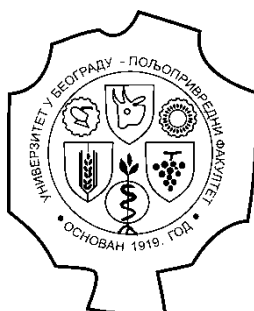


UDC: 63

ISSN 1450-8109

**JOURNAL
OF
AGRICULTURAL SCIENCES**
Belgrade 2017, Vol. 62, No. 1

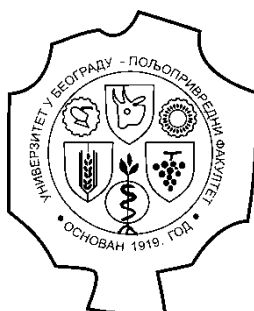


**Published by
University of Belgrade
Faculty of Agriculture
Republic of Serbia**

UDC: 63

ISSN 1450-8109

**JOURNAL
OF
AGRICULTURAL SCIENCES**
Belgrade 2017, Vol. 62, No. 1



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University of Belgrade-Faculty of Agriculture
11081 Belgrade-Zemun, Nemanjina 6, PO Box 14, Serbia
Tel: + 381 11 4413-555/467; Fax: + 381 11 2193-659; E-mail: redakcija@agrif.bg.ac.rs
URL: <http://www.agrif.bg.ac.rs/>

DTP Service: Snežana Spirić

Printed by the Faculty of Agriculture, Belgrade-Zemun
Circulation: 100

Publishing is supported by the Ministry of Education, Science and Technological Development
of the Republic of Serbia, Belgrade

According to the opinion of the Ministry of Science of the Republic of Serbia
No. 413-00-1928/2001-01 dated November 6, 2001 this
Journal is exempt from general tax liability

Frequency: Four times per year

Abstracting and Indexing
CAB Abstracts, AGRICOLA, SCIndeks

Number of institutions the Journal is exchanged with: 80

JOURNAL OF AGRICULTURAL SCIENCES
Vol. 62, No. 1, 2017

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EVALUATION OF DURUM WHEAT GENOTYPES BASED ON DROUGHT TOLERANCE INDICES UNDER DIFFERENT LEVELS OF DROUGHT STRESS

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Abstract: Objectives of this study were to assess durum wheat genotypes for drought tolerance and to study relationships among different drought tolerance indices under different drought stress conditions. The total of twenty-two durum wheat lines was evaluated in a RCBD experiment with three replications for three cropping seasons (2008–2009; 2009–2010 and 2010–2011). Different drought indices such as tolerance (TOL), mean productivity (MP), mean relative performance (MRP), stress susceptibility index (SSI), modified severity stress index (SSSI), geometric mean productivity (GMP), stress tolerance index (STI), yield stability index (YSI), relative efficiency index (REI) and drought response index (DRI) were determined based on yields under drought and non-drought conditions. The studied genotypes showed considerable variation in performance and tolerated various drought conditions that could be exploited in the durum wheat breeding program. The screening of genotypes for drought tolerance in environments with a greater value of stress intensity (SI) will be more efficient in the grouping of indices and genotype selection. The indices were classified into groups (G1 and G2). The group G1, which consisted of the indices REI, STI, MRP, GMP, DRI and YSI, distinguished genotypes with higher yield in different levels of drought stress. The durum breeding line nos. 1, 11, 10, 13, 8, 9, and 12 were superior based on the group G1 and could be regarded for further evaluation in drought-prone environments.

Key words: durum wheat, drought tolerance indices, stress intensity.

Introduction

Precipitation is the most important factor for agricultural production, whereas drought is the serious restriction to crop production (Boyer, 1982). In Iran, wheat is produced in the areas with a variable amount and distribution of

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rainfall. Drought stress occurs in grain filling, when evapo-transpiration is high due to higher air temperature. The respective yield of lines under drought and non-drought conditions is the first beginning point in the determination of characteristics related to stress for choosing genotypic materials in breeding for drought conditions (Clarke et al., 1992). The study of genotypes under stress conditions is the major work of breeders to improve yield productivity and drought tolerance (Benmahammed et al., 2010). Various yield-based drought resistance indices have been proposed. Tolerance index (TOL) (Rosielle and Hamblin, 1981), mean productivity (MP) (McCaig and Clarke, 1982), stress susceptibility index (SSI) (Fischer and Maurer, 1978), modified stress severity index (SSSI) (Singh et al., 2011), geometric mean productivity (GMP) and stress tolerance index (STI) (Fernandez, 1992) are used under different conditions. The use of grain yield per se is the most applicable method for assessing drought resistance. Compared to yield potential, stability of yield seems more relevant to adaptation and stress tolerance in crops (Blum et al., 1990; Van Ginkel et al., 1998; Panthuan et al., 2002). The breeding for yield potential is often negatively associated with yield stability because of GE interaction (Calderini and Slafer, 1999). However, selection based on multi-trials (including years and sites) will be more useful to select genotypes with high stress tolerance and yield productivity (Yau et al., 1991; Ceccarelli et al., 1992; Kirigwi et al., 2004).

According to other studies on detection of drought tolerant genotypes, it has been suggested that by screening genotypes in non-drought conditions, both adaptability and high yield potential are accessible (Panthuan et al. 2002; Kirigwi et al. 2004). On the contrary, under stress environments, selection of genotypes with high yield performance can be favored (Ceccarelli et al., 1992). Many of scientists believe that to enhance more effective selection, trials must be conducted in drought and non-drought conditions (Clarke et al., 1992; NasirUd-Din et al., 1992; Fernandez, 1992; Byrne et al., 1995; Rajaram and Van Ginkel, 2001). According to Trethowan et al. (2002), selection in alternating stress and non-stress drought environments has led to an increasing progress in the development of wheat adaptation in dry areas globally. However, selection for drought tolerance should be made using drought tolerance indices based on yield under both conditions, when the aim is to look for widely adapted genotypes (Sio-se Maedeh et al., 2006).

To identify high yielding durum wheat genotypes in variable years, 22 durum genotypes were evaluated for improving tolerance to drought stress by using different drought resistance indices. This study aimed to assess drought tolerance in durum wheat breeding lines and to find relationships among different indices of drought tolerance under various levels of drought.

Materials and Methods

The trials were carried out at Sararood station of the Dryland Agricultural Research Institute, Kermanshah, Iran (1351 masl, 34°20' N, 47°19' E) on a clay loam soil with the long-term average rainfall of 415 mm, during 2008–2011. The total of twenty durum wheat breeding lines along with two check cultivars i.e., Zardak (local durum wheat) and Saji (new cultivar) were evaluated in a randomized complete block design (RCBD) with three replications. Meteorological conditions were completely different across experimental years (Figure 1). Different levels of rainfall provided an opportunity to assess the response of durum genotypes to different levels of drought stress. However, due to variable climatic conditions in the region, crops experience different levels of drought stress, which complicates selection of ideal genotypes. For this, the drought stresses were applied to evaluate the genotypes based on different levels of stress. Accordingly, calculations of the drought indices were performed based on the two different datasets as follows:

Dataset A: the first year (Y1: 2008–2009 with 288.3 mm of precipitation) represented stress conditions and the second year (Y2: 2009–2010 with 453.9 mm of precipitation) represented non-stress conditions,

Dataset B: the third year (Y3: 2010–2011 with 342.5 mm of precipitation) represented stress conditions and Y2 represented non-stress conditions.

Drought resistance indices were obtained using the following equations:

$$(1) \text{ Stress susceptibility index (SSI)} = \frac{1 - (Y_s/Y_p)}{1 - (\bar{Y}_s/\bar{Y}_p)} \text{ (Fischer and Maurer, 1978),}$$

where Y_s and Y_p are the yields of a given genotype under stress and non-stress conditions, respectively, $\bar{Y}_s$ and $\bar{Y}_p$ are the mean yields of all genotypes under stress and non-stress conditions, respectively, and $1 - (\bar{Y}_s/\bar{Y}_p)$ is the stress intensity (SI).

$$(2) \text{ Modified stress severity index (SSSI)} = (1 - \frac{Y_s}{Y_p}) - (1 - \frac{\bar{Y}_s}{\bar{Y}_p}) \text{ (Sing et al., 2011).}$$

$$(3) \text{ Mean productivity (MP)} = \frac{Y_p + Y_s}{2} \text{ (Hossain et al., 1990).}$$

$$(4) \text{ Tolerance (TOL)} = Y_p - Y_s \text{ (Hossain et al., 1990).}$$

$$(5) \text{ Stress tolerance index (STI)} = \frac{Y_p + Y_s}{(\bar{Y}_p)^2} \text{ (Fernandez, 1992).}$$

$$(6) \text{ Geometric mean productivity (GMP)} = \sqrt{Y_p \times Y_s} \text{ (Fernandez, 1992).}$$

(7) Yield stability index (YSI) = $\frac{Y_s}{Y_p}$ (Boslama and Schapaugh, 1984).

(8) Mean relative performance (MRP) = $\left(\frac{Y_s}{Y_p}\right) + \left(\frac{Y_p}{Y_s}\right)$ (Raman et al., 2012).

(9) Relative efficiency index (REI) = $\left(\frac{Y_s}{Y_p}\right) \times \left(\frac{Y_p}{Y_s}\right)$ (Raman et al., 2012).

(10) Drought response index (DRI) (Bidinger et al., 1987) describing the response of genotypes to drought stress by fitting multiple regression of grain yield in stress conditions on grain yield and days to flowering in non-stress conditions: $\hat{Y}_s = a - bF + cY_p$,

where $\hat{Y}_s$ is the regression estimate of yield in stress conditions, Y_p is the yield under non-stress conditions, F is the days to flowering in non-stress conditions, a is the intercept, b and c are the regression coefficients. Then, the DRI was calculated by the following equation:

$$DRI = \frac{(Y_s - \hat{Y}_s)}{SE(\hat{Y}_s)}, \text{ where SE indicates the standard error.}$$

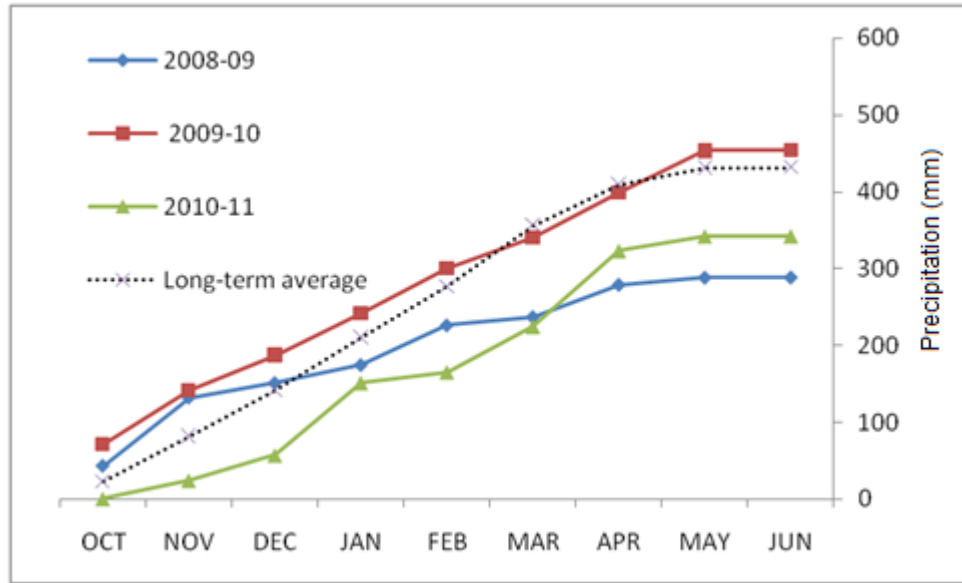


Figure 1. Precipitation of three cropping seasons (2008–2009, 2009–2010 and 2010–2011) compared with the long-term average (20 years).

A combined analysis of variance was performed for partitioning of total variance to its components. A biplot based on principle component analysis (PCA) was applied to classify the drought tolerance indices and characterize the tested genotypes based on the indices using IBM SPSS statistics version 19 software (IBM Corp., Armonk, NY, USA).

Results and Discussion

Results of the analysis of variance of grain yield data showed a highly significant genotype $\times$ year (G $\times$ Y) interaction (Table 1).

Table 1. Combined analysis of variance for grain yield of 22 genotypes in A and B datasets. Dataset A: Y1 (2008–2009) as stress and Y2 (2009–10) as non-stress conditions and Dataset B: Y2 as non-stress and Y3 (2010–2011) as stress conditions.

Source of variations	df	Mean squares	
		A	B
		SI ^o = 0.79	SI = 0.69
Year (Y)	1	702569000**	10884900**
Block/Y	4	243902	21855.2
Genotype (G)	21	880443 ^{ns}	251921 ^{ns}
G $\times$ Y	21	629463**	156768**
Residual	84	214233	60742.6

**significant at the 1% level of probability, ns: non-significant, SI: stress index.

The mean yields of genotypes ranged from 618 kg ha⁻¹(G20) to 1845 kg ha⁻¹(G1) in the first year, from 3495 kg ha⁻¹(G22) to 6611kg ha⁻¹(G10) in the second year and from 1474 kg ha⁻¹(G9) to 2169 kg ha⁻¹(G21) in the third year (Table 2). The ranking of genotypes according to grain yield in each year was different indicating different responses of genotypes to different levels of drought (Table 2). This finding justified the utilization of stress indices and multivariate methods to describe the behavior of genotypes under stress and non-stress conditions (Benmehamad et al., 2010).

Table 2. Mean grain yield (kg ha^{-1}) of 22 durum genotypes and their ranks in each cropping season.

	Code	2008–2009		2009–2010		2010–2011	
		Mean	Rank	Mean	Rank	Mean	Rank
1	Bcr/Gro1//Mgn11	1845.4	1	6461.4	2	1766.7	13
2	Adnan-2	1053.2	16	6275.3	6	1664.3	18
3	Waha	1245.1	9	5858.7	11	1809.5	9
4	Stj3//Bcr/Lks4/3n"er-3	991.7	18	5881.8	10	1988.1	4
5	Stj3//Bcr/Lks4/3/Ter-3	889.8	19	5800.8	13	1640.5	19
6	Geromtel-1	1033.3	17	5703.6	18	1704.8	16
7	Ammar-6	1228.7	11	6304.5	5	1845.2	8
8	Ammar-8	1429.6	6	5754.0	15	1785.7	10
9	Ammar-1	1278.2	8	5753.1	16	1473.8	22
10	Ammar-9	1701.4	3	6611.4	1	1933.3	5
11	Mgn13/Aghrass2	1827.3	2	6460.5	3	2081.0	2
12	Ter-1//Mrf1/Stj2	1231.5	10	5751.3	17	1719.1	15
13	Mgn13/Ainzen-1	1527.1	5	6328.1	4	1916.7	6
14	Ter-1/3/Stj3//Bcr/Lks4	1346.3	7	5429.5	20	1776.2	11
15	EDUYT-15	863.9	20	6071.2	8	1678.6	17
16	EDUYT-18	1113.0	14	6027.2	9	1914.3	7
17	EDUYT-42	756.5	21	5754.5	14	1776.2	12
18	EDUYT-47	1116.0	13	5847.1	12	1750.0	14
19	EDUYT-54	1111.1	15	5319.3	21	1631.0	20
20	EDUYT-70	618.1	22	6151.3	7	2035.7	3
21	Saji	1575.0	4	5456.8	19	2169.1	1
22	Zardak	1204.2	12	3495.2	22	1561.9	21
Mean		1226.7		5840.8		1801.0	
S.E.		69.6		134.3		36.7	

No significant correlation was found between mean yields in different years indicating that the genotypes did not respond similarly to different years (Table 3).

Ys (yield under stress conditions), Yp (yield under non-stress conditions), MP (mean productivity), GMP (geometric mean productivity), TOL (tolerance), STI (stress tolerance index), SSI (stress susceptibility index), YSI (yield stability index), SSSI (modified susceptibility stress index), MRP (mean relative performance), REI (relative efficiency index) and DRI (drought response index).

Table 3. Correlation between drought indices and grain yield in two datasets – A: Y2 as non-stress conditions and Y1 as stress conditions; and B: Y2 as non-stress conditions and Y3 as stress conditions.

Data set		Ys	Yp	MP	GMP	TOL	STI	SSI	YSI	SSSI	MRP	REI
A	Yp	0.203										
B		0.405										
A	MP	0.593**	0.909**									
B		0.596**	0.976**									
A	GMP	0.938**	0.523*	0.829**								
B		0.805**	0.868**	0.956**								
A	TOL	-0.306	0.870**	0.585**	0.035							
B		0.142	0.963**	0.880**	0.701**							
A	STI	0.940**	0.523*	0.831**	0.996**	0.035						
B		0.829**	0.844**	0.940**	0.997**	0.668**						
A	SSI	-0.829**	0.371	-0.048	-0.588**	0.778**	-0.592**					
B		-0.304	0.736**	0.573**	0.315	0.886**	0.263					
A	YSI	0.828**	-0.373	0.046	0.586**	-0.779**	0.591**	-1.00**				
B		0.302	-0.737**	-0.575**	-0.317	-0.888**	-0.266	-1.00**				
A	SSSI	-0.850**	0.247	-0.159	-0.673**	0.668**	-0.652**	0.932**	-0.932**			
B		-0.415	0.657**	0.477*	0.2	0.834**	0.152	0.982**	-0.983**			
A	MRP	0.939**	0.528*	0.834**	0.998**	0.04	0.999**	-0.588**	0.587**	-0.650**		
B		0.817**	0.858**	0.950**	1.00**	0.687**	0.998**	0.294	-0.296	0.181		
A	REI	0.940**	0.522*	0.830**	0.996**	0.034	1.00**	-0.593**	0.592**	-0.652**	0.999**	
B		0.830**	0.843**	0.940**	0.997**	0.667**	1.00**	0.262	-0.265	0.151	0.998**	
A	DRI	0.925**	0.0	0.394	0.805**	-0.466*	0.801**	-0.861**	0.860**	-0.869**	0.802**	0.802**
B		0.978**	0.552**	0.720**	0.890**	0.307	0.904**	-0.129	0.127	-0.253	0.897**	0.904**

This can be supported by the significant $G \times Y$ interactions for grain yield (Table 1). Thus, there is no guarantee that a genotype selected in a year with high rainfall will produce high yields in years with low rainfall (severe and mild drought stresses) and vice versa. Therefore, indirect selection in a drought-prone environment based on the results of optimum conditions will not be efficient. These results are in agreement with those of Ceccarelli and Grando (1991), Bruckner and Froberg (1987), and Sio-Se Mardehet al. (2006), who found that landraces of barley and wheat with a low yield potential were more productive under stress conditions. The lack of response to improved environmental conditions may be related to a lack of adaptation to high-moisture conditions (Clarke et al., 1992). These interactions

complicate the breeding process by introducing unknown factors that modulate the yield response depending on the availability of water. It is important, therefore, to differentiate between genotypes that have high yields in drought conditions simply because of their high inherent yield potential and those that have greater drought tolerance per se.

A linear regression model was fitted to the relationship between genotypic mean yields and total annual rainfall which showed that genotypic mean yield increased with increasing rainfall ($R^2 = 0.82$) (Figure 2). Among genotypes, the grain yield of the old cultivar Zardak was found to be the lowest, and that of the new cultivar Saji was equal or superior to the breeding lines in a low rainfall year, while under high rainfall conditions Saji was not superior to the breeding lines. It can be concluded that the breeding lines were better than the new cultivar in favorable conditions, whereas they were not better than the new cultivar in stress conditions. A similar result for oat (*Avena sativa*) was reported by Akcura and Ceri (2011).

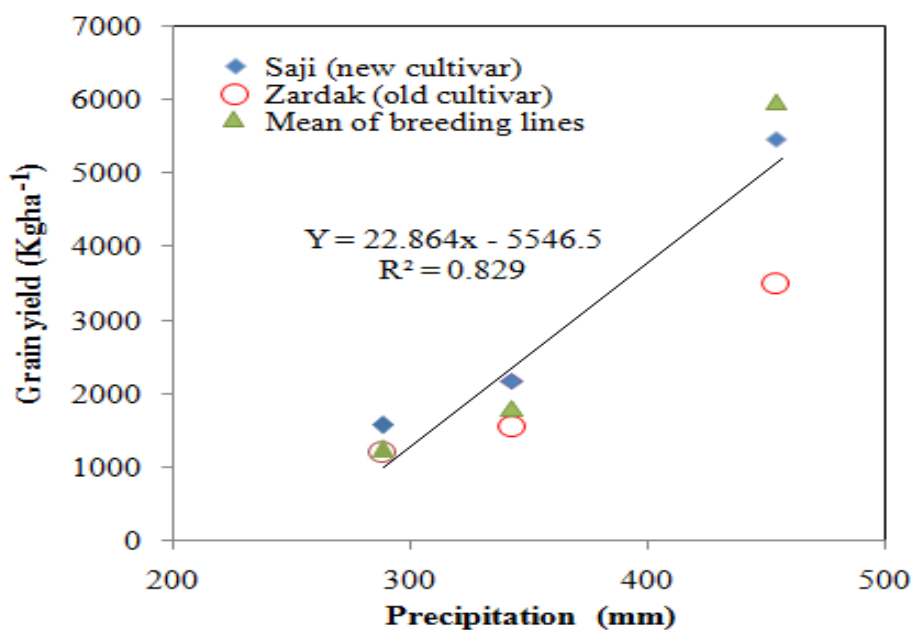


Figure 2. Regression between genotype (mean of breeding lines and cultivars) grain yield and total annual rainfall in different years.

The drought tolerance indices take into account yield potential and yield under drought conditions in different formulas. Using one drought index may not lead the breeder to the best selection. Therefore, selection based on a combination of several indices may provide a more useful criterion for drought tolerance

improvement. Principal component analysis and biplot technique were used to identify superior genotypes based on the indices for each dataset. For the two A and B datasets, the stress intensity (SI) values were 0.79 and 0.69, respectively, which provides an opportunity to evaluate the durum genotypes under both mild and severe stresses under rainfed conditions. The first two PCs accounted for 98.2% and 99.4% of total variation for datasets A and B, respectively. PC analysis in different datasets showed that the dataset A with the highest SI value was the best one for distinguishing genotypes by indices (Figures 3 and 4). In the dataset A, the indices were separated into two groups: TOL, SSI and SSSI (Group G1), and Ys, REI, STI, MRP, GMP, DRI and YSI (Group G2) (Figure 3).

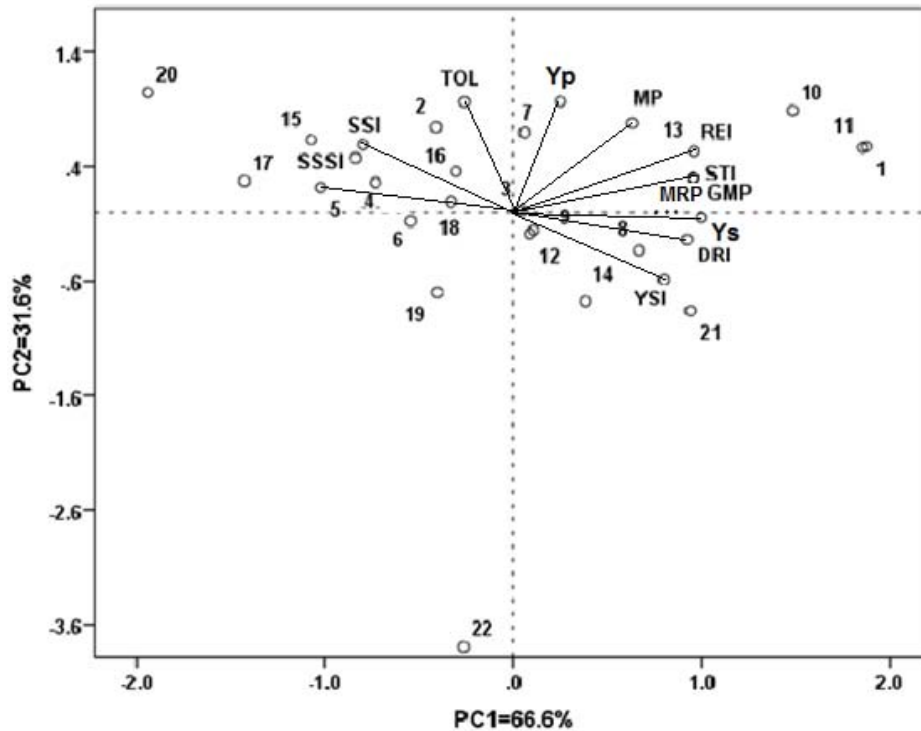


Figure 3. Biplot based on the first two principal component axes (PC1 and PC2) for 22 genotypes across drought indices in the dataset A. Ys (yield under stress conditions), Yp (yield under non-stress conditions), MP (mean productivity), GMP (geometric mean productivity), TOL (tolerance), STI (stress tolerance index), SSI (stress susceptibility index), YSI (yield stability index), SSSI (modified susceptibility stress index), MRP (mean relative performance), REI (relative efficiency index) and DRI (drought response index). The numbers represent genotypes.

Concerning the group G1, the genotype nos. 16, 2, 18, 4, 5, 15, 17, 20 were found to be susceptible to drought conditions, while the genotype nos. 1, 11, 10, 13, 8, 9, and 12 belonging to the group G2 were tolerant. Raman et al. (2012) found a strong positive correlation between the indices REI, MRP, STI, GMP and MPI belonging to the same group and the indices DYI, TOL, SSI and SSSI were classified in another group. They reported a highly negative correlation between these two groups. Sio-Se Mardeh et al. (2006) concluded that GMP and STI were able to discriminate a group of cultivars only under moderate drought stress conditions.

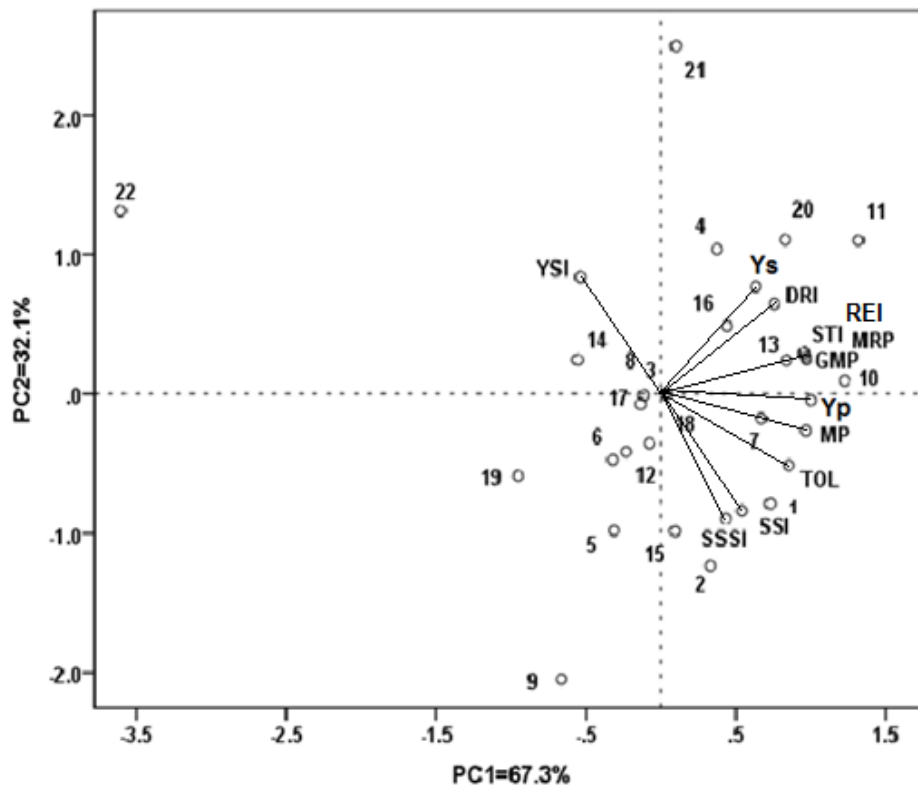


Figure 4. Biplot based on the first two principal component axes (PC1 and PC2) for 22 genotypes across drought indices in the dataset B. Ys (yield under stress conditions), Yp (yield under non-stress conditions), MP (mean productivity), GMP (geometric mean productivity), TOL (tolerance), STI (stress tolerance index), SSI (stress susceptibility index), YSI (yield stability index), SSSI (modified susceptibility stress index), MRP (mean relative performance), REI (relative efficiency index) and DRI (drought response index). The numbers represent genotypes.

A positive correlation between TOL and Yp in the A and B datasets, and a negative correlation between TOL and Ys in the dataset A suggests that selection based on TOL will result in increased yield under optimal conditions (Table 3). Similar results were reported by Sio-Se Mardeh et al. (2006), Clarke et al. (1992) and Rosielle and Hamblin (1981). Hossain et al. (1990) used MP as a resistance criterion for wheat cultivars in moderate stress conditions. Correlations of MP and GMP with Yp and Ys were positive in the two datasets (Table 3). As described by Hohls (2001), selection for MP should increase yield in both stress and non-stress environments unless the correlation between yields in contrasting environments is highly negative. In our experiment, there was no significant correlation between genotypic yields in different conditions. SSI has been widely used by researchers to identify sensitive and resistant genotypes (Clarke et al., 1992; Fischer and Maurer, 1978). SSI showed a negative correlation with Ys in both datasets (Table 3). Similar results were reported by Sio-Se Mardeh et al. (2006).

