plot size of 4 m x 6 m. The cropping seasons were Kharif (June– September) and Rabi (October–March). Cropping scheme used was as under:

Rabi crops were sown during the last week of November during 2010-11 and 2011-12 and Kharif crops were sown in last week of June during 2011 and 2012 in the same field. All cultural and management practices were carried out uniformly as and when required. Grain yield of each crop was recorded at maturity, whereas, for qulfa and podina biomass yield of economic value was noted. After harvesting of each crop, composite soil samples were collected for analysis of pH, EC_e and SAR. All the soil analysis was carried out following the method of U.S. Salinity Laboratory Staff (1954). In order to appraise the economic feasibility of different crop rotation total income was estimated by using existing price of each crop in local markets. Net income was derived subtracting the total expenses from total income and benefit: cost ratio (BCR) was computed by dividing gross income with total expenses (Shah et al., 2013). Collected data was subjected to analysis of variance following the method of Steel et al. (1997) to sort out significant differences among treatments at 5 % probability level.

3 RESULTS

3.1 RABI 2011-12

Data in Table 1 revealed that during first Rabi season (2011-12) maximum yield was produced by wheat crop (2.04 and 1.93 t ha^{-1}) in T_1 and T_2 respectively followed by ispagol with grain yield of 0.39 and 0.38 t ha^{-1} in T_3 and T_4 respectively. While minimum yield of 0.31 t ha^{-1} was recorded in ajwain. With respect to economic value maximum return of Rs. 55716 and 51618 ha^{-1} was earned by wheat crop in T_1 and T_2 respectively which was statistically non-significant ($p < 0.05$) with economic value of ajwain (Rs. 49600). Minimum economic value was observed in ispagol with Rs. 36486 ha^{-1}.

Treatments	Crop rotation	Rabi crops	Kharif crops
T_1	Wheat-Rice	Wheat (*Triticum aestivum* L.)	Rice (*Oryza sativa* L.)
T_2	Wheat-Sesamum	Wheat (*Triticum aestivum* L.)	Sesamum (*Sesamum indicum* L.)
T_3	Ispagol-Rice	Ispagol (*Plantago ovata* Forssk.)	Rice (*Oryza sativa* L.)
T_4	Ispagol-Qulfa	Ispagol (*Plantago ovata* Forssk.)	Qulfa (*Portulaca oleracea* L.)
T_5	Tukhum-e-blangoo-Qulfa	Tukhum-e-blangoo (*Lallemantia royleana* Benth. in Wall.)	Qulfa (*Portulaca oleracea* L.)
T_6	Ajwain-Niazboo	Ajwain (*Carum copticum* (L.) Link.)	Niazboo (*Ocimum basilicum* L.)
T_7	Saunf-Podina	Saunf (*Foeniculum vulgare* Mill.)	Podina (*Mentha piperita* L.)

Table 1: Yield and economic analysis of rabi crops 2011-12

Treatments (Crop Rotation)		Crop	Yield (t. ha^{-1})	Economic Value (Rs.)
T_1	Wheat-rice	Wheat	1.93 A	51618 AB
T_2	Wheat-sesamum	Wheat	2.04 A	55716 A
T_3	Ispagol-rice	Ispagol	0.39 B	39778 CD
T_4	Ispagol-qulfa	Ispagol	0.38 B	36486 D
T_5	Tukhum-e-blangoo-qulfa	Tukhum-e-blangoo	0.36 B	41233 CD
T_6	Ajwain-niazboo	Ajwain	0.31 B	49600 AB
T_7	Saunf-podina	Saunf	0.33 B	45433 BC

Means sharing the same letters are statistically similar at $p \leq 0.05$

3.2 KHARIF 2012

During Kharif season (2012) the highest yield of 2.32 and 2.24 t ha^{-1} was ensued by rice in T_1 and T_3 respectively followed by grain yield of 0.42 t ha^{-1} produced by niazbo which was statistically ($p < 0.05$) alike with yield of podina, qulfa and sesamum. As far as economic value of kharif crops was concerned maximum economic value (Rs. 71852 ha^{-1}) was observed in rice crop however it was statistically insignificant ($p < 0.05$) with qulfa and niazbo. Minimum economic value was recorded in sesamum (Rs. 37742 ha^{-1}).

3.3 RABI 2012-13

Data (Table 3) depicted that during 2nd Rabi season maximum yield was produced by wheat crop. Wheat produces the yield of 2.21 and 1.99 t ha^{-1} in T_2 and T_1 respectively. Minimum yield was produced by ajwain (0.32 t ha^{-1}) which was statistically ($p < 0.05$) non-significant with yield of ispagol and saunf. Data regarding the economic value showed that maximum economic value (Rs. 58049 ha^{-1}) was obtained by wheat crop followed by ajwain and both crops remain statistically ($p < 0.05$) non-

Table 2: Yield and economic analysis of Kharif crops 2012

Treatments (Crop Rotation)		Crop	Yield (t ha^{-1})	Economic Value (Rs. ha^{-1})
T_1	Wheat-rice	Rice	2.32 A	71852 A
T_2	Wheat-sesamum	Sesamum	0.38 B	37742 B
T_3	Ispagol-rice	Rice	2.24 A	69481 A
T_4	Ispagol-qulfa	Qulfa	0.31 B	69404 A
T_5	Tukhum-e-blangoo-qulfa	Qulfa	0.34 B	68455 A
T_6	Ajwain-niazboo	Niazboo	0.42 B	66868 A
T_7	Saunf-podina	Podina	0.35 B	49519 B

Means sharing the same letters are statistically similar at $p \leq 0.05$

Table 3: Yield and economic analysis of Rabi crops 2012-13

Treatments (Crop Rotation)		Crop	Yield (t ha^{-1})	Economic Value (Rs. ha^{-1})
T_1	Wheat-rice	Wheat	1.99 B	52285 B
T_2	Wheat-sesamum	Wheat	2.21 A	58049 A
T_3	Ispagol-rice	Ispagol	0.42 C	42445 C
T_4	Ispagol-qulfa	Ispagol	0.40 C	40153 C
T_5	Tukhum-e-blangoo-qulfa	Tukhum-e-blangoo	0.37 C	45900 C
T_6	Ajwain-niazboo	Ajwain	0.32 C	52267 B
T_7	Saunf-podina	Saunf	0.34 C	54767 AB

Means sharing the same letters are statistically similar at $p \leq 0.05$

Table 4: Yield and economic analysis of Kharif crops 2013

Treatments (Crop Rotation)		Crop	Yield (t ha^{-1})	Economic Value (Rs. ha^{-1})
T_1	Wheat-rice	Rice	2.08 A	88542 A
T_2	Wheat-sesamum	Sesamum	0.25 C	37500 C
T_3	Ispagol-rice	Rice	1.98 A	84292 A
T_4	Ispagol-qulfa	Qulfa	0.61 B	73833 AB
T_5	Tukhum-e-blangoo-qulfa	Qulfa	0.66 B	79833 A
T_6	Ajwain-niazboo	Niazboo	0.53 B	61333 B
T_7	Saunf-podina	Podina	0.30 C	36120 C

Means sharing the same letters are statistically similar at $p \leq 0.05$

significant from each other. While minimum economic value (Rs. 40153 ha^{-1}) was showed by ispagol.

3.4 KHARIF 2013

Data in table 4 displayed that during 2nd Kharif season maximum yield of 2.08 and 1.98 t ha^{-1} was produced by rice crop in T_1 and T_3 respectively followed by qulfa crop, while minimum yield (0.25 t ha^{-1}) was recorded in sesamum. Economic value data showed that maximum economic return of Rs. 88542 ha^{-1} was achieved by rice crop followed by qulfa crop, however, difference between to crop was not large enough to reach a level of significance ($p < 0.05$). Minimum economic value (Rs. 36120 ha^{-1}) was observed in podina crop.

3.5. SOIL PROPERTIES

Soil analysis data (Table 5) at the end of study showed that soil properties were also considerably affected by type of crop used in crop rotation. Among all the rotation rice-wheat crop rotation showed a minimum value (8.36) for soil pH followed by ispagol-rice rotation with pH value of 8.39. Maximum pH value (8.44) was noted in tukhum-e-blangoo-qulfa rotation. Similarly, rice crop also improved the salinity indicators i.e EC_e and SAR of soil. Minimum value of EC_e (3.87 dS m^{-1}) and SAR (20.96 mmol l^{-1}) $^{1/2}$ were noted in rice wheat crop rotation. Whereas, maximum value (4.50 dS m^{-1}) of EC_e was observed in saunf-podina rotation and for SAR maximum value (24.80) was observed where ajwain-niazboo rotation was used.

3.6 ECONOMIC ANALYSIS

Economic analysis data (Table 6) at the end of 2 years study showed that different crop rotation in salt affected soils had significant effect on gross income, net income and benefit: cost ratio (BCR). Maximum net income (208352 Rs. ha^{-1}) and BCR (4.72) was recorded by rice-wheat crop rotation and minimum net income (128207 Rs. ha^{-1}) and BCR (3.10) was observed in wheat sesamum rotation.

4 DISCUSSION

Decision of suitable crop rotation scheme in salt af-

Table 5: Effect of different crop rotation on soil chemical properties at the end of experiment

Treatments (Crop Rotation)		pH_s	% decrese over initial value	EC_e (dS m^{-1})	% decrese over initial value	SAR (mmol l^{-1})$^{1/2}$	% decrese over initial value
T_1	Wheat-rice	8.36	3.35	3.87	25.57	20.96	24.41
T_2	Wheat-sesamum	8.40	2.89	4.32	16.92	22.4	19.22
T_3	Ispagol-rice	8.39	3.00	3.92	24.61	21.76	21.52
T_4	Ispagol-qulfa	8.43	2.54	4.46	14.23	23.8	14.17
T_5	Tukhum-e-blangoo-qulfa	8.44	2.42	4.44	14.61	24.12	13.01
T_6	Ajwain-niazboo	8.41	2.77	4.38	15.76	24.80	10.56
T_7	Saunf-podina	8.42	2.65	4.50	13.46	23.36	15.75

Table 6: Effect of different crop rotation on net income and benefit: cost ratio (BCR) at the end of study

Treatments (Crop Rotation)		Cost of production (Rs. ha^{-1})	Gross income (Rs. ha^{-1})	Net income (Rs. ha^{-1})	Benefit: costratio (BCR)
T_1	Wheat-rice	55945	264297	208352	4.72
T_2	Wheat-sesamum	60800	189007	128207	3.10
T_3	Ispagol-rice	61356	235995	174639	3.84
T_4	Ispagol-qulfa	55987	219876	163889	3.92
T_5	Tukhum-e-blangoo-qulfa	65344	235421	170077	3.60
T_6	Ajwain-niazboo	58650	230068	171418	3.92
T_7	Saunf-podina	55182	185839	130657	3.36

fected soil is very complex intrinsically region dependent task and should be based on salinity tolerance, economic value of the crop, impact on environment, generated revenue, available resources, food security and market conditions (Dogliotti et al., 2014 ; Smajgl et al., 2016). Crop rotation scheme on a farm is justified by preserving soil quality (Brankatschk and Finkbeiner, 2015; Nemecek et al., 2015), increased economic benefits (Dhuyvetter et al., 1996) improved soil properties (Peterson and Westfall, 2004), environmental perspective (Reckling et al., 2015), ensuring long term yield and soil fertility (Hennessy, 2006; Dury et al., 2011). Different crop rotation models have been evaluated in terms of agronomic as well as farm profitability in combination with their impact on soil health (Hulugalle et al., 2002; Popp et al., 2005).

Crop diversification in a cropping sequence generally reduces risk of failure through more stable yield and market price diversification as low income by one crop can be offset by high income of another crop in a given year (Meyer-Aurich et al., 2006; Nemecek et al., 2008). Zentner et al. (2002a) also reported that farmers who adopted diversified crop rotation earned more income.

Therefore, in this study we proposed the seven crop rotations for salt affected soil which should be acceptable to local farmers, can generate the income and contribute to food security and rural development. In developing countries one of most common factor for fast adoptability of a cropping sequence by farming community is expected market price and resulting net economic return of selected crop (Hurine et al., 2000; Salassi et al., 2013).

Nevertheless, viability and adoptability of cropping system does not depend on crop yield but also on efficiency in use of available resources (Moreno et al., 2011). Net return generated by a rotation is very critical because selection of a suitable crop affects the affects economic benefits (Jatoe et al., 2008; Martin and Hanks, 2009). In general, farmers tend to pursue activities which maximizes their farm profitability, reduced physical labor and financial risk, and are convenient and enjoyable (Bowman and Zilberman, 2013).

Salt stress rapidly reduces the plant growth due to osmotic stress and ion toxicity (accumulation of toxic Cl^- and Na^+ in cells of shoot) and ultimately the final yield of crops (Munns and Tester, 2008; Tamimi et al., 2016). The ability of a plant to tolerate salinity is a vital factor in plant productivity (Momayezi et al, 2009). Considering individual year yields, wheat was the most high yielding crop in Rabi season, while rice perform better among Kharif crops. These crops tolerate the salinity mainly two mechanisms, osmotic tolerance and ion exclusion (Munns and Tester, 2008; Roy et al, 2014) while, yield of other crops was low due to higher salinity sodicity problem and inefficient tolerant mechanisms. Also, rice-wheat rotation showed a preventive impact on soil salinity/sodicity build up and consequently crop yield was increased. Rice and wheat crop are previously reported as salt tolerant and can utilize to maximize the productivity and profitability of salt affected soils.

Economic circumstances of developing countries are compelling farmers to cultivate crops that generate high income, leading to cereal-dominated crop rotations (Sieling & Christen, 2015).

Economic performance of a crop rotation contributes to its adaptation and continuity but in general economic analysis studies of rotations are scarce (González et al., 2002). Many researchers study the economic performance of different crop rotations (Chen, 2009; Chen and Chen, 2011; Zhu et al., 2011).

Rice-wheat rotation is a popular crop rotation in southern and eastern Asia with an area of 24 to 27 million hectares (Wassmann et al., 2004). According to economic analysis of present study, rice wheat cropping scheme exhibited more benefit per hectare of crop and this rotation may provide maximum economic benefit to farmers in comparison to other. One of the plausible explanation for yield and revenue advantage of rice and wheat crops under salt affected soil might be their more salinity tolerance in comparison with other crops used in rotation (Yeo et al, 1990; Purushottam et al., 2012; Saringi et al., 2015; Hasan et al., 2015) coupled with market

value and commercialization of these crops (Singh et al., 2014). More revenue generated by rice wheat crop rotation may be due to prevailing socio-economic conditions of the region (Livingston et al., 2012; Pare et al., 2015; Liu et al., 2016) as both crops are used as staple commodities and their market prices were less variable as compared to other crops in rotation. So adequate and high quality yield of rice and wheat with substantial net return contributes the rice- wheat rotation dominating all other rotations. According to González et al. (2013) wheat earned the income of US$1051 ha^{-1} and barley US$711 ha^{-1}, which are economically attractive and more than bean and sugar beet. Comparable results have been reported by Mellado et al. (2000), Stanger et al. (2008), and Hirzel (2011). Dominant and profitable crop rotations with reduced risk depend on prevailing conditions of specific regions (DeVuyst and Halvorson, 2004; Saharawat et al., 2010). Sánchez-Girón et al. (2004) also evaluated the economic performance of different crop rotations wheat-barley, wheat-vetch and barley-vetch for sixteen years and concluded that economic return of crop rotation depend upon the factors beyond control like market price and climate of the region that influence the variable like cost of production, quality and quantity of yield, and net income which are the main sources of variability or economic uncertainty. In a long term study of fifteen years Stanger et al. (2006) evaluated the effect of different nitrogen levels on seven crop rotation i.e. continuous alfalfa (*Medicago sativa* L.) (AA), corn-soybean-corn-oat with alfalfa seeding-alfalfa (CSCOaA), continuous corn (*Zea mays* L.) (CC), corn-alfalfa (CA), corn-corn-oat (*Avena sativa* L.) with alfalfa seeding-alfalfa-alfalfa (CCOaAA), corn-corn-corn-alfalfa-alfalfa (CCCAA) and corn-soybean (*Glycine max* (L.) Merr.) (CS). They reported that maximum return was earned by corn-soybean rotation at all nitrogen levels. Similarly Singh et al. (2011) study the productivity and economic performance of different crop rotation like rice-potato-green gram, rice-pea, rice-wheat, rice-wheat-sesbania, rice-lentil + mustard -cowpea, rice-chickpea, rice-lentil-cowpea, rice-maize + pea - cowpea, rice-mustard-green gram and rice-wheat-green gram. They concluded that rice-potato-green gram cropping system gave the highest productivity net return and benefit: cost ratio.

Similar findings were reported by Kaur et al. (2007) that paddy-based crop rotations were beneficial for salt affected soil and they also recommended the fallow-wheat cropping sequence as an alternative cropping scheme. In similar studies, the highest net income was obtained from wheat-rice rotations (Guan et al., 2011; Zentner et al., 2002), the rice-potato-sunflower sequence (Jaiswal et al., 1993) and rapeseed-common vetch + sunflower-wheat (Dogan et al., 2008). Our results are reinforced by earlier findings that not only the sequence, but also the choice of crops in the rotation influences the economic margin (Wilson et al., 2003; Jatoe et al., 2008). Economic benefits of different crop rotation have been reported by several researchers (Tzilivakis et al. 2005; Chen et al., 2015; Babulicova, 2016; Aminifar et al., 2017) which are in agreement with our findings.

4.1. SOIL PROPERTIES

Hence along with direct income generated, long term environmental impacts and sustainability of a cropping sequence must be also taken into account when determining its suitability for salt affected soil. Rotation type had substantial effects on soil health and qualities, results showed that all cropping sequences lowered the soil pH, EC_e and SAR at the end of study. Rice-wheat rotation showed maximum reduction of 3.35 % in soil pH, which may be ascribed to leaching of soluble salts and $CaCO_3$ resulting a rapid fall in pH value (Cui et al., 2012; Fu et al., 2014; Neugschwandtner et al., 2014; Mahmood et al., 2016). Results of this study are in agreement with finding of Abro and Mahar (2007) who found a significant decrease in salinity indicators i.e. pH, EC_e and SAR after the rice harvest, however, a slight increase in pH and EC_e were recorded while, the SAR levels decreased further after wheat harvest. Similarly, Gehad (2003) also reported rice wheat rotation as most suitable cropping pattern during reclamation of salt affected soil. Data also showed that rice wheat cropping pattern reduces EC_e and SAR upto 25.57 and 24.41 % respectively, which could be explained due to leaching effect of irrigation as rice crop required the frequent irrigation (Iost et al., 2007; Fu et al., 2012). Our results are supported by series of findings that cropping pattern revealed an absolute temporal trend on soil properties (Fu et al., 2014; Lazicki et al., 2016; King and Hofmockel, 2017). Similar findings were reported by Fu et al. (2012) and Liu et al. (2013) that the rice-barley cropping sequence decreased soil salinity after a reclamation time of more than 10 years. Furthermore, cultivation of rice favored the addition of soil organic matter and decreases the Na, Ca and Mg contents (Chen et al., 2011; Zhang and He, 2004) which tends to converge soil pH to neutral and improved the soil properties (Zou et al., 2011). In study conducted by Fu et al. (2014) they found that rice-barley crop rotation had more ameliorative effect on soil properties and significantly decreased the pH value than cotton-barley crop rotation system at the same year.

5 CONCLUSION

Utilization and remediation of marginally salt affected soil is a global challenge for sustainable agriculture. Optimizing the agricultural production from salt affected soil requires appropriate farmer's decision based on economic and environmental constraints. Finally results generated by current study suggested that rice-wheat crop rotation is the most suitable and economically attractive cropping scheme in salt affected soil which has potential to provide better long-term income to farmers, improve soil health and combat soil deterioration caused by salinity.

6 REFERENCES

Abro, S.A., Mahar, A.R. (2007). Reclamation of saline-sodic soils under rice-wheat crop rotation. *Pakistan Journal of Botany, 39*(7), 2595-2600.

Aminifar, J., Ramroudi, M., Galavi, M. and Mohsenabadi, G. (2017). Productivity and economic efficiency of wheat in rotation with cotton. *Iran Agricultural Research, 36*(2), 55-60.

Babulicova, M. (2016). Enhancing of winter wheat productivity by the introduction of field pea into crop rotation. *Agriculture, 62*(3), 101-110. https://doi.org/10.1515/agri-2016-0011

Bhatti, U.A., Khan, M.M. (2012). Review Soil management in mitigating the adverse effects of climate change. *Soil and Environment, 31*(1), 1-10.

Bowman, M.S., Zilberman, D. (2013). Economic Factors Affecting Diversified Farming Systems. *Ecology and Society, 18*(1), 33-48. https://doi.org/10.5751/ES-05574-180133

Brady, N.C., Well, R.R. (2008). *The nature and properties of soils*. Pearson-Prentice Hall, Upper Saddle River NJ p 990.

Brankatschk, G., Finkbeiner, M. (2015). Modeling crop rotation in agricultural LCAs challenges and potential solutions. *Agricultural Systems, 138*, 66-76. https://doi.org/10.1016/j.agsy.2015.05.008

Bremer, E., Janzen, H.H., Ellert, B.H., McKenzie, R.H. (2008). Soil organic carbon after twelve years of various crop rotations in an Aridic Boroll. Soil *Science Society of America Journal, 72*, 970-974. https://doi.org/10.2136/sssaj2007.0327

Chen, C., Anton B., Reza, K., Afshara, Karnes, N. (2015). Intensification of dryland cropping systems for bio-feedstockproduction: Evaluation of agronomic and economic benefits of Camelina sativa. I*ndustrial Crops and Products, 71*, 114-121. https://doi.org/10.1016/j.indcrop.2015.02.065

Chen, L.M., Zhang, G.L., Effland, W.R. (2011). Soil characteristic response times and pedogenic thresholds during the 1000-year evolution of a paddy soil chronosequence. Soil Science *Society of America Journal, 75*, 1-14. https://doi.org/10.2136/sssaj2011.0006

Chen, Q.G. (2009). Research on the effect of input on yield and economic benefits of super rice: Based on the investigation of the 568 households which be-longed to ten counties in two southward provinces and one northward province. *Chinese Journal of Agrometeorology, 30*, 295-300.

Chen, Q.G., and Chen, Y.Z. (2011). Comparison of economical benefits between super rice and conventional rice in different regions: Based on an Investigation of 413 farm households in Zhejiang and Hunan provinces. *Hybrid Rice, 26*(3), 61-67.

Cui, J., Liu, C., Li, Z.L., Wang, L., Chen, X.F., Ye, Z.Z., Fang, C.M. (2012). Long-term changes in topsoil chemical properties under centuries of cultivation after reclamation of coastal wetlands in the Yangtze Estuary, China. *Soil and Tillage Research, 123*, 50-60. https://doi.org/10.1016/j.still.2012.03.009

Dagar, J.C. (1995). *Characteristics of halophytic vegetation in India*. In: Khan, M.A. and Ungar, I.A. pp. 255-276. Biology of Salt Tolerant Plants. (Eds.), University of Karachi, Karachi. 476 pp.

Dhuyvetter, K.C., Thompson, C.R., Norwood, C.A., Halvorson, A.D. (1996). Economicsof dryland cropping systems in the Great Plains: a review. *Journal of Production Agriculture, 9*, 216-222. https://doi.org/10.2134/jpa1996.0216

Dhuyvetter, K.C., Thompson, C.R., Norwood, C.A., Halvorson, A.D. (1996). Economic of dry land cropping system in the Great Plains. *Journal of Production Agriculture, 9*, 216-222. https://doi.org/10.2134/jpa1996.0216

Dogan, R., Goksoy. T. A., Yagdi, K. and Turan, M.Z. (2008). Comparison of the effects of different crop rotation systems on winter wheat and sunflower under rain-fed conditions. *African Journal of Biotechnology, 7*(22), 4076-4082.

Dogliotti, S., Rodrıguez, D., Lopez-Ridaura, S., Tittonell, P., Rossing, W. (2014). Designing sustainable agricultural production systems for a changing world: methods and applications. *Agricultural Systems, 126*, 1-2. https://doi.org/10.1016/j.agsy.2014.02.003

Dury, J., Bergez, J.E., Schaller, N., Garcia, F., Reynaud, A. (2011). Models to support cropping plan and crop rotation decisions. A review. *Agronomy for Sustainable Development, 32* (2), 567-580. https://doi.org/10.1007/s13593-011-0037-x

Fu, Q. Nengfei, D., Chen L., Yicheng, L., Bin, G. (2014). Soil development under different cropping systems in a reclaimed coastal soil chronosequence. *Geoderma, 230*, 50-57. https://doi.org/10.1016/j.geoderma.2014.03.026

Fu, Q.L., Liu, C., Ding, N.F., Lin, Y.C., Guo, B., Luo, J.F., Wang, H.L. (2012). Soil microbial communities and enzyme activities in a reclaimed coastal soil chronosequence under rice-barley cropping. *Journal of Soils and Sediments, 12,* 1134-1144. https://doi.org/10.1007/s11368-012-0544-7

Gehad, A. (2003). *Deteriorated soils in Egypt: management and rehabilitation-report; executive authority for land improvement project* (EALIP). Ministry of Agriculture and Land Reclamation, Cairo.

Gnanamanickam, S.S. (2009). Rice and its importance to human life. *Progress in Biological Control, 8*, 1-11. https://doi.org/10.1007/978-90-481-2465-7_1

Gonzalez U., Pablo, U., Juan, H., Ingrid, M.G. (2013). Economic evaluation of a crop rotation portfolio for irrigated farms in central Chile. *Chilean Journal of Agricultural* Re-

search, 73(3), 243-249. https://doi.org/10.4067/S0718-58392013000300006

González, J., Francisco, E., Foster, W. (2002). Selección de portfolios de rotaciones culturales económicamente óptimos para la Precordillera Andina de la VIII Región. *Agricultura Técnica, 62*, 583-595. https://doi.org/10.4067/S0365-28072002000400010

Goplen, J.J., Jeffrey, A.C., Craig, C.S., Roger, L.B., Fritz, R. B., Lisa M. B., and Jeffrey, L. G. (2018). Economic Performance of Crop Rotations in the Presence of Herbicide-Resistant Giant Ragweed. *Agronomy Journal, 110*(1), 260-268. https://doi.org/10.2134/agronj2016.09.0536

Guan, G., Tu, S., Yang, J., Zhang, J., Yang, L. (2011). A field study on effects of nitrogen fertilization modes on nutrient uptake, crop yield and soil biological properties in rice-wheat rotation system. *Agricultural Sciences in China, 10*, 1254-1261. https://doi.org/10.1016/S1671-2927(11)60117-X

Hasan, A., Hafiz, R.H., Nurealam S., Khatun, M., Rabiul, I., Abdullah, A.M. (2015). Evaluation of Wheat Genotypes for Salt Tolerance Based on Some Physiological Traits. *Journal of Crop Science and Biotechnology, 18*(5), 333-340. https://doi.org/10.1007/s12892-015-0064-2

Hennessy, D.A. (2006). On monoculture and the structure of crop rotations. American *Journal of Agricultural Economics, 88*, 900-914. https://doi.org/10.1111/j.1467-8276.2006.00905.x

Hirzel, J. (2011). *Fertilización de cultivos en Chile*. Instituto de Investigaciones Agropecuarias INIA, Centro Regional de Investigación Quilamapu, Chillán, Chile.

Huirne, R.B.M., Meuwissen, M.P.M., Hardaker, J.B., Anderson, J.R. (2000). Risk and risk management in agriculture: an overview and empirical results. *International Journal of Risk Assessment and Management, 1*, 125-136. https://doi.org/10.1504/IJRAM.2000.001491

Huirne, R.B.M., Meuwissen, M.P.M., Hardaker, J.B., Anderson, J.R. (2000). Risk and risk management in agriculture: an overview and empirical results. *International Journal of Risk Assessment and Management, 1*, 125-136. https://doi.org/10.1504/IJRAM.2000.001491

Hulugalle, N.R., Entwistle, P.C., Weaver, T.B., Scott, F., Finlay, L.A. (2002). Cotton- based rotation systems on asodic vertosol under irrigation: effects on soil quality and profitability. *Australian Journal of Experimental Agriculture, 42*(3), 379-387. https://doi.org/10.1071/EA00118

Iost, S., Landgraf, D., Makeschin, F. (2007). Chemical soil properties of reclaimed marsh soil from Zhejiang Province P.R. China. *Geoderma, 142*, 245-250. https://doi.org/10.1016/j.geoderma.2007.08.001

Jaiswal, V.P., Singh, J.P., Karamjit, S., Shekhawat, G.S., Khuran, S.M.P., Pandey, S.K., Chandla, V.K. (1993). *Production potential and profitability of some potato based cropping sequences in north-western plains. Potato:* present and future. Proceedings of the National Symposium held at Modipuram. pp. 101-105.

Jat, R.A., Dungrani, R.A., Arvadia, M.K. and Kanwar L.S. (2012). Diversification of rice (Oryza sativa L.) based cropping systems for higher productivity, resource-use efficiency and economic returns in south Gujarat, India. *Archives of Agronomy and Soil Science, 58*(6), 561-57. https://doi.org/10.1080/03650340.2010.533172

Jatoe, J., Yiridoe, E., Weersink, A., Clark, S. (2008). Economic and environmental impacts of introducing land use policies and rotations on Prince Edwards Island potato farms. *Land Use Policy 25*, 309-319. https://doi.org/10.1016/j.landusepol.2007.08.005

Jinni, D., Joseph, B. (2017). Physiological mechanism of salicylic acid for alleviation of salt stress in rice. *Rice Science, 24*(2), 97-108. https://doi.org/10.1016/j.rsci.2016.07.007

Karlen, D.L., Varvel, G.E., Bullock, D.G., Cruse, R.M. (1994). Crop rotations for the 21st century. *Advance in Agronomy, 53,* 1-44. https://doi.org/10.1016/S0065-2113(08)60611-2

Kaur R, Malik, R., Paul, M. (2007). Long-term effects of various crop rotations for managing salt-affected soils through a field scale decision support system-a case study. *Soil Use and Management, 23*, 52-62. https://doi.org/10.1111/j.1475-2743.2006.00055.x

Kaye, N.M., Mason, S.C., Jackson, D.S., Galusha, T.D. (2007). Crop rotation and soil amendments alters sorghum grain quality. *Crop Science, 47*, 722-729. https://doi.org/10.2135/cropsci2006.05.0346

Kishk, M.A. (2000). Sustainable land use in poor countries and poor communities: a mirage of real water. In: *Soil and sustainable agriculture in the new century*. Egyptian Soil Science Society (ESSS)-Golden Jubilee Congress 1950-2000, Cairo. (Oct 23-25).

Knowler, D., Bradshaw, B. (2007). Farmers' adoption of conservation agriculture: a review and synthesis of recent research. *Food Policy, 32*, 25-48. https://doi.org/10.1016/j.foodpol.2006.01.003

Lacerda, C.F., Sousa, G.G., Silva, F.L.B., Guimaraes, F.V.A., Silva, G.L., Cavalcante, L.F. (2011). Soil salinization and maize and cowpea yield in the crop rotation system using saline waters. *Engenharia Agricola, 31*, 663-675. https://doi.org/10.1590/S0100-69162011000400005

Liu, C., Xu, J.M., Ding, N.F., Fu, Q.L., Guo, B., Lin, Y.C., Li, H., Li, N.Y. (2013). The effect of long term reclamation on enzyme activities and microbial community structure of saline soil at Shangyu, China. *Environmental Earth Sciences, 69*, 151-159. https://doi.org/10.1007/s12665-012-1943-1

Liu, X., Heikki, L., Tuomo, P., Yulia, P., Reimund, R., Taru, P. (2016). Dynamic economic modelling of crop rotations with farm management practices under future pest pressure. *Agricultural Systems, 144*, 65-76. https://doi.org/10.1016/j.agsy.2015.12.003

Livingston, M., Roberts, M.J., Zhang, Y. (2012). *Optimal sequential plantings of corn and soybeans underprice uncertainty.* North Carolina State University, Working paper.

Ma, H., Chong, K., Deng. X.W. (2007). Rice research: Past, present and future. *Journal of Integrative Plant Biology, 49*, 729-730. https://doi.org/10.1111/j.1744-7909.2007.00515.x

Mahmoud, A., Ouda, S., Abd El-Hafez, S. (2016). High water consuming crops under control: I. Case of rice crop. In: *Major crops and water scarcity in Egypt*. Springer Publishing House, pp 69-82. https://doi.org/10.1007/978-3-319-21771-0_5

Mellado, M., Madariaga, R. and Granger, D. (2000). Opala-INIA, a new spring bread wheat cultivar for the South Central

area of Chile. *Agricultura Técnica, 60,* 415-418. https://doi.org/10.4067/S0365-28072000000400009

Meyer-Aurich, A., K. Janovicek, W. Deen, and A. Weersink. (2006). Impact of tillage and rotation on yield and economic performance in corn based cropping systems. *Agronomy Journal, 98*, 1204-1212. https://doi.org/10.2134/agronj2005.0262

Momayezi, M.R., Zaharah, A.R., Hanafi, M.M., Mohd, R. (2009). Agronomic characteristics and proline accumulation of Iranian rice genotypes at early seedling stage under sodium salts stress. *Malaysian Journal of Soil Science, 13,* 59-75.

Munns, R., Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology, 59*, 651-681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Nel, A.A., Loubser, H.L. (2004). The impact of crop rotation on profitability and production risk in the Eastern and North Western Free State. *Agrekon, 43*(1), 101-111. https://doi.org/10.1080/03031853.2004.9523638

Nemecek, T., Hayer, F., Bonnin, E., Carrou'ee, B., Schneider, A., Vivier, C. (2015). Designing eco-efficient crop rotations using life cycle assessment of crop combinations. *European Journal of Agronomy, 65*, 40-51. https://doi.org/10.1016/j.eja.2015.01.005

Nemecek, T., Richthofen, J.v., Dubois, G., Casta, P., Charles, R. and Pahl, H. (2008). Environmental impacts of introducing grain legumes into European crop rotations. *European Journal of Agronomy 28*, 380-393. https://doi.org/10.1016/j.eja.2007.11.004

Neugschwandtner, R.W., Liebhard, P., Kaul, H.P., Wagentristl, H. (2014). Soil chemical properties as affected by tillage and crop rotation in a long-term field experiment. *Plant Soil Environment, 60*(2), 57-62. https://doi.org/10.17221/879/2013-PSE

Ouda, S. A.H., Abd El-Hafeez, Z., Hamdy, K. (2016). *Combating Deterioration in Salt-Affected Soil in Egypt by Crop Rotations*. SpringerLink. https://doi.org/10.1007/978-3-319-33660-2_6

Pare, M.C., Jean, L., Denis, P. (2015). Best management practices in Northern agriculture: A twelve-year rotation and soil tillage study in Saguenay-Lac-Saint-Jean. *Soil and Tillage Research, 150*, 83-92. https://doi.org/10.1016/j.still.2015.01.012

Peterson, G.A., Westfall, D.G. (2004). Managing precipitation use in sustainable dry land agro ecosystems. *Annals of Applied Biology, 144*, 127-138. https://doi.org/10.1111/j.1744-7348.2004.tb00326.x

Popp, M., Manning, P., Counce, P., Keisling, T. (2005). Rice-soybean rotations: opportunities for enhancing whole farm profits or water savings. *Agricultural System, 86*, 223-238. https://doi.org/10.1016/j.agsy.2004.09.008

Purushottam, K., Singh, D.K., Bharadwaj, A.K. (2012). On-farm Evaluation of Agronomic Management Practices under Rice-wheat Cropping System. Extended Summaries. *Third International Agronomy Congress, New Delhi,* pp.1295-1296.

Rajarm, S., Braun, H. S. (2006). Wheat yield potential. *Farm policy Journal, 3*(1), 103-107.

Reckling, M., Hecker, J.M., Bergkvist, G., Watson, C. A., Zander, P., Schlafke, N., Stoddard, F. L., Eory, V., Topp, C. F., Maire, J. (2015). A cropping system assessment framework evaluating effects of introducing legumes into crop rotations. European *Journal of Agronomy, 76*, 186-197. https://doi.org/10.1016/j.eja.2015.11.005

Robertson, G.P., Swinton, S.M. (2005). Reconciling agricultural productivity and environmental integrity: A grand challenge for agriculture. *Frontiers in Ecology and the Environment. 3,* 38-46. https://doi.org/10.1890/1540-9295(2005)003[0038:RAPAEI]2.0.CO;2

Roy, S.J., Negrao, S., Tester, M. (2014). Salt resistant crop plants. *Current Opinion in Biotechnology, 26*, 115-124. https://doi.org/10.1016/j.copbio.2013.12.004

Saharawat, Y.S., Singh, B., Malik, R.K., Ladha, J.K., Gathala, M., Jat, M.L. and Kumar, V. (2010). Evaluation of alternative tillage and crop establishment methods in a rice-wheat rotation in North Western IGP. *Field Crops Research 116*, 260-267. https://doi.org/10.1016/j.fcr.2010.01.003

Salassi , M.E., Michael, A. D., Kurt M. G. (2013). Economically optimal crop sequences using risk-adjusted network flows: Modeling cotton crop rotations in the southeastern United States. *Agricultural Systems, 118*, 33-40. https://doi.org/10.1016/j.agsy.2013.02.006

Sanchez-Giron, V., Serrano, A., Hernanz, J.L. and Navarrete, L. (2004). Economic assessment of three long-term tillage systems for rain fed cereal and legume production in semiarid central Spain. *Soil and Tillage Research, 78*, 35-44. https://doi.org/10.1016/j.still.2004.01.001

Sarangi, S.K., Maji, B., Singh, S., Burman, D., Mandal, S., Sharma, D.K., Singh, U.S.,Ismail, A.M., Haefele, S.M. (2015). Improved nursery management furtherenhances productivity of stress tolerant rice varieties in coastal rainfedlowlands. *Field Crop Research, 174*, 61-70. https://doi.org/10.1016/j.fcr.2015.01.011

Shah, M.A., Manaf, A., Hussain, M., Farooq, S., Zafar-ul-Hye, M. (2013). Sulphur fertilization improves the sesame productivity and economic returns under rainfed conditions. *International Journal of Agriculture and Biology, 15*, 1301-1306.

Sieling, K., Stahl, C., Winkelmann, C., Christen, O. (2005). Growth and yield of winter wheat in the first years of a monoculture under varying N fertilization in NW Germany. *European Journal of Agronomy, 22*, 71-84. https://doi.org/10.1016/j.eja.2003.12.004

Singh, R.K., Jitendra, S.B., Triyugi, N., Yeshwant, S. and Kalyan, S. (2011). Integrated assessment of diversification of rice-wheat cropping system in Indo-Gangetic plain. *Archives of Agronomy and Soil Science, 57*(5), 489-506. https://doi.org/10.1080/03650341003641771

Singh, Y.P., Nayak, A.K., Sharma, D.K., Gautam, R.K., Singh, R.K., Singh, R., Mishra,V.K., Paris, T., Ismail, A.M. (2014). Farmer's participatory varietal selection: asustainable crop improvement approach for the 21stcentury. *Agroecology and Sustainable Food Systems, 38*, 427-444. https://doi.org/10.1080/21683565.2013.870101

Smajgl, A., Ward, J., Pluschke, L. (2016). The water-food-energy nexus-realising a new paradigm. *Journal of Hydrology, 533*, 533-540. https://doi.org/10.1016/j.jhydrol.2015.12.033

Stanger, T.F., J.G. Lauer, and J. Chavas. (2008). The profitability

and risk of long-term cropping systems featuring different rotations and nitrogen rates. *Agronomy Journal, 100,* 105-113. https://doi.org/10.2134/agronj2006.0322

Stanger, T.F., Joseph, G. L. and Jean, P. C. (2006). The Profitability and Risk of Long-Term Cropping Systems Featuring Different Rotations and Nitrogen Rates. *Agronomy Journal, 100*(1), 105-113 https://doi.org/10.2134/agronj2006.0322

Steel, R.G.D., Torrie, J.H., Dickey, D.A. (1997). *Principles and Procedures of Statistic: A Biometrical Approach*. 3rd edition, pp: 400-428. Mc Graw Hill book Co. Inc. New York, USA.

Sumner, M. E. (2000). *Handbook of Soil Science:* Boca Raton. CRC Press. 2 148pp.

Tamimi, N., Chris, B., Helena, O., Bettina, B., Stephanie, S., Yung S.H., Sandra, M. S., Mark, T., Sonia, N. (2016). Salinity tolerance loci revealed in rice using high-throughput non-invasive phenotyping. *Nature Communications, 7*(13342), 1-11. https://doi.org/10.1038/ncomms13342

Tanaka, D.L., Anderson, R.L., Rao, S.C. (2005). Crop sequencing to improve use of precipitation and synergize crop growth. *Agronomy Journal, 97*, 385-390. https://doi.org/10.2134/agronj2005.0385

U.S. Salinity Lab. Staff. (1954). *Diagnosis and Improvement of Saline and Alkali Soils*. USDA Handbook 60, Washington DC, USA.

Varvel, G.E. (2000). Crop rotation and nitrogen effects on normalized grain yields in a long-term study. *Agronomy Journal, 92*, 938-941. https://doi.org/10.2134/agronj2000.925938x

Wassmann, R., Neue, H.U., Ladha, J.K., Aulakh, M.S. (2004). Mitigating greenhouse gas emissions from rice-wheat cropping systems in Asia. Environment, *Development and Sustainability, 6,* 65-90. https://doi.org/10.1023/B:ENVI.0000003630.54494.a7

Wilson, P., Gibbons, J., Ramsden, S. (2003). The impact of cereal prices and policy on crop rotations and supply response. *Journal of Agricultural Economics, 54,* 313-323. https://doi.org/10.1111/j.1477-9552.2003.tb00065.x

Wyk, B.E.v., Wink, M. (2004). *Medicinal Plants of the World*. Timber Press, Oregon.

Yao, R.J., Yang, J.S., Zhang, T.J., Gao, P., Yu, S.P., Wang, X.P. (2013). Short-term effect of cultivation and crop rotation systems on soil quality indicators in a coastal newly reclaimed farming area. *Journal of Soils and Sediments, 13*(8), 1335-1350. https://doi.org/10.1007/s11368-013-0739-6

Yazar, A. (2008). *Guidelines on crop management under saline conditions including seed treatments technology, crop selection and rotation*. Cukurova University, Faculty of Agriculture, Adana, Turkey.

Yeo, A.R., Yeo, M.E., Flowers, S.A., Flowers, T.J. (1990). Screening of rice (Oryza sativa) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. *Theoretical and Applied Genetics, 79*(3), 377-384. https://doi.org/10.1007/BF01186082

Zentner, R.P., Lafond, G.P., Derksen, D.A., Campbell, C.A. (2002). Tillage method and crop diversification: effect on economic returns and riskiness of cropping systems in a Thin Black Chernozem of the Canadian Prairies. *Soil and Tillage Research, 67*, 9-21. https://doi.org/10.1016/S0167-1987(02)00028-4

Zhang, M., He, Z. (2004). Long-term changes in organic carbon and nutrients of an Ultisol under rice cropping in southeast China. *Geoderma, 118,* 167-179. https://doi.org/10.1016/S0016-7061(03)00191-5

Zhu, S.G., Sai, S.Q., Zhang, Y.J. (2011) Investigation and analysis on the production costs and benefits of nine kinds of grain and oil crops in northern China. *Agricultural Economy, 1*, 32-34.

Zou, P., Fu, J., Cao, Z. (2011). Chronosequence of paddy soils and phosphorus sorption- desorption properties. *Journal of Soils and Sediments, 11*, 249-259. https://doi.org/10.1007/s11368-010-0301-8

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Effects of cassava flour on the stickiness properties of wheat bread dough: unleavened, leavened and frozen dough

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Effects of cassava flour on the stickiness properties of wheat bread dough: unleavened, leavened and frozen dough

Abstract: Cassava utilization in the form of cassava-wheat bread is increasing in Africa. However, information on stickiness properties of dough handling under normal and frozen conditions is limited. In view of this the gluten contents and water absorption of doughs, and stickiness of unleavened, leavened and leavened-frozen doughs processed from 0 to 30 % cassava flour substitution level (CFSL) as compared to wheat flour were determined. The gluten contents of flour blends (6.88–13.00 %) decreased significantly ($p < 0.05$) with increasing CFSL. Water absorption capacity (WAC) was ranged from 59.57–61.70 % and showed positive correlation with gluten contents (r = 0.595, $p < 0.05$). Cassava variety (CV) and CFSL had significant $p < 0.05$) influence on stickiness of unleavened (34.14–122.17 g), leavened (13.53–83.94 g) and leavened frozen (126.88–146.82 g) dough. Irrespective of CV and CFSL, frozen dough had the highest stickiness. Gluten content and WAC had significant ($p < 0.01$) negative influence on stickiness in unleavened (r = -0.445 and -0.437, respectively) and leavened (r = -0.457 and -0.434, respectively) doughs. The variation in stickiness was influenced by gluten contents and CFSL. The unfrozen dough and frozen dough exhibited higher stickiness in lower and higher gluten content flour blends, respectively.

Key words: cassava; composite flours; gluten; stickiness; wheat

Učinki tapioke na lepljivost pšeničnega krušnega testa: nevzhajano, vzhajano in zmrznjeno vzhajano testo

Izvleček: Uporaba tapioke, škroba pridobljenega iz manioke (kasave) (*Manihot esculenta* Crantz) narašča v obliki priprave mešanega kruha s pšenico na afriški celini, a je kljub temu zelo malo podatkov o lepljivosti navadnega in zmrznjenega testa. V povezavi s tem je bila glede na vsebnost glutena in absorpcijo vode v testu določena leplivost nevzhajanega, vzhajanega in zmrznjenega vzhajanega testa, narejenega iz mešane moke, v keteri so pšenično moko nadomestili z od 0 do 30 % tapioke (CFSL)v primerjavi s testom iz čiste pšenične moke. Vsebnost glutena je v mešanicah moke značilno upadala z naraščanjem dodatka tapioke (6,88–13,00 %; $p < 0,05$). Sposobnost absorbcije vode (WAC) je bila v območju od 59,57 do 61,70 % in je pokazala pozitivno korelacijo z vsebnostjo glutena (r = 0,595, $p < 0,05$). Sorta manioke (CV) in delež tapioke v mešani moki (CFSL) sta imela značilen učinek ($p < 0,05$) na lepljivost nevzhajanega (34,14–122,17 g), vzhajanega (13,53–83,94 g) in vzhajanega zmrznjenega testa (126,88–146,82 g). Ne glede na CV in CFSL je imelo vzhajano zmrznjeno testo največjo lepljivost. Vsebnost glutena in WAC sta imeli značilni negativni učinek ($p < 0,01$) na lepljivost nevzhajanega (r = -0,445 in -0,437) in vzhajanega testa (r = -0,457 in -0,434). Na spremenljivost lepljivosti testa sta vplivali vsebnost glutena in CFSL. Nezmrznjena in zmrznjena testa so pokazala večjo lepljivost pri manjših in večjih vsebnostih glutena v mešanicah moke.

Ključne besede: manioka; sestavljene moke; gluten; lepljivost; pšenica

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1 INTRODUCTION

Stickiness, a surface related property, is a tendency of dough to adhere to contact surface of equipment and hands during mixing and kneading. This tendency affect dough handling (Villanueva et al., 2018). Moreover, sticky dough is considered a problem to high speed mixing, and can cause disruption to production schedule, and subsequent loss of quality. Stickiness is associated with physical factors such adhesive force, combined effects of adhesive and cohesive forces (Hoseney and Smewing, 1999; Král et al., 2018) and viscoelasticity.

Stickiness properties of dough are influenced by several factors. The most important are extent of mixing and water quantity (Ahmed and Thomas, 2018). However, studies have shown that excessive water plays the most significant role in dough stickiness. Water acts as plasticizer in dough system due to its influence on molecular mobility (Liu et al., 2018a). Some properties of dough such as surface tension and solvation are dependent on the plasticizing effect of water (Fonseca-Florido et al., 2018). Constituents of food systems such as proteins, in particular, glutenin and gliadins (Stone et al., 2018), alpha-amylase activity (Zadeike et al., 2018), and proteolytic enzyme activity (Zadeike et al., 2018) are also reported to affect stickiness of dough. Therefore, information on the water absorption capability and intrinsic composition of base material is necessary to estimate the stickiness of resulting dough.

Compressive force is applied during mixing of ingredients to form dough and kneading of resulting dough. Force of adhesion between the contact surface and dough may result in stickiness depending on the strength and cohesion forces of the dough. Stickiness has been reported to be dependent on the rheological properties of the dough. Grausgruber et al. (2003) proposed that if the dough is strong and elastic, the adhesive force is overcome, and the dough will separate from the surface (i.e., the dough is not sticky). On the other hand, if the dough is viscous, it will flow and not overcome the adhesive force (i.e. the dough is sticky). Therefore, understanding the stickiness properties of dough from a formulation is important for its handling and machination. In this regard scientific report on the stickiness properties of composite wheat-cassava flour dough is virtually absent.

Rheological tests on doughs are used as quality indicators of the gluten and starch polymers molecular structure in ascertaining the dough's functional behavior. The viscoelastic network of the dough is dependent on gluten development properties during mixing of wheat flour, and can influence the handling characteristics of dough during processing. The inclusion of cassava flour into wheat flour in bread making is an important issue in the sustainable utilization of cassava. Increasing acceptance of bread from composite cassava-wheat flour would stir interest in the storage of the composite flour dough through freezing. However, frozen storage of the dough could have additional effect on its subsequent handling and machination during bakery process. Cassava flour consists mainly of starches and some minor amounts of fibres. In dough system, starches impart high water binding capacity (Kaushik et al., 2015) but favours more starch-starch interaction than wheat gluten protein-protein. Thus, incorporation of cassava flours in frozen wheat dough system may lead to reduced gluten network deteriorations and ameliorate the rate of ice crystal formation during freezing which are detrimental and contribute to gluten network disruptions. However, there is limited information on dough stickiness characteristics in the cassava-wheat frozen dough system. Also, baking ingredients such as yeast, sugar, salt, and fat can influence stickiness of dough. Differences in chemical constituents and flour particle size (Sakhare et al., 2014) can affect the stickiness of the dough. Ascertainable stickiness based purely on raw material and water, and subsequently on developed dough is a reflection of industrial quality acceptance criteria based on raw material. In the present article, stickiness measurements were conducted on three doughs: (1) dough made from mixture of flour and water, (2) developed dough with ingredients, and (3) frozen developed dough. The hypothesis: (1) stickiness of the wheat dough decreases with increasing percentage of cassava flour concentration and (2) there is a variation in stickiness of the three different doughs.

2 MATERIALS AND METHODS

2.1 SOURCE OF MATERIALS

The wheat flour (white flour, Golden Cloud, Pietermartzburg, South Africa) was obtained from the South African market. Six cassava varieties (Bangweulu, Katobamputa, Mweru, Kariba, Kampolombo and Chila) were planted at Mansa Root and Tuber Research Station, a branch of Zambian Agriculture Research Station (ZARI), Mansa District, Luapula Province, Zambia. They were harvested from each block after 18 months of planting.

2.2 CASSAVA FLOUR

The cassava roots were processed into flour using the method of Eriksson et al. (2014). The particle size dis-

tribution at 90 % (D90) finer particles of cassava flours was determined as described in Patwa et al. (2014).

2.3 BLENDING OF WHEAT-CASSAVA FLOUR

Three levels of wheat: cassava (90:10, 80:20, 70:30) composite flours were prepared as described in Aboaba and Obakpolor (2010). Wheat flour (100 %) was used as a control in the analysis.

2.4 CHEMICAL ANALYSIS

Protein content was determined as described in Nuwamanya et al. (2010) using the Dumas combustion method of nitrogen content analysis (Leco Truspec Model FP-528, St Joseph Mi, USA). Percentage protein was calculated as % N x 6.25. The moisture, lipid and fibre contents were determined as described in (AOAC, 2012) methods 925.10, 920.39 and 962.09, respectively. The amylose content was determined using a Megazyme amylose/amylopectin assay kit (K-AMYL 12/16 Megazyme International, Ireland). The gluten content was determined by hand washing method using 2 % sodium chloride solution by taking about 10 g flour sample as described in (AACC, 2011) Method 38-10. Water absorption capacity was determined with Brabender Farinograph (Model 820603, Brabender OHG, Duisberg, Germany) at 30 ± 0.2 °C using a 300 g mixing bowel operated at 63 rev min^{-1} according to AACC (2011) Method 54-21 of constant dough mass method.

2.5 DOUGH PREPARATION

The unleavened dough (flour and water only) was prepared as described in Grausgruber et al. (2003) with modification. A 20 g flour was mixed with water at absorption rates from Farinogram. The mixture was kneaded until the dough was formed. The leavened dough (250 g wheat flour, 25 g sugar, 3 g salt, 5 g baking fat and 2.5 g baker's yeast) was produced from white bread wheat flour dough baking method as described in AACCI (2000) Method 10 to 10.03. The formed dough was divided into portions of 20 g. Then the portions were subjected to freezing with air temperature in convection at −40 °C for 35 min until the geometric dough centre reached −18 °C. After freezing, the doughs were wrapped in polyethylene plastic bags and stored at frozen temperature of -18 °C for three weeks.

2.6 STICKINESS OF DOUGHS

Dough stickiness was determined according to the procedure of Sangnark and Noomhorm (2004) using a Texture Analyzer (TA-XT2, Stable Micro Systems Ltd., England) using 5 g dough for each test. The adhesive test was evaluated at speed 0.5 mm s^{-1}, and post-test speed 10 min s^{-1} with a 25 mm perspex cylinder probe at applied force of 80 g (0.785 N) with trigger type: Button. The recorded parameters were peak positive force, stickiness, work of adhesion, and peak positive area.

2.7 DATA ANALYSIS

A completely randomized design comprising of two factors cassava variety and blend ratio (cassava concentration) was used. Triplicate data were analyzed by two-way ANOVA, and Pearson's correlations were performed using GenStat 18th Edition software and mean differences were determined using Fisher's Least Significance Difference (LSD) test at 5 % significant level.

3 RESULTS AND DISCUSSION

3.1 PROXIMATE COMPOSITION OF CASSAVA AND WHEAT FLOURS

The moisture content of the cassava flours ranged from 10.43 to 11.76 % compared to 13.37 ± 0.15 % for wheat flour (Table 1). The moisture content of the composite flour blends ranged from 13.13 to 13.83 % (Table 2), and increased with increase in CFSL (r = 0.37, $p < 0.001$). The protein content of the cassava flours was in the range 1.21 – 1.87 % (Table 1). The protein content of the cassava flours was very low compared to that of wheat flour (11.03 ± 0.27 %). Wheat flour proteins contain about 85 % gluten proteins (glutenins and gliadins) (Avramenko et al., 2018; Ribeiro et al., 2018), while cassava flour protein is gluten-free (Chakrabarti et al., 2017). The lipid content of cassava flour ranged between 0.15 and 0.63 %. The lipid contents in all cassava flour varieties were significantly (p 0.05) lower than in wheat flour (1.72 ± 0.16 %). The lipids reinforce gluten structure through lipid-protein interactions (Avramenko et al., 2018). The fibre content (0.03 – 0.60 %) of the cassava flour was significantly ($p < 0.05$) lower than that of the wheat flour (2.90 ± 0.10 %). Leavened aerated bread can only be

baked from wheat flour because of viscoelastic dough making properties of wheat gluten proteins (Ceresino et al., 2018). Blending of cassava flour with wheat flour influences the blended dough rheological properties.

3.2. PARTICLE SIZE OF CASSAVA VARIETIES AND WHEAT FLOURS

Cassava varieties flour average particles size ranged from 250.43 – 333.43 µm (Table 1) and varied among varieties. The highest and the lowest particle size of cassava flour were recorded in 'Bangweulu' and 'Mweru', respectively. The average particle size of the wheat flour was low (206.67 ± 6.81 µm) compared to the particle size of cassava flours. Particle size is influenced by the milling technique applied and inherent hardness differences of wheat grain and cassava flour varieties (Liu et al., 2015). Flour particle size influences water absorption capacity of the flour which can affect dough quality (Wang et al., 2017).

3.3. AMYLOSE CONTENT

The amylose contents in cassava varieties were in the range 16.04 – 26.95 % and for wheat flour was 20.83 ± 0.45 % (Table 1). Similar cassava amylose contents have been reported, 19.50 - 20.30 % (Morante et al., 2016), 22.60 ± 1.30 (dos Santos et al., 2018), and 17.06 - 25.72 % (Liu et al., 2019). The amylose content is the basis of classifying starches into waxy, semi-waxy, normal/regular and high-amylose types when amylose content is 0 – 2 %, 3 – 15 % 20 – 35 %, and higher than 40 % of the total starch, respectively (Tester et al., 2004; Morante et al., 2016; Botticella et al., 2018). The result shows all the cassava flour varieties including control sample (wheat flour) were generally classified as normal regular starches.

3.4. GLUTEN CONTENT AND WATER ABSORPTION CAPACITY

The dry gluten content of the wheat sample was 13.00 ± 0.87 % (Table 2), and decreased with increased CFSL (r = -0.839, $p < 0.05$). The gluten content followed a decreasing trend as cassava flour addition increased. Thus gluten at 30 % CFSL < 20 % CFSL < 10 % CFSL < Control. However, higher cassava flour inclusion (30 % CFSL) favored more starch-starch interaction than protein-protein which possibly limits gluten development resulting in weak structures and thus varied recovery of gluten among the varieties. Gluten positively correlated with protein content (r = 0.703, $p < 0.05$). The mixing of wheat flour with water transforms gluten proteins (gliadins and glutenin) into viscoelastic gluten structures, that ultimately determines the quality of the dough and final bread product (Sissons and Smit, 2018). Cassava flour has no gluten type proteins as found in wheat and hence has a diluent effect against a wheat gluten development. Similar was observed in Collar and Armero (2018). Inclusion of cassava flour favours more starch-starch interaction which decreases migration of water to proteins resulting in weak gluten structure. Flour particle size had a significant negative correlation (r = -0.53, $p < 0.05$) with gluten development implying that smaller particles hydrate faster and thereby promote migration of excess water to the gluten network. Water absorption capacity (WAC) results for the flour blends at 10 %, 20 %, and 30 % were in the range 60.43 – 62.10 %, 61.03 – 61.50 %, and 59.57 – 60.33 %, respectively, and negatively correlated with CFSL (r = -0.652, $p < 0.05$) suggesting that higher CFSL resulted in decreasing WAC, in part, due to large particle size of cassava flour with low water absorption capacity. There was a weak positive correlation between WAC and protein (r = 0.337, $p < 0.01$), lipid (r = 0.359, $p < 0.01$) and fibre (r = 0.356, $p < 0.01$) contents. The high protein and fibre levels in wheat flours

Table 1: Moisture, protein, lipid, and amylose contents, and particle size of cassava flours from six cassava varieties grown in Zambia

Variety	Moisture (%)	Protein (%)	Lipid (%)	Fibre (%)	Amylose (%)	Size (µm)
Bangweulu	11.02(1.00)^{ab}	1.87(0.78)^{b}	0.40(0.04)^{bc}	0.60(0.49)^{b}	22.22(2.78)^{ab}	312.01(0.00)^{a}
Katobamputa	11.05(1.46) ^{ab}	1.45(0.03)^{ab}	0.41(0.05)^{bc}	0.15(0.15)^{a}	26.95(2.30)^{b}	282.53(0.02)^{c}
Mweru	11.76 ± (1.61)^{b}	1.78(0.28)^{ab}	0.59(0.18)^{cd}	0.05(0.06)^{a}	17.95(8.02)^{a}	250.43(0.03)^{b}
Kariba	11.18 ± (0.72)^{ab}	1.43(0.41)^{ab}	0.63(0.06)^{d}	0.04(0.02)^{a}	16.04(1.16)^{a}	332.52(0.02)^{e}
Kampolombo	10.69 ± (0.62)^{a}	1.58(0.15)^{ab}	0.32(0.20)^{ab}	0.03(0.02)^{a}	18.47(7.30)^{a}	334.43(0.01)^{e}
Chila	10.43 ± (0.37)^{a}	1.21(0.09)^{a}	0.15(0.04)^{a}	0.15(0.05)^{a}	16.15(3.88)^{a}	278.49(0.00)^{c}
Wheat (control)	13.37 ± (0.15)^{d}	11.03(0.27)^{c}	1.72(0.16)^{c}	2.90(0.10)^{c}	20.83(0.45)^{ab}	206.67(6.81)^{a}

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p < 0.05$ by LSD test.

Table 2: Moisture, water absorption capacity, and crude gluten contents of cassava-wheat flour blends

Variety	CFSL (%)	Moisture (%)	Water absorption capacity (%)	Gluten (%)
Bangweulu	10	13.23(0.31)^{ab}	60.43(1.62)^{abcd}	10.41(0.04)^{de}
Katobamputa	10	13.47(0.06)^{bcd}	62.10(0.10)^{e}	11.25(0.01)^{ef}
Mweru	10	13.27(0.15)^{ab}	61.53(0.20)^{e}	10.41(0.02)^{de}
Kariba	10	13.37(0.06)^{abc}	61.37(1.19)^{cde}	11.26(0.01)^{ef}
Kampolombo	10	13.27(0.05)^{ab}	61.63(0.46)^{e}	11.28(0.01)^{ef}
Chila	10	13.27(0.06)^{ab}	61.57(0.30)^{e}	11.28(0.01)^{ef}
Bangweulu	20	13.13(0.15)^{a}	61.10(0.87)^{bcde}	9.56(0.02)^{cd}
Katobamputa	20	13.83(0.06)^{ef}	61.40(0.20)^{cde}	8.62(0.87)^{bc}
Mweru	20	13.27(0.21)^{ab}	61.03(1.00)^{bcde}	8.67(0.85)^{bc}
Kariba	20	13.67(0.06)^{cde}	61.53(0.50)^{e}	7.77(0.87)^{ab}
Kampolombo	20	13.23(0.15)^{av}	61.50(0.20)^{de}	12.15(0.03)^{fg}
Chila	20	13.37(0.15)^{abc}	61.37(0.55)^{cde}	10.40(0.02)^{de}
Bangweulu	30	13.40(0.20)^{abcd}	59.90(0.53)^{a}	8.66(0.02)^{bc}
Katobamputa	30	13.67(0.59)^{cde}	59.57(0.06)^{a}	8.06(1.01)^{bc}
Mweru	30	13.47(0.15)^{ab}	59.67(0.35)^{a}	6.92(0.87)^{a}
Kariba	30	14.00(0.10)^{f}	60.17(0.15)^{ab}	6.88(0.86)^{a}
Kampolombo	30	13.43(0.16)^{abcd}	60.33(0.58)^{abc}	9.23(0.51)^{c}
Chila	30	13.70(0.10)^{def}	60.07(0.90)^{ab}	10.36(0.01)^{de}
Wheat	100	13.37(0.15)^{abc}	61.70(0.61)^{e}	13.00(0.87)^{g}

All values are means of three replications. Data in the parenthesis are the standard deviations.
Within the same column, the values with different letters are significantly different at $p < 0.05$ by LSD test. CFSL = Cassava flour substitution level

are significant contributors toward water absorption. The protein contents were generally very low in cassava with insignificant difference among the cassava varieties ($p > 0.05$). The fibre contents of the cassava varieties (≤ 0.6 %) were low compared to wheat flours (2.9 %) ($p < 0.05$) and hence the contribution of cassava fibre to WAC was likely low. Nevertheless, the difference in fibre content can bring a difference in water absorption of wheat flours. A study by Struck et al. (2018) found that addition of almond fibre significantly reduced WAC of wheat flour. In a similar study on potato-wheat flour, higher protein contents increased WAC of wheat flour (Sarker et al., 2008). The WAC of the flour blends showed significant correlations with gluten content (r = 0.595, p > 0.05), an indication that high gluten content resulted in a high WAC. There was a negative correlation of WAC with flour particle size (r = -0.264, $p < 0.001$), which indicates that smaller particle size had higher water hydration capacity.

3.5. STICKINESS CHARACTERISTIC

Table 3 shows results for stickiness and peak positive force of unleavened, leavened and frozen leavened doughs. Table 4 show results for work of adhesion and peak positive area of unleavened, leavened and frozen leavened doughs.

The stickiness parameters include work of adhesion, peak positive force and positive area. The work of adhesion is generated during compression. The bonding between adhesive (dough) and adhered (probe surface) is essential for stickiness, however, the mechanism of failure of this bond is equally important (Fig. 1). The clear failure of adhesive and the adhered surface is termed adhesive failure while the failure within the adhesive with residue on the adhered surface is known as cohesive failure (Kilcast and Roberts, 1998;Adhikari et al., 2001). The strength of the dough is influenced by covalent or ionic bonding developing (Dobraszczyk, 1997) upon hydration and kneading.

