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Original scientific paper

EVALUATION OF YIELD AND YIELD-RELATED TRAITS FOR WATERLOGGING TOLERANCE IN MUNGBEAN GENOTYPES USING MULTIVARIATE TECHNIQUES

Nazmun Nahar Shibly[1], M. Rafiqul Islam[1*], Mehfuz Hasan[3], M. Nasimul Bari[1] and Jalal Uddin Ahmed[2]

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Abstract: Waterlogging is a major constraint of mungbean production in the tropical and subtropical regions of the world and can cause a significant yield loss. The study evaluated 100 mungbean genotypes for tolerance to waterlogging employing rigorous field screening procedures. Three-week-old seedlings of 100 mungbean genotypes were subjected to waterlogging for 3 days maintaining a waterlogging depth of 2.5 cm. Waterlogging tolerance was evaluated during the periods of recovery and final harvest considering relative performance (values of waterlogging relative to non-waterlogging controls) of 18 plant traits. All the genotypes showed a wide range of variation in relative values. Some genotypes subjected to waterlogging produced plenty of adventitious roots that contributed to foliage development and chlorophyll increment, which resulted in better shoot growth, and eventually yield of mungbean increased. Nine plant traits highly associated in waterlogged conditions were used in cluster analysis. The genotypes within cluster 6 and cluster 7 performed better regarding almost all plant traits whereas cluster 4 performed very poorly. Discriminant function analysis showed that function 1 and function 2 explained 54.5% and 32.2%, respectively and altogether 86.7% variation in the genotypes. The harvest index and straw dry matter mostly explained the total variance in function 1. Dry matter of root, shoot and straw explained the maximum variance in function 2. Root dry matter played the most dominant role in explaining the maximum variance in the genotypes. The genotypes IPSA-10 and VC 6379 (23-11) showed a better degree of tolerance to waterlogging concerning yield and associated morpho-physiological traits.

Key words: genetic variability, waterlogging tolerance, growth, yield, multivariate analysis.

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Introduction

Mungbean (*Vigna radiata* L. Wilczek), a short-duration grain legume commonly produced in many cropping systems of South and Southeast Asia, is cultivated in more than 6 million ha in the world of which 90% is in Asia (Noble et al., 2020; Kaur et al., 2015). The crop is considered highly suitable for its widespread diversification to traditionally rice monoculture or rice-rice cropping systems in smallholder farms (Campbell-Ross et al., 2019; Malik et al., 2013; Graham and Vance, 2003). The inclusion of mungbean after harvesting rainfed *aman* rice predominantly improves soil fertility and reduces pest and disease incidences (Sravan and Murthy, 2018; Porpavai et al., 2011; Yaqub et al., 2010). Regardless of its various advantages, the South Asian farmers are now reluctant to grow mungbean due to an incessant increase of waterlogging spells caused by intense and unpredictable rainfall under climatic extremes (Nair et al., 2019; Hidangmayum et al., 2018; Hirabayashi et al., 2013). Waterlogging caused by excessive rainfall restricts stand establishment and root and shoot growth which may result in the total loss of crop yield (Islam, 2016; Toker and Mutlu, 2011). Therefore, the sustainability of mungbean production largely depends on the responsiveness of the crop to waterlogging stress and subsequent stress management practices (Witcombe et al., 2008).

Waterlogging is usually caused by heavy rainfall and is overstated due to poor surface drainage in addition to direct flood, particularly in clay soil which is common in many coastal regions where mungbean cultivation is intense (Sarkar et al., 2017). Mungbean cannot withstand waterlogging particularly during the early stages of crop growth (Singh and Singh, 2011). As a result, the yield of mungbean may be reduced up to 45% depending on the stages of encountering waterlogging (Normile, 2008). Although there are strong shreds of evidence that mungbean genotypes exhibit a wide range of variation in the waterlogging-induced changes in morpho-physiological traits (Islam et al., 2007), a substantial yield loss was reported in many studies on mungbean (Amin et al., 2016; Kumar et al., 2013; Ahmad et al., 2003) and other legumes (Islam et al., 2009; Solaiman et al., 2007; Pociecha et al., 2008; Celik and Turhan, 2011).

Interestingly, the response of mungbean genotypes to waterlogging varied depending on the sensitivity of the genotypes. The crop can withstand waterlogging for a considerable period by producing numerous adventitious roots (Islam et al., 2010). Waterlogging-tolerant genotypes maintained a higher assimilation rate, leaf chlorophyll content, plant height, greater leaf number and area, faster root growth, and production of numerous root nodules during the waterlogging period. The recovery of the aforementioned traits after the recession of waterlogging is reported to be comparatively faster in mungbean (Kumar et al., 2013; Islam et al., 2010; Islam et al., 2008). Genotypic differences in transient waterlogging of mungbean were observed by Islam et al. (2005). Since climate-induced unfavorable ecosystems caused by heavy rainfall are an enormous threat to mungbean productivity (Ceccarelli et al., 2010), the identification of suitable mungbean genotype(s) tolerant to waterlogging from a large number of genetic resources having diverse growth habits as well as yield and yield-related morpho-physiological traits could be a useful option. Considering the genetic variability of waterlogging tolerance in mungbean, a model screening technique was developed and some waterlogging-tolerant genotypes with related morphological plant traits were identified under the semi-controlled environment (Islam et al., 2007). However, screening of a large number of genotypes against waterlogging under variable and heterogeneous field conditions has not been adequately performed hitherto. Therefore, the study aims at evaluating 100 mungbean genotypes following a standard protocol and selecting waterlogging-tolerant mungbean genotypes based on yield and yield-related traits under waterlogged and non-waterlogged conditions through various multivariate techniques.

Materials and Methods

Location and climate

The study was conducted at the field research site of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706 from March 2016 to June 2016 (Figure 1).

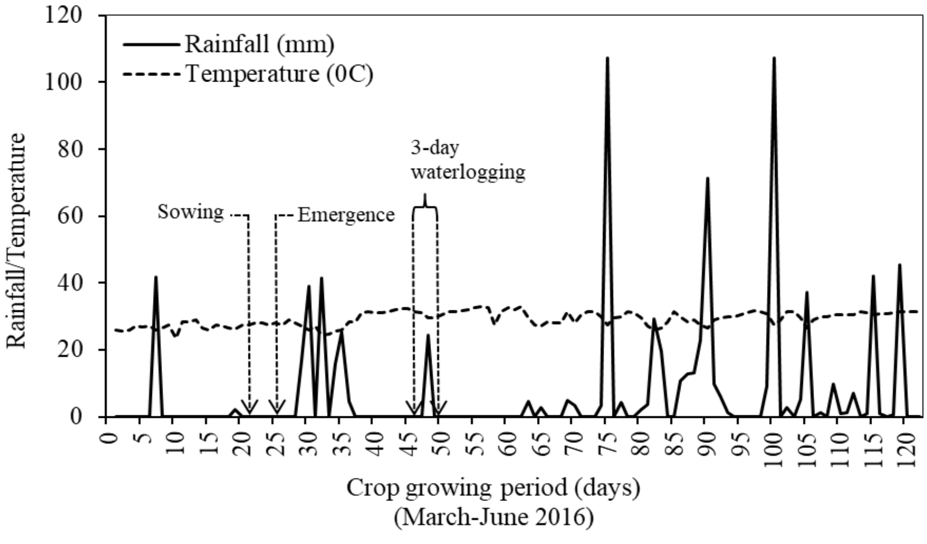


Figure 1. Trends of rainfall and average temperatures during the mungbean growing period.

The location is between 24°05' N latitude and 90°16' E longitude with an elevation of 8.4 m above the mean sea level. It is characterized by the subtropical climate having intermittent rainfall at the early stage and frequent intense rainfall at the later stages of crop growth. The average temperature ranged between 24.4^{o}C and 33.0^{o}C, which was highly favorable for mungbean cultivation. Just after the emergence of mungbean seedlings, frequent heavy rainfall occurred for a week, which was not unfavorable for seedling establishment. However, the following day of imposition of waterlogging treatment, more than 24 mm of rainfall occurred and created transient waterlogging conditions for which the field remained saturated about a week. This created unfavorable soil physical conditions and more adversely affected the waterlogged plants compared to non-waterlogged control plants. Consequently, the recovery of plants from waterlogging damage was delayed.

Planting materials and experimental design

A total of 100 mungbean genotypes available in the Germplasm Unit of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706 were used in the study. Most of the genotypes were collected from AVRDC, Taiwan (56), followed by various organizations of Bangladesh (23), India (9), Philippines (4), France (2), Pakistan (2) and one each from Hong Kong, Indonesia, and the USA. The genotypes and two waterlogging situations (waterlogging and non-waterlogging) were the treatment variables. For each replication, the field was divided into four small plots by 7.5×2 m^2. Each plot contained 25 lines and each line represented individual genotypes. To prevent the side leakage of water from the waterlogged plot, the boundaries of the plot were raised and deeply covered with polythene sheets. The experimental design was a two-factorial randomized complete block design (RCBD) with three replications.

Raising of seedlings

Before sowing, seeds were treated with Vitavax-200. Line to line and plant to plant distances were maintained at 30 and 10 cm, respectively. Two to three seeds were sown in every hill at the 2–3-cm depth to maintain a uniform size of the seedling. Recommended fertilizer doses were used at the time of final land preparation and top dressing. Fertilizer urea at 50 kg ha^{-1}, triple superphosphate at 85 kg ha^{-1} and muriate of potash at 35 kg ha^{-1} were applied. Half of the urea was applied during final land preparation and the rest of urea was applied at 30 days after emergence (DAE).

Waterlogging treatment imposition

Waterlogging treatment was applied on 3-week-old seedlings and waterlogging depth was maintained at 2.5 cm for 3 days. Thereafter, the excess water was removed from the field. Three days of the water retaining period were considered as the waterlogging period. After the removal of water, the soil remained nearly saturated for 7 days. On the other hand, optimum soil moisture was maintained in non-waterlogged control plants.

Measuring morpho-phenological traits

Plant sample collection and data measurement were done from both waterlogged and non-waterlogged plants during the recovery stage. Five plants of each genotype in the row were selected for data recording. The morphological and growth parameters viz. plant height, the number of leaves, leaf area, branches per plant, root dry matter, total shoot dry matter, root: shoot ratio and nodule dry matter, physiological parameter viz. SPAD value were recorded at 49 DAE. Plant height was measured from the above ground level to the shoot portion of the plant. SPAD value was measured at proper sunlight just before harvesting the plants under field conditions. Different plant components were dried in an oven at 80°C for at least 72 hours and then dry weights were recorded. Phenological data i.e. days to flowering and days to maturity were also recorded. Days to flowering were considered when about 50% of flowers were opened.

Harvesting and recording yield data

Yield and yield components such as the number of pods per plant, seeds per pod, pod length, straw weight and seed yield were recorded at variable dates. The crop was finally harvested when 95% of the pods turned black. Both waterlogged and non-waterlogged plants were uprooted carefully by hand and then bundled and tagged. In each time of picking, pods were threshed carefully in the laboratory and kept under the open sunlight for drying. When the pod walls became brittle, the seeds were separated, cleaned, and then dried under sunlight for a desirable moisture level (12%).

Data analysis

Data on waterlogging induced changes of the genotypes were compared in this study. The ranges and mean values of different plant traits were estimated by Microsoft Office Excel-2013. Analysis of waterlogging tolerance of mungbean genotypes was performed using the program SPSS 16.0 following the procedure described by Rojas et al. (2000) and Islam et al. (2007). However, instead of absolute values, the relative values of plant traits were used and thus the comparison was more meaningful towards identifying genotypes or groups of genotypes tolerant to waterlogging (Islam et al., 2007). Eighteen qualitative variables were considered in the descriptive analysis. Based on higher correlation coefficient values between seed yield and other plant traits, nine plant traits were selected and used in the multivariate analysis and discriminant function analysis (DFA). The non-hierarchical procedure (k-means) of cluster analysis was used for grouping the genotypes according to the relative tolerance of the genotypes to waterlogging (Rojas et al., 2000).

Results and Discussion

Waterlogging tolerance of plant traits

A total of 18 quantitative plant traits were studied at 21 days after the removal of waterlogging stress. The range and mean of all the traits of 100 genotypes under waterlogged and non-waterlogged conditions are illustrated in Table 1. The quantitative traits are presented as absolute as well as relative values (values of waterlogging relative to non-waterlogging controls) to understand the degree of waterlogging tolerance in each plant character of the genotypes. The relative values indicate that plant height, the number of leaves and leaf area showed a wide range of variations in waterlogging tolerance in the genotypes. However, remarkable changes were found in the relative values of root and shoot dry matter where the reduction was comparatively much less in roots. This is probably because of the quicker root development of waterlogged plants after the initial damage of roots. The development of adventitious roots in waterlogged plants is the most common as observed in Eurasian species (Visser et al., 2015), tomato (Vidoz et al., 2010). In this study, a comparatively wider range with high relative root: shoot ratio up to 1.86 was observed, which indicates that some genotypes produced numerous roots under waterlogged conditions. This is in agreement with an earlier study conducted by Islam et al. (2007) in a semi-controlled environment. The genotypic differences in producing adventitious roots were also evident in tomato (Ezin et al., 2010). The genotypic differences in root nodule production were much more conspicuous where the relative value rose to 6.4, although the mean was 1.00. This indicates that some genotypes produced abundant root nodules during the recovery period. The information on the recovery of nodulation from waterlogging damage has not been well documented until now, although the decreases in the number and weight of root nodules following waterlogging are commonly observed in grain legumes (Pampana et al., 2016) and soybean (Saputro et al., 2018).

Table 1. The range and mean for quantitative characteristics of 100 mungbean genotypes subjected to waterlogging.

Plant characters	Control[1]		Waterlogging[2]	
	Range	Mean[3]	Range	Mean[3]
Plant height (cm)	20.9–51.5	37.3±0.62	12.5–32.4 (0.34–0.96)	22.5±0.44 (0.61)
Leaf number (plant^{-1})	7.00–24.00	13.9±0.33	3.2–13.8 (0.29–0.98)	7.8±0.20 (0.58)
Leaf area (cm^2)	219.9–753.0	456.9±12.51	88.7–404.7 (0.26–0.98)	275.5±7.72 (0.63)
Root DM (g plant^{-1})	0.22–0.82	0.54±0.01	0.15–0.69 (0.33–1.53)	0.44±0.01 (0.84)
Total shoot DM (g plant^{-1})	2.72–8.96	4.96±0.13	1.36–5.59 (0.25–1.03)	3.35±0.10 (0.70)
Root: shoot ratio	0.06–0.20	0.11±0.01	0.07–0.25 (0.65–1.86)	0.14±0.01 (1.24)
Nodule DM (g plant^{-1})	0.00–0.21	0.08±0.01	0.00–0.20 (0.00–6.40)	0.08±0.01 (1.00)
SPAD value	39.3–59.5	48.9±0.39	23.7–55.1 (0.42–1.05)	42.1±0.56 (0.86)
Days of flowering	38.0–50.0	43.7±0.32	35.0–55.0 (0.87–1.23)	45.5±0.45 (1.04)
Days of maturity	78.0–92.0	83.8±0.35	78.0–92.0 (0.91–1.10)	84.28±0.41 (1.01)
Branches (no.) plant^{-1}	1.00–7.00	3.22±0.12	0.40–3.20 (0.13–0.98)	1.83±0.06 (0.61)
Pods per plant	2.20–42.60	20.17±0.84	0.70–21.80 (0.21–0.89)	10.80±0.46 (0.55)
Seeds per pod	6.45–15.68	11.89±0.20	4.90–10.40 (0.45–0.89)	7.20±0.10 (0.62)
Pod length (cm)	6.33–10.88	8.70±0.10	5.94–9.90 (0.74–0.99)	7.62±0.09 (0.88)
1000-seed weight (g)	19.18–59.41	38.92±0.89	11.00–53.00 (0.44–1.30)	34.75±0.82 (0.91)
Straw weight (g plant^{-1})	2.61–27.39	16.93±0.49	2.23–21.38 (0.21–0.94)	8.42±0.36 (0.50)
Seed yield (g plant^{-1})	0.60–9.81	5.44±0.20	0.21–5.79 (0.15–0.88)	2.96±0.13 (0.55)
Harvest index	0.07–0.38	0.24±0.01	0.05–0.44 (0.48–1.69)	0.26±0.01 (1.10)

[1]plants were allowed to grow at optimum soil moisture throughout the growing period. [2]plants were allowed to grow at optimum soil moisture up to 21 DAE and then exposed to waterlogging for 3 days and after the termination of waterlogging, moist soil conditions remained for around 7 days and then again optimum soil moisture was maintained. Data were collected from both control and waterlogged plants at 49 DAE. [3] mean ± standard error. Parentheses expressed the relative values as a ratio of waterlogged to non-waterlogged plants.

Mungbean plants turned into yellow immediately after waterlogging indicating the destruction of leaf chlorophyll content. This is the most common response of waterlogging in many plant species (Bansal et al., 2019; Saputro et al., 2018; Sarkar et al., 2017; Pociecha et al., 2008). However, the average relative SPAD value (0.86) of waterlogged plants indicated better recovery during the post-waterlogging period. The waterlogging delayed flowering and plants took 2 days more to flower but their maturity durations were prompt. The delay of flowering in waterlogged mungbean plants was also reported earlier (Islam et al., 2019; Amin et al., 2016). Among yield contributing traits, the number of pods per plant showed a wide range of variations in relative performance, although it was the worst affected showing a 45% reduction due to waterlogging. The number of seeds per pod, branches per plant and straw weight were also remarkably affected by waterlogging. Such reductions were not much significant in pod length and seed weight. However, waterlogging-induced average seed yield reduction was 45% in mungbean genotypes. Earlier it was reported that waterlogging reduced seed yield primarily by reducing the number of pods per plant and pod setting (Ahmad et al., 2003). However, there was a wide range of variations in seed yield among the genotypes. Waterlogged plants were capable of recovering depressed growth and yield traits affected by waterlogging and seed production attained 15–88% of the non-waterlogged controls. Ploschuk et al. (2018) explained that plant species used to generate a set of adaptive responses to withstand waterlogging and can produce significant yield at the early stage of waterlogging. In this study, a recovery in root and shoot growth, leaf chlorophyll index, and better translocation of assimilates into seeds as well as better pod settings contributed greatly to seed yield in some genotypes.

Relationship between the root and other plant traits

In our earlier studies, the adverse effect of waterlogging was more pronounced in roots compared to other plant traits at the early stage of waterlogging in mungbean and the recovery rate was faster in the root system of tolerant genotypes due to the development of numerous adventitious roots (Islam et al., 2007, 2010). It is now recognized that the development of adventitious roots on the soil surface or into the soil is a common feature of waterlogged tolerant plants (Pedersen et al., 2020). Therefore, an understanding of the root system development and its relationship with other plant traits as well as their contribution to seed yield is important to identify traits of interest for grouping of a large number of genotypes subjected to waterlogging. Table 2 illustrates the correlation coefficient with the regression equation of root dry matter (DM) with other plant traits. All the morphological plant traits had a significant correlation with root DM in both waterlogged and non-waterlogged control plants except for root: shoot ratio in waterlogged conditions. However, the relationships between morphological traits and root DM were comparatively stronger in waterlogged plants. Waterlogged plants produced numerous adventitious roots which promoted the growth and development of other plant traits faster than non-waterlogged control plants. Such evidence was also reported by Rich et al. (2012). The relationship between root DM and shoot DM was comparatively stronger (r=0.76) in waterlogged plants indicating a higher contribution of root to shoot growth, and then foliage development and other morphological traits. The results are also similar to those observed by Islam et al. (2007) who claimed that the above fact is one of the adaptive strategies of mungbean to waterlogging. SPAD chlorophyll index showed a significant relationship with root DM (r=22) in waterlogged plants but the relationship was insignificant in the control plants.

Table 2. The functional equation between root dry matter and other plant characters under waterlogged and non-waterlogged conditions.

Characters	Regression equation and correlation coefficient			
	Control		Waterlogging	
Morphological				
Plant height	y = 16.06x + 28.62	r= 0.34**	y = 17.06x + 14.91	r= 0.46**
Leaf number	y = 6.12x + 10.60	r= 0.24*	y = 6.71x + 4.81	r= 0.39**
Leaf area	y = 446.10x + 214.9	r= 0.48**	y = 413.30x + 92.75	r= 0.63**
Branches per plant	y = 0.03x + 0.46	r= 0.24*	y = 1.72x + 1.07	r= 0.32**
Total shoot DM	y = 5.72x + 1.85	r= 0.58**	y = 6.15x + 0.63	r= 0.76**
Root: shoot ratio	y = 0.09x + 0.06	r= 0.44**	y = 0.04x + 0.12	r= 0.18
Nodule DM	y = 0.09x + 0.03	r= 0.20*	y = 0.09x + 0.03	r= 0.22*
Physiological:				
SPAD value	y = 0.99x + 48.30	r= 0.03	y = 11.57x + 37.00	r= 0.24*
Phenological				
Days of flowering	y = -1.20x + 44.38	r=- 0.05	y = -5.68x + 47.96	r= -0.15
Days of maturity	y = 2.61x + 82.36	r= 0.04	y = -0.28x + 84.40	r= 0.01
Yield and yield contributing				
Pods per plant	y = 17.37x + 10.74	r= 0.23**	y = 17.72x + 2.96	r= 0.45**
Seeds per pod	y = 3.44x + 10.02	r= 0.28*	y = 1.10x + 6.71	r= 0.13
Pod length	y = -0.07x + 8.74	r= -0.01	y = 0.01x + 7.61	r= 0.00
1000-seed weight	y = -1.19x + 39.56	r= -0.01	y = 15.04x + 28.09	r= 0.22*
Straw weight	y = 10.53x + 11.21	r= 0.28**	y = 15.80x + 1.43	r= 0.51**
Yield	y = 5.08x + 2.68	r= 0.33**	y = 5.54x + 0.51	r= 0.51**
Harvest index	y = 0.04x + 0.22	r= 0.09	y = 0.03x + 0.25	r= 0.03

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed).

This indicates that the development of the adventitious roots of waterlogged plants stimulates leaf greenness probably via an increase in nutrient uptake and assimilates accumulation in leaf. Among yield and yield contributing traits, pods per plant, straw dry weight and seed yield showed strong relationships with root DM in waterlogged and non-waterlogged plants but the relationship was much stronger under the waterlogged conditions. For instance, the correlation coefficient between seed yield and root DM was 0.51 in waterlogged plants against 0.33 in non-waterlogged plants. Seed weight had a significant positive relationship with root DM under waterlogged conditions indicating the contribution of adventitious roots to increase seed size. All these relationships indicate that the traits having more pronounced responses to waterlogging and wider variations among genotypes were mainly the root and shoot DM, leaf area, leaf chlorophyll index which eventually may contribute to variations in seed yield by increasing the number and size of seeds in waterlogged plants.

Relationship between seed yield and other plant traits

Seed yield had significant positive correlations with all the morphological traits under waterlogged and non-waterlogged control conditions, but correlations were stronger in waterlogged plants (Table 3).

Table 3. The functional equation between yield and other plant characters under waterlogged and non-waterlogged conditions.

Plant characters	Regression equation and correlation coefficient			
	Control		Waterlogging	
Morphological				
Plant height	y = 1.02x + 31.80	r = 0.33**	y = 1.61x + 17.69	r= 0.47**
Leaf number	y = 0.58x + 10.73	r = 0.36**	y = 0.76x + 5.52	r= 0.49**
Branches per plant	y = 0.11x + 2.62	r= 0.19	y = 0.19x + 1.27	r= 0.38**
Leaf area	y = 21.62x + 34.20	r= 0.35**	y = 25.06x + 201.3	r= 0.42**
Root DM	y = 0.02x + 0.42	r= 0.33**	y = 0.05x + 0.30	r= 0.51**
Total shoot DM	y = 0.23x + 3.69	r= 0.36**	y = 0.34x + 2.34	r= 0.46**
Root: shoot ratio	y = -0.00x + 0.12	r= 0.04	y = -0.00x + 0.14	r= 0.00
Nodule DM	y = 0.00x + 0.06	r= 0.07	y = 0.01x + 0.05	r= 0.19
Physiological				
SPAD value	y = 0.30x + 47.22	r= 0.15	y = 2.17x + 35.69	r= 0.50**
Phenological:				
Days of flowering	y = -0.50x + 46.47	r= -0.33**	y = -0.67x + 47.45	r= -0.19
Days of maturity	y = 0.13x + 83.08	r= 0.07	y = 0.02x + 84.20	r= 0.01
Yield and yield contributing				
Pods per plant	y = 3.23x + 2.59	r= 0.78**	y = 2.94x + 2.10	r= 0.82**
Seeds per pod	y = 0.28x + 10.34	r= 0.30**	y = 0.17x + 6.69	r= 0.22*
Pod length	y = 0.14x + 7.92	r= 0.30**	y = 0.12x + 7.25	r= 0.18
1000-seed weight	y = 0.74x + 34.86	r= 0.17	y = 2.48x + 27.40	r= 0.39**
Straw weight	y = 1.43x + 9.14	r= 0.59**	y = 1.81x + 3.07	r= 0.64*
Harvest index	y = 0.02x + 0.14	r= 0.63**	y = 0.03x + 0.17	r= 0.52**

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed).

The degree of relationships is useful in selecting traits of interest in any stressful situation. Yield is a multidimensional trait and is affected by the plant characteristics (Filipovic et al., 2014). The closer associations between the plant height, number of leaves, leaf area, root DM, and total shoot DM with seed yield suggest a greater contribution of these traits to increase seed yield of mungbean under waterlogged conditions. Yucel et al. (2006) also determined a positive and significant relationship between seed yield and plant height. A strong significant correlation between SPAD value and grain yield (r=0.50) was observed in waterlogged plants but the relationship was non-significant in control plants. This indicates that waterlogged plants showed a rapid recovery in chlorophyll content that eventually significantly contributed to increasing the yield of mungbean. Phonological traits showed comparatively weaker relationships with seed production. However, the relationship was more negative for days to flowering (r=-0.33) under control conditions compared to waterlogging. This indicates that waterlogged plants took a comparatively longer time to flower. Among yield and yield contributing traits, pods per plant showed the highest correlation coefficient with seed yield in both waterlogged (r=0.82) and control plants (r=0.78). A comparatively higher relationship under waterlogged conditions may be attributed to the rapid recovery of waterlogged plants and pod development. The highest correlations between the number of pods plant^{-1} and seed yield were reported by Ivanovska et al. (2007) and Ali et al. (2003) in *Brassica napus*. Among the other yield contributing traits, a significant relationship between seed weight and seed yield (r=0.39) under waterlogging conditions indicating a better assimilate translocation from shoot to grain possibly contributed to increasing seed yield in waterlogged plants.

