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THE IMPACT OF A PULSED ELECTROMAGNETIC FIELD ON THE SEED PROTEIN CONTENT OF SOYBEAN

Vojin H. Đukić[1*], Zlatica J. Miladinov[1], Gordana K. Dozet[2], Gorica T. Cvijanović[2], Jegor A. Miladinović[1], Predrag D. Ranđelović[1] and Olga L. Kandelinskaja[3]

[1]Institute of Field and Vegetable Crops,
Maksima Gorkog 30, 21000 Novi Sad, Serbia
[2]Megatrend University, Faculty of Biofarming,
Bačka Topola, Maršala Tita 39, 24300 Bačka Topola, Serbia
[3]Institute of Experimental Botany of the National Academy of Sciences of Belarus,
Академическая 27, 220072, Minsk, Belarus

Abstract: Many studies show that in the last 20 years, the increase in production per hectare has been achieved mainly due to the creation of new varieties and the development of plant breeding. Innovations in plant breeding are the main way to find new traits, values and tolerances, which are the only ones that can respond to the increased demand for yield and more efficient production. In addition to high and stable yields, it is very important that the soybean seed has a satisfactory technological quality. Therefore, the aim of this study was to examine how the application of pulsed electromagnetic fields (PEMFs) affected the protein yield of soybean seed depending on the year, exposure time and frequency. The field trials were conducted in the experimental field of the Institute of Field and Vegetable Crops in Novi Sad, Serbia in 2010–2013. For this research, the seeds of a medium-sized variety Valjevka exposed to a pulsed electromagnetic field (PEMF) using a pulse generator and a strip applicator were used. A low frequency pulsed electromagnetic field (16, 24, 30, 72 Hz) was used at the exposure times of 0, 30, 60, and 90 minutes. The results of the research show that the application of this method can increase the yield of protein in soybean seed for more than 20%, which is a significant increase, especially in organic production, where the use of seed treatment agents is very restricted. However, this measure can also have an inhibitory effect if an adverse combination of exposure time and frequency strength is selected.

Key words: proteins, pulsed electromagnetic field, soybeans.

[*]Corresponding author: e-mail: vojin.djukic@ifvcns.ns.ac.rs

Introduction

The magnetic field as an active factor in agriculture has more than 50-year history. Important applied investigations were made in Russia, whose results were the creation of technological lines of magnetic systems for pre-processing vegetative planting seeds (Ivanovich et al., 2013). Krilov and Tarakonova (1960) were among the first investigators to report the effect of magnetic field on plants. They found that their effect was similar to auxin in the seed germination process. Today, the magnetic field is an ecological technique used in sustainable agriculture, which has a little environmental impact, while contributing to an increased yield of cultivated plants (Bilalis et al., 2013). This has a better influence on seed germination (Djukic et al., 2017), absorption and assimilation of nutrients by plants (Bilalis et al., 2013), photosynthetic activity (Shine et al., 2011), enzymatic activity (Ramakrishna and Kumari, 2012) and in the second place ultimately aims to increase the yield of cultivated plants (Shine et al., 2011). Soybean is a crop of great world importance due to the widespread use of its products and their economic value (Filho et al., 2004). Seed protein and oil content are the two main seed quality traits in soybean. Soybean contains 40 to 42% of good quality protein and approximately 20 to 22% of oil, expressed on a dry matter basis (Bhangu and Virk, 2019).

Therefore, the aim of the study was to investigate the influence of pulsed yield electromagnetic fields on the protein content of soybean and whether their effect depended on the weather conditions during vegetation.

Materials and Methods

Weather conditions

In 2010, the lowest temperatures (18.4°C) and the highest rainfall were (684.4 mm) recorded during the soybean vegetation period with a favorable monthly distribution (Table 1). The year of 2011 was characterized by increased temperatures during the vegetation period (19.4°C), especially in August (23.1°C) and September (20.4°C), and with the least amount of precipitation (210.5 mm), as well as a marked deficiency in August (1.5 mm). In 2012, the highest average temperatures were recorded during the soybean vegetation period (20.5°C), with very high middle temperatures in May (17.5°C), June (23.0 °C), July (25.2°C), August (24.6°C) and September (19.8°C). There were 226.8 mm of rainfall during the soybean vegetation period this year, with very low amounts in June (27.5 mm) and August (3.5 mm). The average temperature in 2013 was 18.7°C and the precipitation amount was 448.2 mm, with pronounced deficiencies in July (34.1 mm) and August (26.7 mm).

Table 1. Average monthly temperatures and precipitation during the vegetation period of soybean (2010–2013).

Month	Average monthly temperatures (°C)				Precipitation (mm)			
	2010	2011	2012	2013	2010	2011	2012	2013
IV	12.3	13.2	13.0	13.4	63.7	22.8	82.8	35.8
V	16.9	16.8	17.5	17.4	113.7	62.4	52.2	118.1
VI	20.2	20.9	23.0	20.2	171.8	36.9	27.5	125.7
VII	23.1	22.1	25.2	22.3	99.0	61.5	47.7	34.1
VIII	21.9	23.1	24.6	22.8	168.5	1.5	3.5	26.7
IX	16.1	20.4	19.8	15.7	67.7	25.4	13.1	107.8
Average	18.4	19.4	20.5	18.7	684.4	210.5	226.8	448.2

Plant materials

In order to determine PEMF of soybean seed protein content, the field trials were conducted during four growing seasons (2010, 2011, 2012 and 2013) at the Rimski Šančevi experimental field (45°20′ N 19°51′ E) near Novi Sad, Serbia. The plant material used in this experiment was the medium early cultivar of soybean Valjevka. The trial was conducted on the humus soil type as a randomized block design with four replications under the conditions of dry farming. The plot size was 10 m^2. The inter-row spacing of 50 cm and the intra-row spacing of 5 cm were applied. There were no significant disease and insect attacks. The seed was exposed to a pulsed electromagnetic field (PEMF) using the impulse generator and strip. Low frequencies (16, 24, 30 and 72 Hz) of PEMF lasting for 0, 30, 60 and 90 minutes were used. Immediately after the seed exposure to the PEMF, sowing was carried out at the optimum time. After sprouting, seedlings were counted to determine the percentage of germinated seeds. Standard soybean cultivation practices were applied during the vegetation period. Harvesting was performed at the technological maturity stage. Samples and moisture were measured using a combine harvester for experimental plots with a small work operation (Wintersteiger elite), and yield was calculated at 14% moisture.

Protein yield was calculated based on the yield and protein content of the seed. The yield of soybean seeds obtained is expressed in kg ha^{-1} at 14% moisture. Protein content in the same seed was determined by the NMR method, according to Granlund and Zimmerman (1975). Protein yield was calculated as the product of the seed yield and protein content.

Statistical analysis

The experimental data were analyzed using the software STATISTICA 10, according to the completely randomized design. Analysis of variance (ANOVA) and comparisons of means were calculated using the least significant difference (LSD) test, at the 5% level of significance. Also, the correlation between the tested traits was determined.

Results and Discussion

The effect of PEMF on soybean seed protein content depended on the year (A), duration of exposure (B) and frequency (C), and year-by-duration of exposure interaction (A × B), year-by-frequency interaction (A × C) and exposure interaction-by-frequency interaction (B × C), as shown in Table 2.

Table 2. The effect of a pulsed electromagnetic field (PEMF) on protein yield in soybean seed (kg/ha).

Years A	Time B (min)	Frequency C (Hz) 16	24	30	72	Average AxB	Average A
2010	0	1464.2	1464.2	1464.2	1464.2	1464.20^{c}	
	30	1830.3	1491.9	1433.9	1466.7	1555.70^{ab}	
	60	1439.3	1778.3	1691.4	1509.0	1604.49^{a}	1539.36
	90	1551.6	1731.1	1451.1	1398.4	1533.03^{b}	
	Average AxC	1571.34^{b}	1616.37^{a}	1510.14^{c}	1459.58^{d}		
2011	0	1413.4	1413.4	1413.4	1413.4	1413.42^{a}	
	30	1582.9	1427.0	1379.8	1419.8	1452.36^{a}	
	60	1387.3	1544.4	1434.5	1455.6	1455.44^{a}	1435.50
	90	1474.1	1548.7	1378.8	1281.5	1420.77^{a}	
	Average AxC	1464.42^{ab}	1483.39^{a}	1401.62^{b}	1392.56^{bc}		
2012	0	908.5	908.5	908.5	908.5	908.54^{b}	
	30	1078.6	941.5	895.4	917.9	958.35^{ab}	
	60	926.5	1038.4	1022.7	995.7	995.85^{a}	953.43
	90	1017.0	1050.5	891.0	845.4	950.97^{ab}	
	Average AxC	982.66^{a}	984.74^{a}	929.42^{b}	916.90^{b}		
2013	0	1342.7	1342.7	1342.7	1342.7	1342.66^{bc}	
	30	1482.7	1423.2	1300.5	1433.1	1409.86^{b}	
	60	1387.3	1486.8	1476.0	1435.0	1446.29^{a}	1391.31
	90	1456.8	1481.2	1298.6	1228.9	1366.41^{bc}	
	Average AxC	1417.37^{a}	1433.47^{a}	1354.45^{b}	1359.93^{b}		
Average B*C	0	1282.21	1282.21	1282.21	1282.21	Average B	1282.21
	30	1493.60	1320.92	1252.38	1309.38		1344.07
	60	1285.10	1461.96	1406.16	1348.84		1375.52
	90	1374.86	1452.87	1254.89	1188.56		1317.80
	Average C	1358.94	1379.49	1298.91	1282.24		

Factory	Year (A)	Time (B)	Frequency (C)	AxB	AxC	BxC
	**	**	**	**	**	**

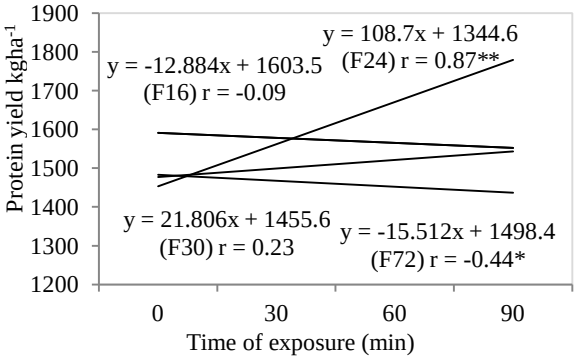
Factors	2010		2011		2012		2013	
	$LSD_{0.05}$	$LSD_{0.01}$	$LSD_{0.05}$	$LSD_{0.01}$	$LSD_{0.05}$	$LSD_{0.01}$	$LSD_{0.05}$	$LSD_{0.01}$
AxB	67.76	97.34	93.58	109.50	72.02	97.26	80.82	95.55
AxC	44.415	55.920	69.80	83.14	57.29	79.78	55.86	67.86

** – significance at 0.01, * – significance at 0.05, - ns (no significance) probability level.

Soybean meal is the most commonly used source of protein for poultry and swine feeds in the world, with 67% of the animal feed market (Pettigrew et al., 2002). In order for a feed ingredient to be considered an important component of an industry feeding program, it must have several fundamental qualities, and above all, it must contain a high proportion of protein. Therefore, the aim of breeding not only soybean but also other plants from the *Fabaceae* family is to produce varieties with the highest protein content in the grain (Jayalakshmi et al., 2018). Applied production technology also has an impact on the chemical composition of the grain (Dekhane et al., 2011; Kang et al., 2012). Various methods have been used to increase protein content. One of them is using the electromagnetic field (Crnobarac et al., 2002). The effect of PEMF on protein yield in soybean depended on year, exposure time, and frequency. The application of PEMF led to increasing the protein yield by 2.89–8.77%. The best effect was achieved in 2012. This year was characterized by water deficit and high temperatures at critical stages for soybean development. The beneficial effects of the electromagnetic field, especially in poor agro-meteorological conditions, can be attributed to their influence in increasing the root volume and surface area, which allows the crop to utilize more moisture during periods of active growth when water is often a limiting factor for successful crop production. Therefore, the seed treatment using the electromagnetic field before sowing can be considered as a pre-sowing measure in reducing the effect of water deficit in arid and semi-arid regions, thereby helping to increase production. Increases in root volume and surface area can reach up to 70% and 65% respectively, allowing the crop utilization for more than 60% of moisture during periods of active growth (Mridha and Nagarajan, 2014). In addition to agro-meteorological conditions, the success of this method depends on the exposure time and the frequency applied. The best results were obtained at the 60-min exposure time. On average, during investigation years, protein yield increased by 6.78% over a 60-minute exposure period, by 4.08% over a 90-minute exposure period, and by 2.18% over the 30-minute exposure time compared to the control. In terms of frequency, at the lowest frequency value of 16 Hz, the average increased the protein yield by 7.39% during the investigation years. With increasing radiation intensity at 24 Hz, the protein yield increased by 9.19%. A similar result was observed in the example of maize (Muraji et al., 1998). The best results were achieved with low frequencies, 10 and 20 Hz, respectively. Wang et al. (2006) concluded that low frequencies have the best impact on plant growth and development because they increase the intake of N, P, K and Mg chemical elements. Nitrogen can increase plant tolerance at high temperatures and water deficit. Potassium affects the water regime of plants while magnesium plays an important role in regulating protein biosynthesis. In the example of insufficient magnesium, the proportion of non-protein nitrogen compounds increases at the expense of the protein (Kastori et al., 1979). A further increase in the PEMF

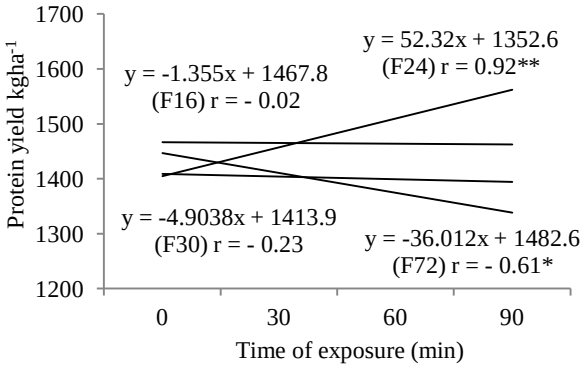
frequency led to a sudden decrease in yield, so that at 72 Hz, there was no effect at all. However, for the successful application of this method, it is of great importance to find the right combination of the exposure time and frequency. In soybean studies, on average, the best combination was 16 Hz*30 minutes, increasing the yield by 14.15%. Marinkovic et al. (2006) point out that, in addition to the activation of the enzyme complex, electromagnetic rays have a beneficial effect on the structure of free water molecules and on the overcoming resistance in the transport of energy and matter in the plant. In this case, energy savings are achieved and the intensity of decomposition of the produced organic matter is reduced, which increases the yield and improves the quality of the plant products.