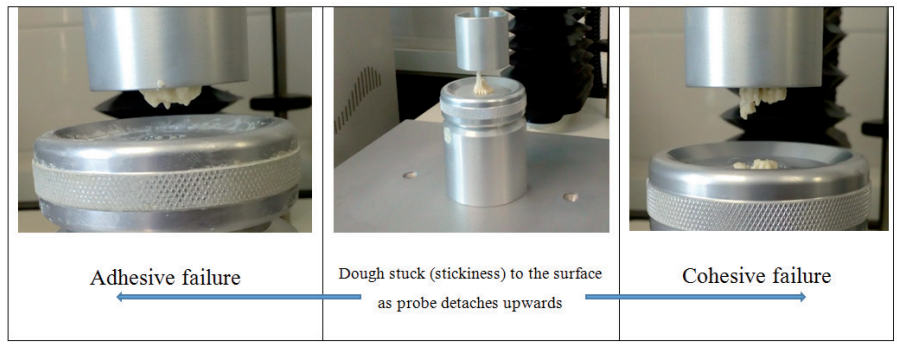

Figure 1: Schematic presentation of stickiness and observed mechanism of failure between the dough (adhesive) and probe surface (adhered)

3.6. STICKINESS OF UNLEAVENED DOUGH

The peak positive force of unleavened dough were in the range 0.44 – 0.77 N, 0.61 – 1.26 N, and 0.57 – 1.20 N, at 10, 20 and 30 % cassava flour level, respectively (Table 3), and increased significantly ($p < 0.05$) with increase in CFSL (r = 0.678, $p < 0.05$). The peak positive force is the maximum force required to pull a compression surface (probe) from a sample after the compression. Doughs with stickiness above the 1N value were characteristic of dough handling difficulties. The work of adhesion of the unleavened dough were ranged from -35.40 to -90.70 g. s, -68.40 to -90.50 g.s, and -33.10 to -90.30 g.s, at 10, 20 and 30 % CFSL, respectively. The control sample (-72.50 ± 10.31 g.s) significantly ($p < 0.05$) increased at higher cassava flour levels. The work of adhesion correlated positively with force (r = 0.515, $p < 0.05$) and weak positive with gluten (r = 0.137, $p < 0.0001$). The adhesion is influenced by cohesion forces which are governed by chemical bonds due to crosslinking of the polymers, glutenin and gliadins within the dough resulting in cohesive and viscoelastic gluten (Guo et al., 2018). The positive area for wheat sample in unleavened dough was 0.03 ± 0.01 N.s, and increased with increase in CFSL (r = 0.321, $p < 0.05$). Positive area exhibited strong positive correlation with both stickiness and peak positive force (r = 0.779, $p < 0.05$) and work of adhesion (r = 0.710, $p < 0.05$). This means that area of displacement is larger in sticky doughs, and may result into adhesive and cohesive failure. The peak positive area is the maximum area of displacement in the dough as the probe (contact surface) detaches upwards from the surface of the dough (Fig 1).

The unleavened dough of flour blends had stickiness in the range 34.14 – 74.10 g, 49.58 – 77.20 g, and 57.91 – 122.17 g, at 10, 20 and 30 % CFSL, respectively, and increased significantly with CFSL (r = 0.678, $p < 0.05$). The wheat sample exhibited stickiness of 42.63 g and was observed to increase with increase in CFSL. Stickiness correlated positively with work of adhesion (r = 0.515, $p < 0.05$), peak force (r = 1.000, $p < 0.05$) and positive area (r = 0.779, $p < 0.05$) (Table 5). This implies that unleavened sticky doughs were associated with higher work of adhesion. Higher stickiness values were characterized with high positive forces. The stickiness of unleavened dough correlated negatively with gluten (r = -0.445, $p < 0.01$), protein (r = -0.592, $p < 0.05$), WAC (r = -0.437, $p < 0.01$), and positively with flour particle size (r = 0.412, $p < 0.05$) suggesting that unleavened doughs with higher gluten and protein content, and high hydration capacity gave lower stickiness values. The larger flour particle sizes hydrate slowly, thus limiting development of gluten structure resulting in high stickiness. High positive area values were characteristic of sticky doughs. Similar was observed in a related study by Amonsou et al. (2013) on adhesiveness of marama bean protein. The reduction in work of adhesion and peak area resulted in decreased stickiness. The differences in varieties could be attributed to variations in amylose contents. The unleavened dough stickiness exhibited negative correlation with amylose contents (r = -0.340, $p < 0.01$). This suggests that higher amylose varieties were less sticky.

3.7. STICKINESS OF LEAVENED DOUGH

The peak positive force (PPF) of leavened dough ranged 0.25 – 0.34 N, 0.21 – 0.50 N, and 0.25 – 0.82 N, at 10, 20 and 30 % CFSL, respectively. The positive force for the control sample was 0.19 N, and was observed to increase with CFSL, and was lower than the positive force of the unleavened dough. The work of adhesion (WA) increased in the leavened dough ranging from -130.20 to -194.40 g.s, -123.90 to -210.40 g.s, and -72.80 to -234.80 g.s, at 10, 20, and 30 % CFSL, respectively. The WA for the wheat was -167.2 g.s, and increased with increasing CFSL. WA for *leavened* dough had positive correlation (r = 0.287, $p < 0.001$) with gluten (Table 5).

Table 3: Stickiness and peak positive force of unleavened, leavened and frozen leavened doughs from composite flours

		Stickiness (g)				Peak positive force (N)			
Variety	Dough type	Wheat (100 %)	10 % CFSL	20 % CFSL	30 % CFSL	Wheat (100 %)	10	20	30
Bangweulu	ULD	42.68(0.61)$^{f-j}$	47.44(7.60)$^{g-k}$	61.95(12.17)^{klm}	57.91(3.66)^{jkl}	0.42(0.01)$^{d-h}$	0.47(0.07)$^{e-i}$	0.61(0.11)^{ijk}	0.57(0.03)^{hij}
	LD	19.26(6.68)^{ab}	26.29(3.02)$^{a-e}$	21.69(2.12)^{abc}	32.16(1.78)$^{b-h}$	0.19(0.07)^{a}	0.26(0.03)^{abc}	0.21(0.02)^{ab}	0.32(0.17)$^{a-e}$
	FLD	150.60(1.66)$^{w-z}$	137.30(9.89)$^{t-w}$	128.98(6.98)^{stu}	93.78(11.36)^{qr}	1.48(0.02)$^{u-x}$	1.35(0.09)$^{r-u}$	1.26(0.07)^{qrs}	0.92(0.11)^{op}
Katobamputa	ULD	42.68(0.61)$^{f-j}$	69.43(3.05)$^{l-o}$	49.58(6.48)$^{h-k}$	67.85(5.67)^{lmn}	0.42(0.01)$^{d-h}$	0.68(0.03)$^{j-m}$	0.49(0.06)$^{f-i}$	0.67(0.05)^{kjl}
	LD	19.26(6.68)^{ab}	25.49(0.98)$^{a-e}$	38.83(10.28)$^{e-i}$	83.94(9.77)^{opq}	0.19(0.07)^{a}	0.25(0.01)^{abc}	0.38(0.10)$^{c-g}$	0.82(0.09)^{mno}
	FLD	150.60(1.66)$^{w-z}$	153.12(13.42)^{xyzA}	179.52(42.29)^{B}	120.91(6.79)^{s}	1.48(0.02)$^{u-x}$	1.50(0.13)^{vwx}	1.76(0.41)^{z}	1.19(0.07)^{q}
Mweru	ULD	42.68(0.61)$^{f-j}$	45.32(9.56)$^{g-j}$	74.12(5.31)$^{m-p}$	103.88(9.02)^{r}	0.42(0.01)$^{d-h}$	0.44(0.09)$^{e-h}$	0.73(0.05)$^{k-n}$	1.02(0.08)^{p}
	LD	19.26(6.68)^{ab}	13.53(10.69)^{a}	23.11(2.75)$^{a-d}$	25.07(8.26)$^{a-e}$	0.19(0.07)^{a}	0.22(0.03)^{ab}	0.23(0.03)^{ab}	0.25(0.08)^{abc}
	FLD	150.60(1.66)$^{w-z}$	162.89(6.89)^{zA}	133.66(13.53)$^{s-v}$	146.82(6.88)$^{v-y}$	1.48(0.02)$^{u-x}$	1.60((0.07)^{xy}	1.31(0.13)$^{q-t}$	1.44(0.07)$^{t-w}$
Kariba	ULD	42.68(0.61) $^{f-j}$	74.10(14.47)$^{m-p}$	75.61(2.76)$^{m-p}$	62.09(1.14)^{klm}	0.42(0.01)$^{d-h}$	0.73(0.14)$^{k-n}$	0.74(0.03)$^{k-n}$	0.61(0.01)^{ijk}
	LD	19.26(6.68)^{ab}	28.76(1.08)$^{a-f}$	35.36(5.44)$^{c-i}$	36.87(0.12)$^{c-i}$	0.19(0.07)^{a}	0.28(0.01)$^{a-d}$	0.35(0.05)$^{b-g}$	0.36(0.00)$^{b-g}$
	FLD	150.60(1.66)$^{w-z}$	126.88(12.70)^{stu}	136.79(10.59)$^{d-i}$	93.67(8.77)^{qr}	1.48(0.02)$^{u-x}$	1.24((0.12)^{qrs}	1.34(0.10)$^{r-u}$	0.92(0.09)^{op}
Kampolombo	ULD	42.68(0.61) $^{f-j}$	57.00(19.30)^{jkl}	76.34(5.58)$^{m-p}$	85.55(5.94)^{pq}	0.42(0.01)$^{d-h}$	0.56(0.18)^{hij}	0.75(0.05)$^{k-n}$	0.84(0.06)^{no}
	LD	19.26(6.68) $^{w-z}$	35.15(2.15)$^{c-i}$	38.67(4.96)$^{s-w}$	42.45(2.39)$^{f-j}$	0.19(0.07)^{a}	0.34(0.02)$^{b-f}$	0.38(0.05)$^{c-g}$	0.42(0.025)$^{d-h}$
	FLD	150.60(1.66)	154.14(5.54)^{yzA}	135.02(7.53)$^{d-i}$	138.21(17.31)$^{u-x}$	1.48(0.02)$^{u-x}$	1.51(0.05)^{wxy}	1.32(0.07)$^{q-u}$	1.36(0.17)$^{s-v}$
Chila	ULD	42.68(0.61) $^{f-j}$	78.48(2.21)$^{n-q}$	77.20(3.43)$^{m-p}$	122.17(8.41)st	0.42(0.01)$^{d-h}$	0.77(0.02)$^{k-n}$	0.76(0.03)$^{k-n}$	1.20(0.08)^{qr}
	LD	19.26(6.68)^{ab}	34.14(1.34)$^{b-h}$	50.68(7.45)^{ijk}	36.1(1.34)$^{c-i}$	0.19(0.07)^{a}	0.33(0.02)$^{a-f}$	0.50(0.07)^{ghi}	0.35(0.01)$^{b-g}$
	FLD	150.60(1.66)^{xyz}	205.66(19.98)^{C}	168.63(30.88)^{AB}	140.15(4.69)$^{u-y}$	1.48(0.02)$^{u-x}$	2.02(0.19)^{A}	1.65(0.30)^{yz}	1.37(0.05)$^{s-w}$

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are lsignificantly different at $p < 0.05$ by LSD test. ULD = Unleavened dough. LD = Leavened dough, FLD = Frozen leavened dough

Table 4: Work of adhesion and peak positive area of unleavened, leavened and frozen leavened doughs from composite flours

		Work of adhesion				Peak positive area			
Variety	Dough type	Wheat (100 %)	10 % CFSL	20 % CFSL	30 % CFSL	Wheat (100 %)	10 % CFSL	20 % CFSL	30 % CFSL
Bangweulu	ULD	−72.5(10.31)$^{h–m}$	−65.5(11.40)$^{i–m}$	−83.7(24.27)$^{g–l}$	−90.3(12.57)$^{g–k}$	0.03(0.01)$^{a–e}$	0.04(0.02)$^{a–f}$	0.06(0.03)$^{a–i}$	0.06(0.00)$^{a–h}$
	LD	−167.2(91.22)$^{b–e}$	−194.4(69.98)^{abc}	−123.9(41.89)$^{e–h}$	−209.8(32.69)^{abc}	0.01(0.01)^{ab}	0.01(0.00)^{ab}	0.00(0.00)^{a}	0.01(0.00)^{a}
	FLD	−31.7(16.75)^{lm}	−60.4(11.30)$^{i–m}$	−90.8(11.74)$^{g–k}$	−26.4(30.52)^{m}	0.21(0.00)$^{l–o}$	0.17(0.003) $^{i–o}$	0.14(0.02)$^{f–n}$	0.11(0.02)$^{a–l}$
Katobamputa	ULD	−72.5(10.31)$^{h–m}$	−65.1(4.22)$^{i–m}$	−91.50(2.09)$^{g–j}$	−78.3(8.40)$^{g–m}$	0.03(0.01)$^{a–e}$	0.19(0.04)$^{k–o}$	0.03(0.01)$^{a–e}$	0.07(0.01)$^{a–j}$
	LD	−167.2(91.22)$^{b–e}$	−178.9(23.10)^{bcd}	−186.0(35.27)^{abc}	−156.7(27.55)$^{c–f}$	0.01(0.01)^{ab}	0.01(0.00)^{ab}	0.03(0.02)$^{a–d}$	0.11(0.04)$^{b–l}$
	FLD	−31.7(16.75)^{lm}	−40.5(5.79)$^{i–m}$	−73.2(37.31)$^{h–m}$	−30(2.49)^{lm}	0.21(0.00)$^{l–o}$	0.13(0.03)$^{d–m}$	0.21(0.07)$^{l–o}$	0.13(0.002)$^{d–m}$
Mweru	ULD	−72.5(10.31)$^{h–m}$	−90.7(9.49)$^{g–k}$	−91.4(9.63)$^{g–j}$	−33.1(7.86)^{lm}	0.03(0.01)$^{a–e}$	0.06(0.05)$^{a–h}$	0.10(0.04)$^{a–k}$	0.42(0.19)^{qr}
	LD	−167.2(91.22)$^{b–e}$	−180.7(40.44)$^{a–d}$	−191.4(27.43)^{abc}	−207.4(13.03)^{abc}	0.01(0.01)^{ab}	0.32(0.43)^{pq}	0.23(0.02)$^{m–p}$	0.01(0.00)^{a}
	FLD	−31.7(16.75)^{lm}	−36.3(7.21)^{klm}	−60.2(40.09)$^{i–m}$	−63.9(10.75)$^{i–l}$	0.21(0.00)$^{l–o}$	0.14(0.01)$^{e–n}$	0.10(0.01)$^{a–k}$	0.16(0.01)$^{i–o}$
Kariba	ULD	−72.5(10.31)$^{h–m}$	−65.2(14.11)$^{i–m}$	−75.1(8.10)$^{h–m}$	−102.6(7.17)$^{f–i}$	0.03(0.01)$^{a–e}$	0.26(0.12)^{op}	0.14(0.07)$^{f–n}$	0.05(0.02)$^{a–g}$
	LD	−167.2(91.22)$^{b–e}$	−173.9(8.83)$^{b–e}$	−178.9(31.16)^{bcd}	−234.8(31.45)^{a}	0.01(0.01)^{ab}	0.01(0.00)^{a}	0.01(0.01)^{ab}	0.02(0.00)^{ab}
	FLD	−31.7(16.75)^{lm}	−70.7(13.27)$^{h–m}$	−40.3(4.42)$^{j–m}$	−49.8(8.02)$^{i–m}$	0.21(0.00)$^{l–o}$	0.16(0.03)$^{h–o}$	0.10(0.01)$^{a–k}$	0.07(0.01)$^{a–j}$
Kampolombo	ULD	−72.5(10.31)$^{h–m}$	−67.8(20.51)$^{i–m}$	−68.4(10.33)$^{i–m}$	−77.3(3.21)$^{g–lm}$	0.03(0.01)$^{a–e}$	0.09(0.09)$^{a–k}$	0.15(0.02)$^{h–o}$	0.16(0.05)$^{h–o}$
	LD	−167.2(91.22)$^{b–e}$	−130.2(38.75)$^{d–g}$	−207.1(19.67)^{abc}	−219.9(17.05)^{ab}	0.01(0.01)^{ab}	0.02(0.00)^{ab}	0.02(0.00)^{abc}	0.02(0.01)^{abc}
	FLD	−31.7(16.75)^{lm}	−62.6(14,93)$^{i–m}$	−67.4(31.43)$^{i–m}$	−56.9(23.97)$^{i–m}$	0.21(0.00)$^{l–o}$	0.18(0.01)$^{k–o}$	0.16(0.02)$^{h–o}$	0.13(0.01)$^{c–m}$
Chila	ULD	−72.5(10.31)$^{h–m}$	−35.4(14.57)^{lm}	−77.7(7.53)$^{g–m}$	−39.4(9.61)$^{j–m}$	0.03(0.01)$^{a–e}$	0.50(0.14)^{r}	0.15(0.01)$^{g–n}$	0.46(0.05)^{r}
	LD	−167.2(91.22)$^{b–e}$	−181.8(7.12)$^{a–d}$	−210.4(26.40)^{abc}	−72.8(49.36)$^{b–e}$	0.01(0.01)^{ab}	0.02(0.02)^{ab}	0.03(0.01)$^{a–e}$	0.01(0.01)^{ab}
	FLD	−31.7(16.75)^{lm}	−60.2(5.00)$^{i–m}$	−30.3(2.28)^{lm}	−55.2(5.85)$^{i–m}$	0.21(0.00)$^{l–o}$	0.24(0.03)^{nop}	0.17(0.02)$^{j–o}$	0.16(0.01) $^{h–o}$

All values are means of three replications. Data in the parenthesis are the standard deviations. Within the same column, the values with different letters are significantly different at $p < 0.05$ by LSD test.
ULD = Unleavened dough, LD = Leavened dough, FLD = Frozen leavened dough

The peak positive area (PPA) of wheat sample had lower value (0.01 ± 0.01 N.s) than that of wheat sample from unleavened dough. The stickiness of the leavened dough were in the range 13.53 – 35.15 g, 21.69 – 50.68 g, and 25.07 – 83.94 g, at 10, 20 and 30 % CFSL, respectively, and varied significantly ($p < 0.05$) according to CFSL (r = 0.578, $p < 0.05$). The wheat sample had stickiness of 19.26 ± 6.68 g which increased with increasing CFSL. The stickiness of leavened dough negatively correlated with protein (r = -0.465, $p < 0.05$). In a related study, Gujral et al. (2018) reported that blending gluten-free flours with wheat resulted in protein weakening due to increased starch-starch, and starch-protein interaction resulting in low level of gluten formation. The gluten content showed negative correlation with stickiness in leavened dough (r = -0.457, $p < 0.01$) similar to that observed in unleavened dough (r = -0.445, $p < 0.01$). The stickiness increased with reduced gluten contents in flour blends in both unleavened and leavened doughs. High CFSL were associated with low gluten contents and yielded high stickiness. The negative correlation of stickiness with WAC in leavened (r = -0.434, $p < 0.01$) was similar with WAC in unleavened (r = -0.437, $p < 0.01$) doughs. This implies that higher WAC produced less sticky doughs. Similar was observed in the work of Amonsou et al. (2013) in which pure gluten isolates were characterized with lower forces of adhesion (low stickiness) as moisture content increased. The amylose contents in the leavened dough did not influence stickiness (r = 0.078, $p > 0.01$). This may suggest that addition of leavening ingredients reduced the influence of amylose contents on stickiness. In the current study, the amylose contents of the flours were classified as normal or regular starches, and have been reported to be highly susceptible to enzymatic hydrolysis (Adefegha et al., 2018). The positive correlation of stickiness with flour particle size in leavened (r = 0.423, $p < 0.01$) was similar with flour particle size in unleavened (r = 0.412, $p < 0.01$) doughs. There was reduction in stickiness upon inclusion of ingredients (yeast, salt, fat and sugar). The stickiness trend was unleavened > leavened dough. The development of dough is important in baking since it combines the ingredients and develops a unique viscoelastic gluten network. Chen et al. (2018) reported that salt increases dough mixing resistance, and decreases dough stickiness during processing as higher levels of salt induced stronger gluten interactions via sulfhydryl-disulfide cross-linking. Salt has been identified as an ingredient in the dough that influences the level of protein–protein interactions and strength of the gluten network by changing the level of gluten hydration. Salt shields around the protein surface and thus induce charge on amino acids on the protein's surface, thus reducing the thickness of the electric double layer, and strengthening gluten interactions, which would subsequently yield a stronger network (Avramenko et al., 2018). Fat enhances dough plasticity (Mert and Demirkesen, 2016), softens and improve smoothness of the dough (Öztürk and Ova, 2018) which would probably contribute to reduced surface tension between probe surface and dough. Adhesion is negligibly small in smooth surfaces (McFarlane and Tabor, 1950; Liu et al., 2018). Dough stickiness may result in chewy bread that adheres to the mouth. Often the dough would seem unbaked, and would thus contribute to decreased consumer acceptance (Caramanico et al., 2018). Grausgruber et al. (2003) classified sticky and non-sticky dough as: stickiness greater than 90 g results in sticky dough, and less than 80 g produces non-sticky dough.

3.7. STICKINESS OF FROZEN LEAVENED DOUGH

The peak positive force (PPF) for frozen dough showed higher levels of peak force in the range 1.24 – 2.02 N, 1.26 – 1.65 N, 0.92 – 1.44 N at 10, 20 and 30 % CFSL, respectively, and significantly decreased with increase in CFSL (r = -0.409, $p < 0.01$). The PPF for the wheat sample was 1.48 ± 0.02 N. The forces were generally higher at 10 and 20 %, and lower at 30 % CFSL. These variations were due to differences in gluten content. The PPF showed weak positive correlation with gluten (r = 0.325, $p < 0.01$), implying that strong gluten content doughs would require higher forces to detach from the adhering surfaces. The WA for frozen dough ranged from -36.30 to -70.70, -30.30 to -90.80 and -26.40 to -63.90 g.s, at 10, 20 and 30 % CFSL, respectively, and decreased significantly ($p < 0.001$) with increase in CFSL. The WA value (31.7 ± 16.75 g.s) for wheat sample was lower than those of unleavened and leavened doughs. This implies that probe surface would require small amount of work to adhere to sticky doughs (sticky doughs easily adheres to surfaces). There was significant ($p < 0.05$) increase of PPA in frozen dough compared to unfrozen leavened dough. This implies adhesive material (dough) displaced from the dough increased. The wheat sample for frozen dough exhibited higher PPA value (0.21 ± 00) and exhibited insignificant changes ($p > 0.05$) in flour blends.

There was significant increase in the stickiness of the frozen dough compared to unfrozen doughs. The stickiness was in the range 126.88 – 205.66 g, 128.98 – 179.52 g and 93.67 – 146.82 g, at 10, 20 and 30 % CFSL, respectively. The stickiness of wheat sample (150 ± 60 g) decreased significantly (r = -0.409, $p < 0.05$) with increase in CFSL. Stickiness of frozen doughs was higher at 10 % cassava flour level, and generally lower at subsequent blend ratios. This trend is opposite to that of unfrozen

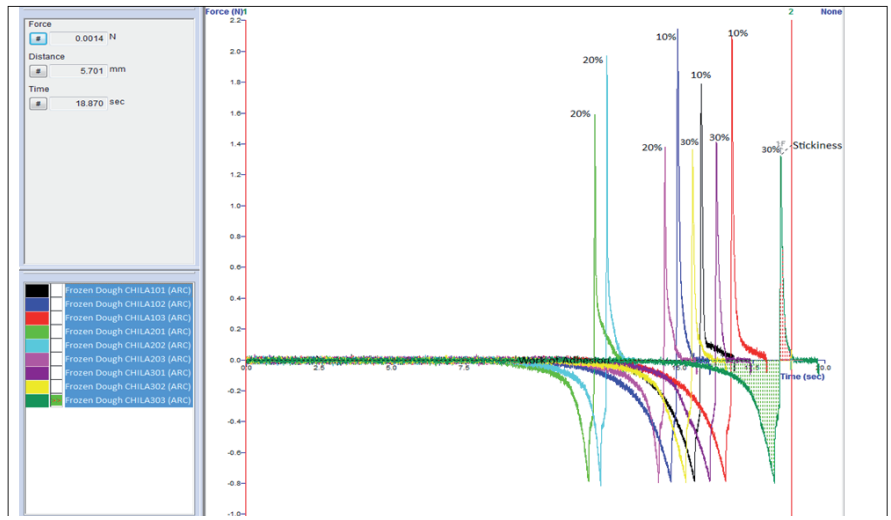

Figure 2: Typical curves for frozen dough of wheat-cassava blend flour at 10, 20 and 30 % cassava flour concentration levels. Cassava variety: Chila

doughs, in which higher CFSL doughs gave lower stickiness. This variation could suggest that depolymerization of gluten during frozen storage generates low molecular mass compounds which are hydrophobic nature. During frozen storage, the water solidifies into ice through crystallization, and subsequent expansion of solid water can cause physical rupture of protein (disulfide) films, thus limiting protein-protein interactions leading to weakening of gluten. During thawing the ice melts and separates irreversibly away from the starch–gluten matrix which reduces interaction of water with hydration sites of gluten–starch system. Hence, the resulting water phase is in weak interaction with the gluten structure (Zhao et al., 2013;Ma et al., 2016). The unbound water probably might have contributed to increased adhesion. Similar was observed by Amonsou et al. (2013) as high moisture content doughs exhibited higher force of adhesion. Moreover, development of gluten structure is the function of disulphide bonds in glutenin and gliadins, and thus depleting disulphide bonds weakens the gluten matrix. The developed gluten matrix undergo deterioration exhibited through molecular changes during frozen storage (Wang et al., 2018). Zhao et al. (2013) reported free sulfhydryl groups increased in the wheat dough during frozen storage time, which indicated decrease of number of disulphide bonds. The higher peak stickiness (Fig 2) in low levels of cassava flour could be as the result of increased number of low molecular mass oligomers due to depolymerisation of glutenin which occurs via the breakage of interchain disulphide bonds, and thus weakening the viscoelasticity resulting in higher stickiness. Also, the decrease in stickiness at higher CFSL could be ascribed to high contents of starches in cassava flour with higher water binding capacity than wheat gluten.

4. CONCLUSION

The stickiness of wheat related blends are dependent on water absorption and gluten development. The stickiness in the unleavened and leavened doughs increased with increasing CFSL. The opposite was observed in the frozen dough in which the stickiness decreased with increasing cassava flour level. Cassava flour acted as diluent against gluten content in wheat, and caused excess water in the aqueous phase at higher CFSL. In the frozen dough, the deterioration of gluten might have led to increased unbound water leading to higher stickiness values at lower CFSL. The stickiness of unleavened dough decreased upon inclusion of ingredients. Therefore, leavened exhibited lower stickiness values than unleavened dough. The frozen dough would be recommended for re-kneading to re-develop the desired consistency with reduced stickiness. Future investigation should focus on the effect of dough improvers (ingredients) on the stickiness of frozen dough.

5 REFERENCES

AACC. (2011). AACC *International, Approved methods of American Association of Cereal Chemists.* 10th ed. ST. Paul, MN : AACC International.

AACCI. (2000). AACCI methods 10-10.03 (Optimized Straight-Dough Bread-Making Method). In: *Approved*

Table 5: Correlation coefficient of stickiness with water absorption, amylose and gluten contents in unleavened and leavened dough

Parameter	CFSL	ST	WA	PPF	PA	WAC	Amy	Pro	Lip	Fib	D90	Glu
Unleavened dough												
CFSL	1											
ST	0.678*	1										
WA	−0.055	0.515*	1									
PPF	0.678*	1.000*	0.515*	1								
PA	0.321	0.779*	0.710*	0.779*	1							
WAC	−0.652*	−0.437*	−0.082	−0.437*	−0.186	1						
Amy	−0.099	−0.340**	−0.199	−0.340**	−0.388	−0.119	1					
Pro	−0.772*	−0.592*	−0.014	−0.592*	−0.401	0.337**	0.152	1				
Lip	−0.741*	−0.620*	−0.128	−0.620*	−0.443	0.359**	0.138	0.957	1			
Fib	−0.760*	−0.613*	−0.027	−0.613*	−0.411	0.356**	0.157	0.982	0.935	1		
D90	0.644*	0.412*	−0.099	0.412**	0.188	−0.264	−0.159	−0.830	−0.787	−0.809	1	
Glu	−0.839*	−0.445*	0.137	−0.445*	−0.159	0.595*	0.082	0.703	0.620	0.704	−0.531*	1
Leavened dough												
CFSL	1											
ST	0.578*	1										
WA	−0.239	−0.192	1									
PPF	−0.051	−0.091	−0.057	1								
PA	0.278	−0.123	−0.107	−0.101	1							
WAC	−0.652*	−0.434**	0.270	0.042	−0.268	1						
Amy	−0.099	0.078	0.087	−0.184	−0.112	−0.119	1					
Pro	−0.772*	−0.465*	0.154	−0.117	−0.134	0.337**	0.152	1				
Lip	−0.741*	−0.520*	0.207	−0.084	−0.059	0.359**	0.138	0.957	1			
Fib	−0.760*	−0.466*	0.174	−0.139	−0.177	0.356**	0.157	0.982	0.935	1		
D90	0.644*	0.423**	−0.104	−0.072	−0.148	−0.264	−0.159	−0.830	−0.787	−0.809	1	
Glu	−0.839*	−0.457*	0.287	−0.025	−0.403	0.595*	0.082	0.703	0.620	0.704	−0.531*	1

ST = Stickiness, WA = Work adhesion, PPF = Peak Positive Force, WAC = Water absorption capacity, PA = Positive area, Amy = Amylose, Pro = Protein, Lip = Lipid, Fib = Fibre, Glu = Gluten. $p < 0.05$*, $p < 0.01$**, $p < 0.001$***

Table 6: Correlation coefficient of stickiness with water absorption, amylose and gluten contents in frozen leavened dough

Parameter	CFSL	ST	WA	PPF	PA	WAC	Amy	Pro	Lip	Fib	D90	Glu
CFSL	1											
ST	-0.409*	1										
WA	-0.243	0.034	1									
PPF	-0.409*	1.000*	0.034	1								
PA	-0.629*	0.566*	-0.009	0.566*	1							
WAC	-0.652*	0.344**	0.134	0.344**	0.365	1						
Amy	-0.099	0.012	0.013	0.012	0.085	-0.119	1					
Pro	-0.772*	0.130	0.397**	0.130	0.545	0.337	0.152	1				
Lip	-0.741*	0.034	0.365**	0.034	0.427	0.359	0.138	0.957	1			
Fib	-0.760*	0.102	0.381**	0.102	0.559	0.356	0.157	0.982	0.935	1		
D90	0.644*	-0.330	-0.420*	-0.330**	-0.540	-0.264	-0.159	-0.830	-0.787	-0.809	1	
Glu	-0.839*	0.325**	0.164	0.325**	0.645	0.595	0.082	0.703	0.620	0.704	-0.531	1

ST = Stickiness, WA = Work adhesion, PPF = Peak Positive Force, WAC = Water absorption capacity, PA = Positive area, Amy = Amylose, Pro = Protein, Lip = Lipid, Fib = Fibre, Glu = Gluten. $p < 0.05$*, $p < 0.01$**, $p < 0.001$***

methods of American Association of Cereal Chemists. 10th ed. ST. Paul , MN : AACC International.

Aboaba, O and Obakpolor, E. (2010). The leavening ability of bakers yeast on dough prepared with composite flour (wheat/cassava). *African Journal of Food Science, 4*(6), 330-329.

Adefegha, SA, Olasehinde, TA and Oboh, G. (2018). Pasting alters glycemic index, antioxidant activities, and starch-hydrolyzing enzyme inhibitory properties of whole wheat flour. *Food Science & Nutrition, 6*(6), 1591-1600. https://doi.org/10.1002/fsn3.711

Adhikari, B, Howes, T, Bhandari, B and Truong, V. (2001). Stickiness in foods: a review of mechanisms and test methods. *International Journal of Food Properties, 4*(1), 1-33. https://doi.org/10.1081/JFP-100002186

Amonsou, EO, Taylor, JR and Minnaar, A. (2013). Adhesive potential of marama bean protein. *International Journal of Adhesion and Adhesives, 41*, 171-176. https://doi.org/10.1016/j.ijadhadh.2012.11.002

Avramenko, N, Tyler, R, Scanlon, M, Hucl, P and Nickerson, M. (2018). The chemistry of bread making: The role of salt to ensure optimal functionality of its constituents. *Food Reviews International, 34*(3), 204-225. https://doi.org/10.1080/87559129.2016.1261296

Botticella, E, Sestili, F, Sparla, F, Moscatello, S, Marri, L, Cuesta-Seijo, JA, Falini, G, Battistelli, A, Trost, P and Lafiandra, D. (2018). Combining mutations at genes encoding key enzymes involved in starch synthesis affects the amylose content, carbohydrate allocation and hardness in the wheat grain. *Plant Biotechnology Journal, 16*(10), 1723-1734. https://doi.org/10.1111/pbi.12908

Caramanico, R, Marti, A, Vaccino, P, Bottega, G, Cappa, C, Lucisano, M and Pagani, MA. (2018). Rheological properties and baking performance of new waxy lines: Strengths and weaknesses. LWT - *Food Science and Technology, 88*, 159-164. https://doi.org/10.1016/j.lwt.2017.09.035

Chakrabarti, T, Poonia, A and Chauhan, AK. (2017). Process optimization of gluten free cookies using cassava flour. *International Journal of Food Science and Nutrition, 2*(5), 190-195.

Chen, G, Ehmke, L, Sharma, C, Miller, R, Faa, P, Smith, G and Li, Y. (2018). Physicochemical properties and gluten structures of hard wheat flour doughs as affected by salt. *Food Chemistry, 275*, 569-576. https://doi.org/10.1016/j.foodchem.2018.07.157

Collar, C and Armero, E. (2018). Kinetics of in vitro starch hydrolysis and relevant starch nutritional fractions in heat-moisture treated blended wheat-based bread matrices: impact of treatment of non-wheat flours. *European Food Research and Technology, 244*(11), 1977-1984. https://doi.org/10.1007/s00217-018-3109-1

Dobraszczyk, B. (1997). The rheological basis of dough stickiness. *Journal of Texture Studies,28*(2), 139-162. https://doi.org/10.1111/j.1745-4603.1997.tb00108.x

dos Santos, TPR, Franco, CML, Demiate, IM, Li, X-H, Garcia, EL, Jane, J-l and Leonel, M. (2018). Spray-drying and extrusion processes: Effects on morphology and physicochemical characteristics of starches isolated from Peruvian carrot and cassava. *International Journal of Biological Macromolecules, 118* Part A, 1346-1353. https://doi.org/10.1016/j.ijbiomac.2018.06.070

Eriksson, E, Koch, K, Tortoe, C, Akonor, P and Oduro-Yeboah, C. (2014). Evaluation of the physical and sensory characteristics of bread produced from three varieties of cassava and wheat composite flours. *Food and Public Health, 4*(5), 214-222.

Grausgruber, H, Hatzenbichler, E and Ruckenbauer, P. (2003). Analysis of repeated stickiness measures of wheat dough using a texture analyzer. *Journal of Texture Studies, 34*(1), 69-82. https://doi.org/10.1111/j.1745-4603.2003.tb01056.x

Gujral, HS, Sharma, B and Khatri, M. (2018). Influence of replacing wheat bran with barley bran on dough rheology, digestibility and retrogradation behavior of chapatti. *Food Chemistry, 240*, 1154-1160. https://doi.org/10.1016/j.foodchem.2017.08.042

Guo, X, Sun, X, Zhang, Y, Wang, R and Yan, X. (2018). Interactions between soy protein hydrolyzates and wheat proteins in noodle making dough. *Food Chemistry, 245*, 500-507. https://doi.org/10.1016/j.foodchem.2017.10.126

Hoseney, R and Smewing, J. (1999). Instrumental measurement of stickiness of doughs and other foods. *Journal of Texture Studies, 30*(2), 123-136. https://doi.org/10.1111/j.1745-4603.1999.tb00206.x

Kaushik, R, Kumar, N, Sihag, MK and Ray, A. (2015). Isolation, characterization of wheat gluten and its regeneration properties. *Journal of Food Science and Technology, 52*(9), 5930-5937. https://doi.org/10.1007/s13197-014-1690-2

Kilcast, D and Roberts, C. (1998). Perception and measurement of stickiness in sugar-rich foods. *Journal of Texture Studies, 29*(1),81-100. https://doi.org/10.1111/j.1745-4603.1998.tb00155.x

Král, M, Pokorná, J, Tremlová, B, Ošťádalová, M, Trojan, V, Vyhnánek, T and Walczycka, M. (2018). Colored Wheat: Anthocyanin Content, Grain Firmness, Dough Properties, Bun Texture Profile. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 66*(3), 685-690. https://doi.org/10.11118/actaun201866030685

Liu, C, Liu, L, Li, L, Hao, C, Zheng, X, Bian, K, Zhang, J and Wang, X. (2015). Effects of different milling processes on whole wheat flour quality and performance in steamed bread making. *LWT-Food Science and Technology, 62*(1), 310-318. https://doi.org/10.1016/j.lwt.2014.08.030

Liu, R, Sun, W, Zhang, Y, Huang, Z, Hu, H and Zhao, M. (2019). Preparation of starch dough using damaged cassava starch induced by mechanical activation to develop staple foods: Application in crackers. *Food Chemistry, 271*(1), 284-290. https://doi.org/10.1016/j.foodchem.2018.07.202

Liu, X, Wang, L, Qiao, Y, Sun, X, Ma, S, Cheng, X, Qi, W, Huang, W and Li, Y. (2018). Adhesion of liquid food to packaging surfaces: Mechanisms, test methods, influencing factors and anti-adhesion methods. *Journal of Food Engineering, 228*, 102-117. https://doi.org/10.1016/j.jfoodeng.2018.02.002

Ma, S, Li, L, Wang, X-x, Zheng, X-l, Bian, K and Bao, Q-d. (2016). Effect of mechanically damaged starch from wheat flour on the quality of frozen dough and steamed bread. *Food Chemistry, 202*, 120-124. https://doi.org/10.1016/j.foodchem.2016.01.075

McFarlane, J and Tabor, D. (1997). Adhesion of solids and the effect of surface films. Proceedings of the Royal Society of London. Series A. *Mathematical and Physical Sciences, 202*(1069), 224-243. https://doi.org/10.1098/rspa.1950.0096

Mert, B and Demirkesen, I. (2016). Evaluation of highly unsaturated oleogels as shortening replacer in a short dough product. *LWT - Food Science and Technology, 68*, 477-484. https://doi.org/10.1016/j.lwt.2015.12.063

Morante, N, Ceballos, H, Sánchez, T, Rolland-Sabaté, A, Calle, F, Hershey, C, Gibert, O and Dufour, D. (2016). Discovery of new spontaneous sources of amylose-free cassava starch and analysis of their structure and techno-functional properties. *Food Hydrocolloids, 56*(3), 383-395. https://doi.org/10.1016/j.foodhyd.2015.12.025

Öztürk, E and Ova, G. (2018). Evaluation of Cocoa Bean Hulls as a Fat Replacer On Functional Cake Production. *Turkish Journal of Agriculture-Food Science and Technology, 6*(8), 1043-1050. https://doi.org/10.24925/turjaf.v6i8.1043-1050.1934

Patwa, A, Malcolm, B, Wilson, J and Ambrose, KR. (2014). Particle size analysis of two distinct classes of wheat flour by sieving. *Transactions of the American Society of Agricultural and Biological Engineers, 57*(1), 151-159. https://doi.org/10.13031/trans.57.10388

Ribeiro, M, Nunes, FM, Rodriguez-Quijano, M, Carrillo, JM, Branlard, G and Igrejas, G. (2018). Next-generation therapies for celiac disease: The gluten-targeted approaches. *Trends in Food Science & Technology, 75*(6): 56-71. https://doi.org/10.1016/j.tifs.2018.02.021

Sakhare, SD, Inamdar, AA, Soumya, C, Indrani, D and Rao, GV. (2014). Effect of flour particle size on microstructural, rheological and physico-sensory characteristics of bread and south Indian parotta. *Journal of Food Science and Technology, 51*(12), 4108-4113. https://doi.org/10.1007/s13197-013-0939-5

Sangnark, A and Noomhorm, A. (2004). Chemical, physical and baking properties of dietary fiber prepared from rice straw. *Food Research International, 37*(1), 66-74. https://doi.org/10.1016/j.foodres.2003.09.007

Sarker, MZI, Yamauchi, H, Kim, S-J, Matsumura-Endo, C, Takigawa, S, Hashimoto, N and Noda, T. (2008). A farinograph study on dough characteristics of mixtures of wheat flour and potato starches from different cultivars. *Food Science and Technology Research, 14*(2), 211-216. https://doi.org/10.3136/fstr.14.211

Sissons, M and Smit, J. (2018). Small-scale methods to assess the gluten properties of durum wheat. *Cereal Chemistry, 95*(3), 456-468. https://doi.org/10.1002/cche.10048

Stone, AK, Lam, RS, Hopkins, EJ, Hucl, P, Scanlon, MG and Nickerson, MT. (2018). Effect of organic acids and NaCl on the rheological properties of dough prepared using Pembina and Harvest CWRS wheat cultivars. *Cereal Chemistry, 95*(3), 478-485. https://doi.org/10.1002/cche.10050

Struck, S, Straube, D, Zahn, S and Rohm, H. (2018). Interaction of wheat macromolecules and berry pomace in model dough: Rheology and microstructure. *Journal of Food Engineering, 223*(4), 109-115. https://doi.org/10.1016/j.jfoodeng.2017.12.011

Tester, RF, Karkalas, J and Qi, X. (2004). Starch-composition, fine structure and architecture. *Journal of Cereal Science, 39*(2), 151-165. https://doi.org/10.1016/j.jcs.2003.12.001

Villanueva, M, Pérez-Quirce, S, Collar, C and Ronda, F. (2018). Impact of acidification and protein fortification on rheological and thermal properties of wheat, corn, potato and tapioca starch-based gluten-free bread doughs. *LWT - Food Science and Technology, 96*, 446-454. https://doi.org/10.1016/j.lwt.2018.05.069

Wang, N, Hou, GG and Dubat, A. (2017). Effects of flour particle size on the quality attributes of reconstituted whole-wheat flour and Chinese southern-type steamed bread. *LWT - Food Science and Technology, 82*, 147-153. https://doi.org/10.1016/j.lwt.2017.04.025

Wang, P, Zou, M, Gu, Z and Yang, R. (2018). Heat-induced polymerization behavior variation of frozen-stored gluten. *Food Chemistry, 255*, 242-251. https://doi.org/10.1016/j.foodchem.2018.02.047

Zadeike, D, Jukonyte, R, Juodeikiene, G, Bartkiene, E and Valatkeviciene, Z. (2018). Comparative study of ciabatta crust crispness through acoustic and mechanical methods: Effects of wheat malt and protease on dough rheology and crust crispness retention during storage. *LWT - Food Science and Technology, 89*, 110-116. https://doi.org/10.1016/j.lwt.2017.10.034

Zhao, L, Li, L, Liu, G-q, Chen, L, Liu, X, Zhu, J and Li, B. (2013). Effect of freeze-thaw cycles on the molecular weight and size distribution of gluten. *Food Research International, 53*(1), 409-416. https://doi.org/10.1016/j.foodres.2013.04.013

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Field evaluation of the relative susceptibility of six pear varieties to the pear psylla (*Cacopsylla pyricola* (Foerster, 1848))

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Field evaluation of the relative susceptibility of six pear varieties to the pear psylla (*Cacopsylla pyricola* (Foerster, 1848))

Abstract: The pear psylla, *Cacopsylla pyricola* (Foerster, 1848) (Hemiptera: Psyllidae), is one of the most detrimental pests in commercial pear orchards. Varieties with low infestation level to pear psylla would offer to integrated psyllid management. The natural infestation level of six pear varieties to pear psylla was studied under field conditions during three successive years. The pear varieties consisted of 'Comice', 'Buerre Giffard', 'Bonne Louise', 'Felestini', 'Shahmiveh', and 'Sebri'. Psyllid population was sampled weekly by limb jarring method and selecting 10 leaves randomly per tree. The results indicated that the size of the psyllid population on the tested pear varieties was significantly different ($F_{5, 30} = 816.18$, $p < 0.0001$). 'Shahmiveh' and 'Sebri' showed high and low susceptible, respectively, to pear psylla infestation. The natural infestation level of *C. pyricola* was 37.01 % and 35.8 % lower on 'Bonne Louise' and 'Sebri', respectively, than on 'Shahmiveh'. These varieties may be used for crossing in breeding programs to develop plant resistance to *C. pyricola* and may be exploited in integrated psyllid management.

Key words: pear psylla; population; relative susceptibility, variety

Ovrednotenje relativne občutljivosti šestih sort hrušk na malo hruševo bolšico (*Cacopsylla pyricola* (Foerster, 1848))

Izvleček: Mala hruševa bolšica, *Cacopsylla pyricola* (Förster, 1848) (Hemiptera: Psyllidae), je eden izmed najbolj uničujočih škodljivcev v komercialnih nasadih hrušk. Sorte z majnim deležem okužbe z bolšico bi lahko uporabili pri integriranem upravljanju s hruševo bolšico. Naravna okužba s hruševo bolšico je bila preučevana na šestih sortah hrušk na prostem v treh zaporednih letih. Sorte hrušk so bile:'Comice', 'Buerre Giffard', 'Bonne Louise', 'Felestini', 'Shahmiveh', in 'Sebri'. Populacija bolšic je bila vzorčena tedensko s stresanjem vej in naključno izbiro 10 listov na drevo. Rezultati so pokazali, da so bile velikosti populacij bolšice na preučevanih sortah hrušk značilno različne ($F_{5, 30} = 816{,}18$, $p < 0{,}0001$). Sorti 'Shahmiveh' in 'Sebri' sta pokazali veliko in manjšo občutljivost na okužbo s hruševo bolšico. Naravni okužbi s hruševo bolšico sta bili na sortah 'Bonne Louise' in 'Sebri' za 37,01 % in 35,8 % manjši kot na sorti 'Shahmiveh'. Ti sorti bi lahko uporabili za križanja v žlahtniteljskih programih za razvoj odpornosti hrušk na bolšico in jih uporabili pri integriranem upravljanju z bolšico.

Ključne besede: hruševa bolšica; populacija; relativna občutljivost; sorta

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1 INTRODUCTION

The pear psylla, *Cacopsylla pyricola* (Förster, 1848) (Hemiptera: Psyllidae), is a main insect pest of the planted pear (*Pyrus communis* L.) in the production areas of Iran (Emami, 2016), North America and Europe (Bell, 2013) with the greatest economic importance (Emami et al., 2014b). The immature psyllids and adults feed by sucking out the plant sap. Psyllids feeding cause to excrete large amounts of honeydew on leaves and fruits, pear tree growth inhibition, leaf necrosis, diminished fruit size, young fruit russetting and premature fruit fall, resulting in considerable losses in crop yield (Emami et al., 2014b). Pear psylla also carries the disease pear decline, which can affect tree health and lead to death of the tree (Sule et al., 2007). Heavy and prolonged feeding and the injection of toxic saliva by enormous populations can cause partial to whole defoliation of pear trees, reducing vitality and inhibiting the formation of fruit buds in the following season. Psyllid invasion is the greatest pest management problem of pear orchards in Iran (Emami et al., 2014a). Since the pear psyllid are able to develop resistance to chemical insecticides (Pree et al., 1990) the domain of effective insecticides for its suppression is limit and the used concentrations are continuously increasing, on the contrary consumers request lower insecticide utilization on the pear crop. Hence investigate on varieties with durable and natural resistance to pear psyllid is an efficient and sustainable strategy for inclusion in pear psylla integrated pest management program. Chang and Philogene (1976) showed that pear psyllid laid more eggs on 'Bosc' cultivar than 'Anjou', 'Bartlett', 'Kieffer' and *Pyrus ussuriensis* Maxim. Cultivar 'Kieffer' was significantly less desirable as hosts for pear psyllid. Quarta and Puggioni (1985) surveyed on 93 cultivars and 43 selections grown in the variety testing trial. They showed that the most common varieties are all very susceptible to pear psylla and only 12 % showed a low susceptibility. Shaltiel-Harpaz et al. (2013) evaluated two pear accessions to pear psylla and indicated that evaluated accessions are more resistant than the commercial cultivar 'Spadona'. The present study was done to evaluate the relative susceptibility of six pear varieties to the pear psylla under natural infestation conditions with the purpose to employ in the psyllid management.

2 MATERIALS AND METHODS

2.1 SITE AND PLANTS

Field assays were carried out in a 1-ha commercial pear orchard placed at Mobarakeh research station (Isfahan, Iran), during the three successive growing seasons (2010-2012) on 25 year-old pear trees. The pear varieties consisted of 'Comice', 'Buerre Giffard', 'Bonne Louise', 'Felestini', 'Shahmiveh', and 'Sebri'. They were nearly an average of 4 m in height, with 5 m spacing between plants. Management activities including suppression of other pests, fertilization, pruning and irrigation were performed alike on all pear trees. No insecticides were utilized during the years of study on the pear trees.

2.2 EXPERIMENTAL DESIGN

The experiment was organized in a completely randomized block design with three replicates.

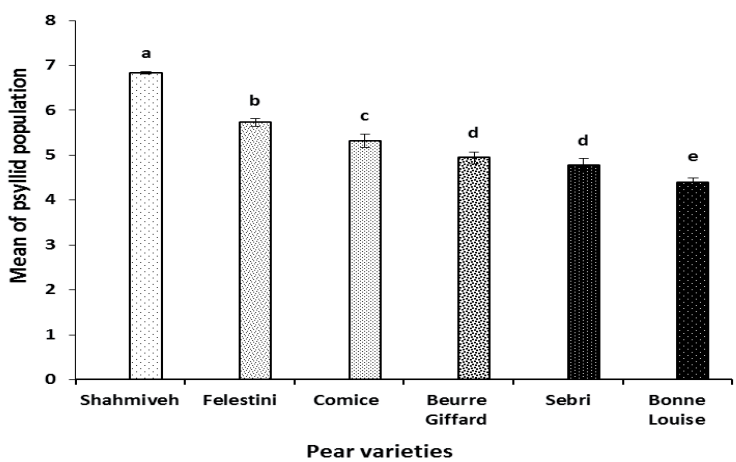

Figure 1: Mean (± SE) of psyllid population (eggs + nymphs + adults) on the pear varieties under natural infestation conditions in 2010. Columns with different letters differ significantly at $p < 0.05$ (Duncan's multiple range test).

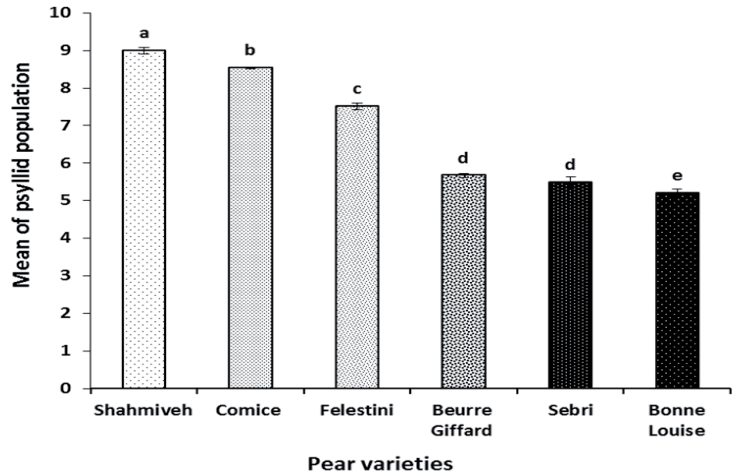

Figure 2: Mean of psyllid population (eggs + nymphs + adults) on the pear varieties under natural infestation conditions in 2011. Columns with different letters differ significantly at $p < 0.05$ (Duncan's multiple range test).

2.3 SAMPLING

Adults of pear psylla were randomly collected from four branches using the limb beating technique (Burts and Retan, 1973). Sampling was done in the morning when adult flight was limited. Each branch was tapped three times with a piece of hard rubber hose and all psyllid falling on the beat tray were counted. Populations of eggs and nymphs in pre bloom stage were counted by getting shoot samples. Four young branches per tree, approximately 25 cm in length, were randomly sampled from different geographical directions. Following foliar expansion of the buds, 10 leaves were randomly selected per tree. Samplings were done weekly. The samples were separately put into nylon bag, moved to the laboratory in an ice box, carefully inspected under a binocular and pear psyllid eggs and nymphs were enumerated and registered.

2.4 DATA ANALYSES

The data were square root (x ± 0.5) transformed before analysis to standardize the variance, and then subjected to one-way ANOVA. The comparison of psyllid population was performed using Duncan's multiple range test (DMRT) ($p < 0.05$). All analyses were performed using SAS statistical software version 9.1. (SAS Institute Inc. 2004).

3 RESULTS

3.1 THE PEAR PSYLLID POPULATION ON THE VARIETIES IN THE FIRST YEAR

There was a significant difference among varieties in term of pear psyllid population ($F_{5, 10} = 128.15$, $p < 0.0001$). The comparison of population means showed that the pear varieties were categorized into five groups (Fig. 1). The natural infestation levels of *C. pyricola* on 'Bonne Louise' and 'Shahmiveh' was the lowest and the highest, respectively (Fig. 1).

3.2 THE PEAR PSYLLID POPULATION ON VARIETIES IN THE SECOND YEAR

The pear psyllid population showed a significant difference among tested pear varieties ($F_{5, 10} = 725.34$, $p < 0.0001$). According to the comparison of means of pest population, the pear varieties were placed into five groups (Fig. 2). The natural infestation level of *C. pyricola* on 'Bonne Louise' was nearly two times lower than on 'Shahmiveh' (Fig. 2).

3.3 THE PEAR PSYLLID POPULATION ON VARIETIES IN THE THIRD YEAR

A significant difference was found among tested varieties in relation to pear psyllid population ($F_{5, 10} = 217.17$, $p < 0.0001$). Means comparison of pest population revealed that the pear varieties were listed into five groups (Fig. 3). The natural infestation level of *C. pyricola* on 'Sebri' and 'Beurre Giffard' was the same and both varieties had the lowest population than others (Fig. 3).

3.4 COMBINED ANALYSIS OF VARIANCE FOR THREE YEARS

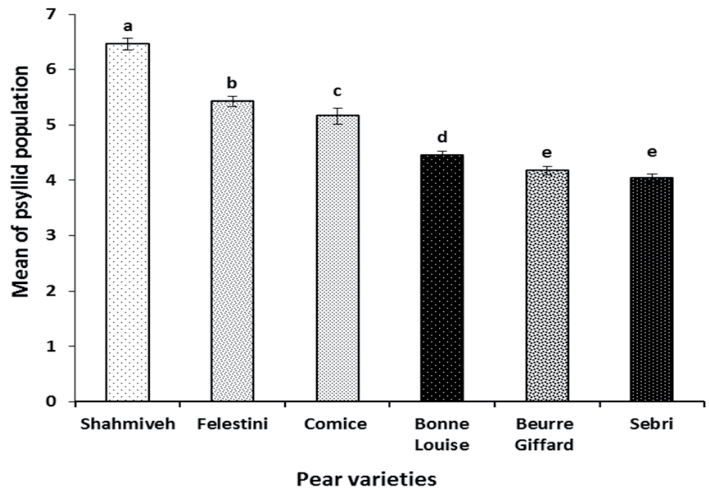

Figure 3: Mean of psyllid population (eggs + nymphs + adults) on the pear varieties under natural infestation conditions in 2012. Columns with different letters differ significantly at $p < 0.05$ (Duncan's multiple range test).

Pest population had a significant difference among experimental varieties ($F_{5, 30} = 816.18$, $p < 0.0001$). The comparison of population means demonstrated that the pear varieties categorized into five groups (Fig. 4). The natural infestation level of *C. pyricola* was 37.01 % and 35.8 % lower on 'Bonne Louise' and 'Sebri', respectively, than on 'Shahmiveh' (Fig. 4).

4 DISCUSSION

C. pyricola is presently a very tiresome problem in pear-growing regions. It appears with two to five population peaks from spring to autumn depending upon latitude (Horton, 2008; Hodkinson, 2009; Emami, 2016). The cost of chemical control per hectare is annually high (Bell, 2013). Pear trees with persistent resistance would improve the economic and environmental durability of pear production by decreasing producer costs and insecticides use. Host resistance in pear tree to the pear psyllid (*Cacopsylla* sp.) can be described under natural infestation conditions on the basis of the pest population size at specific periods (Bell and Puterka, 2004). We noticed, three years study revealed that the size of the psyllid population on the tested pear varieties was significantly different (Fig. 1-4). No immunity to pear psyllid was found.

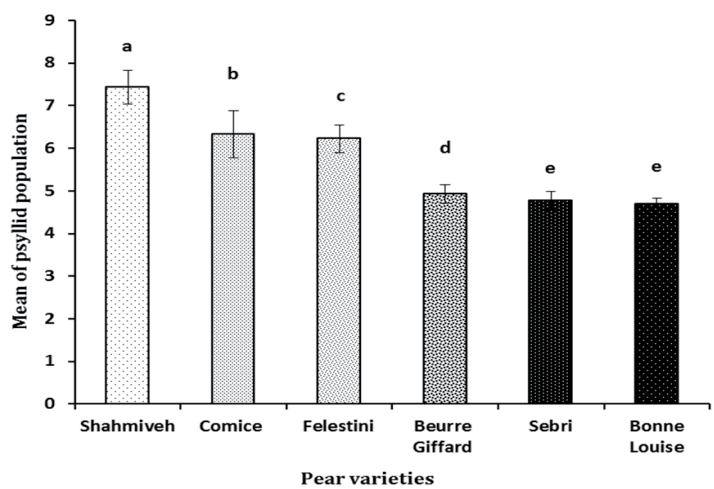

Figure 4: Mean of psyllid population (eggs +nymphs + adults) on the pear varieties under natural infestation conditions in three years study. Columns with different letters differ significantly at $p < 0.05$ (Duncan's multiple range test).

Although none were immune but there was a variation in varieties in their susceptibility to the psyllid. Plants are often varying in their susceptibility to the associated psylla species, arranging across a spectrum from highly susceptible to close resistant (Hodkinson, 2009). In our trial, 'Shahmiveh' variety was very susceptible and vulnerable to psyllid attack (Fig. 1-4). Radjabi (1989) described that the intensity of the pear psyllid population and injuries of pear psylla was high on 'Shahmiveh' variety. Variation in susceptibility can happen across varieties within single species of host plant (Hodkinson, 2009). 'Felestini' and 'Comice' varieties, which were observed in our research, were susceptible varieties. 'Comice' is one of the main pear varieties in many countries and was reported as a susceptible host plant to pear psyllid (Bellini and Nin, 2002; Fischer, 2009). Nin et al. (2015) reported that 'Comice' suffered medium injuries to psylla attack and classified into medium susceptible class. Cross breeding between 'NY10353' as male parent and 'Comice' as female parent has shown a great degree of psylla resistance in controlled conditions (Musacchi et al., 2005; Pasqualini et al., 2006). Behavioral investigations mention that pear psyllid adults use tactile cues to discover chemicals within the tree or on its surface proper for host choice, feeding, and oviposition (Ullman and McLean 1988; Horton, 1990; Horton and Krysan, 1991). Berrada et al. (1995) reported that under field conditions 'Comice' variety was classified as susceptible variety to pear psylla. Westigard et al. (1981) reported that russeted varieties are less susceptible to damage due to honeydew than are smooth-skinned varieties such as 'Comice'. On the contrary, Gerard et al. (1993) showed that resistance is not directly proportional to leaf cuticle thickness, the resistant 'NY' for example has a lower constituent of cutin than the susceptible 'Bartlett' variety. The psyllid population size on 'Sebri', 'Bonne Louise' and 'Buerré Giffard' varieties were lower than 'Shahmiveh' and 'Comice' (Fig. 1-4). Difference in psyllid development prosperity among host species and varieties can generally be explained by variations in the primary attractiveness of the foliage, differential oviposition ratios, larval survival ratios and larval development time (Hodkinson, 2009). Braniste et al. (1994) described 'Buerré Giffard' was slightly attacked by psylla. Horton and Krysian (1991) demonstrated that psyllid is more selective in its oviposition behaviors than in its settling and probing behaviors; i.e., probing is not probably to be a symptom of a variety's acceptability. They also reported that the cues excite feeding activity vary from those for egg laying. Here, the pear psyllid adults had settling and probing activities on later pear varieties but psyllid population did not increase on these than 'Shahmiveh' and 'Comice'. The pear psyllid can colonize and feed on non- desired host plants, but they do not lay eggs (Pasqualini et al., 2006). 'Sebri' variety showed medium susceptible to pear psylla infestation and damage. This finding is in accordance with Radjabi (1989) who described 'Sebri' is a partially resistant host plant to pear psylla damage. Braniste et al. (1994) described 'Buerré Giffard' was slightly attacked by psylla. Aksic et al. (2015) reported that the levels of polyphenolics are most likely responsible for pear resistance to psylla. Resistance of deciduous plants to phloem feeding insects is supposed to result from a combination of structural and excited physical and chemical guards (Eyles et al., 2007). Host resistance has long been regarded as the best alternative and ecologically secure outlook to chemical control of pear psyllid (Civolani et al., 2013). Some of these varieties may be exploited in organic farming combined with biological control. It is suggested that additional studies should be carried out to analyze the morphological and chemical structure of pear cultivars to further characterize the resistance of these cultivars.

5 REFERENCES

Aksić M.M.F., Dabić D.C., Gašić U.M., Zec G.N., Vulić T.B., Tesić Z.L., Natić M.M. (2015). Polyphenolic Profile of Pear Leaves with Different Resistance to Pear Psylla (Cacopsylla pyri). *Journal of Agricultural and Food Chemistry, 63*, 7476-7486. https://doi.org/10.1021/acs.jafc.5b03394

Bell R.L., Puterka G.J. (2004). Modes of host plant resistance to pear psylla: a review. *Acta Horticulturae, 663*, 183-188. https://doi.org/10.17660/ActaHortic.2004.663.26

Bell R.L. (2013). Host resistance to pear psylla of breeding program selections and cultivars. *Horticultural Science, 48*, 143-145. https://doi.org/10.21273/HORTSCI.48.2.143

Bellini E., Nin S. (2002). Breeding for new traits in pear. *Acta Horticulturae, 596,* 217-224. https://doi.org/10.17660/ActaHortic.2002.596.31

Berrada S., Nguyen T.X., Lemoine J., Vanpoucke J., Fournier D. (1995). Thirteen pear species and cultivars evaluated for resistance to Cacopsylla pyri (Homoptera: Psyllidae). *Environmental Entomology, 24,* 1604-1607. https://doi.org/10.1093/ee/24.6.1604

Braniste N., Amzar V., Radulescu M., Sugar D. (1994). Resistance sources to Psylla sp. *Acta Horticulturae, 367*, 54-63. https://doi.org/10.17660/ActaHortic.1994.367.6

Burts E.C., Retan H.A. (1973). *Detection of pear psylla*. Washington State University, Cooperative Extension Service Mimeo 3069, Pullman, WA, 2 pp.

Chang J.F., Philogene B.J.R. (1976). The development and behavior of the Psylla pyricola on different rootstocks and cultivars. *Phytoprotection, 57*, 127-149.

Civolani S., Grandi G., Chicca M., Pasqualini E., Fano E.A., Musacchi S. (2013). Probing behaviour of Cacopsylla pyri on a resistant pear selection. *Journal Applied Entomology, 137*, 365-375. https://doi.org/10.1111/jen.12003

Emami M.S. 2016. Field evaluation of two biorational compounds in the control of pear psylla, Cacopsylla pyricola

(Förster), on pear trees. *Archives of Phytopathology and Plant Protection, 49*, 11-18. https://doi.org/10.1080/03235408.2016.1147122

Emami M.S., Shishehbor P., Karimzadeh J. (2014a). The influences of plant resistance on predation rate of Anthocoris nemoralis (Fabricius) on Cacopsylla pyricola (Förster). *Archives of Phytopathology and Plant Protection, 47*, 2043-2050. https://doi.org/10.1080/03235408.2013.868695

Emami M.S., Shishehbor P., Karimzadeh J. (2014b). Functional response of *Anthocoris nemoralis* (Hemiptera: Anthocoridae) to the pear psylla, Cacopsylla pyricola (Hemiptera: Psyllidae): effect of pear varieties. *Journal of Crop Protection, 3*, 597-609.