Multivariate analysis

Two multivariate methods viz. k-means clustering and discriminant function analysis (DFA) were performed for grouping the genotypes and for identifying the desirable trait(s) of interest related to waterlogging tolerance in 100 mungbean genotypes. The k-means method is widely used among many other techniques for its high-performance quality of clustering with minimum time (Zeebaree et al., 2017; Gayathri et al., 2015). Cluster analysis is the multiple variable method of clustering objects having similar characteristics. The clusters are similar but differences within the clusters are greater (El-Hanjouri and Hamad, 2015). In this study, the traits selected for the multivariate analysis were leaf area, root DM, total shoot DM, SPAD value, branches per plant, pods per plant, straw dry weight, seed yield, and harvest index. These were selected as they were highly correlated with each other and mostly contributed to seed yield. The DFA performs a multivariate test of differences between the groups. It was performed for selecting discriminating variables contributing to the discriminant functions, determining the inter-cluster distance, and graphical illustration of the position of the variable (Verma et al., 2016).

K-means cluster analysis

In k-means clustering, k denotes the number of clusters and the number is usually anonymous but can be chosen by the user (El-Hanjouri and Hamad, 2015). We considered the relative plant traits in the cluster analysis as they exhibited greater variations among genotypes. We arbitrarily grouped the genotypes into seven clusters using k-means non-hierarchical cluster analysis (Table 4). The maximum number of genotypes was included in cluster 7 (35) followed by clusters 5 (18), 1 (12), 3 (11), 6 (11), 2 (10) and 4 (3).

Table 4. The list of 100 mungbean genotypes within 7 clusters classified by k-means cluster analysis based on nine plant characters.

Clustering	No. of genotypes	Genotypes
Cluster 1	12	GK-8, VC-6153 (B-19), IPSA-9, BARI mung 4, VC 6371-94, IPSA-6, Vo-1258 B-G, Vo-1337-A-G, VC 1160 A, VC6163 (B-33), GK-5, Vo1102 A-G
Cluster 2	10	BINA-6, GK-3, BINA-7, VC-6173-B, IPSA-19, VC 6153 (B 20), GK-27, GK-60, Vo 1061 A-G, Vo 1108 B-G
Cluster 3	11	GK-32, IPK-2558-97, VC 6372 (45-8-1), IPSA-24, GK-48, GK-10, VC 6144, IPK 1038-94, GK-23, IPSA-15, ACC 12890054
Cluster 4	3	Vo1353 B-G, VC 1137 A, Vo 1073 A-G
Cluster 5	18	BINA-2, GK-58, VC 6173A, NM 54, Vo1359 B-G, GK-7, VC 6141 (A 90), BUmug 4, IPSA-13, Vo 1396 B-G, BARI mung-2, Vo 1551 B-G, Vo1133 A-G, Vo 1665 A-G, ML-267, ACC12890056, Barisal local, Vo1487 B-G
Cluster 6	11	ACC 12890085, IPSA-10, Vo1472 B-G, GK-3, VC-6173 (B-10), GK-6, BINA-5, IPSA-20, VC 6370-92, GK-65, CO3
Cluster 7	35	VC-3160A-89, IPSA-5, GK-35, GK-1, PDM-11, Vo 1613 A-G, IPSA-10, IPSA-12, GK-50, Vo1183 A-G, Vo 1073 B-G, ACC 12910110, VC-6173 (B-12), Vo1139 B-G, GK-14, BARI mung 6, GK-46, VC 6367 (44-55-2), GK-56, VC 6372 (45-8), GK-21,GK-37, GK-55, BARI mung 5, GK-63, VC 1163, VC 6379 (23-11), Vo1279 A-G, Vo 1319 A-G, GK-16, Vo1368 A-G, GK-29, Vo 1341 B-G, ML 613, Vo 1262 A-G

The mean relative values of nine plant traits of the genotypes within each cluster are presented in Table 5. The genotypes within cluster 1 were characterized by poor relative plant performance regarding leaf area, root and shoot dry matter, SPAD value, branches per plant. The cluster 2 genotypes performed poorly, even much worse in some plant traits including root and shoot dry matter, pods per plant and straw dry weight but showed the highest harvest index. This group of genotypes gave better seed yield compared to that of cluster 1 possibly because of much higher harvest index. The cluster 3 genotypes performed moderately in almost all plant traits. The cluster 4 genotypes showed the lowest relative value of all plant traits except for the leaf area. About 79% of seed yield reduced due to waterlogging in this group of genotypes. The cluster 5 genotypes were categorized also as moderate in terms of morphological traits like leaf area, root and shoot dry matter but yield and yield traits were poor. The cluster 6 genotypes were characterized by the highest relative morphological traits like leaf area, root DM, total shoot DM, SPAD value and yield attributes like pods per plant and straw DM as well as branches per plant. Particularly, the genotypes within this cluster produced the extraordinary amount of adventitious roots. The genotypes within cluster 7 also performed better in terms of morphological and yield traits. However, the genotypes within this group produced the highest yield with the relative value of 0.71. A much higher harvest index (1.33) indicates that the highest yield performance of this group of genotypes was attributed to greater biomass partitioning into the grain. The clustering pattern of the genotypes revealed that the relative performance of the genotypes within clusters 6 and 7 in respect of all plant traits was outstanding compared to genotypes clustered into other groups.

Table 5. The comparison profile of the 7 groups of mungbean genotypes classified by k-means clustering.

Variables in terms of relative values*	Cluster						
	1	2	3	4	5	6	7
Leaf area	0.46	0.39	0.74	0.40	0.60	0.83	0.70
Root dry matter	0.62	0.60	0.90	0.43	0.86	1.07	0.91
Total shoot dry matter	0.52	0.44	0.73	0.42	0.72	0.89	0.77
SPAD value	0.85	0.87	0.86	0.73	0.85	0.90	0.87
Branches per plant	0.45	0.59	0.84	0.42	0.48	0.79	0.61
Pods per plant	0.59	0.41	0.45	0.23	0.41	0.71	0.66
Straw weight	0.43	0.36	0.50	0.32	0.57	0.67	0.49
Seed yield	0.39	0.58	0.44	0.21	0.42	0.64	0.71
Harvest index	0.95	1.42	0.92	0.69	0.80	0.98	1.33

*The relative values are the ratio of the performance of each variable under waterlogging and control conditions.

Discriminant function analysis

The six discriminant functions that differentiated among clusters were obtained by the stepwise procedure. Table 6 summarizes the contribution of each of six canonical discriminant functions for explaining the variance along with their Eigenvalues and canonical correlation coefficient. The larger Eigenvalue (5.0) in function 1 explains that a high variance was noted in the dependent variables. We used functions 1 and 2 having Eigenvalues greater than 1 and reflected 86.7% of the total variation. It gives an idea of the relative performance of the plant traits subjected to waterlogging and indicates that the traits associated with these functions are more useful in differentiating the genotypes. Function 1 alone explained 54.5% of the total variance and function 2 explained 32.2% of the total variance. The high canonical correlation values of functions 1 and 2 also indicate a greater degree of association between the discriminant functions and the dependent variables. The dissimilarities between the clusters were dealt using Wilks' test. The smallest value of Wilks' Lambda in function 1 indicates the greater importance of the independent variables to this function. The Chi-square values are high in functions 1 and 2 indicating a high percentage of variance in the dependent variables within these two functions.

Table 6. The summary of canonical discriminant functions.

Eigenvalues					Wilks' Lambda			
Function	Eigenvalue	% variance	Cumulative %	Canonical correlations	Wilks' Lambda	Chi-square	df	Sig.
1	5.0a	54.5	54.5	0.91	0.015	384.0	42	0.000
2	3.0a	32.2	86.7	0.86	0.092	219.1	30	0.000
3	0.62a	6.8	93.5	0.62	0.366	92.6	20	0.000
4	0.34a	3.7	97.2	0.50	0.592	48.2	12	0.000
5	0.25a	2.7	99.8	0.44	0.791	21.6	6	0.001
6	0.02a	0.2	100.0	0.12	0.985	1.4	2	0.509

The variables that mostly contributed to the discriminatory functions along with their coefficients within each function are presented in Table 7. The coefficients of harvest index and straw dry weight were 1.09 and 0.96 respectively in function 1 that indicates these two variables contributed mostly to explain the total variance within function 1.

Table 7. Standardized canonical discriminant function coefficients of the plant characters that mostly contributed to grouping mungbean genotypes.

Discriminating variables (in relative terms)	Function					
	1	2	3	4	5	6
Root DM	0.46	0.43	0.24	0.05	-0.11	0.23
Total shoot DM	0.39	0.39	-0.15	-0.03	-0.28	0.28
Branches per plant	0.35	0.10	0.61	-0.68	0.03	-0.13
Pods per plant	0.33	0.09	-0.74	-0.20	0.60	0.09
Straw weight	0.96	0.40	0.82	1.93	1.55	0.01
Seed yield	-0.38	-0.37	-0.75	-1.32	-1.69	-0.86
Harvest index	1.09	-0.50	0.82	1.31	1.18	0.55

The other variables that also contributed are branches per plant and pods per plant. In contrast, root and shoot dry matter, and straw dry weight were mostly responsible for the variation in function 2. However, seed yield showed an equal negative contribution to the variability within functions 1 and 2. From Table 8, it is observed that root DM was placed at the top of the list of discriminatory variables with the correlation coefficient of 0.43 within function 1. It indicates that root DM played the most dominant role out of 9 variables in explaining the maximum variance in 100 genotypes by stepwise DFA. Seed yield and harvest index were the secondary important variables in explaining the variation in function 1. Remarkably, the harvest index played the most negative dominant role in explaining the maximum variation within function 2.

Table 8. The structure matrix representing correlations between 9 discriminating variables and standardized canonical discriminant functions of 100 mungbean genotypes.

Plant characters	Functions					
	1	2	3	4	5	6
Root DM	0.43*	0.41	0.08	0.06	-0.259	0.38
Harvest index	0.48	-0.79*	0.03	0.02	-0.139	0.35
Pods per plant	0.38	-0.01	-0.72*	-0.25	0.5	-0.07
Branches per plant	0.20	0.12	0.51	-0.65*	0.33	-0.34
Shoot DM	0.38	0.41	-0.11	-0.03	-0.45*	0.34
Leaf area[a]	0.18	0.17	0.04	0.07	-0.20*	0.06
Straw weight	0.16	0.39	-0.02	0.31	0.06	-0.85*
Yield	0.56	-0.27	-0.14	0.10	-0.31	-0.70*
SPAD[a]	0.12	-0.18	-0.01	-0.03	-0.15	-0.20*

Variables ordered by the absolute size of correlations within the function; *the largest absolute correlation between each variable and any discriminant function; [a]the variable not used in the analysis.

The data were analyzed based on pairwise Mahalanobis distance (D^2) to measure the genetic variability among the genotypes and the average inter-cluster distances. D^2 analysis showed that the seven clusters were statistically different from each other at 0.01 levels (Table 9). The highest distance existed between cluster 7 and cluster 5 followed by clusters 4 and 1 and cluster 6 had a higher distance with clusters 2, 4 and 1. The genotypes within clusters 6 and 7 performed well under waterlogging conditions having less distance from each other. The causes of the highest distance between clusters 5 and 7 as well as clusters 6 and 2 were poor harvest index in cluster 5 but higher harvest index in cluster 2. In contrast, the genotypes were worst affected by waterlogging belonging to clusters 1 and 4. On the other hand, the most similar clusters were clusters 3 and 5 with the lowest distance performed moderately. Figure 2 represents the relative position of the genotypes within the clusters. This is a graphical illustration of how the genotypes are classified into seven clusters according to the first two discriminatory functions i.e. functions 1 and 2. The clustering of the genotypes derived from the k-means cluster analysis is indicated by the closed circles. Function 1 separated clusters 6 and 7 from other clusters. Genotypes situated at the right side of the diagram are characterized by higher harvest index and grain yield under waterlogging conditions. The genotypes that are at the left side produced lower harvest index and seed yield based on X ordinate. On the other hand, the genotypes on the upper side of the diagram produced higher root dry matter and had higher harvest index compared to the genotypes that are scattered at the bottom of the diagram based on Y ordinate.

Table 9. Pairwise Mahalanobis distance (D^2) between the final cluster means.

Cluster number	Pairwise group comparisons						
	1	2	3	4	5	6	7
1	-						
2	13.12**	-					
3	11.94**	17.21**	-				
4	7.80**	16.22**	15.57**	-			
5	9.60**	24.37**	5.69**	13.08**	-		
6	26.52**	31.18**	8.53**	30.64**	16.94**	-	
7	26.70**	17.73**	15.77**	28.74**	32.31**	12.30**	-

**The distance differing at the probability of 0.01.

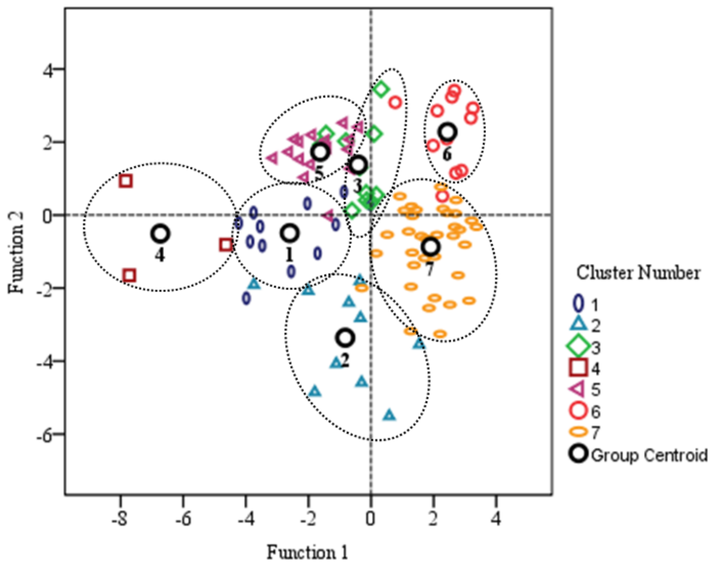
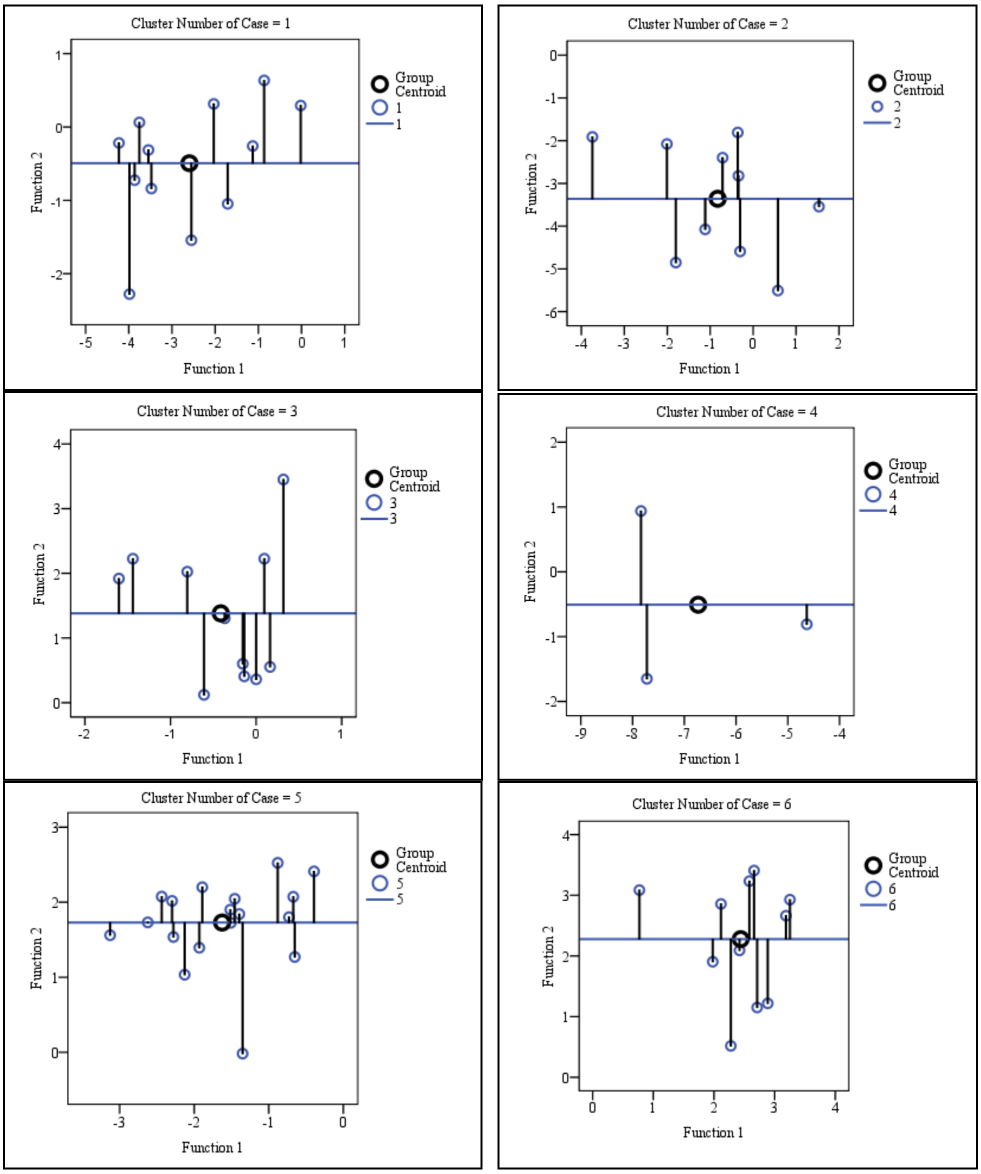


Figure 2. The graphic illustration of the discriminatory analysis of seven clusters of mungbean genotypes.

Representative genotypes

Figure 3 showed the orientation of genotypes within every 7 clusters individually. The relative position of genotypes indicated the cumulative response of variables representing functions 1 and 2. The group centered represented the optimum value in each cluster that resulted from the cumulative effects of all genotypes oriented within that cluster.



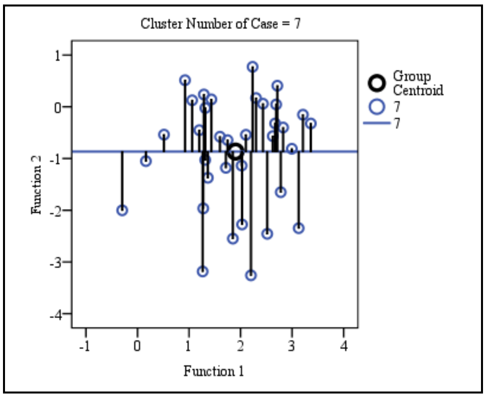


Figure 3. The graphical illustration of genotypes within clusters by DFA based on nine plant characters.

The deviation of the genotypes in the response to discriminating variables was very close to the group centered and might be considered as the most representative (might not be best) of the group. Accordingly, the genotype Vo 1258 B-G in cluster 1, GK-27 in cluster 2, IPK-2558-97 in cluster 3, VC 1137 A in cluster 4, BUmug 4 in cluster 5, IPSA-10 in cluster 6 and VC 6379 (23-11) in cluster 7 were most representative in each cluster.

Conclusion

The study reveals that mungbean genotypes showed a wide range of variation in waterlogging tolerance regarding morpho-physiological characters and yield performance under field conditions. The quick development of adventitious roots in waterlogged plants enhanced waterlogging tolerance by recovering other depressed plant characters. The recoveries of plant traits significantly varied depending on the genotypes. The various multivariate techniques were effectively applied to observe the degree of waterlogging tolerance in the tested genotypes. Thus, the genotypes IPSA-10 and VC 6379 (23-11) were obtained as waterlogging-tolerant considering various yield and yield-related traits. However, the genotypes that showed such tolerance need further evaluation for affirmation of their tolerance to waterlogging.

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OCENA PRINOSA I OSOBINA BILJKE POVEZANIH SA PRINOSOM U POGLEDU TOLERANCIJE NA PREVLAŽIVANJE ZEMLJIŠTA KOD GENOTIPOVA MUNGO PASULJA KORIŠĆENJEM MULTIVARIJANTNIH TEHNIKA

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R e z i m e

Prevlaživanje zemljišta je najčešća i najveća smetnja za proizvodnju mungo pasulja u tropskim i suptropskim regionima sveta i može uzrokovati značajan gubitak prinosa. Studijom je ocenjeno 100 genotipova mungo pasulja u pogledu tolerancije na prevlaživanje zemljišta preciznim i detaljnim ispitivanjima u polju. Mlade tronedeljne biljke bile su izložene prevlaživanju zemljišta tokom 3 dana održavanjem dubine vode na 2,5 cm. Tolerancija na prevlaživanje zemljišta ocenjivana je tokom perioda oporavka i nakon žetve imajući u vidu relativne vrednosti (odnos vrednosti sa prevlaženog zemljišta i iz kontrole bez prevlaživanja) 18 osobina biljaka. Svi genotipovi su pokazali širok raspon varijacija relativnih vrednosti. Neki genotipovi sa prevlaženog zemljišta razvili su mnoštvo adventivnih korenova, što je doprinelo razvoju lišća i rastu sadržaja hlorofila, a rezultiralo bujnijim rastom i konačno, povećanjem prinosa mungo pasulja. U analizi grupisanja korišćeno je 9 osobina biljaka na koje je prevlaživanje imalo visok uticaj. Genotipovi unutar klastera 6 i 7 bolje su se pokazali, uzimajući u obzir skoro sve osobine biljaka, dok su oni iz klastera 4 bili lošiji. Pomoću diskriminante analize, sa funkcijom 1 i funkcijom 2 objašnjava se 54,5 odnosno 32,2% varijanse, što je ukupno 86,7% promenljivosti genotipova. Žetveni indeks i prinos suve materije zrelog stabla objašnjavaju najveći deo ukupne varijacije funkcije 1. Prinos suve materije korena, izdanaka i stabla u žetvi objašnjava najveći deo ukupne varijacija funkcije 2. Suva materija korena igrala je najdominantniju ulogu u objašnjenju maksimalne varijacije genotipova. Genotipovi IPSA-10 i VC 6379 (23-11) pokazali su veći stepen tolerancije na prevlaživanje zemljišta u pogledu prinosa i morfo-fizioloških osobina, koje su povezane sa prinosom.

Ključne reči: genetska varijabilnost, tolerancija na prevlaživanje, rast, prinos, multivarijantna analiza.

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WEED CONTROL AND PRODUCTIVITY OF MAIZE (*ZEA MAYS* L.)

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Abstract: Field trials were conducted at the Teaching and Research Farm of Kwara State University, Malete, to determine the weed control method that will be more effective in controlling weeds and give higher grain yield and cash returns in the production of maize. The experiment consisted of 9 treatments: Primextra + Aminicome at 1.5 + 1.5 kg ha^{-1} (metolachlor 375 g a.i. ha^{-1} + atrazine 375 g a.i. ha^{-1} + 2,4 – D 900 g a.i. ha^{-1}), Primextra + Aminicome at 2.0 + 2.0 kg ha^{-1} (metolachlor 500 g a.i. ha^{-1} + atrazine 500 g a.i. ha^{-1} + 2,4 – D 1200 g a.i. ha^{-1}), Primextra + Aminicome at 2.5 + 2.5 kg ha^{-1} (metolachlor 750 g a.i. ha^{-1} + atrazine 750 g a.i. ha^{-1} + 2,4 – D 1500 g a.i. ha^{-1}), Primextra + Guard force at 1.5 + 0.03 kg ha^{-1} (metolachlor 375 g a.i. ha^{-1} + atrazine 375 g a.i. ha^{-1} + nicosulfuron 1.2 g a.i. ha^{-1}), Primextra + Guard force at 2.0 + 0.05 kg ha^{-1} (metolachlor 500 g a.i. ha^{-1} + atrazine 500 g a.i. ha^{-1} + nicosulfuron 2.0 g a.i. ha^{-1}), Primextra + Guard force at 2.5 + 0.07 kg ha^{-1} (metolachlor 750 g a.i. ha^{-1} + atrazine 750 g a.i. ha^{-1} + nicosulfuron 2.8 g a.i. ha^{-1}), Primextra at 1.5 kg ha^{-1} (metolachlor 375 g a.i. ha^{-1} + atrazine 375 g a.i. ha^{-1}) + one supplementary hoe weeding (SHW) at 6 weeks after sowing (WAS), two hand weedings at 3 and 6 weeks after sowing (WAS) and a weedy check. These treatments were laid out in a randomized complete block design (RCBD) with three replicates. Data collected were subjected to analysis of variance using the Statistical Analysis Software (SAS) package, after which means were separated using Duncan's Multiple Range Test (DMRT). Results showed that treatment combinations of Primextra at 1.5 Kg ha^{-1} + one SHW at 6 WAS, two hoe weedings at 3 and 6 WAS, Primextra + Aminicome at 2.0 + 2.0 kg ha^{-1} and Primextra + Guard force at 2.0 + 0.05 kg ha^{-1} gave effective weed control, higher grain yield and cash returns. They are therefore recommended for application in rotation by farmers in Malete.

Key words: integrated weed management, southern Guinea savanna, maize, yield, cash returns.

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Introduction

Maize (*Zea mays* L.) was cultivated previously on a subsistence scale in Africa. However, it has gradually become an important commercial crop and serves as raw material for many agro-allied industries (Iken and Amusa, 2004). Ogunsami et al. (2005) reported that growing maize by small-scale farmers can overcome hunger in the households and the aggregate effect could double food production in Africa.

The demand for maize is high and this creates an opportunity to increase production per unit area. FAO (2017) reported that 822.7 million metric tons of maize were produced worldwide in 2008 and Nigeria produced 7.3 million tons in 2009. However, this figure was increased to 10.7 and 10.5 million metric tonnes in 2015 and 2017 respectively.

According to Khan et al. (2003), the average production of maize in Africa is still abysmally low, ranging between 1.3 and 1.5 tons/ha and unless the present trends are reversed, Africa will have the world's largest net deficit in cereals in the near future (Mwangi,1995).

