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THE VARIABILITY OF GRAIN YIELD, SEED MORPHOMETRIC AND VIGOUR TRAITS OF EARLY MATURING HYBRID MAIZE

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Abstract: Breeding for yield and quality requires the assessment of the seed metrics and vigour traits. This study, therefore, assessed the variability and inter-dependence of grain yield (GY), seed morphometric and vigour traits in hybrid maize. Seeds of 75 early maturing hybrid maize varieties were evaluated for morphometric traits and quality in four replicates. A field trial laid out in a randomised complete block design with three replicates was also conducted in Ibadan, Nigeria, to determine the grain yield of the hybrids. Data collected on the GY, seed dimension and quality were subjected to analysis of variance. The least significant difference was used to separate means. Relationships among the GY, seed morphometric and vigour traits were determined using correlation coefficients, while principal component (PC) analysis was performed for variability among the hybrids. Significant differences ($P<0.001$) were found in the GY, seed dimension and vigour traits. Four of the nine highest yielding hybrids had ECT higher than 30.0 μsg^{-1} cm^{-1}. The GY correlated with seed diameter (SDT) (0.40^{**}), seed width (SWD) (0.36^{**}), seed length (SLG) (0.35^{**}), seed area (SAR) (0.30^{**}) and seed vigour (SVI) (0.30^{**}). The SAG correlated with SDT, SLG, seed thickness (STH) and SAR. All the seed vigour traits correlated with one another. The PC I explained GY, SDT, SWD, SLG, SAR and SVI, indicating their importance in GY improvement. Seed angle, length and diameter were versatile in maize varietal selection. Identified high yielding hybrids with seed morphometric and vigour qualities can be explored by seed companies as innovation in the seed production business.

Key words: eigenvalues, electrical conductivity, maize yield, seed metrics, seed vigour.

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Introduction

Maize is among the few staple crops grown in almost all the sub-regions of Africa because a fairly dependable improved technology exists for producing the crop in the region (Abalu, 2001). The maize belt in Africa is broad, extending from Southern Africa through Eastern Africa and across the savannah of West Africa. The crop is also adapted to the forest agro-ecologies and derived savannah (Badu-apraku et al., 2010). Maize accounts for about 15% of the total energy intake, representing 72 kg per capita maize consumption of the rural communities in West and Central Africa (FAOSTAT, 2014). Maize yield is still low in Africa compared to that obtainable in the developed countries. Therefore, many scientific efforts have been directed to improve the maize yield in Africa (Badu-apraku et al., 2011). Crop productivity can be improved by planting good quality seeds. Moreover, other production inputs and improved farming technologies are beneficial when high vigour seeds are sown (Goggi et al., 2008; Farshadfar et al., 2012).

Seed quality is mostly determined by seed vigour, which has the potential to influence crop performance through the rapid and uniform establishment and development of normal seedlings. There are several seed quality tests, but none is universally accepted for all kinds of seeds (Powell and Matthews, 2005). A standard germination test informs the farmers about the number of seeds that will produce normal seedlings. A higher germination percentage indicates higher seed vigour (ISTA, 1995). Likewise, the conductivity test measures the leakage of electrolytes into the water in which seeds are soaked to provide the level of seed vigour. High-vigour seeds can reorganise their broken membranes more rapidly and repair any damage better than low-vigour seeds. The test has been widely used in agriculture to measure seed viability and vigour in many crops (AOSA, 1983).

Another aspect of maize seed quality is the seed physical appearance with respect to colour, shape and arrangement, which are essential in designing the seed processing equipment for the handling, conveying, separation, drying, storing and processing of maize seed (Tarighi et al., 2011). Seed size, shape and other physical qualities are important determinants of moisture imbibition and germination of seeds (Balkaya and Odabas, 2002) and grain grading quality. They have also been reported to influence the grain yield of the crop (Kesavan et al., 2013; Zhang et al., 2014; Chen et al., 2016**)**. However, breeding efforts on maize have chiefly been focused on yield, but not on seed quality improvement. Seed morphometric analysis involves measuring the dimensions (such as length, width, masses, angle, ratio and area) of seeds. It has been widely used to discriminate cultivars of many crops (Geetha et al., 2011; Grillo et al., 2011; Daniel et al., 2012). Geetha et al. (2011), Grillo et al. (2011), Sumathi and Balamurugan (2013) observed that this technique gives information that could be visually obtained repeatedly and faster.

This study, therefore, assessed the variability in and inter-dependence of grain yield, seed morphometric and vigour traits in early maturing hybrid maize. The study will be useful for maize breeders in planning maize improvement programmes, seed companies to discover modern packaging techniques and fabricators to explore novelty in designing machines for maize production.

Materials and Methods

Experimental location and materials

The trial was conducted in Ibadan, Nigeria (3.56° E; 7.33° N and 168 m above sea level). The location lies in the rainforest-savanna-transition agro-ecology of Nigeria. The mean annual rainfall and temperature of the trial site were 158.3 cm and 25.9°C, respectively. The description of the test location for the period of the trial is shown in Figure 1.

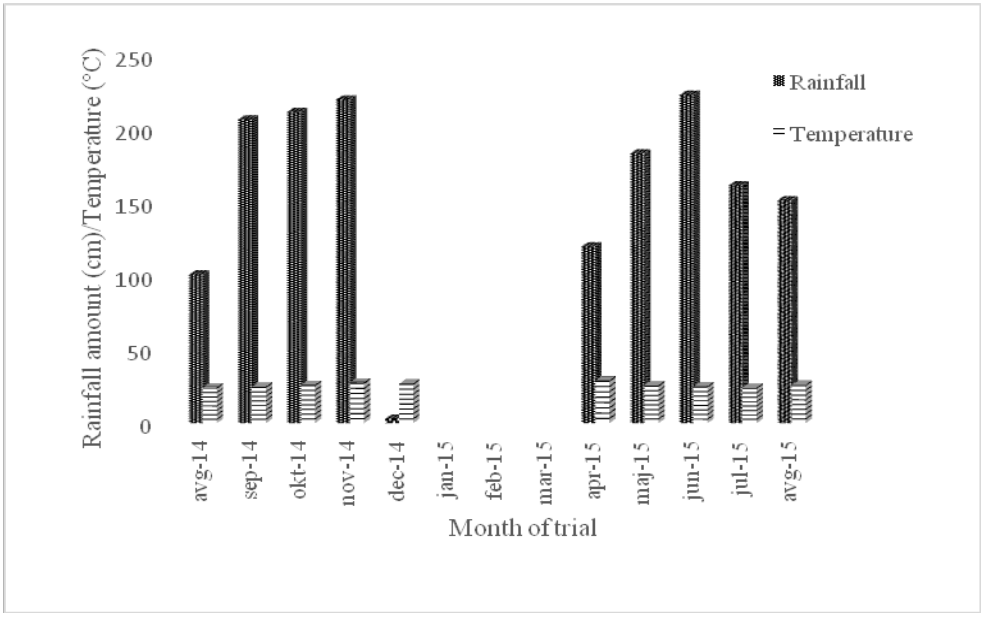

Figure 1. Rainfall and temperature pattern of the site in the period of the field trial.

Seventy-five single cross hybrids of white endosperm maize were evaluated for grain yield and seed quality in a research field and a seed testing laboratory, respectively. The test seeds were obtained from the first filial generation of the single cross hybrid developed in the test location.

Field evaluation for grain yield

The field trial was laid out in a randomised complete block design with three replicates. Each plot consisted of two rows of 5 m long and 0.75 m apart, where plants were spaced 0.5 m in a row. Three seeds were sown and later thinned two weeks after planting (WAP) to two stands per hill to attain a plant population density of 53,333 plants ha^{-1}. Standard cultural practices for field maintenance of maize were applied as recommended by IAR&T (2010). This included ploughing and harrowing of land before planting, applying 60 kg ha^{-1} of N:P:K 15:15:15 fertiliser at 2 WAP and urea as top-dressing at 30 kg N ha^{-1}, two weeks later. The maintenance also involved keeping the field weed-free using herbicides (before plant emergence at 5.0 l ha^{-1} each of paraquat (N, N'-dimethyl-4, 4'-bipyridinuim dichloride) and atrazine (2-Chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) and hoeing. The crop was protected against pests and diseases by hand picking and destruction of pests. The field evaluation was conducted from August to December 2014 and from April to August 2015. Ears of the plants were harvested when dry. The grains were shelled and weighed, and then the moisture content was determined using a digital moisture tester. The yield data across the two planting seasons were pooled to compute the grain yield using grain moisture content = 15%, harvested plot area = 7.5 m^2, and 1 ha = 10,000 m^2 as follows:

$$\text{Grain yield (kg ha}^{-1}) = \frac{\text{GWT (kg)}}{7.5\ \text{m}^2} \times \frac{(100-\text{MC})}{(100-15\%)} \times 10{,}000\ \text{m}^2 \quad (1)$$

where GWT = grain weight and MC = grain moisture content at harvest.

Seed morphometric analysis

Whole and intact 40 seeds were obtained at 10 per ear column from four consecutive ear rows in the middle of the uppermost or only ear of each hybrid maize plant. The seeds were subjected to seed morphometric and quality analyses in four replicates in a laboratory in Ibadan, Nigeria. Ten seeds from each replicate were viewed under a USB microscope one after the other with their embryo axis facing the lens of the camera under the light. The light on the USB microscope was calibrated (×35 magnification) to obtain the brightest image before it was used for measuring the parameters. Morphometric data were taken as described by Grillo et al. (2011) and Geetha et al. (2011), as follows:

- Seed angle (SAG) was the angle created in between two lines touching each other at the tip where the seed is attached to the husk;
- Seed diameter (SDT) was the length of the line drawn across the circle made around the seed;
- Seed thickness (STH) was measured between digital vernier callipers, as the distance between both flat sides of the seed;

- Seed width (SWD) was measured across the middle, and at the right angle to the seed length;
- Seed length (SLG) was the distance between the base of the embryo axis to the tip of the endosperm of the seed;
- Estimations were made on seed area (SAR) as the seed length × seed width.

Seed vigour analyses

Samples were drawn from each seed lot for the standard germination test (SGT), seedling vigour index (SV) and electrical conductivity (EC).

Standard germination test: One hundred seeds were sowed per replicate at 5 cm deep in moistened sterilised river-bed sand inside plastic germination bowls in four replicates under ambient environment. Emerged normal seedlings were counted daily from four to seven days after planting (DAP) according to the procedure laid out by ISTA rules for seed testing. Data were collected on emergence to estimate germination percentage (ISTA, 2009).

Germination percentage (%)

$$= \frac{\textbf{Number of seedlings emerged at 7DAP}}{\textbf{Total number of seeds sowed}} \times 100 \quad (2)$$

Seedling vigour index: The seedling vigour index was estimated using data from the SGT. Ten normal seedlings were randomly selected and carefully uprooted from each replicate at 7 DAP. The seedling lengths were measured for the SVI determination with the germination data in SGT described according to ISTA (2009), using the formula:

$$\text{SVI} = \frac{\textbf{Seedling length} \times \textbf{Germination percentage}}{\textbf{100}} \quad (3)$$

The seedling length was measured as the distance between the base of the plant and the tip of the leaves when folded upward.

Electrical conductivity test: Three replicates of 100 whole and intact seeds per hybrid were weighed. The seeds of each replication were placed in a 200-ml conical flask, and 75 ml of de-ionised water was added. All the flasks were covered by polythene to avoid contaminations and left at ambient temperature in the laboratory for 24 hours. The electrical conductivity of leachates was measured by using a conductivity meter and conductivity per gram of seed weight (µscm^{-1} g^{-1}). The EC was calculated as:

$$\frac{\textbf{Solution conductivity } (\mu\text{scm}^{-1}) \times \textbf{ control conductivity } (\mu\text{scm}^{-1})}{\textbf{Initial weight of seeds (g)}} \quad (4)$$

Data analysis

Data collected on grain yield were subjected to the two-way analysis of variance (ANOVA), while data on seed morphometric and vigour traits were analysed using one-way ANOVA. The least significant difference was obtained to separate pairs or groups of means. The relationships among the grain yield, seed morphometric and vigour traits were determined using Spearman's correlation coefficients, while principal component analysis was performed to detect the contributions of each trait to variability among the hybrids.

Results and Discussion

Mean squares, coefficients of variation and range values for the grain yield and seed parameters of the hybrid maize

Significant differences (*P<0.01*) were observed in GY, all the dimension and vigour traits of the seeds of the hybrid maize (Table 1). Coefficients of variation (CVs) were equal to or less than 15% for all the seed traits except SVI. It was higher for GY (23.03%). Moreover, the CV was less than 10.00% for all the morphometric traits and germination percentage. The minimum and maximum values for the seed morphometric and vigour traits were also shown in Table 1.

Table 1. Analysis of variance for grain yield, seed morphometric and vigour traits of hybrids of early maize inbred lines.

Source of variation	Grain yield (kg^{-1})	Seed morphometric trait						Vigour trait		
		Angle (°)	Diameter (cm)	Thickness (cm)	Width (cm)	Length (cm)	Area (cm^2)	GP (%)	Seedling vigour index	EC (μsg^{-1} cm^{-1})
Genotype (df=74)	2584109.00***	99.04***	3.28***	0.95***	1.21 ***	3.62***	435.16***	1036.61**	50.97**	20.30**
Block (df=2)	2176083.30	-	-	-	-	-	-	-	-	-
Error	408025.70	27.36	0.25	0.16	0.12	0.46	31.45	0.04	0.00	100.347
CV (%)	23.03	6.66	8.66	9.45	4.82	7.99	9.28	4.88	30.60	15.05
Minimum	2263.80	63.55	6.39	3.07	5.46	6.37	35.05	23.00	4.04	2.50
Maximum	5554.60	92.92	11.25	5.73	8.93	11.15	99.58	100.00	20.10	77.30

GP and EC mean germination percentage and electrical conductivity, respectively. **, *** mean significant at *P<0.001* and *0.0001*, respectively.

There have been several reports of variation in grain yields of early maturing hybrid maize due to their genetic potentials (Badu-apraku et al., 2010; Badu-apraku et al., 2011; Ogunniyan et al., 2018). Similarly, the significant variation among the

hybrids in some seed parameters shows variability in the seed vigour quality. Similar results were found in maize for seed vigour, particularly the SVI and EC, and seed morphometry (Peterson et al., 1995; Varga et al., 2012). The low CVs of all the seed morphometric traits in this study show precisions in the experimentation and data collection process. Considering the fairly large number of entries (75 hybrids), the low CVs may also mean consistency of an individual hybrid or suggests that selection through seeds should employ multiple traits. However, the large CVs for the GY and SVI indicate that the parameters may be considered for selection. Wide ranges for the traits also buttressed the nomination of the seed traits as selection indices. Large variability has also been found in the analysis of seedling vigour (Adetumbi, 2013; El-abady, 2015; Ogunniyan et al., 2017).

Grain yield and seed quality traits of the hybrid maize

Grain yield (GY) of the 75 hybrids ranged from 2263.8 kg ha^{-1} to 5554.6 kg ha^{-1} with a mean of 3674 kg ha^{-1} (Appendix 1). However, nine hybrids had GY higher than or equal to 4500 kg ha^{-1}, while eight had GY less than 3000 kg ha^{-1} (Table 2). There were significant differences among the selected 17 high or low yielding hybrids with respect to all the seed traits except SAG and SWD. Three hybrids (BD74-171×BD74-128, BD74-399×BD74-128 and BD74-170×BD74-152) among the eight high yielding hybrids had high values of SAG, SDT, STH, SWD, SLG, SAR, GP, SVI and EC, while four of the nine highest yielding hybrids had EC higher than 30.0 μsg^{-1} cm^{-1}. Although the three high grain yielding hybrids had the high seed morphometric and vigour traits, the high EC recorded in the hybrids is not a desired trait. Four high yielding hybrids (BD74-170×TZEI4, TZEI1×BD74-399, TZEI188×BD74-171 and TZEI136×BD74-399) had high values for all the seed morphometric traits, GP and SVI, but low value for EC (less than 15%). Therefore, the hybrids were more promising than the others due to their high yield potential, high seed morphometric traits, GP and SVI, but a low EC value. This implies that hybrids with high GY potential have big-sized kernels and will be better suited for mechanised farming. Only TZEI7×TZEI2 hybrid consistently had high values for seed morphometric and seedling vigour traits among the low grain yielding hybrids. Similarly, only one hybrid (TZEI 22×TZEI106) had high EC in the low yielding hybrid category.

The high germination and seedling vigour traits coupled with low electrical conductivity identified in the promising hybrids suggest that they can be recommended for seed companies for multiplication and production. Only TZEI7×TZEI2 (about 12.5%) of the low yielding category of the hybrid maize consistently had high values for seed morphometric and vigour traits. This showed the importance of shapes in the final weight of the grains. It has been reported that

seed dimension affects seed germination, emergence, seedling vigour and the resultant yield in crop plants and that the large seed improves germination and seedling vigour (Varga et al., 2012; Kesavan et al., 2013; Zhang et al., 2014; El-abady, 2015). Also, seeds of similar dimension are planted or processed using the same equipment because of their uniformity, therefore, the seed morphometric trait becomes an important factor to seed industries as it facilitates processing, grading and packaging. Peterson et al. (1995) have reported that flat seeds have few tendencies to mechanical damage. Four of the nine highest yielding hybrids had EC higher than 30.0 μsg^{-1} cm^{-1}, while only one (TZEI 22× TZEI 106) had high EC. It, therefore, implies that seed vigour parameters can be used concurrently for high precision in seed selection activities.

Table 2. Mean values for grain yield, seed morphometric and vigour traits of eight highest and nine lowest grain yielding hybrids of early maize inbred lines.

Hybrid	GY ($kg\ ha^{-1}$)	SAG (°C)	SDT (cm)	STH (cm)	SWD (cm)	SLG (cm)	SAR (cm^2)	GP (%)	SVI	EC ($\mu sg^{-1}\ cm^{-1}$)
The highest grain yielding										
BD74-170× TZEI 4	**5554.6**	**75.9**	**9.6**	**3.8**	**7.6**	**9.2**	**70.1**	**56.3**	**9.4**	**15.3**
TZEI 1× BD74-399	**5161.5**	**76.6**	**9.2**	**3.8**	**7.4**	**8.9**	**65.9**	**62.0**	**7.4**	**2.6**
BD74-171× BD74-128	5140.4	70.7	10.5	3.2	6.7	10.5	70.5	23.3	4.0	58.6
BD74-170× BD74-55	5051.5	77.0	9.1	4.3	7.3	8.8	64.8	30.0	4.9	61.1
TZEI 188 × BD74-171	**4941.8**	**77.6**	**8.6**	**4.4**	**6.8**	**8.4**	**57.6**	**51.0**	**6.0**	**6.8**
TZEI 136× BD74-399	**4836.6**	**80.0**	**8.3**	**3.4**	**7.0**	**8.6**	**60.1**	**48.3**	**5.8**	**2.5**
BD74-179×BD74-55	4654.3	72.9	8.8	3.1	7.0	8.5	59.6	31.7	4.6	44.4
BD74-170× BD74-152	4533.7	74.1	8.8	4.3	6.1	8.7	53.3	59.0	12.8	23.5
BD74-399× BD74-128	4495.5	73.2	10.4	3.5	7.5	10.4	77.9	36.7	5.8	34.1
The lowest grain yielding										
TZEI 22× TZEI 106	2869.9	84.5	8.1	4.8	7.0	7.6	53.0	23.0	4.1	77.3
TZEI 136× TZEI 3	2869.2	78.0	8.9	4.1	7.2	9.1	65.5	58.7	7.3	8.4
TZEI 98× BD74-55	2847.9	79.4	8.8	3.5	7.3	9.1	66.3	65.7	12.9	13.5
BD74-399× TZEI 3	2786.3	71.7	7.5	3.5	6.2	7.2	44.0	100.0	20.1	13.1
TZEI7× TZEI2	2717.5	76.6	11.3	4.0	8.9	11.2	99.6	75.0	9.1	9.8
TZEI 188 × TZEI 2	2686.8	84.3	7.0	4.2	6.3	7.0	43.8	57.0	11.3	8.1
TZEI 188 × TZEI 106	2576.5	88.6	7.4	4.4	6.9	7.3	50.5	60.3	11.9	11.3
BD74-152× TZEI 136	2263.8	79.2	7.0	4.0	5.9	6.8	39.7	56.0	13.0	11.9
LSD	430.4	8.44	0.8	0.6	0.6	1.1	9.1	14.3	2.8	16.2

GY: grain yield; SAG: seed angle; SDT: seed diameter; STH: seed thickness; SWD: seed width; SLG: seed length; SAR: seed area; GP: germination percentage; SVI: seedling vigour index; EC: electrical conductivity and LSD: Least significant difference. Hybrids highlighted in bold had high grain yield, seed morphometric trait and vigour (low electrical conductivity values).

Relationship between grain yield, seed germination and morphometric traits of the hybrid maize

Various positive and negative significant correlations were found among the GY, seed dimension and vigour traits of the hybrid maize (Table 3). The GY had a highly significant correlation with SAG, while it was positively correlated with SDT, SWD, SLG, SAR and SVI. This shows that both seed dimension and vigour parameters contributed to the GY of the maize. The SAG had significantly negative correlations with three out of five seed traits, namely SDT, SLG and SAR, but it was positively correlated with STH (0.27^{*}). Hence, SAG is vital in the dimension determination and selection in maize. Highly significant positive correlations were found among the SDT, SWD, SLG and SAR. Similarly, SDT, SWD and SLG positively correlated with one another. The SDT is a versatile seed dimension trait for its relationship with SWD, SLG and SAR because SWD and SLG are factors of SAR. The three traits are important in discriminating and selecting hybrid maize seeds. The GP had a highly significant correlation with SVI (0.83^{***}), while correlations of EC with GP (-0.67^{***}) and SVI (-0.45^{***}) were highly significant. It can also be deduced from this result that seed vigour is independent of GY, as shown in the non-correlation of GY with GP, SVI and EC.

Table 3. Spearman's correlations of grain yield, seed morphometric and vigour traits of hybrids of the early maize inbred lines.

	GY	SAG	SDT	STH	SWD	SLG	SAR	GP	SVI
SAG	-0.28^{*}								
SDT	0.40^{***}	-0.58^{***}							
STH	-0.18	0.27^{*}	-0.16						
SWD	0.16^{***}	-0.06	0.69^{***}	0.03					
SLG	0.35^{***}	-0.63^{***}	0.94^{***}	-0.22	0.62^{***}				
SAR	0.30^{**}	-0.44^{***}	0.93^{***}	-0.15	0.84^{***}	0.93^{***}			
GPT	0.09	0.09	-0.11	-0.02	-0.04	-0.12	-0.10		
SVI	0.30^{**}	0.12	-0.21	0.05	-0.16^{*}	-0.20	-0.20	0.83^{***}	
EC	-0.20	-0.21	0.23^{*}	-0.07	0.00	0.23^{*}	0.17	-0.67^{***}	-0.45^{***}

GY: grain yield; SA: seed angle; SDT: seed diameter; STH: seed thickness; SWD: seed width; SLG: seed length; SAR: seed area; GP: germination percentage; SVI: seedling vigour index and EC: electrical conductivity. *, **, *** mean significant at *P<0.05, 0.001* and *0.0001*, respectively.

Principal component analysis

The first four PCs explained a total of 81.47% of the total variance for the GY, seed germination and morphometric parameters (Table 4). The eigenvalue of PC I was 4.5 and was higher than the other three PCs with the values of 2.7, 1.3, and 1.3, respectively. PC I was responsible for about 37.73% of the total variation and

was loaded with seed diameter (0.868), seed length (0.860) and seed area (0.849). The second PC was responsible for about 22.16% of the total variation and was majorly loaded with seed vigour index (0.678) and germination percentage (0.662). PCs III and IV captured about 11.0% each of the total variation, while seed thickness (0.663), seed angle (0.602), and seed width (0.534) were more prominent in PC III, and electrical conductivity (0.543) was the only parameter prominent in PC IV.

Table 4. The decomposition of contributions of grain yield, seed morphometric and vigour traits of the hybrids of the inbred lines of early maturing maize into the first four principal component axes.

Trait	Eigenvectors			
	PC I	PC II	PC III	PC IV
Grain yield	0.503	-0.100	-0.293	-0.121
Seed angle	-0.468	-0.321	**0.602**	-0.130
Seed diameter	**0.868**	0.446	0.014	0.088
Seed thickness	-0.225	-0.044	**0.663**	0.391
Seed width	0.674	0.377	0.534	-0.038
Seed length	**0.860**	0.443	-0.068	0.070
Seed area	**0.849**	0.469	0.202	0.012
Germination percentage	-0.463	**0.662**	-0.016	-0.502
Seedling vigour I	-0.634	**0.678**	-0.121	-0.150
Electrical conductivity	0.351	-0.463	-0.246	**0.543**
Eigenvalue	4.528	2.659	1.309	1.281
Percent variance (%)	37.73	22.16	10.91	10.67
Cumulative percent variance (%)	37.73	59.89	70.80	81.47

PC is the principal component.

The first two PCs had the greatest discriminating ability and captured greater than 50% of the contribution of the various parameters to variability in the maize. PC I captured three traits, namely SDT, SLG, and SAR, which are important in determining the performance of the maize. The three seed parameters had a value greater than the value for the GY. This result proved the resourcefulness of PC I and buttressed the relatedness of the traits in agreement with the findings of Ogunniyan (2016), who has also reported the usefulness of PC I in associating traits that can be improved to obtain a higher yield of crops. The discriminating ability of the six traits has supported that both seed dimension and vigour parameters contributed to the GY of the maize as recorded through correlation analysis. Therefore, this study has identified high yielding, white kernel hybrid maize with high germination and seed vigour that can be further multiplied or used to improve seed vigour of other inbred lines in maize breeding programmes.

Conclusion

Four early maturing hybrids with high grain yield ability: BD74-170×TZEI4, TZEI1×BD74-399, TZEI188×BD74-171 and TZEI136×BD74-399 were revealed in this study and recommended for seed companies to explore their yield potential, high values for seed morphometric and vigour quality traits. This study emphasises the importance of multiple traits for higher precision in maize varietal selection using seeds. Seed diameter, seed length and seed area are important dimension traits useful in discriminating and selecting hybrid maize seeds.

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Appendix 1. Mean values for grain yield, seed morphometric and vigour traits of the hybrids of maize inbred lines.