Eyles A., Jones W., Riedl K., Cipollini D., Schwartz S., Chan K., Herms D.A., Bonello P. (2007). Comparative phloem chemistry of Manchurian (*F. mandshurica)* and two North American ash species (*F. americana* and *F. pennsylvanica*). *Journal of Chemical Ecology, 33*, 1430-1448. https://doi.org/10.1007/s10886-007-9312-3

Fischer M. (2009). Pear breeding. pp.1-26. In: S. Mohan Jain and P. M. Priyadarshan [Eds.], *Breeding Plantation* Tree Crops: Temperate Species. Springer, New York, 290 pp.

Gerard H.C., Fett W.F., Moreau R.A., Osman F.S., Miller R.L. (1993). Chemical and enzymic investigation of the leaf cuticle of pear genotypes differing in resistance to pear psylla. *Journal of Agricultural and Food Chemistry, 41*, 2437-2441. https://doi.org/10.1021/jf00036a043

Hodkinson I.D. (2009). Life cycle variation and adaptation in jumping plant lice (Insecta: Hemiptera: Psylloidea): a global synthesis. *Journal of Natural History, 43*, 65-179. https://doi.org/10.1080/00222930802354167

Horton D.R., Krysan, J.L. (1991). Host acceptance behavior of pear psylla (Homoptera: Psyllidae) affected by plant species, host deprivation, habituation, and egg load. *Annals of the Entomological Society of America, 84*, 612-627. https://doi.org/10.1093/aesa/84.6.612

Horton D.R. (1990). Oviposition by overwintering morph of pear psylla (Homoptera:Psyllidae) with information on conditioning. *Environmental Entomology, 19*, 357-361. https://doi.org/10.1093/ee/19.2.357

Horton D.R. (2008). Pear psylla, *Cacopsylla pyricola* (Foerster) (Hemiptera: Psyllidae). pp. 2772-2775. In: J. L. Capinera (Ed.), *Encyclopedia of Entomology*. 2nd. Ed. Vols. 1-4. Springer, Dordrecht, The Netherlands, 4346 pp.

Musacchi S., Ancarani V., Gamberini A., Giatti B., Sansavini S. (2005). Progress in pear breeding at the University of Bologna. *Acta Horticulturae, 671*, 191-194. https://doi.org/10.17660/ActaHortic.2005.671.24

Nin S., Ferri A., Sacchetti P., Picardi E., Cantini C., Giordani E. (2015). Susceptibility of European pear germplasm to *Cacopsylla pyri* under mediterranean climatic conditions. *Scientia Horticulturae, 185*, 151-161. https://doi.org/10.1016/j.scienta.2015.01.031

Pasqualini E., Civolani S., Musacchi S., Ancarani V., Dondini L., Robert P., Baronio P. (2006). *Cacopsylla pyri* behaviour on new pear selections for host resistance programs. *Bulletin of Insectology, 59*, 27-37.

Pree D.J., Archibald K.W., Ker K.W., Cole K. J. (1990). Occurrence of Pyrethroid resistance in pear psylla (Homoptera: Psyllidae) populations in southern Ontario. *Journal of Economic Entomology, 83*, 2159-2163. https://doi.org/10.1093/jee/83.6.2159

Quarta R., Puggioni D. (1985). Survey on the variety susceptibility to pear Psylla. *Acta Horticulturae, 159*, 77-86. https://doi.org/10.17660/ActaHortic.1985.159.10

Radjabi Gh. (1989). I*nsects attacking rosaceous fruit trees in Iran: Homoptera.* Vol. 3. Plant Pests and Diseases Research Institute, Tehran, Iran, 256 pp.

SAS Institute Inc. (2004). *SAS/STAT user's guide*. Version 9.1 ed. SAS Institute, Cary, North Carolina, 5121 pp.

Shaltiel-Harpaz L., Soroker V., Kdoshim R., Hason R., Sakalsky T., Hatib K., Bar-Ya'akov I., Holland D. 2013. Two pear accessions evaluated for susceptibility to pear psylla Cacopsylla bidens (Šulc) in Israel. *Pest Management Science, 70*, 234-239. https://doi.org/10.1002/ps.3543

Sule S., Jenser G., Szita E. (2007). Management of pear decline caused by *Candidatus Phytoplasma pyri* in Hungary. *Bulletin of Insectology, 60,* 319-320.

Ullman D.E., McLean D.L. (1988). Feeding behavior of the winterform pear psylla, *Psylla pyricola* (Homoptera: Psyllidae), on reproductive and transitory host plants. Environmental *Entomology, 17*, 675-678. https://doi.org/10.1093/ee/17.4.675

Westigard P.H., Allen R.B., Gut L.J. (1981). Pear psylla: relationship of early-season nymph densities to honeydew-induced fruit damage on two pear cultivars. *Journal of Economic Entomology, 74*, 532-534. https://doi.org/10.1093/jee/74.5.532

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Performance of popcorn introductions for agronomic characters, grain yield and popping qualities in the forest and derived savannah agro-ecologies of Nigeria

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Performance of popcorn introductions for agronomic characters, grain yield and popping qualities in the forest and derived savannah agro-ecologies of Nigeria

Abstract: The study focus on the evaluation of popcorn lines for their yield and agronomic potentials. Genetic materials were evaluated under irrigation in a three-replicate in a Randomized Complete Block Design (RCBD) with a commercial variety as check. Two seeds were planted per hole using two-row plots of 5 m long with inter and intra-row spacing of 0.75 m x 0.5 m, respectively in two locations viz: Ibadan and Ikenne representing the forest and savannah agro-ecologies of Nigeria respectively. Genotypes (G) differed significantly ($\rho \leq 0.01$) for almost all the characters measured except for ear aspect. Location (L) as well as G x L interaction effects were also well pronounced on all agronomic characters measured except for days to silking and ears per plant. Popcorn 33-1-Y, large pearl shaped, Popcorn 40-Y and Popcorn 34-Y were high yielding with a potential of above 2.0 t ha^{-1}. These materials were found to be fairly resistant to major foliar diseases of the tropical humid ecologies. They are recommended for further evaluation across different agro-environments for possible propagation by popcorn farmers in Nigeria to boost production.

Keywords: grain yield; introductions; popcorn; popping; forest; derived; savannah; Nigeria

Predstavitev uspešnosti uvajanja pokovke na osnovi njenih agronomskih lastnosti, pridelka zrnja in kakovosti nabrekanja v gozdnih in prehodno-savanskih agroekosistemih Nigerije

Izvleček: Raziskava je bile osredotočena na ovrednotenje linij pokovke glede na njen pridelek in agronomske lastnosti. Genetski material je bil ovrednoten ob namakanju v naključnem bločnem poskusu s tremi ponovitvami v primerjavi s komercialno sorto. Po dve semeni sta bili posejani v vrstah na ploskvah dolžine 5 m, z medvrstno razdaljo 0,75 m in znotrajvrstno razdaljo 0,5 m, na lokacijah Ibadan in Ikenne, ki predstavljata gozdne in prehodno savanske agroekosisteme Nigerije. Genotipi (G) so se značilno razlikovali ($\rho \leq 0.01$) v skoraj vseh merjenih lastnosti z izjemo storža. Vplivi lokacije (L) kot tudi medsebojni vpliv lokacije in genotipa so se dobro izrazili v vseh merjenih agronomskih lastnostih z izjemo dni do lasenja in števila storžev na rastlino. Linije pokovke Popcorn 33-1-Y, Large Pearl Shaped', Popcorn 40-Y in Popcorn 34-Y so imele velik pridelek, s potencialom čez 2.0 t ha^{-1}. Te linije so bile tudi sorazmerno dobro odporne na večino listnih bolezni, značilnih za vlažne tropske razmere. Priporočamo jih za nadaljne ovrednotenje v različnih pridelovalnih okoljih za nadaljne razmnoževanje pri pridelovalcih pokovke v Nigeriji za povečanje njene pridelave.

Ključne besede: pridelek zrnja; introdukcija; pokovka; nabrekalne lastnosti; gozd; prehodna savana; Nigerija

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1 INTRODUCTION

Popcorn (*Zea mays* L. *everta*) is a special type of flint maize that has the ability to pop into consumable flakes when heated due to the thickness of the endosperm. The kernel pops upon heating as a result of the unique quality of the endosperm that makes it resist the steam pressure generated until it reaches explosive proportion (Acquaah, 2007). It is one of the most widely used groups of maize all over the world including in Nigeria (Mani and Dadari, 2003). Its demand has increased sharply from mid-seventies, stimulating production of the crop in some areas of the Nigeria's savannas and few other metropolitan cities (Iken, 1993). Popcorn varieties exist in various sizes and colour, it yields are lower, usually about half that of maize hybrids (Ziegler et al., 1984) which may be as a result of the small kernel size or its mass.

Popcorn growth requirements are similar to those for dent corn and the cultural practices for both breed of corn are the same except for some minor modifications in respect of popcorn. Ziegler (2001) noted that such modifications may have to do with timely planting due to the slow germination of popcorn and harvest maturity to enable optimum popping ability. Broccoli and Burak (2004) reported high significant differences ($\rho \leq 0.01$) for all characters evaluated except kernel density before expansion among fourteen (14) popcorn hybrids evaluated in two years across three environments. Genotype x Environment (G x E) interaction was also observed to be significant for the two main traits (yield and expansion volume) while the relationship between grain yield and expansion volume was negative. However, a strong and positive association was observed between expansion volume and kernel thickness, suggesting that this trait can be taken as a morphological variable which influence the expansion volume.

Oz and Kapar (2011) also evaluated 18 single cross popcorn hybrids and four commercial popcorn cultivars for yield and quality traits over three cropping seasons (2006, 2007 & 2008). They reported that the genotypes differed significantly ($\rho \leq 0.01$) for grain yield, plant height, tasseling time, grain moisture, % of unpopped kernel and popping volume. The study identified 10 single cross hybrids ('TCM-05-01', 'TCM-05-02', TCM-05-03, TCM-05-04, TCM-05-05, TCM-05-06, TCM-05-09, TCM-05-10, and TCM-05-12) with high grain yield and quality traits superior to those of their commercial cultivars. In their own study, Derera et al., (2014) investigated the popping ability of 119 F_1 hybrids and one standard check using two popping methods viz: microwave and hot air methods. Although the study revealed that popping quality does not depend on the method adopted for popping, the authors reported significant ($\rho \leq 0.01$) variability among the hybrids for popping quality traits (flake volume, popping fold, unpopped kernels and grain moisture) which indicated prospect for selection of hybrids with good popping ability. They further reported preponderance of additive gene action for all popping quality traits, thereby creating an opportunity to effectively improve these traits through selection.

The initial step in achieving improvement in popcorn yield and quality attributes is to evaluate the existing germplasm for their agronomic performances and yield potentials as well as popping attributes. The objectives of this study therefore, were to: (i) assess 19 popcorn lines for yield, phenotypic attributes, reaction to prevailing pests and diseases and (ii) identify superior lines that could be used for the development of commercial hybrids for the popcorn industry, thus enhancing popcorn productivity.

2 MATERIALS AND METHODS

The experimental materials which comprised nineteen (19) popcorn lines and a commercial variety as check (Table 1) were evaluated under irrigation using a Randomized Complete Block Design (RCBD). The plot size was two (2) rows, 5 m long with inter and intra-row spacing of 0.75 x 0.5 m, respectively, replicated three (3) times. The trials were established using two (2) seeds per hole at two (2) locations viz: Ibadan (Longitude $7^0$22'N Latitude $3^0$50'E) and Ikenne (Longitude $6^0$53'N Latitude $3^0$42'E). Fertilizer application was carried out 3 weeks after planting (WAP) at the dosage rate of 180 kg ha^{-1} of NPK 20-10-10 and was top-dressed with 100 kg ha^{-1} of urea 2 weeks before anthesis. Weed control was done chemically with the application of 5 l ha^{-1} pre-emergence herbicides (5 grams per litre Metolachlor and 170 grams per litre Atrazine a.i). Two supplementary hoe-weedings at 6 and 10 WAP, respectively were carried out to keep the field clean of weeds. Agronomic data and disease rating (under natural infestation) were recorded on each line from each replication. Data on days to tasseling and silking; plant and ear aspects as well as husk cover using 1-5 rating where 1 = excellent, 2 = very good, 3 = good, 4 = fair and 5 = poor, were collected on whole plot basis. Plant and ear heights (cm) were based on the mean of measurements collected from five random plants in a plot while grain yield was estimated from cob mass/plot (kg) and later converted to t ha^{-1} after adjusting to 12 % moisture content. Diseases scored included streak, rust, blight, curvularia and infestation of armyworm insects, using a rating of 1-5, where 1 = 1-10 % infection, 2 = 11-29 % infection, 3 = 30-45 % infection, 4 = 46-60 % infection and 5 = 61 % and above infection respectively. Data

Table 1: List of popcorn lines and their source

S/N	Materials	Grain colour	Source
1.	Small Pearl Shaped	Yellow	IAR&T
2.	Popcorn 2-S0	Yellow	IAR&T
3.	Popcorn 34-Y	Yellow	IAR&T
4.	Popcorn 37-Y	Yellow	IAR&T
5.	Popcorn 20-Y	Yellow	IAR&T
6.	Popcorn 44-Y	Yellow	IAR&T
7.	Popcorn 32-Y	Yellow	IAR&T
8.	Large Pearl Shaped	Yellow	IAR&T
9.	Popcorn 9-Y	Yellow	IAR&T
10.	Popcorn 66-Y	Yellow	IAR&T
11.	Popcorn 33-1-Y	Yellow	IAR&T
12.	Popcorn 40-Y	Yellow	IAR&T
13.	Popcorn 52-Y	Yellow	IAR&T
14.	Popcorn 36-Y	Yellow	IAR&T
15.	Popcorn 4-Y	Yellow	IAR&T
16.	Popcorn 3-Y	Yellow	IAR&T
17.	Popcorn-18-Y	Yellow	IAR&T
18.	Popcorn-6-Y	Yellow	IAR&T
19.	Eruwa Local (Check)	Yellow	IAR&T

were also collected on descriptive attributes including the colour of various plant parts such as stem, leaf, mid-rib, leaf blade, anther and silk. Others were broadness of leaves, leaf orientation and nature of anthesis. Palatability and general acceptability ratings were carried out on a scale of 1-5 where 1 = excellent, 2 = very good, 3 = good, 4 = fair and 5 = poor.

Data collected on quantitative characters were subjected to analyses of variance (ANOVA) using SAS version 9.0. Means of attributes for which popcorn lines differ significantly were separated using least significant difference (LSD) as outlined by Steel and Torrie (1980).

The format of Analysis of variance model used can be expressed as follows:

$Yij = \mu + Ej + Gi + GEij + Eijk$

Where:

μ = population mean

E*j* = effect of the ith environment

G*i* = effect of the jth genotype

GE*ij* = interaction effect of ith environment and jth treatment

E*ijk* = error term

3 RESULTS AND DISCUSSION

The results of the mean squares from the combined analyses of variance (ANOVA) for agronomic characters in the 19 popcorn lines showed that expression of almost all the characters differed significantly ($\rho \leq 0.01$) from one location (L) to the other except days to silking and ears/plant while the genotype (G) differed significantly ($\rho \leq 0.01$) for all the characters except ear aspect and cob length (Table 2). Genotype x Location (GL) interaction on the other hand had a pronounced effect ($\rho \leq 0.01$) on all the morphological characters measured except cob length, with location showing a greater magnitude of variability in terms of larger mean squares for most of the characters. These results revealed a wide range of variability among the popcorn lines with respect to most of the agronomic traits measured, indicating the possibility of exploiting the existing variation among the lines for improving popcorn population for enhanced yield and other agronomic attributes through selection. The significant G x L interaction for grain yield and some other agronomic traits indicates differences in genotypic performance for these traits from one location to another which may be attributed to differences in environmental conditions of the testing sites. This underscores the necessity for evaluating new genetic materials across a number of environments where they are intended for cultivation to determine more accurately, the genetic potentials under varying environmental conditions and to detect stability and adaptation for proposed ecologies of cultivation.

The significant differences observed among the lines for popping volume indicate the existence of adequate genetic variability for this trait which could be exploited in the development of hybrid varieties for the popcorn industry. In an earlier study, Oz and Kapar (2011) also reported significant difference in popping volume among popcorn genotypes evaluated for three years which is similar to results obtained in this study. In other words, the presence of genetic variability among these lines will give way for effective selection programme to enhance improvement for high popping quality.

Although the results of the mean squares from combined ANOVA for insect and diseases rating showed that the popcorn lines did not differ significantly for diseases and insect rating, however, the effect of prevailing environmental conditions in the two locations was significant ($\rho \leq 0.01$) for all the parameters except leaf blight and *Curvularia* leaf spot. G x L effect was also significant for streak ($\rho \leq 0.05$). The mean values across the locations revealed that the popcorn lines were generally resistant to leaf blight and *Curvularia.* (Table 3).

Means for agronomic characters of the popcorn lines showed that small pearl shaped was the earliest to mature, recording about 54 days to attain silking while Popcorn 52-Y was the latest to attain maturity, recording 66 days to silking (Table 4). Two other lines (Popcorn 66-Y, Popcorn 4-Y) were also early maturing while large pearl

Table 2: Mean squares from combined ANOVA for agronomic characters, grain yield and yield components in 19 popcorn lines

Source of variation	df	Days to silking	Ear height	Husk cover	Ear aspect	Ears/Plant	Cob length	Cob width	No. of row/cob	Grain yield
Location (L)	1	50.67	110548.25**	54.75**	39.38**	0.00079	251.43**	11.12**	120.08**	3.60**
Rep (Env)	4	66.51	161.86	1.11	0.17	0.02	6.85	0.30	0.12	0.04
Genotype (G)	18	68.49**	460.88**	1.47**	0.83	0.05**	7.63	0.76**	4.73**	1.52**
G x L	18	37.76**	285.14**	1.62**	1.60**	0.05**	6.76	0.78**	5.54**	0.26**
Pooled Error	72	13.68	62.38	0.49	0.54	0.01	5.50	0.34	2.69	0.08

*, ** Significantly different at 0.05 and 0.01 levels of probability respectively

shaped, Popcorn 6-Y and Popcorn 52-Y were the latest to attaining maturity. Popcorn 36-Y was the tallest while Popcorn 6-Y was the shortest. Popcorn 33-1-Y was the highest yielding among the lines across the two locations followed by 'Large Pearl Shaped, Popcorn 40-Y and Popcorn 34-Y, while the poorest for grain yield was 'Eruwa'

Table 3: Mean performance (across location) for insect and disease rating of 19 popcorn lines and check

Popcorn lines	Streak (1-5)	Rust (1-5)	Blight (1-5)	*Curvularia* (1-5)	Armyworm (1-5)
Popcorn 44-Y	2.33	1.67	1.00	1.00	1.33
Popcorn 18-Y	3.17	2.00	1.00	1.00	1.00
Popcorn 9-Y	2.50	2.00	1.00	1.00	1.00
Popcorn 34-Y	1.83	1.83	1.00	1.00	1.17
Popcorn 4-Y	2.83	1.83	1.00	1.00	1.00
Popcorn 66-Y	2.17	1.50	1.00	1.00	1.33
Small Pearl Shaped	2.67	1.83	1.00	1.00	1.17
Popcorn 40-Y	2.83	1.67	1.00	1.00	1.17
Popcorn 20-Y	1.83	1.83	1.33	1.17	1.17
Large Pearl Shaped	2.83	2.17	1.00	1.00	1.00
Popcorn 2-So	2.33	1.83	1.00	1.00	1.33
Popcorn 3-Y	2.83	1.83	1.00	1.00	1.00
Popcorn 32-Y	2.67	1.67	1.00	1.00	1.00
Popcorn 37-Y	2.33	1.83	1.00	1.00	1.17
Popcorn 33-1-Y	2.67	1.50	1.00	1.00	1.17
Popcorn 6-Y	2.00	1.50	1.00	1.00	1.00
Popcorn 52-Y	2.83	2.00	1.00	1.00	1.17
Popcorn 36-Y	2.17	2.00	1.00	1.00	1.17
Eruwa local (check)	3.00	1.83	1.00	1.00	1.00
Mean	2.57	1.81	1.02	1.03	1.12
F-Test					
Location (L)	112.01**	48.04**	0.04	0.08	1.72**
Genotype (G)	1.12	0.21	0.04	0.04	0.09
G x L	1.29*	0.33	0.04	0.04	0.09
Pooled Error	0.67	0.26	0.04	0.04	0.12
CV%	31.77	28.44	18.41	19.95	31.09
LSDα0.05	0.96	0.59	0.22	0.24	0.40

*, ** Significantly different at 0.05 and 0.01 levels of probability respectively

Table 4: Mean Performance for agronomic characters, grain yield and yield components in 19 Popcorn lines and check

Popcorn Lines	Days to 50 % silking	Ear height (cm)	Husk cover (1-5)	Ear aspect (1-5)	Ears/plant (no)	Cob Length (cm)	Cob Width (cm)	No. of rows/ cob (no)	Grain yield (t ha^{-1})
Popcorn 44-Y	62	86	3	3	1	14	7	13	1.50
Popcorn 18-Y	60	76	3	4	1	16	7	12	1.27
Popcorn 9-Y	62	90	3	3	1	14	8	14	1.57
Popcorn 34-Y	57	101	3	3	1	16	7	13	2.15
Popcorn 4-Y	55	87	3	3	1	15	7	13	1.55
Popcorn 66-Y	54	78	2	3	1	18	8	13	1.85
Small Pearl Shaped	54	73	4	3	1	14	7	13	1.05
Popcorn 40-Y	58	95	2	2	1	14	7	12	2.36
Popcorn 20-Y	61	80	3	3	1	14	7	15	1.53
Large Pearl Shaped	63	84	2	3	1	17	7	13	2.48
Popcorn 2-So	55	90	2	3	1	15	8	14	1.18
Popcorn 3-Y	58	80	2	4	1	14	8	13	1.77
Popcorn 32-Y	58	83	3	4	1	15	8	15	1.16
Popcorn 37-Y	56	82	3	4	1	15	7	13	1.05
Popcorn 33-1-Y	58	101	3	3	1	17	8	14	2.61
Popcorn 6-Y	63	75	3	3	1	15	8	13	1.13
Popcorn 52-Y	66	85	4	3	1	15	7	15	1.46
Popcorn 36-Y	59	102	3	3	1	14	7	13	1.91
Eruwa local (check)	60	89	3	4	1	14	7	14	1.01
Mean	58.70	86.16	2.69	3.20	3.20	15.07	7.42	13.33	1.61
CV %	6.30	9.17	26.09	22.88	22.58	15.56	7.80	12.30	17.04
LSD α 0.05	4.48	9.14	0.82	0.83	0.12	2.71	0.66	1.86	0.31

local (check). The relatively low grain yield recorded for small pearl shaped, Popcorn lines 66-Y, 4-Y, 2-S_0 and '37-Y' may not be unconnected with earliness in maturity. However, line '66-Y' combined earliness with average grain yield indicating that this line is a promising candidate for further testing or as parent in a hybridization programme.

Popcorn 33-1-Y gave the highest yield with a yield advantage of 38.7 % over the reference check ('Eruwa' local) with mean yield of 1.01 t ha^{-1}, but with the lowest popping volume of 866.7 cm$^{3.}$ On the other hand, the lowest yielding line ('Eruwa' local) had a popping volume of 2590 cm^3, ranking 4th among the 19 popcorn lines evaluated for popping potential. This suggests that popping expansion is inversely proportional to grain yield, which corroborates the findings of several authors who reported a negative association between grain yield of popcorn and popping volume (Dofing et al., 1991; Burak and Broccoli, 2001; Vijayabharathi et al., 2009). The implication of this is that the two important traits will be difficult to improve simultaneously. The use of molecular breeding approach might therefore be the best option in this situation, where the QTLs for popping expansion and grain yield can be mapped, followed by selection for yield while DNA markers can be used to retain favourable alleles for popping expansion.

Hussain et al., (2010) described grain yield as a combined outcome of the inherent genetic potential and the interaction of genotype with the environment, therefore interactive means of G x L effect for grain yield and ears per plant are presented in Table 5. The lines changed rank for grain yield from one location to the other with Popcorn 36-Y, Popcorn 18-Y, Popcorn 34-Y and 'Large Pearl Shaped' being largely responsible for the significant G x L effect for this trait. This shows that grain yield was found to be genetically diverse as a result of its significant differences when character means were combined across the two environments. Most of the lines performed better in Ibadan compared to Ikenne with respect to grain yield except for three lines (Popcorn 9-Y, Popcorn 4-Y and Popcorn 6-Y). However, Popcorn lines 9-Y, 6-Y, 40-Y and 37-Y exhibiting similar performance in terms

Table 5: Interactive effect of Location by Genotype for grain yield and ears/plant

Grain Yield					Ears/plant			
Popcorn lines	Ibadan	Ikenne	∑RSI	Rank	Ibadan	Ikenne	∑RSI	Rank
Popcorn 44-Y	1.70(9)	1.31(11)	20	9th	1.11(6)	1.06(2)	8	1st
Popcorn 18-Y	1.60(11)	0.94(17)	28	12th	1.11(7)	1.00(4)	11	2nd
Popcorn 9-Y	1.57(12)	1.57(8)	20	9th	0.75(18)	1.00(6)	24	12th
Popcorn 34-Y	2.60(3)	1.70(4)	7	4th	1.08(9)	1.00(7)	16	8th
Popcorn 4-Y	1.47(13)	1.63(7)	20	8th	1.12(5)	1.00(8)	13	5th
Popcorn 66-Y	1.95(6)	1.74(3)	9	5th	1.23(2)	1.00(9)	11	2nd
Small Pearl Shaped	1.19(16)	0.91(18)	34	16th	0.91(14)	1.07(1)	15	7th
Popcorn 40-Y	2.42(4)	2.31(2)	6	2nd	1.14(4)	1.00(10)	14	6th
Popcorn 20-Y	1.71(8)	1.34(10)	18	8th	0.96(13)	0.93(19)	32	
Large Pearl Shaped	3.31(1)	1.64(5)	6	2nd	0.90(15)	1.00(11)	26	13th
Popcorn 2-So	1.25(14)	1.11(14)	28	12th	0.68(19)	1.00(12)	31	17th
Popcorn 3-Y	1.89(7)	1.64(6)	13	6th	0.82(16)	1.00(13)	29	15th
Popcorn 32-Y	1.23(15)	1.09(15)	30	14th	1.15(3)	1.00(14)	17	9th
Popcorn 37-Y	1.10(19)	1.00(16)	35	17th	0.78(17)	1.00(15)	32	18th
Popcorn 33-1-Y	2.76(2)	2.46(1)	3	1st	1.37(1)	1.00(16)	17	9th
Popcorn 6-Y	1.12(18)	1.13(13)	31	15th	1.09(8)	1.06(3)	11	2nd
Popcorn 52-Y	1.64(10)	1.28(12)	22	11th	0.99(11)	0.97(18)	29	15th
Popcorn 36-Y	2.27(5)	1.55(9)	14	7th	1.04(10)	1.00(17)	27	14th
Eruwa local (check)	1.17(17)	0.84(19)	36	18th	0.96(12)	1.00(5)	17	9th
LSD α 0.05		0.10				0.04		

*, ** Significantly different at 0.05 and 0.01 levels of probability, respectively

of grain yield at the two locations can be considered to be relatively stable across these locations compared to other popcorn lines. This superiority of performance may be attributed to efficient irrigation system enjoyed by the plants in Ibadan compared to Ikenne during the evaluation exercise. Similarly, occurrence of streak was very predominant in Ikenne compared to Ibadan, which also might have contributed to the low grain yield recorded in Ikenne. Moreover, Ikenne is a hot spot for maize foliar diseases because of its hot humid nature which favours sporulation and accumulation of different kinds of disease inoculums. Similarly, streak transmitting vectors (*Cicadulina* spp.) is prevalent in Ikenne thereby lending itself as screening site by many maize scientists. Lukuyu et al., (2002) in their study, reported the negative impact of maize streak virus on grain yield and seed quality of maize. In an earlier report, Bosque-Perez et al., (1998) studying the effect of maize streak virus disease on the growth and yield of maize reported that varieties differed significantly in the amount of loss, disease severity and incidence, which can also be related to this study. G x L effect for ears per plant showed that Popcorn 33-1-Y, Popcorn 32-Y, 'Small Pearl', Popcorn 9-Y, Popcorn 66-Y and Popcorn 40-Y were responsible for the significant interaction between genotype and location, due to the wide range observed in their ranking between the two locations.

The popcorn lines differed significantly ($\rho \leq 0.01$) with respect to general acceptability, popping volume and expansion ratio but not for 100-grain mass, taste and flavor (Table 6). Mean performance for popping characters and acceptability ratings revealed that small pearl shaped had the largest popping expansion followed by Popcorn 20-Y while the lowest popping volume of 73.3 cm^3 was recorded for Popcorn 3-Y. Similarly, the largest expansion ratio was recorded for small pearl shaped followed by Popcorn 20-Y while Popcorn 3-Y gave the smallest expansion ratio. Popcorn 18-Y was rated best for taste among the 19 popcorn lines while Popcorn 3-Y was the least. Popcorn 4-Y and Popcorn 36-Y were the best for flavor while Popcorn 32-Y was the poorest. Similarly, Popcorn 9-Y and Popcorn 3-Y. Popcorn 44-Y, Popcorn

Table 6: Mean performance for popping ability and consumer acceptability ratings of 19 popcorn lines

Popcorn lines	100-grain mass (kg)	Popping Volume (cm^3)	Expansion Ratio ($cm^3 kg^{-1}$)	Taste (1-5)	Flavour (1-5)	General acceptability
Popcorn 44-Y	0.01	1340.0	10308	2	2	2
Popcorn 18-Y	0.02	1190.0	9154	1	2	2
Popcorn 9-Y	0.02	930.0	7154	2	3	3
Popcorn 34-Y	0.01	1516.7	11667	2	2	2
Popcorn 4-Y	0.01	2766.7	21282	2	2	2
Popcorn 66-Y	0.02	2040.0	15692	2	3	3
Small Pearl Shaped	0.01	3080.0	23692	3	2	3
Popcorn 40-Y	0.02	836.7	6436	2	3	3
Popcorn 20-Y	0.01	2830.0	21769	2	2	2
Large Pearl Shaped	0.02	14.96.7	11513	2	2	2
Popcorn 2-So	0.01	2370.0	18231	2	3	3
Popcorn 3-Y	0.02	73.3	564	3	3	3
Popcorn 32-Y	0.01	1670.0	12846	3	3	3
Popcorn 37-Y	0.01	1836.7	14128	2	3	2
Popcorn 33-1-Y	0.02	866.7	6667	3	3	3
Popcorn 6-Y	0.01	1776.7	13667	2	3	3
Popcorn 52-Y	0.01	2546.7	19590	2	2	2
Popcorn 36-Y	0.01	1526.7	11744	2	2	2
Eruwa local (check)	0.02	2590.0	19923	3	3	3
Mean	0.02	1751.75	13475.03	2.32	2.54	2.56
F-Test						
Genotype (G)	$0.13x10^{-4}$	1923419.88**	113811827**	0.39	0.34	0.45*
Error	$0.81x10^{-5}$	31813.74	1882470	0.22	0.24	0.24
CV%	19.60	10.18	10.18	20.31	19.33	18.96
LSDα0.05	0.005	295.36	2272	0.78	0.81	0.80

*, ** Significantly different at 0.05 and 0.01 levels of probability, respectively

34-Y and Popcorn 4-Y were rated to be the best for general acceptability while Popcorn 32-Y was rated to be the least acceptable popcorn lines.

4 CONCLUSION

Five of the popcorn lines (small pearl shaped, Popcorn 66-Y, Popcorn 4-Y, Popcorn 2-S_0 and Popcorn 37-Y) were early maturing and so could be utilized to develop source population for inbred line extraction for the formation of early maturing popcorn hybrid. Four other lines (Popcorn 33-1-Y, large pearl shaped, Popcorn 40-Y and Popcorn 34-Y) expressed high yield potential (2.0 t ha^{-1}) while another seven ('Small Pearl', 'Eruwa' local, Popcorn 4-Y, Popcorn 66-Y, Popcorn 20-Y, Popcorn 2-S_0 and Popcorn 52-Y) expressed large popping volume of over 2000 cm^3. These materials therefore look promising as parents for the development of future commercial hybrid popcorn for tropical and sub-tropical agro-ecology.

5 REFERENCES

Acquaah, G. (2007). *Principles of Plant Genetics and Breeding*. UK: BLACKWELL.

Ashman, R.B. (1991). Registration of three popcorn (maize) parental lines HP62-02, HP72-11 and Hp 68- 07. *Crop Science, 315*, 1402-1403. https://doi.org/10.2135/cropsci1991.0011183X003100050100x

Bosque-Perez, N., Olojede, S. & Buddenhagen, I. (1998). Effect of Maize Streak Virus disease on the growth and yield of maize as influenced by varietal resistance levels and plant stage at time of challenge. *Euphytica, 101*, 307. https://doi.org/10.1023/A:1018345921770

Broccoli, A.M. & Burak, R. (2004). Effects of genotype × environment interactions in popcorn maize yield and grain quality. *Spanish Journal of Agricultural Research, 2*, 85-91. https://doi.org/10.5424/sjar/2004021-64

Derera, J., Phumelele, J. & Muthulisi, S. (2014). Assessment of popping ability of new tropical popcorn hybrids. *Australian Journal of Crop Science, 8*, 831-839.

Gökmen, S. (2004). Effects of moisture content and popping method on popping characteristics of popcorn. *Journal of Food Engineering, 65*, 357-362. https://doi.org/10.1016/j.jfoodeng.2004.01.034

Hussain, N., Qamar-ur-Zaman, M., Amjad, N. & Abdul, A. (2010). Response of maize varieties under agro-ecological conditions of deraismail khan. *Journal of Agricultural Research, 48*(1), 59-63.

Iken, J.E. (1993). Popcorn Production and Utilization. In Fakorede, M.A., Alofe, O.O. and Kim, S.K. (eds). *Maize Improvement Production and Utilization in Nigeria*.

Lukuyu, B.A., Murdoch, A.J., McLeod, A. & Doward, P. (2002). *The impact of Maize streak virus disease on quality and yield of maize forage outputs of the maize-dairy production systems in central highlands of Kenya*. Output of Research project R7955; Livestock Production Programme.

Mani, H. & Dadari, S.A. (2003). Growth and yield analysis of irrigated popcorn (*Zea mays everta*) grown in Kadawa as affected by sowing date and intra-row spacing using correlation co-efficient. ASSET - Series A: *Agriculture & Environment, 3*, 63-70.

Obajimi, A.O., Adenle, V. & Salami, A. (1996). Incidence, distribution and control of maize downy mildew in South Western Nigeria. In: *1998 Report of the in-house review of the Inst. of Agric. Res. and Training, Ibadan. Nigeria*. Pp 39-42.

Olakojo, S.A. & Iken, J.E. (2002). Development of popcorn varieties for high grain yield and adaptation. ASSET - Series A: *Agriculture & Environment, 2*, 63-68.

Olakojo, O.O. (2016). Genotypic and agronomic performance of drought tolerant yellow quality protein maize hybrid maize (*Zea mays* L.) in savanna agro-ecologies of south-western Nigeria. *Nigerian Journal of Genetics, 31*, 54-64.

Omidiji, M.O., Kogbe J.O.S., Shoyinka, S.A., Olunuga, B.A., Salawu, R.A. & Somuji, O. (1992). *Striga* on maize in Ogun State Nigeria. *Maize newsletter.* Pp. 1-2

Phumelele, J. (2012). *Genetic analysis of agronomic and quality trait in popcorn hybrids: Development of popcorn breeding*. M.Sc thesis, University Of KwaZulu-Natal, Pietermaritzburg, South Africa.

Rangel, R.M., Amaral Júnior do, A.T., Scapim, C.A., Freitas Júnior, S.P. & Pereira, M.G. (2008). Genetic parameters in parents and hybrids of circulant diallel in popcorn. *Genetics and Molecular Research, 7*, 1020-1030. https://doi.org/10.4238/vol7-4gmr502

Sakın, M.A., Gokmen, S., Yildirim, A., Belen, S. & Kandemır, N. (2005). Effects of cultivar type on yield and quality of popcorn (*Zea mays* everta). *New Zealand Journal of Crop and Horticultural Science, 33*, 17-23. https://doi.org/10.1080/01140671.2005.9514326

Steel, R.G.D. & Torrie, J.H. (1980). P*rinciple and procedures of statistics,* New York: McGraw-Hill Books Co.

Ziegler, K.E., Ashman, R.B. White, G.M. & Wysong, D.B. (1984). *Popcorn Production and Marketing. Cooperative Extension,* Purdue University. USA.

Ziegler, K.E. (2001). Popcorn. In: Hallauer, A.R., (editor) *Specialty Corns*. CRC Press, USA. pp. 199-234. https://doi.org/10.1201/9781420038569.ch7

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The effect of a new non-toxic water-soluble selenorganic substance on antioxidant protection and development of seedlings of oilseed radish (*Raphanus sativus* L. var. *oleiferus* Metzg.)

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The effect of a new non-toxic water-soluble selenorganic substance on antioxidant protection and development of seedlings of oilseed radish (*Raphanus sativus* L. var. *oleiferus* Metzg.)

Abstract: The effect of 2,6-dipyridinium selenabicyclo[3.3.1]nonandibromide (996 zh) on the level of lipid peroxidation (LPO), on the activity of glutathione reductase (GR) and on the morphometric parameters of oilseed radish seedlings under normal conditions and under stress (200 mmol NaCl) has been studied. It has been established that the substance 996 zh at a concentration of 100 μm exerted an antioxidant effect reducing the level of lipid peroxidation and increasing the activity of GR. In connection with that the germinating ability of seeds and the biomass of the roots and stems of seedlings increased, both under normal conditions and under stress conditions.

The concentration of the substance 996 zh of 1000 μmol had a toxic effect, increasing the LPO level in normal conditions, but neutralized the effect of stress due to the addition of NaCl. This concentration had a slight inhibitory effect on germinability and on root development in seedlings. However, the same concentration of the substance 996 zh (1000 μmol) had a positive effect on the development of shoots under both normal and stressed conditions.

Key words: selenium compounds; oilseed radish; glutathione reductase; lipid peroxidation

Učinek nove nestrupene vodotopne organske spojine selena na antioksidacijsko zaščito in razvoj sejank oljne redkve (*Raphanus sativus* L. var. *oleiferus* Metzg.)

Izvleček: V raziskavi je bil preučevan učinek 2,6-dipiridinium selenabiciklo[3.3.1]nonandibromida (996 zh) na peroksidacijo lipidov (LPO), aktivnost glutation reduktaze (GR) in morfometrične parametre sejank oljne redkve v normalnih razmerah in pod slanostnim stresom (200 mmol NaCl). Ugotovljeno je bilo, da je spojina 996 zh pri koncentraciji 100 μmol pokazala antioksidacijski učinek, kar je zmanjšalo peroksidacijo lipidov in in povečalo GR. V povezavi s sposobnostjo kalitve semen sta se biomasi korenin in stebel povečali, tako v normalnih kot v stresnih razmerah. Koncentracija 996 zh 1000 μmol je imela toksičen učinek, povečala je LPO v normalnih razmerah, a nevtralizirala učinek stresa po dodatku NaCl. Ta koncentracija je imela rahel zavirani učinek na kalitev in razvoj korenin sejank. Kakorkoli, ista koncentracija spojine 996 zh (1000 μmol) je imela pozitivni učinek na razvoj poganjkov v normalnih in stresnih razmerah.

Ključne besede: spojine selena; oljna redkev; glutation reduktaza; peroksidacija lipidov

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1 INTRODUCTION

Eastern Siberia covers the territory of the Central and Northern Taiga of Russia. Features of crop production in this region are dependent on harsh climatic conditions. Severe winter is a problem because freezing the soil and covering it with ice cause low oxygen conditions. Despite this, crop production in this region is developing and playing a big role in the life of the region and the country as a whole (Surinet al., 2018).

Due to the fact that the climate in this region is continental and sharply continental, the main focus of agronomy production is on spring crops such as wheat, rye, barley and oats. In addition to these main crops, cruciferous field crops are now widespread. Oilseed radish (*Raphanus sativus* L. var. *oleiferus* Metzg.) is promising for outspread due to its cold resistance, short growing season and accumulation of large yields of high-protein green mass.It is grown as a fodder, green manure, oilbearing and strip culture. To more fully use the potential of the culture, the efficiency of cultivation and the expansion of its distribution areas, it becomes necessary to develop its cultivation technology and identify the most important biological features (Dorofeevet al., 2013).

In the course of growth and development, oilseed radish, like many Siberian plants, is exposed to various stressors, the most common of which are agro-climatic conditions like drought, cold, salinisation and contamination with heavy metals. As a result of exposure to these unfavorable environmental factors in plant cells, the production of reactive oxygen species (ROS) increases, which at high concentrations have a negative impact on all vital processes in the cell (Gill &Tuteja, 2010; Gill et al., 2013). To prevent negative effects on the cells of crops, science has developed and recommended use of a large number of complex and highly targeted substances, including ones containing selenium (Alfthanet al., 2014).

Most of the currently used selenium substances are based on inorganic selenium compounds, their complexes, selenium-containing amino acids and their derivatives due to the availability of these compounds. However, these substances have high toxicity and can have a long-term negative impact on the environment when released into soils and water bodies (Škrabanja, 2017).This fact does not allow them to be used effectively enough both as adaptogens in animal breeding and agriculture, and as preventive substances in regions characterized by lack of selenium (for example, as food additives to products of mass consumption, in the composition of multivitamin and mineral complexes).The development of available methods for the synthesis of organoselenium compounds led to a shift from the use of non-effective selenium compounds to the use of low-toxic organoselenium substances (Nogueiraet al., 2004). Earlier, we first proposed and successfully implemented the use of selenium dihalides compounds in the synthesis of selenorganic compounds (Potapov & Amosova, 2003). Introduction of new selenium-containing electrophilic reagents – selenium dichloride and selenium dibromide – to the organic synthesis has significantly expanded the possibilities of obtaining new organic selenium compounds (Potapovet al., 2016). Based on this approach, we obtained previously unknown 2,6-dipyridinium selenabicyclo[3.3.1]nonandibromide (996 zh). The composition and structure of the new compound are unambiguously proved by NMR methods on 1H and 13C nuclei and are confirmed by the elemental analysis data. At the next stage of work, it was necessary to check the biological activity of the obtained substance 996 zh.

Currently, it is shown that selenium plays a big role in protecting cells from ROS because it is a part of the enzymes of the glutathione antioxidant system. Thus, the purpose of our research was to study the effect of different concentrations of 2,6-dipyridinium selenabicyclo[3.3.1]nonan 2,6-dipyridyl selelenabicyclo[3.3.1] nonandibromide (996zh) on the antioxidant protection (GR, LPO) and morphometric indicators of oilseed radish sprouts both under normal and stressed conditions.

2 MATERIALS AND METHODS

2.1 SYNTHESIS

^{1}H (400.1 MHz), ^{13}C (100.6 MHz) NMR spectra were recorded on a Bruker DPX-400 spectrometer in 1–10% solution in D_2O, referenced to HMDS (^{1}H and ^{13}C NMR, internal).

A solution of pyridine (2 g, 25 mmol) in 5 ml of acetonitrile was added dropwise to a solution of 2,6-dibrom-9-selenabicyclo[3.3.1]nonan (3.48 g, 10 mmol) (Accurso et al., 2011) in 25 ml of acetonitrile. The reaction mixture was stirred for 6 hours at room temperature. The solvent was removed on a rotary evaporator, the residue was washed with CCl_4 (3 x 5 ml), dried in vacuum to constant mass. Product Compound 996 zh (4.97 g, 98 % yield), colorless crystals, purity > 98 %. ^{1}H NMR (400 MHz, D_2O) δ 2.34 – 2.41 (m, 2H, SeCHCH2), 2.54 – 2.59 (m, 4H, BrCHCH), 3.07 – 3.17 (m, 2H, BrCHCH), 3.41 – 3.44 (m, 2H, SeCH), 5.83 – 5.89 (m, 2H, BrCH), 8.09 (t, 4H, *m*-H_{pyr}), 8.54 (t, 2H, *p*-H_{pyr}), 9.02 (d, 4H, *o*-H_{pyr}). 13C NMR (100 MHz, D_2O) δ 25.57, 28.19, 29.37, 74.60, 128.68, 143.15, 146.46. Found: C, 42.84; H, 4.41; Br, 31.72; N, 5.54; Se, 15.49. Calc. for $C_{18}H_{22}Br_2N_2Se$: C, 42.80; H, 4.39; Br, 31.64; N, 5.55; Se 15.63.

2.2 PLANT MATERIAL

Studies were carried out in laboratory conditions on oilseed radish seeds (*Raphanus sativus* L. var. *oleiferus* Metzg.) of lines of Irkutsk State Agricultural Academy, with laboratory germinability of 80-98 %, weighing 1,000 seeds 9.5 g. Seeds were germinated on wet filter paper in Petri dishes at a constant temperature of 23 °C, in the dark, for 4 days, wetting them with the test solutions. The number of seeds in one cup was 30 pcs. The experiment was repeated 3 times.

2.3 EVALUATION OF GERMINABILITY AND MASS OF SEEDLINGS

Germinability was analyzed according to the All-Union State Standard 10-14-86 "Oilseed Radish Seeds. Varietal and sowing qualities". These indicators were determined in accordance with All-Union State Standard 12038-84 "Seeds of agricultural crops. Methods for determining germinability" (Dorofeev et al., 2013). The mass of shoots and roots was determined using the gravimetric analysis.

2.4 DETERMINATION OF PROTEIN CONTENT

Protein contentwas determined by the degree of binding to the Coomassie blue dye (CBB G250 "Sigma") according to the Bradford method (Bradford, 1976).

2.5 DETERMINATIONS OF GLUTATHIONE REDUCTASE ACTIVITY

Glutathione reductase activity (EC 1.6.4.2) was measured according to the method described by Nigmatullina et al. (2014). The activity of glutathione reductase was determined by the change in absorption at 340 nm, caused by the oxidation of NADPH in 3.5 min with an interval of 1 s on the spectrophotometer. The enzyme activity was calculated using the extinction coefficient for NADP+ at a wavelength of 340 nm, equal to 6.22 $mmol^{-1}$ cm^{-1}.

2.6 EVALUTION OF DIENE CONJUGATES

Analysis of the content of the primary products of lipid peroxidation – diene conjugates (DC) –was carried out according to the method (Placer, 1968) in our modification. The measurement was performed on a spectrophotometer at a wavelength of 203 nm. The obtained optical density (D) was used to calculate the concentration of diene conjugates (recalculated per 1 g wet mass) using an extinction coefficient equal to 2.2×10^5 mol^{-1} cm^{-1}.

Salinisation was chosen as a stress, which was created with NaCl, a concentration of 200 mmol was taken from literature data (Ahmad et al., 2015). This concentration causes stress, since it significantly increases the level of lipid peroxidation by almost two times compared with the control (Table 1).

2.7 STATISTICS

The data are presented as arithmetic mean values of quantities and their standard deviations, which were obtained in three independent experiments, calculated using Microsoft Excel. The statistical significance of the differences of the compared average values was evaluated using the Mann-Whitney U-test.

3 RESULTS AND DISCUSSION

The effect of the new selenium-containing substance 996 zh on the morphometric and biochemical parameters of oil radish under normal conditions and in salt conditions was studied. The objective of the work was to identify which concentrations of the substance exhibit antioxidant activity. This was done using various concentrations (1000, 500, 200, 100, 50, 10 μmol). Antioxidants are mainly used for organisms under stress (Alfthan et al., 2014). Therefore, the work of substance 996 zh was tested both under normal and stressful conditions, which allowed us to more fully trace the antioxidant effect of substance 996 zh. Based on the results of the work, 1000 μmol and 100 μmol concentrations were chosen. A concentration of 100 μmol showed a good antioxidant effect under both normal and stressful conditions. A concentration of 1000 μmol showed an antioxidant effect under stress. Selenium is known to play a large role in antioxidant plant protection (Mugesh et al., 2001).In connection with this, at the first stage of our work, we determined the content of diene conjugates and the activity of glutathione reductase. According to the data obtained, it can be seen that the substance 996 zh at a concentration of 100 μmol reduces the level of lipid peroxidation, under both normal and stressed conditions (Table 1). The concentration of the substance 996 zh 1000 μmol under normal conditions had a toxic effect; it increased the level of LPO by two times compared with the control. A similar manifestation regarding the effect of selenium was observed on broccoli plants with low sulfur content

Table 1: The effect of substance 996 zh on the content of diene conjugates (LPO) and the activity of glutathione reductase under normal conditions and under stress conditions

№	Variant	Diene conjugates, nmol on 1 gram wet mass	Glutathione reductase activity, mmol min^{-1} mg^{-1}
1	H_2O (control)	0.72 ± 0.07	7.81 ± 2.4
2	1000 μmol 996 zh	1.14 ± 0.03*	8.39 ± 1.4
3	100 μmol 996 zh	0.50 ± 0.02*	38.53 ± 3.51*
4	200 mmol NaCl (control for stress)	1.30 ± 0.02*	4.8 ± 0.05
5	200 mmol NaCl +1000 μm 996 zh	0.83 ± 0.05**	4.19 ± 0.62
6	200 mmol NaCl +100 μm 996 zh	0.68 ± 0.03**	7.36 ± 5.39

* Differences from control $p \leq 0.01$; ** Difference from control for stress $p \leq 0.0$. Value represents mean ± standard error of three replicates.

in the nutrition environment (Tianet al., 2017). The same concentration (1000 μmol) neutralized the effect of stress almost to the control level (Table 1). A similar positive effect of selenium on oxidative stress created by heavy metals, salinisation, cooling on various crops (cucumber, sunflower) was shown by Saidi (Saidi et al., 2014) and Hawrylak-Nowak (Hawrylak-Nowak et al., 2010).

The key parameters for the antioxidant protective potential are the redox status of glutathione, which under normal conditions is significantly shifted towards the reduced form. Support of the redox status of glutathione is provided by a number of enzymes, the main of which is glutathione reductase (GR) (Gill et al., 2013). Glutathione reductase catalyzes the conversion of oxidized glutathione (GSSG) to reduced glutathione (GRH) and is an important component of the ROS detoxification system in plants (Gill et al., 2013). Selenium is known to affect the activity of enzymes of the glutathione plant protection system (Mugeshet al., 2001).In this regard, in our work, we investigated the effect of 996 zh on the activity of glutathione reductase (GR) in plants grown under normal conditions and under stress (200 mmol NaCl).

According to Table 1, it can be seen that in some variants (3; 4; 6) there is a relationship between the content of diene conjugates and the activity of glutathione reductase, namely, with an increase in the activity of GR, the level of LPO decreases and, conversely, with a decrease in the activity of GR, the level of LPO increases (Table 1). Earlier it was shown that salt stress (150 mmol NaCl) leads to a strong increase in the GR activity in salt-tolerant plants (Ahmad et al., 2015).Oil radish does not belong to salt tolerant plants, therefore, we presume that the activity of GR during salinity of chlorides did not increase, but decreased.It is interesting, that the concentration of the substance 996 zh 100 μmol restored the activity of GR to the level of control even under stress condition. Under normal conditions, the effect of this concentration increased the activity of GR by several times as compared with the control; that was reflected in a decrease in the level of LPO. The concentration of 1000 μmol of substance 996 zh under normal and stressful conditions did not show significant differences.

Evaluation of the level of LPO and the activity of GR showed that the substance 996 zh exhibits an antioxidant effect under both normal and stressed conditions. Morphometric parameters of the organism as a whole are known to depend on the biochemical processes occurring in the cells. Seed germinability is an important indicator for crops, as it affects density of sowing and evenness of plant stand. Germinability is determined by soil and climatic conditions, growing technology and fertilizer systems. It is known that the germinability of oilseed radish in Eastern Siberia can vary from 40-90% depending on agro-climatic conditions (Dorofeevet al., 2013).That is why increasing oilseed radish seeds germinability is a pressing issue in regions with a sharply continental climate (Kashevarovet al., 2016). Therefore, we determined the effect of the substance 996 zh on the germinability of the given culture under both normal and stressed conditions. Concentrations of the substance 996 zh, used in the work, showed the same results both under normal conditions and under salinisation conditions (Fig. 1). The concentration of 1000 μm inhibits germinability and the concentration of 100 μmol activates it. The data obtained may reflect the effect of 996 zh on the activity of GR and the level of LPO. Under normal conditions, the concentration of the substance 996 zh 1000 μmol increased the level of LPO and had almost no effect on the activity of GR; that caused the inhibition of germinability. A similar effect was observed at salinisation; the concentration of the substance 996 zh 1000 μmol increased the level of LPO in comparison with the control, but reduced it compared to the control for stress. The concentration of the substance 996 zh 1000 μmol did not affect the activity of GR (Table 1). As a result, germinability decreased (Fig. 1). It is noted that selenium concentration from 150-300 μmol has a negative effect on germinability and germinative energy for such crops

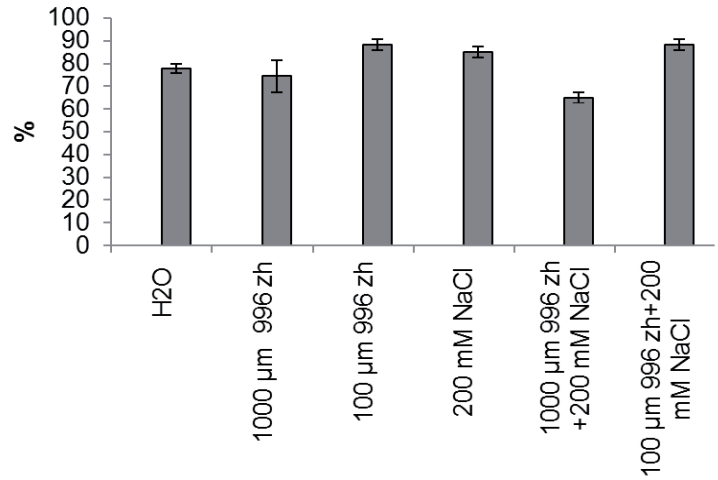

Figure 1: The effect of 996 zh on the oilseed radish seeds germinability under normal conditions and under stress conditions. Value represents mean ± standard error of three replicates.

as wheat, oats, and rye. For barley, these concentrations were destructive (Sindirevaet al., 2013).

The opposite phenomenon was observed when exposed to a concentration of the substance 996 zh 100 µmol (Fig. 1). The level of LPO in seedlings decreased both under normal and stressed conditions, while the GR activity increased (Table 1).It is known that GR reduces the oxidized form of glutathione to the reduced form. In this regard, an increase in GR activity indicates that more reduced glutathione is formed in the cells, which is involved in protecting cells from oxidative damage (Sao et al., 2017).Therefore, the germinability of seeds increased (Fig. 1). It has been shown that when exposed to low concentrations with preparations containing selenium (50 µmol) on seeds of cultivated plants, seed germinability also increases (Nikonov et al., 2009). For pulses (beans, chickpea, soybean), selenium concentration from 10-60 µmol had a positive effect on germinability and germinative energy (Kokorina et al., 2015; Chernenko et al., 2017).

In addition to germinability, there are a number of indicators that are not rated by standards, but are of great importance for assessing the quality of seeds. The biomass of the roots and stems of seedlings is an important indicator, as it reflects the further development of plants (Hajibolandet al., 2015; Sao et al., 2017).

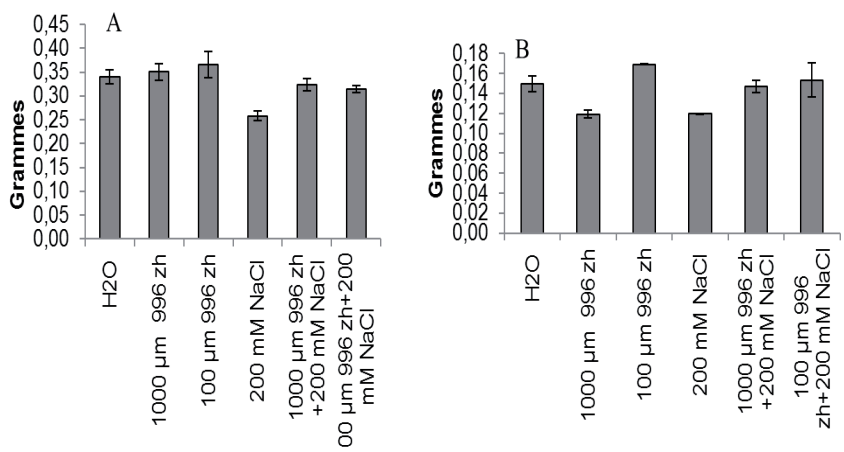

Figure 2: The effect of 996 zh on the mass of the roots (A) and shoots (B) of seedlings of oilseed radish under normal conditions and under stress conditions. Value represents mean ± standard error of three replicates.

The effect of substance 996 zh on seedling biomass showed that both for roots and for stems under stress, both concentrations 1000-100 μmol have a positive effect increasing the mass of roots and stems compared to the control for stress (Fig. 2). The concentration of 1000 μmol, under normal conditions, inhibited the development of the roots of seedlings that affected their mass. However, the same concentration of the substance 996 zh had a positive effect on the development of the stems and, accordingly, on their mass (Fig. 2). This suggests that as the seedlings develop, the growth of the root system slows down when the concentration of the substance 996 zh is 1000 μmol. The concentration of the substance 100 μmol had a positive effect on the biomass of the seedlings; especially, the effect was on the mass of the roots (Fig. 2).

An increase in the biomass of the aboveground and underground parts of wheat is observed when processing with selenium nanoparticles (Yurkova & Omelchenko, 2015). The effect of selenium in concentrations of 10^{-6} and 10^{-7} also had a stimulating effect on the roots and stems of soybean seedlings; the concentration of 10^{-4} was excessive and had an inhibitory effect (Kokorinaet al., 2015).

Thus, the studied new non-toxic water-soluble seleniumorganic substance 996 zh at a concentration of 1000-100 μmol stimulates the antioxidant protection of seedlings, while improving their morphometric parameters, both under normal and stressed conditions. Therefore, on the basis of this substance, it is possible to create preparations for the treatment of seeds of agricultural crops.

4 ACKNOWLEDGMENT

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5 REFERENCES

Accurso, A. A., Cho, S. H., Amin,A., Potapov, V. A., Amosova, S. V., Finn, M. G.(2011). Thia-, Aza-, and Selena[3.3.1]bicyclononane Dichlorides: Rates vs Internal Nucleophile in Anchimeric Assistance. *The Journal of Organic Chemistry*, *76*, 4392–4395. https://doi/10.1021/jo102440k

Alfthan, G., Eurola, M., Ekholm, P.,Venäläinen, E. R., Root, T., Korkalainen, K., Hartikainen, H., Salminen, P., Hietaniemi, V., Aspila, P., Aro, A. (2015). Effects of nationwide addition of selenium to fertilizers on foods, and animal and human health in Finland: From deficiency to optimal selenium status of the population. *Journal Of Trace Elements In Medicine And Biology, 31*, 142–7. https://doi/10.1016/j.jtemb.2014.04.009

Ahmad, P., Hashem, A., Abd-Allah, E. F., Alqarawi, A. A., John, R., Egamberdieva, D., Gucel, S. (2015). Role of Trichoderma harzianum in mitigating NaCl stress in Indian mustard (*Brassica juncea* L) through antioxidative defense system. *Frontiers in Plant Science*, *6*, 868. https://doi/10.3389/fpls.2015.00868

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Chernenko, K. I., Balakova, A. A., Shmarina, Y. G., Ryaskova, K.E. (2017). Prospects for the use of leguminous crops in the creation of selenium-enriched crop production. *Unique research of the XXI century*, *1*, 26–29.

Dorofeev, N. V., Bojarkin, E. V., Peshkova, A. A. (2013). Factors defining field germination of oilseed radish seeds. *Journal of Stress Physiology & Biochemistry, 9*, 159–168.

Gill, S. S., Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, *48*, 909–30. https://doi/10.1016/j.plaphy 2010.08.016

Gill, S. S., Anjum, N. A., Hasanuzzaman, M., Gill, R., Trivedi, D. K., Ahmad, I., Pereira, E., Tuteja, N. (2013). Glutathione and glutathione reductase: a boon in disguise for plant abiotic stress defense operations. *Plant Physiology and Biochemistry, 70*, 204–12. https://doi:10.1016/j.plaphy.2013.05.032

Kashevarov, N. I., Mustafin, A. M., Kharchevnikov, V. V. (2016). *Radish oil in Siberia*. Novosibirsk: publishing house of the RAS. FASE Russia. FSBSI Siberian scientific research Institute of feed. 129 p.p.

Kokorin, A. L., Petrova, N. A., Dem–Roy, G. B. (2015). Effect of trace elements of sprouted seeds of soybean varieties of the Northern ecotype. *News of Saint-Petersburg state agrarian University*, *40*, 28–33.

Mugesh, G., Panda, A., Singh, H. B., Punekar, N. S., Butcher, R.J. (2001). Glutathione Peroxidase-like Antioxidant Activity of Diaryl Diselenides: A Mechanistic Study. *Journal Of The American Chemical Society*, *123*, 839–850. https://doi/10.1021/ja994467p

Nikonov, I. N., Ivanov, L. I., Kovalenko, L. V., Folmanis, G. E.(2009). The influence of nanoscale selenium on the growth of agricultural crops. *Promising material*, *4*, 54–57.

Nigmatullina, L. R., Rumyantseva, N. I., Kostyukova, Y. A. (2014). The effect of D,L –buthionine - S,R -sulfoximine on the ratio of glutathione forms and the growth of Tatar buck-

wheat calli. *Ontogenesis*, *45*, 50–62. https://doi.org/10.1134/S1062360414010056

Nogueira, C. W., Zeni, G., Rocha, J. B. T. (2004). Organoselenium and organotellurium compounds. *Toxicology and pharmacology. Chemistry Revie, 104*, 6255–6286. https://doi.org/10.1021/cr0406559

Hajiboland, R., Sadeghzadeh, N., Ebrahimi, N., Sadeghzadeh, B., Mohammadi, S. A. (2015). Influence of selenium in drought-stressed wheat plants under green house and field conditions. *Acta agriculturae Slovenica*, *105*, 175 – 191. https://doi:10.14720/aas.2015.105.2.01

Hawrylak-Nowak, B., Matraszek, R., Szymańska, M. (2010). Selenium modifies the effect of short-term chilling stress on cucumber plants. *Biological Trace Element Research*, *138*, 307–15. https://doi.org/10.1007/s12011-010-8613-5

Placer, Z. (1968). Lip peroxidation systeme im biologischen material. *Nahrung*, 12, 679. https://doi.org/10.1002/food.19680120615

Potapov, V. A.,Amosova, S.V. (2003). New methods for preparation of organoselenium and organotellurium compounds from elemental chalcogens. *Russian Journal of Organic Chemistry*, *39*, 1373–1380. https://doi.org/10.1023/B:RUJO.0000010549.08131.18

Potapov, V. A., Musalov M. V., Musalova M. V., Amosova S. V. (2016). Recent Advances in Organochalcogen Synthesis Based on Reactions of Chalcogen Halides with Alkynes and Alkenes. *Current Organic Chemistry, 20*, 136145. https://doi.org/10.1002/chin.201618224

Saidi, I., Chtourou, Y., Djebali, W. (2014). Selenium alleviates cadmium toxicity by preventing oxidative stress in sunflower (*Helianthus annuus*) seedlings. *Journal of Plant Physiology*, *171*, 85–91. https://doi/10.1016/j.jplph.2013.09.024

Sao, A., Saraf, P., Bagchi, D. (2017). Effect of salinity on *Brassica rapa* var. *toria* (BRSRT) under selenium defence: A trial to assess the protective role of selenium. *Acta agriculturae Slovenica*, *109*, 577 – 592. https://doi:10.14720/aas.2017.109.3.09

Sindirepa, A. V., Stepanova, O. V., Serebrennikova, A. A. (2013). Influence of selenium and iodine on growth and development of grain crops. Materials XI all-Russian scientific and practical conference of students, postgraduates and young scientists, which was held in Perm, Russia. *Publishing house of Perm national research Polytechnic University*, 233–241.

Škrabanja, V. (2017). An effective way of adding selenium to everyday food with a focus on plant resources. *Acta agriculturae Slovenica*, *109*, 147 – 155. https://doi:10.14720/aas.2017.109.1.14

Surin, N. A., Lyakhov, N. E., Gerasimov, S. A., Lepshin, A. G. (2018). Evaluation of collection samples of spring barley in breeding for productivity and quality of grain in Eastern Siberia. *The Achievements of Science and Technology, Agriculture*, *32*, 41-44.