Among the factors attributed to the difference between potential and actual yields of maize in Africa is weed infestation. Maize is highly sensitive to weed competition especially at the early stages of development (Hall et al., 1992). Weeds do not only cause severe yield losses, but also require farmers and families to spend more of their time on weeding. Manual weed control remains the predominant method of weed control by small-holder farmers in Africa (Chikoye et al., 2002). Past research works have revealed that two hoe weedings at 3 and 6 WAS resulted in effective weed control and higher maize yields (Imoloame, 2016 and 2017). Despite the advantages of this method, it is time-consuming, laborious and expensive per hectare. It is reported that weeding one hectare of land planted with maize may require as much as 25–40 man-days, representing approximately 50–80% of labour budget (Darkwa et al., 2001; Chikoye et al., 2002). This is buttressed by the findings of Ekeleme (2009) that 25–55% of the total cost of production cost is spent on labour and weeding operations.

Chemical weed control has been reported to be a better alternative to manual weeding despite criticism that it leaves toxic residues in the environment. This is because it is cheaper, faster, minimizes drudgery, gives better control of weeds and increases biological yield of crops (Chikoye et al., 2004; Ali et al. 2003; Haider et al. 2009). However, this weed control method is being used indiscriminately by Nigerian farmers as most of them are illiterates and there is lack of information on the correct doses of herbicides to apply. These problems have the potential of causing environmental pollution, herbicide-resistant weeds, herbicide residues in crops and health hazards (Best-Ordinioha, 2017). It is therefore important to come up with the correct minimum herbicide rates of the common herbicides applied in maize in Malete.

An integration strategy that combines low doses of herbicide and hand hoeing will not only cut down the herbicide dose used, but it has been found to be environmentally friendly, more effective and efficient for weed control compared with the use of one single method (Kadil and Kordy, 2013; Imoloame, 2017 and 2018). There is a dearth of information that compares the performance of herbicide at low dose integrated with one SHW at 6WAS with the application of a combination of pre-and post-emergence herbicides for weed control in maize. This is very important as the outcome of the study may provide information on the minimum application rates of the commonly used herbicides and better weed management options that can serve as an alternative to hoe weeding for more effective and profitable weed control in maize in Malete and southern Guinea savanna of Nigeria. The hypothesis of this study is that pre-emergence application of a combination of a low dose of herbicides plus one SHW at 6 WAS will provide most effective and season-long weed control, higher grain yield and cash returns in the production of maize. Therefore, the objectives of this study are to determine:

1. the weed management strategy that will be more effective for weed control and that will increase maize grain yield.
2. the weed management strategy that will be more profitable in the production of maize.

Materials and Methods

Site description

The experiment was conducted during the 2017 and 2018 cropping seasons at the Kwara State University Teaching and Research (T&R) Farm, Malete (Lat.08^{o} 71$^{'}$N; Long.04^{o} 44$^{o'}$E), Nigeria. The experimental site was characterized by two peaks of rainfall in June and September and the soil was sandy loam with low water-retaining capacity.

Treatments and experimental design

The experiment consisted of nine treatments: Primextra + Aminicome at 1.5 + 1.5 kg ha^{-1} (metolachlor 375 g a.i. ha^{-1} + atrazine 375 g a.i. ha^{-1} + 2,4 – D 900 g a.i. ha^{-1}), Primextra + Aminicome at 2.0 + 2.0 kg ha^{-1} (metolachlor 500 g a.i. ha^{-1} + atrazine 500 g a.i. ha^{-1} + 2,4 – D 1200 g a.i. ha^{-1}), Primextra + Aminicome at 2.5 + 2.5 kg ha^{-1} (metolachlor 750 g a.i. ha^{-1} + atrazine 750 g a.i. ha^{-1} + 2,4 – D 1500 g a.i. ha^{-1}), Primextra + Guard force at 1.5 + 0.03 kg ha^{-1} (metolachlor 375 g a.i. ha^{-1} + atrazine 375 g a.i. ha^{-1} + nicosulfuron 1.2 g a.i. ha^{-1}), Primextra + Guard force at

2.0 + 0.05 kg ha^{-1} (metolachlor 500 g a.i. ha^{-1} + atrazine 500 g a.i. ha^{-1} + nicosulfuron 2.0 g a.i. ha^{-1}), Primextra + Guard force at 2.5 + 0.07 kg ha^{-1} (metolachlor 750 g a.i. ha^{-1} + atrazine 750 g a.i. ha^{-1} + nicosulfuron 2.8 g a.i. ha^{-1}), Primextra at 1.5 (metolachlor 375 g a.i. ha^{-1} + atrazine 375 g a.i. ha^{-1}) + one supplementary hoe weeding (SHW) at 6 weeks after sowing (WAS), two hand weedings at 3 and 6 weeks after sowing (WAS) and a weedy check.

These treatments were laid out in a randomized complete block design (RCBD) with three replicates. Data collected were analyzed using the Statistical Analysis Software (SAS) package. Means were separated using Duncan's Multiple Range Test (DMRT) at 5% level of probability.

The gross area used for the experiment was 567 m^2. This was plowed, harrowed and later demarcated into plots measuring 4 m x 4 m each. Three treated seeds of the maize variety SUWAN -1-SR were sown per hole spaced at 75 cm x 25 cm, on the 14th and 11th of July, 2017 and 2018 respectively. The seedlings that emerged were thinned to one plant/stand to give a plant population of 53,333 per hectare. The application of pre-emergence herbicide (metolachlor + atrazine) was done a day after sowing, while that of post-emergence herbicides (nicosulfuron and 2, 4-D) was carried out at 6 WAS. The sprayer used for herbicide application was calibrated to deliver 208 l ha^{-1} of herbicide solution. Fertilizer was applied in two split doses; one at planting and the other at 6 WAS at the rate of 120 kg N, 60 kg P and 60 kg K. Insecticide 'Strong Force' (methomyl 90%) as the active ingredient was applied to control armyworm (*Spodoptera exempta*) at the rate of 10g/15 liters of water. Harvesting of maize was done on the 13th and 12th of November 2017 and 2018 respectively.

The following parameters were measured:

Weed dry matter (g m^{-2})

Weed dry matter was determined by harvesting weeds from one square meter quadrat, randomly placed in three locations within each plot. The weeds were put in well-labeled envelopes which were later oven-dried at a temperature of 80^{o}C for 2 days to constant weight before the final weights were taken. The weed dry matter was taken at 6 and 12 WAS.

Weed cover score

Weed cover score was determined at 6 and 12 WAS by visual observation using a scale of 0–9, where 0 means weed-free plots and 9 complete weed cover of plots.

Weed density (no m^{-2})

Weed density was determined at 6 and 12 WAS by counting the number of weed species within a quadrat (1 m^2), randomly placed in three locations within each plot and the total number of weed species per unit area was recorded.

Shannon-Weiner species diversity index H’

This is a mathematical measure of species diversity in a given community and it is based on the species richness (the number of species present) and species abundance (the number of individuals per species). It is calculated using the formula below:

Shannon Weiner diversity index, $H' = \sum_n^s = 1Pi \text{ In } Pi$,

Pi = Proportion of (ni/N) and it is the number of individuals of one particular species (n) divided by the total number of all individuals in the sample (N),

S = The total number of species found in the community,

In = Naparian log (2.303 x $\log_{10}$).

Leaf area (cm^2)

Leaf area of maize was determined at 6 and 12 WAS by using the expression. Leaf area (LA) = Length (L) width (W) x 0.75. The leaf area was obtained by measuring the length and width of leaves from five randomly selected plants from each plot and the average of these measurements was multiplied by a factor of 0.75 to give the leaf area per plant.

Grain yield (kg ha^{-1})

Grain yield was determined by weighing the grains with a moisture level of 13%, harvested from each net plot and was converted to kilogram per hectare using the equation below:

$$\text{Grain yield} = \frac{\text{Grain yield per net plot x } 10{,}000m^2}{\text{Net plot size } (m^2)}$$

Economic analysis

Information on the cost of all the cultural practices from land preparation to harvesting and processing was collected from Kwara State Agricultural Development Programme (KWASADP), Ilorin, an agency responsible for extension services in Kwara State, Nigeria. The average price of 1 kg of maize in 2018 was obtained from the open market to calculate the income/revenue. The economic assessment was done for different treatments to determine the most cost-effective or profitable method of weed management for the production of maize.

The economic analysis was carried out using partial budgeting (Okoruwa et al., 2005) to calculate the gross margin (profit). The benefit: cost ratio was also determined as follows:

GM = TR – VC;

TR = (Ys X Ps);

VC = M + L;

where: GM = Gross margin/ha for each treatment;

TR = Total revenue (Naira (₦)/United States Dollars ($) for each treatment;

VC = Variable cost (Naira ₦/$) for each treatment;

Ys = Maize grain yield (Kg ha^{-1}) for each treatment;

Ps = Price of maize per kg;

M = Value of material input (seeds, fertilizer, insecticide, herbicides etc.);

L = Value of labour (land preparation, planting, insecticide and herbicide, fertilizer application, harvesting, processing and packaging).

Also, the benefit-cost ratio was determined using the following equation:

Benefit-cost ratio = I/TCP

where TCP is the total cost of production and I is income.

Results and Discussion

Rainfall figures

Total rainfall of 1014.8 and 1451.1 mm was recorded in 2017 and 2018 respectively. The two peaks of rainfall occurred in August and September in 2017, while May and September recorded the highest rainfall in 2018 (Figure 1).

Effect of weed control treatments on weed dry matter and weed cover score

Weed control treatments had a significant ($p<0.05$) effect on weed dry matter and weed cover score in 2017 and 2018 in Malete (Table 1). In 2017, Primextra at 1.5 kg ha^{-1} + one SHW at 6 WAS resulted in weed dry matter that was significantly ($P<0.05$) lower than the weedy check but was comparable with other herbicide treatments and hoe weeding at 3 and 6 WAS, while in 2018, all the treatment combinations significantly reduced weed dry matter compared to the weedy check at 6 WAS. At 12 WAS in 2017, plots treated with Primextra at 1.5 kg ha^{-1} + one SHW at 6 WAS, two hoe weedings at 3 and 6 WAS and Guard force at lower rates resulted in weed dry matter that was significantly ($P < 0.05$) lower than the weedy check but which was not different from other herbicide treatments, whereas in 2018, hoe weeding at 3 and 6 WAS significantly reduced weed dry matter. This performance was comparable with other treatments except for Primextra + Aminicome at 1.5 + 1.5 kg ha^{-1} and all the rates of Primextra + Guard force and the weedy check (Table 1).

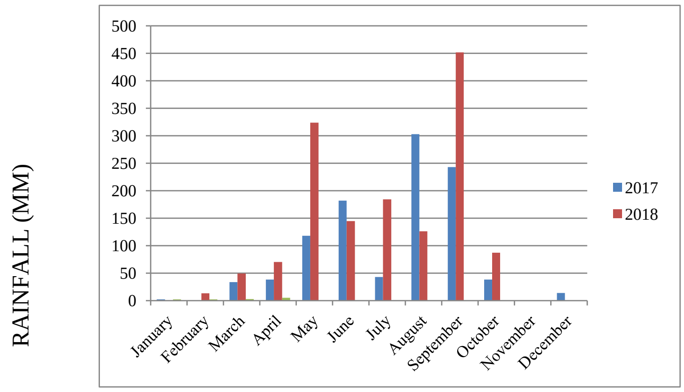

Figure 1. Rainfall figures for 2017 and 2018 rainy seasons (mm).
Source: Lower Niger River Basin and Rural Development Authority, Hydrology Section, Ilorin, Kwara State.

Table 1. The effect of weed control methods on weed dry matter in maize in 2017 and 2018.

		Weed dry matter (g m^{-2})			
Treatment	Rate (kg ha^{-1})	6 WAS[1]		12 WAS	
		2017	2018	2017	2018
P+A	1.5 + 1.5	75.3^{ab2}	293.8^{b}	79.8^{ab}	2519.7^{a}
P+A	2.0 + 2.0	91.4^{ab}	367.1^{b}	94.7^{ab}	1458.9^{bc}
P+A	2.5 + 2.5	159.4^{ab}	362.0^{b}	173.6^{ab}	1027.7^{bc}
P+GF	1.5 + 0.03	58.0^{b}	445.3^{b}	59.1^{b}	1572.9^{ab}
P+GF	2.0 + 0.05	60.1^{ab}	347.1^{b}	68.3^{b}	1060.7^{ab}
P+GF	2.5 + 0.07	88.8^{ab}	238.7^{b}	91.4^{ab}	1745.8^{ab}
P+ 1SHW	1.5	44.0^{b}	382.3^{b}	53.8^{b}	505.8^{bc}
3 + 6 WAS	-	62.5^{ab}	123.4^{b}	70.4^{b}	340.2^{c}
Weedy check	-	195.5^{a}	976.5^{a}	214.6^{a}	2518.6^{a}

WAS = Weeks after sowing; Means having the same letter(s) are not significantly different at 5% level of probability according to Duncan's Multiple Range Test (DMRT). P = Primextra; A = Aminicome; GF = Guard force; SHW = Supplementary hoe weeding.

The same trend was observed regarding weed cover as all herbicide treatments, two hoe weedings and Primextra at 1.5 kg ha^{-1} + one SHW at 6 WAS caused a significant reduction in weed cover in comparison with weedy check in 2017, while in 2018, it was only hoe weeding at 3 and 6 WAS that had a significant and positive influence on weed cover at 6 WAS. At 12 WAS in 2017, all herbicide treatments, two hoe weedings and a combination of herbicide and one SHW at 6 WAS sustained a significant (P< 0.05) reduction in weed cover than the weedy check. However, in 2018, all the treatment combinations caused a significant (P < 0.05) reduction in the weed cover except for Primextra + Aminicome at 2.5+2.5 and Primextra + Guard forceat 2.5 + 0.07 kg a ha^{-1} which had significantly higher weed cover that was comparable with the weedy check (Table 2). Generally, the amount of weed biomass and weed cover recorded under the treatments was greater in 2018 than in 2017. The treatment combination of Primextra at 1.5 kg ha^{-1} + one SHW at 6 WAS, Primextra + Aminicome at 2.0 + 2.0 kg ha^{-1} and two hand weedings at 3 and 6 WAS were consistent in providing more effective and season-long weed control in maize plots.

Table 2. The effect of weed control methods on weed cover score in maize in 2017 and 2018.

Treatment	Rate (kg ha^{-1})	Weed cover score 6 WAS[1] 2017	Weed cover score 6 WAS[1] 2018	Weed cover score 12 WAS 2017	Weed cover score 12 WAS 2018
P+A	1.5 + 1.5	4.3^{b2}	7.0^{a}	4.0^{b}	6.2^{b}
P+A	2.0 + 2.0	4.3^{b}	7.7^{a}	4.3^{b}	4.2^{bc}
P+A	2.5 + 2.5	5.7^{b}	7.7^{a}	5.0^{b}	7.5^{ab}
P+GF	1.5 + 0.03	2.7^{b}	8.3^{a}	3.5^{b}	6.0^{b}
P+GF	2.0 + 0.05	3.0^{b}	7.7^{a}	3.3^{b}	6.3^{b}
P+GF	2.5 + 0.07	5.7^{b}	8.7^{a}	4.3^{b}	6.8^{ab}
P+ 1SHW	1.5	4.7^{b}	8.0^{a}	5.7^{b}	1.8^{c}
3 + 6 WAS	-	5.0^{b}	2.7^{b}	5.7^{b}	1.2^{c}
Weedy check	-	10.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}

WAS = Weeks after sowing, 1 – Means having the same letter(s) are not significantly different at 5% level of probability according to Duncan’s Multiple Range Test (DMRT). P = Primextra; A = Aminicome; GF = Guard force; SHW = Supplementary hoe weeding.

These weed control methods can be applied in rotation in maize fields for weed control. Imoloame (2014) reported that two hand weedings and a combination of herbicide + hand weeding at 6 WAS significantly reduced weed infestation in soybean production. The rotation of the above methods of weed control will help to minimize the chances of herbicide-resistant weeds or weed flora shift. The higher amount of weed biomass observed in 2018 compared to 2017 could be due to the higher amount of rainfall in that year.

Diversity Index (H^1) of weeds under different treatments

Table 3 shows that a total of 16 weed species were observed across treatments. This number is broken down into 9 grass, 5 broadleaved and 2 sedge weed species. It also shows the diversity index ($H^'$) of weed species under different treatments. The weed flora diversity (1.7) was the highest in plots with Primextra + Aminicome at 2.0 + 2.0 kg ha^{-1}, while the lowest diversity was (0.6) in plots treated with Primextra + Guard force at 2.0 + 0.03 kg ha^{-1}.

Table 3. Shannon Weiner diversity index ($H^'$) at 12 WAS in maize in 2018.

Species	Weed form	P+ A 1.5+1.5	P+A 2.0+ 2.0	P+A 2.5 +2.5	P+GF at 1.5+0.03	P+GF at 2.0+0.05	P+GF at 2.5+0.07	3 + 6 WAS	P at 1.5 + SHW	Weedy check
Brachiaria alata	G	18	18	3	-	-	7	-	-	1
Paspalum scrobiculatum	G	59	59	64	62	76	12	158	33	123
Cyperus esculentus	G	-	-	-	-	-	-	-	1	-
Commelina benghalensis	BL	3	3	-	-	-	3	-	-	2
Pycreus lanceolatus	BL	-	-	-	-	-	-	-	31	15
Rottboellia cochinchinensis	G	33	33	28	-	2	4	-	-	1
Digitaria horizontalis	G	65	65	108	117	85	60	55	6	8
Hyptis suaveolens	BL	-	-	-	4	1	4	-	-	1
Gomphrena Celosiodes	BL	-	-	-	-	-	-	-	-	-
Grass (unidentified)	G	-	-	-	-	-	-	-	-	-
Dactyloctenium aegyptium	G	-	-	-	-	-	-	5	-	2
Broad leaf (unidentified)	BL	-	-	-	-	-	-	-	-	1
Chloris pilosa	G	-	-	-	-	-	-	-	-	1
Cyprus rotundus	S	-	-	-	-	-	-	-	-	3
Kyllinga squamulata	S	-	-	-	-	-	-	-	1	11
Kyllinga erecta	S	-	-	-	-	-	-	-	21	-
Seteria barbata	G	-	-	-	-	-	-	-	-	8
Shannon weiner index (H')		1.3	1.3	1.8	0.762	0.6	1.1	0.7	1.3	1.1

The Shannon – Weiner diversity index ($H^'$) ranged from 0 to 4.6. A value near 0 indicates that every species in the sample is the same, while a value near 4.6 would indicate that the numbers of individuals are evenly distributed between all the species (Husnatulyusra, 2012). Therefore the Shannon – Weiner diversity index

(H') recorded ranging from 0.6 to 1.7 under each treatment indicates that the individual number of present weed species is not evenly distributed since H' is near 0. The low Shannon Weiner diversity index value (close to 0), explains the dominance of *Paspalum scrobiculatum* across treatments and *Digitaria horizontalis* in the plots treated with Primextra + Aminicome and Primextra + Guard force at all rates. The dominance of *Paspalum scrobiculatum* across treatments suggests the ineffectiveness of the various weed options to control this species throughout the season and it is an indication of weed ability to easily adapt to the environment. The prevalence of the two grass weed species mentioned above in the plots treated with Primextra + Aminicome at all the rates was expected as the post-emergence herbicide has a narrow spectrum of activity for the effective control of only broadleaved but not grass weeds. This result is similar to the findings of Imoloame (2017), who reported the inability of tank mixture of metolachlor + atrazine and pendimethalin + atrazine at 1.0 + 2.0 kg a.i. ha^{-1} plus one SHW at 6 WAS to fully control *Paspalium scrobiculatum*. This information is very useful as it will help in the formulation of a better weed strategy for its effective control.

Effect of weed control treatments on leaf area

There was a significant ($p<0.05$) difference in the leaf area among treatments (Table 4).

Table 4. The effect of weed control methods on leaf area in maize in 2017 and 2018.

		Leaf area (cm^2)			
Treatment	Rate (kg ha^{-1})	6 WAS[1]		12 WAS	
		2017	2018	2017	2018
P+A	1.5 + 1.5	139.3[a]	275.1[ab]	152.7[a]	351.3[abc]
P+A	2.0 + 2.0	117.0[abc]	288.3[ab]	138.1[a]	386.6[ab]
P+A	2.5 + 2.5	112.9[abc]	278.3[ab]	105.4[bc]	334.8[bc]
P+GF	1.5 + 0.03	129.3[ab]	287.9[ab]	132.2[ab]	352.4[abc]
P+GF	2.0 + 0.05	81.3[c]	318.0[a]	108.0[bc]	392.8[ab]
P+GF	2.5 + 0.07	88.1[c]	274.6[ab]	102.4[bc]	325.0[c]
P+ 1SHW	1.5	127.9[ab]	295.0[ab]	141.6[a]	380.7[abc]
3 + 6 WAS	-	109.9[abc]	307.1[a]	123.4[ab]	398.4[a]
Weedy check	-	91.5[c]	273.4[ab]	90.3[c]	333.3[bc]

WAS = Weeks after sowing, 1 – Means having the same letter(s) are not significantly different at 5% level of probability according to Duncan's Multiple Range Test (DMRT). P = Primextra; A = Aminicome; GF = Guard force; SHW = Supplementary hoe weeding.

In 2017 and at 6 WAS, plots treated with Primextra + Aminicome at 1.5 + 1.5 kg ha^{-1}, Primextra + Aminicome at 2.0 + 2.0 kg ha^{-1}, Primextra at 1.5 kg ha^{-1} + one

SHW at 6 WAS, Primextra + Guard force at 1.5 + 0.03 kg ha^{-1} and two hand weedings, produced significantly ($p < 0.05$) larger leaves than the weedy check and Primextra + Guard force at higher rates. At 12 WAS, in 2017, Primextra at 1.5 kg ha^{-1} + one SHW and Primextra + Aminicome at 1.5 + 1.5 kg ha^{-1}, as well as Primextra + Aminicome at 2.0 + 2.0. kg ha^{-1} resulted in crops with significantly larger leaves which were statistically different from other treatment combinations and significantly larger than the weedy check. However, in 2018 and at 6 WAS, two hoe weedings at 3 and 6 WAS and Primextra + Guard force at 2.5 + 0.05 kg ha^{-1} gave rise to crops with leaf area that was comparable with other treatment combinations but significantly ($p < 0.05$) greater than the weedy check. –In the same year and at 12 WAS, the highest leaf area was detected in the treatment with two hand weedings. The larger leaf area of maize in the plots treated with Primextra + Aminicome at 1.5 + 1.5 kg ha^{-1}, Primextra + Aminicome at 2.0 + 2.0 kg ha^{-1}, Primextra + Guard force at 1.5 + 0.03, Primextra + Guard force at 2.5 + 0.05 kg ha^{-1}, Primextra at 1.5 kg ha^{-1} + one SHW at 6 WAS and two hand weedings provided a larger surface for the interception of a greater amount of light for increased photosynthesis and higher yields.

Effect of weed control treatments on yield and yield components

Primextra at 1.5 kg ha^{-1} + one SHW at 6 WAS and two hoe weedings at 3 and 6 WAS in 2017 produced the highest maize grain yields which were not statistically different from the other herbicide treatments but were significantly ($p < 0.05$) different from the weedy check (Table 5).

Table 5. The effect of weed control methods on 100-seed weight and grain yield in maize in 2017 and 2018.

Treatment	Rate (kg ha^{-1})	100-seed weight (g)		Grain yield kg ha^{-1}	
		2017	2018	2017	2018
P+A	1.5 + 1.5	19.8[a]	21.5[a]	736.5[ab]	1527.2[b]
P+A	2.0 + 2.0	18.4[a]	21.2[a]	433.9[ab]	3122.5[a]
P+A	2.5 + 2.5	17.4[a]	19.5[a]	871.0[ab]	1834.5[ab]
P+GF	1.5 + 0.03	20.9[a]	19.9[a]	1038.2[ab]	2491.3[ab]
P+GF	2.0 + 0.05	20.0[a]	21.5[a]	1160.4[ab]	2793.4[ab]
P+GF	2.5 + 0.07	19.9[a]	20.3[a]	977.8[ab]	2401.7[ab]
P+ 1SHW	1.5	19.6[a]	20.0[a]	1416.2[a]	2878.7[a]
3 + 6 WAS	-	21.0[a]	19.3[a]	1317.8[a]	3140.9[a]
Weedy check	-	16.6[a]	20.5[a]	331.1[b]	1444.5[b]

1 – Means having the same letter(s) are not significantly different at 5% level of probability according to Duncan's Multiple Range Test (DMRT). P = Primextra; A = Aminicome; GF = Guard force; SHW = Supplementary hoe weeding.

While in 2018, a similar trend was observed as Primextra at 1.5 kg ha^{-1} + one SHW at 6 WAS, two hand weedings at 3 and 6 WAS and Primextra + Aminicome at 2.0 + 2.0 kg ha^{-1} and other herbicide combinations resulted in grain yield values significantly ($p < 0.05$) higher than the weedy check and Primextra + Aminicome at 1.5 + 1.5 kg ha^{-1}. The significantly higher grain yields produced from the above-mentioned plots were a result of the ability of the above weed control methods to consistently provide season-long weed control, which could have increased the amount of growth resources available to maize, which in turn led to the production of significantly larger leaves for enhanced photosynthesis and grain yield. The above treatment combinations can serve as an alternative to hoe weeding which could be applied in rotation for effective weed control and higher grain yields in maize. The weedy check produced significantly lower yields as a result of the intense competition between the maize crop and the weeds particularly *Paspalum scrobiculatum* and *Digitaria horizontalis* for growth resources.

