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SADRŽAJ MAGNEZIJUMA U ZEMLJIŠTU I KRMIVU U ZAVISNOSTI OD TIPA ZEMLJIŠTA I KRMNOG USEVA

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Sažetak: Cilj rada je da prikaže uticaj tipa zemljišta i krmnog useva na sadržaj magnezijuma u zemljištu i krmnim biljkama, kao i obezbeđenost krmiva magnezijumom sa aspekta prevencije bolesti „tetanije” kod životinja. Ispitivanja su izvedena na poljoprivrednim površinama černozema i humogleja, na kojima su zasnovane proizvodnje lucerke i crvene deteline. Za određivanje sadržaja Mg u biljnom materijalu, kao i ukupnog sadržaja u zemljištu primenjena je digestija uzoraka u zatvorenom mikrotalasnom sistemu pod visokim pritiskom na aparatu Milestone Ethos 1 i determinacija na aparatu ICP-OES Vista Pro-Axial Varian. Prosečan ukupan sadržaj Mg u zemljištu ispitivanih lokaliteta iznosio je 0,64%. Ukupan sadržaj Mg u černozmu je bio viši u odnosu na humoglej. Sadržaj ukupnog Mg je rastao sa dubinom zemljišta. Konstatovana je značajna pozitivna korelaciona veza između ukupnog sadržaja Mg u zemljištu i pH vrednosti zemljišta, kao i sadržaja CaCO₃. Prosečna vrednost sadržaja Mg u ispitivanim krmnim usevima iznosila je 0,28%. Viši sadržaj Mg u suvoj materiji biljaka je konstatovan na humogleju. Crvena detelina je, u proseku, imala značajno veći sadržaj Mg. Sa aspekta obezbeđenosti biljaka magnezijumom, može se smatrati da ne postoji potencijalna opasnost od pojave bolesti „tetanije” kod životinja, jer je odnos K/(Ca+Mg) ispod 2,2, a sadržaj K i Mg u suvoj materiji biljaka izvan kritičnih vrednosti.

Cljučne reči: lucerka, crvena detelina, černozem, humoglej.

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Uvod

Magnezijum se u zemljištu pojavljuje u obliku silikata, karbonata, nitrata, sulfata i bikarbonata, kao i adsorbovan na površinu adsorptivnog kompleksa. U litosferi ukupna količina varira u intervalu 0,50–3,50 %, a u pedosferi 0,10–1,50 % (Džamić i Stevanović, 2000). Zemljišta povoljnijih fizičko-hemijskih osobina odlikuju se većim sadržajem ovog elementa (Popović, 1989), dok su peskovita i kisela zemljišta deficitarna u magnezijumu jer na ovim zemljištima dolazi do njegovog ispiranja. Prema Popoviću (1989) ukupan sadržaj magnezijuma u sloju 0–20 cm kreće se u zavisnosti od tipa zemljišta: gajnjača 1,31%, černozem karbonatni 1,45%, černozem beskarbonatni 1,26%, degradirani černozem 1,13%, smonica 1,41% i aluvijum karbonatni 2,44%.

Pripada grupi neophodnih i konstitucionih elemenata. Biljke usvajaju magnezijum u obliku Mg^{2+} jona. Ulazi u sastav hlorofila, gde je sa azotom vezan u porfirinskom jezgru, te učestvuje u izgradnji ćelijskih membrana. Najveće zahteve za magnezijumom imaju leguminoze, koje imaju veliku asimilacionu površinu i intenzitet fotosinteze, te ga zahtevaju u većim količinama za biosintezu hlorofila. Ima važnu ulogu u fiksaciji molekularnog azota, jer se pretpostavlja da stvara vezu između gvožđe-protein kompleksa i ATP-a. Prekomernom upotrebom kalijumovih đubriva ili u prisustvu većih količina fosfora, sumpora, kalcijuma i molibdena moguće je smanjenje koncentracije magnezijuma u biljkama (Tesar, 1981).

Nedostatak Mg može kod stoke da izazove bolest „tetaniju” (hipomagneziju). Za manifestaciju ove bolesti važan je odnos $K/(Ca+Mg)$ u krmivu. Kada je količina Mg ispod 0,2%, a K više od 2,5% i odnos $K/(Ca+Mg)$ veći od 2,2, može doći do pojave ove bolesti (Gross, 1973).

Medicago sativa L. (lucerka) je višegodišnja leguminoza, koja se smatra vodećom i najvažnijom krmnom kulturom za proizvodnju kvalitetne stočne hrane, a koristi se u svežem i konzervisanom stanju kao seno, senaža, silaža, brašno, peleti i pasta (Jakšić et al., 2013).

Trifolium pratense L. (crvena detelina) u našoj zemlji je druga po važnosti višegodišnja krmna leguminoza i za razliku od lucerke bolje podnosi kiselija zemljišta u brdsko-planinskim područjima, a za ishranu stoke koristi se kao zelena krma, putem ispaše (sama ili u smeši sa travama), te u konzervisanom stanju kao seno, silaža i dehidrirana u brašno (Jakšić et al., 2013).

Cilj rada je ispitivanje uticaja tipa zemljišta i krmnog useva na sadržaj magnezijuma u zemljištu i krmnim biljkama, kao i obezbeđenost krmiva magnezijumom sa aspekta prevencije bolesti „tetanije” kod životinja.

Materijal i metode

Ispitivanja su izvedena na tipu zemljišta černoze, podtipu na lesu i lesolikim sedimentima, varijetet karbonatni u Novom Sadu i tipu zemljišta humoglej, podtip karbonatni u Hetinu (klasifikacija Škorić et al., 1985). Na ispitivanim površinama je zasnovana proizvodnja lucerke i crvene deteline.

Uzorkovanje zemljišta i biljnog materijala je izvršeno tokom maja 2011. godine, u drugoj proizvodnoj godini lucerke i crvene deteline. Uzorci zemljišta su uzeti u narušenom stanju, agrohemijskom sondom sa dve dubine: 0–30 cm i 30–60 cm. Uzorci su uzeti sa sedam lokaliteta (ponavljanja). Jedan reprezentativan uzorak zemljišta sastojao se od 15 do 25 pojedinačnih uzoraka. Prikupljeni uzorci zemljišta su vazdušno sušeni i samleveni mlinom za zemljište do veličine čestica < 2 mm prema SRPS/ISO 11464:2004.

Uzorci biljnog materijala za hemijske analize su uzeti neposredno nakon kosidbe iz pokošene mase. Krma se skidala ručno, u početku cvetanja, sečenjem biljaka na visini od 5 cm. Uzorci biljnog materijala su vazdušno osušeni i samleveni mlinom za biljni materijal.

Za određivanje sadržaja Mg, K i Ca u biljnom materijalu, kao i ukupnog sadržaja u zemljištu primenjena je digestija uzoraka u zatvorenom mikrotalasnom sistemu pod visokim pritiskom sa postepenim grejanjem do 180°C na aparatu Milestone Ethos 1. Digestija uzorka je urađena sa koncentrovanom HNO₃ i H₂O₂ (5 HNO₃ : 1 H₂O₂). Odnos uzorka i rastvora bio je 1:12 za uzorke zemljišta i 1:24 za uzorke biljnog materijala. Određivanje koncentracije Mg u razorenim uzorcima je vršeno na aparatu ICP-OES Vista Pro-Axial Varian.

Sadržaj humusa u zemljištu određen je metodom po Tjurinu (JDZP, 1966). Sadržaj kalcijum-karbonata u zemljištu je određen volumetrijskom metodom ISO 10693:1995. Određivanje pH vrednosti zemljišta je izvršeno potenciometrijskom metodom ISO 10390: 2005.

Sadržaj ispitivanih elemenata je izražen na vazdušno suvi uzorak.

Podaci dobijeni u ispitivanju obrađeni su metodama deskriptivne statistike. Ispitivanje značajnosti razlika između tretmana je izvršeno analizom varijanse (ANOVA), a povezanosti pojava korelaciono-regresionom analizom. Značajnost razlika je testirana Fišerovim testom najmanje značajne razlike (engl. *Fisher's LSD test*).

Statistička obrada podataka je urađena upotrebom statističkog programa STATISTICA 12.6 (StatSoft, Inc. Corporation, Tulsa, OK, USA).

Rezultati i diskusija

Černoze pripada redu automorfni, te klasi humusno akumulativnih zemljišta, s građom profila Amo-AC-C. Razvija se u semiaridnom stepskom

području, na karbonatnim ilovastim, ređe peskovitim rastresitim supstratima (Miljković, 1996). Tamnosmeđe je boje, sa izraženom zrnastom strukturom i karakterističnim krotovinama i pseudomicelijama. Karbonati se javljaju od površine ili u prelaznom horizontu. Osnovne hemijske osobine zemljišta ispitivanih lokaliteta prikazane su u tabeli 1. Prema pH vrednosti, određenoj u suspenziji sa 1M KCl, zemljišta černozema pripadaju klasi neutralnih i slabo alkalnih zemljišta. Sadržaj CaCO_3 je bio veoma različit te su zemljišta ovih lokaliteta bila slabo do jako karbonatna. Veći sadržaj CaCO_3 je u dubljem sloju, te je i pH vrednost dubljeg sloja viša. Prema sadržaju humusa zemljišta su slabo humozna i humozna. Sadržaj humusa opada sa dubinom zemljišta. Dozet (2010) je u ispitivanju černozema Srema zabeležila sadržaj CaCO_3 od 0% do 17,7%, sadržaj humusa od 1,36% do 5,18% i pH vrednost u intervalu od 5,61 do 7,49.

Tabela 1. Osnovna hemijska svojstva zemljišta ispitivanih lokaliteta.

Table 1. Basic soil chemical properties at the tested sites.

Tip zemljišta/ Soil type	Dubina/Depth (cm)	pH-KCl	pH-H ₂ O	CaCO ₃ (%)	Humus (%)
Černozem	0-30 ($\bar{x}\pm\text{sd}$) (min-max)	7,38 $\pm$ 0,24 (6,92-7,69)	8,11 $\pm$ 0,18 (7,66-8,31)	5,47 $\pm$ 0,34 (0,34-16,42)	2,81 $\pm$ 0,44 (2,19-3,40)
	30-60 ($\bar{x}\pm\text{sd}$) (min-max)	7,57 $\pm$ 0,21 (6,94-7,80)	8,23 $\pm$ 0,16 (7,74-8,41)	13,29 $\pm$ 9,26 (0,17-29,90)	2,15 $\pm$ 0,50 (1,15-2,85)
Humoglej	0-30 ($\bar{x}\pm\text{sd}$) (min-max)	6,43 $\pm$ 0,74 (5,16-7,35)	7,30 $\pm$ 0,67 (6,22-8,15)	0,91 $\pm$ 1,24 (0,08-4,21)	3,59 $\pm$ 0,45 (3,07-4,93)
	30-60 ($\bar{x}\pm\text{sd}$) (min-max)	7,20 $\pm$ 0,60 (5,76-7,69)	8,04 $\pm$ 0,47 (6,94-8,39)	11,42 $\pm$ 11,70 (0,08-37,50)	2,08 $\pm$ 0,78 (1,23-3,65)

Humoglej pripada redu hidromorfni, te klasi glejnih zemljišta sa građom profila Amo,a-Gso. Molični horizont je dublji od 50 cm, sa znacima hidromorfizma. Matični supstrat čine razni sedimenti, transportovani rekama, tako da se mogu obrazovati na fluvijativnim nanosima uglavnom težeg mehaničkog sastava, pretaloženom lesu i eolskom pesku. Prema pH vrednosti zemljišta, ispitivani lokaliteti humogleja pripadaju kiselom, slabo kiselom, neutralnom i slabo alkalnom zemljištu. Prema sadržaju CaCO_3 , zemljišta su bila slabo do jako karbonatna. Veći sadržaj CaCO_3 je u dubljem sloju, te je i pH vrednost dubljeg sloja viša. Prema sadržaju humusa, lokaliteti su bili slabo humozni do humozni. Sadržaj humusa opada sa dubinom zemljišta. Belić et al. (2011) su u ispitivanju humogleja Banata, koje je obuhvatilo i zemljište Hetina, takođe zabeležili velik interval sadržaja CaCO_3 od 0% do 38,3%, sadržaj humusa je bio od 1,5% do 5,23%, dok je pH vrednost bila u intervalu od 5,57 do 8,75.

Ukupan sadržaj magnezijuma u zemljištu kreće se u velikom intervalu od 0,05 do 0,5% (Mengel i Kirby, 2001; Maguire i Cowan, 2002). Donja granica je svojstvena peskovitim, a gornja glinovitim zemljištima (Kastori et al., 2016). Džamić i Stevanović (2000) navode da se ukupan sadržaj Mg u zemljištu kreće od 0,1 do 1,5%, a Vukadinović i Vukadinović (2011) od 0,1 do 1,0%, dok u karbonatnim zemljištima te vrednosti mogu biti i znatno više. Prosečan ukupan sadržaj magnezijuma u zemljištu ispitivanih lokaliteta iznosio je 0,64% (tabela 2). Prema Popoviću (1989), ukupan sadržaj magnezijuma u černozeu kreće se oko 1,45%. Bođa (2001) je u ispitivanju sadržaja Mg u različitim tipovima zemljišta Vojvodine konstatovao sledeći sadržaj: černozeu 0,22–0,40%, humoglej 0,08–0,13%, gajnjača 0,10–0,28% i solončak 0,08–0,25%.

Tabela 2. Ukupan sadržaj Mg u zemljištu ispitivanih lokaliteta černozeu i humogleja pod lucerkom i crvenom detelinom (%).

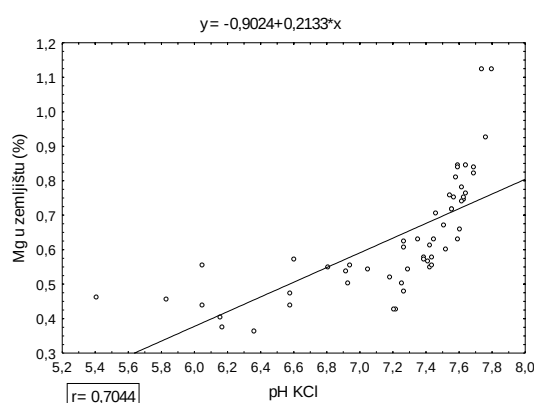
Table 2. Total Mg content in chernozem and humogley soil of the tested sites under alfalfa and red clover (%).

Tip zemljišta <i>Soil type</i>	Dubina/Depth (cm)	Krmni usev/ <i>Forage crop</i>		Prosek <i>Average</i>
		Lucerka <i>Alfalfa</i>	Crvena detelina <i>Red clover</i>	
Černozeu	0–30	0,69	0,55	0,62
	30–60	0,83	0,72	0,78
	Prosek	0,76 ^b	0,64 ^a	0,70 ^b
Humoglej	0–30	0,43	0,51	0,47
	30–60	0,69	0,66	0,68
	Prosek	0,56 ^a	0,59 ^a	0,58 ^a
Prosek <i>Average</i>	0–30	0,56	0,53	0,55 ^a
	30–60	0,76	0,69	0,73 ^b
		0,66 ^A	0,61 ^A	0,64

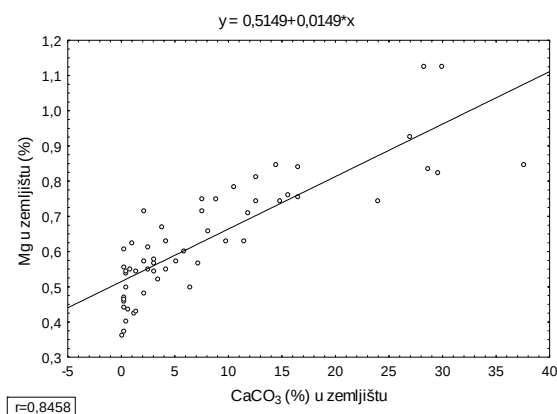
Tretmani označeni istim slovima nemaju statistički signifikantne razlike (Fišerov test na nivou značajnosti 0,05).

Značajne razlike u sadržaju ukupnog Mg konstatovane su između černozeu i humogleja. Ukupan sadržaj Mg u černozeu je bio za 0,12% viši u odnosu na humoglej. Viši sadržaj Mg u černozeu u odnosu na humoglej, u proseku za 0,23%, zabeležio je i Bođa (2011). Konstatovane su signifikantne razlike u sadržaju ukupnog Mg između površinskog i dubljeg sloja zemljišta, što je u skladu sa rezultatima drugih autora, te mineraloškim sastavom lesa koji je bogat karbonatima i predstavlja najčešći matični supstrat za zemljišta Vojvodine. U ispitivanju Popovića (1985) sadržaj ukupnog Mg je rastao sa dubinom u većini ispitivanih tipova zemljišta.

Kisela zemljišta često su siromašna magnezijumom usled konkurencije sa H^+ jonima, te dolazi do njegovog ispiranja (Kastori et al., 2016; Jakšić, 2014). Zbog toga se dešava da je obezbeđenost biljaka magnezijumom na kiselim zemljištima nedovoljna. U ovom ispitivanju konstatovana je značajna pozitivna korelaciona veza između sadržaja Mg i pH vrednosti zemljišta (slika 1). Povećanjem kiselosti smanjuje se sadržaj Mg u zemljištu. Takođe, sadržaj Mg u zemljištu je u značajnoj pozitivnoj korelaciji sa sadržajem $CaCO_3$ (slika 2), što je u skladu sa istraživanjima drugih autora da karbonatna zemljišta imaju veće količine magnezijuma (Vukadinović i Vukadinović, 2011).



Slika 1. Zavisnost sadržaja Mg u zemljištu (%) i pH vrednosti zemljišta (0–60cm), $p < 0.05$.
Figure 1. Dependence of Mg content in soil (%) and soil pH in the 0–60-cm soil layer, $p < 0.05$.



Slika 2. Zavisnost sadržaja Mg i $CaCO_3$ u zemljištu (%), u sloju zemljišta 0–60cm, $p < 0.05$.
Figure 2. Dependence of Mg and $CaCO_3$ contents in soil (%), in the 0–60-cm soil layer, $p < 0.05$.

Prosečna vrednost sadržaja magnezijuma u ispitivanim krmnim usevima iznosila je 0,28% (tabela 3). Sadržaj magnezijuma u biljkama varira od 0,08% do 0,30%, a prema istraživanjima optimalnih koncentracija magnezijuma u stočnoj hrani sa stanovišta kvaliteta, potrebno je najmanje 0,2% Mg u suvoj materiji lucerke (Werk i Schlf, 1971). Lucerka u uslovima adekvatne mineralne ishrane sadrži najmanje 0,30% Mg (Koenig et al., 1999). S obzirom na to da leguminoze imaju najveće zahteve za magnezijumom jer poseduju veliku asimilacionu površinu i izražen intenzitet fotosinteze u odnosu na druge kulture i njegov sadržaj u suvoj materiji lucerke i crvene deteline je veći.

Tabela 3. Sadržaj Mg u suvoj materiji lucerke i crvene deteline (%).

Table 3. Mg content in alfalfa and red clover dry matter (%).

Tip zemljišta <i>Soil type</i>	Lucerka <i>Alfalfa</i>	Crvena detelina <i>Red clover</i>	Prosek <i>Average</i>
Černozem ($\bar{x} \pm sd$) (min–max)	0,23 $\pm$ 0,03 <i>a</i> (0,21–0,28)	0,28 $\pm$ 0,02 <i>b</i> (0,25–0,32)	0,26 <i>a</i>
Humoglej ($\bar{x} \pm sd$) (min–max)	0,27 $\pm$ 0,03 <i>b</i> (0,24–0,31)	0,33 $\pm$ 0,02 <i>c</i> (0,31–0,37)	0,30 <i>b</i>
Prosek/ <i>Average</i>	0,25 <i>A</i>	0,31 <i>B</i>	0,28

Tretmani označeni istim slovima nemaju statistički signifikantne razlike (Fišerov test na nivou značajnosti 0,05).

U ovom ispitivanju crvena detelina je imala veći sadržaj Mg u suvoj materiji u odnosu na lucerku. Prema Lukiću (2000), sadržaj Mg u lucerki varira od 0,08% do 0,30%, dok je prema rezultatima Caddela et al. (2004), prosečan sadržaj u senu iznosio 0,40%. Prema istraživanju Markovića et al. (2009), sadržaj Mg u biljkama lucerke je iznosio 0,68%. U ispitivanju hemijskog sastava lucerke, uzetih sa 12 različitih lokacija u Vojvodini, Živkov-Baloš et al. (2011) su utvrdili sadržaj magnezijuma od 0,26%. Sadržaj Mg u suvoj materiji crvene deteline kreće se u intervalu od 0,24% do 0,81% (Vučković, 2004). U istraživanjima koja su sprovedi Ignjatović et al. (2001), prosečan sadržaj Mg u fazi cvetanja prvog otkosa crvene deteline iznosio je 0,51%. Kastori et al. (2016) navode da crvena detelina sa 1 t prinosa iznosi 5,2 kg Mg, a lucerka senom iznosi 2,7 kg Mg. U ispitivanjima sadržaja MgO u senu lucerke iznete su količine od oko 30 kg/ha (Savić i Jekić, 1975).

Uticaj tipa zemljišta na koncentraciju magnezijuma u krmnim usevima bio je veoma značajan, jer je viši sadržaj Mg u suvoj materiji biljaka konstatovan na humogleju u odnosu na rezultate sa černozema.

Nedostatak Mg u krmivu može kod stoke da izazove bolest „tetaniju” (hipomagneziju). Za manifestaciju ove bolesti važan je odnos K/(Ca+Mg). Kada je količina Mg ispod 0,2%, a K viša od 2,5% i odnos K/(Ca+Mg) veći od 2,2, može

doći do pojave ove bolesti (Gross, 1973). Nedovoljan sadržaj Mg u biljkama može biti posledica primene visokih doza kalijumovih đubriva. U ispitivanim uzorcima lucerke i crvene deteline pomenuti odnos je ispod 2,2 (tabela 4), a sadržaj K i Mg u suvoj materiji biljaka izvan kritičnih vrednosti, tako da se može smatrati da ne postoji potencijalna opasnost od pojave „tetanije”.

Tabela 4. Sadržaj kalcijuma i kalijuma u suvoj materiji lucerke i crvene deteline (%).
Table 4. Ca and K contents in alfalfa and red clover dry matter (%).

Tip zemljišta <i>Soil type</i>	Krmni usev <i>Forage crop</i>	K (%)	Ca (%)	K/(Mg+Ca)
Černozem	Lucerka/ <i>Alfalfa</i>	1,61	1,47	0,95
	Crvena detelina/ <i>Red clover</i>	1,79	1,26	1,16
Humoglej	Lucerka/ <i>Alfalfa</i>	1,69	1,16	1,18
	Crvena detelina/ <i>Red clover</i>	1,75	1,18	1,15

Zaključak

Prosečan ukupan sadržaj Mg u zemljištu ispitivanih lokaliteta iznosio je 0,64%. Ukupan sadržaj Mg u černozmu je bio viši u odnosu na humoglej. Sadržaj ukupnog Mg je rastao sa dubinom zemljišta.

Konstatovana je značajna pozitivna korelaciona veza između ukupnog sadržaja Mg u zemljištu i pH vrednosti zemljišta, kao i sadržaja CaCO_3 .

Prosečna vrednost sadržaja Mg u ispitivanim krmnim usevima iznosila je 0,28%. Viši sadržaj Mg u suvoj materiji biljaka je konstatovan na humogleju u odnosu na černozem. Crvena detelina je, u proseku, imala značajno veći sadržaj Mg u odnosu na lucerku.

Sa aspekta obezbeđenosti biljaka magnezijumom, može se smatrati da ne postoji potencijalna opasnost od pojave bolesti „tetanije” kod životinja, jer je odnos $\text{K}/(\text{Ca}+\text{Mg})$ ispod 2,2, a sadržaj K i Mg u suvoj materiji biljaka izvan kritičnih vrednosti.

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Literatura

- Belić, M., Nešić, L., Ćirić, V., Vasin, J., Milošev, D., & Šeremešić, S. (2011). Characteristics and classification of gleyic soils of Banat. *Ratarstvo i povrtarstvo*, 48, 375-382.

- Boda, V. (2001). *Dinamika magnezijuma u zemljištima Vojvodine*. Poljoprivredni fakultet, Univerzitet u Novom Sadu.
- Caddel, J.L., Zhang, H., & Wise, K. (2004). Responses of alfalfa, red clover, and white clover to soil pH and lime treatments, Preuzeto sa <http://www.plantmanagementnetwork.org/pub/fg/research/2004/treat/Forage and Grazinglands>.
- Dozet, D. (2010). *Sadržaj nikla u zemljištima Srema*. Poljoprivredni fakultet, Univerzitet u Novom Sadu.
- Džamić, R., & Stevanović, D. (2000). *Agrohemija*. Beograd: Pertenon.
- Gross, C.F. (1973). *Ann. Meeting Soil Conserv. Soc. Amer.*, Hot Springs, Ark.
- Ignjatović, S., Vučetić, J., Lugić, Z., & Dinić, B. (2001). Uticaj faze razvića na sadržaj makro i mikroelemenata u crvenoj i beloj detelini. *Agricultural Science Research Journal*, 62 (220), 309-316.
- ISO 10390 (2005). *Soil quality - Determination of pH*.
- ISO 10693 (1995). *Soil quality - Determination of carbonate content - Volumetric method*.
- Jakšić, S.P., Vučković, S.M., Vasiljević, S., Grahovac, N., Popović, V., Šunjka, D.B., & Dozet, G.K. (2013). Akumulacija teških metala u *Medicago sativa* L. i *Trifolium pratense* L. na kontaminiranom fluvisolu. *Hemijska industrija*, 67 (1), 95-101.
- Jakšić, S. (2014). *Uticaj krmnog useva, tipa i plodnosti zemljišta na produktivnost i hemijski sastav kabaste stočne hrane*. Poljoprivredni fakultet, Univerzitet u Beogradu.
- JDPZ (1966). *Priručnik za ispitivanje zemljišta. Knjiga I. Hemijske metode ispitivanja zemljišta*. Beograd.
- Kastori, R., Maksimović, I., Ilin, Ž., & Putnik-Delić, M. (2016). *Magnezijum u ishrani biljaka*. Poljoprivredni fakultet, Univerzitet u Novom Sadu.
- Koenig, R., Kitchen, B., Hurst, C., & Barnhill, J. (1999). Phosphorus and potassium fertilization of irrigated alfalfa in Utah. U: *Western Nutrient Management Conference*, Salt Lake City, Utah, 57-62.
- Lukić, D. (2000). *Lucerka*. Naučni institut za ratarstvo i povrtarstvo. Novi Sad.
- Maguire, M.E., & Cowan, J.A. (2002). Magnesium chemistry and biochemistry. *Bio Metals*, 15, 203-210.
- Marković, J., Štrbanović, R., Cvetković, M., Anđelković, B., & Živković, B. (2009). Uticaj faze razvića na koncentraciju mineralnih elemenata u listu, stablu i celoj biljci lucerke (*Medicago sativa* L.). *Biotechnology in Animal Husbandry*, 25 (5-6), 1225-1231.
- Mengel, K., & Kirkby, E.A. (2001). *Principles of plant nutrition*. Dordrecht: Kluwer Academic Publishers.
- Miljković, N. (1996). *Osnovi pedologije*. Prirodno-matematički fakultet, Institut za geografiju, Univerzitet u Novom Sadu. Novi Sad.
- Popović, Ž. (1985). *Agrohemija i fertilizacija*. Poljoprivredni fakultet, Univerzitet u Beogradu. Beograd.
- Popović, Ž. (1989). *Agrohemija*. Naučna knjiga. Beograd.
- Savić, B., & Jekić, M. (1975). *Agrohemija*. Svjetlost. Sarajevo.
- SRPS ISO 11464 (2004). *Kvalitet zemljišta - Prethodna obrada uzoraka za fizičko-hemijske analize*. StatSoft, Inc. Corporation. *STATISTICA 12.6*. Tulsa, OK, USA.
- Škorić, A., Filipovski, G., & Ćirić, M. (1985). *Klasifikacija zemljišta Jugoslavije*. Akademija nauka i umjetnosti Bosne i Hercegovine. Sarajevo.
- Tesar, M.B. (1981). High yield alfalfa research. U: *Weed, Seed and Fertilizer Conference*. Michigan State University, Lansing, 1-4.
- Vučković, S. (2004). *Travnjaci*. Poljoprivredni fakultet, Univerzitet u Beogradu. Beograd.
- Vukadinović, V., & Vukadinović, V. (2011). *Ishrana bilja*. Poljoprivredni fakultet, Sveučilište Josipa Jurja Strossmayera. Osijek.
- Werk, O., & Schifer, P. (1971). Untersuchungen zum Mg- und Na-Gehalt von Weidefutter. *Mitteilungen der DLG*, 14, 368-372.
- Živkov-Baloš, M., Mihaljev, Ž., & Čupić, Ž. (2011). Content of trace elements and some radionuclides in lucerne (*Medicago sativa* L.). *Biotechnology in Animal Husbandry*, 27 (3), 591-598.

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MAGNESIUM CONTENT IN SOIL AND ROUGHAGES DEPENDING ON SOIL TYPE AND FORAGE CROPS

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A b s t r a c t

The aim of this study is to examine the effects of soil type and forage crops on the content of magnesium in soil and roughages, as well as forage quality regarding Mg content for the prevention of animal disease 'tetany'. The trial was carried out on chernozem and humogley under alfalfa and red clover. Samples for determination of Mg content in plant and total Mg content in soil were digested using the apparatus Milestone Ethos 1. The content of Mg was determined using the apparatus ICP-OES Vista Pro-Axial Varian. The average total Mg content in soil of the tested sites was 0.64%. Total Mg content in chernozem was higher than in humogley. A higher total Mg content was in the deeper horizon. A significant positive correlation was found between total Mg content in soil and soil pH as well as the content of CaCO₃. The average Mg content in forage crops was 0.28%. A higher Mg content in the dry matter was observed in crops grown on humogley. Mg content was significantly higher in red clover. There was no risk for the occurrence of animal disease 'tetany' regarding Mg content in crops, because the ratio K/(Ca+Mg) was below 2.2, and K and Mg content in the dry matter of crops was below the critical value.

Key words: alfalfa, red clover, chernozem, humogley.

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GENETIC DIVERGENCE OF NIGERIAN AND INDIAN PEARL
MILLET ACCESSIONS BASED ON AGRONOMICAL AND
MORPHOLOGICAL TRAITS

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Abstract: The study assessed the genetic diversity of pearl millet accessions grown in Nigeria and India based on morpho-agronomic traits in order to identify genotypes with superior characters which could be utilized in breeding programmes. Twenty-four pearl millet accessions were grown and evaluated for agronomic and morphological traits during the dry and wet seasons of 2015–2016. Data collected on the accessions using standard descriptors were analysed statistically. IP22281 had the highest mean plant height (108.90 cm) while NGB00531 recorded the lowest (61.02 cm). Significant intra-specific variation existed in number of leaves per plant, leaf length, leaf width, number of nodes and internode length, however, stem girth was similar for the accessions. Tillering was generally poor with the highest value (1.60 tillers per plant) found in NGB00531. A significant positive correlation occurred between plant height, number of leaves, leaf length and leaf width. Panicles emerged between 44 and 56 days and NGB00548 had the shortest maturity time. Also, panicle length and peduncle diameter varied significantly for the accessions. The highest grain yield and 1000-grain weight were recorded in NGB00616 and the lowest yield and weight were recorded in IP22269. The principal component analysis grouped the accessions into four clusters, comprising mixtures of Nigerian and Indian members. Similarly, the dendrogram grouped the accessions into two main groups which were sub-divided into smaller clusters with accessions from Nigeria and India in the same cluster. The study concludes that variations in morpho-agronomic and yield characters among the accessions studied could be harnessed for crop improvement. The clustering pattern of these accessions indicated their genetic relatedness, possibly from the same progenitor, but separation by geographical or ecological isolation mechanisms.

Key words: genetic diversity, grain yield and yield components, morphological variation, pearl millet.

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Introduction

Genetic variability is the prerequisite of plant evolution and crop improvement. Analysis of genetic variability in crop species is an important component of crop improvement programmes, as it provides information about characters that can be useful for various breeding purposes (Animasaun et al., 2015). In the wake of climatic change with its impending danger of food scarcity and insecurity, lives of nearly a billion poor people in dryland areas of Africa and Asia depend on cereals that can be grown in drought and water-stressed environments. The poor rural people whose main occupation is farming still await their salvaging crops whose genetics is well understood to develop hybrids of improved yield and stable crops for such conditions (CGIAR, 2012).

Pearl millet (*Pennisetum glaucum* L. R. Br), also known as *P. americanum* and sometimes called bulrush or cattail millet, is the most important cereal of the genus *Pennisetum*. This crop plays a significant role in tropical agriculture. Pearl millet can be grown in a vast range of environmental conditions including those characterized by drought and poor soil (FAO and ICRISAT, 1996). It is grown on approximately 40 million hectares in the arid and semi-arid regions of Asia and Africa where it serves as a staple food and plays a vital role in food security (Rai et al., 2009). Even, under abiotic stress conditions, pearl millet would comparatively produce higher grain yield than sorghum, which is another dryland crop (Hash et al., 2003; Khairwal et al., 2007). In the West African Sahel region and the Indian subcontinent, pearl millet is grown almost exclusively to feed millions of people who live in agroclimatic zones with severe stress limitations to crop production due to heat and inadequate rainfall. Furthermore, pearl millet has been suggested as a new feed grain crop for the south-eastern and mid-southern regions of the United States with acidic soil, low fertility and drought (Kennedy et al., 2002; Davis et al., 2003). In addition, it has a relatively short growing season (60–90 days) that allows double cropping after leguminous crops have been harvested (FAO and ICRISAT, 1996).