Tian, M., Hui, M., Thannhauser, T. W., Pan, S., Li, L. (2017). Selenium-Induced Toxicity Is Counteracted by Sulfur in Broccoli (*Brassica oleracea* L. var. *italica*). *Frontiers in Plant Science, 8*, 1425. https://doi.org/10.3389/fpls.2017.01425

Yurkova, I. N., Omelchenko, V. V. (2015). Influence of nanoparticles of selenium and sodium Selenite on the growth and development of wheat plants. *Scientific notes of V. I. Vernadsky Crimean Federal University Series Biology, Chemistry*, *1*, 99–106.

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Deteriorative changes in maize kernels due to *Aspergillus flavus* Link. and *Fusarium verticillioides* (Sacc.) Nirenberg

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Deteriorative changes in maize kernels due to *Aspergillus flavus* Link. and *Fusarium verticillioides* (Sacc.) Nirenberg

Abstract: The study aimed at measuring changes in chemical composition of maize kernels due to *Aspergillus flavus* Link. and *Fusarium verticillioides* (Sacc.) Nirenberg infection. The samples of maize kernels were incubated at 28 °C for 7, 14, 21, and 28 days. The samples were analysed for mycotoxin, moisture, crude fat, crude protein, crude ash, and crude fibre. Maize kernels inoculated with *A. flavus* and *F. verticillioides* exhibited a significant decrease in crude fat. Aflatoxin B_1 (AFB_1) contamination increased in maize kernels inoculated with *A. flavus*, and fumonisin B_1 (FB_1) in kernels inoculated with *F. verticillioides*. Crude ash and crude fibre content showed no changes. Incubation time significantly affected AFB_1 and FB_1 contamination levels, moisture, crude fat, and crude protein contents. AFB_1 and FB_1 contamination were significantly correlated with crude fat degradation. The tested strains had similar deteriorative effects on maize kernels. The significant changes in the proximate composition were only observed in maize kernels with mycotoxin contamination above the regulatory limit of 10 μg kg^{-1}, thus not fit for human consumption.

Keywords: aflatoxin; fumonisin; maize kernel; mycotoxin; proximate components; fungal species

Kvarjenje koruznih zrn zaradi okužb z glivama *Aspergillus flavus* Link. in *Fusarium verticillioides* (Sacc.) Nirenberg

Izvleček: V raziskavi so bile merjenje spremembe v kemični sestavi koruznih zrn zaradi okužbe z glivama *Aspergillus flavus* in *Fusarium verticillioides*. Vzorci koruznih zrn so bili inkubirani pri temperature 28 °C za 7, 14, 21, in 28 dni. V vzorcih je bila analizirana vsebnost mikotoksinov, vode, celukopnih beljakovin, maščob, vlaknin in pepela. Koruzna zrna, okužena z glivama *A. flavus* in *F. verticillioides,* so imela značilen upad celokupnih maščob. Kontaminacija z aflatoksinom B_1 (AFB_1) se je v koruznih zrnih povečala po inokulaciji z glivo *A. flavus*, s fumonizinom B_1 (FB_1) pa po inokulaciji z glivo *F. verticillioides*. Pri vsebnostih celokupnega pepela in vlaknin ni bilo nobenih sprememb. Čas inkubacije je značilno vplival na vsebnost AFB_1 in FB_1, vsebnost vode, celokupnih maščob in beljakovin. Kontaminacija z AFB_1 in FB_1 je bila značilno povezana z degradacijo celokupnih maščob. Testirani sevi so imeli podoben kvaren učinek na koruzna zrna. Značilne spremembe v zgradbi koruznih zrn so bile ugotovljene pri njihovi kontaminaciji z mikotoksini nad predpisano vrednostjo 10 μg kg^{-1}, kar ni primerno za prehrano ljudi.

Ključne besede: aflatoksin; fumonizin; koruzna zrna; kemijska sestava; mikotoksin; vrste gliv

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1 INTRODUCTION

Maize is highly susceptible to fungal infection. Consequently, the quality of the maize kernels deteriorates (Begum et al., 2013). Fungal development can cause a considerable modification in the chemical composition of stored grains (Kakde and Chavan, 2011). Fungal infection in grains is associated with losses in carbohydrates, proteins and lipids while moisture content and free fatty acid increase. Fungi produce hydrolytic enzymes including peroxidase, amylase, pectinases, proteases and lipases. These enzymes degrade biochemical components such as fats, protein, and carbohydrates leading to the loss of dry matter (Begum et al., 2013). Bhattacharya and Raha (2002) reported a decrease in carbohydrates and fat content in maize kernels and soya beans due to post-harvest fungal infection. Jain (2008) reported a rapid increase in free fatty acids in damaged grains due to fungal infestation. Embaby and Abdel-Galil (2006) observed a reduction in carbohydrates, sugars and crude fat due to *Fusarium* in legume grains. Kakde and Chavan (2011) concluded that *Aspergillus flavus* was responsible for the maximum depletion of fat content and reducing sugars in safflower, soya bean and sesame.

Aspergillus flavus and *Fusarium verticillioides* are commonly occurring maize pathogens that can easily survive on dead plant materials as saprotrophs. They also cause aflatoxin and fumonisin contamination (Probst et al., 2014), especially in maize kernels that provide a good natural substrate for the fungi (Perrone et al., 2014). Nutrient composition is a key factor affecting mycotoxin production in maize kernels (Ma et al., 2015). Inherent materials in maize kernels such as starch, proteins and lipids represent significant carbon and nitrogen sources potentially available during seed infection by fungi (Mellon et al., 2002). Saccharides provide the primary carbon source for mycelial growth and mycotoxin production (Mellon et al., 2005). Fanelli and Fabbri (1989), Wilson et al. (2004), and Mellon et al. (2005) reported a relationship between lipid degradation and AFB_1 production. Glucose, ribose, xylose, and glycerol are also good substrates for growth and aflatoxin production by *A. flavus* (Liu et al., 2016).

Maize serves as an important dietary staple in Sub Saharan Africa. Consequently, the nutritive value of maize is of importance. Maize is vulnerable to infection by toxigenic fungi (Abbas et al., 2006). The high temperature and high relative humidity experienced in most parts of Sub Saharan Africa, coupled with poor grain storage conditions predispose maize to toxigenic fungal attack (Oyekale et al., 2012). Consequently, it is necessary to investigate its nutritive integrity and the subsequent mycotoxin contamination during fungal infection. The objective of this study was to evaluate the effect of *A. flavus* and *F. verticillioides* infection on the proximate composition of maize kernels.

2 MATERIALS AND METHODS

2.1 INOCULUM PREPARATION

Aspergillus flavus Link. (strain PPRI1314-UKZN) and *F. verticillioides (*Sacc.) Nirenberg (strain MRC826) were obtained from the Department of Plant Pathology, School of Agriculture, Earth and Environmental Sciences, University of KwaZulu-Natal, South Africa. The fungi were plated on potato dextrose agar (Merck, Darmstadt, Germany) at 25 °C for five days, after which conidia were harvested by flooding a single culture with either Triton X-100 solution (*A. flavus*) or distilled water (*F. verticillioides*) and scraping the surface mycelia with a sterile scraper. The resulting suspensions were filtered through cheesecloth. The spore concentration was counted using a Neubauer hemocytometer, and diluted using distilled water to obtain a spore concentration of 4×10^6 cells ml^{-1} (Hruska et al., 2014).

2.2 PREPARATION OF MAIZE SAMPLES

The maize kernels were surface sterilised by immersing the kernels in a 5 % (v/v) sodium hypochlorite (NaClO) solution and stirring for one minute. The maize kernels were thereafter rinsed twice with distilled water. The moisture content (MC) of the maize kernels was then adjusted to 205 g kg^{-1} dry matter (DM) by soaking samples in distilled water for 2 hours. The samples were thereafter put in sealed plastic bags and refrigerated at a temperature of 4 °C for 72 hours to ensure uniform moisture distribution.

2.3 INOCULATION AND INCUBATION OF MAIZE

Maize was retrieved from cold storage and allowed to equilibrate to room temperature. A total of 45 samples of maize kernels each of mass 3 kg was weighed into sterilised plastic bags. Five ml of spore suspension from *A. flavus or F. verticillioides* were sprinkled on the samples and mixed manually before being transferred to the incubator. Five ml of distilled water was sprinkled on control samples. All samples were incubated at a temperature of 28 °C and sampling was done after 0, 7, 14, 21, and 28 days, respectively. The incubated samples were analysed

for aflatoxin and fumonisin content, and proximate composition.

2.4 ANALYSIS OF THE CHEMICAL COMPOSITION OF MAIZE KERNELS

Aflatoxin and fumonisin analysis were done using a liquid chromatography-tandem mass spectroscopy (LC-MS/MS) as outlined by de Kok et al. (2007). Two hundred and fifty grams of each sample was ground using a Retsch Rotor Mill (SK 1, Germany). Twenty five grams of the ground maize sample was mixed with 80 ml of acetonitrile and 20 ml of water and left to stand for 2 hours. The extract was filtered and diluted four-times with distilled water. Twenty µl of the diluted extract was injected into the LC-MS/MS for analysis.

The liquid chromatography (LC) had an ultra-performance liquid chromatography, ethyle bridge hybrid column (Aquity, UPLC BEH C18 1.7 µm; 2.1×100 mm column). The mobile phase A and mobile phase B were 0.1 % formic acid in water and 0.1 % formic acid in acetonitrile, respectively. The LC flow rate was 0.4 ml min^{-1}. The eluent from the LC column was directed to the mass spectrometer. The electrospray source was operated in a positive ionisation multiple reaction monitoring (MRM) mode. The MRM transitions monitored for AFB_1 were 313 $m.z^{-1}$, 241 $m.z^{-1}$, 50 V, and 47 V for parent ion, product ion, cone voltage, and collision voltage, respectively. The MRM transitions monitored for FB_1 were 722 m z^{-1}, 334 m z^{-1}, 50 V, and 40 V for parent ion, product ion, cone voltage, and collision voltage, respectively. The data acquired were analysed using Waters Masslynx™ software. The limit of detection for the LC-MS/MS was 0.5 µg kg^{-1}, whereas the quantification limit was 2 µg kg^{-1}.

The proximate components including MC, crude ash, crude fibre, crude fat, and crude protein, were anaysed using AOAC methods (AOAC, 2012).

2.5 DATA PREPARATION AND STATISTICAL ANALYSIS

A two-factor full-factorial design was used in this experiment, with the first factor at two levels and the second factor at five levels. The factors studied were fungal species (*A. flavus, F. verticillioides*) and incubation period (0, 7, 14, 21, and 28 days).

The data was subjected to analysis of variance (ANOVA) at 5 % significance level to determine the effect of *A. flavus* and *F. verticillioides* on mycotoxin contamination (aflatoxin and fumonisin), crude fat, crude fibre, crude protein and MC of grains. Where a significant result was obtained, the mean comparison was done using Duncan's Multiple Range Test. The correlation between mycotoxin contamination and proximate components was established using regression analysis. The analysis was done using GenStat® 17th Edition (VSN International Ltd, Hemel Hempstead, United Kingdom).

3 RESULTS

The proximate composition and mycotoxin concentration at the start and during the experiment are presented in Table 1. No AFB_1 and Fumonisin B_1 (FB_1) was detected in the maize kernels prior to fungal infection. In this study, crude fat and crude protein content decrease with time while AFB_1, FB_1 and MC increased. The crude ash and crude fibre was unchanged with time.

The MC was significantly ($p < 0.05$) affected by incubation period and fungal species (Table 1). The MC increased with increasing time of incubation. The highest increase in MC was observed in samples inoculated with *A. flavus* (205 to 289 g kg^{-1}, Table 1). The MC of samples inoculated with *F. verticillioides* ranged from 205 to 261 g kg^{-1}. The lowest increase in MC was observed in the control samples, ranging from 205 to 228 g kg^{-1}.

There were no mycotoxins detected in the samples before incubation. Mycotoxin contamination was significantly ($p < 0.05$) affected by fungal species and the incubation duration. The levels of both AFB_1 and FB_1 increased with the incubation duration. The control samples showed no aflatoxin contamination at the end of day seven. However, 1 µg kg^{-1} of AFB_1 was detected in the control samples on day 14, increasing to 21 µg kg^{-1} and 141 µg kg^{-1} on day 21 and day 28 respectively. FB_1 contamination was not detected in any of the control samples. The maize kernels inoculated with *A. flavus* resulted in AFB_1 contamination ranging from 409 µg kg^{-1} on day 7 to 10,508 µg kg^{-1} on day 28, while those inoculated with *F. verticillioides* resulted in FB_1 contamination ranging from 212 µg kg^{-1} on day 7 to 2,447 µg kg^{-1} on day 28 (Table 1).

The crude fat content of maize kernels was 39 ± 0.5 g kg^{-1} before incubation. The crude fat content decreased with increased fungal incubation time, while the fat content for the control samples was unchanged. Both the fungal species and the length of time of incubation significantly affected the crude fat content ($p < 0.05$). The greatest reduction in crude fat content was observed in the samples inoculated with *A. flavus*. The crude fat content ranged from 39 g kg^{-1} on day zero to 19 g kg^{-1} on day 28. The crude fat content for samples inoculated with *F. verticillioides* ranged from 39 to 31 g kg^{-1}, whereas that of the control samples ranged from 39 to 37 g kg^{-1} (Table

Table 1: Variation of chemical composition of maize with A. flavus and F. verticillioides and incubated for 7, 14, 21, and 28 days (dry matter basis)

Taxon	Time (day)	MC (g kg^{-1})	Mycotoxin (µg kg^{-1})	Crude ash (g kg^{-1})	Crude fat (g kg^{-1})	Crude fibre (g kg^{-1})	Crude protein (g kg^{-1})
Control	0	205^{g}	0^{a}	11.8^{ab}	39.7^{g}	45^{a}	79.4^{f}
Control	7	209^{fg}	0^{a}	11^{a}	39^{g}	44^{a}	79^{f}
Control	14	213^{fg}	1^{b}	11^{a}	39^{g}	44^{a}	79^{f}
Control	21	218^{e}	21^{b}	11^{a}	38^{fg}	45^{a}	78^{f}
Control	28	228^{e}	141^{b}	11^{a}	37^{ef}	45^{a}	76^{df}
A. flavus	0	205^{g}	0^{a}	11^{a}	39^{g}	45^{a}	79^{f}
A. flavus	7	227^{e}	409^{b}	12^{a}	36^{e}	45^{a}	75^{cd}
A. flavus	14	243^{d}	1,259^{b}	12^{a}	33^{cd}	45^{a}	74^{bc}
A. flavus	21	274^{b}	3,032^{b}	12^{a}	31^{b}	44^{a}	73^{b}
A. flavus	28	289^{a}	10,508^{b}	11^{a}	19^{a}	45^{a}	71^{a}
F. verticillioides	0	205^{g}	0^{a}	11^{a}	39^{g}	45^{a}	79^{f}
F. verticillioides	7	217^{f}	212^{b}	11^{a}	36^{e}	45^{a}	77^{e}
F. verticillioides	14	225^{ef}	604^{b}	12^{a}	35^{d}	45^{a}	75^{c}
F. verticillioides	21	253^{cd}	1,240^{b}	11^{a}	33^{c}	45^{a}	74^{bc}
F. verticillioides	28	261^{c}	2,447^{b}	11^{a}	31^{b}	45^{a}	73^{b}
Significance Level							
Fungal taxon	<.001	<.001	0.082	<.001	0.677	<.001	
Time	<.001	<.001	0.349	<.001	0.991	<.001	
Fungal taxon × Time	<.001	<.001	0.061	<.001	0.241	<.001	
CV	0.013	0.433	0.001	0.017	0.002	0.004	
SE	0.041	994.27	0.009	0.048	0.014	0.036	
LSD $_{(p \leq 0.05)}$	0.123	2,880.3	0.026	0.139	0.04	0.104	

Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($p < 0.05$). nd = not detected

1). The mean crude fat content of maize kernels inoculated with *A. flavus* and *F. verticillioides* were significantly ($p < 0.05$) different across all treatments.

The crude protein content decreased with incubation time. Both fungal species and the length of time of incubation significantly affected ($p < 0.05$) crude protein content of samples across treatments. The crude protein content of the maize kernels inoculated with *A. flavus* decreased from 79 to 71 g kg^{-1} over the 28 days of incubation. A reduction in the crude protein content from 79 to 73 g kg^{-1} was observed in the samples inoculated with *F. verticillioides*. The crude protein content of the control samples was fairly stable, ranging from 79 to 76 g kg^{-1}.

The crude fibre content of the samples was relatively stable across all treatments. The fungal species and the incubation time had no significant effect ($p > 0.05$) on the crude fibre content of the maize kernels. The crude fibre content of the samples ranged from 45 to 44 g kg^{-1} (Table 1). Similarly, there was no significant difference in the crude ash content of the maize kernel samples across all treatments. Both the fungal species and the incubation time had no significant effect ($p > 0.05$) on the crude ash content of maize kernels. The crude ash content of the maize kernels ranged between 11 and 12 g kg^{-1} (Table 1).

The relationship between mycotoxin contamination and both crude fat and crude protein was best described by second order polynomial equations shown in Table 2. A high coefficient of determination (R^2) was observed

Table 2: Regression equations for crude fat and crude protein of maize kernels contaminated with AFB_1 and FB_1

Mycotoxin	Equation	R^2
AFB_1 vs Crude fat	$1 \times 10^{-7}x^2 - 0.003x + 38.57$	0.986
FB_1 vs Crude fat	$1 \times 10^{-6}x^2 - 0.0061x + 38.74$	0.963
AFB_1 vs Crude protein	$1 \times 10^{-7}x^2 - 0.002x + 77.45$	0.821
FB_1 vs Crude protein	$1 \times 10^{-6}x^2 - 0.0065x + 78.84$	0.944

between mycotoxin contamination and crude fat content (R^2 = 0.986 for AFB_1 and R^2 = 0.963 for FB_1). The coefficient of determination between crude protein content and both AFB_1 and FB_1 was 0.821 and 0.944 respectively. No correlation was observed between mycotoxin contamination and either crude ash or crude fibre content.

4 DISCUSSION

The MC of the maize kernels increased with incubation time. A similar observation was made by Islam (2016) on stored black gram (*Vigna mungo* (L.) Hepper). The increase in moisture content with incubation time is attributed to respiration by maize kernels and fungi (Magan et al., 2004). The mycelial growth increased with time as evidenced by the progressive increase in AFB_1 and FB_1. The increased mycelial biomass escalated the respiration of fungi, hence, high MC on day 28 compared to the minimal change in MC at the start of the experiment. The increase in MC was higher for maize kernels inoculated with *A. flavus* compared to *F. verticillioides*. The incubation temperature of 28 °C was optimal for the growth of *A. flavus* (Pratiwi et al., 2015) but unfavourable for *F. verticillioides* whose optimum temperature is around 25 °C (Garcia et al., 2012).

The AFB_1 and FB_1 contamination of maize kernels increased over time because of the increasing mycelial biomass. There was a high AFB_1 contamination as compared to FB_1 contamination. *Aspergillus flavus* grows faster than *F. verticillioides* at the incubation temperature of 28 °C (Garcia et al., 2012; Pratiwi et al., 2015). Aflatoxin B_1 contamination observed in the control samples could have been caused by internal infection (Mellon et al., 2007).

The greatest depletion of crude fats occurred in maize kernels inoculated with *A. flavus*. This observation is consistent with the findings by Kakde and Chavan (2011) who reported that *A. flavus* was responsible for the maximum depletion of fat content in cereals and oilseeds. Embaby and Abdel-Galil (2006) also observed a reduction in crude fat content in legume seeds due to *Fusarium* sp. *Aspergillus flavus* and *F. verticillioides* produce lipases that hydrolyse fats into fatty acids, which are subsequently degraded to provide a carbon and energy source (Kinderlerer, 1993).

The decrease in crude protein content observed in this study agrees with the findings of Reed et al. (2007) who associated changes in the protein content of maize with fungal degradation. The depletion of protein is attributed to its utilisation during the growth and metabolism of fungi (Bhattacharya and Raha, 2002). Liu et al. (2016) reported that amino acids such as glutamate, aspartate and arginine significantly promote AFB_1 production by *A. flavus* indicating protein utilisation. Results from this study are in tandem with previous research findings (Bhattacharya and Raha, 2002; Rheeder et al., 2009; Liu et al., 2016) that associated protein depletion with fungal deterioration.

Hydrolytic enzymes produced by *A. flavus* and *F. verticillioides* break down fats and proteins for use in fungal growth and development, which in turn creates conducive conditions for the production of mycotoxins (Liu et al., 2016). Fats are preferred over proteins as carbon substrates, hence, the high correlation between fats and mycotoxin contamination (Mellon et al., 2007).

5 CONCLUSION

Aspergillus flavus and *F. verticillioides* caused significant ($p < 0.05$) changes in the levels of crude fat and crude protein content of maize kernels. Although aflatoxin contamination was highly correlated with the depletion of crude fats, such changes can also be caused by *F. verticillioides,* which produces FB_1. The proximate composition of maize samples with allowable mycotoxin contamination (< 10 μg kg^{-1}) was similar to uncontaminated maize kernels. Significant changes in proximate components were observed at mycotoxin contamination levels higher than the regulatory limit of 10 μg kg^{-1}, thus not fit for human consumption.

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7 REFERENCES

Abbas, H. K., Cartwright, R. D., Xie, W., & Shier, W. T. (2006). Aflatoxin and fumonisin contamination of corn (maize, *Zea mays*) hybrids in Arkansas. *Crop Protection, 25*(1), 1-9. https://doi.org/10.1016/j.cropro.2005.02.009

AOAC. (2012). *Official Methods of Analysis of AOAC International*. Gaithersburg, USA: AOAC International.

Begum, M. A. J., Venudevan, B., & Jayanthi, M. (2013). Storage fungi in groundnut and the associate seed quality deterioration-A Review. *Plant Pathology Journal, 12*(3), 127-134. https://doi.org/10.3923/ppj.2013.127.134

Bhattacharya, K., & Raha, S. (2002). Deteriorative changes of maize, groundnut and soybean seeds by fungi in storage. *Mycopathologia, 155*(3), 135-141. https://doi.org/10.1023/A:1020475411125

de Kok, A., Spanjer, M., Scholten, J., Rensen, P., & Kearney, G. (2007). *Rapid multi-mycotoxin analysis using AC-*

QUITY UPLC and Quattro Premier XE. Retrieved from http://www.waters.com/waters/library.htm?locale=en_ZA&lid=1512802&cid=511436.

Embaby, E., & Abdel-Galil, M. (2006). Seed borne fungi and mycotoxins associated with some legume seeds in Egypt. *Journal of Applied Sciences Research, 2*(11), 1064-1071.

Fanelli, C., & Fabbri, A. (1989). Relationship between lipids and aflatoxin biosynthesis. *Mycopathologia, 107*(2-3), 115-120. https://doi.org/10.1007/BF00707547

Garcia, D., Barros, G., Chulze, S., Ramos, A. J., Sanchis, V., & Marín, S. (2012). Impact of cycling temperatures on Fusarium verticillioides and Fusarium graminearum growth and mycotoxins production in soybean. *Journal of the Science of Food and Agriculture, 92*(15), 2952-2959. https://doi.org/10.1002/jsfa.5707

Hruska, Z., Rajasekaran, K., Yao, H., Kinkaid, R., Darlington, D., Brown, R. L., Bhatnagar, D., & Cleveland, T. E. (2014). Co-inoculation of aflatoxigenic and non-aflatoxigenic strains of Aspergillus flavus to study fungal invasion, colonization, and competition in maize kernels. *Frontiers in Microbiology, 5*(3), 122. https://doi.org/10.3389/fmicb.2014.00122

Islam, M. (2016). *Effect of biotic and abiotic factors on quality of black gram seed*. Ph.D. thesis, Sher-e-Bangla Agricultural University, Bangladesh.

Jain, P. (2008). *Microbial degradation of grains, oil seeds, textiles, wood, corrosion of metals and bioleaching of mineral ores*. Retrieved from http://nsdl.niscair.res.in/jspui/bitstream/123456789/558/1/MicrobialDegradation.pdf

Kakde, R. B., & Chavan, A. M. (2011). Deteriorative changes in oilseeds due to storage fungi and efficacy of botanicals. *Current Botany, 2*(1), 17-22.

Kinderlerer, J. L. (1993). Fungal strategies for detoxification of medium chain fatty acids. *International Biodeterioration & Biodegradation, 32*(1-3), 213-224. https://doi.org/10.1016/0964-8305(93)90053-5

Liu, J., Sun, L., Zhang, N., Zhang, J., Guo, J., Li, C., Rajput, S. A., & Qi, D. (2016). Effects of nutrients in substrates of different grains on aflatoxin B1 production by Aspergillus flavus. *BioMed Research International, 2016*(2016), 1-10. https://doi.org/10.1155/2016/7232858

Ma, H., Zhang, N., Sun, L. & Qi, D. (2015). Effects of different substrates and oils on aflatoxin B1 production by Aspergillus parasiticus. *European Food Research and Technology, 240*(3), 627-634. https://doi.org/10.1007/s00217-014-2364-z

Magan, N., David, A., & Sanchis, V. (2004). The role of spoilage fungi in seed deterioration. In D. Aurora (Eds.), *Fungal Biotechnology in Agricultural, Food and Environmental Application* (pp. 311-333) New York, NY: Marcel Dekker. https://doi.org/10.1201/9780203913369.ch28

Mellon, J. E., Cotty, P.J., & Dowd, M. K. (2007). Aspergillus flavus hydrolases: their roles in pathogenesis and substrate utilization. *Applied Microbiology and Biotechnology, 77*(3), 497-504. https://doi.org/10.1007/s00253-007-1201-8

Mellon, J. E., Dowd, M. K., & Cotty, P. J. (2002). Time course study of substrate utilization by Aspergillus flavus in medium simulating corn (*Zea mays*) kernels. *Journal of Agricultural and Food Chemistry, 50*(3), 648-652. https://doi.org/10.1021/jf011048e

Mellon, J. E., Dowd, M. K., & Cotty, P. J. (2005). Substrate utilization by Aspergillus flavus in inoculated whole corn kernels and isolated tissues. *Journal of Agricultural and Food Chemistry, 53*(6), 2351-2357. https://doi.org/10.1021/jf040276g

Oyekale, K., Daniel, I., Ajala, M., & Sanni, L. (2012). Potential longevity of maize seeds under storage in humid tropical seed stores. *Nature and Science, 10*(8), 114-124.

Perrone, G., Haidukowski, M., Stea, G., Epifani, F., Bandyopadhyay, R., Leslie, J. F., & Logrieco, A. (2014). Population structure and Aflatoxin production by Aspergillus Sect. Flavi from maize in Nigeria and Ghana. *Food Microbiology, 41*(1), 52-59. https://doi.org/10.1016/j.fm.2013.12.005

Pratiwi, C., Rahayu, W. P., Lioe, H. N., Herawati, D., Broto, W., & Ambarwati, S. (2015). The effect of temperature and relative humidity for Aspergillus flavus BIO 2237 growth and aflatoxin production on soybeans. *International Food Research Journal, 22*(1), 82.

Probst, C., Bandyopadhyay, R., & Cotty, P. (2014). Diversity of aflatoxin-producing fungi and their impact on food safety in sub-Saharan Africa. *International Journal of Food Microbiology, 174*(1), 113-122. https://doi.org/10.1016/j.ijfoodmicro.2013.12.010

Reed, C., Doyungan, S., Ioerger, B., & Getchell, A. (2007). Response of storage molds to different initial moisture contents of maize (corn) stored at 25 C, and effect on respiration rate and nutrient composition. *Journal of Stored Products Research, 43*(4), 443-458. https://doi.org/10.1016/j.jspr.2006.12.006

Rheeder, J., Shephard, G., Vismer, H., & Gelderblom, W. (2009). *Guidelines on mycotoxin control in South African foodstuffs: from the application of the hazard analysis and critical control point (HACCP) system to new national mycotoxin regulations* (Medical Research Council Policy Brief). Retrieved from http://www.mrc.ac/policybriefs/mycotoxinguidelines.

Wilson, R. A., Calvo, A. M., Chang, P. K., & Keller, N. P. (2004). Characterization of the Aspergillus parasiticus Δ12-desaturase gene: a role for lipid metabolism in the Aspergillus-seed interaction. *Microbiology, 150*(9), 2881-2888. https://doi.org/10.1099/mic.0.27207-0

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Association studies between grain yield and agronomic traits of a MARS maize (*Zea mays* L.) population under drought and non-stress condition

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Association studies between grain yield and agronomic traits of a MARS maize (*Zea mays* L.) population under drought and non-stress condition

Abstract: The study aimed at examining the associations between yield and other traits under drought stress and non-stress conditions. A total of 150 MARS testcrosses were evaluated under both conditions at the International Institute of Tropical Agriculture substation for two years under during the dry season. Genotypic and phenotypic correlation, multiple stepwise regression and path co-efficient analyses were carried out to examine the relationship among the traits under both environments. Results showed anthesis-silking interval, days to silking, husk cover and plant aspect were significantly associated with yield under drought condition at both genotypic and phenotypic levels. Yield was positively correlated with plant and ear height but had a negative correlation with plant and ear aspect at both levels under well-watered condition. Regression analysis showed that ears per plant, plant aspect, ear aspect, days to silking, leaf death and plant height had a direct effect on yield, contributing a total of 71.1 % of observed variation under drought, while ears per plant, ear aspect, plant aspect, days to pollen shed, days to silking and plant height contributed about 31.42 % to yield under well-watered conditions. The study concluded that these traits be used as selection criteria as it will aid improvement of maize yield.

Key words: maize; association; grain yield; drought; well-watered; MARS; testcross

Raziskava povezav med pridelkom zrnja in agronomskimi lastnostmi populacij koruze (*Zea mays* L.) v razmerah suše in v nestresnih razmerah

Izvleček: Namen raziskave je bil preučiti povezave med pridelkom zrnja in drugimi lastnostmi koruze v razmerah suše in v nestresnih razmerah. Na International Institute of Tropical Agriculture je bilo ovrednotenih 150 križanj v obeh razmerah v dveh letih, v sušni sezoni. Genotipska in fenotipska korelacija, postopna multipla regresija in koeficient korelacije med neposredno odvisnimi spremenljivkami so bili opravljeni za preučitev razmerja med lastnostmi v obeh rastnih razmerah. Rezultati so pokazali, da so bili znaki kot so obdobje anteza--sviljenje, dnevi do sviljenja in pokritost storža značilno povezani s pridelkom v sušnih razmerah na genotipski in fenotipski ravni. Pridelek je bil v pozitivni korelaciji z višino rastlin in višino storžev na rastlini, a je bil v negativni korelaciji z drugimi aspekti rastline in storža v razmerah dobre oskrbe z vodo. Analiza regresije je pokazala, da so imeli znaki kot so število storžev na rastlino, aspekt rastline in storža, dnevi do sviljenja, smrtnost listov in višina rastlin neposreden učinek na pridelek in so prispevali skupaj 71,1 % opažene spremenljivosti v sušnih razmerah, medtem ko so ti isti znaki prispevali le 31,42 % vpliva na pridelek v razmerah dobre oskrbe z vodo. Na osnovi te raziskave je bilo zaključeno, da bi lahko te lastnosti (znake) uporabili kot selekcijske kriterije, ki bi pomagali izboljšati pridelek koruze.

Ključne besede: koruza; povezava; pridelek zrnja; suša; dobra oskrba z vodo; MARS; testna križanja

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1 INTRODUCTION

Maize (*Zea mays* L.) is presently the second most abundant crop in the world (Ort and Long, 2014) and it has a higher yield potential compared with rice and wheat theoretically, being a C_4 plant (Gong et al., 2015). Despite its potential, the average grain yield of maize in the West and Central Africa (WCA) sub region is estimated to be 1.8 t ha^{-1} (www.fao.org), this is quite low compared with the yield recorded by many other regions where maize is grown, both in the developing and developed regions of the world (Semagn et al., 2015). Maize yields in Africa are considerably lower than the world average because the cultivation of maize is often prone to abiotic stresses such as drought and low soil fertility in addition to biotic stresses principal among which are *Striga* species and stem borers (FAO, 2010). Drought stress is the most restrictive agronomic problem confronting maize production, reducing crop yields particularly in regions of the world faced with water limitation, a region with an appreciable number of resource poor farmers (Mhike et al., 2013). In comparison to other abiotic stress factors, drought is the causative factor for the major losses recorded in crop production (Ober, 2008). Although maize has it origin in the tropics, it is extremely prone to drought and heat, particularly at silk emergence and/or when flowers are ready for pollination (Boyer and Westgate, 2004; Lobell et al., 2011, 2014; Frey et al., 2015). A number of studies have reported a significant decrease in the ear traits and also in the commercial value of maize under drought (Edmeades et al., 1995; Ti-da et al., 2006; Mohammadai et al., 2012). The change in the climate is expected to increase the occasions of drought in Africa (Williams and Funk, 2011), together with the fact that maize production is extending into regions that are predisposed to drought stress (Bankole et al., 2017). The world's grain supply is inadequate compared with the demand for food and feed. Furthermore, a gross limitation in crop production worldwide has been predicted as a result of changes in climate, particularly extreme temperatures and drought (Cooper et al., 2014; Frey et al., 2015; Horton et al., 2015). Consequently, it becomes more difficult for a small-scale producer of maize with little or no access to irrigation facilities, who plants varieties that are susceptible to drought stress in sub-Saharan Africa to survive these myriads of challenges (Derera et al., 2008).

Some maize producers have adjusted their planting dates to fit the rainy season which enables the planting periods to correspond with the outset of the rains. Others producers have substituted maize with tree crops because they are more tolerant of changes in temperature and rainfall regimes (Barimah et al., 2014). Subsequently, improvement of crops for tolerance to drought has become imperative of crops under the changing environmental conditions.

The conventional breeding method which exploits inherent genetic variation and uses selection as a tool to integrate desirable traits into adapted genotypes seems to be the most common method in breeding for drought tolerance (Xoconostle-Cazares et al., 2011). The comparative performance of genotypes under drought stress and non-stressed conditions seem to be a common criterion for identifying desirable genotypes for erratic rain-fed conditions (Nouri et al., 2011). Selection for traits such as yield under drought condition proves more challenging as a result of low heritability for the trait under stress conditions (Edmeades et al., 1999; Venuprasad et al., 2007; Ziyomo and Bernado, 2013) rendering the selection process quite ineffective. However, some secondary traits such as anthesis silking interval and ears per plant show high estimates of heritability and genetic correlations with grain yield under drought stress (Bolaños and Edmeades, 1996; Bänziger and Lafitte, 1997; Badu-Apraku et al., 2004). Therefore, an estimate of correlation and path coefficient between the primary trait and other component traits influencing yield is requisite for selection of superior maize genotypes for a successful breeding programme

Correlation coefficient analyses are useful for selecting the traits that influence grain yield simultaneously (Menkir, 2008). It usually exploits the degree of association among continuous traits (Malik et al., 2005). Despite the usefulness of these estimates in the understanding of complex traits such as grain yield, direct and indirect influences of these traits on productivity are not defined (Baretta et al., 2016). In this context, a method was proposed by Wright (1921) which partitions correlation coefficients into the components of direct and indirect effects known as path coefficient analysis. The analysis not only partitions the correlation coefficient into direct and indirect effects, it also provides the information on the actual contribution of a trait on the yield (Dewey and Lu, 1959).

Several maize breeders have used this method for identifying secondary traits as opposed to the use of genetic correlation (Barros et al., 2010; Chinnadurai and Pothraj, 2011; Begum et al., 2016; Baretta et al., 2016, Talabi et al., 2017; Matin et al., 2017). However, the use of path coefficient analysis to identify and validate secondary traits for selection of improved grain yield under water stress and non-stress condition remains a relevant research focus in maize breeding (Bolaños and Edmeades, 1993, 1996; Bänziger and Lafitte, 1997; Badu-Apraku et al., 2004). The study reported herein examined the nature of inter-trait associations between grain yield and other traits under managed drought stress and non-stress con-

ditions, using genotypic and phenotypic correlations, step-wise multiple linear regression and path co-efficient analyses.

2 MATERIALS AND METHOD

2.1 DEVELOPMENT OF THE MARS POPULATION

The details of the development of the marker assisted recurrent selection (MARS) population and data collected have been provided in an earlier report (Bankole et al., 2017). Briefly, a MARS population was developed by crossing two elite drought tolerant maize inbreds (DTPL-W-C7-S2-7-1-1-1-1-B-5-B*4 and Babangoyo/MO17LPA/Babangoyo-23-4-3-3-B*6) selected for desirable agronomic traits, resistance to foliar diseases. The resulting F_1 was selfed to generate F_2 bulk seeds, which was grown in 50 rows of 5 m length with a spacing of 0.75 m and self-pollinated to generate 300 $F_{2:3}$ lines. A total of 250 $F_{2:3}$ lines from this population were planted each in a row and crossed to an inbred tester of the opposite heterotic group. The testcrosses were evaluated under drought stress (DS) and well watered (WW) conditions at the International Institute of Tropical Agriculture (IITA) Ikenne substation during the dry season in 2014 and 2015.

2.2 EXPERIMENTAL LAYOUT AND CULTURAL PRACTICES FOR DROUGHT AND WELL-WATERED EXPERIMENTS

A trial which consist of 150 testcrosses of randomly selected S_1 lines from a MARS population were evaluated under drought and well-watered conditions at Ikenne during the 2014 and 2015 dry seasons. The testcrosses were arranged in a lattice design with two replications. Each of testcross was planted in a single row of 5 m and 0.75 m spacing between the rows and the plants were spaced 0.25 m apart in the rows. In the DS trial, irrigation was withdrawn from six weeks after planting up to the harvest to elicit drought stress at flowering and grain filling stages, whereas the well-watered trial received irrigation until physiological maturity. Compound fertilizer was application at the rates of 60 kg N, 60 kg P, and 60 kg K ha^{-1} was done at the time of sowing and an additional 60 kg N ha^{-1} was added four weeks later. Gramaxone and atrazine were applied for each of the trial as pre-emergence herbicides at 5.0 l ha^{-1} and supplemented with manual weeding to keep the experiments free from weed.

2.3 DATA COLLECTION

Days to anthesis (DP) and silking (DS) were recorded in each of the plot as the number of days from sowing to when half of the plants were shedding pollen grains and showed emerged silks, respectively. Anthesis-silking interval (ASI) was computed as the interval in days between silking and anthesis. Plant height (PH) and ear height (EH) were measured in centimetres as the distance from the base of the plant to the height of the first tassel branch and the node bearing the upper ear, respectively. Plant aspect (PA) was rated on a scale of 1 to 5, where 1 = excellent overall phenotypic appeal and 5 = poor overall phenotypic appeal. Ear aspect (EA) was scored on a scale of 1 to 5, where 1 = clean, uniform, large, and well-filled ears and 5 = rotten, variable, small, and partially filled ears. Visual leaf death (LD) was scored only under drought at 12 weeks after planting (WAP) on a scale of 1 to 9, where 1 = less than 10 % senesced leaf and 9 = more than 80 % senesced leaf area below the ear. The number of ears per plant (EPP) was the proportion of total number of ears divided by the number of harvested plants. All ears harvested from each plot were shelled to determine percentage moisture and grain yield (GY) adjusted to 15 % moisture.

2.4 STATISTICAL ANALYSES

SAS version 9.3 (SAS institute 2011) was used for the regression analyses. The multiple stepwise regression was then used to describe the contributory relationship among the traits under both the drought stress and well-watered environments using the protocol proposed by Mohammadi et al., 2003. The first and second order traits were organized into the path coefficient analysis based on their contribution to the total variation in grain yield with the use of the multiple stepwise regression analysis. Initially all measured traits were regressed on GY and the first order traits were identified by their significant percentage contribution to GY at 5 % probability level. The other traits that made contributions to GY through the first order traits were classified as second order traits. Estimates of genotypic and phenotypic correlation coefficient between GY and other agronomic and yield related traits were carried out using SAS version 9.3 version (SAS Institute, 2011). The Delta method was used to compute the standard errors as proposed by Holland (2006). The genotypic correlation greater than twice the value of its standard error was considered to be significant statistically (Kolawole et al., 2018)

3 RESULTS AND DISCUSSION

The genotypic and phenotypic correlations between grain yield, the primary trait of selection, and other agronomic traits for the MARS testcrosses under drought stress and well-watered conditions are presented in Tables 1 and 2 respectively. For some of the traits such as ear aspect it was not possible to estimate genotypic correlation because of the null values of genetic variance which was identified as 0.00. The results revealed that though the direction and magnitude of the estimates of both correlations were alike for almost all measured traits, the estimates of genotypic correlation were a little higher than those of the phenotypic correlation. This suggests the presence of environmental influence which is responsible for a reduction in estimates of phenotypic correlation despite the strong association between the traits considered. Similar results were reported by Gazal et al., (2018), who observed higher values for genotypic correlation compared with those of the phenotypic correlation.

Significant ($\rho \leq 0.01$) but negative genotypic and phenotypic correlations were observed between grain yield (GY) and flowering traits viz: anthesis silking interval (ASI), days to anthesis (DP) and silking (DS) under drought stress condition. This observation suggests that improvement in GY is associated with a reduction in ASI, DS, PA and EA under drought stress condition (Table 1).

Significant ($\rho \leq 0.01$) but positive genotypic and phenotypic correlations were observed between grain yield (GY) and plant height (PH), and also with ear height (EH). The association between GY and plant aspect (PA) well as between GY and ear aspect (EA) was significant and negative at both genotypic and phenotypic correlations under well-watered condition (Table 2). The improvement in the yield of grains can therefore be associated with an increase in the PH and EH under well-watered conditions which suggests that both traits are good predictors of GY. Lodging is however increased when selection is done for increased PH under non-stressed conditions and this will have a negative impact on GY (Talabi et al., 2017).

Association between ASI, DP, DS, PH, EA and GY under drought stressed and non-stressed conditions have been earlier reported (Bolanos and Edmeades, 1996; Ribaut et al., 1997; Messmer et al., 2009; Zheng et al., 2009 and Liu et al., 2011). Of all the secondary traits measured under drought stress condition only ASI showed significant association with GY at the genotypic level whereas these traits were weakly or not correlated under well-watered condition. This indicates that ASI is a secondary trait that provides the most important adaptive mechanism for drought tolerance in maize. The strong observed correlations of GY with ASI under drought conditions, was in line with the findings of Betran et al. (2003), Cattivelli et al. (2008). Bolanos and Edmeades (1996) reported strong genetic correlations between GY and ASI (r = -0.60) under drought stress and suggested that selection done for GY under drought stress was less efficient than selection for ASI.

The results of the stepwise multiple regression analysis under drought stress conditions showed that EPP, PA, EA, DS, LD and PH had a direct effect which was significant on grain yield with a contribution of about 71.1 % to the total observed variation (Table 3). EPP accounted for the largest proportion of the contribution to grain yield under drought stress (52 %) which is an indication that it is the most important trait in determination of grain yield under drought conditions. With a cumulative contribution of 71.1 % to the observed variation in grain yield, this suggests that these traits can be used in selection programmes as secondary traits for yield improvement under drought stress. Grain yield is therefore expected to increase in response to an increase in any of the traits. Badu-Apraku et al. (2012a) had earlier identified EA, PA, ASI, PH, EH, DS and EPP among some extra early maturing inbred lines as secondary traits to be considered during selection under drought conditions.

Under well-watered condition, EPP also had the highest contribution of 31.42 % to grain yield followed by EA, PA, DP, DS and PH which contributed 15.94, 5.37, 3.13, 10.7 and 0.037 % respectively with a cumulative total of 56.97 %. Similar to drought stress condition, a contribution of these traits was highly significant ($\rho \leq 0.01$) except DS which was significant at 5 % probability level. This is an indication of the level to which these traits are able to influence yield under well-watered condition. EPP has proven to be a trait to be considered when improvement is being carried out for grain yield under both drought stress and well-watered conditions (Bazinger et al., 2000).

Grain yield is complex being an effect of the interrelatedness of several other plant components that influences growth and development, thus deducing with reference to the method of accumulation, combination of its processes through its life cycle proves difficult (Anjum et al., 2011). Tadesse et al. (2018) hypothesized that selection based on simple correlation without taking into cognisance the direct effect of the one variable (independent) on the other (dependent) is ineffective in determining the actual interrelatedness among traits. The use of path coefficient analysis will therefore aid the breeder in deciphering the cause, consequences and importance of the variables therefore providing a more effective means of interpreting the association.

The partitioning of genotypic correlation coefficient with path coefficient analysis showed that DS, PH, PA,

Table 1: Estimates of genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficient and standard errors between grain yield and other agronomic traits for testcrosses of S_1 lines derived from three cycles of marker assisted recurrent selection evaluated under drought stress condition in 2014 and 2015.

	Days to anthesis	Days to silking	Anthesis silking interval	Plant height	Ear height	Husk cover	Plant aspect	Ear aspect	Ears per plant	Grain yield
Days to anthesis		1.00**	1.00*	-1.00	0.00	1.00**	1.00	0.00	-0.80	1.00*
Days to silking	0.80**		1.00*	-1.00	-1.00	0.70*	0.90	0.00	-0.60	-1.00**
Anthesis silking interval	0.20**	0.80**		-1.00	0.00	0.00	1.00	0.00	-1.00	-1.00**
Plant height	-0.40**	-0.50**	-0.40**		1.00	-1.00	-1.00	0.00	0.00	0.00
Ear height	0.00	-0.50**	0.00	0.80**		-1.00	0.00	0.00	1.00	0.00
Husk cover	0.20**	0.30**	0.00	-0.30**	-0.30**		0.80	0.00	-0.30	-0.60*
Plant aspect	0.50**	0.50**	0.40**	-0.60**	0.00	0.30**		0.00	-1.00	-1.00
Ear aspect	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00
Ear per plant	-0.30**	-0.50**	-0.50**	0.00	0.40**	-0.40**	-0.60**	0.00		1.00
Grain yield	-0.50**	-0.60**	-0.50**	0.00	0.00	-0.40**	-0.70**	0.00	0.70	

*, ** Significant at 0.05 and 0.01 probability levels respectively

Table 2: Estimates of genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficient and standard errors between grain yield and other agronomic traits for testcrosses of S1 lines derived from three cycles of marker assisted recurrent selection evaluated under well-watered condition in 2014 and 2015

	Days to anthesis	Days to silking	Anthesis silking interval	Plant height	Ear height	Husk cover	Plant aspect	Ear aspect	Ear per plant	Grain yield
Days to anthesis		1.00**	-1.00	0.40	0.30	-0.20	0.00	-0.10	-0.10	-0.01
Days to silking	0.90**		-1.00	0.50	0.30	0.12	0.00	-0.01	-0.30	-0.10
Anthesis silking interval	-0.50**	-0.10**		0.02	-1.00	1.00	-1.00	1.00	-1.00	-0.20
Plant height	-0.10**	-0.10**	0.01		0.90**	-0.40**	0.00	-1.00*	-0.30	0.90**
Ear height	-0.10**	-0.10**	0.01	0.80		-0.10	0.00	-1.00*	0.50	0.90**
Husk cover	-0.10**	-0.00**	0.10	-0.00	0.01		0.00	0.30	0.10	-0.30
Plant aspect	0.00	0.00	0.10	0.00	0.00	0.00			0.00	-1.00*
Ear aspect	0.15**	0.20**	-0.00	-0.20**	-0.20**	0.10*	0.00		-0.70	-1.00**
Ear per plant	-0.20**	-0.20**	0.02	0.20**	0.20**	-0.00	0.00	-0.10		1.00
Grain yield	-0.30**	-0.30**	0.02	0.40**	0.30**	-0.01	-0.48	-0.50**	0.60**	

*,** Significant at 0.05 and 0.01 probability levels.

Table 3: Multiple stepwise regression of other agronomic traits on grain yield of marker assisted recurrent selection maize testcrosses evaluated under drought stress and well-watered conditions at Ikenne in 2014 and 2015

Drought stress condition				Well-watered condition			
Trait	R^2	Percent contribution to grain yield	F- value	Trait	R^2	Percent contribution to grain yield	F- value
Ear per plant	0.52	51.72	607.47**	Ear per plant	0.31	31.42	285.03**
Plant aspect	0.61	9.69	142.45**	Ear aspect	0.47	15.94	188.00**
Ear height	0.67	5.25	89.32**	Plant aspect	0.53	5.37	70.45**
Days to silking	0.69	2.70	49.78**	Days to anthesis	0.56	3.13	43.97**
Leaf death	0.70	0.96	18.21**	Plant height	0.57	1.07	15.34**
Plant height	0.71	0.80	15.59**	Days to silking	0.57	0.37	5.37*

EA, LD and EPP as having a direct effect on GY. These six first order traits were recognized as being important in contributing to the observed variation in GY as shown from the path coefficient analysis (Fig 1) thus signifying them as potential secondary traits used as a selection criterion under drought stress. These findings corroborate those of Talabi et al. (2016) who identified EA, EPP, PA, STGR, DS, EHT and SL as traits with direct significant traits affecting GY under drought stress conditions.

EPP had the highest direct effect (0.37) on GY followed by DS (0.22) under drought stress condition. However, only the effects of EPP and PH were positive while the other direct effects were negative. Four traits (DP, ASI, EH and HC) also made contributions to GY but the contributions were indirect through DS (DP and ASI), PH (EH and HC), PASP (DP and EH) and EPP (DP, ASI, EH). EH and HC also contributed indirectly to grain yield through LD. ASI a known secondary trait contributed to GY indirectly through the six first order traits indicating that it should be considered as trait of importance during selection under drought stress. DP had the highest indirect positive effect on GY through DS (0.74) followed by EH through PH (0.73). The high coefficient reported for EPP corroborates the findings of Badu Apraku et al. (2018) who reported the highest path coefficient for EPP among extra early maize cultivars under drought stress conditions.

Under well-watered condition, the first order traits include EPP, EA, PA, DP and PH (Fig 2) with EPP (0.56) having the highest direct effect among the traits followed by EA (-0.40). Similar to the result under drought stress, only EPP and PH had positive direct effect on GY while other first order traits were also negative. None of the second order traits which include DP, ASI, EH and HC made contributions through all the first order traits simultaneously. DS had the highest contribution to GY through DP (0.91) followed by the contributions of EH through PH (0.87).

Increased EPP together with some other traits had been identified as a secondary trait used as a selection criterion (Bazinger et al., 1997) under drought stress conditions, including PH which also had a direct positive effect on GY. Selection for increased EPP may be accomplished without an adverse effect under well-watered condition, but selection for increased plant height though may aid the plant to synthesize more assimilates resulting in production of more grains but usually at a price. Increased plant height usually predisposes the plant to lodging which adversely affects grain production. The proportion of the variation in the dependent variable that is influenced by the other variables (independent) is less than half under drought as reflected by the coefficient of determination. The high positive and direct effect of plant

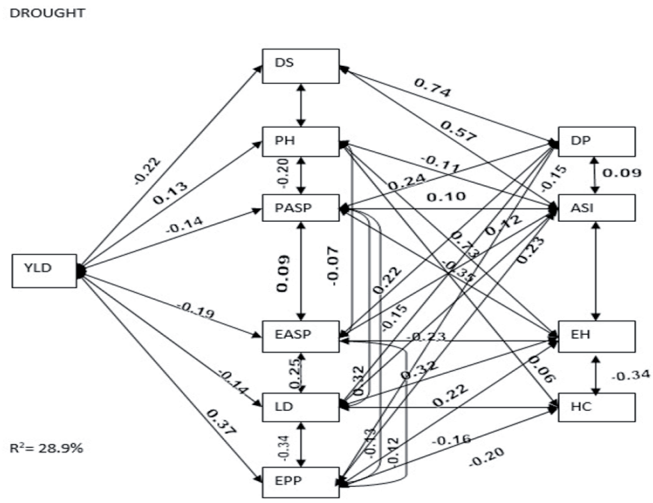

Figure 1: Path analysis diagram showing contributing associations of measured traits of testcrosses of S1 lines of marker assisted recurrent selection evaluated under drought conditions at Ikenne in 2014 and 2015. YLD, yield; ASI, anthesis–silking interval; DP, days to anthesis; DS, days to silking; EASP, ear aspect; EPP, ears per plant; PASP, plant aspect; PH, plant height; EH, ear height; LD, leaf death; HC, husk cover.

height on the yield corroborates the findings of Adesoji et al. (2015) who reported the highest positive and direct effect of plant height to grain yield in a maize population grown under legume incorporation and nitrogen. Therefore, the use of PH as a selection criterion may not be practical due to its non-beneficial effect on production of grains.

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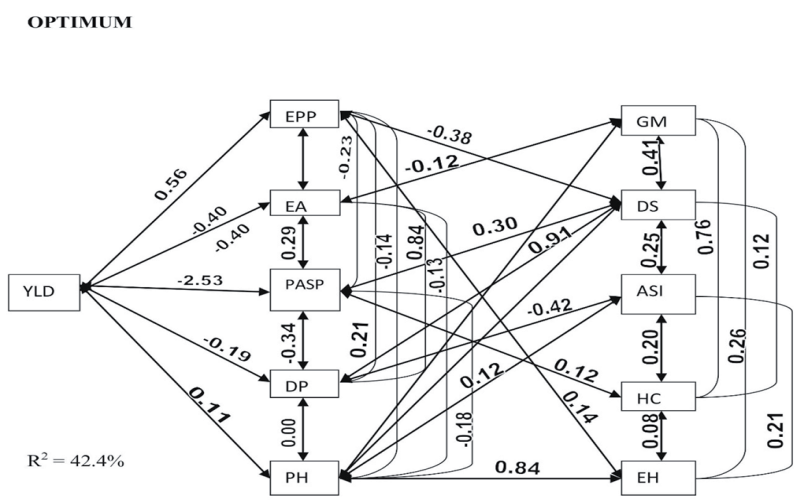

Figure 2: Path analysis diagram showing contributing associations of measured traits of testcrosses of S1 lines of marker assisted recurrent selection evaluated under well-watered conditions at Ikenne in 2014 and 2015. YLD, yield; ASI, anthesis–silking interval; DP, days to anthesis; DS, days to silking; EASP, ear aspect; EPP, ears per plant; PASP, plant aspect; PH, plant height; EH, ear height; HC, husk cover.

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The high positive and direct effect of plant height on the yield corroborates the findings of Adesoji et al. (2015) who reported the highest positive and direct effect of plant height to grain yield in a maize population grown under legume incorporation and nitrogen. Therefore, the use of PH as a selection criterion may not be practical due to its non-beneficial effect on production of grains.

4 CONCLUSION

It can be concluded that though varying results were obtained with the use of correlation with respect to the measured traits associated with grain yield under both conditions, similar results were however obtained for the MARS testcrosses under both conditions with the use of multiple stepwise regression and path coefficient analysis. The coefficient of determination however showed variation: with the use of regression the dependent trait influenced the first order traits more under drought condition but reverse was the case with the use of path analysis. Ear aspect, plant aspect and ears per plant were identified as secondary traits that will aid selection for improved yield of maize. Leaf death; which is not one of the observed traits under well-watered condition was also identified as a secondary trait under drought stress. These traits are rated based on phenotypic appeal therefore their determination is highly subjective. Extra caution should therefore be taken when scoring to increase precision.

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6 REFERENCES

Anjum S.A., L.C. Wang, M. Farooq, M. Hussain, L.L. Xue and C.M. Zou (2011). Brassinolide application improves the drought tolerance in maize through nodulation of enzymatic antioxidant and leaf gas exchange. *Journal of Agronomy and crop science, 197,* 177-185. https://doi.org/10.1111/j.1439-037X.2010.00459.x

Asima Gazal, Zahoor Ahmed Dar, Ajaz Ahmad Lone, Nida Yousuf and Shazia Gulzar (2018). Studies on Maize Yield under Drought Using Correlation and Path Coefficient Analysis. *Internantional Journal of Current Microbiology and Applied Science, 7*(01), 516-521. https://doi.org/10.20546/ijcmas.2018.701.062

Badu-Apraku B., A. O. Talabi, B. E. Ifie, Y. C. Chabi, K. Obeng-Antwi, A. Haruna, and R. Asiedu (2018). Gains in Grain Yield of Extra-Early Maize during Three Breeding Periods under Drought and Rainfed Conditions. *Crop Science*. https://doi.org/10.2135/cropsci2018.03.0168

Badu-Apraku, B., R.O. Akinwale, J. Franco, and M. Oyekunle. (2012a). Assessment of reliability of secondary traits in selecting for improved grain yield in drought and low nitrogen environments. *Crop Science, 52*, 2050-2062. https://doi.org/10.2135/cropsci2011.12.0629

Badu-Apraku, B., M.A.B. Fakorede, A. Menkir, A.Y. Kamara, L. Akanvou, and Y. Chabi. (2004). Response of early maturing maize to multiple stresses in the Guinea savanna of West and Central Africa. *Journal of Genetics and Breeding, 58*, 119-130.

Bankole F., A. Menkir, G. Olaoye, J. Crossa, S. Hearne, N. Unachukwu and M. Gedil (2017). Genetic gains in grain yield and other traits in a maize population improved using marker assisted recurrent selection. Frontiers in plant science. https://doi.org/10.3389/fpls.2017.00808

Bänziger M., H.R. Lafitte (1997a). Efficiency of secondary traits for improving maize for low-nitrogen target environments. *Crop Science, 37*, 1110-1117. https://doi.org/10.2135/cropsci1997.0011183X003700040013x

Barata C, M.J. Carena (2006). Classification of North Dakota maize inbred lines into heterotic groups based on molecular and testcross data. *Euphytica, 151*, 339-349. https://doi.org/10.1007/s10681-006-9155-y

Baretta D, M. Nardino, I.R. Carvalho and R. Nornberg (2016). Path analysis for morphological characters and grain yield of maize hybrids. Australian. *Journal of Crop Science, 10,* 1655-1661. https://doi.org/10.21475/ajcs.2016.10.12.p7707

Barima, Y.S. D. Angaman, K. N'gouran, N. KoffI, F. Kardel, C. DE Cannière, R. Samson, (2014). Assessing atmospheric particulate 25 matter distribution based on Saturation Isothermal permanent magnetization of herbaceous and tree leaves in a tropical urban environment. *Science of the Total Environment,* 975-982. https://doi.org/10.1016/j.scitotenv.2013.10.082

Barros L.B., Moreira R.M.P., Ferreira J.M. (2010) Phenotypic,

additive genetic and environment correlations of maize landraces populations in family farm systems. *Scientia Agricola (Piracicaba, Braz.), 67*, 685-691. https://doi.org/10.1590/S0103-90162010000600010

Bänziger, M., G.O. Edmeades, D. Beck, and M. Bellon. (2000). *Breeding for Drought and Nitrogen Stress Tolerance in Maize: From Theory to Practice*. Mexico, D.F.: International Maize and Wheat Improvement Centre (CIMMYT)

Begum, S., A. Ahmed, S.H. Omy, M.M. Rohman. and M. Amiruzzaman (2016). Genetic Variability, Character Association and Path Analysis in Maize (*Zea mays* L.). *Bangladesh Journal of Agricultural Research, 41*, 173-182. https://doi.org/10.3329/bjar.v41i1.27682

Betrán F. J., D. Beck, M. Bänziger and G.O. Edmeades (2003). Genetic analysis of inbred and hybrid grain yield under stress and non-stress environments in tropical maize. *Crop Science, 43*, 807-817. https://doi.org/10.2135/cropsci2003.0807

Bolaños J., G.O. Edmeades (1996). The importance of the anthesis-silking interval in breeding for drought tolerance in tropical maize. *Field Crops Research, 48,* 65-80. https://doi.org/10.1016/0378-4290(96)00036-6

Bolaños J., G.O. Edmeades (1993). Eight cycles of selec¬tion for drought tolerance in tropical maize II. Responses in reproductive behaviour. *Field Crop Research, 31*, 253-268. https://doi.org/10.1016/0378-4290(93)90065-U

Boyer J. and M. Westgate (2004). Grain yields with limited water. *Journal of Experimental Botany, 55*, 2385-2394. https://doi.org/10.1093/jxb/erh219

Byrne, P.F., J. Bolanos, G.O. Edmeades, D.L. Eaton (1995). Gains from selection under drought versus multilocation testing in related tropical maize populations. *Crop Science, 35*, 63-69. https://doi.org/10.2135/cropsci1995.0011183X003500010011x

Cattivelli L., F. Rizza, F.W. Badeck, E. Mazzucotelli, A.M. Mastrangelo, E. Francia E., C. Mare, A. Tondelli, A.M. Stanca (2008). Drought tolerance improvement in crop plants: An integrative view from breeding to genomics. *Field Crop Research, 105,* 1-14. https://doi.org/10.1016/j.fcr.2007.07.004

Cooper M., C. Gho, R. Leafgren, T. Messina (2014). Breeding drought-tolerant maize hybrids for the US corn-belt: discovery to product. *Journal of Experimental Botany, 65,* 6196-6204. https://doi.org/10.1093/jxb/eru064

Derera J., Tongoona, P., Vivek, B.S., Laing, M.D. (2008). Gene action controlling grain yield and secondary traits in southern African maize hybrids under drought and non-drought environments. *Euphytica, 162*, 411 422. https://doi.org/10.1007/s10681-007-9582-4

Dewey, D.R., and K.H. Lu. (1959). A correlation and path co-efficient analysis of components of crested wheat seed produc¬tion. *Agronomy Journal, 51*, 515-518. https://doi.org/10.2134/agronj1959.00021962005100090002x

Edmeades, G.O., Bolaños, J., Chapman, S.C., Lafitte, H.R. and M. Bänziger (1999). Selection improves drought tolerance in tropical maize populations: Gains in biomass, grain yield and harvest index. *Crop Science, 39*, 1306-1315. https://doi.org/10.2135/cropsci1999.3951306x

Edmeades G.O., Banziger, M., Chapman, S.C., Ribaut, J.M. and J. Bolanos (1995). Recent Advances in Breeding for Drought Tolerance in Maize. In B. Badu-Apraku et al. (ed.) *Contributing to food self-sufficiency: Maize Research and Development in West and Central Africa.* Proc. of Regional Maize Workshop. 28 May-2 June 1995. IITA-Cotonou, Benin Republic. IITA, Ibadan, Nigeria. pp. 24-41.

FAO. (2010). The state of food insecurity in the world. Addressing food insecurity in protracted crises. Available at http://www.fao.org/publications/sofi /en/ (verified 10

Frey F.P., Urbany, C., Hüttel, B., Reinhardt, R. and B. Stich (2015). Genomewide expression profiling and phenotypic evaluation of European maize inbreeds at seedling stage in response to heat stress. BMC *Genomics, 16*,123. https://doi.org/10.1186/s12864-015-1282-1

Gong F, Wu, X., Zhang, H., Chen, Y. and W. Wang (2015). Making better maize plants for sustainable grain production in a changing climate. *Frontiers in Plant Science, 6*, 835. https://doi.org/10.3389/fpls.2015.00835

Holland J.B. (2006). Estimating genotypic correlations and their standard errors using multivariate restricted maximum likelihood estimation with SAS Proc MIXED. *Crop science, 46*, 642-654. https://doi.org/10.2135/cropsci2005.0191

Horton D.E., Johnson, N.C., Singh, D., Swain, D.L., Rajaratnam, B. and N.S. Diffenbaugh (2015). Contribution of changes in atmospheric circulation patterns to extreme temperature trends. *Nature, 522*, 465-469. https://doi.org/10.1038/nature14550

Kolawole A.O., Menkir, A. Blay, E. Ofori, K. and J. Kling (2018). Genetic advance in grain yield and other traits in two tropical maize composites developed via reciprocal recurrent selection. *Crop Science 58*:2360-2369 https://doi.org/10.2135/cropsci2018.02.0099

Lobell D.B., M. Bänziger, C. Magorokosho B. Vivek (2011). Non-linear heat effects on African maize as evidenced by historical yield trials. *Nature Climate Change, 1,* 42-45. https://doi.org/10.1038/nclimate1043

Lobell D.B., Roberts, M.J. Schlenker, W. Braun, N. and B.B. Little (2014). Greater sensitivity to drought accompanies maize yield increase in the U.S. Midwest. *Science, 344*, 516-519. https://doi.org/10.1126/science.1251423

Malik H.N., Malik, S.I. Hussain, M. Chughtai, S.R. and H.I. Javed (2005). Genetic correlation among many quantitative characters in maize (*Zea mays)* hybrids. *Journal of Agriculture and social sciences, 1*(3), 262-265.