Hybrid	Grain yield (kg ha^{-1})	SAG (°C)	SDT (cm)	STH (cm)	SWD (cm)	SLG (cm)	SAR (cm^2)	GP (%)	SVI	EC (µsg^{-1} cm^{-1})
TZEI 1×TZEI 2	2959.9	80.3	7.2*	3.5	6.3	8.0	50.9	41.7	5.68*	28.2
TZEI 1×TZEI 98	3235.5	77.0	9.4	5.4**	7.5	9.2	68.8	48.3	6.62	16.2
TZEI 1×TZEI 106	3299.4*	78.3	8.2	4.1	7.0	8.8	61.1	40.3	8.00	9.8*
TZEI 7×TZEI 2	2717.5	76.6	11.3**	4.0	8.9**	11.2**	99.6**	75.0**	9.10	9.8*
TZEI 7×TZEI 3	3996.2*	79.5	9.3	4.0	7.2	8.7	62.6	53.3	6.35	6.7*
TZEI 7×TZEI 4	2966.2	76.8	9.0	3.6	7.1	9.6**	68.9	44.3	9.61	23.4
TZEI 7×TZEI 98	3373.6*	71.8	10.2**	3.4*	7.3	10.5**	76.5	35.0*	4.98*	22.5
TZEI 22×TZEI 2	3211.8	84.2	7.8*	4.9**	6.7	7.5	49.8*	37.7*	5.62*	12.2
TZEI 22×TZEI 3	3038.7	83.1	8.1	3.8	7.3	8.4	61.4	61.7	11.66**	9.0*
TZEI 22×TZEI 98	3226.5	75.8	9.5**	3.9	7.3	9.2	66.7	50.7	10.38	31.9
TZEI 22×TZEI 106	2869.9	84.5	8.1	4.8**	7.0	7.6	53.0	23.0*	4.13*	77.3**
TZEI 136×TZEI 2	3318.5*	74.5	9.1	3.5	7.3	9.1	66.3	45.7	4.20*	18.5*
TZEI 136×TZEI 3	2869.2	78.0	8.9	4.1	7.2	9.1	65.5	58.7	7.30	8.4*
TZEI 136×TZEI 98	3828.0*	83.8	8.7	3.7	6.9	8.4	57.6	64.3	9.90	8.6*
TZEI 188×TZEI 2	2686.8	84.3	7.0*	4.2	6.3	7.0*	43.8*	57.0	11.32**	8.1*
TZEI 188×TZEI 3	3436.0*	72.7	8.6	4.0	7.1	8.5	60.3	63.0	9.11	4.7*
TZEI 188×TZEI 106	2576.5	88.6**	7.4*	4.4	6.9	7.3*	50.5*	60.3	11.91**	11.3*
BD74-152×TZEI 1	3202.2	82.2	6.6*	4.4	5.5*	6.4*	35.1*	84.3**	14.46**	8.8*
BD74-152×TZEI 7	3215.2	77.8	8.4	4.1	7.6**	8.3	62.6	27.0*	4.04*	58.9**
BD74-152×TZEI 22	3392.1*	89.2**	7.3*	4.2	6.8	6.6*	45.1*	54.0	11.34**	7.5*
BD74-152×TZEI 136	2263.8	79.2	7.0*	4.0	5.9*	6.8*	39.7*	56.0	12.96**	11.9*
BD74-152×TZEI 188	3736.1*	84.2	8.5	4.2	7.5	8.3	62.4	95.3**	18.75**	7.5*
BD74-147×TZEI 7	4078.9*	79.4	9.0	3.8	6.9	8.2	56.4	61.0	7.19	5.7*
BD74-147×TZEI 22	3492.8*	90.0**	7.7*	5.2**	7.4	7.9	58.5	63.7	8.47	3.7*
BD74-147×TZEI 188	3457.5*	77.7	9.0	3.8	8.0**	8.9	71.4**	77.0**	15.22**	2.7*
BD74-31×TZEI 1	3249.8*	82.0	7.2*	4.1	6.2*	7.1*	44.4*	90.0**	17.34**	3.5*
BD74-31×TZEI 7	3772.4*	76.4	7.3*	3.7	6.1*	7.8	48.3*	99.7**	17.24**	2.9*
BD74-31×TZEI 188	3026.0	81.6	7.5*	4.3	6.6	7.7	50.7*	65.7	9.44	2.5*
BD74-55×TZEI 1	2916.4	80.4	7.6*	4.0	6.5	7.4	47.6*	68.0	7.68	3.2*
BD74-55×TZEI 7	3359.5*	80.7	7.3*	4.0	6.3	7.1*	45.2*	44.0*	4.57*	20.1
BD74-55×TZEI 22	3432.3*	89.0**	8.4	5.2**	8.1**	7.5	60.7	52.3	9.36	3.0*
BD74-55×TZEI 136	3466.3*	80.0	7.8*	4.5	5.9	7.3*	43.4*	67.0	13.34**	4.1*
BD74-55×TZEI 188	4160.5**	84.4	9.0	3.4*	8.0**	8.7	70.0**	78.7**	10.07	2.6*
TZEI 1×BD74-170	3849.3*	72.9	10.3**	3.5	8.2**	10.1**	82.9**	62.0	6.76	3.1*
TZEI 1×BD74-171	4135.9**	76.3	8.6	4.2	6.8	8.5	57.8	62.7	8.80	3.9*
TZEI 1×BD74-399	5161.5**	76.6	9.2	3.8	7.4	8.9	65.9	62.0	7.38	2.6*
TZEI 7×BD74-170	4184.6**	72.8	9.6**	3.3*	7.4	9.5	69.9**	55.3	5.90*	5.2*
TZEI 7×BD74-171	3570.1*	68.8*	9.9**	4.7**	7.1	9.8**	69.9**	40.3	6.12	15.5
TZEI 7×BD74-179	4078.7*	80.9	8.8	5.6**	7.4	8.4	62.2	56.3	8.33	4.8*

Continued – Appendix 1. Mean values for grain yield, seed morphometric and vigour traits of the hybrids of maize inbred lines.

Hybrid	Grain yield (kg ha-1)	SAG (°C)	SDT (cm)	STH (cm)	SWD (cm)	SLG (cm)	SAR (cm2)	GP (%)	SVI	EC (µsg-1 cm-1)
TZEI 136×BD74-399	4836.6**	80.0	8.3	3.4*	7.0	8.6	60.1	48.3	5.81*	2.5*
TZEI 188×BD74-170	4354.9**	77.2	10.9**	3.7	8.3**	10.9**	90.1**	47.0	6.67	15.0
TZEI 188×BD74-171	4941.8**	77.6	8.6	4.4	6.8	8.4	57.6	51.0	6.01	6.8*
TZEI 188×BD74-175	3897.0*	71.4	7.8*	5.7**	6.4	7.6*	48.7*	40.3	7.85	11.2*
TZEI 4×BD74-152	3737.4*	87.7*	7.2*	4.2	6.6	6.5*	43.0*	41.7	6.44	8.9*
TZEI 98×BD74-31	3069.9	71.9	8.3	3.1*	6.6	8.1	53.8	31.7*	5.53	19.0
TZEI 98×BD74-55	2847.9	79.4	8.8	3.5	7.3	9.1	66.3	65.7	12.87**	13.5
TZEI 106×BD74-55	3564.5*	83.9	9.0	3.9	7.9**	8.5	66.7	59.0	12.30**	22.8
BD74-170×TZEI 2	4251.1**	63.6*	9.0	3.4*	6.3	9.6**	60.3	43.0	7.30	52.6
BD74-170×TZEI 3	3891.4*	79.2	9.8**	4.2	7.5	9.7**	72.9**	36.3*	5.47*	67.8**
BD74-170×TZEI 4	5554.6**	75.9	9.6**	3.8	7.6**	9.2	70.1**	56.3	9.40	15.3
BD74-170×TZEI 98	4317.6**	77.0	9.3	3.7	6.8	9.8**	66.9	44.3	8.05	19.1
BD74-171×TZEI 2	3789.4*	73.7	10.4**	4.4	7.2	10.5**	74.4**	56.3	9.11	24.3
BD74-171×TZEI 3	3214.9	80.1	9.2	3.9	7.0	8.7	61.5	87.3**	18.09**	11.7*
BD74-171×TZEI 4	4003.5**	89.4**	8.8	4.5	7.5	8.9	66.7	36.3*	4.77*	33.9
BD74-171×TZEI 106	3739.0*	85.8	7.5*	4.0	6.5	7.0*	45.1*	51.7	7.58	21.3
BD74-179×TZEI 3	3662.6*	80.6	8.3	3.4*	6.6	8.2	54.0	59.0	9.61	22.0
BD74-179×TZEI 4	3172.7	83.5	8.5	4.0	6.8	7.7	52.6	33.7*	5.58*	43.9
BD74-179×TZEI 98	4115.3**	79.2	8.9	3.3*	7.4	8.4	61.9	24.3*	4.31*	71.0**
BD74-175×TZEI 98	3548.1*	71.9	8.7	3.8	6.4	8.6	55.6	41.7	7.04	19.1
BD74-399×TZEI 2	3634.9*	71.0	9.1	3.4*	7.0	8.9	62.3	85.7**	14.28**	38.1
BD74-399×TZEI 3	2786.3	71.7	7.5*	3.5	6.2*	7.2*	44.0*	100.0**	20.10**	13.1
BD74-399×TZEI 98	3458.3*	83.2	6.4*	4.1	5.6*	6.4*	35.9*	53.7	10.61	42.7
BD74-170×BD74-152	4533.7**	74.1	8.8	4.3	6.1*	8.7	53.3*	59.0	12.78**	23.5
BD74-170×BD74-55	5051.5**	77.0	9.1	4.3	7.3	8.8	64.8	30.0*	4.85*	61.1**
BD74-170×BD74-128	4236.3**	67.8*	10.0**	4.3	7.0	9.8**	67.9	60.3	9.30	47.4
BD74-171×BD74-152	3535.1*	71.2	9.5**	4.5	7.3	9.4	68.6	55.0	10.31	38.1
BD74-171×BD74-31	3347.1*	75.0	9.8**	4.6	7.7**	9.7**	74.6**	40.3	8.24	40.7
BD74-171×BD74-55	4233.7**	92.9**	7.2*	3.5	6.6	7.4	48.4*	24.0*	4.08*	76.6**
BD74-171×BD74-128	5140.4**	70.7	10.5**	3.2*	6.7	10.5**	70.5**	23.3*	4.04*	58.6**
BD74-179×BD74-147	3547.4*	81.4	9.2	3.8	7.5	8.9	67.2	95.0**	16.44**	12.4
BD74-179×BD74-55	4654.3**	72.9	8.8	3.1*	7.0	8.5	59.6	31.7*	4.62*	44.4
BD74-175×BD74-55	4138.2**	78.1	8.3	4.3	6.7	8.0	53.2	36.7*	4.38*	57.7**
BD74-399×BD74-147	4312.1**	72.0	9.8**	4.4	8.0**	9.7**	77.3**	58.0	5.48*	13.8
BD74-399×BD74-55	3130.8	80.8	9.1	4.9**	7.8**	8.7	67.3	30.7*	4.18*	50.6
BD74-399×BD74-128	4495.5**	73.2	10.4**	3.5	7.5	10.4**	77.9**	36.7*	5.76	34.1
Mean	3674.1	78.56	8.7	4.1	7.0	8.5	60.5	54.1	8.8	21.9
LSD	430.4	8.44	0.8	0.6	0.6	1.1	9.1	14.3	2.8	16.2

GY, SAG, SDT, STH, SWD, SLG, SAR, GP, SVI and EC mean grain yield, seed angle, seed diameter, seed thickness, seed width, seed length, seed area, germination percentage, seedling vigour and electrical conductivity, respectively. * and ** mean lower and higher than mean values, respectively.

PROMENLJIVOST PRINOSA ZRNA, MORFOMETRIJSKIH I OSOBINA ŽIVOTNE SPOSOBNOSTI SEMENA RANOG HIBRIDNOG KUKURUZA

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R e z i m e

Oplemenjivanje radi prinosa i kvaliteta zahteva procenu pokazatelja i osobina životne sposobnosti semena. Stoga je ovom studijom procenjena promenljivost i međuzavisnost prinosa zrna (PZ), morfometrijskih i osobina životne sposobnosti semena kod hibridnog kukuruza. Semena 75 linija ranog hibridnog kukuruza ocenjena su u pogledu morfometrijskih osobina i kvaliteta u četiri ponavljanja. Poljski ogled izveden u potpuno slučajnom blok sistemu sa tri ponavljanja takođe je sproveden u Ibadanu (Nigerija), kako bi se utvrdio prinos zrna hibrida. Podaci prikupljeni o PZ, dimenziji i kvalitetu semena obrađeni su analizom varijanse. Najmanje značajna razlika korišćena je za poređenje srednjih vrednosti. Odnosi između PZ, morfometrijskih i osobina životne sposobnosti semena utvrđeni su pomoću koeficijenata korelacije, dok je analiza glavnih komponenti (GK) primenjena radi utvrđivanja promenljivosti među hibridima. Značajne razlike ($P<0,001$) utvrđene su kod PZ, dimenzija semena i osobina životne sposobnosti semena. Četiri od devet hibrida sa najvišim prinosom imali su ECT veći od 30,0 μsg^{-1} cm^{-1}. PZ je korelirao sa prečnikom semena ($0,40^{**}$), širinom semena ($0,36^{**}$), dužinom semena ($0,35^{**}$), površinom semena ($0,30^{**}$) i životnom sposobnošću semena ($0,30^{**}$). Ugao semena korelirao je sa prečnikom semena, dužinom semena, debljinom semena i površinom semena. Sve osobine životne sposobnosti semena korelirale su jedna s drugom. Prvom glavnom komponentom objašnjen je prinos zrna, prečnik semena, širina semena, dužina semena, površina semena i životna sposobnost semena, ukazujući na njihovu važnost kod poboljšanja prinosa zrna. Ugao, dužina i prečnik semena bili su promenljivi kod izbora varijeteta kukuruza. Semenske kompanije mogle bi istražiti identifikovane hibride visokog prinosa sa morfometrijskim i kvalitetima životne sposobnosti semena kao inovaciju u proizvodnji semena.

Ključne reči: svojstvene vrednosti, električna provodljivost, prinos kukuruza, pokazatelji u vezi sa semenom, životna sposobnost semena.

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THE EFFECTS OF FLOWER REMOVAL AND EARTHING UP ON TUBER YIELD AND QUALITY OF POTATO (*SOLANUM TUBEROSUM* L.)

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Abstract: A field experiment was conducted in Eastern Tigray, Ethiopia, during the summer season to determine the effects of flower removal and earthing up time on the tuber yield and quality of potato (*Solanum tuberosum* L.). The experiment comprised three flower removal stages and five earthing up time treatments, which were laid out in a randomized complete block design (RCBD) of a 5x3 factorial arrangement with three replications. Data collected on tuber yield and quality parameters were analyzed using SAS version 9.2. The interaction of flower removal stages and earthing up time treatments affected marketable and unmarketable tuber number and yield, total tuber number and yield, large-sized tuber weight, and number of large-sized tubers. The medium and small-sized tubers were also affected by main treatments but not by their interaction treatments. Similarly, dry matter content was significantly ($p<0.05$) affected by flower removal alone, but not by earthing up time and its interaction with flower removal. Generally, the highest marketable tuber yield (30.25 t ha^{-1}), large-sized tuber weight (424.9 g), the number of large-sized tubers (5), and total tuber yield (30.96 t ha^{-1}) were recorded in the treatment of potato flower removed at the bud stage and earthed up at 15 days after complete emergence. Therefore, the removal of potato flowers at the bud stage and earthing up at 15 days after complete emergence and common cultivation can be practiced for better tuber yield and quality of potato.

Key words: dry matter content, earthing up, flower removal, tuber yield, tuber quality.

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important food crops in the world. China is the biggest producer of potatoes worldwide, with about one-third of the world's potatoes produced in China and India. According to FAOSTAT (2019),

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over 370 million metric tons of potatoes were produced worldwide. Potatoes are an essential crop and are recommended as a food security crop by the United Nations. Potatoes can grow in different climate conditions. They take less time to grow and need less input than other vegetables, and they can be replanted as seed potatoes. Potatoes provide high food energy and complex carbohydrates while taking up a smaller unit of land than other alternatives. Potato has been identified as a cheap source of the human diet since it produces more food value per unit time in terms of the supply of carbohydrates, quality protein (lysine), minerals, nutrient salts and several B-group vitamins and a large amount of vitamin C (Horton, 1987). Due to these merits, potato ranks first in the expansion of production in developing countries.

Ethiopia is endowed with suitable climatic and edaphic conditions for quality potato production. About 70% of the available agricultural land is located at an altitude of 1800–2500 m a.s.l and receives an annual rainfall of more than 600 mm, which is suitable for potato production (Solomon, 1987). According to FAOSTAT (2016), the area under potato cultivation was about 51,698 ha in 2005/2006 producing 509,716 tonnes of tuber yields; in 2014/2015, the area under potato crop increased to 67,362 ha, and its productivity was about 921,832 tonnes. Its national productivity was 13.7 tonnes per hectare in the production years of 2014/2015 (FAOSTAT, 2016), which is still far less than that of other countries such as New Zealand (50.2 t ha^{-1}) and North America (41.2 t ha^{-1}). The main contributing factors for underproduction and underutilization of potato are lack of high yielding and disease-tolerant varieties, unavailability of quality seed and poor agriculture practices. However, the production could be increased by applying better agronomic practices or management, such as earthing up at the appropriate time and removing flowers at the proper stage, which contributed to a substantial amount of crop yield.

Proper earthing up increases tuber yield by creating favourable conditions for tuber initiation and development and prevents the greening of tubers. Poor ridging around a potato plant could expose the tuber to sunlight, high temperatures, diseases, and insect damage, which could affect the yield and quality of the tuber (Gebremedhin et al., 2008). In addition, the removal of potato flower has a great impact on tuber yield and quality. Flowers and tubers would compete to acquire assimilates and pruning of flowers or berries would increase transferred assimilates into underground structures and increase tuber yield (Almekinders and Struik, 1996). Hence, there is an attempt to increase tuber yield and quality of potato by promoting improved techniques and applying proper agronomic practices in the production areas. Flowers are less valuable economically in potato production. However, in Eastern Tigray, Ethiopia, many farmers who grow potatoes frequently do not consider earthing up and flower removal. This results in low and erratic tuber yield and quality. Thus, potato tuber production with appropriate time of

earthing up and flower removal for maximum yield and better quality is not well known. Therefore, the objective of this study was to determine the effects of flower removal and earthing up on the tuber yield and quality of potato (*Solanum tuberosum* L.) in Eastern Tigray, Ethiopia.

Materials and Methods

Description of the study area

The field experiment was conducted in Maymegelta, Eastern Tigray, Ethiopia, for the 2018 summer season. The site is located at an altitude of 2492 m above sea level and lies at 14^0 15` to $14^0$ 30` N latitude and 39^0 30` to $39^0$ to 45` E longitude. The mean annual rainfall is 475 mm, which ranges from 350 to 600 mm. The texture of the topsoil of the study area is sandy loam with organic matter of 0.45% and pH of 6.15.

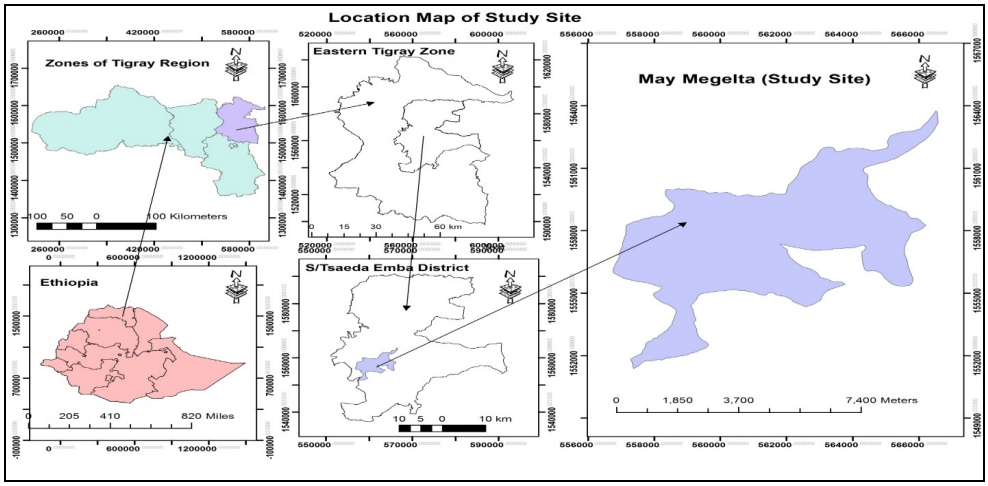


Figure 1. The map of the study area of Maymegelta, Saesie Tsaeda Emba, Eastern Tigray.

Experimental treatments and design

To study the effect of flower removal and earthing up time on tuber yield and quality of potato, a 5x3 factorial experiment based on a randomized complete block design with three replications was conducted. The first factor was three flower removal stage treatments: normal growth potato (growth of potato was not disturbed, allowed to flower, and set fruit (control)), flower removal at the bud

stage (potato was not allowed to produce flowers and all flower clusters were nipped off at the bud stage before opening); and flower removal after full opening (flowers were removed when fully opened). The second factor was five earthing up time treatments including control (no earthing up), earthing up at 15 days, 30 days, 45 days, and 60 days. The treatments of earthing up were applied after complete emergence, and common cultivation was applied for all treatments equally. The size of the unit plot was 3x2.4 m, and each included four rows. The well-sprouted local seed tubers of potato were planted at a depth of 12 cm, and plants were spaced 30cm apart in each plot. The row distances in plots, the path between plots within each block and distance between blocks were 75 cm, 50 cm, and 1 m, respectively.

Experimental procedures

The experimental site was plowed, and the ridge prepared well as per the recommended practices. Potato tubers of the promising local variety named Shashemene were prepared and separated. Hand weeding, side dressing and other agronomic practices were applied uniformly to all treatments. Phosphorus was applied in the form of DAP at the planting time at a rate of 195 kg ha^{-1} and nitrogen in the form of urea was applied in a split dose, at planting and after full emergence at a rate of 165 kg ha^{-1} (EARO, 2004). Initial light irrigation was applied five days after planting. Subsequent irrigation was given at a seven-day interval depending upon the climatic conditions and soil type. For the earthing up treatments, before applying treatments, the first cultivation was applied for all treatments, then the soil was uniformly put around the plant up to 20 cm high at the different times according to earthing up treatments except control. Other agronomic practices and pest management were applied uniformly for all treatments based on the national recommendation (EARO, 2004).

Data collected

Marketable tuber number per hill was counted as a marketable tuber based on its size category, greater or equal to 25g, and free from disease (Lung'aho et al., 2007). Unmarketable tuber number per hill was counted as an unmarketable tuber based on its size category, < 25g, including disease and insect attacks (Lung'aho et al., 2007). Total tuber number was determined as the sum of marketable tuber number and unmarketable tuber number per hill. Marketable tuber yield (tha^{-1}) was free from diseases, and greater than or equal to 25 g (Lung'aho et al., 2007).

Unmarketable tuber yield (tha^{-1}) was determined by weighing diseased, and small-sized (< 25g) tubers from the net plot area. Total tuber yield (tha^{-1}) was determined as the sum of the weights of marketable and unmarketable tubers from

the net plot area and was calculated based on ton per hectare. Tuber size distribution in weight (g) was recorded by weighing the number of tubers that were categorized into small (<39 g), medium (39 –75 g) and large (>75 g), according to Lung'aho et al. (2007). Tuber size distribution was recorded by counting the number of tubers that were categorized into small (<39 g), medium (39–75 g) and large (>75 g) according to Lung'aho et al. (2007). Tuber dry matter content (%) was determined randomly by selecting five potato tubers from each plot, chopping them into small 1–2 cm cubes, mixing them thoroughly, and two fresh sub-samples of 200 g were prepared. Each sub-sample was placed in a paper bag and put in an oven at 70^{o}C until a constant dry weight was attained. Each sub-sample was immediately weighed, and the mean recorded as dry weight. Percent dry matter content for each subsample was calculated based on the following formula:

$$\text{Tuber dry matter content (\%)} = \frac{\text{Tuber dry weight (g)}}{\text{Tuber fresh weight (g)}} \text{X } 100 \quad (1)$$

Methods of data analysis

All the relevant data collected from the experimental plots was subjected to analysis of variances (ANOVA) and computed using SAS computer software program version 9.2. Significant treatment means were compared using the least significant difference (LSD) test at $p< 0.05$ probability level.

Results and Discussion

Marketable tuber number

Marketable tuber number was significantly affected ($p<0.05$) by the interaction of flower removal and time of earthing up treatments. As shown in Table 1, the highest number of marketable tuber (11.56) was recorded in the potato flower removed at the bud stage and earthed up at 30 days after full emergence. However, there was no significant difference in the treatments of the potato flower removed at the bud stage and earthed up at 15 days (11.5), the potato flower removed at the full opened stage and earthed up of at 15 days (11.5), and potato without removed flower and earthed up at 15 days after complete emergence (11.49). On the contrary, the lowest marketable tuber number (6.83) was found in the potato without flower removal and earthing up (Table 1). It is believed that preventing the growth of reproductive organs could avoid competition for assimilates. Thus, most of the tubers in which flowers were removed at the bud stage attained marketable tuber size, and the number of marketable tubers increased accordingly. However, there is no evidence showing the interaction effect between flower removal and earthing up on marketable tuber numbers. Tekaligen (2005)

reported that the highest marketable tuber number was obtained from potato plants when their flowers were removed at the bud stage before opening, followed by potato whose flowers were removed after being fully opened compared to the normal potato plant.

Unmarketable tuber number

The analysis of variance indicated that unmarketable tuber number was significantly ($p < 0.05$) affected by their interaction (Table 1). The highest unmarketable tuber number (1.33) was found in the potato treatment without removing flowers and earthing up, while there was no significant difference in potatoes when flowers were removed at the full opening stage without earthing up (1.32); and potato without removed flowers and earthed up at 60 days after complete emergence (1.21). On the other hand, the lowest unmarketable tuber number (0.69) was found in potato without removed flowers and earthed up at 15 days after full emergence. However, there was no significant difference in the result obtained from potato without removed flowers and earthed up at 30 days after full emergence (0.79); and potato with flowers removed at the full opening stage and earthed up at 15 days after full emergence (0.79) (Table 1).

Total tuber number

A significant difference ($p < 0.05$) in the total tuber number was obtained due to interaction and the main effect of flower removal and time of earthing up (Table 1). The highest total tuber number (12.4) was recorded in potato flowers removed at the bud stage and earthed up at 30 days after full emergence. Hence, there was no significant difference in the result found in potato with flowers removed at the bud stage and earthed up at 15 days after full emergence (12.3); and the treatment of potato with flowers removed at the full opening stage and earthed up at 15 days after full emergence (12.29). On the contrary, the lowest total tuber number was also observed in potato without removed flower and earthing up (8.16). However, there was no significant difference in the result found in potato without removed flower and earthed up at 60 days after full emergence (9.31) as well as in the treatment of potato with flowers removed at the full opening stage and earthing up at 60 days (9.42) (Table 1). This may be because when potato flowers are removed at the right stage and earthing up at the right time, it increases the number of tubers. A similar work was reported by Bizuayehu and Tekaligen (2008) that the existence of flower buds decreased productivity by reducing the tuber number. This could be due to high gibberellic acid activity, which leads to reduced partitioning of assimilates to tubers while encouraging stolon elongation and reducing the tuber number.

Table 1. The interaction effect of flower removal and earthing up on marketable tuber number (MTN), unmarketable tuber number (UmTN) and total tuber number (TTN) of potato grown in Eastern Tigray, Ethiopia.

Treatments		Parameters		
Flower removal stage	Earthing up time	MTN	UmTN	TTN
Normal growth/ Without flower removal	Control	6.83f	1.33a	8.16f
	at 15 days	11.49a	0.69f	12.19abc
	at 30 days	11abc	0.79ef	11.79abcd
	at 45 days	10.5abcd	0.93bcd	11.43abcd
	at 60 days	8.1ef	1.21a	9.31f
Flower removal at the bud stage	Control	10.55abcd	1.01b	11.57abcd
	at 15 days	11.5a	0.78def	12.3ab
	at 30 days	11.56a	0.83cde	12.4a
	at 45 days	11.16abc	0.92bced	12.09abc
	at 60 days	9.93cd	0.97bc	10.9cd
Flower removal after the full opening stage	Control	9.33de	1.32a	10.66de
	at 15 days	11.5a	0.79ef	12.29ab
	at 30 days	11.41ab	0.81def	12.22abc
	at 45 days	10.16bcd	0.86cde	11.03bcd
	at 60 days	8.41e	1.0b	9.42ef
LSD (0.05)		1.29	0.13	1.34
Level of significance		*	*	*
CV (%)		7.73	8.29	7.37

Values followed by the same letter/s are not significantly different at 0.05% probability level.

Marketable tuber yield

Marketable tuber yield of potato was significantly affected ($p < 0.05$) by the interaction of flower removal and time of earthing up treatments. The highest marketable tuber yield (30.25 t ha^{-1}) was recorded in the potato treatment with flowers removed at the bud stage and earthing up at 15 days after full emergence. However, there was no significant difference in the potato treatment with flowers removed at the bud stage and earthing up at 30 days after full emergence (30.1 t ha ^{1}). On the other hand, the lowest marketable tuber yield (8.63 tha^{-1}) was found in the potato treatment without removing flowers and earthing up, but there was no significant difference in the potato treatment without removing flowers and earthing up at 60 days after full emergence (Figure 2). The result of the highest marketable tuber yield achieved because of flower removal may be due to the absence of competition for a limiting factor between developing flowers and tubers. It was also speculated that in the absence of reproductive parts, presumably since developing tubers were the pre-dominant sinks, many assimilates were diverted to the tubers, which would otherwise be utilized for flower and fruit

production (Tekaligan, 2005). As a result, most of the initiated tubers in this study increased in size and attained marketable size in agreement with the findings of Bartholdi (1940).

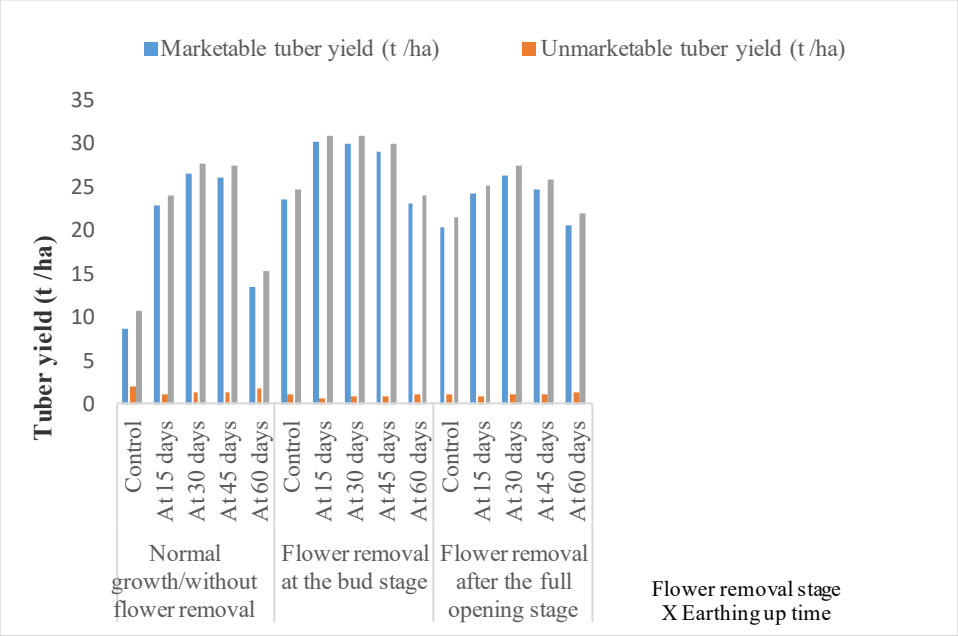


Figure 2. The interaction effect of flower removal and earthing up time on the marketable tuber yield, unmarketable tuber yield and total tuber yield of potato grown in Eastern Tigray, Ethiopia.