Pearl millet is an excellent forage crop with low hydrocyanic acid content and considerable amounts of protein, calcium, phosphorous and other minerals. The grain contains higher protein than wheat or rice (Poncet et al., 1998) and more essential amino acids than corn (Adeola et al., 1994; Amato and Forrester, 1995). In addition, pearl millet contains more oil than other cereals (Hill and Hanna, 1990; Sullivan et al., 1990). Feeding tests conducted on cattle, swine, and chicken have shown that pearl millet is at par with maize and superior to sorghum in feed formulations. Hence, pearl millet is used as a feed ingredient for broilers (Davis et al., 2003), laying hens (Kumar et al., 1991; Collins et al., 1997), ducks (Adeola et al., 1994), and cattle (Hill and Hanna, 1990). Also, it has been demonstrated that pearl millet is suitable for ethanol production and the plant of choice for biofuel in

areas where other crop production is not economical for resource-limited farmers (Gulia et al., 2007; Dowling et al., 2013).

In Nigeria, in spite of the considerable economic importance of pearl millet, it has received relatively little research attention and is still regarded as an ‘orphan’ crop. Therefore, there is a need for a better understanding of the genetic diversity of this crop in relation to accessions available in other regions, in particular, India. The gene pool in cultivated pearl millet contains an enormous range of genetic variability with no incompatibility and few linkage problems (Wilson et al., 2004). The genetic relationship among genotypes of the pearl millet cultivated in Nigeria and India has not been well investigated. The understanding of the crop genetics and variability among the available germplasm could open a window of greater potentials for the sustainable utilization for fodder and grain production in the present erratic climatic changes. Intra-specific genetic diversity studies and morphological character correlation in pearl millet germplasm could offer possibilities for the improvement of the crop and its hybrids. Thus, the success of a breeding programme for pearl millet would require adequate information on variations in growth and reproductive traits of the available accessions which could be explored by the breeders. Therefore, the present work characterized pearl millet accessions from Nigeria and India based on morpho-agronomic traits with a view to assess the genetic diversity among the accessions and to provide information that would enhance crop improvement.

Materials and Methods

Plant material

A total of twenty-four accessions of pearl millet were used for the investigation. The seeds of 10 accessions were collected from the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Oyo State, Nigeria and another 10 from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. In addition, 4 unclassified accessions were obtained from the farmer’s field at Bardoli, Surat District, Gujarat State of India. The names of the accessions and source of collection are presented in Table 1.

Agro-ecological description of the experimental location

The morpho-agronomic characterization of twenty-four accessions of pearl millet was conducted in the screenhouse facility at C. G. Bhakta Institute of Biotechnology (CGBIBT), Uka Tarsadia University (UTU), Bardoli, Gujarat State, India in the period 2013–2015. UTU is located at latitude 21.04°N, longitude

073.03°E and at an elevation of 39 feet above mean sea level. The climate of Gujarat varies from tropical monsoon in the south to temperate in the north with annual average maximum temperatures of 33°C and minimum of 22°C. The annual average rainfall is about 1117.6 mm with the mean precipitation of 66 days. The annual maximum and minimum average relative humidity values are 77% and 54% respectively. The estimated length of daylight is 12.5 h and the wind speed is 4 mph (Indian Metrological Society, 2015).

Table 1. Accession name and source of pearl millet used for the study.

SN	Accession	[†] Collection source
1	IP3616	ICRISAT
2	IP3495	ICRISAT
3	NGB00463	NACGRAB
4	BALKUVE	GUJARAT*
5	BAJARA	GUJARAT*
6	NGB00616	NACGRAB
7	IP4133	ICRISAT
8	NGB01263	NACGRAB
9	IP22281	ICRISAT
10	IP12556	ICRISAT
11	NGB00458	NACGRAB
12	NGB00531	NACGRAB
13	IP22271	ICRISAT
14	NGB00476	NACGRAB
15	IP22268	ICRISAT
16	TAYABI	GUJARAT*
17	JALGONE	GUJARAT*
18	NGB00551	NACGRAB
19	IP17862	ICRISAT
20	IP3122	ICRISAT
21	NGB00528	NACGRAB
22	IP22269	ICRISAT
23	NGB00528	NACGRAB
24	NGB00537	NACGRAB

[†]NACGRAB: National Centre for Genetic Resources and Biotechnology, Ibadan, Nigeria.

[†]ICRISAT: International Crops Research Institute for the Semi-Arid Tropics, India.

*Accession obtained from the farmer's field within the state of Gujarat, India.

Morpho-agronomic traits

Dry seeds of each pearl millet accessions were grown in pots filled with soil (clay, sand and topsoil in the ratio of 3:1:1) for morphological characters and

genetic diversity assessment. The experiment was carried out in the randomized complete block design (RCBD) with five replicates. Watering was done every four days with automated overhead fogging irrigation facility, and humidity was regulated by an auto-humidifier system, while weeding was carried out by hand-picking throughout the period of evaluation. After seedling emergence, plants were thinned and a plant was chosen and tagged from a pot for observation and data collection. Data were collected on morpho-agronomic characters such as plant height, number of leaves per plant, leaf length, leaf width, number of nodes, internode length, stem girth, number of tillers per plant, and number of leaves per tiller. Other characters considered were: days to panicle emergence, panicle length and 1000-grain weight based on the crop descriptor index (IBPGR and ICRISAT, 1993). The growth and grain yield related characters were considered at maturity for each accession.

Statistical analysis

Data collected on quantitative growth and reproductive traits (days to panicle emergence, panicle size, and grain weight) of the accessions were subjected to analysis of variance (ANOVA), and agronomic trait correlations were determined using the Statistical Package for the Social Sciences (SPSS) version 17. The mean values of the analysed traits were separated by Duncan's multiple range test (DMRT). The intervariety divergence was calculated using the unweighted pair-group method of arithmetic averages (UPGMA) to evaluate the degree of genetic diversity in the accessions.

Results and Discussion

The mean values and standard errors of a mean between qualitative and quantitative variations were observed in vegetative and reproductive parameters. A comparison of the mean for the morpho-agronomic traits evaluated at maturity in two years (pooled data) showed significant differences (Table 2). Plant height varied significantly across the accessions. IP22281 was the tallest accession (108.90 cm), closely followed by IP22269 (104.10 cm) while NGB00531 had the lowest plant height (67.46 cm) (Table 2). The results obtained here were similar to those documented by Al-Suhaibani (2011). The difference in plant height among the accessions could be due to a combination of genetic variability and environmental effects. The mean number of leaves per plant also varied significantly and ranged from 7.00 to 10.90 (Table 2), and the highest number of leaves per plant (10.90) occurred in accession IP3616. Expectedly, NGB00531 which was the shortest accession also had the lowest number of leaves per plant. Leaf length differed statistically for the accessions – the shortest leaf (21.1 cm) was

found in a Nigerian accession (NGB00463), and the longest (57.4 cm) in IP3616, followed by IP3132 (51.4 cm), which are both ICRISAT accessions (Table 2).

Table 2. The mean values of morpho-agronomic traits and vegetative characters of twenty-four accessions of pearl millet at maturity.

Accession	PH (cm)	NL	LL (cm)	LW (cm)	SG (cm)
IP3616	103.06±22.66 ^{abc}	10.90±43.85 ^a	57.4±5.33 ^{bc}	2.08±0.11 ^{cdefghi}	0.75±0.5 ^{cde}
IP3495	77.92±12.46 ^{cdef}	7.60±0.89 ^c	38.2±2.5 ^{bcde}	1.66±0.11 ^{hijk}	0.58±0.02 ^{de}
NBG00463	80.54±17.57 ^{bcdef}	9.40±1.67 ^{ab}	21.1±7.37 ^e	1.80±0.11 ^{fghijk}	0.72±0.05 ^{cde}
BALKUVE	71.90±4.25 ^{def}	7.80±1.10 ^c	33.4±5.91 ^{cdef}	2.10±0.15 ^{bcdefghi}	0.56±0.05 ^{de}
BAJARA 2	92.22±9.76 ^{bcde}	9.20±1.92 ^{ab}	37.9±17.4 ^{bcde}	2.52±0.18 ^{abcde}	0.88±0.12 ^c
NGB00616	72.42±23.26 ^{def}	8.20±0.84 ^{bc}	44.4±11.9 ^{abcd}	1.97±0.21 ^{efghij}	0.64±0.05 ^{cde}
IP4133	80.64±35.68 ^{bcdef}	8.40±1.14 ^b	38.0±15.3 ^{bcde}	2.26±0.18 ^{cdefgh}	0.67±0.07 ^{cde}
NGB01263	73.68±17.10 ^{def}	10.80±1.10 ^a	41.4±12.6 ^{bcde}	1.93±0.23 ^{efghijk}	0.74±0.07 ^{cde}
IP22281	108.90±17.52 ^a	10.00±1.22 ^a	41.1±12.8 ^{bcde}	1.74±0.22 ^{ghijk}	0.65±0.08 ^{cde}
IP12556	83.16±12.80 ^{bcdef}	7.60±1.14 ^c	33.1±5.63 ^{cdef}	1.67±0.14 ^{hijkl}	0.49±0.05 ^e
NGB00458	72.40±21.91 ^{def}	7.20±1.30 ^d	33.1±5.62 ^{cdef}	1.42±0.14 ^{kl}	0.50±0.47 ^e
NGB00531	67.46±9.75 ^e	7.00±1.00 ^d	30.5±5.36 ^{abc}	1.10±0.16 ^l	0.51±0.04 ^e
IP22271	89.62±12.78 ^b	8.00±1.58 ^{bc}	28.1±3.79 ^{de}	1.66±0.14 ^{hijkl}	0.54±0.03 ^e
NGB00476	94.46±26.11 ^{bcde}	10.20±1.48 ^a	37.0±5.34 ^{bcde}	1.56±0.19 ^{ijkl}	0.58±0.06 ^{de}
IP222868	103.04±12.60 ^b	8.40±1.52 ^b	42.3±3.67 ^{bcde}	1.91±0.16 ^{efghijk}	0.81±0.11 ^{cd}
TAYABI	84.64±13.91 ^{bcdef}	7.80±1.1 ^{0^c}	36.6±7.79 ^{cde}	1.94±0.24 ^{efghijk}	0.62±0.05 ^{cde}
JALGONE	91.12±32.02 ^{bcde}	8.20±1.30 ^{bc}	33.7±4.52 ^{cdef}	2.14±0.35 ^{bcdefghi}	0.66±0.05 ^{cde}
NGB00551	108.40±12.67 ^{bc}	8.40±1.34 ^{bc}	33.4±4.84 ^{cdef}	1.46±0.20 ^{ijkl}	0.56±0.03 ^{de}
IP17862	82.82±14.87 ^{bcdef}	10.00±1.58 ^a	41.3±4.67 ^{bcde}	2.00±0.19 ^{efghijk}	0.60±0.02 ^{de}
IP3132	99.88±19.75 ^{abcd}	9.20±2.17 ^{ab}	51.4±15.1 ^c	1.83±0.15 ^{fghijk}	0.64±0.02 ^{de}
NGB00528	95.42±19.34 ^{ab}	7.80±1.30 ^c	37.0±9.77 ^{bcde}	1.92±0.18 ^{efghijk}	0.51±0.03 ^e
IP22269	104.10±19.19 ^{ab}	8.20±1.48 ^{bc}	42.4±16.6 ^{bcde}	2.34±0.16 ^{bcdefg}	0.63±0.03 ^{cde}
NGB00523	85.82±14.87 ^{bcdef}	10.40±0.55 ^a	33.5±4.81 ^{cdef}	2.96±0.22 ^a	0.70±0.04 ^{cde}
NGB00537	103.46±24.69 ^a	9.60±2.30 ^{ab}	46.5±5.78 ^{abc}	2.05±0.19 ^{defghij}	0.53±0.06 ^e

Means followed by different letters along a column are significantly different at the 0.05 probability level; PH = Plant height, NL = Number of leaves per plant, LL = Leaf length, LW = Leaf width, SG = Stem girth, NON = Number of nodes, INL = Internode length, NTP = Number of tillers per plant, NLT = Number of leaves per tiller.

In terms of leaf width, accession NGB00523 had the broadest leaves (2.96 cm), IP3616, BALKUVE, BAJARA 2, IP4133, JALGONE, IP17862, IP22296, NGB00523 and NGB00537 had leaf width greater than 2.00 cm while NGB00531 had significantly narrower leaves (Table 2). All the accessions had stem girth less than 1.0 cm, but nevertheless the highest stem girth (0.88 cm) was recorded in BAJARA 2, an unclassified accession from the farmer's field (Table 2). The number of nodes per plant was highest in accessions NGB00463 and BALKUVE

which had 5.20 and 5.10 nodes per plant respectively, while other accessions had between 2 and 4 nodes per plant (Table 2). The highest internode length (24.08 cm) occurred in Nigerian accession NGB00537 (Table 2). To corroborate the present results, according to Hash et al. (2003) and Burson et al. (2015), gene actions and environmental influences have great effects on vegetative and yield performance of cereals. Furthermore, it has been demonstrated that particular genotypes perform best in particular environments because of favoured interactions (Mano and Takeda, 1995). However, at maturity, due to senescence, gene interactions and a switching mechanism from vegetative to reproductive stages drastically reduced growth rate.

Table 2. Continuation.

Accession	NON	INL	NTP	NLT	NON
IP3616	4.00±.29 ^{abcdefg}	15.84±4.46 ^{bc}	0.20±0.45 ^c	1.50±0.63 ^{bc}	4.00±.29 ^{abcdefg}
IP3495	4.00±0.36 ^{abcdefg}	18.64±2.92 ^b	0.40±0.55 ^c	1.60±0.60 ^{bc}	4.00±0.36 ^{abcdefg}
NBG00463	5.20±0.35 ^a	17.44±3.60 ^b	0.40±0.55 ^{bc}	1.40±0.49 ^{bc}	5.20±0.35 ^a
BALKUVE	5.10±0.40 ^{ab}	16.64±2.18 ^b	0.63±0.15 ^{bc}	1.90±0.69 ^{bc}	5.10±0.40 ^{ab}
BAJARA 2	3.60±0.56 ^{cdefg}	10.60±9.61 ^c	0.60±0.55 ^{abc}	1.10±0.48 ^{bc}	3.60±0.56 ^{cdefg}
NGB00616	3.20±0.41 ^{fgh}	12.94±5.53 ^c	0.80±0.84 ^{abc}	2.20±0.69 ^b	3.20±0.41 ^{fgh}
IP4133	4.20±0.55 ^{abcdef}	14.12±6.81 ^b	1.00±0.71 ^b	2.10±0.52 ^{bc}	4.20±0.55 ^{abcdef}
NGB01263	2.50±0.50 ^{hi}	11.44±5.54 ^c	1.00±0.71 ^{abc}	2.30±0.66 ^b	2.50±0.50 ^{hi}
IP22281	4.00±0.25 ^{abcdefg}	10.56±3.17 ^c	0.80±0.84 ^{abc}	2.40±0.71 ^b	4.00±0.25 ^{abcdefg}
IP12556	4.70±0.26 ^{abcd}	15.46±2.52	1.60±0.45 ^b	1.40±0.61 ^{bc}	4.70±0.26 ^{abcd}
NGB00458	4.70±0.21 ^{abcd}	12.96±1.96 ^c	0.20±0.45 ^{bc}	1.20±0.46 ^{bc}	4.70±0.21 ^{abcd}
NGB00531	4.70±0.26 ^{abcd}	10.62±3.46 ^c	1.60±0.89 ^b	2.20±0.41 ^b	4.70±0.26 ^{abcd}
IP22271	4.20±0.41 ^{abcdef}	12.08±2.56 ^c	0.00±0.00 ^c	0.00±0.00 ^c	4.20±0.41 ^{abcdef}
NGB00476	4.50±0.40 ^{abcdef}	13.92±1.89 ^b	0.60±0.89 ^{abc}	1.60±0.54 ^{bc}	4.50±0.40 ^{abcdef}
IP222868	4.60±0.40 ^{abcde}	15.24±3.81 ^b	0.40±0.89 ^{bc}	0.60±0.42 ^{bc}	4.60±0.40 ^{abcde}
TAYABI	4.10±0.31 ^{abcdefg}	19.46±3.95 ^b	0.40±0.89 ^{bc}	1.80±0.74 ^{bc}	4.10±0.31 ^{abcdefg}
JALGONE	3.50±0.34 ^{defgh}	17.90±3.14 ^b	0.20±0.45 ^c	1.20±0.62 ^{bc}	3.50±0.34 ^{defgh}
NGB00551	4.00±0.21 ^{abcdefg}	16.38±3.87 ^b	0.40±0.55 ^{bc}	1.10±0.45 ^{bc}	4.00±0.21 ^{abcdefg}
IP17862	4.30±0.30 ^{abcdef}	11.02±1.42 ^c	0.00±0.00 ^c	0.50±0.50 ^{bc}	4.30±0.30 ^{abcdef}
IP3132	3.30±0.26 ^{efgh}	13.28±2.24 ^c	0.60±0.89 ^{abc}	1.20±0.51 ^{bc}	3.30±0.26 ^{efgh}
NGB00528	3.90±0.27 ^{abcdefg}	13.44±3.70 ^c	0.00±0.00 ^c	0.00±0.47 ^{bc}	3.90±0.27 ^{abcdefg}
IP22269	3.70±0.44 ^{cdefgh}	10.02±1.31 ^c	0.40±0.89 ^{bc}	1.10±0.58 ^{bc}	3.70±0.44 ^{cdefgh}
NGB00523	3.80±0.53 ^{bcdefgh}	13.84±2.52 ^c	0.20±0.45 ^c	1.50±0.63 ^{bc}	3.80±0.53 ^{bcdefgh}
NGB00537	3.60±0.45 ^{cdefgh}	24.08±52.57 ^a	1.60±1.52 ^b	1.60±0.47 ^{bc}	3.60±0.45 ^{cdefgh}

Means followed by different letters along a column are significantly different at the 0.05 probability level; PH = Plant height, NL = Number of leaves per plant, LL = Leaf length, LW = Leaf width, SG = Stem girth, NON = Number of nodes, INL = Internode length, NTP = Number of tillers per plant, NLT = Number of leaves per tiller.

Tillering was generally poor among the accessions evaluated, and the highest number of tillers per plant (1.60) was found in IP12556 and NGB00537 (Table 2). The maximum number of leaves per tiller (2.30) was recorded in NGB01263, and lower number was observed in other accessions, but neither the number of tillers per plant nor the number of leaves per tiller was found in accessions NGB00528 and IP22271 (Table 2).

Stem base was basically green in most of the accessions, however, purple stem base was observed in the seedlings of NGB00616, JALGONE, NGB000551, IP22269 and NGB00523, which gradually changed to light purple at maturity (Figure 1). Leaf blades were razor-sharp, typically green, with white midribs and thin ligule margins in all the accessions. The occurrence of the stem base pigmentation at the juvenile stage, which gradually disappeared at maturity, may likely be due to the presence of mutant chlorophyll, which eventually normalized in the course of growth. A similar scenario was documented by Ukwunguwu et al. (2003), who noticed a gradual disappearance of the base colouration in juvenile rice, as it reached maturity.

The correlation coefficients of the vegetative traits of the investigated accessions showed that plant height correlated significantly and positively with the number of leaves and leaf width (Table 3). The correlation was also found between plant height, leaf length and stem girth (Table 3). The number of leaves significantly and positively correlated with leaf length, leaf width, stem girth, number of tillers per plant and number of leaves per tiller (Table 3). Furthermore, a significant and positive correlation was found between leaf length and leaf width, stem girth, number of tillers per plant and number of leaves per tiller (Table 3).

Table 3. Correlation coefficients of quantitative vegetative traits of twenty-four accessions of pearl millet evaluated at maturity.

	PH	NL	LL	LB	SG	NON	INL	NTP	NLT
PH	1.000								
NL	0.468*	1.000							
LL	0.393*	0.870**	1.000						
LB	0.494*	0.702*	0.654*	1.000					
SG	0.397*	0.949*	0.879**	0.687*	1.000				
NI	0.020	-0.419*	-0.390	-0.434*	-0.393*	1.000			
NLL	0.105	-0.151	-0.133	-0.106	-0.227	0.164*	1.000		
NTP	0.261	0.821**	0.781*	0.497*	0.862*	-0.370	-0.223*	1.000	
NLT	0.297**	0.915**	0.843**	0.569*	0.942**	-0.376*	-0.204	0.939**	1.000

** – significant at the 0.01 level; * – significant at the 0.05 level; PH – Plant height, NL – Number of leaves, LL – Leaf length, LW – Leaf width, SG – Stem girth, NON – Number of nodes, INL – Internode length, NTP – Number of tillers per plant and NLT – Number of leaves per tiller.

The high (> 0.900) significant and positive correlations obtained for number of leaves and stem girth, number of leaves and number of leaves per tiller, stem girth and number of leaves per tiller, number of tillers per plant and number of leaves per tiller showed that the traits were genetically linked and/or under the influence of pleiotropy. The high and positive correlations between agronomic traits can be useful in breeding programmes, allowing simultaneous improvement of both characters through correlated responses to selection. This finding is congruent with those of Azeez (2010) and Al-Suhaibani (2011), who obtained positive significant correlations among growth and seed yield characters studied in sesame and pearl millet, respectively.

The mean values and standard errors of a mean between quantitative seed characters and seed related characters of the accessions are shown in Table 4. The accessions varied significantly for the following characters: days to panicle emergence, peduncle length, panicle diameter and 1000-grain weight. Panicles emerged from 44 to 56 days, and the accession NGB00458 was the first to flower at 44 days after sowing, followed by IP2281 and IP22269 at 47 days, while NGB00463 was the last to flower at 56 days after sowing (Table 4). Differences in flowering time could be due to photoperiodic sensitivity. It has been noted that flowering in wild relatives of cultivated plants is influenced by photoperiodism as observed in *Digitaria exilis* (Aliero and Morakinyo, 2002). Peduncle lengths of 11.54 and 11.50 cm were the highest, obtained for IP12556 and NGB00476, respectively, and the shortest peduncle length was observed in IP3616 (Table 4). Panicle length was between 6.54 and 13.63 cm, but it was statistically similar in NGB00463 (12.53 cm), BALKUVE (12.56 cm) and NGB00616 (12.10 cm) (Table 4). The highest peduncle diameter of 2.11 cm was found in IP3132 (Table 4). TAYABI (2.09 cm), IP22271 (2.06 cm) and IP17862 (2.05 cm) differed statistically from other accessions with a peduncle diameter below 2.0 cm.

Meanwhile, accessions NGB00616, NGB00551, NGB00532 and NGB00537, which are all Nigerian accessions, were superior in terms of early maturity and panicle size. Panicle and yield related traits may be influenced by environment as pointed out by Al-Suhaibani (2011). Variations observed in flowering and panicle characters were remarkable, correlating with earlier work (Barbosa et al., 2003), and could be of significant consideration in a breeding programme. Considering grain weight, NGB00616 produced the heaviest grains (10.13 g/1000 grain) (Table 4). The 1000-grain weight was similar for other accessions, except for IP22269, which had the smallest weight (8.08 g/1000 grain). With respect to 1000-grain weight, the results in this study were similar to those reported by other authors (Zerbini and Thomas 2003; Al-Suhaibani, 2011).

Table 4. The mean values of seed related characters of twenty-four Indian and Nigerian accessions of pearl millet.

Accession	DPE	PL (cm)	PNL (cm)	PND (cm)	1000GW (g)
IP3616	47.51±4.21 ^{c-f}	5.37±0.96 ^f	10.01±1.04 ^{c-i}	1.53±0.01 ^{d-i}	9.24±1.02 ^b
IP3495	51.42±4.18 ^{a-f}	8.93±0.54 ^{bcd}	7.13±0.89 ^{jk}	1.62±0.05 ^{c-i}	9.66±1.06 ^b
NBG00463	56.91±10.12 ^a	6.33±0.98 ^{ef}	12.53±0.97 ^{a-e}	1.80±0.09 ^{a-g}	9.69±0.98 ^b
BALKUVE	49.06±6.01 ^{b-f}	6.67±0.31 ^{ef}	12.56±1.02 ^{a-e}	1.94±0.07 ^{a-d}	9.86±1.04 ^b
BAJARA 2	52.53±6.87 ^{a-e}	8.32±1.01 ^{de}	12.62±0.86 ^{a-d}	1.78±0.04 ^{a-h}	9.33±0.92 ^b
NGB00616	49.41±5.16 ^{b-f}	10.56±0.64 ^{abc}	12.10±0.58 ^{a-e}	1.76±0.18 ^{a-h}	10.13±1.02 ^a
IP4133	50.20±7.11 ^{a-f}	7.70±0.43 ^{de}	8.97±0.66 ^{g-k}	1.37±0.04 ^{ghi}	9.94±0.97 ^b
NGB01263	55.84±11.13 ^{ab}	10.85±1.07 ^{ab}	11.65±1.06 ^{b-g}	1.45±0.07 ^{f-i}	9.57±1.01 ^b
IP22281	47.21±6.81 ^{def}	10.54±0.87 ^{abc}	6.54±0.65 ^k	1.68±0.06 ^{a-i}	9.73±0.89 ^b
IP12556	54.56±12.01 ^{a-d}	11.54±1.01 ^a	9.16±0.45 ^{f-j}	1.27±0.04 ⁱ	9.43±0.69 ^b
NGB00458	44.72±9.21 ^f	10.54±0.84 ^{abc}	13.10±1.05 ^{abc}	1.58±0.08 ^{d-i}	9.09±0.88 ^b
NGB00531	49.71±4.05 ^{a-f}	9.72±0.56 ^{bcd}	12.34±0.99 ^{a-e}	1.48±0.08 ^{e-i}	9.55±0.73 ^b
IP22271	50.53±6.14 ^{a-f}	10.85±1.02 ^{ab}	13.39±1.12 ^{ab}	2.06±0.10 ^{abc}	9.89±0.91 ^b
NGB00476	52.44±4.89 ^{a-e}	11.50±0.91 ^a	7.83±0.57 ^{ijk}	1.63±0.07 ^{b-i}	9.40±1.01 ^b
IP222868	47.91±5.90 ^{c-f}	8.13±0.59 ^{de}	9.52±0.96 ^{f-j}	1.84±0.05 ^{a-f}	9.47±0.96 ^b
TAYABI	52.86±5.31 ^{a-e}	8.14±0.97 ^{de}	13.59±1.04 ^{ab}	2.09±0.09 ^{ab}	9.73±0.79 ^b
JALGONE	49.11±6.34 ^{b-f}	7.54±0.66 ^{de}	11.43±0.78 ^{b-g}	1.77±0.09 ^{a-h}	9.80±0.94 ^b
NGB00551	50.14±7.71 ^{a-f}	8.15±0.81 ^{de}	14.33±1.11 ^a	1.68±0.07 ^{a-i}	9.60±1.02 ^b
IP17862	49.21±5.15 ^{b-f}	7.98±0.71 ^{de}	10.42±0.96 ^{d-h}	2.05±0.05 ^{abc}	9.52±0.99 ^b
IP3132	46.34±6.71 ^{ef}	6.48±0.67 ^{ef}	9.55±0.87 ^{f-j}	2.11±0.11 ^a	9.87±1.00 ^b
NGB00528	54.86±4.73 ^{abc}	7.80±0.38 ^{de}	8.94±0.81 ^{g-k}	1.88±0.08 ^{a-f}	9.25±0.78 ^b
IP22269	47.21±6.61 ^{def}	8.43±0.72 ^{cde}	8.82±0.74 ^{h-k}	1.34±0.05 ^{hi}	8.08±0.99 ^c
NGB00523	50.57±4.76 ^{a-e}	8.69±0.77 ^{bcd}	12.67±1.08 ^{c-h}	1.96±0.06 ^{a-e}	9.21±0.73 ^b
NGB00537	52.50±7.31 ^{a-e}	9.22±1.01 ^{bcd}	13.63±1.02 ^a	1.97±0.07 ^{a-d}	9.26±1.05 ^b

Means followed by different letters along a column are significantly different at the 0.05 probability level according to Duncan's multiple range test; DPE – Days to panicle emergence, PL – Peduncle length, PNL – Panicle length, PND – Panicle diameter, 1000GW – 1000-grain weight.

The first three principal component axes accounted for 98.89% of the total variation, out of which PCA 1 accounted for 74.41%. PCA analysis separated the accessions into four groups (Figure 1). The first quadrant (I) had eight mixtures of Nigerian and Indian pearl millet accessions 1. Cluster 2 consisted of eight mixtures of ICRISAT and NACGRAB accessions in quadrant II with accessions IP22271 and NGB00528 farther apart. In quadrant III, combination of IP3132 and IP3616 accessions formed cluster 3. IP22269, IP222868, NGB00523 and NGB00537 accessions were located in the fourth quadrant to form cluster 4. Interestingly, both Indian and Nigerian accessions clustered together. Though accessions in cluster 1

were more closely related, those of clusters 2–4 were more diffused, which implies they are distant relatives, possibly separated by geographical isolation. Meanwhile, Azeez (2010) is of the opinion that accessions in the same cluster are genetically similar or related. Cultivation of pearl millet in the arid and semi-arid regions, during breeding for new cultivars and lines, could increase the genetic differentiation under artificial selection conditions, and consequently increase the genetic distance as opined by Xie et al. (2009).

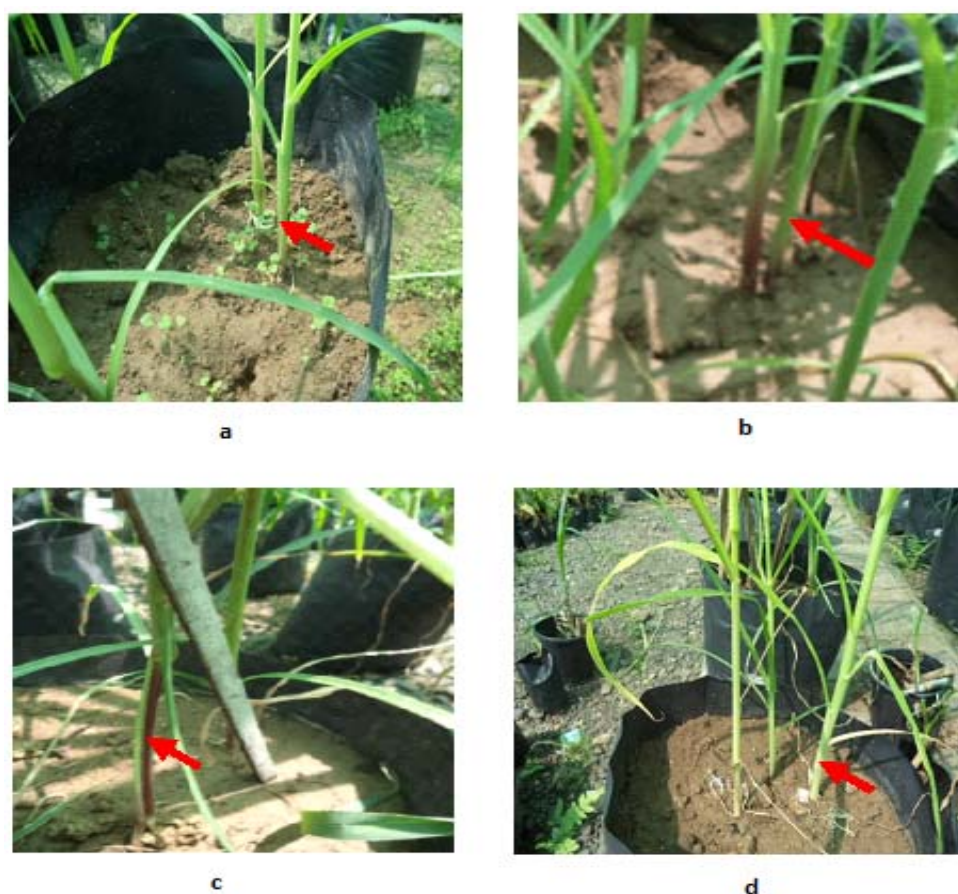


Figure 1. (a) green stem base colour of most pearl millet accessions at 4WAP; (b) purple stem colouration of NGB00616, JALGONE, NGB000551, IP22269 and NGB00523; (c) purple stem base colouration at maturity; (d) green stem base colouration of all other pearl millet accessions at maturity.

The genetic dissimilarity coefficients between the accessions varied and ranged from 0.05 to 0.95. The accessions were separated into two major groups I

and II (Figure 2). The group I was sub-divided into two sub-groups (Ia and Ib), with subgroup Ia splitting into two clusters (Ia1 and Ia2). The cluster Ia1 consisted of eight pearl millet accessions. The three of the accessions: TAYABI, JALGONE and BALKUVE, which were unimproved and sourced from the farmers' field, clustered together, and were similar to IP12556, IP17862, NGB00551, NGB00476 and NGB00463. It has been demonstrated by the present study that morphologically similar accessions may be genetically different, and it is clear that most morpho-agronomic traits could be influenced by environmental factors. The spatial locations of the accessions in a cluster signified their genetic distance and closeness. JALGONE and BALKUVE were the most related with the closest genetic distance and the similarity index above 90% among the accessions. The other cluster Ia2 comprised of BAJARA 2, NGB00523, NGB00537, IP22269, IP22281 and IP222868 genotypes, similar at a genetic distance less than 5.0. These results are in agreement with the findings of Burson et al. (2015), who observed that accessions of pearl millet did not necessarily congregate into the same cluster based on their geographical origins. Contrarily, Swain and Dikshit (1997) observed that clustering pattern of accessions was such that genetic diversity might not be necessarily compatible with geographic diversity, as reflected in this study.