The results showed yield ranks in each year indicating that G11, G10, G13 and G1 had mean ranks below 5 during three years (Table 2). These genotypes were already identified by the group G2 of indices as the most drought tolerant (Figure 3). Therefore, selection based on this group of indices will enhance yield productivity in durum genotypes under different levels of drought stress.

Conclusion

Screening of durum wheat genotypes for drought tolerance in environments with a higher value of stress intensity (SI) proved to be more efficient for grouping of drought tolerance indices and genotype selection. The group G1 of indices (Ys, REI, STI, MRP, GMP, DRI and YSI) distinguished genotypes with higher yields under different levels of drought stress. The studied genotypes showed considerable variability in yield and tolerance to different levels of drought stress, which could be exploited for crop improvement. Among the examined materials, the breeding line nos. 1, 11, 10, 13, 8, 9, and 12 with higher yield productivity under different levels of stress can be included in further considerations for durum wheat breeding programs for drought-prone environments.

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Received: June 5, 2016

Accepted: February 14, 2017

PROCENA GENOTIPOVA DURUM PŠENICE NA OSNOVU INDEKSA
TOLERANTNOSTI NA SUŠU PRI RAZLIČITIM NIVOIMA STRESA
IZAZVANOG SUŠOM

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R e z i m e

Ciljevi ovog istraživanja bili su da se procene genotipovi durum pšenice za tolerantnost na sušu i da se prouče veze između različitih indeksa tolerantnosti na sušu u različitim uslovima stresa uslovljenog sušom. Ukupno dvadeset i dve linije durum pšenice procenjene su u ogledu po slučajnom blok sistemu u tri ponavljanja za tri vegetacione sezone (2008–2009; 2009–2010 i 2010–2011). Različiti indeksi suše kao što su tolerantnost (engl. *tolerance* [TOL]), srednja produktivnost (engl. *mean productivity* [MP]), srednji relativni učinak (engl. *mean relative performance* [MRP]), indeks osetljivosti na stres (eng. *stress susceptibility index* [SSI]), indeks promenjenog intenziteta stresa (engl. *modified severity stress index* [SSSI]), geometrijska srednja produktivnost (engl. *geometric mean productivity* [GMP]), indeks tolerantnosti na stres (engl. *stress tolerance index* [STI]), indeks stabilnosti prinosa (engl. *yield stability index* [YSI]), indeks relativne efikasnosti (engl. *relative efficiency index* [REI]) i indeks odgovora na sušu (engl. *drought response index* [DRI]) određeni su na osnovu prinosa u sušnim uslovima i u odsustvu suše. Ispitivani genotipovi su pokazali značajnu varijaciju u učinku i tolerisali su različite sušne uslove, koji se mogu iskoristiti u programu oplemenjivanja durum pšenice. Ispitivanje genotipova za tolerantnost na sušu u sredinama sa višom vrednošću intenziteta stresa (SI) biće efikasnije pri grupisanju indeksa i selekciji genotipova. Indeksi su bili klasifikovani u grupe (G1 i G2). Grupa G1, koja se sastojala od indeksa REI, STI, MRP, GMP, DRI i YSI, istakla je genotipove sa višim prinosom pri različitim nivoima stresa izazvanog sušom. Oplemenjivačke linije durum pšenice brojeva 1, 11, 10, 13, 8, 9, i 12 bile su superiornije na osnovu grupe G1 i mogle bi se uzeti u obzir za dalju evaluaciju u sredinama izloženim suši.

Ključne reči: durum pšenica, indeksi tolerantnosti na sušu, intenzitet stresa.

Primljeno: 5. juna 2016.
Odobreno: 14. februara 2017.

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EVALUATION OF MAIZE GRAIN YIELD IN DROUGHT-PRONE ENVIRONMENT

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Abstract: Multi-location trial data obtained between 2007 and 2014 involving open pollinated varieties (OPVs) and hybrid maize (*Zea mays* L.) were analysed using GGE (Genotype and Genotype x Environment) and AMMI (Additive Main Effect and Multiplicative Interaction) models to assess their performance and suitability as cultivars, and identify promising genotypes and sites for further evaluations in the different locations of the southern Guinea savanna of Nigeria. The experiments were set up as a randomized complete block design with three replications in all the locations. The plot size consisted of two rows, 5m long with inter and intra row spacing of 0.75m x 0.4m for the early maturing varieties and 0.75m x 0.5m for intermediate/late maturing varieties and hybrids. Two seeds were planted/hill to give a plant population of approximately 66,000 plants/ha (early) and 54,000 plants/ha (intermediate/late). Data were collected on agronomic and yield parameters. The environment accounted for 84.80% and 90.42% of the total variation in grain yield of OPVs and hybrids, respectively. TZE-Y-DT STR C₄ (early OPV) and white-DT-STR-SYN (intermediate/late OPV), TZE-W-Pop-DT STR-C₅ (early maturing hybrids) and TZEEI 3 x TZEEI 46 (extra early genotype) were the most stable and high yielding. The core test locations for evaluation of early OPVs, intermediate/late OPVs and hybrids are Ilorin/Ballah, Ejiba/Mokwa and Kishi/Badeggi, respectively. This study recommends that fewer but better locations that provide relevant information should be used for conducting multi-location trials and TZE-Y-DT STR C₄, white-DT-STR-SYN, TZE-W-Pop-DT STR-C₅ and TZEEI 3 x TZEEI 46 should be further evaluated on farmer fields.

Key words: genotype, grain yield, hybrids, maturity, maize, location.

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Introduction

Maize (*Zea mays* L.) is the most important staple food for over 300 million people in the Sub-Saharan Africa (SSA) countries (Beyene et al., 2016), making it an essential food crop for global food security. However, recent changes in climatic factors, which often manifest in form of irregular rainfall pattern, excessive temperatures and abrupt cessation of rains during the growth period, have militated against attainment of optimum maize productivity in drought-prone areas of the west and central Africa (WCA). Although maize productivity, especially in the Nigerian savannas, is affected by biotic (parasitic weed – *striga*, stem borer) and abiotic (drought, low nitrogen) stress factors, drought stress alone constitutes the greatest yield limiting factor in maize production in most of WCA including Nigeria. Empirical data show that maize yield loss due to moisture deficit can be as high as 80%, especially if it occurs during the flowering period (Bolanos and Edmeades, 1993; Cairns et al., 2013) while a range of 40–90% yield loss has also been reported for maize in studies conducted in WCA (Menkir and Akintunde, 2001; Olaoye, 2009). Consequently, a reduction in drought susceptibility through the cultivation of maize drought tolerant genotypes will provide added stability to rural economics and reduce the level of chronic food deficit in more marginal production areas (Edmeades et al., 1997). In other words, the development and deployment of maize genotypes with capacity to remain productive under moisture deficit conditions of drought-prone ecologies will likely boost maize production beyond its present level.

The initial strategy in combating drought in WCA focused on the deployment of extra-early and early maturing maize genotypes to the marginal rainfall areas. However, recent breeding efforts by maize scientists at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria have resulted in the development of maize varieties (open pollinated varieties and hybrids) of different maturity with capacity to tolerate moisture deficit conditions. These varieties have demonstrated the superiority for grain yield over the existing genotypes especially under moisture deficit conditions. Furthermore and in order to ensure that resource-poor farmers benefit from growing these varieties, recently developed genotypes have been evaluated in multi-environments in the Nigerian savannas, including the southern Guinea savanna (SGS), through funds provided by Bill and Melinda Gates for the Drought Tolerant Maize for Africa (DTMA) project and facilitated by the Maize Improvement Programme of the IITA.

The contribution of DT maize varieties in averting crop failure or increasing crop productivity especially in semi-arid and low moisture areas of the world is well documented (Jensen, 1994; Ngure, 1994; Njoroge, 1994). The authors have attributed the gains either to the use of DT maize genotypes or the adoption of early and extra-early maize varieties for cultivation in marginal rainfall areas.

However, varietal evaluation in multi-locations and/or years has heavy financial implication in terms of physical and human resources, identification of locations which correctly classifies the genotypes with respect to their yielding ability as well as those with the similarity of performance for grain yield will reduce the number of testing sites to manageable size without losing information on yield potential. The study reported here employed data collected on DT maize genotypes across several locations and years (2007–2014) in the Nigerian SGS with the objectives to (1) identify promising candidates for further evaluation in farmers' fields, (2) classify the testing sites for their discriminating ability and (3) identify maize varieties that could serve as a replacement for the existing varieties.

Materials and Methods

Description of experimental materials and sites

The genetic materials used were comprised of DT maize germplasm obtained from the Maize Improvement Programme (MIP) of the IITA, Ibadan, Nigeria. This study was conducted in the drought-prone ecologies of Nigerian SGS which comprised many farming communities in four states: Kwara, Kogi, Niger and the northern fringes of Oyo state. The communities included Ilorin, Oke-Oyi and Ballah (Kwara State), Ejiba (Kogi State), Mokwa and Badeggi (Niger State) and Kishi (Oyo State), all in the SGS of Nigeria. The coordinates of locations ranged between latitude $8^{\circ}26' - 9^{\circ}30'N$ and longitude $3^{\circ}50' - 6^{\circ}56'E$ with an altitude between 143 and 430m asl. Ferruginous tropical soils on crystalline acid rocks are the soil types across the locations. Rainfall distribution at each location is bimodal with an annual rainfall of 1100–1400mm. However, the distribution is highly unpredictable, with an early false start around April, but which often ceases abruptly for few weeks and ends not later than mid-October of every year.

Crop establishment and maintenance

The experimental materials were evaluated during the 2007–2014 late cropping seasons. Each set was planted as a separate experiment, but adjacent to each other, as a randomized complete block design with three replications in all the locations. The plot size consisted of two rows, 5m long with inter and intra row spacing of 0.75m x 0.4m for the early maturing varieties and 0.75m x 0.5m for intermediate/late maturing varieties and hybrids. Three seeds were planted/hill but later thinned to 2/hill to give a plant population of approximately 66,000 plants/ha (early) and 54,000 plants/ha (intermediate/late). Crop management practices included weed control with a pre-emergence application of the herbicide (Primextra at 2.5kg ai/ha) and supplementary hoe weeding. The fertilizer

application was carried out as split-dosage at the rate of 80kgN/ha, 60K₂O/ha and 60P₂O₅ at three weeks after planting (3WAP) and at anthesis (7WAP), using compound fertilizer (NPK 20:10:10).

Data collection

Data were collected on agronomic and yield parameters including days to mid-anthesis and silking, plant and ear heights (cm), stand count, ear aspect, cob weight and grain weight at harvest.

Data analysis

Using extrapolated grain yield data, genotypes (37 OPVs and 99 hybrids) common to a specific period were selected and subjected to individual as well as combined analysis of variance of the three-way mixed effects model where a genotype and locations were considered as fixed effect and year effect was considered as random. The statistical model used is $Y_{ijkl} = \mu + B_i + G_j + E_k + F_l + (GE)_{jk} + (GF)_{jl} + (EF)_{kl} + (GEF)_{jkl} + e_{ijkl}$

where:

Y_{ijk} = performance of genotype j in the k^{th} environment;

μ = grand mean;

B_i = block effect;

G_j = main effect of the j^{th} genotype;

E_k = main effect of the k^{th} year;

F_l = main effect of the l^{th} location;

$(GE)_{jk}$, $(GF)_{jl}$, $(EF)_{kl}$, $(GEF)_{jkl}$ = interaction effects;

e_{ijkl} = random error term.

Genotype x Environment (GE) interaction for each trait was determined. Pertinent means were separated using least significant difference (LSD) at $p \geq 0.05$ while significant GE interactions were further analysed using the GGE biplot to identify superior genotypes, ideal genotypes, genotypes with a specific adaptation as well as an ideal location for future testing of the varieties using GGE biplot software (Version 7.0).

Results and Discussion

Results from the AMMI model for grain yield showed significant differences among the levels of all sources of variation except the genotypes. The environment accounted for the largest total mean squares followed by principal component (PC) axes 1 and 2 accounted for both OPVs and hybrids across the trial years (Table 1).

The large mean squares contributed by the environment indicated that the SGS region of Nigeria was highly variable from location to location and a highly significant GE for grain yield justified the use of the GGE biplot to decompose the GE to determine the yield potential and stability of the genotypes.

Table 1. AMMI model for grain yield (t/ha) of selected DT maize genotypes evaluated between 2007 and 2014 in the SGS of Nigeria.

	Early OPVs		Intermediate/late OPVs			
	2007–2012		2007–2009		2010–2012	
	DF	MS	DF	MS	DF	MS
Genotypes	13	992973	7	203103	14	296439
Environments	5	13786592**	6	9670266**	5	26099623**
Interactions	65	731092	42	268452	70	460989
IPC1	17	1539730**	12	461174**	18	915409**
IPC2	15	1014934**	20	390917*	16	490596*
Residuals	33	185503	20	91586	36	220621
	Early maturing hybrids		Extra early maturing hybrids			
	2009–2014		2009–2013			
	DF	MS	DF	MS		
Genotypes	21	433073	21	433073		
Environments	6	70578713**	6	70578713**		
Interactions	126	679831	126	679831		
IPC1	26	2367911**	26	2367911**		
IPC2	24	595193**	24	595193**		
Residuals	76	129058	76	129058		

DF=degree of freedom; MS=mean sum of square; **significant at $p=0.001$; *significant at $p\leq 0.05$.

This is in agreement with the findings of Badu-Apraku et al. (2011) who reported 83.4%, 1.5% and 11% contributions of environment, genotype and G x E to the total variation in grain yield of maize in West and Central Africa, respectively. The findings of this study are consistent with the reports of Yan et al. (2010), who identified essential test locations for oat breeding in Canada, Setimela et al. (2007), who evaluated early to medium open pollinated maize in southern African communities and Badu-Apraku et al. (2011), who targeted early maize cultivars in West Africa countries.

Mean location grain yield across years for the selected genotypes depicted similar performance among each group and trial year. The early OPVs had higher grain yield compared to the intermediate/late OPVs and similar performance in each cropping year (Table 2). A similar trend was observed for early and extra early hybrids (Table 3). This is a deviation from the norm because yield is usually sacrificed for earliness although the early OPVs selected are new generation of advanced lines that are being improved as opposed to the intermediate/late OPVs.

Table 2. Maize grain yield (kg ha⁻¹) of selected drought tolerant OPVs in the SGS of Nigeria.

Genotype	2007	2008	2009	2010	2011	2012	Sed
Early DT OPVs							
TZE Comp 3 DT CO F3	6903.29	6526.52	7186.19	7541.14	6931.80	7197.76	460.60
TZE-Y DT STR C4	6998.44	6871.12	7853.23	6500.37	7294.55	7478.19	485.13
TZE Comp 3 DT CO F2	6602.32	6772.69	6392.81	6554.37	6613.07	6480.76	599.38
EVDY-Y 2000 STR CO	6343.40	6156.45	6105.75	6603.83	6295.77	6360.21	418.57
TZE Comp 3 DT CO F5	6625.77	6135.54	6444.80	6433.99	6175.13	6092.28	612.64
TZE-W DT STR C4	5263.41	5370.96	6026.94	5632.39	5122.77	3407.53	715.90
TZE Comp 3 DT CO F4	6021.73	6197.69	6292.22	5531.34	6004.34	5379.29	666.55
Sed	1290.49	1344.99	1372.19	1387.81	1280.29	1558.48	
Intermediate/late DT OPVs							
DT-SR-W CO F2	5337.27	6154.12	5523.26	-	-	-	558.21
DT-SYN-1-W	5081.27	5018.44	5479.03	-	-	-	429.04
SUWAN-1-SR-SYN	5032.29	4381.98	5093.78	-	-	-	256.41
TZB-SR	4794.45	4937.62	4257.63	-	-	-	432.45
TZL Comp 1-W- C6 F2	4859.11	5442.28	5461.32	-	-	-	606.23
TZUTSY-W-STR-SYN	5188.29	5082.76	5072.84	-	-	-	406.50
White DT STR SYN	5900.21	5025.81	5628.41	-	-	-	401.82
DT-STR-Y-SYN 2	-	-	-	5064	4867	5158	438.30
IWD C3 SYN F2	-	-	-	4993	5160	5472	533.60
TZL COMP3 C3 DT	-	-	-	5771	4846	5650	507.70
IWD C3 SYN/DT-SYN-1-W	-	-	-	5489	5727	5179	549.00
(White DT STR SYN/IWD C3 SYN)F2	-	-	-	5738	5489	5176	525.30
DT-STR-W C2	-	-	-	5811	5306	5288	607.90
DT-STR-W SYN C2	-	-	-	5404	5519	5629	571.00
(White DT STR SYN/TZL Comp1-W)F2	-	-	-	4712	5604	6648	776.30
TZL Comp4 C3 DT	-	-	-	6383	5725	6015	715.60
Sed	481.53	676.88	696.39	942.63	590.51	838.57	

Table 3. Maize grain yield (kg ha⁻¹) of selected drought tolerant hybrids in the SGS of Nigeria.

Genotype	2009	2010	2011	2012	2013	2014	Sed
Early maturing DT hybrid							
TZE-Y Pop DT STR C4 x TZEI 11	8118.64	8557.72	8887.17	8863.61	9250.60	8924.43	720.84
TZEI 24 x TZEI 17	8824.10	9094.45	8808.21	8390.31	9226.72	8884.67	427.68
TZEI 8 x TZEI 17	8964.43	7965.20	9164.89	8969.90	8015.25	7737.73	664.36
TZE-W Pop DT C4 STR C5	8968.49	10467.07	10349.65	9789.20	10088.46	9664.60	581.25
DT -W STR Synthetic	8861.87	8910.48	8324.60	9716.30	10139.42	8855.42	686.91
DTE STR-Y Syn Pop C2	8910.42	8963.26	9355.23	9299.35	9563.91	9367.93	651.87
2012 TZE-Y DT C4 STR C5	9632.42	9865.03	9322.40	9754.20	8821.86	9182.60	612.25
DTE STR-W Syn Pop C2	9677.82	10098.13	9601.83	8624.57	8372.74	8633.66	554.45
Sed	1689.29	1729.71	1757.48	1808.88	2012.04	1945.52	
Extra early white DT hybrid							
TZEE-W Pop STR C5 x TZEEI 14	11439	10498	9955	8787	11742	-	775.20
(TZEEI 29 x TZEEI 21) x (TZEEI 14 x TZEEI 37)	10784	8920	10256	9661	9836	-	1775.20
(TZEEI 21 x TZEEI 14) x TZEEI 29	9576	8354	11183	9507	10428	-	925.20
TZEEI 3 x TZEEI 46	9899	9864	10557	9358	9316	-	1331.20
TZEEI 29 x TZEEI 21	9499	9062	10622	9451	9000	-	681.90
(TZEEI 29 x TZEEI 21) x TZEEI 55	10645	9096	10218	8267	8282	-	826.90
(TZEEI 29 x TZEEI 37) x TZEEI 13	9763	9850	9322	8588	8827		1109.00
TZEE-W Pop STR C5 x TZEEI 46	9208	9001	8609	9498	8986	-	965.60
(TZEEI 4 x TZEEI 14) x (TZEEI 29 x TZEEI 49)	12605	12308	10616	11264	11986	-	1442.70
(TZEEI W Pop STR C5 x TZEEI 29	10552	9675	8993	9265	9761	-	993.00
TZEEI 4 x TZEEI 49) x TZEEI 29	9297	10363	9413	10512	8320	-	776.50
TZEEI 29 x TZEEI 21) x (TZEEI 4 x TZEEI 14)	10047	10165	9457	8635	9239	-	1218.5
Sed	2133.30	2016.70	1628.20	2013.60	1915.60	-	

The GGE biplot for the early OPVs explained 74.7% of genotype main effects and G x E interaction. The PC1 and PC2 components explained 51.9 and 22.8% of genotype main effects and G x E interaction, respectively (Figure 1) while 80.1% (Figure 2A) and 94.4% (Figure 2B) of the GGE biplot for the intermediate/late OPVs explained the genotype main effects and G x E interaction.

Based on the reports of Yan and Tinker (2006) and Setimela et al. (2007), the small circle is the average-environment axis (AEA), and the arrow pointing to it is used to indicate the direction of the AEA and the locations that have shorter vectors are less informative in contrast to those with longer vectors whereas the most representative locations are those locations with smaller angles with the AEA. Therefore, Ballah is the most discriminating location while Ilorin is the most representative location (Figure 1). The implication of the above is that a promising OPV selected in one of these locations will also be suitable for production in the other locations within the SGS of Nigeria.

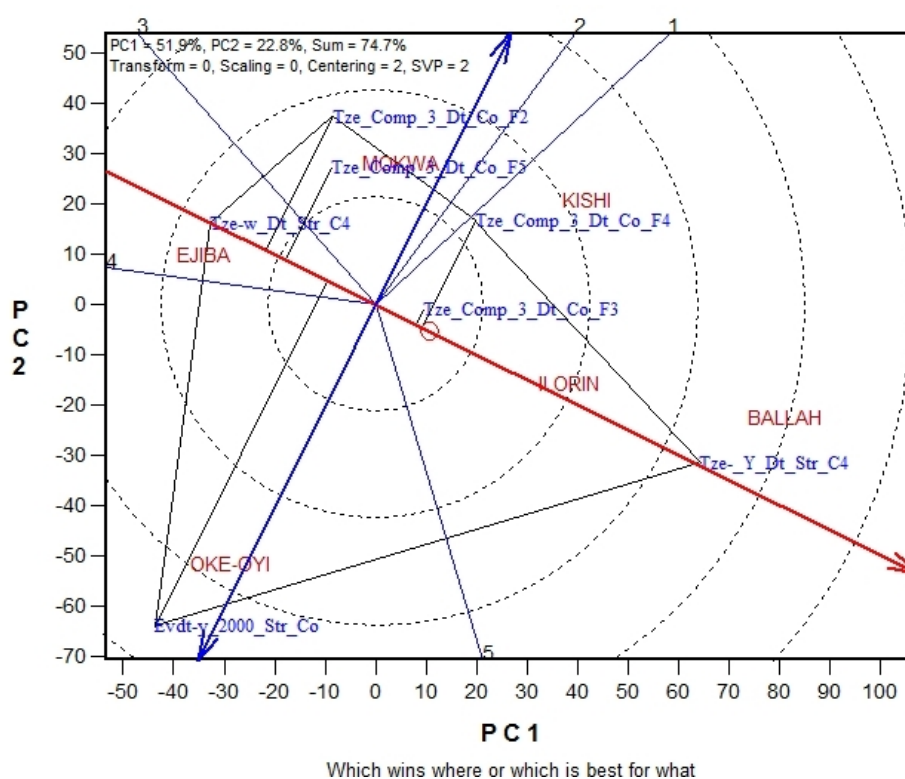


Figure 1. The polygon view of the GGE biplot showing which early OPVs won in which location.

Yan (2001) defined ideal genotype to have high projection towards the double-headed line and near zero projection to the line with the AEA. In Figure 1, the early OPV, TZE-Y-DT STR_C4, had the highest but unstable grain yield performance across the environments, TZE-Comp-3-DT-Co F₃ was highly stable with low yielding as compared to the former while TZE-Comp-3-DT-Co F₄ was unstable and low yielding across the environments.

Stability across locations for intermediate/late OPVs in 2007–2009 seasons (Figure 2A) shows that TZL-Comp 1-W C₆-F 2 was high yielding but unstable genotype, DT-SR-W-Co_F₂ and White-DT-STR-SYN were the most stable and high yielding genotypes.

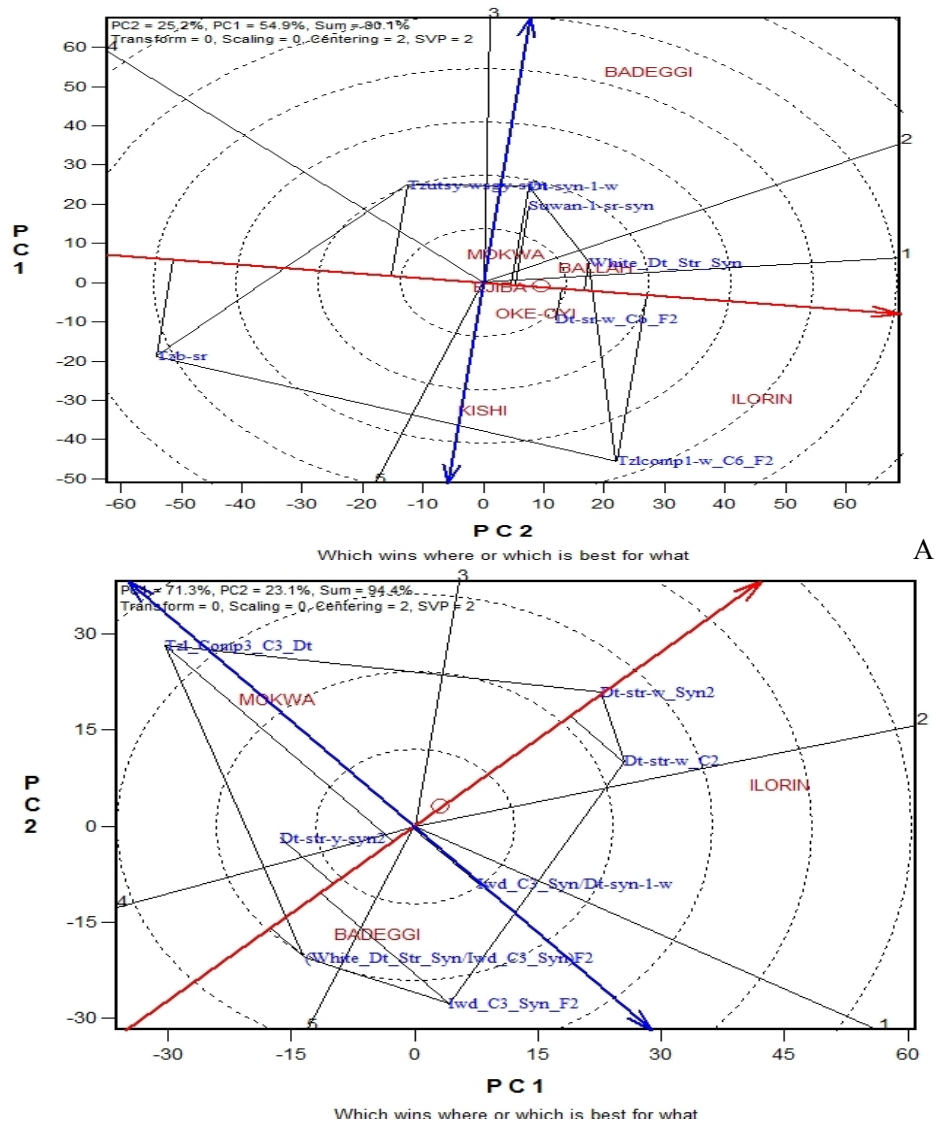


Figure 2. The polygon view of the GGE biplot showing which OPVs won in which location for intermediate/late DT OPVs between 2007 and 2009 (A) and between 2010 and 2012(B).

The 2010–2012 evaluation shows that DT-STR-W-SYN 2 and DT-SR-W-C₂ were high yielding but less stable. Genotype White-DT-STR-SYN/IWD-C₃-SYN F₂ was the most stable but low yielding (Figure 2B). Yield performance consists of mean yield and stability concepts (Karimizadeh et al., 2013), therefore plant breeders should explore genotypes that indicate yield stability as well as high yield across environments. This study will project TZE-Y-DT STR C₄ (early OPV) and White-DT-STR-SYN (intermediate/late OPV) with acceptable stability and good grain yields as the best OPVs.

The early hybrid genotypes in 2009–2011 (Figure 3A) trials showed that the highest mean performance was obtained from TZE-Y-Pop-DT-STR-C₄ x TZEI 11 but relatively unstable while in 2012–2014 (Figure 3B), TZE-W-Pop-DT-C₄-STR-C₅, 2012 TZE-Y-DT-C₄ STR-C₅ and DTE-STR-Syn-Pop-C₂ were the high performed hybrids, while the most stable genotype was 2012 TZE-Y-DT-C₄ STR-C₅. Also, Figure 4A shows that hybrid TZEEI-W-Pop-STR-C₅ x TZEEI 14 was the highest yielding genotype but unstable compared to TZEEI-3 x TZEEI 46 while in 2011–2012 (TZEEI 4 x TZEEI 14) x (TZEEI 29 x TZEEI 49) was the only outstanding hybrid in terms of performance and stability (Figure 4B).

The polygons are divided into several sectors and some of these sectors have locations within them suggesting the possibility of different mega-environments existing for the genotypes. The mega-environments for early OPVs were Ballah, Ilorin and Kishi (Figure 1) while Ilorin, Kishi, Oke-Oyi and Ejiba made up the first mega-environment; Badeggi and Mokwa constituted the second mega-environment for the intermediate/late maturing OPVs during 2007–2009 cropping periods (Figure 2A).

Figure 3A shows two mega-environments in 2009–2011 trials which included: Badeggi, Ejiba and Ilorin as a mega-environment with TZE-Y-Pop-DT-STR-C₄ x TZEI11 as the best performing hybrid and Mokwa as the second mega-environment and TZEI-24 x TZEI-17 as the best yielding hybrid. The 2012–2014 genotype evaluation was divided into five mega-environments and each with the best performing hybrid (Figure 3B). The extra early trial shows that Kishi was the most discriminating and representative in both trials while Ballah was close to Kishi during the 2009–2010 growing seasons. Hybrid TZEEI-W-Pop-STR-C₅ x TZEEI 14 was the highest yielding genotype but unstable while TZEEI-3 x TZEEI 46 was the most stable and high yielding genotype (Figure 4A). Similarly, in 2011–2012, (TZEEI 4 x TZEEI 14) x (TZEEI 29 x TZEEI 49) was high yielding hybrid but unstable. Three of the hybrid genotypes were stable but had low yield (Figure 4B).