Observing the investigation, it can be established that the strongest linear dependence was determined at the frequency of 24 Hz, which means that with increasing the time of soybean seed exposure, this frequency achieved a favorable effect on protein yield (Figures 1, 2, 3, and 4).



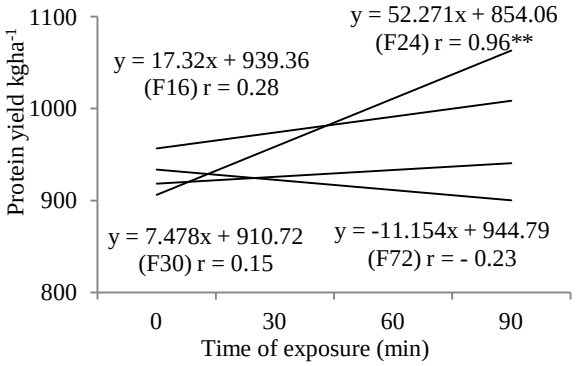
F16, F24, F30 and F72: frequencies for PEMF (Hz)

Figure 1. The effect of PEMF on soybean protein yield in 2010.



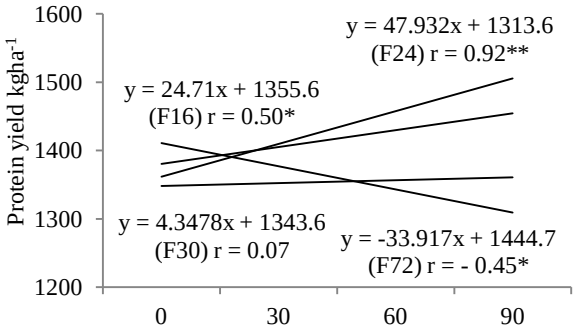
F16, F24, F30 and F72: frequencies for PEMF (Hz)

Figure 2. The effect of PEMF on soybean protein yield in 2011.



F16, F24, F30 and F72: frequencies for PEMF (Hz)

Figure 3. The effect of PEMF on soybean protein yield in 2012.



F16, F24, F30 and F72: frequencies for PEMF (Hz)

Figure 4. The effect of PEMF on soybean protein yield in 2013.

The value of the correlation coefficient ranged from 0.87 to 0.96, which means that with increasing the exposure time at this frequency, the protein yield increased significantly. In 2012 and in the year with the most unfavorable agro-ecological conditions, the highest linear dependence ($r^2 = 0.96$) was achieved, while in 2013 and in the year with the best agro-ecological conditions, the highest negative linear dependence ($r^2 = -0,45$) was achieved. Failure to choose the right combination can result in a significant decrease in yield. With a combination of 72 Hz*90 min, the protein yield was reduced by 7.88%. Djukic et al. (2017) point out that the application of PEMP, in addition to the positive, can have an inhibitory effect if a favorable combination of the exposure time and frequency strength is not chosen. Attention must also be paid to the trait of the plant being observed. For example, regarding soybean, it was found that the combination of 16 Hz*30 min had the best effect on seed germination, while the best effect was obtained by using the combination of 24 Hz*30 min.

Conclusion

The results show that the use of the pulsed electromagnetic field as a pre-sowing treatment can increase the yield of protein in soybean. This is a good basis for this measure to be used, especially in organic production, where the use of seed treatments is very restricted. However, in addition to its positive impact, this measure can also have an inhibitory effect resulting in decreasing the yield.

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UTICAJ PULSIRAJUĆEG ELEKTROMAGNETNOG POLJA NA SADRŽAJ PROTEINA U SEMENU SOJE

Vojin H. Đukić[1*], Zlatica J. Miladinov[1], Gordana K. Dozet[2], Gorica T. Cvijanović[2], Jegor A. Miladinović[1], Predrag D. Ranđelović[1] i Olga L. Kandelinskaja[3]

[1]Institut za ratarstvo i povrtarstvo,
Maksima Gorkog 30, 21000 Novi Sad, Srbija
[2]Megatrend Univerzitet, Fakultet za biofarming, Bačka Topola,
Maršala Tita 39, 24300 Bačka Topola, Srbija
[3]Institut za eksperimentalnu botaniku Nacionalne akademije nauka Belorusije,
Академическая 27, 220072, Minsk, Belorusija

R e z i m e

Mnoga istraživanja pokazuju da se u poslednjih 20 godina povećanje proizvodnje po hektaru ostvaruje uglavnom zahvaljujući stvaranju novih sorti i razvoju oplemenjivanja biljaka. Inovacije u oplemenjivanju biljaka su glavni put za iznalaženje novih osobina, vrednosti i tolerantnosti, koje jedine mogu da odgovore povećanom zahtevu za prinosom i efikasnijom proizvodnjom. Osim visokih i stabilnih prinosa, veoma je važno da seme soje poseduje i zadovoljavajući tehnološki kvalitet. Zbog toga je cilj ovog istraživanja bio da se ispita kako primena pulsirajućeg elektromagnetnog polja (PEMP) deluje na sadržaj proteina u semenu soje u zavisnosti od godine, vremena trajanja ekspozicije i jačine frekvencije. Poljski ogled je izveden na eksperimentalnom polju Instituta za ratarstvo i povrtarstvo u Novom Sadu u periodu od 2010. do 2013. godine. Za ovo istraživanje korišćeno je seme srednjerane sorte Valjevka koje je izlagano pulsirajućem elektromagnetnom polju (PEMP) pomoću generatora impulsa i trakastog aplikatora. Korišćeno je pulsirajuće elektromagnetno polje niskih frekvencija (16, 24, 30, 72 Hz) u vremenu ekspozicije od 0, 30, 60 i 90 minuta. Rezultati istraživanja pokazuju da primena ove metode može povećati prinos proteina u semenu soje do 20%, što predstavlja značajno povećanje i dobru osnovu da se ova mera počne koristiti, pre svega u organskoj proizvodnji, gde je primena sredstava za tretiranje semena veoma ograničena međutim, ova mera može imati i inhibitorni efekat ako se izabere nepovoljna kombinacija vremena ekspozicije i jačine frekvencije.

Ključne reči: proteini, pulsirajuće elektromagnetno polje, soja.

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[*]Autor za kontakt: e-mail: vojin.djukic@ifvcns.ns.ac.rs

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THE EFFECTS OF GOAT MANURE AND SUGARCANE MOLASSES ON THE GROWTH AND YIELD OF BEETROOT (*BETA VULGARIS* L)

Priyadarshani W. M. Nadeeka and Thayamini H. Seran*

Department of Crop Science, Faculty of Agriculture, Eastern University, Sri Lanka

Abstract: A pot experiment was conducted to study the effect of goat manure and sugarcane molasses on the growth and yield of beetroot (*Beta vulgaris L.*) in sandy regosol. The experiment was laid out in a completely randomized design (CRD) with six treatments. The treatments included inorganic fertilizer (T1), 10 t/ha of goat manure alone (T2) and also 10 t/ha of goat manure and 50% triple superphosphate (TSP) with 1–4 t/ha of sugarcane molasses (T3-T6). The results showed that plant growth parameters (leaf length, leaf petiole length, leaf width, leaf number, fresh weight and dry weight of leaves) were significantly varied among the treatments. There was a significant difference in the diameter of beetroot among the treatments. Significant differences ($P<0.05$) were observed in fresh weight of beetroot and total plant among the treatments. Fresh weight of root yield and total yield of beetroot per plant were increased in 10 t/ha goat manure, 2 t/ha sugarcane molasses and 50% TSP (T4) and 10 t/ha goat manure, 3 t/ha sugarcane molasses and 50% TSP (T5) when compared to the control treatment (T1). The total yield of beetroot per m^2 was 1,792.62 g in T4 and 1,402.68 g in T1. The root yield of beetroot was increased in T4 in comparison to T5. It can be concluded that 10 t/ha of goat manure with 2 t/ha of sugarcane molasses and 50% TSP could be applied for obtaining a high yield of beetroot in sandy regosol.

Key words: beetroot, goat manure, molasses, yield.

Introduction

Beetroot (*Beta vulgaris* .L) belongs to Chenopodiaceae family. It is grown widely in Sri Lanka. Beetroot is good for health as it contains minerals, vitamins, fiber and medicinal properties. This crop is one of the most noteworthy vegetables with regard to antioxidant properties, primarily owing to the presence of betatains (Ullah and Khan, 2008; Nahla et al., 2018). In some countries, beetroot pigment is commercially used as red food dye in diverse products. *Beta vulgaris* var. rubra has considerable anticancer properties (Kapadia et al., 1996). Beetroot is an annual vegetable crop grown for its root, and commonly grown beet varieties are detroit

*Corresponding author: e-mail: thayaminis@esn.ac.lk

dark red and crimson globe. In Sri Lanka, it is grown in all agroecological regions but effectively cultivated in Nuwara Eliya, Badulla, Kandy and Matale districts. Beets prefer well-drained fertile sandy loams to silt loams with high organic matter for better root development.

Inorganic fertilizers release nutrients quickly, therefore, farmers widely use it to supply nutrients for vegetable crops to obtain a high yield, but excessive use of these fertilizers is toxic to human health and causes nutrient loss, groundwater contamination and a decrease in effective microbial communities (Chen, 2006). On the other hand, the use of organic manure improves soil physical and chemical properties and provides favorable conditions for crop growth with less environmental impact (Suthamathy and Seran, 2009). The application of organic manures can increase crop yield and soil fertility more than chemical fertilizer (Murmu et al. 2013). Hence, the residual effects of organic manure or compost application increased crop production for at least one growing season (Eghball et al., 2004; Imthiyas and Seran, 2017).

The use of locally available organic manures with a reduced level of inorganic fertilizers could give a greater seed yield than inorganic fertilizers alone (Seran, 2016; Raveenthira and Seran, 2020). Ojeniyi and Adegboyega (2003) reported that the application of goat manure remarkably increases nitrogen (N), phosphorous (P), potassium (K), calcium (Ca) and magnesium (Mg) in the leaf of celosia plant. Sugarcane molasses are ones of the economically important agricultural crops grown in tropical regions, and they are produced annually in large amounts (De la Rosaa et al., 2019). They are a byproduct of sugar industries which have been used in agriculture as an organic source of fertilizer. It contains sugar, minerals, vitamins, ash and polyphenols (Takara et al., 2007; Jain and Venkatasubramanian, 2017; De la Rosaa et al., 2019). The use of sugarbeet molasses in crop cultivation accelerates nutrient uptake efficiency and soil biological activity (Samavat and Samavat, 2014). The application of organic manures improves the water holding capacity of soil and supplies both macro and micro plant nutrients for achieving better crop yield. Therefore, this experiment was aimed to determine the effect of the combined application of goat manure and sugarcane molasses with a reduced level of phosphorus (P) inorganic fertilizer on the growth and yield of beetroot and also to find out the optimum rate of molasses with 10 t/ha of goat manure as a basal application for a high yield of beetroot in sandy regosol. The finding is useful to beetroot growers for obtaining high yield with less use of inorganic fertilizer.

Materials and Methods

A pot experiment was carried out in the Crop Farm of Eastern University, Sri Lanka in 2018–2019. This area comes under the agroecological zone of the low country dry zone. The annual rainfall ranges from 1800 to 2100 mm. The annual mean temperature is 32±3˚C and the humidity level nearly 60%–80%. The type of

soil is sandy regosol. A pot experiment was laid out in a completely randomized design (CRD) with six treatments and ten replications. Treatments included in this experiment are as follows.

T1: Recommended NPK inorganic fertilizers;
T2: 10 t/ha GM + 0 t/ha SM + 50% TSP;
T3: 10 t/ha GM + 1 t/ha SM + 50% TSP;
T4: 10 t/ha GM + 2 t/ha SM + 50% TSP;
T5: 10 t/ha GM + 3 t/ha SM + 50% TSP;
T6: 10 t/ha GM + 4 t/ha SM + 50% TSP;
GM: Goat manure;
SM: Sugarcane molasses;
50% TSP: Half dosage of recommended tripe super phosphate.

Sugarcane molasses were collected from the Galoya Plantation (Pvt) Ltd, Hingurana, Ampara, Sri Lanka and goat manure was obtained from Animal farm, Eastern University, Sri Lanka. According to the treatments, goat manure (1.73% N, 0.47 P_2O_5, 1.67% K_2O) and sugarcane molasses (0.53% N, 0.043 P_2O_5, 1.48% K_2O) were taken and mixed with soil to prepare the soil mixture. The polybags (30 cm height and 30 cm diameter) were filled with soil mixture, and holes were made at the side and base of each polybag to facilitate the drainage of water.

Seeds of beetroot cv. crimson globe were collected from the Department of Agriculture, Nuwaraeliya, Sri Lanka. Two seeds were placed in each polybag, but only one healthy seedling was maintained in each polybag one week after seedling emergence. Inorganic fertilizers as basal (165 kg/ha urea, 270 kg/ha tripe superphosphate, 125 kg/ha muriate of potash) and top dressing (165 kg/ha urea, 125 kg/ha muriate of potash) were applied to control treatment (T1) as recommended by the Department of Agriculture, Sri Lanka. Other treatments, that is, T2-T6, 10 t/ha goat manure and 0–4 t/ha sugarcane molasses with half dosage of recommended tripe superphosphate (TSP) were applied as described above. Irrigation was done twice a day in the morning and evening by using a watering can. Weeding was done manually.

In this experiment, leaf, root and yield parameters in all treatments were collected at harvesting time. Plant height was measured using a meter scale in each plant. The number of leaves per plant was counted. Length and width of leaf, petiole length and edible root length (cm) were measured with the aid of a ruler while the diameter of the edible root was measured with the aid of a vernier calliper. The chlorophyll content of leaves was measured using a chlorophyll meter (SPAD 502 plus). The shoot (above-ground level plant part) and edible root portion of each plant in all treatments were separated and their fresh weights (g) were measured. These materials were cut into small pieces, separately dried at 105^0C for 1 hour in an oven, and their dry weight (g) was recorded using an electronic

balance. Harvesting was done at 75 days after planting. Total crop yield (g/m^2) and marketable root yield (g/m^2) were calculated. All the collected data for each parameter were analyzed by analysis of variance using the SAS 9.1 statistical software package. The treatment mean was compared by the Tukey's test at the 5% significance level.