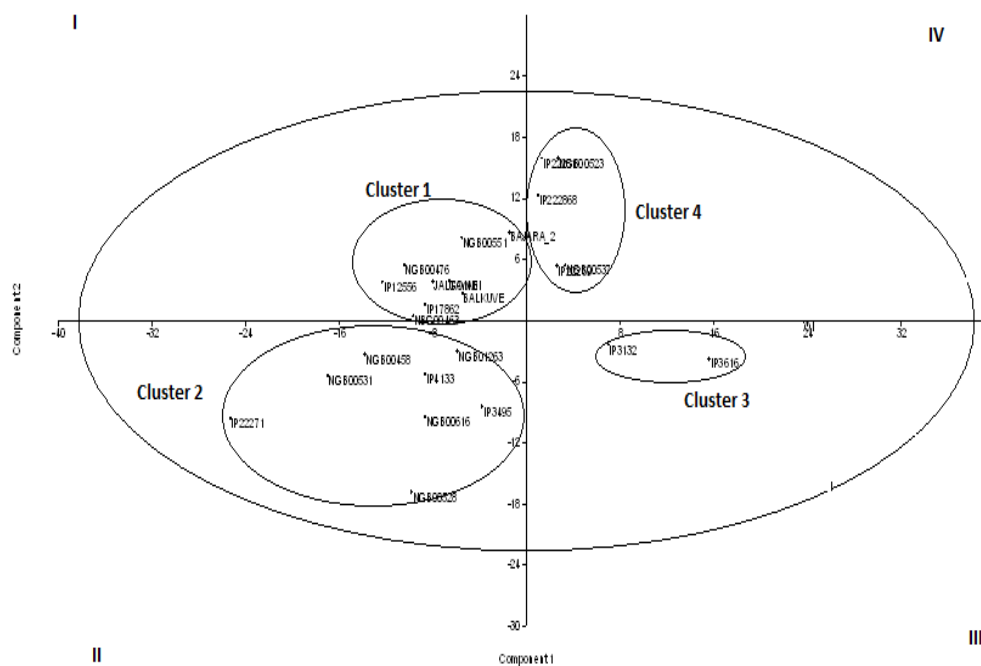


Figure 2. The ordination of twenty-four accessions of pearl millet on principal component axes PCA 1 versus PCA 2, based on cluster analysis of vegetative and reproductive traits.

The subgroup (Ib) separated into two distinct clusters Ib1 and Ib2 (Figure 2). Three closely related accessions (NGB00458, NGB00531 and IP22271) constituted the cluster Ib1, while cluster Ib2 was made up of five accessions of two closely related ICRISAT genotypes (IP3495 and IP4133) and the remaining three members were NACGRAB accessions (NGB00528, NGB01263 and NGB00616). The other major group (group II) included only two accessions: IP3132 and IP3616. Clustering of pearl millet accessions together regardless of their source supports the possibility of a common progenitor elucidated by Jauhar (1981). In contrast, the origin of collections has been identified to also influence the clustering pattern (Fasakin, 2002). Accessions may cluster based on geographical origin or genetic difference and further small clusters could be based on similar characteristics, pedigree relation or close area of cultivation within the main group (Ercan et al., 2002). Co-existence of farmers' field accessions with those from NACGRAB and ICRISAT was expected because these accessions could interbreed, thus limiting potential recombination between them, consequently reducing genetic diversity.

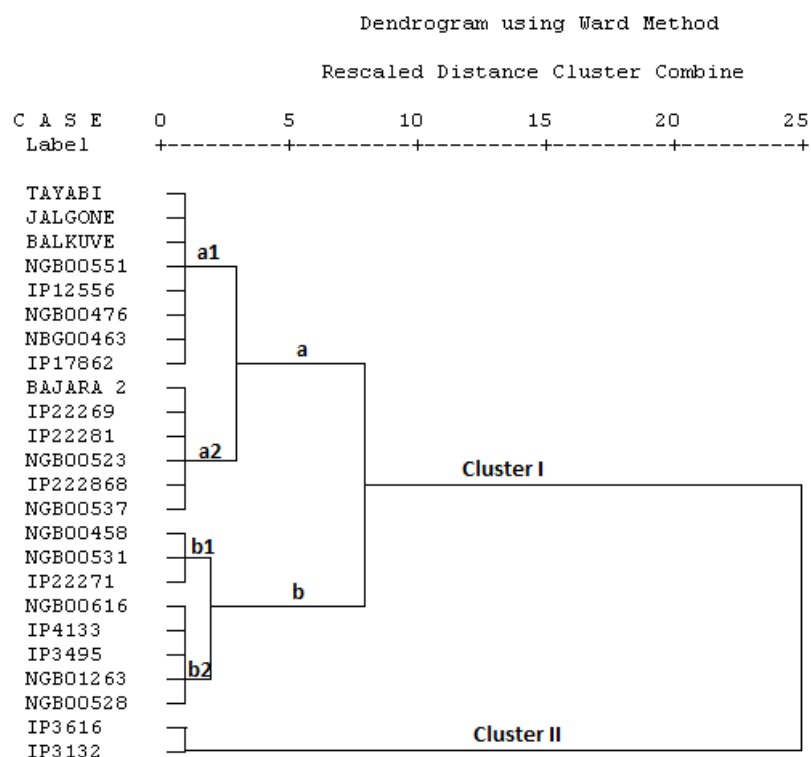


Figure 2. Dendrogram generated using minimum dissimilarity distance based on the UPGMA clustering method, showing genetic distance for vegetative and seed related traits among twenty-four accessions of pearl millet.

Conclusion

An accurate assessment of genetic diversity is important in crop breeding and improvement. Adequate genetic information would identify potential parental combinations to create segregating progenies with maximum genetic variability for further selection. The present study showed the existence of the large morphological diversity in the Nigerian and Indian accessions of pearl millet. Clustering of pearl millet accessions from Nigeria with the accessions from India has confirmed that they are genetically related, and possibly from the same progenitor, but could have been separated by geographical or ecological isolation mechanisms. To further understand a genetic relationship among the accessions, a combination of morphological and molecular characterizations is recommended. This would give more valid genetic variability information for a robust crop improvement programme.

References

- Adeola, O., Rogler, J. C., & Sullivan, T.W. (1994). Pearl millet in diets of White Pekin ducks. *Poultry Science*, 73, 425-435.
- Aliero, A.A., & Morakinyo, J.A. (2002). Phenotypic correlation between vegetative and floral characters in Acha (*Digitaria exilis* and *D. Iburua*) varieties. *Moor Journal of Agriculture Research*, 3, 130-136.
- Al-Suhaibani, N.A. (2011). Better forage and grain yield quality of pearl millet (*Pennisetum glaucum* L.) under different irrigation water supplies and planting densities. *World Applied Sciences Journal*, 15 (8), 1136-1143.
- Amato, S.V., & Forrester, R.R. (1995). Evaluation of pearl millet as a feed ingredient for broiler rations. In: I. D. Teare, (Ed.) *First national grain pearl millet symposium* (pp. 125-128). Tifton, GA, USA.
- Animasaun, D.A., Morakinyo, J.A., Mustapha, O.T., & Krishnamurthy, R. (2015). Assessment of genetic diversity in accessions of pearl millet (*Pennisetum glaucum*) and napier grass (*Pennisetum purpureum*) using microsatellite (ISSR) markers. *Iranian Journal of Genetics and Plant Breeding*, 4 (1), 25-35.
- Azeez, M.A. (2010). Genetic diversity and hybridization studies in sesame (Genera *Sesamum* and *Ceratotheca*) for improved seed yield and oil quality. *Un-published Ph. D. Thesis*. University of Ilorin, Ilorin, Nigeria.
- Barbosa, S., Davide, L.C., & Pereira, A.V. (2003). Cytogenetics of hybrids between *Pennisetum purpureum* Schumacher, *Pennisetum glaucum* L. and *Pennisetum glaucum* L. eseus genitores. *Journal of Agrotechnology and Science*, 27, 26-35.
- Burson, L.B., Renganayaki, K., Dowling, C.D., Hinze, L.L., & Jessup, R.W. (2015). Genetic Diversity among pentaploid buffelgrass Accessions. *Crop Science*, 55, 637-1645.
- CGIAR (2012). Research programme on Dryland Cereals. *Dryland Cereals Publication*, 2012, 6-9.
- Collins, V.P., Cantor, A.H., Pescatore, A.J., Straw, M.L., & Ford, M.J. (1997). Pearl millet in layer diets enhances egg yolk n-3 fatty acids. *Poultry Science*, 76, 326-330.
- Davis, A.J., Dale, N.M., & Ferreira, F.J. (2003). Pearl millet as an alternative feed ingredient in broiler diets. *Journal of Applied Poultry Research*, 12, 137-144.

- Dowling, C.D., Burson, B.L., Foster, J.L., Tarpley, L., & Jessup, R.W. (2013). Confirmation of pearl millet-napier grass hybrids using EST-Derived Simple Sequence Repeat (SSR) markers. *American Journal of Plant Sciences*, 4, 1004-1012.
- Ercan, G.A., Melik, T.K., Kenan, T., Mehmet, B., & Ziya, F.M. (2002). Characterization of Turkish sesame (*Sesame indicum* L.) land races using agronomic and morphological descriptors. *Akdenis Universitesi Ziraat Fakultesi Dergisi*, 15 (2), 45-52.
- FAO and ICRISAT (1996). The world Sorghum and millet economics; facts, trends and outlook. Food and Agricultural Organization of the United Nations: Rome, Italy and International Crops Research Institute for the Semi-Arid Tropics: Patanchera 502 324, Andhra Pradesh, India pp. 23-26.
- Fasakin, K. (2002). Cultivar characterization, crop management practices and chemical composition of Bungus cultivars (*Ceratotheca sesamoides* Endl.) An un-published Ph.D. Thesis University of Ilorin, Ilorin, Nigeria.
- Gulia, S.K., Wilson, J.P., Singh, B., & Carter, J. (2007). Progress in grain pearl millet research and market development. In: J. Janick, (Ed.), *Issues in new crops and new uses*. (pp. 196-203). ASHS Press, Alexandria, VA.
- Hash, C.T., Bhasker Raj, A.G., Lindup, S., Sharma, A., Beniwal, C.R., Folkertsma, R.T., Mahalakshmi, V., Zerbini, E., & Blümmel, M. (2003). Opportunities for marker-assisted selection (MAS) to improve the feed quality of crop residues in pearl millet and sorghum. *Field Crops Research*, 84, 79-88.
- Hill, G.M., & Hanna W.W. (1990). Nutritive characteristics of pearl millet grain in beef-cattle diets. *Journal of Animal Science*, 68, 2061-2066.
- IBPGR & ICRISAT 1993. Descriptors for pearl millet (*Pennisetum glaucum* (L.) R. Br). International Board for Plant Genetic Resources, Rome, Italy; International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India.
- Jauhar, P.P. (1981). Cytogenetics and breeding of pearl millet and related species. In: A.A. Sandberg, (Ed.), *Progress and topics in cytogenetics*. (pp. 1-145). Alan R Liss Inc., New York.
- Kennedy, C., Bell, P., Caldwell, D., Habetz, B., Rabb, J., & Alison, M.A. (2002). Nitrogen application and critical shoot nitrogen concentration for optimum grain and seed protein yield of pearl millet. *Crop Science*, 42, 1966-1973.
- Khairwal, I.S., Rai, K.N., Diwakar, B. Sharma, Y.K., Rajpurohit, B.S., Nirwan, B., & Bhattacharjee, R. (2007). *Pearl millet: Crop management and seed production manual*. Patancheru, Andhra Pradesh, India: ICRISAT.
- Kumar, A.M.R., Reddy, V.R., Reddy, P., & Reddy, P.S. (1991). Utilization of pearl millet (*Pennisetum typhoides*) for egg production. *British Poultry Science*, 32, 463-469.
- Mano, Y., & Takeda, K. (1995). Varietal variation effects of some major genes on salt tolerance in barley seedlings. *Bulletin of Research Institute Bioresources*, 3, 71-81.
- Poncet, V., Lamy, F., Enjalbert, J., Joly, H., Sarr, A., & Robert, T. (1998). Genetic analysis of the domestication syndrome in pearl millet (*Pennisetum glaucum* L., Poaceae): inheritance of the major characters. *Heredity*, 81, 648-658.
- Rai, K.N., Gupta, S.K., Bhattacharjee, R., Kulkarni, V.N.M Singh, A.K., & Rao, A.S. (2009). Morphological characteristics of ICRISAT-bred pearl millet hybrid seed parent. *Journal of SAT Agricultural Research*, 7, 56-71.
- Sullivan, T.W., Douglas, J. H., Bond, P. L., & Andrews D. J. (1990). Nutritional value of pearl millet in broiler diets. *Poultry Science*, 69, 132-136.
- Swain, D., & Dikshit, U.N. (1997). Genetic divergence in Rabi sesame (*Sesmmun indicum* L.). *Indian Journal of Genetics*, 57 (3), 296-300.
- Ukwunguwu, M.N., Bakare, S.O., Kuta, D.D., & Dachi, S.N. (2003). Evaluation of accessions of Acha in Badaggy. *NCRI Annual Report: 2003*, 148-157.
- Wilson, J.P., Hess, D.E., Hanna, W.W., Kumar, K.A., & Gupta, S.C. (2004). *Pennisetum glaucum* subsp. *monodii* accessions with Striga resistance in West Africa. *Crop Protection*, 23, 865-870.

- Xie, X.M., Zhou, F., Zhang, X.Q., & Zhang, J.M. (2009). Genetic variability and relationship between MT-1 elephant grass and closely related cultivars assessed by SRAP markers. *Journal of Genetics*, 88, 281-290.
- Zerbini, E., & Thomas, D. (2003). Opportunities for improvement of nutritive values in sorghum and pearl millet residues in south Asia through genetic enhancement. *Field Crop Research*, 84, 3-15.

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GENETIČKA DIVERGENTNOST GENOTIPOVA NIGERIJSKOG I
INDIJSKOG BISERNOG PROSA ZASNOVANA NA AGRONOMSKIM I
MORFOLOŠKIM OSOBINAMA

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R e z i m e

Ovim istraživanjem ocenjivana je genetička divergentnost genotipova bisernog prosa uzgajanih u Nigeriji i Indiji na osnovu morfo-agronomskih osobina kako bi se identifikovali genotipovi sa superiornim svojstvima koji bi se mogli iskoristiti u programima za oplemenjivanje. Dvadeset četiri genotipa bisernog prosa uzgajano je i evaluirano u pogledu agronomskih i morfoloških osobina tokom suve i vlažne sezone 2015–2016. godine. Podaci prikupljeni o genotipovima korišćenjem standardnih deskriptora statistički su analizirani. IP22281 je imao najvišu srednju visinu biljke (108,90 cm), dok je kod NGB00531 zabeležena najniža (61,02 cm). Značajna varijacija u okviru vrste postojala je kod broja listova po biljci, dužini lista, širini lista, broju nodija i dužini internodija, međutim, obim stabljike je bio sličan za ove genotipove. Bokorenje je generalno bilo slabo sa najvišom vrednošću (1,60 bokora po biljci) uočenom kod NGB00531. Značajna pozitivna korelacija javila se između visine biljke, broja listova, dužine lista i širine lista. Metlice su se pojavljivale između 44. i 56. dana i NGB00548 je imao najkraće vreme sazrevanja. Dužina metlice i prečnik cvetne drške varirali su značajno među genotipovima. Najveći prinos zrna i masa 1000 zrna zabeleženi su kod NGB00616, dok su najniži prinos i masa zabeleženi kod IP22269. Analizom glavnih komponenti genotipovi su grupisani u četiri klastera, obuhvatajući nigerijske i indijske genotipove. Pored toga, dendrogramom su genotipovi grupisani u dve glavne grupe, koje su dalje podeljene u manje klastere sa genotipovima iz Nigerije i Indije u istom klasteru. Istraživanjem se zaključuje da bi varijacije u morfo-agronomskim osobinama i osobinama prinosa među proučavanim genotipovima mogle biti iskorišćene za poboljšanje useva. Obrazac grupisanja ovih genotipova u klastere ukazao je na njihovu genetičku povezanost, moguće od istog pretka, ali i na odvojenost mehanizmima geografske ili ekološke izolacije.

Ključne reči: genetička divergentnost, prinos zrna i komponente prinosa, morfološka varijacija, biserno proso.

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KARAKTERISTIKE RASTA I RODNOSTI SORTI JAPANSKE ŠLJIVE

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Rezime: U periodu od tri godine (2013–2015) kod deset sorti japanske šljive na području Beograda proučavane su karakteristike rasta i rodnosti: inicijalno i finalno zametanje plodova, prinos po stablu, površina poprečnog preseka debla, koeficijent rodnosti i masa ploda. Inicijalno zametanje plodova je variralo u rasponu 5,6–44,4%, a finalno zametanje 2,6–23,3%. Visok prinos po stablu (preko 10 kg) imale su sorte Obilnaja i Morettini 355, dok su nizak prinos (ispod 5 kg) imale sorte Strival, Black Amber, Early Angeleno i Autumn Giant. Najmanju bujnost je imala sorta Golden Plum, a najveću Strival. Većina sorti je imala krupan ili vrlo krupan plod (preko 50 g). Prinos je bio u jakoj pozitivnoj korelaciji sa inicijalnim i finalnim zametanjem plodova ($r = 0,84^{**}$, odnosno $r = 0,92^{**}$). Masa ploda je bila u srednje jakoj negativnoj korelaciji sa inicijalnim i finalnim zametanjem plodova ($r = -0,67^{*}$, odnosno $r = -0,63^{*}$).

Ključne reči: *Prunus salicina*, zametanje plodova, prinos, bujnost, koeficijent rodnosti, masa ploda.

Uvod

Japanska šljiva (*Prunus salicina* Lindl. i njeni hibridi sa drugim diploidnim vrstama šljive) učestvuje sa preko 70% u ukupnoj svetskoj proizvodnji šljive, koja je u 2014. godini iznosila 11,3 miliona tona (FAOSTAT, 2017). Samo u Evropi, čiji je udeo u svetskoj proizvodnji šljive oko 25%, dominira evropska šljiva (*Prunus domestica* L.). U Srbiji se skoro isključivo gaji evropska šljiva, dok su japanske šljive vrlo malo zastupljene.

Japanske šljive imaju kratko biološko zimsko mirovanje i rano cvetaju, tako da su osetljive na pozne prolećne mrazeve. Pored toga, one su osetljivije od evropskih šljiva i na zimske mrazeve. Szabó (2008) navodi da u toku zime kritične temperature (LT_{50}) za izmrzavanje cvetnih pupoljaka evropske šljive iznose oko -30°C , dok su kod japanske šljive one oko -25°C . Zbog veće osetljivosti na mrazeve, gajenje japanske šljive je ograničeno na područja sa toplijom klimom.

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Unutrašnji kvalitet ploda sorti japanske šljive je lošiji u odnosu na sorte evropske šljive. One imaju niži sadržaj šećera, viši sadržaj kiselina, kao i slabije izraženu aromu (Milatović et al., 2011). Kod većine sorti japanske šljive koštica se ne odvaja od mesa. Međutim, one se odlikuju atraktivnim izgledom ploda (naročito velikom krupnoćom), imaju veću čvrstoću i bolju transportabilnost plodova u odnosu na evropske šljive. Njihovi plodovi se koriste isključivo za potrošnju u svežem stanju.

Sorte japanske šljive su pretežno samobesplodne i potrebno ih je gajiti sa odgovarajućim sortama oprašivačima (Beppu et al., 2002; Halász et al., 2007; Sapir et al., 2008; Guerra et al., 2010a; Guerra i Rodrigo, 2015). Utvrđeno je da unošenje društava medonosnih ili solitarnih pčela u voćnjak u vreme cvetanja može značajno povećati zamaćanje i prinos japanske šljive (Calzoni i Speranza, 1998; Sapir et al., 2007).

Sorte japanske šljive su proučavane u Evropi u ekološkim uslovima različitih zemalja: Mađarske (Szabó i Nyéki, 2002); Hrvatske (Vrsaljko, 2008), Italije (Liverani et al., 2010) i Turske (Son, 2010a, 2010b). U Srbiji su ove sorte proučavane u uslovima beogradskog Podunavlja (Milatović et al., 2011) i Šumadije (Milatović et al., 2013).

Cilj ovog rada je bio ispitivanje karakteristika rasta i rodosti većeg broja sorti japanske šljive. Dobijeni rezultati mogu biti korisni za izbor najboljih sorti pogodnih za gajenje u beogradskom području.

Materijal i metode

Ispitivanja su obavljena u kolekcionom zasadu šljive Poljoprivrednog fakulteta iz Beograda na Oglednom dobru „Radmilovac”, u periodu 2013–2015. godine. Zasad je podignut 2008. godine. Ispitivanjima je obuhvaćeno 10 sorti japanske šljive: Autumn Giant, Blackamber, Burbank, Early Angeleno, Golden Plum, Morettini 355, Obilnaja, Santa Rosa, Strival i T.C. Sun. Sve sorte su kalemljene na sejancu džanarike (*Prunus cerasifera* Ehrh.) i zastupljene su u kolekciji sa po pet stabala. Uzgojni oblik je slobodan, a rastojanje sadnje je 4,5 x 3 m.

Za određivanje zamaćanja plodova u fazi „balona“ su obeležene po tri grane od svake sorte sa po najmanje 200 cvetova. U vreme cvetanja na njima su izbrojani cvetovi. Broj inicijalno zamaćenih plodova je utvrđen tri nedelje po precvetavanju, a broj finalno zamaćenih plodova 2–3 nedelje pred berbu. Prinos je utvrđen merenjem mase ubranih plodova i izražen je u kg po stablu. Površina poprečnog preseka debla (PPPD) je izračunata na osnovu prečnika debla koji je meren na visini od 30 cm iznad spojnog mesta. Kumulativni koeficijent rodosti (KKR) je izračunat kao odnos kumulativnog prinosa po stablu za tri godine (2013–2015) i PPPD u poslednjoj godini ispitivanja (2015) i izražen je u kg po cm². Masa ploda je određena na uzorku od 30 plodova po sorti.

Podaci su statistički obrađeni metodom analize varijanse za dvofaktorijski ogled. Značajnost razlika između srednjih vrednosti je utvrđena pomoću Dankanovog testa višestrukih intervala za verovatnoću 0,05. Između ispitivanih pokazatelja rodnosti (inicijalno i finalno zamaštanje, prinos i masa ploda) izračunati su Pirsonovi koeficijenti korelacije, a njihova značajnost je testirana pomoću t-testa.

Rezultati i diskusija

U tabeli 1 prikazani su podaci za inicijalno i finalno zamaštanje plodova u periodu od tri godine (2013–2015).

Tabela 1. Inicijalno i finalno zamaštanje plodova (%) sorti japanske šljive (2013–2015).

Table 1. Initial and final fruit set (%) in Japanese plum cultivars (2013–2015).

Sorta <i>Cultivar</i>	Inicijalno zamaštanje <i>Initial fruit set</i>				Finalno zamaštanje <i>Final fruit set</i>			
	2013.	2014.	2015.	Prosek <i>Average</i>	2013.	2014.	2015.	Prosek <i>Average</i>
Autumn Giant	5,3	12,8	5,9	8,0 d	3,0	3,1	2,6	2,9 c
Black Amber	4,1	7,7	5,1	5,6 d	2,0	4,1	1,8	2,7 c
Burbank	19,1	44,4	39,5	34,3 ab	11,5	20,8	15,9	16,1 ab
Early Angeleno	2,5	34,1	10,5	15,7 cd	1,1	16,5	4,0	7,2 bc
Golden Plum	1,2	12,2	5,7	6,4 d	0,8	4,2	2,9	2,6 c
Morettini 355	46,8	29,3	42,6	39,6 ab	27,1	17,2	25,6	23,3 a
Obilnaja	22,9	56,8	53,5	44,4 a	11,4	17,9	33,9	21,1 a
Santa Rosa	19,8	21,7	12,9	18,1 cd	18,0	11,9	7,5	12,5 abc
Strival	5,9	39,5	21,7	22,4 bcd	3,1	11,7	13,5	9,4 bc
T.C. Sun	9,0	61,5	12,1	27,5 abc	4,7	31,1	7,1	14,3 ab

Prosečno inicijalno zamaštanje plodova je variralo od 5,6% kod sorte Black Amber do 44,4% kod sorte Obilnaja, dok je prosečno finalno zamaštanje plodova variralo od 2,6% kod sorte Golden Plum do 23,3% kod sorte Morettini 355. Kod većine sorti vrednosti inicijalnog i finalnog zamaštanja plodova su bile niže u 2013. godini u odnosu na ostale dve godine. Razlog za to su niže temperature u periodu cvetanja sorti japanske šljive u ovoj godini (prva polovina aprila).

Prema klasifikaciji koju je dao Szabó (2003) za japanske šljive, sorte Golden Plum, Black Amber i Autumn Giant su imale mali stepen finalnog zamaštanja plodova, ispod 5%. Sorte Strival i Early Angeleno su bile u kategoriji srednjeg zamaštanja (5–10%), dok su ostale sorte imale visok stepen zamaštanja, iznad 10%.

Kod japanske šljive zametanje plodova obično se kreće u intervalu 0–15% (Szabó i Nyéki, 2002; Jia et al., 2008; Guerra et al., 2010b). Ono je prosečno manje u odnosu na evropske šljive (Kemp, 1996; Hjeltneš i Nornes, 2007; Glišić et al., 2012; Jaćimović et al., 2012; Nikolić et al., 2012).

Zametanje plodova japanske šljive značajno varira iz godine u godinu zbog toga što je ona samobesplodna vrsta koja cveta rano u proleće kada su vremenski uslovi često nepovoljni zbog niskih temperatura, kiše i vetra. Ovakvi uslovi mogu ograničiti let pčela i transfer polena (Calzoni i Speranza, 1998). U našem istraživanju takvi uslovi su zabeleženi naročito u 2013. godini, kada je i zametanje plodova bilo najmanje. Pored toga, utvrđeno je da je atraktivnost cvetova japanske šljive za pčele i genetički uslovljena i da zavisi od količine polena, boje antera, produkcije nektara i mirisa (Radice et al., 2010).

Prosečan prinos po stablu se kretao od 4,3 kg kod sorte Strival do 12,8 kg kod sorte Obilnaja (Tabela 2). Visok prosečan prinos po stablu (preko 10 kg) imale su sorte Obilnaja i Morettini 355, dok su nizak prinos (ispod 5 kg) imale sorte Strival, Black Amber, Early Angeleno i Autumn Giant. Posmatrano po godinama, kod većine sorti prinos je bio najniži u 2013. godini, u kojoj je bilo zabeleženo i najmanje zametanje plodova. S druge strane, kod većine sorti najviši prinos je bio u 2015. godini koja se odlikovala povoljnim vremenskim uslovima u periodu pred cvetanje i za vreme cvetanja (kraj marta i prva polovina aprila).

Tabela 2. Prinos po stablu, površina poprečnog preseka debla i kumulativni koeficijent rodosti sorti japanske šljive.

Table 2. Yield per tree, trunk cross-sectional area and cumulative yield efficiency in Japanese plum cultivars.

Sorta <i>Cultivar</i>	Prinos (kg po stablu) <i>Yield (kg per tree)</i>				PPPD ¹ <i>TCSA</i> (cm ²)	KKR ² <i>CYE</i> (kg/cm ²)
	2013.	2014.	2015.	Prosek <i>Average</i>		
Autumn Giant	2,5	4,4	7,5	4,8 c	80,3 a	0,18
Black Amber	1,2	4,2	8,0	4,5 c	64,7 b	0,21
Burbank	4,9	9,7	10,5	8,4 b	85,7 a	0,29
Early Angeleno	1,7	7,6	4,8	4,7 c	54,9 bc	0,26
Golden Plum	0,7	5,0	11,7	5,8 bc	49,8 c	0,35
Morettini 355	7,4	6,5	23,1	12,3 a	65,8 b	0,56
Obilnaja	5,0	6,7	26,8	12,8 a	56,6 bc	0,68
Santa Rosa	6,5	6,3	4,6	5,8 bc	50,8 c	0,34
Strival	1,7	5,8	5,3	4,3 c	88,0 a	0,15
T.C. Sun	3,8	10,1	7,5	7,1 bc	53,8 bc	0,40

¹PPPD – Površina poprečnog preseka debla u 2015. godini./*TCSA – Trunk cross-sectional area in 2015.*

²KKR – Kumulativni koeficijent rodosti (2013–2015)/*CYE – Cumulative yield efficiency (2013–2015).*

Najmanju bujnost, izraženu preko površine poprečnog preseka debla u poslednjoj godini istraživanja, imala je sorta Golden Plum (49,8 cm²). Najveću bujnost je imala sorta Strival (PPPD od 88,0 cm²). Statistički značajno veću PPPD u odnosu na ostale sorte imale su Strival, Burbank i Autumn Giant.

Kumulativni koeficijent rodnosti (KKR) je značajan pokazatelj produktivnosti sorti, koji kombinuje prinos i bujnost. Kod ispitivanih sorti KKR je imao vrednosti od 0,15 kg/cm² kod sorte Strival do 0,68 kg/cm² kod sorte Obilnaja. Sorte koje imaju manju PPPD i veći KKR se mogu saditi na manjem rastojanju.

Rezultati za rodnost pojedinih sorti su u skladu sa rezultatima prethodnih istraživanja (Liverani et al., 2010; Son, 2010b; Milatović et al., 2013). Kod sorti japanske šljive postignuti su prosečno manji prinosi u odnosu na sorte evropske šljive gajene u istim uslovima (Milatović et al., 2016). U periodu ispitivanja nisu zabeleženi jači zimski mrazevi, kao ni pozni prolećni mrazevi koji bi mogli dovesti do izmrzavanja generativnih organa šljive. Kao glavni razlog slabije rodnosti ispitivanih sorti japanske šljive može se navesti slabo zametanje plodova. Ono može biti posledica samobesplodnosti ovih sorti, kao i lošijih vremenskih uslova u periodu cvetanja u pojedinim godinama (posebno u 2013).

Krupnoća ploda je veoma značajna osobina kod sorti japanske šljive čiji su plodovi namenjeni za potrošnju u svežem stanju. Većina ispitivanih sorti je imala krupan do vrlo krupan plod sa prosečnom masom iznad 50 g (Tabela 3). Najmanju masu ploda imala je najranija sorta Morettini 355 (39,5 g), a najveću pozna sorta Golden Plum (95,7 g).

Tabela 3. Masa ploda (g) sorti japanske šljive (2013–2015).

Table 3. Fruit weight (g) of Japanese plum cultivars (2013–2015).

Sorta <i>Cultivar</i>	2013.	2014.	2015.	Prosek <i>Average</i>
Autumn Giant	79,1	78,4	75,4	77,6 b
Black Amber	90,3	93,1	82,0	88,5 a
Burbank	57,0	63,9	44,3	55,1 d
Early Angeleno	67,2	72,1	55,0	64,8 c
Golden Plum	75,0	105,9	106,1	95,7 a
Morettini 355	37,9	43,2	37,4	39,5 e
Obilnaja	37,5	40,3	46,6	41,5 e
Santa Rosa	41,8	47,5	38,7	42,7 e
Strival	43,5	32,5	53,6	43,2 e
T.C. Sun	80,0	55,3	83,5	72,9 bc

Naši rezultati za krupnoću ploda pojedinih sorti japanske šljive u skladu su sa rezultatima koje su dobili Szabó i Nyéki (2002) u uslovima Mađarske, Son (2010a) u uslovima Turske, kao i Milatović et al. (2011, 2013) u uslovima Srbije.

Između ispitivanih pokazatelja rodosti izračunati su Pirsonovi koeficijenti korelacije, a njihove vrednosti su prikazane u tabeli 4.

Tabela 4. Korelacioni matriks između ispitivanih osobina.

Table 4. Correlation matrix among characteristics studied.

Osobina <i>Trait</i>	Finalnozametanje <i>Final fruit set</i>	Prinos po stablu <i>Yield per tree</i>	Masa ploda <i>Fruit weight</i>
Inicijalno zametanje <i>Initial fruit set</i>	0,95**	0,84**	-0,67*
Finalno zametanje <i>Final fruit set</i>	-	0,92**	-0,63*
Prinos po stablu <i>Yield per tree</i>		-	-0,44

**Statistički veoma značajno ($P < 0,01$)/Statistically very significant ($P < 0.01$).

*Statistički značajno ($P < 0,05$)/Statistically significant ($P < 0.05$).

Prinos je bio u jakoj pozitivnoj korelaciji sa inicijalnim i finalnim zametanjem plodova ($r = 0,84^{**}$, odnosno $r = 0,92^{**}$). Masa ploda je bila u srednje jakoj negativnoj korelaciji sa inicijalnim i finalnim zametanjem plodova ($r = -0,67^{*}$, odnosno $r = -0,63^{*}$). Pored toga, jaka pozitivna korelacija je utvrđena između inicijalnog i finalnog zametanja plodova ($r = 0,95^{**}$).