Economic evaluation of different weed control methods in maize production

The highest revenues (₦267,492.00/$?743.03), (₦257,700.00/$715.83) and (₦ 237,228.00/$658.00) were obtained from plots treated with Primextra at 1.5 kg ha^{-1} + one SHW, followed by two hand weedings at 3 and 6 WAS and Primextra + Guard force at 2.0 + 0.05 kg ha^{-1}, while the weedy check resulted in the lowest revenue (₦ 111,504.00/ $ 309.73) (Table 6). Plots that gave higher revenues produced higher yields of maize. The most expensive weed control method (₦ 173,900.00/ $ 451.70) was the treatment combination of Primextra + Aminicome at 2.5+ 2.5 kg ha^{-1}, while the lowest cost (₦ 143,900.00/ $ 399.72) was incurred under the weedy check in which weeds were not controlled at all. The next plot treated with herbicides with the lowest cost in the production of maize was Primextra at 1.5 kg ha^{-1} + one SHW. This is at variance with the findings of Imoloame (2014, 2017, 2018) that hoe weeding is most expensive compared with chemical and integrated weed control methods. This demonstrates the fact that chemical weed control becomes more expensive as application rates are increased. The treatment that is the most profitable in the production of maize is Primextra at 1.5 kg ha^{-1} + one SHW (₦ 109,592.00/$302.42) followed by two hoe weedings at 3 and 6 WAS (₦ 93,700.00/$D260.00), Primextra + Guard force at 2.0+0.05 kg ha^{-1} (₦ 70,328.00/ $ 195.36) and Primextra + Aminicome at 2.0 + 2.0 kg ha^{-1} (₦ 63,640.00/$ 176.78) in the descending order. The other treatments like Primextra + Aminicome at 1.5 + 1.5 kg ha^{-1}, Primextra + Aminicome at 2.5 + 2.5 kg ha^{-1} and the weedy check resulted in losses. This could be due to the ability of these methods of weed control to increase the grain yield of maize, compared with the other treatments like Primextra + Aminicome at 1.5 + 1.5 kg ha^{-1}, 2.5 + 2.5 kg ha^{-1} and the weedy check. Similarly, these methods of weed control, Primextra at 1.5 kg ha $^{-1}$ + one SHW at 6 WAS, two hoe weedings at 3 and 6 WAS, Primextra + Guard force at 2.0 + 0.05 kg ha^{-1} and Primextra + Aminicome at 2.0 + 2.0 kg ha^{-1} had a higher benefit: cost ratio, implying that they were more economical and profitable in the production of maize in Malete, Nigeria.

Table 6. The profitability of different weed control methods in the production of maize in Malete in naira (₦) and US dollars ($) in 2017 and 2018.

Farm operations/hectare	P+A 1.5+1.5	P+A 2.0+2.0	P+A 2.5+2.5	P+GF at 1.5+0.03	P+GF at 2.0+0.05	P+GF at 2.5+0.07	3 & 6 WAS	P at 1.5+ISHW	Weedy check
Land preparation	18,000.00 (50.00)	18,000.00 (50.00)	18,000.00 (50.00)	18,000.00 (50.00)	18,000.00 (50.00)	18,000.00 (50.00)	18,000.00 (50.00)	18,000.00 (50.00)	18,000.00 (50.00)
Seeds	4,200.00 (11.00)	4,200.00 (11.00)	4,200.00 (11.00)	4,200.00 (11.00)	4,200.00 (11.00)	4,200.00 (11.00)	4,200.00 (11.00)	4,200.00 (11.00)	4,200.00 (11.00)
Planting	6,000.00 (16.70)	6,000.00 (16.70)	6,000.00 (16.70)	6,000.00 (16.70)	6,000.00 (16.70)	6,000.00 (16.70)	6,000.00 (16.70)	6,000.00 (16.70)	6,000.00 (16.70)
Fertilizer application	9,000.00 (25.00)	9,000.00 (25.00)	9,000.00 (25.00)	9,000.00 (25.00)	9,000.00 (25.00)	9,000.00 (25.00)	9,000.00 (25.00)	9,000.00 (25.00)	9,000.00 (25.00)
Cost of fertilizer (NPK and urea)	75,000.00 (209.00)	75,000.00 (209.00)	75,000.00 (209.00)	75,000.00 (209.00)	75,000.00 (209.00)	75,000.00 (209.00)	75,000.00 (209.00)	75,000.00 (209.00)	75,000.00 (209.00)
Cost of the first hoe weeding	-	-	-	-	-	-	10,000.00 (27.78)	10,000.00 (27.78)	-
Cost of the second hoe weeding	-	-	-	-	-	-	10,000.00 (27.78)	-	-
Cost of herbicide application (Pre- and post-emergence)	8,000.00 (22.00)	8,000.00 (22.00)	8,000.00 (22.00)	8,000.00 (22.00)	8,000.00 (22.00)	8,000.00 (22.00)	8,000.00 (22.00)	8,000.00 (22.00)	8,000.00 (22.00)
Cost of herbicide	13,500.00 (37.50)	13,500.00 (37.50)	13,500.00 (37.50)	13,500.00 (37.50)	13,500.00 (37.50)	13,500.00 (37.50)	13,500.00 (37.50)	13,500.00 (37.50)	13,500.00 (37.50)
Cost of pesticide application	3,300.00 (9.20)	3,300.00 (9.20)	3,300.00 (9.20)	3,300.00 (9.20)	3,300.00 (9.20)	3,300.00 (9.20)	3,300.00 (9.20)	3,300.00 (9.20)	3,300.00 (9.20)
Cost of pesticide	8,000.00 (22,20)	8,000.00 (22,20)	8,000.00 (22,20)	8,000.00 (22,20)	8,000.00 (22,20)	8,000.00 (22,20)	8,000.00 (22,20)	8,000.00 (22,20)	8,000.00 (22,20)
Labour cost for harvesting, processing and bagging	20.000.0 (55.60)	20.000.0 (55.60)	20.000.0 (55.60)	20.000.0 (55.60)	20.000.0 (55.60)	20.000.0 (55.60)	20.000.0 (55.60)	20.000.0 (55.60)	20.000.0 (55.60)
Total cost of production (VC)	165,700.0 (429.67)	167,900.0 (437.80)	173,900.0 (451.70)	162.700.0 (420.60)	166,900.0 (463.60)	170,700.0 (474.20)	164,000.0 (455.60)	157,900.0 (438.61)	143,900.0 (399.72)
Average yield/ha	1,289.00	1929.5	1352.8	1,764.8	1,976.9	1,689.8	2,147.5	2,229.1	929.2
Selling price (TR)	154.680.0	231,540.0	162.336.0	211,776,0	237,228.0	202,776	257,700.0	267,492.0	111,504.0
Profit (GM)	-11,020.0 (-30.61)	63,640.00 (176.78)	-11,564.0 (-32.12)	49,076.0 (126.32)	70,328.0 (195.36)	320.76 (89.10)	93,700.0 (260.28)	109,592.0 (304.42)	-32,396.0 (-89.98)
Benefit: cost ratio	0.933	1.379	0.933	1.302	1.421	1.00	1.571	1.694	0.775

1. The average price of maize in the open market in 2018 = ₦120/kg. 2. The prices in parenthesis are in United States dollars (USD $), while the ones not in parenthesis are in naira (₦). 3. The exchange rate between the naira and the US dollars = ₦1=USD360.

Conclusion

The findings show that Primextra at 1.5 kg ha^{-1} + one SHW at 6 WAS, two hand weedings at 3 and 6 WAS, Primextra + Aminicome at 2.0 + 2.0 and Primextra + Guard force at 2.0 + 0.05 kg ha^{-1} are comparable in their performance in promoting effective weed control, better growth and higher yield of maize. Their applications also resulted in higher cash returns and are, therefore, recommended to farmers as alternatives to hand weeding for the profitable production of maize in Malete.

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SUZBIJANJE KOROVA I PRODUKTIVNOST KUKURUZA (*ZEA MAYS* L.)

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R e z i m e

Poljski ogledi su sprovedeni na Nastavno-istraživačkom imanju Državnog univerziteta u Kvari, Malete, kako bi se odredio metod suzbijanja korova koji će biti efikasniji u suzbijanju korova i uz pomoć kojeg će se postići viši prinos zrna i prihod u proizvodnji kukuruza. Ogled se sastojao od 9 tretmana: Primextra + Aminicome u dozi od 1,5 + 1,5 kg ha^{-1} (metolahlor 375 g a.s. ha^{-1} + atrazin 375 g a.s. ha^{-1} + 2,4 – D 900 g a.s. ha^{-1}), Primextra + Aminicome u dozi od 2,0 + 2,0 kg ha^{-1} (metolahlor 500 g a.s. ha^{-1} + atrazin 500 g a.s. ha^{-1} + 2,4 – D 1200 g a.s. ha^{-1}), Primextra + Aminicome u dozi od 2,5 + 2,5 kg ha^{-1} (metolahlor 750 g a.s. ha^{-1} + atrazin750 g a.s. ha^{-1} + 2,4 – D 1500 g a.s. ha^{-1}), Primextra + Guard force u dozi od 1,5 + 0,03 kg ha^{-1} (metolahlor 375 g a.s. ha^{-1} + atrazin 375 g a.s. ha^{-1} + nikosulfuron 1,2 g a.s. ha^{-1}), Primextra + Guard force u dozi od 2,0 + 0,05 kg ha^{-1} (metolahlor 500 g a.s. ha^{-1} + atrazin 500 g a.s. ha^{-1} + nikosulfuron 2,0 g a.s. ha^{-1}), Primextra + Guard force u dozi od 2,5 + 0,07 kg ha^{-1} (metolahlor 750 g a.s. ha^{-1} + atrazin 750 g a.s. ha^{-1} + nikosulfuron 2,8 g a.s. ha^{-1}), Primextra u dozi od 1,5 kg ha^{-1} (metolahlor 375 g a.s. ha^{-1} + atrazin 375 g a.s. ha^{-1}) + jedno dodatno okopavanje 6 nedelja posle setve, dva ručna plevljenja 3 i 6 nedelja posle setve i kontrola bez uklanjanja korova. Ovi tretmani bili su postavljeni u randomiziranom potpunom blok dizajnu sa tri ponavljanja. Prikupljeni podaci su obrađeni analizom varijanse uz pomoć softverskog paketa za statističku analizu (SAS), posle čega su srednje vrednosti odvojene korišćenjem Dankanovog testa višestrukog poređenja. Rezultati su pokazali da se kombinacijama tretiranja primenom Primextra u dozi od 1,5 kg ha^{-1} + jedno dodatno okopavanje 6 nedelja posle setve, dva okopavanja 3 i 6 nedelja posle setve, Primextra + Aminicome u dozi od 2,0 + 2,0 kg ha^{-1} i Primextra + Guard force u dozi od 2,0 + 0,05 kg ha^{-1} postiže efikasno suzbijanje korova, veći prinos zrna i novčani prihod. Poljoprivrednici u Maleteu ih zato preporučuju za primenu u rotaciji.

Ključne reči: integralna kontrola zakorovljenosti, savana južne Gvineje, kukuruz, prinos, novčani prihod.

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Effect of nitrogen levels and weed management methods on weed abundance and yield of upland rice (*Oryza sativa* L.)

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Abstract: Weed infestation and inherent low soil fertility are among the major factors attributed to the low yield of rice in Nigeria. Field trials were therefore conducted to evaluate the effect of nitrogen application levels and weed control methods on growth and yield of upland rice (var. NERICA 2) at the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta (07^{o} 15'N, 03^{o} 25'E) during 2015 and 2016 cropping seasons. Three nitrogen (N) levels (0, 60 and 90 kg/ha) were evaluated and they constituted the main plot treatments, while three weed control treatments, viz: pre-emergence application of Orizo Plus® (propanil plus 2, 4-D) at 2.0 kg a.i ha^{-1}, Orizo Plus® at 2.0 kg a.i ha^{-1} followed by supplementary hoe weeding (SHW) at 6 weeks after sowing (WAS) and three hoe-weeding regimes at 3, 6 and 9 WAS, and a weedy check constituted the sub-plot treatments. All the treatments in different combinations were laid out in a randomized complete block design with a split-plot arrangement with three replicates. Results indicated a significant ($p \leq 0.05$) increase in weed density and dry matter with an increase in N application level from 0 to 90 kg ha^{-1}. Similarly, crop vigour and plant height increased significantly ($p \leq 0.05$) with increasing N application levels up to 90 kg ha^{-1}. However, 60 and 90 kg N ha^{-1} were at par in increasing the number of tillers, leaf area index and yield attributes of rice. All the weed control methods resulted in a significant ($p \leq 0.05$) reduction in weed density and dry matter with subsequent increase in rice growth and yield than the weedy check. Pre-emergence application of Orizo Plus® followed by SHW at 6 WAS and three hoe-weeding regimes resulted in significantly ($p \leq 0.05$) lower weed density and dry matter, and a higher number of tillers, panicle weight and grain yield than a sole application of Orizo Plus®. With Orizo Plus® followed by one SHW or three

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hoe-weeding regimes, increasing N application levels resulted in a significant ($p \leq 0.05$) increase in grain yield of rice. However, with Orizo Plus® applied alone, increasing N application levels did not increase rice grain yield. These results suggest that Orizo Plus® at 2.0 kg a.iha^{-1} followed by one SHW at 6 WAS integrated with N application at 90 kg ha^{-1} is adequate to effectively control weeds and increase rice yield in the rainforest-savannah transition zone of Nigeria.

Key words: grain yield, hoe-weeding, integrated weed management, Orizo Plus®, weed competition.

Introduction

Rice is the world's most important food crop and a major source of food for half of the world population (Kumar and Ladha, 2011). It provides 27% of dietary energy supply and 20% of dietary protein intake in the developing countries (GRiSP, 2013). Rice cultivation is the principal activity and source of income for about 100 million households in Asia and Africa (Seck et al., 2012; GRiSP, 2013). It is planted to about 154 million hectares annually (11% of the world's cultivated land) with a production of about 741 million tons (FAO, 2012; Pode, 2016). Nigeria is the largest rice-producing country in Africa producing about 5.8 million tons (Udemezue, 2018). Rice production in Nigeria is already a highly strategic and priority element for food security and poverty alleviation. However, there is lopsidedness in the present level of production and yield of rice in Nigeria as compared to its demand and consumption pattern. Reports have indicated that only an average yield of 1.8 tons per hectare of rice is realized in Nigeria (Cadoni and Angelucci, 2013; Adigun et al., 2017). This is far below the potential yield and global average production of 5.4 t ha^{-1} (GRiSP, 2013). Production constraints such as inherent low soil fertility and poor weed management are among the major factors attributed to the low yields of rice in Nigeria (Adigun et al., 2017). Tropical soils are generally poor in organic matter and are adversely affected by sub-optimal soil fertility, hence, productivity and sustainability decline over time (Bationo et al., 2006; Oikeh et al., 2014). In addition, the heavy downpour and high relative humidity favour rapid and luxuriant weed growth which results in high yield reduction between 48% and100% (Waddington et al., 2010; Adeyemi et al., 2017). Weed control methods currently employed to avoid such losses are predominantly hoe-weeding and herbicide application. However, these methods are inadequate and have several drawbacks. Hoe-weeding is tedious, inefficient, time-consuming, associated with high labour demands and often too expensive for the average farmer to afford (Imoloame, 2014; Adigun et al., 2017). Herbicide use, on the other hand, does not provide season-long weed control. Moreover, there are not many herbicides that can control different kinds of weeds with one application (Balasubramanian et al., 2007; Chauhan and Opena, 2013). Rice being a closely spaced crop also makes mechanical weed control difficult, thus resulting in high yield reduction (Johnson et al., 2018).

In response to this, therefore, the development of integrated weed management (IWM) is pivotal to combat weed menaces in rice production. IWM involves the reduction of weed interference through a multi-disciplinary action, while acceptable crop yields and environment, social and economic health are maintained (Tohari et al., 2017). The number of hoe-weeding regimes and the amount of herbicides used could be reduced if they were integrated with agronomic practices that enhance crop competitiveness with weeds (Chauhan and Johnson, 2010). Supply and availability of nutrients are of immense significance among these agronomic practices because they influence weed dynamics and weed-crop interference (Camara et al., 2003; Mahajan and Timsina, 2011). Of all nutrients, plant response to nitrogen (N) fertilizer is the most widely observed (Camara et al., 2003). N fertilizer has been reported to play an important role in the competitive balance between weeds and rice (Mahajan and Timsina, 2011). However, some weeds are more effective in utilizing the available excess nutrients than the crops (Blackshaw et al., 2005). Under weedy conditions, fertilizer application has been reported to stimulate weed growth so greatly that the crop growth is suppressed (Mahajan and Timsina, 2011). However, if fertilizers are applied at the proper rate and time, they could enhance crop growth to smother infesting weed species. There is a paucity of information on the interaction of N application levels and weed control methods in the upland rice system in Nigeria, and the influence of N fertilizer on weed interference in the crop has not been yet fully understood (Zoschke and Quadranti, 2002). We hypothesized that efficient weed management and optimum yield of upland rice can be achieved through the integration of appropriate N rate and weed control methods. An early application of N could enhance the growth and competitiveness of rice against weeds. Early weed control provided by herbicide application could further give rice an advantage over weeds. The objective of this study was therefore to evaluate the effects of N levels and weed control methods on weed-crop competition, growth and yield of upland rice in the forest-savanna transition zone of Southwestern Nigeria.

Materials and Methods

Field trials were conducted at the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta, Nigeria (07 15’N; 03 25’E) in the forest-savanna transition zone of South-western Nigeria during 2015 and 2016 cropping seasons. In both trials, the land was first plowed and disc harrowed, then it was pulverized and leveled manually before marking into various plots. Gross and net plot sizes were (4.5 x 3.0) m^2 and (3.0 X 3.0) m^2, respectively. Three nitrogen application levels (0, 60 and 90 kg ha^{-1}) constituted the main plot treatments while the subplot treatments were three weed control methods: pre-emergence application of propanil plus 2, 4-D (Orizo Plus®, Proficol Calle, Baranquilla, Colombia) at 2.0 kg a.i ha^{-1} Orizo Plus® at 2.0 kg a.i ha^{-1} followed by supplementary hoe-weeding (SHW) at 6 weeks after sowing (WAS) and three hoe-weeding regimes at 3, 6 and 9 WAS, and a weedy check. All treatments in different combinations were set up in a randomized complete block design with a split-plot arrangement and three replicates. Rice seed (Var. NERICA 2) was sown manually by the drilling method at the inter-row spacing of 75 cm in both years. Nitrogen was applied as urea in two equal doses at 3 and 6 WAS by banding between the crop rows. Herbicide treatments were applied pre-emergence, one day after sowing with a knapsack sprayer (CP 15) with a spraying volume of 250 L ha^{-1} using a deflector nozzle at a pressure of 2.1 kg cm^2. Hoe-weeding was done using a West African hoe in both years.

Data on weed density and weed dry matter were collected at 12 WAS in both years using a 50 cm × 50 cm quadrat placed randomly at three spots in each plot. Weeds sampled from the quadrat were counted and oven-dried at 70^0C for 72 hours, after which they were weighed and expressed in g m^{-2}. Observation on rice growth and yield attributes such as crop vigour score and plant height per plot (cm), leaf area index (LAI), number of tillers (number/m^2) at 9 WAS, panicle length (cm), panicle weight (g/plant), number of grains per panicle and grain yield (t ha^{-1}) at harvest were recorded. Crop vigour score was accessed by a visual estimate based on the scale 0–10, where 0 represents a completely dead plant and 10 represents the most vigorous plant (Tunku et al., 2007; Adigun et al., 2017). LAI was calculated following the formula of Watson (1947, as follows:

$$LAI = \frac{\text{Leaf area per plant } (cm^2)}{\text{Ground area per plant } (cm^2)} \quad (1)$$

The grain yield of rice was obtained after harvesting the plants in each plot. The resulting grain weight in kg $plot^{-1}$at 12.5% moisture content was expressed in t/ha. Data collected were subjected to analysis of variance (ANOVA) using the GENSTAT discovery package. Treatment means were separated using the least significant difference (LSD at $p \leq 0.05$).

Results and Discussion

Effect of nitrogen levels and weed management methods on weed type, weed density and weed dry matter. The major weed species at the experimental sites in 2015 and 2016 were *Tridax procumbens*, *Euphorbia heterophylla*, *Commelina benghalensis*, *Gomphrena celosioides*, *Digitaria horizontalis*, *Eleusine indica*, *Rottboellia cochinchinensis* and *Cyperus rotundus* (Table 1).

Table 1. Weed species composition of the experimental plots.

Weed species	Plant family	Photosynthetic pathway
Grasses		
Axonopus compressus (Sw.) P. Beauv	Poaceae	C4
Cynodon dactylon (L) Gaertn	Poaceae	C4
Digitaria horizontalis (Willd.)	Poaceae	C4
Eleusine indica (*Gaertn*)	Poaceae	C4
Panicum maximum (Jacq)	Poaceae	C4
Paspalum scrobiculatum (Linn.)	Poaceae	C4
Rottboellia cochinchinensis (Lour.) Clayton	Poaceae	C4
Broadleaves		
Amaranthus spinosus Linn.	Amaranthaceae	C4
Boerhavia diffusa (Linn).	Nyctaginaceae	C4
Centrosema pubescens (Linn.)	Fabaceae	C3
Chromolaena odorata (L.) R.M. King and Robinson	Asteraceae	C3
Commelina benghalensis (Burn.)	Commelinaceae	C3
Euphorbia heterophylla (Linn).	Euphorbiaceae	C4
Gomphrena celosioides (Mart.)	Amaranthaceae	C3
Tridax procumbens (Linn).	Asteraceae	C3
Sedge		
Cyperus esculentus (Linn).	Cyperaceae	C4
Cyperus rotundus (Linn).	Cyperaceae	C4

Nitrogen levels had a significant effect ($p \leq 0.05$) on grasses, broadleaves and total weed density, and weed dry matter (Table 2). There was a significant ($p \leq 0.05$) increase in grasses, broadleaves and total weed density, and weed dry matter with increasing N application levels from 0 to 60 and 60 to 90 kg ha^{-1}. However, the density of sedges was not affected by N application levels (Table 2). This result is in agreement with that of Mahajan and Timsina (2011), who reported that weed response to nitrogen application varies with different species. A similar density of sedges at 0, 60 and 90 N kg ha^{-1} may be attributed to the fact that sedges have higher efficiency in the use of N compared to grasses and broadleaf weeds (Li, 1993). The higher efficiency in N use by sedges could have resulted in increased density of these groups of weed species even at low or no N application. In this study, total weed density increased by 50.4% and weed dry matter by 10.7% with the application of 60 kg ha^{-1} compared to plots where N was not applied. Furthermore, an increase in N application levels from 60 to 90 kg ha^{-1} resulted in 10.6 and 8% increases in total weed density and weed dry matter, respectively in both years.

Weed control methods had a significant ($p \leq 0.05$) effect on weed density and dry matter in both years of experimentation (Tables 2). All the weed control methods reduced grasses, broadleaves, sedges and total weed density, and weed dry matter significantly ($p \leq 0.05$) compared to the weedy check (Table 2). All the weed control methods were significantly ($p \leq 0.05$) at par in reducing the density of grasses and sedges in both years. However, application of Orizo Plus® at 2.0 kg a.i ha^{-1} followed by hoe-weeding and three hoe-weeding regimes significantly ($p \leq 0.05$) reduced broadleaves and total weed density as well as weed dry matter better than the sole application of Orizo Plus® in both years (Table 2).

Table 2. Effect of nitrogen levels and weed management methods on weed density and dry matter.

Nitrogen level (kg ha^{-1})	Weed density (no m^{-2})								Weed dry matter (g m^{-2})	
	Grasses		Broadleaves		Sedges		Total			
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
0	49.0	55.0	41.1	45.1	30.4	22.4	115.6	110.0	106.7	96.7
60	73.6	79.6	54.6	58.6	32.7	24.7	172.8	167.0	117.7	107.7
90	85.9	91.9	64.6	68.6	37.4	29.4	191.3	185.0	126.2	116.2
Lsd	2.5	8.5	4.3	8.3	ns	ns	14.5	8.5	10.5	0.5
Weed control methods										
Orizo Plus®	30.4	36.4	17.7	21.7	20.8	12.8	72.9	66.9	88.2	78.2
Orizo Plus® + SHW	23.6	29.6	13.3	17.3	17.0	9.0	57.9	51.9	65.4	55.4
3 hoe-weeding regimes	19.9	25.9	18.6	22.6	11.8	3.8	54.3	48.3	66.3	56.3
Weedy check	203.3	209.3	162.3	166.3	85.0	77	454.6	449	247.9	237.9
Lsd	4.5	10.5	7.8	11.8	12.5	4.5	20.5	14.5	13.2	3.2
Interaction	ns	ns	ns	ns	ns	ns	13.5	7.5	16.6	6.6

SHW – supplementary hoe-weeding; Lsd – Least significant difference; ns – Not significant.

Better weed control obtained with the pre-emergence application of Orizo Plus® followed by supplementary hoe-weeding can be attributed to the initial weed control of the herbicide, as well as the effectiveness of the supplementary hoe-weeding in controlling subsequent weeds that emerged, thereby sustaining efficient weed control until harvest similar to three hoe-weeding regimes. This clearly underscores the usefulness of integrated weed management as a substitute for the single weed control method or multiple hoe-weeding, particularly where labour is limited and expensive and land under cultivation is large. This result is in conformity with those of Imoloame (2014) and Adigun et al. (2017), who reported superior weed control efficiency of herbicide application plus supplementary hoe-weeding to sole herbicide application. Although the sole application of Orizo Plus® reduced the detrimental effect of weed infestation to a great extent compared with the weedy plot, it did not provide season-long weed control. As the herbicide was taken up mainly through the root and coleoptile, the weeds were killed before, at or shortly after emergence. The excellent germicidal activity of Orizo Plus® effectively controlled the first flush of the weeds but did not control the later flushes of the weeds. Previous studies also reported late-season weed infestation after pre-emergence herbicide application (Jonas et al., 2016; Adigun et al., 2017).

Effect of nitrogen levels and weed management methods on yield and yield attributes of upland rice in 2015 and 2016 cropping seasons

Crop vigour score and plant height of rice increased significantly ($p \leq 0.05$) with increasing N application rates from 0 to 90 kg ha^{-1} in 2015 and 2016 (Table 3). The improvement in rice vegetative growth with increasing N application levels observed in this study may be attributed to the effect of applied N for chlorophyll formation which allows plants to convert solar energy to sugars used for growth (Brady and Weil, 2002).

Table 3. Effect of nitrogen levels and weed management methods on rice growth parameters.

Nitrogen level (kg ha^{-1})	Crop vigour score		Plant height (cm)		Number of tillers (no m^{-2})		Leaf area index	
	2015	2016	2015	2016	2015	2016	2015	2016
0	3.3	5.3	71.0	74.0	51.3	49.3	1.6	1.3
60	5.1	7.1	79.0	82.0	60.0	58.0	1.9	1.6
90	6.7	8.7	94.0	97.0	66.0	64.0	1.9	1.6
Lsd	0.5	0.9	2.5	5.5	8.4	6.4	0.4	0.1
Weed control methods								
Orizo Plus®	7.2	9.2	91.6	94.6	54.6	52.6	1.8	1.5
Orizo Plus® + SHW	6.6	8.6	93.0	96.0	70.0	68.0	2.0	1.7
3 hoe-weeding regimes	7.1	9.1	94.3	97.3	70.0	68.0	2.2	1.9
weedy check	1.3	1.2	50.0	53.0	42.0	40.0	1.2	0.9
Lsd	1	1.3	5.5	8.5	10.3	8.3	0.5	0.2
Interaction	ns	ns	ns	ns	ns	ns	ns	ns

SHW– supplementary hoe-weeding; Lsd – Least significant difference; ns – Not significant.