Unmarketable tuber yield

The analysis of variance indicated that the unmarketable tuber yield was significantly ($p < 0.05$) affected by interaction effects of flower removal and earthing up time treatments. The highest unmarketable tuber yield (2.1t ha^{-1}) was found in potato without removed flowers and earthing up, while there was no significant difference in the treatment of potato without removing flower and earthing up at 60 days after full emergence (1.83t ha^{-1}). However, the lowest unmarketable tuber yield (0.71t ha^{-1}) was found in the treatment of potato with flowers removed at the bud stage and earthing up at 15 days after full emergence. There was no statically significant difference in the treatment of potato with flowers removed at the bud stage and earthing up at 30 days after full emergence (0.83t ha^{-1}) (Figure 2). Moreover, a higher number of tubers affected by disease,

malformed and pre-harvest sprouting on tubers were observed in potato without removed flower and earthing up. This result agreed with Tafi et al. (2010), who reported that soil added to the plant affected the potato product structure. This is due to the appropriate time of the soil addition for active physiological growth stages that create favorable soil environment for tuber yield.

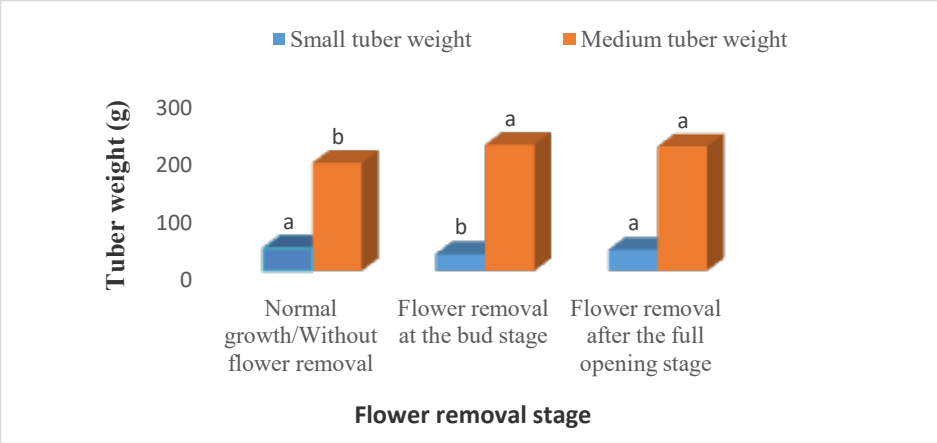
Total tuber yield

The analysis indicates that total tuber yield was significantly ($p<0.05$) influenced by the interaction effect of flower removal and earthing up time. Figure 2 indicates that the highest total tuber yield (30.96 t ha^{-1}) was recorded in the potato treatment with flowers removed at the bud stage and earthing up at 15 days after complete emergence. However, there was no significant difference in the result obtained from the treatment of potato with flowers removed at the bud stage and earthing up at 30 days after full emergence (30.93t ha^{-1}). On the contrary, the lowest total tuber yield (10.74 t ha^{-1}) was obtained from the potato treatment without removing flowers and earthing up. However, no significant difference was found in the potato treatment without removing flower and earthing up at 60 days after full emergence (15.37 t ha^{-1}) (Figure 2). This seems to indicate that flower and fruit development had a depressing effect on tuber development, which may be due to active competition for assimilating among flowers and fruits and developing tubers (Tekalign, 2005). Ali (2016) also reported that potato florescence removal increased tuber yield by 13% compared to the treatment without florescence removal. Similarly, Nazari (2010) and Tekalign (2005) also reported that tuber yield would be increased by 9 and 18 percent when the florescence of potato was removed compared to unremoved flowers. The result is also in agreement with research done by Hassen et al. (2013) on anchote accessions, when the flower bud removal treatment increased root yield by 15.87%. This is due to the intense competition that exists between the reproductive and root parts.

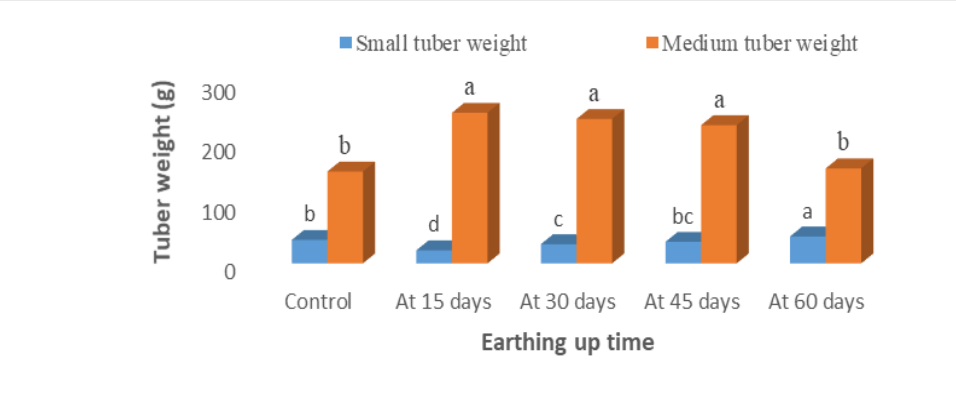
Potato tuber size distribution in weight

A. Small-sized tuber weight (<39 g): The result has shown that small-sized tuber weight was significantly ($p<0.05$) affected by flower removal and the time of earthing up treatments, but it was not significantly ($p>0.05$) affected by their interaction effects. As indicated in Figure 3, the largest small-sized tuber weight (38.67g) was obtained from the treatment of potato without removing flowers, but there was no significant difference in the treatment of potato with flowers removed at the full opening stage (36.24g). In contrast, the smallest weight of small-sized tubers (29.06 g) was recorded in the treatment of potato with flowers removed at the bud stage. In the case of earthing up, the largest small-sized tuber weight was

found in potato earthed up at 60 days after full emergence (44.83 g), whereas the smallest weight of small-sized tubers was also recorded in potato earthed up at 15 days after full emergence (21.71g) (Figure 3).



(a)



(b)

Figure 3. The effects of flower removal (a) and earthing up time (b) on small-sized tubers (<39 g) and medium-sized tubers in weight (39–75 g) of potato grown in Eastern Tigray, Ethiopia.

Medium-sized tuber weight (39–75 g): The analysis of variance results has shown that medium-sized tuber weight was significantly ($p < 0.05$) influenced by the main effect of flower removal and earthing up time. The largest medium-sized tuber weight was recorded in the treatment of potato with flowers removed at the

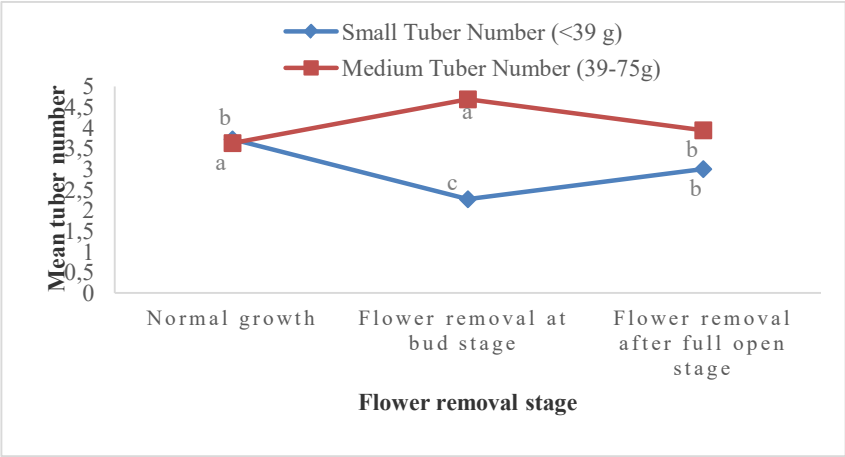
bud stage (217.71g), but non-significant difference was observed in the treatment of potato with flowers removed at the full opening stage (214.94 g). On the contrary, the smallest medium-sized tuber weight was found in the treatment of potato without removing flowers (186.47 g). The result of earthing up has also shown that the largest medium-sized tuber weight (250.59 g) was recorded in the treatment of potato earthed up at 15 days after complete emergence. However, there was no significant difference in the treatment of potato earthed up at 30 days (240.33 g) and 45 days after full emergence (230.03 g). On the other hand, the smallest medium-sized tuber weight (152.89 g) was recorded in control (without earthing up), but there was no significant difference in the treatment of potato earthed up at 60 days after full emergence (158.03 g) (Figure 3). Earthing up at 15 days after complete emergence might create favorable conditions for producing a good yield of medium-sized tubers of potato. This result is supported by the report of Qadir et al. (1999), who confirmed that earthing up at 15 days after complete plant emergence resulted in better yield performance.

Large-sized tuber weight (>75 g): The analysis of variance revealed that interaction effects of flower removal and earthing up time significantly ($p<0.05$) influenced large-sized tuber weight. As indicated in Table 2, the highest large-sized tuber weight (424.9 g) was obtained from the treatment of potato with flowers removed at the bud stage and earthing up at 15 days after full emergence. However, there was no significant difference in the treatment of potato with flowers removed at the bud stage and earthing up at 30 days (412.5 g); and at 45 days after full emergence (409.32 g). Hence, the lowest large-sized tuber weight (113.79 g) was obtained from the potato treatment without removing flowers and earthing up, but there was no significant difference in the result found in the potato treatment without removing flowers and earthing up at 60 days after full emergence (171.43 g) (Table 2). This could be early earthing up during the active growth period of the plant that improved the soil conditions for nutrient absorption; plants absorbed the sufficiently available resources and increased their photosynthetic efficiency that ultimately increased the number of large-sized tubers. The result supported the observations of Qadir et al. (1999) that earthing up at 15 days after complete plant emergence resulted in better yield.

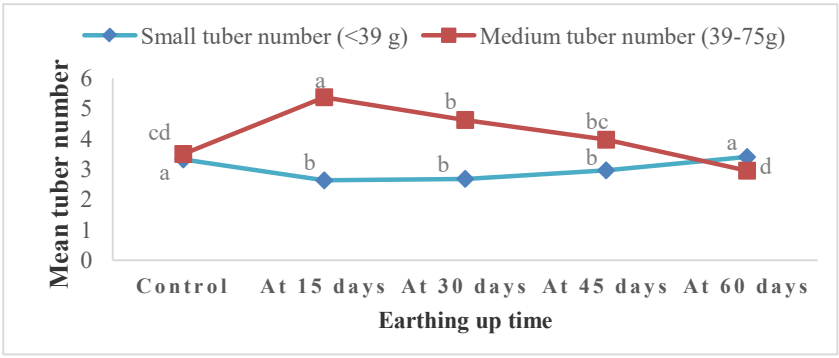
Number of tuber size distribution

A number of small-sized tubers (<39 g): The main factors of flower removal and earthing up time significantly ($p<0.05$) affected the number of small-sized tubers. As indicated in Figure 4, the highest small-sized tuber number (3.72) was obtained from the treatment of potato without removing flowers, whereas the lowest small-sized tuber number (2.27) was recorded in the treatment of potato with flower removed at the bud stage. In the case of earthing up, the highest

number of small-sized tubers (3.4) were found in potato earthed up at 60 days after full emergence, but there was no significant difference in potato non-earthing up (3.32). On the contrary, the lowest small-sized tuber number (2.63) was recorded in the treatment of potato earthed up at 15 days after full emergence. However, there was no significant difference among treatments of potato earthed up at 30 days (2.68) and at 45 days after full emergence (2.96) (Figure 4).



(a)



(b)

Figure 4. The effects of flower removal (a) and earthing up time (b) on small-sized tuber number (<39 g) and medium-sized tuber number (39–75g) of potato grown in Eastern Tigray, Ethiopia.

B. The number of medium-sized tubers (39–75 g): Flower removal and earthing up time significantly ($p<0.05$) influenced the number of medium-sized

tubers, but the two factors did not interact to influence the number of medium-sized tubers. As indicated in Figure 4, the highest number of medium-sized tubers was obtained from the treatment of potato with flowers removed at the bud stage (4.69), whereas the lowest number of medium-sized tubers (3.63) was found in the potato treatment without removing flowers. In the case of earthing up, the highest number of medium-sized tubers (5.37) was recorded in the treatment of potato earthed up at 15 days after full emergence. On the other hand, the lowest number of medium-sized tubers was found in the treatment of potato earthed up at 60 days after full emergence (2.95), but there was no significant difference in potato non-earthed up (3.5) (Figure 4). This might be earthing up at 15 days after complete plant emergence that created favorable conditions for plant growth and ultimately a greater number of medium-sized tubers produced. This result is in line with the findings of Qadir et al. (1999), who confirmed that earthing up at 15 days after complete plant emergence resulted in better yield performance.

C. The number of large-sized tubers (>75g): The analysis of variance indicated that interaction effects of flower removal and earthing up time significantly ($p< 0.05$) affected the number of large-sized tubers (Table 2).

Table 2. The interaction effect of flower removal and earthing up on the weight of large-sized tubers (>75g) and the number of large-sized tubers (>75g) of potato grown in Eastern Tigray, Ethiopia.

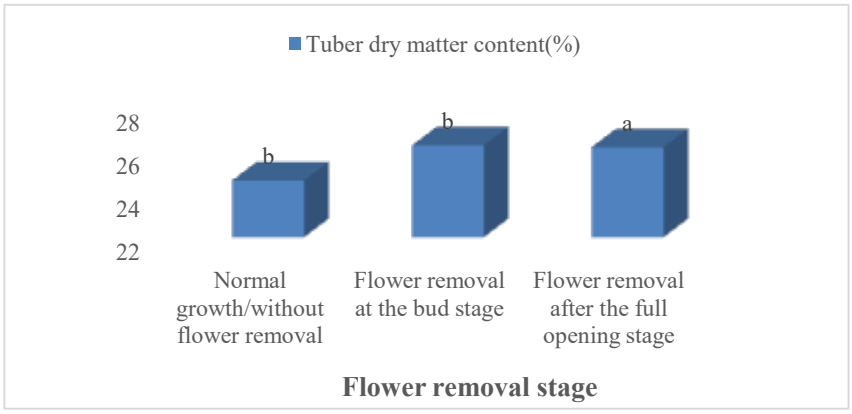
Treatments		Parameters	
Flower removal stage	Earthing up time	Weight of large-sized tubers (>75g)	Number of large-sized tubers (>75g)
Normal growth/ without flower removal	Control	113.79e	1.63g
	At 15 days	257.83d	3.39e
	At 30 days	324.78cd	4.27cd
	At 45 days	325.42cd	4.28bcd
	At 60 days	171.43e	2.5f
Flower removal at the bud stage	Control	357.18abc	4.32bcd
	At 15 days	424.9a	5.38a
	At 30 days	412.5ab	5.32a
	At 45 days	409.32ab	5.09ab
	At 60 days	340.17bc	4.34bcd
Flower removal after the full opening stage	Control	298.58cd	3.97de
	At 15 days	323.03cd	4.25cd
	At 30 days	370.92abc	4.88abc
	At 45 days	342.17bc	4.06de
	At 60 days	296.24cd	3.74de
LSD (0.05)		76.41	0.82
Level of significance		*	*
CV (%)		13.72	10.88

Values followed by the same letter/s are not significantly different at 0.05% probability level.

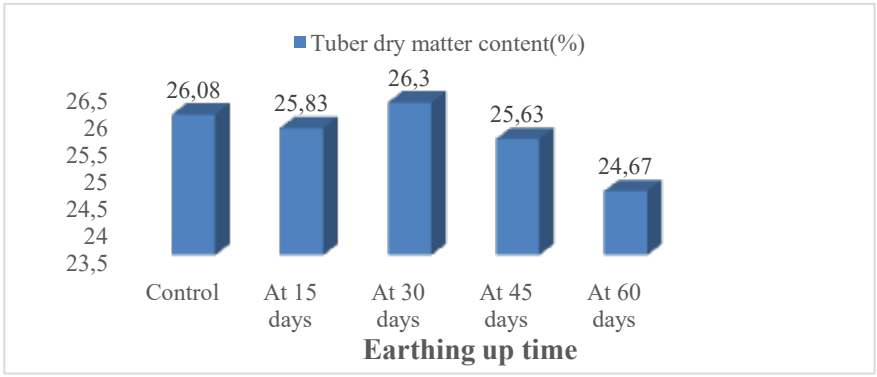
The highest number of tubers (5.38) was found in the treatment of potato with flowers removed at the bud stage and earthing up at 15 days after full emergence. However, there was no significant difference in the treatment of potato with flowers removed at the bud stage and earthing up at 30 days after full emergence (5.32). On the contrary, the lowest number of large-sized tubers was recorded in the potato treatment without removing flowers and earthing up (1.63). The treatment of potato with flowers removed after the full opening and earthing up at 60 days after full emergence (3.74); and the treatment of potato with flowers removed after the full opening without earthing up (3.97) showed non-significant results (Table 2). This could be due to early earthing up during the active growth period of the plant that improved the soil conditions for nutrient absorption; plants absorbed the sufficiently available resources and increased their photosynthetic efficiency that ultimately increased the number of large-sized tubers. The result agreed with the research finding of Almekinders and Struik (1996). The authors have reported that flowers and tubers of potato compete to attract assimilates and pruning of flowers would increase assimilate transition to underground structures to increase the yield of the tuber. The result also supported the observations of Qadir et al. (1999) that earthing up at 15 days after complete plant emergence resulted in a better yield of large-sized tubers.

Dry matter content

The analysis of variance indicated that the main factor of flower removal significantly ($p<0.05$) affected the dry matter content of the potato tuber. However, earthing up time and their interaction had no influence. As indicated in Figure 5, the highest dry matter content was recorded in the treatment of potato with flowers removed at the bud stage (26.27). However, there was no significant difference in the result recorded in the treatment of potato with flowers removed after the full opening (24.64). On the contrary, the lowest dry matter content was found in the potato treatment without removing flowers (26.19) (Figure 5). The increase in values of the dry matter content of tubers may be due to the largest proportion of assimilates being diverted to the developing tubers rather than to flower production. Consequently, more carbohydrate could be accumulated in the tubers as dry matter. The result supported the observations of Tekaligen (2005), who reported that removing flowers significantly increased the tuber dry matter content of potato. Hassen et al. (2013) also reported that an increase in dry matter content because of flower bud removal in different anchote accessions might be due to the flow of an ample amount of assimilates to the sink, which would have otherwise contributed to fruit development, ultimately resulting in high dry matter content in roots. In the case of earthing up, the highest value of the dry matter content of tubers was recorded in the treatment of potato earthing up after 30 days (26.3). However, it was a non-significant difference among all the treatments of earthing up.



(a)



(b)

Figure 5. The effects of flower removal (a) and earthing up time (b) on the dry matter content of the potato grown in Eastern Tigray, Ethiopia.

Conclusion

The investigation revealed that the highest marketable tuber yield (30.25 t ha^{-1}), weight of large-sized tubers (424.9 g), number of large-sized tubers (5.38) and total tuber yield (30.96 t ha^{-1}) were recorded in the potato treatment with flowers removed at the bud stage and earthing up at 15 days after complete emergence. However, there was no significant difference in the result obtained from the treatment of potato with flowers removed at the bud stage and earthing up at 30 days after full emergence. Therefore, removing potato flowers at the bud stage and earthing up at 15 days after complete emergence can be practiced for better tuber yield and quality of potato.

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UTICAJ UKLANJANJA CVETOVA I ZAGRTANJA NA PRINOS KRTOLA I KVALITET KROMPIRA (*SOLANUM TUBEROSUM* L.)

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R e z i m e

Poljski ogled je sproveden u istočnom Tigraju (Etiopija), tokom letnje sezone kako bi se utvrdili uticaji uklanjanja cvetova i zagrtanja na prinos krtola i kvalitet krompira (*Solanum tuberosum* L.). Ogled je obuhvatao tri faze uklanjanja cvetova i pet tretmana zagrtanja, koji su postavljeni u potpuno slučajnom blok sistemu faktorskog rasporeda 5x3 sa tri ponavljanja. Podaci prikupljeni o prinosima i parametrima kvaliteta krtola analizirani su korišćenjem verzije SAS 9.2. Interakcija uklanjanja cvetova i zagrtanja uticala je na broj tržišnih i netržišnih krtola i prinos krtola, ukupan broj i prinos krtola, masu velikih krtola i broj velikih krtola. Na krtole srednje i male veličine uticali su glavni tretmani, ali ne i njihove interakcije. Slično tome, na sadržaj suve materije značajno je uticalo ($p<0,05$) samo uklanjanje cvetova, ali ne i vreme zagrtanja i njegova interakcija sa uklanjanjem cvetova. Generalno, najveći prinos tržišnih krtola (30,25t ha^{-1}), masa velikih krtola (424,9 g), broj velikih krtola (5), i ukupni prinos krtola (30,96t ha^{-1}) zabeleženi su u tretmanu krompira sa uklanjanjem cvetova krompira u fazi pupoljka i zagrtanjem 15 dana posle potpunog nicanja. Stoga se uklanjanje cvetova u fazi pupoljka i zagrtanje 15 dana posle potpunog nicanja i uobičajena kultivacija mogu primeniti radi boljeg prinosa i kvaliteta krtola krompira.

Ključne reči: sadržaj suve materije, zagrtanje, uklanjanje cvetova, prinos krtola, kvalitet krtola.

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THE FRUIT METRIC TRAIT CHARACTERIZATION OF SCARLET EGGPLANT USING THE HIGH-THROUGHPUT TOMATO ANALYZER SOFTWARE

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Abstract: Scarlet eggplant (*Solanum aethiopicum* [L.]) is an indigenous, underutilized fruit vegetable in Africa. Preference for fruit shape and size is high among growers and consumers. Fruit metric traits are important for yield improvement. Fruit metric descriptors are important contributors to variation, phenotypic and genotypic variation, and heritability. However, the measurement of these traits is cumbersome and subjective. Forty-three accessions were evaluated in 2016 and 2017. At maturity, 5 fruits were randomly harvested from each accession, digitalized and processed using the Tomato Analyzer software. Sixteen fruit metric traits were automatically generated and submitted for analysis of variance and multivariate analysis. The accessions differed over fruit size and shape due to genetic make-up. Fruit metric trait variation among *S. aethiopicum* groups was less influenced by the environment. The cv. Gilo group has oblong fruits, the cv. Shum group fruits are circular and ovoid; the cv. Kumba group fruits are less circular, lobed and flattened. AE/113 (C3), FUO 1 (C1) and FUO 5 (C2) Gilo groups are promising for fruit size. There were phenotypic plasticity and overlapping for fruit metric traits between the Gilo and Shum groups due to a common genome. The Tomato Analyzer software was able to discriminate accessions based on fruit phenomic traits, and the information could be used to establish commonalities between groups.

Key words: *Solanum aethiopicum*, fruit area, fruit size, genetic diversity, heritability.

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Introduction

Scarlet eggplant (*Solanum aethiopicum* [L.]) has 4 cultivar groups (Aculeatum, Gilo, Kumba and Shum), with overlapping of morphological and agronomic traits (Levin, 2005; Adeniji et al., 2013), and it is able to tolerate varying environments (Shippers, 2002; Adeniji et al., 2013). Consumer choice for fresh fruit depends on external appearance, where cream or white fruit color at physiological ripeness, fruit shape, fruit length, fruit width and degree of fruit lobbing are factors determining purchase (Adeniji and Aloyce, 2012).

Fruit shape and size development depend on processes taking place during fruit formation. The assessment of variation in scarlet eggplant using morphological descriptors and agronomic traits contributes to understanding differentiation in phenotypes (Polignano et al., 2010; Adeniji et al., 2012, 2013). Based on morphological descriptors, fruits of scarlet eggplant are categorized into 10 shapes (Anonymous, 1996). Within the characterization of morphological traits, fruit shape is a non-ordered trait, usually coded as oblate, flattened, heart-shaped, pyriform and ellipsoidal round, oval, and squat in tomato (*Solanum lycopersicum* [L.]) (Anonymous, 1996) and eggplant (Anonymous, 2003). This does not provide realistic information on fruit shape. The phenotypic measurement of physical fruit characteristics is laborious, time-consuming, and unreliable. Tomato and scarlet eggplant groups belong to the family Solanaceae. Some fruits of each plant are similar in size and shape. Fruit shape attributes can be measured with precision using scanned images of fruit sections (Brewer et al., 2007; Gonzalo and van der Knaap, 2008). The Tomato Analyzer software has been used for fruit morphometric and metric trait analysis in tomato and eggplant (Rodriguez et al., 2010; Hurtado et al., 2013). This technique allows for the precise measurement of fruit size and shape.

The market demand for the scarlet eggplant fruit is associated with its nutritional health benefits. Challenges in realizing the best yield of this crop include the absence of commercial varieties with consumer-preferred fruit metric traits. The availability of this information could provide a phenotypic classification of fruit characteristics essential for discrimination among accessions. In addition, information on fruit metric traits, through diversity analysis, will add to the existing information on morphological and agronomic trait characterization of scarlet eggplant groups, hasten the identification of pollen parents and promising accessions for field evaluation. This study was undertaken to identify fruit metric descriptors that are the most important contributors to fruit metric variation among scarlet eggplant groups, evaluate the magnitude of variation for fruit metric traits, estimate heritability and identify promising accessions for genetic enhancement.

Material and Methods

Forty-one accessions and a variety DB_3 (check) of scarlet eggplant (Table 1) were obtained from the southwest, north-central and northeast regions in Nigeria between August 2015 and July 2016. Seedling production and field planting took place between May and September 2016 and 2017 at the Teaching and Research Farm of the Department of Crop Science and Horticulture, Federal University Oye Ekiti, Nigeria (longitude 7°07'N, latitude 5°49'E, altitude 554.4 m asl). The physico-chemical properties of the soil (0–25 cm depth) from the research field indicated a pH of 5.7 (in H_2O, 1:1), OM (%) 0.82, N (%) 0.08, available phosphorus (Bray Method) 219.33, exchangeable Mg (C mol·kg^{-1}) 0.19, exchangeable K (C mol·kg^{-1}) 0.48, exchangeable Na (C mol·kg^{-1}) 0.07, exchangeable Ca (C mol·kg^{-1}) 2.93, ECEC, 3.68, Zn (mg·kg^{-1}) 1.12, Cu (mg·kg^{-1}) 1.01, Mn (mg·kg^{-1}) 107.7, Fe (mg·kg^{-1}) 180.07, sand 68%, silt 20%, clay 11%, with a sandy loam textural class. The location is characterized by an annual temperature of 24.2°C, precipitation averages of 1,313 mm annually, with September having the highest rainfall, avg. 241 mm.

Table 1. The accessions of *Solanum aethiopicum* and the place of collection.

Sn	Accession code	Place of collection	Sn	Accession code	Place of collection
Acc 1	AE/192	NaGRAB[a], Nigeria	Acc 23	AE/138	NaGRAB, Nigeria
Acc 2	AE/132	NaGRAB, Nigeria	Acc 24	AE/138-2	NaGRAB, Nigeria
Acc 3	AE/1437	NaGRAB, Nigeria	Acc 25	AE/0737	NaGRAB, Nigeria
Acc 4	AE/138	NaGRAB, Nigeria	Acc 26	AE/38	NaGRAB, Nigeria
Acc 5	AE/001	NaGRAB, Nigeria	Acc 27	AE/38-2	NaGRAB, Nigeria
Acc 6	AE/01473	NaGRAB, Nigeria	Acc 28	FUO 8	FUOYE, Nigeria
Acc 7	AE/1370	NaGRAB, Nigeria	Acc 29	FUO 9	FUOYE, Nigeria
Acc 8	AE/128	NaGRAB, Nigeria	Acc 30	EX SIVON	AVRDC[c], Taiwan
Acc 9	AE/113	NaGRAB, Nigeria	Acc 31	FUO 10	FUOYE, Nigeria
Acc 10	FUO 1	FUOYE[b], Nigeria	Acc 32	DB3	AVRDC, Taiwan
Acc 11	FUO 2	FUOYE, Nigeria	Acc 33	MM 1133	AVRDC, Taiwan
Acc 12	FUO 3	FUOYE, Nigeria	Acc 34	SOS	INRA[d], France
Acc 13	FUO 4	FUOYE, Nigeria	Acc 35	FUO 11	FUOYE, Nigeria
Acc 14	AE/130	NaGRAB, Nigeria	Acc 36	FUO 12	FUOYE, Nigeria
Acc 15	AE/1472	NaGRAB, Nigeria	Acc 37	FUO 13	FUOYE, Nigeria
Acc 16	AE/1475	NaGRAB, Nigeria	Acc 38	FUO 14	FUOYE, Nigeria
Acc 17	AE/30	NaGRAB, Nigeria	Acc 39	S.INT	INRA, France
Acc 18	FUO 5	FUOYE, Nigeria	Acc 40	FUO 15	FUOYE, Nigeria
Acc 19	FUO 6	FUOYE, Nigeria	Acc 41	FUO 16	FUOYE, Nigeria
Acc 20	FUO 7	FUOYE, Nigeria	Acc 42	FUO 17	FUOYE, Nigeria
Acc 21	AE/100	NaGRAB, Nigeria			
Acc 22	AE/1473-2	NaGRAB, Nigeria			

[a]NaCGRAB = National Center for Genetic Resources and Biotechnology; [b]FUOYE = Federal University Oye Ekiti, Nigeria; [c]INRA = French Institute for Agricultural Research; [d]AVRDC = Asian Vegetable Research and Development Center.