Results and Discussion

Plant height

Figure 1 shows the average plant height of the beetroot crop in each treatment. There was a significant variation ($P<0.01$) in plant height among the treatments. Plants treated with T5 (10 t/ha of goat manure, 3 t/ha of sugarcane molasses and 50% of TSP) exhibited maximum plant height (27.4 cm), whereas T1 (inorganic fertilizers as control) had a minimum value of 19.36 cm among the treatments. A significant variation in plant height was observed between T1 and T5, but the difference between T2 (10 t/ha of goat manure alone) and T5 was not significant. Among the treatments, the shortest plant was observed in the control (T1) treatment where NPK inorganic fertilizers were applied at the recommended rate. In addition to primary nutrients, sufficient amounts of secondary macronutrients such as calcium, magnesium and sulfur are important for plant growth. Ojeniyi and Adegboyega (2003) have stated that goat manure also releases essential micronutrients to the soil, and it was found to significantly increase the leaf growth of celosia.

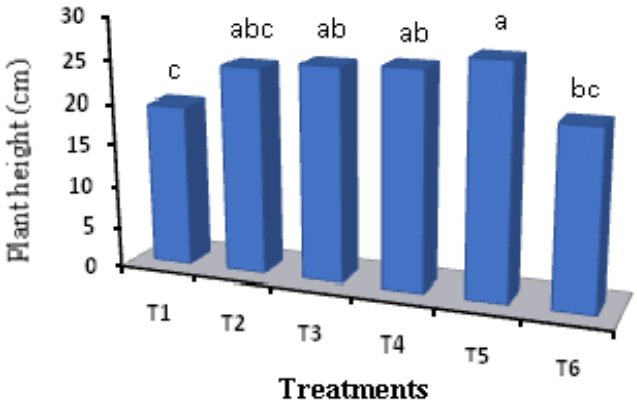

Figure 1. The effect of goat manure, molasses and 50% TSP application on the average plant height of beetroot.

The maximum plant height recorded in T5 may be probably due to favorable application of goat manure and molasses which contain potassium and other nutrients. Nitrogen (N), phosphorus (P) and potassium (K) are the major nutrients essential for plant growth and yield. The high concentration of these primary nutrients is necessary for plants as any deficiency of the essential nutrients will preclude healthier plant growth (Gholizadeh et al., 2009). T6 treatment (10 t/ha of goat manure, 4 t/ha of sugar masses and 50% TSP) produced significantly shorter plants as compared to T5. This may be due to excess potassium ion availability from the high concentration of K fertilizer applied. This result is supported by the findings of Taiz and Zeiger (2006), who suggested that excess ions in nutrient solutions decreased plant growth. Shaibur et al. (2008) reported that potassium ions reduced calcium and magnesium concentrations in rice seedlings.

Number of leaves

There was no considerable difference ($P>0.05$) in an average number of leaves produced per plant. The minimum value of 6.4 was obtained in T2, while the maximum number of leaves (9.2) was recorded in T5 (Figure 2). This may be due to the high nutrient supply, particularly nitrogen and potassium in the combined application of goat manure and molasses. In this study, the number of leaves per plant was increased by increasing potassium supply to a certain limit then declined as observed in the lower number of leaves in T6 compared to T5. Molasses supply the ion at a higher rate. Serio et al. (2001) mentioned the lower rate of lettuce leaf production under the higher electrical conductivity value in soilless conditions. The leaf surface area is important for the physiological activity of the plant.

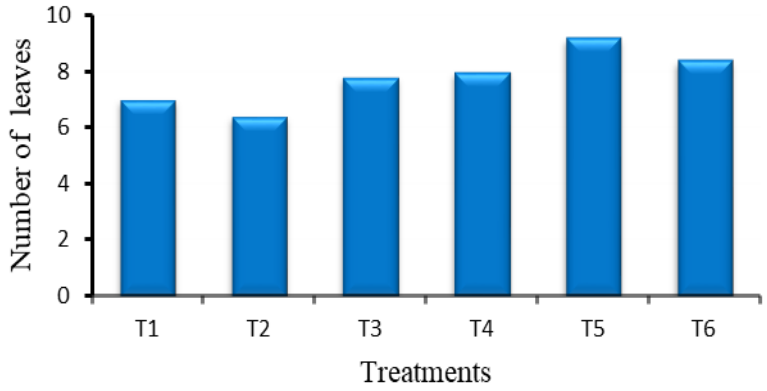

Figure 2. The effect of goat manure, molasses and 50% TSP application on the average number of beetroot leaves.

Leaf length

Statistically analyzed data revealed that there was a remarkable difference ($P<0.0001$) in the length of the 1st leaf of the plant among the treatments (Table 1). This ranged from 9.20 cm to 13.36 cm, whereas it was 8.90 cm – 10.30 cm for the length of the 2nd leaf. These lengths were higher in T5 compared to other treatments. The application of molasses may provide the plant nutrients probably potassium for the leaf growth. The result obtained in this study was in agreement with the findings of Shabala (2003) and Fromm (2010), who have mentioned that potassium serves a vital role for osmoregulation, cell expansion, stomatal movements and photosynthesis. In the other study, the increasing potassium fertilizer application resulted in decreased phosphorus (Baghour et al., 2001; Lin and Yeh, 2008) and Ca and Mg concentrations in leaves (Sabreen and Saiga, 2004; Lin and Yeh, 2008). The inadequate primary nutrient supply reduces photosynthesis, leaf production and plant growth (Zhao et al., 2005), which is an indication that the plant requires a balanced supply of plant nutrients for better leaf growth.

Table 1. The effect of goat manure, molasses and 50% TSP application on the average lengths of leaf and petiole of the beetroot plant.

Treatment	1st leaf length (cm)	2nd leaf length (cm)	Petiole length of the 1st leaf (cm)	Petiole length of the 2nd leaf (cm)
T1	11.50±0.59b	09.90±0.50	10.60±1.80ab	11.00±1.67ab
T2	09.20±0.70b	09.40±0.62	08.10±0.33b	08.60±1.12b
T3	09.50±0.74b	09.80±0.25	11.00±1.04ab	11.30±0.86ab
T4	11.00±0.88b	09.50±0.74	12.20±0.37ab	11.70±1.11ab
T5	13.36±0.42a	10.30±1.82	13.80±1.46a	12.10±0.84a
T6	10.50±0.94b	08.90±0.62	10.20±0.46ab	10.00±0.22ab
F test	P<0.0001	P>0.05	P<0.05	P<0.05

The value represents the mean ± standard error of replicates. Means followed by the same letters in each column are not significantly different according to the Tukey's test at the 5% significance level.

Leaf petiole length

There was a substantial variation ($P<0.05$) in the petiole length of the beetroot plant among the treatments. Petiole lengths of the 1st leaf (13.80 cm) and the 2nd leaf (12.10 cm) were the longest in T5 compared to other treatments (Table 1). Potassium is identified as the dominant osmoticum (Shabala et al., 2000), and it is important for cell enlargement and stomatal function (Shabala, 2003). Plants make use of calcium ion for strengthening cell walls (Hepler, 2005). In addition to N, P and K nutrients, the application of goat manure also supplies Ca, Mg and other micronutrients to soil for plant growth as stated by Ojeniyi and Adegboyega (2003).

Leaf width

A significant difference (P<0.001) was observed in the average leaf width of the plant among the treatments. The higher value of 6.24 cm was attained in T5, and a lower value of 5.04 cm was noted in T6 (Table 2). Leaf width was considerably higher (P<0.05) in the T5 treatment than in other treatments except for T4. This finding was supported by the result of Shabala et al. (2000), who stated that potassium plays a role in cell enlargement and leaf expansion. The T6 treatment had a lower value of leaf width than T5. A high potassium concentration may cause a deficiency of calcium and magnesium (Nguyen et al., 2017). As a result of low calcium availability, a decrease of plant height, leaf area and plant growth was observed by Leal and Prado (2008).

Table 2. The effect of goat manure, molasses and 50% TSP application on the average leaf width and shoot weight of the beetroot plant.

Treatment	Leaf width (cm)	Fresh weight of shoot (g)	Dry weight of shoot (cm)
T1	5.08±0.12b	14.27±0.67abc	2.42±0.45ab
T2	5.20±0.15b	10.55±0.49c	1.62±0.40b
T3	5.28±0.13b	12.47±0.89bc	1.97±0.29b
T4	6.20±0.12a	16.55±0.91ab	2.72±0.40ab
T5	6.24±0.11a	17.55±0.37a	3.02±0.39a
T6	5.04±0.09b	16.77±0.95ab	2.79±0.07ab
F test	P<0.001	P<0.01	P<0.05

The value represents the mean ± standard error of replicates. Means followed by the same letters in each column are not significantly different according to the Tukey's test at the 5% significance level.

Shoot weight of the beetroot plant

Fresh and dry weights of the shoot (above-ground level plant part) per plant are given in Table 2. Significant differences in both fresh (P<0.01) and dry (P<0.05) weights of the shoot were observed among the treatments. The values of fresh (17.55 g) and dry (3.02 g) shoot weights obtained in T5 were higher than those in other treatments while lower values (10.55 g and 1.62 g respectively) were recorded in T2. The T1 treatment using inorganic fertilizers alone produced 14.27 g of fresh and 2.42g of dry shoot weights. A similar shoot weight was recorded between T1 and T2. It may be due to potassium availability to plants. Potassium performs significant roles in the physiological processes, such as photosynthesis, translocation of photoassimilates, transportation of water and nutrients, nutrient balance, etc., in plants (Marschner, 2012; Hasanuzzaman et al., 2018).

Chlorophyll content

There was a significant variation ($P<0.05$) in the chlorophyll content of leaves, as shown in Figure 3. The leaf chlorophyll content ranged from 35.40 (T2) to 45.17 (T5) and a notable difference ($P<0.05$) was observed between T2 and T5 treatments. This difference may be due to the required amount of nutrients received by plants. Chlorophyll is necessary for photosynthesis to produce essential carbohydrates as a food source for the plants (Hynninen and Leppakases, 2002). Guo et al. (2019) have stated that increased potassium supply interferes with the uptake of the other nutrients, particularly nitrogen and potassium uptake, and optimal potassium enhances the nutritional function of NO_3^- in wheat plants.

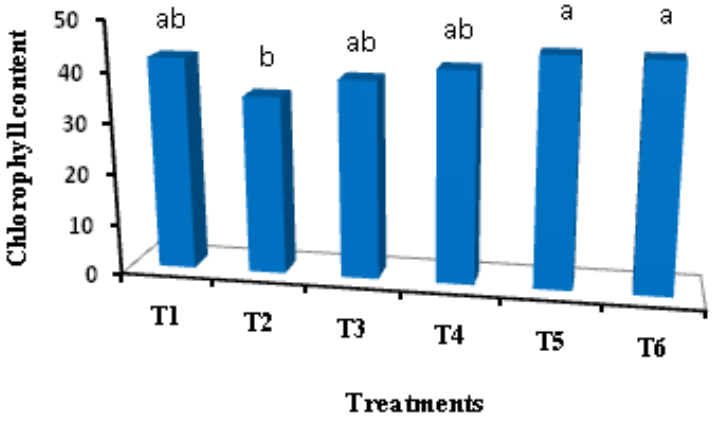

Figure 3. The effect of goat manure, molasses and 50% TSP application on the average chlorophyll content of beetroot leaves.

Diameter of the edible root

The diameter value of the edible root (beetroot) in each treatment is shown in Table 3. According to the statistical analysis, there was a considerable difference ($P<0.01$) in the diameter of beetroot among the treatments. A high average value (4.04 cm) of the root diameter was attained in T4 (10 t/ha of goat manure, 2 t/ha of sugar molasses and 50% TSP). This value, however, was statistically similar to those in T1 and T5 treatments.

The reason may be due to the sufficient amount of potassium supplied by molasses for increasing the diameter of the beetroot grown in sandy regosol soil. The potassium fertilizer application increased the total sugar in beetroot plants grown in sandy calcareous soil (Abdel-Motagally and Attia, 2009). In this study, T5 and T6 treatments showed lower values of the root diameter than the value

obtained in T4. A higher rate of potassium provides the adverse effect on calcium (Leal and Prado, 2008), but calcium is very important for fundamental physiological functions in plant structure and signalling (Gilliham et al., 2011). Domingues et al. (2016) reported that the dry mass of the shoot and root was high in plants grown with high calcium concentrations.

Table 3. The effect of goat manure, molasses and 50% TSP application on the average diameter of beetroot (edible root).

Treatment	Diameter of beetroot (cm)	Fresh weight of edible root (g)	Fresh weight of plant (g)
T1	3.12±0.13ab	27.84±1.51c	42.12±1.94bc
T2	2.64±0.34b	22.29±0.64d	32.84±0.37d
T3	2.92±0.20b	27.04±0.77cd	39.52±1.29cd
T4	4.04±0.20a	37.28±1.36a	53.83±1.78a
T5	3.51±0.17ab	33.21±1.37ab	50.76±1.34a
T6	3.40±0.27ab	30.18±0.31bc	46.96±1.07ab
F test	P<0.01	P<0.0001	P<0.0001

The value represents the mean ± standard error of replicates. Means followed by the same letters in each column are not significantly different according to the Tukey's test at the 5% significance level.

Fresh weight of the edible root

The fresh weight of the edible root (beetroot) per plant is indicated in Table 3. There was a remarkable difference (P<0.0001) in the fresh weight of the beetroot among the treatments. The fresh weight of the root ranged from 22.29 g to 37.28 g. The combined application of 10 t/ha of goat manure, 2 t/ha of sugar molasses and 50% TSP significantly (P<0.05) had a higher value of beetroot than the other treatments except for T5. The lowest fresh weight was recorded in T2. Romheld and Kirkby (2010) mentioned the positive effect of the potassium fertilizer application on the yield of sugar beet. Mg deficient plants are less competent to translocate sucrose to the root via the phloem (Hermans et al., 2006; Farhat et al., 2016). Potassium nutrient accumulates more in roots than in the shoot of the beetroot plant (Granjeiro et al., 2007). Chen et al. (2018) reported that magnesium deficiency did not influence the dry weight of the shoot but reduced the dry weight of the root. The application of farmyard manure with reduced inorganic fertilizers improves soil fertility and crop yield (Seran, 2016).