However, this mega-environment pattern needs verification through other multi-environment trials for this target region. Regarding this pattern, genotypes TZE-Y-DT-STR-C₄ and White-DT-STR-SYN were the most favorable genotypes, having specific adaptability for these mega-environments. These inferences about

polygon view patterns are mostly, but not totally, validated from the original data. However, the model outcome is worthwhile for recommendation purposes as demonstrated by several authors (Setimela et al., 2007; Badu-Apraku et al., 2008, 2011, 2013; Badu-Apraku and Lum, 2010).

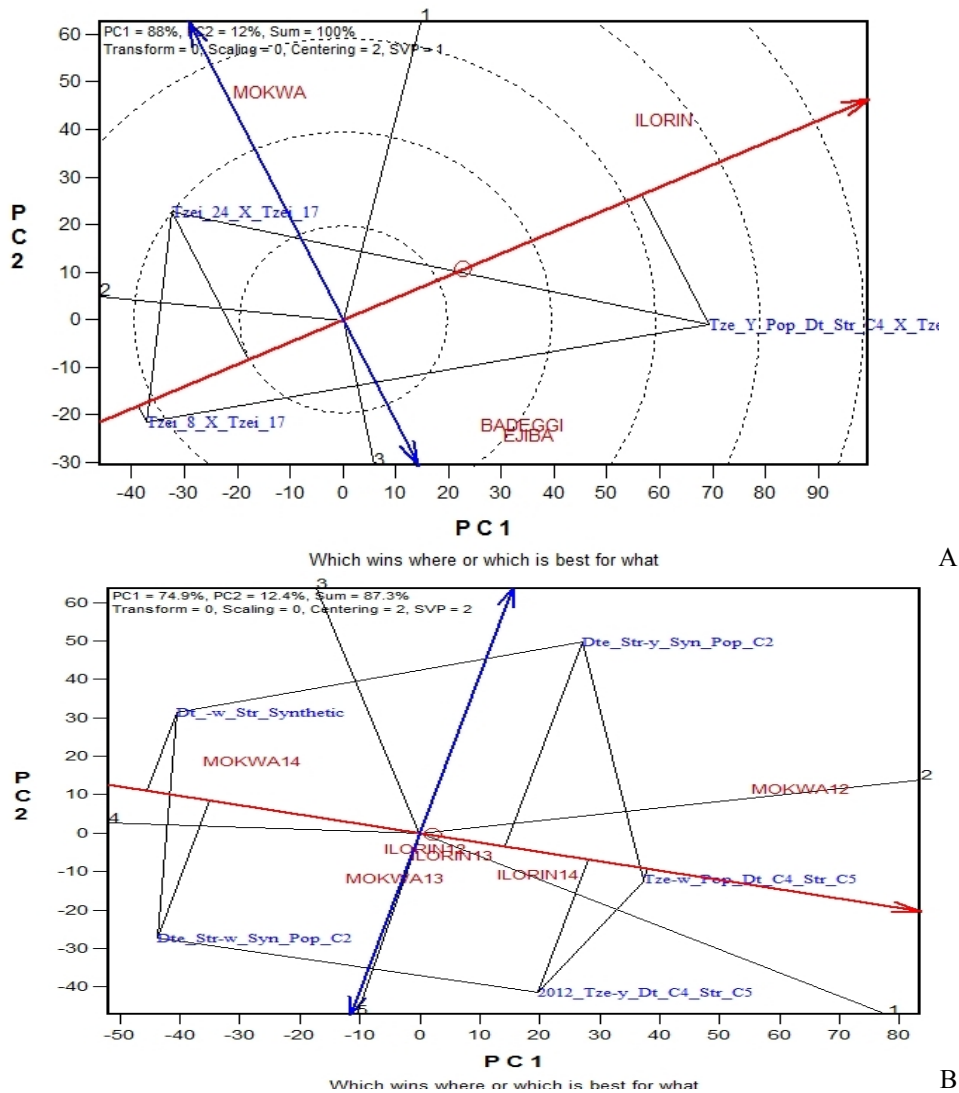


Figure 3. The polygon view of the GGE biplot showing which OPVs won in which locations for early maturing DT hybrids between 2009 and 2011 (A) and between 2012 and 2014 (B).

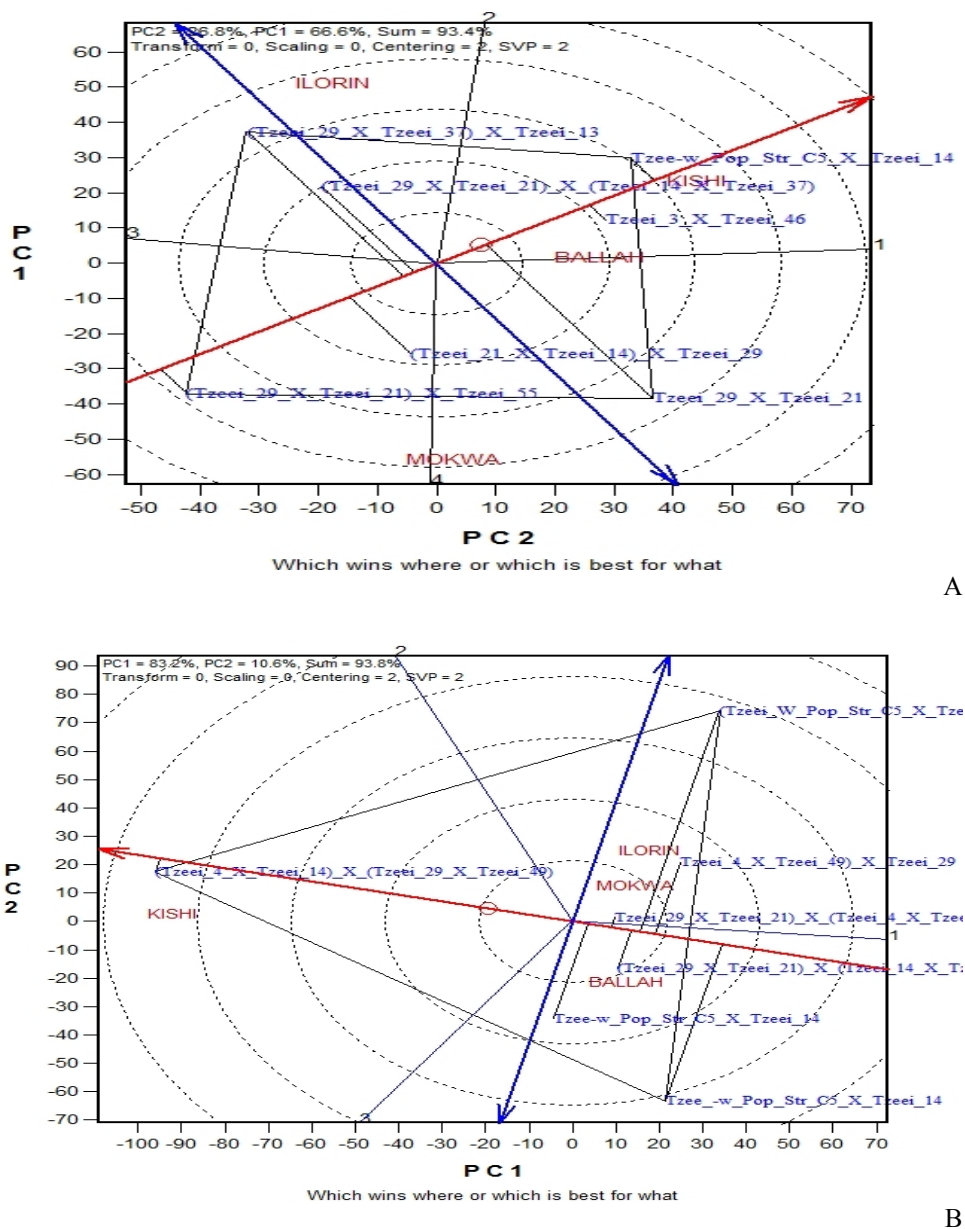


Figure 4. The polygon view of the GGE biplot showing which OPVs won in which location for extra early white DT hybrid maize between 2009 and 2010 (A) and between 2011 and 2013 (B).

Conclusion

This study concludes that fewer but better locations that provide relevant information should be used for conducting multi-location trials. Thus, Ilorin/Ballah, Ejiba/Mokwa and Kishi/Badeggi are core test locations for evaluation of early OPVs, intermediate/late OPVs and hybrids, respectively although these patterns need verification through other multi-environment trials. The following promising genotypes are recommended for further evaluation on farmer's fields: TZE-Y-DT-STR-C₄ (early OPV), White-DT-STR-SYN (intermediate/late maturing OPVs), TZE-W-Pop-DT STR-C₅ (early maturing hybrids) and TZEEI 3 x TZEEI 46 (extra early genotype) for the SGS agro-ecological region of Nigeria.

Acknowledgements

Financial support of the DTMA project for this study is gratefully acknowledged. The authors are also grateful to the United Nations University-Institute for Resources in Africa (UNU-INRA) for granting the first author financial support and the enabling environment during his Visiting Scholar Programme. This manuscript is culled from his working paper submitted to UNU-INRA.

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Received: August 1, 2016

Accepted: February 14, 2017

PROCENA PRINOSA ZRNA KUKURUZA U SREDINI SKLONOJ SUŠI

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R e z i m e

Podaci višelokacijskih ogleda dobijeni između 2007. i 2014. godine uključujući slobodno oprašujuće populacije (engl. *open pollinated varieties* [OPVs]) i hibrid kukuruza (*Zea mays* L.) analizirani su korišćenjem modela GGE (engl. *Genotype and Genotype x Environment*) i AMMI (engl. *Additive Main Effect and Multiplicative Interaction*) kako bi se ocenio njihov učinak i pogodnost kao populacija, i identifikovali obećavajući genotipovi i mesta za dalju evaluaciju na različitim lokacijama savane južne Gvineje u Nigeriji. Ogledi su postavljeni po metodi slučajnog blok sistema u tri ponavljanja na svim lokacijama. Veličina parcele se sastojala od dva reda, dužine 5m sa rastojanjem od 0,75m x 0,4m između i unutar redova za rane populacije i 0,75m x 0,5m za srednje i kasne populacije i hibride. Zasejana su dva semena po kućici, kako bi se dobila gustina populacije od oko 66.000 biljaka/ha (rane) i 54.000 biljaka/ha (srednje/kasne). Prikupljeni su podaci o agronomskim parametrima i parametrima prinosa. Uslovi spoljašnje sredine su činili 84,80% odnosno 90,42% ukupne varijacije u prinosu zrna slobodno oprašujućih populacija, odnosno hibrida. TZE-Y-DT STR C₄ (rani OPV) i beli-DT-STR-SYN (srednji/kasni OPV), TZE-W-Pop-DT STR-C₅ (rani hibridi) i TZEEI 3 x TZEEI 46 (veoma rani genotip) bili su najstabilniji i dali su visok prinos. Glavne proučavane lokacije za evaluaciju ranih slobodno oprašujućih populacija, srednjih/kasnih slobodno oprašujućih populacija i hibrida su Ilorin/Balah, Ejiba/Mokva odnosno Kiši/Badegi. Ovo istraživanje preporučuje da manji broj, ali boljih lokacija, koje obezbeđuju relevantne informacije, treba da budu korišćene za sprovođenje višelokacijskih ogleda. Genotipove TZE-Y-DT STR C₄, bela-DT-STR-SYN, TZE-W-Pop-DT STR-C₅ i TZEEI 3 x TZEEI 46 treba dalje procenjivati na poljoprivrednim površinama.

Ključne reči: genotip, prinos zrna, hibridi, zrelost, kukuruz, lokacija.

Primljeno: 1. avgusta 2016.
Odobreno: 14. februara 2017.

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CONTENT OF SOME ANTIOXIDANTS IN INTERCROPPED MAIZE AND SOYBEAN GRAIN

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Abstract: Intercropping, as a combination of different crops at the same time and the same field, enables interaction of their roots, improving plant growth and stress tolerance, thus improving nutritional quality of produced grains. The investigation was aimed to examine the effect of different cropping systems: intercropping in combination with alternating rows and alternating strips of maize and soybean, as well as single cropping, combined with different fertilization regimes (conventional, application of organic fertilizer, bio-fertilizer and control) on the antioxidant content (glutathione [GSH], phenolics and yellow pigment [YP]) in red maize and black soybean grain. Black soybean is richer in antioxidants than red maize. Season expressed the highest influence on the level of GSH, phenolics and YP in maize and soybean, while cropping system and fertilization regime influenced GSH and phenolics. The antioxidant level in grains with greater weight corresponded with an increased GSH level for maize, as well as an increased GSH and phenolic level for soybean, while smaller grains were characterised by the increased YP content. Generally, antioxidant content was increased mainly by alternating strips in maize grain and by alternating rows in soybean grain. Bio-fertilizer had the highest impact on an increase in GSH in maize grain and YP in soybean grain, while organic fertilizer was important for acquiring of GSH and phenolics in soybean grain.

Key words: antioxidant content, intercropping, red maize, black soybean.

Introduction

Sustainable agriculture combines various measures aimed to produce high quality and healthy crops, together with preservation of an agro-ecosystem. Produced crop yields are more nutritious and free from agrochemicals and their

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residues. This type of agriculture includes a combination of different crops at the same field, application of organic fertilizers and bio-fertilizers, facilitating better utilization of time, space and nutrients, i.e. soil potential, with minimal inputs.

Intercropping, as a combination of different crops at the same time and the same field, enables interaction of their roots, by the root exudates, and interaction with soil micro-organisms (Zhang et al. 2013). Roots of intercrops have greater root development, going deeper than roots of sole crops (Yang et al., 2010). Also, intercropping enables increased resistance to various diseases and pests. For instance, in soybean intercropped with maize, resistance to red crown rot was increased, by enhancing of phenolic acid concentration in root exudates, which constrain *C. parasiticum* growth (Gao et al., 2014). Root exudates create a specific environment for development of soil microorganisms, improving soil chemical and microbial properties. Thus, when pepper was intercropped with green garlic, growth of actinomycetes was improved, with inhibition of fungi growth, together with increased activity of invertase, alkaline phosphatase and catalase (Ahmad et al., 2013). Moreover, some biochemical pathways of intercrops could be altered, like phenylpropanoid and organosulfur biosynthesis in Chinese onion, when it is intercropped with cucumber (Yang et al., 2013).

Application of bio-fertilizers which contain promoting microorganisms could enhance crop growth and stress tolerance. Aroca and Ruiz-Lozano (2009) found that pulses, inoculated with rhizobial bacteria had improved growth during drought conditions, due to the bacteria induced regulation of plant hormones, like abscisic acid and ethylene. Pandey et al. (2016) also confirmed a positive impact of rhizobacteria on crop growth and stress tolerance, by increasing of the antioxidant defence and nutrient absorption.

The additional health effect on humans could be achieved by consumption of food produced from specific genotypes, such as red grain maize or black soybean. Red maize is characterised by about 20% higher protein content than white or yellow genotypes, with an increased anthocyanin and flavonoid level (Žilić et al., 2011a), while black soybean contains two times higher phenolic level in grain than yellow grain genotypes (Žilić et al., 2011b). Some other important antioxidants for plants and human health include glutathione and yellow pigments (mainly β -carotene), which play an important role in the antioxidative defence and stress signalling (Foyer and Noctor, 2005; Grodstein et al., 2007).

The aim of experiment was to examine effects of different cropping systems: intercropping and single cropping and fertilization regimes (conventional, application of organic fertilizer and bio-fertilizer) on the antioxidant content (glutathione, phenolics and yellow pigment) in maize and soybean grain.

Materials and Methods

The experiment was conducted during the 2011 and 2012 vegetative period, at Zemun Polje (44°52'N 20°20'E), on a slightly calcareous chernozem, with 53.0% sand, 30.0% silt, 17.0 % clay, 3.3% organic matter, 7.0 pH KCl and 7.17 pH H₂O. Chemical analysis showed that soil contained 37.45 ppm N, 10.70 ppm P, 107.40 ppm K, 327.95 ppm Mg, 0.65 ppm Fe and < 0.02 ppm Zn in the 0–30 cm layer. Red grain maize (variety Rumenka) and black grain soybean (variety Dukat) were grown in three different cropping systems: as a single crop (SC), in alternating rows of both crops (AR) and in alternating strips (3 rows of each species – AS), with a 70-cm inter-row distance, as well as with a 22- and 4-cm intra-row distance for maize and soybean, respectively. The crops were sown on the 11th May 2011 and 2012. The elementary plot encompassed 4 × 4.2 m in a completely randomized block design with 4 replications. The effect of fertilization regimes included the incorporation of bio-fertilizer Uniker (11 l ha⁻¹), organic fertilizer Ofert (3 t ha⁻¹), urea (163 kg ha⁻¹) and control – without fertilization. Bio-fertilizer Uniker contains the following bacteria: *Bacillus megaterium*, *B. licheniformis* and *B. subtilis*. Ofert contains a minimum of 2.2% N, 4.8% P₂O₅, 2.8 % K₂O, 60% organic matter, C/N 12.17, and 1.08% Mg.

After harvesting, grain weight was measured from 4x10 grains and expressed in g per grain. Then grains were milled and the content of total glutathione (GSH) was determined by the method of Sari Gorla et al. (1993), water soluble phenolics were determined by the method of Simić et al. (2004) and expressed in µg of 3-hydroxy-4-methoxycinnamic acid g⁻¹ and yellow pigment (YP) was determined by the American Association of Cereal Chemists Method (AACC, 1995) and expressed in µg of β-carotene g⁻¹.

Significant differences between treatment means were determined by the Fisher's least significant difference (LSD) test at the 0.05 probability level, after the analysis of variance (ANOVA) using a two-factorial RCB design. Differences with p<0.05 were considered as significant. The interdependences between the grain weight of maize and soybean and examined antioxidants were processed by regression analysis.

Meteorological conditions: The vegetative period of 2012 could be considered as unfavourable with an unequal distribution of precipitation (the lowest value was observed in August), followed by the high average temperatures in July and August (Table 1), indicating drought stress during the grain filling period. Oppositely, 2011 could be considered as a relatively moderate year, with lower average temperatures and higher amounts of monthly precipitation.

Table 1. Average monthly air temperatures and precipitation sums for the vegetative period (April–September) of 2011 and 2012 at Zemun Polje.

	Month	IV	V	VI	VII	VIII	IX	Aver./Σ
T aver.	2011	13.4	16.8	21.5	23.3	23.9	21.6	20.1
	2012	14.5	17.9	24.6	27.1	26.2	22.1	22.1
Σ precip.	2011	14.9	89.6	26.2	44.0	66.0	32.6	273.3
	2012	64.2	66.4	17.5	30.7	5.8	26.0	210.6

Results and Discussion

According to the results presented in Table 2, a significant variation in GSH and phenolic content was induced by the all examined factors (cropping system, fertilization and year) and their interactions, while a significant variation in YP was present under the influence of year and its interaction with the other two factors in grain of both crops.

Table 2. Analysis of variance for the effect of cropping system, fertilization and year on contents of glutathione (GSH), phenolics and yellow pigment (YP) in maize and soybean grain.

Source of variation	df	GSH (nmol g ⁻¹)		Phenolics (μg g ⁻¹)		YP (μg g ⁻¹)	
		MS	LSD _{0.05}	MS	LSD _{0.05}	MS	LSD _{0.05}
Maize grain	Replications	3					
	CS	2	17971*	103.6	69507*	256.2	1.26
	F	3	30025*	97.35	155560*	231.2	0.01
	Y	1	182040*	85.55	1604530*	179.8	58.83*
	CS X F	11	13558*	101.3	70821*	253.5	0.28
	CS x Y	5	45839*	89.41	190247*	132.4	812682*
	F x Y	7	51930*	62.29	32997*	139.8	11.447*
	CS x F x Y	23	20961*	39.05	131688*	51.4	4.256*
	CV (%)		19.01		28.02		18.25
	Average		552.53		915.51		8.58
	Min		337.55		625.62		5.82
	Max		798.34		1518.07		11.60
Soybean grain	Replications	3					
	CS	2	107441*	191.7	1478188*	606.8	29.9
	F	3	19297*	203.1	342795*	638.7	12.9
	Y	1	772211*	154.5	7849883*	503.7	2595.3*
	CS X F	11	53879*	188.3	625479*	592.6	17.5
	CS x Y	5	58088*	86.82	9159254*	276.0	768821*
	F x Y	7	145032*	146.1	1409855*	491.3	402.0*
	CS x F x Y	23	76714*	66.1	841822*	81.9	137.77*
	CV (%)		28.65		25.00		32.03
	Average		695.96		2577.91		26.10
	Min		291.46		1082.35		15.69
	Max		1181.54		3392.04		49.36

*Significant at the 5% probability level; df: degrees of freedom; MS: mean squares; LSD_{0.05}: least significant difference at the 0.05 probability level; CS: cropping system; F: fertilization; Y: year; CV: coefficient of variation.

It was already mentioned that GSH and phenolics play an important role in soybean nutritional quality (Dragičević et al., 2010). The highest variation and the highest average values of examined antioxidants were noticed in soybean grain compared to maize grain. This could be related to the high antioxidant level, particularly phenolics, present in the black grain soybean (Astadi et al., 2009; Žilić et al., 2011b).

Among fertilization regimes, the bio-fertilizer had the highest impact on an increase in GSH in maize grain (Figure 1) and YP in soybean grain (Figure 2), with the highest average values of 608.90 nmol g⁻¹ and 28.43 µg g⁻¹, respectively, while organic fertilizer was important for acquiring of GSH and phenolics in soybean grain (average values of 744.31 nmol g⁻¹ and 2861.84 µg g⁻¹, respectively). Taie et al. (2008) also acquired the highest phenolic content in soybean fertilized with compost and bio-fertilizer, compared to fertilization with compost only and conventional fertilization. Dragičević et al. (2013) obtained the elevated β-carotene content in intercropped soybean and maize grain under the influence of bio-fertilizer.

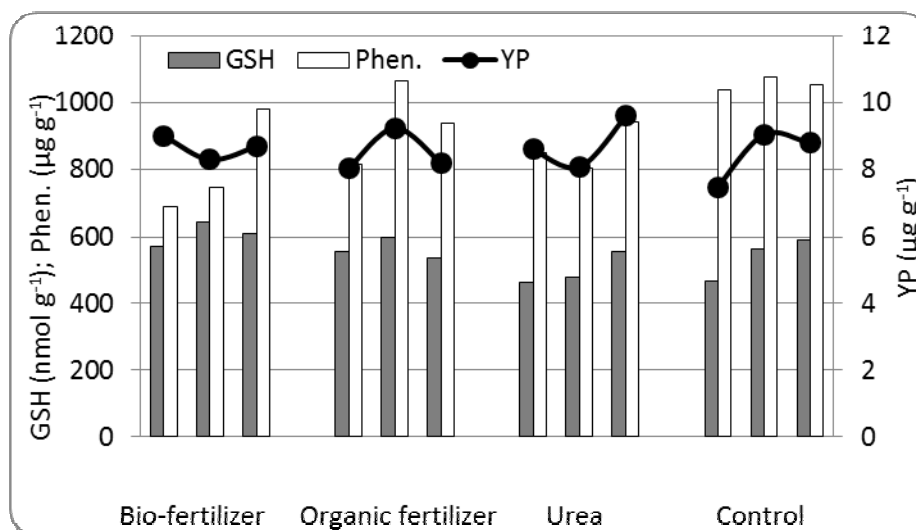


Figure 1. The effect of different cropping systems (SC – single crop, AR – alternating rows, AS – alternating strips) and fertilizer types on variation in glutathione (GSH), soluble phenolics (Phen.) and yellow pigment (YP) in maize grain (average for 2011–2012).

Rhizobacteria provide cross protection against numerous stresses by increasing of the antioxidant defence, nutrient absorption, etc., what is of particular importance during drought conditions (Pandey et al., 2016) which were

present during 2012. This could be one of the reasons for the increased antioxidant content in grain of maize and soybean from bio-fertilizer treatment. Urea and control treatments were characterised by higher YP and phenolic content in maize grain ($8.75 \mu\text{g g}^{-1}$ and $1055.13 \mu\text{g g}^{-1}$, respectively). When cropping systems were compared, alternating strips mainly had the highest impact on the increase of GSH (urea and control) and phenolics (bio-fertilizer), while alternating rows were important for GSH increase (bio-fertilizer and organic fertilizer), as well as phenolics and YP (organic fertilizer and control) in maize grain (Figure 1). Alternating rows had the highest effect on the increase of GSH (organic fertilizer, urea and control), phenolics (bio-fertilizer, organic fertilizer and control) and YP (all four cropping systems), while a single crop increased mainly GSH (bio-fertilizer) and phenolics (urea) in soybean grain (Figure 2). Dragičević et al. (2013) also emphasized alternating rows as an important intercropping system for GSH and phenolic increase. Other than that, the highest values of GSH content in maize grain and YP in soybean grain were noticed in AR + bio-fertilizer combination. The highest GSH and phenolic content in soy grain was noticed in AR + organic fertilizer treatment combination, and in maize grain the highest YP was noted in AS + urea combination. Similarly, the highest phenolic content was observed in AR + control combination.

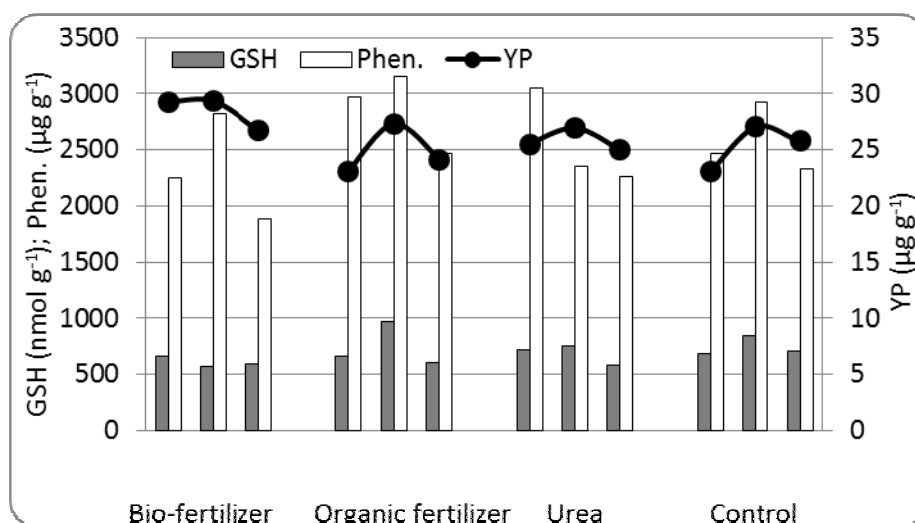


Figure 2. The effect of different cropping systems (SC – single crop, AR – alternating rows, AS – alternating strips) and fertilizer types on variation in glutathione (GSH), soluble phenolics (Phen.) and yellow pigment (YP) in soybean grain (average for 2011–2012).

Irrespective of the present variations in the content of examined antioxidants, relations with the yield parameter, such as seed weight indicated that increased seed weight was followed by the higher GSH and lower YP contents in maize and soybean grain (Figures 3 and 4).

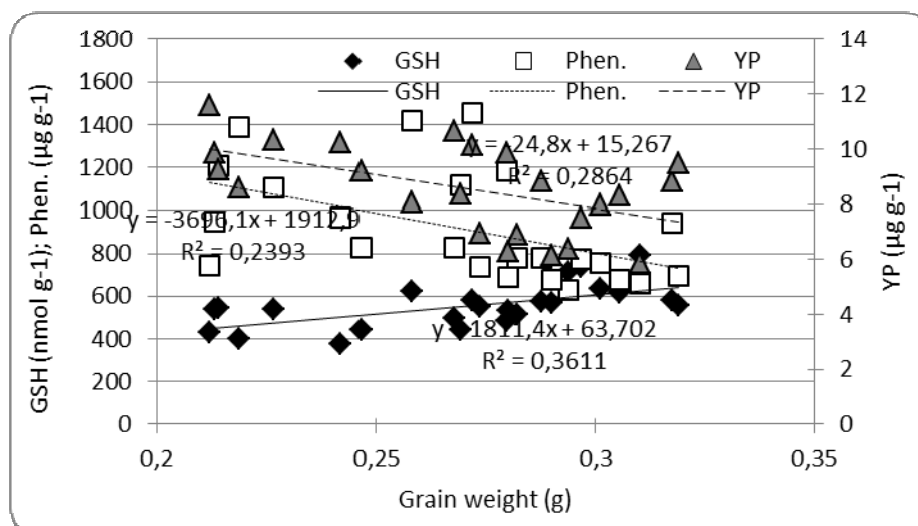


Figure 3. Interdependence between grain weight and glutathione (GSH), phenolics (Phen.) and yellow pigment (YP) content in maize grain.