Matin, M.Q., Uddin, M., Rohman, M., Amiruzzaman, M., Azad, A. and B. Banik (2017). Genetic Variability and Path Analysis Studies in Hybrid Maize (*Zea mays* L.). *American Journal of Plant Sciences, 8,* https://doi.org/10.4236/ajps.2017.812209

Menkir, A. (2008). Genetic variation for grain mineral content in tropical adapted maize inbred lines. *Food chemistry, 110*, 454-464. https://doi.org/10.1016/j.foodchem.2008.02.025

Messmer R., Fracheboud, Y., Banziger, M., Vargas, M., Stamp, P. and J.M. Ribaut (2009). Drought stress and tropical maize: QTL-by-environment interactions and stability of QTLs across environments for yield components and secondary traits. *Theoretical and Applied Genetics, 119*, 913-930. https://doi.org/10.1007/s00122-009-1099-x

Mhike X., Okori, P., Magorokosho, C. and K. Semagn (2013). QTL mapping for morpho-physiological traits of MARS

targeted maize biparental crosses under moisture stress and non-stress environments. *11th African Crop Science Proceedings, Sowing innovations for sustainable food and nutrition security in Africa., Uganda, 14-17 October, 2013,* pp.553-558 ref.19 Entebbe.

Mohammadai H., Soleymani, A. and M. Shams (2012). Evaluation of Drought Stress Effects on Yield Components and Seed Yield of Three Maize Cultivars (Zea mays L.) in Isfahan region. *International Journal of Agriculture and Crop Sciences, 4*(19), 1436-1439.

Mohammadi R., Amri, A. and M. Nachit (2011). Evaluation and Characterization of International Durum Wheat Nurseries under Rainfed Conditions in Iran. *International Journal of Plant Breeding, 5*(2), 94-100.

Mohammadi R, Armion, M. Kahrizi, D. and A. Amri (2010). Efficiency of screening techniques for evaluating durum wheat genotypes under mild drought conditions. *Journal of Plant Production, 4*(1), 11-24.

Mohammadia, S.A., Prasanna, B. M. and N.N. Singh (2003). Sequential path model for determining interrelationship among grain yield and related characters in maize. *Crop Science, 43*, 1690-1697. https://doi.org/10.2135/cropsci2003.1690

Najafian G. (2009). Drought tolerance indices, their relationships and manner of application to wheat breeding programmes. In: Mohammadi R, Haghparast R (Eds) Plant Science in Iran. *Middle Eastern and Russian Journal of Plant Science and Biotechnology, 3*(Special Issue 1), 25-34.

Nouri A, Etminan, A. Teixeira da Silva, J.A. and R. Mohammadi (2011). Assessment of yield, yield-related traits and drought tolerance of durum wheat genotypes (Triticum turjidum var. durum Desf.). *Australian Journal of Crop Science, 5*(1), 8-16.

Ober E.S. (2008). Breeding for improved drought tolerance and water use efficiency in HGCA (Eds): *Arable cropping in a changing climate HGCA Higham*, pp 28 - 37.

Olaoye G. (2009). Evaluation of new generations of maize streak virus (msv) resistant varieties for grain yield, agronomic potential and adaptation to Southern Guinea savanna ecology of Nigeria. *Journal of Tropical Agriculture, Food, Environment and Extension, 8*(2), 104-109. https://doi.org/10.4314/as.v8i2.51107

Ort D. and S. Long (2014). "Limits on Yields in the Corn Belt." *Science, 344*, 484-85. https://doi.org/10.1126/science.1253884

Rajaram S. and M. Van Ginkle (2001). Mexico, 50 years of international wheat breeding. In Bonjean A.P., and Angus W.J. (eds) *The world Wheat Book, A History of Wheat Breeding*, Paris, France. Lavoisier Publishing, pp 579-604.

Ribaut J.M., Jiang, C., Gonzalez-de-Leon, D., Edmeades, G.O. and D.A. Hoisington (1997). Identification of quantitative trait loci under drought conditions in tropical maize. 2. Yield components and marker-assisted selection strategies. *Theoretical and Applied Genetics, 94*, 887-896. https://doi.org/10.1007/s001220050492

SAS Institute (2009). *SAS system for Windows v. 9.3*. SAS Inst. Inc., Cary, NC.

Semagn K, Beyene, Y., Babu, R., Nair, S. Gowda, M.,Das, . B., Tarekegne, A., Mugo, S., Mahuku, G., Worku, M., Warburton, M.L., Olsen, M. and B.M. Prasanna (2015). Quantitative trait loci mapping and molecular breeding for developing stress resilient maize for sub-Saharan Africa. *Crop Science, 55*, 1-55. https://doi.org/10.2135/cropsci2014.09.0646

Sio-Se Mardeh A., Ahmadi, A., Poustini, K. and V. Mohammadi (2006). Evaluation of drought resistance indices under various environmental conditions. *Field Crop Research, 98*, 222-229. https://doi.org/10.1016/j.fcr.2006.02.001

Tadesse L., Mekbib, F., Wakjira, A. and Z. Tadele (2018). Correlation and path coefficient analysis of yield and quality components of garden cress (Lepidium sativum L.) genotypes in Ethiopia. *Journal of Plant Breeding and Crop Science, 10*(10), 290-297. https://doi.org/10.5897/JPBCS2018.0757

Talabi, A.O., Badu-Apraku, B. and M.A.B. Fakorede. (2017). Genetic variances and relationship among traits of an early-maturing maize population under drought-stress and low N environ¬ments. *Crop Science, 57*, 681-692. https://doi.org/10.2135/cropsci2016.03.0177

Tardieu F. and R. Tuberosa (2010). Dissection and modeling of abiotic stress tolerance in plants. *Current Opinion in Plant Biology, 13,* 206-212. https://doi.org/10.1016/j.pbi.2009.12.012

Ti-da G.E., Fang-Gong, S.U.I. and B.A. Ping (2006). Effects of water stress on the protective enzymes and lipid peroxidation in roots and leaves of summer maize. *Agricultural Science China, 5*, 291-298. https://doi.org/10.1016/S1671-2927(06)60052-7

Venuprasad R., Lafitte, H.R. and G.N. Atlin (2007). Response to direct selection for grain yield under drought stress in rice. *Crop Science, 47*, 285-293. https://doi.org/10.2135/cropsci2006.03.0181

Williams A.P. and C. Funk. (2011). A westward extension of the warm pool leads to a westward extension of the Walker circulation, drying eastern Africa. *Climate Dynamics, 37*, 2417-2430. https://doi.org/10.1007/s00382-010-0984-y

Wright, S. (1921). Correlation and Causation. *Journal of Agricultural Research, 20*, 557-585.

Xoconostle-Cazares B, Ramirez-Ortega, F.A., Flores-Elenes, L. and R. Ruiz Medrano (2011). Drought tolerance in crop plants. *American Journal of Plant Physiology*. ISSN 1557-4539

Zheng J., Wu, A.Z., A.Z. Zheng, A.Z., Wang, Y.F., Cai, R., Shen, X.F., Xu, R.R., Liu, P., Kong, L.J. and S.T. Dong (2009). QTL mapping of maize (*Zea mays*) stay-green traits and their relationship to yield. *Plant Breeding, 128*, 54-62. https://doi.org/10.1111/j.1439-0523.2008.01529.x

Ziyomo C. and R. Bernardo (2013). Drought tolerance in maize: indirect selection through secondary traits versus genome wide selection. *Crop Science, 53*, 1269-12. https://doi.org/10.2135/cropsci2012.11.0651.

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Biochar application in alkaline soil and its effect on soil and plant

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Biochar application in alkaline soil and its effect on soil and plant

Abstract: Scientists reported that biochar can improve soil properties in acidic soils, while in alkaline soils were shown negative results. A field study was done to evaluate the effect of biochar application solely in alkaline soil compared with biochar composts with farm yard manure (BC-FYM) and sulfur (BC-S). The results revealed that using solely biochar decreased yield of potatoes tubers to more than 6 % and 10 % using mineral and organic fertilization, respectively. This was attributed to the alkalinity effect of biochar and raises the soil pH, which might precipitate macro and micro elements in soil and become unavailable for plant absorption. While using mixtures of BC-FYM and BC-S were shown to enhance yield productivity of potatoes tubers 11.7 % and equal to control under mineral fertilization; and 25.13 % and 10.53 % using organic fertilization, respectively. Mixture of BC-FYM and BC-S proved to have the ability for recovering the alkalinity effect of biochar, improve nutrients availability in soil and increase crop yield of potatoes. In general, mixing biochar with FYM was efficient, economical and environmentally sound solution in alkaline soils.

Keywords: biochar; alkaline soil; potatoes; nutrient availability; crop yield

Uporaba oglja na alkalnih tleh in učinek na tla in rastlino

Izvleček: Znanstveniki poročajo, da uporaba oglja izboljša lastnosti kislih tal, medtem ko so učinki na alkalnih tleh negativni. V poljskem poskusu so bili ovrednoteni učinki uporabe samo oglja v primerjavi z njegovo kombinacijo s hlevskim gnojem (BC-FYM) in žveplovimi spojinami (BC-S). Rezulati so pokazali, da je uporaba samo oglja zmanjšala pridelek krompirja za več kot 6 %, oziroma 10 %, ko je bilo gnojeno z mineralnimi in organskimi gnojili. To je bila posledica alkalnega učinka oglja preko dviga pH tal, kar je lahko oborilo mikro in makro elemente in jih naredilo nedostopne za prevzem v rastline. Uporaba mešanic BC-FYM in BC-S je povečala pridelek gomoljev krompirja za 11,7 %, kar je bilo enako kontrolnemu obravnavanju pri gnojenju z mineralnimi (25,13 %) in organskimi (10,53 %) gnojili. Mešanica BC-FYM in BC-S je imela dokazano sposobnost blaženja alkalnega učinka oglja, kar je izboljšalo dostopnost hranil v tleh in povečalo pridelek gomoljev krompirja. Nasplošno je bilo mešanje oglja s FYM učinkovita, ekonomsko in okoljsko dobra rešitev na alkalnih tleh.

Ključne besede: oglje; alkalna tla; krompir; dostopnost hranil; pridelek

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1 INTRODUCTION

Biochar is a carbon(C) -rich product obtained by thermal decomposition of biomass at relatively high temperatures (<700 °C) and absence of oxygen, in a process known as pyrolysis (Verheijen et al. 2010). Biochar claimed to have potential benefits for soil including water holding capacity (Busch et al., 2012; Busscher et al., 2010; Kammann et al., 2012; Karhu et al., 2011), water infiltration (Asai et al., 2009; Ippolito et al., 2012), soil water availability (Baronti et al., 2014), nutrient retention (Clough et al., 2013; Ventura et al., 2013), hydraulic conductivity (Buss et al., 2012), and soil aeration (Case et al., 2012; Cayuela et al., 2013), increased microbial activity (Lehmann et al., 2011; Warnock et al., 2007), shifts in microbial diversity (Jin, 2010), increase in electrical conductivity (Husson, 2012) and immobilization of contaminants such as trace elements (especially Cu) (Borchard et al., 2012; Buss et al., 2012; Ippolito et al., 2012) or pesticides (Gomez-Eyles et al., 2013; Graber et al., 2012). However, significant increase in soil fertility, plant growth and yield was reported due to biochar application in tropical and subtropical soils (Asai et al., 2009; Atkinson et al., 2010; Glaser et al., 2002; Lehmann and Rondon, 2006; Lehmann and Steiner, 2009a; Major et al. 2010). This was attributed to the liming effect of biochar which decrease significantly soil acidity, resulting in better conditions for growing crops (Steiner et al. 2007; Yuan and Xu2011). The application of biochar in alkaline soils showed different effects: the application of biochar solely lead to reduction in crop yield in alkaline soil. This was reported by many scientists (Ding et al., 2010; Graber and Elad, 2013; Jin, 2010; Taghizadeh-Toosi et al., 2011) who referred this effect to nutrients adsorption onto biochar surface (e.g. the adsorption of ammonium, phosphate and other cations). Consequently, to avoid the alkalinity effects of biochar, different suggestions were proposed, such as enhancement of biochar with organic or mineral nutrients (Alburquerque et al., 2012; Bruun et al., 2011; Gathorne-Hardy et al., 2009; Joseph et al., 2013a, b), composting BC with compost (Fischer and Glaser, 2012; Steiner et al., 2010), charge the porous biochar matrix with nutrients, stimulate microbial colonization (Pietikäinen et al., 2003), reduce noxious pyrogenic materials during production of BC (Tuomela et l., 2000), or increase the biochar surface reactivity using enhanced oxidative ageing (Cheng and Lehmann, 2009b; Zimmerman, 2010) as well as DOC adsorption (Prost et al., 2012). Thus, in the present study, we aimed to reduce alkalinity effect of BC through composting BC with farmyard manure (BC-FYM) and sulfur (BC-S) for enhancing the elements availability, crop yield and crop quality in alkaline soil as compared with freshly produced biochar (BC) under recommended mineral and organic fertilizer conditions.

2 MATERIALS AND METHODS

2.1 PRODUCTION OF BIOCHAR

Eggplant shoots were used to synthesize BC under low oxygen conditions using small-scale unit. The unit was designed as described by Abd el-hafez et al (2014). Briefly, barrel with a diameter of 55 × 85 cm was served as a burning barrel. For the lid, a well tight lid of the burning barrel with another half barrel inverted and supported with 20 cm diameter chimney tube was used to

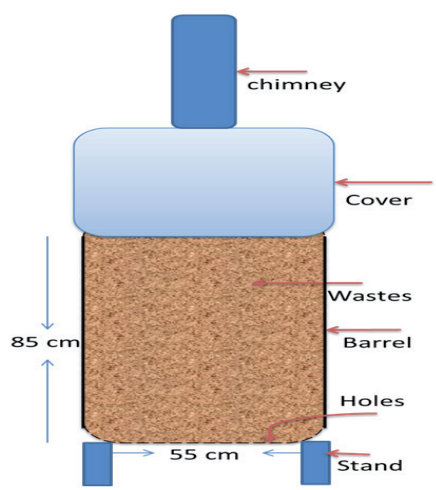

Figure 1: Scheme of designed unit for biochar production.

Table 1: Physical and chemical characteristics of the experimental soil

	Particle size distribution %						
Soil depth	Coarse sand	Fine sand	Silt	Clay	Texture class	OM %	$CaCO_3$ %
0 - 20	6.18	19.4	37.3	36.6	Clay loam	2.80	1.85
20 - 50	13.2	26.0	33.3	29.5	Clay loam	2.70	2.20
50 - 70	10.5	20.5	35.2	33.8	Clay loam	2.38	2.25

			Soluble anions (mmol l^{-1})				Soluble cations (mmol l^{-1})			
Soil depth	pH	EC (dS m^{-1})	CO_2^{--}	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+
0 - 20	7.93	3.41	0.00	1.80	26.70	4.50	9.20	6.40	19.90	0.50
20 - 50	8.01	3.52	0.00	2.20	25.30	6.50	10.00	5.50	18.00	0.50
50 - 70	8.02	2.32	0.00	1.70	14.60	3.70	9.00	3.20	10.50	0.30

cover the burning barrel (Fig. 1). The unit was stroked from the bottom in addition to three metal sheets were placed at the bottom of the unit to guarantee that the air derives up regularly. The produced biochar was denoted as BC. Then, BC was mixed and composted with farmyard manure (BC-FYM) and/or sulfur (BC-S) with a ratio of 1:1 (mass/mass) for 3 months. The stack was covered, stirred and moisturized every week. The final products were added to soil during soil preparation two week before sowing day. Different physical and chemical analyses were done on initial unfertilized soil as described below (Table 1).

2.2 EXPERIMENT SETUP

The investigation was carried out in Dokki site El-Giza governorate, Egypt which is situated at 30° 03` N latitude, 31° 20` E longitude during winter time of 2015 and 2016 to explain the effect of modified BC on growth and yield of potatoes (*Solanum tuberosum* L.) grown in alkaline soil. A field experiment was done at a clay loam soil, around 250 m^2 were roared and cleaned from weeds. This land was divided into plots (3 x 3.5 m), These treatments were evaluated at two kinds of fertigation (mineral and organic). Split plot design was used in this experiment as follows: main plots were divided into 1) mineral fertigation and 2) organic fertigation, while sub-main plots were used the different BC treatments including (☐) control, (☐) BC, (☐) BC-FYM, and (☐) BC-S. BC dose was fixed at 12 Mg ha^{-1} for each kind of BC. Each treatment was replicated three times. Required quantities of BC were added to the selected treatment plots and were mixed thoroughly with the soil using spade at January two weeks before sowing date. The recommended dose of NPK nutrients were added to all mineral fertilization treatments (including control) through ammonium sulphate, mono superphosphate and potassium sulphate, respectively. While full doses of P were applied as basal with BC, 50 % of the N and K doses were applied as basal and the remaining 50 % were top-dressed after 1 month from planting. Organic fertilization (30 t h^{-1}) was applied according to N % before sowing with two weeks. Each plot was divided into three rows (width 90 cm and highest 30 cm). A tunnel was made in each row and tubers ('Spunta') were planted by hand at 10 cm depth and 25 cm spacing between tubers, then tunnels were covered with soil and the field was irrigated using drip irrigation. The soil was irrigated when required, and was kept weed-free by hand weeding.

2.3 EXPERIMENTAL ANALYSIS

Total N of biochar was determined in the supernatant of digested biochar by mixture of sulfuric and salicylic acid using Kjeldahl method according to Jones J. Benton. (1991), while total C of biochar was measured following ASTM1762-84 (American Standard of Testing Material, 2001). EC and pH of biochar was determined as described by Masulili et al (2010). Briefly, 1 g of material was dissolved in 100 ml de-ionized water under heating to 90 °C and stirred for 20 minutes. Then the suspensions were cooled to room temperature which after EC and pH was measured using EC and pH-meter (Masulili et al., 2010). To determine P and K soil samples were digested using hydrochloric and nitric acid (Cottenie et al., 1982), while for N determination another mixture of acids were used for digestion as described by Jones J. Benton. (1991). Nutrients accumulated in tubers were determined af-

Table 2: Chemical characteristics of materials used in the experiment

Parameter	FYM	Compost	BC	BC-FYM	BC-S
EC μS cm^{-1}	456	2080	1085	846	818
pH	7.00	7.6	7.6	7.4	6.9
OC %	34.80	12.5	38.20	37.78	36.63
C:N ratio	24.26	12.5	509.3	47.52	466.6
N %	1.40	1.00	0.075	0.795	0.0785
P %	0.071	0.41	0.074	0.136	0.044
K %	0.085	0.32	0.003	0.042	0.002
Fe %	1.90	0.35	1.21	0.906	0.61
Mn mg kg^{-1}	423.60	61.9	257.75	269.70	121.65
Zn mg kg^{-1}	77.25	79.2	81.25	107.15	0.60
Cu mg kg^{-1}	39.40	24.1	45.35	43.30	30.55
B mg kg^{-1}	19.15	32.6	14.40	14.00	8.30

ter digestion using mixture of sulfuric and perchloric acid (5:1). P was determined in the solution digested using inductively coupled plasma (ICP- JY ULTIMA). Chemical analysis results for materials used are in Table 2.

2.3.1 Quantitative determination of surface acidic groups

Biochar surface acid functional groups were determined according to the description of Boehm titration method (Boehm et al., 1964 and Mukherjee et al., 2011). Briefly, about 0.5 g of coarse biochar sample was added to 50 ml of each of three 0.05 M bases of $NaHCO_3$, Na_2CO_3 and NaOH. Then, the mixtures beside control solution without any material were shaken for 24 h. Thereafter, the mixtures were filtered through a 42 Whatman filter paper to remove solids. Then, a 1 ml of suspension from each filtrate was added to 10 ml of HCl (0.05 M) to guarantee complete neutralization of bases and then back-titrated with NaOH (0.05 M). Phenolphthalein color indicator was used to identify the endpoint. The total surface acidity was calculated as the moles neutralized by NaOH, and the carboxylic acid groups as the moles neutralized by $NaHCO_3$, and the lactonic groups as those neutralized by Na_2CO_3. The difference between moles neutralized by NaOH and Na_2CO_3 was considered as phenolic groups content (Rutherford et al., 2007).

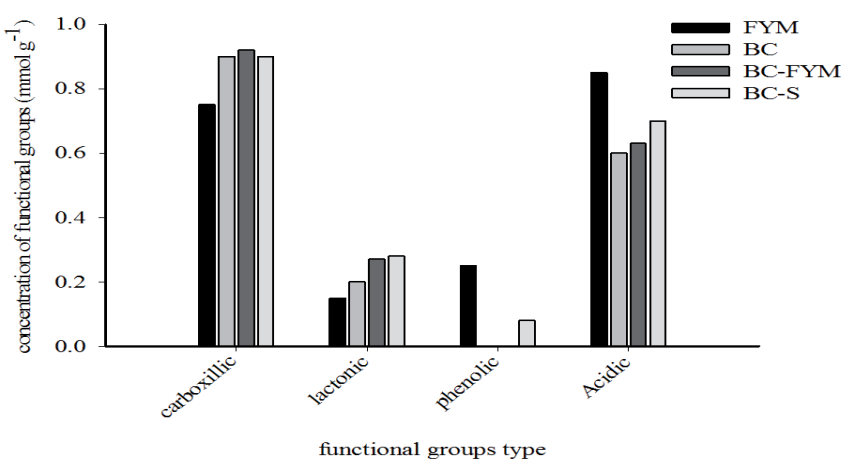

Figure 2: Functional groups concentration on the surface of used materials

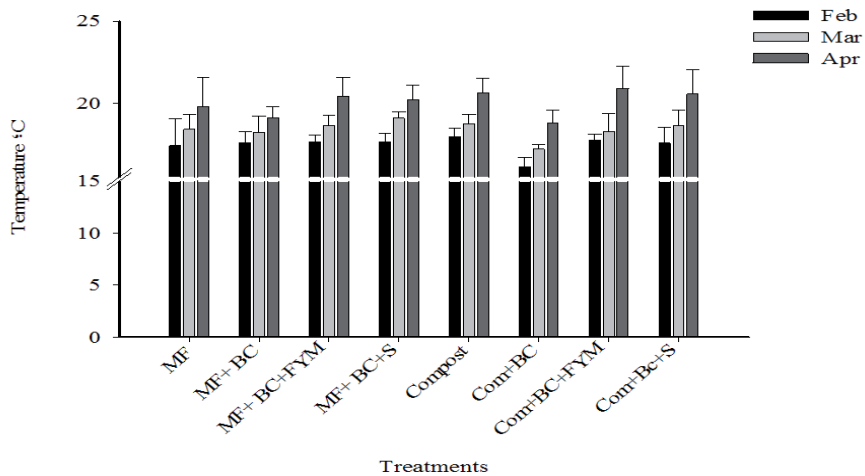

Figure 3: Influence of biochar on soil temperature during three months of potato cultivation in alkaline soil

2.3.2 Determination of total carbohydrates

Acid hydrolysis of tubers (0.2 g) was done in sealed tube using 10.0 ml H_2SO_4 solution (1.0 M). The sealed tubes were boiled in water bath for 10 h. After complete hydrolysis, suspension was neutralized by a known amount of barium carbonate and the precipitate was filtered through whatman No.1 filter paper. The filtrate was made up to a known volume. Total carbohydrates were determined in acid using phenol-sulfuric acid method as described by Dobois et al. (1965) as follows: A known volume of filtrate (1.0 ml) was transferred into a clean dry test tube. 1.0 ml of phenol solution (5 %) and 5.0 ml of H_2SO_4 were added. The yellow orange color was measured at 490 nm using spectrophotometer against blank.

3 RESULTS AND DISCUSSION

3.1 BIOCHAR CHARACTERIZATION

Yield of BC (as the mass ratio of biochar recovered after pyrolysis and the initial feedstock) was approximately 35 %, while BC carbon content was recorded 38.2 %. Biochar pH (extracted according to Masulili et al., 2010) was slightly decreased using FYM and S from 7.6 for BC to 7.4 and 6.9 for BC-FYM and BC-S, respectively. These finding were already proven by Boehm titration method which is commonly used technique to determine the acidic oxygen surface functional groups on carbon samples. The total acidic groups were slightly higher in BC-S and BC-FYM than BC (Fig. 2). This was attributed to the acidic products resulted from decomposition of FYM or formation of $SO_4^=$ anions during hydration of sulfur in BC-FYM and BC-S, respectively. This might explain how pH values of BC-FYM and BC-S were decreased compared to BC alone.

3.2. INFLUENCE OF BIOCHAR APPLICATION ON SOIL PROPERTIES.

3.2.1. Soil temperature

McCormack et al. (2013) reported that biochar enhances soil microbial activity by enhancing soil aggregation and porosity, pH, moisture retention and soil temperature, as well as nutrient retention. This work studies the effect of BC and modified-BC application on soil properties such as nutrient availability, soil temperature and chemical characteristics. The influence of different treatments of biochar on soil temperature during field study is shown in Fig. 3. Soil temperature was measured monthly during potato growing season (Quartz digi-thermo thermometer). The results revealed that soil temperature was higher using BC-FYM and BC-S by 0.9 to 2.1 °C as compared with control or biochar only. This might be attributed to the high energy release during decomposition of FYM or sulfuric acid that resulted from hydration of sulfur. It was also mentioned that biochar has positive effect on soil biota (Lehmann et al., 2011; Warnock et al., 2007) which might increase soil temperature. In general, soil temperature has an important role creating a healthier and more active soil environment. Soil biota plays an important role in soil nutrient cycling (McLaughlin et al., 1988; Frossard et al., 2000). Phosphate-solubilizing bacteria enhance P transfer from soil to plants: soil biota may contain a significant amount of P, typically 10–50 kg

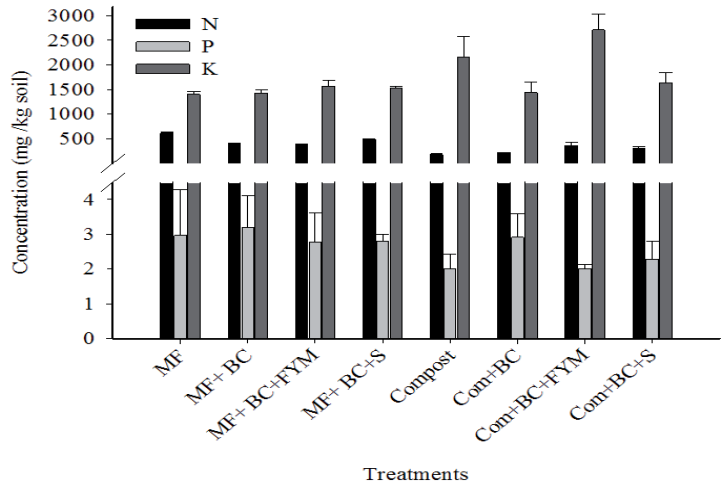

Figure 4: The influence of biochar and modified biochar on nutrient availability of macronutrients in alkaline soil cultivated with potatoes

P ha^{-1}, or 1–10 % of the total P, and around 10–15 % of soil organic P (Brookes et al., 1984; Richardson, 2001), so soil biota considered a major factor controlling organic and inorganic P concentrations in temperate soils (Seeling and Zasoski, 1993). All these findings showed that BC plays an important role in nutrient availability and yield due to its effect on soil temperature and soil biota. Soil temperature increased from February to April due to climatic conditions (Fig. 3).

3.2.2. Soil nutrient availability

Glaser et al. (2002) and Lehmann et al. (2011) reported that biochar used as a soil amendment to enhance soil fertility and plant growth, since it has shown potential as a sustainable amendment to improve chemical properties of soil. BC also was found to have a positive effect on soil nutrient availability (Mengel and Kirkby, 2001). In this study we investigate effect of biochar treatments on soil nutrients availability during field study on potatoes. Available NPK were measured in soil 70 days after planting (Fig. 4). The results revealed in general, that using solely biochar lowered the nutrient availability in soil, since biochar application generally raises soil pH (Hass et al., 2012) which reduces the availability of nutrients in alkaline soil. Modified biochar (BC-FYM or BC-S) showed higher nutrient availability than control despite the sig-

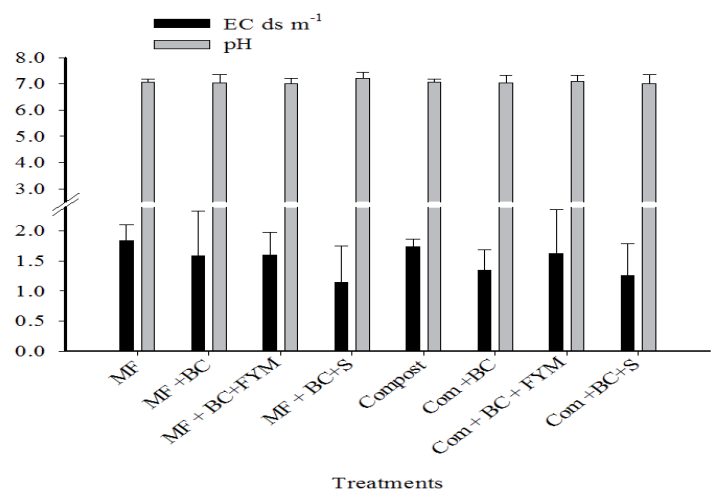

Figure 5: Influence of BC addition onto soil characteristics (EC and pH)

nificance was low in some cases. This might be attributed to excess of the amount of nutrients that exist in FYM composted with biochar or/and decomposition of organic matter or sulfur had led to decrease of soil pH which release more nutrients adsorbed or precipitated into soil solution. Biochar was also found to have an important role in fertilizer use efficiency due to adsorption of nutrients on its surface and keep it from leaching (Blackwell et al., 2010; Laird et al., 2010). In addition, BC was found to improve soil biota, such as arbuscular mycorrhizal fungi (AMF), which enhance nutrient availability in soil (Warnock et al., 2007).

3.2.3. Chemical characteristics

Figure 5 shows the influence of materials studied application onto soil chemical characteristics (EC and pH). The pH level of alkaline soils would be affected by biochar application and the possible increase of soil pH in alkaline soil is harmful for plant growth (Liu and Zhang, 2012). While the results revealed that EC and pH of soil weren't affected significantly by the addition of BC or modified BC (BC-FYM or BC-S). This was attributed to the amount of added BC amendment (12 Mg ha^{-1}), which was not enough to change the pH number. These results were agreed with those obtained by Somchai-Butnan et al. (2015) who found that soil pH was not affected by biochar amendment except in the soil amended with the highest rate of flash carbonization (FC) biochar excess than 12 Mg ha^{-1}. Biochar relatively reduced soil EC, this might be attributed to the high adsorption capacity of biochar which enhance the mutual form and reduce the soluble form of salts.

3.3. INFLUENCE OF BIOCHAR APPLICATION ON PLANT CHARACTERISTICS.

3.3.1. Crop yield

Influence of studied materials on the productivity of potatoes tubers is shown in Fig. 6. The results revealed that solely addition of biochar decreased the yield of potatoes more than 6 % and 10 % as compared with control using mineral and organic fertilization, respectively. This was attributed to the alkalinity effect of biochar which reduce the availability of some nutrients; consequently the total yield was reduced. These results are similar as those obtained by Van Zwieten et al. (2010), who reported that the application of biochar 1 with pH value of 9.4 and biochar 2 with pH value of 8.2 both increased the pH of ferrosol (initial pH at 4.2), but only biochar 2 increased the pH value of calcareous soil (initial pH at 7.67). Also Fellet et al. (2011) reported that application of BC in mine tailing soil had led to excess in soil pH from 8.13 to 10.2 at 10 % biochar application rate. Treatment BC-FYM resulted in yield increase for 11 and 25 % in both mineral and organic fertilization. This was similar to the results reported by Glaser et al. (2002) who concluded the fact that crop yield is increased using biochar combined with mineral or organic fertilizers. BC-FYM application with organic fertilization produced higher yield than mineral fertilization due to the acidity effect of compost which decreases alkalinity of BC. It benefits in releasing nutrients slowly in available form for plant absorption during growth period. This led to minimizing nutrient leaching from soil rather than mineral fertilization. Also, we found that the yield was much higher using BC-FYM than BC-S with both types of fertigation. This

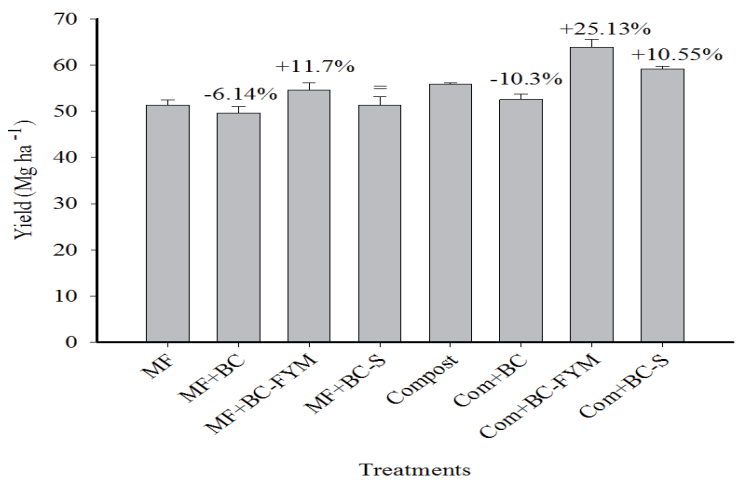

Figure 6: Biochar addition efficacy on yield of potato tubers cultivated in alkaline soil

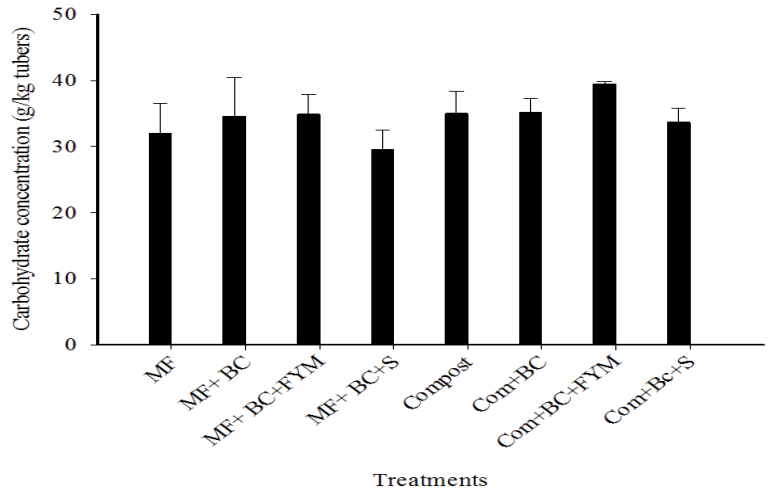

Figure 7: Biochar addition efficacy on carbohydrate concentration of potatoes tubers cultivated in alkaline soil

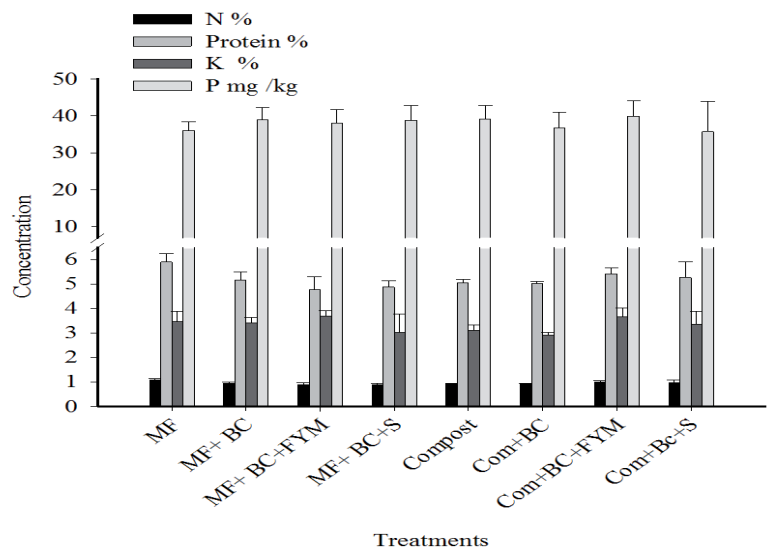

Figure 8: Biochar addition efficacy on content of macronutrients in tubers

might be attributed to the additional amounts of N exist in BC-FYM and/or higher nutrient availability due to higher microbial activity, such as arbuscular mycorrhizal fungi (AMF) (Warnock et al., 2007). So we recommend using BC in alkaline soils after composting with FYM to enhance crop productivity and soil chemical characteristics. More work is needed to state the adequate amount of BC added.

3.3.2. Yield component

Total carbohydrates in potatoes tubers were measured to study the efficiency of biochar addition on yield component grown in alkaline soil compared with modified biochar (BC-FYM or BC-S) (Figure 7). Total carbohydrate was significantly increased using BC-FYM or BC-S as compared with using BC solely, while there wasn't any significance with control. This was attributed to the excess amount of potassium in FYM found in BC-FYM, which are responsible for carbohydrate transferring from leaves to tubers. Since BC has a high adsorption capacity for K ions (Lehmann et al., 2003) because of its high porosity and surface/volume ratio and can improve plant nutrients uptake and P, Ca, K availability (Chan et al., 2007; Yamato et al., 2006). Elemental concentration of N P K in potatoes tubers weren't significantly affected by biochar treatments (Fig. 8). Consequently, protein con-

tent wasn't also affected by biochar addition, with the exception of BC-FYM and BC-S using organic fertilization where a significant increase in tubers protein content was measured than in control and BC solely.

4 CONCLUSION

Biochar proved to enhance soil chemical and physical properties, while this effect was negative in alkaline soils since literature reported that BC raise soil pH. Composting biochar with other materials (FYM and Sulfur) was suggested to modify biochar action in alkaline soil. A field experiment was conducted using modified BC (BC-FYM or BC-S) compared with BC solely addition to alkaline soils under mineral and organic fertilization. BC-FYM proved to be the best treatment, since BC-FYM increased crop yield of potatoes 11 and 25 % compared to control under mineral and organic fertilizers, respectively. BC-FYM was recorded higher amount of carbohydrate and protein as compared with BC solely especially under organic fertilization. Yield content of elements (N P K) in potatoes tubers weren't affected significantly with biochar application as found in carbohydrate concentration. So we recommend composting biochar with FYM before using it in the alkaline soil to enhance crop productivity and soil chemical characteristics. More work is needed to state the adequate amount of BC added.

5 REFERENCES

Abdelhafez, A.A., Li, J. & Abbas, M.H.H. (2014). Feasibility of biochar manufactured from organic wastes on the stabilization of heavy metals in a metal smelter contaminated soil. *Chemosphere, 117*, 66-71.https://doi.org/10.1016/j.chemosphere.2014.05.086

Alburquerque, J.A., Salazar, P., Barrón, V., Torrent, J., Campillo, M.C., Gallardo, A. & Villar, R. (2012). Enhanced wheat yield by biochar addition under different mineral fertilization levels. *Agronomy Sustainable Development, 33*(3), 475-484. https://doi.org/10.1007/s13593-012-0128-3

American Standard of Testing Material. (2001). *Standard Test Method for Chemical Analysis of Wood Charcoal*. ASTM D 1762-84.

Asai, H., Samson, B. K., Stephan, H. M., Songyikhangsuthor, K., Homma, K., Kiyono, Y., Inoue, Y., Shiraiwa, T. & Horie, T. (2009). Biochar amendment techniques for upland rice production in northern Laos. *Field Crops Research, 111,* 81-84. https://doi.org/10.1016/j.fcr.2008.10.008

Atkinson, C.J., Fitzgerald, J.D. & Hipps, N.A. (2010). Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *Plant Soil, 337*, 1-18. https://doi.org/10.1007/s11104-010-0464-5

Baronti, S., Vaccari, F.P., Miglietta, F., Calzolari, C., Lugato, E., Orlandini, S., Pini, R.,Zulian, C. & Genesio, L. (2014). Impact of biochar application on plant water relations in Vitis vinifera L.. *European Journal of Agronomy, 53*, 38-44. https://doi.org/10.1016/j.eja.2013.11.003

Bateman, E. J., & Baggs, E. M. (2005). Contributions of nitrification and denitrification to N2O emissions from soils at different water-filled pore space. *Biology and Fertility of Soils, 41*, 379- 388. https://doi.org/10.1007/s00374-005-0858-3

Blackwell, P., Krull, E., Butler, G., Herbert, A. & Solaiman, Z. (2010). Effect of banded biochar on dry land wheat production and fertilizer use in south-western Australia: an agronomic and economic perspective. *Australian Journal of Soil Research, 48*, 531-545. https://doi.org/10.1071/SR10014

Boehm, H.P., Diehl, E., Heck, W. & Sappok, R. (1964). Surface oxides of carbon. Angewandte Chemie *International Edition, 3,* 669-77. https://doi.org/10.1002/anie.196406691

Borchard, N., Prost, K., Kautz, T., Moeller, A. & Siemens, J. (2012). Sorption of copper (II) and sulphate to different biochar before and after composting with farm yard manure. *European Journal of Soil Science, 63*, 399-409. https://doi.org/10.1111/j.1365-2389.2012.01446.x

Brookes, P.C., Powlson, D.S. & Jenkinson, D.S. (1984). Phosphorus in the soil microbial biomass. *Soil Biology and Biochemistry, 16*,169-175. https://doi.org/10.1016/0038-0717(84)90108-1

Bruun, E.W., Müller-Stöver, D., Ambus, P. & Hauggaard-Nielsen, H. (2011). Application of biochar to soil and N2O emissions: potential effects of blending fast-pyrolysis biochar with anaerobically digested slurry. *European Journal of Soil Science, 62*, 581-589. https://doi.org/10.1111/j.1365-2389.2011.01377.x

Busch, D., Kammann, C., Grünhage, L. & Müller, C. (2012). Simple biotoxicity tests forevaluation of carbonaceous soil additives: establishment and reproducibility offour test procedures. *Journal of Environmental Quality, 41*, 1023. https://doi.org/10.2134/jeq2011.0122

Buss, W., Kammann, C. & Koyro, H.W. (2012). Biochar reduces copper toxicity in Chenopodium quinoa Willd. in a sandy soil. *Journal of Environmental Quality, 41*, 1157. https://doi.org/10.2134/jeq2011.0022

Busscher, W.J., Novak, J.M., Evans, D.E., Watts, D.W., Niandou, M.A.S. & Ahmedna, M. (2010). Influence of pecan biochar on physical properties of a Norfolk loamy sand. *Soil Science, 175*, 10-14. https://doi.org/10.1097/SS.0b013e3181cb7f46

Butnan, S., Deenik, J.L., Toomsan, B., Antal, M.J. & Vityakon, P. (2015). Biochar characteristics and application rates affecting corn growth and properties of soils contrasting in texture and mineralogy. *Geoderma*, 237-238, 105-116. https://doi.org/10.1016/j.geoderma.2014.08.010

Case, S.D.C., McNamara, N.P., Reay, D.S. & Whitaker, J. (2012). The effect of biochar addition on N2O and CO2 emissions from a sandy loam soil - the role of soil aeration. *Soil Biology and Biochemistry, 51*,125-34. https://doi.org/10.1016/j.soilbio.2012.03.017

Cayuela, M.L., Sánchez-Monedero, M.A., Roig, A., Hanley, K., Enders, A. & Lehmann, J. (2013). Biochar and denitrification in soils: when, how much and why does biochar reduce N2O emissions? *Scientific Reports, 3,* 1732. https://doi.org/10.1038/srep01732

Chan K.Y., Van Zwieten L., Meszaros I., Downie A. & Joseph S.

(2007). Agronomic values of green waste biochar as a soil amendment. *Australian Journal of Soil Research, 45*, 629-634. https://doi.org/10.1071/SR07109

Cheng, C.-H. & Lehmann, J. (2009). Ageing of black carbon along a temperature gradient. *Chemosphere, 75*, 1021-1027. https://doi.org/10.1016/j.chemosphere.2009.01.045

Clough, T., Condron, L., Kammann, C. & Müller, C. (2013). A review of biochar and soil nitrogen dynamics. *Agronomy, 3*, 275-293. https://doi.org/10.3390/agronomy3020275

Cottenie A., Verloo, M., Kiekens, l., Velghe, G. & Camerlynck, R. (1982). *Chemical Analysis of Plants and Soils*. Lab. of Analytical and Agro. State, University Ghent. Belgium.

Ding, W., Meng, L., Yin, Y., Cai Z. & Zheng, X. (2007). CO2 emission in an intensively cultivated loam as affected by long term application of organic manure and nitrogen fertilizer. *Soil Biology and Biochemistry, 39*, 669-679. https://doi.org/10.1016/j.soilbio.2006.09.024

Ding, Y., Liu, Y.X., Wu, W.X., Shi, D.Z., Yang, M. & Zhong, Z.K. (2010). Evaluation of Biochar effects on nitrogen retention and leaching in multi-layered soil columns. *Water Air Soil Pollution, 213*, 47-55. https://doi.org/10.1007/s11270-010-0366-4

Dubois, M., Cilles, K.A., Hamilton, J.K., Rober, P.A. & Smith, F. (1965). Colorimetric method for determination of sugars related substances. *Analytical Chemistry, 28*, 350-365. https://doi.org/10.1021/ac60111a017

Fellet, G., Marchiol, L., DelleVedove, G. & Peressotti, A. (2011). Application of biochar on mine tailings: Effects and perspectives for land reclamation. *Chemosphere, 83*, 1262-1267. https://doi.org/10.1016/j.chemosphere.2011.03.053

Fischer, D. & Glaser, B. (2012). Synergisms between compost and biochar for sustain-able soil amelioration. In: Kumar, S., Bharti, A. (Eds.), *Management of Organic Waste*. Rijeka, In Tech., 167-198. https://doi.org/10.5772/31200

Frossard, E., Condron, L.M., Oberson, A., Sinaj, S. & Fardeau, J.C. (2000). Processes governing phosphorus availability in temperate soils. *Journal of Environmental Quality, 29*, 15-23. https://doi.org/10.2134/jeq2000.00472425002900010003x

Gathorne-Hardy, A., Knight, J. & Woods, J. (2009). Biochar as a soil amendment positively interacts with nitrogen fertiliser to improve barley yields in the UK. IOP Conf. Ser. Earth. *Environmental Science, 6*, 372052. https://doi.org/10.1088/1755-1307/6/37/372052

Glaser, B., Lehmann, J. & Zech, W. (2002). Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal - a review. *Biology and Fertility of Soils, 35*, 219-230. https://doi.org/10.1007/s00374-002-0466-4

Gomez-Eyles, J.L., Beesley, L., Moreno-Jiménez, E., Ghosh, U. & Sizmur, T. (2013). The potential of biochar amendments to remediate contaminated soils. In: Ladygina, N., Rineau, F. (Eds.), *Biochar and Soil Biota*. CRC Press, Boca Raton. https://doi.org/10.1201/b14585-5

Graber, E.R. & Elad, Y. (2013). *Biochar Impact on Plant Resistance to Disease.* CRC Press, Boca Raton. https://doi.org/10.1201/b14585-3

Graber, E.R., Tsechansky, L., Gerstl, Z. & Lew, B. (2012). High surface area biochar negatively impacts herbicide efficacy. *Plant Soil, 353*(1-2), 95-106. https://doi.org/10.1007/s11104-011-1012-7

Harsono, S. S., Grundmann, P. & Siahaan, D. (2015). Role of Biogas and Biochar Palm Oil Residues for Reduction of Greenhouse Gas Emissions in the Biodiesel Production. *Energy Procedia, 65*, 344 - 351. https://doi.org/10.1016/j.egypro.2015.01.063

Hass, A., Gonzalez, J.M., Lima, I.M., Godwin, H.W., Halvorson, J.J. & Boyer, D.G. (2012). Chicken manure biochar as liming and nutrient source for acid Appalachian soil. *Journal of Environmental Quality, 41*, 1096-1106. https://doi.org/10.2134/jeq2011.0124

Husson, O. (2012). Redox potential (Eh) and pH as drivers of soil/plant/microorganism systems: a trans disciplinary overview pointing to integrative opportunities for agronomy. *Plant Soil, 362,* 389-417. https://doi.org/10.1007/s11104-012-1429-7

Ippolito, J.A., Laird, D.A. & Busscher, W.J. (2012). Environmental benefits of biochar. *Journal of Environmental Quality,* 41, 967. https://doi.org/10.2134/jeq2012.0151

Jin, H. (2010). *Characterization of Microbial Life Colonizing Biochar and Biochar-Amended Soils*. Cornell University Graduate School.

Jones J.B. (1991). *Kjeldahl Method for Nitrogen Determination*. Athens, GA: Micro- Macro Publishing,

Joseph, S., Graber, E., Chia, C., Munroe, P., Donne, S., Thomas, T., Nielsen, S., Marjo, C., Rutlidge, H., Pan, G., Li, L., Taylor, P., Rawal, A. & Hook, J. (2013a). Shifting paradigms: development of high-efficiency biochar fertilizers based on nano-structures and soluble components. *Carbon Management, 4*, 323-343. https://doi.org/10.4155/cmt.13.23

Joseph, S., Van Zwieten, L., Chia, C., Kimber, S., Munroe, P., Lin, Y., Marjo, C., Hook, J.,Thomas, T., Nielsen, S., Donne, S. & Taylor, P. (2013b). Designing Specific Biochars to Address Soil Constraints: A Developing Industry. In: Ladygina, N., Rineau, F. (Eds.), *Biochar and Soil Biota*. CRC Press, Boca Raton. https://doi.org/10.1201/b14585-7

Kammann, C., Ratering, S., Eckhard, C. & Muller, C. (2012). Biochar and hydrochar effects on greenhouse gas (carbon dioxide, nitrous oxide, and methane) fluxes from soils. *Journal of Environmental Quality, 41*, 1052-66. https://doi.org/10.2134/jeq2011.0132

Karhu, K., Mattila, T., Bergström, I. & Regina, K. (2011). Biochar addition to agricultural soil increased CH4 uptake and water holding capacity - results from a short-term pilot field study. *Agriculture, Ecosystems & Environment, 140,* 309-13. https://doi.org/10.1016/j.agee.2010.12.005

Laird, D., Fleming, P., Wang, B., Horton, R. & Karlen, D. (2010). Biochar impact on nutrient leaching from a Midwestern agricultural soil. *Geoderma, 158*, 436-442. https://doi.org/10.1016/j.geoderma.2010.05.012

Lehmann J., Da Silva J.P. jr., Steiner C., Nehls T., Zech W. & Glaser B. (2003). Nutrient availability and leaching in an archaeological anthrosol and a ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant Soil, 249*, 343-357. https://doi.org/10.1023/A:1022833116184

Lehmann, J., Gaunt, J. & Rondon, M. (2006). Bio-char sequestration in terrestrial ecosystems-a review. *Mitigation and*

*Adaptation Strategies for Global Chan*ge, *11*, 403-427. https://doi.org/10.1007/s11027-005-9006-5

Lehmann, J., Rillig, M., Thies, J., Masiello, C., Hockaday, W. & Crowley, D. (2011). Biochar effects on soil biota - a review. *Soil Biology and Biochemistry, 43*, 1812-1836. https://doi.org/10.1016/j.soilbio.2011.04.022

Lehmann, J. & Steiner, C. (2009a). A*mazonian Dark Earths: Wim Sombroek's Vision*. Springer Netherlands, Dordrecht.

Lehmann, J. & Steiner, J. (2009b). *Biochar for Environmental Management*. Published by Earth scan Publishers Ltd (ISBN 978-1-84407-658-1).

Liu, X.H. & Zhang, X.C. (2012). Effect of biochar on pH of alkaline soils in the Loess Plateau: Results from incubation experiments. *International Journal of Agriculture and Biology, 4*, 745-750.

Major, J., Rondon, M., Molina, D., Riha, S.J. & Lehmann, J. (2010). Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. *Plant Soil, 333*, 117-128. https://doi.org/10.1007/s11104-010-0327-0

Masulili, A., Utomo, W.H. & Syechfani, M.S. (2010). Rice Husk Biochar for Rice Based Cropping System in Acid Soil 1. The Characteristics of Rice Husk Biochar and Its Influence on the Properties of Acid Sulfate Soils and Rice Growth in West Kalimantan, Indonesia. *Journal of Agricultural Science, 2*(1), 39-47. https://doi.org/10.5539/jas.v2n1p39

McCormack, S.A., Ostle, N., Bardgett, R.D., Hopkins, D.W. & Vanbergen, A.J. (2013). Biochar in bioenergy cropping systems: impacts on soil faunal communities and linked ecosystem processes. *GCB Bioenergy, 5*, 81-95. https://doi.org/10.1111/gcbb.12046

Mclaughlin, M.J., Alston, A.M. & Martin, J.K. (1988). Phosphorus cycling in wheat pasture rotations .II. The role of the microbial biomass in phosphorus cycling. Australian *Journal of Soil Research, 26*(2), 333 - 342. https://doi.org/10.1071/SR9880333

Mengel, K. & Kirkby, E.A. (2001). *Principles of Plant Nutrition*, 5th edition. Kluwer Academic Publishers, Dordrecht. https://doi.org/10.1007/978-94-010-1009-2

Mukherjee, A., Zimmerman, A.R. & Harris, W. (2011). Surface chemistry variations among a series of laboratory-produced biochars. *Geoderma, 136*, 247-255. https://doi.org/10.1016/j.geoderma.2011.04.021

Nishio, M. (1996). *Microbial Fertilizers in Japan*. FFTC Extension Bulletin. Food and Fertilizer Technology Center, Taipei City.

Pietikäinen, J., Kiikkilä, O. & Fritze, H. (2003). Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. *Oikos, 89*,231-242. https://doi.org/10.1034/j.1600-0706.2000.890203.x

Prost, K., Borchard, N., Siemens, J., Kautz, T. & Séquaris, J. (2012). Biochar affected by composting with farmyard manure. *Journal of Environmental Quality, 42*(1), 164-172. https://doi.org/10.2134/jeq2012.0064

Richardson, A.E. (2001). Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Australian Journal of Plant Physiology, 28*, 897-906. https://doi.org/10.1071/PP01093

Rondon, M., Lehmann, J., Ramírez, J. & Hurtado, M. (2007). Biological nitrogen fixation by common beans (Phaseolus vulgaris L.) increases with bio-char additions. *Biology and Fertility of Soils, 43*, 699-708. https://doi.org/10.1007/s00374-006-0152-z

Rutherford, D.W., Wershaw, R.L. & Reeves, J.B. (2008). *Development of acid functional groups and lactones during the thermal degradation of wood and wood components.* Denver: U.S. Department of the Interior, U.S. Geological Survey, 2007- 5013. https://doi.org/10.3133/sir20075013

Schulz, H., Dunst, G. & Glaser, B. (2014). No Effect Level of Co-Composted Biochar on Plant Growth and Soil Properties in a Greenhouse Experiment. *Agronomy, 4*, 34-51. https://doi.org/10.3390/agronomy4010034

Seeling, B. & Zasoski, R.J. (1993). Microbial effects in maintaining organic and inorganic solution phosphorus concentrations in a grass land topsoil. *Plant Soil, 148*, 277-284. https://doi.org/10.1007/BF00012865

Steiner, C., Das, K.C., Melear, N. & Lakly, D. (2010). Reducing nitrogen loss during poultry litter composting using biochar. *Journal of Environmental Quality, 39*, 1236. https://doi.org/10.2134/jeq2009.0337

Steiner, C., Teixeira, W.G., Lehmann, J., Nehls, T., de Macedo, J.L.V., Blum, W.E.H. & Zech, W. (2007). Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered central Amazonian upland soil. *Plant and Soil, 291*, 275-290. https://doi.org/10.1007/s11104-007-9193-9

Taghizadeh-Toosi, A., Clough, T.J., Sherlock, R.R. & Condron, L.M. (2011). Biochar adsorbed ammonia is bioavailable. *Plant Soil*, 57-69. https://doi.org/10.1007/s11104-011-0870-3

Tan, W.L, Rosenani, A.M, Ahmad, S.H. & Ishak, C.F. (2012). *Oil palm empty fruit bunch biochar: characterization and potential usage as soil amendment for its nutrient retention capacity in soil*, The 4th International Biochar Congress, 12.

Tuomela, M., Vikman, M., Hatakka, A. & Itävaara, M. (2000). Biodegradation of lignin in a compost environment: a review. Bioresource *Technology, 72*,169-183. https://doi.org/10.1016/S0960-8524(99)00104-2

Van Zwieten, L., Kimber, S., Morris, S., Chan, K.Y., Downie, A., Rust, J., Joseph, S. & Cowie, A. (2010). Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. *Plant Soil, 327*, 235-246. https://doi.org/10.1007/s11104-009-0050-x

Ventura, M., Sorrenti, G., Panzacchi, P., George, E. & Tonon, G. (2013). Biochar reduces short-term nitrate leaching from a horizon in an apple orchard. *Journal of Environmental Quality, 42*, 76-82. https://doi.org/10.2134/jeq2012.0250

Verheijen, F.G.A., Jeffery, S., Bastos, A.C., van der Velde, M. & Diafas, I. (2010). *Biochar Application to Soils - A Critical Scientific Review of Effects on Soil Properties, Processes and Functions*. EUR 24099 EN, Luxembourg.

Warnock, D., Lehmann, J., Kuyper, T. & Rillig, M. (2007). Mycorrhizal responses to biochar in soil - concepts and mechanisms. *Plant Soil, 300,* 9-20. https://doi.org/10.1007/s11104-007-9391-5

Yamato M., Okimori Y., Wibowo I.F., Anshori S. & Ogawa M. (2006). Effects of the application of charred bark of Acacia mangium on the yield of maize, cowpea and peanut, and soil chemical properties in South Sumatra, Indonesia.

Soil Science and Plant Nutrition, 52, 489-495. https://doi.org/10.1111/j.1747-0765.2006.00065.x

Yuan, J.H. & Xu, R.K. (2011). The amelioration effects of low temperature biochar generated from nine crop residues on an acidic ultisol. *Soil Use and Management, 27*, 110- 115. https://doi.org/10.1111/j.1475-2743.2010.00317.x

Zimmerman, A.R. (2010). Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). *Environmental Science and Technology, 44*, 1295-1301. https://doi.org/10.1021/es903140c

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Entomopathogenic fungus, *Lecanicillium lecanii* R. Z are & W. Gams anchored into MCM-41: A new and effective bio-insecticide against *Brevicoryne brassicae* (Linnaeus, 1758) (Hom: Aphididae) to protect cabbages

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Entomopathogenic fungus, *Lecanicillium lecanii* R. Z are & W. Gams anchored into MCM-41: A new and effective bio-insecticide against *Brevicoryne brassicae* (Linnaeus, 1758) (Hom: Aphididae) to protect cabbages

Abstract: *Brevicoryne brassicae* is a significant pest of cultivated cabbages and vegetable crops in the world. The present study was carried out to examine a potential strategy to enhance the insecticidal activity of *Lecanicillium lecanii* for cost-effective management of *B. brassicae*. The insecticidal efficacy of pure entomopathogenic fungus (PEF) and MCM-41 (Mobil Composition of Matter) *L. lecanii* were assessed against the cabbage aphid under laboratory and greenhouse conditions. The fungus was supported on MCM-41 and was completely characterized by Scanning Electron Microscope (SEM), thermogravimetric analysis (TGA) and Fourier transform infrared (FT-IR) techniques. LC_{50} values of PEF and MCM-41@fungus were 1.9×10^6 and 2.5×10^4 and 2.0×10^7 and 2.0×10^5 conidia/ml on adults of *B. brassicae* under laboratory and greenhouse conditions, respectively. Bioassays demonstrated that MCM-41@fungus significantly decreased LC_{50} values of entomopathogenic fungus and it was more toxic than *L. lecanii* at adult stage of the pest. The results showed that pure *L. lecanii* and its nano-formulation could play key roles as bio-pesticides in *B. brassicae* management programs.

Key words: *Brevicoryne brassicae*; *Lecanicillium lecanii*; MCM-41@fungus; virulence

Entomopatogena gliva, *Lecanicillium lecanii* R. Zare & W. Gams, vključena v MCM-41: Novi učinkoviti bio-insekticid za zatiranje mokaste kapusove uši (*Brevicoryne brassicae* (Linnaeus, 1758) (Hom: Aphididae)) pri zaščiti zelja

Izvleček: Mokasta kapusova uš (*Brevicoryne brassicae*) je pomemben škodljivec zelja in drugih zelenjadnic širom po svetu. Raziskava je bila izvedena za preučitev potencialne strategije povečanja insekticidne aktivnosti glive *Lecanicillium lecanii* za učinkovito in poceni zatiranje mokaste kapusove uši. Insekticidna učinkovitost čistega pripravka entomopatogene glive (PEF) in njene vključitve v MCM-41 (Mobil Composition of Matter)@*L. lecanii*) je bila ocenjena na kapusovi mokasti uši v laboratoriju in v rastlinjaku. Gliva, ki je bila vključena v MCM-41, je bila podrobno opisana z vrstičnim elektronskim mikroskopom (SEM), termogravimetrično analizo (TGA) in Fourierjevo transformacijsko unfrardečo tehniko (FT-IR). LC_{50} vrednosti za odrasle osebke mokaste kapusove uši so bile za PEF in MCM-41@gliva 1,9 × 106 in 2,5 × 104 ter 2,0 × 107 in 2,0 × 105 konidijev/ml v laboratoriju, oziroma rastlinjaku. Biotest je pokazal, da je kombinacija MCM-41@gliva značilno zmanjšala LC_{50} vrednosti entomopatogene glive in, da je bila bolj toksična za odrasle uši kot gliva sama. Rezultati so pokazali, da lahko imajo čiste kulture glive *L. lecanii* in njeni nano pripravki ključno vlogo kot biopesticidi v programih biološkega uravnavanja mokaste kapusove uši.

Ključne besede: *Brevicoryne brassicae*; *Lecanicillium lecanii*; MCM-41@gliva; virulenca

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1 INTRODUCTION

Cabbage (*Brassica oleracea* L. var. *capitata*) is one of the significant vegetables throughout the world such as Iran. Brassica crops are attacked by many species of insect pests that reduce the quantity and quality of them (Neupane, 1999). *Brevicoryne brassicae* (Lineus, 1758), (Hom: Aphididae) is one of the serious pests of crucifer crops throughout the world such as Iran (Mousavi Anzabi et al., 2013). The pest causes severe damage by direct feeding or indirect via transfer of viral diseases that could be seriously led to plant destruction (Abdu-Allah, 2012). Heavily infested plants become covered with a mass of aphids that can finally lead to leaf decay and plant death (Griffin & Williamson, 2012). Today, applying chemical insecticides are still considered the foremost and the most important action to manage insect pests. Nevertheless, relying on chemical insecticides has been resulted in adverse effects on environmental and human health. Abuse of non-selective chemical insecticides can destroy the natural enemies and beneficial organisms and induce problems such as development of pest resistance (Sharma & Gupta, 2009). Therefore, these adverse effects supported the development of alternative pest management tactics in which microbial controls may play principle roles (Collantes et al., 1986; Llanderal-Cázares et al., 1996). Entomopathogenic fungi are efficient microbial control agents of Homopteran pests (Goettel et al., 2008). *Lecanicillium lecanii* R. Zare & W. Gams is an entomopathogenic fungus that its mycelium produces a cyclodepsipeptide toxin called bassianolide (Suzuki et al., 1977; Kanaoka et al., 1978). Insect mortality is caused by secrete of mycotoxins and extreme fungal growth (Burges, 1981). In spite of the potential of entomopathogenic fungi in the pest control, these bio-control agents have some defects (including sensitivity to environmental factors such as moisture, light, and temperature) that have limited their applications in storage, greenhouse and field conditions. So, these problems will be overcome through recent technological advances such as nanotechnology that will permit future use of entomopathogenic fungi in crop production systems and the nano-formulation approaches can improve their efficacy and pathogenicity. The hexagonal array of uniform mesoporous of MCM-41 with particular properties such as exceptionally high surface areas and high pore volume (Rath & Parida, 2011) attracted our interest in applying it in plant protection and *B. brassicae* management. Since it has 1D uniform mesopores, the entomopathogenic fungi can be grafted into the MCM-41. Consequently, we selected MCM-41 as a support which belongs to the M41S family that mainly made up of silica, SiO_2 (Rath & Parida, 2011; Shylesh & Singh, 2005). Silica has unique benefits as a support like extraordinary thermal and chemical stability, ease of handling, and abundance of exposed silanol (Si-OH) groups (Abdollahi-Alibeik & Pouriayevali, 2012).

2 MATERIALS AND METHODS

2.1 INSECT REARING

Adults of *B. brassicae* were collected from the cabbage fields in West Azarbaijan Province, Urmia, Iran and then transferred to cabbage plants var. capitata growing on a mixture of soil, sand and manure in plastic plant pots in the greenhouse at 22 ± 2 °C, 70 ± 5 % RH and a photoperiod of 12 L: 12 D. These colonies were rearing on cabbages for 3-4 generations. Cabbages were replaced with new 4-7 leaf stage cabbage plants at every eight days. Trials were carried out with cohorts of apterous adults. Seedlings used for producing leaf disks for entomopathogenic fungus bioassays in Petri dishes.