Thus, plants were taller and more vigorous as a result of the production of more photosynthates needed for more vegetative growth. Similarly, number of tillers, leaf area index, panicle length, panicle weight, number of grains per panicle and grain yield of rice increased with increasing N application rates from 0 to 60 kg ha^{-1}. A further increase in N application levels from 60 to 90 kg ha^{-1}, however, did not result in a significant ($p \leq 0.05$) increase in these parameters (Tables 3 and 4). This result showed that N-use efficiency increased with increasing N application up to 60 kg Nha^{-1} with a subsequent increase in yield of rice, whereas increasing N application up to 90 kg ha^{-1} led to luxuriant vegetative growth similar to the observation of Aminifard et al. (2012). In this study, it was observed that weed density and dry matter increased consistently with increasing N application up to 90 kg ha^{-1}, thus resulting in increased weed competition which must have limited yield increase. Hence, the crop did not respond further to increasing N application from 60 to 90 kg ha^{-1}. This showed that the increase in N application favoured weed growth at the expense of the crop. Weeds have been reported to exhibit high growth rates, water and N-use efficiency which confers them higher competitiveness with crops (Ampong-Nyarko and De Datta, 1993; Harbur and Owen, 2004).

Pre-emergence application of Orizo Plus® followed by supplementary hoe-weeding at 6 WAS and three hoe-weeding treatments resulted in similar crop vigour score, plant height, number of tillers, leaf area index, panicle length, panicle weight, number of panicles and grain yield, all of which were significantly ($p \le 0.05$) higher than those recorded with the sole application of Orizo Plus® (Table 4). A similar result was reported by Adigun et al. (2017) who reported that pre-emergence application of butachlor followed by hoe-weeding resulted in superior growth and yield of rice compared to hoe-weeding and the sole application of butachlor.

Table 4. Effect of nitrogen levels and weed management methods on yield and yield attributes of rice.

Nitrogen level (kg ha^{-1})	Panicle length (cm $plant^{-1}$)		Panicle weight (g $plant^{-1}$)		Number of grains		Grain yield (t ha^{-1})	
	2015	2016	2015	2016	2015	2016	2015	2016
0	21.3	18.9	45.6	47.9	140.8	145.8	1.5	1.8
60	24.2	21.8	53.7	56	157.8	162.8	3.0	3.3
90	22.4	20.0	58.2	60.5	186.6	191.6	3.3	3.6
Lsd	0.9	1.5	4.5	6.8	15.7	20.7	0.5	0.8
Weed control methods								
Orizo Plus®	25.1	22.7	54.0	56.3	170.6	175.6	2.4	2.7
Orizo Plus® + SHW	26.0	23.6	64.8	67.1	206.7	211.7	4.0	4.3
3 hoe-weeding regimes	27.9	25.5	65.9	68.2	200.7	205.7	4.0	4.3
Weedy check	17.1	14.7	24.7	27.0	80.6	85.6	0.5	0.8
Lsd	1.5	1.7	5.3	7.6	20.6	25.6	0.5	0.6
Interaction	ns	ns	ns	ns	ns	ns	0.9	1.2

SHW – supplementary hoe-weeding; Lsd – Least significant difference; ns – Not significant.

In this study, the rice crop capitalized on the changes in weed competition across the weed control methods with crop growth, yield and yield attributes showing an opposite response to weed density and dry matter. A significant ($p \le 0.05$) increase in grain yield with a reduction in weed density and dry matter may be attributed to the adequate supply and use of growth resources occasioned by reduced weed infestation that led to increased assimilation during the crop growth period and its subsequent partitioning at maturity (Matloob et al., 2015).

The interactive effect of nitrogen application levels and weed management methods on weed infestation and rice grain yield

With increasing N application levels from 60 to 90 kg ha^{-1}, weed density and dry matter were similar in plots treated with Orizo Plus® plus supplementary hoe-weeding and plots hoe-weeded thrice at 3, 6 and 9 WAS, while rice grain yield increased significantly ($p \leq 0.05$) with increasing N application in these plots (Figure 1).

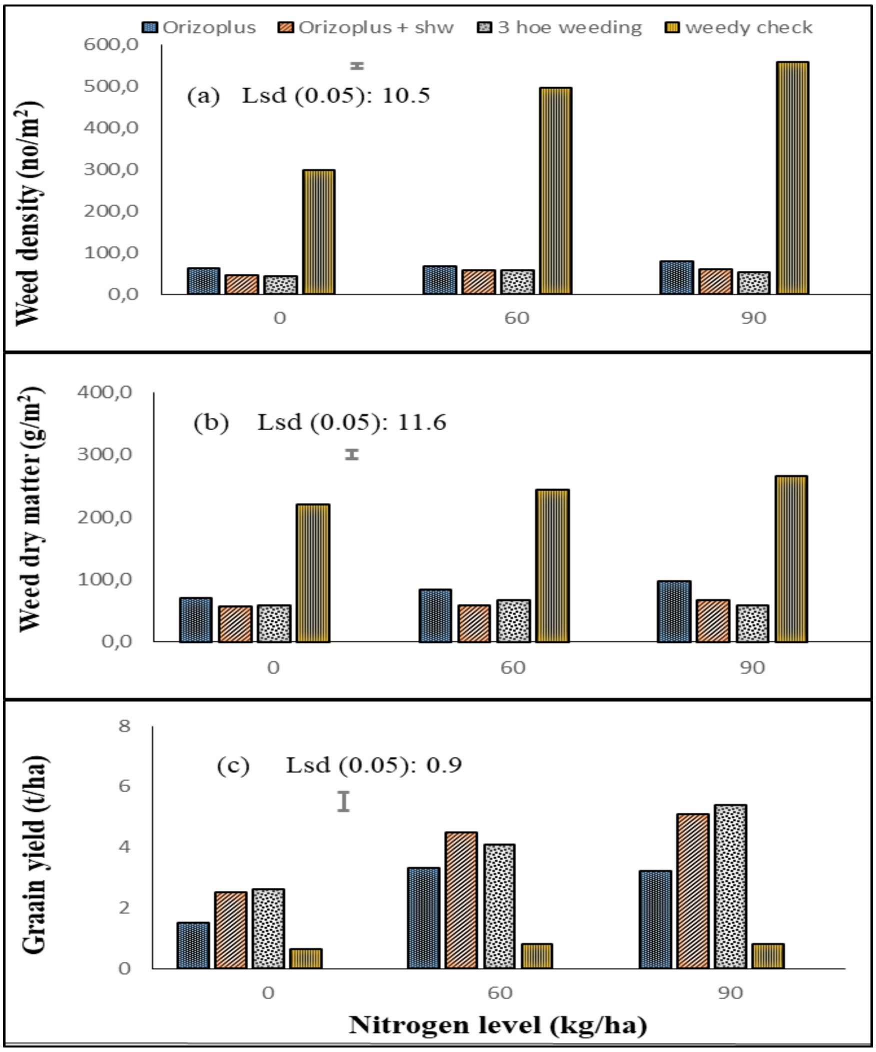

Figure 1. Nitrogen level × weed control method interaction on (a) weed density,(b) weed dry matter and (c) grain yield in upland rice (Data average for 2015 and 2016).

However, in weedy plots and plots treated with Orizo Plus® alone, weed density and dry matter increased with increasing N application levels from 60 to 90 kg ha^{-1}, while rice grain yield had no response to increasing N application rates. This response may be attributed to high weed infestation in weedy plots and poor weed control with the sole application of Orizo Plus® which made the increased N application favour weeds at the detriment of the crops. On the other hand, with Orizo Plus® followed by supplementary hoe-weeding or three hoe-weeding treatments, good weed control was achieved, hence, the N application favour the crop. This result suggests that, in the presence of weeds, increased N application levels increased weed infestation rather than crop yield. This result has corroborated the earlier findings of Blackshaw et al. (2003), who indicated that many weed species are more responsive to increased N application levels than crops.

Conclusion

The result of this study demonstrated that rice grain yield was improved with the application of 90 $kgNha^{-1}$ when weeds were controlled either by pre-emergence application of Orizo Plus® at 2.0 kg a.i ha^{-1} followed by supplementary hoe-weeding at 6 WAS or three hoe-weeding regimes at 3, 6 and 9 WAS. However, under poor weed control conditions (sole application of Orizo Plus® at 2.0 kg a.i ha^{-1}), increasing N application did not improve rice grain yield but rather increased vegetative growth and weed infestation at the detriment of crop yield. It is therefore concluded that pre-emergence application of Orizo Plus® at 2.0 kg a.i ha^{-1} followed by supplementary hoe-weeding at 6 WAS integrated with N application at 90 kg ha^{-1}will provide adequate weed control and optimum yield of rice in the rainforest-savannah transition zone of Nigeria.

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UTICAJ DOZA AZOTA I METODA KONTROLE ZAKOROVLJENOSTI NA BUJNOST KOROVA I PRINOS PLANINSKOG PIRINČA (*ORYZA SATIVA* L.)

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R e z i m e

Zakorovljenost udružena sa niskom plodnošću zemljišta su među glavnim faktorima koji se pripisuju niskom prinosu pirinča u Nigeriji. Sprovedeni su poljski ogledi, kako bi se procenio uticaj primene azota i metoda kontrole zakorovljenosti na rast i prinos planinskog pirinča (sorta NERICA 2) na Naučno-istraživačkom imanju Federalnog poljoprivrednog univerziteta, Abeokuta (07^o 15'N, 03^o 25'E) tokom žetve u 2015. i 2016. godini. Tri doze azota (N) (0, 60 i 90 kg/ha) vrednovane su kao glavni tretmani, dok su tri tretmana kontrole zakorovljenosti: primena preparata Orizo Plus® (propanil plus 2, 4-D) sa dozom od 2,0 kg a.s ha^{-1} pre nicanja, Orizo Plus® sa dozom od 2,0 kg a.s ha^{-1} praćen dodatnim okopavanjem 6 nedelja posle setve, kao i sa tri okopavanja 3, 6 i 9 nedelja posle setve i kontrola bez uklanjanja korova, predstavljala tretmane potparcela. Svi tretmani u različitim kombinacijama postavljeni su u potpuno slučajnom blok dizajnu sa rasporedom podeljenih parcela sa tri ponavljanja. Rezultati su pokazali značajan ($p \leq 0,05$) porast zakorovljenosti i suve materije sa povećanjem primenjene doze azota od 0 do 90 kg ha^{-1}. Pored toga, životna sposobnost useva i visina biljke su se povećali značajno ($p \leq 0,05$) sa povećanjem primenjene doze azota do 90 kg ha^{-1}. Međutim, efekti 60 i 90 kg N ha^{-1} bili su jednaki pri povećanju broja bokora, indeksa lisne površine i karakteristika prinosa pirinča. Sve metode suzbijanja korova vodile su do značajnog ($p \leq 0,05$) smanjenja zakorovljenosti i suve materije sa naknadnim povećanjem rasta i prinosa pirinča u odnosu na kontrolu. Primena preparata Orizo Plus® pre nicanja uz dodatno okopavanje 6 nedelja posle setve kao i sa tri okopavanja rezultirala je značajno ($p \leq 0,05$) nižom zakorovljenošću i suvom materijom i većim brojem bokora, težinom metlice i prinosom zrna u odnosu na samostalnu primenu preparata Orizo Plus®. Primenom preparata Orizo Plus® sa jednim i tri okopavanja povećavanje nivoa primene azota dovelo je do značajnog

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($p \leq 0,05$) porasta prinosa zrna pirinča. Međutim, samo primenom preparata Orizo Plus®, povećanje doze primenjenog azota nije povećalo prinos zrna pirinča. Ovi rezultati sugerišu da Orizo Plus® sa 2,0 kg a.s ha^{-1} sa jednim okopavanjem 6 nedelja posle setve u sadejstvu sa primenom azota u količini od 90 kg ha^{-1} je adekvatan za efikasno suzbijanje korova i povećanje prinosa pirinča u zoni prelaska prašume u savanu u Nigeriji.

Ključne reči: prinos zrna, okopavanje, integralna kontrola zakorovljenosti, Orizo Plus®, kompeticija korova.

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OROBANCHE (*OROBANCHE* SPP.) IN LENTIL (*LENS CULINARIS* MEDIC.): HOW HUGE ARE THE LOSSES OF YIELD, QUALITY, MARKETING PRICES AND PROFITABILITY?

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Abstract: This research aimed to inspect the impacts of severe parasitic plants (*Orobache* spp.) on grain yield, some quality characteristics, marketing price and profitability loss of red lentil in the major lentil growing area of south-east Anatolia. Farmer field trials were carried out in two neighbouring fields planted with Yerli Kırmızı (landrace) and Firat-87 varieties of lentil employing a split-plot experimental design in the Yollar basi location of Viransehir in the 2018–2019 crop growing season. Lentil varieties were placed into main plots and the broomrape infestations (i.e. 0, 5, 10, and 15 plants m^{-2}) in the subplots respectively. Grain yield, hectolitre weights and 1000-kernel weights and protein contents (%) were scored. All grain samples were presented to randomly chosen grain purchasers in the local commodity market and marketing price offers were scored respectively. Results showed that broomrape infestation from zero to 15 broomrapes m^{-2} reduced the grain yield significantly from 2033.33 kg ha^{-1} to 833.33 kg ha^{-1} by 59%. Although being non-significant, Firat-87 (1512, 5 kg ha^{-1}) was found to be higher yielding than Yerli Kırmızı (1325 kg ha^{-1}). Regression equations between grain yield reductions vs. broomrape infestation ratios turned out to be significant and reliable with high coefficients of determinations for both varieties. Some visual purchasing criteria such as hectolitre and 1000-kernel weights were not affected seriously. Purchasers offered very similar marketing prices for pulse grains with all severity levels. The economic loss was huge ($555 ha^{-1}). Regression equations derived from grain yield vs. infestation densities were found to be reliable with high coefficients of determinations and can be perfectly used for yield estimates under various levels of broomrape infestations. It was

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concluded that an infestation over 15% might result in a disastrous yield loss in lentil production. Therefore, some agronomic measures must be taken quickly.

Key words: red lentil, *Orobanche*, grain yield, quality, marketing prices, profitability.

Introduction

Lentil (*Lens curinalis* Medic.) is an important food legume crop used both as food and feed due to its protein-rich grain and straw. Worldwide, lentil is cultivated on 3.85 million hectares (m ha) with 3.59 million tons (mt) production under rain fed conditions (Erskine et al., 2011).

Lentil is also a nutritious food legume. The seed has a relatively high content of protein (22%), carbohydrates and calories (3250 kcal kg^{-1}) including fast cooking characteristics (Muehlbauer, 1985; Saskatchewan Agriculture and Food, 2005). Lentil (*Lens culinaris* Medic.) is a cool-season annual cash crop, classified as a grain legume or pulse in south-east Anatolia (Ozberk et al., 2006).

Turkey is one of the major lentil producer ranking third globally after Canada and India with 410000 tons of annual production on 214,788 ha (TUİK, 2014). Green lentil is grown in the central and transitional zones of Turkey while red lentil is grown in the south-east of Anatolia (Ozberk et al., 2006).

By far, the most economically damaging root parasitic weeds in Europe and neighboring regions are members of the *Orobachaceae*, mainly species belonging to the genera *Orobanche* and *Phelipanche* (broomrapes) (Joel et al., 2007a; Parker, 2009). In Turkey, 36 species of Orobanche have been recorded (Gilli, 1987). However, only four of them have resulted in considerable damage to crops. *Phelipanche ramosa* (L) Pomel (syn. *O. ramosa* L.) *Phelipanche aegyptiaca* (Pers) Pomel (syn. *O. aegyptiaca* Pers.) (Joel et al., 2007; Parker, 2009) and *O. crenata* Forsks also result in damage in lentil (Kıtıkı et al., 1993; Uludag and Demir, 1997; Aksoy and Uygur, 2003; Bulbul and Uygur, 2009). In a survey carried out in neighbouring Diyarbakir province, it was found that the most abundant broomrape species infesting lentil plants were: *Orobanche crenata* Forsk., and *Phelipanche aegyptiaca* (Pers.) Pomel (syn. *Orobanche aegyptiaca* Pers.). The frequency of the occurrence of broomrape species ranged between 12% and 41% (Ozaslan et al., 2017). In legumes, the most intensive growth of *Orobanche* usually coincides with the flower and pot stages of the host. Broomrapes spend most of their life cycle underground, where they undergo processes of germination, haustorial differentiation from the radicle, haustorial penetration of the host formation of vascular connection with the host, utilization of host nutrients and the storage of resources in a parasite organ called the tubercle or nodule (Fernandez-Aparicio et al., 2011). This results in flower dropping and low amount of pod formation due to an increase in competition receiving more plant nutrients. The wilting symptoms occur due to the reduced water uptake because of a deficiency of energy in the host roots, as a result of the flow of carbon hydrates to the parasite (Anonymous, 1997).

Yield losses due to *Orobanche* ranged from 5 to 100% (Aksoy et al., 2016; Erskine et al., 2011; Bulbul et al., 2009; Australian Department of Agriculture and Food, 1999; Anonymous, 1997). An economical loss of lentil of about 60 million euros is estimated annually (Uludağ and Demirci, 2005; Bulbul et al., 2009). Only in the Middle East, it could reach about 1.3 and 2.6 billion dollars (Aly, 2007). Several strategies have been developed for the control of broomrape, ranging from cultural practices to chemical control (Habimana et al., 2014; Joel et al., 2007b; Parker and Riches, 1993), but none with unequivocal success. Good broomrape control can be achieved in faba bean by glyphosate at low rates. However, insufficient selectivity was found in lentil (Arjona-Berral et al., 1988). Lentil tolerates pre- and post-emergence treatment of other herbicides suitable for broomrape control, such as imazapic and imazetgapyr (Jurada-Exposito et al., 1997). Imazapic ($10gl^{-1}$) is widely used in SE Anatolia. Phytotoxicity has been observed at higher doses of some herbicides and depends on the level of water stress and lentil cultivar (Henson and Hill, 2001). A number of cultural practices including solarisation, delay sowing, hand weeding, no-tillage, nitrogen fertilization, intercropping, rotations can contribute to seed bank demise. Other strategies such as trap plants, suicidal germination, and activation of systematic acquired resistance, biocontrol or target site herbicide resistance are promising solutions that are being explored but none of them is the sole solution of the problem (Habimana et al., 2014). The only methods currently available for farmers are the use of resistant varieties and chemical control (Rubiales and Fernadez-Aparicio, 2011). Resistance breeding is hampered by the scarcity of proper sources of resistance and of a reliable and practical screening procedure (Fernandez-Aparicio et al., 2007b).

This study aimed to assess the effect of broomrape damage on grain yield, some quality characteristics, marketing price of lentil in the local commodity markets and finally the loss of net income.

Materials and Methods

This study was carried out in the farmer fields in Viransehir location in S. Urfa in the 2018–2019 growing season, wherein broomrape infestations are common. *O. crenata* Forsk. (white flowering), *P. ramosa* (pale blue flowering and branched) and *P. aegyptica* Pers. (blue flowering) were found to be wide-spreading in lentil fields (75,51%) in the Eastern Mediterranean region and Southeastern Anatolia (Bulbul et al., 2009; Temel et al., 2012). *O. aegyptica* Pers. was prevalent in the Viranşehir region (Uludağ and Demir, 1997; Bayaa et al., 1998).

A preceding crop was wheat in Viransehir. Disharrow + float + drill planting + roller combination was employed for planting management. Two farmer fields with 10 ha each in Viranşehir were planted by a drill with a 120 kg ha^{-1} seed rate in both fields on the 30th of October in 2018. A 50 kg ha^{-1} di ammonium phosphate rate (DAP, 18 and 46% of N and P) was applied at sowing. Annual rainfall was quite satisfactory with 712.6 mm and its distribution throughout the cropping season was also well-balanced. All other necessary agronomical measures were taken for healthy crop production. Chemical weed control for narrow-leaf weeds was employed. However, pest control was not applied. A field trial was carried out under moderate volunteering broomrape infestation. Field plots were assigned at the flowering stage of broomrape and the early pod stage of lentil. One m^2 plots with 0, 5, 10 and 15 broomrape plants surrounded by the strip at the corners built up a block. Three blocks for each cultivar were assigned as shown above (Picture 1).

Picture 1. *Orobanche* plants at flowering and plots surrounded by strips.

A split-plot experimental design was employed. Lentil varieties were placed into main plots and the broomrape infestations (i.e. 0, 5, 10, and 15 plants m^{-2}) in the subplots respectively (Table 1).

Subplots were randomly selected taking into account the number of flowering broomrape plants placed in the one m^2 acreage. Numbers of broomrape flowers were counted in each plot at the flowering and pod filling stages of lentil and the assignment of plots was performed. Plots were harvested by hand and threshed by a single plant thresher for weighting grain yield on the 30th of May in 2019. The highest yield loss for both cultivars was calculated as:

HYL% = [1- (mean grain yield of plots with 15 broomrapes/mean grain yield of zero broomrapes)] x 100.

Table 1. The field randomization plan of the study.

1st field			2nd field		
Yerli Kırmızı*			Fırat-87*		
0**	5	10	15**	10	5
5**	10	0	10**	5	15
10**	15	5	5**	0	10
15**	0	15	0**	15	0
Block1	Block2	Block3	Block1	Block2	Block3

*main plot, **subplot.

Hectolitre and 1000-kernel weights and protein (%) content of lentil grains were also scored through the methods given by Williams et al. (1986).

Seed samples of the experiment with an increasing amount of broomrape damage were presented to randomly chosen 3 grain purchasers in the local commodity market in mid-June in 2019. ANOVA and mean separation were performed for marketing price estimates employing a split-split-plot experimental design with 3 replications. Purchasers, varieties and infestation ratios were placed into main plots, subplots and sub-subplots respectively. Coefficients of correlation among such characteristics were estimated. Regression relations between grain yield, hectolitre weight and 1000-kernel weights vs. broomrape infestations were further investigated (Finlay and Wilkinson, 1963; Eberhard and Russel, 1966). The economic loss (US\$ ha^{-1}) was calculated as:

EL = HYL (%) x mean grain yield of non-damaged grains x mean marketing price of non-damaged grains.

The JMP statistical software was employed for statistical analysis and figures.

Results and Discussion

O. aegyptica Pers. (blue flowering) and *O. ramosa* (pale blue flowering and branched) were dominating species with a little amount of *O. crenata* Forsk (white flowering) in Viranşehir in 2018–2019. The sowing time was earlier than that of a common practice. The adopted practice is to plant lentil in late October or *early* December. The grain yields of lentil crop under various broomrape infestations are given in Table 2.

The analysis of variance (not given here) for grain yield indicated that there were significant differences between broomrape infestation ratios (F=232.27**) and infestation ratios x varieties interaction (F=5.30*), but no difference was found between cultivars (F=17.30, p>0.0532). Table 3 shows the mean differences of significant variables through LSD.

Although being non-significant, Firat-87 seemed to be higher yielding than Yerli Kırmızı. Increasing infestation ratios reduced grain yield significantly. Plots free from broomrape gave the high yield (2033.33 kg ha^{-1}), whereas the plots with 15 broomrape plants reduced grain yield to 833.33 kg ha^{-1} dramatically.

Table 2. Grain yields of cultivars under various broomrape infestations.

Replication	Broomrape infest ratio (no m^{-2})	Yerli Kırmızı Yield (kg ha^{-1})	Fırat-87 Yield (kg ha^{-1})
1	0	2000	2250
1	5	1500	1650
1	10	1200	1500
1	15	950	750
2	0	1950	2350
2	5	1450	1800
2	10	1350	1600
2	15	850	950
3	0	1700	1950
3	5	1300	1450
3	10	900	1150
3	15	750	750

Table 3. LSD groups for significant variables for grain yield of lentil.

Varieties/ratios	0	5	10	15	Average/groups (kg ha)
Fırat-87	2183.33a	1633.33c	1416.66d	816.66f	1512.00a
Yerli kırmızı	1883.33b	1416.66d	1150.00e	850.00f	1325.00a
Average/groups (kg/ha)	2033.33a	1525.00b	1283.33c	833.33d	

HYL% = [1-(833.33 /2033.33)] x 100 = 59%. Grain yield loss seems to be dramatic due to the increasing amount of broomrape infestation.

ANOVA for 1000-kernel weights (not given here) indicated the absence of any significant source of variation. Although being non-significant, there was a slight difference between varietal means in favour of Firat-87. Although being non-significant, there were some differences among the means of 1000 KW depending upon infestation ratios in favour of less infestation. ANOVA for hectolitre weights showed that there was not any significant source of variation. Although being non-significant, the mean of Firat-87 seemed to be slightly higher than that of Yerli Kırmızı. Although being non-significant, increasing infestation ratios reduced the hectolitre weights slightly. The negative influence of broomrape infestation on lentil quality was discussed by the Australian Department of Agriculture and Food (1999). However, broomrape infestation in our study did not affect any quality characteristics.

Regressions between grain yield vs. broomrape infestation turned out to be significant (F=76, 45**) for Firat-87 and Yerli Kırmızı (F=74, 78**) indicating the presence of the effects of broomrape infestation on grain yield. Regression equations were estimated as y=1830**-67.33**x with R^2%=87.02 for Yerli Kırmızı and y=2160**-86.33x** with R^2 % =87.27 for Firat-87. A high coefficient of determination indicated the reliability of equations. Yield reductions due to increasing broomrape infestations are given in Figures 1 and 2.

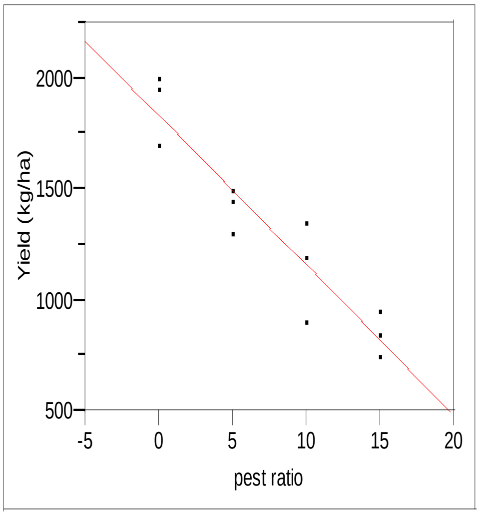

Figure 1. The regression line and equation for Yerli Kırmızı.