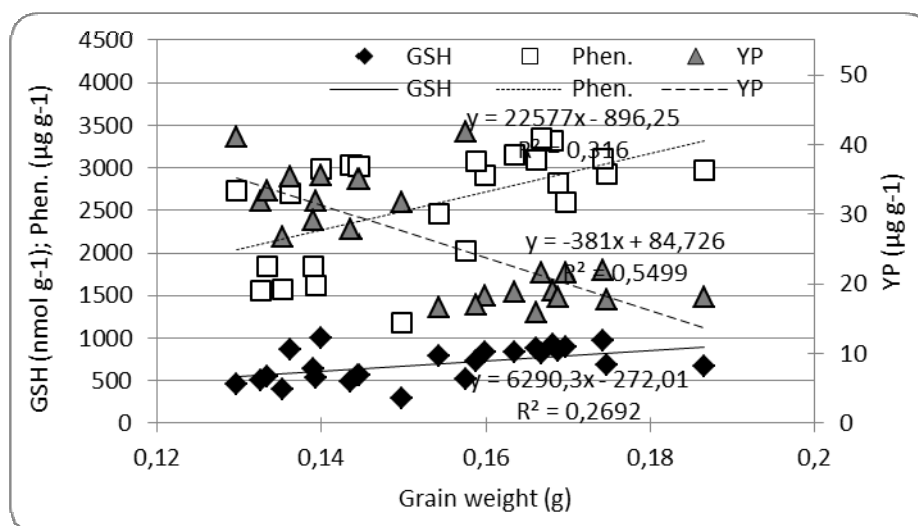


Figure 4. Interdependence between grain weight and glutathione (GSH), phenolics (Phen.) and yellow pigment (YP) content in soybean grain.

Nevertheless, higher grain weight was followed by the decreased phenolic content in maize grain and increased phenolic content in soybean grain. All regression coefficients were significant. Similarly to results obtained for maize grain, Yafang et al. (2011) and Shen (2009) also found higher phenolic level and antioxidant activity of small rice grains. Moreover, grain weight depends on many different factors, so Konopka et al. (2012) found slightly higher content of free phenolics and total carotenoids in wheat grain with smaller kernel weight, produced with organic fertilizers, in comparison to the conventionally produced, with the application of NPK fertilizer.

Conclusion

Based on the results obtained, it could be concluded that black soybean is richer in antioxidants than red maize. Season had the highest influence on the level of GSH, phenolics and yellow pigment in maize and soybean level, while the cropping system and fertilization regime influenced GSH and phenolics. The antioxidant level in grains with greater weight corresponded with the increased GSH level for maize, as well as increased GSH and phenolic level for soybean, while smaller grains were characterised by the increased YP content. Generally, the antioxidant level was increased mainly by alternating strips and/or alternating rows in maize grain and by alternating rows in soybean grain. The bio-fertilizer had the highest impact on an increase in GSH in maize grain and yellow pigment in soybean grain, while organic fertilizer was important for acquiring of GSH and phenolics in soybean grain.

Acknowledgements

This study was supported by the Ministry of Education and Science of the Republic of Serbia (Project TR-31037). The authors are grateful to Biljana Noro for a tremendous effort devoted to conducting the experiment.

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Received: December 6, 2016

Accepted: February 27, 2017

SADRŽAJ NEKIH ANTIOKSIDANATA U ZRNU KUKURUZA I SOJE IZ ZDRUŽENOG USEVA

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R e z i m e

Združeni usev, kao kombinacija različitih useva, koji se gaje u isto vreme i na istom polju, omogućava interakciju njihovih korenova, poboljšava rast i tolerantnost na stres, poboljšavajući tako nutritivni kvalitet proizvedenog zrna. Cilj istraživanja je bio da se ispita efekat različitih sistema gajenja: združeni usev kombinujući naizmenične redove i naizmenične trake kukuruza i soje, kao i pojedinačne useve, zajedno sa različitim režimima đubrenja (konvencionalni, upotreba organskog đubriva, bio-đubriva i kontrola) na sadržaj antioksidanta (glutathiona [GSH], fenola i žutog pigmenta [YP]) u zrnu crvenog kukuruza i crne soje. Zrno crne soje je bogatije antioksidantima od crvenog kukuruza. Sezona je pokazala najveći uticaj na sadržaj GSH, fenola i YP kod kukuruza i soje, dok su sistem gajenja i đubrenje uticali na promene u sadržaju GSH i fenola. Sadržaj antioksidanata u zrnima sa većom masom je odgovarao povećanom nivou GSH kod kukuruza, kao i povećanju nivoa GSH i fenola kod soje, dok su zrna manje mase imala veći sadržaj YP. Uopšteno, sadržaj antioksidanata je uglavnom bio povećan u zrnu kukuruza pri gajenju u naizmeničnim trakama, a u zrnu soje pri gajenju u naizmeničnim redovima. Bio-đubrivo je pokazalo najveći uticaj na povećanje sadržaja GSH u zrnu kukuruza i YP u zrnu soje, dok je organsko đubrivo ispoljilo uticaj na nakupljanje GSH i fenola u zrnu soje.

Ključne reči: sadržaj antioksidanata, združeni usev, crveni kukuruz, crna soja.

Primljeno: 6. decembra 2016.
Odobreno: 27. februara 2017.

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BIOCONTROL POTENTIALS OF CRUDE EXTRACTS OF SOIL FUNGI ON *AMARANTHUS HYBRIDUS* AND *PHYLLANTHUS AMARUS*

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Abstract: In a pot experiment, two fungal strains from soil, *Aspergillus fumigatus* and *Penicillium citrinum*, were evaluated for their mycoherbicidal properties on *Amaranthus hybridus* and *Phyllanthus amarus* using the biomass reduction method. The experiment was set up in a completely randomised block design made up of two weed species exposed to 20 treatments which consisted of the concentrated crude extracts of *Aspergillus fumigatus* and *Penicillium citrinum* at three levels as well as positive and negative controls, each in three replications. The percentage biomass yields of *Amaranthus hybridus* and *Phyllanthus amarus* were determined at 1, 3, 5 and 7 days after application and biomass reductions were calculated. Data collected were subjected to analysis of variance and significant means were separated using Duncan's multiple range test. Infections of the weeds occurred within 48 hours after the application of the crude extract treatments. Significant differences ($p \leq 0.001$) were observed in the percentages of biomass yields of the two weed species, especially at the highest inocula concentration (5% w/v) of the crude extracts. The percentages of biomass yields of *Amaranthus hybridus* were 88.58% and 88.91%, while 69.79% and 81.34% were recorded for *Phyllanthus amarus* after the application of the concentrated extracts of *Aspergillus fumigatus* and *Penicillium citrinum*, respectively. The study shows that the concentrated crude extracts of both *Aspergillus fumigatus* and *Penicillium citrinum* had the potentials for use as biocontrol agents, with the fact that extracts of *Penicillium citrinum* had the greatest impact on the biomass yields of the two test weeds.

Key words: soil fungi, mycoherbicidal properties, crude extracts, biocontrol and weeds.

Introduction

The management of weeds requires the use of suitable techniques and approaches to reduce economic expenses and increase crop productivity. *Amaranthus hybridus* and *Phyllanthus amarus* are weed species belonging to

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Amaranthaceae and *Euphorbiaceae* family, respectively. The two weed species are annual herbs that reproduce from seeds and can grow up to 60cm to 80cm high. *Amaranthus hybridus* is highly hybridised and exhibits a wide range of colour variations. It is cultivated or harvested from the wild and is eaten as a vegetable in some parts of West Africa, while *Phyllanthus amarus* is a common weed of cultivated fields which is very widespread in West Africa. Several efforts have been made to reduce the menace of weed infestation by adopting different control measures, but chemical control has been internationally accepted to be most effective in reducing weed infestations. There are increasing constraints in the use of synthetic herbicides because weeds are becoming resistant, some products are being removed from sale as a result of re-evaluation, and rules governing usage are being tightened. There is also an increasing public demand for organic produce, free from synthetic residues. Bioherbicides serve a more important role as a complimentary component in successful integrated management strategies (Hoagland et al., 2007), and not as a replacement for synthetic herbicides and other weed management tactics (Singh et al., 2006). Although the research on mycoherbicides started in the 1940s in the developed countries where soil and rhizospheric fungi have been screened for bioactive compounds with specific interest in secondary metabolites produced biotechnologically for agrochemical industry, there has been a dearth of information on the bioherbicidal potentials of indigenous soil fungi in soils of the southern Guinea savanna (SGS) agro-ecological zone of Nigeria. This study was therefore conducted to evaluate the biocontrol potentials of *Aspergillus fumigatus* and *Penicillium citrinum* isolated from a SGS soil on *Amaranthus hybridus* and *Phyllanthus amarus*.

Materials and Methods

Description of the experimental site and collection of soil samples

The experiment was conducted in the screen house of the Faculty of Agriculture, University of Ilorin, Kwara state, Nigeria. Soil samples were collected randomly from 0–15 cm depth in a farmland near the screen house cropped with Citrus and Moringa plants with the use of a soil auger into polyethylene bags. The samples were bulked, thoroughly mixed and air-dried.

Determination of some physicochemical properties of the soil

Moisture content was determined using the weight loss-on-ignition method; pH of the soil sample in water and pH in 1N KCl (1:2.5) were determined as described by Bates (1954); percent organic carbon and organic matter were determined using the Walkley-Black method as described by Jackson (1996); total nitrogen determination was made by the micro Kjeldahl distillation method as

described by AOAC (1999) and; determination of available phosphorus in soil was made by the Bray 1 method (Bray and Kurtz, 1954).

Isolation of fungi

The serial dilution agar plating method as described by Onyegeme-Okerenta et al. (2009) was adopted. Ten-fold serial dilutions of the soil suspension from each soil samples were made in sterile water. Potato dextrose agar (PDA) medium was used for the isolation of the fungi from the soil samples. Sterilized PDA plates were inoculated with 10^{-3} dilutions of soil samples and incubated for 10 days at $28^{\circ}\text{C} \pm 2$. After ten days, the growth of the different fungal strains was observed. Each fungal isolate was then sub-cultured on fresh medium to obtain pure cultures.

Production of fungal inocula

Fungal inocula of *Aspergillus fumigatus* and *Penicillium citrinum* were produced on Czapek Dox broth. Mycelial discs of pure cultures were obtained using a 10mm sterile cork borer. The medium, prepared in 2-liter flasks, was then inoculated with twenty of the 10mm mycelial discs of pure fungal cultures and incubated in a rotary incubator at 150 rpm, 28°C for 7 days. Harvested mycelial batches from the flasks were then filtered using Whatman filter paper No. 40 and oven dried at 80°C for 24 hr. The dry weights were recorded in order to determine mycelial biomass (referred to as dry mycelium equivalents). The fermentation product was homogenized in 3–4 L aliquots with an electric blender prior to spray application on the weeds.

Pot experiment

The experimental design used was a completely randomized design (CRD) made up of twenty treatments which consisted of two weed species treated with the crude extracts of *Aspergillus fumigatus* and *Penicillium citrinum* at three levels with positive and negative controls each in three replications.

The viable weed seeds were obtained from the wild, surface sterilized with 0.05% NaOCl for 5 minutes and rinsed with sterile distilled water. They were germinated (28°C) on moistened filter paper in Petri dishes and were later transferred into pots containing sterilized soils supplemented with the addition of poultry droppings. The different concentrations of the crude extracts were 1.0 $\times$, 0.5 $\times$, and 0.1 $\times$, where 1.0 $\times$ concentration contained equivalent of 50g of mycelium (dry weight basis) L^{-1} and a positive control containing the surfactant only and a negative control containing only sterile water. The sprays were applied with the use of hand held sprays. After treatment, plants were monitored at daily intervals for disease development for 7 days. Plants were excised at the soil line, oven-dried for

24 hours at 85°C and the percentage of biomass reduction was determined at 0, 3, 5, and 7 days after application (DAA).

Data analysis

The reductions in biomass of the selected weeds in the pot experiment were calculated for each of the treatments. The data were subjected to analysis of variance at 5% probability level. The significant means were separated using Duncan's multiple range test.

Results and Discussion

Physicochemical analyses of the soil

Table 1 shows some physicochemical properties and nutrient status of the soil used for the study. The characteristics of the soil agree with the assertions of Osundare (2009) that soils of the southern Guinea savanna are mostly classified as Alfisol with majority of them falling within the sandy-loam textural class. The fertility levels of these soils were reported to be usually low creating the need for constant augmentation through the addition of either organic or inorganic fertilizers. The pH of the soil used for this study was slightly acidic while the available phosphorus level was within the high range. The physicochemical properties of soil and its nutrient status influence the microbial population both quantitatively and qualitatively because soil microorganisms just like higher plants depend entirely on soil for their nutrition, growth and activity (Rohilla and Salar, 2012).

Table 1. Some physicochemical properties of soil.

Source of soil sample	Organic matter (%)	Organic carbon (%)	pH		Nitrogen (%)	Available phosphorus (ppm)	Moisture content (%)
			Water	KCl			
*Unilorin T & R Farm	0.86	0.50	6.3	5.9	0.06	7.12	19.05

*University of Ilorin, Teaching and Research Farm.

Effects of the crude extracts of *Aspergillus fumigatus* and *Penicillium citrinum* on *Amaranthus hybridus* and *Phyllanthus amarus*

Tables 2 and 3 show the main and the interactive effects of the crude extract formulations of *Aspergillus fumigatus* and *Penicillium citrinum* on the biomass yields of *Amaranthus hybridus* and *Phyllanthus amarus*, respectively. Significant differences ($p \leq 0.001$) were observed in the reduction of the biomass yields of the weed species at 5 and 7 days after the application of the treatments as shown in Table 2.

Table 2. Main effects of the crude extract formulations of *Aspergillus fumigatus* and *Penicillium citrinum* on the biomass of *Amaranthus hybridus* and *Phyllanthus amarus* at various days after application.

Treatments	Biomass yield reduction (g) of the selected weeds at various days after application (DAA)			
	1	3	5	7
Weeds				
<i>Amaranthus hybridus</i>	0.92	0.49	0.26	0.41
<i>Phyllanthus amarus</i>	0.62	0.47	0.41	0.20
SED	0.18	0.11	0.04	0.04
LSD	0.36	0.21	0.08***	0.09***
Levels				
Crude extract formulation of <i>Aspergillus fumigatus</i> (% w/v)				
5	2.86	1.46	0.617	0.36
2.5	1.94	1.05	0.462	0.35
0.5	1.56	0.72	0.595	0.30
0 (+ ve)	0.38	0.39	0.68	1.31
0 (- ve)	0.40	0.42	0.52	1.19
Crude extract formulation of <i>Penicillium citrinum</i> (% w/v)				
5	0.29	0.16	0.11	0.04
2.5	0.28	0.15	0.09	0.06
0.5	0.30	0.20	0.21	0.07
0 (+ve)	0.07	0.11	0.13	0.25
0 (-ve)	0.16	0.20	0.19	0.25
SED	2.86	1.46	0.62	0.36
LSD	0.40	0.24	0.09	0.10
Interaction	0.81***	0.48***	0.18***	0.20
Weeds × Levels				
SED	0.56	0.53	0.12	0.14
LSD	1.14	0.67	0.25**	0.29**

It was observed that the highest concentrations of the crude extracts of *Aspergillus fumigatus* and *Penicillium citrinum* were more efficient than the lower concentrations. The highest concentrations of the crude extract (5% w/v) of *Aspergillus fumigatus* and *Penicillium citrinum* had 72.74% and 78.68% biomass yield reduction, respectively. It was observed that the crude extracts of *Penicillium citrinum* significantly ($P \leq 0.001$) reduced the biomass of the weed species and it was more effective than the crude extracts of *Aspergillus fumigatus*. Gupta (1998) reported that the aggressiveness and percentage rot of *Penicillium citrinum* were found to be directly correlated with the amount of citrinin production. However, the efficacy of the highest concentrations began to reduce at 5 and 7 days after the application of the crude extracts as shown in Table 2. This might be attributed to the age of the plants as reported by Wolf (2011) and Boyette et al. (2014) that the efficacy of mycoherbicides decreases as the plants mature. Ghorbani et al. (2002)

reported that the efficacy of *Ascochyta caulina* was substantially reduced on older plants when applied on common lambsquarters (*Chenopodium album*).

Table 3. Interactive bioherbicidal effects of the different levels of the crude extract formulations of *Aspergillus fumigatus* and *Penicillium citrinum* on the biomass of four common weeds at various days after application.

Weeds	Treatments	Levels (% w/v)	Biomass yield reduction (g) of the selected weeds at various days after application (DAA)			
			1	3	5	7
<i>Amaranthus hybridus</i>	Crude extract (<i>Aspergillus fumigatus</i>)	5	3.09	1.48	0.49	0.26
“	“	2.5	2.63	1.40	0.45	0.34
“	“	0.5	2.16	0.92	0.73	0.35
“	Crude extract (<i>Penicillium citrinum</i>)	5	0.32	0.17	0.12	0.80
“	“	2.5	0.37	0.19	0.10	0.38
“	“	0.5	0.28	0.20	0.12	0.74
“	“	0 (+ve)	0.07	0.12	0.13	0.31
“	“	0 (-ve)	0.08	0.14	0.18	0.29
<i>Phyllanthus amarus</i>	Crude extract (<i>Aspergillus fumigatus</i>)	5	2.63	1.43	0.75	0.46
“	“	2.5	1.26	0.70	0.47	0.35
“	“	0.5	0.97	0.53	0.46	0.25
“	“	0 (+ve)	0.06	0.46	0.58	0.15
“	“	0 (-ve)	0.23	0.33	0.40	0.13
“	Crude extract <i>Penicillium citrinum</i>	5	0.25	0.15	0.10	0.12
“	“	2.5	0.19	0.10	0.07	0.15
“	“	0.5	0.31	0.20	0.31	0.08
“	“	0 (+ve)	0.06	0.46	0.58	0.15
“	“	0 (-ve)	0.23	0.33	0.40	0.13
		SED	0.56	0.53	0.12	0.14
		LSD	1.14	0.67	0.25**	0.29**

The percentage reduction in biomass yields of the weed species at 5% w/v concentration was in the order: *Phyllanthus amarus* 60.40% at 3DAA > *Amaranthus hybridus* 52.29% at 7DAA > *Phyllanthus amarus* 47.90% at 5DAA. The higher percentage reduction in the biomass yield of *Phyllanthus amarus* might be attributed to the composition of the plant. The morphological classification is the most important and useful in weed control as morphological characters of a plant are closely related to herbicidal absorption, retention, and translocation. The weeds belonging to the same group are likely to have the same kind of response to specific herbicides or cultural or mechanical methods. Significant differences ($p \leq$

0.001) were observed in the interactive effects of the different levels of application of crude extracts and the weed species (Table 3).

The two highest concentrations (5% w/v, 2.5 % w/v) of crude extracts were more efficient than the lowest concentration (0.5 % w/v). There were no significant differences between the positive and negative controls. The 5% w/v and 2.5% w/v concentrations of *Penicillium citrinum* crude extracts were more efficient than 0.5% positive and negative controls in reducing the biomass of both *Amaranthus hybridus* and *Phyllanthus amarus*.

At 7 days after application of the crude extracts, the highest percentage reductions in biomass yields (88.91% and 81.34%) were observed on *Phyllanthus amarus* while 88.58% and 69.79% were recorded for the reduction in biomass yield of *Amaranthus hybridus* by the highest crude extract formulation of *Penicillium citrinum* and *Aspergillus fumigatus* respectively. Graham et al. (2004), Peng and Wolf (2008) reported that disease response to an increased dose of bioherbicide agents may be non-linear and substantially higher inoculum doses can be required for noticeable efficacy improvement.

Conclusion

This study has shown that crude extracts of both *Penicillium citrinum* and *Aspergillus fumigatus* have the potentials to be used in controlling weeds as shown by reduction in the biomass yields of both *Amaranthus hybridus* and *Phyllanthus amarus* with application of crude extracts of the soil fungi. The crude extract formulations of *Penicillium citrinum* are more effective on the weed species than the crude extract formulation of *Aspergillus fumigatus*. Further studies for determination of the effective biological metabolites responsible for the herbicidal properties of the fungal crude extracts are recommended.

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Received: October 5, 2016

Accepted: February 14, 2017

POTENCIJALI SIROVIH EKSTRAKATA ZEMLJIŠNIH GLJIVA U
BIOKONTROLI KOROVA
AMARANTHUS HYBRIDUS I *PHYLLANTHUS AMARUS*

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R e z i m e

Proučavana su mikoherbicidna svojstva dva soja gljiva iz zemljišta *Aspergillus fumigatus* i *Penicillium citrinum* na korovske vrste *Amaranthus hybridus* i *Phyllanthus amarus*, u ogledu u sudovima, korišćenjem metoda redukcije biomase. Ogled je postavljen po slučajnom blok sistemu sačinjenom od dve vrste korova sa 20 tretmana, koji su se sastojali od koncentrovanih sirovih ekstrakata dobijenih iz zemljišnih gljiva *Aspergillus fumigatus* i *Penicillium citrinum* na tri nivoa, kao i od pozitivne i negativne kontrole, svaki u tri ponavljanja. Prinosi biomase korova *Amaranthus hybridus* i *Phyllanthus amarus* izraženi u procentima smanjenja su određeni posle 1, 3, 5. i 7. dana od primene. Prikupljeni podaci su obrađeni analizom varijanse i značajne srednje vrednosti odvojene su korišćenjem Dankanovog testa višestrukog opsega. Infekcije korova su se javile u toku 48 sati nakon primene tretmana sa sirovim ekstraktima. Uočene su značajne razlike ($p \leq 0,001$) u procentima smanjenja prinosa biomase dve korovske vrste, posebno pri najvećoj koncentraciji inokuluma (5% w/v) sirovih ekstrakata. Prinos biomase korova *Amaranthus hybridus* bio je 88,58% odnosno 88,91% manji u odnosu na kontrolu, dok je 69,79% odnosno 81,34% smanjenja zabeleženo za korov *Phyllanthus amarus* posle primene koncentrovanih ekstrakata dobijenih iz gljiva *Aspergillus fumigatus* i *Penicillium citrinum*. Istraživanje pokazuje da su koncentrovani sirovi ekstrakti dobijeni iz gljiva *Aspergillus fumigatus* i *Penicillium citrinum* imali potencijale da se upotrebe kao biokontrolni agensi, uz činjenicu da su ekstrakti dobijeni iz gljive *Penicillium citrinum* imali najveći uticaj na smanjenje prinosa biomase dve vrste ispitivanih korova.

Ključne reči: zemljišne gljive, mikoherbicidna svojstva, sirovi ekstrakti, biokontrola i korovi.

Primljeno: 5. oktobra 2016.
Odobreno: 14. februara 2017.

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INFLUENCE OF UV LIGHT EXPOSURE ON MINERAL COMPOSITION
AND BIOMASS PRODUCTION OF *MYCOMEAT* PRODUCED
FROM DIFFERENT AGRICULTURAL SUBSTRATES

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Abstract: The wild and mutant strain of *Pleurotus sajor caju* was cultured on different agricultural substrates. Treatment 1 contained agricultural substrates alone. Treatment 2 contained the mutant strain of the mushroom plus agricultural waste. Treatment 3 contained the wild strain of the mushroom plus agricultural waste. The mutant strain of *Pleurotus sajor caju* cultured on groundnut shell had the highest iron content while the mutant strain cultured on palm kernel meal had the highest biomass production, 10.5 g/L and 17.20 g/L after 7 and 14 days respectively. The proximate analyses of the feed variety (*mycomeat*) revealed the rich nutritional content which may be explored for feed ingredients in livestock production. The findings do not only support the bioremediation of agricultural waste to produce high-value bio-products, but also provide evidence that improvement of microorganism strains represents a viable way to enhance the nutritional value of fermented products.

Key words: agricultural wastes, mutant, *mycomeat*, *Pleurotus*.

Introduction

Agricultural wastes are produced in large quantities during the processing of agricultural products or after harvest and their disposal in developing countries is currently a major economic and ecological challenge. They are usually allowed to accumulate in large quantities, allowed to decay or are burnt indiscriminately which could impact negatively on the environment as well as pose a serious threat to human health. Mushrooms refer to fruiting bodies of macrofungi (Das, 2000)

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and they are highly nutritious (Kumari et al., 2011; Aina et al., 2012). Chang and Miles (1989) coined *mycomeat* to refer to fungal protein obtained through the conversion of food processing biomass wastes; most times via solid state fermentation (SSF). Edible fungi, mainly mushrooms, can be cultured for their fruiting body, metabolites such as an enzyme, or *mycomeat* (containing both the growth substrate and the mycelia of the fermenting fungi). Chiu et al. (2000) have earlier noted that production of medicinal or edible mushrooms is a successful way of agro-waste recycling. Mushroom has been found to suppress breast cancer (Grube et al., 2001). Ultra violet radiation is one of the best physical methods of strain improvement for better performance (Oloke et al., 2012). The microbiological quality of feed is of great importance to its safety. Adetunji and Adejumo (2017) earlier studied the proximate and amino acid profile of *mycomeat* produced from different agricultural substrates. The present study evaluated the nutritional value of a feed variety, *mycomeat*, produced through solid state fermentation from various agricultural wastes using wild and mutant strains of *P. sajor caju*.

Materials and Methods

Pleurotus sajor caju LMU 01 was procured from NIHORT, Ibadan, Nigeria. The cultures were sub-cultured periodically after every 4 weeks and incubated at $25 \pm 1^\circ\text{C}$ for 10 days on potato dextrose agar (PDA) slants and stored at 4°C . A fresh PDA plate to grow the organisms was prepared. After the growth of the organisms, a cork borer was used to obtain several mycelia plugs from the culture into a sterile PDA plate. The sterile plate containing several mycelia plugs was placed under UV lamp at 300 nm wavelength at a distance of 30 cm to the plates. At time interval of 30 min, 5 mycelia plugs were withdrawn and used as inoculants for solid state fermentation studies. The mycelia plugs from the domesticated type culture served as the control (Adetunji and Oloke, 2013).

The basal medium for seed culture was made (w/v) of glucose 2.0%, malt extract 2.0% and peptone 0.1%. The pH was initially adjusted to 5 before sterilizing by autoclaving. *P. sajor caju* was transferred to the medium by punching out 0.7 mm diameter agar discs from a culture grown on PDA plates, and five discs were used to inoculate 100 mL of liquid media. The seed culture was grown in a 250 mL Erlenmeyer flask at 25°C on a rotary shaker incubator at 100 rpm for 3 days. Agricultural wastes (wheat bran [WB], groundnut shell, *Moringa oleifera* seed shell, corn bran [CB], corn cob [CC], palm kernel meal [PKM], rice bran [RB] and cassava peel [CP] meal) which served as substrates were prepared according to the method of Akintunde and Akintunde (2002) with a little modification. The moisture content of the different agricultural wastes was maintained at 60% and apportioned into three sets each. Treatment 1 contained agricultural substrate waste

alone. Treatment 2, in addition to agricultural waste, contained the mutant strain of the mushroom exposed to UV for 30 min. Treatment 3, in addition to agricultural waste, contained the wild strain of the mushroom. They were filled into the wide-mouthed transparent jars in triplicates, corked with cotton wool and sterilized in the autoclave at 121°C for 15 min. The sterilized substrates were inoculated with 10% (v/v) seed cultures on different agricultural substrates. The set-up was incubated in the dark and monitored daily until full ramification was obtained.

The mycelia were obtained by boiling the cultures with boiling water for about 0.5 h to separate the mycelia from the fermented substrate, and filtered immediately with eight layers of gauze. The filter residue was washed three times with boiled water. Then the yield of biomass in SSF was determined gravimetrically after drying at 70°C overnight to a constant weight.

The potassium (K) and sodium (Na) contents of the *mycomeat* were determined using a flame spectrophotometer (Jenway PFP7, UK). The phosphorus content was determined by a UV spectrophotometer (Jenway 6305, UK). The magnesium (Mg) and calcium (Ca) contents were determined by the EDTA titration method while the iron (Fe), manganese (Mn) and copper (Cu) contents were determined using an atomic absorption spectrophotometer (AA320N, PEC Medical, USA) according to the procedure of AOAC (2000). The values of the determined minerals were reported in mg/L. The design of the study was a 3x8 factorial arrangement in a completely randomized design. The mean values obtained for mineral composition and biomass production were subjected to an analysis of variance using SPSS (Version 21). Significant means were analyzed using Duncan's multiple range tests at $\alpha = 0.05$.

Results and Discussion

Using the mutant strain, the *mycomeat* produced from groundnut shell (0.14 mg/L) had the highest iron content while rice bran wild (0.00 mg/L) and cassava peel (CP) control (0.00 mg/L) yielded the least value (Table 1). Conversely, the wild strain using groundnut shell as substrate produced the highest value of calcium content (108.24 mg/L), followed by the mutant strain produced from corn cob, CC (98.52 mg/L) while the wild strain produced from the palm kernel meal, PKM (36.49 mg/L), obtained the least value. The mutant strain produced from the wheat bran, WB (244.52 mg/L), obtained the highest value of magnesium while the wild strain produced from the CC (36.09 mg/L) obtained the lowest values.

The manganese content ranged from 0.04 to 0.23 mg/L. Similar values were obtained by the wild strain produced from the WB (0.04 mg/L), groundnut shell control (0.04 mg/L), the mutant strain produced from groundnut shell (0.03 mg/L), the wild strain produced from groundnut shell (0.04 mg/L), the mutant strain produced from *M. oleifera* (0.04 mg/L), the wild strain produced from *M. oleifera*

(0.03 mg/L), the wild strain produced from corn bran, CB (0.03 mg/L), CC control (0.03 mg/L), the mutant strain produced from CC (0.04 mg/L), the wild strain produced from CC (0.03 mg/L), PKM control (0.03 mg/L), CP control (0.04 mg/L) and the mutant strain produced from CP (0.04 mg/L).