2.2 MICROORGANISM AND CULTURE MEDIA

The entomopathogenic fungus, *Lecanicillium lecanii* (IRAN229) was provided from Mycology collections of Urmia University, Urmia, Iran. Fungal species was cultured in potato dextrose agar (PDA) at 25 ± 1 °C. After 2 weeks (enough growth and sporulation of fungus), conidia were scrapped to make an aqueous suspension with 0.02 % Tween-80. The conidia suspension was filtered through three layers of sterile cheesecloth to remove mycelium and was enumerated with an improved Neubauer haemacytometer (Weber Scientific International Ltd., United Kingdom). Before the bioassay tests, spore viability percentage was determined by inoculating plates of PDA with the suspension. After 24 h, the germination rate was observed under a light microscope. Germination was considered positive when the length of germ tube was as long as the width of the conidia (Hall, 1981). The germination rate of *L. lecanii* conidia was 97 %.

2.3 NANO STRUCTURE ANALYSIS

The particle morphology was surveyed by a scanning electron microscope (SEM) (Day Petronic Company, Tehran, Iran), using FESEM-TESCAN MIRA3. The thermo gravimetric analysis (TGA) curves were recorded on a Shimadzu DTG-60 instrument (University of Kurdistan, Sanandaj, Iran). Fourier transform infra-

red (FT-IR) spectra were recorded with KBr pellets on a Nexus 670 FT-IR spectrometer (Medical Sciences of Urmia University, Urmia, Iran).

2.4 PREPARATION OF SILICEOUS MCM-41

Mesoporous Si-MCM-41 was synthesized through Sol-gel tactic according to Cai et al., (2001). The synthesis procedure of Si-MCM-41 was as follow: In a typical procedure, to a solution containing 480 ml deionized water and 3.5 ml NaOH (2M) which was stirred at 80 ˚C, 1.0 g (2.74 mmol) of surfactant cetyltrimethylammonium bromide (CTAB) was added, when the solution became homogeneous, 5 ml of TEOS was slowly added dropwise into the solution, giving rise to a white slurry. The resulting mixture was refluxed for 2h at the same temperature under continuous stirring. The collected product was filtered, washed with deionized water and dried in an oven at 70˚C followed by calcination at 550˚C for 5 h with a ramp 2˚C min^{-1} to remove the residual surfactants. Finally, we obtained the mesoporous Si-MCM-41.

2.5 PREPARATION OF MCM-41-ENTOMOPATHOGENIC FUNGUS

In a 100 ml round bottom flask, a mixture of MCM-41-Cl (1 g), entomopathogenic fungus (10 ml of determined concentrations) and Et_3N (1.5 ml) in H_2O (50 ml) were stirred under room temperature for 24 h. Then, the final product was dried at room temperature (Abdollahi-Alibeik & Pouriayevali, 2012).

2.6 BIOASSAYS

2.6.1 Aphid-dipping in the laboratory and greenhouse conditions

The fresh leaves used in aphid-dipping under laboratory conditions came from cabbages grown in the research field of Urmia University, Urmia, Iran. For greenhouse trials, cabbages were grown in pots under greenhouse conditions.

To investigate adult sensitivity, each one-day-old aphid was dipped in spore suspensions of pure *L. lecanii* and MCM-41@fungus treatments separately under laboratory and greenhouse conditions that determined by preliminary dose setting experiments. The concentration ranges for PEF and MCM-41@fungus in laboratory and greenhouse conditions were 10^4-10^8 and 10^2-10^6 and 10^5-10^9 and 10^3-10^7conidia/ml, respectively. For each trial, adults were dipped in the spore suspensions and MCM-41@fungus treatments separately. For controls, the aphids were immersed in 0.02 % Tween-80 aqueous solution. In the laboratory, when the water had evaporated and the aphids were dry, they were transferred into Petri dishes with fresh cabbage leaves kept at 22 ± 2 °C, 70 ± 5 % RH and a photoperiod of 12 L: 12 D. In the greenhouse, aphids that were immersed in the spore suspensions of PEF and MCM-41@fungus treatments separately were placed on fresh cabbage leaves. Mortality was recorded after 7 days. There were three replicates of 20 adults per fungal isolate, MCM-41@fungus and control. In MCM-41@fungus experiments, 0.1 g of each nanocomposite was dispersed in 100 ml distilled water containing 0.02 % Tween-80 until water absorbance was stabilized. After shaking and product dispersion, adult

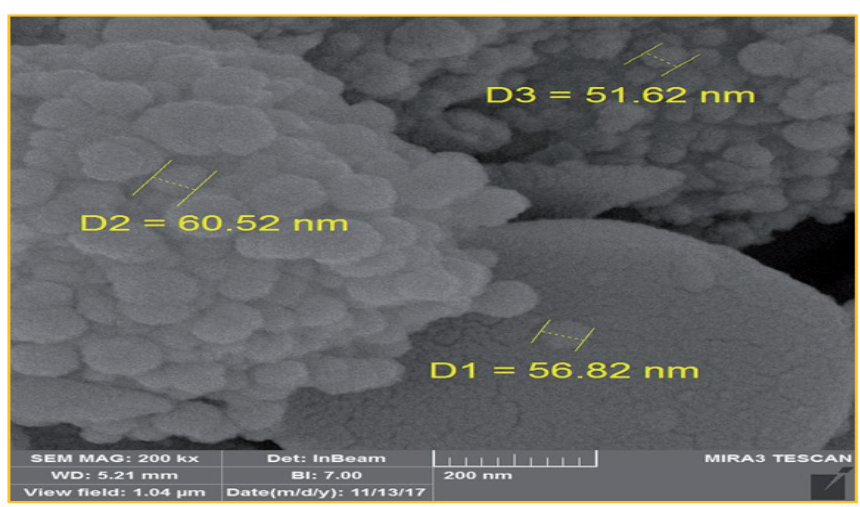

Figure 1: SEM image of MCM-41@fungus

aphids were dipped in the corresponding solutions for 10 s.

2.7 DATA ANALYSIS

In order to determine LC_{50} values, the data were analyzed utilizing the probit procedures with SPSS for Windows® release 24.

3 RESULTS

The Scanning Electron Microscope analysis is an important device for the analysis of the surface morphology of a mesoporous structure. The SEM micrograph of MCM-41@fungus mesoporous is shown in Fig.1. Notably, the particles observe to be in nano range (50-60 nm). The morphology of the mesoporous demonstrates homogeneous, regular, and spherical morphology.

Thermogravimetric analysis (TGA) was employed to select the mass changes of functionalized mesoporous MCM-41.TGA curves for MCM-41 (a) and MCM-41@fungus (b) are depicted in Fig.2. According to the TGA curve b, an initial mass loss was seen at temperatures below 200 °C because of the removal of physically and chemically adsorbed water molecules inside the pores channels and surface hydroxyl groups. Additionally, at temperatures above 200 °C, large mass losses were occurred which were mainly due to the decomposition of

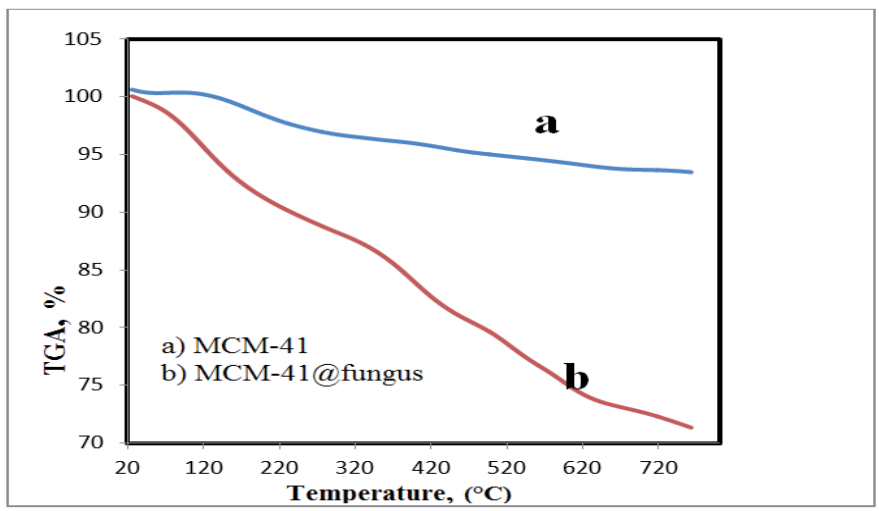

Figure 2: TGA thermograms of MCM-41(a) and MCM-41@fungus (b)

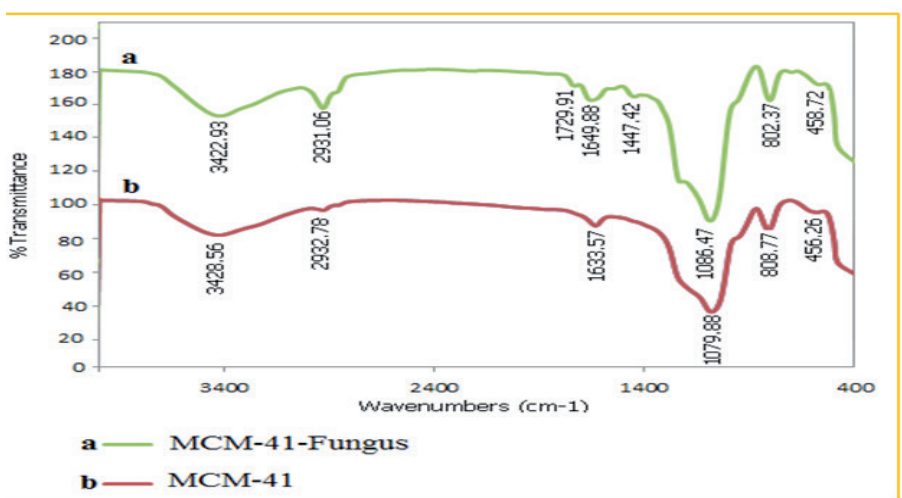

Figure 3: FT-IR spectra of MCM-41@fungus (a) and MCM-41 (b)

Table 1: Toxicity of *Lecanicillium lecanii* and MCM-41@fungus to one-day-old adults of *Brevicoryne brassicae* under laboratory and greenhouse conditions

Experimental conditions	Treatments	Slope ± S. E.	χ^2 (df)	LC_{50} (conidia/ml)	LC_{90} (conidia/ml)
Laboratory	PEF	3.84 ± 0.04	1.58 (3)	1.9×10^6 (6.5×10^5-6.5×10^6)	2.0×10^{10} (1.4×10^9-3.3×10^{12})
	MCM-41@fungus	4.80 ± 0.05	1.53 (3)	2.5×10^4 (9.3×10^3-8.3×10^4)	1.4×10^8 (1.2×10^7- 1.4×10^{10})
Greenhouse	PEF	3.60 ± 0.03	1.26 (3)	2.0×10^7 (7.1×10^6- 6.6×10^7)	1.5×10^{11} (1.2×10^{10}- 1.9×10^{13})
	MCM-41@fungus	4.52 ± 0.05	1.22 (3)	2.0×10^5 (7.8×10^4-6.1×10^5)	8.4×10^8 (8.8×10^7-5.1×10^{10})

PEF: Pure Entomopathogenic Fungus (= non-formulated fungus), 95 % fiducial limit (FL) is shown in parenthesis

covalently bonded organics (200-500 °C) and silanol groups (> 500 °C). This showed that the grafting of fungus had occurred on the inner pore channels of Si-MCM-41.

As shown in Figure 3, curve a, demonstrates stretching vibrations at 1447 cm^{-1} (C-N), 1729 cm^{-1}(C=O) and a broad band at around 3422 cm^{-1} (O-H and N-H) which is related to the fungus wall. Curve b, displays the absorption band at 3428 cm^{-1} is attributed to the stretching vibration of the O-H groups. The bands at 1079, 808 and 456 cm^{-1} are attributed to the symmetric and asymmetric stretching vibrations of the mesoporous framework (Si-O-Si). By comparing the FT-IR spectrums of MCM-41@fungus (a) and MCM-41 (b) in figure 3, the presence of fungus surrounding the MCM-41 can be clearly acclaimed.

LC_{50} values of PEF and MCM-41@fungus were 1.9×10^6 and 2.5×10^4 conidia/ml on adults under laboratory conditions and 2.0×10^7 and 2.0×10^5 conidia/ml in greenhouse conditions, respectively (Table 1). It is obvious that there is a difference between PEF and MCM-41@fungus, as inferred by the confidence limits of LC_{50} values (Table 1).

4 DISCUSSION

MCM-41 has attracted great attention because of its large pore size, high thermal stability and extremely high surface area especially above 1000 $m^2 g^{-1}$ (Nikoorazm et al., 2014). The exceptionally high internal surface area, porous structure and uniformity of MCM-41 make it as a cost-efficient host material for a variety of supported catalysts (Nikoorazm et al., 2014). In this study, fungus was grafted into MCM-41 which led to the synthesis of MCM-41@fungus. During the synthesis, MCM-41 was functionalized with the fungus. Functionalization of MCM-41 was performed by the hydroxyl group of entomopathogenic fungus. Some studies have revealed the efficacy of *L. lecanii* in pest control (Ghaffari et al., 2017; Alavo, 2015; Schreiter et al., 1994; Ramanujam et al., 2017). Our findings confirm *L. lecanii* pathogenicity against adults of *B. brassicae* so it will be an efficient candidate for integrated *B. brassicae* management strategies. According to LC_{50} values, a significant reduction was seen in the amount of entomopathogenic fungus found in the formulated ones. LC_{50} values of PEF and MCM-41@fungus were 1.9×10^6 and 2.5×10^4 conidia/ml on adults of *B. brassicae* under laboratory and 2.0×10^7 and 2.0×10^5 conidia/ml in greenhouse conditions, respectively. Bioassays demonstrated that nano-formulated *L. lecanii* significantly decreased LC_{50} values of fungus and it was more efficient than non-formulated one at adult stage of the pest under laboratory and greenhouse conditions. These results were consistent with the results of Sabbour (2014 b) that studied the efficacy of nano-destruxin of *M. anisopliae* (Metchnikoff) Sorokin (against adult females of *Hetiracris littoralis* Rambur under laboratory conditions. She demonstrated that in nano-formulated samples, LC_{50} values were significantly decreased. The toxicity bioassays of PEF and MCM-41@fungus indicated that adult stage was more sensitive to nano-formulated *L. lecanii* than pure entomopathogenic fungus (i.e. MCM-41@fungus exhibited lower LC_{50} value compared with PEF). Based on our findings, *B. brassicae* adults were more susceptible to infection by MCM-41@fungus than PEF. Based on our knowledge, this is the first research revealing the susceptibility of *B. brassicae* adults to MCM-41@entomopathogenic fungus. The experimental protocol followed in the present study allowed us to provide evidence for MCM-41@entomopathogenic fungus infection.

5 CONCLUSION

Finally, laboratory and greenhouse bioassays have clearly exhibited the pathogenicity of MCM-41@*L. leca-*

nii for adults of *B. brassicae*. These bio-control agents should be considered for the development of a new and environmentally compatible approach for cabbage aphid management in terms of preventing *B. brassicae* infestations. Further research is required to determine the biological activities of MCM-41@*L. lecanii* and its persistence after greenhouse and field applications and other factors that may improve its performance against *B. brassicae* throughout its adult stage.

6 REFERENCES

Abdollahi-Alibeik, M., & Pouriayevali, M. (2012). Nanosized MCM-41 supported protic ionic liquid as an efficient novel catalytic system for Friedlander synthesis of quinolones. *Catalysis Communications, 22*, 13-18. https://doi.org/10.1016/j.catcom.2012.02.004

Abdu-Allah, G. (2012). Aphicidal activity of Imidacloprid and Primicarb compared with certain plant extracts on *Brevicoryne brassicae* L. and *Aphis craccivora* Koch. *Assiut Journal of Agricultural Science, 43*, 104-114.

Alavo, T. B. (2015). The insect pathogenic fungus Verticillium lecanii (Zimm.) Viegas and its use for pests control: A review. *Journal of Experimental Biology, 3,* 337-345. https://doi.org/10.18006/2015.3(4).337.345

Burges, H.D. (1981). *Microbial control of pests and plant diseases 1970-1980*. Academic Press,London, 949 pp.

Cai, Q., Luo, Z.S., Pang, W.Q., Fan, Y.W., Chen, X.H., & Cui, F.Z. (2001). Dilute solution routes to various controllable morphologies of MCM-41 silica with a basic medium. *Chemistry of materials, 13*, 258-263. https://doi.org/10.1021/cm990661z

Collantes, L. G., Raman, K., & Cisneros, F. H. (1986). Effect of six synthetic pyrethroids on two populations of potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), in Peru. *Crop Protection, 5*, 355-357. https://doi.org/10.1016/0261-2194(86)90116-X

Ghaffari, S., Karimi, J., Kamali, S., & Moghadam, E. M. (2017). Biocontrol of Planococcus citri (Hemiptera: Pseudococcidae) by *Lecanicillium longisporum* and *Lecanicillium lecanii* under laboratory and greenhouse conditions. *Journal of Asia-Pacific Entomology, 20*, 605-612. https://doi.org/10.1016/j.aspen.2017.03.019

Goettel, M.S., Koike, M., Kim, J.J., Aiuchi, D., Shinya, R., & Brodeur, J. (2008). Potential of *Lecanicillium* spp. for management of insects, nematodes and plant diseases. *Journal of Invertebrate Pathology, 98*, 256-261. https://doi.org/10.1016/j.jip.2008.01.009

Griffin, R.P., & Williamson, J. (2012). *Cabbage, Broccoli and other cole crop insect pests HGIC 2203*, Home and Garden information center. Clemson cooperative extension, Clemson University, Clemson, SC.

Hall, R. A. (1981). The fungus *Verticillium lecanii* as a microbial insecticide against aphids and scales. In: Burges HD (ed.) *Microbial Control of Pests and Plant Diseases*. Academic Press, London, New York.

Kanaoka, M., Isogai, A., Murakoshi, S., Ichinoe, M., Suzuki, A., & Tamura, S. (1978). Bassianolide, a new insecticidal cyclodepsipeptide from *Beauveria bassiana* and *Verticillium lecanii. Agricultural and Biological Chemistry, 42*, 629-635. https://doi.org/10.1271/bbb1961.42.629

Llanderal-Cazares, C., Lagunes-Tejada, A., Carrillo-Sanchez, J. L., Sosa-Moss, C., Vera-Graziano, J., & Bravo-ojica, H. (1996). Susceptibility of *Phthorimaea operculella* (Zeller) to insecticides. *Journal of Entomological Science, 31*, 420-426. https://doi.org/10.18474/0749-8004-31.4.420

MousaviAnzabi, S.H., Nouri-Ghanbalani, G., Eivazi, A., & Ranji, A. (2013). Resistance Components of Canola, *Brassica napus* L. Genotypes to Cabbage Aphid *Brevicoryne brassicae* (L.). *Applied Research in Plant Protection, 2*, 85-100.

Neupane, F. P. (1999). Field Evaluation of Botanicals for the Management of Cruciferous Vegetable Insect Pests. Nepal *Journal of Science and Technology, 1*, 77-84.

Nikoorazm, M., Ghorbani-Choghamarani, A., Ghorbani, F., Mahdavi, H., & Karamshahi, Z. (2014). Bidentate salen Cu(II) complex functionalized on mesoporous MCM-41 as novel nano catalyst for the oxidative coupling of thiols into disulfides using urea hydrogen peroxide (UHP). *Journal of Porous Materials* https://doi.org/10.1007/s10934-014-9892-6

Ramanujam, B., Krishna, J., & Poornesha, B. (2017). Field evaluation of entomopathogenic fungi against cabbage aphid, *Brevicoryne brassicae* (L.) and their effect on coccinellid predator, *Coccinella septempunctata* (Linnaeus). *Journal of Biological Control, 31*,168-171. https://doi.org/10.18311/jbc/2017/16350

Rath, D., & Parida, K. M. (2011). Copper and nickel modified MCM-41 an efficient catalyst for hydrodehalogenation of chlorobenzene at room temperature. *Industrial and Engineering Chemistry Research, 50*, 2839-2849. https://doi.org/10.1021/ie101314f

Sabbour M. M. (2014). Evaluating toxicity of extracted nano-Destruxin against the desert locust Schistocerc agregaria in Egypt. *The Journal of Egyptian Academic Society Environmental Development, 15,* 9-17.

Schreiter, G., Butt, T.M., Beckett, A., Vestergaard, S., & Moritz, G. (1994). Invasion and development of *Verticillium lecanii* in the western flower thrips, *Frankliniella occidentalis*. *Mycological Research, 98*, 1025-1034. https://doi.org/10.1016/S0953-7562(09)80429-2

Sharma, A., & Gupta, R. (2009). Biological activity of some plant extracts against *Pieris brassicae* (Linn.). *Journal of Biopesticides, 2* 26-31.

Shylesh, S., & Singh, A. P. (2005).Vanadium-containing ordered mesoporous silicates: Does the silica source really affect the catalytic activity, structural stability, and nature of vanadium sites in V-MCM-41? *Journal of Catalysis, 233*, 359-371. https://doi.org/10.1016/j.jcat.2005.05.001

Suzuki, A., Kanaoka, M., Isogai, A., Tamura, S., Murakoshi, S., & Ichinoe, M. (1977). Bassianolide, a new insecticidal cyclodepsipeptide from *Beauveria bassiana* and *Verticillium lecanii. Tetrahedron Letters, 18,* 2167-2170. https://doi.org/10.1016/S0040-4039(01)83709-6

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Diversity of endophytic fungal community associated to the roots of *Argania spinosa* (L.) Skeels growing in the arid and semi-arid regions of Algeria

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Diversity of endophytic fungal community associated to the roots of *Argania spinosa* (L.) Skeels growing in the arid and semi-arid regions of Algeria

Abstract: Current study identified endophytic fungi associated to *Argania spinosa* (argan) roots and revealed diverse haplotype diversity by the sequencing of internal transcribed spacer (ITS). 586 operational taxonomic units were identified and these operational taxonomic units (OTUs) could be assigned to fungal functional diversity such as endophytes, ectomycorrhiza and putative pathogens. Ascomycota phylum was abundant. Beside Ascomycota phylum, Basidiomycota members were also found in argan roots. *Geopora, Sebacina, Knufia, Tomentella, Penicillim* had high relative abundance. Our results highlighted a non-nested assemblage of fungi. Current non-nested findings also confirm that fungi have similar pattern found in other habitats. Pairwise analysis mirrored segregation pattern between same and different functional fungal group. Fungi in semi-arid conditions are non-randomly structured. Members of Ascomycota phylum had high Z-scores. This is the first molecular study conducted in arid and semi-arid habitats of Algeria aiming to identify fungi associated with roots in argan tree. Given the fact that deserts are among harsh environments and fungi associated to desert plants may have implications for biodiversity and ecosystem functioning.

Key words: *Argania spinose*; fungi, diversity; internal transcribed spacer; endophytes; ectomycorrhiza

Raznolikost endofitskih glivnih združb povezanih s koreninami argana (*Argania spinosa* (L.) Skeels), v sušnih in polsušnih območjih Alžirije

Izvleček: Namen raziskave je bil določiti endofitske glive, ki so povezane s koreninami argana (*Argania spinosa*) in odkriti raznolikost različnih haplotipov s sekvenciranjem ITS DNK. Določenih je bilo 586 operacijskih taksonomskih enot in te enote (Operational Taxonomic Unit, OTUs) lahko pripišemo funkcionalni raznolikosti gliv kot so endofiti, ektomikorizne glive in potencialni patogeni. V koreninah argana so bili najbolj pogosti predstavniki zaprtotrosnic (Ascomycota), poleg njih so bili najdeni tudi predstavniki prostotrosnic (Basidiomycota). Rodovi *Geopora, Sebacina, Knufia, Tomentella, Penicillim* so imeli veliko relativno pogostnost. Rezultati raziskave so osvetlili nepovezanost skupin gliv. Podobni vzorci nepovezanih skupin gliv so bili najdeni tudi v drugih habitatih. Analiza parov je pokaza vzorce segregacije med enakimi in različnimi funkcionalnimi skupinami gliv. Glive polsušnih območij niso naključno organizirane. Predstavniki zaprtotrosnic imajo velikokrat normalno porazdelitev. To je prva molekularna raziskava v sušnih in polsušnih habitatih Alžirije, katere namen je bil določiti glive, ki so povezane s koreninami argana. Puščave so med najbol ekstremnimi okolji in glive, ki so povezane s puščavskimi rastlinami so pomemben del raznolikosti in delovanja teh ekosistemov.

Ključne besede: *Argania spinose*; glive; raznolikost; ITS; endofiti; ektomikoriza

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1 INTRODUCTION

Fungi are important component of ecosystem and play pivotal role in ecosystem functioning such as carbon and nutrient cycling (Courty et al., 2010; Treseder, 2004). Among fungi there are diverse functional groups such as mycorrhizal, endophytic and pathogenic fungi. These fungi interact with diverse plant species (Smith & Read, 2008), and such interactions are vital for survival and growth of plant species (Finlay, 2008).

Among harsh ecosystem, desert ecosystems represent one of the challenging habitats for microorganism, as water affects microbial activity, which in turn could play crucial role in ecosystem functioning (Austin et al., 2004; Collins et al., 2008). Argan tree (*Argania spinosa* (L.) Skeels) is a slow growing endemic plant species in distributed in north-west Africa (Díaz-Barradas et al., 2010). This tree species is known for its ecological importance because it creates a favourable microclimate for the development of other plant species and protects soils against erosion. In addition, it plays a socio-economic role in the regions where it grows. Each part of the plant including leaves and fruits are used as source of forage for cattle, whereas timber is widely used for fuel purpose, furthermore argan oils have therapeutic properties to cure scars and serve as an anti-ageing agent (Charrouf & Guillaume, 2009). In Algeria, there is decline in population of argan plant due to ecological and anthropogenic factors including climate change and grazing pressure (Charrouf & Guillaume, 2009; Díaz-Barradas et al., 2010).

Given the drastic effect of climate change and anthropogenic factors, it is important to explore the fungal community of desert plant species, such as argan tree. This plant has strong and deep root system (Kenny & De Zborowski, 2007), which harbours high fungal diversity (Sellal, 2016). Previous focus had been paid to study fungal endophytes in arid and semi-arid habitats. For instance, revealed high colonization of fungal endophytes in semi-arid. Martínez-García et al. (2011) also highlighted impact of shrubs on root associated fungi and highlighted importance of selective pressure in determining root associated fungi.

To our knowledge fungal diversity in arid and semi-arid is rarely explored and understood owing to correct identification and most of the fungal strains are non-cultivable in laboratory conditions and laboratory based culturing method may not capture the real fungal diversity (Zhang et al., 2016). Nonetheless, a development in a high throughput sequencing technology provides an excellent platform to explore below ground functional fungal diversity (Buee et al., 2009; Fortuna Miguel et al., 2010).

Nestedness or species-species interaction networks describing the interactions between species is important structural ecological property, and nestedness has revealed positive influence on diversity against catastrophic effect. Nestedness has been proposed to assess community assembly which can further push our understanding about community structure and interactions therein (Ulrich et al., 2009).

We hypothesize fungal community may lack nestedness pattern but could show other non-random pattern such as segregation and aggregation. We propose that limited resources, such as water paucity and nutrients availability generate the segregation pattern.

Key objectives of current study were to: I) identify root associated functional endophytic fungal diversity; II) assess how different fungal taxonomic genera are organize in roots? III) find potential fungal OTUs segregation and aggregation pattern.

2 MATERIAL AND METHODS

2.1 STUDY AREAS AND SAMPLING

The study was carried out in three different climatic regions of Algeria: Tindouf, Mostaganem and Chlef. The area of Tindouf is a desert region in the south-west of Algeria (28°29′56.47″N 8°07′09.72″W). Mostaganem (35°48’09.81″N 0°03’59.30″E) and Chlef (36°09′46.95″N 1°20′12.22″E) are situated in the north-west of Algeria (Table1)

At each region, five healthy specimens (20-30 m apart) of *Argania spinosa* were randomly selected. Four replications in cardinal directions of each tree were collected and homogenised to form a single sample in cardinal directions. In each direction the top litter (20-40 cm) was removed to eliminate part of the dry/not decomposed leaf litter, and samples (soils and argan roots) were collected at 0-30 cm depth and pooled. Fine roots were excavated and traced from the originating tree to ensure identity. Samples were kept in plastic bags and stored at 4 °C until processing.

2.2 DNA EXTRACTION FROM ROOTS AND PCR

After roots surface sterilization by soaking in 70 % ethanol (7:3, v/v, 1 min), 3 % sodium hypochlorite (3 min) and 70 % ethanol (7:3, v/v, 1 min) and were then rinsed twice for 1 min in sterile water , genomic DNA was extracted from field roots, using the genomic DNA Kit (Nucleo Spin Soil). The manufacturer’s protocol was modified in that 250 mg of roots was ground by hand

Table 1: Sites characteristics

Location	Chlef	Mostaganem	Tindouf
Coordinates	36°09′46.95″N 1°20′12.22″E	35°48’09.81’N 0°03’59.30″E	28°29′56.47″N 8°07′09.72″W
Mean annual temperature	18.6° C	18.3° C	23.4° C
Mean annual precipitation	394 mm	436 mm	30 mm
Altitude	119 m	35 m	537 m
Associated plants			*Acacia tortilis* Forssk. *Acacia raddiana* Forssk.
			Anabasis articulata Forssk., *Asphodelus* sp.
			Aristida pungens Desf., *Calotropis procera* Aiton
		Sonchus arvensis L.	*Chrysocomoides cassini* Desf.
		Malva sylvestris L.	*Euphorbia guyoniana* Boiss. & Reut. *Faidherbia albida* Delile, *Genista saharae* Coss. & Durieu
		Avena sativa L.	
		Echium vulgare L.	
		Hordeum vulgare L.	*Helianthemum lippii* L.
		Bromus sp.	*Moricandia arvensis* L.,
		Plantago laceolata L.	*Marrubium deserti* Noë
		Antenis coatula L.	*Nolletia. Retama monosperma* L.
	Oxalis sp.	*Chrysanthemum* sp.	*Rhus tripartitus* L., *Zizyphus lotus* Lam., *Zilla spinosa* L.
	Olea europea L.	*Cenaurea napifolia* L.	

with pestle in a mortar containing 700 µl of lysis solution before transfer to Eppendorf tube and incubated at 70 °C for 10 min. To study the effect of DNA dilution on PCR inhibitors, after extraction, DNA was diluted 1:1, 1:10, 1:20 and 1:100 with ultra-pure water, in order to obtain DNA containing less PCR inhibitors. Fungal sequences were amplified using the primers ITS1F-ITS4 (White et al., 1990) to target a complete rDNA ITS region. The PCRs were carried out in a final volume of 25 µl, containing 12.5 µl PPP Master Mix (Top-Bio, Prague, Czech Republic) 1 µl of each primer solution (10 µM), 1 µl of DNA template and 9.5 µl ultra-pure water. The PCR conditions were as follow: initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 52 °C for 45 s and extension at 72 °C for 1min 30 s, with a final extension at 72 °C for 10 min. All PCR reactions were run in Eppendorf PCR cycler. PCR products were examined on a 1 % (w:v) agarose gel with an ethidium bromide staining in and compared to a 100 bp DNA ladder. PCR products (only DNA diluted 1:20 and 1:100 were used in this step) were purified using QIAquick® PCR purification Kit (Qiagen).

2.3 NESTED PCR

The products of the first PCR were diluted 1:1000 with ultra-pure water and used as template DNA for the second PCR amplification using various combinations between gITS7 (Ihrmark et al., 2012) and ITS4 (White et al., 1990) including Illumina adapters. Each PCR reactions (25 µl) contained 12,5 µl PPP Master Mix (Top-Bio, Prague, Czech Republic) 0.5 µl of each primer solution (10 µM), 1µl of DNA template and 10.5 µl ultra-pure water with the following cyclin conditions: initial denaturation at 94 °C for 4 min, followed by 25 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s and extension at 72 °C for 30 s, with a final extension at 72 °C for 10 min. PCR products were examined on a 1 % (w:v) agarose gel with ethidium bromide staining in the presence of 100 bp DNA ladder. PCR products were pooled and purified using QIA quick PCR purification Kit (Qiagen). DNA was quantified with Nano Drop (Thermo Scientific) and pooled before sequencing. Amplicons were sequenced on sequenced on Illumina MiSeq 2×250 bp platform in the laboratory of fungal biology, Institute of

microbiology, The Czech Academy of Sciences, Prague, Czech Republic. Species area curve was generated in PC-ORD version 5 (McCune and Mefford, 2006). Curves were generated based on samples.

2.4 BIOINFORMATICS

Sequencing data was analysed in Seed software (Větrovský & Baldrian, 2013), version 2.0.4. Following parameters were carried out: after rarefying the reads and demultiplexing of the samples based on their unique barcodes, removing low-quality sequences and sequences shorter than 40 bp, ITS sequences were extracted from the sequences, the contigs were chimaera-cleaned/clustered by the Usearch tool, version 8.1.1861, (Edgar & Flyvbjerg, 2015) at the similarity level 97 % and the most abundant sequences were compared while following blast tool version 2.2.26 + (Altschul et al., 2008). Sequencing similarity was matched against the sequences in GenBank database (environmental sequences, metagenomes and unidentified organisms excluded).

2.5 STATISTICAL ANALYSIS

To assess fungal community assemblage in argan plant species we carried out a nestedness analysis based on Nestedness Metric Based On Overlap And Decreasing Fill (NODF metric) (Almeida-Neto et al., 2008) implemented in Aninhado software (Guimaraesjr & Guimaraes, 2006). We used present and absent data to compute NODF metric in Aninhado. Fungal OTUs present and absent data was permuted 1000 times and significance was assessed while following null models in Aninhado software. NODF values were inferred according to *p*-values of null models.

To discern pair wise fungal OTUs association in argan plant roots collected from sampling regions, non-random association between fungal OTUs was assessed. In order to remove a rare species bias, relationship between fungi having relative frequency of 7 was calculated. Unclassified/unidentified fungal species were also not kept in pairwise analysis as this can lead to false conclusion on OTU community. Presence/absence matrix was randomized and computed to get C-scores and *p*-values respectively. Values were inferred while following fixed-fixed null model. All the calculations were carried out in the Pairs software (Ulrich, 2008).

3 RESULTS

3.1 FUNGAL COMMUNITY COMPOSITION

Fungal community was diverse, as 1220 fungal OTUs including singletons were recorded. We removed singletons from our analysis, none the less it resulted in 586 OTUs belonging to 65 different genera. Ten most relatively frequent genera belonged to all three functional groups - endophytic, pathogenic and ectomycorrhizal fungi (Fig.1).

Some genera, such as *Geopora*, *Sebacina*, *Knufia*, *Tomentella* and *Penicillim* had high relative abundance in terms of sequence abundance reads (Fig.2).

Ascomycota was the most abundant phylum in all regions (461 reads) comparing to Basidiomycota (125 reads) ($p = 0.0001$).

The number of reads of OTUs varies between the

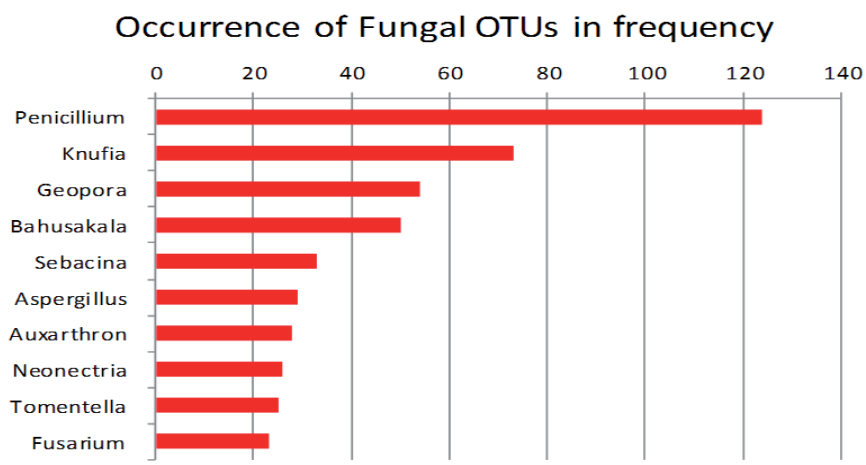

Figure 1: Relative frequency of different functional fungal genus

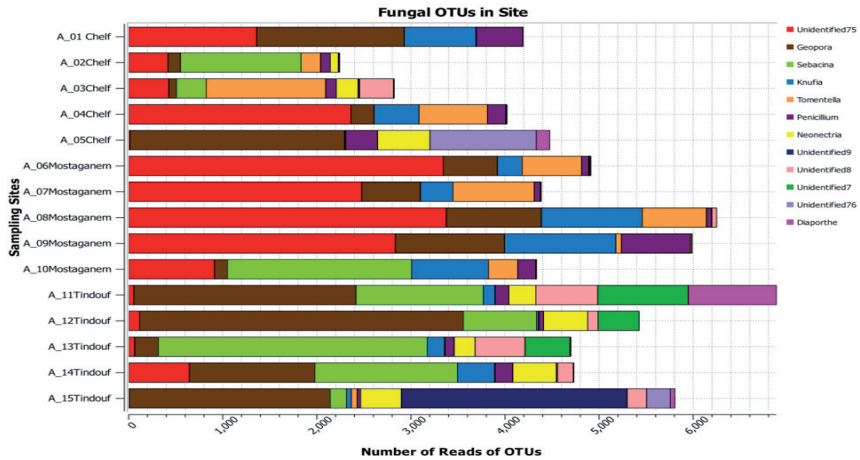

Figure 2: Relative abundance of fungal genus

three regions and from one tree to another in the same region. In fact, Mostaganem was the richest region with fungal community. The genus *Neonectria* was absent in all samples of Mostaganem region. (Figure 2). *Sebacina* was the most abundant genus in Tindouf and absent in all samples of Mostagenem, *Geopora* came in the second position in the same region. However the undefined fungi were the most important fungi in Chlef and Mostaganem ($p = 0.001$).

Sample based curve captured maximum diversity for common fungal OTUs (Fig. 3a), and rarefaction revealed increase in OTU richness, whereas we did not obtain a plateau curve for rare fungal taxa (Figure 3a, Figure 3b).

3.2. FUNGAL COMMUNITY ASSEMBLAGE/NESTEDNESS ANALYSIS

According to our expectations, we detected low lev-

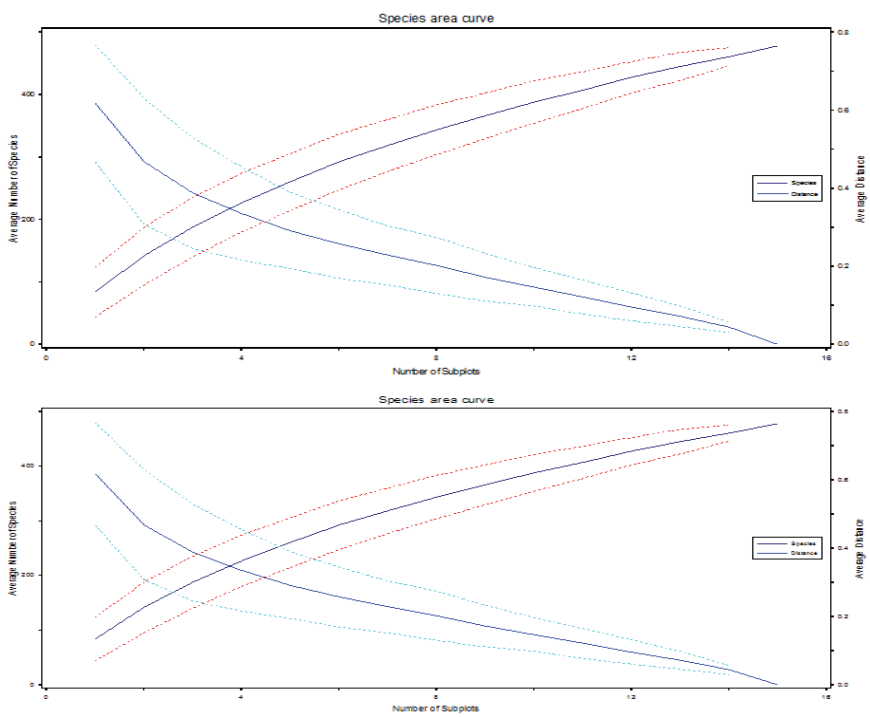

Figure 3a, b: Rarefaction curve for all fungal OTUs. a) Species area curve without singletons. b) Species area curve with singletons. X-axis = number of subplots and Y-axis = OTUs and Sorenson distance of OTUs

Table 2: Species pair results based on Z and P values

Species 1	Species 2	Z -score	P-value
Phomopsis sp.	*Tricholoma* sp.	4.9	6×10^{-7}
Tricholoma sp.	*Pseudogymnoascus* sp.	4.9	6×10^{-7}
Knufia sp.4	*Phomopsis* sp.	4.6	3.5×10^{-6}
Tomentella sp.	*Phomopsis* sp.	4.5	4.2×10^{-6}
Pseudogymnoascus sp	*Bahusakala* sp.2	4.4	8.7×10^{-6}
Knufia sp.4	*Pseudogymnoascus* sp.	3.7	1.4×10^{-4}
Knufia sp.4	*Diaporthe* sp.	3.7	2.0×10^{-4}
Knufia sp.3	*Phomopsis* sp.	3.3	8.8×10^{-4}
Tomentella sp.	*Pseudogymnoascus* sp.	3.1	1.6×10^{-3}
Tricholoma sp.	*Embellisia* sp.	3.1	1.7×10^{-3}
Pyrenochaeta sp.	*Penicillium* sp.6	2.9	3.2×10^{-3}
Tricholoma sp.	*Diaporthe* sp.	2.8	3.8×10^{-3}
Knufia sp.3	*Diaporthe* sp.	2.8	4.8×10^{-3}
Knufia sp.3	*Embellisia* sp.	2.7	5.9×10^{-3}
Phomopsis sp.	*Pseudogymnoascus* sp.	-1.9	4.7×10^{-2}

el of nestedness as revealed by analysis. NODF values for whole fungal community were significantly not higher than expected by chance (NODF = 16, $p = 0.7$).

3.3 GENUS PAIR ANALYSIS

19 species depicted significant non-random association and majority of species showed segregation pattern, while 5 species revealed positive co-occurrence (Table 2). Competitive interactions were predominant at phylum and subphylum level. Positive interactions between the fungi at same and different genera were observed (Table 2).

4 DISCUSSION

Using high throughput sequencing, we explored fungal communities in semi-arid region. Majority of fungal OTUs belonged to Ascomycota phylum. Culturing based study also revealed dominancy of Ascomycota fungi in desert covered by *Artemisia herba-alba* Asso and *Zygophyllum dumosum* Boiss. (Grishkan & Nevo, 2010). Current results are also in line with study conducted in semi-arid areas. Based on high throughput sequencing technology (Wehner et al., 2014), highlighted abundance of Ascomycota phylum in semi-arid. Our results support notions that desert plant *Argania spinosa* harbour diverse fungal communities. We reported fungal OTUs representing different functional diversity such as mycorrhizal, endophytic and pathogens. It is not uncommon to report and document such functional diversity in semi-arid habitat. It has been well documented occurrence of mycorrhizal, endophytic and pathogens diversity in semi-arid (Porras-Alfaro et al., 2008). We highlighted dominancy of Ascomycota phylum and fungal OTUs such as *Penicillium* and *Fusarium*. Indeed, semi-arid supports high endophytic fungal diversity, as strong evidences suggest prevalence and dominancy of Ascomycota, *Penicillium* and *Fusarium* (Gonzalez-Teuber et al., 2017) Fungal OTUs i.e. *Geopora* which are identified and characterized in present research could be compared with other habitats, as *Geopora* formed mycorrhizal association with *Pinus* species (Flores-Renteria et al., 2014), and samples collected in dry season revealed dominancy of *Geopora* genus fungi (Gordon & Gehring, 2011). This shows adaptability and occurrence of *Geopora* in various environmental conditions argan plant supports high fungal diversity, as Basidiomycota members were also recorded. Usually Basidiomycetes fungi are found in relative moist habitat (Buee et al., 2009), therefore fungal diversity explored could be linked other habitats. Research carried out in secondary temperate forest documented occurrence of endophytic and ectomycorrhizal fungi in single host plant species (Frossard et al., 2015). *Sebacina* genus was among abundant fungal genus detected in argan root samples from Tindouf. There is strong evidence which supports ubiquitous nature of Sebacinales and such emerging evidences indicate ubiquitous occurrence

of fungi in various habitats. Our results did not capture rare fungal diversity (Figure 3b). The plausible explanation for such absence of rare fungal diversity is overexploitation of argan plant species and overexploitation may stem local extinction of rare fungal taxa.

Fungal community depicted non-nested assemblage and on the contrary fungi revealed competitive interactions (Table 2). Current non nested findings also confirm that fungi have similar pattern as compare to fungi in forests. We found support for our hypothesis that fungi may lack nested pattern and it could also lead to conclusion that argan root do not support facilitative interactions. Similar results were documented by some other authors (Bahram et al., 2014; Roy-Bolduc et al., 2016). Since, argan plants are cultivated and thus under human management and grazing pressure (Charrouf & Guillaume, 2009; Díaz-Barradas et al., 2010), we cannot exclude anthropogenic factors causing a non-nested assemblage of fungi. Another explanation is that host plants were under abiotic stress, which in turn may had generated non nested pattern.

Pair wise analysis mirrored segregation pattern between same and different functional fungal group. Several mechanisms are proposed to reveal such non-random occurrence pattern and competitive interactions between fungal OTUs could generate segregation pattern (Chen et al., 2000; Chilvers et al., 1987).

Competitive interactions between fungal OTUs in semi-arid have revealed that fungal communities were non-randomly structured in semi-arid. Our results are in line with (Wehner et al., 2014) who highlighted the abundance of Ascomycota phylum in semi-arid. We also showed that majority of fungal OTUs belonging to Ascomycota phylum had Z- scores -2 above 2 (Table2), showing statistical significance (Ulrich, 2008). Fungal pairs having endophytic and mycorrhizal mode of life style had high Z-scores, which indicated strong signal of competition between them. It is important to mention that previous studies focused on fungal groups and highlighted the prevalence of segregation patterns between ectomycorrhizal and endophytic fungi (Pickles et al., 2012; Saunders et al., 2010).

Positive interactions between same and different fungal OTUs suggested facilitative interactions between fungi. Facilitative interactions between fungi could be due to fact that fungi having similar functional requirements may occupy the same habitat which in turn could result in facilitative interactions. Species may sort according to shared requirements (Leibold et al., 2004), this may generate positive interactions between species. Given the fact, different functional genus in root occur due to different ecological and physiological requirements; henceforth there is high probability of positive interactions between fungi and facilitative interactions are well documented between different functional fungal groups (Wagg et al., 2008).

5 CONCLUSIONS

The present study is the first study to assess fungi associated with *Argania spinosa* roots growing in the arid and semi-arid climate. We revealed non nested assemblage pattern at community level, whereas pair wise association showed non-random pattern. Quite significant numbers of fungal OTUs were explored.

Future studies may carry out abundance-based data to assess pair wise association between fungi. We provide framework and initial study while using present-absent data to reveal non-random association of fungi in argan roots. Furthermore, inoculation experiments may be conducted to confirm facilitative and competitive interactions. Perhaps most striking feature of our research was high fungal diversity associated to single desert plant species.

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7 REFERENCE

Austin, A. T., Yahdjian, L., Stark, J. M., Belnap, J., Porporato, A., Norton, U., Schaeffer, S. M. (2004). *Water pulses and biogeochemical cycles in arid and semiarid ecosystems*. [Research Support, Non-U.S. Gov't https://doi.org/10.1007/s00442-004-1519-1

Bahram, M., Harend, H., & Tedersoo, L. (2014). Network perspectives of ectomycorrhizal associations. *Fungal Ecology, 7*, 70-77. https://doi.org/10.1016/j.funeco.2013.10.003

Buee, M., Reich, M., Murat, C., Morin, E., Nilsson, R. H., Uroz, S., & Martin, F. (2009). Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. [Research Support, Non-U.S. Gov't]. *New Phytol, 184*(2), 449-456. https://doi.org/10.1111/j.1469-8137.2009.03003.x

Charrouf, Z., & Guillaume, D. (2009). Sustainable Development in Northern Africa: The Argan Forest Case. *Sustainability, 1*(4), 1012-1022. https://doi.org/10.3390/su1041012

Collins, S. L., Sinsabaugh, R. L., Crenshaw, C., Green, L., Porras-Alfaro, A., Stursova, M., & Zeglin, L. H. (2008). Pulse dynamics and microbial processes in aridland ecosystems.

Journal of Ecology, 96(3), 413-420. https://doi.org/10.1111/j.1365-2745.2008.01362.x

Courty, P.-E., Buée, M., Diedhiou, A. G., Frey-Klett, P., Le Tacon, F., Rineau, F., Garbaye, J. (2010). The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. *Soil Biology and Biochemistry, 42*(5), 679-698. https://doi.org/10.1016/j.soilbio.2009.12.006

Díaz-Barradas, M. C., Zunzunegui, M., Ain-Lhout, F., Jáuregui, J., Boutaleb, S., Álvarez-Cansino, L., & Esquivias, M. P. (2010). Seasonal physiological responses of Argania spinosa tree from Mediterranean to semi-arid climate. *Plant and Soil, 337*(1-2), 217-231. https://doi.org/10.1007/s11104-010-0518-8

Edgar, R. C., & Flyvbjerg, H. (2015). Error filtering, pair assembly and error correction for next-generation sequencing reads. [Evaluation Studies]. *Bioinformatics, 31*(21), 3476-3482. https://doi.org/10.1093/bioinformatics/btv401

Finlay, R. D. (2008). *Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium*. [Research Support, Non-U.S. Gov't. https://doi.org/10.1093/jxb/ern059

Flores-Renteria, L., Lau, M. K., Lamit, L. J., & Gehring, C. A. (2014). *An elusive ectomycorrhizal fungus reveals itself: a new species of Geopora (Pyronemataceae) associated with Pinus edulis.* [Research Support, Non-U.S. Gov't https://doi.org/10.3852/13-263

Fortuna Miguel, A., Stouffer Daniel, B., Olesen Jens, M., Jordano, P., Mouillot, D., Krasnov Boris, R., Bascompte, J. (2010). Nestedness versus modularity in ecological networks: two sides of the same coin? *Journal of Animal Ecology, 79*(4), 811-817. https://doi.org/10.1111/j.1365-2656.2010.01688.x

Frossard, A., Ramond, J.-B., Seely, M., & Cowan, D. A. (2015). Water regime history drives responses of soil Namib Desert microbial communities to wetting events. *Scientific Reports, 5*, 12263. https://doi.org/10.1038/srep12263

Gonzalez-Teuber, M., Vilo, C., & Bascunan-Godoy, L. (2017). Molecular characterization of endophytic fungi associated with the roots of Chenopodium quinoa inhabiting the Atacama Desert, Chile. *Genom Data, 11*, 109-112. https://doi.org/10.1016/j.gdata.2016.12.015

Gordon, G. J., & Gehring, C. A. (2011). *Molecular characterization of pezizalean ectomycorrhizas associated with pinyon pine during drought.* [Research Support, Non-U.S. Gov't. https://doi.org/10.1007/s00572-010-0349-8

Research Support, U.S. Gov't, Non-P.H.S.]. *Mycorrhiza, 21*(5), 431-441. https://doi.org/10.1007/s00572-010-0349-8

Grishkan, I., & Nevo, E. (2010). Spatiotemporal distribution of soil microfungi in the Makhtesh Ramon area, central Negev desert, Israel. *Fungal Ecology, 3*(4), 326-337. https://doi.org/10.1016/j.funeco.2010.01.003

Guimaraesjr, P., & Guimaraes, P. (2006). Improving the analyses of nestedness for large sets of matrices. *Environmental Modelling & Software, 21*(10), 1512-1513. https://doi.org/10.1016/j.envsoft.2006.04.002

Ihrmark, K., Bodeker, I. T., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Lindahl, B. D. (2012). New primers to amplify the fungal ITS2 region--evaluation by 454-sequencing of artificial and natural communities. [Research Support, Non-U.S. Gov't]. FEMS *Microbiol Ecol, 82*(3), 666-677. https://doi.org/10.1111/j.1574-6941.2012.01437.x

Kenny, L., & De Zborowski, I. (2007). *Atlas de l'arganier et de l'arganeraie*. Rabat, Maroc: IAV Hassan II.

Leibold, M. A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J. M., Hoopes, M. F., . . . Gonzalez, A. (2004). The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters, 7*(7), 601-613. https://doi.org/10.1111/j.1461-0248.2004.00608.x

Loro, M., Valero-Jiménez, C. A., Nozawa, S., & Márquez, L. M. (2012). Diversity and composition of fungal endophytes in semiarid Northwest Venezuela. *Journal of Arid Environments, 85*, 46-55. https://doi.org/10.1016/j.jaridenv.2012.04.009

Martínez-García, L. B., Armas, C., Miranda, J. d. D., Padilla, F. M., & Pugnaire, F. I. (2011). Shrubs influence arbuscular mycorrhizal fungi communities in a semi-arid environment. *Soil Biology and Biochemistry, 43*(3), 682-689. https://doi.org/10.1016/j.soilbio.2010.12.006

Pickles, B. J., Genney, D. R., Anderson, I. C., & Alexander, I. J. (2012). Spatial analysis of ectomycorrhizal fungi reveals that root tip communities are structured by competitive interactions. [Research Support, Non-U.S. Gov't]. *Mol Ecol, 21*(20), 5110-5123. https://doi.org/10.1111/j.1365-294X.2012.05739.x

Porras-Alfaro, A., Herrera, J., Sinsabaugh, R. L., Odenbach, K. J., Lowrey, T., & Natvig, D. O. (2008). Novel root fungal consortium associated with a dominant desert grass. [Research Support, U.S. Gov't, Non-P.H.S.]. *Appl Environ Microbiol, 74*(9), 2805-2813. https://doi.org/10.1128/AEM.02769-07

Roy-Bolduc, A., Laliberte, E., & Hijri, M. (2016). High richness of ectomycorrhizal fungi and low host specificity in a coastal sand dune ecosystem revealed by network analysis. *Ecol Evol, 6*(1), 349-362. https://doi.org/10.1002/ece3.1881

Saunders, M., Glenn, A. E., & Kohn, L. M. (2010). Exploring the evolutionary ecology of fungal endophytes in agricultural systems: using functional traits to reveal mechanisms in community processes. *Evol Appl, 3*(5-6), 525-537. https://doi.org/10.1111/j.1752-4571.2010.00141.x

Sellal, Z. (2016). Arbuscular Mycorrhizal fungi species associated with rhizosphere of Argania spinosa (L.) Skeels in Morocco. *International Journal of Pure & Applied Bioscience, 4*(1), 82-99. https://doi.org/10.18782/2320-7051.2201

Smith, S. E., & Read, D. (2008). 1 - The symbionts forming arbuscular mycorrhizas *Mycorrhizal Symbiosis* (Third Edition) (pp. 13-41). London: Academic Press. https://doi.org/10.1016/B978-012370526-6.50003-9

Treseder, K. K. (2004). A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO2 in field studies. *New Phytologist, 164*(2), 347-355. https://doi.org/10.1111/j.1469-8137.2004.01159.x

Ulrich, W. (2008). *Pairs - a FORTRAN program for studying pair wise species associations in ecological matrices* [8]. Pairs.

Ulrich, W., Almeida-Neto, M., & Gotelli, N. J. (2009). A consumer's guide to nestedness analysis. *Oikos, 118*(1), 3-17. https://doi.org/10.1111/j.1600-0706.2008.17053.x

Větrovský, T., & Baldrian, P. (2013). Analysis of soil fungal communities by amplicon pyrosequencing: current approaches to data analysis and the introduction of the pipeline SEED.

Biology and Fertility of Soils, 49(8), 1027-1037. https://doi.org/10.1007/s00374-013-0801-y

Wagg, C., Pautler, M., Massicotte, H. B., & Peterson, R. L. (2008). The co-occurrence of ectomycorrhizal, arbuscular mycorrhizal, and dark septate fungi in seedlings of four members of the Pinaceae. [Research Support, Non-U.S. Gov't]. *Mycorrhiza, 18*(2), 103-110. https://doi.org/10.1007/s00572-007-0157-y

Wehner, J., Powell, J. R., Muller, L. A. H., Caruso, T., Veresoglou, S. D., Hempel, S., . . . van der Heijden, M. (2014). Determinants of root-associated fungal communities within Asteraceae in a semi-arid grassland. *Journal of Ecology, 102*(2), 425-436. https://doi.org/10.1111/1365-2745.12197

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetique. In A. Press (Ed.), *PCR protocols* (pp. 315). San Diego: Academic Press. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zhang, T., Jia, R.-L., & Yu, L.-Y. (2016). Diversity and distribution of soil fungal communities associated with biological soil crusts in the southeastern Tengger Desert (China) as revealed by 454 pyrosequencing. *Fungal Ecology, 23*, 156-163. https://doi.org/10.1016/j.funeco.2016.08.004

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Winter wheat variability according to local conditions

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Winter wheat variability according to local conditions

Abstract: The objectives of our experiments are the description of the phenotypic and genotypic variability by the main agriculture-value traits of the new winter wheat lines according to their interactions with different environmental conditions. Five new winter wheat lines were investigated at field experiment during three years by parameters of grain productivity and quality, uptaking of macro- and microelements and heavy metals from soil under different relief conditions.

Our investigations confirmed statement about more perspective direction for exploiting local sources for winter wheat improvement and closely relation between concentration of nutrient substances in plants, their loss from soil and peculiarities of relief, genotype and limits of adaptation. We developed high-adaptive line 213 ('Leana'), which provides us higher than standard grain yield under all conditions. All lines showed higher grain productivity under favorable conditions than control. Variability of traits was higher under south slope conditions (unfavorable conditions) rather than on other (proper conditions). Only line 156 was identified by good protein content and composition under every condition for gliadin and glutenin components. Influence of relief on microelements and heavy metals uptake to the winter wheat plants is not so important as for macroelements and, in consequence, for grain productivity and quality.

Key words: agrolandscape; growing conditions; winter wheat.

Spremenljivost ozimne pšenice glede na lokalne razmere

Izvleček: Namen poskusov je bil opisati fenotipsko in genotipsko spremenljivost glavnih agronomskih lastnosti linij ozimne pšenice glede na njihove interakcije z različnimi okoljskimi razmerami. Pet novih linij ozimne pšenice je bilo preučevano v poljskem poskusu v treh zaporednih letih glede na parametre pridelka in kakovosti zrnja, privzem makro in mikrohranil ter težkih kovin iz tal v različnih reliefnih razmerah.

Raziskava je potrdila spoznanja o bolj perspektivnih smernicah za izkoriščanje lokalnih danosti za izboljšanje ozimne pšenice in tesno povezanost med koncentracijo hranil v rastlini in njihovo izgubo iz tal, kot tudi posebnosti reliefa in genotipa in omejenosti prilagajanja. Vzgojili so zelo prilagodljivo linijo 213 ('Leana'), ki daje večji pridelek zrnja od standarda v vseh razmerah. Vse linije so imele v ugodnih razmerah večji pridelek zrnja kot kontrola. Spremenljivost lastnosti je bila na neugodnih južnih legah večja kot na drugih, ustreznih razmerah. Samo linija 156 je bila prepoznana po dobri vsebnosti in sestavi proteinov v vseh razmerah glede na vsebnost gliadina in glutenina. Vpliv reliefa na prevzem mikroelementov in težkih kovin pri ozimni pšenici ni tako pomemben kot za makroelemente in posledično za pridelek ter kakovost zrnja.

Ključne besede: kmetijska pokrajina; rastne razmere; ozimna pšenica

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1 INTRODUCTION

One of the main problem of bread wheat improvement at new century is using of local sources for improvement in breeding programs. Regarding the annual production of about 757 million tons (in 2017) (USDA, 2018), bread wheat (*Triticum aestivum* L.) occupied the first place as a major cereal crop in the worlds trade. Winter wheat is not only a world's leading cereal crop but also the most important food crop in Ukraine, which has occupied leading position in the national agriculture, taking about 48 % area under cereals and contributing 38 % of the total food grain production (Nazarenko, 2016). As for the quality traits winter wheat is the main stable crop for our country and provides more than 20 % of calories and proteins. Focused on only yield traits we have to understand that any high yield has no sense without proper quality for food and fodder demands (Shewry et al., 2012). During the 19th century, traditional varieties were mainly landraces that were well suitable to their local conditions. But from the beginning of the 20th century, general situation was rapidly changing, the majority of genotypes were out of this process, first of all for regions with specific conditions (Bordes et al., 2008). In the last 60 years plant breeding programs in terms of intensive agriculture led to the total replacement of landraces by modern semi-dwarf and high-yielding varieties, correlating with a decrease in wheat genetic diversity and needs in special requirements for realization their potential, but local sources were not taken into account at this process (Kharytonov et al., 2017; Nazarenko, 2017). Wheat improvement, which bases on the principal of ecological adaptability and taking into account special interactions between environment and genotypes, special abilities for changeability under unfavorable conditions provides us new approaches for formation stable agroecological systems without great losses at productivity (as an example local programs of INRA (France) (Bordes et al., 2011).

All efforts in past research on wheat improvement both at the genotype level and by technology, have been focused on obtaining more highly adaptive forms, not taking into account specific local conditions. Moreover, only a limited set of initial material was actively used. Higher priority was given to geographical areas and only at the end of the 20th century the efforts were focused on agro-ecological districts (Dawson et al., 2011).

Key attention we pay to major groups of agronomic-value traits, grain productivity (and formation of this trait) and grain quality. These traits in interaction with environment actually determine the overall wheat genotypes suitability for farming (Gepts & Hancock, 2006). Winter wheat yield has the most important and complex character affected directly or indirectly by genome systems present in plant (Rangare et al., 2010) as well as interaction with environment (Tester & Langridge, 2010; Serpolay et al., 2011). Thus, ecological assessment (part of evaluation process in breeding program for measurements of adaptation for new lines and varieties under difference regional conditions) of new wheat lines with high yield and quality genetic potential under difference condition (local and landscape), it`s components (Slafer & Andrade, 1993) and interaction (Sperling et all, 2001) have become a permanent task in the plant farming and breeding programs (Reif et al., 2005; Tuberosa & Salvi, 2006).

Regarding the fact of instability in agroecosystems related to types of agroecodistricts and relief type at interaction we investigated lines requirements under different types of slopes. One of the main nature factors for evaluation is a land relief, which is determined by balance of moisture, character of winter wheat growth and development, differences in seasons conditions (Andrusevich et al., 2018).

Grain storage proteins include about 60–80 % of the total protein in wheat grains and metabolic proteins, remaining part consists of the albumins and globulins (15–20 %) (Dai et al., 2015). Grain storage proteins are produced by plants during the effective filling phase of plant development (Shewry et al., 2012; Bonnot et al., 2017; Khalili et al., 2018). Gluten proteins represent major protein fraction of the grain and are responsible for unique properties of the dough. Major determinants of wheat quality are storage proteins. Proteins from wheat flour combined with water formed gluten, which holds gas produced by yeast during baking. Gluten proteins are gliadins and glutenins (Anjum et al., 2007; Katyal et al., 2017). For proper protein composition we need available N and S in the soil, which highly influence quality characteristics (Tribo et al., 2003; Chope et al., 2014). There is a strongly negative correlation between grain yield and grain protein (Slafer & Andrade, 1993; Oury & Godin, 2007).

The objectives of our experiments are the description of the phenotypic and genotypic (part of dispersion, which determined only by genotype, not by climate or other factors) variability of the main agriculture-value traits of the new winter wheat lines according to their interactions with different environmental conditions. One of the main tasks is how reactions on specific conditions of our region of local breeding lines are different from national officially released varieties. Second purpose is evaluation of agronomic-value traits variability of local sources and local possibilities for successful creation new material for winter wheat improvement under special conditions of region, possibilities of new genotypes in

formation of ecological adaptation (Nazarenko & Bezus, 2018).