Fresh weight of the beetroot plant

There was a significant variation (P<0.0001) in the total fresh weight of the plant among the treatments. The total fresh weight of the beetroot plant in each treatment is shown in Table 3. The maximum value of 53.83 g was recorded in T4,

whereas a minimum value of 32.84 g was attained in T2. Abdel-Motagally and Attia (2009) stated that the fresh weight of beetroot was increased with increasing potassium rates. A similar result was reported by Chandraju et al. (2008), who noted that the application of molasses enhances the primary nutrient uptake and yield of leafy vegetables. In plants grown with a low ion level of magnesium, total biomass is lower than that of plants with sufficient magnesium ions (Cakmak and Kirkby, 2008), and potassium deficiency results in a severe reduction in photosynthetic CO_2 fixation and use of photosynthates (Cakmak, 2005). The application of goat manure with EM could give a higher yield of vegetable cowpea (Seran and Shahardeen, 2012).

Beetroot yield

Marketable root yield (g/m^2) and total crop yield (g/m^2) of beetroot are given in Figure 4 and Figure 5, respectively. According to the statistical analysis, there were remarkable differences ($P<0.0001$) in root yield and total crop yield among the treatments. The highest root yield (1241.51g) and total crop yield (1792.62 g) of beetroot were obtained in the T4 treatment. These values, however, were not different from T5.

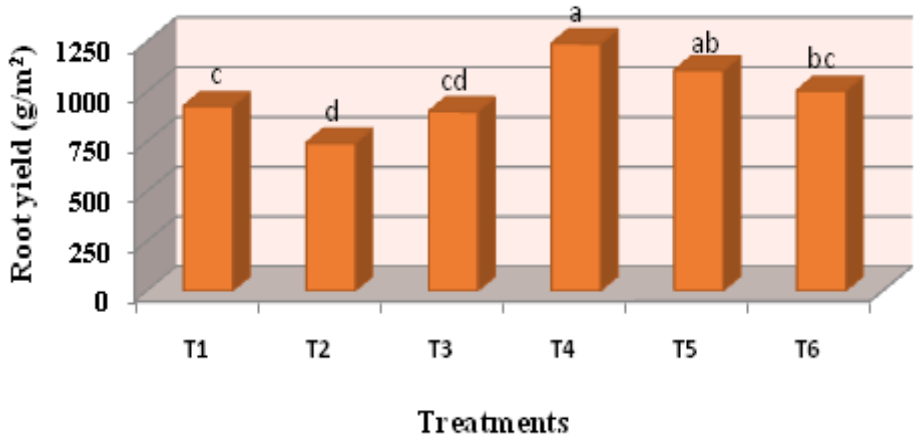

Figure 4. The effect of goat manure, molasses and 50% TSP application on the marketable root yield of beetroot crop.

Plants treated with T1 (control treatment) gave significantly lower values of the crop and root yields than plants treated with T4 and T5. This result supported the findings of Çakmak (2005) and Hermans et al. (2006), who have indicated that potassium and magnesium have important functions in photosynthesis and biomass

distribution among the plant parts. Magro et al. (2015) have observed that the use of organic compost increases root production.

Plants treated with T1 (control treatment), T2 (10 t/ha of goat manure alone) and T3 (10 t/ha of goat manure, 1 t/ha and 50% TSP) gave significantly lower values ($P<0.05$) of root yield and crop yield than those in T4. Mohammadi and Brimvandi (2009) reported that the application of molasses increased available potassium and total nitrogen but reduced available phosphorus in soil. The excess potassium application may influence calcium and magnesium deficiency (Nguyen et al., 2017). In this study, goat manure, molasses and triple superphosphate provide essential macro and micronutrients for the growth and yield of beetroot in sandy regosol. Further, we suggest that naturally available phosphate fertilizer can be used in crop cultivation as a substitute for triple superphosphate (inorganic fertilizer) as stated by Sithamparam and Seran (2014).

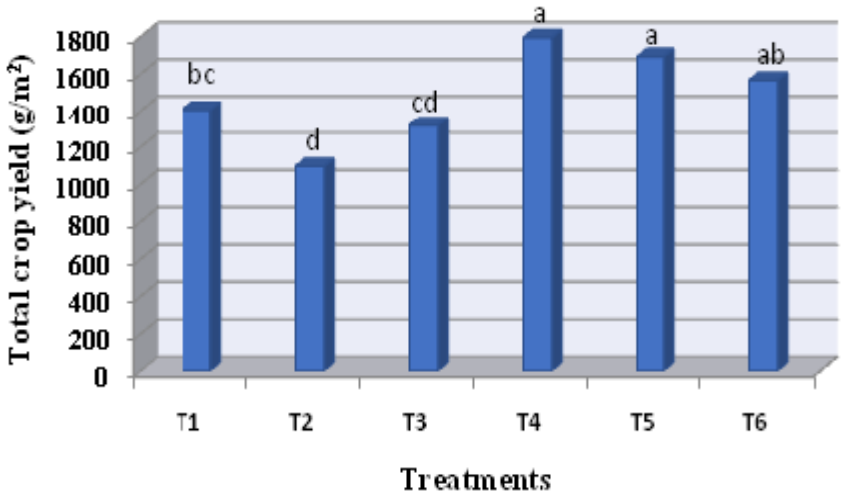

Figure 5. The effect of goat manure, molasses and 50% TSP application on the total yield of the beetroot crop.

Conclusion

The results revealed that the application of 10 t/ha of goat manure, 2–3 t/ha of sugarcane molasses and 50% TSP significantly increased marketable root yield and total crop yield of beetroot when compared to the recommended NPK inorganic fertilizers. Marketable root yield was high (1241.51g) in the T4 treatment (10 t/ha of goat manure, 2 t/ha of sugarcane molasses and 50% TSP) followed by T5 (10 t/ha of goat manure, 3 t/ha of sugarcane molasses and 50% TSP). The marketable

root yield and total yield of the beetroot crop in T4 were increased by 33.8% and 27.8% respectively as compared to those in the control treatment. The result proves that goat manure combined with sugarcane molasses and TSP is a suitable source of nutrients for improving soil fertility and the yield of beetroot. This study concludes that the combined application of 10 t/ha of goat manure with 2 t/ha of sugarcane molasses and 50% TSP is more suitable for sandy regosol to obtain a high yield of beetroot than the recommended chemical fertilizer. Moreover, it can be suggested that organic or natural phosphorus resources can be used as a replacement for inorganic phosphorous fertilizer since organic manure is eco-friendly. A further study is necessary to evaluate the quality of beetroot.

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UTICAJ KOZJEG STAJNJAKA I MELASE ŠEĆERNE TRSKE NA RAST I PRINOS CVEKLE (*BETA VULGARIS* L)

Priyadarshani W. M. Nadeeka i Thayamini H. Seran*

Odsek za ratarstvo, Poljoprivredni fakultet, Istočni univerzitet, Šri Lanka

R e z i m e

Ogled je sproveden u sudovima kako bi se proučio uticaj kozjeg stajnjaka i melase šećerne trske na rast i prinos cvekle (*Beta vulgaris L.*) u peskovitom regosolu. Ogled je postavljen u potpuno slučajnom dizajnu (PSD) sa šest tretmana. Tretmani su uključivali neorgansko đubrivo (T1), 10 t/ha samo kozjeg stajnjaka (T2), a takođe 10 t/ha kozjeg stajnjaka i 50% trostrukog superfosfata (TSP) sa 1–4 t/ha melase šećerne trske (T3–T6). Rezultati su pokazali da su parametri rasta biljaka (dužina lista, dužina peteljki lista, širina lista, broj listova, sveža masa i suva masa listova) značajno varirali među tretmanima. Među tretmanima je postojala značajna razlika u prečniku cvekle. Značajne razlike ($P<0,05$) primećene su u svežoj masi cvekle i cele biljke u zavisnosti od tretmana. Prinos sveže masa korena i ukupni prinos cvekle po biljci povećani su kod tretmana sa 10 t/ha kozjeg stajnjaka, 2 t/ha melase šećerne trske i 50% TSP (T4) i 10 t/ha kozjeg stajnjaka, 3 t/ha melase šećerne trske i 50% TSP (T5) u poređenju sa kontrolnim tretmanom (T1). Ukupan prinos cvekle po m^2 iznosio je 1.792,62 g u tretmanu T4 i 1.402,68 g u tretmanu T1. Prinos korena cvekle povećan je u tretmanu T4 u poređenju sa tretmanom T5. Može se zaključiti da se 10 t/ha kozjeg stajnjaka sa 2 t/ha melase šećerne trske i 50% TSP može primeniti za dobijanje visokog prinosa cvekle u peskovitom regosolu.

Ključne reči: cvekla, kozji stajnjak, melasa, prinos.

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*Autor za kontakt: e-mail: thayaminis@esn.ac.lk

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EFFECTS OF SPATIAL ARRANGEMENT AND POPULATION DENSITY ON THE GROWTH AND YIELD OF SESAME (*SESAMUM INDICUM* L.) IN A SESAME/MAIZE INTERCROP

Afe A. Isaac[1*], Alabi K. Oyebisi[1], Ogundare S. Kayode[2] and Adeniji S. Mojisola[1]

[1]Department of Crop Production, Kwara State University, Malete, Nigeria
[2]Division of Agricultural Colleges, Ahmadu Bello University, Kabba, Nigeria

Abstract: A field experiment was carried out at the Teaching and Research Farm, Kwara State University, Malete, Nigeria. The aim of the experiment was to investigate the growth and yield of sesame (*Sesamum indicum* L.) as affected by the row arrangement and population density of maize (*Zea mays* L.). The full population of sesame in two-row arrangements (1:1) and (2:2) was combined with four population densities of maize viz: 100S:100M; 100S:75M; 100S:50M and 100S:25M (where S and M represented sesame and maize, respectively). Sole crops of sesame and maize at full population were included in the treatments as control. The number of pods per plant (NPP), length of fruit zone (LFZ), and yield of sesame were significantly ($P \leq 0.5$) influenced by the interactive effect of population ratios and row arrangements. These variables increased as the population of associated maize decreased. All variables measured in maize were influenced by population density and row arrangement except for the number of cobs per plant (NCP), cob length (CL), and cob circumference (CC). Regardless of spatial arrangement and population density, the aggressivity (A) value was positive for sesame and negative for maize. The competitive ratio (CR) values were also higher in sesame than in maize. Land equivalent ratio (LER) and land equivalent coefficient (LEC) values, for all population ratios tested, indicated the intercropping advantage with the highest value recorded at a full population of sesame mixed with fifty percent population of maize in a 2:2-row arrangement and hence, recommended for adoption.

Key words: competitive ratio, grain yield, intercropping efficiency, intercropping, row arrangement.

[*]Corresponding author: e-mail: adeafe22@yahoo.com

Introduction

The simultaneous growing of two or more crops on the same piece of land to produce greater yield is common in the tropical farming system. In the crop mixture, each component crop must have adequate space to maximize cooperation and reduce competition for optimum yield. This makes spatial arrangement and population density an important factor to achieve optimum yield. Plant density manipulates the microenvironments and could affect the growth, development, and yield due to the interception of available photosynthetic active radiation. Exceeding the plant population above the optimum gave did not increase the yield due to a decrease in radiation use efficiency (Purcell et al., 2002), competitive shading within the leaf canopy architecture (Hiyane et al., 2010) with a consequent reduction of intercepted radiation by the middle and lower stem leaves particularly during the silking stage in maize (Chistopher et al., 2009; Li and Wang, 2010).

Seran and Brintha (2010) opined that the full population of each crop at the intercrop would significantly reduce yield due to overcrowding. Muoneke et al. (2007) reported a reduction in soybean seed yield with increasing maize plant density. In intercropping involving sesame and grain legumes, Iftikhar et al. (2006) reported that sesame appeared to be a dominant crop and hence utilized the resources more aggressively than the dominated legumes at the intercrop. In another study, Tamiru et al. (2019) reported that population density and spatial arrangement significantly influenced the yield of sesame when intercropped with maize. This study did take into consideration the varying population of maize for optimum yield in the mixture.

According to Sevgi et al. (2004), the population density was reported to influence the growth and yield component of sesame significantly. Similarly, Ijoyah et al. (2016) reported that the yield and yield components of both maize and sesame decreased with increasing plant density of sesame. In another study, Kolawole et al. (2015) observed that the growth and yield of maize were not affected when the two crops were simultaneously planted but the yield of sesame, being a weak competitor, decreased, particularly, when introduced two weeks after maize.

Intercropping sesame with maize is a common practice among smallholder farmers in the southern Guinea Savannah agro-ecological zone of Nigeria. Although several studies have been carried out on sesame/maize intercropping, reports from these studies were inconsistent and did not take into consideration the optimum plant population of maize to be intercropped with sesame at the appropriate spatial arrangement for optimum productivity. This research was therefore carried out to determine the suitable population density of maize and appropriate spatial arrangement for intercropping, and also examined the competitive behavior of the intercrop, considering sesame as the main crop.

Materials and Methods

Study location

The experiment was carried out between July to November of the 2017 cropping season at the Teaching and Research Farm of the Kwara State University, Malete, in the southern Guinea Savannah agro-ecological zone of Nigeria. Six composite soil samples at a depth of 0–20 cm were collected in a zigzag pattern with a soil auger to determine the physical and chemical properties. Soil particles were analysed using the hydrometer method (Bouyoucous, 1962 modified by Gee and Bauder, 1986). Total nitrogen was determined using the Kjeldahl digestion method as described by Bremner and Mulvaney (1982). Available P was determined by Bray 1 method (Anderson and Ingram, 1998). Soil pH was determined electrometrically in a 1:1 soil water suspension. Total soil organic carbon was determined using the acid dichromate wet-oxidation procedure of the Walkley and Black method (1934), and soil organic matter was determined by multiplying with a factor (1.72). Exchangeable bases (Ca, Mg, Na and K) were extracted with 1M ammonium acetate (1M NH4OAc) solution buffered at pH 7.0, as described by Anderson and Ingram (1998). The exchangeable sodium (Na^+) and potassium (K^+) contents of the filtrates were determined by the flame photometer while the exchangeable calcium (Ca^+) and magnesium (Mg^+) were determined by the EDTA titration method and were read using the atomic absorption spectrophotometer (AAS). The cation exchange capacity of the soil was determined with 1M NH4OAc (1M ammonium acetate), buffered at pH 7.0 (Rhoades, 1982).