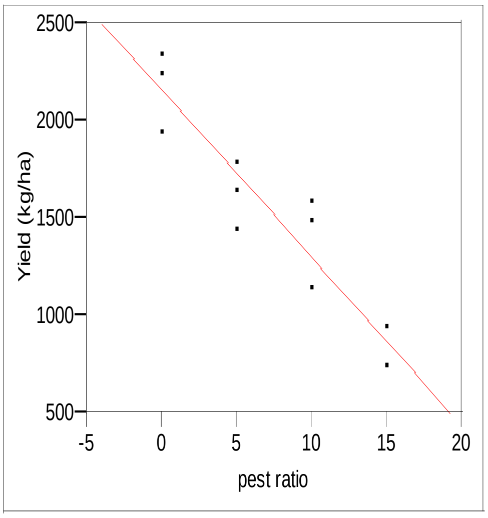

Figure 2. The regression line and equation of Fırat-87.

Analysis of variance (not given here) for marketing prices indicated the absence of any significant sources of variation. Replications ($F=0.12^{ns}$), purchasers ($F=4.48^{ns}$, p>0.09), varieties ($F=5,30^{ns}$, p>0.06) and infestation ratios ($F=0.11^{ns}$) turned out to be non-significant. Although being non-significant, purchasers seemed to be offering marketing prices subjectively employing visual characteristics. Moreover, Fırat-87 tended to receive a higher marketing price offer (0.468 US$kg^{-1}) than Yerli Kırmızı (0.460 US$kg^{-1}). Increasing broomrape infestations did not affect marketing prices negatively.

The economical loss (EL) for the experiment was approximately = 0.59x 2.033 ton ha^{-1} x US$ 462.06 ton^{-1} = US$ 554.22ha^{-1}

Relatively early planting of lentil suffered from broomrape severely (Temel et al., 2012). Volunteering broomrape plants developed very fast and attacked young lentil seedlings. When lentil is planted late into humid soil, plants can escape from severe broomrape attacks and late planting of early maturing type of lentil was recommended by Temel et al. (2012). There were some statistically significant differences among the varieties showing a response to broomrape infestation. Fırat-87 was found to be high-yielding. Yerli Kırmızı (Landrace) was the yield-limiting variety among entries. Late planting of Altın Toprak-98 was recommended for the farmers in the region because of its high-yielding performance and early maturing ability (Temel et al., 2012). Broomrape infestation can reduce grain yield severely as pointed out (Anonymous, 1997). Fifteen broomrapes m^{-2} reduced grain yield by 59% compared to non-damaged plots. Grain yield reduction was 51.5% in a study carried out in the same region by Ozberk et al. (2016). Regression between grain yield vs. broomrape infestations for both varieties turned out to be significant giving F=74.78** and 76.45** respectively. Regression equations with high coefficients of determinations can be used for yield estimates under various broomrape infestations. Both 1000-kernel weights and hectolitre weights seemed to be slightly decreasing depending on the increasing broomrape infestations as indicated (Ozberk et al., 2016) earlier.

Analysis of variance for marketing price offers indicated that broomrape infestation and replications (purchasers) were found to be non-significant. The presence of broomrapes until 15 per m^2 did not affect negatively on some quality traits and marketing prices. Another study carried out in the same region indicated the absence of any marketing price differences among the grains suffering from the various amounts of broomrape infestation (Ozberk et al., 2016).

Broomrape infestation did not affect any visual quality characteristics such as 1000-kernel weights and hectolitre weight as obtained by Ozberk et al. (2016). Comparing the grain yields of the highest broomrape infested plots versus those of non-damaged plots, the losses showed the importance of broomrape management in the red lentil growing belt of the country. The economic loss was about US$555 ha^{-1}. Confirming our findings, this was also huge (US$ 396.77 ha^{-1}) under 8–9 broomrape m^{-2} infestations in a similar region (Ozberk et al., 2016). Breeding resistance is the most economic, feasible and environmentally friendly method of control. However, resistance to broomrape is difficult to access due to the scarcity of complex nature and low heritability. Breeding for resistance is a difficult task (Rubiales, 2003). So far, no source of resistance has been available in lentil (Muehlbauer et al., 2006). Nevertheless, Fernandez-Aparicio et al. (2008) have observed a wide range of responses to crenate broomrape resistance under field conditions. Low infection rates seemed to be based on a combination of various escape and resistance mechanisms. Moreover, some in-vitro screening techniques may be used to rank and identify lentil accessions with potential broomrape resistance. There are numbers of cultural practices including delayed sowing, hand weeding, no-tillage, nitrogen fertilization, intercropping, and trap plants such as Brussels sprout, cabbage, broccoli, canola, turnip and crop rotations to contribute to seed bank demise. Taking into account simplicity in application, planting time modifications can be employed as agronomical measures. However, there is a conflict between early planting and late planting. Late planting of early-maturing types of lentil are recommended (Temel et al., 2012; Rubiales and Fernandez-Aparicio, 2011), whereas farmer practice in the region is to plant lentil relatively early for the emergence and rapid vigorous seedling development. Therefore, plant can compete with broomrape development as partially suggested by Silim et al. (1999). Crop rotation, solarisations and the use of the trap plant formerly used to be planted in large scale in the region such as *Linum usitatissimum* L. are other possible measures (Aksoy and Uygur, 2003). An increase in farmer awareness was also recommended urgently (Ozaslan et al., 2017).

Conclusion

It was concluded that 15% of broomrape infestation resulted in early 60% yield loss with the US$ 555 ha^{-1} economic loss. It is predicted that 20% or slightly more of broomrape infestation might result in a disaster giving the zero amount of grain at harvest. Thus, immediate agronomical measures given above must be taken in the region and neighboring countries.

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VOLOVOD (*OROBANCHE* SPP.) U SOČIVU (*LENS CULINARIS* MEDIC.): KOLIKO SU VELIKI GUBICI PRINOSA, KVALITETA, TRŽIŠNE CENE I PROFITABILNOSTI?

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R e z i m e

Cilj ovog istraživanja bio je da se ispitaju uticaji veoma štetnih parazitskih biljaka (*Orobache* spp.) na prinos zrna, neke karakteristike kvaliteta, tržišnu cenu i gubitak profitabilnosti crvenog sočiva u glavnoj oblasti uzgajanja sočiva u jugoistočnoj Anatoliji. Poljski ogledi su sprovedeni u dva susedna polja zasejana sortama sočiva Yerli kırmızı (landrace) i Firat-87 korišćenjem oglednog dizajna podeljenih parcela u lokalitetu Yollar basi Viransehira u vegetacionom periodu useva 2018–2019. Sorte sočiva su postavljene u glavne parcele, a zakorovljenost volovodom (tj. 0, 5, 10, i 15 biljaka m^{-2}) je stavljena u potparcele. Izračunati su prinos zrna, hektolitarska masa, masa hiljadu zrna i sadržaj proteina (%). Svi uzorci zrna predstavljeni su slučajno izabranim kupcima zrna na lokalnom tržištu robe i ocenjene su ponude tržišnih cena. Rezultati su pokazali da zakorovljenost volovodom od 0-15 biljaka m^{-2} značajno smanjuje prinos zrna od 2033.33 kg ha^{-1} do 833.33 kg ha^{-1} za 59%. Iako nije značajno, sorta Firat-87 (1512.5 kg ha^{-1}) je imala viši prinos nego sorta Yerli Kırmızı (1325 kg ha^{-1}). Regresione jednačine odnosa smanjenja prinosa useva prema zakorovljenosti volovodom pokazale su se značajnim i pouzdanim sa visokim koeficijentima determinacije za obe sorte. Neki vizuelni kriterijumi kao što je hektolitarska masa i masa hiljadu zrna nisu bili ozbiljno oštećeni. Kupci su ponudili vrlo slične tržišne cene za zrna mahunarki sa svim nivoima oštećenja. Ekonomski gubitak je bio ogroman ($555 ha^{-1}). Regresione jednačine izvedene iz prinosa zrna nasuprot gustinama zakorovljenosti bile su pouzdane sa visokim koeficijentima determinacije i mogu se savršeno koristiti za procene prinosa pri različitim nivoima zakorovljenosti volovodom. Zaključeno je da zakorovljenost od preko 15% može dovesti do katastrofalnih gubitaka prinosa u proizvodnji sočiva. Stoga se neke agronomske mere moraju brzo preduzeti.

Ključne reči: crveno sočivo, *Orobanche*, prinos zrna, kvalitet, tržišne cene, profitabilnost.

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BACTERIAL INOCULATION: A TOOL FOR RED CLOVER GROWTH PROMOTION IN POLLUTED SOIL

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Abstract: Red clover (*Trifolium pratense* L.) seeds were inoculated with several plant growth-promoting bacteria (PGPB) and sown in the substrate contaminated with polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organometallic derivatives of tin (OT). The aim was to determine if selected PGPB strains can promote the growth of red clover in the substrate contaminated with several organic pollutants. The influence of bacteria on red clover growth (height, root length and biomass) was monitored during the three-month experimental period. The most significant improvements of seedling height were noted in the treatment with *Bacillus amyloliquefaciens* D5 ARV and *Pseudomonas putida* P1 ARV. Root growth was positively affected by *Serratia liquefaciens* Z-I ARV. The same isolates significantly affected biomass production. Those isolates caused total biomass increases of 70%, 48% and 33% compared to control. Bacterial strains used in this study were already confirmed as PGPB by biochemical testing, as well as by an *in vivo* test of mixed inoculums on several woody plants grown in the coal-mine overburden site. This work is the first-time record on their individual effects on one plant species. Obtained results confirmed that inoculation with several PGPB strains can enhance red clover growth in polluted soil.

Key words: organic pollutants, red clover, revegetation, plant growth-promoting bacteria.

Introduction

Organic contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organometallic derivatives of tin (OT) represent a global problem. The main anthropogenic sources of PAHs, PCBs and

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OTs are industrial sector, agricultural practice, waste incineration and incomplete combustion of fossil fuels (Karličić et al., 2014; Skála et al., 2018). Broad use of those substances resulted in their accumulation in soil and water bodies representing a serious threat to wildlife, human health and the whole ecosystem. Jeelani et al. (2017) quote that 25% of global soils are highly degraded by the presence of PAHs and heavy metals, whereas 44% are referred to as moderately degraded. Those contaminants are characterized as toxic and persistent (Kummerová et al., 2012) and the remediation of contaminated soil is qualified as urgent.

Plant ability to take up organic and inorganic pollutants from soil is employed through the process of phytoremediation. They are capable to hyperaccumulate, immobilize and convert contaminants into simpler or volatile compounds (Ahmad et al., 2017). Plant mechanisms are strongly supported by the microbial activity in the root zone (Mesa et al., 2017). Some of the microorganisms inhabiting the root zone are capable to mobilize or immobilize contaminants and they are included in degradation and detoxification of pollutants through the process of bioremediation (Ahmad et al., 2017; Ite et al., 2019). Microbial-assisted phytoremediation takes advantage of the plant-microorganism partnership and coexistence under the same stressful conditions with one main goal – economical and effective soil purification (Pinto et al., 2018). Phyto/Bioremediation applies to a broad range of chemicals such as heavy metals, PCBs, PAHs, OTs, pesticides/insecticides (Rohrbacher and St-Arnaud, 2016; Jeelani et al., 2017).

On the other hand, organic pollutants are well known as phytotoxic. Plants are especially susceptible to the presence of pollutants during the germination and root formation stages (Kummerová et al., 2012). Such effects complicate the establishment of vegetation cover which is a precondition of phytoremediation. That is why contaminant-tolerant plant species, with flexibility in stress conditions, ability to grow quickly and produce high biomass are highly recommended (Hou et al., 2015; Eskandary et al., 2017). Similarly, the root zone microorganisms play a critical role in the plants' survival under stressful conditions (Backer et al., 2018). Among rhizosphere inhabitants, plant growth-promoting bacteria (PGPB) attracted special attention. This group of bacteria is capable to promote plant growth in contaminated soils, to increase plant resistance to environmental stresses (De Souza et al., 2015), to change the availability of organic and metal pollutants (Ahmad et al., 2017). They also play a great role in nutrient acquisition by nitrogen fixation, solubilization of phosphorus, or other unavailable forms of nutrients and siderophore production (Pii et al., 2015). PGPB produce plant hormones (auxins, cytokinins, gibberellins) which are in charge of growth regulation and stress response (Backer et al., 2018). A wide range of plant stimulating mechanisms of PGPB ensured them a significant place in current agricultural practice. They are considered as a "green and clean" technology (Ramakrishna et al., 2019) which can be a proper alternative for mineral fertilizers and pesticides, two main pollutants of conventional agriculture. Even though the main field of their study and application is food production, PGPB find their place in numerous fields related to plant production (forestry, horticulture) or remediation. Those bacteria possess the natural potential to alleviate the impacts of toxic contaminants in soil (Backer et al., 2018), and help in the establishment of vegetation.

Red clover (*Trifolium pratense* L.), fast-growing legume suitable for remediation of contaminated soils (Gkorezis et al., 2016), was chosen as a test plant in the study. The objective was to estimate the effects of bacterial inoculation on red clover growth in the substrate contaminated with PAHs, PCBs and OT substances. Several bacterial strains were used for inoculation and we searched for the most effective ones in terms of red clover growth promotion.

Materials and Methods

The substrate used in the experiment was soil from Tivat City Park, Montenegro. Chemical analyses of this substrate were performed by a gas chromatography-mass spectrometry method and they revealed the presence of PAHs, PCBs and OT substances (Table 1).

Table 1. Concentrations of PAHs, PCBs and OT (mg/kg) in the soil of Tivat City Park (Jovičić Petrović et al., 2014, Karličić et al., 2014).

Contaminant	Soil sample	MAC* mg/kg
PAH	5.97	0.6
PCB 28	<0.005	
PCB 52	0.016	
PCB 101	0.030	
PCB 118	0.030	0.004
PCB 138	0.024	
PCB 153	0.018	
PCB 180	0.010	
ORGANOTINS		
MONOBUTYLTIN	0.005	
DIBUTYLTIN	0.008 ± 0.0009	
MONOFENIL TIN	< 0.004	0.005
TRIBUTILTIN	0.026 ± 0.0032	
DIPHENILTIN	< 0.004	
TRIPHENILTIN	< 0.004	

Maximum allowed concentration (MAC)* statutory values in Montenegro (Official Gazette of RCG, No. 18/97).

Bacterial material. Strains used in this study were: *Psedomonas putida* P1 ARV (agricultural soil), *Serratia liquefaciens* Z-I ARV (isolated from the soil of

Tivat City Park), *Ensifer adhaerens* 10_ARV (isolated from the soil of Tivat City Park), and *Bacillus amyloliquefaciens* D5 ARV (isolated from the overburden site of coal-mine Kolubara). The strains were identified by molecular methods, and characterized as PGPB based on indoleacetic acid, siderophore production, phosphate solubilization ability and *in vivo* tests on London plane (*Platanus x acerifolia*), black locust (*Robinia pseudoacacia*) and Scots pine (*Pinus sylvestris*) grown in the coal-mine overburden used as a substrate (Karličić et al., 2015; Karličić et al., 2017).

Seed sterilization. The seeds of red clover were surface sterilized by emersion in 70% ethanol for 2 min followed by a 15-min exposure to 2% NaOCl. Seeds were washed properly with sterile distilled water (5-10 x) and soaked for the next half an hour to antibiotics solution (600 mg L^{-1} penicillin, and 250 mg L^{-1} streptomycin). Seeds were washed, and the final step was drying in aseptic conditions.

Inoculum preparation. Separate bacterial strains were grown in nutrient broth aerobically at 28±2°C/48h/100 rpm (BIOSAN, Latvia). *Ensifer adhaerens* 10_ARV was grown in Fjodorov medium (Anderson, 1958) at 28±2°C/72h/100 rpm. The bacterial suspensions were centrifuged at 6000 *x g* for 10 min (5804 R, Eppendorf, Germany), and diluted in distilled water to achieve 10^8 CFU mL^{-1}. The mixed inoculum was prepared by mixing bacterial strain-specific inoculums in the 1:1:1:2 (*Ensifer adhaerens* 10_ARV) ratio.

Seed inoculation and pot experiment. Sterilized seeds were inoculated with selected strains by 1h immersion at 100 rpm/h (BIOSAN, Latvia) and the treatments were as follows:

Z-I ARV: Seeds inoculated with *S. liquefaciens* Z-I ARV;
10_ARV: Seeds inoculated with *E. adhaerens* 10_ARV;
D5 ARV: Seeds inoculated with *B. amyloliquefaciens* D5 ARV;
P1 ARV: Seeds inoculated with *P. putida* P1 ARV;
MIX: Seeds inoculated with mixed inoculum;
CONPS: Noninoculated seeds grown in polluted soil.

Prepared seeds were sown in 0.5-dm^3 plastic pots filled with the substrate. Afterwards, the plants were cultivated under controlled conditions in the growth chamber, exposed to sunlight for 12 h daily (14 000 lux; MH Philips 600W) with a maximum temperature of 30°C and a minimum temperature of 20°C. Soil moisture was kept at 60% of the soil field capacity.

The experimental period lasted for three months and the aboveground biomass of plants was harvested three times, about 3 cm above soil level, approximately every 25th day. The height of the seedlings was measured prior to every cut. Mown grass was dried at 60°C until constant mass and dry matter (DM) of the harvests was determined. Root length was measured at the end of the experiment. Fifty seeds were sown on each pot and the experiment was set up as a completely randomized design with three replications.

Statistical analyses. Data were analyzed by two-way analyses of variance (ANOVA) followed by the Fisher's Least Significant Difference (LSD) test. The analyses were conducted using the SPSS 22 software package (SPSS Inc., Chicago, IL, USA).

Results and Discussion

This work examines the potential of several PGPB to promote red clover growth in soil polluted with organic contaminants. Red clover seeds were inoculated and sown in the substrate with an increased content of PAHs, PCBs and OTs according to the regulation of the Republic of Montenegro. Even though the regulation of The Republic of Serbia is less rigorous on this issue, concentrations of total PAHs and several PCBs were marked as elevated. According to the 2010 Statute (Official Gazette of RS, No. 88/2010), MAC of total PAHs is 1 mg kg^{-1}. In the case of PCBs, the MAC value is 0.02 mg kg^{-1}.

The effect of applied treatments on red clover seedlings was monitored through seedling height (Table 2), root length (Table 3) and biomass (Table 4) in the three-month experimental period.

After the first mowing, the highest values of height were noted on seedlings inoculated with *B. amyloliquefaciens* D5 ARV, followed by *P. putida* P1 ARV treatment. All other treatments did not induce significantly better growth compared to CONPS. The second mowing showed that the treatment with *B. amyloliquefaciens* D5 ARV kept the highest values of height. Other treatments gave the same results as in the time of the first cut. The third mowing showed that *P. putida* P1 ARV seedlings were significantly higher compared to others. The remaining treatments did not induce the promotion of this growth parameter.

Table 2. Height (cm) of red clover seedlings grown on the soil contaminated with organic pollutants.

Treatment	Red clover seedling height (cm)			$LSD_{0.01}$
	Mowing			
	I	II	III	
Z-I ARV	7.90 ± 1.21^{b}	6.50 ± 1.54^{b}	8.60 ± 0.96^{b}	0.191
10 ARV	7.83 ± 1.13^{b}	6.75 ± 1.47^{b}	9.00 ± 0.66^{c}	
D5 ARV	9.53 ± 1.59^{d}	7.95 ± 0.97^{c}	8.50 ± 0.50^{ab}	
P1 ARV	8.50 ± 1.31^{c}	5.75 ± 0.44^{a}	9.70 ± 1.19^{d}	
MIX	7.30 ± 0.98^{a}	6.75 ± 1.35^{b}	8.30 ± 0.40^{a}	
CONPS	7.68 ± 1.16^{b}	6.75 ± 1.11^{b}	9.00 ± 0.45^{c}	
$LSD_{0,01}$	0.292			

± shows standard deviation; different small caps represent significant statistical differences between treatments (p=0.01).

The data of the root length were recorded at the end of the experiment (Table 3). The only isolate that significantly influenced root length was *S. liquefaciens* Z-I ARV. The presence of all others isolates and their mixed inoculum did not induce significant root development.

Table 3. Root length (cm) of red clover seedlings grown on the soil contaminated with organic pollutants.

Treatment	Root length (cm)
Z-I ARV	8.63 ± 2.86^{c}
10 ARV	6.87 ± 1.07^{b}
D5 ARV	7.15 ± 1.48^{b}
P1 ARV	5.73 ± 1.30^{a}
MIX	6.02 ± 1.46^{ab}
CONPS	6.26 ± 1.43^{ab}
$LSD_{0,01}$	0.896

± shows standard deviation; different letters represent significant statistical differences between treatments based on the LSD test (p=0.01).

After the first mowing, the highest biomass production (Table 4) was noted in the case of seedlings inoculated with *B. amyloliquefasiens* D5 ARV, *P. putida* P1 ARV and *S. liquefaciens* Z-I ARV. The presence of other isolates did not induce significantly different results compared to the CONPS treatment. The second mowing showed similar results. At the end of the experiment, inoculation did not show any effects on seedling biomass production.

Table 4. Dry aboveground biomass (g) of red clover seedlings grown on the soil contaminated with organic pollutants.

Treatment	Dry aboveground biomass (g)			$LSD_{0.01}$
	Mowing			
	I	II	III	
Z-I ARV	1.03 ± 0.12^{bC}	0.85 ± 0.09^{bB}	0.60 ± 0.09^{aA}	0.133
10 ARV	0.80 ± 0.13^{aB}	0.47 ± 0.10^{aA}	0.72 ± 0.03^{aB}	
D5 ARV	1.33 ± 0.21^{cC}	1.07 ± 0.19^{cB}	0.77 ± 0.06^{aA}	
P1 ARV	1.07 ± 0.19^{bB}	0.89 ± 0.09^{bcA}	0.80 ± 0.23^{aA}	
MIX	0.74 ± 0.10^{aB}	0.53 ± 0.08^{aA}	0.61 ± 0.17^{aAB}	
CONPS	0.67 ± 0.06^{aB}	0.50 ± 0.03^{aA}	0.69 ± 0.08^{aB}	
$LSD_{0,01}$		0.202		

± shows standard deviation; different small caps represent significant statistical differences between treatments; different large caps represent significant statistical differences between mowing based on the LSD test (p=0.01).

Analyses of data within a treatment obtained after every mowing showed that the first mowing gave the highest yield. The yields obtained in the other two mowings were significantly lower in most cases (Table 4). Plant growth can cause nutrient depletion considering limited pot resources. Besides, the main reason to apply bacterial inoculants on seeds is to support the plant growth in the earlier stages of plant ontogenesis. Those microbes affect germination and early growth which is critical for the success of revegetation. Further research is needed to evaluate the potential effects of subsequent application of the bacterial inoculants in soil, in later phases of plant growth.

Red clover inoculated with *B. amyloliquefasiens* D5 ARV produced the highest aboveground dry biomass through the whole experimental period. Also, *P. putida* P1 ARV and *S. liquefaciens* Z-I ARV significantly elevated biomass yield (Figure 1). This effect is highly desirable in the case of phytoremediation based on phytoextraction where biomass represents the storehouse of pollutants.

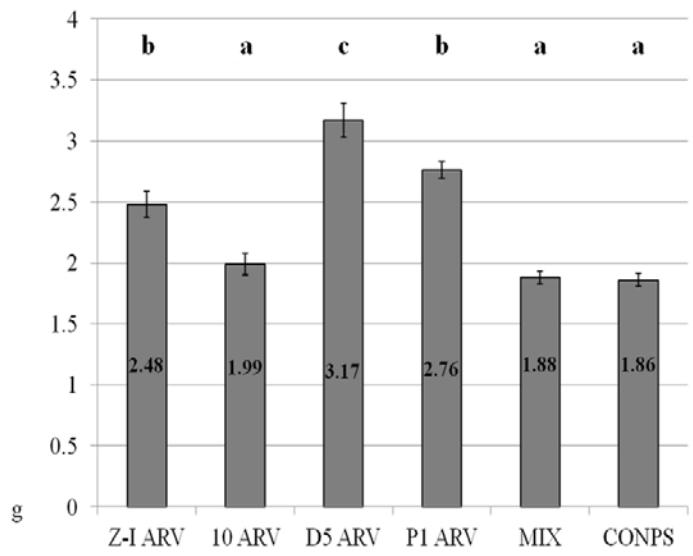

Figure 1. Total dry aboveground biomass (g) of red clover seedlings grown on the soil contaminated with organic pollutants. Different letters represent significant statistical differences between treatments based on the LSD test (p=0.05).

Even though red clover is used in bioremediation frequently (Gkorezis et al., 2016; Nazarov et al., 2017), Sverdrup et al. (2003) recorded its sensitivity on PAHs presence which resulted in the fresh and dry weight decrease. Such a problem can be successfully alleviated by PGPB. Bacterial strains used in this study were already confirmed as growth promoters of several woody species. Previously those strains were used in the form of mixed inoculums and this is the first time that their individual effects were studied on one plant species.

The overview of the presented results showed that, in the presence of *B. amyloliquefasiens* D5 ARV and *P. putida* P1 ARV, seedlings were higher and produced more biomass in comparison to control. Karličić et al. (2017) referred to the ability of those two isolates to produce ammonia, siderophores and indoleacetic acid (1.5 µg ml^{-1} and 1.2 µg ml^{-1} respectively). *P. putida* P1 ARV was capable to solubilize inorganic phosphates, too (Karličić et al. 2017). *S. liquefaciens* Z-I ARV induced higher biomass production and supported root growth. This isolate showed similar PGP characteristics as the previous two, but it possessed higher indoleacetic acid production (8.4 µg ml^{-1}) abilities (Karličić et al., 2017).