One seed of each accession was planted in each cell of multipot seedling trays filled with sterilized soil. Water was applied to seedlings with a watering can in the morning and evening for 4 weeks. Prior to the nursery establishment, the field was plowed. Fourteen days later, the soil was harrowed, and ridges made 1 m apart. Seedlings, with adhering soil, were transplanted to the field by hand. The experiment was arranged in a randomized complete block design with 4 replications. Each accession was allotted to a 2-row plot 4 m long and 1 m between rows, with 0.45 m between plants.

Prior to planting, the soil was fertilized with 20N-10P-10K at the rate of 90 $kg \cdot ha^{-1}$ N, 45 $kg \cdot ha^{-1}$ P_2O_5, and 45 $kg \cdot ha^{-1}$ K_2O. Urea fertilizer (46% N) was applied at a total of 120 $kg \cdot ha^{-1}$ in 3 splits at 1 week after transplanting, at flowering and 3 weeks thereafter. Ridomyl® (Matalxyl) WP (fungicide) was applied against damping-off in the field at the rate of 20 g/15 L of water 12 days after transplanting. Selecron® EC (insecticide) was applied 2 weeks after transplanting at 20 mL/20 L of water to control cutworms and other insects. The experiment was furrow-irrigated every 2 days for the first 2 weeks after transplanting, then once a week thereafter. Weeding was carried out manually with hoes.

At maturity, 5 fruits were randomly picked from each accession in a replicate during 2016 and 2017; 20 fruit for each accession. A longitudinal cut was made on each fruit, and fruit halves were digitalized with an HP Scanjet G4010 photo scanner (Hewlett-Packard, Palo Alto, CA) at a resolution of 300 dpi. Before morphometric measurement, a default setting was established for fruit blockiness and proximal and distal fruit end shape. Scanned images were exported to Tomato Analyzer, ver. 3, software (Rodriguez et al., 2010) for measuring morphometric traits. Data were measured for 15 fruit metric traits (Table 2) automatically received from Tomato Analyzer. The fruit morphometric names and measurements were defined by the manufacturer. Morphometric data for fruit descriptors were analyzed for the means, coefficients of variation and ranges. A combined analysis of variance was applied to detect differences among accessions for each fruit morphometric trait. Accession was treated as a fixed trait; the year was a random trait in PROC GLM of SAS (ver. 9.4, SAS Institute, Inc., Cary, NC). Means were separated using the Tukey's HSD test. Variances due to the phenotype (σ^2p) and the genotype (σ^2g) and coefficients of variation associated with the phenotype (PCV) and genotype (GCV) were estimated according to Syukur and Rosidah (2014).

To identify fruit metric attributes with high contribution to variability, the principal component analysis was performed on entry means using the PROC PCA procedure of SAS. A dendrogram was constructed using the unweighted pair group method of analysis with a squared Euclidean distance option (Sokal and Michener, 1958; Ward, 1963) in SPSS (ver. 16.0, IBM Corporation, UK).

Table 2. The phenomic characterization of scarlet eggplant fruits.

Fruit metric trait	Measurement and description
Fruit perimeter	cm
Fruit area	cm^3
Fruit height mid-width	Fruit mid-width height (cm) was calculated as ½ of the fruit's width.
Fruit maximum width	Fruit maximum horizontal distance (cm).
Fruit width mid-height	Fruit width mid-height (cm) was calculated as ½ of the fruit's height.
Fruit maximum height	Maximum height of the fruit (cm) (vertical distance of the fruit).
Fruit shape index internal	Fruit shape index internal computed as the ratio of internal ellipse fruit height to width, a value >1 is elongated, equal to 1 is round, and <1 is short.
Fruit shape index external II	Calculated as the ratio of the fruit height mid-width to its width mid-height.
Proximal eccentricity	Proximal eccentricity determined as the ratio of fruit height of the ellipse to the distance between the ellipse value close to unity is round fruit, less than unity is pear-shaped.
Distal eccentricity	Distal eccentricity measured as the ratio between the vertical axis of the ellipse and distance from the top of the ellipse to the bottom of the fruit, round bottom fruit have distal eccentricity close to 1, slightly pointed fruit have value <1.
Fruit eccentricity	The ratio between internal ellipses of the maximum height.
Fruit curve height	The height measured along a curved line through the fruit (passing through the midpoints of opposing pairs of points on either side of the distal and proximal points).
Fruit shape eccentricity I	Describe how top or bottom heavy a fruit is calculated according to the formula described by Rodríguez et al. (2010).
Fruit shape eccentricity II	Fruit shape eccentricity II is calculated according to the formula described by Rodríguez et al. (2010).
Lobeness	Computed as the standard deviation of distance from the center of the fruit perimeter multiplied by 100.

Results and Discussion

Significant mean squares were recorded among accessions for traits associated with fruit perimeter, area, width mid-height, maximum width, height mid-width, and maximum height. The year and the accession by year interaction were not significant (Table 3). A similar trend was found for traits associated with fruit shape (Table 4). The range of variation was high in fruit perimeter, fruit lobeness, fruit curvature, fruit shape index, curved fruit width, maximum fruit height, maximum fruit width, and fruit area. The accessions of the scarlet eggplant Gilo group (accessions 9, 18 and 10) were best for fruit perimeter, width, and area (Table 5). Accessions 9 (C3), 10 (C1) and 18 (C2) could be pollen parents for improving fruit size through intraspecies hybridization within Gilo, between Gilo and Shum, and Gilo and Kumba groups. Accessions 14 (C3), 19 (C2) 17, had high fruit mid-width height and maximum fruit width values (Table 6), so they are promising for wide fruits. Fruit distal eccentricity was the lowest for Accession 24

(C3a), but the highest for Accession 27 (C3a) (Table 6). Entries with mean values close to 0 corresponded to a pointed fruit tip end, and the fruit with a mean closer to 1 had a round distal fruit end. The scarlet eggplant fruit with a pointed tip end will enhance the packaging and arrangement of fruits in trays. Fruit proximal eccentricity value was low in Accession 18 (C2b) and high in Accession 27 (C3a) (Gilo). Fruit shape index refers to the internal ellipse drawn around the seed area. A fruit shape index of a value greater than 1 indicates an elongated fruit, equal to 1 indicates a round fruit, and less than 1 indicates a squat fruit.

Table 3. Mean squares for fruit metric traits among *Solanum aethiopicum* groups for which significant differences ($P < 0.05$) were found.

Source	Fruit						
	Perimeter	Width mid-height	Max. width	Height mid-width	Max. height	Area	Lobeness
Accession (A)	*	**	*	**	*	*	**
Year (Y)	ns	ns	ns	ns	ns	ns	ns
A × Y	ns	ns	ns	ns	ns	ns	ns

ns, *, ** not significant or significant at P<0.05 or P<0.01, ANOVA.

Table 4. Mean squares for fruit shape among *Solanum aethiopicum* groups for which significant differences ($P < 0.05$) were found.

Source	Fruit							
	Curve height	Shape index internal	Eccentricity	Shape eccentricity I	Shape eccentricity II	Proximal eccentricity	Distal eccentricity	Shape index external II
Accession (A)	*	*	*	*	**	**	*	**
Year (Y)	ns	ns	ns	ns	ns	ns	ns	ns
A × Y	ns	ns	ns	ns	ns	ns	ns	ns

ns, *, ** not significant or significant at P<0.05 or P<0.01, ANOVA.

Accessions with the round fruit are more frequent than accessions with pear-shaped fruits. The fruit shape eccentricities I and II were high in Accessions 10, 18 and 41 (Gilo) (Table 6). Fruit lobes measure the degree of an uneven shape of a fruit. Accessions 1, (C2b) 3, (Outlier) 4 (Outlier), 10 (C1b), 17 (Outlier), 18 (C2b), 32 (C1a), 35 (C4), 37 (C4), 38 (C4), 39 (C1) and 41(C1a) exhibited unevenly shaped fruits and were less circular. Large similarity and overlapping were noticed among accessions for fruit perimeter, fruit area, fruit width mid-height, maximum fruit width, fruit height mid-width and maximum fruit height. The foregoing corresponds to phenotypic plasticity in scarlet eggplant groups (Shippers, 2002; Adeniji et al., 2012, 2013), and other eggplant relatives (Kaushik et al., 2016). This is important for conservation and selection.

Table 5. The mean separation for some fruit size traits among accessions of the African eggplant (*Solanum aethiopicum*) group.

Accession code	Fruit perimeter	Area	Fruit width mid-height	Fruit maximum width	Fruit height mid-width	Fruit maximum height	Fruit curved height
1[a]	7.64l-p[b]	2.94i-l	2.02h-l	2.02l-r	0.86pq	1.21qr	2.14j-p
2	5.06t	1.93k-l	1.58j-m	1.57q-r	1.39l-p	1.37pqr	1.74o-q
3	8.04l-p	2.91h-l	1.93h-l	2.06l-r	0.35pq	0.65st	1.82opq
4	10.34f-i	1.34mn	1.26m	1.67o-t	1.49k-o	1.49n-r	4.70c
5	11.87de	8.70c	3.71bcd	3.70cde	2.80bcd	2.87b-e	3.12e-i
6	10.52e-h	4.91fgh	2.96d-g	2.94fgh	1.82g-l	1.88l-p	2.36i-o
7	16.12b	3.93g-j	2.45e-j	2.88f-i	1.47k-o	2.15g-l	4.58
8	8.18j-o	1.56mn	1.91h-l	2.02i-s	0.97op	1.10qrs	2.05l-p
9	48.3a	6.01ef	3.03c-g	4.57ab	2.72b-e	2.86cde	11.02a
10	20.5b	24.62a	3.90bc	3.94bc	7.52a	7.63a	7.75b
11	9.54i-j	2.52i-l	2.40e-j	2.67f-j	1.15m-p	1.39pqr	2.67g-m
12	8.22j-n	3.84g-k	3.17 c-f	2.41o-l	1.35l-p	1.69n-q	1.84o-q
13	8.12l-p	2.69i-l	2.41e-j	2.24l-p	1.16l-p	1.49n-r	2.06l-p
14	12.40d	11.66b	4.09ab	4.98a	3.22b	3.40b	3.76
15	6.80o	2.80i-l	1.89h-l	1.93o-t	1.43l-p	1.43o-r	1.47op
16	10.10ghi	6.18def	3.03c-g	3.05efg	2.46def	2.47e-i	2.90e-i
17	14.80c	1.50mn	4.98a	4.98a	0.27r	0.67st	2.41g-o
18	20.51b	25.31a	3.91bc	3.70cde	7.52a	7.62a	7.75b
19	11.73de	8.13cd	4.21ab	4.21bc	2.14e-i	2.34e-k	2.62f-n
20	10.09ghi	5.11fg	3.17bcd	3.22	1.72i-m	1.99 i-m	2.54f-o
21	7.86l-p	3.73g-k	2.40e-h	2.40g-l	1.55k-n	1.55n-r	1.05 l-p
22	8.19j-o	1.68lmn	1.25m	1.87o-t	1.06n-p	1.12rst	2.51 g-o
23	9.33h-k	3.64g-k	1.25m	2.57f-l	1.85i-l	1.93l-o	2.73f-l
24	7.30m-s	2.30j-l	1.87h-l	1.87o-t	1.79i-l	1.79l-p	2.00l-p
25	6.75p-s	2.60i-l	1.57j-m	1.57q-t	1.81i-l	1.83l-p	1.95l-q
26	9.31hijk	3.77g-k	3.03c-g	3.09efg	1.44l-o	1.44l-o	1.81m-p
27	5.98st	0.82n	2.20g-j	2.20l-r	0.33qr	0.33qr	0.61t
28	6.98n-s	2.83i-l	1.60j-m	1.60p-t	1.87g-l	1.87g-l	1.87l-p
29	11.64def	8.22c	3.83bcd	3.84cd	2.33d-h	2.33d-h	2.58e-h
30	8.23j-m	3.71g-k	2.28f-i	2.29l-q	1.64j-n	1.64j-n	1.69n-p
31	11.71def	8.57c	2.67e-g	2.69f-i	3.18bc	3.18bc	3.18bcd
32	7.52m-p	2.85i-l	1.73i-l	1.77o-t	2.51def	2.51def	2.67e-g
33	8.43jkl	3.72g-k	2.26g-i	2.32i-p	1.87g-l	1.87g-l	2.01i-n
34	7.17 m-s	8.21c	1.93h-l	2.12l-j	2.02f-k	2.02f-k	2.11h-m
35	6.20rst	2.25j-l	2.00h-l	2.02l-s	1.38l-p	1.38l-p	1.55n-r
36	9.27h-k	2.69i-l	1.50klm	1.64o-t	2.23d-j	2.23d-j	2.29f-j
37	10.98efg	7.32cde	2.24g-j	2.24l-p	3.27b	3.27b	3.27bc
38	7.51m-p	2.93t-l	1.39lm	1.51rst	2.5def	2.50def	2.71def
39	7.51m-p	2.93h-l	1.39lm	1.51rst	2.5def	2.50def	2.71def
40	6.98i-l	4.49f-i	2.03h-j	2.08l	2.63cde	2.63cde	2.79c-f
41	7.07m-s	2.39j-l	1.24m	1.31t	2.39e-g	2.39d-g	2.48e-j
42	7.99l-p	4.29f-i	2.53e-i	2.51g-l	2.04f-k	2.04f-k	2.14h-h

[a]refers to Table 1 for the accession name; [b]values in columns followed by the same letter are not significantly different.

Table 6. The mean separation for some fruit shape traits among accessions of the African eggplant (*Solanum aethiopicum*) group.

Accession code	Fruit shape eccentricity I	Fruit shape eccentricity II	Fruit shape index internal	Fruit eccentricity	Proximal eccentricity	Distal eccentricity	Fruit shape index external II	Lobeness
1[a]	0.56mn[b]	0.43opq	1.62bn	0.58l	0.89ab	0.86abc	0.42	10.28c-f
2	0.77i-m	0.77m-o	1.10g-m	0.79ab	0.89ab	0.87abc	0.78b-f	7.11i-m
3	0.27no	0.19pqr	0.67k-n	0.45m	0.99ab	0.81abc	0.18f	16.13ab
4	0.89h-l	0.89h-n	2.76a	0.79ab	0.89ab	0.88abc	2.49a	12.55c
5	0.77i-m	0.75m-0	0.85i-n	0.77a-e	0.88ab	0.87abc	0.75c-f	4.76nop
6	0.65klm	0.54mpo	1.90b-e	0.68g-k	0.88ab	0.89abc	0.53def	7.33g-l
7	0.68j-m	0.62l-o	0.81j-n	0.74a-h	0.88ab	0.88abc	0.62c-f	6.63k-n
8	0.68j-m	0.60mno	1.30d-l	0.68g-k	0.88ab	0.89abc	0.60c-f	6.80k-n
9	1.08g-j	1.02c-j	1.07i-m	0.78a-d	0.89ab	0.88abc	1.02b-f	6.80k-n
10	2.02a	2.06a	2.23ab	0.78a-d	0.87ab	0.87abc	2.07ab	17.98a
11	0.56mn	0.96f-j	0.63lmn	0.65 i-l	0.88ab	0.88abc	0.46ef	9.43f-h
12	0.58lmn	0.46opq	0.62lmn	0.59l	0.88ab	0.89abc	0.47ef	9.36f-i
13	0.85i-m	0.82h-o	1.07g-m	0.74a-i	0.88ab	0.87abc	0.82b-f	3.00opq
14	0.82i-m	0.78h-o	0.92k-m	0.76d-f	0.71cd	0.88abc	0.77c-f	3.91opq
15	0.71j-m	0.71j-o	0.74j-n	0.79ab	0.89ab	0.87abc	0.71c-f	7.70h-l
16	0.68j-m	0.77h-o	0.87k-n	0.71c-h	0.63jkl	0.87abc	0.73c-f	3.13pq
17	1.46cde	1.42c-e	1.47c-h	0.76 a-f	0.88ab	0.87abc	1.42	10.28c-f
18	1.91ab	1.92a	1.98bcd	0.78abc	0.88ab	0.73cd	1.93a-c	16.14ab
19	0.79i-m	0.73j-o	0.87k-n	0.70f-i	0.88ab	0.88abc	0.73c-f	9.09e-i
20	0.63klm	0.55m-p	0.87k-n	0.69f-i	0.89ab	0.82abc	0.55def	8.09f-l
21	0.64klm	0.68j-o	0.82i-n	0.79ab	0.89ab	0.87ab	0.84b-f	8.58e-i
22	0.60lm	0.56m-p	1.41d-h	0.72c-h	0.89ab	0.87ab	0.52def	8.38e-k
23	0.83i-m	0.79h-n	0.90l-r	0.75a-g	0.89ab	0.88ab	1.31a-f	4.25opq
24	0.82i-m	0.79h-o	0.93i-m	0.76a-f	0.89ab	0.78c	1.04b-f	4.15opq
25	1.16e-h	1.16d-h	1.25d-c	0.79ab	0.89ab	1.16a-f	1.16a-f	8.07f-l
26	0.58lmn	0.47opq	0.67lmn	0.63jkl	0.63jkl	0.88ab	0.47ef	9.15e-i
27	0.95h-k	1.06d-i	2.19abc	0.78a-d	0.78a-d	0.91a	1.06b-f	9.60
28	1.16e-h	1.16d-h	1.18c-l	0.80a	0.80a	0.88abc	1.17a-f	7.72h-l
29	0.67klm	0.60m-p	0.77j-n	0.71d-i	0.71d-i	0.88abc	0.60c-f	6.76k-n
30	0.86h-l	0.62l-o	1.13i-m	0.74a-h	0.74a-h	0.83abc	0.79b-f	4.87m-p
31	0.001h-l	0.01	0.79mn	0.80a	0.80a	0.89 abc	1.18a-f	7.72h-l
32	1.35def	1.35cdef	1.44d-i	0.79ab	0.79ab	0.89 abc	1.34a-f	10.02c-g
33	0.86h-l	0.82h-n	1.07i-m	0.74a-h	0.74a-h	0.88 abc	0.82b-f	3.39opq
34	1.06fgh	1.04e-j	1.24e-l	0.77a-e	0.77a-e	0.88 abc	1.04b-f	2.28q
35	0.76i-m	0.68j-o	0.87i-n	0.70f-i	0.70f-i	0.88 abc	0.68c-f	6.08l-o
36	1.39de	1.47bcd	1.85b-f	0.77a-e	0.77a-c	0.88 abc	1.46a-f	10.25c-f
37	1.46ced	1.46bcd	1.46c-h	0.80a	0.80a	0.89 abcd	1.47a-f	11.75cd
38	1.65bcd	1.73abc	1.86b-f	0.78a-d	0.78a-d	0.91a	1.74a-e	12.11c
39	1.78a-c	1.79ab	1.82b-g	0.73b-g	0.73b-h	0.88 abc	1.80a-d	14.78b
40	1.31efg	1.29d-g	1.56ghi	0.78a-d	0.78a-d	0.88 abc	1.33a-f	6.74k-n
41	1.89ab	1.92a	2.20ab	0.76a-f	0.76a-f	0.88 abc	1.72a-c	16.59ab
42	0.85h-m	0.81h-n	0.931j-m	0.75a-g	0.75a-g	0.88 abc	0.81b-j	3.93pq

[a]refers to Table 1 for the accession name; [b]values in columns followed by the same letter are not significantly different.

The CV (%) value < 20% is considered to be good, indicating the accuracy of the experiments (Table 7). These traits are relevant for characterization, documentation, conservation and crop improvement. It is essential to partition observed variability into genotypic, phenotypic and environmental effects for the selection of superior genotypes. This is important in determining the additive proportion of phenotypic variability. The variance due to the genotypic effect was low in magnitude, while the phenotypic variance was large (Table 7).

Table 7. Components of genetic variation and broad-sense heritability estimates for fruit metric and shape traits among the *Solanum aethiopicum* group.

Fruit metric trait	Coefficient of variation (%)	Genotypic variance	Phenotypic variance	Phenotypic coefficient of variation	Genotypic coefficient of variation	Broad-sense heritability
Fruit perimeter	4.72	46.07	69.02	45.47	33.70	67
Fruit area	14.06	12.73	25.80	100.70	99.70	49
Fruit width mid-height	12.71	0.85	0.92	39.0	38.0	92
Fruit maximum width	9.67	0.85	0.90	37.0	37.0	94
Fruit height mid-width	9.68	3.99	16.05	59	58	24
Fruit maximum height	8.25	1.90	1.93	62	61	98
Fruit curved height	8.29	3.30	3.38	62	61	98
Fruit shape index internal	15.03	0.03	0.03	39.0	39.0	100
Fruit shape eccentricity I	12.22	0.22	0.22	50.43	49.28	100
Fruit shape eccentricity II	14.00	0.23	0.25	54	53	92
Fruit eccentricity	3.28	0.004	0.009	12.82	11.31	44
Proximal eccentricity	5.71	0.002	0.004	22	16	50
Distal eccentricity	3.60	0.0005	0.0014	4.30	2.57	36
Fruit shape index external II	4.82	0.26	0.43	64.92	45.00	60
Lobeness	9.43	29.50	119.29	134.17	66.72	25

Values for genotypic and phenotypic variances were very close to or equal in magnitude for few traits. For all traits, the phenotypic coefficient of variation (PCV) was, in most cases, large compared to the genotypic coefficient of variation (GCV). The difference in magnitude between PCV and GCV was very close. Estimates of the coefficient of variation for phenotypes were low (distal eccentricity) and high (curved fruit height). The GCV values were not like PCV

values, the maximum difference between the GCV and PCV was found in fruit lobes. Broad-sense heritability values were greater than 80% for fruit area, fruit width mid-height, fruit width (maximum), fruit height mid-width, fruit height (maximum), fruit curved height, fruit shape eccentricities I and II, and fruit lobes. A moderately low estimate for GCV for fruit metric traits implies that the improvement in this trait may be achieved to a reasonable extent. Low PCV and GCV values for fruit traits correspond to less phenotypic gain under selection. High PCV values indicate the extent of phenotypic improvement through selection to enhance the potentiality of fruit size. The difference between the PCV and the GCV was low for traits, inferring a low influence of environmental factors compared to genetic factors. In a separate study, the magnitude of GCV and PCV was close for fruit size in bell pepper (*Capsicum annuum* [L.]), another member of the Solanaceae, implying that the selection of fruit size will be worthwhile for the improvement in fruit yield (Sharma et al., 2013). Moderate to high variability for fruit yield indicated a possibility for the improvement through selection. Heritability values were moderately high for fruit curved height and low for lobeness, distal eccentricity and fruit mid-width. The magnitude of variability for fruit metric traits among scarlet eggplant groups is important for breeding, selection, and conservation. The phenotypic and genotypic coefficients of variation for all fruit metric characters indicate that the fruit metric traits are heritable.

The PC analysis indicated 4 of 15 PC axes had eigenvalues >1.3 and were responsible for most of the total variation among accession means (Table 8). The discriminating ability of eigenvalues was high for PC axes 1 and 2, which collectively explained most fruit metric variation. The first PC was frequently correlated with the large and elongated fruit. There were positive coefficients for fruit maximum height and height mid-width, fruit shape eccentricity I and fruit shape eccentricity II with equal loading, and fruit area. Distal eccentricity and proximal eccentricity had equal loading with negative coefficients on PC 1. The second PC axis had a high frequency of positive than negative eigen coefficients. Circular and curved fruit shapes predominate with positive coefficients (fruit shape index and curved fruit shape index), and these traits had negative coefficients on PC 1. Negative and equal coefficients occurred for fruit mid-width height and fruit maximum width. Traits associated with fruit size had negative coefficients on PC 2. The magnitude of variance concentrated in PCs 1 and 2 was attributed to fruit size and shape (maximum height, height mid-width, and area, curved width, fruit shape eccentricity I and fruit shape eccentricity II). These traits could form a focal point for characterization and conservation. Accessions with large-sized fruits are characterized by a low number of fruits per fruit cluster and total fruits harvested on an individual plant basis (Adeniji et al., 2012, 2013). Accessions 31 (C4) and 41 (C1a) were characterized by very small fruit size and preferred fruit taste. Accessions 9 (C3), 17 (Outlier) and 19 (C2b) are possible pollen sources for

fruit width. Proximal and distal fruit end shapes are indicators of fruit size. Among members of the Gilo, the distal end of the fruit is pointed. The Kumba group is characterized by a depressed distal fruit end. Fruit end shape is important for packaging, ease of transportation over a long distance, and display in stores. Fruit shapes in the Gilo group are oval, pear-shaped and oblong. Fruits of the Kumba group are deeply lobed, less circular, furrowed and flattened compared to the Gilo group (Adeniji, 2013; Plazas et al., 2014), and fruits of the Shum group are circular. Accessions of the Kumba group are more lobed compared to the Shum group accessions, and the latter are more uniform and ovoid. Traits with eigenvectors greater than >0.25 are important contributors to the variation observed for PC 1.

Table 8. Eigenvalues and vectors, correlation coefficients of fruit phenomic traits for the first four principal component axes.

Variable	PC 1	PC 2	PC 3	PC 4
Fruit perimeter	0.24	-0.22	0.40	0.28
Fruit area	0.35	-0.15	-0.14	-0.07
Fruit width mid-height	0.19	-0.40	-0.16	-0.11
Fruit maximum width	0.19	-0.40	0.26	-0.04
Fruit height mid-width	0.37	-0.02	-0.15	-0.007
Fruit maximum height	0.37	-0.04	0.12	-0.03
Fruit curved height	0.29	-0.10	0.30	0.03
Fruit shape eccentricity I	0.26	0.33	0.17	-0.29
Fruit shape eccentricity II	0.26	0.34	0.16	-0.25
Fruit shape index internal	0.13	0.40	0.08	0.14
Fruit eccentricity	0.12	0.19	-0.05	0.43
Fruit proximal eccentricity	-0.27	0.08	0.39	0.13
Fruit distal eccentricity	-0.27	0.04	0.41	0.08
Fruit shape index external II	0.11	0.27	0.02	0.48
Fruit lobeness	0.22	0.23	0.05	0.01
Eigenvalue	6.12	3.18	1.62	1.34
Proportion	0.38	0.20	0.10	0.08
Cumulative (%)	38	58	68	77

The projection of accessions on a 2-dimensional plot of principal component axes 1 by 2 (Figure 1) indicated the spread of accessions into 4 quadrants alongside traits responsible for ordination. Fruits of accessions ordered in quadrant 1 are circular and curved with oval seed shape (positive coefficients on PCs 1 and 2 for fruit shape eccentricities I and II, fruit curved height, fruit shape index and lobeness) (Figure 1). Accession 10 (Q1) was widely separated from others, indicating the highest contribution to phenotypic variability in this quadrant.

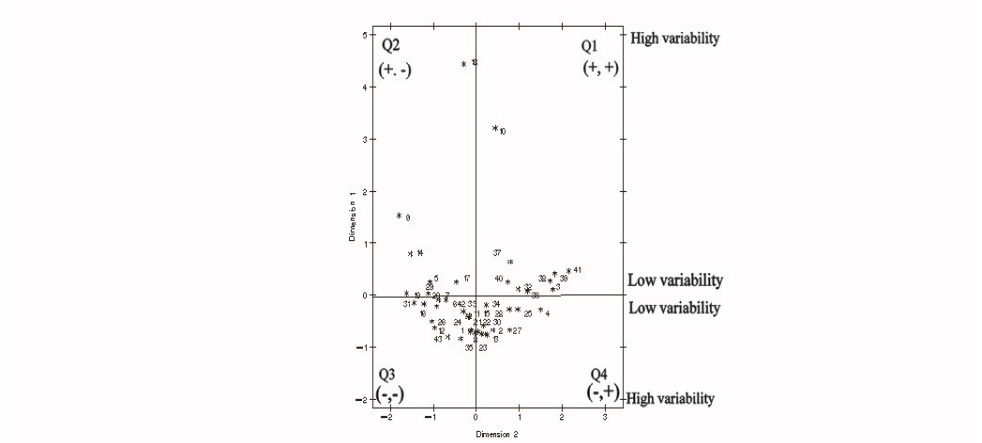

Figure 1. The ordination of 43 accessions from the *Solanum aethiopicum* group derived from the unweighted pair group method of analysis clustering of correlation coefficients for 15 phenomic traits by the squared Euclidean distance and the Ward's method.

Q1 (Quadrant 1) PC1: PC2 (+, + coefficients), Q2 (Quadrant 2) PC1: PC2 (+, - coefficients), Q3 (Quadrant 3) PC1: PC2 (-, - coefficients), Q4 (Quadrant 4) PC1: PC2 (+, - coefficients).