The meteorological data during the period of the experiment were obtained at the Lower Niger River Basin Development Authority; Ilorin. The rainfall was measured using the rain gauge at regular intervals, usually in the morning by measuring the water collected in a graduated measuring jar of 0.1mm. The air temperature was measured using a maximum thermometer that was mounted at an angle of about 2^0 from a horizontal position such that the mercury column rested against the constriction without gravity.

Planting materials

Maize variety, ACR9931-DMSR-Y, and sesame X-Sudan were, respectively, obtained from the International Institute of Tropical Agriculture, Ibadan, and the National Cereal Research Institute, Badeji, Nigeria.

Experimental design

Full populations of sesame at two-row arrangements (1:1 and 2:2) were combined with four population densities of maize as 2 × 4 factorial combinations in a split-plot design and replicated three times. Sole crops each of sesame and maize were included in the treatments as the control. The treatments were: 100S: 100M; 100S: 75M; 100S: 50M; and 100S: 25M (where S and M represented sesame and maize respectively). Spatial arrangements with one row of sesame alternated with one row of maize (1:1) and two rows of sesame alternated with two rows of maize (2:2) constituted the main plots while population density was assigned to the sub-plot treatments. Plant population per hectare was determined as:

$$\text{Plant population} = \frac{\text{Area} \times \text{number of plants per stand}}{\text{Intra-row spacing} \times \text{inter-row spacing}}$$

Details of population ratios and the corresponding plant population are presented in Table 1.

Table 1. Component population ratios.

Population ratios	Spacing cm (+)		Plant	Population/ha	Total plant population/ha
S:M	S	M	S	M	
100:100	75 x 20	75 x 50	133,333	53,333	186,666
100:75	75 x 20	75 x 60	133,333	40,000	173,333
100:50	75 x 20	75 x 100	133,333	26,666	160,000
100:25	75 x 20	75 x 125	133,333	13,333	146,666
100:00	75 x 20	75 x 50	133,333	-	133,333
00:100	75 x 20	75 x 50	-	53,333	53,333

S = sesame; M = maize; + = two plants were maintained per stand.

Cultural practices

The land was ploughed and harrowed twice. Each plot size measured 4.0 m × 4.0 m with 0.5m between plots and 1.0 m between blocks. Four seeds of sesame were planted on the 28th of July but thinned to two plants per stand at two weeks after planting. Component maize at its respective plant populations was introduced to sesame at two weeks after planting (Mkamilo, 2004). Pendimethalin [N-(ethyl propyl)-3,4 dimethyl-2,6 dinitrobenzene amine], a pre-emergence herbicide, was applied at the rate of 1.5 litres/ha using a knapsack sprayer. The herbicide was applied immediately after the sesame crop was planted. This was followed by hoe weeding at a three-week interval. N-P-K (20:10:10) fertiliser was applied to sesame

at the rate of 150kg/ha (3 bags) at three weeks after planting while 400kg/hectare (8 bags) was applied to maize at 3 and 6 weeks after planting.

Harvesting

Sesame was harvested from the 2.0 m inner rows at physiological maturity before shattering of the seeds. At physiological maturity, 75% of the capsules on the main stem have white colour seeds, and dark brown at the tip of the capsule (Langham et al., 2008). The harvested crops were made to stand erect until properly dried and were thereafter manually threshed and winnowed. Maize was harvested at 12WAP, at this period, the leaves and the silk were dried, and the kernels became hard.

Sampling and data collection

The following variables were collected from five tagged plants at the inner rows for the two crops.

1. Maize: Days to 50% tasselling, plant height at maturity, number of cobs/pant, cob length and circumference, number of grains per cob, stem girth at 8 WAP, the weight of 100 grains and grain yield per hectare.
2. Sesame: plant height, number of pods per plant, number of branches per plant, length of fruit zone at maturity, stem girth, and seed yield per hectare.

Evaluating the efficiency of intercropping

The land equivalent ratio (LER) was determined as described by Willey (1985) using the equation:

$$LER = \frac{\text{Intercrop yield of crop a}}{\text{Sole crop yield of crop a}} + \frac{\text{Intercrop yield of crop b}}{\text{Sole crop yield of crop b}}$$

The land equivalent coefficient (LEC), a measure of the level of interaction and productive potential at the intercrop, was estimated as proposed by Adetiloye et al. (1983).

LEC = (Ls x Lm), where Ls and Lm are the partial LER for sesame and maize, respectively. The partial LER is the ratio of the intercrop yield to the sole crop yield.

The competitive ratio (CR) was calculated using the formula proposed by Willey and Rao (1980). The CR describes the degree of competition among

component crops in the mixture. A higher CR value indicates a higher competitive ability.

CR (sesame) = Ls x Zsm/Lm x Zms,
CR (maize) = Lm x Zms/Ls x Zsm,
where: Ls and Lm are the partial LER for sesame and maize, respectively;
Zsm and Zms = proportions of sesame and maize, respectively.

Aggressivity (A)

The value of A was calculated by the formula proposed by McGilchrist (1965). A is a measure of how much a relative yield increase of one species (dominant) is greater than of the other species (dominated or recessive) in the mixture. The A value for component crops a and b is usually the same, but the dominant crop gives a positive value, while the dominated crop gives a negative value. A high A value is an indication of a higher difference between the actual and expected yield.

$$Aab = \frac{Yab}{Yaa \times Zab} - \frac{Yba}{Ybb \times Zba}$$

where:
Aab = Aggresivity value for component crop a,
Yab = Intercrop yield of crop a,
Yba = Intercrop yield of crop b,
Zab and Zba = Sown proportions of crops a and b in the intercrop.

Statistical analysis

All data collected, analysed by the analysis of variance (ANOVA) using the SAS statistical model and treatment means where significant, were separated using the Duncan Multiple Range Test at the 5% probability level.

Results and Discussion

The textural class of soil at the experimental site is sandy loam with the soil reaction that is moderately acidic (Table 2).

The highest rainfall (302.7mm) during the experiment was during August with the temperature range between 33^0C and 25.5^0C. The mean rainfall and temperature during the period of the experiment were 155.82 mm and 27.90^0C, respectively (Table 3).

Table 2. Pre-planting physical and chemical properties of the soil.

Particle size (%)	
Sand	87.60
Clay	5.22
Silt	7.18
Textural class	Sandy loam
pH (water)	5.48
Bulk density (g/cm^3)	1.63
Organic matter (%)	1.81
Total nitrogen (%)	0.14
Available P (mg/kg)	2.61
Exchangeable cation (Cmol/kg)	
Ca^+	0.32
Mg^{2+}	2.98
K^{2+}	0.92
Na^+ (mmol/L)	0.47

Table 3. Meteorological data for the experimental site (June–December 2017).

Months	Rainfall (mm)	Temperature (^{o}C)	Relative humidity (%)
June	184.90	33.00	74.50
July	42.80	26.50	74.50
August	302.70	26.00	51.00
September	215.90	25.50	52.00
October	38.20	28.50	75.00
Total	779.10	139.50	32.00
Mean	155.82	27.90	65.40

Source: Lower Niger River Basin Development Authority (Hydrology section) Ilorin, Nigeria.

The effect of spatial arrangement and population density on the growth and yield of sesame is presented in Table 4. The plant height, stem girth and the number of branches per plant of sesame were not influenced by spatial arrangement and population density of component maize but the length of fruit zone, the number of pods per plant and seed yield per hectare were significantly influenced. In the 1: 1row arrangement, the length of fruit zone in the sole stand was significantly higher than the intercrops, except in the 100: 75 population ratio, whereas in the 2: 2 row arrangement, there was no significant difference between the sole crop and the intercrops except where full populations of both crops were mixed. The number of pods per plant increased as the population of component maize decreased. Irrespective of the arrangement, the lowest numbers of pods were recorded where the full population of sesame was mixed with the full population of maize. The seed yield followed a similar trend. Regardless of the arrangements, significantly lower seed yield was recorded in the intercrops compared to their respective sole crop stand. There was no significant difference in the seed yield when the full

population of sesame was mixed with the full population and 75% population of maize as well as 50% and 25% populations of maize in the 1:1 row arrangement. This observation is different in the 2:2 row arrangement, where the similarity in seed yield was observed where 75% and 50% populations of maize were combined with a full population of sesame.

Table 4. Effects of spatial arrangement and population density on the growth and yield of sesame.

Spatial arrangements	Population ratios S:M	Plant height (cm)	Stem girth (cm)	Length of fruit zone (cm)	Number of branches per plant	Number of pods per plant	Yield (Kg/ha)
1:1	100:100	106.64	7.40	66.29b	3.05	26.39c	404.49e
	100:75	105.59	7.53	66.97b	3.15	27.80c	417.56e
	100:50	105.91	7.49	75.95a	3.16	34.60ab	519.69c
	100:25	103.12	7.82	61.71c	3.28	34.73ab	521.64c
	100:00	101.38	7.89	74.43a	3.31	36.75ab	543.72b
2:2	100:100	106.66	7.40	65.63b	3.06	28.00c	402.56e
	100:75	105.64	7.72	74.43a	3.16	31.07bc	466.67d
	100:50	105.93	7.84	75.71a	3.25	31.67bc	475.68d
	100:25	106.97	7.87	74.43a	3.28	34.68ab	519.69c
	100:00	102.42	7.94	74.95a	3.32	40.34a	605.76a
SA × PD		Ns	Ns		Ns	*	*

Values with the same letter(s) in the same column are not significantly different at the p≤ 5% level of probability by the Duncan Multiple Range Test. Ns = non-significant; * = significant; S = sesame; M = maize.

The growth and yield of maize as influenced by spatial arrangement and population density are presented in Table 5. The number of cobs per plant, cob length, and circumference of maize were not significantly influenced by spatial arrangement and population density. Plant height, stem girth, number of grains per cob, weight of grains, and yield were significantly influenced by spatial arrangement and population density. In the intercrops, the tallest plants were observed where a full population of sesame was mixed with a full population of maize in the two spatial arrangements. This, however, is not superior to other population ratios, particularly in the 1:1 row arrangement. The girth of the stem increased as the population pressure decreased. In the 1:1 row arrangement, the girth of the stem in 100S: 100M was found to be significantly thinner compared to other population ratios, including the sole crop stand. The similar stem girth was recorded in the 2:2 row arrangements.

The sole stand in the two-row arrangements had a significantly higher number of grains per cob and grain yield than their respective intercrops. In the two arrangements, where two rows of maize were followed by two rows of sorghum,

similar numbers of grains per cob were produced except in a combination involving the full population of both crops where significantly fewer numbers of grains were recorded. The least grain weight of 140.33 gram was obtained where the full population of sesame was mixed with the full population of maize. Regardless of spatial arrangement, the component population ratio of 100S:25M recorded the lowest grain yield/ha. Irrespective of the arrangement, the sole stand was superior in grain yield compared to the intercrops. A significantly lower yield was recorded in 100S:25 M in the two-row arrangements. There was no significant difference in seed yield between 100S:75M and 100S:50M in the 1:1 row arrangement, but 100S:75M was superior to 100S:100M in the 2:2 row arrangement.

Table 5. The effect of spatial arrangement and population density on the growth and grain yield of maize.

Spatial arrangements	Population ratios S:M	Plant height (cm)	Stem girth (cm)	Number of cobs/ plant	Cob length (cm)	Cob circum-ference (cm)	Number of grains/ cob	Weight of 1000 grains (g)	Grain yield (kg/ha)
1:1	100:100	145.64bc	11.80c	1.67	9.87	10.13	328.81d	140.33e	1662.02d
	100:75	131.85c	13.44abc	1.68	9.89	10.16	370.00b	176.43cd	1830.83c
	100:50	136.90bc	14.17ab	1.68	10.00	10.19	357.60bc	162.75de	1745.76cd
	100:25	143.68b	14.23ab	1.69	10.20	10.22	366.29bc	157.40ef	985.31e
	00:100	140.74bc	14.45a	1.70	10.23	10.24	406.00a	183.70bc	2862.76a
2:2	100:100	145.10b	12.03bc	1.66	9.63	10.28	329.51d	146.66ef	1658.15d
	100:75	159.74a	12.20bc	1.69	10.18	10.40	369.91bc	199.49ab	1942.50b
	100:50	132.42c	12.68abc	1.69	10.19	10.15	369.53bc	199.46abc	1873.52c
	100:25	135.07bc	12.98abc	1.70	10.25	10.19	344.91cd	184.28bc	1008.68e
	00:100	142.71bc	14.18ab	1.71	10.28	10.68	414.40a	203.86a	2890.88a
SA x PD			*	Ns	Ns	Ns	*	*	*

Values with the same letter(s) in the same column are not significantly different at the p≤ 5% level of probability by the Duncan Multiple Range Test. Ns = non-significant; * = significant; M = maize; S = sesame.

The efficiency of intercropping as measured by land equivalent ratio (LER), land equivalent coefficient (LEC), aggressivity (A), and competitive ratio (CR) is presented in Table 6. Regardless of spatial arrangement and population density, all the intercrops demonstrated intercropping advantages over their respective sole crops. LER and LEC values increased as the population of component maize decreased from the full population to the 50% population and thereafter decreased. The highest LER and LEC values, 1.51 and 0.56, respectively were obtained when the 50% population of maize was intercropped with the full population of sesame in the treatment where two rows of sesame were followed by two rows of maize. The A values were positive for sesame and negative for maize, and they were found to be higher in the 1:1 row arrangement than in the 2:2 row arrangement

except in the 100S:50M population ratio. The CR values for sesame were higher than for maize in all the mixtures with the highest value recorded in 100S:25M population ratios.

Table 6. Evaluation intercropping advantages as influenced by the spatial arrangement and population density in the sesame/maize system.

Spatial arrangement	Population ratios	Partial LER	Partial LER	LER	LEC	A		CR	
	S:M	Sesame	Maize	-	-	S	M	S	M
1:1	100:100	0.74	0.58	1.32	0.43	0.16	–0.16	1.28	0.78
	100:75	0.77	0.64	1.41	0.49	0.13	–0.13	1.60	0.62
	100:50	0.87	0.61	1.48	0.53	0.26	–0.26	2.80	0.36
	100:25	0.96	0.34	1.30	0.33	0.62	–0.62	10.67	0.09
	100:00	1.00	1.00	1.00	0.25			-	
	00:100	1.00	1.00	1.00	0.25				
2:2	100:100	0.66	0.57	1.23	0.38	0.09	–0.09	1.16	0.86
	100:75	0.77	0.67	1.44	0.52	0.10	–0.27	1.54	0.64
	100:50	0.86	0.65	1.51	0.56	0.21	–0.28	2.77	0.36
	100:25	0.86	0.34	1.20	0.29	0.52	–0.77	9.55	0.10
	100:00	1.00	1.00	1.00	0.25	-	-	-	-
	00:100	1.00	1.00	1.00	0.25	-		-	-

LER = land equivalent ratio; LEC = land equivalent coefficient; A= aggressivity; CR = competitive ratio; S = sesame; M = maize.