2 MATERIALS AND METHODS

Experiments were conducted at the experimental fields of Dnipropetrovsk State Agrarian and Economic University. The field`s geographic coordinates are: 48°30'N lat. and 35°15' E long. The experimental fields are lied down on 245 meters above the sea level. Weather conditions for hydrothermal indicators in the years of research (2015 – 2017) varied, which made possible to obtain objective results, but in general, they were typical. Air temperature during winter wheat growing season (September - July) is 8.5 °C, the average rainfall is 511 mm for the location of the research fields (air temperature during winter wheat growing season 2015 (September - July) was 9.8 °C, the average rainfall is about 622.8 mm; air temperature in season 2016 was 10.4 °C, the average rainfall is about 512.0 mm; air temperature in season 2017 was 9.2 °C, the average rainfall is about 533.8 mm.

The field station of Dnipro State Agrarian and Economic University has been used for many years (start from 60th years of XX century) as a field for intensive agricultural farming and researches (Kharytonov et.al., 2017). It is located far away from the city Dnipro (about 28 km) which is enough to avoid industrial or town air pollution effects. The research fields occupy an area of 60 hectares and are situated acrossed three types of landscapes. One of them is of 30 m deep valley with a slope of > 7°, the other two have the slopes up to 3°. Comparison of the received information in aspects of the crop yield and quality due to the landscape peculiarities gives us the arguments to classify the agricultural resource potential for different type of agrolandscapes. Investigation was performed on flat (full-height normal soil), on the northern exposition slope (low eroded soil), on the southern exposure slope (middle level of erosion). Special attention was paid to the differences on several agronomical-value traits (grain yield, protein and main protein components content in grains, assimilation of main macro- and microelements).

Winter wheat seeds were obtained from department of breeding and seedfarming of Dnipro State Agrarian and Economic University (line number 130 ('Giant', seeds of variety 'Kalinova' were subjected by gamma-rays, 100 Gy), 156 ('Leroy', seeds of 'Kolos Mironovschiny' was treated by nitrosomethilurea 0.0125% at water solution during 18 hours), 157 ('Sonechko', 100 Gy), 211 ('Deada', 'Kolos Mironovschiny', nitrosomethilurea 0.0125%) 213 ('Leana', 'Favoritka', nitrosomethilurea 0.0125%) (Nazarenko et al., 2018). The recommended intensive agronomic practice was followed (N 180 kg ha^{-1}, P 60 kg ha^{-1}, K 40 kg ha^{-1}). Evaluation of total grain yield of five lines per plot was calculated from 2015 to 2017 years. The control was national standard variety Podolyanka. The trial was set up at a randomized block design method with three replications and with a plot size of 10 m^2 in 3 replications (Bhutta et al., 2005). Coefficients of nutrient elements utilization were calculated as the ratio of element content in yield to the content of element in the soil and from fertilizers (in percent). Agrochemical analysis of soils for content of nutrient elements was provided too ($N\text{-}NO_3$, mg kg^{-1} 18.7 – 32.8, P_2O_5 14.8 – 27.1, K_2O 134 – 235).

The nitrogen and phosphorus concentration in plant samples was estimated using Kjeldahl method. Total P concentrations of the applied residues were determined by sulfuric acid digestion (Thomas et al., 1967). Potassium was determined with flame photometry. Trace elements were determined with method of atomic absorption spectrophotometry. The protein content and contents of gliadin and glutenin were identified on device CNS Model Flash EA 1112 (for protein content) and RP-HPLS (for gliadins and glutenins).

Mathematical processing of the results was performed by the method of analysis of variance, the variability of the mean difference was evaluated by Student's t-test, factor analyses were conducted by module ANOVA. In all cases standard tools of the program Statistica 8.0 were used.

3 RESULTS AND DISCUSSION

Winter wheat lines (Tables 1 and 2) are sensitive to cultivation under different conditions with statistical certainty, but not within as wide a range of varieties and with some differences for one line. These differences in terms of growing conditions are expressed in grain productivity and the ability to use basic nutrients from the soil. Regarding the grain productivity, Table 1 shows that higher yields are typical for the lines on the slope of the northern exposure, especially for lines 157, 213, however, the remaining lines also exceeded the standard in terms of yielding capacity. In all cases, the new lines needed larger doses of fertilizer to form the yield bigger than the standard. All of the new lines belong to intensive winter wheat type (Nazarenko et al., 2018). Efficiency in utilization of main nutrient elements are depended on following factors, such as genotype ($F = 14.75$; $F_{critical} = 3.16$; p-level 0.01), type of landscape ($F = 8.70$; $F_{critical} = 3.34$; p-level 0.01) and quantity of mineral nutrients ($F = 4.16$; $F_{critical} = 4.01$; p-level 0.02) available for plants.

Table 1: Yield and utilization of main nutrient macroelements by winter wheat plants

		Uptake from soil kg ha^{-1}			For 1 ton of grain, kg		
Variety, line	Yield, t ha^{-1}	N	P	K	N	P	K
Flat							
Podolyanka	8.8	273.7	83.6	213.0	31.1	9.5	24.2
130	10.5*	342.2	111.6	247.5	32.5	10.6	23.5
156	9.1*	299.5	98.6	220.9	32.8	10.8	24.2
157	11.2*	380.4	103.2	272.6	33.9	9.2	24.3
211	10.2*	352.2	98.5	246.6	34.7	9.7	24.3
213	11.5*	402.6	110.1	281.0	35.1	9.6	24.5
Average	10.2	341.8	100.9	246.9	33.4	9.9	24.2
Slope of noth exposition							
Podolyanka	8.4	261.2	79.8	200.8	31.1	9.5	23.9
130	10.4*	317.2	103.0	249.6	30.5	9.9	24.0
156	9.8*^	301.8	97.0	235.2	30.8	9.9	24.0
157	11.9*^	389.1	103.5	285.6	32.7	8.7	24.0
211	11.5*^	389.9	108.1	273.7	33.9	9.4	23.8
213	11.7*	402.5	107.6	279.6	34.4	9.2	23.9
Average	10.6	343.6	99.8	254.1	32.2	9.4	23.9
Slope of south exposition							
Podolyanka	7.2^	201.6	64.8	146.2	28.0	9.0	20.3
130	8.1^	251.1	76.1	190.4	31.0	9.4	23.5
156	8.3^	257.3	80.5	200.9	31.0	9.7	24.2
157	7.2^	234.0	64.1	175.0	32.5	8.9	24.3
211	6.9^	224.3	60.7	167.7	32.5	8.8	24.3
213	8.5*^	255.9	72.3	173.4	30.1	8.5	20.4
Average	7.7	237.4	69.8	175.6	30.9	9.1	22.8

*- is significantly from control on 5 % level, ^- is significantly from flat on 5 % level

After analyzing the special needs of the lines (Table 1) in nutrients used by winter wheat plants for growth and development and the number of elements required to obtain 1 ton of grain, it was found that the need for basic nutrients primarily depends on the genetically determined characteristics of these lines in the formation of the grain crop yield (Lazrak et al., 2010).

Nitrogen utilization by winter wheat plants on flat sites were from 273.7 (national standard 'Podolyanka') to 402.6 kg ha^{-1} (line 213), phosphorus 83.6 (national standard 'Podolyanka') – 111.6 (line 130) kg ha^{-1}, potassium 213.0 (national standard 'Podolyanka') – 281.0 kg ha^{-1} (line 130) during three years. We can see that difference in demands for different elements depend on winter wheat lines peculiarities and more adaptable genotypes have fewer demands for mineral sources (Ladoni et al., 2017). Some of the lines needed more nitrogen (line 213), but others (line 130) needed more phosphorus to form the grain. From 31.1 (variety Podolyanka) to 35.1 kilograms of nitrogen is required to obtain 1 ton of winter wheat, 9.5 (variety Podolyanka) – 10.8 (line 156) kg of phosphorus and from 23.5 (line 130) for 24.5 kg (line 213) of potassium.

From the above data, we could find out that the most productive line 213, in general, needs more nitrogen and phosphorus than other genotypes. The same line needs only more nitrogen than other lines for the formation of 1 ton of grain, phosphorus is more significant in the context of the same indicator for the line 156. Potassium is not so important and is required at about the same level for all lines and variety Podolyanka. From the results of the protein components study, winter wheat lines with large requirements for phosphorus provide good grain quality (Bonnot et al., 2017).

In more favorable conditions of a northern exposure, differences in yielding capacity are not so signifi-

Table 2: Coefficient of nutrient elements utilization from soil on different types of relief, %

	Flat			Slope of north exposition			Slope of south exposition		
Variety, line	N	P	K	N	P	K	N	P	K
Podolyanka	62.9	57.0	17.0	69.1	89.5	20.3	74.7	87.9	23.0
130	53.3	61.6	15.7	59.1	86.8	17.5	60.9	85.4	18.0
156	59.0	60.7	15.7	60.0	89.0	17.5	60.5	85.1	18.6
157	60.6	45.1	13.9	65.2	76.6	17.0	74.1	80.4	20.0
211	63.0	50.3	16.5	64.3	69.0	17.5	71.1	72.3	19.8
213	64.8	57.1	15.8	69.7	83.9	17.3	78.4	88.4	23.9
Average	59.8	54.9	15.8	63.5	82.2	18.0	68.3	82.2	19,9

cant between the lines comparing to the upland soils. For the two lines – 130 and 213, yielding capacity remained at about the same level as for the upland soils, for the other three lines yielding capacity is higher than on the upland soils, but in any case, all of the lines exceed the control for yielding capacity. It is required within 216.2 - 402.5 kg ha^{-1} of nitrogen, phosphorus 79.8 – 108.1 kg ha^{-1}, potassium 200.8 – 285.6 kg ha^{-1} from the soil for crop formation.

The reaction of individual genotypes to the growing conditions shows the same situation as for the previous conditions, only line 213 differs by specific high phosphorus needs. The similar situation was observed on features of the use of mineral substances on the formation of 1 ton of grain. Line 213 has shown the ability to use less nutrients for crop formation in contrast to other lines, which allows it to provide higher yields in adverse conditions.

According to the results of the grain yield and the use of macronutrients from the soil, all lines can be divided into two groups. The first group (line 213) provides a higher yield than the standard in all cases, which sharply distinguishes it from the rest of the lines (and varieties in previous studies). The yields in the second group (all other lines) are inferior to standard in adverse conditions, because the standard belongs to the semi-intensive varieties and has an advantage due to low demands for mineral nutrition in adverse conditions compared to intensive ones. Cases like the line 213 (when intensive variety exceeds the variety of standard in adverse conditions) have not been observed and this indicates the possibilities of obtaining a highly adaptive intensive genotype in the framework of the intensive varieties that had previously relied possible only theoretically (Nazarenko & Lykholat, 2018).

In accordance with the data on the use of macronutrients from the soil, it can be concluded that there is mutual influence of the type of agricultural landscape (in the sense of terrain features) and genotype on this parameter. It can be concluded that plants use nutrients more rationally in the harsher conditions of the southern exposure than in other variants. It shows wide adaptability of winter wheat plants in different agro-ecological conditions. In the case of the phosphorus use by plants, the differences in the results on the slopes of the northern and southern exposure are not so significant and reliable on average. The consumption of this macronutrient is more dependent on the special needs of individual lines.

The total intake of 6 elements is summarized in tables 3 and 4. Thus, according to the previous studies, the

Table 3: Intake of microelements and heavy metals with winter wheat grains and straw under different agrolandscape conditions mg kg^{-1}

Relief element	Zn	Mn	Cu	Pb	Ni	Fe
Grain						
Flat	20.1	19.2	2.8	1.9	2.9	38.9
Slope of north exposition	18.9	26.9	5.0	2.0	1.7	39.4
Slope of south exposition	22.7	24.1	4.3	2.0	1.9	30.1
Straw						
Flat interfluve	4.7	16.9	3.1	3.1	2.9	75.9
Slope of north exposition	2.9	14.5	2.5	2.5	1.9	26.7
Slope of south exposition	2.1	9.2	2.1	2.7	1.2	24.7

Table 4: General uptake of microelements and heavy metals with yield under different relief conditions, g ha^{-1}

Relief element	Fe	Zn	Mn	Cu	Ni	Pb
Flat interfluve	5723.0	1219.2	1437.6	354.2	312.6	212.3
Slope of north exposition	3211.1	1213.4	2348.1	445.2	211.3	279.2
Slope of south exposition	1824.2	1111.1	1116.5	243.9	163.2	211.6
Average	2517.7	1181.2	1647.7	347.8	229.0	234.4
Cv, %	980.7	60.8	622.5	100.8	76.3	38.8

wheat grain contains more essential microelements than straw, while the situation with undesirable elements is the opposite. Thus, lead and nickel could be found more in the straw. This is not unusual because it is determined by the biological laws of plant development. This is one of the natural mechanisms for avoiding the high concentration of adverse elements in the reproductive organs of the plant and ensure the provision of the necessary substances. No statistically significant relations between concentrations of trace elements, heavy metals and relief conditions were detected. Data on the concentration of these elements depending on the growing conditions are inconsistent and there is no statistically significant difference (Table 3, 4) (type of landscape ($F = 1.17$; $F_{critical} = 2.91$; p-level 0.04)) as in previous studies (Nazarenko & Lykholat, 2018).

On the other hand, a decrease in the content of some microelements (Zn, Mn, Fe) in straw under any terrain conditions should be noted (which is fully consistent with previous studies in this area). The intake of microelements from soil to wheat grain varied for iron from 1.824.2 to 5.723.0 g ha^{-1}, zinc 1.111.1 – 1.219.2 g ha^{-1}, manganese 1.116.5 – 2.348.1 g ha^{-1}, copper 243.9 - 445.2 g ha^{-1}, lead 211.6 – 279.2 g ha^{-1}, nickel 163.2 – 312.6 g ha^{-1}. The content of microelements in grain from the southern exposure slope was significantly lower than that obtained from the upland soils and northern exposure slope.

It is impossible to determine any differences in this situation from the data of cultivation in different conditions, unlike previous studies (Kharitonov et al., 2017), where such a relation was observed. That is why, it can be concluded that the dependence of this feature primarily bases on the genotype of this line, and not on the growing conditions (Han et al., 2017).

Higher protein content (statistically) has been recognized in winter wheat grains of two lines 157 and 213 ('Leana') (Table 5). We cannot identify any differences between growing conditions and we have to conclude that it's depended only on genotype of winter wheat line, not from the growth conditions in our experiment like as in previous investigations (Snapp & Kravchenko, 2015). Both lines (157 and 213) have a good composition of protein under any conditions (and good quality of protein), but not superior.

Lines 130 and 156 were determined as superior in gliadin and glutenin content in all terrain conditions. Similar varieties in this quality have been identified as holders of high baking characteristics in previous studies (Bordes et al., 2008; Bonnot et al., 2017), which gives grounds for the highlighting of these genotypes.

Thus, in general, lines 157 and 213 (the latter also holds for the high level of grain productivity and stability

Table 5: Protein content in winter wheat grains depending on line and agrolanscape, %

	Flat			Slope of north exposition			Slope of south exposition		
Variety, line	gliadin	glutenin	protein	gliadin	glutenin	protein	gliadin	glutenin	protein
Podolyanka	13.5	0.024	0.61	13.6	0.020	0.62	13.5	0.021	0.63
130	13.3	0.031	0.72	13.5	0.033	0.73	13.3	0.033	0.75
156	13.6	0.033	0.74	13.7	0.034	0.76	13.5	0.032	0.75
157	14.3*	0.025	0.65	14.5*	0.023	0.64	14.1*	0.024	0.62
211	13.5	0.024	0.66	13.7	0.024	0.65	13.4	0.023	0.66
213	13.9*	0.023	0.67	14.1*	0.024	0.63	14.0*	0.024	0.65
Average	13.7	0.027	0.68	13.9	0.026	0.67	13.6	0.026	0.68
Cv, %	2.6	15.9	7.1	2.6	21.8	8.7	1.9	19.2	8.7

*- is significantly on 5 % level.

of this feature under all conditions; a high level of adaptability) can be highlighted in the complex on the content and quality of protein. Only line 156 showed the quality of protein under all conditions.

Thus, like as in previous studies (Bordes et al., 2011) grain quality is depended on genotype only, not from agrolandscape types. This statement is appropriate for new lines and intensive varieties (Nazarenko & Lykholat, 2018), but not for older varieties (Kharitonov et al., 2017). Besides the quality, grain productivity is depended on both genotype and growth conditions, like as at previous investigations (Serpolay et al., 2011; Milev et al., 2014). Differences in relief type (not only climate conditions for region (Bhutta et al., 2005; Tsenov et al., 2015; Hatfield & Dold, 2018) are new local factor for varieties phenotypic variability, which has a great influence on general grain productivity (Gepts & Hancock, 2006; Kharitonov et al., 2017). Unlike varieties in previous studies (Kharitonov et al., 2017; Nazarenko & Lykholat, 2018), one of the new lines (213) has shown a consistently high (higher than control) yield in any relief conditions.

4 CONCLUSIONS

Field experiments on yield and grain quality of main cereal crops are usually limited to a one type (flat) of agrolandscape, one or two points in geographical zone and are measured on number of officially released varieties (well-known, not new lines). These ecological surveys are conducted without any record on genotype special demands in nutrition sources and ecological requirements to realize potential yield and grain quality traits. The wide phenotypic variability for the most of the agricultural-value traits investigated is indicative of the large diversity of the genotypes and genotype-environment interactions, mutual influences of landscape, local climate conditions in terms of agroecological district and genotype peculiarities.

Finally, our investigations confirmed statement about more perspective directions for exploiting local sources for winter wheat improvement and closely relation between concentration of nutrient substances in plants, their loss from soil and peculiarities of relief, genotype and limits of adaptation. Generally, north exposition gives winter wheat more preferable conditions for growth and development, but one high-adaptive line 213 ('Leana') was determined, which provided higher than standard grain yield under all conditions, include unfavorable. Protein content and quality depends on genotype, not on relief conditions. Yields of new lines obtained base on local sources as a result of selection under local conditions, and are less depends on growth conditions (type of relief) than officially released varieties.

5 REFERENCES

Andrusevich, K.V., Nazarenko, M.M., Lykholat, T.Yu., & Grigoryuk, I.P. (2018). Effect of traditional agriculture technology on communities of soil invertebrates. *Ukrainian journal of Ecology, 8*(1), 33-40. https://doi.org/10.15421/2018_184

Anjum, F.M., Khan, M.R., Din, A., Saeed, M., Pasha, I., & Arshad, M.U. (2007). Wheat gluten: high molecular weight glutenin subunits - structure, genetics, and relation to dough elasticity. *Journal of Food Science, 72*(3), 56-63. https://doi.org/10.1111/j.1750-3841.2007.00292.x

Bhutta, W.M., Akhtar, J., Anwar-ul-Haq, M., & Ibrahim, M. (2005). Cause and effect relations of yield components in spring wheat (*Triticum aestivum* L.) under normal conditions. *Caderno de Pesquisa Serie Biologia, Santa Cruz do Sul, 17*(1), 7-12.

Bordes, J., Branlard, G., Oury, F.X., Charmet, & Balfourier, G. F. (2008). Agronomic characteristics, grain quality and flour rheology of 372 bread wheats in a worldwide core collection. *Journal of Cereal Science, 48*(3), 569-579. https://doi.org/10.1016/j.jcs.2008.05.005

Bordes, J., Ravel C., Le Gouis J., Lapierre A., Charmet G., & Balfourier F. (2011). Use of a global wheat core collection for association analysis of flour and dough quality traits. *Journal of Cereal Science, 54*, 137-134. https://doi.org/10.1016/j.jcs.2011.03.004

Bonnot, T., Bance, E., Alvarez, D., Davanture, M., Boudet, J., Pailloux, M., Zivy, M., Ravel, C., & Martre, P. (2017). Grain subproteome responses to nitrogen and sulfur supply in diploid wheat *Triticum monococcum* ssp. Monococcum. *The Plant Journal, 91*(5), 894-910. https://doi.org/10.1111/tpj.13615

Chope, G.A., Wan, Y., Penson, S.P., Bhandari, D.G., Powers, S.J., Shewry, P.R., & Hawkesford, M.J. (2014). Effects of genotype, season, and nitrogen nutrition on gene expression and protein accumulation in wheat grain. *Journal of Agricultural Food Chemistry, 62*, 4399-4407. https://doi.org/10.1021/jf500625c

Dai, Z., Plessis, A., & Vincent, J. (2015). Transcriptional and metabolic alternations rebalance wheat grain storage protein accumulation under variable nitrogen and sulfur supply. *Plant Journal, 83*, 326-343. https://doi.org/10.1111/tpj.12881

Dawson, J. C., Rivire, P., & Berthellot, J. F. (2011). Collaborative Plant Breeding for Organic Agricultural Systems in Developed Countries. *Sustainability, 3*, 1206-1223. https://doi.org/10.3390/su3081206

Han, Z., Walter, M.T., & Drinkwater, L.E. (2017) N2O emissions from grain cropping systems: a meta-analysis of the impacts of fertilizer-based and ecologically-based nutrient management strategies. *Nutrient Cycling in Agroecosystems, 107* 335-355.

Hatfield, J. L., & Dold, C. (2018). Agroclimatology and Wheat

Production: Coping with Climate Change. *Frontier Plant Sciences, 9*, 1-5. https://doi.org/10.3389/fpls.2018.00224

Khalili, M., Naghavi, M., & Yousefzadeh, S. (2018). Protein pattern analysis in tolerant and susceptible wheat cultivars under salinity stress conditions. *Acta agriculturae Slovenica, 111*(3), 545-558. https://doi.org/10.14720/aas.2018.111.3.03

Gepts, P., & Hancock, J. (2006). The future of plant breeding. *Crop Science, 46*, 1630-1634. https://doi.org/10.2135/cropsci2005-12-0497op

Katyal, M., Virdi, S.V., Kaur, A., Singh, N., Kaur, S., Ahlawat, A.K., & Singh, A.M. (2016). Diversity in quality traits amongst Indian wheat varieties I: Flour and protein characteristics. *Food Chemistry, 194*, 337-344. https://doi.org/10.1016/j.foodchem.2015.07.125

Kharytonov, M.M., Pashova, V. T., Mitsik, O.O., Nazarenko, M.M., & Bagorka, M.O. (2017). Estimation of winter wheat varieties suitability for difference growth of landscape conditions. *Annals of the Faculty of Engineering Hunedoara, 15*(4), 187-191.

Ladoni, M., Basir, A., Robertson, P.G., & Kravchenko, A.N. (2016) Scaling-up: cover crops differentially influence soil carbon in agricultural fields with diverse topography. *Agriculture Ecosystems & Environment, 225*, 93-103. https://doi.org/10.1016/j.agee.2016.03.021

Lazrak E.G, Mari J.F, & Benoit M. (2010) Landscape regularity modeling for environmental challenges in agriculture. *Landscape Ecology, 25*, 1-15. https://doi.org/10.1007/s10980-009-9399-8

Mba, C., Guimaraes, E.P., & Ghosh, K. (2012). Re-orienting crop improvement for the changing climatic conditions of the 21st century. *Agriculture & Food Security, 7*, 1-17. https://doi.org/10.1186/2048-7010-1-7

Milev, G., Nankov, N. , Iliev, I., Ivanova A., & Nankova, M. (2014). Growing Wheat (Trititcum aestivum L.) by the Methods of Organic Agriculture Under the Conditions of Dobrudzha Region, Bulgaria. *Turkish journal of agricultural and natural sciences, 1*, 849-857.

Nazarenko, M. (2016). Parameters of winter wheat growing and development after mutagen action. Bulletin of Transilvania University of Brasov - series II - Forestry, Wood Industry, Agricultural, *Food Engineering, 9*(2), 109-116.

Nazarenko, N.N. (2017). Specific Features in the Negative Consequences of a Mutagenic Action. *Russian Journal of Genetics: Applied Research, 7*(2), 195-196. https://doi.org/10.1134/S2079059717020083

Nazarenko M., & Lykholat, Y. (2018). Influence of relief conditions on plant growth and development. Dnipro university bulletin. Geology. *Geography, 26*(1), 143-149. https://doi.org/10.15421/111815

Nazarenko, M., Lykholat Y., Grigoryuk I., & Khromykh N. (2018). Optimal doses and concentrations of mutagens for winter wheat breeding purposes. Part I. Grain productivity. *Journal of Central European Agriculture, 19*(1), 194-205. https://doi.org/10.5513/JCEA01/19.1.2037

Nazarenko, M., & Bezus R. (2018). Interactions between agrolandscape and winter wheat agronomical-value traits. *Bulletin of Transilvania University of Brasov - series II - Forestry, Wood Industry, Agricultural, Food Engineering, 11*(60), 141-150.

Oury, F. X., & Godin, C. (2007). Yield and grain protein concentration in bread wheat: how to use the negative relationship between the two characters to identify favourable genotypes? *Euphytica, 157*(1-2), 45-57. https://doi.org/10.1007/s10681-007-9395-5

Rangare, N.R., Krupakar, A., Kumar, A., & Singh, S. (2010). Character association and component analysis in wheat (*Triticum aestivum* L.). *Electronic Journal of Plant Breeding, 1*, 231-238.

Reif, J.C., Zhang, P., Dreisigacker, S., & Warburton, M.L. (2005). Wheat genetic diversity trends during domestication and breeding. *Theoretical and Applied Genetics, 110*, 859-864. https://doi.org/10.1007/s00122-004-1881-8

Serpolay, E., Dawson, J.C., Chable, V., Lammerts Van Bueren, E.,Osman, A., Pino, S., Silveri, D., & Goldringer, I. (2011). Phenotypic responses of wheat landraces, varietal associations and modern varieties when assessed in contrasting organic farming conditions in Western Europe. *Organic Agriculture, 3*, 12-18.

Shewry, P.R., Mitchell, R.A.C., & Tosi, P. (2012). An integrated study of grain development of wheat (cv. Hereward). *Journal of Cereal Science, 56*, 21-30. https://doi.org/10.1016/j.jcs.2011.11.007

Slafer, G.A., & Andrade, F.H. (1993). Physiological attributes related to the generation of grain yield in bread wheat cultivars released at different eras. *Field Crops Research, 31,* 351-367. https://doi.org/10.1016/0378-4290(93)90073-V

Snapp, S.S., & Kravchenko, A. (2015) Cover crop and tillage systems effect on soil CO2 and N2O fluxes in contrasting topographic positions. *Soil and Tillage Research, 154*, 64-74. https://doi.org/10.1016/j.still.2015.06.015

Tsenov, N., Atanasova, D., Stoeva, I. & Tsenova, E. (2015). Effects of drought on grain productivity and quality in winter bread wheat. *Bulgarian Journal Agricultural. Sciences, 21*, 592-598.

Tester, M., & Langridge, P. (2010). Breeding technologies to increase crop production in a changing world. *Science, 327,* 818-822. https://doi.org/10.1126/science.1183700

Thomas, R.L.; Sheard, R.W., & Mayer, J.R. (1967). Comparison of conventional and automated procedures for nitrogen, phosphorus and potassium analysis of plant material using a single digestion. *Agronomy Journal, 59*, 240-243. https://doi.org/10.2134/agronj1967.00021962005900030010x

Tribo, E., Martre, P., & Tribo-Blondel, A.M. (2003). Environmentally induced changes in protein composition in developing grains of wheat are related to changes in total protein content. *Journal of Experimental Botany, 54*, 1731-1742. https://doi.org/10.1093/jxb/erg183

Tuberosa, R., & Salvi, S. (2006). Genomics-based approaches to improve drought tolerance of crops. *Trends in Plant Science, 11*, 405-412. https://doi.org/10.1016/j.tplants.2006.06.003

USDA. (2018) *World Agricultural Supply and Demand Estimates*. Retrieved from https://www.usda.gov/oce/commodity/wasde/latest.pdf.

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Unravelling efficient applications of agriculturally important microorganisms for alleviation of induced inter-cellular oxidative stress in crops

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Unravelling efficient applications of agriculturally important microorganisms for alleviation of induced inter-cellular oxidative stress in crops

Abstract: Abiotic stresses like high temperature, cold, freezing, drought, salinity, flooding or oxidizing agents cause significant loss in the crop yield and quality. Abiotic stresses cause reactive oxygen species (ROS) production such as singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\bullet-}$), hydroxyl radical (OH^-), etc., that leads to a significant reduction of crop yield. A major source of ROS production in plants through aerobic metabolism is chloroplast, mitochondria, and peroxisome. The tripartite interactions involving *Trichoderma*- Phytopathogen-Host have received less attention in contrast to the plant–antagonist, plant–pathogen or pathogen–antagonist interactions. This article explores the possibilities of employing thermotolerant strains of agriculturally important microorganisms (AIMs) for alleviating the oxidative stress induced due heat stress in crops by modulating oxidative and defense network of the host.

Key words: heat stress; ROS; AIMs; abiotic stress; crop protection

Pojasnitev učinkovite uporabe kmetijsko pomembnih mikroorganizmov pri blaženju oksidacijskega stresa v celicah kmetijskih rastlin

Izvleček: Abiotski stresorji kot so visoke temperature, mraz, zmrzovanje, suša, slanost, poplave ali oksidacijska sredstva povzročajo znantne izgube pridelka in kakovosti kmetijskih rastlin. Abiotski stresorji povzročajo nastanek reaktivnih zvrsti kisika (ROS) kot so singletni kisik ($^1O_2$), vodikov peroksid (H_2O_2), superoksidni radikal ($O_2^{\bullet-}$), hidroksilni radikal (OH^-), itd., kar vodi k znantnemu zmanjšanju pridelka kmetijskih rastlin. Glavni vir nastanka ROS v rastlinah je aerobna presnova v kloroplastih, mitohondrijih in peroksisomih. Tripartitne interakcije, ki vključujejo glivo iz rodu *Trichoderma*-fitopatogena in gostitelja so bile deležne manj pozornosti v nasprotju s sistemi kot so rastlina- antagonist, rastlina–patogen ali interakcije pathogen – antagonist. V članku so prikazane možnosti uporabe termotolerantnih sevov kmetijsko pomembnih mikroorganismov (AIMs) za blaženje oksidacijskega stresa, ki ga v kmetijskih rastlinah sproži vročinski stres z modulacijo oksidativnega in obrambnega odziva gostitelja.

Ključne besede: vročinski stres; ROS; AIMs; abiotski stres; zaščita kmetijskih rastlin

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1 ABIOTIC STRESSES IN PLANTS

Plants are frequently exposed to a plethora of unfavorable environmental conditions, thereby posing a serious threat to sustainable crop production (Bhatnagar-Mathur et al., 2008). In continuously changing environment, plants are constantly challenged by various abiotic stresses (drought, salinity, temperature extremes, heavy metal toxicity, high-light intensity, nutrient deficiency, UV-B radiation, ozone, etc.) which cause considerable losses in the yield and quality of a crop (NAAS, 2017; Hasanuzzaman et al., 2012). Abiotic stress is best defined as any aspect exerted by the environment on the optimal functioning of an organism. Abiotic stresses like heat, cold, freezing, drought salinity, flooding or oxidizing agents usually cause protein dysfunction (NAAS, 2013; Wang et al., 2004).

A number of abnormal environmental factors are collectively termed as abiotic stresses. Abiotic stresses alter plant metabolism leading to harmful effects on growth, development, and productivity. If the stress becomes very high and continues for an extended period, it may lead to an unbearable metabolic load on cells, reducing growth, and in severe cases, resulting in plant death (Keswani, 2015). However, plant stress may vary depending on the types of stress factors and on the prevailing period. In nature, plants may not be completely free from abiotic stresses. They are expected to experience some degree of stress by different factors (Keswani, 2019). Some environmental factors, such as air temperature, can become stressful in just a few minutes; others, such as soil water content, may take few days to weeks, and factors such as soil mineral deficiencies/overload may take months to become stressful (Taiz & Zeiger, 2006). These stresses are associated with the production of ROS, capable of inducing cellular damage by proteins degradation, enzymes inactivation and alterations in the gene expression (Kumar et al., 2017; Choudhury et al., 2013). Abiotic stresses remain the greatest constraint to worldwide crop production. It has been estimated that more than 50 % of yield reduction is the direct result of abiotic stresses (Keswani, 2015; Acquaah, 2007; Camejo et al., 2005). Abiotic stress leads to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity (Mishra et al., 2015; Wang et al., 2001).

2 GLOBAL WARMING

Global warming is a gradual increase in the global atmospheric temperature of the Earth, usually caused by the greenhouse effect due to higher levels of CO_2, CFCs and other pollutants (Chitara et al., 2017; Broecker, 1975). Warmer temperatures may make many crops grow more quickly, but warmer temperatures could also reduce yields. Crops tend to grow faster in warmer conditions. However, for some crops (such as grains), faster growth reduces the amount of time that seeds have to grow and mature. Extreme temperature and rainfall can inhibit crops growth (Karl et al., 2009). It was reported that for every 1 °C rise in temperature the decline in rice yield would be about 6 % (Saseendran et al., 2000).

3 CLIMATE CHANGE AND ITS IMPACT ON AGRICULTURE

Agriculture depends on the favorable climate, hence is among the sectors of the global economy where most concern currently lies in the context of climate change in order to maintain global food security (Mertz et al., 2009). The Inter-Governmental Panel on Climate Change (IPCC), fourth assessment report (Field et al., 2014) stated that human-induced climate change is real, and identified agriculture as a critical sector. Climate change is likely to affect crop productivity directly through changes in crop environment and indirectly through the prevalence of agriculture insect and pest, associated impact on soil fertility and biological function and agriculture biodiversity can also be observed (Lobell & Burke, 2010).

4 HIGH TEMPERATURE STRESS

Heat stress is often defined as a rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development. A transient temperature elevation, typically 10-15° C above ambient, is generally considered to be a heat shock or heat stress (Willits & Peet, 1998). In many areas of the world, heat stress due to higher temperatures is a serious threat to crop production worldwide (Lorenzoni et al., 2001). Transient or constantly high temperatures cause a range of morpho-anatomical, physiological and biochemical changes in plants that affect plant growth and development and can result in a drastic reduction in economic output (Keswani, 2015; Wahid et al., 2007) (Figure 1). As plants lack the capability of locomotion as a means of responding to changes in their environment, they are exposed to various environmental stresses and must adapt to them in other ways. The most typical kind of stress plants receives from their surroundings is temperature stress. Each plant species has its own optimum temperature for growth, and its distribution is de-

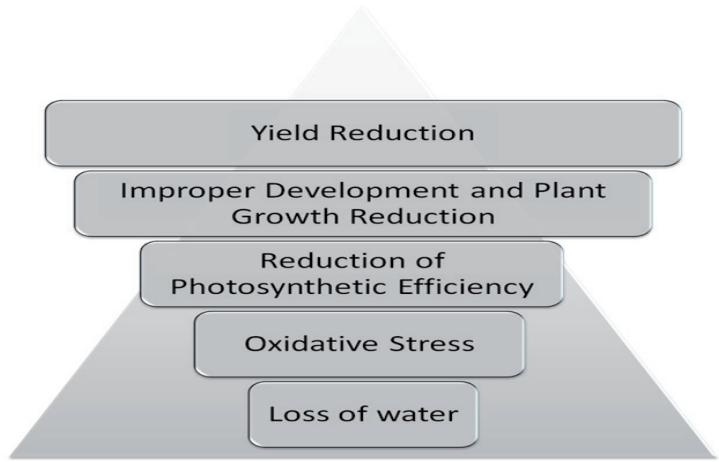

Figure 1: Correlation of heat stress with biochemical and physiological parameters of host

termined to a major extent by the temperature zone in which it can survive (Ram et al., 2018; Sasaki, 1997).

Severe cell injury and even cell death can occur in minutes at very high temperatures, which can be attributed to a catastrophic collapse of cellular organization (Schoffl et al., 1997). High temperature direct injuries include protein denaturation, aggregation, and increased membrane lipid fluidity. Indirect or slower heat injuries include chloroplast and mitochondrial inactivation of enzymes, protein synthesis inhibition, protein degradation, and membrane integrity loss (Singh et al., 2016; Singh et al., 2017).

At very high temperatures, severe cellular injury and even cell death may occur within minutes, which could be attributed to a catastrophic collapse of cellular organization (Schoffl et al., 1997). High temperature causes both direct and indirect or slower heat injuries. Direct injuries include protein aggregation, denaturation, and increased membrane fluidity whereas, indirect or slower heat injuries include enzymes inactivation in both chloroplast and mitochondria, protein degradation, inhibition of protein synthesis and loss of membrane integrity (Howarth, 2005). High temperatures can also cause considerable pre- and post-harvest damages, including scorching of leaves and twigs, sunburns on leaves, branches and stems, leaf senescence and abscission, shoot and root growth inhibition, fruit discoloration and damage, and reduced yield (Guilioni et al., 1997; Vollenweider & Günthardt-Goerg, 2005).

5 PRODUCTION OF ROS AND THEIR HEALTH IMPACT ON PLANT

Oxygen supports the aerobic life of plants, providing them with enormous energy benefits, but challenges them through endless ROS formation (Figure 2). However, certain environmental stresses or genetic defects cause ROS production to exceed the management capacity. In plants, ROS play two divergent roles: at lower concentrations, it acts as signaling molecules to activate defense responses under stress, while exacerbating damage to the cellular component at higher concentrations. If abiotic stress is imposed on the plant for a longer duration than, through enhanced ROS production, can pose a severe threat to cells by causing the lipids peroxidation, proteins oxidation, damage to nucleic acids, enzyme inhibition, programmed cell death (PCD) pathway activation and ultimately cell death (Mittler, 2002; Sharma & Dubey, 2005). Oxidative stress is essentially a regulated process, and the equilibrium between ROS and anti-oxidative capacity determines the fate of the plant. The enhanced ROS production is, however, kept under tight control by versatile and cooperative ROS-scavenging antioxidant mechanisms that modulate intracellular ROS concentration (Apel & Hirt, 2004). Oxygen activation or reduction results in reactive ROS including singlet oxygen ($^1O_2$), superoxide ($O^{2\bullet-}$), hydrogen peroxide (H_2O_2) and radical hydroxyl ($HO^\bullet$). The imbalance between the production of ROS and its detoxification through enzymatic and non-enzymatic reactions causes oxidative stress. Photo-oxidative damage to DNA, proteins and lipids, and ultimately cell death occurs as a result of higher net ROS formation. ROS act as signaling molecules for development and growth, defense responses of pathogens such as systemic acquired resistance and hypersensitive reaction, production of the stress hormone, acclimation and programmed cell death (Singh et al., 2019b; Apel & Hirt, 2004). The first step in the O_2 reduction results in the formation of superoxide ($O_2^{\bullet-}$) or hydroperoxide ($HO^\bullet_2$) radicals. $O_2^{\bullet-}$

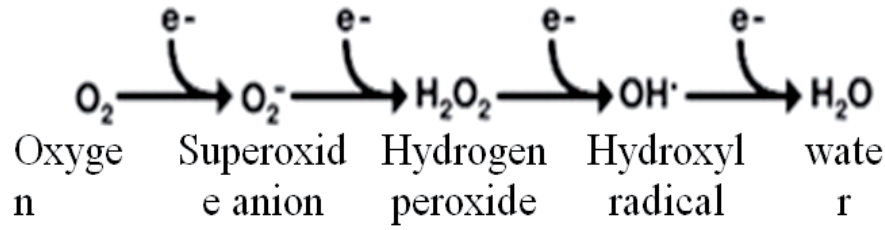

Figure 2: Production of ROS by a multistep reduction of molecular oxygen

is very unstable and has a short half-life of 2 to 4 min. The second step leads to the formation of a relatively stable molecule hydrogen peroxide (H_2O_2) with a half-life of 10 min. H_2O_2 can migrate from the sites of synthesis to adjacent compartments and even neighboring cells due to longer half-life (Bienert et al., 2006). The oxidizing capacity of $O_2^{\bullet-}$ and H_2O_2 makes them potentially harmful to the cell environment surrounding them. $O_2^{\bullet-}$ may inactivate significant metabolic enzymes that contain Fe-S clusters and alter catalytic activity (Halliwell, 2006; Van Breusegem et al., 2001). $HO^{\bullet}_2$ (a protonated form of $O_2^{\bullet-}$) is mostly found in cellular acidic environments. $HO^{\bullet}_2$ may cross biological membranes and initiate oxidation of lipid by extraction of protons from polyunsaturated fatty acids. In most biological systems, the enzyme superoxide dismutase (SOD) rapidly converts $O_2^{\bullet-}$ to H_2O_2. By oxidizing the thiol group of enzymes, H_2O_2 can inactivate them (Halliwell, 2006). $O_2^{\bullet-}$ and H_2O_2 can cause more prominent destruction when interacting with metal ions during the so-called Haber-Weiss reaction to form the highly reactive hydroxyl radical (Kehrer, 2000).

$HO^{\bullet}$ can react virtually anything that comes into contact with and damages it (Halliwell, 2006). $HO^{\bullet}$ is highly reactive; cells do not have $HO^{\bullet}$ detoxification enzyme and are dependent on mechanisms that reduce $HO^{\bullet}$ production. These mechanisms include the elimination of $O_2^{\bullet-}$ and H_2O_2 and/or metal ions sequestration that catalyze the Haber-Weiss reaction with metal-binding proteins, such as ferritins or metallothioneins (Hintze & Theil, 2006).

6 PLANT ORGANELLES INVOLVED IN ROS PRODUCTION

Chloroplast, mitochondria, and peroxisome are organelles with a high oxidizing metabolic activity or an intense rate of electron flow and are the major source plant ROS production (Figure 3). Together with these organelles, peroxidases and amine oxidases present in cell walls and NADPH oxidase in the plasma membrane, often producing ROS in response to stress. Due to photosynthetic electron transport, oxygen is continuously produced inside the chloroplast and at the same time removed by reduction and assimilation (Keswani, 2015; Chawla et al., 2013; Shoeb et al., 2013; Tripathy & Oelmüller, 2012).

An unavoidable leakage of electrons onto O_2 from the electron transport activities of mitochondria, chloroplasts and plasma membranes or from a diversity of metabolic by-products in different cellular compartments can lead to the formation of ROS in plants (Keswani et al., 2016b; Sharma et al., 2012).

6.1 CHLOROPLAST

Different forms of ROS are generated from several locations in chloroplast. In chloroplasts, the main sources of ROS are electron transportation chains in Photosystem I (PSI) and Photosystem II (PSII). ROS production by these sources is enhanced in plants by limiting CO_2 fixation conditions, such as drought, salt, and temperature stresses, as well as by combining these conditions with high-light stress. In case of ETC overload, because NADP supply decreases due to stress conditions, the electrons leak from ferredoxin to O_2, reducing it to $O_2^{\bullet-}$ occurs in the case of overloading of the ETC (Elstner, 1991). This process is called the Mehler reaction.

6.2 MITOCHONDRIA

Mitochondria can produce ROS at multiple ETC sites. In mitochondria, the flavoprotein region of NADH dehydrogenase segment (complex I) of the respiratory chain directly reduces oxygen to $O_2^{\bullet-}$ (Arora et al., 2002). In ETC, the primary ROS formed by a monovalent reduction is $O_2^{\bullet-}$. It is quickly converted into the relatively stable and membrane-permeable H_2O_2, either by the MnSOD (a mitochondrial form of SOD) or APX. In Fenton reaction, H_2O_2 can be further converted to

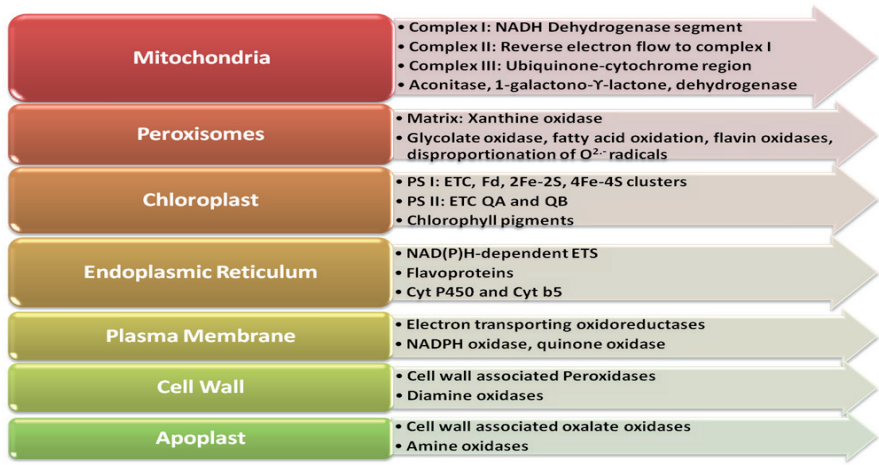

Figure 3: Plant organelles involved in ROS production

extremely active hydroxyl radical (OH•) (Sharma et al., 2012).

6.3 PEROXISOMES

Peroxisomes are probably the major sites of intracellular H_2O_2 production, as a result of their essentially oxidative type of metabolism (Luis et al., 2006). The main metabolic processes responsible for the generation of H_2O_2 in different types of peroxisomes are the glycolate oxidase reaction, the fatty acid β-oxidation, the enzymatic reaction of flavin oxidases, and the disproportionation of $O_2^{\bullet-}$ radicals (Baker & Graham, 2013). During photorespiration, the oxidation of glycolate by glycolate oxidase in peroxisomes accounts for the majority of H_2O_2 production (Noctor et al., 2002).

6.4 PLASMA MEMBRANES

The electron transporting oxido-reductases are ubiquitous at plasma membranes and lead to the generation of ROS at the plasma membrane (Kovačić, 2003). NADPH oxidase catalyzes the transfer of electrons from cytoplasmic NADPH to O_2 to form $O_2^{\bullet-}$. $O_2^{\bullet-}$ is dismutated to H_2O_2 either spontaneously or through SOD activity. It was proposed that NADPH oxidase play a key role in plant production and accumulation of ROS under stress conditions (Apel & Hirt, 2004).

7 ROS SIGNALLING IN PLANTS

In plant cells, ROS are continuously produced as a consequence of aerobic metabolism in all the intracellular organelles, in particular in the chloroplast, mitochondria and peroxisomes (Bisen et al., 2015; Apel & Hirt, 2004). The chloroplast is the main source of ROS in plants. Insufficient energy dissipation during photosynthesis can lead to the formation of a chlorophyll triplet state that can transfer its excitation energy onto O_2 to make $^1O_2$ (Logan, 2008). $O_2^{\bullet-}$ is produced by the photosynthetic electron transport chain (ETC) via the reduction of O_2 (Mehler reaction) (Apel & Hirt, 2004), which is subsequently converted to H_2O_2 by superoxide dismutase (Foyer & Noctor, 2000). The photo-production of ROS is largely affected by physiological and environmental factors, including high light intensity and drought stress (Asada, 2006). Proline accumulation is a widespread phenomenon in higher plants in response to various environmental stresses and is demonstrated to be protective for plants under adverse conditions (Keswani et al., 2016a). Proline so accumulated is proposed to act as a compatible osmolyte, free radical scavenger, cell redox balancer, a potential inhibitor of programmed cell death (PCD), cytosolic pH buffer and stabilizer for subcellular structures during various stresses (Gill & Tuteja, 2010; Kishor et al., 2005; Trovato et al., 2008). Under supra optimal temperature, free proline is known to accumulate in different crops (Rasheed et al., 2011). It is, therefore, considered to be a useful component for evaluating the degree of heat stress (Kuo et al., 1986).

8 EXPLORING THE ROLE OF *TRICHODERMA* SPP. IN BIOTIC STRESS MANAGEMENT

The three-partite interactions involving plants,

Trichoderma, phytopathogen and host has received less interest in contrast to the plant–antagonist, plant–pathogen or pathogen–antagonist interactions (Keswani et al., 2013; Singh et al., 2016; Singh et al., 2017). There are certain intricacies in studying a complex system even *in vitro*. Studies have shown some of the molecular or morphological features involved in plant–antagonist–pathogen interactions by application of gene reporter systems (Lu et al., 2004) and proteomics (Marra et al., 2006). The crosstalk during the three-partite interactions requires research that investigates the alteration in gene expression in each partner singly and afterward in all combinations. The main focus of the studies published so far in three way interaction has been related to molecular changes pathogen attack and/or plant response (Hammond-Kosack & Parker, 2003; Jones & Dangl, 2006). Various defense factors, signal molecules, virulence and avirulence factors have been identified in plant (Canovas et al., 2004; Ramonell & Somerville, 2002), and in microbes (Kazemi-Pour et al., 2004; Smolka et al., 2003). However, the molecular bases of interaction systems that may generate beneficial effects on plant are mostly unknown. Furthermore, the influence of biocontrol agent in the plant and pathogen has not yet been studied by using proteomics, though this technique offers an effective tool to examine such biological processes (Keswani et al., 2013; Woo et al., 2006).

In order to analyze the differential protein produced during the three partite interactions between Plant-*Trichoderma*-pathogen, (Singh et al., 2019a; Marra et al., 2006) investigated the interactions of *Trichoderma*, plant and different fungal pathogens by using proteomic techniques. During the three-way crosstalk, the alterations in each partner's proteomes were studied and the most attractive differential spots were analyzed via peptide mass finger-printing (PMF) (Bisen et al., 2016). Several proteins expressed differently have been found in the *Trichoderma atroviride* Karsten proteome during the three partite interactions with foliar pathogen *Botrytis cinerea* Pers.: Fr and bean leaves or with roots and the soil borne pathogen *Rhizoctonia solani* Kühn. Results demonstrated that in the three-partite interaction may be regulated by disease related factors and plant proteome-specific pathogenesis related proteins (PR proteins). In addition to that, the plant responses to a pathogen attack are qualitatively and quantitatively affected by the presence of antagonist (Bisen et al., 2016). LC MS/MS previously identified a protein with PPIase activity in the *Trichoderma harzianum* Rifai proteome (Suárez et al., 2005). The *in silico* study of the data from plant–*Trichoderma* and plant–*Botrytis* interactions exposed many homologues to PR-proteins. Conserved domains such as Nucleotide Binding Sites (NBS), Leucine Rich Repeats (LRR) and SGT1-specific domain (SGS) were found along with preserved Bet v I PR sequences and Barwin-protein families. For instance, thaumatin-like protein and tobacco PR-4 family with a Barwin domain involved in the *Magnaporthe grisea* (Hebert) Barr. plant defense response (Kim et al., 2004) were accumulated. Various proteins from the *Trichoderma atroviride* interaction proteome showed exciting similarities to those of ABC transporters and fungal hydrophobin. In the pathogen proteome, virulence factors such as cyclophilins were also regulated in the interaction with the antagonist and as well as with the plant. In *Trichoderma*–plant–pathogen in situ interaction, Gfp-tagged mutant strain *Trichoderma atroviride* was used to study the expression of the living producer (Lu et al., 2004). Specifically, incitement of *Trichoderma* genes encoding for diverse cell wall degrading enzymes in the vicinity of the pathogens *Pithyum ultimum* Trow and *Rhizoctonia solani* was observed by fluorescence microscopy. During the three partite interactions, purified colloidal chitin and the fungal pathogen activated the transformants and appeared to fluoresce during the early phases of contact. A direct visualization of the gene encoding the mycoparasitic expression *in vivo* is possible for the first time with this approach. The authors suggested that the induction of mycoparasitism involved compounds released by the host cell walls. In addition, T's endo-and exochitinase (nag1 and chit42) contribution of *Trichoderma atroviride* was also present in mycoparasitism other than basic host hyphae degradation.

9 BIOPROSPECTING THE ROLE OF *TRICHODERMA* SPP, IN ALLEVIATION OF INDUCED OXIDATIVE STRESS

Genus *Trichoderma,* competent of rhizosphere fungi are widely used in commercial formulations as biofertilizers and biopesticides due to multiple beneficial effects on plant growth and resistance to disease (Tucci et al., 2011). Various mechanisms of action, such as antibiotic production (Keswani et al., 2014; Vinale et al., 2008) or hydrolytic enzymes (Benítez et al*.,* 2004) and nutrients competition (Elad, 2000) were associated with the antifungal properties of *Trichoderma* spp. Abiotic stress often limits the growth and productivity of major crop species, reducing yields under ideal growing conditions to less than half of that possible (Boyer, 1982) (Figure 4). *Trichoderma* spp. is also known to be able to induce biotic and abiotic stress resistance in plant and thereby encouraging plant growth (Harman et al., 2004). *T*he ability of Trichoderma to alleviate abiotic stress is well known, although there is still a lack of specific knowledge of mechanisms that control multiple plant (Bisen et al*.,*

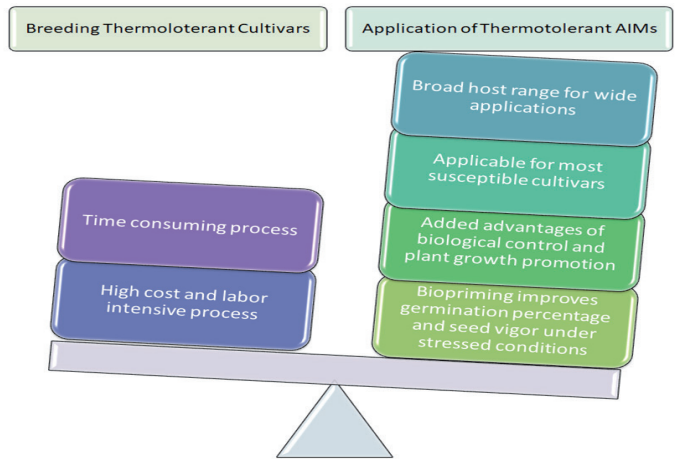

Figure 4: Relative advantages of using thermotolerant agriculturally important microrrganisms in comparison to breeding thermotolerant cultivars

2016; Chitara et al., 2017; Ram et al., 2018). Regardless of the stress condition, either osmotic, salinity, or suboptimal temperature the *Trichoderma harzianum* T22 treated seeds germinated rapidly and more uniformly than the untreated seeds (Mastouri et al., 2012). Some *Trichoderma* spp. are able to cope with extreme environments, facilitating their presence in diverse geographical regions, from Caribbean countries to Antarctica (Hermosa et al., 2012). T22 enhances the water tolerance of tomato seedlings by enhancing the antioxidant defense mechanism and enhancing ascorbate and glutathione-recycling enzyme activity (Mastouri et al., 2012).

Hence, these studies point to a possibility of employing thermotolerant strains of agriculturally important microorganisms in alleviation of heat stress in crops by relocating them in the rhizosphere for modulation of oxidative and defense network of the host, rendering it additional endurance to heat stress.

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11 REFERENCES

Acquaah, G. (2007). Principles of plant breeding and genetics. Malden, MA USA: *Blackwell Publishing*.

Apel, K., & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Reviewof Plant Biology*, *55*, 373-399. https://doi.org/10.1146/annurev.arplant.55.031903.141701

Arora, A., Sairam, R., & Srivastava, G. (2002). Oxidative stress and antioxidative system in plants. *Current cience*, 1227-1238.

Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant physiology, 141(2)*, 391-396. https://doi.org/10.1104/pp.106.082040

Baker, A., & Graham, I. A. (2013). *Plant peroxisomes: biochemistry, cell biology and biotechnological applications:* Springer Science & Business Media.

Bhatnagar-Mathur, P., Vadez, V., & Sharma, K. K. (2008). Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. *Plant cell reports, 27(3)*, 411-424. https://doi.org/10.1007/s00299-007-0474-9

Bienert, G. P., Schjoerring, J. K., & Jahn, T. P. (2006). Membrane transport of hydrogen peroxide. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1758(8)*, 994-1003. https://doi.org/10.1016/j.bbamem.2006.02.015

Bisen, K., Keswani, C., Mishra, S., Saxena, A., Rakshit, A. & Singh, H.B. (2015) Unrealized Potential of Seed Biopriming for Versatile Agriculture. In: *Nutrient Use Efficiency: from Basics to Advances.* Eds. A, Rakshit, H.B. Singh, A. Sen. Springer India, pp. 193-206. https://doi.org/10.1007/978-81-322-2169-2_13

Bisen, K., Keswani, C., Patel, J.S., Sarma, B.K. & Singh, H.B. (2016). Trichoderma spp.: *Efficient Inducers of Systemic Resistance in Plants.* In: Microbial-mediated Induced Systemic Resistance in Plants. Eds. Choudhary, D.K. and Verma, A. Springer Singapore, pp. 185-195. https://doi.org/10.1007/978-981-10-0388-2_12

Boyer, J. S. (1982). Plant productivity and environment. *Science, 218*(4571), 443-448. https://doi.org/10.1126/science.218.4571.443

Broecker, W. S. (1975). Climatic change: are we on the brink of a pronounced global warming? *Science, 189*(4201), 460-463. https://doi.org/10.1126/science.189.4201.460

Camejo, D., Rodríguez, P., Morales, M. A., Dell'Amico, J. M.,

Torrecillas, A., & Alarcón, J. J. (2005). High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. *Journal of plant physiology, 162*(3), 281-289. https://doi.org/10.1016/j.jplph.2004.07.014

Canovas, F. M., Dumas-Gaudot, E., Recorbet, G., Jorrin, J., Mock, H. P., & Rossignol, M. (2004). Plant proteome analysis. *Proteomics, 4*(2), 285-298. https://doi.org/10.1002/pmic.200300602

Chawla, S., Jain, S., & Jain, V. (2013). Salinity induced oxidative stress and antioxidant system in salt-tolerant and salt-sensitive cultivars of rice (Oryza sativa L.). *Journal of plant biochemistry and biotechnology, 22*(1), 27-34. https://doi.org/10.1007/s13562-012-0107-4

Chitara, M.K., Keswani, C., Bisen, K., Singh, V., Singh, S.P., Sarma, B.K. & Singh H.B. (2017). Improving Crop Performance under Heat Stress using Thermo tolerant Agriculturally Important Microorganisms. In: *Advances in PGPR Research*. Eds. Singh, H.B., Sarma, B.K., Keswani, C. CABI, Wallingford, UK, pp. 296-305. https://doi.org/10.1079/9781786390325.0296

Choudhury, S., Panda, P., Sahoo, L., & Panda, S. K. (2013). Reactive oxygen species signaling in plants under abiotic stress. *Plant signaling & behavior, 8*(4), e23681. https://doi.org/10.4161/psb.23681

Elad, Y. (2000). Biological control of foliar pathogens by means of Trichoderma harzianum and potential modes of action. *Crop protection, 19*(8-10), 709-714. https://doi.org/10.1016/S0261-2194(00)00094-6

Elstner, E. F. (1991). Metabolisms of oxygen activation in different compartments of plant cells. *Active Oxygen/Oxidative Stress and Plant Metabolism.*, 13-25.

Field, C.B. (Ed.) (2014). Climate change 2014-Impacts, adaptation and vulnerability: Regional aspects. Cambridge University Press, UK.

Foyer, C. H., & Noctor, G. (2000). Tansley Review No. 112 Oxygen processing in photosynthesis: regulation and signalling. *The New Phytologist, 146*(3), 359-388. https://doi.org/10.1046/j.1469-8137.2000.00667.x

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry, 48*(12), 909-930. https://doi.org/10.1016/j.plaphy.2010.08.016

Guilioni, L., Wery, J., & Tardieu, F. (1997). Heat stress-induced abortion of buds and flowers in pea: is sensitivity linked to organ age or to relations between reproductive organs? *Annals of Botany, 80*(2), 159-168. https://doi.org/10.1006/anbo.1997.0425

Halliwell, B. (2006). Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant physiology, 141*(2), 312-322. https://doi.org/10.1104/pp.106.077073

Hammond-Kosack, K. E., & Parker, J. E. (2003). Deciphering plant-pathogen communication: fresh perspectives for molecular resistance breeding. *Current opinion in biotechnology, 14*(2), 177-193. https://doi.org/10.1016/S0958-1669(03)00035-1

Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., & Lorito, M. (2004). Trichoderma species-opportunistic, avirulent plant symbionts. *Nature reviews microbiology, 2*(1), 43. https://doi.org/10.1038/nrmicro797

Hasanuzzaman, M., Hossain, M. A., da Silva, J. A. T., & Fujita, M. (2012). Plant response and tolerance to abiotic oxidative stress: antioxidant defense is a key factor. In: *Crop stress and its management*: Perspectives and strategies, Eds. B.: Venkateswarlu, A. K. Shanker, C. Shanker, M. Maheswari. Springer- Netherlands. p. 261-315. https://doi.org/10.1007/978-94-007-2220-0_8

Hermosa, R., Viterbo, A., Chet, I., & Monte, E. (2012). Plant-beneficial effects of Trichoderma and of its genes. *Microbiology, 158*(1), 17-25. https://doi.org/10.1099/mic.0.052274-0

Hintze, K., & Theil, E. (2006). Cellular regulation and molecular interactions of the ferritins. *Cellular and molecular life sciences, 63*(5), 591. https://doi.org/10.1007/s00018-005-5285-y

Howarth, C. (2005). Genetic improvements of tolerance to high temperature. In: *Abiotic stresses-plant resistance through breeding and molecular approaches*. Eds. M. Ashraf, P. J. C. Harris p. 277-300: The Haworth Press: New York.

Jones, J. D., & Dangl, J. L. (2006). The plant immune system. *Nature, 444*(7117), 323. https://doi.org/10.1038/nature05286

Karl, T. R., Melillo, J. M., Peterson, T. C., & Hassol, S. J. (2009). Global climate change impacts in the United States: Cambridge University Press.

Kazemi-Pour, N., Condemine, G., & HugouvieuxCottePattat, N. (2004). The secretome of the plant pathogenic bacterium Erwinia chrysanthemi. *Proteomics, 4*(10), 3177-3186. https://doi.org/10.1002/pmic.200300814

Kehrer, J. P. (2000). The Haber-Weiss reaction and mechanisms of toxicity. *Toxicology, 149*(1), 43-50. https://doi.org/10.1016/S0300-483X(00)00231-6

Keswani, C., Singh, S.P. & Singh, H.B. (2013). A Superstar in Biocontrol Enterprise: Trichoderma spp.. *Biotech Today,3*(2), 27-30. https://doi.org/10.5958/2322-0996.2014.00005.2

Keswani, C., Mishra, S., Sarma, B. K., Singh, S. P., & Singh, H. B. (2014). Unraveling the efficient applications of secondary metabolites of various Trichoderma spp.. *Applied microbiology and biotechnology, 98*(2), 533-544. https://doi.org/10.1007/s00253-013-5344-5

Keswani, C.(2015). *Proteomic studies of thermotolerant strain of Trichoderma spp.*. Ph.D thesis. Banaras Hindu University, Varanasi, India, pp.126

Keswani, C., Bisen, K., Singh, V., Sarma, B.K. & Singh H.B. (2016a) Formulation Technology of Biocontrol Agents: Present Status and Future Prospects. In: Bioformulations: for Sustainable Agriculture. Eds. Arora, N.K. Springer, New Delhi, India, pp. 35-52. https://doi.org/10.1007/978-81-322-2779-3_2

Keswani, C., Bisen, K., Singh, S.P., Sarma, B.K. & Singh H.B. (2016b) A proteomic approach to understand the tripartite interactions between plant-Trichoderma-pathogen: investigating the potential for efficient biological control. In: *Plant, Soil and Microbes Vol. II: Mechanisms and Molecular Interactions.* Eds. Hakeem K.R., Akhtar, M.S. Springer, USA, pp. 79-93. https://doi.org/10.1007/978-3-319-29573-2_5

Keswani, C., (Eds.). *Bioeconomy for Sustainable Development* (2019). Springer-Nature, Singapore. P. 392

Kim, S. T., Kim, S. G., Hwang, D. H., Kang, S. Y., Kim, H. J., Lee, B. H., Kang, K. Y. (2004). Proteomic analysis of pathogen-responsive proteins from rice leaves induced by rice blast fungus, Magnaporthe grisea. *Proteomics, 4*(11), 3569-3578. https://doi.org/10.1002/pmic.200400999

Kishor, P. K., Sangam, S., Amrutha, R., Laxmi, P. S., Naidu, K., Rao, K., Sreenivasulu, N. (2005). Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. *Current science*, 424-438.

Kovačić, P. (2003). Mechanism of drug and toxic actions of gossypol: focus on reactive oxygen species and electron transfer. *Current medicinal chemistry, 10*(24), 2711-2718. https://doi.org/10.2174/0929867033456369

Kumar, G., Patel, J.S., Maharshi, A., Mukherjee, A., Keswani, C., Singh, S.P., Singh, H.B. & Sarma, B.K. (2017). PGPR-Mediated Defence Responses in Plants under Biotic and Abiotic Stresses. In: *Advances in PGPR Research*. Eds. Singh, H.B., Sarma, B.K., Keswani, C. CABI, Wallingford, UK, pp. 427-438.