Značajna korelacija između inicijalnog i finalnog zametanja plodova utvrđena je u prethodnim istraživanjima kod višnje (Rakonjac et al., 2010) i breskve (Milatović et al., 2010). Takođe, značajna korelacija između prinosa i zametanja plodova utvrđena je kod breskve (Milatović et al., 2010) i višnje (Alrgei et al., 2016).

Zaključak

Na osnovu proučavanja karakteristika rodosti kod deset sorti japanske šljive na području Beograda u periodu od tri godine može se zaključiti da se po visokom zametanju plodova i prinosu ističu sorte Obilnaja i Morettini 355. Međutim, ove sorte imaju plod manje krupnoće. S obzirom na to da su plodovi japanske šljive namenjeni isključivo za potrošnju u svežem stanju, posebno su interesantne sorte koje imaju vrlo krupan plod, a to su: Golden Plum, Black Amber, Autumn Giant, T.C. Sun i Early Angeleno. Međutim, ove sorte imaju slabu do umerenu rodost. Glavni razlog njihove slabije rodosti je slabo zametanje plodova.

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Literatura

- Alrgei, H., Guffa, B., Fotirić-Aksić, M., Rakonjac, V., Nikolić, D., Čolić, S., & Đorđević, B. (2016). The tree growth effect on fertility and fruit quality traits in 'Oblačinska' sour cherry genotypes. *Acta Horticulturae*, 1139, 243-248.
- Beppu, K., Yamane, H., Yaegaki, H., Yamaguchi, M., Kataoka, I., & Tao, R. (2002). Diversity of S-RNase genes and S-haplotypes in Japanese plum (*Prunus salicina* Lindl.). *Journal of Horticultural Science and Biotechnology*, 77 (6), 658-664.
- Calzoni, G.L., & Speranza, A. (1998). Insect controlled pollination in Japanese plum (*Prunus salicina* Lindl.). *Scientia Horticulturae*, 72 (3), 227-237.
- FAOSTAT (2017). <http://faostat.fao.org/Datum> pristupa 23.01.2017.
- Glišić, I., Cerović, R., Milošević, N., Đorđević, M., & Radičević, S. (2012). Initial and final fruit set in some plum (*Prunus domestica* L.) hybrids under different pollination types. *Genetika*, 44 (3), 583-593.
- Guerra, M.E., & Rodrigo, J. (2015). Japanese plum pollination: A review. *Scientia Horticulturae*, 197, 674-686.
- Guerra, M.E., Wunsch, A., Rodrigo, J., & López-Corrales, M. (2010a). Influence of pollination on the low fruit set in Japanese plum. *Acta Horticulturae*, 874, 189-192.
- Guerra, M.E., Wunsch, A., López-Corrales, M., & Rodrigo, J. (2010b). Flower emasculation as the cause for lack of fruit set in Japanese plum crosses. *Journal of the American Society for Horticultural Science*, 135 (6), 556-562.
- Halász, J., Hegedűs, A., Szabó, Z., Nyéki, J., & Pedryc, A. (2007). DNA-based S-genotyping of Japanese plum and pluot cultivars to clarify incompatibility relationships. *HortScience*, 42 (1), 46-50.
- Hjeltnes, S.H., & Nornes, L. (2007). Pollination and pollen germination of some new plum cultivars. *Acta Horticulturae*, 734, 317-319.
- Jaćimović, V., Radović, M., Bogavac, M. & Božovic, Đ. (2012). Impact of honey bees (*Apis mellifera* L.) on pollination and yield of cultivated plum varieties. *Agriculture and Forestry*, 58 (2), 151-157.
- Jia, H.J., He, F.J., Xiong, C.Z., Zhu, F.R., & Okamoto, G. (2008). Influences of cross pollination on pollen tube growth and fruit set in Zuili plums (*Prunus salicina*). *Journal of Integrative Plant Biology*, 50 (2), 203-209.
- Kemp, H. (1996). Pollination results of apple, Malus, pear, plum and cherry of the International Working Group on Pollination. *Acta Horticulturae*, 423, 243-298.
- Liverani, A., Giovannini, D., Versari, N., Sirri, S., & Brandi, F. (2010). Japanese and European plum cultivar evaluation in the Po valley of Italy: yield and climate influence. *Acta Horticulturae*, 874, 327-335.
- Milatović, D., Nikolić, D., & Đurović, D. (2010). Variability, heritability and correlations of some factors affecting productivity in peach. *Horticultural Science*, 37, 79-87.
- Milatović, D., Đurović, D., & Zec, G. (2011). Ispitivanje stonih sorti šljive na području Beograda. *Voćarstvo*, 45, 101-108.
- Milatović, D., Đurović, D., & Đorđević, B. (2013). Evaluation of Japanese plum cultivars in Serbia. *Acta Horticulturae*, 981, 173-176.
- Milatović, D., Đurović, D., Zec, G., & Radović, M. (2016). Phenological traits, yield and fruit quality of plum cultivars created at the Fruit Research Institute in Čačak, Serbia. In: D. Kovačević (Ed.) *Book of proceedings, VII international scientific agriculture symposium "Agrosym 2016"* (pp. 789-795). Jahorina, Bosnia and Herzegovina.
- Nikolić, D.T., Rakonjac, V.S., & Fotirić-Aksić, M.M. (2012). The effect of pollinizer on the fruit set of plum cultivar Čačanska Najbolja. *Journal of Agricultural Sciences*, 57, 9-18.

- Radice, S., Giordani, E., Nencetti, V., & Bellini, E. (2010). Phenological expression in *Prunus salicina* Lindl. genotypes and its relationship with insect attraction and pollination. *Acta Horticulturae*, 874, 151-156.
- Rakonjac, V., Fotirić Akšić, M., Nikolić, D., Milatović, D., & Čolić, S. (2010). Morphological characterization of 'Oblačinska' sour cherry by multivariate analysis. *Scientia Horticulturae*, 125 (4), 679-684.
- Sapir, G., Goldway, M., Shafir, S., & Stern, R.A. (2007). Multiple introduction of honeybee colonies increases cross-pollination, fruit-set and yield of 'Black Diamond' Japanese plum (*Prunus salicina* Lindl.). *Journal of Horticultural Science and Biotechnology*, 82 (4), 590-596.
- Sapir, G., Stern, R.A., Shafir, S., & Goldway, M. (2008). S-RNase based S-genotyping of Japanese plum (*Prunus salicina* Lindl.) and its implication on the assortment of cultivar-couples in the orchard. *Scientia Horticulturae*, 118 (1), 8-13.
- Son, L. (2010a). Determination on quality characteristics of some important Japanese plum (*Prunus salicina* Lindl.) cultivars grown in Mersin-Turkey. *African Journal of Agricultural Research*, 5, 1144-1146.
- Son, L. (2010b). Yield and fruit weight of Japanese plum (*Prunus salicina* Lindl.) cultivars in Turkey. *Journal of the American Pomological Society*, 64 (2), 106-109.
- Szabó, Z. (2003). Plum (*Prunus domestica* L.). In: P. Kozma, J. Nyéki, M. Soltész & Z. Szabó (Eds.), *Floral biology, pollination and fertilisation in temperate zone fruit species and grape*. (pp. 383-410). Budapest: Akadémiai Kiadó.
- Szabó, Z. (2008). Frost susceptibility and tolerance of flower buds and flowers in stone fruits. In: J. Nyéki, M. Soltész and Z. Szabó (Eds.), *Morphology, biology and fertility of flowers in temperate zone fruits*. (pp. 83-116). Budapest: Akadémiai Kiadó.
- Szabó, Z., & Nyéki, J. (2002). Japanese plums in Hungary. *Acta Horticulturae*, 577, 65-71.
- Vrsaljko, A. (2008). Ekologija Ravnih kotara za uzgoj kinesko-japanskih sorti šljiva. *Pomologia Croatica*, 14, 235-252.

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GROWTH AND PRODUCTIVITY CHARACTERISTICS OF
JAPANESE PLUM CULTIVARS**Dragan P. Milatović*, Dejan B. Đurović and Gordan N. Zec**University of Belgrade, Faculty of Agriculture,
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A b s t r a c t

Growth and productivity characteristics including: initial and final fruit set, yield per tree, trunk cross-sectional area, yield efficiency and fruit weight were studied in ten cultivars of Japanese plums in the Belgrade area for the period of three years (2013-2015). Initial fruit set ranged from 5.6 to 44.4%, and the final fruit set from 2.6 to 23.36%. The high yield per tree (over 10 kg) was obtained in cultivars 'Obilnaja' and 'Morettini 355', whereas the low yield (under 5 kg) was obtained in cultivars 'Strival', 'Black Amber', 'Early Angeleno' and 'Autumn Giant'. The lowest vigor was observed in the cultivar 'Golden Plum', and the highest in the cultivar 'Strival'. The majority of cultivars had a very large fruit (over 50 g). The yield was strongly positively correlated with initial and final fruit set ($r = 0.84^{**}$; $r = 0.92^{**}$, respectively). Fruit weight was moderately negatively correlated with initial and final fruit set ($r = -0.67^{*}$; $r = -0.63^{*}$, respectively).

Key words: *Prunus salicina*, fruit set, yield, vigor, yield efficiency, fruit weight.

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UTILIZATION OF AFRICAN STAR APPLE (*CHRYSOPHYLLUM ALBIDUM*) KERNEL MEAL IN BROILER DIETS

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Abstract: The effect of feeding graded levels (0, 5, 10, 15, 20, 25 and 30%) of untreated African star apple kernel meal (ASAKM) on the performance and blood profile of broilers was investigated in an 8-week trial. Two hundred and ten one-week-old Cobb strain broiler chicks were randomly allotted to 7 diets with 3 replicates of 10 chicks per replicate in a completely randomized design. Data on minerals, vitamins, amino acids and quantification of some anti-nutrients were assayed. The results showed that the kernel was high in nutrients, especially carbohydrate as a source of energy but contains high levels of anti-nutritional or toxic factors. Performance traits ($p < 0.05$) of broilers fed ASAKM gave poor results though without mortality. The increment of dietary levels of the untreated ASAKM did not negatively influence blood composition and most of the measured biochemical indices, the parameters were better ($p < 0.05$), or had values statistically similar with the control diet compared with those on the test diets ($p > 0.05$). Increasing levels of ASAKM in diets caused elevation of AST, ALT, ALP and a significant increase in blood urea levels in the raw kernel meal based diets relative to the control diet ($p < 0.05$). Based on the performance of the birds fed the raw ASAKM, it may be concluded that broilers could barely tolerate 5% raw ASAKM in their diets with minimum adverse effects. However, the values of the blood parameters particularly the red blood cells favoured erythropoiesis, suggesting that the African star apple kernel meal had no detrimental effect on the health status of the birds. Subsequent research should process the raw star apple seed meal before dietary inclusion for optimum results.

Key words: ASAKM, poultry, performance, serum biochemical, haematological parameters.

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Introduction

The need to develop cheap and readily available alternate feeding materials to support the growth of food animals in developing countries, Nigeria inclusive, is receiving great attention. This is due to the daily increase in human population which creates high demand on animal protein in diets of the populace. Feed, which has always been a major limiting factor in the growth of the poultry industry, accounts for about 70–75% of the total cost of intensive production. Evaluation of unconventional feeding stuff for use by farm animals to mitigate competition among humans, animals and industries for the limited conventional feeding materials is receiving attention by researchers to solve the problem.

African star apple (*C. albidum*) is an indigenous wild fruit tree with enormous potentials for plantation establishment, and the fruit/seed could serve as an alternative carbohydrate or energy foodstuff (Okigbo, 1978; Okafor, 1981). *C. albidum* is reported to be an important tree in the agro-forestry system and is cultivated in Nigeria, Uganda, Niger, Cameroon, Ivory Coast republics on commercial scale (Adewusi and Bada, 1997).

Preliminary investigations (Ajewole and Adeyeye, 1991) found that *C. albidum* seed contains valuable nutrients such as crude protein, carbohydrate, crude fat, crude fibre, mineral matter in concentrations of 8.75, 83.38, 3.45, 2.42 and 2.00% respectively warranting the potentials of the seed as a good novel feedstuff in animal feed. However, like numerous plants, the tree and products contain many anti-nutrients or phytochemicals including cyanogens, flavonoids, oxalate, phenolics, phytate, saponins, tannins, terpenoids which have been established by research to have deleterious effects on nutrition by diminishing nutrient bioavailability or utilization in the consumer if ingested in high levels solely or in diets (Ojiako and Igwe, 2008). The phytotoxins are also implicated in eliciting adverse toxic biochemical and physiological responses in the body (Igile, 1996). Early works by Annongu and Abimbola, (2003) and Amaefule et al. (2006) observed that monogastric animals can utilize non-conventional feedstuffs successfully in their diets. It is for this reason that this study investigated the use of star apple seed kernel (*Chrysophyllum albidum*) in nutrition of broilers to determine its acceptability and optimum level of replacing maize.

Materials and Methods

Experimental diets and management of birds

African star apple fruits for this trial were collected from a plantation in Osun State of Nigeria. The seeds were removed from the fruits, washed, sun dried and further dehulled to obtain the kernels. The kernels were milled for inclusion in diet

mixtures. Seven diets were formulated to comprise graded levels of African star apple kernel meal (ASAKM) at inclusion rates of 0, 5, 10, 15, 20, 25 and 30% in replacing maize in the diets of the birds. Two hundred and ten day-old Cobb strain broiler chicks from Zartech commercial farms, Oyo State, Nigeria were fed for one week on a standard broiler starter diet before the commencement of the experiment for both the starter and finisher phases. The birds were randomly allotted to 7 diets with 3 replicates of 10 chicks per replicate using a one-way analysis of variance. Experimental diets and drinking water were given to the chicks *ad libitum* in a study that lasted for 8 weeks. The composition of the experimental diets on as-fed basis (for broiler starter and finisher phases) and their analysed nutrient contents are presented in Tables 1 and 2.

Table 1. Composition of the experimental diets for broiler starter (kg/100kg diet).

Parameter (%)	Inclusion levels of African star apple kernel meal						
	0	5	10	15	20	25	30
Maize	56.46	53.63	50.81	47.99	45.17	42.34	39.52
African star apple kernel meal	0.00	2.83	5.65	8.47	11.29	14.12	17.94
Soybean meal	38.04	38.04	38.04	38.04	38.04	38.04	38.04
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Bone meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Broiler premix	0.30	0.30	00	0.30	0.30	0.30	0.30
Total	100	100	100	100	100	100	100
Calculated nutrient analysis of the diet							
Metabolizable energy kcal/kg	3132.74	3118.63	3104.57	3090.52	3076.46	3062.35	3077.62
Crude protein	22.99	22.95	22.90	22.85	22.80	22.75	22.70
Ether extract	3.74	3.63	3.52	3.40	3.29	3.18	3.06
Crude fibre	4.02	3.95	3.87	3.79	3.72	3.64	3.57
Ash	1.07	1.04	1.00	0.96	0.93	3.18	3.06
Analysed nutrient composition of the diet (%)							
Dry matter	90.15	90.37	90.13	90.18	90.67	90.19	90.08
Crude protein	23.20	21.91	22.41	21.98	22.78	23.64	22.99
Ether extract	2.47	2.90	2.70	2.71	2.46	2.36	2.02
Crude fibre	2.64	2.62	2.30	2.77	2.70	3.31	3.06
Ash	8.64	8.11	7.34	9.45	8.59	9.33	7.13
NFE	63.05	64.46	67.53	63.09	63.47	61.36	64.80

Table 2. Composition of the experimental diets for broiler finisher (kg/100kg diet).

Parameter (%)	Inclusion levels of African star apple kernel meal						
	0	5	10	15	20	25	30
Maize	63.91	60.71	57.52	54.32	51.13	47.93	44.74
African star apple kernel meal	0.00	3.20	6.39	9.59	12.78	15.98	19.17
Soybean meal	30.19	30.19	30.19	30.19	30.19	30.19	30.19
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Lysine	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Limestone	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Broiler premix	0.30	0.30	0.30	0.30	0.30	0.30	0.30
	100	100	100	100	100	100	100
Calculated nutrient analysis of the diet							
Metabolizable energy kcal/kg	3153.71	3137.76	3121.86	3105.90	3089.99	3074.04	3058.14
Crude protein	20.00	19.74	19.49	19.23	18.97	18.71	18.60
Ether extract	3.77	3.64	3.51	3.38	3.25	3.13	3.00
Crude fibre	3.71	3.63	3.54	3.46	3.37	3.28	3.20
Ash	1.17	1.13	1.09	1.05	1.00	0.96	0.92
Analysed nutrient composition of the diet (%)							
Dry matter	89.06	89.34	89.30	89.32	89.30	89.15	89.26
Crude protein	19.80	20.51	21.11	21.59	21.57	21.68	21.49
Ether extract	2.21	2.16	2.14	2.09	2.13	2.10	2.04
Crude fibre	2.03	2.50	3.02	3.23	3.47	3.48	3.46
Ash	6.94	7.13	7.52	6.56	6.97	6.46	6.52
NFE	69.02	67.70	66.53	66.53	65.86	66.28	66.49

Data collection/Response criteria

In the course of the experiment, data were recorded on the feed intake, daily weight gain, feed conversion ratio and the mortality rate. At the end of the experiment, one broiler per replicate in all the dietary treatments was taken at random and blood collected from the wing web using syringes and sterilized needles in accordance with the ethical requirements of the University of Ilorin for the procedures using animals in experiments. Blood samples collected in sample bottles with the anti-coagulant (EDTA) were used to assay for the following haematological parameters: packed cell volume (PCV), red blood cell (RBC), haemoglobin (Hb), white blood cell (WBC). The mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were calculated. Blood for serum biochemistry was taken in test tubes without anti-coagulant for the analysis of blood glucose level, serum total protein, albumin,

globulin and albumin/globulin was calculated. The lipid profile (triglyceride, total cholesterol, high density lipoprotein [HDL]) and low density lipoprotein (LDL), blood urea and creatinine, enzyme activities (aspartate aminotransferase [AST]), alanine aminotransferases (ALT) and alkaline phosphate (ALP) were also assayed. The sera were also used for the determination of blood electrolytes, calcium (Ca^{2+}), sodium (Na^+), phosphorus (P), potassium (K^+) and magnesium (Mg^{2+}).

Chemical analyses

Chemical analysis was carried out in the Central Research Laboratory and Department of Biochemistry, University of Ilorin. Blood metabolites were assayed for in the haematological and clinical chemistry laboratories of the Faculty of Veterinary Medicine, University of Ilorin, Kwara state, Nigeria using appropriate equipment and analytical kits.

Statistical analysis

Data were analysed by analysis of variance (ANOVA) according to the one-way classification design model. Differences between treatment means were separated using Duncan's multiple range test as described by Steel and Torrie (1980).

Results and Discussion

Results on the chemical composition of the minerals, vitamins, amino acids (Tables 3, 4 and 5) in the African star apple seed kernels obtained from the western part of Nigeria confirmed that African star apple tree and products contained reasonable amounts of valuable nutrients and the kernels were particularly high in carbohydrate as a source of energy. This finding suggests that the kernels could serve as a useful alternative energy source in the diet of animals. Findings on the nutritional content of *C. albidum* in this investigation agreed with past reports (Ajewole and Adeyeye, 1991; Edem and Miranda, 2011; Ukana et al., 2012) that the star apple tree, its fruits and seed are high in nutrients which could be used as cheap alternative sources of nutrients in the nutrition of man and domestic animals. Conversely, the phytochemicals (Table 6) present in the seed are high in anti-nutritional or toxic factors, namely flavonoids, saponins, phenolics including tannins, anti-mineral elements (oxalate and phytates), terpenoids as well as the undetermined cyanogens in this study. These phytochemicals have been shown to elicit adverse effects on nutrition and health of man and livestock with possible physiological abnormalities especially when consumed in amount exceeding normal threshold limits (Kersten et al., 1991; Igile, 1996). For example, the effect of saponins on chicks has been reported to reduce growth, feed efficiency and

interfere with the absorption of dietary lipids, cholesterol, bile acids and vitamins A and E respectively (Jenkins and Atwal, 1994).

Table 3. Proximate and mineral composition of African star apple kernel meal.

Parameters	% concentration
Dry matter	91.38 $\pm$ 5.17
Crude protein	8.71 $\pm$ 0.49
Crude fat	3.10 $\pm$ 0.18
Mineral matter	4.79 $\pm$ 0.27
Crude fibre	7.42 $\pm$ 0.42
Soluble carbohydrate	67.36 $\pm$ 3.81
Metabolizable energy (Kcal/kg)	2,939
Nutrient minerals	% concentration
Calcium (Ca)	0.077 $\pm$ 0.00438
Phosphorus (P)	0.46 $\pm$ 0.02602
Potassium (K)	0.038 $\pm$ 0.00014
Magnesium (Mg)	0.008 $\pm$ 0.00042
Sodium (Na)	0.815 $\pm$ 0.04610
Manganese (Mg)	0.062 $\pm$ 0.00353
Iron (Fe)	0.821 $\pm$ 0.04638
Zinc (Zn)	0.075 $\pm$ 0.00424
Chloride (Cl)	0.006 $\pm$ 0.00028

Values for each parameter are taken from an average of two (2) determinants.

Table 4. Vitamin composition of African star apple kernel meal.

Water soluble vitamins (%)	
Vitamin B1 (thiamin)	0.0013 $\pm$ 0.00014
Vitamin B2 (riboflavin)	0.0028 $\pm$ 0.00014
Vitamin B3 (niacin)	0.0080 $\pm$ 0.00042
Vitamin C (ascorbic acid)	0.3983 $\pm$ 0.00042
B-carotene	0.3662 $\pm$ 0.02249
Fat soluble vitamins (%)	
Vitamin A (retinol)	0.693 $\pm$ 0.03917
Vitamin E (tocopherol)	0.957 $\pm$ 0.05416
Vitamin D and K	ND

ND, not detected.

The cyanide ions inhibit several enzyme systems; depress growth through interference with certain essential amino acids and utilization of associated nutrients. They also cause acute toxicity, neuropathy and death (Osuntokun, 1972). Oxalate and phytate bind minerals essential to the body and interfere with their

metabolism in addition to causing muscular weakness, paralysis, gastrointestinal tract irritability, hypocalcaemia, nephrotic lesions in the kidney when consumed in an unprocessed food (Ojiako and Igwe, 2008; Blood and Radostits, 1989). Too much of soluble oxalate in the body prevents the absorption of soluble calcium ions which are bound to form insoluble calcium-oxalate complex resulting to kidney stones (Adeniyi et al., 2009). Tannins in poultry diets reduce dry matter intake, body weight gain, feed efficiency and nutrient digestibility (Hassan et al., 2003)

Table 5. Amino acid profile of African star apple kernel meal.

Amino acid	g/100g sample
Alanine	3.00 ± 0.17
Arginine	3.79 ± 0.21
Aspartic acid	6.30 ± 0.36
Cystine	0.73 ± 0.04
Glutamic acid	7.27 ± 0.41
Glycine	3.42 ± 0.19
Histidine	1.63 ± 0.09
Isoleucine	2.62 ± 0.15
Leucine	7.18 ± 0.41
Lysine	2.86 ± 0.16
Methionine	0.64 ± 0.04
Phenylalanine	3.19 ± 0.18
Proline	3.05 ± 0.17
Threonine	2.30 ± 0.13
Tryptophan	0.68 ± 0.04
Valine	2.98 ± 0.17

Table 6. Quantitative analysis of African star apple kernel meal.

Anti-nutrient	(mg/kg)
Flavonoids	26.54 ± 1.50
Oxalate	0.80 ± 0.05
Phytate	131.00 ± 7.41
Saponins	12.15 ± 0.69
Tannins	1.04 ± 5.88
Terpenoids	1.37 ± 7.75
Total phenolics	7.91 ± 4.48

The result of the impact of feeding ASAKM in the diets of broilers on the performance traits is shown in Table 7. Increasing levels of the raw kernel meal in the broiler's diets resulted in decreased ($p < 0.05$) feed consumption, body weight gain and increased poor feed conversion ratio observed in the birds and these findings corroborate the reports of Igile (1996) that phytochemicals elicit toxic

biological responses with possible physiological implications. Similarly, the reduced weight gains observed in the test groups are in line with the findings of Jenkins and Atwal (1994) that saponins reduce growth, feed efficiency and interfere with the absorption of dietary lipids, cholesterol, bile acids, vitamins A and E in chicks. An early report by Kumar (1992) has also stated that the utility of *C. albidum* in monogastric poultry is limited due to the presence of phytotoxins. The observed insignificant differences between the control diet value and values of mortality rate of ASAKM diets ($P>0.05$) may suggest that poultry could tolerate high inclusion of the test feedstuff in their diet and better if the test ingredient is adequately processed.

Table 7. Performance characteristics of broilers fed raw *C. albidum* kernel meal.

Parameter (%)	Inclusion level of African star apple kernel meal							SEM
	0	5	10	15	20	25	30	
Initial live weight (g/)	99.04	99.04	99.04	99.52	99.52	100.47	99.04	1.09
Final live weight (g)	1910.1 ^a	1255.7 ^b	885.0 ^c	479.2 ^d	474.1 ^d	395.0 ^e	388.83 ^e	4.99*
Weight gain (g/day)	32.34 ^a	20.66 ^b	14.04 ^c	6.78 ^d	6.69 ^d	5.29 ^d	5.15 ^d	0.65*
Feed intake (g/day)	116.87 ^a	99.37 ^b	91.32 ^b	71.40 ^c	69.90 ^c	68.36 ^c	65.23 ^c	1.45*
Feed conversion ratio	3.61 ^d	4.84 ^d	6.63 ^c	10.53 ^b	10.32 ^b	12.84 ^a	13.23 ^a	0.54*
Mortality rate (%)	0.00	0.00	0.33	0.00	0.33	0.33	0.00	0.35NS

SEM = Standard error of mean; NS = Non significant ($P>0.05$); *Significant ($p<0.05$); ^{a,b,c,d,e}Means with different superscripts along the same row are significantly different.

The result on the blood composition is presented in Table 8. There were significant ($p<0.05$) differences in the haematological parameters as the level of the ASAKM increased in the diet except for the mean cell haemoglobin (MCH).

Table 8. Blood composition of broilers fed raw *C. albidum* kernel meal.

Parameters (%)	Inclusion levels of African star apple kernel meal							SEM
	0	5	10	15	20	25	30	
RBC ($\times 10^{12}/l$)	3.12 ^d	3.08 ^d	6.33 ^{ab}	6.52 ^a	5.83 ^c	5.95 ^c	6.09 ^b	0.10*
Hb (g/dl)	6.80 ^d	6.60 ^d	11.80 ^c	12.30 ^{bc}	12.90 ^b	13.20 ^a	13.40 ^a	0.25*
PCV (%)	16.00 ^d	16.00 ^d	35.00 ^a	37.00 ^a	35.00 ^a	36.00 ^a	37.00 ^a	1.25*
MCV (fl)	51.28 ^c	51.94 ^c	55.29 ^b	56.74 ^b	60.03 ^a	60.50 ^a	60.75 ^a	0.95*
MCH (pg)	21.79	21.42	18.6	18.86	22.15	22.35	22.00	0.83NS
MCHC (g/dl)	42.50 ^a	41.25 ^a	33.71 ^{bc}	33.24 ^c	36.85 ^b	36.66 ^b	36.22 ^{bc}	0.90*
WBC ($\times 10^9/l$)	16.10 ^a	15.50 ^a	10.55 ^b	9.52 ^b	8.19 ^c	6.13 ^d	5.89 ^d	0.58*

The haemoglobin (Hb) increased in response to increasing dietary levels of the ASAKM except for the birds at the 5% inclusion level of the test diet. There was a consistent increase in the mean cell volume (MCV) in response to increasing

dietary inclusion levels of the ASAKM. The red blood cell (RBC) and the packed cell volume (PCV) did not follow a particular trend. However, the trend in the values of the haematological parameters revealed that the rich mineral content of the dietary African star apple kernel meal positively influenced haematopoiesis, hence favouring blood production.

Serum biochemical parameters are presented in Table 9. Blood glucose level, serum total protein and globulin increased ($p < 0.05$) as the dietary levels of the African star apple kernel meal increased across the treatments. Similarly, dietary inclusion of the star apple kernel meal at graded levels did not exert any adverse effect on the lipid composition of the broilers as values of the triglyceride, total cholesterol, HDL and LDL diminished concomitantly with increasing dietary levels of ASAKM ($p < 0.05$). Blood electrolytes (Ca^{2+} , Na^+ , K^+ , Cl^- , HCO_3^-) tended to increase with a corresponding increase in ASAKM meal levels in diets suggesting that the test feedstuff contained adequate nutrient minerals to maintain the electrolyte balance in the birds.

Table 9. Serum biochemical indices of broilers fed raw *C. albidum* kernel meal.

Parameter (%)	Inclusion levels of African star apple kernel meal							SEM
	0	5	10	15	20	25	30	
Glucose (mmol/l)	7.10 ^d	7.50 ^c	7.90 ^b	8.10 ^b	8.80 ^a	8.70 ^a	8.90 ^a	0.29*
Total protein (g/l)	31.00 ^c	36.00 ^{bc}	39.00 ^{ab}	40.00 ^{ab}	42.00 ^{ab}	44.00 ^a	45.00 ^a	1.17*
Albumin (g/l)	27.00	28.00	26.00	27.00	24.00	23.00	18.00	1.96NS
Globulin (g/l)	4.00 ^d	8.00 ^d	13.00 ^c	13.00 ^c	18.00 ^b	21.00 ^b	27.00 ^a	1.31*
A/G ratio	8	3	2	2	1	1	0	
Total cholesterol (mmol/l)	2.40 ^a	0.92 ^b	0.40 ^c	0.30 ^{cd}	0.20 ^d	0.40 ^c	0.30 ^{cd}	0.05*
HDL cholesterol (mmol/l)	0.40 ^a	0.30 ^b	0.20 ^c	0.10 ^d	0.10 ^d	0.10 ^d	0.10 ^d	0.02*
LDL cholesterol (mmol/l)	2.10 ^a	0.80 ^b	0.20 ^c	0.20 ^c	0.10 ^c	0.10 ^c	0.10 ^c	0.04*
Triglyceride (mmol/l)	1.30 ^a	0.60 ^b	0.50 ^{bc}	0.50 ^{bc}	0.40 ^c	0.30 ^d	0.30 ^d	0.04*
Calcium (mmol/l)	2.10 ^c	2.10 ^c	2.20 ^{bc}	2.40 ^{bc}	2.30 ^{bc}	2.50 ^b	2.90 ^a	0.27*
Sodium (mmol/l)	138 ^c	140 ^{bc}	145 ^a	140 ^{bc}	138 ^{bc}	143 ^{ab}	142 ^{ab}	0.88*
Potassium (mmol/l)	4.30 ^{cd}	4.40 ^{bcd}	4.50 ^{bc}	4.30 ^{cd}	4.10 ^d	4.70 ^{ab}	4.90 ^a	0.26*
Chloride (mmol/l)	70.00 ^d	83.00 ^c	88.00 ^{bc}	70.00 ^d	75.00 ^d	92.00 ^b	105.00 ^a	1.12*
Bicarbonate (mmol/l)	24.00	25.00	25.00	26.00	25.00	26.00	25.00	0.82NS
AST(IU/L)	14.00 ^g	29.00 ^f	38.20 ^e	59.30 ^d	72.15 ^c	82.00 ^b	94.00 ^a	1.24*
ALT(IU/L)	7.00 ^f	16.00 ^e	50.20 ^d	57.60 ^c	60.91 ^c	64.31 ^b	69.00 ^a	1.84*
ALP(IU/L)	104.6 ^f	124.7 ^e	156.5 ^d	160.8 ^d	197.6 ^a	183.7 ^c	189.20 ^b	0.95*
Urea (mmol/l)	1.20 ^d	1.30 ^{cd}	1.40 ^{bcd}	1.40 ^{bcd}	1.60 ^{abc}	1.70 ^{ab}	1.80 ^a	0.08*
Creatinine (mmol/l)	0.74 ^a	0.41 ^b	0.37 ^{bc}	0.35 ^{bc}	0.27 ^c	0.25 ^c	0.13 ^d	0.03*

A/G = Albumin globulin ratio; HDL = High density lipid; LDL = Low density lipid; ^{a,b,c,d,e,f} Means with different superscripts along the same row are significantly different.

Activities of AST, ALT and ALP were elevated as the quantity of raw African star apple kernel meal increased in diet ($p < 0.05$), which may attest to the fact that phytotoxins in the untreated kernel meal cause high enzyme activities in the blood. The increment in ALT activity as recorded in diets 2 to 7 compared with the control diet suggests the likelihood of liver damage by direct feeding of the raw kernel meal. Similarly, a significant increase in the serum urea levels of the birds fed the test ingredient is an indication of poor utilization of protein in the ASAKM. Based on results of the performance characteristics and the blood metabolites, broilers could barely tolerate 5% raw ASAKM meal diets. The appropriate processing method is therefore recommended.

Conclusion

The findings from this study imply that the birds could barely tolerate beyond 5% inclusion level of the ASAKM, based on the result of the performance characteristics, however, the haematological parameters revealed that the rich mineral content of the dietary African star apple kernel meal positively influenced erythropoiesis. There is the need to adequately process the ASAKM for optimum utilization by the birds.