Table 1. Interactive effect of agricultural substrates and strain improvement on mineral composition of *mycomeat*.

Substrates	Strain	Fe	Ca	Mg	Mn	P	K	Cu	Na
WB	Control	0.05g	68.18f	216.45c	0.23a	263.84k	3.88g	0.006def	4.36a
	Mutant	0.02l	47.45q	244.52a	0.09ef	265.79h	3.35i	0.004ef	0.00b
	Wild	0.01m	54.73m	220.46b	0.04h	265.60i	4.63e	0.004ef	0.00b
Groundnut shell	Control	0.10d	88.77c	92.19q	0.04h	229.86p	3.59h	0.003g	0.00b
	Mutant	0.14a	47.42q	98.19p	0.03h	237.97o	4.36f	0.003g	0.00b
	Wild	0.04i	108.24a	166.37f	0.04h	141.25t	3.08j	0.02a	0.00b
<i>M. oleifera</i> seed shell	Control	0.02j	52.29o	138.28i	0.07g	240.96n	1.54m	0.002g	0.00b
	Mutant	0.11c	63.25i	122.26l	0.03h	237.98o	3.10j	0.011b	4.38a
	Wild	0.13b	66.89g	142.31h	0.03h	252.41l	2.59k	0.002g	4.38a
CB	Control	0.003op	46.23r	184.39e	0.08f	329.09b	4.37f	0.009bc	0.00b
	Mutant	0.06f	72.97e	142.29h	0.08f	345.10a	4.88d	0.006def	0.00b
	Wild	0.002pq	58.37j	136.27j	0.03h	277.19f	5.13c	0.002g	0.00b
PKM	Control	0.05gh	41.34s	210.42d	0.03h	302.69c	4.87d	0.02a	0.00b
	Mutant	0.01m	55.96l	126.26k	0.16c	256.28k	5.15c	0.02a	0.00b
	Wild	0.02j	36.49u	156.33g	0.09ef	267.53g	4.64e	0.02a	0.00b
RB	Control	0.00q	38.91t	118.24n	0.12d	294.59d	3.85g	0.002g	0.00b
	Mutant	0.01n	52.39n	88.22s	0.20b	292.86e	3.62h	0.016a	4.38a
	Wild	0.00q	48.66p	102.21o	0.17c	245.23m	2.05l	0.007fg	0.00b
CP meal	Control	0.00 ^q	75.41 ^d	70.18 ^u	0.04 ^h	206.68 ^q	6.42 ^a	0.002 ^s	0.00 ^b
	Mutant	0.02 ^{kl}	98.57 ^b	90.22 ^r	0.04 ^h	182.11 ^s	6.43 ^a	0.003 ^{fg}	4.36 ^a
	Wild	0.07 ^c	57.16 ^k	120.24 ^m	0.12 ^d	191.56 ^r	6.16 ^b	0.003 ^s	4.37 ^a
	SEM	0.01	3.91	11.08	0.01	12.81	0.26	0.001	0.41

Means with different superscripts within the same column are significantly different, P = 0.05, SEM = standard error of the mean; CB = corn bran; WB = wheat bran; RB = rice bran; CC = corn cob; PKM = palm kernel meal.

The mutant strain produced from CB (345.10 mg/L) obtained the highest value of phosphorus while the wild strain produced from CC (106.78 mg/L) obtained the least value. Potassium content was low for *M. oleifera* control (1.54 mg/L) and the wild strain produced from rice bran, RB (2.05 mg/L). The copper content was generally low across the treatments. Sodium was only observed in WB control (4.36 mg/L), the mutant strain produced from *M. oleifera* (4.38 mg/L), the

wild strain produced from *M. oleifera* (4.38 mg/L), CC control (4.35 mg/L), the mutant strain produced from RB (4.38), the mutant strain produced from CP (4.36 mg/L) and the wild strain produced from CP (4.37 mg/L). The groundnut shell (0.094) and *M. oleifera* (0.087) obtained significant higher iron values. Significant lower values of calcium content were reported for PKM (44.60), RB (46.66) and WB (56.79) (Figure 1).

WB obtained the highest value of magnesium (227.14), followed by PKM (164.34). CC (53.45) obtained the least value. Manganese content ranged between 0.16 (RB) and 0.03(CC). Phosphorus content was higher for CB (317.13), RB (277.56) and PKM (275.50). Potassium content ranged between 6.34 (CP meal) and 3.17 (CC and RB). The copper content was generally low. PKM obtained the highest mean (0.022), while CP meal (0.003) obtained the least value. Groundnut seed shell meal, CB, and PKM obtained no values for sodium. *M. oleifera* (2.92) and CP meal (2.91) recorded the highest means, followed by RB (1.46), CC (1.45) and WB (1.45). Strain improvement enhanced mineral contents of the *mycomeat* produced except for magnesium and phosphorus.

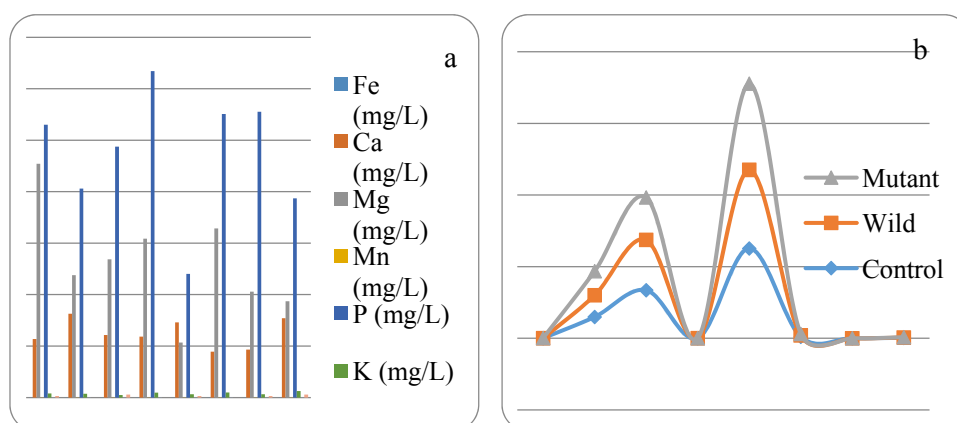


Figure 1. Effects of agricultural substrates and strain type of *Pleurotus sajor caju* on mineral composition of *mycomeat*, A = effect of agricultural substrates on mineral composition of *mycomeat*; B = effect of strain type of *Pleurotus sajor caju* on mineral composition of *mycomeat*.

The mutant strain of the *mycomeat* produced on PKM had the best biomass yield after 7 days (10.50 g/L) and 14 days (17.20 g/L). The main effects of agricultural substrates and strain types on biomass production are presented in Figure 2 while the interactive effect of agricultural substrates and strain improvement on biomass production of *mycomeat* is presented in Table 2. The *mycomeat* produced on palm kernel meal obtained the biomass yield after 7 days

(7.66 g/L) and 14 days (10.03 g/L) while CP obtained the least values. Strain improvement enhanced biomass production.

The low yield by *M. oleifera* seed shell and cassava peel could be due to the inhibitory activities of antibacterial compounds and hydrocyanide acid of *M. oleifera* and cassava peel respectively against the fungi. *M. oleifera* had been previously reported to express anti-microbial properties while cassava is rich in hydrocyanide (Tewe 1994; Ola-Fadunsin and Ademola, 2014).

Table 2. Interactive effect of agricultural substrates and strain improvement on biomass production of *mycomeat*.

Substrates	Strain	7 days (g/L)	14 days (g/L)
WB	Control	0.00 ^j	0.00 ^k
	Mutant	4.20 ^{de}	9.60 ^c
	Wild	2.10 ^g	4.30 ^{fg}
Groundnut shell	Control	0.00 ^j	0.00 ^k
	Mutant	7.20 ^b	13.40 ^b
	Wild	3.20 ^f	6.20 ^e
<i>Moringa oleifera</i> seed shell	Control	0.00 ^j	0.00 ^k
	Mutant	0.03 ^j	0.08 ^k
	Wild	0.00 ^j	0.02 ^k
CB	Control	0.00 ^j	0.00 ^k
	Mutant	1.20 ^{ghi}	4.30 ^{fg}
	Wild	0.90 ^{hij}	2.10 ^{hi}
CC	Control	0.00 ^j	0.00 ^k
	Mutant	3.50 ^{ef}	7.80 ^d
	Wild	1.60 ^{gh}	4.30 ^{fg}
PKM	Control	0.00 ^j	0.00 ^k
	Mutant	10.50 ^a	17.20 ^a
	Wild	8.30 ^b	10.40 ^c
RB	Control	0.00 ^j	0.00 ^k
	Mutant	6.20 ^c	10.40 ^c
	Wild	4.60 ^d	7.80 ^d
CP meal	Control	0.00 ^j	0.00 ^k
	Mutant	0.02 ^j	0.05 ^{jk}
	Wild	0.00 ^j	0.01 ^k
SEM		0.06	0.09

Means with different superscripts within the same column are significantly different, P = 0.05, SEM = standard error of the mean; WB = wheat bran; CB = corn bran; CC = corn cob; PKM = palm kernel meal; RB = rice bran; CP = cassava peel.

Strain improvement resulted in better biomass yield in the present study. Strain improvement in the field of microbiology is carried out to improve the microbial productivity, to change unused co-metabolites, to improve the use of

carbon and nitrogen sources and to improve the morphology of cells in order to separate the cells and its products.

The values reported in this study for Fe, Ca, Mg, Mn, Na, and Cu are lower than the values reported by Bamigbose et al. (2013). The variation could be attributed to the substrates used. The substrate used in the previous study was richer in these minerals than the substrates used in the present study. Minerals perform structural, physiological, catalytic and regulatory functions in animals. Calcium, phosphorus, and magnesium form structural components of the body organs and tissues. Sodium, potassium, calcium and magnesium in the blood, cerebrospinal fluid, and gastric juice are known to provide maintenance of osmotic pressure, acid-base balance, transmission of nerve impulses and membrane permeability (Suttle, 2010).

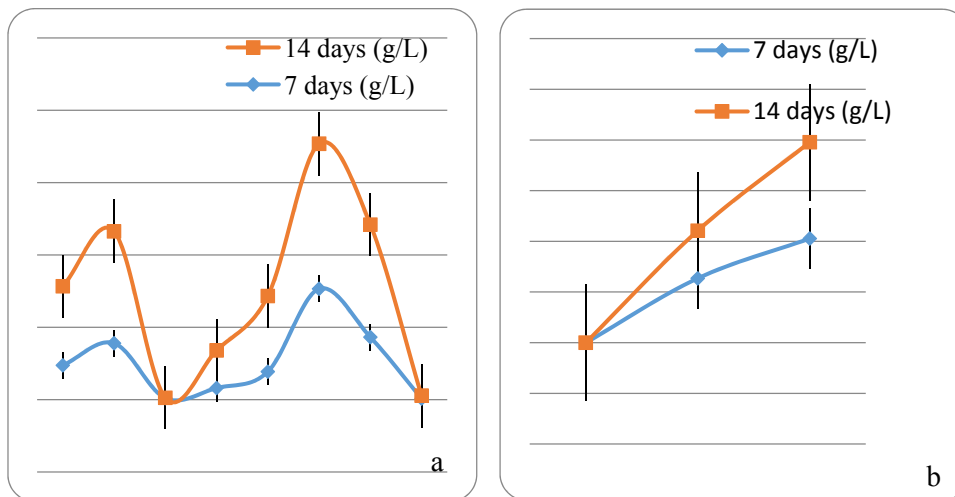


Figure 2. Effects of agricultural substrates and strain type of *Pleurotus sajor caju* on biomass yield of *mycomeat*; A = effect of agricultural substrates on biomass yield of *mycomeat*; B = effect of strain type of *Pleurotus sajor caju* on biomass yield of *mycomeat*.

The values of Mg in the present study are higher than the values reported in the MAFF (1990). The increase in the present study could be attributed to the fermentation and fungal growth. Lack of magnesium in the diet could result in hyperirritability and convulsions. Magnesium is largely protein-bound and functions as a catalyst of a wide array of enzymes. The formation of bone and its maintenance are the most important functions of phosphorus. It is a component of deoxy and ribonucleic acids, essential for cell growth and differentiation (Suttle, 2010).

Conclusion

Strain improvement enhanced iron, calcium, manganese, potassium, copper, and sodium content of *mycomeat*. PKM, groundnut seed shell meal and RB enhanced biomass production of *mycomeat* while *M. oleifera* seed shell meal and CP meal did not. This study supports the bioremediation of agricultural wastes to produce high-value bio-products. It provides evidence that improvement of microorganism strains represents an important means of enhancing nutritional values of fermented products.

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Received: September 29, 2016

Accepted: February 9, 2017

UTICAJ ULTRALJUBIČASTOG ZRAČENJA NA MINERALNI SASTAV I
PROIZVODNJU BIOMASE *MIKOMESA* DOBIJENOG OD RAZLIČITIH
POLJOPRIVREDNIH PODLOGA

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R e z i m e

Divlji i mutirani soj *Pleurotus sajor caju* gajen je na različitim poljoprivrednim podlogama. Tretman 1 se sastojao samo od poljoprivrednog otpada. Tretman 2 je sadržao mutirani soj pečurke i poljoprivredni otpad. Tretman 3 je sadržao divlji soj pečurke i poljoprivredni otpad. Mutirani soj *Pleurotus sajor caju* koji je uzgajan na ljuskama kikirikija imao je najviši sadržaj gvožđa, dok je mutirani soj uzgajan na sačmi od palminog jezgra imao najveću proizvodnju biomase, 10,5 g/L odnosno 17,20 g/L posle 7 odnosno 14 dana. Standardne analize različitih varijanata mikomesa (engl. *mycomeat*) otkrile su bogati hranljivi sadržaj koji bi se mogao koristiti kao sastojak hraniva u stočarskoj proizvodnji. Dobijeni rezultati ukazuju na to da je, pored bioremedijacije poljoprivrednog otpada, moguće dobiti visoko vredne bioproizvode, a takođe pružaju dokaze da poboljšanje sojeva mikroorganizama predstavlja održiv način da se poveća hranljiva vrednost fermentisanih proizvoda.

Ključne reči: poljoprivredni otpad, mutant, *Pleurotus*.

Primljeno: 29. septembra 2016.

Odobreno: 9. februara 2017.

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PROCENA POTENCIJALNOG ZDRAVSTVENOG RIZIKA USLED
PRISUSTVA TOKSIČNIH METALA U VODI ZA PIĆE IZ INDIVIDUALNIH
BUNARA U SELU DUBRAVICA U BRANIČEVSKOM OKRUGU

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Rezime: Da bi se proverio sadržaj makro- i mikroelemenata u vodi za piće u selu Dubravica u Braničevskom okrugu, koje nema centralizovan vodovodni sistem, izvršena je analiza voda koje su uzorkovane iz dva individualna bunara, indirektnom metodom preporučenom od strane Rajkovića i saradnika. Ispitivanja su pokazala da se: Al, Fe, Cr, Pb i U nalaze u koncentracijama višim od MDK vrednosti dozvoljene Pravilnikom o higijenskoj ispravnosti vode za piće. Da bi se utvrdila opasnost od prisustva toksičnih metala u povišenoj koncentraciji urađena je procena kratkoročnog i dugoročnog potencijalnog kancerogenog rizika. Na osnovu rezultata ispitivanja koji su dobijeni za ispitivane uzorke vode, ne postoji potencijalni kratkoročni kancerogeni rizik po zdravlje ljudi. Na osnovu rezultata dobijenih za dugoročni potencijalni rizik za pojavu i razvoj kancerogenih oboljenja, kod stanovnika sela koji koriste vodu za piće I uzorka izražen je rizik za nastajanje kancera od Cr. U slučaju Pb, rizik od pojave kancera u slučaju I uzorka vode za piće javlja se kod 44 od 1000 stanovnika, a u slučaju II uzorak kod 183 od 1000 stanovnika. Na osnovu izračunate procene dugoročnog zdravstvenog rizika od prisustva U u vodi za piće zapaža se da u slučaju konzumiranja oba uzorka vode postoji potencijalni rizik od nastajanje kancera: u slučaju I uzorka vode kod 24,5 stanovnika, a u slučaju II uzorka 10,3 stanovnika od 1000 stanovnika.

Ključne reči: voda za piće, makroelementi, mikroelementi, kratkoročni i dugoročni zdravstveni rizik, toksični metali.

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Uvod

Hemijske supstance koje se nalaze u vodi za piće podeljene su na organske i neorganske supstance, a kao posebna grupa se izdvajaju pesticidi, dezinfekciona sredstva i sporedni proizvodi dezinfekcije.

Sa aspekta uticaja na zdravlje, hemijske supstance mogu se podeliti na:

1. supstance čiji je unos u organizam poželjan, jer učestvuju u mnogim oksido-redukcionim i metabolitičkim procesima u organizmu;
2. supstance koje na organizam deluju toksično, ukoliko se u vodi za piće nađu u većim količinama od dozvoljenih;
3. supstance koje mogu imati kancerogeno dejstvo na organizam.

Za toksične i kancerogene supstance u vodi za piće neophodno je znati njihove granične vrednosti ispod kojih se neće ispoljiti negativni efekti na zdravlje. Kako je do sada u vodi za piće identifikovano više od 1000 hemijskih supstanci organskog i neorganskog porekla, nije moguće da se za svaku od njih odrede preporučene granične vrednosti, pa se stoga pri određivanju hemijskih supstanci za koje se izračunavaju preporučene granične vrednosti polazi od dva osnovna kriterijuma: 1. relativno česta pojava u vodi za piće u značajnim koncentracijama i 2. prisustvo hemijskih supstanci koje su potencijalno opasne za ljudsko zdravlje.

Po preporukama SZO (Commission of EC, 1994; WHO, 1984) granične vrednosti za hemijske supstance u vodi za piće izračunavaju se na osnovu tolerišućeg dnevnog unosa i na osnovu relativne kancerogenosti za potencijalno kancerogene supstance. Izračunavanje graničnih vrednosti za hemijske supstance u vodi za piće na osnovu tolerišućeg dnevnog unosa, vrši se po formuli:

$$GV = \frac{TDI \cdot bW \cdot P}{C} \quad (1)$$

gde je: GV, preporučena granična vrednost (mgkg^{-1} ili μgkg^{-1}); TDI, tolerišući dnevni unos koji predstavlja procenjenu količinu supstance u hrani ili vodi izraženu na jedinicu telesne mase mgkg^{-1} ili μgkg^{-1} koja se svakodnevno konzumira tokom trajanja života bez rizika za zdravlje; bW, telesna masa (izražena u kg); P, deo prihvatljivog dnevnog unosa koji pripada vodi za piće. Upotrebljavaju se vrednosti u opsegu 1 do 100% u zavisnosti od stepena izloženosti preko hrane i vazduha; C, dnevno konzumiranje vode za piće, koje po preporukama SZO iznosi 2L za odrasle, 1L za dete do 10 kg telesne mase i 0,75L za dete do 5 kg telesne mase.

Tolerišući dnevni iznos se izračunava po formuli:

$$TDI = \frac{NOAEL \text{ ili } LOAEL}{UF} \quad (2)$$

gde je: NOAEL, nivo bez opaženih neželjenih efekata definisan kao najveća doza ili koncentracija hemijske supstance pri kojoj tokom opservacija i eksperimenata nisu opaženi neželjeni efekti na zdravlje; LOAEL, najniži opaženi

nivo neželjenih efekata definisan kao najniža doza ili koncentracija hemijske supstance pri kojoj su tokom eksperimenata i opservacija opaženi neželjeni efekti na zdravlje; UF, faktor nesigurnosti čija vrednost zavisi od prirode toksičnog efekta, obima i vrste populacije koju treba zaštititi, kvaliteta informacija o toksičnosti hemijske supstance i uvek se određuje od slučaja do slučaja. Ukupan faktor UF ne sme da prekorači 10.000, jer u protivnom dobijene vrednosti za TDI postaju u velikoj meri nepouzdanе i netačne, tako da gube svaku verodostojnost. Za supstance u vodi za piće za koje vrednost faktora UF prelazi 1000, preporučene granične vrednosti se upotrebljavaju kao privremene (Knežević, 1995).

Grupa hemijskih elemenata pod nazivom „teški metali” je vrlo heterogena sa hemijskog, fiziološkog i ekološkog aspekta (Phipps, 1981). Termin „težak metal” se više od 60 godina koristi u hemiji i zaštiti životne sredine, a predložen je širok spektar značenja od strane različitih autora. Različita objašnjenja i klasifikovanja metala kao teških se sreću u literaturi, od kojih se neka zasnivaju na gustini metala, neka na atomskom broju ili atomskoj masi, a neka na hemijskim karakteristikama i toksičnosti metala (Duffus, 2002).

Osim toga, ovaj izraz se sve češće koristi i za toksične metale, tj. elemente koji ispoljavaju svoju toksičnost i pri niskim koncentracijama. Postoje četiri grupe elemenata od interesa za prehrambenu industriju, nauku i nutricioniste: 1. esencijalni za ljude (Cu, Ca, Fe, K, Mg); 2. esencijalni za biljke i jednu ili više životinjskih vrsta, ali ne i za čoveka (As, Cd, Ni), 3. toksični ili sa terapijskom upotrebom (Al, Ba, Hg) i 4. teški metali koji deluju isključivo toksično i nisu biogeni (Cd, Pb, Hg).

Toksični metali ne mogu da se unište i iščeznu, mogu samo da se rastvore, a da zatim nastave svoj put i premeste iz jedne sredine koju su zagađivali u neku drugu i da tako uđu u biološki lanac ishrane. Toksični metali se tako mogu pronaći na onim površinama gde ih ranije nije bilo, ali je nemoguće pretpostaviti da li bi u tom slučaju njihove koncentracije bile iznad ili ispod propisanih granica (Harvey, 2000; Mao et al., 1995; Rajković, 2002).

Aluminijum nije bitan za rad živih ćelija, ali je u većim količinama štetan po zdravlje ljudi. Američka Uprava za hranu i lekove (engl. Food and Drug Administration [FDA ili USFDA]) klasifikovala je Al kao GRAS (engl. Generally Regarded As Safe), generalno prepoznat kao bezbedan (za upotrebu), iako postoji veliki broj studija koje su pokazale vezu između unošenja Al i neurološke demencije kod bubrežnih bolesnika. Studije iz poslednjih 10–15 godina, inače perioda kada se „zvanično” prestalo sa posmatranjem Al kao toksičnog, pokazuju loš uticaj Al na ljudsko zdravlje. Studije posebno naglašavaju njegovo učešće kod Alchajmerove bolesti, Parkinsonove bolesti i amiotropne lateralne skleroze (Lu Gerigova bolest), jer se smatra da je Al jedan od etioloških činilaca. Njegov patofiziološki mehanizam nije potpuno razjašnjen, ali je kod bolesnika uočen mnogo viši sadržaj Al u mozgu nego kod zdravih osoba. Al^{3+} se najverovatnije

vezuje, preko fosfatnih i $-OH$ grupa za DNK i RNK stvarajući aluminijско-proteinske komplekse sa nukleinskim kiselinama. Rezultat toga je kompromitovana genska modulacija, transkripcija i sinteza proteina. Takođe, Al^{3+} ometa katalitičku aktivnost heksokinaze, smanjuje enzimsku aktivnost ATP, aktivnost koja reguliše membranski transport K^+ i Na^+ (jonska pumpa).

Al^{3+} se vezuje za albumine plazme (~80%) verovatno na istom mestu na kome se vezuje i Ca^{2+} . Eliminise se iz organizma urinom i fecesom. Resorpcija Al u gastrointestinalnom traktu čoveka (pod normalnim uslovima) procenjuje se na 0,1–0,3%, a skoro sav resorbovani Al je iz zdravog organizma ekskretovan urinom. Nakon resorpcije Al^{3+} se brzo eliminise iz krvi u druge telesne tečnosti i pripremljen je za ekskreciju, ali kasnije se brzina eliminacije smanji.

Prema ulozi koju ima u organizmu, gvožđe pripada mineralnim supstancama, a prema količini u kojoj se nalazi u organizmu pripada grupi mikroelemenata. Fe u vodu najčešće dospeva iz zemljišta kroz koje protiče voda, tzv. primarno gvožđe, i najčešće se susreće kod voda poreklom iz reni-bunara i dubokih arteških bunara, ili cevi kroz koje voda protiče, tzv. sekundarno gvožđe. Dozvoljena koncentracija Fe u vodi za piće iznosi od $0,3 \text{ mgL}^{-1}$ do $0,45 \text{ mgL}^{-1}$ (Službeni list SRJ, br. 44/99), a povećana koncentracija u vodi nema direktnog uticaja na zdravlje ljudi, ali potpomaže razvoj Feruginoznih bakterija koje stvaraju neprijatan miris i ukus vode, a mogu dovesti i do začepljenja i prskanja cevi što omogućava sekundarno zagađenje vode.

Hrom se u vodi uglavnom nalazi u obliku soli, pri čemu soli Cr (VI) imaju veću rastvorljivost od soli Cr (III). Eksperimentalna istraživanja na životinjama su pokazala da je Cr (VI) toksičan i kancerogen (pripada 1. grupi kancerogena). Preporučena granična vrednost za Cr (VI) iznosi $0,05 \text{ mgL}^{-1}$, dok granična vrednost za Cr (III) nije određena. Međutim, u pravilniku se navodi samo *ukupni* Cr čija MDK vrednost za redovne prilike iznosi $0,05 \text{ mgL}^{-1}$ (Službeni list SRJ, br. 42/98 i 44/99). U organizam se najviše unosi hranom, dok vodom i vazduhom manje. Cr se uglavnom apsorbuje kroz gastrointestinalni trakt i u stanju je da prođe kroz ćelijsku membranu. Cr može da ošteti pluća, želudac, jetru i bubrege. Cr je sastojak mnogih enzima i spada u mikroelemente neophodne za život. Olakšava prelazak glukoze iz krvi u ćelije. Smanjuje potrebe za insulinom. Smanjuje rizik od infarkta pošto spušta nivo holesterola u krvi.

Olovo u vodu može dospeti iz lemljivih spojeva cevi, upotrebom vodovodnih cevi od Pb (koje se više ne ugrađuju, ali su i dalje u upotrebi u nekim naseljima ili delovima naselja), a količina Pb u vodi za piće može se smanjiti kontrolom korozije i podešavanjem vrednosti pH vode u sistemu za distribuciju.

Na osnovu novijih eksperimentalnih istraživanja, Pb i njegova neorganska jedinjenja svrstana su u 2. grupu kancerogena (WHO, 1994; Unterwood, 1987, Huges, 1972; Arena and Dorw, 1986), što znači da su verovatno kancerogena za čoveka, pa je granična vrednost od $0,05 \text{ mg/dm}^3$ smanjena na $0,01 \text{ mgL}^{-1}$. Pb

napada mozak, srce, pluća, želudac, jetru, bubrege i creva, a uz to izaziva oštećenje fetusa i anemiju.

Neorgansko Pb je za biljke manje toksično od bakra i jedinjenja žive. Većina beskičmenjaka koji žive pri dnu, kao i planktonskih vrsta, ne izvlači Pb ni iz hrane ni iz vode, i ono je za ove organizme manje toksično od Cu, Cd, Zn i Hg, ali je otrovnije od Ni, Co i Mn. Međutim, organska jedinjenja olova u velikim količinama u vodi dospevaju iz veštačkih izvora. Uprkos činjenici da se organska jedinjenja Pb u vodi retko akumuliraju, treba naglasiti da su ona veoma otrovna i da predstavljaju određenu opasnost.

Uran se u prirodi nalazi kao smeša tri izotopa: uran-238, uran-235 i uran-234 čija rasprostranjenost u prirodi iznosi: 99,28%, 0,71% odnosno 0,006%. Svi uranovi izotopi su radioaktivni sa poluvremenom raspada: $^{238}\text{U} - 4,5 \cdot 10^9$ godina, $^{235}\text{U} - 7,07 \cdot 10^8$ godina i $^{234}\text{U} - 2,5 \cdot 10^5$ godina. Radioaktivnost urana može prouzrokovati probleme čovekovom organizmu, kao što je npr. kancer nekoliko godina po njegovom izlaganju, ali najveća opasnost preči od njegove hemijske toksičnosti, koja se manifestuje u veoma kratkom vremenu (nekoliko nedelja ili meseca) pri kontaktu sa njim. Toksičnost urana sastoji se od dva toksikološka mehanizma: prvi je neradijacioni, hemijsko-toksični, koji karakteriše teške metale kao što su: Pb, Hg, Cd i Bi, a drugi je jonizacioni, usled α -emisije, što karakteriše i ostale prirodne i veštačke radionuklide različitih emisija.

Rastvorni uran pokazuje istu hemijsku toksičnost kao i rastvorno Pb, ali je 20 puta otrovniji od rastvornog W. Zbog toga je koncentracija U u vodi za piće limitirana na $2 \mu\text{g L}^{-1}$ (0,002 ppm) (Domingo, 1995; Maynard, Down and Hodge, 1953). Uran vodi poreklo iz prirodnih izvora (Harmsten and Haan, 1980) ili je antropogenog porekla: iz različitih industrijskih grana, nekontrolisanom upotrebom organskih i mineralnih đubriva i pesticida i iz otpadnih muljeva. Migracioni potencijal U zavisi od fizičko-hemijskih svojstava zemljišta i zemljišnih rastvora i oksidacionih proizvoda U. Na mobilnost rastvorenih proizvoda urana, dominantno utiču pH vrednost, Eh i prisustvo kompleksirajućih organskih i neorganskih agenasa u lokalnim podzemnim vodama zemljišta.