The overall results of the study showed the superiority of intercropping sesame with maize over their respective sole crops as measured by LER, LEC, A, and CR indices. The reduction in the number of pods per plant and seed yield of sesame as observed in this study agreed with the findings of earlier researchers on sesame/maize intercropping (Kolawole et al., 2015; Ijoyah et al., 2016; Ajibola and Kolawole, 2019; Tamiru et al., 2019). The extent of reduction varied significantly among the population ratios and spatial arrangements, possibly due to differences in competition for natural resources. This competition was found to be higher at high population densities of both crops. This is evident in the reduction in the number of pods and branches per plant of sesame, cob length, and circumference, number of grains per cob, weight of grains of maize, and consequent lower yield of both crops compared to their respective sole stands.

Generally, when plants compete for light, they tend to grow taller by producing more nodes for the light interception. High plant density, according to Huang (2008) and Feng et al. (2010), increases the length of the basal internodes and reduces mechanical tissue and cortical thickness in maize. In the present study, there was an increased plant height accompanied by thinner stem girth, low aggressivity, and competitive ratio values at higher population densities, which further lends credence to this observation. In a much earlier study, Seran and

Brintha (2010) had observed that a reduction in seed yield in the intercrops where the full population of both crops was combined was due to overcrowding. This overcrowding, according to Purcell et al. (2002) and Hiyane et al. (2010), could decrease radiation use efficiency and competitive shading within the leaf canopy architecture.

The non-significant effects of plant density on plant height and the number of branches per plant of sesame of the population ratio tested as observed in the present study did not conform to the earlier reported work involving sesame and cowpea (Iftikhar et al., 2006) and sesame and maize (Ijoyah et al., 2016). This non-significant effect could be attributed to the differences in the time of introduction of component crops, planting patterns, and varietal differences among sesame genotypes. In the earlier studies, sesame and the component crops were planted at the same time and with maize planted on the top of the ridge and sesame by the side of the ridge. This planting pattern and time of introduction had given maize an advantage over sesame to shade the sesame and maize was able to intercept more light in view of the fact that it is naturally taller than sesame, either sole or intercropped.

The intercropping advantages, as measured by LER and LEC and observed in this study, have been reported by other researchers (Ijoyah et al., 2016). The implication is that growing sesame and maize in the mixture enables them to utilize natural resources better than when grown alone. This complementarity could be due to differences in the growth habit of the two crops, time of introduction of maize, and spatial arrangement employed. The A value was positive for sesame and negative for maize in all population ratios tested regardless of the spatial arrangement. The CR values were also found to be higher in sesame than in maize irrespective of population ratios and spatial arrangements. This indicated that sesame was more competitive and dominated maize with the implication that maize yield was lower than the expected yield, possibly due to poor utilization of natural resources. This is in agreement with the work of Afe (2014) on the sesame/cowpea intercrop, but contrary to the finding of Kolawole et al. (2015), who reported that maize was more competitive than sesame in a sesame/maize system. The discrepancy could be due to the time of introduction of maize and population ratios employed. In the earlier study, sesame and maize were simultaneously sown on the same day, whereas, in the present study, sesame was sown two weeks before maize. Planting sesame two weeks ahead of maize and with the corresponding full population gave sesame early establishment and apparently sesame was able to utilize the natural resources at the early stage better than the component maize. This is corroborated by the non-significant difference in the height of sesame and a significant difference in the maize height. Earlier, Ofori and Stern (1987) have reported a significant decrease in the yield of the latter introduced crop in *Phaseolus* beans/millet and maize/cowpea mixtures.

Conclusion

Intercropping significantly influenced the number of pods produced per plant and the seed yield of sesame. The number of grains per cob and the grain yield of maize were also influenced. Sesame was found to be more competitive and it dominated maize, possibly due to better utilization of natural resources. Based on LER and LEC values, the full population of sesame mixed with 50% population of maize irrespective of the arrangement is most suitable for intercropping in southern Guinea savannah ecological zone of Nigeria.

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UTICAJ PROSTORNOG RASPOREDA I GUSTINE USEVA KUKURUZA NA RAST I PRINOS SUSAMA (*SESAMUM INDICUM* L.) GAJENOG U ZDRUŽENOM USEVU SA KUKURUZOM

Afe A. Isaac[1*], Alabi K. Oyebisi[1], Ogundare S. Kayode[2] i Adeniji S. Mojisola[1]

[1]Odsek za ratarstvo, Univerzitet u Državi Kvara, Malete, Nigerija
[2]Odeljenje poljoprivrednih koledža, Univerzitet Ahmadu Bello, Kaba, Nigerija

R e z i m e

Poljski ogled izveden je na imanju za nastavu i istraživanje Univerziteta u Državi Kvara, Malete, Nigerija. Cilj ovog ogleda bio je da se istraži rast i prinos susama (*Sesamum indicum* L.) na koje utiče raspored i broj biljaka kukuruza u redu kukuruza (*Zea mais* L.). Broj biljaka susama u čistom usevu u dvoredom rasporedu (1:1) i (2:2) kombinovan je sa četiri gustine populacija kukuruza, 100S:100K; 100S:75K; 100S:50K i 100S:25K (gde S i K predstavljaju susam, odnosno kukuruz). Čisti usevi susama i kukuruza u punoj gustini uključeni su u tretmane kao kontrola. Na broj mahuna po biljci (BMB), dužinu plodne zone (DPZ) i prinos susama značajno je uticala ($P \leq 0,5$) interakcija prostornog rasporeda i broja biljaka u redu. Ove promenljive su se povećavale kako se gustina kukuruza u združenom usevu smanjivala. Na sve parametre izmerene kod kukuruza uticali su gustina populacije i raspored redova, osim broja klipova po biljci (BKB), dužine klipa (DK) i prečnik klipa (PK). Bez obzira na prostorni raspored i broj biljaka, vrednost agresivnosti (A) bila je pozitivna za susam i negativna za kukuruz. Vrednosti indeksa kompetitivnosti (KO) takođe su bile veće kod susama nego kod kukuruza. Vrednosti indeksa efikasnosti korišćenja zemljišta (EKZ) i koeficijenta korišćenja zemljišta (KKZ) za sve ispitivane odnose populacija, ukazale su na prednost združivanja useva sa najvišom vrednošću zabeleženom pri punoj gustini susama pomešenog sa polovinom broja biljaka kukuruza u odnosu na čist usev u rasporedu redova 2:2. Zbog toga se ovaj tretman preporučuje za korišćenje u praksi.

Ključne reči: konkurentski odnos, prinos zrna, efikasnost združene setve, združena setva, prostorni raspored.

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[*]Autor za kontakt: e-mail: adeafe22@yahoo.com

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HISTOPATHOLOGY OF *COLLETOTRICHUM SP*. IN INFECTED MANGO (*MAGNIFERA INDICA* L.) FRUITS

Oluwole O. Oladele*

Department of Biology, Federal University of Technology,
PMB 704, Akure, Nigeria

Abstract: Postharvest losses as a result of anthracnose infection remain a serious threat to mango producers. Hence, histopathology of mango fruits after being artificially infected with spores of *Colletotrichum* sp. was investigated. Fruits at the physiologically mature stage were wounded (cut) in the peels and inoculated with a spore suspension of *Colletotrichum* sp. (8.04×10^3 conidia $m1^{-1}$) and incubated at 28±2ºC for five days to allow pathogen establishment. The infected peel was then carefully cut with a razor blade and dehydrated in series in different grades (50, 70, 80, 90 and 100%) of ethyl alcohol for 1½ hours each. Histopathological studies were carried out on the infected peel tissue excised from inoculated fruits using standard procedures while unwounded peels of fruits that were not artificially inoculated served as control. Sections were examined by light microscopy to observe histopathological differences between the infected and non-infected fruits. Results from this study revealed that only the wounded peel showed symptoms of anthracnose infection as a result of the artificial inoculation, but the unwounded peel showed no disease symptoms. This showed that the fungus infected the mango fruits through the peel wounds. Besides, the disorganization of the cells and the rupture of the cell walls were observed microscopically, thus indicating disease establishment in the infected fruits. Therefore, mango producers should avoid mechanical damage to fruits during harvesting since this work confirms that the fungus infects mango fruits through wounds.

Key words: infection process, mango anthracnose, wounds, microscopy, inoculation.

Introduction

Mango (*Mangifera indica* L.) is a member of the family Anacardiaceae and one of the most important and widely cultivated fruits of the tropical world. It is a dominant species of fruit produced worldwide, followed by pineapples, papaya and avocado (FAO, 2013). Mangoes are cultivated mostly for their edible fruit (Singh

*Corresponding author: e-mail: prophetoladele2014@gmail.com

and Saini, 2017), rich in vitamins A and C, and they have a very good food value (Lauricella et al., 2017). The fruit is a large, fleshy drupe, containing an edible mesocarp which is resinous and an outer thin leathery part is the epicarp which is thick yellowish to reddish in color. The endocarp, the innermost, is hard and stony. In fact, the mesocarp together with the epicarp forms the pericarp or peel of the fruit (Tharanathan et al., 2006).

The two major fungal diseases of mango fruits are anthracnose caused by *Collectotrichum gloesporioides* or *C. acutatum*, characterized by the appearance of sunken black spots on the surface of the fruit during ripening (Afanador-Kafuri et al., 2003) and powdery mildew caused by *Oidium mangiferae*, evident by the formation of whitish, superficial, powdery fungal growth mainly on leaves, the stalk of panicles, flowers and fruits (Diedhlou et al., 2007). However, anthracnose is the most serious, causing considerable losses for the mango industry and is widely distributed all over mango growing regions in the world (Angasu et al., 2014). Namely, postharvest losses due to *C. gloeosporioides* (Penz and Sacc.) remains one of the biggest concerns for mango producers (Fivaz, 2009). Unfortunately, not much had been done on anthracnose disease of mango in the area of research in Nigeria. Besides, there is little or no information on the histopathology and pathogen infection of *C. gloeosporioides* in mango fruits. Thus, this work was conducted to study the histopathology of mango fruits after artificial inoculation with *Colletotrichum* sp. spores.

Materials and Methods

Isolations from infected fruits

Isolations were made from anthracnose lesions characterized by the appearance of black spots on the symptomatic mango fruits. The symptomatic rind was cut without surface sterilization, cultured on plates of solidified malt extract agar (MEA, oxoid) and then incubated at 28±2ºC for 10 days. Pure cultures of the obtained fungus were used to prepare the spore suspension.

Preparation of spore suspension

A ten-day-old agar slant culture of *Colletotrichum* sp. (the test isolate) on MEA was used to prepare the spore suspension. Sterile water was poured into the slant and shaken vigorously to dislodge the spores from the vegetative hyphae. The wash water was collected in a sterilized beaker, serially diluted to $\times 10^3$ and 1ml of the spore suspension was placed on the calibrated hemocytometer (Model 1280) slide and viewed under ×40 objective light binocular microscope (model Olympus

CX40). The spore count was measured under four different fields and calculated as follows:

Cells/ml = (n) × 10^3,

where n = average cell count per square of the four corner squares counted using *in vitro* inoculation and pathogenicity assay.

The spore suspension of *Colletotrichum* sp. (8.04×10^3 conidia $m1^{-1}$) was used to inoculate fresh mango fruits, cut 1mm at the equator and incubated at 28±2ºC and 75±5% of relative humidity (RH) inside sterilized desiccators. The infected fruits, observed after 5 days of incubation for anthracnose infection, were then used for histopathological studies. Fruits that were not inoculated served as control. Also, after 5 days of incubation, the pathogen was re-isolated, and its identity was confirmed.

Histopathology

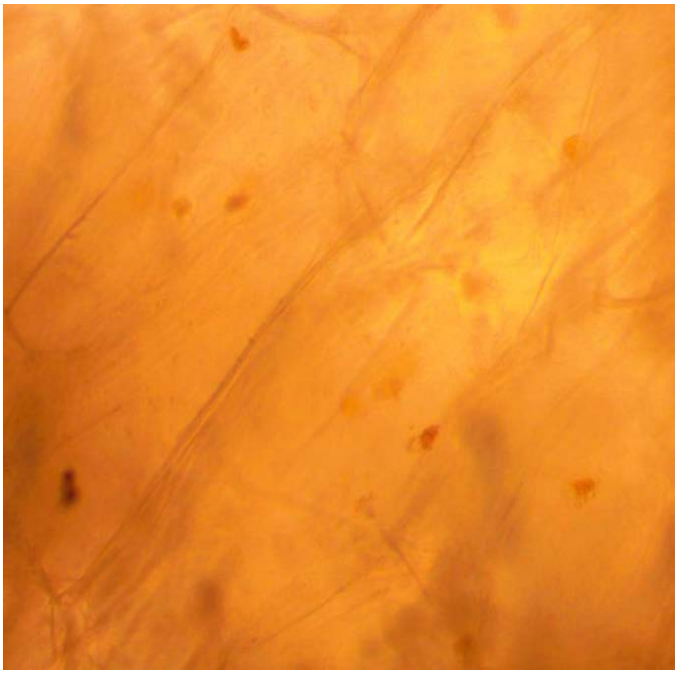
Histopathological studies were carried out on the peel tissue excised from inoculated fruits. The peel was carefully cut from the diseased portion of inoculated fruit with a new razor blade and dehydrated in series in different grades of ethyl alcohol for 1½ hours each (Lamb, 1981) and was later cleared with 100% xylene before being impregnated in molten paraffin wax overnight. The embedded tissues were sectioned to form ribbons (sections) using an HM 325 microtome. The ribbons were made to float on warm water (45ºC) and mounted on a slide and dried in an oven at 40ºC for 2 hours.

Before staining, the section was cleared in 100% xylene by dipping the section in different percentages of alcohol (50, 70, 80, 90 and 100%) to remove the wax, and finally washed in running tap water (hydration) to remove the alcohol. The hydrated sections were stained first with haematoxylin for 4 minutes, and the stained section differentiated in 1% acid alcohol and washed again in running tap water before counterstaining in eosin for 2 minutes. The counterstained section was washed in running tap water and dehydrated in different percentages of alcohol and then cleared in 100% xylene before adding Canada balsam. The preparation was left in the oven at 40ºC for 1 hour and then observed under the microscope (model Olympus CX40) fitted with a digital camera and photographs were taken. The procedure was also repeated on non-inoculated fruits.