References

- Adeniyi, S.A., Orjiekwe, C.L., & Ehiagbonare, J.E. (2009). Determination of alkaloids and oxalates in some selected food samples in Nigeria. *African Journal of Biotechnology*, 8 (1), 110-112.
- Adewusi, H.G., & Bada, S.O. (1997). Preliminary information on the Ecology of *Chrysophyllum albidum* in West and Central Africa. *Proceeding of a national workshop on the potentials of the star apple in Nigeria*. (pp. 16-25). Ibadan, Nigeria.
- Ajewole, K., & Adeyeye, A. (1991). Seed oil of white star apple (*Chrysophyllum albidum*) physiochemical characteristics and fatty acid composition, *Journal of Science, Food and Agriculture*, 54, 313-315.
- Amaefule, K.U., Okechukwu, S.O., Ukachukwu, S.N., Okoye, F.C., & Onwudike, O.C. (2006). Digestibility and nutrient utilization of pigs fed graded levels of brewers dried grain based diets. *Livestock Research and Rural Development*, 18, 1-10.
- Annongu, A.A., & Abimbola, S. F. (2003). Biochemical evaluation of Gmelina arborea fruit meal as swine feedstuff. *Biokemistri* 15 (1), 1-6.
- Blood, D.C., & Radostits, O.M. (1989). *Veterinary Medicine*, 7th Edition, Ballieretindall, London, pp. 589-630.
- Edem, C.A. & Miranda, I.D. (2011). Chemical evaluation of proximate composition, ascorbic acid and anti-nutrients content of African star apple (*Chrysophyllum africanum*) fruit. *IJRRAS*, 9 (1), 146-149.
- Hassan, I.A., Elzuber, E.A., & Tinay, H.A. (2003). Growth and apparent absorption of minerals in broiler chicks fed diets with low or high tannin content. *Tropical health production*, 35, 189-196.
- Igile, G.O. (1996). Phytochemical and biological studies on some constituents of *Vernonia amygdalia* leaves. Ph.D. thesis, dept. of biochemistry, University of Ibadan, Nigeria.

- Jenkins, K.J., & Atwal, A.S. (1994). Effects of dietary saponins on faecal bile acids and neutral sterols and availability of vitamins A and E in the chick. *Journal of Nutritional Biochemistry*, 5, 134-137.
- Kersten, G.F., Spiekstra, A., Beuvery, F.C., & Crommelin, D.J. (1991). On the structure of immune-Stimulating saponin-lipid complexes (iscoms), *Biochimicaet Biophysica Acta*, 1062 (2), 165-171.
- Kumar, R. (1992). Antinutritional factors, the potential risks of toxicity and methods to alleviate them. In Andrew Speedy and Pierre-Luc Puglise (eds). *Proceedings of the FAO Expert Consultation* held at the Malaysian Agricultural Research and Development Institute (MARDI) (pp. 14-18). Kuala lumpur, Malaysia.
- Ojiako, O.A., & Igwe, C.U. (2008). The nutritive, anti- nutritive and hepatotoxic properties of *Trichosanthes anguina* (snake tomato) fruits from Nigeria. *Pakistan Journal of Nutrition*, 7, 85-89.
- Okafor, J.C. (1981). Woody plants of nutritional importance in traditional farming system of the Nigeria humid tropics. Ph.D. dissertation. University of Ibadan, Nigeria.
- Okigbo, B.N. (1978). *Cropping systems and related research in Africa*. Association for the Advancement of Agricultural Sciences in Africa (AASA) 10th Anniversary issue.
- Osuntokun, B.O. (1972). Cassava diet and cyanide metabolism in Wistar rats. *British Journal of Nutrition*, 24, 797-805.
- Steel, R.G.D., & Torrie, J.H. (1998). *Principles and Procedures of statistics, a biometrical approach* (2ndEdn.). McGraw-Hill, New York.
- Ukana, D.A., Aniekan, E.A., & Godwin, N.E. (2012). Evaluation of proximate compositions and mineral elements in the star apple peel, pulp and seed. *Journal of Basic Applied Sciences Research*, 2 (5), 4839-4843.

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KORIŠĆENJE SAČME OD SEMENA AFRIČKOG HRIZOFILUMA
(*CHRYSOPHYLLUM ALBIDUM*) U ISHRANI BROJLERA

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R e z i m e

U osmonedeljnom eksperimentu ispitivan je uticaj različitih nivoa (0, 5, 10, 15, 20, 25 i 30%) netretirane sačme od semena hrizofiluma (engl. *African star apple kernel meal* – ASAKM) u ishrani brojlera na proizvodne parametre i profil krvi. Eksperiment je postavljen po modelu slučajnog plana, sa dve stotine i deset jednonedeljnih brojlerskih pilića (hibrid *Cobb*), raspoređenih u sedam tretmana (obroka), po deset pilića u tretmanu i sa tri ponavljanja za svaki tretman. Ispitivane su količine minerala, vitamina, amino kiselina i nekih antinutritivnih materija. Rezultati su pokazali da je seme bogato hranljivim materijama, posebno ugljenim hidratima kao izvorom energije, ali da sadrži i visoke nivoe antinutritivnih ili toksičnih faktora. Upotreba sačme od afričkog hrizofiluma uticala je negativno na proizvodne rezultate ($p < 0,05$), ali bez smrtnosti pilića. Povećanje nivoa netretirane sačme od semena afričkog hrizofiluma u ishrani nije negativno uticalo na sastav krvi i većinu praćenih biohemijskih parametara, koji su bili bolji ($p < 0,05$) ili su imali vrednosti statistički slične tretmanu sa kontrolnim obrokom ($p > 0,05$). Povećanje nivoa sačme od semena afričkog hrizofiluma u obroku izazvalo je porast AST, ALT, ALP i značajno povećanje nivoa uree u krvi za obrok sa svežom sačmom u odnosu na kontrolni obrok ($p < 0,05$). Na osnovu produktivnosti ptica kojima je davana sveža sačma od semena afričkog hrizofiluma, moglo bi se zaključiti da brojleri mogu tolerisati najviše do 5% ovog hraniva, sa minimalnim neželjenim efektima. Međutim, vrednosti parametara krvi, posebno crvenih krvnih zrnaca koje sugerišu na favorizovanje eritrocitopoeze, ukazuju da sačma od semena afričkog hrizofiluma nije štetno uticala na zdravlje ptica. U narednim istraživanjima treba koristiti obrađenu sačmu u cilju dobijanja optimalnih rezultata.

Ključne reči: ASAKM, živina, proizvodni rezultati, biohemijski i hematološki parametri seruma.

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EFFECTS OF MUCUNA SEED EXTRACT ON SPERM FUNCTIONAL
INTEGRITIES AND SEMINAL OXIDATIVE STRESS
INDICES OF VITRIFIED GOAT SEMEN

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Abstract: Effects of mucuna seed extract (MSE) in tris-extenders on sperm functional integrities and seminal oxidative stress indices of vitrified sperm of West African Dwarf (WAD) goat bucks were investigated. Varying levels (0, 0.25, 0.5, 0.75, 1 g/100 mL of diluent) of MSE in tris-extenders were diluted with semen specimens. Diluted semen specimens were vitrified and preserved in liquid nitrogen. The results showed that sperm functional integrities (acrosome and membrane integrities) in MSE extenders were comparable. However, seminal oxidative stress indices (malondialdehyde [MDA] and acrosin activity) differed ($P < 0.05$) among the various treatments and the control. The results revealed that semen vitrified with MSE had reduced ($P < 0.05$) MDA concentrations compared to the control. Higher ($P < 0.05$) acrosin activities were recorded in semen vitrified with MSE compared to the control except at 0.25g MSE and optimal values were recorded at 1g MSE. The findings indicated that MSE in the tris-extenders reduced MDA concentration with congruent elevated acrosin activity and consequently maintained sperm functional integrities of vitrified spermatozoa of the WAD goat.

Key words: antioxidant, freezing, mucuna, oxidative stress, sperm integrity.

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Introduction

Cryopreservation is a major development made in artificial insemination to preserve sperm cells in order to multiply important genetic material in species of economic interest (Blash et al., 2005). The utilization of this proven technology to preserve sperm cells as a method of improving goat production (*Capra hircus*) is necessary to alleviate the problem of protein malnutrition. The protocols of cryopreservation, however, can cause harmful effects on sperm viability (Pegg, 2007; Bagchi et al., 2008). The conventional constituents of semen extenders such as milk and egg yolk are animal products (Correa et al., 2006; Purdy, 2006) and they are sometimes susceptible to microbial damage. In addition, an enzyme that coagulates egg yolk in the bulbourethral gland (Iritani and Nishikawa, 1961) or SBUIII (a protein fraction) that interacts with milk components in extenders causes reduced viability of buck sperm cells (Leboeuf et al., 2000). Therefore, semen diluents void of these constraints could be a possible way to preserve goat semen. Inclusion of plant products like fruit juices, coconut water and coconut milk as constituents of semen extenders has recently been proved to improve the quality of cryopreserved spermatozoa (Daramola et al., 2016 a, b, c). The seeds of *Mucuna pruriens* are sources of important compounds such as antioxidants (Sharma et al., 1978). The seeds contain flavonoids, magnesium, copper, zinc, manganese and iron (Misra and Wagner, 2007). Daramola et al. (2015) recently investigated the potential effect of *Mucuna pruriens* seed powder on spermograms of West African Dwarf goats and observed improved testicular measurements and sperm quality. However, the protective effects of MSE on stored semen have not been reported. The objective of this experiment was therefore to assess the effects of MSE in extenders on sperm functional integrities and oxidative stress indices of WAD goats (*Capra hircus*) during vitrification.

Materials and Methods

Experimental location and animal management

The Directorate of University Teaching and Research Farm, Federal University of Agriculture, Abeokuta in south-western part of Nigeria was used for the research. The animals used for this study consisted of four WAD goat bucks aged 3–5 years. The bucks had access to the concentrate feed and guinea grass (*Panicum maximum*) under intensive system.

Preparation of mucuna seed extract

Dried seeds of *Mucuna pruriens* were ground into granules. The granules (518g) were then soaked in 1750 mL of methanol for 17 hours with intermittent

stirring. The soaked mucuna granules were filtered and the resultant extract solution was concentrated on a water bath by evaporation at 54°C. The extract was then transferred into a beaker for use with a tris-based extender.

Semen collection, dilution and vitrification

An artificial vagina was used to collect semen specimens from the bucks. A total of four semen specimens from the bucks were pooled (Bucak and Tekin, 2007). The pooled semen specimens were extended at room temperature using a tris-based extender. The specimen was divided into 5 equal portions, diluted with varying levels of MSE (0, 0.25, 0.50, 0.75, 1.00g/100 mL of the diluent) in tris-extenders. The tris-extenders consisting of 2 M tris-hydroxymethyl-aminomethane (2.42 g), 0.5 M glucose (1 g), 0.0005 M penicillin (0.028 g), egg yolk (20 ml) and distilled water made up 100 ml and were supplemented with the varying levels of MSE. The diluted semen specimens were vitrified at room temperature using separate cryoprotectants as described by Srirattana et al. (2013) with some changes. Phosphate-buffered saline (PBS) was supplemented with bovine serum albumin (20%) (v/v) as a holding medium (HM). Ethylene glycol (EG), dimethyl sulfoxide (DMSO) and HM were used to prepare vitrification solution I (VS-I) and vitrification solution II (VS-II), respectively. VS-I was made by mixing 0.2 M EG (12.5%), 0.15 M DMSO (12.5%) and the HM in the ratio of 1:1:6 while VS-II was made by mixing 0.4 M EG (25%), 0.3 M DMSO (25%) and the HM in the ratio of 1:1:2. The semen specimen previously diluted was added to the separate cryoprotectants sequentially for each treatment. The specimen (1 mL) was first added to 1 mL of HM for 10 min. The mixture (diluted semen specimen + HM) was then placed in 1 mL of VS-I for 4 min, and the mixture (diluted semen specimen + HM + VS-I) was finally put into 1 mL of VS-II for 1 min. Following equilibration in VS-II, the specimen was loaded into 0.2 mL straws and treatment was replicated four times (4 straws per treatment) and preserved in liquid nitrogen for 7 days.

Evaluation of sperm functional indices

Following storage for 7 days, thawing of the straws was carried out in a water bath at 38°C and assessed for acrosome integrity and membrane integrity. Assessment of acrosome integrity was carried out in a formalin citrate solution consisting of 2.9% sodium citrate (96 mL) and 37% formaldehyde (4 mL) as described by Ahmad et al. (2003) to identify the acrosome that showed a normal apical ridge of sperm cells. Membrane integrity of the sperm cells was evaluated in a hypo-osmotic solution consisting of 9 g fructose plus 4.9 g sodium citrate mixed with 1000 mL of distilled water as described by Zubair et al. (2013) to

identify swelled spermatozoa with a characteristic coiled tail as the intact plasma membrane.

Evaluation of seminal oxidative stress indices

The malondialdehyde concentrations in the preserved semen were evaluated using a thiobarbituric acid reactive substance as described by Pipan et al. (2014) while evaluation of the acrosin activity using the specific substrate for acrosin consisting of N-a-benzoyl-DL-arginine *p*-nitroanilide (BAPNA) was carried out as described by Rosatti et al. (2004).

Statistical analysis

Estimations were performed for the stored semen specimens for each treatment consisting of four straws and four different measurements made for each straw (n=16). One-way analysis of variance in the SAS 2002 package was used to analyze the data obtained and all significant means were separated by Duncan's multiple range test (Duncan, 1955).

The model used for the experiment included:

$$Y_{ij} = \mu + E_i + \sum_{ij},$$

where Y_{ij} = Dependent variables,

μ = Population mean,

E_i = Effect due to the j^{th} level of MSE inclusion (0, 0.25, 0.50, 0.75, 1.00),

$\sum_{ij}$ = Experimental error.

Results and Discussion

The sperm functional integrities (acrosome and membrane integrities) of WAD goat semen vitrified with MSE are presented in Figures 1 and 2, respectively. The results showed that the acrosome integrity and membrane integrity in MSE extenders were comparable. However, seminal oxidative stress indices such as malondialdehyde and acrosin activity differed ($P < 0.05$) among the various treatments and the control (Figures 3 and 4). The results revealed that semen vitrified with MSE had reduced ($P < 0.05$) malondialdehyde concentrations compared to the control (Figure 3) while higher ($P < 0.05$) acrosin activities were observed in semen vitrified with MSE compared to the control except at 0.25g MSE and optimal values were recorded at 1g MSE (Figure 4).

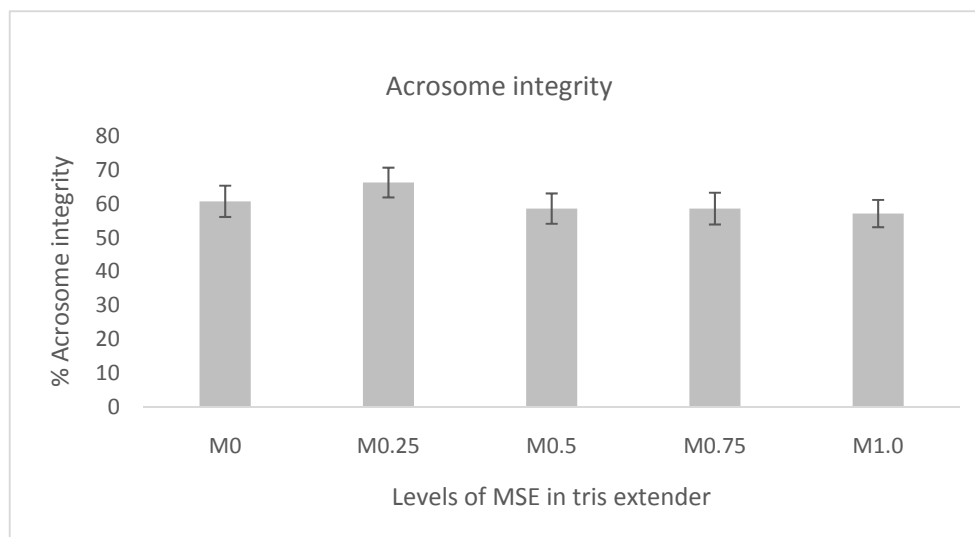


Figure 1. Acrosome integrities of semen vitrified with MSE extenders (n=16).
MSE = Mucuna seed extract.

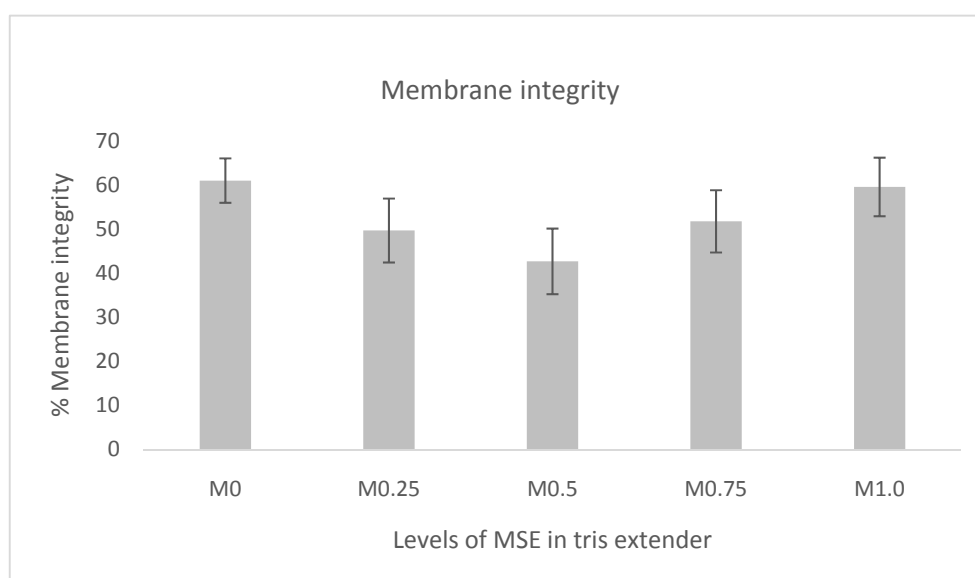


Figure 2. Membrane integrities of semen vitrified with MSE extenders (n=16).
MSE = Mucuna seed extract.

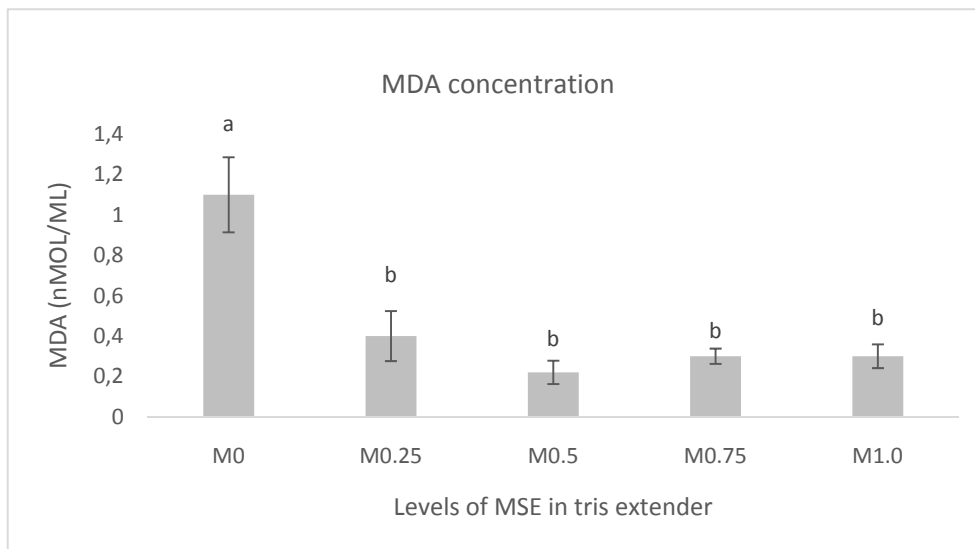


Figure 3. Malondialdehyde concentrations of semen vitrified with MSE extenders (n=16).

^{a,b} Means with different superscripts differ significantly ($P < 0.05$).
MSE = Mucuna seed extract; MDA = Malondialdehyde.

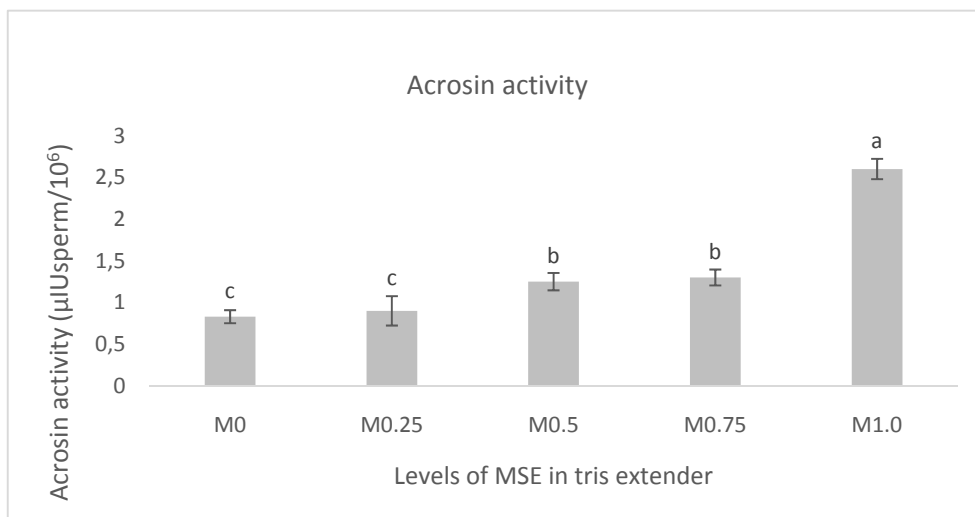


Figure 4. Acrosin activity of semen vitrified with MSE extenders (n=16).

^{a,b,c} Means with different superscripts differ significantly ($P < 0.05$).
MSE = Mucuna seed extract.

The reduced MDA and congruent elevated acrosin activity in extenders supplemented with MSE might probably be due to protective action of the antioxidant in *Mucuna pruriens* seed against oxidative stress during the freezing process. *Mucuna pruriens* seeds contain many bioactive components such as alkaloids, coumarins, flavonoids and alkylamines which perform an important function in elevating the antioxidant ability (Misra and Wagner, 2007). In addition, seeds of *Mucuna pruriens* have antioxidant, lipid reducing and neuroprotective functions (Sharma et al., 1978; Longhi et al., 2011). Moreover, phenolic substances have high antioxidant potential that is attributed to their ability to reduce oxides, which perform a vital function in the adsorption or neutralization of free radicals (Basile et al., 2005). The total phenolic content (24 ± 0.2 g/100 g) that translates to 6.48 g of total phenolic in 100 g of mucuna seed has been observed in *M. pruriens* extract (Longhi et al., 2011). *M. pruriens* with its characteristic hydrogen-donating ability possesses an excellent reducing power and its extract has been observed to possess the potential scavenging activity on the free radical-scavenging activity (Longhi et al., 2011).

Production of free radicals and reactive oxygen species during the oxygen metabolism leads to body cell damage (Grace, 1994). Although the mechanisms by which free radicals obstruct activities of cells have not been well elucidated yet, cell membrane damage by lipid peroxidation seems to be an important event to reckon with (Nijveldt et al., 2001; Lobo et al., 2010). Flavonoids like other antioxidants exert their effects on cell functions by different mechanisms including electron donation, metal ion chelating, co-antioxidants, or by gene expression regulation (Krinsky, 1992; Misra and Wagner, 2007). Oxidation of flavonoids by radicals leads to a more stable, less reactive radical that stabilizes the reactive oxygen species (Nijveldt et al., 2001). The high reactivity of the hydroxyl group of the flavonoids makes radicals inactive (Korkina and Afanas'ev, 1997). The protective action on spermatozoa in this study could therefore be linked to the antioxidant property of flavonoids in *M. pruriens* (Misra and Wagner, 2007).

The protective action of extenders supplemented with MSE on spermatozoa in this study could further be linked to the antioxidant property of metals such as magnesium, copper, zinc, manganese and iron that are abundant in MSE (Misra and Wagner, 2007). Metal ions perform important functions in different cellular activities including homeostasis (Bertini and Cavallaro, 2008). *M. pruriens* is rich in copper (Misra and Wagner, 2007), and as an antioxidant, copper scavenges or neutralizes free radicals and helps to reduce some of the damage they cause (Araya et al., 2006; Bonham et al., 2002). The role of copper as an antioxidant supported by higher content of MDA compared to copper deficiency and reduced MDA with the increasing copper intake has been reported (Duan et al., 2010). Furthermore, copper as an antioxidant acts as a cofactor of some enzymes, such as Cu/Zn SOD with a protective effect on spermatozoa against peroxidative damage of its cellular

enzymes and structures from reactive oxygen species (Bray and Bettger, 1990; Menella and Jones, 1991; Sikka, 2001; Tabassomi and Alayi-Shoushtari, 2013). Reports have revealed that copper ion is important for optimal superoxide dismutase activity that leads to less free radical production during spermatogenesis (Abdul-Rasheed, 2010).

Moreover, in addition to copper as an antioxidant, findings suggest that zinc has antioxidant properties and protects cells from oxidative damage (Ho and Ames, 2002; Sakaguchi et al., 2002). Zinc performs a vital role in cell functions and as an important constituent of several proteins involved in the defense against oxidative stress (Song et al., 2009). The mechanisms by which zinc performs antioxidative activity involve the protection of sulfhydryl groups of proteins and reduction of free radical formation through the prevention mechanisms or antagonism of redox-active transition metals, such as iron and copper (Bray and Bettger, 1990). The synergetic antioxidant property of zinc and copper in MSE probably contributed to the survival of spermatozoa cryopreserved in this study.

Frozen spermatozoa are susceptible to reactive oxygen species and lipid peroxidation produced in sperm metabolism (Watson, 2000). The protective effect of antioxidants on the plasma membrane of frozen sperm has been observed in both metabolic activity and cellular viability (Cheema et al., 2009). Supplementation of manganese (Mn) in a semen extender during freezing of semen revealed a protective ability and explained the increase for sperm quality characteristics and the decrease in malondialdehyde concentration due to its chain breaking antioxidant property and inhibitory effect on oxidative stress damage (Cheema et al., 2009). The importance of Mn^{2+} has been observed in cryopreserved bull semen, and the supplementation of an egg-yolk-citrate extender with Mn^{+} glycerol dilutor was found to improve the quality/fertility of cryopreserved semen (Barber et al., 2005). The protective action of extenders supplemented with MSE against reactive oxygen species and lipid peroxidation damage on spermatozoa in this study could further be linked to the antioxidant property of Mn in MSE.

The sustained integrities (acrosome and membrane) in this study could also be attributed to important components of mucuna seeds that are necessary for cell survival in freezing conditions. Mucuna seed is rich in magnesium (Misra and Wagner, 2007). The inclusion of magnesium fumarate in Biosolvens extender improves sperm quality in boar semen (Szczęśniak-Fabiańczyk et al., 2003). Magnesium is important for the synthesis of different substances that have energy-rich bonds in form of adenosine triphosphate (Durlach, 1988). Insufficient magnesium makes a cell unable to keep up the appropriate number of high-energy molecules (adenosine triphosphate) to maintain cell function. The sustained viability of the spermatozoa subjected to vitrification in this study could be due to the magnesium stabilizing effect on the membrane and functional integrity of sperm cells (Durlach, 1988).

Conclusion

The findings revealed that MSE in the tris-extenders reduced MDA concentration with congruent elevated acrosin activity and consequently maintained sperm functional integrities of cryopreserved spermatozoa of the WAD goat. The study therefore indicated the protective effect of MSE against oxidative stress damage due to the components of its antioxidant. Vitrified semen specimens of WAD goat bucks with MSE in the tris-extenders would be good enough for a successful artificial insemination programme.

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References

- Abdul-Rasheed, O.F. (2010). Association between seminal plasma copper and magnesium levels with oxidative stress in Iraqi infertile men. *Oman Medical Journal*, 25 (3), 168-172.
- Ahmad, Z., Anzar, M., Shahab, N., Ahmad, S., & Andrabi, M.H. (2003). Sephadex and sephadex ionexchange filtration improves the quality and freezability of low-grade buffalo semen ejaculates. *Theriogenology*, 59, 1189-1202.
- Araya, M., Pizarro, F., Olivares, M., Arredondo, M., Gonzalez, M., & Méndez, M. (2006). Understanding copper homeostasis in humans and copper effects on health. *Biological Research*, 39, 183-187.
- Bagchi, A., Woods, E.J., & Critser, J.K. (2008). Cryopreservation and vitrification: Recent Advances in fertility preservation technologies. *Expert Review Medical Devices*, 5, 359-370.
- Barber, S.J., Parker, H.M., & McDaniel, C.D. (2005). Broiler breeder semen quality as affected by trace minerals in vitro. *Poultry Science*, 1, 100-105.
- Basile, A., Ferrara, L., Del Pozzo, M., Mele, G., Sorbo, S., Bassi, P., & Montesano, D. (2005). Antibacterial and antioxidant activities of ethanol extract from *Paullinia cupana* Mart. *Journal Ethnopharmacology*, Philadelphia, 102 (1), 32-36.
- Bertini, I., & Cavallaro, G. (2008). Metals in the "omics" world: copper homeostasis and cytochrome c oxidase assembly in a new light. *Journal of Biological Inorganic Chemistry*, 13, 3-14.
- Bonham, M., Jacqueline, M., Bernadette, M.H., & Strain, J.J. (2002). The immune system as a physiological indicator of marginal copper status? *British Journal of Nutrition*, 87, 303-403.
- Bray, T.M., & Bettger, W.J. (1990). The physiological role of zinc as an antioxidant. *Free Radical Biology and Medicine*, 281-291.
- Bucak, M.N., & Tekin, N. (2007). Protective effect of taurine, glutathione and trehalose on the liquid storage of ram semen. *Small Ruminant Research*, 73, 103-108.
- Cheema, R.S., Bansal, A.K., & Bilaspuri, G.S. (2009). Manganese provides antioxidant protection for sperm cryopreservation that may offer new consideration for clinical fertility. *Oxidative Medicine and Cellular Longevity*, 2 (3), 152-9.
- Correa, M.N., Lucia, T., Bianchi, I., Schmitt, E., Bordignon, J., Rech, D.C., Peruzzo, I.A., & Deschamps, J.C. (2006). Swine semen cooled at 5°C with PIGPEL-5 extender: effects on semen quality in vitro and fertility estimators in vivo. *Animal Reproduction*, 3, 41-48.

- Daramola, J.O., Adekunle, E.O., Oke, O.E. Ogundele, O., Saanu, E.A., & Odeyemi, A.J. (2015). Effect of vitamin E on sperm and oxidative stress parameters of West African Dwarf goat bucks. *Tropical and Subtropical Agroecosystems*, 19:151-158.
- Daramola, J.O., Adekunle, E.O., Onagbesan, O.M., Oke, O.E., Ladokun, A.O., Abiona, J.A., Abioja, M.O., Oyewusi, I.K., Oyewusi, J.A., Isah, O.A., Sogunle, O.M., & Adeleke, M.A. (2016a). Protective effects of fruit-juices on sperm viability of West African Dwarf goat bucks during cryopreservation. *Animal Reproduction*, 13 (1), 7-13.
- Daramola, J.O., Adekunle, E.O., Oke, O.E., Onagbesan, O.M., Oyewusi, I.K., & Oyewusi, J.A. (2016b). Effects of coconut (Cocos nucifera) water with or without egg-yolk on viability of cryopreserved buck spermatozoa. *Animal Reproduction*, 13 (2), 57-62.
- Daramola, J.O., Adekunle, E.O., Iyasere, O.S., Oke, O.E., Sorongbe, T.A., Iyanda, O.A., Kehinde, A.R., Aluko, S.P., Olaoye, I.O., Gbadebo, O.E., Falolu, L.I., Olukayode, E.O., Ajayi, R.A., Enikannaye, O.J., & Osunjaiye, E.D. (2016c). Effects of coconut milk alone or supplementation with pyridoxine in tris-extenders on viability of buck spermatozoa during vitrification. *Small Ruminant Research*, 136, 208-213.
- Duan, L., Cheng, Y., & Jin, Y. (2010). Effect of copper intake and copper-zinc ratio on rat lipid peroxidation in copper deficiency. *Journal of hygiene research*, 39, (1) 25-28.
- Duncan, B.D. (1955). Multiple range test and multiple F-test. *Biometrics*, 11, 1-42.
- Durlach, J. (1988). Magnesium in clinical practice. Published in 1988 by John Libbey & Company Ltd, 80/84 Bondway, London SW8 1SF, England (01) 582 5266. Pg 1-39. Available at: <http://www.mgwater.com/durex01.shtml>
- Grace, P.A. (1994). Ischaemia-reperfusion injury. *British Journal of Surgery*, 81, 637-47.
- Ho, E., & Ames, B.N. (2002). Low intracellular zinc induces oxidative DNA damage, disrupts p53, NFkappa B, and AP1 DNA binding, and affects DNA repair in a rat glioma cell line. *Proc Natl Acad Sci USA*. 99: 16770-5.
- Iritani, A., & Nishikawa, Y. (1961). Studies on the egg-yolk coagulating factors in goat semen: II Properties of the coagulating factor and influential conditions for coagulation. In: Proceedings of Silver Jubilee Lab. Anim. Husbandry, (pp. 97-104) Kyoto University.
- Korkina, L.G., & Afanas'ev, I.B. (1997). Antioxidant and chelating properties of flavonoids. *Advance Pharmacology*, 38, 151-63.
- Krinsky, N.I. (1992). Mechanism of action of biological antioxidants. *Proceedings of Society for the Experimental Biology and Medicine*, 200, 248-54.
- Leboeuf, B., Restall, B., & Salomon, S. (2000). Production and storage of goat semen for Artificial insemination. *Animal Reproduction Science*, 62, 113-141.
- Lobo, V., Patil, A., Patak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, 4 (8):118-26.
- Longhi, J.G., Perez, E., Jair José de Lima, J.J., & Cândido, L.M.B. (2011). *In vitro* evaluation of *Mucuna pruriens* (L.) DC. antioxidant activity Brazilian Journal of Pharmaceutical Sciences. *Brazilian Journal of Pharmaceutical Sciences*, 47 (3).
- Menella, M.R.F., & Jones, R. (1991). Properties of superoxides in metal-ion-catalysed lipid peroxidation reaction in semen. *Biochemical Journal*, 80, 289-297.
- Misra, L., & Wagner, H. (2007). Extraction of bioactive principle from *Mucuna pruriens* seeds. *Indian Journal Biochemistry and Biophysics*, 44, 56-60., 44, 56-60.
- Nijveldt, R.J., Nood, E., Hoorn, D.E.C., Boelens, P.G., Norren, K., & Leeuwen, P.A.M. (2001). Flavonoids: a review of probable mechanisms of action and potential applications. *American Journal of Clinical Nutrition*, 74, 418-25.
- Pegg, D.E. (2007). Principles of cryopreservation. *Methods in Molecular Biology*, 368, 39-57.
- Pipan, M.Z., Mrkun, J., Kosec, M., Svete, A.N., & Zrimšek, P. (2014). Superoxide dismutase: A predicting factor for boar semen characteristics for short-term preservation. *Biomedical Research International*, 2014, 105280.
- Purdy, P.H. (2006). A review on goat sperm cryopreservation. *Small Ruminant Research*, 63, 215-225.