Production of indoleacetic acid is one of the most important characteristics of PGPB due to the great role of this hormone in plant-microbe interactions. Even a small amount of indoleacetic acid produced by rhizobacteria is enough to induce a positive effect on plant growth (Teixeria et al., 2007). The higher amount does not guarantee a positive plant response and may be the reason for plant growth inhibition (Gamalero and Glick, 2015). This could be the explanation for disappointing results obtained in the treatment with *E. adhaerens* 10_ARV. Biochemical testing marked this isolate as the most promising one. It produced a high amount of indoleacetic acid (44.5 µg ml^{-1}), solubilized inorganic phosphates, produced ammonia and siderophores (Karličić et al., 2017). This emphasizes the significance of numerous factors (amount of endogenous auxins, plant species and phase of plant growth) that need to be taken into account for successful PGPB application.

Higher biomass accumulates higher amounts of pollutants and speeds up the soil purification process (Jiang et al., 2015). Ficko et al. (2010) assert that plants with the ability to storage low PCB concentration could still extract a valuable quantity of PCBs with large shoot biomass. This is the aspect that can be significantly improved by PGPB. Rostami et al. (2017) showed that *Sorghum bicolor* inoculation with *Pseudomonas aeruginosa* resulted in higher shoot (21.27%) and root biomass (14.5%) at the pyrene concentration of 150 mg kg^{-1}. They also showed that such a combination reduced 66–82% of pyrene after the 90-day experimental period. In our study, the presence of *S. liquefaciens* Z-I ARV induced higher biomass production (an increase of 33%), *P. putida* P1 ARV induced an increase of 48%, while *B. amyloliquefaciens* D5 ARV caused an increment of biomass that reached 70%. Eskandary et al. (2017) reported the shoot and root biomass increase of *Festuca arundinacea* inoculated with *B. licheniformis* ATHE9 and *B. mojavensis* ATHE13 under polluted soil conditions. Those authors related 95% PAH degradation to the synergistic effect of *B. mojavensis*, *B. licheniformis* and the plant.

The contaminated soils are the main pools of microorganisms suitable for bioremediation (Karličić et al., 2016). In this study, *S. liquefaciens* Z-I ARV and *E. adhaerns* 10_ARV originated from the soil that was the object of interest. Among them, the presence of *S. liquefaciens* Z-I ARV gave promising results. We also used *P. putida* P1 ARV and *B. amyloliquefaciens* D5 ARV of a completely different origin, but they showed the capacity to improve red clover growth under given circumstances.

Revegetation is a process that occurs naturally and slowly (Panchenko et al., 2018), but it can be speeded up by taking measures that reconcile *in situ* requirements. Two crucial factors that need to be optimized are plant species, adapted to the presence of high levels of contaminants, and microbial community capable to survive and influence plant growth and tolerance under given circumstances (Hou et al., 2015). This can be achieved by plant inoculation with proper PGPB. The right combination enhances metabolic processes in the soil, causing faster and more complete soil recovery. The results of the presented work show that some of the tested combinations may be qualified as the “right” since applied PGPB stimulated growth and biomass production of red clover grown in the substrate burden with a high presence of organic pollutants. Obtained data imply that red clover used in combination with PGPB can achieve better growth in polluted soil, and thus it has a better potential for use in revegetation of contaminated soil.

Conclusion

The wide use of different organic and inorganic pollutants causes deterioration of soil. The most vulnerable are plant species, tightly attached to the substrate, which accumulate toxic substances in their biomasses. Some of them are very resistant and highly appreciated for revegetation and remediation of contaminated soils. Soil microorganisms are ever-present residents well known as plant helpers. The obtained results pointed out at three isolates, *B. amyloliquefaciens* D5 ARV, *Pseudomonas putida* P1 ARV and *S. liquefaciens* Z-I ARV, whose individual application resulted in significant plant growth promotion. Among them, the most effective was *B. amyloliquefaciens* D5 ARV, which substantially raised red clover biomass production. The biochemical tests, especially those for indoleacetic acid production, pointed out at *E. adhaerens* 10_ARV, but this strain failed *in vivo*. This shows the complexity of factors that modulate plant-microbe interactions, and indicate the necessity for additional selection through *in vivo* tests, particularly in such specific substrates. A significant rise of biomass production caused by the presence of PGPB strains will certainly encourage further research in the estimation of remediation utility of such combinations.

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BAKTERIJSKA INOKULACIJA: POSTUPAK ZA STIMULACIJU RASTA CRVENE DETELINE GAJENE U ZAGAĐENOM ZEMLJIŠTU

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R e z i m e

Seme crvene deteline (*Trifolium pratense* L.), inokulisno sa nekoliko bakterija stimulatora biljnog rasta (PGPB), posejano je u supstrat kontaminiran policikličnim aromatičnim ugljovodonicima (PAHs), polihlorovanim bifenilima (PCBs) i organometalnim derivatima kalaja (OT). Cilj je bio da se utvrdi da li selektovane PGPB mogu promovisati rast crvene deteline u supstratu kontaminiranom sa nekoliko organskih zagađujućih materija. Uticaj bakterija na rast crvene deteline (visina, dužina korena i biomasa) praćen je tri meseca. Najveća visina je zabeležena kod biljaka inokulisanih sa *Bacillus amyloliquefaciens* D5 ARV i *Pseudomonas putida* P1 ARV. Rast korena je stimulisan od strane *Serratia liquefaciens* Z-I ARV. Ovi izolati su značajno uticali i na produkciju biomase. Ukupna biomasa dobijena tokom celog ogleda je za 70%, 48% i 33% veća u odnosu na kontrolu. Bakterijski sojevi korišćeni u ovoj studiji su prethodno potvrđeni kao PGPB kroz biohemijske i *in vivo* testove mešanog inokuluma na nekoliko drvenastih vrsta gajenih u jalovini. Ovaj rad prvi put beleži njihove pojedinačne efekte na jednu biljnu vrstu. Dobijeni rezultati potvrđuju da inokulacija sa nekoliko PGPB sojeva može ubrzati rast crvene deteline u zagađenom zemljištu.

Ključne reči: organske zagađujuće materije, crvena detelina, revegetacija, bakterije stimulatori biljnog rasta.

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VARIATION IN HEALTH PROMOTING COMPOUNDS OF BLUEBERRY FRUIT ASSOCIATED WITH DIFFERENT NUTRIENT MANAGEMENT PRACTICES IN A SOILLESS GROWING SYSTEM

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Abstract: The objective of this study was to determine and compare the content of total anthocyanins (TACY), total phenolics (TPC) and total antioxidant activity (TAA) of the fruit of 'Bluecrop' highbush blueberry grown under different nutrient management practices in a soilless production system. A field study was carried out in a highbush blueberry plantation situated near Belgrade (Serbia), during the period of 2016–2017. The orchard was planted in the spring of 2016 in 50 l polypropylene pots with 5-year-old nursery plants. Each pot was filled with the mix of pine sawdust (60%), white peat (30%) and perlite (10%), and placed at a distance of 0.8 m within the row and 3.0 m between the rows (4,170 bushes ha^{-1}). The following fertilizer treatments were evaluated: 1. organic fertilizers (Org); 2. mineral fertilizers (Min) and 3. a combination of organic and mineral fertilizers (Org-Min). Soluble NPK fertilizers were applied with irrigation water, whereas granulated mineral and pelleted organic fertilizers were mixed with the substrate. Fruit samples were collected in triplicate at the beginning of ripening, full maturity and the end of the harvest season. No significant effect of harvest time on each of the tested parameters was observed, whereas the content of TACY did not even differ under various fertilizer treatments. TPC in the fruit significantly increased in Org and Org-Min treatments (139.8 and 139.3 mg eq GA 100 g^{-1} FW, respectively) compared to Min treatment (122.7 mg eq GA 100 g^{-1} FW), while a considerably high TAA level was found only in berries under Org-Min treatment (0.53 mg asc g^{-1} FW).

Key words: *V. corymbosum*, fertilizers, harvest time, total phenolics, total anthocyanins, antioxidant activity.

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Introduction

Northern highbush blueberry (*Vaccinium corymbosum* L.) is presently one of the very popular commercial crops in the Republic of Serbia covering the total area of around 1,500 ha. The majority of blueberries are soil-grown, but a soilless growing system has also gained popularity in recent years. Some of the advantages of soilless culture are related to easier control of irrigation and nutrient management; manipulation with plant growth to better control of shoot length, fruit to shoot ratio, and fruit quality (Voogt et al., 2014). This production system also enables higher planting densities (4,170–5,500 plants ha^{-1}) aiming to reach maximum production per unit area (Milivojević et al., 2017a).

The initial focus in a soilless culture system is on balanced and precise fertilizer applications which can improve the nutrient status and regulate plant development and yield of blueberries. The major concern in the application of nitrogen (N) fertilizers is N availability, which is highly related to productivity (Miller et al., 2006). It is generally accepted that blueberry plants mainly use ammonium (NH_4) form of N and therefore fertilizers supplying N as ammonium (urea, ammonium sulfate) are preferable for blueberries. In a field study of Vargas and Bryla (2015) on northern highbush blueberry (*V. corymbosum* L.), all ammoniacal N and 25% to 50% of organic N were available for blueberry plants during the same year when they were applied. In the case of phosphorus (P), symptoms of deficiency are not commonly seen, and field plants seldom respond to P applications (Hanson and Hancock, 1996). Potassium (K) deficiency can be due to a number of factors: reduced root function, flooding, poor drainage, high N levels, drought and very acid soils (Retamales and Hancock, 2018). Since fruit is an important sink for K in the plant, leaf K levels are greatly influenced by fruit load. Calcium (Ca) nutrition has also recognized effects on the fruit quality (texture, firmness and ripening rate). Among microelements, iron (Fe) deficiency is common in blueberries. The most effective means to correct Fe deficiency is to adjust substrate pH within the optimal range of 4.2–5.5, which, among the other things, can be achieved using adequate fertilizers.

It is important to underline that deficiency or imbalance of mineral nutrients can affect some physiological and metabolic modifications, such as lower photosynthetic activity, displacement and unusual circulation of organic compounds, as well as the accumulation of primary and secondary metabolite products (Fotirić Akšić et al., 2019). Thus, the understanding of the effects of different nutrients on the overall quality of blueberries could lead to the adoption of specific cultivation and nutrient management practices to meet certain demands of this fruit species.

Blueberries are reported to be an excellent source of bioactive compounds with high antioxidant activity and potential health benefits exhibiting anti-diabetic properties, decreasing blood pressure and blood cholesterol, and inhibiting the development of cancer cells in the breast and colon (Calò and Marabini, 2014; Pertuzatti et al., 2016). Most researches have correlated the high antioxidant capacity with the phenolic content in the fruit, especially anthocyanins and flavanols, as well as hydroxycinnamic acids (Castrejón et al., 2008; You et al., 2011; Milivojević et al., 2012; Milivojević et al., 2016a; Milivojević et al., 2017b). However, a wider knowledge of the effect of different fertilizers on the variation of some bioactive compounds is imperative to control and manipulate preharvest changes in the blueberry fruit. Therefore, this study aimed to compare the effect of organic and mineral fertilizers on the content of total anthocyanins and total phenolics, as well as expressed antioxidant activity for the 'Bluecrop' highbush blueberry cultivar grown as a soilless culture.

Materials and Methods

Description of the experimental site

The field study was carried out in the 'Bluecrop' highbush blueberry plantation situated near Belgrade (44°45' N, 20°35' E, 112 m a.s.l.) during the period of 2016–2017. The climate of the region is temperate continental, with a mean annual air temperature of 10.8°C and mean annual precipitation of 650 mm. The orchard was planted in the spring of 2016 in 50 l polypropylene pots with 5-year-old nursery plants. The growing media was pine sawdust (60%), white peat (30%) and perlite (10%). Each pot, filled with the mix, was placed at a distance of 0.8 m within the row and 3.0 m between the rows (4,170 bushes ha^{-1}).

Experimental design and treatments

The following nutrient treatments were examined in this study: 1) organic fertilizer application (pelleted and water-soluble fertilizers); 2) mineral fertilizer application (granulated and water-soluble fertilizers); 3) combined application of organic and mineral fertilizers. Plants were fertilized with 64 kg ha^{-1} N, 42 kg ha^{-1} P, 52 kg ha^{-1} K in the treatment 1 (Org); 85 kg ha^{-1} N, 45 kg ha^{-1} P, 64 kg ha^{-1} K in the treatment 2 (Min), and with 72 kg ha^{-1} N, 48 kg ha^{-1} P, 68 kg ha^{-1} K in the treatment 3 (Org-Min).

At the beginning of intensive vegetative growth, pelleted fertilizers (I: 6% N, 15% P, 3% K, 2% MgO, 10% CaO, 55.2% organic matters; II: 3% N, 3% P, 7% K, 2% MgO, 60% organic matters) were applied with 50 g and 30 pot^{-1}, respectively in the treatment 1; granulated NPK (14% N; 14% P and 17% K) and ammonium sulphate (20% N; 24% S) fertilizers, both with 50 g pot^{-1}, were applied in the treatment 2; while organic fertilizer (6% N, 15% P; 3% K; 2% MgO; 10% CaO, 55.2% organic matters) and granulated ammonium sulphate (20% N; 24% S), both with 50 g pot^{-1}, were applied in the treatment 3. During the spring, when the plants were in the vegetative (i.e. sprout) stage of production, pelleted fertilizer (10% N, 3% P, 3% K, with 72% organic matters) was mixed with substrate two times (application rate of 50 g pot^{-1} per each) in the Org treatment, whereas in the Min and Org-Min treatments, water-soluble NPK fertilizer (20% N, 20% P and 20% K) was applied through the irrigation system 4 times a week, from the end of April to the end of May. Fertigation with water-soluble NPK fertilizer (12% N, 12% P and 36% K) was applied to the plants that had been in the cropping stage of vegetation (4 times a week, from the beginning of June to the middle July) in the Min and Org-Min treatments. At this stage of vegetation, 50 g pot^{-1} of pelleted fertilizer (10% N, 3% P, 3% K, with 72% organic matters) was mixed with the substrate and additionally, potassium (12% K) foliar feeding was applied 2 times a week only in the Org treatment.

The trial was set up in a completely randomized design with 3 replications and 10 bushes pots^{-1} per replication for each fertilizer treatment. A sample of 30 randomly selected fruits from all around of the bush (from each fertilizer treatment and replication) was collected at the beginning, mid and the end of the harvest season and used for analyzing the content of total anthocyanins (TACY) and phenolics (TPC), as well as total antioxidant activity (TAA). Each sample was pooled to obtain a composite sample. For the extraction of phenolics, fruits were homogenized in 80% methanol (1:3 w/v). The homogenates were centrifuged at 10,000 x *g* for 10 min. Three replications of supernatants were prepared for spectrophotometric analysis (Thermo Scientific Multiskan Spectrum, Vantaa, Finland). The TACY was measured with the modified pH differential absorbance method (Cheng and Breen, 1991). The absorbance was measured at 510 and 700 nm in 0.025 M potassium chloride buffer at pH 1.0 and 0.4 M sodium acetate buffer at pH 4.5. Results were expressed as micrograms of cyanidin-3-glucoside (ε=26900l·mol^{-1}·cm^{-1}) equivalents per 100 g of fresh weight (μg g cy-3-g eq/100 g FW).

The TPC was determined according to the Folin-Ciocalteu procedure (Singleton and Rossi, 1965) using gallic acid (GA) as a standard. Results were read at 724 nm and expressed as milligrams of gallic acid equivalent per 100 g of fresh weight (mg GAE 100 g^{-1} FW). Determination of TAA was done following the ABTS method of Arnao et al. (1999) and results were expressed as milligrams of ascorbic acid equivalent per gram of fresh weight (mg eq asc g^{-1} FW).

Statistical analysis

The data obtained in the research were processed applying the Fisher model of variance analysis (ANOVA, F test) and the statistics software package STATISTICA version 8.0 (StatSoft, Inc., Tulsa, OK, USA). The analyses were performed in three replications and the obtained values for two years were expressed as the means ± standard error. Significant differences among the means of the treatments were determined by LSD test at a level of $P \leq 0.05$.

Results and Discussion

The determination of TPC allows one to estimate the content of all compounds belonging to the subclass of phenolic compounds (Pertuzatti et al., 2016). Besides the genetic background as the main factor determining the phenolic composition of the blueberries, the interaction with the environmental conditions and the cultivation systems also influence the phenolic concentration (Vittori et al., 2018). In our study, the analysis of the TPC in the fruit of ‘Bluecrop’ highbush blueberry grown as a soilless culture showed that the effect of fertilizer treatments was more marked than the effect of harvest time (Table 1). The implementation of organic fertilizers in Org and Org-Min treatments had a stimulating impact on TPC in the fruit (139.8 and 139.3 mg eq GA 100 g^{-1} FW, respectively). Numerous studies also confirm the fact that blueberries grown under the organic cultivation system have more polyphenols as well as other antioxidant compounds than blueberries grown under the conventional system (Wang et al., 2008; You et al., 2011; Gupta-Elera et al., 2012). Higher values of TPC were also found in strawberries treated with biofertilizers (Pešaković and Milivojević, 2014) which can be explained by highly intensive mineralizing processes in the substrate and the increased physiological functions and activity of the plant root. Wang et al. (2008) found the mean values for TPC of 319.3 and 190.3 mg eq GA 100 g^{-1} FW in blueberries from organic and conventional cultures, respectively. According to Kim et al. (2013), the ‘Bluecrop’ highbush blueberry cultivar grown in Korea exhibited higher TPC (205.2 mg eq GA 100 g^{-1} FW) compared to results in the present study. Concerning the influence of other factors, Milivojević et al. (2017b) underlined the predominant effect of the modified microclimate under a grey hail protection net, rather than the influence of the cultivation cycle on the accumulation of total phenolics in cv. ‘Bluecrop’.

Among the secondary plant metabolites found in blueberries, the flavonoid subclass (anthocyanins) has received the most attention. Up to 60% of the total phenolic content in highbush blueberries is accounted for by anthocyanins, which are responsible for the blue colours in fruit and are dependent on environmental pH values (You et al., 2011; Retamales and Hancock, 2018). Pertuzatti et al. (2016) have reported that only five types of anthocyanins are found in blueberries as follows: delphinidin, malvidin, petunidin, peonidin and cyanidin. In the present study, TACY was not significantly affected by fertilizer treatment and harvest time. However, fertilizer treatment × harvest time interaction showed a significantly higher accumulation of TACY at the mid and end of the ripening season in Org and Org-Min treatments. In addition to the above said, a significantly higher accumulation of TACY was also observed at the beginning and mid of the ripening season in the Min treatment.

Similarly, Connor et al. (2002) found that delaying fruit harvest could have a markedly positive influence on levels of anthocyanins in blueberry fruit. The same authors reported statistically significant genotype × environment interactions for both total anthocyanin and phenolic content; however, they did not discuss the differences between regions that may be caused by agronomic practices and climatic factors, such as differences in mineral nutrients available, ultraviolet intensity, temperature during fruit ripening, and water stress.

Table 1. Average contents of total anthocyanins (TACY) and total phenolics (TPC) in the fruit of ‘Bluecrop’ highbush blueberry affected by fertilizer treatment and harvest time.

Treatments	FT/HT		TACY (μg cy-3-g eq g^{-1} FW)	TPC (mg GAE 100 g^{-1} FW)
Fertilizer treatments (FT)	Org		563.8 ± 31.5	139.8 ± 6.08a
	Min		541.0 ± 30.0	122.7 ± 4.25b
	Org-Min		577.4 ± 28.4	139.3 ± 3.46a
Harvest time (HT)	Beginning		517.7 ± 25.3	125.4 ± 5.26
	Mid		602.4 ± 25.8	138.9 ± 4.90
	End		562.1 ± 32.1	137.6 ± 4.99
FT × HT	Org	Beginning	455.6 ± 43.6d	123.0 ± 10.2
		Mid	600.9 ± 28.9ab	144.3 ± 4.22
		End	634.8 ± 9.61a	152.1 ± 9.71
	Min	Beginning	593.6 ± 13.8ab	115.2 ± 4.23
		Mid	557.7 ± 56.4abcd	125.9 ± 11.35
		End	471.7 ± 59.1cd	127.2 ± 5.15
	Org-Min	Beginning	504.0 ± 25.8cd	138.1 ± 8.66
		Mid	648.4 ± 43.5a	146.4 ± 3.77
		End	579.7 ± 43.3abc	133.4 ± 3.31
F test	FT		ns	*
	HT		ns	ns
	FT × HT		*	ns

Data are presented as the means of 2-year values and 3 replications in each year ± standard error. Values within each column followed by the same letter are not significantly different at $P \leq 0.05$ (LSD test). *Significant at $P \leq 0.05$; ns – not significant.

Milivojević et al. (2016a) also noted the variations in TACY and TPC across the harvests in the fruit of cv. ‘Duke’ achieving higher concentrations in the 1st and 2nd harvest, whereas no significant differences were observed in cv. ‘Bluecrop’. This discrepancy may be explained by variation in solar radiation and air temperature throughout the ripening season of the mentioned cultivars as the factors known to impact phytochemical biosynthesis. Changes in TPC during maturation of four tested cultivars were also reported by Castrejón et al. (2008), with higher concentrations of total phenolics in unripe green berries. After this stage, TPC was decreasing during color break and ripening, and then in cv. 'Bluecrop', values were maintained stable until reaching horticultural maturity.

The phytochemical content of blueberries, particularly anthocyanins and other polyphenols, is responsible for the total antioxidant activity (TAA). The contributions of TPC and TACY to expressed TAA of highbush blueberries were previously reported by Milivojević et al. (2016a, b). In the mentioned studies, a significant variation in TAA was found among the tested cultivars and growing seasons. Besides these factors, TAA can be affected by location, cultural management, maturity, and postharvest handling and storage. In our study, a considerable variation in TAA levels of cv. 'Bluecrop' was caused by applied fertilizer treatments. Significantly higher TAA was registered in the Org-Min treatment (0.53 mg asc g^{-1} FW) compared to the other tested treatments (Table 2).

Table 2. Average levels of total antioxidant activity (TAA) in the fruit of 'Bluecrop' highbush blueberry affected by fertilizer treatment and harvest time.

Treatments	FT/HT		TAA (mg eq asc g^{-1} FW)
Fertilizer treatments (FT)	Org		0.38 ± 0.04b
	Min		0.38 ± 0.03b
	Org-Min		0.53 ± 0.04a
Harvest time (HT)	Beginning		0.37 ± 0.05
	Mid		0.41 ± 0.04
	End		0.40 ± 0.02
FT × HT	Org	Beginning	0.24 ± 0.06
		Mid	0.46 ± 0.07
		End	0.42 ± 0.03
	Min	Beginning	0.44 ± 0.01
		Mid	0.32 ± 0.07
		End	0.37 ± 0.05
	Org-Min	Beginning	0.44 ± 0.11
		Mid	0.46 ± 0.08
		End	0.40 ± 0.02
F test	FT		*
	HT		ns
	FT × HT		ns

Data are presented as the means of 2-year values and 3 replications in each year ± standard error. Values within each column followed by the same letter are not significantly different at $P \leq 0.05$ (LSD test). *Significant at $P \leq 0.05$; ns – not significant.

This result may be attributed to the TPC increment owing to the synergistic effect of organic and mineral fertilizers, although the impact of cultivation management on antioxidants is pretty controversial. On the one hand, Wang et al. (2008) found that cv. 'Bluecrop' grown organically yielded significantly higher total phenolics, total anthocyanins and antioxidant activity (ORAC), whereas, on the other hand, Sablani et al. (2010) indicated that total anthocyanins content, phenolic content and total antioxidant activity of blueberries were not altered by the agricultural production system. Generally, the organic production system is in most cases considered a more stressful production system, due to the insufficient supply of mineral nitrogen and other nutrients, which leads to a higher accumulation of primary and secondary metabolic products (Fotirić Akšić et al., 2019).

No significant effects of harvest time and fertilizer treatment × harvest time interaction on TAA levels were observed in our study. Previous findings of Milivojević et al. (2016b) showed that TAA levels of cv. ‘Bluecrop’ grown in soil were increased linearly from the 1^{st} to the 4^{th} harvest. These discrepancies could be influenced by pre- and post-harvest factors such as environmental characteristics (temperature, humidity and solar intensity), agro-technical conditions (soil, water supply, use of fertilizers or manure), where each of them or their combination may predominantly affect biomolecule activity and availability.

Conclusion

The changes in the content of blueberry polyphenols and antioxidant activity in cv. ‘Bluecrop’, occurring as a result of the application of different fertilizers in a soilless growing system, point to the fact that the most prominent effect was produced by the organic fertilizers, either applied alone (Org treatment) or in combination with mineral fertilizers (Org-Min treatment). Some inconsistent results were also found when comparing TACY and TPC in the blueberry fruit, affected by fertilizers and harvest time.

Considering the positive effect of organic fertilizers, as well as a stimulating impact of combined application of organic and mineral fertilizers on the expressed total antioxidant activity, a partial substitution of mineral by organic fertilizers may be recommended in advancing the existing technology of soilless blueberry production. Detailed research on some other bioactive compounds that also contribute to the expressed antioxidant activity, such as sugars, vitamins and enzymes should be also performed in the future.

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VARIRANJE SADRŽAJA ZDRAVSTVENO KORISNIH KOMPONENTI U PLODU BOROVNICE POVEZANO SA RAZLIČITIM NAČINIMA ISHRANE U SISTEMU GAJENJA U SUPSTRATU

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R e z i m e

Cilj ovog istraživanja bio je da se odredi i uporedi sadržaj ukupnih antocijana i ukupnih fenola, kao i antioksidativna aktivnost u plodu sorte visokožbunaste borovnice ’Bluecrop’ gajene u supstratu pod uticajem različitih načina ishrane. Poljsko istraživanje je izvedeno u proizvodnom zasadu borovnice koji se nalazi u blizini Beograda (Srbija), tokom perioda 2016–2017. godine. Zasad je podignut u proleće 2016. godine sadnjom petogodišnjih biljaka u saksije od polipropilenske folije zapremine 50 l. Svaka saksija je ispunjena supstratnom smešom sastavljenom od strugotine četinara (60%), belog treseta (30%) i perlita (10%). Primenjeno rastojanje sadnje je 0,8 m u redu i 3,0 m između redova (4.170 žbunova po ha). Ispitivana je primena sledećih đubriva: 1. organska đubriva (Org); 2. mineralna đubriva (Min) i 3. kombinovana primena organskih i mineralnih đubriva (Org-Min). Rastvorljiva NPK đubriva su primenjivana kroz sistem za navodnjavanje, dok su granulisana mineralna i peletirana organska đubriva mešana sa supstratom. Uzorci plodova su uzimani u 3 ponavljanja na početku, sredini i na kraju sezone berbe. Vreme berbe nije ispoljilo značajan uticaj na testirane parametre, dok se sadržaj ukupnih antocijana nije razlikovao čak ni pod uticajem primenjenih đubriva. Sadržaj ukupnih fenola u plodu bio je značajno povećan u Org i Org-Min tretmanima (139,8, odnosno 139,3 mg ekv. gal. kis./100 g sv.m.pl.), dok je značajno veća ukupna antioksidativna aktivnost ustanovljena u plodovima iz Org-Min tretmana (0,53 mg ask./g sv.m.pl.).