Results and Discussion

The examination of several cross-sections of the peel tissue of the mango fruits using a light microscope revealed histopathological differences between the

healthy (uninfected) and the diseased mango fruits. Microscopic observation showed that the tissues of the healthy fruit had closely adhered cells. The cells were in perfect cohesion, and the cell walls were also intact (Figure 1). However, the *Colletotrichum* sp. colonized the tissues of the inoculated mango fruits, and showed long, thickening fungal hyphae in some tissues of the infected fruits and the cells had become disrupted (Figure 2) while in other tissues, many conidia were observed in the cells of the infected fruits (Figure 3). Meanwhile, the fungal hyphae appeared in the form of spherical vesicles in some cells (Figure 4).



X400

Figure 1. The photomicrograph of a cross-section of the uninoculated and healthy mango fruit showing intact cell walls.

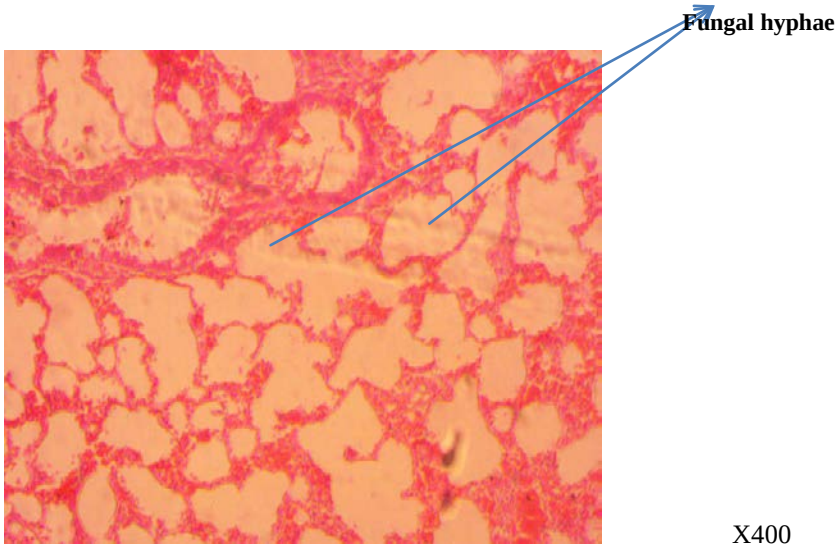


Figure 2. The photomicrograph of a cross-section of the inoculated mango fruit with *Collectotrichum* sp. showing the fungal hyphae in distorted cells.

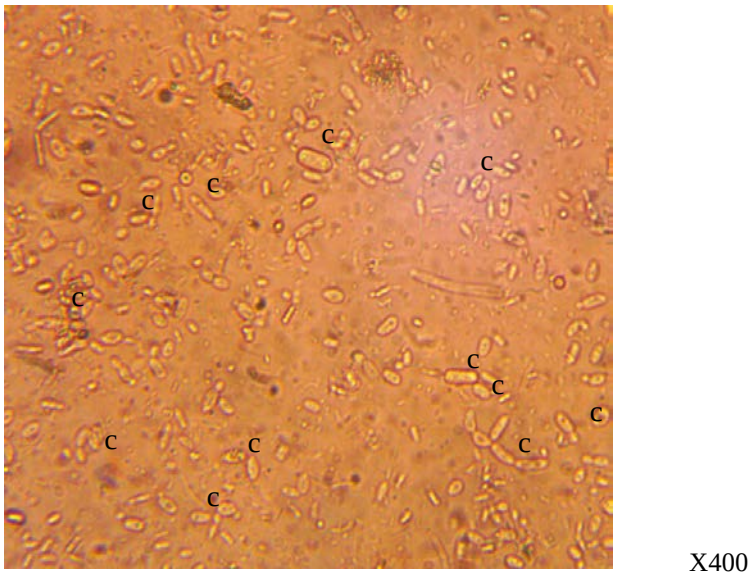


Figure 3. The photomicrograph of a cross-section of the infected mango fruit with *Collectotrichum* sp. showing the fungal conidia (**c**) in ruptured cells.

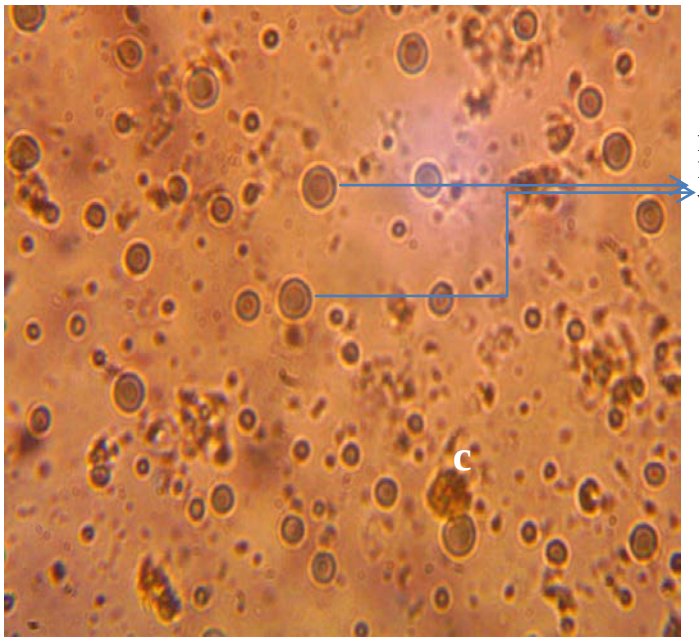


Figure 4. The photomicrograph of a cross-section of the infected mango fruit with *Collectotrichum* sp. showing fungal hyphae as spherical vesicles in disrupted cells.

Results from this study revealed that only the wounded peel showed symptoms of anthracnose infection, but the unwounded peel showed no disease symptoms. This showed that *Colletotrichum* sp. infected the mango fruits through cuts and by using its conidia. This was in conformity with the report of Jeffries et al. (1990) that *Colletotrichum* species penetrate host plant through wounds, natural openings or directly via appressorium. Arauz (2000), in his own work, has further reported and confirmed that *C. gloeosporioides* mainly infect through conidia that get easily dispersed by rain. Similar observations were made by Ferreira et al. (2005) but related to *C. fimbriata*. They also found aleurioconidia in *Eucalyptus* plants.

In fact, observation from this work also revealed the presence of hyphae and conidia in the infected cells distorting the cell structure. Hyphae were seen growing within and between the cells. This could not but be connected with the ability of the pathogenic fungi to invade the infected tissues causing changes in the cell structure and plasmolysis and dissolution of the infected cells (Pandey et al., 2012). O'Connell et al. (2000) have also reported that dissolution of the cell wall could be connected with hyphae penetration, after which the pathogen grows beneath the cuticle and inside the periclinal and anticlinal wall of the epidermal cells, causing dissolution of the cell wall.

Similarly, microscopic observation from this study also showed the fungal hyphae in the form of spherical vesicles. This was equally the observations of several authors who reported the occurrence of empty fungal hyphae of pathogens surrounded by amorphous material in the cells of many plant species (Bélanger et al., 2003; Rodrigues et al., 2003). After penetration, they form a spherical infection vesicle. Hyphae grow from this vesicle and subsequently colonize other host cells (O'Connell et al., 1985; Mould et al., 1991).

Conclusion

Mango producers should avoid mechanical damage to fruits during harvesting since this work has shown that the fungus infects mango fruits through wounds. Similarly, harvested fruits can be stored in plastics or bowls instead of inside woven baskets to protect the fruits against being wounded. Besides, knowledge of specialized infection structures such as hyphae and infection vesicles of *Colletotrichum* sp. as observed in this study may be an excellent tool for studying the infection processes of other post-harvest pathogens.

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HISTOPATOLOGIJA PLODA MANGA (*MAGNIFERA INDICA* L.) ZARAŽENOG GLJIVOM *COLLETOTRICHUM SP*.

Oluwole O. Oladele[*]

Odsek za biologiju, Federalni tehnološki univerzitet,
PMB 704, Akure, Nigerija

R e z i m e

Gubici koji se javljaju nakon berbe, kao rezultat antraknoze, predstavljaju ozbiljnu pretnju za proizvođače manga. Stoga je istražena histopatologija plodova manga posle veštačke infekcije sporama *Colletotrichum* sp. Kore plodova u fiziološki zreloj fazi su povređene, inokulisane suspenzijom spora *Colletotrichum* sp. ($8{,}04 \times 10^3$ konidija $m1^{-1}$) i plodovi su inkubirani na 28±2ºC tokom pet dana kako bi se razvila infekcija. Zaražena kora je zatim pažljivo isečena oštricom žileta i dehidrirana u nizu rastvora različitih koncentracija (50, 70, 80, 90 i 100%) etil alkohola u trajanju od 1,5 časa. Histopatološka ispitivanja zaraženog tkiva kore inokulisanih plodova izvršena su pomoću standardnih postupaka, dok je kora nepovređenih plodova, koji nisu veštački inokulisani, služila kao kontrola. Isečci su pregledani svetlosnim mikroskopom, kako bi se uočile histopatološke razlike između zaraženih i nezaraženih plodova. Rezultati ove studije pokazali su da se antraknoza javila samo na povređenoj kori nakon veštačke inokulacije, dok se simptomi bolesti nisu javili na nepovređenoj kori. Rezultati pokazuju da je gljiva zarazila plodove manga kroz povrede/rane na kori. Pored toga, dezorganizacija ćelija i razgradnja ćelijskih zidova uočeni su mikroskopskim pregledom tkiva, što ukazuje na razvoj bolesti u zaraženim plodovima. Stoga proizvođači manga treba da izbegavaju mehanička oštećenja plodova tokom berbe, jer ovaj rad potvrđuje da gljiva zaražava plodove manga preko povreda/rana.

Ključne reči: proces zaražavanja, antraknoza manga, povrede/rane, mikroskopija, inokulacija.

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[*]Autor za kontakt: e-mail: prophetoladele2014@gmail.com

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EVALUATION OF ERODIBILITY INDICES AND SOIL PROPERTIES AFFECTED BY LAND-USE TYPES IN MBANO, SOUTH-EAST NIGERIA

Donald N. Osujieke[1*], Bernardine N. Aririguzo[2] and Chioma M. Ahukaemere[2]

[1]Department of Soil Science and Land Resources Management, Federal University Wukari, PMB, 1020, Wukari, Taraba State, Nigeria
[2]Department of Soil Science and Technology, Federal University of Technology Owerri, PMB 1526 Owerri, Imo State, Nigeria

Abstract: Soil erosion has been at the forefront of the degradation of soils under different land-use types in southeast Nigeria. Hence, this research aimed to determine the erodibility of the soils under different land-use types in Mbano. The four land-use types selected were oil palm plantation (OP), cassava farm (CF), pineapple orchard (PO) and plantain plantation (PP). However, clay dispersion ratio (CDR), clay dispersion index (CDI) and clay flocculation index (CFI) were the erodibility indices used. A total of 5 composite samples were collected randomly from each type of the land-uses at a depth of 0–20 cm using the free survey. The samples were subjected to laboratory analysis. Data generated were analyzed statistically using a completely randomized design of analyses of variance (ANOVA) and correlation. CDR had an increasing order of 47.9% <48.16% <51.49% <56.24% for soils under CF, PP, PO and OP, while CDI had 36.44%, 29.06%, 40.96% and 49.04% for soils under OP, CF, PO and PP land uses. CFI had 63.62%, 71.24%, 59.14% and 50.96% for soils under OP, CF, PO and PP, respectively. The ANOVA indicated that studied erodibility indices had no significant difference (p=0.05) among the soils under the different land-uses. The research will unite farmers and other land-users in adopting only good conservation practices that will aid the sustainability of the area.

Key words: clay dispersion ratio, clay dispersion index, clay flocculation index, land-uses, soil properties, erosion.

Introduction

Soil erosion depends on the erosivity of the rainfall and erodibility of soil. The soil erodibility depends primarily on the nature and amount of soil aggregates, organic matter content, hydraulic conductivity, root abundance, and particle size

*Corresponding author: e-mail: bigdonax@yahoo.com

distribution. According to Nyakatawa et al. (2001), soil erosion is a major environmental problem worldwide. Water erosion carries soil nutrients, pesticides and other harmful chemicals into rivers, streams and groundwater. Eroded soils are deposited in water systems leading to pollution and siltation, which brings about reduction of water quantity, and eventual siltation and drying up of rivers, water reservoirs and dams. Hence, the aquatic life is eventually eliminated and land productivity reduced drastically. Soil erosion is one of the most serious forms of land degradation in the world (Sohan and Lal, 2001). Elirehema (2001) states that more than 56% of land degradation is caused by soil erosion, raising a global concern on land productivity. Changes in land-use due to urbanization, agricultural expansion, deforestation, and monoculture productions have led to an accelerated and spatial increase in erosion.

Agassi and Bradford (1999) have found that erodibility varies with soil textures, aggregate stability, shear strength, soil structures, infiltration capacity, soil depth, bulk density and soil organic matter. However, soil erosion in areas of very intense rainfall has been estimated using water-dispersible clay and its indices (Igwe and Agbatah, 2008; Calero et al., 2008). Seta and Karathanasis (1996) and Heathwaite et al. (2005) have ascertained that clay and silt dispersions, when soils are submerged in water, affect a lot of soil physical and chemical properties such as shrink-swell for soils with very high clay contents, water-retention characteristics and hydraulic conductivity, crusting and sealing. Soil erodibility can be determined using various soil erodibility indices based on soil characteristics. Erodibility indices like clay dispersion ratio, clay dispersion index, clay flocculation index have been employed by different authors (Oguike and Mbagwu, 2009; Chris-Emenyonu and Onweremadu, 2011) to assess the soil erodibility. Igwe (2005) has remarked that the clay-dispersion ratio and clay dispersion index are good indices for predicting erodibility in soils of south-eastern Nigeria. However, not much information is available on these erodibility indices for the soils of south-eastern Nigeria. According to Igwe and Udegbunam (2008), CFI is a perfect index for predicting soil erodibility and a useful micro-aggregate index. Soils high in CFI are well aggregated and will not easily disperse in water.