Šestovalentni uran, U(VI), postoji u rastvoru kao uranil jon (UO_2^{2+}) koji je mobilniji od četvorovalentnog urana, U(IV), jer lakše gradi rastvorne komplekse sa ligandima prisutnim u zemljišnim rastvorima. Transport rastvornih oblika urana može biti ubrzan razblaživanjem, pošto se time smanjuje njegova koncentracija u podzemnim i površinskim vodama. Ove reakcije uključuju jonsku izmenu i specifičnu adsorpciju urana organskim supstancama, mineralnim glinama, Fe(III) i hidroksida prisutnih u zemljištu (Stojanović et al., 2012).

Ispitivanje sadržaja teških metala u vodi za piće u Srbiji (Petrović et al., 2012) pokazala su da stanovnici naselja u kome postoji centralizovani vodovod piju vodu zadovoljavajućeg kvaliteta, ali i da postoje čitavi regioni u kojima je jako visoka koncentracija toksičnih metala. Podaci Instituta za javno zdravlje

Srbije „Dr Milan Jovanović Batut” za 2012. godinu pokazuju da je oko polovine uzoraka vode za piće iz centralizovanih vodovodnih sistema neispravno za piće.

Naša ispitivanja (Rajković et al., 2012; Milojković, 2014) potvrdila su da je u vodama za piće sadržaj pojedinih toksičnih metala viši od dozvoljenih, propisanih Pravilnikom o higijenskoj ispravnosti vode za piće (Službeni list SRJ, br. 42/98 i 44/99) i Pravilnikom o kvalitetu i drugim zahtevima za prirodnu mineralnu vodu, prirodnu izvorsku i stonu vodu (Službeni list SCG, br. 53/05).

Zbog toga je osnovna namena rada da se ispitivanjem uzoraka vode za piće, uzetih iz individualnih bunara iz sela Dubravica na teritoriji grada Požarevca u Braničevskom okrugu, izvrši procena potencijalnog zdravstvenog rizika usled prisustva toksičnih metala u vodi za piće po stanovništvo naselja.

Materijal i metode

Selo Dubravica u kome je vršeno ispitivanje vode za piće nalazi se severozapadno od Požarevca na 75 m nadmorske visine na 44°41'13.8" severne geografske širine (N) i 21°4'21.6" istočne geografske dužine (E) u širem dunavskom priobalnom pojasu na teritoriji opštine Požarevac (Braničevski okrug).

Po morfološkim karakteristikama Dubravica je selo ravničarskog tipa i pripada podunavskim selima. Prema strukturi učešća aktivnih lica u primarnom sektoru delatnosti, Dubravica pripada mešovitom tipu seoskih naselja (učešće aktivnih lica 25–50%). Na osnovu geografskih odlika i razvojnih predispozicija pripada kategoriji moravskih naselja sa razvijenim osnovnim funkcijama za zadovoljenje dela (svako)dnevni potreba stanovnika. Snabdevanje vodom u naselju obezbeđuje se preko lokalnih vodovoda, dok se kanalisanje otpadnih voda pretežno vrši preko septičkih jama (Službeni glasnik grada Požarevca, 2012).

Za određivanje sadržaja toksičnih metala u vodi za piće korišćena je indirektna metoda određivanja elemenata u vodi za piće, predložena od strane Rajkovića i saradnika (Rajković et al., 2008a; Rajković et al., 2009). Ova metoda je odabrana zbog toga što su rezultati ispitivanja sadržaja teških metala u vodi za piće standardnom metodom određivanja (upotrebom AAS) pokazali da se svi metali nalaze u tragovima, daleko ispod granice detekcije, što nije tačno. To se može najbolje videti na primeru Cd koji je u drugom uzorku vode na samoj granici MDK vrednosti, a standardnom metodom nije ni detektovan. Što se tiče Pb, standardnom metodom nije ni detektovan, a indirektna metoda je pokazala vrednosti Pb koje su daleko iznad MDK vrednosti (Milojković, 2014).

Uzorkovanje, metode ispitivanja i tumačenje rezultata urađeni su u skladu sa pravilnikom (Službeni list SRJ, br. 42/98 i 44/99; Laboratorijski priručnik, 2006). Uzorak I uzet je iz bunara u centru sela na udaljenosti od oko 2,5 km od reke Dunav, dok je II uzorak uzet iz bunara sa ulaza u selo iz pravca grada Požarevca

udaljen oko 1 km u odnosu na uzorak broj I, a oko 3,5 km od reke Dunav. Oba bunara su na dubini od 12 m.

Rezultati ispitivanja voda iz individualnih bunara u selu Dubravica u Braničevskom okrugu prikazani su u našim prethodnim radovima (Rajković et al., 2015a; Rajković et al., 2015b). Analitičke metode i granica detekcije za sve ispitivane parametre prikazane su u tabeli 1 (EPA, 2009).

Tabela 1. Analitičke metode primenjene u radu i njihova granica detekcije.
Table 1. Analytical methods used in the paper and their detection limit.

Pokazatelj	Merna jedinica	Analitička metoda	Granica detekcije	MDK vrednost
Amonijak	mgL ⁻¹	spektrofotometrijski	-	0,50
Boja	mgL ⁻¹ Pt/Co skale	spektrofotometrijski	-	20
Elektroprovodljivost	μS/cm pri 20°C	konduktometrijski	-	2500
Hloridi, kao Cl ⁻	mgL ⁻¹	jon-selektivna elektroda	0,01	250
Kolimorfne bakterije	broj/100 mL		-	0
pH vrednost	-	potenciometrijski	-	6,50–8,00
Miris	-	organoleptički	-	bez
Mutnoća	NTU jedinica	turbidimetrijski	-	4
Oksidativnost	mg O ₂ L ⁻¹	prema Kubel-Tiemannu		<8 mgL ⁻¹
Ukus	-	organoleptički	-	bez
Nitrati, kao NO ₃ ⁻	mgL ⁻¹	jonska hromatografija	0,01	0,5
Nitriti, kao NO ₂ ⁻	mgL ⁻¹	jonska hromatografija	0,01	0,005
Makroelementi	mgL ⁻¹	analiza kamenca pomoću AAS	0,01	*
Mikroelementi	mgL ⁻¹	analiza kamenca pomoću AAS	0,01	*
U	μgL ⁻¹	fluorimetrijski	0,0005	2
Temperatura vode	°C	živin termometar (u °C)	-	
Mineralizacija (180°C)	mgL ⁻¹	uparavanje na vodenom kupatilu	-	

Za ispitivanje voda korišćen je kamenac, koji se izdvaja na grejaču kućnog bojlera prilikom zagrevanja vode, a koji nastaje taloženjem neorganskih nevolatilnih supstanci koje se nalaze u vodi za piće, tokom dužeg vremenskog perioda. Pošto sastav kamenca potiče od prisustva neorganskih jedinjenja u vodi (Rajković et al., 2003), cilj ovih ispitivanja bio je da se određivanjem elementarnog sastava kamenca, utvrdi sadržaj toksičnih metala koji se nalaze u vodi za piće.

Sastav kamenca određen je upotrebom atomskog apsorpcionog spektrofotometra The Perkin-Elmer AAnalyst Model 300, prema standardu JUS B.B8.070 (metode ITNMS: DM 10 – 0/4, 0/6, 0/7, 0/8, 0/9, 0/10, 0/11, 0/12, 0/13 i 0/17) (Rajković et al., 2007; Rajković et al., 2008; Rajković et al., 2008b).

Udeo elemenata, u obliku jedinjenja ili u elementarnom obliku, u % upoređen je sa maksimalno dopuštenim koncentracijama (MDK) neorganskih supstanci u vodi, koje su propisane Pravilnikom o higijenskoj ispravnosti vode za piće (Službeni list SRJ, br. 42/98 i 44/99) i Pravilnikom o kvalitetu i drugim zahtevima za prirodnu mineralnu vodu, prirodnu izvorsku i stonu vodu (Službeni list SCG, br. 53/05).

Kvantitativni sadržaj urana određen je fluorimetrijskom metodom zasnovanoj na linearnoj zavisnosti intenziteta fluoriscencije molekula uranovih jedinjenja od njihove koncentracije. Uticaj ometajućih (interferirajućih) parametara na intenzitet fluorescencije sveden je na najmanju moguću meru tehnikom „standardnog dodatka”, koja je sprovedena nakon ekstrakcije urana sa sinergističkom smešom TOPO (tri-n-oktil fosfin oksid)-etil-acetat. Intenzitet fluorescencije meren je pomoću Fluorimetra 26-000 Jarrel Ash Division (Fisher Scientific Company, Waltham, 1978) (ITNMS, 2004).

Rendgenska difrakciona analiza (RDA) izvršena je tako što je kamenac prethodno usitnjen i frakcija finog praha je dodatno proučena. Snimanje je izvršeno na fluorescentnom spektrometru EDXRF MiniPal 4 X-Ray.

Procena kratkoročnog i dugoročnog potencijalnog kancerogenog rizika

Kratkoročni rizik po zdravlje ljudi

Unos toksičnih elemenata i rizik po zdravlje ljudi, koji je uzrokovan konzumiranjem vode za piće, određeni su na nedeljnom nivou (kratkoročni rizik), preko procenjenog nedeljnog unosa vode (PNU) i koeficijenta rizika po zdravlje ljudi (KR).

Ovi koeficijenti određeni su na osnovu sledećih jednačina (Lin et al., 2015):

$$PNU = \frac{PPV \cdot c \cdot 7}{PTM} \quad \text{i} \quad KR = \frac{PNU}{TNU} \quad (3)$$

gde su: PPV – prosečna potrošnja vode po stanovniku (1,5L dnevno) (Papić et al., 2012), c – koncentracija elemenata u ispitivanim uzorcima vode izražena u $\mu\text{g L}^{-1}$, PTM – prosečna telesna masa stanovnika koja iznosi 75,65 kg (Pavlica et al., 2010), a TNU je tolerantni nedeljni unos toksičnih metala izražen kao $\mu\text{g kg}^{-1}$ telesne mase.

Pri proceni kratkoročnog rizika po ljudsko zdravlje smatra se da visok rizik postoji ukoliko je koeficijent rizika za neki element veći od 1 (Leung et al., 2008; Kostić et al., 2016a; Kostić et al., 2016b; WHO, 1998; WHO, 2011).

Dugoročni, potencijalno kancerogeni, rizik po zdravlje ljudi

Osim kratkoročnog rizika, prouzrokovanog konzumiranjem vode sa povišenim sadržajem toksičnih elemenata, moguće je odrediti i dugoročni, potencijalno kancerogeni, rizik po zdravlje ljudi (Wu and Sun, 2015).

Kao parametri za procenu ovog tipa rizika, određeni su unos toksičnih elemenata oralnim putem (konzumiranjem vode za piće), U_{oral} , kao i koeficijent rizika izazvan oralnim unosom toksičnih elemenata, KR_{oral} , preko sledećih jednačina:

$$U_{\text{oral}} = \frac{PPV \cdot c \cdot 365 \cdot 30}{PTM \cdot 10950} \quad \text{i} \quad KR_{\text{oral}} = \frac{U_{\text{oral}}}{RfD_{\text{oral}}} \quad (4)$$

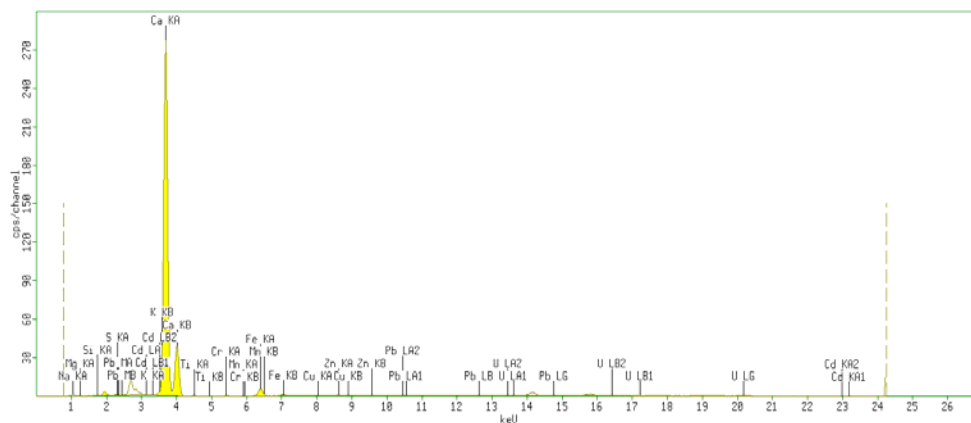
gde je RfD_{oral} – referentna vrednost za unos kancerogenih i potencijalno kancerogenih kontaminanata oralnim putem propisane od strane američke Agencije za zaštitu životne sredine EPA (Momot and Synzynys, 2005; CHMP, 2007), dok su skraćenice PPV i PTM objašnjene kod kratkoročnog rizika po zdravlje ljudi.

Rezultati i diskusija

Rendgenska difrakciona analiza (RDA)

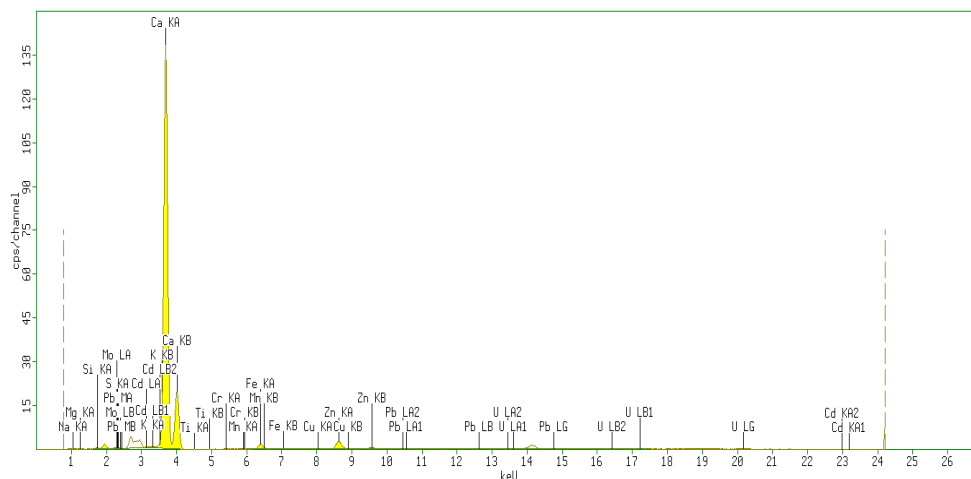
Difraktogrami ispitivanih kamenaca dokazali su prisustvo makro- i mikroelemenata nađenih AAS u kamencu (slike 1 i 2) (Rajković et al., 2015a; Rajković et al., 2015b).

Na difraktogramu je jasan pik koji ukazuje da se kalcijum nalazi u najvećem iznosu, ali da se u uzorcima vode nalazi i određeni broj makro- i mikroelemenata.



Slika 1. RDA difraktogram I uzorka kamenca.

Figure 1. RDA diffractogram of the first sample of limescale.



Slika 2. RDA difraktogram II uzorka kamenca.

Figure 2. RDA diffractogram of the second sample of limescale.

Uticaj prisustva toksičnih elemenata u vodi za piće na zdravlje ljudi

Na osnovu rezultata dobijenih ispitivanjem sadržaja makro- i mikroelemenata u analiziranim uzorcima vode (Rajković et al., 2015a; Rajković et al., 2015b), može se uočiti da je u nekoliko uzoraka zabeležena povećana koncentracija pojedinih elemenata u poređenju sa dozvoljenim vrednostima propisanim pravilnikom (Službeni list SRJ, br. 42/98 i 44/99), što je prikazano u tabeli 2.

Tabela 2. Koncentracija potencijalno toksičnih makro- i mikroelemenata (μgL^{-1}) u uzorcima vode za piće.

Table 2. The concentration of potentially toxic macro- and microelements (μgL^{-1}) in drinking water samples.

Uzorak	Al	Fe	Cr	Pb	U
I uzorak	50	1830	54,5	18,87	3,71
II uzorak	9,49	870	4,04	78,33	1,56
MDK	50	50	50	10	2

Procena kratkoročnog i dugoročnog potencijalnog kancerogenog rizika

Da bi se utvrdila opasnost od prisustva toksičnih elemenata u koncentracijama višim od vrednosti dozvoljene pravilnikom (Službeni list SRJ, br. 42/98 i 44/99), urađena je procena kratkoročnog (tabela 3) i dugoročnog potencijalnog kancerogenog rizika (tabela 4).

Tabela 3. Kratkoročni zdravstveni rizik izražen kroz tolerantni nedeljni unos (TNU), procenjeni nedeljni unos (PNU) i koeficijent rizika (KR) za odabrane toksične metale.

Table 3. Short-term health risk expressed through the tolerable weekly intake (TWI), estimated weekly intake (EWI) and risk coefficient (RC) for selected toxic metals.

Element	TNU (μgkg^{-1})	Uzorak			
		I		II	
		PNU	KR	PNU	KR
Al	7000	6,94	0,001	1,32	0,0002
Fe	5600	254	0,045	120,75	0,02
ukupni Cr	– *	–	–	–	–
Pb	25	2,69	0,105	10,87	0,435
U	0,6	0,515	0,858	0,216	0,361

*Nema dostupnih podataka.

Na osnovu rezultata koji su dobijeni za ispitivane uzorke vode za piće iz individualnih bunara iz sela Dubravica (I i II uzorak) (tabela 3), vidi se da je vrednost koeficijenta rizika po zdravlje manja od 1 za sve potencijalno toksične elemente koji su određeni u vodi za piće. Najveću opasnost za oba uzorka vode predstavlja Pb čija je vrednost za I uzorak 0,105 odnosno čak 0,435 za II uzorak.

Treba istaći da je KR za uran za I uzorak vode za piće svega 14,18% manji od granične vrednosti koeficijenta rizika ($KR = 1$), što ukazuje čak i na eventualnu kratkoročnu opasnost od prisutnog U u vodi za piće u slučaju ovog uzorka vode.

Na osnovu rezultata dobijenih za dugoročni potencijalni rizik za pojavu i razvoj kancerogenih oboljenja (tabela 4), mogu se uočiti razlike za ispitivane metale, u zavisnosti od procenjenog unosa oralnim putem, kao i propisanih referentnih vrednosti (RfD_{oral}) (ATSDR, 19997; EPA, 1989).

Kod stanovnika sela Dubravica koji koriste vodu za piće I uzorka izrazito je izražen rizik za nastajanje kancera od hroma. Pitanje je samo koji je hrom u pitanju, Cr(III) ili Cr(VI), koji se razlikuju po toksičnosti i kancerogenosti. Ukoliko se pretpostavi da se u oba uzorka hrom nalazi u obliku Cr(III), opasnost po zdravlje ljudi ne postoji, a ukoliko se pretpostavi da se radi o Cr(VI), u slučaju I uzorka vode opasnost postoji kod 360, a u slučaju II uzorka vode kod 27 od ukupno 1000 stanovnika. Kako u ovom radu nije utvrđen udeo Cr(III) odnosno Cr(VI) u ukupnoj masi Cr, može se uzeti realna pretpostavka da uticaj prisutnog Cr u vodi za piće ipak nije zanemarljiv i da zdravstveni rizik postoji.

Kada se radi o Pb, situacija je obrnuta. Rizik od pojave kancera u slučaju vode za piće (I uzorak) javlja se kod 44 od 1000 stanovnika, a u slučaju vode za piće (II uzorak) 4,16 puta više (kod 183 od 1000 stanovnika).

U slučaju Fe nije moguće odrediti dugoročni rizik s obzirom na to da se ovaj elemenat ne nalazi na listi potencijalno kancerogenih supstanci (EPA, 2009). Ova činjenica, međutim, ne opravdava povišenu koncentraciju Fe u ispitivanim uzorcima vode za piće iz individualnih bunara, u slučaju I uzorka 36,6 puta, a u slučaju II uzorka 17,4 puta više od MDK vrednosti predviđene pravilnikom (Službeni list SRJ, br. 42/98 i 44/99; Službeni list SCG, br. 53/05).

Tabela 4. Dugoročni zdravstveni rizik izražen kroz oralni unos (U_{oral}) i koeficijent rizika unosa oralnim putem (KR_{oral}) za odabrane toksične metale.

Table 4. Long-term health risk expressed through the oral intake (U_{oral}) and risk coefficient of oral input (KR_{oral}) for selected toxic metals.

Elemenat	RfR_{oral} (mg/dan/kg)	Uzorak			
		I		II	
		U_{oral}	KR_{oral}	U_{oral}	KR_{oral}
Al	– *	–	–	–	–
Fe	– *	–	–	–	–
ukupni Cr	Cr (III)	1,5	$1,08 \cdot 10^{-3}$	$7,2 \cdot 10^{-4}$	$8,01 \cdot 10^{-5}$
	Cr (VI)	0,003	$1,08 \cdot 10^{-3}$	0,36	$8,01 \cdot 10^{-5}$
Pb	0,0085	$3,74 \cdot 10^{-4}$	0,044	$1,55 \cdot 10^{-3}$	0,183
U	0,003	$7,4 \cdot 10^{-5}$	0,0245	$3,1 \cdot 10^{-5}$	0,103

*Ne postoji RfD_{oral} za Fe, s obzirom na to da nije potvrđeno postojanje kancerogenog rizika od strane EPA. Za Al ne postoje literaturni podaci, jer je FDA Al klasifikovala kao GRAS.

Na osnovu izračunate procene dugoročnog zdravstvenog rizika od prisustva urana u vodi za piće zapaža se da u slučaju konzumiranja oba uzorka vode postoji zdravstveni rizik. U slučaju I uzorka vode kod 24,5 stanovnika, a u slučaju II uzorka vode kod 10,3 stanovnika od 1000 stanovnika postoji potencijalni rizik od nastajanje kancera na osnovu prisutne koncentracije urana u vodi za piće u individualnim bunarima u selu Dubravica.

Pošto i Cr, Pb i U kao metu napada u čoveku imaju bubreg, moguća posledica je pojava hronične bubrežne insuficijencije (HBI) kod stanovništva. Zvanični podaci o zdravstvenom stanju stanovništva Braničevskog okruga ukazuju na konstantan broj obolelih i umrlih od HBI u periodu 2011–2014. godine koji se u proseku kreće oko 6,30%. Ovi podaci su u dobroj saglasnosti sa podacima dobijenim o zdravstvenom stanju stanovništva ove oblasti: stopa opšteg mortaliteta (18,39%), bolesti mokraćno-polnog sistema (7,74%), HBI (9,39%), pojava dijabetesa (26,61%) i porast endemske nefropatije (EN) (Zavod za javno zdravlje Požarevac, 2012).

Svi navedeni podaci ukazuju da i prisustvo ovih toksičnih metala može biti uzrok pojave HBI kod stanovništva Braničevskog okruga.

Zaključak

Ispitivanjem voda iz individualnih bunara iz sela Dubravica u Braničevskom okrugu indirektnom metodom, ispitivanjem kamenca, utvrđeno je prisustvo velikog broja makro- i mikroelemenata. Od prisutnih elemenata utvrđeno je da se: Al, Fe, Cr, Pb i U nalaze u koncentracijama koje su više od vrednosti dozvoljene Pravilnikom o higijenskoj ispravnosti vode za piće, tako da mogu da predstavljaju potencijalno toksične elemente po zdravlje stanovništva koji koriste ovu vodu za piće. Najveću opasnost za oba uzorka vode predstavlja Pb čija je vrednost za I uzorak 0,105 i 0,435 za II uzorak.

Treba istaći da je KR za uran za I uzorak vode za piće svega 14,18% manji od granične vrednosti koeficijenta rizika, što ukazuje čak i na eventualnu kratkoročnu (akutnu) opasnost od prisutnog urana u vodi za piće u slučaju ovog uzorka vode.

Na osnovu rezultata dobijenih za dugoročni potencijalni rizik za pojavu i razvoj kancerogenih oboljenja, mogu se uočiti razlike za ispitivane metale, u zavisnosti od procenjenog unosa oralnim putem, kao i propisanih RfD_{oral} vrednosti.

Kod stanovnika sela Dubravica koji koriste vodu za piće I uzorka izrazito je izražen rizik za nastajanje kancera od hroma. Ukoliko se pretpostavi da je u oba uzorka hrom u obliku Cr(III), opasnost po zdravlje ljudi ne postoji. Ukoliko pretpostavimo da se radi o Cr(VI), u slučaju I uzorka vode opasnost postoji kod 360, a u slučaju II uzorka vode kod 27 od ukupno 1000 stanovnika. Kako u radu nije utvrđen udeo Cr(III) odnosno Cr(VI) u ukupnoj masi Cr, može se uzeti realna pretpostavka da zdravstveni rizik od prisutnog Cr u vodi za piće postoji.

Rizik od pojave kancera, na osnovu koncentracije Pb u vodi za piće, u slučaju I uzorka javlja se kod 44 od 1000 stanovnika, a u slučaju II uzorka 4,16 puta više (kod 183 od 1000 stanovnika).

Koncentracija Fe u ispitivanim uzorcima vode za piće iz individualnih bunara, u slučaju I uzorka je 36,6 puta, a u slučaju II uzorka 17,4 puta više od MDK vrednosti predviđene pravilnikom, ali nije moguće odrediti dugoročni rizik s obzirom na to da se Fe ne nalazi na listi potencijalno kancerogenih supstanci (EPA, 2009).

Na osnovu izračunate procene dugoročnog zdravstvenog rizika od prisustva U u vodi za piće zapaža se da u slučaju konzumiranja oba uzorka vode postoji zdravstveni rizik. U slučaju I uzorka vode kod 24,5 stanovnika, a u slučaju II uzorka vode kod 10,3 stanovnika od 1000 stanovnika postoji potencijalni rizik od nastajanje kancera na osnovu prisutne koncentracije U u vodi za piće.

Ovi podaci su u dobroj saglasnosti sa podacima dobijenim o zdravstvenom stanju stanovništva ove oblasti.

Zahvalnica

Ovaj rad je raden u okviru projekta osnovnih istraživanja broj III 43009. Autori se zahvaljuju Ministarstvu nauke i zaštite životne sredine Republike Srbije za učešće u finansiranju ovoga rada.

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Primljeno: 14. oktobra 2016.
Odobreno: 14. februara 2017.

POTENTIAL HEALTH RISK ASSESSMENT DUE TO THE PRESENCE OF
TOXIC METALS IN DRINKING WATER FROM INDIVIDUAL WELLS IN
THE VILLAGE OF DUBRAVICA IN THE BRANICEVO DISTRICT

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A b s t r a c t

In order to verify the content of macro- and microelements in drinking water in the village of Dubravica in the Branicevo district, which has no centralized water supply system, an analysis of the water sampled from two individual wells was conducted using the indirect method recommended by Rajkovic and associates. Tests on the RDA and AAS showed that: Al, Fe, Cr, Pb and U were in concentrations higher than the values allowed by Regulations on the hygienic quality of drinking water. To determine the risk of the presence of toxic metals in the elevated concentrations, the assessment of short-term and long-term potential carcinogenic risks was conducted. Based on the results obtained in the tested water samples, it is noticed that the value of the coefficient of risk (CR) was less than 1 for all potentially toxic metals that were determined in drinking water, which implies that there is no short-term carcinogenic potential risk to human health. Based on the results of the long-term potential risk for the occurrence and development of cancer, the risk of getting cancer of Cr was identified in the inhabitants of the village that used potable water of the first sample. In case of Pb, a risk of developing cancer, concerning the first sample of drinking water, may occur in 44 out of 1000 people and regarding the second sample, in 183 out of 1000 people. Based on the calculated estimates of the long-term health risk related to the presence of U in drinking water, it can be seen that there is a health risk related to the use of both water samples. On the basis of the calculated estimates of the long-term health risk related to the presence of uranium in drinking water, it can be noticed that the use of both water samples may lead to the potential risk of developing cancer: in 24.5 inhabitants regarding the first water sample and in 10.3 out of 1000 inhabitants in the case of the second water sample.

Key words: drinking water, microelements, macroelements, short-term health risk, long-term health risk, toxic metals.

Received: October 14, 2016
Accepted: February 14, 2017

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ANALIZA VISINE I STRUKTURE INVESTICIONIH ULAGANJA U OVČARSKO I KOZARSKO FARME U EVROPSKOJ UNIJI I REPUBLICI SRBIJI

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Rezime: Ovčarska i kozarska proizvodnja razlikuje se od ostalih važnih vrsta stočarske proizvodnje (govedarstva, svinjarstva, živinarstva) po tome što je ona najčešće ekstenzivna, odnosno zahteva relativno nizak nivo ulaganja u objekte, opremu i ostala osnovna sredstva. Cilj rada bio je da se sagledaju karakteristike investicionih ulaganja u ovčarsku i kozarsku proizvodnju u Evropskoj uniji i uporede sa sličnim ulaganjima u Republici Srbiji. Da bi se sagledalo stanje u EU, prvenstveno su se koristili podaci iz odgovarajuće baze podataka FADN-a (engl. *Farm Accountancy Data Network*), kao i sektorske analize koje su provedene za područje EU. Sa druge strane, za sagledavanje potrebnih investicija u ovčarstvo i kozarstvo u Srbiji upotrebili su se raspoloživi tehnički i tehnološki podaci, kao i odgovarajući literaturni izvori. Izvršena je i analiza troškova izgradnje objekata za ovčarsku i kozarsku proizvodnju, kao i proračun investicija u odgovarajuću neophodnu opremu. Uočena je potreba ukupnjavanja ovčarskih i kozarskih farmi u Srbiji u cilju obezbeđenja odgovarajućeg životnog standarda za nosioce ovakvih gazdinstava.