- Rosatti, M.I., Beconi, M.T., & Córdoba, M. (2004). Proacrosin-acrosin activity in capacitated and acrosome reacted sperm from cryopreserved bovine semen. *Biocell*, 28 (3) Mendoza ago. /dic.
- Sakaguchi, S., Iizuka, Y., Furusawa, S., Ishikawa, M., Satoh, S., & Takayanagi, M. (2002). Role of Zn (2+) in oxidative stress caused by endotoxin challenge. *European Journal of Pharmacology*, 451, 309-16.
- Sharma, M.L., Chandhoke, N., Ray Ghatak, B.J., Jamwal, K.S., Gupta, O.P., & Singh, G.B. (1978). Pharmacological screening of Indian medicinal plants. *Indian Journal of Experimental Biology*, 16, 228-35.
- Sikka, S.C. (2001). Relative impact of oxidative stress on male reproductive function. *Current Medicinal Chemistry*, 8, 851-862.
- Song, Y., Leonard, S.W., Traber, M.G., & Ho, F. (2009). Zinc Deficiency Affects DNA Damage, Oxidative Stress, Antioxidant Defenses, and DNA Repair in Rats. *Journal of Nutrition*, 139, 1626-1631.
- Srirattana, K., Sripunya, N., Sangmalee, A., Imsoonthornruksa, S., Liang, Y., Ketudat-Cairns, M., & Parnpai, R. (2013). Developmental potential of vitrified goat oocytes following somatic cell nuclear transfer and parthenogenetic activation. *Small Ruminant Research*, 112 (1-3), 141-146.
- Szcześniak-Fabiańczyk, B., Bochenek, M., Smorag, Z., & Ryszka, F. (2003). Effect of antioxidants added to boar semen extender on the semen survival time and sperm chromatin structure. *Reproductive Biology*, 3 (1), 81-7.
- Tabassomi, M., & Alayi-Shoushtari, S.M. (2013). Effects of *in vitro* copper sulphate supplementation on the ejaculated sperm characteristics in water buffaloes (*Bubalus bubalis*). *Veterinary Research Forum*. Winter, 4 (1), 31-36.
- Watson, P.F. (2000). The causes of reduced fertility with cryo-preserved semen. *Animal Reproduction Science*, 61, 481-492.
- Zubair, M., Akbar Lodhi, L., Ahmad, E., & Muhammad, G. (2013). Osmotic swelling test asscreening for evaluation of semen of bull. *Journal of Entomology and Zoology Studies*, 1 (6), 124-128. from <http://www.entomoljournal.com>

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UTICAJI EKSTRAKTA DOBIJENIH IZ SEMENA STIZOLOBIUMA NA
FUNKCIONALNI INTEGRITET I INDEKSE OKSIDATIVNOG STRESA KOD
VITRIFIKOVANE SPERME JARČEVA

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R e z i m e

Istraženi su uticaji ekstrakta dobijenih iz semena stizolobiuma (engl. *mucuna seed extract* – MSE) u tris-razređivačima na funkcionalni integritet i indekse oksidativnog stresa kod vitrifikovane sperme jarčeva zapadnoafričkih patuljastih koza (engl. *West African Dwarf* – WAD). Sperma je razređena različitim količinama (0, 0,25, 0,5, 0,75, 1 g/100 mL razređivača) ekstrakta iz semena stizolobiuma u tris-razređivačima. Razblaženi uzorci sperme su vitrifikovani i čuvani u tečnom azotu. Rezultati su pokazali da su funkcionalni integriteti sperme (integriteti akrozoma i membrane) u razređivačima sa ekstraktom iz semena stizolobiuma uporedivi. Međutim, indeksi oksidativnog stresa sperme (aktivnosti malondialdehida [MDA] i akrozina) razlikovali su se ($P < 0,05$) među različitim tretmanima i kontrolom. Rezultati su pokazali da je u spermi vitrifikovanoj ekstraktom iz semena stizolobiuma smanjena ($P < 0,05$) koncentracija malondialdehida u poređenju sa kontrolom. Više ($P < 0,05$) aktivnosti akrozina zabeležene su kod sperme vitrifikovane ekstraktom iz semena stizolobiuma u poređenju sa kontrolom osim kod 0,25g MSE i optimalne vrednosti su bile zabeležene kod 1g MSE. Rezultati su ukazali da ekstrakt iz semena stizolobiuma u tris-razređivačima smanjuje koncentraciju malondialdehida sa kongruentnom povišenom aktivnošću akrozina i kao posledica toga održava se funkcionalni integritet vitrifikovane sperme kod jarčeva zapadnoafričkih patuljastih koza.

Ključne reči: antioksidans, zamrzavanje, stizolobium, oksidativni stres, integritet sperme.

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POGODNOST RAZLIČITIH PRAJMER A ZA SPECIFIČNU MOLEKULARNU DETEKCIJU *MONILINIA* SPP.

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Rezime: Vrste roda *Monilinia* su ekonomski značajni patogeni jabučastih i koštičavih vrsta voćaka. U Srbiji su prisutne četiri vrste ovog roda: *M. fructigena*, *M. laxa*, *M. fructicola* i *Monilia polystroma*. Detekcija i identifikacija vrsta roda *Monilinia* je složena, tako da je cilj ovog rada bio da se ispita i standardizuje brza i efikasna metoda molekularne detekcije korišćenjem različitih prajmera u PCR metodi i da se utvrdi njihova specifičnost i mogućnost korišćenja u identifikaciji *Monilinia* spp. U radu su korišćeni izolati *M. fructigena*, *M. laxa*, *M. fructicola* i *M. polystroma* poreklom iz plodova jabuke prikupljenih u Srbiji, kao i referentni izolati iz Italije i Japana. Specifična molekularna detekcija *M. laxa* postignuta je korišćenjem prajmera ITS1Mlx/ITS4Mlx i Ml-Mfg-F2/Ml-Mfc-R1, a vrste *M. fructicola* korišćenjem prajmera ITS1Mfcl/ITS4Mfcl i Mfc-F1/Mfc-R1. Prajmeri ITS1Mfng/ITS4Mfng i ITS1/Mfg-R2, koji su u literaturi opisani kao specifični za *M. fructigena*, pored izolata *M. fructigena* amplifikovali su i DNK izolata *M. polystroma*. Za razdvajanje ove dve vrste, kao i za razlikovanje sve četiri ispitivane vrste roda *Monilinia*, najpogodniji su uzvodni prajmer MO368-5 u kombinaciji sa nizvodnim prajmerima MO368-8R, Laxa-R2 i MO368-10R u direktnoj ili Multiplex PCR metodi.

Ključne reči: *Monilinia fructigena*, *M. polystroma*, *M. fructicola*, *M. laxa*, PCR, Multiplex PCR, molekularna detekcija.

Uvod

Vrste roda *Monilinia* se ubrajaju u ekonomski najštetnije patogene – prouzrokovaoče sušenja cvetova, grančica i smeđe truleži plodova jabučastih i koštičavih voćaka (Wormald, 1954; Byrde i Willetts, 1977). Najznačajnije vrste ovog roda su: *Monilinia fructigena* (Aderh. & Ruhl.) Honey, *M. laxa* (Aderh. & Ruhl.) Honey, *M. fructicola* (G. Winter) Honey i *Monilia polystroma* van Leeuwen (Byrde i Willetts, 1977; Batra, 1991; van Leeuwen et al., 2002). *M. laxa* i *M.*

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fructicola su najzastupljenije i ekonomski najznačajnije na koštičavim voćkama, a *M. fructigena* na jabučastim (Wormald, 1954; Byrde i Willetts, 1977; Batra, 1991).

Gljive iz roda *Monilinia* su rasprostranjene u celom svetu. *M. fructigena* je široko rasprostranjena u Evropi i Aziji i karantinska je vrsta za Severnu Ameriku i Australiju, *M. laxa* je prisutna na svim kontinentima, a *M. fructicola* u Severnoj i Južnoj Americi, Aziji i Australiji (Wormald, 1954; Byrde i Willetts, 1977; Batra 1991; OEPP/EPPO, 2009; CABI, 2016). U Evropi je *M. fructicola* prvi put opisana u Francuskoj 2001. godine (OEPP/EPPO, 2002). Ova vrsta je karantinska gljiva za Evropu (EPPO A2 lista karantinski štetnih vrsta) (OEPP/EPPO, 2009), a u Srbiji se nalazi na IA listi I deo štetnih patogena (Službeni glasnik Republike Srbije, br. 7/2010). Iako je opisana u većini evropskih zemalja, ova vrsta i dalje ima karantinski status jer kao prouzrokovatelj bolesti dovodi do velikih ekonomskih gubitaka u proizvodnji voća (Baker, 2011). Četvrta vrsta, *M. polystroma*, opisana je 2002. godine i do sada je rasprostranjena u Evropi i Aziji (van Leeuwen et al., 2002; OEPP/EPPO, 2009; Vasić et al., 2013; CABI, 2016).

Detekcija i identifikacija vrsta roda *Monilinia* je složena jer ove gljive prouzrokuju slične simptome na zaraženim biljkama, morfološki su slične, a u okviru vrste varijabilne (Munoz et al., 2008; Poniatowska et al., 2013; Vasić et al., 2016). To je razlog zbog koga su molekularne metode našle mesto i u identifikaciji vrsta ovog roda (Carbone i Kohn, 1993; Holst-Jensen et al., 1997; Fulton et al. 1999; Ios i Frey, 2000; Hughes et al., 2000; Cote et al., 2004). Kod vrsta roda *Monilinia* kao i kod ostalih gljiva, najviše su proučavani geni ribozomalnih RNK.

Carbone i Kohn (1993) su utvrdili da između vrsta familije *Sclerotiniaceae*, pa i vrsta roda *Monilinia*, postoji nukleotidna razlika u ITS regionu ribozomalne DNK (rDNK). U okviru sekcije *Juntoriae* pronađeno je pet baznih parova razlike između *M. fructicola* i *M. laxa*, dok je *M. fructigena* imala 13 baznih parova razlike u poređenju sa prethodne dve vrste (Holst-Jensen et al., 1997). Fulton et al. (1999) su utvrdili genetičku razliku u pet baznih parova ITS regiona rDNK između izolata vrste *M. fructigena* i *M. polystroma*. Značajno variranje u genetičkim osobinama utvrđeno je analizom gena male ribozomalne podjedinice (SSU) koje je pokazalo postojanje introna samo kod izolata *M. fructicola*, ali ne i kod *M. laxa* i *M. fructigena* (Fulton i Brown, 1997; Snyder i Jones, 1999). Međutim, kasnije su utvrđeni izolati bez introna u ovom delu genoma i kod ove vrste (Fulton et al., 1999).

Na osnovu ITS regiona rDNK razvijeno je više molekularnih metoda za detekciju i identifikaciju vrsta roda *Monilinia* među kojima je najzastupljenija metoda lančane reakcije polimeraze (PCR) (Förster i Adaskaveg, 2000; Hughes et al., 2000; Ios i Frey, 2000; Boehm et al., 2001; Ma et al., 2003; Gell et al., 2007). Hughes et al. (2000), Ios i Frey (2000) i Gell et al. (2007) dizajnirali su prajmere za specifičnu detekciju *M. fructicola*, *M. fructigena* i *M. laxa*. Förster i Adaskaveg (2000), Boehm et al. (2001) i Ma et al. (2003) napravili su prajmere koji mogu

razdvajati i specifično detektovati *M. fructicola* i *M. laxa*. Snyder i Jones (1999) su na osnovu građe ITS1 regiona razvili metodu analize polimorfizma dužine restrikcionih fragmenata (engl. *Restriction Fragment Length Polymorphism* – RFLP) u kojoj se digestijom PCR produkata sa *MseI* restrikcionim enzimom omogućava razlikovanje *M. laxa* i *M. fructicola*.

Iako je većina molekularnih metoda detekcije i identifikacije vrsta roda *Monilinia* zasnovana na varijabilnosti u ITS regionu rDNK, i osobine drugih genskih regiona su se pokazale uspešnim za karakterizaciju i identifikaciju vrsta ovog roda. Cote et al. (2004) su razvili Multiplex PCR metodu koja omogućava istovremenu pouzdanu detekciju *M. fructigena*, *M. laxa*, *M. fructicola* i *M. polystroma* upotrebom specifičnih prajmera zasnovanih na delu genoma nepoznate funkcije.

Prema podacima OEPP/EPP (2009) prajmeri koje su dizajnirali Hughes et al. (2000), Ioos i Frey (2000) i Cote et al. (2004) uspešno razdvajaju četiri najznačajnije vrste roda *Monilinia*. U našoj zemlji su prisutne i ekonomski značajne *M. fructigena* i *M. laxa*, a od nedavno je utvrđeno prisustvo karantinske *M. fructicola* na plodovima jabuke (Vasić et al., 2012) i nektarine (Hrustić et al., 2013), kao i *M. polystroma* na plodovima jabuke (Vasić et al., 2013). Prisustvo i ove dve vrste roda *Monilinia* u našoj zemlji od kojih je jedna karantinska i druga nova vrsta podstaklo je detaljnija ispitivanja. Cilj ovog rada je bio da se ispita i standardizuje brza i pouzdana metoda molekularne detekcije korišćenjem različitih parova prajmera u PCR metodi i da se utvrdi njihova specifičnost i mogućnost korišćenja u identifikaciji četiri ekonomski značajne vrste gljiva ovog roda.

Materijal i metode

Izolati *Monilinia* spp.

Korišćeni su izolati vrsta roda *Monilinia* iz zaraženih plodova jabuke iz kolekcije Laboratorije za postžetvenu fitopatologiju Katedre za fitopatologiju, Univerziteta u Beogradu – Poljoprivrednog fakulteta i referentni izolati poreklom iz Italije (prof. Mari, Univerzitet u Bolonji) i Japana (dr van Leeuwen, Wageningen, Holandija). Izolati iz Srbije su prethodno okarakterisani i potvrđena je njihova patogenost (Vasić, 2016; Vasić et al., 2016). U ispitivanja su uključeni sledeći izolati vrsta roda *Monilinia*: *M. fructigena* (MFSRB-1, MFSRB-6, MFSRB-3, MFSRB-2, MFSRB-10 i referentni izolat MCG, poreklom iz Italije), *M. laxa* (MLX-SV1, MLX-13, MLX-24 i referentni izolat C-28, poreklom iz Italije), *M. fructicola* (MFC-1, MFC-20, MFC-28 i referentni izolat a-100, poreklom iz Italije) i *M. polystroma* (MPSRB-13, MPSRB-20, MPSRB-2, MP-13 i referentni izolat 0603202046, poreklom iz Japana).

Ekstrakcija DNK i PCR

Iz kultura starih sedam dana gajenih na podlozi od krompir-dekstroznog agara (PDA, EMB Chemicals INC. Gibbstown, NJ, USA) sakupljena je vazдушna micelija, homogenizovana u tečnom azotu i urađena ekstrakcija nukleinskih kiselina po CTAB metodi koju su opisali Day i Shatock (1997). Ekstrakt nukleinskih kiselina čuvan je pri temperaturi od -20°C.

PCR metoda je izvedena korišćenjem univerzalnih prajmera ITS1 i ITS4 (White et al., 1990), kao i specifičnih prajmera ITS1Mfgn, ITS4Mfgn, ITS1Mlx, ITS4Mlx, ITS1Mfcl, ITS4Mfcl (Ioos i Frey, 2000), Mfg-R2, MI-Mfg-F2, MI-Mfc-R1, Mfc-F1, Mfc-R1, (Hughes et al., 2000) i MO368-5, MO368-8R, Laxa-R2 i MO368-10R (Cote et al., 2004). Svaka reakciona PCR smeša, ukupne zapremine 25 µl, sadržala je 12,5 µl 2xPCR master mix (Fermentas, Lithuania), po 1 µl svakog prajmera (10 pmol/µl) (Metabion international AG, Germany), 9,5 µl vode i po 1 µl ekstrahovane DNK, dok je u Multiplex PCR metodi reakciona smeša sadržavala po 0,5 µl svakog prajmera (10 pmol/µl). Kao negativna kontrola korišćena je reakciona PCR smeša uz dodavanje vode umesto ciljane DNK.

Uslovi izvođenja PCR metode sa prajmerima ITS1/ITS4 bili su sledeći: početna denaturacija 2 min pri temperaturi od 94°C, zatim 30 ciklusa: denaturacija 30 s pri 94°C; hibridizacija 30 s pri 55°C; elongacija 30 s pri 72°C i finalna elongacija 10 min pri 72°C. Pozitivnom reakcijom smatrana je pojava amplikona veličine oko 550 bp.

Uslovi izvođenja PCR metode korišćenjem prajmera Ioos i Frey (2000) obuhvatili su početnu denaturaciju 3 min pri temperaturi od 94°C, zatim 30 ciklusa: denaturacija 30 s pri 94°C; hibridizacija 30 s pri 62,5°C; elongacija 1,5 min pri 72°C i finalna elongacija 10 min pri temperaturi od 72°C. U PCR metodi upotrebom prajmera ITS1Mfgn/ITS4Mfgn pozitivnom reakcijom smatrana je pojava amplikona veličine oko 350 bp specifičnih za *M. fructigena*, upotrebom prajmera ITS1Mlx/ITS4Mlx pojava amplikona veličine oko 350 bp specifičnih za *M. laxa* i upotrebom prajmera ITS1Mfcl/ITS4Mfcl pojava amplikona veličine oko 350 bp specifičnih za *M. fructicola*.

Uslovi izvođenja PCR metode sa prajmerima koje su dizajnirali Hughes et al. (2000) bili su: početna denaturacija 2 min pri temperaturi od 94°C, zatim denaturacija 1 min pri 94°C, hibridizacija 1 min pri 59°C, elongacija 1,5 min pri 72°C u 27 ciklusa za prajmere Mfc-F1/Mfc-R1 i 30 ciklusa za prajmere MI-Mfg-F2/MI-Mfc-R1 i ITS1/Mfg-R2 i finalna elongacija 10 min pri temperaturi od 72°C. Korišćenjem prajmera ITS1/Mfg-R2 pozitivnom reakcijom smatrana je pojava amplikona veličine oko 460 bp specifičnih za *M. fructigena*, upotrebom prajmera MI-Mfg-F2/MI-Mfc-R1 pojava amplikona veličine oko 280 bp specifičnih za *M. laxa* i upotrebom prajmera Mfc-F1/Mfc-R1 pojava amplikona veličine oko 280 bp specifičnih za *M. fructicola*.

Uslovi izvođenja PCR metode sa prajmerima koje su opisali Cote et al. (2004) bili su: početna denaturacija 2 min pri temperaturi od 95°C, zatim 35 ciklusa: denaturacija 15 s pri 95°C; hibridizacija 15 s pri 60°C; elongacija 1 min pri 72°C i finalna elongacija 3 min pri 72°C. U PCR metodi korišćenjem prajmera MO368-8R/MO368-5 pozitivnom reakcijom smatrana je pojava amplikona veličine oko 402 bp specifičnih za *M. fructigena* i amplikona veličine oko 425 bp specifičnih za *M. polystroma*, upotrebom prajmera Laxa-R2/MO368-5 pojava amplikona veličine oko 351 bp specifičnih za *M. laxa* i korišćenjem prajmera MO368-10R/MO368-5 pojava amplikona veličine oko 535 bp specifičnih za *M. fructicola*.

Nakon izvedene PCR metode (Biometra GmbH, Germany), vizuelizacija PCR produkata obavljena je elektroforezom u 1,5% agaroznom gelu koji je obojen etidijum bromidom (0,5 µg/ml) i posmatran pomoću UV transiluminatora (Vilber Lourmat, France).

Rezultati i diskusija

Kod svih ispitivanih izolata *Monilinia* spp. upotrebom prajmera ITS1/ITS4 dobijeni su amplikoni očekivanih veličina oko 550 bp, dok kod negativne kontrole nije došlo do amplifikacije, čime je potvrđeno prisustvo nukleinskih kiselina u uzorcima.

Primenom prajmera dizajniranih od strane Ioos i Frey (2000) ITS1Mlx/ITS4Mlx amplikoni veličine oko 350 bp dobijeni su kod svih izolata *M. laxa*, dok kod negativne kontrole, kao i ispitivanih izolata *M. fructigena*, *M. polystroma* i *M. fructicola* nije došlo do amplifikacije. Korišćenjem prajmera ITS1Mfcl/ITS4Mfcl amplikoni veličine oko 350 bp dobijeni su kod svih izolata *M. fructicola*. Ovi prajmeri nisu amplifikovali negativnu kontrolu, kao ni ispitivane izolate *M. fructigena*, *M. polystroma* i *M. laxa*. Upotrebom prajmera ITS1Mfgn/ITS4Mfgn, amplikoni veličine oko 350 bp dobijeni su kod izolata *M. fructigena* i *M. polystroma*, dok kod izolata *M. fructicola* i *M. laxa*, kao i negativne kontrole nije došlo do amplifikacije.

Primenom prajmera dizajniranih od strane Hughes et al. (2000) Ml-Mfg-F2/Ml-Mfc-R1 dobijeni su amplikoni veličine oko 280 bp kod svih izolata *M. laxa*, dok kod negativne kontrole, kao i ispitivanih izolata *M. fructigena*, *M. polystroma* i *M. fructicola* nije došlo do amplifikacije. Primenom prajmera Mfc-F1/Mfc-R1 dobijeni su amplikoni veličine oko 280 bp samo kod izolata *M. fructicola*. Ovi prajmeri nisu amplifikovali negativnu kontrolu, kao ni ispitivane izolate *M. fructigena*, *M. polystroma* i *M. laxa*. Upotrebom prajmera ITS1/Mfg-R2 dobijeni su amplikoni veličine oko 460 bp kod svih izolata *M. fructigena* i *M. polystroma*, dok kod negativne kontrole, kao i ispitivanih izolata *M. laxa* i *M. fructicola* nije došlo do amplifikacije.

Prajmeri specifični za detekciju *M. fructicola* i *M. laxa* (Hughes et al., 2000; Ioos i Frey, 2000) u ovim ispitivanjima bili su specifični za ove dve vrste roda *Monilinia* (tabela 1).

Tabela 1. Pogodnost različitih specifičnih prajmera za molekularnu detekciju *Monilinia* spp.

Table 1. Suitability of different PCR primers for detection of *Monilinia* spp.

Vrsta Species	Izolati Isolates	PCR produkti/PCR products									
		Ioos i Frey (2000)			Hughes et al. (2000)			Cote et al. (2004)			
		A	B	C	A	B	C	A	B	C	D
<i>M. fructigena</i>	MFSRB-1	+	–	–	+	–	–	+	–	–	–
	MFSRB-6	+	–	–	+	–	–	+	–	–	–
	MFSRB-3	+	–	–	+	–	–	+	–	–	–
	MFSRB-10	+	–	–	+	–	–	+	–	–	–
	MFSRB-2	+	–	–	+	–	–	+	–	–	–
	MCG	+	–	–	+	–	–	+	–	–	–
<i>M. polystroma</i>	MP-13	+	–	–	+	–	–	–	–	–	+
	MPSRB-20	+	–	–	+	–	–	–	–	–	+
	MPSRB-2	+	–	–	+	–	–	–	–	–	+
	MPSRB-13	+	–	–	+	–	–	–	–	–	+
	0603202046	+	–	–	+	–	–	–	–	–	+
<i>M. fructicola</i>	MFC-20	–	–	+	–	–	+	–	–	+	–
	MFC-1	–	–	+	–	–	+	–	–	+	–
	MFC-28	–	–	+	–	–	+	–	–	+	–
	a-100	–	–	+	–	–	+	–	–	+	–
<i>M. laxa</i>	MLX-SV1	–	+	–	–	+	–	–	+	–	–
	MLX-13	–	+	–	–	+	–	–	+	–	–
	MLX-24	–	+	–	–	+	–	–	+	–	–
	C-28	–	+	–	–	+	–	–	+	–	–

A: ampikon specifičan za *M. fructigena*; B: ampikon specifičan za *M. laxa*; C: ampikon specifičan za *M. fructicola*; D: ampikon specifičan za *M. polystroma*; + pojava ampikona specifične veličine; – nije došlo do amplifikacije.

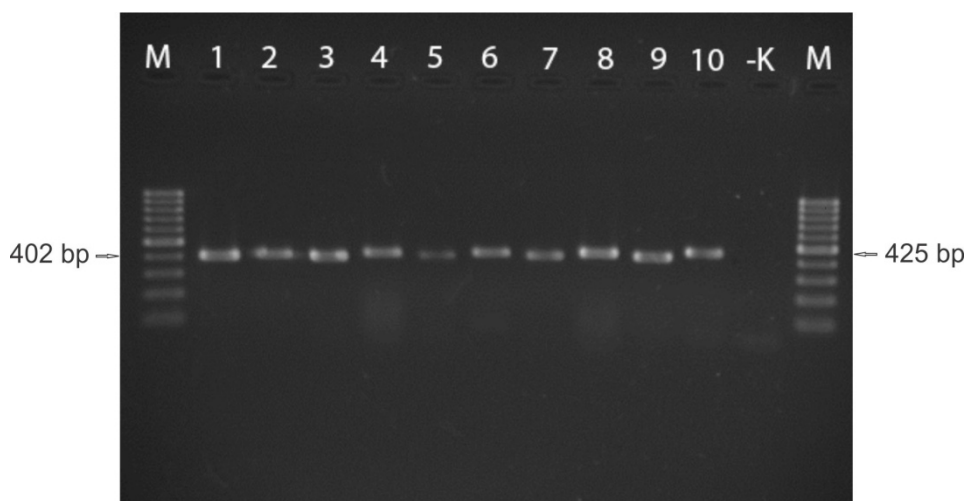
A: *M. fructigena*-specific product; B: *M. laxa*-specific product; C: *M. fructicola*-specific product; D: *M. polystroma*-specific product; '+' and '-' indicate the presence and absence of specific band patterns, respectively.

Međutim, prajmeri koje ovi autori navode kao specifične za *M. fructigena* su u sprovedenim istraživanjima pored izolata *M. fructigena*, amplifikovali i izolate *M. polystroma* (tabela 1). Razlog može biti što su Ioos i Frey (2000) i Hughes et al. (2000) dizajnirali ove prajmere pre opisa *M. polystroma* kao nove vrste (van

Leeuwen et al., 2002). *M. polystroma* se od *M. fructigena* razlikuje u pet nukleotida u sekvenci ITS rDNK regiona (Fulton et al., 1999). Hu et al. (2011) su također pokazali da prajmeri specifični za *M. fructigena* dizajnirani od strane Ioos i Frey (2000) mogu da amplifikuju *M. yunnanensis*, koja je na osnovu molekularnih osobina ITS rDNK veoma slična *M. fructigena* i *M. polystroma*.

Zbog male intraspecijske varijabilnosti ITS rDNK regiona vrsta roda *Monilinia*, ovaj region je pogodan za njihovu identifikaciju (Carbone i Kohn, 1993; Holst-Jensen et al., 1997; Fulton et al., 1999). Kod *M. fructigena* i *M. polystroma* razlika od pet nukleotida u sekvenci ITS rDNK regiona nije dovoljno velika da bi se na osnovu nje mogli dizajnirati specifični prajmeri. Međutim, ova genetička razlika ITS rDNK regiona *M. fructigena* i *M. polystroma* je poslužila za razvijanje druge molekularne metode (PCR-RFLP) za specifičnu detekciju *M. polystroma* zasnovane na digestiji ITS1/ITS4 PCR produkata restrikcijom *Hha* I enzimom (Vasić et al., 2016).

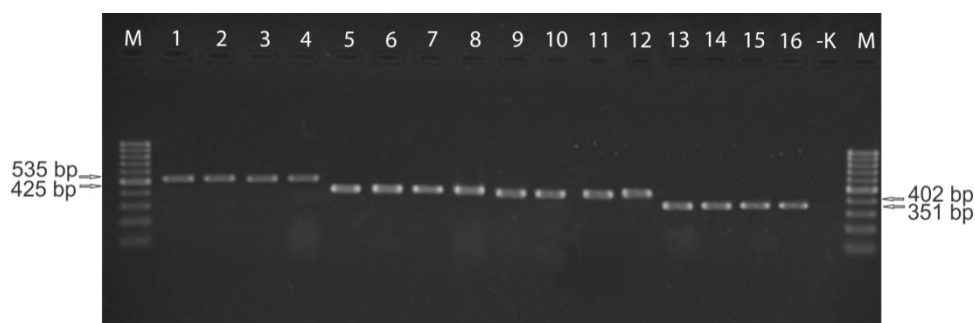
U PCR metodi razvijenoj od strane Cote et al. (2004), korišćenjem prajmera MO368-8R/MO368-5, amplikoni veličine oko 402 bp dobijeni su samo kod izolata *M. fructigena*, dok su amplikoni veličine 425 bp dobijeni samo kod izolata *M. polystroma* (slika 1).



Slika 1. 1,5% agarozni gel sa amplikonima dobijenim korišćenjem prajmera MO368-8R/MO368-5 u PCR metodi (Cote et al., 2004). M-marker 100 bp; 1,3,5,7,9 – izolati *M. fructigena*; 2,4,6,8,10 – izolati *M. polystroma*; -K – negativna kontrola.

Figure 1. 1.5% agarose gel with amplification products obtained with MO368-8R/MO368-5 primers in PCR (Cote et al., 2004). M 100-bp ladder; 1,3,5,7,9 – isolates of *M. fructigena*; 2,4,6,8,10 – isolates of *M. polystroma*; -K – negative control.

Upotrebom prajmera Laxa-R2/MO368-5 ampliconi veličine oko 351 bp dobijeni su samo kod izolata *M. laxa*, dok su prajmerima MO368-10R/MO368-5 ampliconi veličine oko 535 bp dobijeni samo kod izolata *M. fructicola*. Kod negativnih kontrola nije došlo do amplifikacije. U Multiplex PCR metodi dobijeni su ampliconi odgovarajuće veličine kod izolata sve četiri ispitivane vrste (slika 2).



Slika 2. 1,5% agarozni gel sa ampliconima dobijenim Multiplex PCR metodom (Cote et al., 2004). M-marker 100 bp; 1,2,3,4 – izolati *M. fructicola*; 5,6,7,8 – izolati *M. polystroma*; 9,10,11,12 – izolati *M. fructigena*; 13,14,15,16 – izolati *M. laxa*; -K – negativna kontrola.

Figure 2. 1.5% agarose gel with amplification products obtained in Multiplex PCR (Cote et al., 2004). M 100-bp ladder; 1,2,3,4 – isolates of *M. fructicola*; 5,6,7,8 – isolates of *M. polystroma*; 9,10,11,12 – isolates of *M. fructigena*; 13,14,15,16 – isolates of *M. laxa*; -K – negative control.

Prajmeri koje su dizajnirali Cote et al. (2004) na osnovu dela genoma čija je funkcija nepoznata, uspešno su amplifikovali i razdvojili ispitivane izolate *M. fructigena* i *M. polystroma*, kao i ostale dve vrste *M. fructicola* i *M. laxa* (tabela 1). PCR metoda razvijena od strane Cote et al. (2004) se pokazala kao efikasna, pouzdana i brza za detekciju sve četiri vrste roda *Monilinia*.