Entries in the second quadrant were characterized by the large, elongated fruit with positive and negative coefficients on PCs 1 and 2 (fruit height mid-width, fruit maximum width, fruit width mid-height, fruit maximum height, fruit curved height, fruit area and fruit perimeter). Accession 18 (Q2) was widely separated from other entries in the second quadrant. Accessions 18 (Q2) had the highest contribution to fruit metric variability, followed by Accession 9 (Q2). The ordination of entries in the third quadrant is consistent with differences in fruit distal end blockiness, with negative coefficients on both PCs 1 and 2. Fruits that are more triangular, less ellipsoidal and circular, round, and pear-shaped predominate in this quadrant. Ten accessions were dispersed in the fourth quadrant, with traits associated with this dispersion being proximal and distal eccentricity, fruit area index, with negative and positive coefficients, on PCs 1 and 2. No accession was dispersed in the top right and left-hand corner of quadrants 1 and 2, with a maximum contribution to fruit metric variability.

The dendrogram grouped the scarlet eggplant accessions into 4 clusters (C_1, C_2, C_3 and C_4) (Figure 2). The first cluster (C1) was divided into sub-cluster 'a' with 9 accessions and 5 accessions in sub-cluster 'b'. Accessions in the Gilo group predominate in this cluster, they were widely distributed in the first quadrant, with positive correlation coefficients on the plot of principal component axes 1 and 2. Cluster members were related by fruit shape eccentricity I, fruit shape eccentricity II, curved fruit shape 1, and fruit shape index and fruit lobes. Accessions ordered in

cluster 1b were dispersed in quadrants 2 and 3 (Figure 1). Accessions of the Kumba group predominate in this cluster, they are triangular and lobed, less circular, flattened fruit (high values for fruit shape eccentricity indices I and II). Nine accessions were grouped in cluster 2 (C_2) and divided into sub-clusters 'a' and 'b'. Accessions of the Gilo group were interspersed among the Kumba group. This cluster had moderate fruit size (moderate values for fruit perimeter, fruit area, fruit mid-width height, fruit maximum height), and moderately lobed compared to clusters 1 and 4, and fruits are less ovoid and triangular. Moderate values for fruit shape eccentricity indices I and II compared to entries grouped in cluster 1 indicate a preponderance of accessions of the Gilo group in this cluster. Seven accessions among the Gilo and Kumba were grouped in cluster 3. Members of this group are characterized by medium and small fruit sizes (low values for fruit perimeter, fruit area, fruit width mid-height, maximum fruit width, fruit height mid-width) and short fruit. Accessions 24 and 27 (C3a) had small-sized fruits, accessions 30 and 42 (C3b) were oval, and accessions 6 and 14 (C3b) had medium-sized fruits. Members of cluster 3 were less circular (moderately high circular values), fairly lobed compared to clusters 1 and 4. Accessions grouped in cluster 4 were characterized by small fruit compared to clusters 1, 2 and 3. Accessions in cluster 4 had low to moderate fruit perimeter, fruit area and highly lobed fruit. Accessions 31 and 36 (C4) were related to fruit pericarp curvature. Dispersion of accessions on the PC plot is consistent with the grouping of accessions on the dendrogram. Accessions grouped in cluster 1 were dispersed in quadrants 1 and 4. Members of cluster 2 were ordered in quadrants 2 and 3, members of cluster 3 occurred in quadrants 2 and 3. Accessions in cluster 4 were dispersed in quadrants 1, 2, and 3. The out-group (accessions 4, 3 and 17 linked C4 at the lower end) were dispersed in quadrants 1, 4 and 2, respectively. A large number of traits needed to explain total variance for fruit metrics may be associated with duplicate accessions in the germplasm collection (Yada et al., 2010). Phenotypic improvement in fruit perimeter will account for large fruit size. Selection in favor of the fruit area will complement fruit eccentricity (proximal and distal). Accessions with a thick fruit will have a tall fruit shoulder.

The dispersion and ordination (Figure 1), and grouping of the accessions (Figure 2) in the dendrogram (Figure 2) indicate that members of Gilo and Kumba groups displayed fruit traits specific to each group which overlapped. Findings are similar to previous reports of Polignano et al. (2010) and Tümbilen et al. (2011). Accessions from different geographical locations grouped with other entries exhibiting geographic heterogeneity and fruit traits are not specifically assigned to a specific location. The dendrogram indicated 4 distinct clusters with overlapping and plasticity of fruit metric traits and geographical heterogeneity within and among clusters. The high degree of fruit metric variation and overlapping of fruit metric traits may be associated with ecological regions where accessions were

collected. The proximity between Gilo and Shum groups confirm the findings of Shippers (2002) that the Gilo group evolved from the Shum group or that both share a common genome (Polignano et al., 2010; Plazas et al., 2014; Kaushik et al., 2016). A high degree of fruit metric trait diversity observed in the scarlet eggplant genetic resources necessitates conservation strategies for preserving local genetic resources for breeding (Polignano et al., 2010; Tümbilen et al., 2011; Plazas et al., 2014; Kaushik et al., 2016).

Conclusion

The range of variation was high in fruit perimeter, fruit lobeness, fruit curve height, fruit shape index, fruit maximum height, fruit maximum width, and fruit area. These traits are relevant for characterization, documentation, conservation and crop improvement. Broad-sense heritability values were greater than 80% for fruit area, fruit width mid-height, fruit width (maximum), fruit height mid-width, fruit height (maximum), fruit curved height, fruit shape eccentricities I and II, and fruit lobes. The dendrogram and PC plot grouped accessions by shape, size and fruit area. Accessions 9 (C3), 10 (C1) and 18 (C2) could be pollen parents for the improvement in fruit size through intraspecies hybridization within Gilo, between Gilo and Shum, and Gilo and Kumba groups. Using variation present in the eggplant materials, selecting and developing new varieties should be possible. The Tomato Analyzer software characterizes the fruits of scarlet eggplant by groups, and the information could be used to establish commonalities between groups.

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METRIČKA KARAKTERIZACIJA OSOBINA PLODA GRIMIZNOG PLAVOG PATLIDŽANA KORIŠĆENJEM VISOKOPROPUSNOG SOFTVERA ZA ANALIZU PARADAJZA

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R e z i m e

Grimizni plavi patlidžan (*Solanum aethiopicum* [L.]) je autohtono, nedovoljno iskorišćeno plodovito povrće u Africi. Sklonost ka obliku i veličini ploda velika je među uzgajivačima i potrošačima. Metričke osobine ploda važne su za poboljšanje prinosa. Metrički deskriptori ploda značajno doprinose varijacijama, fenotipskim i genotipskim varijacijama i heritabilnosti. Međutim, merenje ovih osobina je komplikovano i subjektivno. Četrdeset i tri genotipa procenjena su u 2016. i 2017. godini. U fazi zrelosti, iz svakog genotipa nasumično je ubrano 5 plodova, digitalizovano i obrađeno pomoću softvera za analizu paradajza. Šesnaest metričkih osobina ploda automatski je generisano i dostavljeno za analizu varijanse i multivarijantnu analizu. Genotipovi su se razlikovali u odnosu na veličinu i oblik ploda zbog genetske predispozicije. Varijacija metričkih osobina ploda među grupama *S. aethiopicum* bila je pod manjim uticajem okoline. Grupa sorte Gilo ima duguljaste plodove, plodovi grupe sorte Shum su kružni i okruglasti; plodovi grupe sorte Kumba su manje kružni, režnjeviti i spljošteni. Grupe AE/113 (C3), FUO 1 (C1) i FUO 5 (C2) Gilo obećavajuće su za veličinu ploda. Postojala je fenotipska plastičnost i preklapanje za metričke osobine ploda između grupa Gilo i Shum zbog zajedničkog genoma. Softver za analizu paradajza je uspeo da razdvoji genotipove na osnovu fenotipskih osobina ploda, a informacije su mogle da se koriste za utvrđivanje zajedničkih osobina između grupa.

Ključne reči: *Solanum aethiopicum*, površina ploda, veličina ploda, genetski diverzitet, naslednost.

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EFFECTS OF THE CARBONATED DRINK AS AN EXTENDER ON SEMEN CHARACTERISTICS, FERTILITY AND HATCHABILITY IN NIGERIAN INDIGENOUS CHICKEN

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Abstract: Semen extenders are liquid diluents that buffer sperm cells and preserve their fertilizing potentials. The commercial carbonated drink (CD) as an extender was evaluated on semen characteristics, fertility and hatchability in Yoruba ecotype chickens (YECs). The fructose of the CD was 1.52±0.05 mg/ml. Under the conditions of 37^0Celsius, 5% and 10% of CD were added to the egg yolk citrate solution to make 100%. Semen was obtained from ten matured Yoruba ecotype chicken cocks with an average weight of 1.8±0.2 kg. The semen was pooled in a test tube and added to the extenders for preservation at 0, 30 and 60 minutes, respectively, in a factorial design layout. Percentage motility of sperm cells was significantly ($p<0.05$) higher in 5% CD inclusion compared with 10% CD inclusion and control. Motility decreased with an increase in preservation time across the treatments. The percentage of dead sperm cells decreased ($p<0.05$) in 5% CD inclusion when compared with 10% CD inclusion and control. The sluggish sperm percentage increased significantly ($p<0.05$) with semen preservation time. Fertility and hatchability of eggs were significantly ($p<0.05$) higher in 5% CD inclusion. It was concluded that carbonated drinks at 5% inclusion in an extender could preserve cock sperm cells for 60 minutes with improved fertility and hatchability of eggs.

Key words: semen, extender, carbonated drink, motility, Yoruba ecotype chickens.

Introduction

Poultry is important livestock in the agricultural economy of Nigeria, both as sources of good quality protein and income generation. Huge resources are committed to importing birds while the Nigerian indigenous chicken (NIC) remained uncharacterized and unimproved (Ige et al., 2012). Nigerian indigenous chickens (NICs) are relatively small-sized birds that produce semen of low volume and high concentration (Akanbi, 2018). Improving the YEC would require

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committed mating, which could be facilitated by artificial insemination (AI). The increasing use of AI in poultry emphasizes the need for qualitative semen. The efficacy of AI could be ascertained by an efficient extender with an improved diluent (Mian et al., 1990). The advantages of the semen extender include the maximum use of semen in short supply and a reduction in the male to female mating ratio. NIC produces about 0.2ml of semen per ejaculate (Akanbi, 2018), which may be difficult to handle in AI. Diluents enhance the spread of semen over many hens (Mian et al., 1990), and the application of an extender is essential to sustain semen quality (Bootwalla and Miles, 1992), which could be preserved without impairing the viability and fertilizing ability of the semen (Lukaszewicz et al., 2008). An appropriate semen extender has to maintain sperm cell motility, fertility capacity and membrane integrity (Rihast et al., 2006).

Generally, an extender will facilitate semen handling, maintain sperm viability, and inhibit pathways detrimental to sperm cell survival. Several sources such as coconut, tomato and carrot (Banerjee, 2011) and *Borassus aetiopium* (Adeyina et al., 2017) have been reported for their semen extending potential, and there are a number of buffered salt solutions available as extenders for chicken semen. These extenders have a different composition, sometimes with special additives such as skimmed milk, egg albumin, glutamine sulphate and carbonic acid (Iaffaldano et al., 2007). The carbonated drink is one of such solutions with the composition that could support semen extending potentials. The carbonated drink contains sugar, mild acid, sodium citrate and other minerals that could serve as minimum essential medium (MEM) for semen survival. The availability and affordability of the carbonated drink (CD) could make it a ready-made additive of importance in semen extenders for reproductive improvement. The need to evaluate the potential of CD in enhancing the viability and fertility of semen for the successful application of AI has necessitated this research.

Material and Methods

The experiment was conducted at the poultry unit of the Teaching and Research Farm, Faculty of Agriculture, University of Ilorin, Ilorin, Nigeria.

The carbonated drink (CD) was obtained from a commercial outlet within the University campus. The drink was contained in a green bottle produced by a globally renowned carbonated drink manufacturer. The solution was colourless and drizzling when opened. The solution was opened and allowed to settle for about five minutes, after which 2 ml of the CD was taken and analyzed for the mineral composition, according to Volpe et al. (2015).

Sodium citrate solution was prepared by weighing and dissolving 2.90g of sodium citrate dehydrate into 100ml of distilled water. The solution was sterilized and kept in the refrigerator, according to Singh (2005). Egg yolk solution was prepared by breaking an egg at the narrow end with a forcep and draining out the

albumin. The egg yolk membrane was broken, and the clear yolk was poured into a measuring cylinder. The egg yolk citrate solution was prepared by adding 40% of the egg yolk into a 60% sodium citrate solution to serve as a control. Treatment one had 40% egg yolk added to a 55% sodium citrate solution and 5% CD, while treatment two had 40% egg yolk added to a 50% sodium citrate solution and 10% CD. The solutions were then placed in a water bath maintained at 37^0Celsius.

Ten matured YEC cocks (average weight 1.8±0.2kg) aged 12 months were obtained from the stock of YEC being raised on the Teaching and Research Farm, University of Ilorin. The birds were trained to be used to the milking procedures and then milked to obtain semen using the massaging method as described by Adeyina et al. (2016). The undiluted semen was pooled into a test tube and analyzed for semen quality and characteristics using the method of Wishart (1995). From the pooled semen, 0.5ml was taken into 2.5ml of the extender in test tubes already placed in the water bath in the ratio of 1:5, according to Adeyina et al. (2017). All the semen solutions were maintained at the same temperature in the water bath. Samples, in triplicate from the semen solutions, were taken on a slide and observed under a microscope (Olympus model x40) at 0, 30 and 60 minutes according to the method described by Wishart (1995). Sixty actively laying hens of YEC (weighing 1.3 ± 0.2kg) were selected and inseminated using semen with 0%, 5% and 10% inclusion of CD. The birds were divided into three treatments and four replicates (five hens per replicate). The insemination was done using 0.1ml of the extended semen at 0, 30 and 60 minutes. The hens were kept individually in a battery cage system and fed with a commercial layer mash of 17.5% CP and 2700kcal/kg M.E, water was offered *ad libitum*. Eggs from the birds were collected after 24 hours following insemination for four days before the insemination was repeated. A total of 150 eggs, 50 eggs /treatment, were set in the incubator.

Table 1. The percentage composition of experimental extenders.

Egg yolk	40	40	40
Sodium citrate (buffer)	60	55	50
Carbonated drink (CD)	0	5	10
Total	100	100	100

The experiment was conducted in line with the university's guideline for the ethical treatment of experimental animals in accordance with the best practices within Institutional Animal Care and Use Committee (IACUC) guidelines.

Statistical analysis

All data obtained were subjected to statistical analysis using the analysis of variance (ANOVA) procedure following a factorial (2x3) model (SAS 1999), and the level of interaction was determined using the same procedure.

Results and Discussion

Table 2 shows the semen characteristics and biochemical properties of undiluted cock semen. The value of the average volume of semen from the cock was within 0.2 and 0.3 ml, corroborating the findings of Akanbi (2018), who reported the semen volume of YEC as 0.3ml. The percentage motility of 98% recorded in this study indicates that YEC sperm is of very good quality in accordance with the value presented for NIC by Ajayi et al. (2014). The sperm concentration of $256x10^6$/ml recorded in this study was higher compared with $248x10^6$/ml reported by Akanbi (2018), supporting the fact that semen of low volume is usually of high concentration. The value of the fructose is higher than 2.0mg/ml reported for cocks by Singh (2005). Fructose is an energy source for semen metabolism, and the high fructose concentration is a reflection of energy required to support the metabolism of a sperm cell with high concentration.

Table 2. Characteristics of undiluted semen.

Parameters	Values ±SD
Volume (ml)	0.2 ± 0.08
Concentration ($x10^6$/ml)	256 ± 18.57
Motility (%)	98 ± 5.64
Sluggish (%)	0 ± 0.0
Dead (%)	2 ± 0.61
Total protein (mg/ml)	2.95 ± 0.51
Fructose (mg/ml)	2.95 ± 0.06
Potassium (mg/ml)	18.83 ± 2.32
Sodium (mg/ml)	34.95 ± 1.07
Magnesium (mg/ml)	2.98 ± 0.33
Calcium (mg/ml)	0.95 ± 0.11
Osmolarity (Osm/L)	388.94 ± 21.55
pH	7.1 ± 0.46

Table 3 shows the chemical composition and the properties of carbonated drink. The presence of minerals is necessary for the maintenance of the certain physiochemical process essential to life. It is believed that this edible CD was formulated to provide the needed energy for the mass activities of sperm cells.

Table 4 shows the effect of the CD and the preservation period on semen quality. There was a significant ($p<0.05$) reduction in sperm cell motility while the percentage of sluggish and dead cells significantly ($p<0.05$) increased with an increase in preservation time. However, the value of motility of 75% at 60 minutes was still within the range of good quality semen. This suggests that carbonated drinks in diluents supported a minimum essential medium (MEM) for survivability. According to Singh (2005), MEM for sperm cell survival includes soluble sugars

and minerals (Fukuhara and Nishikawa, 1993), which were adequately available for up to 60 minutes.

Table 3. Properties of the carbonated drink.

Parameters	Value ± SD
Fructose (mg/ml)	1.52 ± 0.05
Potassium (mg/ml)	7.35 ± 2.13
Sodium (mg/ml)	274.07 ± 10.24
Magnesium (mg/ml)	0.94 ± 0.03
Calcium (mg/ml)	0.94 ± 0.08
Osmolarity (Osm/L)	615.83 ± 20.68
pH	5.6 ± 0.07

Table 4. The effects of carbonated drinks and preservation periods on semen characteristics.

	Carbonated drinks				Preservation periods (minutes)				
Parameters	control	5%CD	10%CD	SEM	0	30	60	SEM	CD*PP
Motility (%)	85.17^{b}	88.00^{a}	85.00^{b}	1.02	97.17^{a}	86.00^{b}	75.00^{c}	3.06	*
Sluggishness (%)	1.83	1.50	1.66	0.67	0.33^{c}	2.04^{b}	3.75^{b}	0.74	*
Dead cell (%)	13.50^{a}	10.50^{b}	13.33^{a}	1.24	2.50^{c}	11.88^{b}	21.25^{a}	2.25	*

a, b, c means with different superscripts within the same row are significantly different ($p<0.05$). S=significant and SEM=standard error of the means.

Table 5 shows the effect of CD and time of preservation on sperm cell motility. Sperm motility was significantly ($p<0.05$) reduced with an increase in preservation time. However, the motility (%) in the 5% inclusion of CD was higher than that of control. At 60 minutes, the motility was still above 70%, signifying that the inclusion of CD supported the good fertilizing ability of the semen (Adeyina *et al.*, 2017). The inclusion of CD at 5% and 10% did not affect the motility at 0 minute compared with the control because the CD is edible and contains minerals supportive of life processes, including semen metabolism due to the presence of MEM. More importantly, the sperm cell motility in 5% and 10% inclusions of CD is of importance to the overall usefulness of CD as an extender.

Table 5. The effect of the CD and preservation time on sperm cell motility (%).

Treatment	Control	5% CD	10% CD
		Periods	
0	96.33^{a}	98.00^{a}	98.00^{a}
30	85.17^{b}	88.00^{b}	85.00^{b}
60	74.00^{c}	78.00 ^{c}	72.00^{c}
SEM	10.27	8.29	9.13

a, b, c means with different superscripts in the same column are significantly different ($p<0.05$). SEM=standard error of the means.

Table 6 shows the effect of the CD and time of preservation on sperm cell abnormality. Sperm cells abnormality significantly ($p<0.05$) increased with the period of preservation, but it was still within the range of 9.9–12.8% for successful AI (Tselusi et al., 1999).

Table 6. The effect of the CD and preservation time on sperm cell abnormality (%).

Treatment	Control	5% CD	10%CD
		Periods	
0	0.67^{c}	0.00^{c}	0.00^{c}
30	1.84^{b}	1.50^{b}	1.67^{b}
60	3.00^{a}	3.00^{a}	3.33^{a}
SEM	0.23	0.40	0.62

a, b, c means with different superscripts in the same column are significantly different ($p<0.05$). SEM = standard error of the means.

Table 7 shows the effect of the CD and time of preservation on semen dead cells. The percentage of dead cells increased with time across the treatments. According to Tselusi et al., (1999), the survivability of semen reduces with time. The inclusion of 5% CD reduced dead cells compared with 10% inclusion and the control at 60 minutes.

Table 7. The effect of the CD and preservation time on dead sperm cells (%).

Treatment	Control	5% CD	10% CD
		Periods	
0	3.00^{c}	2.00^{c}	2.00^{c}
30	13.0^{b}	10.50^{b}	13.34^{b}
60	23.00^{a}	19.00^{a}	24.67^{a}
SEM	4.01	3.82	2.93

a, b, c means with different superscripts in the same column are significantly different ($p<0.05$). SEM = standard error of the means.

Table 8 shows the effect of CD on the fertility and hatchability of eggs. The inclusion of CD at 5% was significantly ($p<0.05$) higher in fertility and egg hatchability compared with that of 10% and the control. This could be due to the maintenance of sperm cell integrity in 5% CD as a result of moderate metabolic activity and energy availability. The CD provides additional energy for sperm cell function, having been constituted with sugar (fructose). The availability of carbonate in the CD could have caused the production of CO_2, which could reversibly immobilize sperm cell. According to Long (2006), CO_2 narcosis is an effective means to maintain the viability and fertilizing ability of spermatozoa. Carbonated water is the water in which bubbles were added (Anon, 2004).

However, 10% CD seems to have resulted in toxicity of the sperm cells, hence, the poor fertility and hatchability in this treatment.

Table 8. The effect of CD on the fertility and hatchability of eggs.

Treatment	Fertility (%)	Hatchability (%)
Control	77.00^{b}	67.00^{a}
5% CD	83.00^{a}	70.00^{a}
10% CD	54.00^{c}	46.00^{b}
SEM	4.83	3.72

a, b, c means with different superscripts in the same column are significantly different ($p<0.05$). SEM = standard error of the means.

Conclusion

Carbonated drinks up to 10% have the capacity of a potential extender for cock semen, and they are supportive of semen storage on a short-term basis. They could preserve cock's sperm cells for up to 60 minutes. It can, therefore, be concluded that CD at 5% inclusion in the egg yolk citrate diluent provides considerably high fertility and hatchability of eggs and could be used in the storage of YEC cock semen for AI over a short distance before usage.

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UTICAJI GAZIRANOG PIĆA KAO RAZREĐIVAČA NA KARAKTERISTIKE SPERME, PLODNOST I IZVODLJIVOST KOD NIGERIJSKIH DOMAĆIH PILIĆA

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R e z i m e

Razređivači sperme su tečni razblaživači koji puferiraju ćelije sperme i čuvaju njihov potencijal oplođenja. Komercijalno gazirano piće (GP) kao razređivač procenjeno je u odnosu na karakteristike sperme, plodnost i izvodljivost kod pilića ekotipa Joruba. Sadržaj fruktoze u gaziranom piću iznosio je 1,52 ± 0,05 mg/ml. U uslovima temperature od 37^0C, 5% i 10% gaziranog pića dodati su citratnom rastvoru žumanceta kako bi se dobilo 100%. Sperma je uzeta od deset zrelih petlova pilića ekotipa Joruba prosečne težine 1,8±0,2 kg. Sperma je sakupljena u epruvetu i dodata je razređivačima radi čuvanja u vremenskom periodu od 0, 30 odnosno 60 minuta u faktorskom dizajn rasporedu. Procenat pokretljivosti spermatozoida bio je značajno ($p<0,05$) veći kod 5% GP u poređenju sa 10% GP u rastvoru i kontrolom. Pokretljivost se smanjivala sa povećanjem vremena čuvanja tokom tretmana. Procenat mrtvih ćelija sperme se smanjio ($p<0,05$) kod 5% GP u poređenju sa 10% GP i kontrolom. Procenat slabo pokretnih ćelija sperme se značajno povećao ($p<0,05$) sa vremenom čuvanja sperme. Plodnost i izvodljivost takođe su bili značajno ($p<0,05$) veći kod 5% GP. Zaključeno je da gazirana pića u količini od 5% u razređivaču mogu da sačuvaju spermatozoide petla tokom 60 minuta uz poboljšanu plodnost i izvodljivost.

Ključne reči: sperma, razređivač, gazirano piće, pokretljivost, pilići ekološkog tipa Joruba.

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EFFECT OF STORAGE PERIOD ON PHYSICAL, CHEMICAL, MICROBIAL, AND SENSORY QUALITIES OF INSTANT *MASA* FLOUR PRODUCED FROM BLENDS OF RICE AND BAMBARA GROUNDNUT

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Abstract: *Masa* is a traditionally fermented meal usually made from cereals. The aim of this research was to produce an enriched meal from rice and Bambara groundnut. The colour, functional properties and microbial quality of composite blend of rice and Bambara groundnut flour, in the ratio of 100:0, 95:5, 90:10, 85:15, 80:20, were evaluated using standard methods. Sensory properties of freshly made *masa* and *masa* prepared from stored flours were also determined. The microbial load of the *masa* flour blend increased over the storage period. Water absorption capacity, swelling capacity and bulk densities increased, while the oil absorption capacity decreased with the storage period. The objective colour result showed a decrease in the lightness (L*) value. Sensory properties of *masa* were not substantially altered with Bambara groundnut inclusion, but the ratings reduced with storage. Instant *masa* may be prepared from flour stored for 4 weeks without considerable changes in quality.

Key words: *masa,* rice, Bambara groundnut, storage, microbial.

Introduction

The knowledge of consumers on the relationship between diet and health has led to a change in the dietary pattern of the African populace. According to Samuel et al. (2015), there are swift and widespread shifts in food consumption patterns towards the western diet and lifestyle. Consumption of snacks such as *masa* produced from cereals contributes significantly to the calorie intake of the populace in many parts of the world, including Africa. *Masa* is a traditional fermented product in Nigeria from millet, maize or rice flour (Badau et al., 2018). Good quality *masa* is round in shape with brown and smooth surfaces (Badau et al., 2018).

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The nutritional value of *masa* may vary with the type and variety of cereals and perhaps the processing conditions used in its preparation. For example, the protein content of rice-*masa* (7.59%) was found to be significantly lower than those of maize (9.56%) and millet (9.21%) (Ayo et al., 2008b). In general, *masa* is very low in protein but may contain up to about 7% to 11% of protein depending on the cereals used and their combinations with legumes. Nkama and Malleshi (1998) reported that the protein content of rice-*masa* increased by 54% when 20% of cowpea was added to enrich the *masa*. Other authors reported a 10% increase in protein content of maize-*masa* enriched with groundnut paste at 20% levels (Ayo et al., 2008a).

The addition of protein-rich leguminous crop such as Bambara groundnut (*Vigna subterranea*) to cereal-based snack is a promising way to enrich the snack and improve the nutritional intake of the populace. Bambara groundnut is a leguminous crop that is rich in protein (15–27%) and carbohydrate (57–67%) (Oyeyinka and Oyeyinka, 2018). It is often referred to as a complete food because of its reasonably high protein content (Adebowale et al., 2002; Arise et al., 2015; Oyeyinka et al., 2015; Sirivongpaisal, 2008) and a good balance of the essential amino acids (Yao et al., 2015). The high levels of lysine (6.5–6.8%) in Bambara groundnut and the considerable amount of methionine (1.8-2.84%), which is normally limiting in most legumes, further confirm the grain as a balanced diet (Aremu et al., 2006; Kudre et al., 2013; Ijarotimi and Esho, 2009). Bambara groundnut is a drought-tolerant crop that has potentials for cultivation during extreme conditions of drought. Bambara groundnut has similar composition to cowpea but has limited utilisation when compared to cowpea (*Vigna unguiculata*), presumably due to the very limited research to unlock the potential of the crop (Oyeyinka et al., 2015). The hard-to-cook defect (HTC) commonly associated with legumes including Bambara groundnut may also explain the limited utilisation of the crop. It has been found that HTC defect results in high energy utilisation and consequently reduces the nutritive value of the grain (Molina and Bressani, 1975; Paredes-López et al., 1991).

Previous studies on *masa* enriched with legumes such as cowpea or groundnut found significant improvement in the nutritional value of the *masa* (Ayo et al., 2008a; Nkama and Malleshi, 1998). Besides the improvement in the nutritional value of *masa*, the addition of legumes to *masa* may affect the physical, chemical, and sensory properties. The sensory properties of maize-based *masa* enriched with 20% and 25% of groundnut paste were found to be superior to those of *masa* without groundnut paste or to those with lower levels of the paste (Ayo et al., 2008a). So far, studies on *masa* have focused on nutritional properties as well as sensory properties of the snack. In this study, instant *masa* flour was produced from blends of rice and Bambara groundnut flours. The physical, chemical, sensory, and microbial qualities of the freshly produced and stored *masa* flour were investigated.

Materials and Methods

Materials

Local rice, Bambara groundnut, corn starch, and polyethene bags were obtained from the Oja Oba market in Ilorin, Kwara State. Materials were transferred into the Food Processing laboratory of the Department of Home Economics and Food Science, University of Ilorin, for further processing.