Ključne reči: ovčarstvo i kozarstvo, ekstenzivna proizvodnja, investicije.

Uvod

Po Statističkom godišnjaku Republike Srbije, 2016, u Srbiji je u 2015. godini bilo 1.789.000 ovaca i 203.000 koza. Na osnovu podataka Popisa poljoprivrede vršenom 2012. godine, od ukupnog broja gazdinstava koja se bave ovčarskom proizvodnjom najveće je učešće gazdinstava sa 3–9 grla ovaca, odnosno 53,21% od ukupnog broja gazdinstava i gazdinstava sa 10–19 grla ovaca čije je učešće 27,27%. Gazdinstva koja imaju 100 i više ovaca ostvaruju učešće od 0,64% od ukupnog broja gazdinstava koja se bave ovčarskom proizvodnjom. Od ukupnog broja gazdinstava koja se bave kozarskom proizvodnjom, prema podacima Popisa poljoprivrede vršenom 2012. godine, najveći je broj gazdinstava koja poseduju

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manje od 10 koza, odnosno oko 95% ukupnog broja gazdinstava. Gazdinstva koja imaju 100 i više koza u Srbiji imaju učešće od 0,09% od ukupnog broja gazdinstava koja se bave kozarskom proizvodnjom.

Karakteristično je za ovčarstvo i kozarstvo da je od svih smeštajnih kapaciteta za smeštaj svih vrsta stoke najbolja iskorišćenost stajskih mesta kod objekata u kojima se drže ostale vrste životinja (gde se vode i ovce i koze) i iznosi 81% (Radivojević i sar., 2014).

Analizirano po regionima uočava se da je broj ovaca i broj koza znatno veći u regionu Srbija Jug, bez obzira na to što se u regionu Srbija Sever nalaze veće površine obradivog poljoprivrednog zemljišta. Najveći broj proizvođača iz ovih oblasti stočarstva bavi se ekstenzivnom proizvodnjom, pa ovakav teritorijalni raspored ovčarske i kozarske proizvodnje proizilazi iz intenzivnosti proizvodnje (Nastić i Potrebić, 2015).

Ovce imaju veliku moć adaptacije, veliku izdržljivost i vrlo su skromne u pogledu smeštaja i upravo zbog toga ovčarstvo je najzastupljenije u onim regionima gde je poljoprivreda najekstenzivnija. Autori su analizirali mogućnosti intenzifikacije proizvodnje njenim prevođenjem na industrijski nivo (Mekić i sar., 2007). Takve promene bi dovele i do rasta broja grla na gazdinstvima, a samim tim i do većih ulaganja u objekte i opremu u ovčarskoj proizvodnji.

Istraživanja pokazuju da je za profitabilnu proizvodnju u Republici Hrvatskoj potrebno osigurati dovoljno površina za držanje od 100 do 300 ovaca ili koza u intenzivnom uzgoju za proizvodnju mleka, odnosno 200 do 500 grla za iste uslove u proizvodnji mesa i mleka kod koza, a samo mleka kod ovaca. Za manja stada, preporučuje se da se na gazdinstvu vrši prerada mleka i proizvode sirevi i druge prerađevine od mleka, kako bi se osigurao zadovoljavajući dohodak. I kod ovakvog načina proizvodnje ne preporučuje se ulagati u stada manja od 60 grla (Grgić i sar., 2014).

Istraživanja koja su vršena u SAD vezana za ekonomske aspekte uzgoja koza u cilju proizvodnje mesa pratila su uticaj promene veličine kozarskih farmi na visinu troškova i tehničku efikasnost ovih farmi. Na osnovu analiza utvrđeno je da kozarske farme mogu biti efikasne u proizvodnji sa više od 60 koza i da sa porastom veličine farme troškovi značajno opadaju. Autori su utvrdili da na efikasnost kozarskih farmi utiče i niz drugih faktora kao što su sistem proizvodnje, lokacija gazdinstva i drugi (Qushim et al., 2016). Dok se u Evropi koze drže prvenstveno zbog proizvodnje mleka, koje se prerađuje u sireve (Aziz, 2010), u državama kao što su Australija ili Novi Zeland kozarstvo je prvenstveno usmereno na proizvodnju mesa.

Razvoj ovčarstva i kozarstva uklapa se sa globalnim stavovima o potrebi da se sa intenzivne pređe na ekstenzivnu stočarsku proizvodnju (Eisler et al., 2014). Sa druge strane, ekstenzivna stočarska (ovčarska) proizvodnja uklapa se sa potrebom za očuvanjem dobrobiti životinja, ali i dovodi do potrebe za novim pristupima koji

se odnose na upravljanje stočarskim (ovčarskim) gazdinstvima (Goddard et al., 2006; Stott et al., 2005). Tako i neka druga istraživanja govore da je jedan od uslova za ekonomičnije poslovanje kozarskih gazdinstava upravo bolja organizacija rada (Kitsopanidis, 2002).

Materijal i metode

U radu su korišćeni rezultati istraživanja domaćih i stranih autora koji se odnose na problematiku optimalnog broja životinja na gazdinstvima koja se bave ovčarskom i kozarskom proizvodnjom. Takođe su korišćeni podaci Republičkog zavoda za statistiku i rezultati Popisa poljoprivrede iz 2012. godine.

Da bi se sagledale karakteristike ovčarskih i kozarskih gazdinstava u Evropskoj uniji, upotrebljeni su podaci iz FADN baze podataka EU (http://ec.europa.eu/agriculture/rica/database/database_en.cfm), bazirani na regulativi Evropske komisije 868/2008, kao i odgovarajuća sektorska analiza ovog tipa stočarske proizvodnje urađena od strane Evropske komisije. FADN metodologija omogućava da se na isti način prikupe i analiziraju tehnički i ekonomski podaci o poljoprivrednim gazdinstvima svih zemalja EU, čime je obezbeđena njihova uporedivost između svih zemalja članica, kao i donošenje odgovarajućih mera agrarne politike na nivou čitave Evropske unije.

Visina ulaganja u objekte i opremu za ovčarsku i kozarsku proizvodnju u Srbiji utvrđena je na osnovu troškova izgradnje objekata i troškova pribavljanja odgovarajuće opreme, pri čemu su uzeti u obzir tipični objekti i oprema koji se u ovim vrstama stočarske proizvodnje koriste u Srbiji.

Rezultati i diskusija

Veličina zemljišnih površina koje koriste gazdinstva usmerena na ovčarsku i kozarsku proizvodnju u EU kreće se u posmatranom periodu (2010–2013. godina) u intervalu od 35 do 37 ha (tabela 1). Način korišćenja zemljišta na ovim gazdinstvima prvenstveno je određen njihovim potrebama za proizvodnjom stočne hrane. Zbog toga u strukturi zemljišnih površina dominiraju usevi koji se koriste kao kabasta stočna hrana, uključujući i sejane livade i pašnjake, kao i prirodne pašnjake na kojima se napasa stoka. Učešće ovih površina u ukupno korišćenoj zemljišnoj površini kreće se od 88,6% do 89%. Sa druge strane, proizvodnja žitarica odvija se na samo 7,0–7,4% korišćenih zemljišnih površina ovih gazdinstava.

Ovakav način korišćenja zemljišta potpuno je u skladu sa sastavom stočnog fonda u pogledu zastupljenosti pojedinih vrsta stoke na gazdinstvu. Na prvom mestu po broju uslovnih grla nalaze se ovce i koze (tabela 2), koje u posmatranom periodu sačinjavaju oko 80% ukupnog broja uslovnih grla. Nakon toga po značaju

se izdvajaju različite kategorije goveda (bez muznih krava) sa približno 15% ukupnog broja uslovnih grla, dok je učešće ostalih kategorija stoke gotovo zanemarljivo. Prema tome, u proseku, ova gazdinstva osim ovaca i koza (za čije su držanje specijalizovana) drže i određeni manji broj grla goveda različitih kategorija.

Tabela 1. Način korišćenja zemljišta.

Table 1. The way of using agricultural areas.

FADN pokazatelji	SE oznaka u FADN metodologiji	Jedinica mere	2010	2011	2012	2013
Ukupno korišćeno poljoprivredno zemljište	SE025	ha	35,6	35,1	37,7	37,3
Žitarice	SE035	ha	2,6	2,6	2,8	2,6
Ostale ratarske kulture	SE041	ha	0,2	0,2	0,2	0,2
Usevi koji se koriste kao kabasta stočna hrana	SE071	ha	31,6	31,1	33,6	33,2
Neobrađeno zemljište	SE072	ha	0,7	0,6	0,6	0,6
Ostalo korišćeno poljoprivredno zemljište	-	ha	0,5	0,6	0,6	0,8
Ukupno poljoprivredno zemljište van proizvodnje	SE074	ha	0,9	0,9	0,8	1,0

Izvor: http://ec.europa.eu/agriculture/rica/database/database_en.cfm.

Tabela 2. Karakteristike stočnog fonda.

Table 2. Characteristics of livestock.

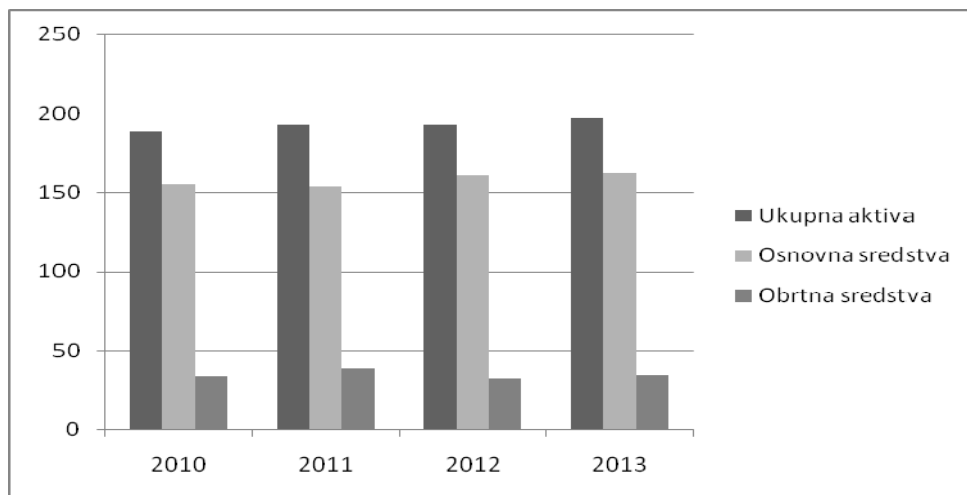
FADN pokazatelji	SE oznaka u FADN metodologiji	Jedinica mere	2010	2011	2012	2013
Ukupan broj uslovnih grla stoke	SE080	Uslovno grlo	30,5	30,5	31,2	29,4
Muzne krave	SE085	Uslovno grlo	0,4	0,5	0,5	0,5
Ostala goveda	SE090	Uslovno grlo	4,7	4,6	4,6	4,4
Ovce i koze	SE095	Uslovno grlo	24,3	24,4	25,1	23,4
Svinje	SE100	Uslovno grlo	0,2	0,2	0,2	0,2
Živina	SE105	Uslovno grlo	0,1	0,1	0,1	0,1

Izvor: http://ec.europa.eu/agriculture/rica/database/database_en.cfm.

Ako se pogleda broj uslovnih grla ovaca i koza po gazdinstvu, onda se može uočiti da se njihov broj kreće od 23,4 do 25,1. Imajući u vidu da jedno uslovno grlo podrazumjeva ukupnu težinu životinja od 500 kg, uz pretpostavku da je prosečna težina jedne ovce ili koze 50kg, onda se prosečan broj ovaca odnosno koza na ovim gazdinstvima nalazio na nivou od preko 200 grla (234 do 251 grlo). Međutim, s obzirom na to da su pod pojmom ovce i koze ovde obuhvaćene sve njihove kategorije, a ne samo odrasla grla, jasno je da je fizički broj grla na ovim gazdinstvima bio još veći.

Ovde se može uočiti činjenica da je broj ovaca i koza na gazdinstvima u zemljama EU znatno veći od broja ovaca i koza na gazdinstvima u Srbiji. Prema statističkim pokazateljima utvrđeno je da je u Srbiji mali broj ovčarskih gazdinstava sa više od 100 grla, a takođe su veoma retka ona gazdinstva koja imaju preko 50 grla koza.

U skladu sa tim, može se očekivati da će i ukupne investicije u osnovna sredstva ovčarskih i kozarskih gazdinstava (koja su u najvećem broju ekstenzivna gazdinstva) biti znatno viša u zemljama EU, nego u Srbiji. Zbog toga su na slici 1 prikazana ulaganja u osnovna sredstva, obrtna sredstva, kao i ukupna aktiva na ovčarskim i kozarskim gazdinstvima u EU.



Izvor: http://ec.europa.eu/agriculture/rica/database/database_en.cfm i proračun autora.

Slika 1. Osnovna i obrtna sredstva ovčarskih i kozarskih gazdinstava u EU (000 EUR).
Figure 1. Fixed and working assets of sheep and goat farms in euros (000 euros).

Može se uočiti da u strukturi ukupne aktive posmatranih gazdinstava dominiraju osnovna sredstva (čija se vrednost kreće od približno 155.000 do približno 160.000 EUR), dok je vrednost obrtnih sredstava znatno niža (32.000

EUR do 38.000 EUR). Prema tome, ukupna vrednost aktive ovih gazdinstava je prosečno gledano na nivou od 190.000 EUR.

Sa druge strane, postavlja se pitanje strukture osnovnih sredstava, odnosno visine investicionih ulaganja u pojedine vrste osnovnih sredstava na ovčarskim i kozarskim gazdinstvima u EU. U tabeli 3 (koja prikazuje podatke za period 2010–2013. godina) kao i na slici 2 (gde su prikazani isključivo podaci za 2013. godinu) može se uočiti dominacija vrednosti zemljišta, nakon toga po značaju dolaze objekti, a najmanji značaj imaju osnovno stado, kao i mehanizacija i oprema.

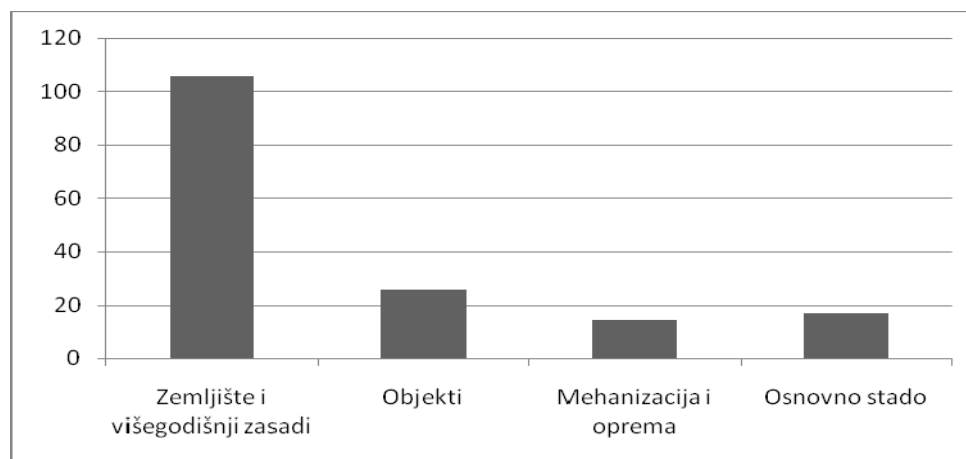
Tabela 3. Struktura osnovnih sredstava.

Table 3. The fixed asset structure.

FADN pokazatelji	SE oznaka u FADN metodologiji	Jedinica mere	2010	2011	2012	2013
Zemljište i višegodišnji zasadi	SE446	EUR'000	100,3	97,5	101,2	105,7
Objekti	SE450	EUR'000	25,9	25,9	27,2	25,7
Mehanizacija i oprema	SE455	EUR'000	13,2	14,6	15,0	14,6
Osnovno stado	SE460	EUR'000	15,7	16,0	17,3	16,7
Ukupna osnovna sredstva	SE441	EUR'000	155,1	154,0	160,7	162,7

Izvor: http://ec.europa.eu/agriculture/rca/database/database_en.cfm.

Tako se, na primer, u 2013. godini (slika 2) na zemljište odnosilo približno 65% ulaganja u osnovna sredstva, na objekte približno 16%, na osnovno stado oko 10%, dok su ulaganja u mehanizaciju i opremu najniža (svega oko 9%).



Izvor: http://ec.europa.eu/agriculture/rca/database/database_en.cfm i proračun autora.

Slika 2. Visina ulaganja u pojedine vrste osnovnih sredstava u 2013. godini (000 EUR).
Figure 2. The amount of investment in certain types of fixed assets in 2013 (000 euros).

Pošto u Srbiji ne postoje raspoloživi podaci o visini ulaganja u ovčarsku i kozarsku proizvodnju, a podaci prikupljeni u okviru FADN sistema još uvek nisu javno dostupni, trenutno se može koristiti samo procena vrednosti pojedinačnih osnovnih sredstava na ovčarskim i kozarskim farmama.

Postojeći objekti za smeštaj ovaca i koza na poljoprivrednim gazdinstvima u Republici Srbiji su potpuno neadekvatni i ne odgovaraju nijednom savremenom standardu. Čak više od 97% smeštajnog kapaciteta u Srbiji je van standarda i ne može se svrstati ni u jednu kategoriju koje tretiraju ovu oblast (Radivojević, 2013).

Objekti za smeštaj ovaca i koza treba da se grade tako da obezbede sve tehnološke parametre koji su grlima potrebni, a posebno u zimskim uslovima i u vreme jagnjenja/jarenja. To znači da se bar neki delovi objekata mogu termički izolovati u smislu da se temperatura održava na nivou potrebne. Za male porodične farme (kakvih je u Srbiji većina), ceo objekat treba da bude takav, jer se praktikuje da se jagnjenje/jarenje obavlja u boksevima u kojima se grla inače drže.

Objekat za smeštaj ovaca/koza se gradi tako da se odvoje tehnološke celine, odnosno hranidbeni hodnik se odvaja od prostora bokseva u kojima su grla. Celo stado se grupiše u manje grupe, ne veće od 20 grla u boksu. Konstrukcija objekta treba da je trajna i stabilna, a može da bude od različitog materijala (drvo, beton, čelik). Isto važi i za popunu zidova, pri čemu se biraju najjeftiniji materijali za popunu koji mogu zadovoljiti normative. Pod bokseva u kojem se drže koze/ovce treba da bude zemljani, a na pod se ubacuje prostirka od slame. Sistem izdubavanja se zasniva na principu sve unutra – sve napolje. To znači da se jednom do dva puta godišnje vrši izdubavanje, uz svakodnevno dodavanje prostirke. Ograde bokseva kao i jaslje su drvene, prenosne, tako da se lako mogu pomerati prateći nivo stajnjaka i prostirke u boksevima.

Za kozu/ovcu treba obezbediti oko $1,5\text{m}^2$ površine poda staje, dok je za jarčeve/ovnove potrebno oko $2,5\text{--}3,0\text{m}^2$ površine poda staje. Ukoliko jarad/jagnjad ostaju sa majkama u istim boksevima, onda je potrebno planirati prostor i za njih. U tom slučaju je potrebno obezbediti još $0,5\text{m}^2$ po mladom grlu, adekvatno njihovom ukupnom broju. Ukupno, za jednu kozu/ovcu i podmladak, uključujući i prostor koji zauzima oprema za ishranu i napajanje (jasje i pojilice), potrebno je obezbediti oko $2,5\text{m}^2$ površine poda staje.

Cena gradnje ovakvih objekata u uslovima gradnje u Srbiji iznosi oko 100 evra/ m^2 . Pod tim se podrazumeva kompletan objekat sa opremom za ishranu i napajanje odraslih grla i jaradi/jagnjadi, ili ukupno oko 250 evra/grlu – plotkinji.

Od ukupnog broja jaradi/jagnjadi oko 20% se ostavlja za odgoj kao podmladak za remont osnovnog stada. Sva ostala grla (muška i ženska) se prodaju. Za mlada grla, namenjena remontu, potrebno je obezbediti oko 1m^2 površine poda staje, dok je sve ostalo isto kao i za odrasla grla.

Pored proizvodnog objekta za koze/ovce, potrebno je izgraditi i izmuzište. Ono može biti u sastavu objekta i zauzima površinu oko $0,2\text{m}^2$ po grlu. Izmuzište je deo objekta za koji su potrebni viši troškovi gradnje, jer zahteva drugačije standarde. Ulaganje u izgradnju izmuzišta se kreće oko 250 evra/m^2 . Ovde treba imati u vidu da izmuzište sa opremom nije neophodno graditi u slučajevima kada je proizvodnja orijentisana na meso, odnosno jagnjad.

Što se tiče objekata za smeštaj kabaste stočne hrane za farmu, treba predvideti nadstrešnicu sa površinom od 3m^3 po grlu. Cena izgradnje ovakve nadstrešnice se kreće oko 20 evra/m^3 .

Deponiju za stajnjak nije potrebno graditi, jer se objekat izdubrava dva puta godišnje, a stajnjak odmah nosi na poljoprivredno zemljište.

U Srbiji je veoma teško obezbediti kvalitetna priplodna grla ovaca i koza, jer postoji malo umatičenih kvalitetnih plotkinja, a potražnja nije mala. Zbog toga se proizvođačima može preporučiti uvoz priplodnih grla. Visina ulaganja u osnovno stado prvenstveno zavisi od rase grla i od stepena graviditeta, ali se kao pravilo može navesti da je cena priplodnih grla iz uvoza duplo viša od cene domaćih priplodnih grla.

Zaključak

Analiza je pokazala da je broj grla ovaca i koza u Srbiji po jednom gazdinstvu veoma mali, najčešće ispod 10 grla. Broj proizvođača koji imaju više od 10 ovaca/koza je gotovo zanemarljiv. Sa druge strane, prosečan broj grla u EU na ovakvim farmama je preko 200, što potvrđuje potrebu ukupnjavanja ovčarskih i kozarskih farmi u Srbiji u cilju obezbeđenja odgovarajućeg životnog standarda za nosioce ovakvih gazdinstava.

U radu je data analiza tehničkih i ekonomskih indikatora za ovčarska i kozarska gazdinstva u EU, dok su za Srbiju (zbog nedostatka odgovarajućih podataka sa terena) dati standardi investicionih ulaganja u objekte i opremu na ovčarskim i kozarskim farmama. Pokazano je da ova gazdinstva u EU obrađuju oko 37 ha zemljišta, prvenstveno u cilju proizvodnje kabaste stočne hrane. Zemljište takođe ima najveću vrednost u okviru osnovnih sredstava, dok je prosečna vrednost osnovnog stada oko 17.000 EUR. Ukupna vrednost imovine ovog tipa gazdinstava u EU je u proseku oko 190.000 EUR, što može biti dobar orijentacioni pokazatelj za gazdinstva u Srbiji.

Zahvalnica

Rezultati istraživačkog rada nastali su zahvaljujući finansiranju Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije, projekat 179028: „Ruralno tržište rada i ruralna ekonomija Srbije – diverzifikacija dohotka i smanjenje siromaštva”.

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Primljeno: 5. januara 2017.
Odobreno: 19. februara 2017.

ANALYSIS OF AMOUNT AND STRUCTURE OF INVESTMENTS INTO THE
SHEEP AND GOAT FARMS IN THE EUROPEAN UNION AND THE
REPUBLIC OF SERBIA

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A b s t r a c t

Sheep and goat production is different from other types of livestock production (cattle, pig, poultry production) because it is most commonly extensive, which means that it demands a relatively low level of investments into buildings, equipment and other fixed assets. This paper aims to analyse characteristics of investments into the sheep and goat production in the European Union and compare them to the similar investments in the Republic of Serbia. In order to analyse the state in the EU, data from the appropriate FADN data base were used, as well as the sector analyses conducted for the area of the EU. On the other hand, in order to analyse necessary investments into sheep and goat production in Serbia, available technical and technological data and appropriate literary sources were used. Analysis of construction costs for buildings used in sheep and goat production was done, as well as the calculation of investments into appropriate necessary equipment. It has been noticed that there is a need for enlargement of the sheep and goat farms in Serbia in order to provide a proper living standard for holders of such enterprises.

Key words: sheep and goat husbandry, extensive production, investments.

Received: January 5, 2017
Accepted: February 19, 2017

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ASSESSMENT OF DRY SEASON GARDEN EGG PRODUCTION AMONG
SMALL-SCALE FARMERS IN EDU LOCAL GOVERNMENT AREA OF
KWARA STATE, NIGERIA

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Abstract: The study was carried out for the purpose of assessing dry season garden egg production among small-scale farmers in Edu Local Government Area of Kwara State. The study adopted a snowballing sampling technique to sample 120 small-scale garden egg farmers. Five research questions were formulated to achieve the objectives of this research work. Descriptive statistics, cost and return analysis and OLS regression were used to analyze data collected. Cost and return analysis shows that the total revenue generated from the sales of irrigated garden egg produce for a typical farmer was ₦ 36,596.5k while the total fixed cost (depreciated) and variable input cost amounted to 1,279.22K to give a net farm income (NFI) of ₦ 24,582.68k. The OLS regression results revealed that the coefficients of farm size and household size were positive and significant at the 1% and 10% levels of probability respectively. This implies that an increase in farm size by 1 ha will increase the production of dry season garden egg by 1 percent. While the results of stochastic production frontier estimate show that the parameters of labour, fertilizer and farm size were highly significant, chemical use was significant at 10% level. This result implies that the farmers were technically efficient in the use of labour, fertilizer, chemical and farm size. Therefore, it is recommended that farmers should be encouraged to form cooperative societies, whereby they can join their resources together to provide a cost-effective irrigation system. Also, government authorities and other non-governmental organizations are encouraged to give farmers fertilizers and other chemicals at subsidized rates. There is a need for revitalization of the marketing board in Nigeria to further strengthen the marketing of agricultural produce and reduce marketing, storage and transportation problems.

Key words: dry season, garden egg, small-scale farmers, Nigeria.

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Introduction

Garden eggplants are fruit vegetables of some varieties which are white and shaped like chicken eggs, hence the name ‘eggplants’ (Chen et al., 2001). The fruits may be pear-shaped, round, long or cylindrical depending on the variety. The plant with the scientific name *Solanum* spp. is a vegetable with the increasing popularity in the world. It is an economic flowering plant belonging to the family *Solanaceae* and genus *Solanum*. It exists in about 1,400 species found around the world most especially in the temperate and tropical regions (Pessarakli et al., 2003). The genus *Solanum* comprises over 1,000 species with at least 100 indigenous African species (Grubben et al., 2004). Production of garden egg is highly concentrated with 85% of the output coming from five (5) countries of which China is the world largest producer (56% of garden egg output), followed by India (26%), Egypt, Turkey and Indonesia (FAO, 2008).

Four cultivar groups are recognized within the *Solanum* species, three of which are important for Africa (Plant Resources of Tropical Africa [PROTA], 2004). They are the Gilo, Kumba, Shum and Aculeatum groups. The first three are the most important in Africa; Gilo and Kumba groups are produced for their fruits, especially in the humid zone of West Africa while Shum is cultivated for its leaves in the savannah area. African garden egg is one of the most commonly consumed fruit vegetable in the tropical Africa, in quantity and value and probably, the third after tomato and onions and before okra. In Nigeria, different local species/varieties are in existence and are grown by different ethnic groups for local consumption and other uses. The fruits can be eaten raw as a vegetable. It could also be boiled, fried and stuffed before consumption (Rice et al., 1987).

Garden egg vegetables are mostly annual crops belonging to the group of plants called horticultural crops which are diverse in nature. However, vegetables can be grouped into fruit and leafy vegetables depending on the nature of their consumable products or parts. Fruit vegetables are those that produce fruit such as okra, tomato, garden egg, etc. On the other hand, leafy vegetables are those whose leaves are the desired parts e.g. lettuce, spinach, cabbage, cauliflower, parsley, etc. Thus, the cultivation of vegetable during the dry season with the aid of irrigation is termed dry season vegetable farming. Consuming high amounts of garden eggs has been found to be beneficial for people with glaucoma because it lowers the eye pressure (Harish et al., 2008; Ozobia et al., 2013).

Eggplant nutritious value is comparable to the values of other common vegetables. Its fresh weight is composed of 92.7% moisture, 1.4% protein, 1.3% fibre, 0.3% fat, 0.3% minerals, and the remaining 4% consists of various carbohydrates and vitamins (A and C). It also contains water (about 92.5%), protein (1%), fat (0.3%), and carbohydrates (6%). Similarly, eggplant contains nutrients such as dietary fiber, folate, ascorbic acid, vitamin K, niacin, vitamin

B6, pantothenic acid, potassium, iron, magnesium, manganese, phosphorus, and copper (USDA, 2009). The crop is usually intercropped with okra, tomato and hot pepper under rain-fed conditions and often results in reduction of yield of both component crops possibly due to similarity in the growth pattern and duration (Norman, 1974). Dry season vegetable farming has its origin in the northern region. It is a major economic activity during the dry season involving many youths (Iorkua et al., 2004).