Vrste roda *Monilinia* su ekonomski značajni patogeni jabučastih i koštičavih voćaka. Dve od četiri ispitivane vrste prisutne u Srbiji imaju karantinski status, *M. fructicola* u Evropi i Srbiji, a *M. fructigena* u Severnoj Americi i Australiji (OEPP/EPPO, 2009; CABI, 2016). Iako je biologija ovih vrsta slična, razlike između njih određuju pojavu i zastupljenost pojedinih vrsta, što se odražava i na ekonomske štete koje nastaju njihovom pojavom (Byrde i Willetts, 1977; van Leeuwen et al., 2002). *M. polystroma* se odlikuje visokom virulentnošću kao *M. fructigena*, ali za razliku od nje intenzivnije i kompleksnije stromatizacije plodova što joj omogućava bolju adaptaciju i održavanje u nepovoljnim uslovima spoljašnje sredine (Vasić et al., 2016), a time i veću postojanost primarnog inokuluma. Zbog toga razvijanje pouzdane metode detekcije doprinosi pravilnoj identifikaciji prouzrokovaca, omogućava praćenje promena u zastupljenosti pojedinih vrsta, što

kao krajnji cilj ima primenu efikasnih mera zaštite biljaka od bolesti koje vrste roda *Monilinia* prouzrokuju.

Zaključak

Primenom PCR metode specifična molekularna detekcija *M. fructicola* postignuta je korišćenjem prajmera ITS1Mfc1/ITS4Mfc1 i Mfc-F1/Mfc-R1, a vrste *M. laxa* korišćenjem prajmera ITS1Mlx/ITS4Mlx i Ml-Mfg-F2/Ml-Mfc-R1. Prajmeri opisani kao specifični za *M. fructigena* amplifikovali su i izolate *M. polystroma*. Za razdvajanje ove dve vrste, kao i za razlikovanje sve četiri vrste roda *Monilinia* najpogodniji prajmeri bili su MO368-5 u kombinaciji sa MO368-8R, Laxa-R2 i MO368-10R.

Zahvalnica

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Literatura

- Batra, L.R. (1991). *World Species of Monilinia (Fungi): Their Ecology*. Berlin, Germany: Biosystematics and Control.
- Baker, R. (2011). Pest risk assesment of *Monilinia fructicola* for EU territory and identification and evaluation of risk management options. *EFSA Journal*, 9, 2119. Preuzeto sa www.efsa.europa.eu/efsajournal
- Boehm, E.W., Ma, Z., & Michailides, T.J. (2001). Species-Specific Detection of *Monilinia fructicola* from California Stone Fruits and Flowers. *Phytopathology*, 91 (5), 428-439.
- Byrde, R.J.W., & Willetts, H.J. (1977). *The Brown Rot Fungi of Fruit. Their Biology and Control*. Oxford: Pergamon Press.
- CABI. (2016). *Monilia polystroma (Asiatic brown rot) Datasheet*. UK: CAB International.
- Carbone, I., & Kohn, L.M. (1993). Ribosomal DNA sequence divergence within internal transcribed spacer 1 of the Sclerotiniaceae. *Mycologia*, 85 (3), 415-427.
- Cote, M.J., Tardif, M.C., & Meldrum, A.J. (2004). Identification of *Monilinia fructigena*, *M. fructicola*, *M. laxa* and *Monilia polystroma* on inoculated and naturally infected fruit using Multiplex PCR. *Plant Disease*, 88, 1219-1225.
- Day, J.P., & Shattock, R.C. (1997). Aggressiveness and other factors relating to displacement of populations of *Phytophthora infestans* in Engli and Wales. *European Journal of Plant Pathology*, 103 (4), 379-391.
- Förster, H., & Adaskaveg, J.E. (2000). Early brown rot infections in sweet cherry fruit are detected by monilinia-specific DNA primers. *Phytopathology*, 90 (2), 171-178.
- Fulton, C.E., van Leeuwen, G.C.M., & Brown, A.E. (1999). Genetic variation among and within *Monilinia* species causing brown rot of stone and pome fruits. *European Journal of Plant Pathology*, 105 (5), 495-500.
- Gell, I., Cubero, J., & Melgarejo, P. (2007). Two different PCR approaches for universal diagnosis of brown rot and identification of *Monilinia* spp. in stone fruit trees. *Journal of Applied Microbiology*, 103 (6), 2629-2637.

- Holst-Jensen, A., Kohn, L., Jakobsen, K. & Schumacher, T. (1997). Molecular phylogeny and evolution of *Monilinia* (Sclerotiniaceae) based on coding and noncoding rDNA sequences. *American Journal of Botany*, 84 (5), 686-701.
- Hrustić, J., Tanović, B., Mihajlović, M., Delibašić, G., Stanković, I., Krstić, B. & Bulajić, A. (2013). First report of brown rot caused by *Monilinia fructicola* on nectarine in Serbia. *Plant Disease*, 97, 147.
- Hu, M.J., Cox, D.C., Shnnabel, G., & Luo, C.X. (2011). *Monilinia* species causing brown rot of peach in China. *Plos One*, 6, 1-14.
- Hughes, K.J.D., Fulton, C.E., McCreynolds, D., & Lane, C.R. (2000). Development of new PCR primers for identification of *Monilinia* species. *EPPO Bulletin*, 30 (3-4), 507-511.
- Ioos, R., & Frey, P. (2000). Genomic variation within *Monilinia laxa*, *M. fructigena* and *M. fructicola*, and application to species identification by PCR. *European Journal of Plant Pathology*, 106 (4), 373-378.
- Ma, Z., Luo, Y., & Michailides, T.J. (2003). Nested PCR assays for detection of *Monilinia fructicola* in stone fruit orchards and *Botryosphaeria dothidea* from pistachios in California. *Journal of Phytopathology*, 151, 312-322.
- Munoz, Z., Moret, A., & Bech, J. (2008). Morphological and molecular characterization of *Monilinia* sp. isolates and pathogenicity on apple. *Agrociencia*, 42, 119-128.
- OEPP/EPPO. (2002). *First report of Monilinia fructicola in France*. EPPO Reporting Service. 003. Preuzeto sa <http://www.EPPO.org>
- OEPP/EPPO. (2009). *Monilinia fructicola*. *Bulletin OEPP/EPPO*, 39, 337-343.
- Poniatowska, A., Michalecka, M., & Bielenin, A. (2013). Characteristic of *Monilinia* spp. fungi causing brown rot of pome and stone fruits in Poland. *European Journal of Plant Pathology*, 135, 855-865.
- Snyder, C.L., & Jones, A.L. (1999). Genetic variation between isolates of *Monilinia fructicola* and *Monilinia laxa* isolated from cherries in Michigan. *Canadian Journal of Plant Pathology*, 21, 70-77.
- van Leeuwen, G.C.M., Baayen, B.P., Holb, I.J., & Jeger, M.J. (2002). Distinction of the Asiatic brown rot fungus *Monilia polystroma* sp. nov. from *M. fructigena*. *Mycological Research*, 106, 441-451.
- Vasić, M. (2016). *Karakterizacija Monilinia spp. patogena plodova jabuke u Srbiji i različiti aspekti njihove kontrole*. Univerzitet u Beogradu – Poljoprivredni fakultet.
- Vasić, M., Duduk, N., Ivanović, M.M., Obradović, A., & Ivanović, M.S. (2012). First report of brown rot caused by *Monilinia fructicola* on stored apple in Serbia. *Plant Disease*, 96, 456.
- Vasić, M., Duduk, N., & Ivanović, M. (2013). First report of brown rot caused by *Monilia polystroma* on apple in Serbia. *Plant Disease*, 97, 145.
- Vasić, M., Duduk, N., Vico, I., Rančić, D., Pajić, V., & Backhouse, D. (2016). Comparative study of *Monilinia fructigena* and *Monilia polystroma* on morphological features, pathogenicity, RFLP analysis and histopathology. *European Journal of Plant Pathology*, 144, 15-30.
- White, T.J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. U M.A. Innis, D.H. Gelfi, J.J. Sninsky, & T.J. White (Ur.), *PCR Protocols: A guide to methods and applications*. (315-322). New York, USA: Academic Press.
- Wormald, H. (1954). *The Brown Rot Diseases of Fruit Trees*. Her majesty'Stationery Office. London.

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SUITABILITY OF DIFFERENT PRIMERS FOR SPECIFIC MOLECULAR DETECTION OF *MONILINIA* SPP.

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A b s t r a c t

Monilinia spp. are economically important pathogens of pome and stone fruits. Four *Monilinia* species are present in Serbia – *Monilinia fructigena*, *M. laxa*, *M. fructicola* and *Monilia polystroma*. As detection and identification of *Monilinia* species are complex, the aim of this research was to evaluate species-specific primers in PCR in order to standardize fast and reliable molecular methods for differentiation between the four *Monilinia* species. Isolates of *M. fructigena*, *M. laxa*, *M. fructicola* and *M. polystroma* from apple fruit and referent isolates from Italy and Japan were used for testing. Specific molecular detection of *M. laxa* was obtained using ITS1Mlx/ITS4Mlx and MI-Mfg-F2/MI-Mfc-R1 primer pairs, and *M. fructicola* using ITS1Mfcl/ITS4Mfcl and Mfc-F1/Mfc-R1 primer pairs. ITS1Mfgn/ITS4Mfgn and ITS1/Mfg-R2 primer pairs, described as *M. fructigena* species-specific, amplified *M. fructigena* and *M. polystroma*, as well. Specific detection of these two species as well as of all four tested *Monilinia* species was obtained using the reverse primer MO368-5 with forward primers MO368-8R, Laxa-R2 and MO368-10R in separate or in Multiplex PCR reactions.

Key words: *Monilinia fructigena*, *M. polystroma*, *M. fructicola*, *M. laxa*, PCR, Multiplex PCR, molecular identification.

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ECONOMIC ANALYSIS OF TOMATO MARKETING IN ILORIN METROPOLIS, KWARA STATE, NIGERIA

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Abstract: Marketing of tomatoes is a complex phenomenon due to their perishable nature, seasonality and bulkiness, and as such, tomato production requires an efficient marketing system. Studies on tomato marketing in the study area are rare in the literature. Thus, this study analysed tomato marketing in Kwara State, Nigeria. A simple random sampling technique was used in the selection of one hundred and twenty tomato marketers. A well-structured questionnaire was used for data collection. Data collected were analysed using descriptive, Herfindahl index, costs and returns analysis and multiple regression analysis. The study revealed that most (70%) of the marketers were female with mean age of 39.5 years. The Herfindahl index of 0.008 revealed that the tomato market tended toward pure competition. Furthermore, tomato marketing is a profitable venture in the study area with a monthly gross margin of ₦310,095. The average rate of returns to total investment was 52.6%. This implies that an average profit of ₦53k was realized on every naira invested in vegetable marketing in the area. Moreover, the regression analysis indicated that about 96% of the total variation in net returns was explained by the independent variables. The study revealed that purchase cost, labour cost, transport cost and marketing experience were important in determining the net returns of tomato marketers. Most (30%) of the respondents identified finance to be their major constraint. This study therefore recommends that tomato marketers should pool their resources to reap the benefits of economies of scale. Also, policies and strategies that lower marketing cost should be vigorously pursued.

Key words: profitability, wholesalers, Herfindahl index, marketing and tomato.

Introduction

Despite the high oil revenue, agriculture is still one of the most important sectors of the Nigerian economy. Agriculture contributes over 40% of Nigeria's

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GDP, employs about 70% of the population and produces about 80% of the food needs (Aye, 2013). Among the wide range of agricultural crops, vegetables occupy an important place because of their economic potentials. The term 'vegetable' applies to those plants and plant parts that are edible, especially leafy or fleshy parts that are usually eaten with staples as main courses or supplementary foods in cooked or raw forms. It is estimated that there are at least ten thousand (10,000) plant species used as vegetables worldwide although only about fifty (50) are of great commercial value (Shing-Jy and Hsiao-Feng, 2003). Vegetables play a very significant role in human nutrition; they contain vitamins, minerals and chemical compounds that are essential for human health. For instance, vitamin A maintains eye health and strengthens the immune system, vitamin B helps convert food to energy, folate reduces the risk of some birth effects and helps prevent heart diseases. Vitamin C increases the absorption of calcium and iron from other food. Vitamin E is a powerful antioxidant that protects the cell from cancer causing agents. Dietary fibre helps move food through the digestive tract and lower blood cholesterol levels (FAO, 2006). The World Health Organization (WHO) places low vegetable intake sixth among its twenty risk factors of global human mortality, just behind better known killers such as tobacco use and high cholesterol (FAO, 2006). It is to this end that a minimum level of 400g per head per day is recommended for the consumption of vegetables by the WHO. However, according to the FAO (2010), vegetable consumption per head per day in Nigeria is as low as 179g compared to the recommended rate. Apart from its nutritional benefits, vegetables also serve as a source of employment for both the rural and urban dwellers directly or indirectly providing smallholder farmers with much higher income and more jobs per hectare than staple crops (AVRDC, 2006).

Among different vegetables grown in Nigeria, tomato clearly stands out as the most important both in scale of production and level of consumption (Adejobiet al., 2011). Tomato (*Solanum lycopersicum*) is grown by most dry season market gardeners who regard it as the principal crop. Tomato is one of the most important vegetable crops both in scale of production and level of consumption. Most other vegetables have restricted demand in Nigeria, demand for tomato is universal. Tomato has the great poverty alleviation capacity. Its production, handling, transportation, distribution and marketing will definitely employ a large number of people. Tomato can be processed and exported to other West African nations or sold within the country. An increase in agricultural productivity depends heavily on its marketability. An efficient market does not only link sellers and buyers in reacting to current situations in supply and demand but rather has a dynamic role to play in stimulating consumption of outputs which are essential elements of economic development (Haruna et al., 2012). Katharina and Stefan (2011) have reported that the concept of marketing subsumes a set of different innovative advertising instruments which aim at having a large effect with a small budget.

Agricultural marketing is defined as the performance of all the activities involved in the flow of agricultural products and services from the initial points of agricultural production until they reach the hands of the ultimate consumers. It is concerned with all that happens to crops after they leave the farm gate; making decisions, taking actions and bearing the responsibility of the action. Agricultural marketing also articulates all processes that take place from when the farmer plans to meet specified demands and market prospects to when the produce finally gets to its consumers (Haruna et al., 2012). Aminu (2009) pointed out that in a typical vegetable marketing, retailers and wholesalers were observed to sell both tomato and onion at the same time in addition to other vegetables like hot pepper, sweet pepper, cabbage, salad and in some cases, chili pepper. The crops were sold in heaps, small baskets and metal containers of varying weights.

Tomato marketing is poorly developed in Nigeria. It is characterized mainly by the problem of seasonality and perishability amongst others. Worst still, in the past, the government paid more attention to production with little attention to the marketing of vegetables such as tomato, pepper, onions, garden eggs, okra and leafy vegetables despite the fact that they need spatial marketing facilities (Idachaba, 2000). Consequently, losses of 40–50 percent occur for many vegetables mainly due to spoilage, inadequate transportation, sorting, improper packaging and handling and lack of storage facilities. Also, another problem with tomato marketing is in the area of standard weights and measurements. These leave the consumer to their luck and haggling abilities in securing a good deal. Thus, this study describes the marketing functions and structure, estimates costs and returns to tomato marketing and determines the factors influencing net returns of tomato marketers.

Materials and Methods

Area of study

The study was carried out in six major vegetable markets within Ilorin metropolis, namely: Oko-olowo, mandate, sango, ipata, tanke and garage-offa markets. Ilorin is the capital of Kwara State, Nigeria. The choice of Kwara State for this study was deemed to be appropriate given its antecedent in agriculture and food marketing. The State lies midway between the Northern and Southern parts of the Country (Figure 1). It has a population of about 2,371,089 with a total landmass of 32,500 square kilometres, most of which is arable (NPC, 2010). About 1,094,232 people of the State are engaged in direct farming, out of whom 26,865 are vegetable farmers. The State has two main climatic seasons, the dry season and wet season with annual rainfall ranging between 1,000 and 1,500 mm while the average temperature lies between 30°C and 35°C. The climate is conducive for growing fruits and vegetables. Common vegetables cultivated include: amaranthus,

okra, pepper, lettuce, rosette, tomato, carrot, cucumber, cabbage and jute mallow (Kwara state diary, 2002). The rainy season lasts between April and October while the dry season starts in November and ends in March of the following year providing ample opportunity for irrigated tomato production and marketing.



Figure 1. A map of Nigeria showing the location of Kwara State.

Data collection and sampling methods

The primary data were used in this study. The data were obtained from a field survey through the use of a well-structured questionnaire, administered through personal interview. The questionnaire was designed to elicit information on marketing activities, functions and structure of tomato marketers, cost and returns, factors determining the net return of tomato marketers and the major constraints confronting marketers in the study area.

Ilorin metropolis is made up of three Local Government Areas (LGAs), namely: Ilorin west, east and south LGAs. A two-stage sampling method was used to select the respondents for the study. The first stage involved the purposive selection of 2 major markets in each of the 3 LGAs that make up Ilorin metropolis. In the second stage, 20 wholesale marketers were randomly selected in each market to make up a sample size of 120 respondents. The response rate was 100%.

Analytical framework

The data derived from the field survey were analysed using descriptive statistics which involved the use of mean, frequency distribution and percentages.

Simple frequency tables and percentages were used to profile the socio-economic characteristics and constraints facing the respondents in the study area.

Herfindahl index

The Herfindahl index was used to determine the structure of tomato marketers.

The Herfindahl index (HI), $HI = \sum Si^2$,

where Si = Market share for respondent i , calculated as: $Si = Qi/Q$,

where Qi = Basket of tomato sold per month by respondent i , and

Q = Total number of baskets sold per month by all respondents.

Gross margin (GM) analysis

According to Olukosi and Erhabor (2005), gross margin (GM) analysis is the difference between gross income (revenue) and total variable cost (TVC) of production. This was used to determine the costs, returns as well as profitability of tomato marketers.

$GM = TR - TVC$,

where: GM = Gross margin, TR = Total revenue, TVC = Total variable cost.

The net return represents the total profit and was determined using:

$Net\ return = TR - TC$,

where: TR = Total revenue, TC = Total cost and

Rate of return to investment (ROR) = $TR - TVC / TVC \times 100$.

The depreciation of the fixed cost was calculated using the straight line method of depreciation: $D = C - S / N$,

where: D = Depreciated amount, C = Initial cost of the assets, S = Salvage value, N = Expected useful life. The salvage value was assumed to be zero.

Multiple regression technique

A multiple regression technique was used to ascertain factors determining net returns of tomato marketers. The implicit form of the equation is: $Q = F(X_1 + X_2 + X_3 + X_4 + X_5 + U)$,

where: Q = Net return (measured in naira),

X_1 = Purchase cost (naira),

X_2 = Labour cost (naira),

X_3 = Transport cost (naira),

X_4 = Tomato marketing experience (years),

X_5 = Storage cost (naira) and

u = Error term assumed to fulfill all the assumptions of the classical linear regression model.

Definition of economic indicators

Labour cost: Labour is human efforts in the production or marketing processes.

It adds to the cost incurred by the trader and therefore it is expected to have a negative effect on tomato traders' profits.

Purchase price: This is the price at which a basket of tomatoes is sold at the farm gate. It adds to the cost incurred by the trader and therefore it is expected to have a negative effect on tomato traders' profits.

Quantity of tomatoes offered for sale: The quantity of tomatoes that a marketer handles defines his/her scale of business operations. *Ceteris paribus*, the higher this scale, the higher the marketing profit because of possible economies of scale. Therefore, the quantity of the tomatoes offered for sale is expected to have a positive effect on marketing profit.

Transportation costs: This is the cost incurred in moving baskets of tomatoes from the point of purchase to the market. It adds to the cost incurred by the trader and therefore it is expected to have a negative effect on tomato traders' profits.

Selling price: This is the value of a basket of tomatoes. Higher selling prices of tomatoes benefit tomato traders and therefore this variable is expected to positively affect marketing profit.

Cost of storage: This is the cost of renting or putting up a warehouse. It adds to the cost incurred by the trader and therefore it is expected to have a negative effect on tomato traders' profits.

Results and Discussion

Socio-economic characteristics of the respondents

Majority (70%) of the marketers were female with an average age of 39.5 years (Table 1). This is contrary to the findings of Haruna et al. (2012), who found out that 88% and 12% of tomato marketers in Bauchi State were males and females, respectively.

The implication of this is that most of the respondents were in their active age being able to go about their business with vigour. The respondents' years of experience ranged between 5 and 30 years with an average of 13.9 years. This indicates that most of the respondents have been involved in tomato marketing for quite a long time. About sixty-four percent (64%) of the respondents were literate. Given this level of literacy, it is expected that information can be disseminated with ease among the respondents. The family size ranged between 3 and 12 persons with an average of 8 persons. Most (79.2%) of the respondents belonged to one cooperative society or the other and so had access to credit facilities. According to Akinsanmi et al. (2005), cooperatives are a vehicle for development since they

provide informal credit to farmers. Members of the cooperative, *ceteris paribus*, are likely to perform better than non-members because of possible economies of scale.

Table 1. Socio-economic characteristics of the respondents.

Variables	Frequency	Percentage
i) Age		
11–30 years	28	23.3
31–50 years	70	58.3
51–70 years	22	18.4
Total	120	100
ii) Marital status		
Married	92	76.7
Single	12	10.0
Divorced	16	13.3
Total	120	100
iii) Household size		
1–10	89	74.2
11–20	31	25.8
Total	120	100
iv) Education status		
No formal education	43	35.8
Formal education	77	64.2
Total	120	100
v) Years of marketing experience		
1–10	39	32.5
11–20	61	50.8
21–30	20	16.7
Total	120	100
vii) Sex		
Male	36	30.0
Female	84	70.0
Total	100	100
viii) Members of cooperative		
Yes	95	79.2
No	25	20.8
Total	100	100

Source: Field survey, 2015/2016.

Marketing functions and practices of respondents

Table 2 shows that the marketers performed the transportation function, and in doing this, 95.5% of them used vehicles as means of transport while 2.7% and 1.8% respectively opted for motorbikes and headloads.

Table 2. Marketing functions and practices.

Variables	Frequency	Percentage
i) Transportation means		
Vehicle	115	95.5
Motorbike	3	2.7
Headload	2	1.8
Total	120	100
ii) Storage facilities		
Shed	69	57.5
Rented shop	35	29.2
Home	16	13.3
Total	120	100
iii) Source of purchase		
Farm	69	57.3
Suburb	2	1.8
Market place	49	40.9
Total	120	100
iv) Distribution channels		
Wholesalers	17	14.1
Retailers	28	23.3
Consumers	51	42.7
Processors/food vendors	24	20.0
Total	120	100
v) Sales (basket sold per week)		
< 50	11	9.1
50--100	14	11.8
101--150	24	20.0
151--200	22	18.2
> 200	49	40.9
Total	120	100
vi) Major source of capital		
Personal savings	60	51.6
Friends and relatives	2	1.8
Cooperative loans	52	43.3
Bank loans	4	3.6
Total	120	100

Source: Field survey, 2015/2016.

Furthermore, analysis of storage function showed that 57.5 % of respondents stored their wares under sheds, 29.2% stored in rented shops while 13.3% claimed to store in their houses. Regarding tomato bulk purchase, 57.3% of the respondents bought directly from the producers' farms, 1.8% opted for suburbs. Data analysed showed that the marketers used diverse channels for carrying out their distributing

function. About 14.1% claimed to supply their wares in bulks. The remaining 23.3%, 42.7% and 20% sold their wares directly to the retailers, final consumers and processors/food vendors, respectively. The major (51.6%) source of capital was personal savings. This was closely followed by cooperative loans (43.3%).

Results of Herfindahl index

The result of the analysis revealed an estimated Herfindahl index (HI) of 0.008.

The highest value obtainable here is 1. A very low (0.008) HI obtained here revealed that the concentration ratio for tomato marketers was very low, thus implying that the market structure of tomato tended toward perfect competition, which is characterized by (1) The product sold is homogenous, (2) There is no barrier to entry into the business, (3) There are many buyers and sellers in the study area.

Costs and returns analysis of tomato marketers

Table 3 indicates that the gross revenue, total variable and fixed costs obtained from tomato marketing per month were ₦899,248, ₦589,153 and ₦7,419 respectively, leaving a gross margin of ₦310,095.

Table 3. Costs and returns to tomato marketers per month in Kwara State.

Item	Costs (₦)	Returns(₦)
Gross revenue (GR)		899, 248
Less Total variable costs (TVC)	589,153	
Tomato purchased	408,528	
Transport	100,556	
Storage	15, 998	
Agent	10, 786	
Labour	53, 285	
Equals		
Gross margin (GR-TVC)	310,095	
Fixed costs		
Sacks	1,580	
Baskets	2,090	
Tables and chairs	1,189	
Shed	2,560	
Total fixed cost	7,419	
Total cost	596,572	
Net return		302,676
Rate of return on investment (GR-TVC/TVCx 100/1)		52.6%

Source: Field survey, 2016. Note: The official *naira* to *dollar* exchange rate was pegged at ₦305 per \$1 while the black market rate was pegged at ₦403 per \$1. On the other hand, 1EUR is ₦330 and ₦470 at the official and black market rates, respectively.

The average rate of returns to total investment was 52.6%. This implies that an average profit of ₦0.53k was realized on every naira invested in vegetable marketing in the area. This signifies that, on the average, the investment was highly profitable.

Results of regression analysis

The double-log functional form was chosen as the best fitted equation (Table 4). The regression analysis revealed a coefficient of multiple determinations (R^2) of 0.957. This implies that the estimated variables explained 95.7% of variation in net returns of the tomato marketers.

Table 4. Results of regression analysis.

Variable	Coefficient	T-ratio
Constant	6.430	9.367
Purchase cost (X_1)	-0.399	4.293***
Labour cost (X_2)	-0.965	-3.821***
Transport cost (X_3)	0.330	2.083**
Marketing experience (X_4)	1.999	7.358***
Storage cost (X_5)	-0.002	-0.091

$R^2 = 0.959$; Adjusted $R^2 = 0.957$; F-statistic = 534.958; *** significant at 1%; ** significant at 5%.

Furthermore, four variables were found to be important in determining the net returns of tomato marketers. Purchase cost (X_1) and labour cost (X_2) were negative and statistically significant at the 1% level of probability. This implies that an increase in these variables will reduce the net returns of marketers. This is in line with a priori expectation. On the other hand, transport cost (X_3) and year(s) of marketing experience (X_4) were positive and significant at the 5% and 1% levels respectively. This suggests that the more the commodity for sale, the higher the transport cost incurred. Thus, the effect of higher transport cost paid was compensated for by better profits made in returns.

The lead equation can be written as:

$$\text{Log } Q = 6.430 - 0.399\log X_1 - 0.965\log X_2 + 0.330\log X_3 + 1.999\log X_4 - 0.002\log X_5.$$

Challenges facing tomato marketers

Table 5 shows the major problem facing marketers was inadequate capital (29.2%). About 23% and 19.2% of respondents claimed the high transportation cost and perishability of the commodity as their major problems, respectively.

Table 5. The most important challenges facing tomato marketers.

Variables	Frequency	Percentage
Perishability	23	19.2
High cost of transportation	28	23.3
Price fluctuation (seasonality)	14	11.6
Inadequate capital	35	29.2
Storage problem	5	4.2
Poor marketing information	10	8.3
Low demand	5	4.2
Total	120	100.0

Conclusion

This study analysed the economic activities of tomato marketers in Kwara State, Nigeria. The results showed that the tomato market was dominated by females, with an average age of 39.5 years. The Herfindahl index of 0.008 revealed that tomato market tended toward pure competition. The costs and returns analysis indicated that tomato marketing was profitable in the study area with a monthly gross margin of ₦310,095. The average rate of returns to total investment was 52.6%. This implies that an average profit of ₦0.53k was realized on every naira invested in vegetable marketing in the area. Furthermore, the regression analysis revealed that purchase cost, labour cost, transport cost and marketing experience were the important variables determining the net returns of tomato marketers. Marketers should strengthen themselves by forming cooperative groups to enjoy the benefit of economies of scale. Policies and strategies that lower the costs of marketing should be vigorously pursued to enhance better market performance and profitability.

References

- Adejobi, A.O., Babatunde, R.O., & Idowu, E.O. (2011). Weight and measurement issues in retail marketing of fresh tomatoes: evidence from Osun State. *Journal of Agricultural Science*, 6 (4), 20-26. Nigeria. Asian Research Publishing network (arpn)
- Akinsanmi, A., & Doppler, W. (2005). Socio-economic and food security of farming families in Southeast Nigeria. *Proceedings of Conference on international agricultural research and development*, University of Honhentiem, Stuttgart, Germany, 1-5.
- Aminu, A. (2009). *Framework for Agricultural Market Analysis: Theories and Application*. Ahmadu Bello University Press Limited, Zaria.
- Asian Vegetable Research Development Centre (AVRDC) (2006). *Vegetables Matter*, AVRDC The World Vegetable Centre, Shanhua, Taiwan.
- Aye, G.C. (2013). *Efficiency and policy analysis in agriculture: Methods and applications*. Saarbrücken, Lambert Academic Publishing.
- Food and Agricultural Organization, FAO (2006). More fruits and vegetables. Accessed online at www.unfao.org/spotlight/archive. July 24, 2009.

- Food and Agriculture Organization (FAO) (2006). Agricultural data FAOSTAT.
- Haruna, U., Sani, M.H., Danwanka, H.A., & Adejo, E. (2012). Economic Analysis of Fresh Tomato Marketers in Bauchi Metropolis of Bauchi State, Nigeria. *Nigerian Journal of Agriculture, Food and Environment*, 8 (30), 1-8.
- Idachaba, F.S. (2000). *Food Policy in Nigeria*. Agricultural Research Bulletin, 2000. 1, 162.
- Katharina, H., & Stefan, H. (2011). Guerrilla Marketing: The Nature of Concept and Propositions for further Research. *Asian Journal of Marketing*, 5 (2), 39-54.
- Kwara State Dairy (2002). Kwara State Ministry of Information, 2002.
- National Population Commission (NPC) (2010). Kwara state Population.
- Olukosi, J.O., & Erhabor, P.O. (2005). *Introduction to Farm Management Economics, Principles and Application*, Agitab publishers Limited, Zaria.
- Shing-Jy, J.T., & Hsiao-Feng, L. (2003). *Handbook of Vegetable Preservation and Processing* Edited by Y. H. Hui, Sue Ghazala, Dee M. Graham, K.D. Murrell, & Wai-Kit Nip CRC Press.
- USDA (2012). National Nutrient Database for Standard ReferenceSR23-Reports by Single Nutrients. Release 88#23. 1-26. US Department of Agriculture. Agricultural. Research Service.

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EKONOMSKA ANALIZA PRODAJE PARADAJZA U METROPOLI ILORIN, DRŽAVA KVARA, NIGERIJA

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R e z i m e

Prodaja paradajza je složena pojava zbog njegove kvarljivosti, sezonskog karaktera i veličine i kao takva, proizvodnja paradajza zahteva efikasan sistem prodaje. Istraživanja o prodaji paradajza u posmatranom području su retka u literaturi. Stoga se ovim istraživanjem analizira prodaja paradajza u državi Kvara u Nigeriji. Tehnika jednostavnog slučajnog uzorka korišćena je pri odabiru sto dvadeset ispitanika koji se bave prodajom paradajza. Dobro strukturiran upitnik korišćen je za prikupljanje podataka. Prikupljeni podaci su analizirani uz pomoć deskriptivnog, Herfindalovog indeksa, analize troškova i povraćaja i analize višestruke regresije. Istraživanje je pokazalo da su većina (70%) ispitanika koji se bave prodajom ženskog pola prosečne starosti od 39,5 godina. Herfindalov indeks od 0,008 ukazao je da tržište paradajza teži ka čistoj konkurenciji. Osim toga, prodaja paradajza je profitabilan poslovni poduhvat u ispitavanom području sa mesečnom bruto maržom od ₦310.095. Prosečna stopa povraćaja ukupne investicije bila je 52,6%. Ovo ukazuje da je prosečan profit od ₦53k ostvaren za svaku nairu koja je uložena u prodaju povrća u ovom području. Pored toga, regresionom analizom se ukazuje da se oko 96% ukupne varijacije neto povraćaja objašnjava nezavisnim varijablama. Istraživanje pokazuje da su troškovi kupovine, troškovi rada, troškovi transporta i markentinško iskustvo važni u određivanju neto povraćaja ispitanika, koji se bave prodajom paradajza. Većina (30%) ispitanika prepoznala je finansije kao glavnu prepreku. Ovim istraživanjem se preporučuje da bi oni koji se bave marketingom paradajza trebalo da udruže svoje resurse, kako bi iskoristili ekonomiju obima. Politike i strategije kojima se smanjuje cena prodaje trebalo bi takođe energično nastaviti.

Ključne reči: profitabilnost, veletrgovci, Herfindalov indeks, prodaja i paradajz.

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ECONOMIC ANALYSIS OF INTERCROPPING OKRA WITH LEGUMES

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Abstract: A field study was carried out in a vegetable research field of the National Horticultural Research Institute, Ibadan, Nigeria during the late raining season of 2015 to determine the appropriate okra/legume-based cropping system that will increase crop yield and farmer's income. The experiment was arranged in a randomized complete block design (RCBD) with three (3) replicates. The treatments comprised intercropping okra with groundnut or cowpea planted at varying spacings (60 cm x 30 cm, 60 cm x 40 cm, 60 cm x 50 cm and 60 cm x 60 cm). Okra was planted at a spacing of 60 cm x 40 cm, the plot size was 3 m x 2.4 m (7.4 m²). Data collected was subjected to analysis of variance (ANOVA) and significant means were separated using the least significant difference (LSD) test at the 5% probability level. Economic analysis of the cropping mixture was carried out using gross margin analysis, monetary advantage index and benefit to cost ratio analysis. Results revealed that the land equivalent ratio (LER) of 1.62 was recorded in okra/groundnut at 60 cm x 40 cm and LER of 1.74 was observed in okra/cowpea at 60 cm x 30 cm. Okra/cowpea intercropping system spaced at 60 cm x 30 cm was the most remunerative (₦859,192/ha), followed by okra/cowpea spaced at 60 cm x 50 cm (₦744,212/ha) while okra/cowpea spaced at 60 cm x 60 cm was the least remunerative. The highest gross margin of ₦2,188,961/ha was obtained in okra/groundnut spaced at 60 cm x 40 cm. The least return was obtained in okra/groundnut spaced at 60 cm x 60 cm (₦700,103/ha). The economic analysis revealed that okra/cowpea spaced at 60 cm x 30 cm produced the highest gross margin of ₦859,192/ha and monetary advantage index of ₦450,447/ha while okra/groundnut spaced at 60 cm x 40 cm gave the highest gross margin of ₦2,188,961/ha and monetary advantage index of ₦924,642/ha.