The preparation of *masa* flour

Masa was prepared from rice as previously described by Ayo et al. (2008a), except that Bambara groundnut was used to enrich the *masa* instead of groundnut paste. Briefly, rice grains (500 g) were sorted to remove foreign matters, washed, and soaked in distilled water (600 ml) at 34^{o}C for 12 hrs. A quarter ($^{1}/_{4}$) of the cooked rice grains was mixed with three-quarters ($^{3}/_{4}$) of milled rice flour to form a batter. The resulting batter was inoculated with bakers' yeast (1% w/w of rice grain) and left to ferment at room temperature (25±2^{o}C) for 16 hrs, and the thick batter obtained was diluted with 10 ml of 20% sodium carbonate solution. Salt (10 g/500 g of rice), sugar (30 g/500 g of rice) and Bambara groundnut flour in varying proportions (5, 10, 15 and 20% w/w of rice grain) were added to the batter and properly mixed. Samples were dried in an oven at 60^{o}C for 5 hrs, cooled and milled into fine flour, mixed with 5% of corn starch, and packaged into a high-density polythene bag. The *masa* flours were stored for two months at room temperature (25±2^{o}C), and the colour and functional properties of the flour, as well as the sensory and microbial qualities of the masa, were assessed bi-weekly from the day of production.

The proximate composition of *masa* flour

The proximate composition (ash, fat, fibre and moisture contents) of the flour was determined using standard methods (AOAC, 2000). Protein content was measured using the Kjeldahl method (6.25 × N), while the total carbohydrate was calculated by difference.

The colour of masa flour

Tristimulus l* a* b* parameters of *masa* flour were determined after standardisation using colour Flex (A60-1014593, USA). Snapshots in triplicate were taken, and values were read directly from a digital print. The average of the readings was computed and recorded.

Functional properties of *masa* flour

Water absorption capacity was determined by the method described by Oyeyinka et al. (2015). One gram of sample was mixed with 10 ml of distilled water. The mixture was left at room temperature (25±2 ^{o}C) for 30 mins and thereafter centrifuged (model 5810R, Eppendorf International, Frankfurt, Germany) for 30 mins. Water absorption capacity was expressed as a gram of water bound per gram of flour. The same procedure was repeated for oil absorption capacity, except that the water was replaced with soybean oil.

Swelling capacity was determined as previously described with slight modifications (Oyeyinka et al., 2019). Flour blends were filled to the 10 ml mark in a 50-ml glass measuring cylinder. Distilled water was added at room temperature to give a total volume of 50 ml. The top of the cylinder was tightly covered, and the contents mixed by inverting the cylinder. The cylinder was then left to stand for additional 4 hrs, and the final volume occupied by the sample was recorded. Swelling capacity was determined by dividing the volume of the flour in water by the initial volume of the flour blends.

The microbial analysis of masa flour

Quantitative bacteriological analysis of the samples was carried out using the total plate count on nutrient agar (NA), and potato dextrose agar (PDA) was used for the fungal count. The counts were expressed as colony-forming units per millimetre (cfu/ml) (Balogun et al*.,* 2016).

Sensory analysis

For sensory evaluation, *masa* was prepared from the instant *masa* flour, and sensory evaluation was carried out to know the most acceptable blend of rice flour and Bambara groundnut flour from the various mixing ratios. The analysis was done using a nine-point hedonic scale questionnaire. The *masa* samples were placed in front of 30 panellists to decide on the most acceptable one by assessing the samples for sourness, appearance, aroma, taste, texture, and overall acceptability. The panellists were selected among the staff and students at the University of Ilorin who are regular consumers of *masa*. Dried *masa* flour was mixed with water to form a batter. The batter was deep-fried in heated soybean oil for 4 mins on one side and turned over for additional 4 mins. Fried *masa* samples were cooled, packaged, and stored at 4^{o}C until needed for analysis for a maximum of 1 week. Freshly made samples and *masa* from stored flours were prepared and used for sensory evaluation. Panel members were provided with water to rinse their mouths after evaluating each sample to prevent carry-over effects.

Statistical analysis

Duplicate samples were prepared, and analyses were done in triplicate. Data were analysed using the one-way analysis of variance (ANOVA), and means were compared using the Fisher's least significant difference (LSD) test ($p \leq 0.05$) using the Statistical Package for the Social Sciences (SPSS) Version 16.0 for Windows (SPSS Inc., Chicago, USA).

Results and Discussion

Effect of storage period on the colour of rice-Bambara *masa* flour

The objective colour parameters of the freshly made and stored rice-Bambara groundnut *masa* flour blends are presented in Table 1. Lightness (L) values (83.99–85.81) for the flour blends containing Bambara groundnut flour were generally lower than that of the control sample (88.11) without Bambara groundnut (Table 1). Although the L values of the enriched samples were lower than the control, the addition of Bambara groundnut up to 20% level did not significantly ($p < 0.05$) change the colour of the samples. The L value result suggests that the *masa* flour without Bambara was whiter in appearance, which could be attributed to variation in the composition of the grains. Enriched *masa* flour samples showed a significant decline in all the colour values (L, a and b) with an increase in the storage period (Table 1), which could be due to the Maillard reaction in the stored flour (Ward et al., 1998; Yeboah-Awudzi et al., 2018). This seems plausible since the presence of protein from Bambara grains and carbohydrate in the *masa* flour may facilitate the Maillard reaction. Previous studies similarly observed a decline in the colour of maize with an increase in the storage period (Paraginski et al., 2014).

Table 1. Colour of rice-Bambara groundnut *masa* flour blend stored for 0 and 8 weeks.

Sample	Week 0			Week 8		
	L	a	b	L	a	b
$R_{100}B_0$	$88.11^{a}\pm1.37$	$7.21^{b}\pm0.05$	$-1.08^{bc}\pm1.45$	$77.29^{a}\pm2.82$	$1.20^{d}\pm0.03$	$-3.75^{a}\pm0.64$
$R_{95}B_5$	$85.42^{b}\pm1.33$	$7.02^{b}\pm0.12$	$-1.06^{c}\pm0.21$	$73.91^{b}\pm2.93$	$1.41^{bc}\pm0.05$	$-2.65^{b}\pm0.33$
$R_{90}B_{10}$	$85.81^{b}\pm1.92$	$7.46^{a}\pm0.14$	$0.05^{a}\pm0.53$	$75.14^{ab}\pm1.13$	$1.76^{a}\pm0.06$	$-0.17^{a}\pm0.45$
$R_{85}B_{15}$	$83.99^{b}\pm1.51$	$7.02^{b}\pm0.10$	$-0.16^{b}\pm0.62$	$77.97^{a}\pm2.81$	$1.56^{b}\pm0.16$	$0.28^{a}\pm0.70$
$R_{80}B_{20}$	$85.24^{b}\pm0.94$	$7.51^{a}\pm0.15$	$1.59^{a}\pm0.06$	$74.92^{ab}\pm0.59$	$1.46^{bc}\pm0.11$	$-0.42^{a}\pm0.30$

Values are means ±SD (N=3). Means with different superscript letters are significantly different across columns ($p < .05$). $R_{100}B_0$: 100% of rice and 0% of Bambara; $R_{95}B_5$: 95% of rice and 5% of Bambara; $R_{90}B_{10}$: 90% of rice and 10% of Bambara; $R_{85}B_{15}$: 85% of rice and 15% of Bambara; $R_{80}B_{20}$: 80% of rice and 20% of Bambara.

Effect of storage period on proximate composition of rice-Bambara *masa* flour

The proximate composition of freshly prepared and stored *masa* flour from blends of rice and Bambara flour is shown in Table 2. Carbohydrate was the major nutrient in the freshly prepared *masa* flour (75.43–82.57%) and stored flour samples (74.13–81.19%). Other nutrients such as ash (0.50–0.87%), fat (0.41–0.95%), fibre (4.28–6.03%) and protein (6.80–9.74%) were generally low (Table 2). Bambara groundnut flour addition to rice flour increased the ash, fibre, fat, and protein contents in the composite flour but decreased the carbohydrate content. The protein content of the composite flour increased by approximately 15%, 21%, 33% and 42% when adding 5%, 10%, 15% and 20% of Bambara groundnut flour, respectively. The increase in protein is expected since legumes, including Bambara groundnut, are known to be good sources of protein (Oyeyinka and Oyeyinka, 2018). This increase in protein represents a good strategy to improve the nutritional intake of *masa*-loving individuals and could further help reduce protein-energy malnutrition prevalent in Africa, including Nigeria.

Table 2. Proximate composition of rice-Bambara groundnut *masa* flour blend stored for 0 and 8 weeks.

Parameters	Week 0					
	$R_{100}B_0$	$R_{95}B_5$	$R_{90}B_{10}$	$R_{85}B_{15}$	$R_{80}B_{20}$	$R_{100}B_0$
Moisture	5.38^{b}±0.36	6.66^{a}±0.00	6.72^{a}±0.02	6.96^{a}±0.01	7.06^{a}±0.04	5.38^{b}±0.36
Fat	0.41^{d}±0.00	0.55^{c}±0.01	0.62^{c}±0.01	0.87^{b}±0.00	0.95^{a}±0.07	0.41^{d}±0.00
Ash	0.50^{c}±0.01	0.61^{b}±0.00	0.66^{b}±0.02	0.68^{a}±0.00	0.74^{a}±0.04	0.50^{c}±0.01
Protein	6.84^{e}±0.03	7.84^{d}±0.02	8.26^{c}±0.01	9.07^{b}±0.28	9.74^{a}±0.01	6.84^{e}±0.03
Fibre	4.28^{a}±0.02	4.44^{b}±0.06	4.62^{c}±0.04	5.25^{d}±0.05	6.03^{e}±0.02	4.28^{a}±0.02
CHO	82.57^{e}±0.37	79.88^{d}±0.02	79.12^{c}±0.04	77.36^{b}±0.09	75.43^{a}±0.02	82.57^{e}±0.37
Parameters	Week 8					
	$R_{100}B_0$	$R_{95}B_5$	$R_{90}B_{10}$	$R_{85}B_{15}$	$R_{80}B_{20}$	$R_{100}B_0$
Moisture	6.50^{b}±0.02	8.47^{a}±0.07	8.54^{a}±1.10	8.84^{a}±0.43	8.37^{a}±0.24	6.50^{b}±0.02
Fat	0.45^{c}±0.02	0.56^{c}±0.00	0.71^{b}±0.02	0.90^{a}±0.01	0.94^{a}±0.05	0.45^{c}±0.02
Ash	0.56^{d}±0.00	0.66^{c}±0.01	0.81^{b}±0.01	0.84^{ab}±0.01	0.87^{a} ±0.02	0.56^{d}±0.00
Protein	6.80^{e}±0.00	7.86^{d}±0.00	8.27^{c}±0.02	9.06^{b}±0.01	9.74^{a}±0.01	6.80^{e}±0.00
Fibre	4.36^{d}±0.00	4.46^{d}±0.01	4.62^{c}±0.03	5.17^{b}±0.08	5.98^{a}±0.04	4.36^{d}±0.00
CHO	81.19^{a}±0.04	77.97^{b}±0.06	77.04^{b}±1.11	75.12^{c}±0.50	74.13^{c}±0.28	81.19^{a}±0.04

Values are means ±SD (N=3). Means with different superscript letters are significantly different across columns ($p < .05$). $R_{100}B_0$: 100% of rice and 0% of Bambara; $R_{95}B_5$: 95% of rice and 5% of Bambara; $R_{90}B_{10}$: 90% of rice and 10% of Bambara; $R_{85}B_{15}$: 85% of rice and 15% of Bambara; $R_{80}B_{20}$: 80% of rice and 20% of Bambara; CHO: Carbohydrate.

The storage of the flour in a high-density polythene bag at room temperature (25±2^{o}C) for a period of 8 weeks did not substantially alter the composition of the flours, except for the moisture values (Table 2). The moisture content of the flours

increased significantly ($p < .05$), suggesting that the polythene bags were not impermeable to water vapour. Similar results were reported for Bambara and cowpea snacks for a period of 4 weeks (Oyeyinka et al., 2018). The ash, fibre, fat and protein contents were almost constant, while the carbohydrate content of the flours reduced with an increase in the storage period. The proximate results were within the range reported for rice-Bambara nut flour extruded flakes (Adebowale et al., 2016).

Effect of storage period on selected functional properties of rice-Bambara *masa* flour

The water absorption capacity (WAC) of the flour blend increased with increasing levels of Bambara groundnut (Figure 1). This could be due to higher polar amino acid residues of proteins in the Bambara flour which presumably have more affinity for water molecules (Yusuf et al., 2008).

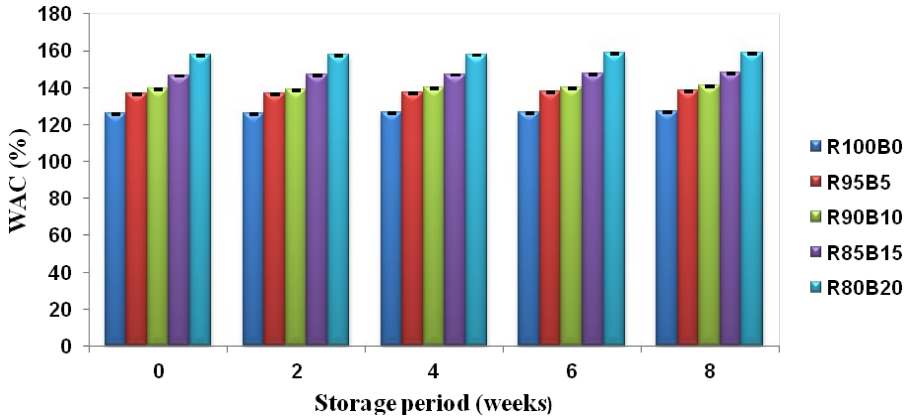

Figure 1. Effect of storage period on the water absorption capacity of *masa* flour blends.

Error bars indicate standard deviation. $R_{100}B_0$: 100% of rice and 0% of Bambara; $R_{95}B_5$: 95% of rice and 5% of Bambara; $R_{90}B_{10}$: 90% of rice and 10% of Bambara; $R_{85}B_{15}$: 85% of rice and 15% of Bambara; $R_{80}B_{20}$: 80% of rice and 20% of Bambara; WAC: Water absorption capacity.

According to Lawal and Adebowale (2004), the major chemical compositions that enhance the WAC of flours are proteins and carbohydrates since these constituents contain hydrophobic part such as polar or charged side chains. Thus, the increased WAC of the blends may also be associated with an increase in the starch content of the flour. This seems plausible since previous studies reported Bambara groundnut to be a good starch source, contributing to increased swelling (Oyeyinka et al., 2015; Oyeyinka et al., 2017). Although the WAC increased

slightly with an increase in the storage period, the increase was not significant ($p \geq 0.05$). The WAC of the samples ranged between 126.08 and 157.74 % (Figure 1), which is similar to the values previously reported for Bambara groundnut during short time storage (Goudoum et al., 2016).

The oil absorption capacity (OAC) of the flour blends (Figure 2) was generally lower than their WAC (Figure 1). The storage period similarly did not significantly ($p < 0.05$) alter the OAC of the flour blends, though there was a slight increase (Figure 2). In general, the OAC decreased with an increase in the percentage of Bambara groundnut flour, suggesting that Bambara groundnut has fewer hydrophobic proteins compared with rice. Although rice is a cereal, its protein has been reported to have surface hydrophobicity, which may increase with heat denaturation (Ju et al., 2001). In addition, earlier studies have indicated hydrophobic proteins have better oil binding properties (Lawal and Adebowale, 2004).

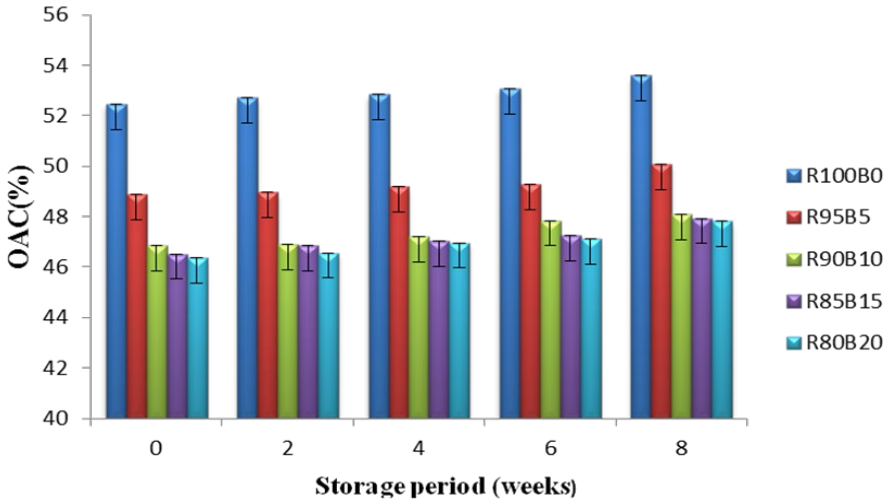

Figure 2. Effect of storage period on the oil absorption capacity of *masa* flour blends.

$R_{100}B_0$: 100% of rice and 0% of Bambara; $R_{95}B_5$: 95% of rice and 5% of Bambara; $R_{90}B_{10}$: 90% of rice and 10% of Bambara; $R_{85}B_{15}$: 85% of rice and 15% of Bambara; $R_{80}B_{20}$: 80% of rice and 20% of Bambara; OAC: Oil absorption capacity. Error bars indicate standard deviation.

The swelling capacity of the flour blends increased with increasing levels of Bambara groundnut flour (Figure 3). However, the swelling index showed an insignificant ($p > 0.05$) increase with an increase in the storage period. The swelling index represents the ability of the flour samples to imbibe water and swell at room temperature. The extent of swelling is determined by the amount of water that starch and protein can absorb before the chains become separated completely

(Chrastil, 1990). Starch is chiefly responsible for swelling, and the degree of swelling depends on the ratio of amylose to amylopectin and the chain length distribution of the amylopectin chain length. Functional properties of cereal and legume flours are important parameters to be considered in developing food products because they contribute to the texture, mouth feel as well as consistency of the product (Bhat and Nabilah, 2014).

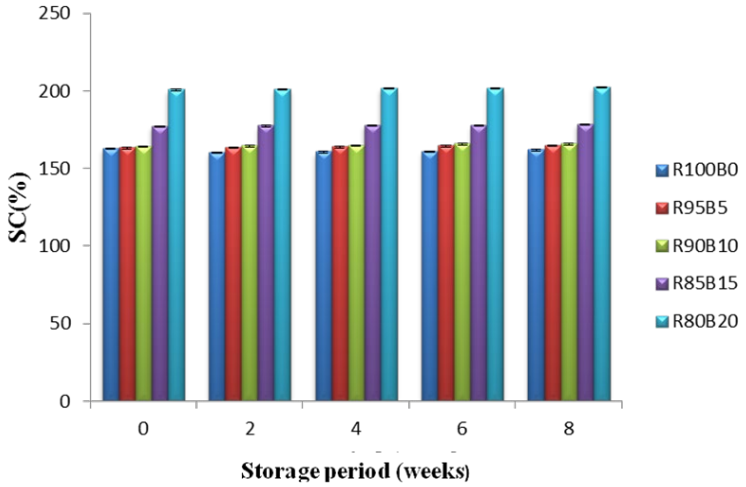

Figure 3. Effect of storage period on the swelling capacity of *masa* flour blends.

$R_{100}B_0$: 100% of rice and 0% of Bambara; $R_{95}B_5$: 95% of rice and 5% of Bambara; $R_{90}B_{10}$: 90% of rice and 10% of Bambara; $R_{85}B_{15}$: 85% of rice and 15% of Bambara; $R_{80}B_{20}$: 80% of rice and 20% of Bambara; SC: Swelling capacity; Error bars indicate standard deviation.

Effect of storage period on the microbial load of rice-Bambara *masa* flour

The total bacterial count (TBC) and total fungal count (TFC) as measured in cfu/g in rice-Bambara *masa* flour samples during storage at room temperature for a period of 8 weeks are presented in Figures 4 and 5, respectively. In general, there was an increase in both TBC and TFC of the flour blends with an increase in the storage period (Figures 4 and 5). Furthermore, the TBC and TFC similarly increased with an increase in the levels of Bambara groundnut. Bambara groundnut is a good source of protein and other nutrients that are needed by microbes for growth. Balogun et al. (2016) also reported an increase in microbial growth with an increase in the storage period of gruel made from fermented maize and soybean. Bacteria seem to grow faster in the flour blends than the fungi. For example, the TBC ranged between 2.1 and 9.05 cfu/g $\times 10^4$, while the TFC ranged between 0 and 3.8 cfu/g $\times 10^4$. These values fall within the range of the maximum permissible level ($>10^4$ to $<10^6$cfu/g) of the total aerobic colony.

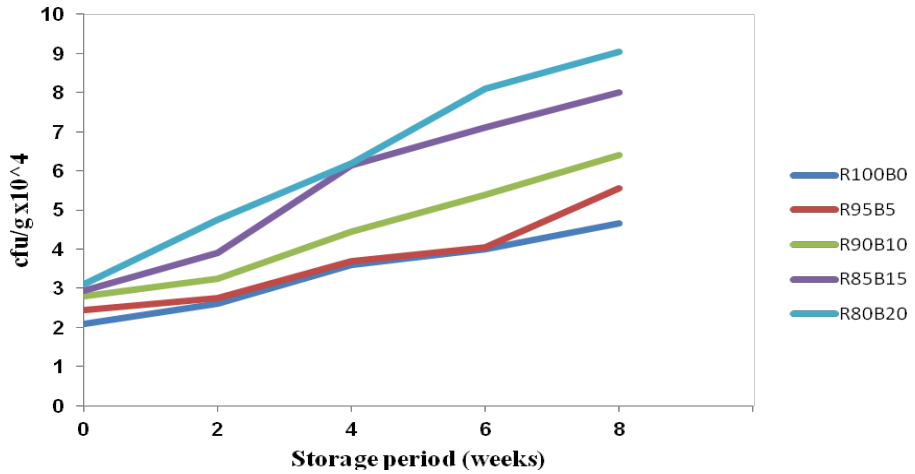

Figure 4. Effect of storage period on the swelling capacity of *masa* flour blends.

$R_{100}B_0$: 100% of rice and 0% of Bambara; $R_{95}B_5$: 95% of rice and 5% of Bambara; $R_{90}B_{10}$: 90% of rice and 10% of Bambara; $R_{85}B_{15}$: 85% of rice and 15% of Bambara; $R_{80}B_{20}$: 80% of rice and 20% of Bambara; SC: Swelling capacity; Error bars indicate standard deviation.

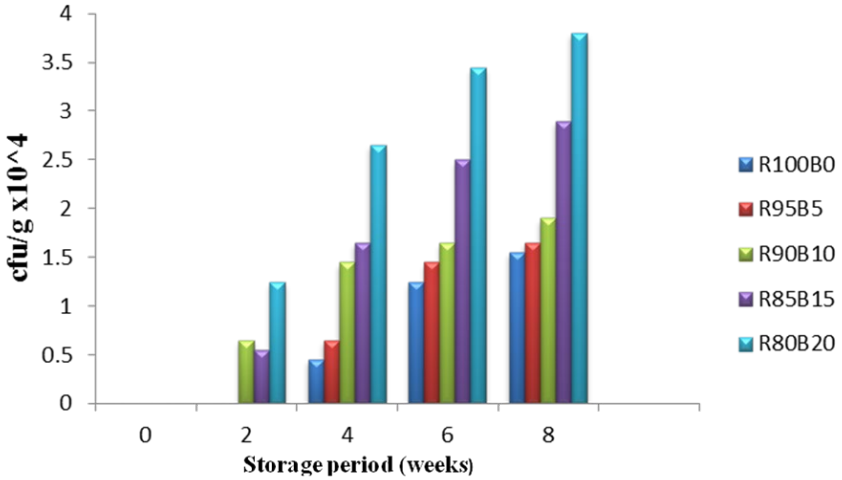

Figure 5. Effect of storage period on the total fungal count of *masa* flour blends.

$R_{100}B_0$: 100% of rice and 0% of Bambara; $R_{95}B_5$: 95% of rice and 5% of Bambara; $R_{90}B_{10}$: 90% of rice and 10% of Bambara; $R_{85}B_{15}$: 85% of rice and 15% of Bambara; $R_{80}B_{20}$: 80% of rice and 20% of Bambara.

Effect of storage period on the sensory properties of rice-Bambara *masa*

Masa samples were prepared from the flour blends, and the sensory quality was assessed using panel members as described above. Mean sensory scores showed that there were no significant differences ($p<0.05$) in sourness, appearance, aroma, and texture, but in taste and overall acceptability among the samples (Table 3). *Masa* flour with 100% of rice had the highest rating for appearance, aroma, taste, texture, and overall acceptability. Panel members consisted of regular consumers of *masa*. Thus, the higher rating recorded for *masa* prepared from 100% of rice flour could be due to the familiarity of the panel members with *masa*. The storage period significantly ($p<0.05$) affected the sensory properties of *masa* (Table 3). The ratings recorded for all the sensory properties reduced significantly with an increase in the storage period. *Masa* prepared from 80% of rice and 20% of Bambara groundnut flour had the highest rating in overall acceptability after the storage period of 8 weeks.

Table 3. Mean sensory scores of rice-Bambara groundnut *masa* flour blend stored for 0 and 8 weeks.

Parameters	Week 0				
	$R_{100}B_0$	$R_{95}B_5$	$R_{90}B_{10}$	$R_{85}B_{15}$	$R_{80}B_{20}$
Sourness	$6.6^{a}\pm1.6$	$6.1^{a}\pm1.4$	$6.7^{a}\pm1.8$	$6.0^{a}\pm1.6$	$6.4^{a}\pm1.7$
Appearance	$7.2^{a}\pm1.6$	$6.7^{a}\pm1.4$	$6.7^{a}\pm1.8$	$6.7^{a}\pm1.9$	$6.9^{a}\pm1.4$
Aroma	$6.9^{a}\pm1.4$	$6.0^{a}\pm1.2$	$6.5^{a}\pm1.4$	$6.9^{a}\pm1.3$	$6.5^{a}\pm1.7$
Taste	$7.3^{a}\pm0.9$	$6.0^{b}\pm1.7$	$6.0^{b}\pm1.6$	$6.6^{a}\pm1.5$	$6.1^{b}\pm1.7$
Texture	$6.6^{a}\pm1.9$	$6.2^{a}\pm1.7$	$6.1^{a}\pm1.9$	$6.1^{a}\pm1.7$	$6.0^{a}\pm1.9$
Overall acceptability	$7.4^{a}\pm1.4$	$7.0^{ab}\pm1.2$	$6.9^{ab}\pm1.5$	$6.7^{ab}\pm1.5$	$6.3^{b}\pm1.5$
Parameters	Week 8				
	$R_{100}B_0$	$R_{95}B_5$	$R_{90}B_{10}$	$R_{85}B_{15}$	$R_{80}B_{20}$
Sourness	$5.2^{b}\pm1.0$	$5.1^{b}\pm1.1$	$5.9^{a}\pm1.1$	$5.8^{ab}\pm0.9$	$5.5^{ab}\pm1.0$
Appearance	$5.1^{b}\pm0.9$	$5.2^{b}\pm1.6$	$5.3^{b}\pm0.9$	$5.6^{ab}\pm1.2$	$6.1^{a}\pm1.2$
Aroma	$4.9^{b}\pm1.5$	$5.6^{ab}\pm1.4$	$5.5^{ab}\pm1.4$	$5.8^{ab}\pm1.2$	$6.4^{ab}\pm1.1$
Taste	$5.2^{a}\pm1.3$	$5.5^{a}\pm1.9$	$5.3^{a}\pm1.3$	$8.6^{a}\pm1.4$	$5.6^{a}\pm1.5$
Texture	$5.5^{a}\pm1.5$	$5.5^{a}\pm1.3$	$5.6^{a}\pm1.9$	$5.4^{a}\pm1.1$	$5.9^{a}\pm1.4$
Overall acceptability	$5.3^{ab}\pm1.8$	$5.3^{ab}\pm1.5$	$4.9^{b}\pm1.6$	$5.5^{ab}\pm0.9$	$6.2^{a}\pm1.5$

Values are means ± SD (N=30). Means with different superscript letters are significantly different across the row ($p < .05$). * Multiple comparison tests were done for week 0 and week 8 separately. $R_{100}B_0$: 100% of rice and 0% of Bambara; $R_{95}B_5$: 95% of rice and 5% of Bambara; $R_{90}B_{10}$: 90% of rice and 10% of Bambara; $R_{85}B_{15}$: 85% of rice and 15% of Bambara; $R_{80}B_{20}$: 80% of rice and 20% of Bambara.

Conclusion

The objective of the study was to determine the physical, chemical, sensory, and microbial qualities of freshly produced and stored *masa* flour from rice and Bambara groundnut flour blends. Bambara groundnut flour addition did not significantly alter the colour of *masa* samples prepared from rice but did change the proximate composition. However, a slight colour change was observed after storage for 8 weeks. There was an improvement in the nutritional value of the masa flour due to the Bambara groundnut addition. Flour functionality improved with the Bambara groundnut addition and did not change significantly with an increase in the storage period. However, the total bacterial and fungal count increased with an increase in the storage period. Although the microbial load was within the range accepted for ready-to-eat foods, better packaging may be required to reduce the proliferation of microorganisms during storage to extend the shelf-life of the *masa* flour. Sensory properties of *masa* were not substantially altered with Bambara groundnut inclusion, but the ratings reduced with an increase in the storage period. This study showed that enriched *masa* flour can be stored for at most 4 weeks before significant changes can be observed in the sensory properties. Conclusively, instant *masa* flour from the rice-Bambara groundnut blend had improved nutritional qualities, with extended shelf life and convenience in terms of handling and less preparation time.