Kuo, C., Chen, H., & Ma, L. (1986). Effect of high temperature on proline content in tomato floral buds and leaves. *Journal of the American Society for Horticultural Science*. 111: 746-750

Lobell, D. B., & Burke, M. B. (2010). On the use of statistical models to predict crop yield responses to climate change. *Agricultural and Forest Meteorology, 150*(11), 1443-1452. https://doi.org/10.1016/j.agrformet.2010.07.008

Logan, B. A. (2008). 10 Reactive oxygen species and photosynthesis. *Antioxidants and Reactive Oxygen Species in Plants*, 250. https://doi.org/10.1002/9780470988565.ch10

Lorenzoni, I., Jordan, A., Favis-Mortlock, D., Viner, D., & Hall, J. (2001). Developing sustainable practices to adapt to the impacts of climate change: a case study of agricultural systems in eastern England (UK). *Regional Environmental Change, 2*(3), 106-117.

Lu, Z., Tombolini, R., Woo, S., Zeilinger, S., Lorito, M., & Jansson, J. K. (2004). In vivo study of Trichoderma-pathogen-plant interactions, using constitutive and inducible green fluorescent protein reporter systems. Applied and Environmental Microbiology, 70(5), 3073-3081. https://doi.org/10.1128/AEM.70.5.3073-3081.2004

Luis, A., Sandalio, L. M., Corpas, F. J., Palma, J. M., & Barroso, J. B. (2006). Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling. *Plant physiology, 141*(2), 330-335. https://doi.org/10.1104/pp.106.078204

Marra, R., Ambrosino, P., Carbone, V., Vinale, F., Woo, S. L., Ruocco, M., Soriente, I. (2006). Study of the three-way interaction between Trichoderma atroviride, plant and fungal pathogens by using a proteomic approach. *Current genetics, 50*(5), 307-321. https://doi.org/10.1007/s00294-006-0091-0

Mastouri, F., Björkman, T., & Harman, G. E. (2012). Trichoderma harzianum enhances antioxidant defense of tomato seedlings and resistance to water deficit. *Molecular plant-microbe interactions, 25*(9), 1264-1271. https://doi.org/10.1094/MPMI-09-11-0240

Mertz, O., Mbow, C., Reenberg, A., & Diouf, A. (2009). Farmers' perceptions of climate change and agricultural adaptation strategies in rural Sahel. *Environmental management, 43*(5), 804-816. https://doi.org/10.1007/s00267-008-9197-0

Mishra, S., Singh, A., Keswani, C., Saxena, A., Sarma, B.K. & H.B. Singh. (2015). Harnessing plant-microbe interactions for enhanced protection against phytopathogens. In: *Plant Microbe Symbiosis- Applied Facets*. Ed. Arora, N.K. Springer, New Delhi, India, pp.111-125. https://doi.org/10.1007/978-81-322-2068-8_5

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in plant science, 7*(9), 405-410. https://doi.org/10.1016/S1360-1385(02)02312-9

NAAS (2013). *Climate Resilient Agriculture in India*. Policy Paper No. 65, National Academy of Agricultural Sciences, New Delhi, India. p. 20

NAAS 2017. Abiotic Stress Management with Focus on Drought, Flood and Hailstorm. Policy Paper No. 87 National Academy of Agricultural Sciences, New Delhi, India. p. 14

Noctor, G., Veljović-Jovanović, S., Driscoll, S., Novitskaya, L., & Foyer, C. H. (2002). Drought and oxidative load in the leaves of C3 plants: a predominant role for photorespiration? *Annals of Botany, 89*(7), 841-850. https://doi.org/10.1093/aob/mcf096

Ram, R.M., Keswani, C., Bisen, K., Tripathi, R., Singh, S.P. & Singh, H.B. (2018). Biocontrol Technology: Eco-Friendly Approaches for Sustainable Agriculture. In: *Omics Technologies and Bio-Engineering: Towards Improving Quality of Life Volume II Microbial, Plant, Environmental and Industrial Technologies*. Eds. Brah, D., Azevedo, V. Academic Press, London, U.K. pp. 177-190. https://doi.org/10.1016/B978-0-12-815870-8.00010-3

Ramonell, K. M., & Somerville, S. (2002). The genomics parade of defense responses: to infinity and beyond. *Current opinion in plant biology, 5*(4), 291-294. https://doi.org/10.1016/S1369-5266(02)00266-2

Rasheed, R., Wahid, A., Farooq, M., Hussain, I., & Basra, S. M. (2011). Role of proline and glycinebetaine pretreatments in improving heat tolerance of sprouting sugarcane (Saccharum sp.) buds. *Plant growth regulation, 65*(1), 35-45. https://doi.org/10.1007/s10725-011-9572-3

Sasaki, T. (1997). *Science of the rice plant* (Genetics): Nobunkyo.

Saseendran, S., Singh, K., Rathore, L., Singh, S., & Sinha, S. (2000). Effects of climate change on rice production in the tropical humid climate of Kerala, India. *Climatic Change, 44*(4), 495-514. https://doi.org/10.1023/A:1005542414134

Schoffl, F., Prandl, R., Hinderhofer, K., & Reindl, A. (1997). Molecular and applied aspects of the heat stress response and of common stress tolerance in plants. *Acta Physiologiae Plantarum, 19*(4). https://doi.org/10.1007/s11738-997-0052-3

Sharma, P., & Dubey, R. S. (2005). Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. *Plant growth regulation, 46*(3), 209-221. https://doi.org/10.1007/s10725-005-0002-2

Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative de-

fense mechanism in plants under stressful conditions. *Journal of botany*, 2012. https://doi.org/10.1155/2012/217037

Shoeb, M., Singh, B. R., Khan, J. A., Khan, W., Singh, B. N., Singh, H. B., & Naqvi, A. H. (2013). ROS-dependent anti-candidal activity of zinc oxide nanoparticles synthesized by using egg albumen as a biotemplate. Advances in Natural Sciences: *Nanoscience and Nanotechnology, 4*(3), 035015. https://doi.org/10.1088/2043-6262/4/3/035015

Singh, H.B, Sarma, B.K. & Keswani, C. (Eds.) (2016). *Agriculturally Important Microorganisms*: Commercialization and Regulatory Requirements in Asia. Springer, Singapore. P. 336. https://doi.org/10.1007/978-981-10-2576-1

Singh, H.B., Keswani, C. & Singh, S.P. (Eds.)(2019a). *Intellectual Property Issues in Microbiology* Springer-Nature, Singapore. P. 425. https://doi.org/10.1007/978-981-13-7466-1

Singh, H.B, Keswani, C., Reddy, M.S., Royano, E.S. & García-Estrada, C. (Eds.)(2019b). *Secondary Metabolites of Plant Growth Promoting Rhizomicroorganisms: Discovery and Applications* Springer-Nature, Singapore. P. 392. https://doi.org/10.1007/978-981-13-5862-3

Singh, V., Ray, S., Bisen, K., Keswani, C., Upadhyay, R. S., Sarma, B. K. & Singh H. B. (2017) Unravelling the Dual Applications of Trichoderma spp. as Biopesticide and Biofertilizer. In: *Advances in PGPR Research*. Eds. Singh H. B., Sarma, B. K., Keswani, C. CABI, Wallingford, UK pp. 364-374.

Smolka, B., Lukac, R., Chydzinski, A., Plataniotis, K. N., & Wojciechowski, W. (2003). Fast adaptive similarity based impulsive noise reduction filter. *Real-Time Imaging, 9*(4), 261-276. https://doi.org/10.1016/j.rti.2003.09.015

Suárez, M. B., Sanz, L., Chamorro, M. I., Rey, M., González, F. J., Llobell, A., & Monte, E. (2005). Proteomic analysis of secreted proteins from Trichoderma harzianum: identification of a fungal cell wall-induced aspartic protease. *Fungal Genetics and Biology, 42*(11), 924-934. https://doi.org/10.1016/j.fgb.2005.08.002

Taiz, L., & Zeiger, E. (2006). Plant physiology. 4th. S*inauer Associate, Sunderland, Mass*., EUA.

Tripathy, B. C., & Oelmüller, R. (2012). Reactive oxygen species generation and signaling in plants. *Plant signaling & behavior, 7*(12), 1621-1633. https://doi.org/10.4161/psb.22455

Trovato, M., Mattioli, R., & Costantino, P. (2008). Multiple roles of proline in plant stress tolerance and development. *Rendiconti Lincei, 19*(4), 325-346. https://doi.org/10.1007/s12210-008-0022-8

Tucci, M., Ruocco, M., De Masi, L., De Palma, M., & Lorito, M. (2011). The beneficial effect of Trichoderma spp. on tomato is modulated by the plant genotype. *Molecular Plant Pathology, 12*(4), 341-354. https://doi.org/10.1111/j.1364-3703.2010.00674.x

Van Breusegem, F., Vranová, E., Dat, J. F., & Inzé, D. (2001). The role of active oxygen species in plant signal transduction. *Plant Science, 161*(3), 405-414. https://doi.org/10.1016/S0168-9452(01)00452-6

Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Marra, R., Woo, S. L., & Lorito, M. (2008). Trichoderma-plant-pathogen interactions. *Soil Biology and Biochemistry, 40*(1), 1-10. https://doi.org/10.1016/j.soilbio.2007.07.002

Vollenweider, P., & Günthardt-Goerg, M. S. (2005). Diagnosis of abiotic and biotic stress factors using the visible symptoms in foliage. *Environmental Pollution, 137*(3), 455-465. https://doi.org/10.1016/j.envpol.2005.01.032

Wahid, A., Gelani, S., Ashraf, M., & Foolad, M. R. (2007). Heat tolerance in plants: an overview. *Environmental and experimental botany, 61*(3), 199-223. https://doi.org/10.1016/j.envexpbot.2007.05.011

Wang, W., Vinocur, B., Shoseyov, O., & Altman, A. (2004). Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends in plant science, 9*(5), 244-252. https://doi.org/10.1016/j.tplants.2004.03.006

Wang, Y., Mopper, S., & Hasenstein, K. H. (2001). Effects of salinity on endogenous ABA, IAA, JA, and SA in Iris hexagona. *Journal of chemical ecology, 27*(2), 327-342. https://doi.org/10.1023/A:1005632506230

Willits, D., & Peet, M. (1998). The effect of night temperature on greenhouse grown tomato yields in warm climates. *Agricultural and Forest Meteorology, 92*(3), 191-202. https://doi.org/10.1016/S0168-1923(98)00089-6

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Ugotavljanje sposobnosti prilagoditve listerij na benzalkonijev klorid z določanjem njegove minimalne inhibitorne koncentracije

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Ugotavljanje sposobnosti prilagoditve listerij na benzalkonijev klorid z določanjem njegove minimalne inhibitorne koncentracije

Izvleček: Bakterije rodu *Listeria* v živilski industriji predstavljajo težavo zaradi njihove razširjenosti in dobre sposobnosti preživetja v neugodnih razmerah. *L. monocytogenes* (E. Murray et al. 1926) Pirie 1940 je za človeka patogena bakterija, medtem ko je v živilsko-predelovalnem okolju od listerij najpogosteje prisotne nepatogene bakterije vrste *L. innocua* Seeliger (ATCC® 33090™) Namen dela je bil določiti, ali se listerije lahko prilagodijo na razkužilo benzalkonijev klorid (BAC). Za prilagoditev smo potrebovali natančno določitev protimikrobne aktivnosti BAC. Z metodo razredčevanja v mikrotitrski ploščici (MTP) smo ugotovili minimalno inhibitorno koncentracijo (MIK_{MTP}) BAC za preiskovane seve. Nato smo z rastnimi krivuljami preverili, ali so tako določene koncentracije zares najmanjše koncentracije BAC, ki vplivajo na rast sevov. Ugotovili smo, da imajo inhibitorni učinek že precej manjše koncentracije BAC kot je MIK_{MTP}, saj so bile minimalne inhibitorne koncentracije določene z rastnimi krivuljami (MIK_{RK}) le 0,1–0,5x MIK_{MTP}. Prilagoditev listerij na BAC smo zato izvedli tako, da smo kot začetno koncentracijo BAC uporabili 0,25x MIK_{RK}. Rezultati so pokazali, da se je kar 50 % sevov uspelo prilagoditi na BAC, in pri sevu *L. monocytogenes* ŽM500 je bila ta prilagoditev celo trajna. Metoda razredčevanja v mikrotitrski ploščici je uporabna za približno določitev protimikrobne aktivnosti razkužila, medtem ko je za natančnejšo določitev aktivnosti razkužila potrebno le-to določiti z drugo metodo, kot je npr. štetje kolonij na trdem gojišču.

Ključne besede: razkužila; benzalkonijev klorid; *Listeria;* metoda razredčevanja v mikrotitrski ploščici; rastna krivulja

Assessing of adaptation ability of Listeria to benzalkonium chloride (BAC) by determination of its minimal inhibitory concentration

Abstract: Bacteria of the genus *Listeria* pose a problem in the food industry due to their wide distribution and their good survival in adverse conditions. *L. monocytogenes* (E. Murray et al. 1926) Pirie 1940 is human pathogen, while *L. innocua* Seeliger (ATCC® 33090™) as not pathogenic bacteria is the most often found listeria in food production environment. Disinfectants represents an important part of *Listeria* management in food processing environments and benzalkonium chloride (BAC) is used frequently. The purpose of the work was to determine whether strains of listeria can adapt to BAC. To carry out the adaptation, a precise determination of antibacterial activity of BAC was needed. Firstly minimum inhibitory concentration (MIC_{MTP}) of BAC was determined with broth microdilution method for each *Listeria* strain. Then, we checked whether MIC_{MTP} was indeed the lowest concentration of BAC, which had an influence on growth of strains with growth curves. We found out that growth inhibitory effect (MIC_{GC}) was achieved at concentrations of BAC that were lower than MIC_{MTP} (0.1-0.5x of MIC_{MTP} values). Adaptation of listeria to BAC was therefore performed by using 0.25x MIC_{GC} as the initial BAC concentration. Results showed that 50 % of the strains were able to adapt to BAC, and in *L. monocytogenes* ŽM500 this adaptation was even stable. The broth microdilution method was useful for approximate assessment of antimicrobial activity of BAC, while for the more precise determination of disinfectant activity it is necessary to determine it by using another method such as plate count method.

Key words: disinfectants; benzalkonium chloride; *Listeria;* broth microdilution method; growth curve

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1 UVOD

Kvarterne amonijeve spojine (KAC) so pogosto uporabljena razkužila, ki se uporabljajo pri mikrobiološkem obvladovanju znotraj zdravstvenih ustanov, veterinarskih ambulant in živilsko predelovalnih obratov. Posebej primerna so predvsem zaradi majhne korozivnosti in toksičnosti, a so kljub temu zelo protimikrobno učinkovita (Wu in sod., 2015). Med KAC uvrščamo tudi razkužilo benzalkonijev klorid (BAC), ki se kot kationsko površinsko aktivno sredstvo pogosto uporablja za medicinsko dezinfekcijo in sanitacijo v živilsko predelovalnih okoljih zaradi svoje dvojne, tako hidrofobne kot tudi hidrofilne, narave. Pogosto se dodaja kot biocid v izdelke za osebno nego, kozmetiko in produkte za dezinfekcijo kože (Liu in sod., 2017). Protimikrobno delovanje BAC temelji na spremembi permeabilnosti celične membrane in motenj v naboju celične membrane, kar povzroči izločanje citoplazemske vsebine v zunanjost celice ter s tem celično smrt (Arias-Moliz in sod., 2015).

Številne študije kažejo na to, da se bakterije vrste *L. monocytogenes* pri izpostavitvi subinhibitornim koncentracijam BAC lahko prilagodijo na večje koncentracije BAC, vendar mehanizem odpornosti še ni popolnoma raziskan. Predvideva se, da so pri razvoju bakterijske odpornosti proti razkužilom pomembni dejavniki membranske izlivne črpalke, za katere se v literaturi uporabljajo tudi izrazi »sistem transportnih proteinov« in »efluksne črpalke« (Gadea in sod., 2017). Določene študije nakazujejo tudi, da je eden izmed možnih mehanizmov za razvoj odpornosti bakterij vrste *L. monocytogenes* proti KAS, sprememba sestave maščobnih kislin v bakterijski membrani (Bisbiroulas in sod., 2011), kar bakterijam omogoča rast v stresnih razmerah. Še več, nekateri strokovnjaki predvidevajo, da bi v teoriji pri prilagoditvi listerij na KAS lahko v bakterijski celici prišlo do aktivacije dveh ali več mehanizmov hkrati, kot so npr. modifikacija strukture in sestave bakterijske membrane, optimizacija tvorbe biofilma, prekomerno izražanje genov, ki kodirajo membranske izlivne črpalke in biorazgradnja razkužila (Gadea in sod., 2017).

Ob pravilni uporabi in primerni koncentraciji so razkužila precej učinkovita pri preprečevanju rasti bakterij. Zaradi različnih razlogov, kot so nepopolno čiščenje delovnih površin, premajhen odmerek uporabljenega razkužila ali zaradi tvorbe biofilma na delovnih površin, pa lahko v stik z bakterijami prihajajo tudi v manjših koncentracijah. V takih razmerah so bakterije izpostavljene sub-letalnim koncentracijam razkužil, kar lahko vodi do prilagoditve bakterij na razkužila (Soumet in sod., 2016). Zaradi prekomerne uporabe KAC se lahko v določenih okoljih tako nevede vršijo selektivni pritiski (v smislu delovanja majhnih koncentracij KAC, ki niso dovolj za baktericidno aktivnost) , ki lahko prispevajo k razvoju proti KAC odpornih bakterij. Zaradi vse večjega števila proti protimikrobnim spojinam odpornih bakterij in široke uporabe KAC, je ključnega pomena oceniti, katera metoda je najbolj primerna za določitev protimikrobne aktivnosti KAC. Trenutno ni posebej predpisane metode za določitev protimikrobne aktivnosti KAC. V ta namen se pogosto uporablja metoda razredčevanja v mikrotitrski ploščici ali metoda razredčevanja v trdnem gojišču, ki sta standardizirani s strani CLSI (Clinical and Laboratory Standards Institute) (Wu in sod., 2015). Pri metodi razredčevanja v mikrotitrski plošči se po inkubacijskem obdobju bakterijska rast preverja bodisi z vizualnim pregledom bodisi s polavtomatskimi ali popolnoma avtomatiziranimi sistemi. Najmanjša koncentracija protimikrobnega sredstva, ki zavre rast bakterij je definirana kot minimalna inhibitorna koncentracija (MIK) (Klančnik in sod., 2010). Prednosti omenjene metode so predvsem v tem, da je metoda enostavna za izvedbo in jo je mogoče enostavno standardizirati pri preiskavah med dvema ali večimi laboratoriji. Kljub temu se v literaturi pojavljajo študije, ki ugotavljajo relevantnost rezultatov, pridobljenih z metodo razredčevanja v mikrotitrski ploščici in katero drugo metodo, ki se danes prav tako pogosto uporablja za določitev protimikrobne aktivnosti preiskovanih spojin. Standardna metoda razredčevanja v mikrotitrski ploščici ima priznano natančnost za en razredčitveni interval, saj metoda za vrednost MIK npr. 1 mg l^{-1} sprejema vrednosti med 0,5 in 2 mg l^{-1}. Lepe in sod. (2013) so v študiji ugotovili šibko korelacijo med rezultati pridobljeni z E-testi in metodo razredčevanja v mikrotirski ploščici, zaradi česar bi bilo zaželeno razviti metodo za še natančnejšo določanje vrednosti MIK. Tudi Klodzinska in sod. (2018) so predpostavili, da bi že samo ob kombiniranju določitve MIK in MBK (mikrobicidne koncentracije) pridobili podrobnejši vpogled v občutljivost bakterij za protimikrobna sredstva in bolj klinično relevantne podatke.

Namen raziskave je bil določiti, kakšna je zmožnost različnih sevov bakterij rodu *Listeria*, da se prilagodijo na BAC in hkrati preveriti ali za določitev protimikrobne aktivnosti BAC ustreza uporaba metode razredčevanja v mikrotitrski ploščici.

2 MATERIAL IN METODE

2.1 SEVI BAKTERIJ RODU *LISTERIA* IN GOJIŠČA

V raziskavo smo vključili 10 sevov bakterij rodu *Listeria* iz mikrobiološke zbirke Laboratorija za živilsko mikrobiologijo na Oddelku za živilstvo Biotehniške fakul-

tete (oznaka sevov ŽM). Uporabili smo 5 sevov bakterij vrste *Listeria monocytogenes* (E. Murray et al. 1926) Pirie 1940) in 5 sevov bakterij vrste *L. innocua* Seeliger (ATCC® 33090™) (Preglednica 1). Bakterije so bile shranjene pri -20 °C v suspenziji glicerola (0,15 ml) in kulture (0,85 ml v tekočem gojišču TSB (angl. Tryptic Soy Broth, Biolife, 4021552, Milano, Italija). Po revitalizaciji smo sev iz gojišča TSB nacepili na selektivno gojišče ALOA (angl. Agar Listeria acc. to Ottaviani & Agosti, Biolife, 4016052), kjer so po 24 h pri 37 °C zrasle modrozelene kolonije, ki so značilne za bakterije vrste *L. innocua,* in modrozelene kolonije s prosojno cono, ki so značilne za bakterije vrste *L. monocytogenes.* Po eno kolonijo smo nacepili na gojišče TSA (angl. Tryptic Soy Agar, Biolife, 4021502) in ponovno 24 h inkubirali pri 37 °C. Pred vsakim eksperimentom smo za pripravo inokuluma z gojišča TSA vzeli eno kolonijo izbranega seva ter jo prenesli v 4 ml gojišča TSB, vsebino premešali na vrtinčnem mešalniku in 4 ure inkubirali pri 37 °C na stresalniku (75 obratov/ minuto). Predvidevali smo, da se je v tem času kultura namnožila do koncentracije 10^8 KE (kolonijskih enot) ml^{-1}. Za nadaljnje delo smo 150 µl tako pripravljene kulture prenesli v 10 ml gojišča TSB ter točno koncentracijo celic določili z metodo štetja kolonij na gojišču TSA.

2.2 BENZALKONIJEV KLORID

Pri raziskovalnem delu smo uporabili benzalkonijev klorid (BAC, ang. Benzalkonium chloride, Sigma Aldrich, B6295, Saint Louis, ZDA). Pri pripravi delovne raztopine za določitev protimikrobne aktivnosti BAC za seve *L. monocytogenes* smo 10 mg BAC raztopili v 1 ml gojišča TSB ter redčenje z gojiščem TSB nadaljevali do koncentracije 100 µg ml^{-1}. Za določitev protimikrobne aktivnosti BAC za seve *L. innocua* smo na enak način pripravili delovno raztopino BAC (5 mg ml^{-1}) ter redčenje z gojiščem TSB nadaljevali do koncentracije 500 µg ml^{-1} saj so podatki iz literature nakazovali, da je minimalna inhibitorna koncentracija (MIK) BAC za seve *L. innocua* nekoliko večja (Korsak in Szuplewska, 2016).

2.3 METODA RAZREDČEVANJA V MIKROTITRSKI PLOŠČICI

Za določitev minimalne inhibitorne koncentracije (MIK) razkužila BAC smo uporabili metodo razredčevanja v mikrotitrski ploščici (Klančnik in sod., 2010) in to koncentracijo označili kot MIK_{MTP}. Po 24 h inkubaciji pri 37 °C smo v vse luknjice mikrotitrske ploščice, ki so vsebovale preiskovan sev listerij in različne koncentracije BAC, dodali 10 µl reagenta INT (2-p-iodofenil-3-p-nitrofenil-5-fentil tetrazolijev klorid, 2 mg ml^{-1} Sigma Aldrich, 18377-56, Saint Luis, ZDA) ter mikrotitrsko ploščico 1 minuto stresali na stresalniku (100 obratov/ minuto) in jo ponovno 20 minut inkubirali pri 37 °C. Vrednost MIK_{MTP} smo določili vizualno glede na obarvanost suspenzije v posamezni luknjici, pri čemer je bila prva neobarvana luknjica določena kot MIK_{MTP} BAC, saj je to pomenilo, da ni več metabolno aktivnih bakterijskih celic, ki bi INT lahko reducirale v rdeč formazan (Klančnik in sod., 2010). MIK_{MTP} smo definirali kot najmanjšo koncentracijo, ki zavira vidno rast testnega seva bakterij (Burt, 2004). Po določitvi vrednosti MIK_{MTP} smo pri tej koncentraciji BAC določili število preživelih bakterij z metodo štetja kolonij na trdem gojišču TSA. Vse preiskave z metodo razredčevanja v mikrotitrski ploščici smo izvedli v 2 ali 3 paralelkah.

2.4 RASTNA KRIVULJA

Z rastno krivuljo smo preverjali, ali je koncentracija BAC določena z metodo razredčevanja v mikrotitrski ploščici (MIK_{MTP}) dejansko najmanjša koncentracija BAC, ki zmanjša rast posameznega seva listerij. Rastno krivuljo smo določili tako, da smo v epruveto z 8,5 ml gojišča TSB dodali 1 ml delovne raztopine razkužila s tako koncentracijo, da je bila končna koncentracija razkužila v 10 ml raztopine enaka določeni MIK_{MTP} BAC, in 0,5 ml inokuluma testnega seva listerij. Vzporedno smo v drugo epruveto z 9,5 ml TSB dodali 0,5 ml inokuluma testnega seva listerij (kontrolni vzorec). Vzorce smo nato 24 h inkubirali pri 37 °C. Vzorčili smo v času 0, 4 in 24 h ter določili število listerij, ki so rasle v gojišču TSB z dodanim BAC, in število listerij, ki so rasle v gojišču TSB brez dodanega BAC z metodo štetja kolonij na trdem gojišču TSA. S pridobljeni podatki smo izrisali grafe, ki so ponazarjali rast posameznih sevov listerij pri MIK_{MTP} BAC. Rastne krivulje smo določili tudi pri manjših koncentracijah BAC, kot je bila določena MIK_{MTP}. Minimalno inhibitorno koncentracijo BAC smo nato določili iz rastnih krivulj in jo označili kot MIK_{RK} pri tisti najmanjši koncentraciji BAC, kjer je bila statistično značilna razlika (Sekcija 2.5) med rastjo posameznega seva v gojišču TSB z dodanim BAC v gojišču TSB brez dodanega BAC. Vse rastne krivulje smo določili v najmanj dveh paralelkah.

2.5 STATISTIČNA OBDELAVA

Rezultate, pridobljene z rastnimi krivuljami, smo primerjali s testom ANOVA (Microsoft Excel). Predpostavili smo, da velja ničelna hipoteza (H_0) in izbrali 0,05 za stopnjo značilnosti α. Iz vzorca smo izračunali

testno statistiko in glede na to, v katero območje je vrednost padla, ovrednotili rezultate. V kolikor je bila verjetnost $p > 0,05$, smo ničelno domnevo obdržali, kar pomeni, da rezultati niso bili statistično značilno različni. V nasprotnem primeru, ko je bila $p < 0,05$, smo ničelno domnevo zavrnili v korist alternativne domneve (H_1), kar je pomenilo, da so rezultati statistično značilno različni (Košmelj, 2007).

2.6 PRILAGODITEV LISTERIJ NA BENZALKONIJEV KLORID

Prilagoditev posameznega seva listerij na BAC smo izvajali v 2 paralelkah in vzporedno v 2 paralelkah preverjali rast istega seva bakterij v samem gojišču TSB (kontrolna vzorca). 150 µl pripravljene prekonočne kulture posameznega seva listerij smo prenesli v 10 ml tekočega gojišča TSB. 150 µl tako pripravljenega inokuluma smo aseptično prenesli v epruveto, kjer je bilo 9 ml gojišča TSB in dodali 1 ml ustrezno razredčene delovne raztopine razkužila BAC. Razredčitve delovne raztopine BAC smo izvedli glede na predhodno določene vrednosti MIK_{RK} tako, da smo prilagoditev posameznega seva listerij začeli pri 0,25 x MIK_{RK}. Seve smo postopoma izpostavljali naraščajočim se koncentracijam BAC vse do 16x MIK_{RK} oz. do koncentracije, pri kateri se je rast bakterij ustavila. Rast bakterij smo pri manjših koncentracijah BAC (0,25 x -2x MIK_{RK}) določili s pojavom motnosti gojišča TSB v epruveti, ki je vsebovala dodatek BAC v primerjavi s kontrolno epruveto, ki je vsebovala gojišče TSB brez dodanega BAC. Pri večjih koncentracijah BAC (> MIK_{RK}) smo rast listerij določili z metodo štetja kolonij na trdnem gojišču, saj motnost ni bila več očitna. V primerih, ko ni bilo zaznane bakterijske rasti, smo vzorce inkubirali do sedem dni ter vsak dan preverjali, če je prišlo do kakšne spremembe v koncentraciji listerij. V kolikor po sedmih dneh ni bilo zaznane rasti, smo prilagoditev zaključili. Pri kontrolnem vzorcu smo v 10 ml TSB dodali 150 µl razredčene prekonočne kulture izbranega seva in sev inkubirali v enakih razmerah kot sev, ki smo ga prilagajali na BAC.

3 REZULTATI IN RAZPRAVA

Glavni namen raziskave je bil določiti sposobnost prilagoditve različnih sevov listerij za benzalkonijev klorid. Prvi korak za nadaljnji poskus prilagoditve je bil ugotoviti, katera je najmanjša koncentracija BAC, ki ima inhibitorni učinek za rast posameznega seva.

3.1 DOLOČITEV KONCENTRACIJE BENZALKONIJEVEGA KLORIDA ZA RAZLIČNE SEVE LISTERIJ

Za izvedbo prilagoditve smo za posamezen sev najprej določili protibakterijsko učinkovitost BAC tako, da smo določili minimalne inhibitorne koncentracije z metodo razredčevanja v mikrotitrski ploščici (MIK_{MTP}). Ker smo ugotovili, da so te koncentracije BAC zelo velike in da lahko delujejo že baktericidno (Preglednica 1), smo natančneje določili minimalne inhibitorne koncentracije iz rastnih krivulj (MIK_{RK}), pri katerih smo rast posameznega seva listerij spremljali pri različnih manjših koncentracijah BAC, kot je bila določena MIK_{MTP}. Rezultati so pokazali, da se MIK_{MTP} in MIK_{RK} med seboj zelo razlikujejo (Preglednica 1). To lahko pojasnimo s tem, da gre pri metodi razredčevanja v mikrotitrski ploščici za »ohlapno«, manj točno metodo, s katero testiramo zelo široko koncentracijsko območje razkužila, kjer je rezultat le približna minimalna inhibitorna koncentracija. Poleg tega z reagentom INT ne moremo določiti števila preživelih bakterij, ampak le ugotovimo pri kateri koncentraciji je bakterij dovolj, da zaznamo spremembo v barvi gojišča zaradi redukcije barvila INT (Berridge in sod., 2005). Na primer pri sevu *L. innocua* ŽM39 je bila MIK_{MTP} BAC 7,81 µg ml^{-1}, medtem ko smo z rastno krivuljo določili, da pride do statistično značilnega zmanjšanja rasti že pri 1,95 µg ml^{-1} BAC (MIK_{RK}) in zato smo to koncentracijo upoštevali kot tisto, kjer se rast tega seva bakterij že zmanjša. Analogno smo za vse druge seve listerij določili, da so MIK_{RK} od 2 x do 8 x manjše od MIK_{MTP} (Preglednica 1).

Rezultati MIK_{MPT} iz preglednice 1 so primerljivi z rezultati, ki so jih pokazali Soumet in sodelavci (2005). Pri rezultatih dobljenih z metodo razredčevanja v mikrotitrski ploščici so ugotovili, da je vrednost MIK za BAC pri bakterijah vrste *L. monocytogenes* v območju med 1,87 in 15 µg ml^{-1}, pri čemer so jo pri 88 sevih od 254 preiskovanih določili pri koncentraciji 3,75 µg ml^{-1}. Tudi vrednosti MIK_{MPT} izbranih sevov bakterij vrste *L. innocua* se v našem primeru skladajo s podatki iz literature. Korsak in Szuplewska (2016) sta pri 90 % preiskovanih sevov bakterij vrste *L. innocua* z uporabo metode razredčevanja v mikrotitrski ploščici določili vrednost MIK za BAC 5 µg ml^{-1}, pri čemer se je območje MIK za preiskovane seve bakterij vrste *L. innocua* gibalo med 2,5 in 40 µg ml^{-1}, kar je v skladu z našimi rezultati MIK_{MPT} (Preglednica 1).

3.2 PRILAGODITEV LISTERIJ NA BENZALKONIJEV KLORID

S prilagoditvijo listerij na BAC smo začeli pri zelo

Tabela 1: Določitev minimalne inhibitorne koncentracije benzalkonijevega klorida z metodo razredčevanja v mikrotitrski ploščici in rastno krivuljo za seve bakterij rodu *Listeria*
Table 1: Determination of minimum inhibitory concentration of benzalkonium chloride by microdilution method and growth curve for *Listeria* strains

	Metoda razredčevanja v mikrotitrski ploščici		Rastna krivulja						
	MIK_{MTP} (μg ml^{-1})	N_{MTP} (KE $ml^{-1)}$	0,5 x MIK_{MTP}		0,25 x MIK_{MTP}		0,125 x MIK_{MTP}		MIK_{RK} (μg ml^{-1})
Sev			C (μg ml^{-1})	N (KE ml^{-1})	C (μg ml^{-1})	N (KE ml^{-1})	C (μg ml^{-1})	N (KE ml^{-1})	
L. innocua ŽM39	7,81	3,5 x 10^3	3,91	< 10	**1,95**	**2,3 x 10^8**	/	/	1,95
L. innocua ŽM40	7,81	1,7 x 10^2	3,91	< 10	1,95	<10	**0,98**	**1,0 x 10^9**	0,98
L. innocua ŽM41	7,81	5,2 x 10^2	3,91	< 10	1,95	*<10*	**0,98**	**4,2x 10^7**	0,98
L. innocua ŽM43	7,81	1,6 x 10^3	3,91	< 10	**1,95**	**2,6x 10^8**	/	/	1,95
L. innocua ŽM68	7,81	6,0 x 10^1	3,91	< 10	1,95	2,6 x 10^2	**0,98**	**4,0 x 10^7**	0,98
L. monocytogenes ŽM51	6,25	< 10	**3,13**	**1,0 x 10^5**	1,56	/	/	/	3,13
L. monocytogenes ŽM58	6,25	< 10	**3,13**	**6,6 x 10^7**	1,56	/	/	/	3,13
L. monocytogenes ŽM69	12,5	< 10	6,25	<10	**3,13**	**1,7 x 10^7**	/	/	3,13
L. monocytogenes ŽM500	6,25	< 10	3,13	7,0 x 10^1	**1,56**	**2,6 x 10^6**	/	/	1,56
L. monocytogenes ŽM520	12,5	< 10	6,25	<10	**3,13**	**9,3 x 10^5**	/	/	3,13

Legenda: MIK MTP: MIK, dobljena z metodo razredčevanja v mikrotitrski ploščici; NMTP: število bakterij, določeno pri metodi razredčevanja v mikrotitrski ploščici; N: število bakterij, določeno pri rastni krivulji; KE: kolonijska enota; C: koncentracija benzalkonijevega klorida, uporabljena pri rastni krivulji; poudarjena pisava: C in N, določena kot tista koncentracija (C), pri kateri je število bakterij (N) manjše kot pri kontrolnem vzorcu, kjer so bakterije rasle v gojišču brez benzalkonijevega klorida (p < 0,05), pri tej koncentraciji smo C definirali kot minimalno inhibitorno koncentracijo, pridobljeno iz rastne krivulje: MIK RK: dosežena MIK

majhni koncentraciji razkužila, to je pri 0,25 x MIK_{RK}, ki smo jo določili glede na podatke iz rastnih krivulj (Preglednica 1). S to izbiro majhne začetne koncentracije BAC smo listerijam omogočili postopno in tudi časovno dovolj dolgo prilagoditev.

Trije preiskovani sevi *L. innocua* so se uspeli prilagoditi na 8 x MIK_{RK} (7,84 μg ml^{-1}), saj pri 16 x MIK_{RK} (15,6 μg ml^{-1}) ni bilo mogoče zaznati preživelih listerij z metodo štetja kolonij na trdnem gojišču (N < 10 KE ml^{-1}), zato smo adaptacijo zaključili (Preglednica 2). Do prilagoditve na 8 x MIK_{RK} (7,84 μg ml^{-1}) BAC je prišlo tudi pri treh sevih *L. innocua* ŽM40, *L. innocua* ŽM41 in *L. innocua* ŽM68. V primeru sevov bakterij vrste *L. monocytogenes* sta se dva seva, *L. monocytogenes* ŽM500 in *L. monocytogenes* ŽM520, uspela prilagoditi na večje koncentracije BAC. Rast seva *L. monocytogenes* ŽM500 je bila pri naraščajočih koncentracijah BAC še vedno zaznana pri 8 x MIK_{RK} (12,5 μg ml^{-1}), medtem, ko se je pri 16 x MIK_{RK} (25 μg ml^{-1}) prilagoditev ustavila. Za razliko od rasti seva *L. monocytogenes* ŽM500 se je rast seva *L. monocytogenes* ŽM520 ustavila že pri 4 x MIK_{RK} (12,5 μg ml^{-1}). Sev *L. monocytogenes* ŽM500 se je prilagodil na 8 x MIK_{RK} (12,5 μg ml^{-1}) in sev *L. monocytogenes* ŽM520 na 4 x vrednost MIK_{RK} (12,5 μg ml^{-1}) (Preglednica 2). Do prilagoditve ni prišlo pri dveh sevih *L. innocua* ŽM39 ter *L. innocua* ŽM43 in treh sevih *L. monocytogenes* ŽM51, *L. monocytogenes* ŽM58 ter *L. monocytogenes* ŽM69.

Podobne rezultate so dobili tudi Aase in sodelavci (2000), ki so ugotovili, da so se sevi, kljub različnim začetnim MIK, med prilagajanjem uspeli prilagoditi na približno enake končne koncentracije BAC. Tudi naši rezultati so pokazali, da sta se tako sev *L. monocytogenes* ŽM500 kot sev *L. monocytogenes* ŽM520 uspela prilagoditi na 12,5 μg BAC ml^{-1}, kljub temu, da so rezultati rastne krivulje pokazali, da je MIK_{RK} seva *L. monocytogenes* ŽM500 za polovico manjša od MIK_{RK} seva *L. monocytogenes* ŽM520. Pri tem je potrebno omeniti, da se končna koncentracija prilagojenih sevov v naši raziskavi razlikuje od koncentracij raziskave Aase in sod. (2000), saj so se sevi *L. monocytogenes* v njihovi raziskavi prilagodili le na 7 μg ml^{-1}. Podobno so ugotovili tudi To in sod. (2002), ki so pokazali, da je MIK prilagojenih sevov ostala v območju med 5-8 μg ml^{-1}. Tudi v primeru prilagoditve bakterij vrste *L. innocua* se je izkazalo, da so se sevi uspeli prilagoditi na enake koncentracije BAC, pri čemer je bila končna koncentracija prilagoditve 7,84 μg BAC ml^{-1} (Preglednica 2), kar je sicer manjša koncentracija kot v primeru bakterij vrste *L. monocytogenes* (Preglednica 2). V nam dostopni literaturi primerljivih podatkov o prila-

Tabela 2: Prilagoditev sevov bakterij rodu *Listeria* na povečane koncentracije benzalkonijevega klorida
Table 2: Adaptation of *Listeria* strains to increased concentrations of benzalkonium chloride

	C (μg ml^{-1})						
Sev	0,25 x MIK$_{RK}$	0,5 x MIK$_{RK}$	1 x MIK$_{RK}$	2 x MIK$_{RK}$	4 x MIK$_{RK}$	8 x MIK$_{RK}$	16 x MIK$_{RK}$
L. innocua ŽM39	0,49	0,98	**1,96**	3,92	7,84	15,68	31,36
L. innocua ŽM40	0,24	0,49	0,98	1,96	3,92	**7,84**	15,68
L. innocua ŽM41	0,24	0,49	0,98	1,96	3,92	**7,84**	15,68
L. innocua ŽM43	0,49	0,98	**1,96**	3,92	7,84	15,68	31,36
L. innocua ŽM68	0,24	0,49	0,98	1,96	3,92	7,84	15,68
L. monocytogenes ŽM51	0,78	1,56	**3,12**	6,25	12,5	25	50
L. monocytogenes ŽM58	0,78	1,56	**3,12**	6,25	12,5	25	50
L. monocytogenes ŽM69	0,78	1,56	**3,12**	6,25	12,5	25	50
L. monocytogenes ŽM500	0,39	0,78	1,56	3,12	6,25	**12,5**	25
L. monocytogenes ŽM520	0,78	1,56	3,12	6,25	**12,5**	25	50

Legenda: MIK: minimalna inhibitorna koncentracija; MIKRK: MIK, dobljena z rastno krivuljo; poudarjeno zapisane vrednosti: največja koncentracija benzalkonijevega klorida, do katere se je sev prilagodil

goditvi bakterij vrste *L. innocua* na BAC ali sorodno KAS nismo zasledili.

3.3 STABILNOST PRILAGODITVE LISTERIJ NA BENZALKONIJEV KLORID

Po prilagoditvi petih sevov bakterij rodu *Listeria* (Preglednica 2) smo preverili, ali je le-ta trajna ali začasna. Seve smo sedemkrat zapored precepili na trdno gojišče TSA brez dodanega BAC (Aase in sod., 2000) in ponovno določili minimalno inhibitorno koncentracijo BAC za vsak sev in vsako izmed precepitev z metodo razredčevanja v mikrotitrski ploščici (MIK$_{MTP}$). Že pri prvi precepitvi se je izkazalo, da se MIK$_{MTP}$ ni ujemala s koncentracijo BAC, na katero so se sevi prilagodili. Ugotovili smo namreč, da se je MIK$_{MTP}$ prilagojenih sevov, določena z metodo razredčevanja v mikrotitrski ploščici po adaptaciji povečala skladno faktorjem prilagoditve in je bila 4-8 x MIK vrednosti, ki je bila sprva določena z metodo razredčevanja v mikrotitrski ploščici (Preglednica 3). Z dobljenimi rezultati smo tako potrdili, da se MIK$_{MTP}$, določena z metodo razredčevanja v mikrotitrski ploščici razlikuje od dejanske MIK določene z rastno krivuljo MIK$_{RK}$. V nadaljevanju smo preverjali vrednosti MIK$_{MTP}$ prilagojenih sevov bakterij rodu *Listeria* za nadaljnjih sedem precepitev. Pri štirih izmed petih prilagojenih sevov bakterij rodu *Listeria* je MIK$_{MTP}$ s precepitvami na trdno gojišče TSA pričela upadati, a je še vedno ostajala večja pri vseh štirih sevih bakterij rodu *Listeria* (25 μg ml^{-1} - 50 μg ml^{-1}) glede na dejansko koncentracijo benzalkonijevega klorida, na katero smo prilagodili seve (6,25 μg ml^{-1} - 12, 5 μg ml^{-1}).

Od petih prilagojenih sevov listerij je bil sev *L. monocytogenes* ŽM500 edini, pri katerem je MIK po sedmih zaporednih precepljanjih ostala nespremenjena. Sklepali smo, da je pri prilagoditvi v primeru štirih sevov bakterij rodu *Listeria* prišlo do razvoja prilagojenih sevov z začasno povečano odpornostjo na benzalkonijev klorid, medtem ko je v primeru seva *L. monocytogenes* ŽM500 pri adaptaciji prišlo do razvoja odpornega seva proti benzalkonijevemu kloridu.

4 SKLEPI

Metoda razredčevanja v mikrotitrski ploščici je uporabna za približno določitev protimikrobne aktivnosti razkužila, medtem ko je za natančnejšo določitev aktivnosti razkužila potrebno le-to določiti z drugo metodo (npr. določitev števila preživelih bakterij z metodo štetja kolonij na trdnem gojišču). Predvidevamo namreč, da se pri prilagoditvi na večje koncentracije benzalkonijevega klorida ne bi prilagodilo tolikšno število sevov listerij, če bi prilagoditev pričeli s tako velikimi koncentracijami, kot smo jih določili z metodo razredčevanja v mikrotitrski ploščici. Prikazani rezultati kažejo na sicer sprejemljivo rutinsko uporabo metode razredčevanja v mikrotitrski ploščici, ki jo je za natančnejše študije potrebno nujno kombinirati z drugimi metodami.

5 VIRI

Aase, B., Sundheim, G., Langsrud, S., & Rorvik, L.M. (2000). Occurrence of and a possible mechanism for resistance to

Tabela 3: Stabilnost prilagoditve na benzalkonijev klorid prilagojenih sevov bakterij rodu *Listeria*
Table 3: Stability of adaptation to benzalkonium chloride-adapted *Listeria* strains

			BAC (µg ml^{-1})				
			L. monocytogenes ŽM500	*L. monocytogenes* ŽM520	*L. innocua* ŽM40	*L. innocua* ŽM41	*L. innocua* ŽM68
		MIK_{MTP}	**6,25**	12,5	7,81	7,81	7,81
		MIK_{RK}	**1,56**	3,125	0,98	0,98	0,98
Neprilagojen sev		n- MIK_{RK}	8x	4x	8x	8x	8x
Prilagojen sev	2 prec.	MIK_{MTP}	**50**	100	62,5	62,5	62,5
	3 prec.	MIK_{MTP}	**50**	100	62,5	62,5	62,5
	4 prec.	MIK_{MTP}	/	/	62,5	31,25	62,5
	5 prec.	MIK_{MTP}	**50**	25	62,5	31,25	62,5
	6 prec.	MIK_{MTP}	**50**	25	/	/	/
	7 prec.	MIK_{MTP}	**50**	25	31,25	31,25	31,25

Legenda: BAC: benzalkonijev klorid, MIKMTP: minimalna inhibitorna koncentracija BAC, določena z metodo razredčevanja v mikrotitrski ploščici, MIKRK: minimalna inhibitorna koncentracija BAC, določena z rastno krivuljo; n-MIKRK: n-kratnik vrednosti MIKRK, določene z rastno krivuljo, na katero so se prilagojeni sevi prilagodili, prec.: precepitev; /: ni določeno, precepitev seva na TSA; odebeljena pisava: razvoj seva, odpornega proti BAC

a quaternary ammonium compound in *Listeria monocytogenes*. *International Journal of Food Microbiology*, 62(1-2), 57-63. https://doi.org/10.1016/S0168-1605(00)00357-3

Arias-Moliz, M.T., Ruiz-Linares, M., Cassar, G., Ferrer-Luque, C.M., Baca, P., Ordinola-Zapata, R., & Camilleri J. (2015). The effect of benzalkonium chloride additions to AH Plus sealer. Antimicrobial, physical and chemical properties. *Journal of Dentistry*, 43(7), 846-854. https://doi.org/10.1016/j.jdent.2015.05.003

Berridge, M. V., Hers,t P. M., & Tan, A.S. (2005). Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction. *Biotechnology Annual Review*, 11, 127-152. https://doi.org/10.1016/S1387-2656(05)11004-7

Bisbiroulas, P., Psylou, M., Iliopoulou, I., Diakogiannis, I., Berberi, A., & Mastronicolis S.K. (2011). Adaptational changes in cellular phospholipids and fatty acid composition of the food pathogen *Listeria monocytogenes* as a stress response to disinfectant sanitizer benzalkonium chloride. *Letters in Applied Microbiology*, 52, 3, 275-280. https://doi.org/10.1111/j.1472-765X.2010.02995.x

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods - a review. *International Journal of Food Microbiology*, 94(3), 223-253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Gadea, R., Fuentes, M.A.F., Pulido, R.P., Galvez, A., & Ortega, E. (2017). Effects of exposure to quaternary-ammonium--based biocides on antimicrobial susceptibility and tolerance to physical stresses in bacteria from organic foods. *Food Microbiology*, 63, 58-71. https://doi.org/10.1016/j.fm.2016.10.037

Košmelj, K. (2007). *Uporabna statistika*. 2. dop. izd. Ljubljana, Biotehniška fakulteta: 239 str. http://www.bf.uni-lj.si/fileadmin/groups/2721/Uporabna_statistika_okt_2007/Uporabna_statistika_01.pdf (januar 2018)

Klančnik, A., Piskernik ,S., Jeršek, B., & Smole Možina, S. (2010). Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. *Journal of Microbiological Methods*, 81(2), 121-126. https://doi.org/10.1016/j.mimet.2010.02.004

Klodzinska, S. Priemel, P. A., Rades, T., & Nielsen, H. M. (2018). Combining diagnostic methods for antimicrobial susceptibility testing - A comparative approach. J*ournal of Microbiological Methods*, 144, 177-185. https://doi.org/10.1016/j.mimet.2017.11.010

Korsak, D., & Szuplewska, M. (2016). Characterization of non-pathogenic *Listeria* species isolated from food and food processing environment. International *Journal of Food Microbiology,* 238, 274-280. https://doi.org/10.1016/j.ijfoodmicro.2016.08.032

Lepe, J. A., Dominguez-Herrera, J., Pachon, J., & Aznar, J. (2013). Determining accurate vancomycin MIC values for methicillin-resistant *Staphylococcus aureus* by the microdilution method. Journal of Antimicrobial Chemotherapy, 69(1), 136-138. https://doi.org/10.1093/jac/dkt308

Liu, J.J., Deng, W.J., Yu, M.Q., Wen, R.Z, Yao, S.Z., & Chen, B. (2017). Rapid analysis of benzalkonium chloride using paper spray mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, 145, 151-157. https://doi.org/10.1016/j.jpba.2017.06.030

Soumet, C., Meheust, D., Pissavin, C., Le Grandois, P., Fremaux, B., Feurer, C., Le Roux, A., Denis, M., & Maris, P. (2016). Reduced susceptibilities to biocides and resistance to antibiotics in food-associated bacteria following exposure to quaternary ammonium compounds. *Journal of Applied Microbiology*, 121, 1275-1281. https://doi.org/10.1111/jam.13247

Soumet, C., Ragimbeau, C., & Maris, P. (2005). Screening of benzalkonium chloride resistance in *Listeria monocyto*ge-

nes strains isolated during cold smoked fish production. *Letters in Aplied Microbiology,* 41(3), 291-296. https://doi.org/10.1111/j.1472-765X.2005.01763.x

To, M.S., Favrin, S., Romanova, N., & Griffiths, M. W. (2002). Postadaptational resistance to benzalkonium chloride and subsequent physicochemical modifications of *Listeria monocytogenes. Applied and Environmental Microbiology*, 68(11), 5258-5264. https://doi.org/10.1128/AEM.68.11.5258-5264.2002

Wu, G., Yang, Q., Long, M., Guo, L., Li, B., Meng, Y., Zhang, A., Wang, H., Liu, S., & Zou, L. (2015). Evaluation of agar dilution and broth microdilution methods to determine the disinfectant susceptibility. *The Journal od Antibiotics*, 68(11), 661-665. https://doi.org/10.1038/ja.2015.51

Jubilantka, zaslužna redna profesorica dr. Venčeslava Šikovec, univ. dipl. inž. agr.

Prof. dr. Venčeslava (ki jo večina pozna pod imenom Slavica) Šikovec je diplomirala 1954. leta in istega leta postala asistentka za vinarstvo na Biotehniški fakulteti, Univerze v Ljubljani. Doktorirala je leta 1964, leta 1967 je postala vnaprej habilitirana docentka za predmet Tehnologija vina in vinarstvo, leta 1972 docentka za isti predmet, leta 1975 izredna profesorica, leta 1978 pa redna profesorica za vinarstvo. Leta 1988 je bila ponovno izvoljena v naziv redne profesorice za predmet Vinarstvo in tehnologija vina, v študijskem letu 2005/06 pa je bila izvoljena v naziv zaslužne profesorice. Na področju vinogradništva in vinarstva je delovala tudi v mednarodnem prostoru, v Avstriji, Nemčiji in Italiji.

V okviru svojega pedagoškega dela je vsa leta poučevala na Oddelku za živilstvo in na Oddelku za agronomijo. Na dodiplomskem študiju je predavala študentom živilstva 3. letnika predmet Tehnologija vina in 4. letnika predmet Enologija (izbrana tehnologija), študentom agronomije pa predmeta Vinarstvo in Kletarstvo. Na podiplomskem študiju živilstva je predavala na magistrskem študiju Znanost o živilih.

Prof. dr. Venčeslava Šikovec se je upokojila konec leta 1990 in do takrat vzgojila številne generacije študentov živilstva in agronomije na Biotehniški fakulteti, Univerze v Ljubljani. Svoje bogato znanstveno-raziskovalno in strokovno znanje je prenašala na študente kot mentorica pri pripravi diplomskih, magistrskih in doktorskih del, saj je v 36 letih poučevanja na Biotehniški fakulteti bila mentorica 38 diplomskim nalogam, somentorica 10 diplomskim nalogam, mentorica 6 magistrskim delom in

1 doktorski disertaciji. Od diplomskih nalog je bila ena nagrajena s Prešernovo nagrado. Do študentov je imela strog, vendar korekten odnos.

Dolga leta je zelo aktivno sodelovala pri pripravi Bitenčevih živilskih dni, ki jih Oddelek za živilstvo še vedno organizira kot obliko podiplomskega izobraževanja.

Tematske celote njenega znanstveno-raziskovalnega dela obsegajo področja mikrobiologije in tehnologije vina. Dolga leta je proučevala selekcijo avtohtone grozdne mikroflore vinogradniških dežel Slovenije, katerih rezultat je odbira in selekcija sojev kvasovk rodu *Saccharomyces* za povretje mirnih vin, penečih vin in vin posebnih kakovosti.

Vrsto let je na področju tehnologije vina proučevala difuzijo antocianov rdečih vinskih sort, fenolni sestav moštov in vin, aminokislinsko sestavo grozdja, zorenje penečih vin na kvasovkah in s tem v povezavi obogatitev penečega vina z manoproteini kvasovk. Poseben poudarek je v svojih raziskavah posvetila tudi zaščiti človeka in njegove okolice v smeri reševanja tehnoloških postopkov, s katerimi bi se zmanjšala uporaba žveplove(IV) kisline in s tem povečala dietetična vrednost vina.

Velik je njen prispevek pri raziskavah tehnološke zrelosti belega in rdečega grozdja v primerjavi z različnimi vzgojnimi oblikami, obremenitvami vinske trte in vremenskimi razmerami v času dozorevanja grozdja, separiranju moštov in dodatku selekcioniranih kvasovk za relativno čisto alkoholno vrenje, tehnološkimi pogledi pri hitrejšemu zorenju vin v povezavi z vrsto posode in dodajanje kisika ter nenazadnje proučevanje zmanjšanja organskih kislin s kemijskim razkisom.

Rezultati vseh teh raziskovanj so publicirani v znanstvenih časopisih in prikazani na mednarodnih, znanstvenih in strokovnih posvetih. V vzajemni bazi podatkov COBISS.SI/COBIB.SI najdemo skupno 159 njenih bibliografskih enot.

Vsa leta službovanja na Biotehniški fakulteti je prof. dr. Venčeslava Šikovec aktivno prenašala svoje bogato strokovno znanje in izkušnje neposredno v prakso. Izpostavili bi izdelavo vinogradniških kart v okviru vinogradniške rajonizacije Slovenije. Vedno je imela posluh za reševanje in odpravljanje praktičnih tehnoloških problemov. Dodatno je redno sodelovala pri domačih in mednarodnih ocenjevanjih vin, tudi kot predsednik mednarodnega ocenjevanja vin v Ljubljani in na Kmetijskem inštitutu Slovenije (KIS).

Rezultat njenega strokovnega dela je poleg trinajstih strokovnih in poljudnih člankov tudi pet strokovnih monografij, kjer na razumljiv način približa znanost o vinu malemu vinogradniku, vinarju in ljubitelju vina. Strokovne monografije so: Malo kletarstvo (1975); Sodobno kletarjenje (1980, 1985); Za vsakogar nekaj o vinu (1984, 1987); Vinarstvo. Od grozdja do vina (1993); Vino, pijača doživetja (1996).

Njena širina se kaže v aktivnem organizacijskem delu pri organizaciji domačih in mednarodnih strokovnih kolokvijev in posvetov, od katerih bi izpostavili vsakoletne Enološke dneve, ki še vedno potekajo v Ljubljani. Bila je stalna članica jugoslovanske delegacije Mednarodne organizacije za trto in vino (O.I.V.), dolga leta predsednica Jugoslovanskega vinogradniško-vinarskega društva, predsednica Strokovnega društva vinogradnikov in vinarjev Slovenije (SDVVS), delegatka Prehrambno tehnološkega odseka pri PSVVS.

Do upokojitve 30. 12. 1990 je bila predstojnica Katedre za vinarstvo. Aktivna je bila tudi pri organizacijskem delu na Biotehniški fakulteti kot članica Sveta oddelka in študijskega odbora. Za svoje življenjsko delo je prejela številna priznanja: Jesenkovo priznanje (1985), Zlato plaketo PSVVS (1985), priznanje in plaketo Svetovnega kongresa Zveze kuharjev (1986), plaketo ob 40. letnici Biotehniške fakultete za dolgoletno delo in prispevek k razvoju fakultete. Jesenkove nagrade so najvišja priznanja za pedagoško, raziskovalno in strokovne dosežke na področju biotehniških ved v Sloveniji. Leta 1985 je prejela naziv Velika dama z viteškim križcem evropskega viteškega reda vina, kot oseba, ki je izjemno prispevala k vinogradniškim in vinarskim zadevam.

Zaslužna prof. dr. Venčeslava Šikovec, upokojena redna profesorica Biotehniške fakultete, je izjemno natančno in dosledno opravljala svoje pedagoško, mentorsko, znanstveno-raziskovalno in strokovno delo. Pomemben je njen prispevek k razvoju pedagoškega dela na Oddelku za živilstvo in Oddelku za agronomijo, znanstveno-raziskovalnega dela na Katedri za vinarstvo in nenazadnje vinarski stroki.

Vsi, ki jo poznamo ali se je še spomnimo, vemo, da je še vedno aktivna, »glasna« zagovornica sodobnih strokovnih stališč, predvsem pa visoko cenjena in nadvse spoštovana oseba v vinogradniško-vinarskih krogih.

Doktorica Šikovec je pogosto omenila svoj odnos, da sta pridelovanje grozdja in vinarstvo neke vrste poezija.

Zaposleni na Oddelku za živilstvo Biotehniške fakultete Univerze v Ljubljani ji iskreno čestitamo ob njeni 90-letnici in ji želimo obilo lepe ustekleničene poezije. Vedno je uživala vino iz majhne steklenice, a se učila iz velike.

Jubilee, Emeritus full professor Dr. Venčeslava Šikovec, university graduate agronomy engineer

Prof. Dr. Venčeslava (known by most as Slavica) Šikovec graduated in 1954 and became assistant professor

of winemaking at the Biotechnical Faculty, University of Ljubljana in the same year. She received her PhD in 1964, in 1967 she became a pre-habilitated assistant professor for the subject Wine Technology and Winemaking, then in 1972 an assistant professor for the same subject, in 1975 an associate professor, and in 1978 a full professor for the subject 'winemaking'. In 1988 she was re-elected as full professor for the subject Winemaking and Wine Technology, and in the academic year 2005/06 she was elected to Emeritus Professor. She has also worked in the field of viticulture and winemaking internationally, in Austria, Germany and Italy.

As part of her teaching work, she was teaching at the Department of Food Science and Department of Agronomy, Biotechnical Faculty, University of Ljubljana. At the undergraduate studies, she taught in the 3rd year students the course of Wine technology (obligatory subject) and in the 4th year the course of Enology (elective subject) and she was teaching the subjects Winemaking and Cellarman's trade for the students of Agronomy department. Within master study programmes she holds lectures at a postgraduate degree Master in Food Science.

Prof. Dr. Venčeslava Šikovec retired at the end of 1990 and by that time had risen many generations of students of Food Science and Agronomy at the Biotechnical Faculty. She transferred her rich scientific achievements and expertise to students as a mentor in the preparation of graduate, master's and doctoral theses during her 36 years of teaching at the Biotechnical Faculty. She was a mentor for 38 diploma's theses, a co-mentor for 10 diploma's theses, a mentor for 6 master's theses and 1 doctoral dissertation. Out of her diploma thesis, one was awarded the Prešeren Award. She had a strict but fair attitude towards the students.

For many years, she has been actively involved in the organisation of Bitenc Food Days that are still organized by the Department of Food Science as a form of postgraduate education.

The thematic sections of her research work cover the fields of microbiology and wine technology. For many years, she has studied the selection of indigenous grape microflora of the wine-growing regions in Slovenia. The most important results of this research include selections of yeast strains of the genus *Saccharomyces* for the production of still wines, sparkling wines and wines of special quality; some strains are commercially used.

In the field of wine technology, she has spent many years studying the diffusion of anthocyanin's of red wine varieties, the phenolic composition of musts and wines, the amino acid composition of grapes, the maturation of sparkling wines on yeast (wine lees), and in this connection the enrichment of sparkling wine with yeast mannoproteins. In her research, special emphasis was placed on the protection of humans and their surroundings with a view of addressing technological processes that would reduce the use of sulphuric (IV) acid and thus increase the nutritional and safety issues of wine.

Her great contribution is also related to the research of technological maturity of white and red grapes in terms of different vine growth forms, loads of vines and weather conditions during the ripening phase of grapes. With regard to processing technologies she investigated the separation of musts and the addition of selected yeasts for relatively pure alcoholic fermentation, she introduced technological perspectives for faster maturation of wines in connection to the type of container and the addition of oxygen, and last but not least the study of the reduction of organic acids by chemical deacidification.

The results of all these studies are published in scientific journals and presented at international, scientific and professional meetings. The COBISS.SI/COBIB.SI mutual database contains a total of 159 bibliographic units.

All years of employment within the Biotechnical Faculty prof. Dr. Venčeslava Šikovec was actively transferred her rich professional knowledge and experiences directly into practice. We would emphasize the production of vineyard maps within the framework of the winegrowing region of Slovenia. She has always had an ear for eliminating or solving practical technological problems. In addition, she regularly participated in domestic and international wine sensory evaluations, acting as President of the International Wine Assessment in Ljubljana and at the Agricultural Institute of Slovenia (KIS).

In addition to thirteen professional and popular articles, the results of her professional work are five professional monographs, where she presented the science of wine in an understandable way winegrowers, winemakers and wine lovers. Professional Monographs are: A Little Cellarman's Trade (1975); Contemporary Cellarman's Trade (1980, 1985); For Everyone Something About Wine (1984, 1987); Winemaking. From Grape to Wine (1993); Wine, the drink of adventure (1996).

Her breadth is reflected in active organizational work in the organization of domestic and international professional colloquiums and consultations, from which we would highlight the annual Oenological Days still held in Ljubljana. She was a permanent member of the Yugoslav delegation of the International Organization of Vine and Wine (O.I.V.), for many years the president of the Yugoslav Viticulture and Wine Society, the president of the Professional Society of Viticulturists and Winemakers of Slovenia (SDVVS), a delegate of the Food Technology Section at the PSVVS.

Until her retirement on December 30th, 1990, she was a chief of the winemaking chair. She was also active

in organizational work at the Biotechnical Faculty as a member of the Board of the Department and the Study Committee. She has received numerous awards for her life's work: the Jesenko Prize (1985), the PSVVS Gold Plaquette (1985), the World Congress of the Chefs' Federation (1986) recognition, and the Plaquette at 40th anniversary of Biotechnical Faculty for many years of work and contribution to faculty development. The Jesenko Prize is the highest recognition for educational, research and professional achievements in the field of biotechnical sciences in Slovenia. In 1985 she was named Supreme Lady with the Knight Cross of the European Knights Wine Order as a person who has made an outstanding contribution to the cause of the Vines and Wines.

As a scientist, Dr. Venčeslava Šikovec has been extremely meticulous and consistent in her teaching, mentoring, scientific research and professional work. Important is her contribution to the development of educational work at the Department of Food Science and the Department of Agronomy, Biotechnical Faculty, scientific research work at the Chair of winemaking and, last but not least, the wine profession.

All of us, who know her or still remember her, know that she is still an active, "loudly" defender of contemporary professional views, and above all, a highly appreciated, respected and exceedingly honoured person in the wine industry.

Dr. Šikovec often mentioned her attitude that grape growing and winemaking is a kind of poetry.

We, the employees from the Department of Food Science at the Biotechnical Faculty, University of Ljubljana sincerely congratulate at her 90 anniversary and wish her a lot of nice bottled poetry. She always enjoyed wine from a small bottle but learn from big one.

prof. dr. Tatjana Košmerl in prof. dr. Rajko Vidrih,
Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za Živilstvo, Katedra za tehnologije rastlinskih živil in vino

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.

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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.

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Postopek oddaje prispevka poteka v petih korakih. Priporočamo, da se avtor pred oddajo najprej seznani s postopkom in se na oddajo prispevka pripravi:

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- Izbrati je treba eno od sekcij,
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- 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.
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Submission process consists of 5 steps. Before submission, authors should go through the checklist and prepare for submission:

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