Key words: intercropping, economic analysis, vegetable, legumes, land equivalent ratio.

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Introduction

Intercropping is a crop management system involving two or more economic species growing together for at least a portion of their respective production cycles and planted significantly close to each other so that inter-specific competition can occur (Usman, 2001). It plays a vital role in subsistence food production in both advanced and emerging countries (Adeoye et al., 2005). It tends to give higher yield than sole crops, greater yield stability and efficient use of nutrients (Seran and Brintha, 2009).

Intercropping systems mostly involve cereal legumes, particularly maize – soybean, maize – cowpea, maize – groundnuts, millet – groundnut, and rice – pulses (Matusso et al., 2012). Cereals and legumes have become a popular combination among farmers probably due to the ability of legumes to combat erosion and raise soil fertility levels (Matusso et al., 2012). Legumes can relocate fixed nitrogen to intercropped cereals through their joint growing period and this nitrogen is an essential resource for the cereals (Bhagad et al., 2006). Development of a feasible and economically viable intercropping system largely depends on the adaptation of planting pattern and selection of compatible crops (Seran and Brintha, 2009). Okra has also been used in the cropping mixture with legumes (Ijoyah and Jimba, 2012).

Okra is one of the most widely known and utilized species of the family *Malvaceae* (Naveed et al., 2009) and an economically important vegetable crop grown in tropical and sub-tropical parts of the world (Oyelade et al., 2003). Okra production constitutes about 4.6 percent of the total staple food production in Nigeria within the period of 1970–2003 (CBN, 2004). It ranks first among other vegetable crops (Babatunde et al., 2007). Its cultivation and production have been widely practiced because of its importance to the economy development and it can be found in almost every market in Africa (Ngbede et al., 2014). The fruit contains some essential vitamins (vitamin C) and mineral salts such as calcium, magnesium, potassium and iron including a varying proportion of water (Shippers, 2000). Okra production worldwide is estimated at six million tonnes per year. India is the world largest producer followed by Nigeria and Sudan (Varmudy, 2011).

Considering the importance of okra, low yield resulting from poor nutrient status of the soil has been one of the major factors limiting its production. A number of research efforts on okra based intercropping system in Nigeria have been made. These include those of Ijoyah and Anyam (2013), Ijoyah and Jimba (2012), and Orluchukwu and Udensi (2013). However, these studies did not reveal the economic viability, particularly in the cropping combination. Evaluating a cropping system is an imperative to select the best performing system among the existing ones in terms of biological productivity and economic potential for a particular area (Seran and Brintha, 2009). Furthermore, in order to adequately

analyse the efficiency of the intercropping system, an economic analysis must be carried out since vegetables vary in yield, price and production cost year-round (Rezende et al., 2005). The objective of this study was therefore to determine appropriate okra/legume-based cropping system that will increase crop yield and consequently farmer's income.

Materials and Methods

The experiment was conducted at the Vegetable Research Farm of the National Horticultural Research Institute (NIHORT), Ibadan, Oyo state, Nigeria ($3^{\circ}56'E$ and $7^{\circ}33'N$ 168 m above sea level) between August and November, 2015. The site lies within the rain forest agro-ecological zone of Nigeria where the monthly rainfall distribution pattern is bimodal with peaks in June and September and annual rainfall between 1250 mm and 1500 mm spanning eight months (March to October) with a dry spell in August. The experiment was arranged in a randomized complete block design (RCBD) with three (3) replicates. The treatments comprised intercropping okra with groundnut or cowpea at a varying spacing (60 cm x 30 cm, 60 cm x 40 cm, 60 cm x 50 cm and 60 cm x 60 cm). Okra (NHAe47-4) obtained from NIHORT was planted at a spacing of 60 cm x 40 cm both in intercrop and sole crop while cowpea (Ife Brown) and groundnut (Kampala) bought from a local market were sown at a different spacing on a plot with dimension of 3 m x 2.4 m (7.4 m²). Land preparation was done by ploughing and harrowing after which planting was done in August. Weeding was done at two and five weeks after sowing. Five plants were randomly tagged in each plot and data was collected on yield and yield components of okra, groundnut and cowpea. Data collected was subjected to analysis of variance (ANOVA) and significant means were separated using the least significant difference (LSD) test at the 5% probability level.

An economic analysis of the performance of the cropping mixture was carried out using gross margin analysis, monetary advantage index and benefit to cost ratio analysis. Thus:

Gross returns = TR – TVC, where: TR = Total revenue, TVC = Total variable cost;

Monetary advantage index = Value of combined intercrops x LER-1/LER;
LER = Land equivalent ratio.

The gross returns were computed for each treatment using the prevailing farm gate price in the study area. Monetary advantage index was used to assess the yield of okra and the legume in the intercropping/sole cropping system. The higher the index value the more profitable is the cropping system (Mahapatra, 2011) while the benefit to cost ratio was employed to ascertain viability of the cropping mixture.

Results and Discussion

Yield and yield components

Intercropping either groundnut or cowpea with okra had a significant effect ($p \leq 0.05$) on number of fruits per plant, fruit yield and dry matter of okra (Table 1). This is because intercropping systems are known to make a more efficient use of growth factors as they capture and make a better use of radiant energy (Matusso et al., 2012), available water and nutrients (Sullivan, 2003), prevent pest and diseases, suppress weeds and maintain and improve soil fertility (Sanginga and Woome, 2009; Seran and Brintha, 2010). Okra sown sole also produced the highest fruit yield of 8.9 t/ha, but this was not significantly different from okra intercropped with groundnut at a spacing of 60 cm x 60 cm that produced 8.1 t/ha and also okra intercropped with cowpea at a spacing of 60 cm x 30 cm that produced 8.1 t/ha. This indicated that adequate space available due to these treatments to the crop during the growth period ultimately enhanced higher fruit yield. Similar types of results were reported by Jha et al. (2000) and Das et al. (2002). Okra intercropped with groundnut at a spacing of 60 cm x 30 cm and 60 cm x 40 cm together with okra intercropped with cowpea at a spacing of 60 cm x 50 cm produced dry matter comparable with that of sole okra (Table 1).

Table 1. Yield and yield components of okra intercropped with groundnut and cowpea at different spacings.

Treatment	Number of fruits/plant	Average fruit weight (g)	Fruit yield (t/ha)	Dry matter (t/ha)
Okra + Groundnut (60 X 30)	11.33c	12.44	5.77d	2.58a
Okra + Groundnut (60 X 40)	12.33bc	13.07	6.77bcd	2.68a
Okra + Groundnut (60 X 50)	13.33abc	11.19	6.22cd	1.51bc
Okra + Groundnut (60 X 60)	12.67bc	15.33	8.08ab	1.29c
Okra + Cowpea (60 X 30)	16.00a	12.18	8.13ab	1.58bc
Okra + Cowpea (60 X 40)	15.00ab	11.87	7.33abcd	1.87b
Okra + Cowpea (60 X 50)	12.00c	13.23	6.58bcd	2.45a
Okra + Cowpea (60 X 60)	12.33c	15.06	7.70abc	1.49bc
Sole okra	16.00a	13.42	8.89a	2.53a
F sig. ($P \leq 0.05$)	*	Ns	**	**

Groundnut sown sole at all different spacings produced comparable seed weight per plant and seed yield, but significantly higher than those intercropped with okra. Likewise, cowpea sown sole produced significantly higher seed weight per plant and seed yield than those intercropped with okra (Tables 2 and 3). These results are in agreement with those reported by Das et al. (2002) as they found a reduction in yield of a base crop due to intercrop competition.

Table 2. Yield and yield components of groundnut at different spacings in sole crop and intercropped with okra.

Treatment	Seed weight/ plant (g)	Number of pods	Seed yield (t/ha)	Dry matter (t/ha)
Okra/Groundnut(60 cm x 30 cm)	202.67bc	208.00	12.14bc	20.41
Okra/Groundnut(60 cm x 40 cm)	292.50abc	200.67	14.63b	23.18
Okra/Groundnut (60 cm x 50 cm)	200.67bc	208.00	8.03bc	18.18
Okra/Groundnut(60 cm x 60 cm)	110.92c	110.33	3.31c	23.13
Sole groundnut (60 cm x 30 cm)	434.03a	316.00	26.04a	23.67
Sole groundnut (60 cm x 40 cm)	338.41ab	267.67	16.92ab	19.26
Sole groundnut (60 cm x 50 cm)	311.40ab	196.33	12.46bc	15.71
Sole groundnut (60 cm x 60 cm)	441.59a	389.67	15.98b	15.54
F sig. ($P \leq 0.05$)	*	ns	**	ns

Table 3. Yield and yield components of cowpea at different spacings in sole crop and intercropped with okra.

Treatment	Seed weight/ plant (g)	Seed yield (t/ha)	1000 seed weight	Dry matter
Okra/Cowpea (60 cm x 30 cm)	119.82f	7.19cd	19.17	34.32
Okra/Cowpea (60 cm x 40 cm)	87.77g	4.39e	21.14	36.62
Okra/Cowpea (60 cm x 50 cm)	167.80d	6.71d	22.35	37.94
Okra/Cowpea (60 cm x 60 cm)	117.85f	3.54f	20.99	41.82
Sole cowpea (60 cm x 30 cm)	148.70e	8.92b	20.97	55.58
Sole cowpea (60 cm x 40 cm)	314.32a	15.72a	19.92	48.43
Sole cowpea (60 cm x 50 cm)	234.65c	9.39b	18.86	29.18
Sole cowpea (60 cm x 60 cm)	253.78b	7.61c	20.09	32.49
F sig. ($P \leq 0.05$)	**	**	Ns	ns

Productivity

In this study, all intercropping systems showed advantages compared with monocropping systems, and all the intercropping systems recorded high LER compared with monocropping systems (Table 4). A similar finding was reported by Susan and Mini (2005) for okra and amaranth intercropping and Seran and Brintha (2009) for radish and vegetable amaranth. LER values exceeding unity indicate yield advantages from intercropping compared with monocropping. From Table 4, LER values of 1.62 from okra/groundnut at 60 cm x 40 cm and 1.74 from okra/cowpea at 60 cm x 30 cm indicated that the area planted with monocultures would need to be 62% and 74% respectively greater than the intercropping area for the two to produce the same combined yields.

Table 4. LER, aggressiveness and percentage land saved in intercropping over the sole crop.

Treatment	Yield (t/ha)	LER	Aggressiveness	Percentage land save (%)
Okra/Groundnut (60 cm x 30 cm)	5.77	1.41	-0.63	29.08
Okra/Groundnut (60 cm x 40 cm)	6.77	1.62	-0.65	38.27
Okra/Groundnut (60 cm x 50cm)	6.22	1.34	0.33	25.37
Okra/Groundnut (60 cm x 60 cm)	8.08	1.12	4.13	17.71
Okra/Cowpea (60 cm x 30 cm)	8.13	1.74	0.65	42.53
Okra/Cowpea (60 cm x 40 cm)	7.33	1.10	3.21	0.00
Okra/Cowpea (60 cm x 50 cm)	6.58	1.46	0.17	31.51
Okra/Cowpea (60 cm x 60 cm)	7.70	1.34	2.36	25.37

Economic analysis of intercropping systems

Okra/cowpea combination at 60 cm x 40 cm incurred the highest cost of production (₦219,828/ha). This was followed by okra/cowpea spaced at 60 cm x 60 cm (₦219,828/ha). The duo of okra/cowpea at 60 cm x 50 cm incurred the least cost (₦187,908/ha) out of the four okra/cowpea combinations (Table 5). For cowpea at different spacing regimes, the highest cost was incurred when cowpea was spaced at 60 cm x 30 cm (₦166,368/ha) followed by cowpea spaced at 60 cm x 40 cm (₦165,828/ha) while the least cost of production was incurred in sole cowpea spaced at 60 cm x 60 cm (Table 5). Higher costs were incurred in the intercrops compared to the sole. This may be attributable to additional costs involved in the procurement of seed and field management.

The highest cost was incurred for okra/groundnut spaced at 60 cm x 40 cm (₦227,039/ha), followed by okra/groundnut spaced at 60 cm x 30 cm (₦208,410/ha) while the least cost was incurred by okra/groundnut spaced at 60 cm x 50 cm (₦194,321/ha) (Table 5). Higher cost of 227,039/ha incurred in okra/groundnut spaced at 60 cm x 40 cm was due to higher cost of harvesting and planting of the crops in the combination. For sole groundnut at different spacing regimes, the highest cost was incurred when sole groundnut was spaced at 60 cm x 30 cm (₦174,810/ha) and was followed by sole groundnut at 60 cm x 40 cm (₦173,039/ha) while the least cost was incurred when sole groundnut was spaced at 60 cm x 60 cm (₦152,697/ha).

Okra/cowpea spaced at 60 cm x 30 cm was the most remunerative (₦859,192/ha), followed by okra/cowpea spaced at 60 cm x 50 cm (₦744,212/ha) while okra/cowpea spaced at 60 cm x 60 cm was the least remunerative. The highest returns in sole cowpea were obtained when cowpea was spaced at 60 cm x 40 cm (₦1,158,972/ha) while the least was obtained in sole cowpea spaced at 60 cm x 60 cm (₦490,812/ha).

For the okra/groundnut crop combination, the highest gross margin of ₦2,188,961/ha was obtained in okra/groundnut spaced at 60 cm x 40 cm followed by okra/groundnut spaced at 60 cm x 30 cm (₦1,819,590/ha) (Table 5). The least return in okra/groundnut mixture was obtained in okra/groundnut spaced at 60 cm x 60 cm (₦700,103/ha).

Table 5. Economic analysis of the okra intercrops with legumes.

Treatment	Total cost ₦/ha	Gross returns ₦/ha	Gross margin ₦/ha	Monetary advantage index	Benefit to cost ratio
Okra/Cowpea 60 cm x 30 cm	199,968	1,059,160	859,192	450,447	5.3
Okra/Cowpea 60 cm x 40 cm	219,828	776,400	556,572	70,581	3.5
Okra/Cowpea 60 cm x 50 cm	187,908	932,120	744,212	293,682	5.0
Okra/Cowpea 60 cm x 60 cm	201,788	728,560	526,772	184,856	3.6
Sole cowpea 60 cm x 30 cm	166,368	748,800	582,432	-	4.5
Sole cowpea 60 cm x 40 cm	165,828	1,324,800	1,158,972	-	8.0
Sole cowpea 60 cm x 50 cm	153,108	788,400	635,292	-	5.2
Sole cowpea 60 cm x 60 cm	147,588	638,400	490,812	-	4.3
Sole okra	89,148	496,000	406,852	-	5.6
Okra/Groundnut 60 cm x 30 cm	208,410	2,028,000	1,819,590	587,702	9.7
Okra/Groundnut 60 cm x 40 cm	227,039	2,416,000	2,188,961	924,642	10.6
Okra/Groundnut 60 cm x 50 cm	194,321	1,464,000	1,269,679	371,463	7.5
Okra/Groundnut 60 cm x 60 cm	207,897	908,000	700,103	97,286	4.4
Sole groundnut 60 cm x 30 cm	174,810	2,256,000	2,081,190	-	12.9
Sole groundnut 60 cm x 40 cm	173,039	2,368,000	2,194,961	-	13.6
Sole groundnut 60 cm x 50 cm	159,521	1,740,000	1,580,479	-	10.9
Sole groundnut 60 cm x 60 cm	152,697	2,240,000	2,087,303	-	14.7

Okra/groundnut system gave better returns compared to the okra/cowpea cropping system. This may be attributable to higher unit price of groundnut compared to cowpea. Based on the performance of the crop combination, intercropping okra with groundnut was found to be the best for better remuneration and yield.

Monetary advantage index (MAI) values were positive in all the cropping systems evaluated indicating a yield advantage in the intercropping systems. The values of MAI were higher in okra/groundnut intercropping system compared to okra/cowpea intercropping system. The highest MAI value of ₦924,642/ha was obtained in okra/groundnut at 60 cm x 40 cm while the minimum MAI value of ₦70,581/ha was obtained in okra/cowpea at 60 cm x 40 cm spacing. This implied that okra/groundnut at 60 cm x 40 cm was the most valuable economic mixture.

The result of the benefit to cost ratio (BCR) analysis indicated that the highest BCR in okra/cowpea mixture (5.3) was obtained when okra/cowpea was spaced at 60 cm x 30 cm, followed by okra/cowpea spaced at 60 cm x 50 cm (5.0) while the

least BCR was obtained in okra/cowpea spaced at 60 cm x 40 cm (3.5). Sole cowpea spaced at 60 cm x 40 cm gave the highest BCR ratio of 8.0 while the lowest BCR ratio of 4.3 was obtained when cowpea was planted at 60 cm x 60 cm.

The highest BCR of 10.6 was obtained in okra/groundnut spaced at 60 cm x 40 cm followed by okra/groundnut spaced at 60 cm x 30 cm while the lowest (4.4) was recorded in okra/groundnut spaced at 60 cm x 60 cm. Sole groundnut at 60 cm x 60 cm produced the highest BCR of 14.7 compared to other spacing when groundnut was planted sole. The benefit to cost ratio obtained for the cropping mixture that was greater than one indicates the viability of the cropping mixture.

Conclusion

The study indicates that sole okra produced higher fruit yield while okra intercropped with groundnut at closer spacing produced a higher number of fruits per plant. Okra intercropped with cowpea at any of different spacings produced significantly higher fruit yield than those intercropped with groundnut except with groundnut at a wider spacing. Intercropping okra with cowpea at 60 cm x 30 cm gave the highest LER value of 1.74 while okra intercropped with groundnut at 60 cm x 60 cm was most aggressive, followed by okra intercropped with cowpea at 60 cm x 40 cm. More land was saved by intercropping okra with cowpea at 60 cm x 30 cm (42.53%). The economic analysis revealed that okra/cowpea spaced at 60 cm x 30 cm produced the highest gross margin of ₦859,192/ha and monetary advantage index of ₦450,447/ha while okra/groundnut spaced at 60 cm x 40 cm gave the highest gross margin of ₦2,188,961/ha and monetary advantage index of ₦924,642/ha.

References

- Adeoye, G.O, Sridhar, M.K.C, Adeoluwa, O.O., & Akinsoji, N.A. (2005). Evaluation of naturally decomposed solid waste from municipal dump sites for their manorial value in southwest Nigeria. *Journal of Sustainable Agriculture*, 26 (4), 143-152.
- Babatunde, R.O., Omotesho, O.A., & Sholotan, O.S. (2007). Socio-economic characteristics and food security status of farming household in Kwara State, North – Central Nigeria. *Pakistan Journal of Nutrition*, 6 (1), 16-21.
- Bhagad, S.B, Chavan, S.A, Zagade, M.V., & Dahiphale, A.V. (2006). Intercropping groundnut and sweet corn at different fertility levels and row proportions. *Indian Journal of Crop Science*, 1 (1-2), 151-153.
- Central Bank of Nigeria (CBN) (2004): Annual Report and Statistical Bulletin, 6, 12, December 2004.
- Das, Anup, Gnanamurthy, A.P., & Kumar, Narendra (2002). Effect of vegetable intercropping and source of nutrient on yield attributing character and yield of pigeonpea. *Indian Journal of Agronomy*, 47 (3), 340-344.
- Ijoyah, M.O., & Jimba, J. (2012). Evaluation of yield and yield components of maize (*Zea mays* L.) and okra (*Abelmoschus esculentus*) intercropping system at Makurdi, Nigeria. *Journal of Biological Environmental Science*, 2 (2), 38-44.

- Jha, G., Singh, D.P., & Thakre, R.B. (2000). Production potential of maize (*Zea mays*) + potato (*Solanum tuberosum*) intercropping as influenced by fertilizer and potato genotype. *Indian Journal of Agronomy*, 45 (1), 59-63.
- Mahapatra S.C. (2011). Study of grass legume intercropping system in term of competition indices and Monetary Advantage Index under acid laterite soil of India. *American Journal of Experimental Agriculture*, 1 (1), 1-6.
- Matusso, J.M.M., Mugwe, J.N., & Mucheru-Muna, M. (2012). Potential role of cereal-legume intercropping systems in integrated soil fertility management in smallholder farming systems of Sub-Saharan Africa. *Research Application summary, Third RUFORUM Biennial Meeting*, Entebbe, Uganda.
- Naveed, A. Ohler, T.A, Khan, A.A., & Khan, I.A. (2009). Generation mean analysis of water stress tolerance in okra (*Abelmoschus esculentus* L.) *Pakistan Journal of Botany*, 41, 195-205.
- Ngbede S.O., Ibekwe H.N., Okpara, S.C. Onyegbule, U.N., & Adejumo L. (2014). An overview of okra production, processing, marketing, utilization and constraints in Ayaragu in Ivo Local Government Area of Ebonyi State, Nigeria. *Greener Journal of Agricultural Sciences*, 4 (4), 136-143.
- Orluchukwu, J.A., & Udensi, E.U. (2013). The effect of intercropping pattern of okra, maize, pepper on weeds infestations and okra yield. *African Journal of Agricultural Research*, 8 (10), 896-902.
- Oyelade, O.J., Ade-Omowaye, B.I.O., & Adeomi, V.F. (2003). Influence of variety on protein, fat contents and some physical characteristics of okra seeds. *Journal of Food Engineering*, 57, 111-114.
- Rezende, B.L.A., Cecílio, Filho, A.B., Catelan, F., & Martins, M.I.E.G. (2005). Análise econômica de consórcios de alface americana × rabanete: um estudo de caso. *Horti Bras*, 23, 853-858.
- Sanginga, N., & Woomer, P.L. (2009). Integrated soil fertility management in Africa: Principles, Practices and Development Process. (Eds.). *Tropical Soil Biology and Fertility*. Institute of the International Centre for Tropical Agriculture. Nairobi.
- Seran, T.H., & Brintha, I. (2010). Review on maize based intercropping. *Journal of Agronomy*, 9 (3), 135-145.
- Seran, T.H., & Brintha, I. (2009). Biological and economic Efficiency of radish (*Raphanus sativus* L.) intercropped with vegetable amaranthus (*Amaranthus tricolor* L.). *The Open Horticulture Journal*, 2, 17-21.
- Shippers, R.R. (2000). *African indigenous vegetables: An overview of the cultivated species*. Natural Resource Institute, E.U. Technical Centre of Agriculture and rural Cooperation, Chathan, UK.
- Sullivan, P. (2003). *Intercropping principles and production practices*. Appropriate Technology Transfer for Rural Areas Publication. Retrieved from <http://www.attra.ncat.org>
- Usman, S.D. (2001). Seed production and quality of okra as affected by sowing time. *Seed Research*, 29 (1), 47-51.
- Varmudy, V. (2011). Marketing survey needed to boost okra exports. Department of economic Vive Kananda College, Puttur Karnataka. Matusso, J.M.M., Mugwe, J.N., & Mucheru-Muna, M. (2012). Potential role of cereal-legume intercropping systems in integrated soil fertility management in smallholder farming systems of sub-Saharan Africa Research Application Summary. *Third RUFORUM Biennial Meeting*, Entebbe, Uganda.

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EKONOMSKA ANALIZA ZDRUŽENE SETVE BAMIJE I LEGUMINOZA

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R e z i m e

Poljski ogled je sproveden na eksperimentalnom polju Nacionalnog instituta za istraživanja u hortikulturi u Ibadanu u Nigeriji krajem kišne sezone 2015. godine kako bi se odredio odgovarajući sistem gajenja zasnovan na bamiji i leguminozama koji će povećati prinos useva i prihod poljoprivrednika. Ogled je organizovan po slučajnom blok dizajnu (engl. *randomized complete block design* – RCBD) sa tri (3) ponavljanja. Tretmani su obuhvatali združenu setvu bamije sa kikirikijem ili vignom sejanih na različitim rastojanjima (60 cm x 30 cm, 60 cm x 40 cm, 60 cm x 50 cm i 60 cm x 60 cm). Bamija je sejana na rastojanju od 60 cm x 40 cm, a veličina parcele bila je 3 m x 2,4 m (7,4 m²). Prikupljeni podaci su podvrgnuti analizi varijanse (ANOVA), i značajne srednje vrednosti su odvojene korišćenjem testa najmanje značajne razlike (engl. *least significant difference [LSD] test*) na nivou verovatnoće of 5%. Ekonomska analiza združivanja useva sprovedena je korišćenjem analize bruto marže, indeksa monetarne prednosti i analize odnosa primanja i troškova. Rezultati su pokazali da je LER indeks (engl. *land equivalent ratio* – LER) od 1,62 zabeležen u združenom usevu bamija/kikiriki na rastojanju od 60 cm x 40 cm, dok je LER indeks od 1,74 uočen u združenom usevu bamija/vigna na rastojanju od 60 cm x 30 cm. Združeni sistem bamija/vigna na rastojanju od 60 cm x 30 cm bio je najunosniji (₦859.192/ha), zatim je sledio združeni usev bamija/vigna sa rastojanjem od 60 cm x 50 cm (₦744.212/ha), dok je združeni usev bamija/vigna na rastojanju od 60 cm x 60 cm bio najmanje unosan. Najviša bruto marža od ₦2.188.961/ha postignuta je u združenom usevu bamija/kikiriki na rastojanju od 60 cm x 40 cm. Najmanji prihod je ostvaren kod združenog useva bamija/kikiriki na rastojanju od 60 cm x 60 cm (₦700.103/ha). Ekonomska analiza je pokazala da je kod združenog useva bamija/vigna pri rastojanju od 60 cm x 30 cm postignuta najviša bruto marža od ₦859.192/ha i indeks monetarne prednosti od ₦450.447/ha, dok je u združenom usevu bamija/kikiriki na rastojanju od 60 cm x 40 cm ostvarena najviša bruto marža od ₦2.188.961/ha i indeks monetarne prednosti od ₦924.642/ha.

Ključne reči: združena setva, ekonomska analiza, povrće, leguminoze, LER indeks.

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Poglavljje „Literatura“ treba da sadrži samo radove citirane u glavnom tekstu. Rad citiran u tekstu treba da sadrži prezime autora i godinu. Ako citat obuhvata jednog autora on se navodi kao Jalikop (2010) ili (Jalikop, 2010). Kada citat obuhvata dva autora on se navodi kao Sadras i Soar (2009) ili (Sadras i Soar, 2009). Ako se u tekstu citiraju više od dva autora posle prezimena prvog autora navodi se skraćenica „et al.“, a zatim godina. Ovakav citat navodi se kao Lehrer et al. (2008) ili (Lehrer et al., 2008). Ako se za određeni problem istovremeno citira više radova onda se oni hronološki nabrajaju. Odvajanje većeg broja citiranih radova van zgrade vrši se zarezmom (,) a u zagradi tačkom i zarezmom (;). Ako se citiraju dva ili više rada istog autora oni moraju biti poredani prema hronološkom redu (1997, 2002, 2006, itd.). Ukoliko se određeni autor pojavljuje nekoliko puta u istoj godini, dodaju se slova (2005a, b, c, itd.). Citate ličnih komunikacija i neobjavljenih podataka treba izbegavati, osim ako je to apsolutno neophodno. Takvi citati bi trebali da se pojave samo u tekstu (npr. Brown, lična komunikacija), ali ne i u spisku referenci.

Literatura koja je citirana u tekstu navodi se u spisku referenci u originalnom obliku, po abecednom redu, bez numeracije. Ako se citira veći broj radova istog autora najpre se navode radovi kada je autor sam, a zatim kada su prisutna dva i više autora. Ako se u nekoj od ovih kategorija javlja veći broj radova, treba ih hronološki srediti po godinama (1997, 2002, 2006, itd.), a ako se u istoj godini javlja veći broj radova dodaju se slova (2005a, 2005b, 2005c, itd.). Literaturni podatak treba da sadrži: prezime autora, početno slovo imena, godinu izdanja u zagradi, naslov rada, naziv časopisa, volumen i broj stranica (prva-poslednja). Prilikom citiranja knjiga navodi se izdavač i mesto izdavanja. Redovi svake reference posle prvog reda moraju biti uvučeni. U časopisu se koristi APA - Publication Manual of the American Psychological Association citatni stil.

Primeri navođenja referenci su sledeći:

Periodičan časopis

Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (*Helianthus annuus* L.). *Journal of Agricultural Sciences*, 54, 1-10.

Knjiga

Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

Poglavlje u knjizi

Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R.M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits*. (pp. 441-514). New York: John Wiley and Sons, Inc.

Zbornik

Behera, T.K., Staub, J.E., Behera, S., Rao, A.R., & Mason, S. (2008). One cycle of phenotypic selection combined with marker assisted selection for improving yield and quality in cucumber. In M. Pitrat (Ed.), *Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae* (pp. 115-121). Avignon.

Teza

Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

Izveštaj

Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

Veb sajt

Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from <http://research.amnh.org/entomology/spiders/catalog/index.html>

Rezime

Rezime na srpskom jeziku (za radove napisane na engleskom jeziku) ili na engleskom jeziku (za radove napisane na srpskom jeziku) navodi se na kraju rada i treba da ima od 100 do 250 reči. Ispred osnovnog teksta rezimea, navodi se naslov rada, puno ime, srednje slovo i prezime svih autora i naziv i adresa ustanove autora. Naslov „Rezime“ piše se razmaknuto i centrirano. Nakon naslova sledi jedan razmak, a zatim tekst rezimea, uvučen jednim tabulatorom. Neposredno nakon teksta rezimea, navode se ključne reči, sa tačkom na kraju. E-mail adresa autora za kontakt navodi se ispod crte, pri dnu stranice.

Tabele

Tabele obeležene arapskim brojevima (1, 2, itd.) praćene naslovom treba da se nalaze na odgovarajućem mestu u tekstu, u fontu 9. Maksimalna širina tabela treba da bude 13 cm. One treba da budu jasne, što jednostavnije i pregledne. Treba izbegavati vertikalne crte, a broj kolona ograničiti tako da tabela ne bi bila preširoka. Takođe, treba izbegavati nepotrebnu upotrebu horizontalnih crta. Naslov tabele, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom iznad tabele. Ispod tabele treba dati detaljno objašnjenje skraćenica, simbola i znakova korišćenih u samoj tabeli. Svaka tabela mora biti pomenuta u tekstu.

Ilustracije

Svi grafikoni, dijagrami i fotografije treba da se nazovu „Slika“ (1, 2, itd.). Prilažu se na odgovarajućem mestu u tekstu. Grafikone i dijagrame treba uraditi fontom 9, u crno-beloj tehnici i sa maksimalnom širinom od 13 cm. Voditi računa da oni budu čitki i jasni i nakon redukcije veličine. Za svaki grafikon i dijagram treba obezbediti detaljnu legendu bez skraćenica. Fotografije moraju biti visokog kvaliteta da bi se tehnički mogle dobro reprodukovati. Prilažu se u „TIF“ ili „JPG“ formatu, u crno-beloj tehnici. Naslov ilustracije, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom ispod ilustracije. Svaka ilustracija mora biti pomenuta u tekstu.

Skraćenice i jedinice

U radu treba koristiti samo standardne skraćenice. Merne jedinice treba izražavati u internacionalnom sistemu jedinica (SI). Kod navođenja jedinica posle broja treba da stoji razmak (osim za % i °C). Skraćenice se mogu koristiti i za druge izraze pod uslovom da se ti izrazi navedu u punom obliku prilikom prvog pominjanja, sa skraćenim oblikom u zagradi. Vrednosti od 1 do 9 mogu se izražavati slovima, a ostali brojevi isključivo numerički.

Nomenklatura

Celokupna nomenklatura (hemijska i biohemijska, taksonomska, genetička itd.) mora biti usklađena sa međunarodnim kodeksima i komisijama, kao što su *International Union of Pure and Applied Chemistry*, *IUPAC-IUB Combined Commission on Biochemical Nomenclature*, *Enzyme Nomenclature*, *International Code of Botanical Nomenclature*, *International Code of Nomenclature of Bacteria* itd.

Formule

Sve formule i jednačine u radu moraju biti urađene pomoću programa „Word Equation“. Pri pisanju formula, radi preglednosti, ostaviti dovoljno praznog prostora oko same formule. Subskripti i superskripti treba da budu jasni. Prilikom pisanja jednačina treba dati smisao svih simbola odmah posle jednačine u kojoj se simbol prvi put koristi. Jednačine treba da budu numerisane arapskim brojevima, serijski u zagradama, na desnoj strani linije. Svaka jednačina mora biti pomenuta u tekstu kao Eq. (1), Eq. (2), itd.

Nakon objavljivanja rada, autoru za kontakt će biti poslat jedan primerak časopisa. Mole se svi budući saradnici da rad pripreme prema datom uputstvu, kako bi olakšali rad redakcije časopisa. Ukoliko se rad ne pripremi po navedenom uputstvu neće biti prihvaćen za objavljivanje.

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