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UTICAJ SKLADIŠTENJA NA FIZIČKE, HEMIJSKE, MIKROBIOLOŠKE I SENZORNE KVALITETE INSTANT BRAŠNA *MASA* DOBIJENOG OD MEŠAVINA PIRINČA I BAMBARA GRAŠKA

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R e z i m e

Masa je tradicionalno fermentisani obrok koji se obično pravi od žita. Cilj ovog istraživanja bio je da se proizvede obogaćeni obrok od pirinča i Bambara graška. Boja, funkcionalna svojstva i mikrobiološki kvaliteti kompozitne mešavine brašna od pirinča i Bambara graška u odnosu 100:0, 95:5, 90:10, 85:15, 80:20, procenjeni su korišćenjem standardnih metoda. Takođe su utvrđena senzorna svojstva sveže pripremljene *mase* i *mase* pripremljene od uskladištenih vrsta brašna. Mikrobno opterećenje mešavine brašna *masa* povećavalo se tokom perioda skladištenja. Sposobnost apsorbovanja vode, sposobnost bubrenja i zapreminske mase povećali su se, dok se sposobnost apsorbovanja ulja smanjivala sa povećanjem perioda skladištenja. Rezultat merenja boje pokazao je smanjenje vrednosti svetline (L*). Senzorna svojstva *mase* nisu bila suštinski izmenjena uključivanjem Bambara graška, ali su ocene smanjene produžavanjem skladištenja. Instant *masa* se može pripremiti od brašna skladištenog tokom 4 nedelje, a da pri tom ne dođe do značajnih promena u kvalitetu.

Ključne reči: *masa,* pirinač, Bambara grašak, skladištenje, mikrobni.

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TESTING THE EFFECTS OF THE PRESENCE OF URANIUM IN DRINKING WATER FROM INDIVIDUAL WELLS IN THE VILLAGE OF DUBRAVICA IN THE BRANIČEVO DISTRICT ON PUBLIC HEALTH

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Abstract: The village of Dubravica is partially located over the western lignite deposits of the Kostolac basin. The examination of the dry residue obtained from drinking water from two individual wells by X-ray diffraction analysis, based on a typical peak, showed the presence of uranium in drinking water. The indirect method by Rajković and associates showed that, in sample I, the concentration of uranium in drinking water was 85.5 percent higher (3.71 μg/L) and that the concentration of uranium in sample II was only 22 percent lower (1.56 μg/L) than the Maximum Allowable Concentration (MAC) values required by the Regulations (2 μg/L). Analysis of the result of the introduction of uranium in the human body has shown that this way brings 0.84 to 2 mg of uranium in the human organism per annum or 0.09 to 0.22 mg of uranium is deposited annually in the kidney. Assessment of the potential health risk due to the presence of uranium in drinking water indicated that the population using drinking water from wells will be threatened by uranium in a short time interval. Regarding the long-term risk, the calculation has indicated that in the first sample of drinking water, about 25 inhabitants, and in the case of the second sample of drinking water, 10 inhabitants out of 1000 inhabitants are endangered. As the kidney is the organ in which uranium is deposited (accumulated) to the greatest extent, its presence causes weakening and failure of kidney function, which can destroy 75 percent of kidney function until the manifestation of the first clinical symptoms.

This phenomenon is observed among the population along the rivers the Kolubara, the Drina, the Sava and the Morava and is called endemic nephropathy.

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The elements found in trace amounts (Pb, Cd, Si), live agents (bacteria and viruses), fungal plant toxins, genetic factors and immune mechanism can be listed as possible causes. However, uranium in drinking water has not been listed so far.

The tests performed in this study clearly show the role of uranium in the epidemic, endemic nephropathy, which is growing and which is not at the acute phase of the disease but has already progressed to renal failure and end-stage kidney disease. Official data on the rise of endemic nephropathy and diabetes and increasing their share in diseases, as well as overall mortality rates, which amount to 18.19%, clearly indicate that the role of uranium in the Braničevo district environment should not be ignored. Since there are settlements on the terrain to be investigated, uranium and its migration through the environment must be monitored as all conditions for its migration are unfortunately favourable.

Key words: drinking water, heavy metals, uranium, endemic nephropathy.

Introduction

Population irradiation by natural radionuclides from drinking water is generally small and occurs due to the dissolution of radionuclides of the uranium and thorium series. One year of consuming drinking water must not contribute more than 5% of the average total irradiation of the population. To evaluate the similarity of drinking water from the radiological point of view, it is necessary to determine the concentration of certain radionuclides in drinking water for the recommended reference dose level of 0.1 mSv (Official Gazette of RS, 2018). Below this reference dose level, water can be used for drinking (for human purposes).

The first edition of recommendations on drinking water quality radiological aspects was printed in 1984 (WHO, 1984). Tolerant levels of radioactive substances in drinking water, recommended in the first edition of the WHO recommendations, were based on, at that moment, the available information on the degree of risk of exposure to ionizing radiation, concentrated mainly in the International Commission on Radiological Protection (ICRP) Publication 26 (ICRP, 1977). According to the ICRP issue, the WHO recommends radiological aspects of drinking water quality (ICRP, 1990). Also, in a separate chapter of a later release of recommendations (WHO, 1993), the WHO discusses the radiological aspects of drinking water quality and establishes methods for quality checking and tolerant levels of some radionuclide content or the total content of α- and β-non stabile radionuclides, from natural and anthropogenic origins.

The recommended reference values for α-unstable radionuclides in drinking water are 0.1 Bq/L and for unstable β-unstable isotopes – 1 Bq/L. Higher values of the specific activity of α- and/or β-unstable radionuclides do not automatically mean that water should not be used for drinking (Official Gazette of FRY, 1998).

The dose of irradiation of the organism from radionuclides in water depends on the amount of the radionuclides and their metabolism and kinetics in the body. Therefore, the calculation of the tolerant (allowed) concentration of radionuclides in drinking water is based on the total amount of radionuclides taken into the body in one year, with consumption of 2L of water per day, taking into account the parameters of metabolism in an adult – *reference man* (ICRP Publication 23, 1975; Papić et al., 2012).

Maximum levels of radioactive contamination of drinking water were determined according to the Annual limits of intake of radionuclides (ALI) in the human body by ingestion and derived concentrations (IC) (Zamora et al., 1998; Official Gazette of RS, 2018). The method of calculating the value of uranium in drinking water is given in Rajković et al. (2008a).

In drinking water, the concentration of individual radionuclides and an unknown (or partially known) mixture of radionuclides must not be higher than the calculated concentration of radionuclides in the environment for 12 consecutive months.

Uranium migration through the environment

From a physical-chemical aspect, the term heavy metal includes metals whose density is higher than 5 g/mL or the regular (atomic) number greater than 20. In addition, the term is more often used for toxic metals, i.e. elements that exert their toxicity at low concentrations. One such metal is uranium (the regular number of 92), the last element found in nature, a heavy metal and a toxic and radioactive element.

Uranium occurs in nature as a mixture of three isotopes: uranium-238 (^{238}U), uranium-235 (^{235}U) and uranium-234 (^{234}U). The prevalence of these isotopes in nature is as follows: ^{238}U (99.282%), ^{235}U (0.712%) and ^{234}U (0.006%). All uranium isotopes are radioactive with a half-life: ^{238}U – $4.5 \cdot 10^9$ years, ^{235}U – $7.07 \cdot 10^8$ years and ^{234}U – $2.5 \cdot 10^5$ years.

Uranium and its isotopes at the same time are radioactive and highly chemically toxic elements. Toxicity of uranium consists of two toxicokinetic mechanisms: the first is non-radiation, chemical toxicity, characterised by heavy metals such as Pb, Hg, Cd and Bi, and the second is ionisation due to α-emissions (radiation), and other natural and artificial radionuclides (ATSDR, 1997). Uranium radioactivity can cause the problems to the human body, such as cancer which can be manifested several years after exposure to uranium. However, the higher risk is of its chemical toxicity, which manifests itself in a very short period of time (weeks or months) after contact with it (for example, kidney, leukaemia, etc.) (Domingo, 1995).

Soluble uranium shows the same chemical toxicity as soluble lead (Rajković, 2001). In this sense, the impacts of uranium and its chemical effect as a toxic element are limited through the following concentrations (Domingo et al., 1987; Domingo, 1995; Maynard et al., 1953; NIOSH, 1994):

- in the air – 0,01 mg/L (0.01 ppm);
- water – $2 \cdot 10^{-3}$ mg/L (0.002 ppm or 2 μg/L);
- lethal dose is 100 mg/kg body weight (BW).

Uranium in drinking water originates from natural sources: the lithosphere (3–4 ppm), volcanic rocks (0.1–5 ppm), sedimentary rocks (0.5–4 ppm), phosphate rocks (30–300 ppm) and soil (1–4 ppm) (Harmsten and DeHaan, 1980); or it is of anthropogenic origin: from different industries (mining, metal smelting, metallurgy, chemical industry, etc.), uncontrolled use of organic and mineral fertilisers and pesticides from sewage sludge.

After 1999, the drinking water used in our country could contain the uranium originating from the ammunition with depleted uranium (DU) used in the NATO bombing (Rajković, 2001). According to official data, about 112 strikes were carried out, of which 98 were in Kosovo and Metohija, 12 in southern Serbia, and two in Montenegro. Of that, 60 percent were civilian targets. In Kosovo, the area of Podujevo with the surrounding villages, the area around Kosovska Mitrovica, the part around Dečani, Đakovica, Prizren and other places were most affected. According to the data of our military experts, a total of 8,112 bullets with depleted uranium were fired (Zaric et al., 2001), while the exact number of fired bullets was never determined with certainty.

Although it is different toxicity of these two forms of uranium, the effect of the presence of depleted uranium in drinking water seems the same as natural uranium – it is a risk to human health (Rajković and Đorđević, 2006).

The migration potential of uranium depends on the physical and chemical properties of soil and soil solution and the oxidation product of uranium. The mobility of the dissolved uranium products predominantly affects pH value, Eh and the presence of complex organic and inorganic agents in a local groundwater area.

Hexavalent uranium U(VI) exists in solution as uranyl ion (UO_2^{2+}) and it is more mobile than four-valent uranium U(IV), as it easily builds soluble complexes with ligands present in the soil solutions. The presence of carbonate and phosphate is also influenced by these processes. The transport of the soluble forms of uranium can be affected by dilution, as this reduces its concentration in groundwater and surface water. These reactions include ion exchange and specific adsorption of uranium organic substances, mineral clays, Fe(III) and hydroxide present in the soil.

The lifecycle of uranium in the environment and the potential risk to human health are shown in Figure 1.

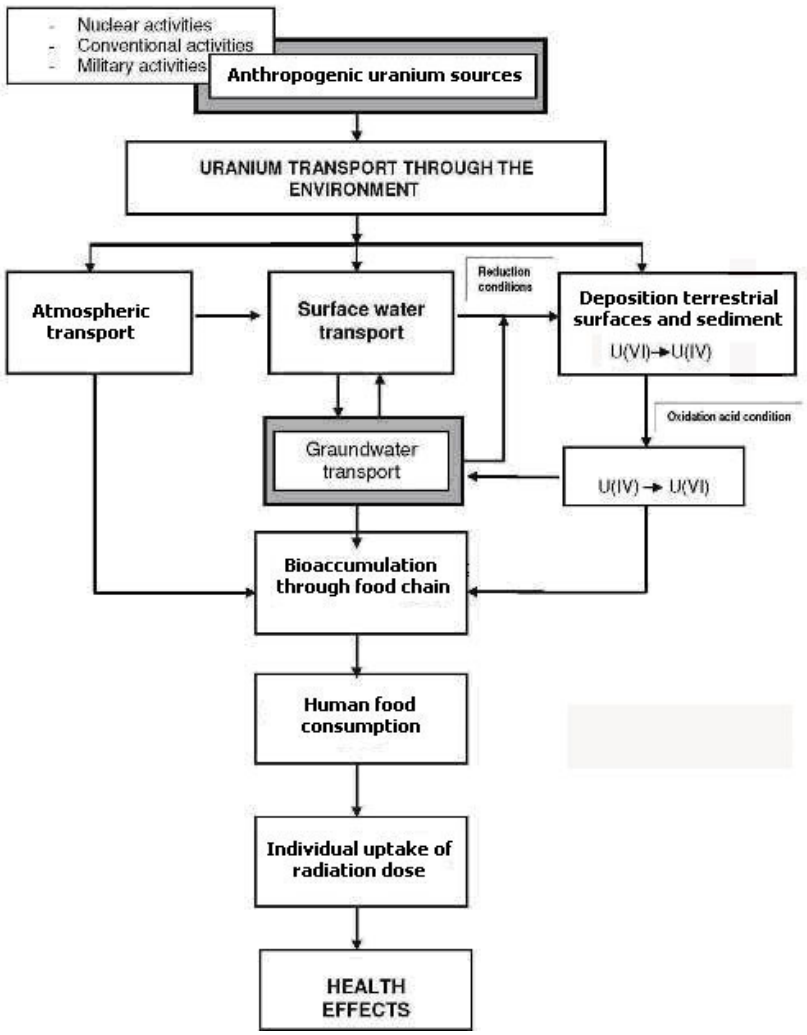

Figure 1. The life cycle of uranium in the environment (Stojanović et al., 2012).

The solubility of natural uranium occurs in two phases (Dong et al., 2006; Laue et al., 2004):

The first phase, the oxidation of metallic uranium to uranium dioxide or uranium(IV) oxide, which builds up in the natural mineral, *uraninite* (UO_2) according to the equations (1) and (2):

$$U_{(s)} + 2H_2O \rightarrow UO_{2(s)} + 2H_2 \quad (1)$$

$$U_{(s)} + O_2 \rightarrow UO_{2(s)} \quad (2)$$

The second phase, the oxidation of uranium dioxide to uranium trioxide, according to equations (3) and (4):

$$UO_{2(s)} + 2H^+ + 1/2O_2 \rightarrow UO_2^{2+}{}_{(aq)} + H_2O \quad (3)$$

$$UO_{2(s)} + 1/2O_2 + 2H_2O \rightarrow UO_3 \cdot 2H_2O \quad (4)$$

The first phase is favourable from the aspect of environmental protection because it results in insoluble products, which, under certain soil conditions (pH value and redox potential), pass to the second phase, which leads to the formation of soluble kinds of products, such as uranyl ions (equation 3) and mineral *schoepite* ($UO_3 \cdot 2H_2O$) (equation 4). $UO_3 \cdot 2H_2O$, under certain conditions, can dismiss UO_2^{2+} ions, which are easily transported and the territorial solutions are included in the food chain (equation 5):

$$UO_3 \cdot 2H_2O_{(s)} + 2H^+ \rightarrow UO_2^{2+}{}_{(aq)} + 3H_2O \quad (5)$$

Uranium oxidised in nature due to the inflow of oxygen, or a rise in its fugacity. The oxygen affinity is such that the first hydrogen sulfide oxidises to sulfate ion, uranium(IV) to uranium(VI), and only at a higher redox potential, Fe(II) to Fe(III), according to equations 6–8:

$$H_2S + 4H_2O \rightarrow SO_4^{2-} + 10H^+ + 8e^-, \quad E^0 = 0{,}303V \quad (6)$$

$$U^{+4} + 2H_2O \rightarrow UO_2^{2+} + 4H^+ + 2e^-, \quad E^0 = 0{,}327V \quad (7)$$

$$Fe^{2+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+ + e^-, \quad E^0 = 1{,}06V \quad (8)$$

The process of precipitation of uranium by reduction is of significant importance since it excludes the uranium from aqueous streams and therefore suspends its expansion process through the environment. The reduction of mobile ions of uranium (U^{6+}) to insoluble forms of uranium (U^{4+}) is carried out when the fugacity of oxygen is declined in the solution so that the above reaction takes place at the expense of the oxidation of iron and sulfur. If there is more Fe^{2+} than oxygen, the oxygen will run out, and uranyl ion will be used as an oxidising agent, which transforms (oxidises) Fe^{2+} to Fe^{3+}, or sulfide to sulfate. Also, uranyl ion reduces to UO_2, as shown in equations 9–11 (Milačić et al., 2004):

$$UO_2^{2+} + 2Fe^{2+} + 3H_2O \rightarrow UO_2^0 + Fe_2O_3 + 6H^+ \quad (9)$$

$$2Fe^{2+} + 1/2O_2 + H_2O \rightarrow FeO(OH) + H^+ \quad (10)$$

$$2UO_2^{2+} + S^{2-} + 4H_2O \rightarrow 2UO_2^0 + SO_4^{2-} + 8H^+ \quad (11)$$

Experiment

In the municipality area of Požarevac, there are three ways of the water supply of the population (Official Gazette of Požarevac, 2012):

1. Central waterpipe supplies in the cities of Požarevac and Kostolac, and rural areas such as Ćirikovac, Klenovnik, Stari Kostolac, Drmno and Bradarac;
2. The supply of local water facilities – public fountains;
3. Individual supplies from wells in the rest of settlements.

For verification the safety of drinking water, in our earlier works are presented and sampled models of drinking water from individual wells from the village of Dubravica in the territory of the city of Požarevac in the Braničevo district (Rajković et al., 2014; Milojković, 2014). Basic and target tests comprised examining drinking water samples taken from rural areas of the Braničevo district, where it is determined whether the water is physico-chemically and microbiologically correct and to determine the presence of heavy metals in drinking water (Rajković et al., 2017).

With a control method of testing the quality of drinking water, additional water tests from the village of Dubravica were carried out. Two samples from different wells are examined by the indirect method for determining toxic elements (heavy and light metal) in drinking water, proposed by Rajković and associates (Rajković et al., 2008b; Rajković et al., 2009). Testing has proved their presence in drinking water, and heavy metals, such as Fe, Ti, Pb, Cd and Cr are found at a concentration that exceeds the Regulations (Official Gazette of FRY, 1998; Rajković et al., 2015a).

According to the Regulation (Official Gazette of the FRY, 1998), drinking water may not contain radionuclides, so that their presence in normal conditions without accidents or incidents is challenging to prove (Official Gazette of FRY, 1999). In drinking water from individual wells from the village of Dubravica revealed the presence of uranium (Rajković et al., 2015b), which is in one sample approaching the allowable concentration envisaged by Regulation on hygienic quality of drinking water (EPA, 2009; Official Gazette of FRY, 1998).

One of the work tasks was to fortify the concentration of uranium in drinking water from individual wells from the village of Dubravica. Since it is a radioactive element, and highly toxic substance, in this paper, we analyse the consequences of the presence of uranium in drinking water on human health caused by long-term consumption of these waters.

Location

The village of Dubravica is located north-west of Požarevac at 75 m above sea level at 44°41'13.8" north black latitude (N) and 21°4'21.6" east longitude (E) beyond the Danube coastal zone in the municipality of Požarevac (Braničevo district).

According to morphological characteristics, the Dubravica village is a lowland type, belonging to the Danube villages. Water supply in the village is provided through the local water supply and channelling wastewater, mainly through septic tanks (Official Gazette of Požarevac, 2012).

Sampling methods, tests and the interpretation of the results are in compliance with Regulations on hygienic safety of drinking water (Civil Engineering Faculty,

2006; Official Gazette of FRY, 1998). Sample I was taken from the location in the centre of a densely populated village at a distance of about 2.5 km from the Danube, while sample II was taken from the entrance to the village from the direction of Požarevac with a distance of about 1 km in relation to sample code I, and about 3.5 km from the Danube. Both wells are at the same depth of 12 m.

Materials and Methods

A sample of scale formed by precipitation on a water-heater surface during a long period of time has been used in this research. The content of all solids, which actually represents scale, has been determined by boiling 1.0 dm^3 of drinking water to obtain the corresponding dry residue. The composition of scale was determined using an atomic absorption spectrophotometer the Perkin-Elmer AAnalystModel 300, according to SRPS B.B8.070 (by methods DM 10 – 0/4, 0/6, 0/7, 0/8, 0/9, 0/10, 0/11, 0/12, 0/13, and 0/17) (the standard method of ITNMS).

The share of the elements, in the form of compounds or the elemental form, in %, is compared with the Maximum Allowable Concentration (MAC) of inorganic substances in water, which are prescribed by the Regulative (Official Gazette of the FRY, 1998) and the Act provided for bottled water for drinking and natural disasters (Official Gazette of the FRY, 1999).

With a control method of testing the quality of drinking water, additional water tests from the village of Dubravica were carried out. Two samples from different wells were examined by the indirect method of determining toxic elements (heavy and light metal) in drinking water, proposed by Rajković and associates (Rajković et al., 2008b; Rajković et al., 2009).

To determine the presence of uranium in drinking water, an X-ray diffraction analysis of scale was carried out. The scale originating from drinking water was previously milled into a fine powder fraction which was further examined. The difractogram was recorded with the Energy Dispersive X-Ray Fluorescence (EDXRF) by the MiniPal 4 X-Ray Fluorescence Spectrometer.

The quantitative content of uranium was determined by the fluorimetric method based on the linear dependence with fluorescence intensity of uranium compounds from their concentration (Anonym, 2004). There was a linear dependence for a vast range of low concentrations (about four orders of magnitude). The decrease in fluorescence intensity was reduced to a minimum using “standard addition” after the extraction of uranium with the synergistic alloy TOPO (tri-n-octyl phosphine oxide) – ethyl acetate.

The fluorescence intensity was measured by the fluorometer 26-000 Ash Jarrah Division (Fisher Scientific Company, Waltham, 1978). It should be noted that the fluorimetric method only gives information about the mass of the uranium

isotope U-238 in the sample and the extrapolation to the total activity of natural isotopes U-234 and U-235 is unreliable (Stojanović and Martinović, 1993).

Results and Discussion

The diffractograms of tested scales (Figures 2 and 3) proved the presence of uranium, which was detected in our previous work (Rajković et al., 2015b).

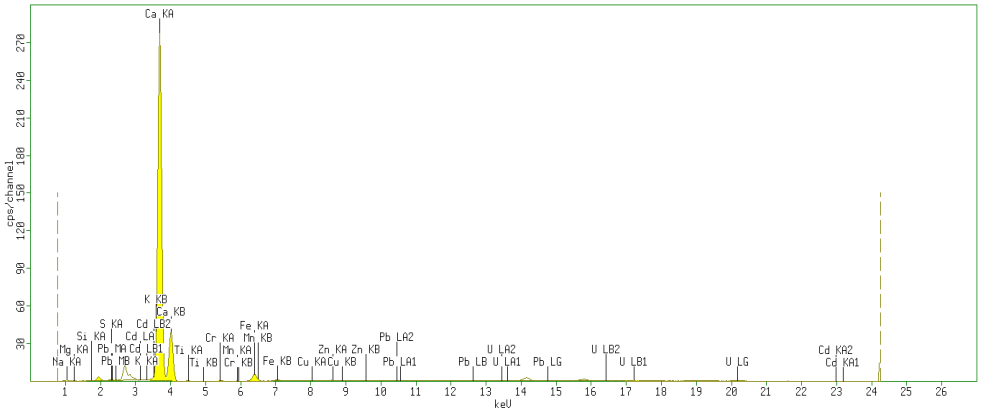

Figure 2. The diffractogram of the scales formed in sample I of drinking water.

On the diffractogram of scale, the pick dots are at 13, 13.5, 16, 17 and 20 keV, which clearly indicates the presence of uranium in the drinking water.

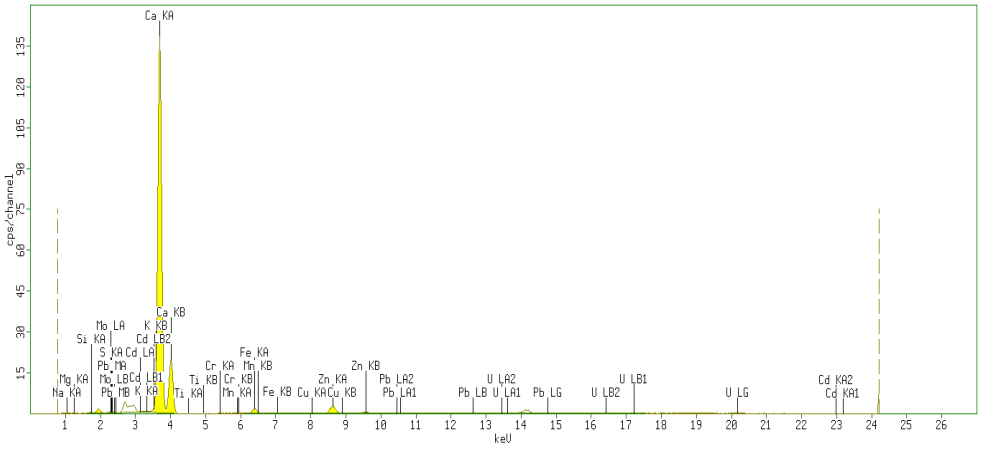

Figure 3. The diffractogram of the scale formed in sample II of drinking water.

Results of the tests of urine composition by this process obtained in our previous paper (Rajković et al., 2015a; Rajkovićet al., 2015b) have indicated the presence of uranium (probably isotopes ^{234}U, ^{235}U, ^{238}U) at concentrations shown in Table 1. They are compared with the maximum permissible concentration of uranium in drinking water based on literature data of 0.002 ppm or 2 μg/L (Domingo et al., 1987; Domingo, 1995; Maynard et al., 1953; NIOSH, 1994).

Table 1. The presence of uranium in the drinking water from individual wells from the village of Dubravica, calculated on the basis of the scale composition.

Scale sample	Uranium (ppm)	The calculated concentration by weight in drinking water (μg/L) (Rajkovićet al., 2015b)	The MAC value in drinking water (ppm or μg/L) (Domingo et al., 1987; Domingo, 1995; Maynard et al., 1953; NIOSH, 1994)
Sample I	8.84	3.71	$2 \cdot 10^{-3}$ mg/L
Sample II	3.29	1.56	(0.002 ppm or 2 μg/L

where: MAC – *Maximum Allowable Concentration.*

Based on the data from Table 1, it can be seen that in the case of the first water sample, this value was exceeded 1.855 times, and in the case of the second sample of water, this value was only 22% smaller. This means that one must be careful about the possible consequences of the long-term use of this water for drinking.

The human organs primarily affected by uranium are kidneys (Harley et al., 1999; Rostker, 1998). They retain the uranium input ranging from 0.05% to 12% with a period of elimination from 6 to 1500 days. Uranium is highly toxic to the kidney, while its radiation effect at a single intake is fragile. Therefore, long-term ingestion of uranium present in drinking water by the people does not mean that it will lead to losing kidney function or chronic disease. Still, it is undoubtedly the first step that leads to progressive and irreversible kidney damage (ATSDR, 1997).

People exposed to uranium have a higher risk of renal disorder than those who are not exposed. Also, before any changes in kidney function are observed, 25% of kidney function can be lost, and more than 75% can be lost before showing severe clinical symptoms (Rajković, 2002).

The earliest recorded data on the maximum permitted concentration of uranium in the kidney, which does not cause a significant deviation from the normal functioning of kidneys, is 3 mg/kg (i.e. 3 ppm) (Rajković et al., 2014). An extensive study of the state laboratory in Oak Ridge recommended maximum concentration of 300 μg/kg (0.3 ppm) of uranium to which the people can be exposed (Edwards, 1999). Because of the chemical toxicity of uranium in soluble form, the amount of uranium of any isotopic composition should not exceed any daily limit of 150 mg of uranium inputted into the body of water and food

(ingestion) (Official Gazette of SFRJ, 1987). For a one-time introduction and timely treatment, changes are reversible (Rajković et al., 2003).

Uranium primarily affects the proximal tubule in the kidney. The dissolved complex of uranyl carbonate decomposes in this acidic environment on the uranyl and hydrogen carbonate ions. As Hg, Cd, and other heavy metals, uranyl ions reduces glomerular function, secretion of organic anion tubes, and reabsorption of filtered glucose and amino acids in the proximal wrapped tube (Harley et al., 1999; DOE, 1999). The dissolved uranyl ion, similar to the ions of heavy metals (e.g., Hg and Cd), reacts with outstretched chelate compounds in the form of a relatively stable and inert complex. These inert complexes were then filtered and extracted in the urine (Figure 7).

Uranium can be detected in the urine for several years (even after 7 years) after ingestion (intake), but when ingesting small doses, it is best to perform a urine test for the first six months, a maximum of one year.

Complete necrosis and renal tubules of the nephron occur in the chronic exposition. Kidney insufficiency occurs slowly. Damages occur on the first tubular cells and then the glomerulus. As the tubular cells regenerate very quickly, but at the same time, they often change (become atypical), the sensitivity of these structures changes (Miler et al., 1998; Hendee and Edwards, 1996; McDiarmid et al., 2000).

Soluble uranium absorbed into the blood circulates through the body and is quickly eliminated through the kidneys into the urine. About 67% of uranium is excreted on the first day without its deposit in the body. About 11% of the initial amounts are deposited in the kidney and excreted with a half-life of 15 days. Out of the remaining 22%, most of it is deposited in the bones (more than 20%), which are the main storage of uranium in the body, and the rest is distributed to other organs and tissues of a man (Rajković et al., 2003) (Figure 4).