Dry season vegetable production also called vegetable farming is the production of vegetable outside the normal growing season using certain infrastructures such as green houses, irrigation, watering can, etc. In most parts of Nigeria, there are two distinct seasons, the rainy season and the dry season. The rainy season is the regular cropping season and this starts in April and stops in October, while the dry season starts in November and ends in March. During the rainy season the production of vegetable is high resulting in the saturation of the market, but during the dry season there is usually the scarcity of this important farm product thereby leading to a high price due to short supply. Garden egg is cultivated all year round in different parts of Nigeria and West Africa and serves as the main source of income for many rural farmers and households. Production is however constrained by a wide range of pests and diseases reducing total production as well as production quality. A great variety of insect species from different orders and families have been recorded on the garden egg of which very few are of economic importance. According to Okito et al. (2004), eggplant is most popular in southern Nigeria particularly in Igbo land, because of both cultural and traditional importance. In Nigeria, though there are no official figures recorded for *Solanum gilo* production, the crop has a wide distribution as a garden crop (Dauda et al., 2005). In order to obtain high yield of this crop in Nigeria, there is a great need to augment the nutrient status of the soil to meet the crop needs (Dauda et al., 2005).

In Nigeria, the dietary importance of vegetables to human beings and its contribution to rural economy need not to be over emphasized. Farming has been the major occupation of people of Edu Local Government Area from time immemorial. They embark on the production of rainfed crops like rice, sorghum, maize, etc. However, during the dry season period, production of vegetable crops like amaranths, tomato, pepper, garden egg, watermelon through irrigation is almost exclusively in the hand of non-indigenes, particularly the Hausa who have a long history of irrigation agriculture. This scenario raises some concern. Why do the indigenes not practice irrigation farming? Do the non-indigenes (Hausa) realize a sustainable income from these irrigation farming practices during the dry season? Hence, the specific objectives of the study are to:

- estimate the costs and returns of dry season garden egg production in the study area;

- identify the determinants of dry season garden egg production in the study area;
- examine the production efficiency of dry season garden egg farming in the study area and
- describe the problem and constraints militating against dry season garden egg farmers in the study area.

Materials and Methods

Study area

This study was carried out in Edu Local Government Area of Kwara State, Nigeria. The Local Government comprises of Lafiagi, Shonga and Tsaragi districts. It is located at latitude 9⁰ north and longitude 5⁰ east with an altitude of 300m above sea level. It is in a derived savannah zone of Nigeria with an average annual rainfall of about 120mm. It has the boundary in the north, east and west by the river Niger, Patigi Local Government Area and Ifelodun Local Government Area. The people of the area are predominantly small-scale farmers characterized by the use of crude farm implements and a farm size of about 1.0 to 3.0 hectares. Crops mostly grown during the raining season are lowland rice, maize, sorghum, groundnut, melon, sugarcane and sweet potato. Vegetables like garden egg, onion, tomatoes, sweet pepper and okra are produced during the dry season. However, since the Local Government Area is surrounded by water (the River Niger), fishing forms an integral part of farming activities most especially in the dry season. There, few farmers own small units of poultry and some farmers also raise sheep and goat.

Data collection and sampling procedure

There are three major rivers that flow through Edu Local Government Area, viz: the River Egwa, the River Tada and the River Belle. Based on this, data for this study was collected using a snowballing technique to identify the garden egg farmers along the bank of the rivers. This technique involves referral of respondents by previously identified garden egg farmers. Hence, 120 small-scale garden egg farmers were sampled to make a total of 120 respondents for the study.

Sources of data collection

The study utilized primary data collected by personal interviews with the aid of a well-structured questionnaire. Data collected includes socioeconomic

characteristics of dry season garden egg farmers in the study area, the output, the cost and return of non-indigene farmers, problem of irrigation farming among others.

Methods of data analysis and model specification

Descriptive statistics such as frequency distribution and percentages were used to analyze the socioeconomic characteristics of the vegetable farmers. Gross margin analysis was used to estimate the costs and returns to dry season garden egg production, while ordinary least square regression (OLS) and the stochastic production frontier were used to identify the determinants of dry season garden egg production and examine the production efficiency of dry season garden egg farming, respectively. The ranking technique, 4-point Likert scale was used to describe the problem and constraints militating against dry season garden egg farmers.

Analytical techniques

Ordinary least square regression model

This was used to examine the determinant of garden egg production in the study area. The implicit form of the model is specified as:

$$Y = f(X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, U) \quad (1)$$

where: Y = Output (kg), X_1 = Age of respondent (years), X_2 = Education (years), X_3 = Size of the farm (ha), X_4 = Extension contact, X_5 = Quantity of water pumped (litres), X_6 = Quantity of fertilizer (kg), X_7 = Size of household, X_8 = Farming experience (years), U = Error term.

Gross margin analysis

This was used to analyze the costs and benefits of garden egg farming during the dry season in the study area. Gross margin is the difference between the gross farm income and the total variable cost. According to Abu et al. (2011), gross margin analysis is a model that is used to estimate the costs, returns, profitability or loss per hectare. This can be expressed as:

$$GM = TR - TVC \quad (2)$$

where: TR = Total revenue in Naira/ha, TC = Total cost in N/ha, GM = Gross margin (N/ha), TR = Total revenue (N/ha), TVC = Total variable costs (N/ha),

$$\text{and NFI} = GM - TFC \quad (3)$$

where: NFI = Net farm income (N/ha), TFC = Total fixed cost (N/ha).

The variable cost includes hired labour, land cost, empty basket, fertilizer and herbicide while the fixed cost includes depreciation of fixed assets computed using the straight-line method of depreciation (3 years).

Estimation of stochastic production frontier model

A stochastic production frontier model was used to estimate the result productivity and the efficiency of the farmers in the study area. The model specification for the production stochastic frontier is specified as:

$$\ln Y = B_0 + B_1 \ln X_1 + B_2 \ln X_2 + B_3 \ln X_3 + B_4 \ln X_4 + B_5 \ln X_5 + V_i - U_i \quad (4)$$

Thus, to estimate the Cobb-Douglas production function, all the input variable and output data were converted into natural log form before analysis (Coelli, 1995),

where: Y = Output of garden egg in the i^{th} farm (kg), X_i = Input vector used in the production, β_i = Unknown parameter vector, $e_i = V_i - U_i$ (error term in composite form), V_i = Random parameter assumed to be identical, normally distributed with zero means and constant $N(0, \sigma^2)$ which were assumed to be independent of the U_i that captured the stochastic effects beyond the farmer control (e.g. weather, natural disaster), U_i = Random variable of the technical inefficiency, X_1 = Labour (man-day), X_2 = Fertilizer quantity (kg), X_3 = Chemical used (herbicides) in kg, X_4 = Quantity of seed used (kg), X_5 = Farm size (hectares of land).

Likert scale analysis

A four-point Likert type scale was used to measure the constraints to garden egg production in the study area. Clason et al. (1994) identified the Likert type scale as comprising single unrelated and independent questions whose responses cannot be combined into a composite scale as in the case of the Likert scale.

The scale used was as follows:

3 = Very severe;

2 = Severe;

1 = Undecided;

0 = Not severe.

Results and Discussion

This section presents the results of the various analyses carried out on the data.

The socioeconomic characteristics of the vegetable farmers in terms of sex, age, household size among others are presented in Table 1. Statistics of the data reported in Table 1 revealed that dry season farmers in the study area were male-dominated (100%).

Table 1. Socioeconomic characteristics (N=120).

Characteristics	Category	Frequency	Percentage
Gender	Male	120	100
	Female	0	0
Age bracket	≤20	13	10.8
	21–25	11	9.2
	26–30	30	25
	31–35	34	28.4
	36–40	22	18.3
	>40	10	8.3
Marital status	Married	96	80
	Single	24	20
Religion	Islam	110	91.7
	Christianity	10	8.3
Educational level	No formal education	18	15
	<i>Quaranic</i> education	38	31.7
	Primary education	28	23.3
	Secondary education	26	21.7
	Tertiary education	10	8.3
Farming experience (years)	≤5	21	17.5
	6–10	56	46.7
	11–15	14	11.6
	>15	29	24.2
Household size	1–4	7	5.83
	5–9	4	3.33
	10–14	38	3.17
	15–20	41	34.17
	>20	30	25
Farm size (ha)	≤0.5	47	47
	0.51–1.0	65	65
	1.01–1.50	18	18
Minor occupation	Farming	92	76.6
	Trading	17	14.2
	Livestock farmers	11	9.2
Income from major occupation (Naira)	>10,000	62	51.7
	11,000–20,000	9	7.5
	21,000–30,000	26	21.6
	31,000–40,000	14	11.7
	>40000	9	7.5
Income from minor occupation (Naira)	<5,000	82	69.3
	11,000–15,000	28	23.3
	<15,000	10	8.3
Extension contacts	Yes	97	80.8
	No	23	19.2

Source: Field survey, 2015.

There were no females that engaged themselves in dry season agricultural practices of garden egg in the study area. This may be due to the nature of rigorous processes and activities related to dry season garden egg irrigation farming in the study area. Findings also revealed that only 10.8% of the respondents were below 20 years of age, while majority of them were at the age of 32.55 (mean age) and the modal age group was between 31 and 35 years. However, based on the educational level of the studied population, larger percentage had 'Quranic' education (31.7%), while 23.3% and 21.7% had primary and secondary school education respectively. Few of the respondents had a higher level of education. Meanwhile, the modal age experience was between 6 and 10 years at 46.7% with the least experience of 11.6%. This indicates that the respondents were active, young and agile. It also implies that majority of the garden egg dry season farmers were experienced in garden egg farming.

Other minor occupations of the farmers included 76.6% farmers, 14.2% and 9.2% traders and livestock farmers respectively. The average monthly incomes from the major occupation differed among farmers on the basis of size of land cultivated and their respective contribution to dry season farming in the study area. Moreover, about 51.7% of the farmers earned at least ₦ 10,000 per month. Also, those in the minor occupation categories earned average monthly income of at least ₦ 5,000. The nature of extension visit or contact varied among the respondents. Only 19.2% of the respondents had no access to extension services.

Table 2 shows the cost and returns of dry season garden egg vegetable production under irrigation.

Table 2. Cost and return analysis of dry season garden egg production/ha.

Total income		₦ 36,596.5k
Less: Variable cost		
	Planting material	₦ 514.25k
	Fertilizer	₦ 1123.903k
	Herbicide	₦ 862.93k
	Labour	₦ 2850.87k
	Land rent	₦ 5351.97k
	Basket	₦ 30.70k
	Total variable cost	₦ 10,734.6k
Gross revenue		₦ 25,861.9k
Less: Fixed cost (depreciation)		
	Farm implement (hoe, cutlass, watering can)	₦ 164.46k
	Pumping machine	₦ 1,114.76k
	Total depreciation	₦ 1,279.22K
Net farm income (NFI = TR – TC)		₦ 24,582.68k

Source: Field survey, 2015.

The total revenue generated from the sales of irrigated garden egg produce for a typical farmer was ₦ 36,596.5k/ha while the total fixed cost (depreciated) and variable input cost amounted to ₦ 1,279.22k/ha to give a net farm income (NFI) of ₦ 24,582.68k/ha. This shows that the dry season garden egg farmers under irrigation practices actually yielded a total amount of ₦ 24,582.68k profit per hectare. Moreover, the production cycle was from November to March, which is 5 months. A large proportion of the variable costs were attributable to the labour and land rent, which accounted for more than 50%. This shows that farmers spent more on inputs such as labour and rent in operating an irrigated dry season farm in the study area. This situation is not unexpected, since all farm operations including land clearing, leveling, channel construction, planting, weeding, watering, and harvesting were accomplished through labour. The costs of planting material (basket) were relatively low. The cost of labour was, however, dominated by the imputed cost of unpaid family labour. Moreover, if most of the farmers choose not to engage in dry season farming, they may be redundant and jobless during the dry season since they are not likely to secure any other profitable employment.

The results in Table 3 reveal that the coefficients of farm size and household size were positive and significant at 1% and 10% levels of probability respectively. This implies that an increase in farm size by 1 ha increased the production of dry season garden egg by 1,507 kg. Hence, an increase in the size of land cultivated will lead to an increase in production output. Also, household size was positively significant (10%). This implies that a unit increase in the household size will lead to an increase in the dry season garden egg production by 205 kg. This suggests that the increase in the household size, which consequently leads to an increase in labour, will result in an increase in production. The quantity of fertilizer used was also significant. This implies that a unit increase in the quantity of fertilizer use will increase garden egg production by 226 kg.

Table 3. OLS regression analysis of determinants of dry season garden egg production in the study area.

Variable	Coefficient	Standard error	t-values
Constant	-462.562	5599.91	-0.83
Age	-79.999	74.48	-1.075
Educational level	288.961	553.572	0.522
Farm size	1,506.797***	514.862	2.927
Extension contact	424.034	1,678.42	0.253
Cost of a pumping machine	-1,465.103***	463.141	-3.163
Fertilizer quantity	226.495*	309.972	1.683
Quantity of labour	133.216	343.56	0.388
Household size	205.495*	118.443	1.735
Experience	73.737	101.532	0.472
R ²		43.7	
F – Statistics		2.856	

***Significant at 1%, **Significant at 5%, *Significant at 10%. Source: Field survey, 2015.

The results are in line with the results of Ahmed et al. (2003), who applied a Cobb-Douglas production function to quantify the contribution of various factors in muskmelon production. They discovered that variables such as family size, use of fertilizer and interaction of variety with pesticide sprays were highly significant for muskmelon productivity. The results are consistent with the findings of Okoye et al. (2008) and Ukoha et al. (2010). Based on the magnitude of the marginal effect of the explanatory variables, the findings reveal that household size, farm size and farming experience were major incentives available to regulate the total factor productivity among garden egg farmers in the study area.

The results of the estimate in Table 4 show that the parameters of labour, fertilizer, farm size and chemical were positively significant. These results imply that the farmers were technically efficient in the use of labour, fertilizer, chemical and farm size. The results also show that seed use was not significant and the negative value indicated that increasing the seed would not have any positive impact on the output. This suggests that the farmers may have easy access to garden egg seeds and the availability of these seeds may not affect the efficiency of production. This also suggests that since they are small-scale farmers constrained by the size of the farm and availability of labour, the availability of more seed may not have any effect on efficiency. However, the result on gamma square was significant and the value of 72.5% indicated that there were differences among production units considered in this study. This also implies that 27.5% of variations in output were largely due to weather conditions, errors in data collection and managerial differences among production units.

Table 4. Maximum likelihood estimate (MLE) of the Cobb-Douglas production functions based on stochastic production frontier function for dry season garden egg farming.

Variable	Coefficient	Standard error	t-ratio
X_0 (constant)	-0.677	0.863	-0.784
X_1 (labour)	0.581***	0.091	6.387
X_2 (fertilizer)	0.193***	0.072	2.65
X_3 (chemical)	0.129*	0.068	1.89
X_4 (seed)	-0.021	0.034	-0.608
X_5 (farm size)	0.451***	0.073	6.15
Diagnostic statistics			
δ^2	0.213***	0.053	4.01

Source: Field survey, 2015. ***Significant at 1%, *Significant at 10%.

Constraints militating against dry season garden egg irrigation farming

A ranking technique was used to rank the various pre-determined constraints of dry season irrigation farming of garden egg. The constraints were analyzed using ordinal scale of measurement such as: very severe (VS), less severe (LS), undecided (U), disagree (D) and not severe at all (NSA). The perception of the respondents towards these constraints is given in Table 5.

Table 5. The result of Likert type scale analysis of constraints militating against dry season garden egg production.

Constraints	Very severe	Less severe	Undecided	Not severe	Total	Mean score	Rank
Inadequate supply of irrigation water	56	35	8	21	246	2.05	3 rd
Pest and disease problems	63	33	6	18	261	2.18	1 st
Insufficient demand of garden egg	57	32	8	23	243	2.03	4 th
Inadequate land for dry season farming	48	30	11	31	215	1.79	6 th
Lack of sufficient garden egg seedling	41	37	13	29	210	1.75	8 th
Land dispute	39	31	32	18	211	1.76	7 th
Lack of storage facility	46	37	16	21	228	1.9	5 th
Insufficient capital for dry season farming	20	38	25	83	161	1.34	11 th
Insufficient market place for garden egg	39	38	17	27	210	1.74	9 th
Unavailability of labour use	21	43	30	26	179	1.49	10 th
High cost of transportation	60	32	8	20	252	2.1	2 nd

Source: Field survey, 2015.

Table 5 shows the results of ranking among the identified constraints. According to the results, constraints related to pests and diseases were ranked 1st. This indicates that the constraints were the main factors discouraging the dry season garden egg farmers to effectively produce at maximum rate. Constraints related to the high cost of transportation ranked 2nd and this suggests that some of the respondents did not have quick access to affordable means of transporting their farm produce to the market place. The farmers were also not satisfied with the rate of water availability for dry season garden egg farming in the study area.

This was ranked 3rd in the constraint categories. This is an indication that the water availability was not sufficient enough for the farmers to irrigate and might have limited their effort to produce more of the produce. Therefore, the problem of insufficient demand for the garden egg vegetable in the production area was also identified but rated 4th in that category. The lack of storage facility was ranked 5th. This implies that the farmers had no adequate storage facilities to store their farm produce. This may however lead to post-harvest losses. Also, insufficient availability of farmland was identified as one of the limiting factors of production in the area and it was rated 6th. Other constraints including: land dispute issues, lack of sufficient garden egg seedling, insufficient market places for the garden egg, unavailability of labour used, insufficient capital for dry season irrigation farming of garden egg were ranked 7th, 8th, 9th, 10th and 11th respectively. In addition, all these afore-mentioned constraints severely influenced the respondents negatively in practicing dry season garden egg production in the study area.

Conclusion

From the foregoing research, dry season garden egg farming may be considered as profitable business venture dominated by male farmers who are in the active age range regarding agricultural production. The quantity of fertilizer use, household size and farm size were the determinants of production. Labour, fertilizer use, chemical use and farm size affected the efficiency of production. Furthermore, farmers were mainly constrained by the availability and cost of water for irrigation, pests and diseases, high costs of transportation and inadequate storage facilities.

Hence, based on the findings of the research, it was recommended that government at all levels should assist dry season farmers through policy reform. This can be done through the construction of dams and other irrigation facilities. Also, there is a need for these farmers to formulate modality that will assist them to benefit from off-season irrigation farming and to sustain food production in the area. For instance, farmers should be encouraged to form groups and cooperatives, where they can pull their resources together to construct cost effective irrigation systems. Government authorities and other non-governmental organizations are encouraged to give farmers fertilizer and other chemicals at subsidized rates. They should also make these inputs accessible to the farmers. The government should also review the land tenure system so as to make farmers accessible to large areas of land. There is a need for revitalization of the marketing board in Nigeria to further strengthen the marketing of agricultural produce and reduce marketing, storage and transportation problems.

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Received: August 30, 2016

Accepted: February 7, 2017

PROCENA PROIZVODNJE PLAVOG PATLIDŽANA TOKOM SUŠNE
SEZONE MEĐU MALIM POLJOPRIVREDNIM PROIZVOĐAČIMA U
LOKALNOJ OBLASTI EDU U DRŽAVI KVARA U NIGERIJ

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R e z i m e

Istraživanje je sprovedeno u cilju ocenjivanja proizvodnje plavog patlidžana tokom sušne sezone među malim poljoprivrednim proizvođačima u lokalnoj oblasti Edu države Kvara. U istraživanju je za uzorkovanje korišćena tehnika 'snežne grudve' kako bi se uzorkovalo 120 malih poljoprivrednih proizvođača plavog patlidžana. Formulirano je pet istraživačkih pitanja, kako bi se postigli ciljevi ovog istraživačkog rada. Deskriptivna statistika, analiza troškova i prihoda i OLS regresija korišćene su za analizu prikupljenih podataka. Analiza troškova i prihoda pokazuje da je ukupan prihod od prodaje navodnjavanog plavog patlidžana za tipičnog poljoprivrednog proizvođača iznosio ₦ 36.596,5k, dok su ukupni fiksni troškovi (amortizovan) i varijabilni trošak inputa iznosili 1.279,22k da bi se dobio neto prihod gazdinstva od ₦ 24.582,68k. Rezultati OLS regresije su pokazali da su koeficijenti veličine gazdinstva i veličine domaćinstva pozitivni i značajni pri nivoima verovatnoće od 1% odnosno 10%. Ovo implicira da će povećanje veličine gazdinstva od 1 ha povećati proizvodnju plavog patlidžana tokom suve sezone za 1%. Dok rezultati procene stohastičke granice proizvodnje pokazuju da su parametri rada, đubriva i veličine gazdinstva veoma značajni, upotreba hemikalija je značajna pri nivou od 10%. Ovakav rezultat implicitno pokazuje da su poljoprivredni proizvođači efikasni prilikom korišćenja rada, đubriva, hemikalija i veličine gazdinstva. Stoga se preporučuje da se poljoprivrednici ohrabre kako bi formirali zadruge, pri čemu mogu da udruže svoje resurse da bi obezbedili isplativ sistem za navodnjavanje. Pored toga, državni organi i druge nevladine organizacije se ohrabruju da daju poljoprivrednicima đubriva i druge hemikalije po subvencionisanim cenama. Postoji potreba za revitalizacijom tržišne organizacije u Nigeriji za dalje jačanje prodaje poljoprivrednih proizvoda i smanjenje problema u vezi sa prodajom, skladištenjem i transportom.

Ključne reči: sušna sezona, plavi patlidžan, mali poljoprivredni proizvođači, Nigerija.

Primljeno: 30. avgusta 2016.
Odobreno: 7. februara 2017.

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Introduction

The introduction should contain all the relevant information on past researches according to the stated problem and what can be achieved by further research. Reviewing the references, the author and the year should be provided, and the mentioned author should be cited in References. The title of the introduction should be centred and bolded, written in lower-case letters, below which using one line spacing, the text of the introduction should follow, justified. Each new paragraph should be indented pressing the tab key. These rules should be applied to all parts of the paper.

Material and Methods

The material and methods should be clearly outlined explaining all applied procedures in the paper. Generally known methods should be presented briefly, and a detailed explanation should be given if there is a deviation from previously published procedures. Papers, which have an experimental character, should provide the way of statistical data processing. This part, as well as the part Results and Discussion, if needed, may comprise certain subparts, too.

Results and Discussion

In the part Results and Discussion data obtained on the basis of observation and conducted experiments should be interpreted. In the comment of the results, references should be quoted at the end of the paper, providing the comparison between the obtained results and previous knowledge of the certain area.

Conclusion

All relevant items achieved in the researched area should be mentioned in the conclusion. Listing of all results with repetition of numbers previously specified in Results and Discussion should be avoided. Conclusion should not contain references.

Acknowledgements

Acknowledgements should contain the title and the number of the project that is the title of the program within which the paper was written, as well as the name of the institution which financed the project or program. It should be placed between the conclusion and references.

References

The References section should contain only papers cited in the main text. The paper cited in the text should contain the last (family) name and the year. If the citation is comprised of one author, it is stated as Jalikop (2010) or (Jalikop, 2010). When the citation is comprised of the two authors it is stated as Sadras and Soar (2009) or (Sadras and Soar, 2009). If more than two authors are cited, after the last (family) name of the first author, the abbreviation "et al." is given, and then the year. This citation is stated as Lehrer et al. (2008) or (Lehrer et al., 2008). If more than one paper are cited simultaneously for a certain problem, they should be listed chronologically. A large number of cited papers out of brackets should be separated by comma (,) and if in brackets, by semicolon (;). If two or more papers of the same author are cited, they must be listed chronologically (1997, 2002, 2006, etc.). If a certain author appears several times for the same year, the letters are added (2005a, b, c, etc.). The citations of personal communication and unpublished papers should be avoided, except that it is an absolute necessity. Such citations should appear in the text only as (Brown, personal communication), and not in the list of References.

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The examples of listing references are the following:

Periodicals

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.

Books

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

Book chapter

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.

Proceedings

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, France.

Thesis

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

Report

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

Web site

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>

Summary

The summary in Serbian is given at the end of the paper and should comprise 100 to 250 words. Before the main text of the summary, as well as in English, the title of the paper, first name, middle initial(s) and last (family) name of all authors and the names and addresses of affiliations should be given. The title of the summary is centred and written separately. Below the title, the text of the summary should follow, without any indentation, and immediately after the text of the summary, the key words are given with the full stop at the end. The e-mail address of the corresponding author should be given at the bottom of the page.

Tables

Tables numbered with Arabic numerals (1, 2, etc.), followed by the title should be placed in the text using 9 font size and a maximum width of 13 cm. They should be clear, simple and unambiguous. The vertical sections should be avoided, and the number of columns should be limited so that the table is not too wide. Also, an unnecessary usage of horizontal sections should be avoided. The title of the table, single spaced above the table, justified, and with the full stop at the end should be given. The detailed explanation of abbreviations, symbols and signs used in the table should be provided below the table. Each table must be mentioned in the text.

Illustrations

All graphs, diagrams and photographs should be titled "Figure" (1, 2, etc.). They should be placed in the text. Graphs and diagrams should be computer drawn, using 9 font size and a maximum width of 13 cm, so that they can be legible and distinct after the size reduction. The overuse of colours and hues should be avoided for aesthetic reasons. The detailed legend without abbreviations for each graph and

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Abbreviations and units

Only standardised abbreviations should be used in the paper. Measure units should be expressed using International System of Units (SI). The abbreviations can be used for other expressions provided these expressions are stated in the full form when appear for the first time with the abbreviated form in the brackets. Values from 1 to 9 can be written in letters, but others numerically.

Nomenclature

The complete nomenclature (chemical and biochemical, taxonomical, genetic etc.) must be adjusted to international codes and commissions, such as *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* etc.

Formulae

All formulae and equations in the paper should be worked out by means of the programme "WORD Equation". An ample space should be left around the formulae for the sake of visibility. Subscripts and superscripts should be clear. Greek letters and other non-Latin symbols should be explained when they are first used. The meaning of all symbols should be given immediately after the equation where these symbols are first used. Equations should be numbered by Arabic numerals, serially in brackets, at the right-hand side. Each equation must be mentioned in the text as Eq. (1), Eq. (2), etc.

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Originalan naučni rad – Rad koji sadrži predhodno neobjavljivane rezultate sopstvenih istraživanja. Obim ovog rada treba da iznosi od 6 do 12 strana.

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Ključne reči

Ključne reči su termini ili fraze koje najbolje opisuju sadržaj članka za potrebe indeksiranja i pretraživanja. Broj ključnih reči može biti od 3 do 10. Navode se ispod sažetka. Naslov „Ključne reči“ piše se boldovano i uvlači jednim tabulatorom. Nakon toga slede dve tačke, a zatim nabrojanje ključnih reči malim slovima, sa tačkom na kraju. Treba izbegavati korišćenje ključnih reči koje se nalaze u naslovu rada. Ključne reči se dostavljaju na srpskom i engleskom jeziku posle sažetaka na oba jezika.

Uvod

Uvod treba da sadrži informacije o dosadašnjim istraživanjima po navedenom pitanju i šta se datim istraživanjem želi postići. Prilikom osvrta na literaturu, navesti autora i godinu, a autora citirati u spisku literature. Naslov „Uvod“ piše se sa prvim velikim slovom, centrirano i boldovano, nakon čega sa jednim razmakom ispod naslova sledi tekst uvoda poravnat po levoj i desnoj margini. Svaki novi pasus uvlači se jednim tabulatorom. Ova pravila važe i za sva ostala poglavlja.

Materijal i metode

Materijal i metode treba izložiti jasno uz objašnjenje svih primenjenih postupaka u radu. Opšte poznate metode izložiti kratko, a detaljnije ih objasniti ukoliko se odstupa od ranije objavljenih postupaka. Za radove eksperimentalnog karaktera obavezno navesti način statističke obrade podataka. U ovom poglavlju, kao i u poglavlju „Rezultati i diskusija“, po potrebi se mogu dati i određena podpoglavlja.

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U poglavlju „Rezultati i diskusija“ interpretiraju se podaci dobijeni na osnovu zapažanja i izvršenih eksperimenata. U komentaru rezultata treba se pozivati na literaturu koja se navodi na kraju rada, čime se obezbeđuje poređenje dobijenih rezultata sa dosadašnjim saznanjima u toj oblasti.

Zaključak

U zaključku treba ukratko navesti najznačajnije rezultate dobijene u radu. Izbegavati nabrojanje svih rezultata istraživanja sa ponavljanjem brojčanih vrednosti koje su prethodno već navedene u poglavlju „Rezultati i diskusija“. Zaključak ne sme da sadrži reference.

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Zahvalnica treba da sadrži naziv i broj projekta, odnosno naziv programa u okviru koga je rad nastao, kao i naziv institucije koja je finansirala projekat ili program.

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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>

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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.

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JOURNAL of Agricultural Sciences / editor
in chief Snežana Oljača. - Vol. 44, No. 1
(1999)- . - Belgrade : Faculty of Agriculture, 1999-
(Belgrade : Faculty of Agriculture). - 24 cm

Tromesečno. - Tekst na srp. i engl. jeziku. -
Je nastavak: Review of Research work at the Faculty
of Agriculture = ISSN 0354-3498
ISSN 1450-8109 = Journal of Agricultural Sciences
COBISS.SR-ID 169380871