Ključne reči: *V. corymbosum*, đubriva, vreme berbe, ukupni fenoli, ukupni antocijani i antioksidativna aktivnost.

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INFLUENCE OF INTEGRATED SOIL FERTILITY MANAGEMENT ON THE VEGETATIVE GROWTH PARAMETERS OF *ZEA MAYS* IN THE GUINEA SAVANNA ECO-ZONE OF GHANA

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Abstract: All over the world, attention has been drawn to the use of eco-friendly biochar application to improve crop productivity. In Ghana, there are available potential feedstocks left unused and can be used for the production of biochar. Therefore, this study investigated the effect of different rates of rice husk biochar and different rates of inorganic nitrogen (N) on the growth of *Zea mays* in Nyankpala, Northern Ghana. Field experiments were conducted in the cropping period of 2012. The treatments involved 4 different rates of inorganic nitrogen fertilizer (0 kg Nha^{-1}, 30 kg Nha^{-1}, 60 kg Nha^{-1} and 90 kg Nha^{-1}) and 3 different rates of rice husk biochar (0 ton ha^{-1}, 2 ton ha^{-1} and 4 ton ha^{-1}). The treatments were allocated in a split-plot design with three replications. The vegetative parameters assessed were the number of leaves, plant height and plant girth. Treatments showed a significant ($p<0.05$) influence on all the traits considered. The combined effects of inorganic nitrogen fertilizer and rice husk biochar significantly influenced the vegetative growth parameters of *Zea mays* with the maximum values recorded at 4 ton ha^{-1}rice husk biochar. Yet, this observation corresponds with 2 ton ha^{-1} rice husk biochar which recorded optimum growth parameters compared to the control. Within the limit of this work, it was concluded that growth parameters of *Zea mays* in the Guinea Savannah Eco-Zone of Ghana could significantly be improved by applying 4 ton ha^{-1} rice husk biochar. Integrated soil fertility management (ISFM), a prudent combination of inorganic fertilizers and residues from various sources to sustain the environment is currently a necessity. The experiment revealed that the application of rice husk biochar can improve the growth parameters of *Zea mays*. Yet, further experiments need to be done using

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higher rates of rice husk biochar to ensure the appropriate rate of biochar application.

Key words: rice husk biochar, maize, inorganic fertilizer, integrated soil fertility management, savanna, vegetative growth.

Introduction

Zea mays is the crop with the highest yields per hectare and the second largest commodity crop after cocoa in Ghana. The crop is high yielding and has diversified uses. About 70% of the total maize was produced by small-scale farmers. Average maize yields fluctuate between 1.2 metric ton ha^{-1} and 1.9 metric ton ha^{-1}, whereas on-station and on-farm trials suggest that yields averaging between 4 and 6 metric ton ha^{-1} of maize are attainable in the country. The maize supply in Ghana has been increasing steadily over the past few years with an average supply at 1.4 million metric ton over the period 2005–2010 (Ragasa et al., 2014). However, human consumption is rising and competing with the poultry industry and to a lesser extent with the livestock industry. Consequently, the production of maize needs to be increased by adopting integrated soil fertility management practices. Lately, a rising consent has emerged on the need for the application of integrated soil fertility management practices to reverse the negative nutrient balances in cropping systems in agriculture in sub-Saharan Africa (SSA) (Vanlauwe et al., 2015). This know-how can improve soil health, keep land alive and sustain its productive capacity. Biochar is a pyrolyzed by-product of biomass produced at high temperatures (>400°C) with little or no available oxygen. According to international biochar initiatives (IBIs), it is persistently utilised for its agricultural and environmental advantages. It is fine-grained, carbon-rich and porous material which basically consists of nano-structured aromatic compounds systematically arranged like graphite (Sohi et al., 2010). Nearly any type of organic resources can be pyrolyzed into biochar including various types of forest residues (sawdust) (Xu et al., 2012), agricultural residues (corn cob, corn stalk, wheat straw, rice straw, stalk of pearl millet, cotton, mustard, soybean, and sugar beet tailing) (Jindo et al., 2014; Yu et al., 2014; Prabha et al., 2015), and agro-industrial waste (paper mill waste, Jatropha husk, coffee husk, coconut shell and cocoa pod husk) (Dume et al., 2015; Prabha et al., 2015; Munongo et al., 2017). The waning in soil productivity due to increased intensity with low fertilizer inputs has been recognised as one of the core causes of food insecurities in Ghana. To attain food sufficiency, there is an urgent need to manage hitches in soil infertility, advancing crop production in these soils that are essential for social and economic explanations. Applying inorganic fertilizers affords an alternative to overcome soil infertility. However, the sole application of inorganic fertilizers causes degradation of soil health and decreases its productive capacity. The application of organic sources reduces the reliance on expensive fertilizers that sustain and reduce nutrient losses and enhances biological N-fixation. When applied repetitively, soil organic matter accumulates, thus providing a capital of nutrients released slowly, increasing soil buffering capacity (balance between cations and anions), as well as capacity to hold up water (Sheahan and Barrette, 2014). The accumulation of nutrients and buffering capacity are slow processes and their potential benefits are likely to become visible in a long-standing case. The objective of the investigation was, however, to assess the effect of rice husk and inorganic nitrogen fertilizer on the maize growth parameters.

Materials and Methods

Site description

The field experiment was established throughout the 2012 cropping season at the experimental fields of Savannah Agriculture Research Institute (SARI), Nyankpala in the Northern region of Ghana. The site is located between latitude 9^{o} 25′ N and longitude 00^{o} 58′ W. The area has a rain forest belt with an average rainfall of about 1100 mm and a mean temperature range of 33^{o} C to 39^{o} C (SARI, 2016). The soil of the experimental site was sandy loam and slightly acidic (Table 1).

Table 1. Initial chemical and physical properties of soil.

Parameters	values
pH (1:1H_2O)	6.22
OC (%)	0.62
N (%)	0.62
P (mg kg^{-1})	4.92
K (mg kg^{-1})	0.16
ECEC (mg kg^{-1})	2.89
% base saturation	97.00
Sand (%)	62.85
Silt (%)	33.82
Clay (%)	3.33
Texture class	Sandy loam

Biological materials

Seeds for this experiment were collected from a local variety of maize (Dorke SR). However, the rice husk was collected in Nyankpala in the northern region of Ghana. Rice husk biochar was obtained after pyrolysis at 500^{o}C using the drum type pyrolyzer (Gul et al., 2015).

Experimental design and treatment

The experiment was carried out in a split-plot design with 12 treatments, each of which was replicated 3 times. Treatments included the application of 0 ton ha^{-1}, 2 ton ha^{-1} and 4 ton ha^{-1} rice husk biochar and four levels of inorganic nitrogen fertilizer: 0 kg Nha^{-1}, 30 kg Nha^{-1}, 60 kg Nha^{-1} and 90 $kgNha^{-1}$. Soil preparing comprised a single ploughing and harrowing. The experimental area was 50 m x 50 m (2500 m^2) and was subdivided into 3 whole plots of 12.8 m x 12.8 m and each whole plot consisted of 4 sub-plots of 6.4 m x 6.4 m with a working path of 2.0 m in the whole plot and a working path of 1 m in the sub-plot. The biochar was mixed thoroughly with soil at a depth of 10 cm using a hoe (Venkateshet al., 2015) before planting whilst the basal application of triple superphosphate and muriate of potash was incorporated before planting to ensure a sufficient amount of essential nutrients in the soil. Nitrogen in the form of urea was split-applied: 1/3 at 1 week after planting and 2/3 weeks after planting (WAP). Four seeds were planted per seed-bed. Refilling was done 1 week after planting. Maize seedlings were thinned to 2 plants per seed-bed and a spacing distance of 80 cm x 40 cm with a plant population size of 62,500 plants ha^{-1}. Weeding was done 2 times within the cropping season; the first at 3 WAP and the second at 7 WAP (Table 2).

Table 2. Details of treatment combination.

Treatment symbol	Treatment combination
T_1	0 ton ha^{-1} Rice husk biochar + 0kg N ha^{-1}
T_2	0 ton ha^{-1} Rice husk biochar + 30kg N ha^{-1}
T_3	0 ton ha^{-1} Rice husk biochar + 60kg N ha^{-1}
T_4	0 ton ha^{-1} Rice husk biochar + 90kg N ha^{-1}
T_5	2 ton ha^{-1} Rice husk biochar + 0kg N ha^{-1}
T_6	2 ton ha^{-1} Rice husk biochar + 30kg N ha^{-1}
T_7	2 ton ha^{-1} Rice husk biochar + 6kg N ha^{-1}
T_8	2 ton ha^{-1} Rice husk biochar + 90kg N ha^{-1}
T_9	4 ton ha^{-1} Rice husk biochar + 0kg N ha^{-1}
T_{10}	4 ton ha^{-1} Rice husk biochar + 30kg N ha^{-1}
T_{11}	4 ton ha^{-1} Rice husk biochar + 60kg N ha^{-1}
T_{12}	4 ton ha^{-1} Rice husk biochar + 90kg N ha^{-1}

Soil sampling and analysis

Initial soil samples were collected from thirty-six (36) plots at a depth of 0–15 cm with a soil auger. The samples were bulked together to form a composite sample. The soil samples collected were air-dried, gently crushed and passed through a 2-mm sieve and taken to the laboratory for physical and chemical analyses. Soil pH was measured in a 1:1 soil to water ratio (McLean, 1983). The Walkey and Black procedure was used to determine soil organic content (Walkey and Black, 1934). The nitrogen content was determined using the Kjeldahl digestion and distillation procedure. The cation exchange capacity was determined by the NH_4OAc method. Calcium and magnesium (Mg) were determined by atomic absorption spectrophotometry while potassium (K) and sodium (Na) were determined by flame photometry.

Characteristics of the biochar

The biochar chemical analysis results presented in Table 3 show that biochar prepared from rice husk that was cultivated in slightly acidic soil also had slightly acidic properties at pH 6.5. The biochar had lower concentrations of Ca, Mg, and Na, however a higher content of ash and organic carbon. The pyrolysis of rice husk increases organic carbon in the materials. Rice husk biochar had ECEC of 13.67 m kg^{-1}.

Table 3. The chemical composition of rice husk biochar.

Parameters	values
pH (1:1H_2O)	6.5
OC (%)	43.5
Ash (%)	58.2
N (%)	0.62
P (mg kg^{-1})	0.57
K (mg kg^{-1})	0.48
ECEC (mg kg^{-1})	13.67
Ca (mg kg^{-1})	2.34
Mg (mg kg^{-1})	0.89
Na (mg kg^{-1})	0.23
Al (mg kg^{-1})	3.4
Fe (mg kg^{-1})	1.2

Data collection

The data were collected every 2 weeks starting from 2 WAP and terminated at 10 WAP. Randomly, 5 maize plants were selected from individual sub-plots and labelled for measurements. Parameters assessed were the plant height measured from the base of the stem to the last leaf using a graduated ruler, number of leaves was determined by counting the leaves on plant and plant girth was measured at the soil level using a slide calliper.

Data analysis

Effects of different treatments were analysed by analysis of variance. The least significant difference (LSD) test was applied to distinguish the treatment differences ($p<0.05$) using the statistical software GenStat 18th edition.

Results and Discussion

Plant height

There was a general increase in plant height starting from 2 WAP to the end of the experiment (8 WAP) for all the treatment levels (Table 4). Mean differences in height among treatments were significant ($p<0.05$) at the end of the experiment. The highest mean plant height (32.34 cm) was recorded at T_{12} whilst the lowest mean plant height (10.74 cm) was recorded at T_1. Similar trends were observed at 4, 6 and 8 WAP where mean plant height of maize was significantly higher with the combination of rice husk biochar and inorganic nitrogen fertilizer. The highest mean heights were observed at T_{12}, $T_{11,}$ T_{10}, T_9, $T_{8,}$ T_7, T_6, T_5 from 2 WAP to the end of the experiment at 8 WAP (Table 4).

Table 4. The influence of integrated soil fertility management on maize plant height (cm).

Treatment	Week after planting (WAP)			
	2 WAP	4 WAP	6 WAP	8 WAP
T_1	10.74a	29.24bc	89.14bn	98.82dq
T_2	11.56c	34.46e	93.83abc	104.93bnr
T_3	13.43d	38.45v	97.43vn	112.45xv
T_4	16.34z	42.33g	100.45k	118.54mb
T_5	14.55q	49.56j	114.65qa	128.45aa
T_6	18.34r	56.04b	129.54tr	133.45cf
T_7	22.43t	61.25v	141.56hi	179.34fj
T_8	28.93s	68.09w	156.83f	189.95pp
T_9	18.45p	65.45t	137.54er	165.76sw
T_{10}	24.45x	69.53k	143.43am	193.45ah
T_{11}	26.59y	73.66s	168.44cff	204.56qw
T_{12}	32.34h	82.78u	174.76gh	220.45xyz
CV%	5.4	9.4	5.6	8.3

Means with the different letter(s) in a column are significantly different at the 5% level of probability.

The significant increase in the plant height of maize could be ascribed to the fact that the added rice husk biochar (58.2%) increased the supply and availability of plant nutrients in the soil. This approves the recommendation that higher accessibility of nutrients increases the vegetative growth of plants (Zoghi et al., 2019).

Plant girth

The effect of rice husk biochar on maize girth at different weeks after planting is described in Table 5. It was clear that rice husk biochar and inorganic nitrogen fertilizer had a significant effect on the plant girth. At 2 WAP, the highest plant girth was recorded in the treatment T_{12} while the lowest was recorded at $T_{1.}$ This trend was, however, similar in all phases.

Table 5. The influence of integrated soil fertility management on maize plant girth (cm).

Treatment	Week after planting (WAP)			
	2 WAP	4 WAP	6 WAP	8 WAP
T_1	0.58bd	0.83ee	2.01c	4.12qw
T_2	0.67as	1.01ab	2.46bv	4.64pk
T_3	0.75ss	1.12cv	2.74vv	4.96mn
T_4	0.88cv	1.21yy	2.91qw	5.33mb
T_5	1.08bv	1.45qq	3.25rt	6.34max
T_6	1.16ng	1.71hi	3.54uh	6.89nv
T_7	1.24qw	1.98pt	3.85ky	7.16xzy
T_8	1.38bb	2.05yg	4.12gg	7.44cj
T_9	1.49rr	2.18jk	4.53py	7.98kt
T_{10}	1.61jj	2.29mm	4.94hh	8.33ree
T_{11}	1.84hn	2.41am	5.25xz	8.76ytr
T_{12}	2.04kk	2.78mx	5.98zv	8.99ghf
CV%	5.4	1.4	2.7	6.7

Means with the different letter(s) in a column are significantly different at the 5% level of probability.

It is clear that there is a noticeable difference in plant girth with the highest rate of rice husk biochar treatments compared to 0 ton ha^{-1} biochar treatments. A comparable result was found by Varela et al. (2013) who stated that an increase in the application of both wood and rice husk biochar improved the stem size of *Ipomoea aquatica*. They, furthermore, stated that the decomposition of wood and rice husk biochar improved the organic matter content in the soil leading to increase in water holding capacity of soil, thus improving stem diameter.

The number of leaves

Observations made on the number of leaves produced by *Zea mays* showed an increase from week 2 to week 8 (Table 6). By week 2, mean differences among treatments had already been significant ($p<0.05$), with T_{12}, $T_{11,}$ T_{10}, T_9, $T_{8,}$ T_7,T_6 and T_5 producing more leaves relative to the treatments in T_1 (Table 6). These differences were still significant ($p<0.05$) by the end of the experiment. Similar trends were, however, observed from 4 WAP to the end of the experiment at 8 WAP.

Plants that received the highest rate of rice husk biochar and inorganic nitrogen fertilizer recorded a greater number of leaves than T_1. The application of biochar improved the fertility of the soil and safeguarded the availability of vital nutrients that boosted the vegetative growth of maize. Our findings corroborate with the findings of Ali et al. (2017).

Table 6. The influence of integrated soil fertility management on the number of leaves of maize.

Treatment	Week after planting (WAP)			
	2 WAP	4 WAP	6 WAP	8 WAP
T_1	4.01a	7.43gh	9.44cdd	10.12yu
T_2	4.15ab	7.46gh	9.68yt	10.27got
T_3	4.16ab	7.49gh	9.67yt	10.65ye
T_4	4.33dc	7.50gh	10.02aa	10.95qa
T_5	5.49cc	8.77kb	10.58ba	11.23ax
T_6	5.78vc	8.84kb	10.89cgd	11.30ax
T_7	5.76vc	8.93kb	11.23q	11.89ws
T_8	5.87dd	8.99kb	11.56we	12.32pz
T_9	6.56ff	10.83vy	11.89rt	13.00nn
T_{10}	6.57ff	11.02bb	12.34pq	13.56ww
T_{11}	6.75wr	11.48hi	12.75mn	13.86uy
T_{12}	6.95qs	11.93xy	12.92am	14.03zx
CV%	0.8	1.6	5.6	5.2

Means with the different letter(s) in a column are significantly different at the 5% level of probability.

Conclusion

The highest vegetative growth parameters were obtained at 4 ton/ha of biochar and inorganic nitrogen fertilizer which were effective at 90 kg N ha^{-1} and 60 kg N ha^{-1}. It was, therefore, concluded that the combination of various sources of inorganic and organic nutrients improved the performance of growth parameters of *Zea mays* rather than the sole use of either the inorganic fertilizers or the organic sources.

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UTICAJ INTEGRISANOG UPRAVLJANJA PLODNOŠĆU ZEMLJIŠTA NA PARAMETRE VEGETATIVNOG RASTA *ZEA MAYS* U EKOZONI GVINEJSKE SAVANE U GANI

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R e z i m e

Širom sveta, skrenuta je pažnja na upotrebu ekološki prihvatljive primene biouglja kako bi se poboljšala produktivnost useva. U Gani postoje dostupne potencijalne sirovine koje ostaju neiskorišćene i mogu se upotrebiti za proizvodnju biouglja. Ovom studijom se stoga istražuje uticaj različitih količina biouglja od pirinčane ljuske i različitih količina neorganskog azota (N) na rast *Zea mays* u gradu Nyankpala, u severnoj Gani. Poljski ekperimenti su sprovedeni za period uzgajanja useva 2012. godine. Tretmani su uključivali 4 različite količine mineralnog azotnog đubriva (0 kg N ha^{-1}, 30 kg N ha^{-1}, 60 kg N ha^{-1} i 90 kg N ha^{-1}) i 3 različite količine biouglja od pirinčane ljuske (0 t ha^{-1}, 2 t ha^{-1} i 4 t ha^{-1}). Tretmani su bili raspoređeni u dizajnu podeljenih parcela sa tri ponovljanja. Ocenjivani vegetativni parametri uključivali su broj listova, visinu biljke i obim biljke. Tretmani su pokazali značajan ($p<0,05$) uticaj na sve razmatrane osobine. Kombinovani uticaji mineralnog azotnog đubriva i biouglja od pirinčane ljuske značajno su uticali na parametre vegetativnog rasta *Zea mays* sa maksimalnim vrednostima zabeleženim pri upotrebi 4 t ha^{-1} biouglja od pirinčanih ljuski. Ipak, ovo zapažanje odgovara količini od 2 t ha^{-1} biouglja od pirinčanih ljuski čijom su upotrebom zabeleženi optimalni parametri rasta u poređenju sa kontrolom. U granicama ovog rada, zaključeno je da se parametri rasta *Zea mays* u ekozoni gvinejske savane u Gani mogu značajno poboljšati primenom 4 t ha^{-1} biouglja od pirinčane ljuske. Trenutno je neophodno integrisano upravljanje plodnošću zemljišta, razumna kombinacija mineralnih đubriva i ostataka iz različitih izvora za održavanje životne sredine. Eksperiment je pokazao da primena biouglja od pirinčanih ljuski može poboljšati parametre rasta *Zea mays*. Ipak, potrebno je da se urade dodatni

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eksperimenti korišćenjem većih količina biouglja od pirinčane ljuske kako bi se osigurala odgovarajuća količina primene.

Ključne reči: biougalj od pirinčanih ljuski, kukuruz, mineralno đubrivo, integrisano upravljanje plodnošću zemljišta, savana, vegetativni rast.

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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.
Pregledni rad – Rad koji sadrži originalan, detaljan i kritički prikaz istraživačkog problema ili područja u kome je autor ostvario određeni doprinos, vidljiv na osnovu autocitata (najmanje 10). Obim ovog rada treba da iznosi od 15 do 20 strana.

Prethodno saopštenje – Originalan naučni rad punog formata, ali manjeg obima ili preliminarnog karaktera (od 2 do 6 strana).
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.

Naslov rada
Naslov rada treba što vernije da opiše sadržaj rada i da ima što manje reči. U interesu je autora da se u naslovu koriste reči prikladne za indeksiranje i pretraživanje. Naslov se piše velikim slovima i centrirano. Ako je rad prethodno bio izložen na nekom skupu u vidu usmenog saopštenja, pod istim ili sličnim naslovom, podatak o tome treba navesti pri dnu prve stranice, posle podataka autora za kontakt.

Imena autora
Navodi se puno ime, srednje slovo i prezime svih autora, u originalnom obliku. Imena se pišu ispod naslova, malim slovima, centrirano i boldovano. Ukoliko su autori iz različitih institucija brojčanom oznakom u superskriptu, iza prezimena, označiti ustanovu u kojoj radi svaki autor. Autor za kontakt označava se zvezdicom u superskriptu, iza prezimena, komandom „insert footnote“, a njegova e-mail adresa navodi se ispod crte pri dnu prve stranice članka.

Naziv ustanove autora
Navodi se pun naziv i adresa ustanove u kojoj je autor zaposlen. Ispisuje se neposredno nakon imena autora, centrirano. Ukoliko su autori iz različitih institucija brojčanom oznakom u superskriptu ispred institucije označava se ustanova u kojoj je zaposlen svaki od navedenih autora.

Sažetak
Sažetak je kratak informativni prikaz sadržaja članka koji čitaocu omogućava da brzo i tačno odredi njegovu relevantnost. U interesu je autora da sažetak sadrži termine koji se koriste za indeksiranje i pretraživanje. Sažetak ne sme da sadrži reference. Sastavni delovi sažetka su cilj istraživanja, metode, rezultati i zaključak. Sažetak treba da ima od 200 do 250 reči. Reč „Sažetak“ piše se boldovano i uvlači jednim tabulatorom, nakon čega slede dve tačke, a zatim tekst sažetka.

Ključne reči
Ključne reči su termini ili fraze koje najbolje opisuju sadržaj članka za potrebe indeksiranja i pretraživanja. Broj ključnih reči može biti od 3 do 10. Navode se ispod sažetka. Naslov „Ključne reči“ piše se boldovano i uvlači jednim

tabulatorom. Nakon toga slede dve tačke, a zatim nabrajanje ključnih reči malim slovima, sa tačkom na kraju. Treba izbegavati korišćenje ključnih reči koje se nalaze u naslovu rada. Ključne reči se dostavljaju na srpskom i engleskom jeziku posle sažetaka na oba jezika.

Uvod

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

Materijal i metode

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

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.

Literatura

Poglavlje „Literatura" treba da sadrži samo radove citirane u glavnom tekstu. Rad citiran u tekstu treba da sadrži prezime autora i godinu. Ako citat obuhvata jednog autora on se navodi kao Jalikop (2010) ili (Jalikop, 2010). Kada citat obuhvata dva autora on se navodi kao Sadras i Soar (2009) ili (Sadras i Soar, 2009). Ako se u tekstu citiraju više od dva autora posle prezimena prvog autora navodi se skraćenica „et al.", a zatim godina. Ovakav citat navodi se kao Lehrer et al. (2008) ili (Lehrer et al., 2008). Ako se za određeni problem istovremeno citira više radova onda se oni hronološki nabrajaju. Odvajanje većeg broja citiranih radova van

zagrade vrši se zarezom (,) a u zagradi tačkom i zarezom (;). Ako se citiraju dva ili više rada istog autora oni moraju biti poređani prema hronološkom redu (1997, 2002, 2006, itd.). Ukoliko se određeni autor pojavljuje nekoliko puta u istoj godini, dodaju se slova (2005a, b, c, itd.). Citate ličnih komunikacija i neobjavljenih podataka treba izbegavati, osim ako je to apsolutno neophodno. Takvi citati bi trebali da se pojave samo u tekstu (npr. Brown, lična komunikacija), ali ne i u spisku referenci.
Literatura koja je citirana u tekstu navodi se u spisku referenci u originalnom obliku, po abecednom redu, bez numeracije. Ako se citira veći broj radova istog autora najpre se navode radovi kada je autor sam, a zatim kada su prisutna dva i više autora. Ako se u nekoj od ovih kategorija javlja veći broj radova, treba ih hronološki srediti po godinama (1997, 2002, 2006, itd.), a ako se u istoj godini javlja veći broj radova dodaju se slova (2005a, 2005b, 2005c, itd.). Literaturni podatak treba da sadrži: prezime autora, početno slovo imena, godinu izdanja u zagradi, naslov rada, naziv časopisa, volumen i broj stranica (prva-poslednja). Prilikom citiranja knjiga navodi se izdavač i mesto izdavanja. Redovi svake reference posle prvog reda moraju biti uvučeni. U časopisu se koristi APA - Publication Manual of the American Psychological Association citatni stil.

Primeri navođenja referenci su sledeći:

Periodičan časopis

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

Knjiga

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

Poglavlje u knjizi

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

Zbornik

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

Teza

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

Izveštaj

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

Veb sajt

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

Rezime

Rezime na srpskom jeziku (za radove napisane na engleskom jeziku) ili na engleskom jeziku (za radove napisane na srpskom jeziku) navodi se na kraju rada i treba da ima od 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 uslovom da se ti izrazi navedu u punom obliku prilikom prvog pominjanja, sa skraćenim oblikom u zagradi. Vrednosti od 1 do 9 mogu se izražavati slovima, a ostali brojevi isključivo numerički.

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

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

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

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