The accumulation of uranium in the bones and other organs is consequently returned to the bloodstream with at least two different half-lives, both longer than the half-life for kidney extraction (ICRP, 1978; Arlt et al., 2002). Since uranium retains in the bones for a long time, it causes significant radiation effects. U(IV) is accumulating in kidney and bone tissue. In other tissues (liver, pancreas, and spleen), 0.03 to 12% of ingested uranium, as U (VI), is bound before redistribution in the kidney and skeletal system. Erythrocytes are the most vulnerable in the blood. Due to membrane damage, their lifespan is significantly shortened (Duraković, 1999).

Due to the toxic effects of radiation, pancytopenia (decreased number of blood cells) occurs, causing infection, fever, spontaneous bleeding from the mucous tissues and later from internal organs, anaemia, weakness, fatty degeneration of the centrilobular liver necrosis (Hendee and Edwards, 1996; Mc Diarmid et al., 2000). In the chronic exposition, neurologic disorders (vertigo, loss of balance) appear.

After a very long period (20–25 years), late effects – malignant changes, osteosarcomas, leukosis, tumours of the liver – occur. Potential mutagenic effects of uranium are still unknown, but it is experimentally demonstrated that the content of uranium in urine is positively correlated with urinary mutagenic effects (Miller et al., 1998).

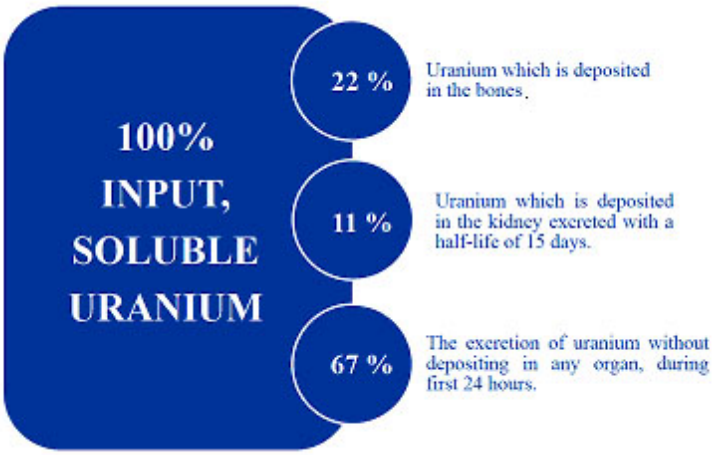

Figure 4. The *flowsheet* diagram of the distribution of uranium in the human body (Rajković et al., 2008a).

Based on the data in Table 1, the input of uranium in the human organism based on the consumption of both samples of drinking water on daily, monthly and annual bases is calculated (Table 2).

Table 2. The input of uranium in the human organism based on the consumption of drinking water.

Scale sample	One glass of water (μg U)	Daily intake (μg U)	Monthly intake (μg U)	Annual intake (mg U)
Sample I	0.74	5.56	166.95	2.00
Sample II	0.31	2.34	70.2	0.84

The data in Table 2 were calculated based on the data that the volume of one glass is 0.2L, and assuming that the person normally drinks 10 glasses of water daily – 1.5L of water (Official Gazette RS, 2018). The calculation is based on the content of the corresponding element in the dry residue. Therefore, the monthly intake is based on 30 days, and the annual intake is based on 12 months (365 days).

Based on the intake of uranium through drinking water (Tables 1 and 2), the distribution of the uranium in the human body can be calculated (Table 3).

Table 3. The distribution of uranium in the human body based on the consumption of drinking water.

Sample	The precipitated uranium in the bones			Uranium deposited in the kidneys		
	day (µg U)	month (µg U)	year (mg U)	day (µg U)	month (µg U)	year (mg U)
Sample I	1.22	36.70	0.44	0.61	18.35	0.22
Sample II	0.51	15.44	0.185	0.26	7.72	0.09

The rapid absorption of uranium for 5 days results in a concentration of uranium (c) in the blood:

$$c = T/(\ln 2)\cdot(k\cdot U)/m\cdot(1 - 2^{\tau/T}) \quad (12)$$

where: T – 15 days is the time for extracting the accumulated uranium, τ – 5 days from the moment of entry, U – an average daily value of the uranium which enters the blood, for example, 2.1 mg/day, for k = 11%, the amount of uranium which is deposited in the blood, ln 2 = 0.693 = 0.29 kg of the total weight of the two kidneys (Harley et al., 1999).

The increasing of uranium concentrations in the kidney, according to this calculation, is shown in Figure 5.

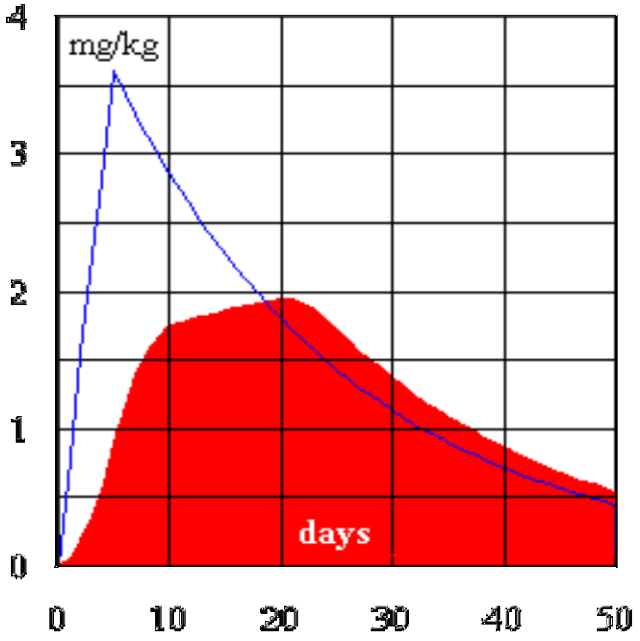

Figure 5. The uranium concentration in the kidney calculated by the model of exposure of Gulf War veterans to depleted uranium (Eckerman et al., 1998).

However, the rapid absorption of ”caught” and ingested uranium in the blood is not a realistic assumption. When also including the period of 5 and 6 days in the calculation (equation 12) (ICRP, 1995), there will be a small absorption of uranium from the lungs and gastrointestinal tract (GI tract). The maximum concentration in the kidneys will be noticed on the 20th day as 90% of the maximum value. This maximum value will be extracted for 15 days (Figure 5 – a tinted area).

Figure 6 shows the organs in which uranium is stored after ingestion through drinking water at a concentration of 1 μg/L.

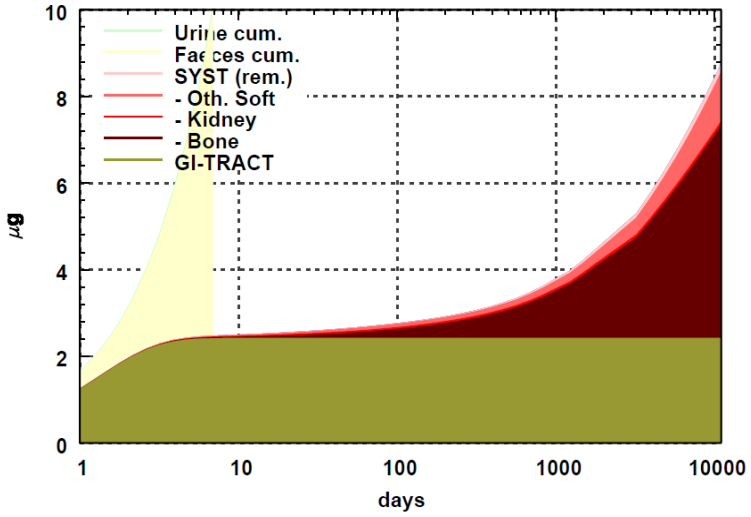

Figure 6. Value of uranium stored in organs after ingestion of 1 μg/L.

As can be seen from Figure 6, a constant amount is retained in the GI tract, while the excretion of uranium takes place via the faeces and of a small extent via urine. Uranium slowly accumulates over time, partly in the lungs but in kidneys and other soft tissues as well, so that its concentration is continuously growing (WISE, 2005).

Figure 7 shows concentrations of uranium in the urine of adults with continuous ingestion of drinking water containing 1 μg/L of uranium and daily intake of 1.4L (at the speed of introduction of 1.4 L/day) (according to ICRP's biokinetics model for uranium (ICRP, 1995)).

Figure 7 indicates that the continuous introduction of uranium through drinking water concentration of only 1 μg/L makesthe concentration of uranium in urine grow rapidly, but it still shows a tendency to rise to about 20 ng/L for adults (WISE, 2005). With increasing concentrations of uranium in drinking water over time, the concentration of uranium increases in the urine.

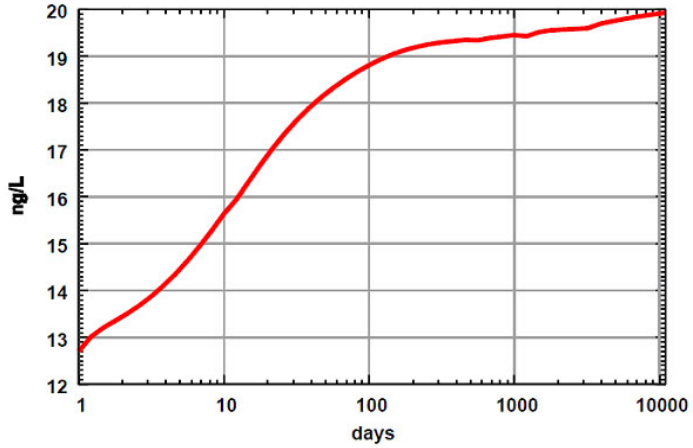

Figure 7. The concentration of uranium in the urine of adults with continual ingestion of 1 µg/L.

United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) takes as a reference value for uranium in drinking water of 1 mBq U-238/kg (UNSCEAR, 2000), which corresponds to 81 ng U_{nat}/L and results in a level of uranium in the urine of about 1.6 ng/L, according to biokinetics model for adults (WISE, 2005).

Roth et al.(2001) sum the years as a conditional value for uranium in the urine of the German population: median values were 11 ng/L for the age of 20 years and 21 ng/L for the age of 50 years. Maximum values are: 21 ng/L for the age of 20 years and 50 ng/L for the age of 50 years (value converted with an intake of 1.4 L/day).

The assessment of the potential health risks due to the presence of uranium in drinking water

Short-term risk

Intake of toxic elements (heavy and light metals) and a risk to human health caused by the consumption of drinking water from individual wells from the village of Dubravica are determined on a weekly basis **(short-term risk)** over *the estimated weekly intake* through water **(PNU)** and *the coefficient of risks to health* **(KR)**.

These coefficients are determined based on the following equation (Lin et al., 2015):

$$\mathbf{PNU} = \mathbf{[PPV \cdot \mathit{c} \cdot 7]/PTM} \quad (13)$$

$$\mathbf{KR} = \mathbf{PNU/TNU} \quad (14)$$

where: **PPV** – the average water consumption per citizen (1.5L per day) (Papić et al., 2012; Official Gazette of RS, 2018), *c* – the concentration of elements in the analysed samples of water expressed in μg/L, **PTM** – the average body mass of the population, which amounts to 75.65 kg (Pavlica et al., 2010). **TNU** is a tolerant weekly intake of toxic metals expressed as μg/kg body weight. As for the oral intake of uranium, it amounts to 0.6 μg/day for each kilogram of body weight (WHO, 1998; WHO, 2011).

When assessing the short-term risk to human health, it is considered to be high risk if there is a risk coefficient (KR) for an element greater than 1 (Kostić et al., 2016).

Potential long-term carcinogenic risks to human health

In addition to **short-term risks** caused by consuming water with a high content of toxic elements, it is possible to determine the **potential long-term, carcinogenic risks to human health** (Wu and Sun, 2015).

To assess this type of risks, the oral intake of toxic elements (by consuming drinking water) $\mathbf{U_{oral}}$, and the risk coefficient caused by the oral intake of toxic elements, $\mathbf{KR_{oral}}$, are defined based on the following equations:

$$\mathbf{U_{oral} = [PPV \cdot \mathit{c} \cdot 365 \cdot 30]/[PTM \cdot 10950]} \quad (15)$$

$$\mathbf{KR_{oral} = U_{oral}/RfD_{oral}} \quad (16)$$

where: **PPV** – the average water consumption per citizen (1.5L per day) (Papić et al., 2012, Official Gazette of RS, 2018), *c* – the concentration of elements in the analysed samples of water expressed in μg/L, **PTM** – the average weight of the population of Serbia, which is 75.65 kg (Pavlica et al., 2010) and $\mathbf{RfD_{oral}}$ reference values for the intake and potentially carcinogenic contaminants prescribed by American agency for environmental protection – EPA (Momot and Synzynys, 2005; CHMP, 2007). The minimum level of risk for long-term intake of toxic metals in the population prescribed by the Agency for Toxic Substances and Disease Registry (ATSDR) is 1.1 mg/day/kg (ATDSR, 1997).

Based on the data on the concentration of uranium in drinking water (samples I and II), short-term and long-term potential health risks for people who use this water are assessed (Table 4).

Based on the results obtained for the tested samples of drinking water from the village of Dubravica, the value of the coefficient of risk KR < 1 for potentially harmful uranium, analysed in drinking water can be seen. It should be noted that the KR for the first sample of drinking water amounted to only 14.18% less than the value of the coefficient of risk (Kostić et al., 2016), suggesting the possible short-term (acute) danger of uranium present in drinking water in this water sample.

Table 4. The assessment of short- and long-term risks to the health of people who use this water for drinking expressed through risk coefficients (KR) and risk coefficients of oral intake (KR_{oral}) for uranium.

Sample	The concentration of uranium in drinking water	**Short-term risk**		**Long-term risk**	
		PNU	**KR**	**U_{oral}**	**KR_{oral}**
Sample I	3.71 µg/L	0.515	0.858	$7.4 \cdot 10^{-5}$	0.0245
Sample II	1.56 µg/L	0.216	0.361	$3.0 \cdot 10^{-5}$	0.0100

where: **TNU** (tolerant weekly entry) – 0.6 µg/kg (WHO, 1998; WHO, 2011), **RfD_{oral}** (reference value of the oral intake of uranium) – 0,003 mg/day/kg (ATDSR, 1997; US EPA, 1989).

However, based on the calculated estimates of long-term health risk, it can be seen that there is a health risk in the case of consumption of both water samples. Of 1000 inhabitants, 24.5 inhabitants (from water sample I) and 10.3 inhabitants (from water sample II) show a potential risk of cancer, based on the present concentration of uranium in drinking water in the village of Dubravica.

In this analysis, the risk of uranium concentration in drinking water during the long-term consumption of drinking water from individual wells in the village of Dubravica is compared with the standard values prescribed by the EPA (Environmental Protection Agency) and the WHO (World Health Organization).

The standard for the concentration of uranium in drinking water has not been regulated for a long time. Only recently, there has been an increased activity to elaborate and establish reference values (standards).

Table 5 shows some examples of current legal standards on the concentration of uranium in drinking water.

Table 5. The standards for uranium concentrations in drinking water.

Standard		Continuous long-term effects after ingestion of uranium		
	Concentration of U in drinking water	Concentration of U in urine*	Concentration of U in the kidneys*	Annual radiation doses**
EPA Standard (US EPA, 2000)	30 µg/L	600 ng/L	$8{,}9 \cdot 10^{-3}$ µg/g	0.018 mSv/year
WHO recommendations (WHO, 2004)	15 µg/L	300 ng/L	$4{,}5 \cdot 10^{-3}$ µg/g	$9 \cdot 10^{-3}$ mSv/year
Sample I	3.71 µg/L	74.2 ng/L	$1{,}1 \cdot 10^{-3}$ µg/g	$2.2 \cdot 10^{-3}$ mSv/year
Sample II	1.56 µg/L	31.2 ng/L	$4{,}6 \cdot 10^{-4}$ µg/g	$9.4 \cdot 10^{-4}$ mSv/year

where: * by application of ICRP biokinetics model for the uranium, based on the introduction of 500L of drinking water per year; ** Annual radiation dose data for natural uranium (U_{nat}) – Natural uranium of isotopic composition (0.71% of isotope ^{235}U).

The standards for uranium content in the drinking water are based upon chemical toxicity and are intended for protecting the kidney. Maximum concentrations obtained for the kidney after 30 years of continuous ingestion of 30 μg/L of drinking water (US EPA standard 2000) are still 30 times lower than the recommended value of 0.3 g/g for a kidney, and the annual radiation is 50 times lower than the value recommended by the ICRP biokinetics model (ICRP, 1995).

The data presented in Table 5, compared with the standards recommended by the EPA and WHO, indicate that with the prolonged ingestion of the drinking water (water samples I and II), the concentration of uranium in the kidneys may not be negligible, even though the concentration of uranium in drinking water is lower than recommended.

These data confirm the first result obtained by analysing the uranium found in the scale of the individual wells. These wells are located in different soil types, which directly affects the quality of water.

Where does uranium found in drinking water in the village of Dubravica come from?

The village of Dubravica is partially located over the western lignite deposit of the Kostolac basin (Official Gazette of Požarevac, 2012). Although coal is mainly composed of organic matter, some trace elements in coal are naturally radioactive and constitute naturally occurring radioactive material (*Normally Occurring Radioactive Material* – NORM). These radioactive elements include U, Th, and their numerous products of disintegration, Ra and Rn (Kisić et al., 2013). As a result, with contents of potentially hazardous trace elements (As, Be, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Sb, Se, Th, and U), the role model of coal from the coal liner fields of Drmno was examined (Životić et al., 2008).

By analysing the geometric middle values, the Kostolac coal basin contains relatively high concentrations of Mn, Ni, Cr, As, Cu, and Co. These concentrations are much higher than average world values. However, the content of other elements is lower compared to the average world values.

Content of other elements close to the world average values. Based on the results in our previous works (Rajković et al., 2015a; Rajković et al., 2015b), it was found that the concentration of Cr in sample I amounted to 54.5 μg/L, which is higher than the allowed value by Regulation, 50.5 μg/L, while in sample II, this value was much lower than this value (4.04 μg/L), which is in agreement with these studies.

The contents of U and Th in the coal of the Kostolac basin were lower than the world average, although there were some parts of the coal with a high content of uranium. Trials of the content of natural radionuclides ^{238}U, ^{226}Ra, ^{232}Th and ^{40}K in samples of the lignite coal basin in Kostolac (WHO, 2004) revealed the content of

the isotope ^{238}U of 0.60 to 70.10 mg/kg and of the isotope ^{232}Th from 0.20 to 2.60 mg/kg. These data perhaps indicate the origin of uranium in drinking water since the drinking water was obtained from the individual wells. This is especially pronounced for sample I (3.71 μg/L) although the uranium value in sample II (1,56 μg/L) was only slightly lower than the value permitted by regulations (2 μg/L).

In this region, a systemic examination of radionuclides in the environment is provided twice a year in residential areas - in the working environment, as well as in the soil (Official Gazette of Požarevac, 2012). Also, the General Urban Plan of Požarevac envisages that in the period of 2016–2020 preparations for relocating (parts of) Dubravica settlements that are located above the western lignite deposits of the Kostolac basin may be made (Official Gazette of Požarevac, 2012). The data obtained in this study clearly indicate that the location and the water from which sample I was taken are in the zone of lignite deposits, while the location from which sample II was taken is not in the zone.

What is especially warning is the occurrence of endemic nephropathy (EN) along the rivers of the Kolubara, the Drina, the Sava and the Morava (Institute of Public Health, Požarevac, 2011). Since the aetiology of endemic nephropathy is unknown, certain elements that are found in trace amounts (Pb, Cd, Si), live agents (bacteria and viruses), fungal and plant toxins, genetic factors and immune mechanisms have been listed as possible causes.

Some experts believe that this insidious disease is caused by aristolochic acid (AA) and ochratoxin A (OTA) found in certain plants.

The disease is undetectable, relatively asymptomatic and quite well-tolerated, sometimes for decades. The general weakness occurs only in the stage of kidney insufficiency when the anaemia expresses because of the retention of nitrogenous products in the blood. Patients complain of vague gastric ailments. Elevated blood pressure is encountered in about 30–40% of elderly patients with advanced stages of the disease. There is no oedema of renal origin. The disease progresses to kidney failure and end-stage kidney disease.

The previous analysis clearly indicates that long-term ingestion of uranium by humans can lead to disturbances in the functioning of kidneys and that the introduction of uranium in elevated concentration is the first step that leads to progressive and irreversible kidney damage. Thus, in addition to all the causes that lead to the occurrence of endemic nephropathy, uranium in the environment should be also taken into account.

Conclusion

Based on tests of drinking water, from the aspect of uranium content from individual wells in the village of Dubravica in Braničevo district, we can draw the following conclusions:

Naturally occurring radioactive materials (NORM) may move out of the ground into the drinking water, so the analysis of drinking water may establish their presence in the environment. Thus, it can indirectly determine the degree of danger to human health, and also explain the origin of many diseases that are now linked to other causes.

The village of Dubravica, according to official data, lies over the western part of lignite deposits of the Kostolac basin, as tests of drinking water unambiguously confirmed based on the different contents of uranium from different locations.

Uranium is a natural element of the soil, and the degree of its migration depends on the physical and chemical properties of soil, soil solution and the oxidation product of uranium. The biggest danger for migration represents U(VI) in the form of uranyl ions because they easily build soluble complexes with ligands present in the soil solution. In this way, uranium enters groundwater from individual wells from which water is used for drinking.

Examining the uranium contents in drinking water from individual wells by the indirect method, based on the deposited lime, found significant differences in the content of uranium, since sample I recorded 2.4 times more uranium in comparison to the second pattern. In addition to uranium, in sample I, higher values of Fe (2.1 times higher), Mn (1.4 times), Ti (not found in sample II), Cr (13.5 times more, one of the causes of EN) were recorded, while, as for the heavy metals, higher contents of Pb (4.15 times), Zn (11 times), Cu (6.4 times) and Cd (1.4 times) were observed in sample II. The contents of elements found naturally in the soil, Si and Al, were higher in sample II: Si (2.7 times), Al (190 times). These data clearly show that the individual wells are found on different soils, and all the elements can affect the EN found in both samples of drinking water.

Uranium as a radioactive element and highly toxic substance is extremely dangerous to human health, so that its allowable concentration always decreases. Thus, the initially permitted concentration of 3 ppm in drinking water has decreased 10 times. Today, this value is 1000 times lower than the original (0.002 ppm) due to the risk of long-term ingestion of uranium, which is shown in this paper.

Although most of uranium is taken out through the urine (67%), the rest is deposited in the soft tissues so that its concentration is continuously growing, even with low concentrations of uranium in drinking water.

In a study examining the effects of chronic ingestion of uranium in the drinking water on people, it was found that kidney function is disturbed by the presence of uranium despite the conclusion that the examination is not done on animals in the laboratory. The fact is that endemic nephropathy (EN) occurs in hot spots along the rivers of the Kolubara, the Drina, the Sava, and the Morava. Also, in Serbia, an inhabitant has kidney problems. Because of this, it is of great importance to monitor the concentration of uranium in drinking water or to

discover the cause of its presence. Evaluation of short-term health risk showed the pattern that drinking water indicates a risk to human health. In contrast, long-term risk assessment indicates a threat to human health for the population in consuming both samples of drinking water.

Official data show that, in the period between the two censuses, the population of the village of Dubrava fell by 15.92%. The reasons can be either migration flows or effects on the quality of the environment, primarily on drinking water.

Toxic effects of radiation lead to the reduction in the number of blood cells which arise due to infection, fever, spontaneous bleeding from mucous membranes and the internal organs, anaemia, loss of appetites with this, fatigue, and also lead to fatty degeneration of the liver with centrilobular necrosis. Neurological outbursts can appear in the chronic exposition. After a very long period (20–25 years), malignant changes, the most common cancers of the bronchi, osteosarcoma, leucosis, and liver tumours can occur. Potential mutagenic effects of uranium are still unknown, but it is experimentally demonstrated that uranium content in urine is positively correlated with urinary mutagenicity.

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ISPITIVANJE POSLEDICA PRISUSTVA URANA U VODI ZA PIĆE IZ INDIVIDUALNIH BUNARA U SELU DUBRAVICA U BRANIČEVSKOM OKRUGU PO ZDRAVLJE STANOVNIŠTVA

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R e z i m e

Selo Dubravica se delimično nalazi iznad zapadnog lignitskog ležišta Kostolačkog basena. Ispitivanjem suvog ostatka dobijenog iz vode za piće iz dva individualna bunara rendgenskom difrakcionom analizom, na osnovu karakterističnih pikova, utvrđeno je prisustvo urana u vodi za piće. Indirektnom metodom Rajkovića i saradnika pouzdano je dokazano da je u prvom uzorku koncentracija urana u vodi za piće za 85,8% viša (3,71 μg/L), dok je u drugom uzorku koncentracija urana svega 22% niža (1.56 μg/L) od vrednosti MDK predviđene Pravilnikom (2 μg/L).

Analiza posledica unošenja urana u čovekov organizam ukazala je na to da se na ovaj način unosi 0,84–2 mg urana u čovekov organizam na godišnjem nivou, odnosno 0,09–0,22 mg deponuje na godišnjem nivou u bubreg.

Procena potencijalnog zdravstvenog rizika usled prisustva urana u vodi za piće ukazala je na to da je stanovništvo koje koristi vodu za piće iz prvog bunara i u kratkom vremenskom intervalu ugroženo od urana. Što se tiče dugoročnog rizika, proračun je ukazao da su u slučaju prvog uzorka vode za piće ugroženo oko 25, a u slučaju drugog uzorka vode za piće 10 stanovnika od 1000 stanovnika. Kako je bubreg organ u kome se uran deponuje u najvećoj meri u čovekovom organizmu, a dejstvo urana dugotrajno, slabljenje i otkazivanje funkcije bubrega može biti toliko da je čak 75% funkcije bubrega uništeno da bi se pokazali prvi klinički simptomi. Ova pojava se zapaža među stanovništvom duž reka Kolubare, Drine, Save i Morave i naziva se endemska nefropatija. Kao mogući uzročnici ističu se elementi koji se nalaze u tragovima (Pb, Cd, Si), živi agensi (bakterije i virusi), gljivični i biljni toksini, genetski faktori i imuni mehanizam, ali ne i uran u vodi za piće.

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Ispitivanja izvršena u ovom radu jasno ukazuju i na ulogu urana u epidemiji endemske nefropatije koja je u porastu i koja nema akutnu fazu već bolest progredira ka bubrežnoj insuficijenciji i terminalnom stadijumu bolesti bubrega.

Zvanični podaci koji govore o porastu endemske nefropatije i šećerne bolesti i porastu njihovog udela u bolestima, kao i stope opšteg mortaliteta koja iznosi 18,19%, nedvosmisleno ukazuju na to da se uloga urana u životnoj sredini Braničevskog okruga ni na koji način ne sme zanemarivati. Zbog konfiguracije terena na kojima se nalaze naselja mora se pratiti uran i njegova migracija kroz životnu sredinu, za čije kretanje postoje, nažalost, svi uslovi.

Ključne reči: voda za piće, teški metali, uran, endemska nefropatija.

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Key words

Key words are terms or phrases which describe best the content of the article for the needs of indexing and browsing purposes. The number of key words should be 3 to 10. They should appear below the abstract. The title of key words should be bolded and indented by pressing the tab key. The colon should be used after the title, and then the list of key words in lower-case letters should be given with the full stop at the end. Key words should be provided in Serbian and English after abstract on both languages.

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 200 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.

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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 diagram should be given. The photographs must be of high quality so that they can technically be well reproduced. They should be submitted in "TIF" or "JPG" format, and they will be printed in black and white. The title of the illustration should be justified, with a full stop at the end, single spaced from the illustration and given below it. Each illustration should be mentioned in the text.

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 prethodno neobjavljivane rezultate sopstvenih istraživanja. Obim ovog rada treba da iznosi od 6 do 12 strana.
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Obavezna poglavlja svakog originalnog naučnog rada i prethodnog saopštenja su sledeća: naslov rada, imena autora, naziv ustanove autora, sažetak, ključne reči, uvod, materijal i metode, rezultati i diskusija, zaključak, zahvalnica, literatura i rezime na srpskom jeziku (ako je rad na engleskom i obrnuto). Pregledni rad mora da sadrži: naslov rada, imena autora, naziv ustanove autora, sažetak, ključne reči, uvod, analizu-diskusiju određene teme, zaključak, literaturu i rezime na srpskom jeziku (ako je rad na engleskom i obrnuto). Ako su radovi na engleskom jeziku, prednost se daje britanskoj varijanti ovog jezika.

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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

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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.

Rezultati i diskusija

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 nabrajanje svih rezultata istraživanja sa ponavljanjem brojčanih vrednosti koje su prethodno već navedene u poglavlju „Rezultati i diskusija“. Zaključak ne sme da sadrži reference.

Zahvalnica

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

Rezime
Rezime na srpskom jeziku (za radove napisane na engleskom jeziku) ili na engleskom jeziku (za radove napisane na srpskom jeziku) navodi se na kraju rada i treba da ima od 200 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

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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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