Therefore, there are needs to provide the erodibility data that will enable agriculturists, engineers and other land-users to know the areas prone to erosion. These will enable them to provide control measures and embrace sustainable land-use practices that will help to check the menace in the future. Hence, the study was undertaken to evaluate the erodibility indices and soil properties affected by different land-use types in Mbano, south-eastern Nigeria.

Materials and Methods

Study area

The sites are situated at Mbano in Imo State, south-eastern Nigeria. The area lies between latitude 5°35' N to 5°48' N and longitude 7°02' E to 7°18' E. A tropical wet and dry season prevails in the study area. The wet season extends from March to October with peaks in June/July and September (Iloeje, 2002). In some years, rainfall may be prolonged while the onset may be delayed in some other years. The area has an annual mean rainfall of 2000 mm and an annual mean temperature of 27°C (NIMET, 2015). The area is geomorphologically plain and nearly flat with a gentle slope, while dominant vegetation types are tropical rainforests (Onweremadu and Uhuegbu, 2007). The study sites comprised four land-use types, including oil palm plantation (OP), cassava farm (CF), pineapple orchard (PO) and plantain plantation (PP).

Soil sampling and analysis

Soil samples were randomly collected at a depth of 0–20 cm from surface soils at different sampling points on each of the four land-use types. The collected samples were air-dried, sieved and subjected to routine laboratory analyses. Particle size distribution was determined by the Bouyoucos hydrometer method (Gee and Or, 2002). Soil pH was determined electrometrically using the glass electrode pH meter in a solid-liquid (water) ratio of 1:2.5 (Hendershot et al., 1993). Exchangeable bases were determined by the neutral ammonium acetate procedure buffered at pH 7.0 (Thomas, 1982). Exchangeable acidity was determined by a method described by McLean (1982). Total carbon was determined by the wet digestion method (Nelson and Sommers, 1982). The soil moisture content at saturation was determined by the Klute method (1986). Soil bulk density was determined by the core method (Grossman and Reinsch, 2002). Total porosity (P_o) was obtained from bulk density (ℓp) values with an assumed particle density value of (ℓs) 2.65 gcm^{-3} as follows, Porosity (Po) = $100 - (\ell p/\ell s) \times 100/1$.

The clay-dispersion indices were calculated as follows:

Clay dispersion ratio (DR) = {(% silt + % clay (H_2O)} / {% silt + % clay (calgon)} × 100;

Clay dispersion index (CDI) = {[% clay (H_2O)] / [% clay (calgon)]} × 100;

Clay flocculation index (CFI) = [% clay (calgon)] - [% clay (H_2O)] / [% clay (calgon)] × 100.

The higher the CDR and CDI, the higher the ability of the soil to disperse while the higher the CFI, the better aggregated the soil. The clay dispersion ratio was used to determine the erodibility of the soils. Hence, soils with a clay dispersion ratio greater than 15% are erodible, and with less than 15% are not erodible.

Statistical analysis

Analysis of variance (ANOVA) for a completely randomized design (CRD) was used to compare the influence of the land-use types. Similarly, the least significant difference (LSD) at the 5% probability level was used for comparison. The correlation was used to determine the relationship between erodibility indices and selected soil properties.

Results and Discussion

Table 1 shows that the textural class of the studied site is predominantly sandy loam and loamy sand. Sand particle dominated among the soil particles of the different land-uses with mean values ranging from 829.60 to 857.56 g/kg. The sandiness of the soils suggests low moisture content and high porosity which indicate high infiltration and erosion. There was no significant difference in sand content among different land-uses, which could be associated with homogeneity in the parent material and climatic conditions. The sandy nature agrees with the findings of Enwezor et al. (1990) and Ahukaemere et al. (2012) that soils reflect the parent material from which they originated. However, soils that have high sand content are prone to erosion due to a low binding agent. There was no difference among soils under OP, CF, PO and PP land-uses in terms of silt particles. The level of silt content is in concurrence with the findings of Osujieke et al. (2017) in soils of south-eastern Nigeria. The OP soils (105.76 g/kg) had the highest clay particle while the PO soils (84.48 g/kg) had the lowest among the studied land-uses. However, the land-uses indicated the low clay content which agrees with the findings of Malgwi et al. (2000) and Wakene (2001) and it is attributed to sorting of soil minerals by biological and/or agricultural activities, clay migration or surface erosion by runoff or combination of these. There was no significant difference ($p = 0.05$) in clay content among the different land-uses. This conforms to the findings of Onweremadu and Mbah (2009) that suggest the effect of climate on the area under the same agro-ecological zone. The presence of clay material provides the required bondage between the varying soil particles resulting in the formation of more stable aggregates which makes them less susceptible to erosion. However, clay absence reduces the capacity of the soil particles to bind together and form aggregates that can resist the shearing force of flowing water, thus making the soils vulnerable to soil erosion. This is in compliance with the finding of Parfitt and Salt (2001), who have indicated that higher clay content of soils reduces erodibility. Hence, soils with low clay content are more prone to erosion and have lower binding forces and poor cohesion. Bulk density had mean values ranging from 0.96 to 1.14 g/cm^3 among the different land-uses. However, bulk density of soils of CF and PP differed significantly ($p= 0.05$) from that of OP.

while the bulk densities of soils of CF, PO, and PP land-use types showed no significant difference. This was in agreement with the work of Lemenih et al. (2005), who have postulated that the significant difference is due to land management practices and land-use history. Heard et al. (1988) have stated that soil bulk density is an important property in relation to water erosion since it determines the level of compaction, structural development, and cohesiveness of soils. However, the soil of PP land-use type had the highest bulk density, which can lead to losses by surface runoff among the land-uses due to a low infiltration rate. This is in concurrence with the findings of Weil and Brady (2016). Soil moisture content was low in all the studied land-uses, which could be associated with high sand content, low organic matter and high porosity.

Table 1. Soil physical properties of the studied land-uses.

Rep	Sand	Silt	Clay	TC	BD	MC	Po
		g/kg			(g/cm^3)		%
OIL PALM PLANTATION (OP)							
1	858.40	31.60	110.00	SL	0.95	9.20	64.20
2	887.60	28.00	84.40	LS	1.02	10.14	62.00
3	811.00	70.00	119.00	SL	0.88	21.61	66.80
4	822.40	66.20	111.40	SL	1.07	7.28	59.60
5	830.00	66.00	104.40	SL	0.90	6.99	66.10
Mean	841.88^{a}	52.36^{a}	105.76^{a}		0.96^{b}	11.04^{a}	63.74^{a}
CASSAVA FARM (CF)							
1	858.00	64.00	78.00	LS	1.02	9.69	61.50
2	862.40	31.60	106.00	SL	1.06	9.64	60.00
3	861.00	60.00	79.00	SL	1.25	7.86	52.80
4	852.40	50.00	97.60	SL	1.09	11.04	58.90
5	854.00	72.00	74.00	LS	1.12	12.07	57.70
Mean	857.56^{a}	55.52^{a}	86.92^{a}		1.11^{a}	10.06^{a}	58.18^{b}
PINEAPPLE ORCHARD (PO)							
1	872.60	31.80	95.60	LS	0.96	15.36	63.80
2	810.00	100.00	90.00	SL	0.96	5.83	63.80
3	862.40	80.00	57.60	LS	1.11	4.45	58.10
4	814.00	91.40	94.60	SL	1.20	12.08	54.70
5	870.00	45.40	84.60	LS	1.10	15.84	58.50
Mean	845.80^{a}	69.72^{a}	84.48^{a}		1.07^{ab}	10.71^{a}	59.78^{ab}
PLANTAIN PLANTATION (PP)							
1	841.00	56.90	102.10	LS	1.11	10.56	58.10
2	811.00	40.00	149.00	SL	1.12	8.33	57.70
3	810.00	114.00	76.00	LS	1.25	12.26	51.20
4	840.00	118.60	41.40	LS	1.05	13.26	60.40
5	846.00	37.60	116.40	SL	1.16	11.74	56.23
Mean	829.60^{a}	66.65^{a}	96.98^{a}		1.14^{a}	11.23^{a}	56.73^{b}
$LSD_{(0.05)}$	31.84^{NS}	37.57^{NS}	31.94^{NS}		0.117	5.646^{NS}	4.609

Rep= replicate; TC= textural class; SL= sandy loam; LS= loamy sand; BD= bulk density; MC= moisture content; Po= porosity; LSD= least significant difference; ^{NS}= not significant.

The result, as indicated in Table 2, shows that the soil pH (H_2O) had mean values ranging from 4.97 to 5.48 among the different land-uses. The soils under the land-use types were moderately–strongly acidic according to the rating of Chude et al. (2011). The CF soil was the most acidic while the PP soil was the least acidic. The acidic nature of the soils of the various land-use types could be attributed to organic acids released by litter decomposition (Jandl et al., 2004) and parent material.

Table 2. Soil chemical properties of the studied land-uses.

Rep	pH	Ca^{2+}	Mg^{2+}	K^+	Na^+	TEB	TEA	ECEC	OC
	(H_2O)				$cmolkg^{-1}$				(%)
OIL PALM PLANTATION (OP)									
1	4.68	0.20	0.28	0.02	0.04	0.54	5.94	6.48	3.42
2	4.69	0.12	0.18	0.03	0.05	0.38	7.14	7.52	2.82
3	5.63	0.26	0.42	0.02	0.03	1.33	1.60	2.93	2.80
4	5.74	0.40	0.12	0.02	0.03	0.57	1.20	1.77	2.78
5	5.78	1.00	1.10	0.02	0.02	2.14	3.30	5.44	2.76
Mean	5.30^{ab}	0.40^{b}	0.42^{b}	0.02^{a}	0.03^{ab}	0.99^{ab}	3.84^{a}	4.83^{a}	2.92^{a}
CASSAVA FARM (CF)									
1	5.05	0.48	0.12	0.01	0.04	0.65	2.56	3.21	2.49
2	5.10	0.22	0.50	0.02	0.08	0.82	3.36	4.18	3.25
3	4.82	0.08	0.60	0.02	0.03	0.73	4.54	5.27	2.66
4	4.96	0.10	0.06	0.01	0.04	0.75	1.20	1.95	2.63
5	4.91	0.18	0.32	0.04	0.06	0.60	4.00	4.60	3.31
Mean	4.97^{b}	0.21^{b}	0.43^{b}	0.02^{a}	0.05^{a}	0.71^{b}	3.13^{a}	3.84^{a}	2.87^{a}
PINEAPPLE ORCHARD (PO)									
1	4.86	0.23	1.45	0.04	0.02	1.74	7.52	9.26	3.38
2	5.66	0.06	1.14	0.02	0.02	1.24	0.32	1.56	3.00
3	5.48	0.04	1.52	0.03	0.01	1.60	4.80	6.40	2.59
4	5.10	0.18	1.12	0.03	0.02	1.35	3.92	5.27	2.94
5	5.54	0.50	0.60	0.03	0.01	1.14	1.80	2.94	2.49
Mean	5.33^{ab}	0.20^{b}	1.17^{a}	0.03^{a}	0.02^{b}	1.41^{a}	3.67^{a}	5.09^{a}	2.88^{a}
PLANTAIN PLANTATION (PP)									
1	5.05	0.36	0.54	0.02	0.01	0.93	3.52	4.45	2.45
2	5.40	1.68	0.52	0.06	0.04	2.30	0.80	3.10	2.35
3	5.64	1.32	0.24	0.03	0.01	1.60	2.96	4.56	2.24
4	5.65	0.86	0.42	0.01	0.03	1.32	0.32	1.64	2.18
5	5.68	1.14	0.50	0.05	0.04	1.73	0.26	1.99	2.20
Mean	5.48^{a}	1.07^{a}	0.44^{b}	0.03^{a}	0.03^{ab}	1.58^{a}	1.57^{a}	3.15^{a}	2.28^{b}
$LSD_{(0.05)}$	0.483	0.440	0.402	0.017^{NS}	0.019	0.627	2.901^{NS}	2.874^{NS}	0.405

Rep= replicate; TEB= total exchangeable bases; TEA= total exchangeable acidity; ECEC= effective cation exchange capacity; OC= organic carbon; $LSD_{(0.05)}$= least significant difference at the 5% probability level; ^{NS}= not significant.

The result also indicated that the pH of PP soils differed significantly (p=0.05) from that of CF soils and had a non-significant difference with OP and PO soils.

The significant difference could be associated with vegetation type, leaching/plant uptake of basic cations and soil management practices. Organic carbon (OC) had means of 2.92%, 2.87%, 2.88%, and 2.28% for soils under OP, CF, PO and PP land-use types, respectively. They were low when compared to critical values of 3.55% for Eastern Nigeria (Akinrinde and Obigbeson, 2000). The generally low level of OC could be attributable to plant uptake, leaching, and climatic factors. However, soils under OP had high organic carbon when compared to that of other land-uses. This could be attributed to the amount of litter deposit and a high rate of decomposition. The OC had no significant difference (p 0.05) among soils under OP, CF, and PO land-uses, but OC of soil under PP differed significantly (p=0.05) from that of soils under OP, CF, and PO land-uses. The difference could be associated with vegetation cover, runoff, and litter size. Morgan (2001) has reported that soil erodibility decreases linearly with increasing soil organic matter content. However, organic matter and chemical constituents of the soil are important because of their influence on the stability of aggregates which reduces erodibility. The total exchangeable bases of the soil were dominated by Mg for soils under OP, CF and PO land-uses while calcium dominated the exchangeable bases for the soil under PP land-use. However, according to the ratings of Landon, calcium, magnesium, potassium, and sodium were very low in soils under the land-use types except for magnesium that was moderate in the soil under PO. The effective cation exchange capacity was generally low according to the rating of Landon. However, total exchangeable acidity dominated over the total exchangeable bases.

Erodibility indices

Table 3 indicates the result of the clay dispersion ratio (CDR), clay dispersion index (CDI), and clay flocculation index (CFI), under the different land-use types. The clay dispersion ratio has mean values ranging from 47.92 to 56.24% for soils under the different land-uses, while the CDI has mean values ranging from 29.06 to 49.04% for soils under the different land-uses. However, CFI had a mean of 63.62%, 71.24%, 59.14%, and 50.96% for soils under OP, CF, PO, and PP land-uses, respectively. The CDR and CFI values were contrary to the finding of Igwe and Udegbunam (2008) in southern Nigeria. The clay dispersion ratio showed no significant difference (p=0.05) among the soils of the different land-use types. Soils of the studied land-use types were found to be sensitive to erosion since they are above 15% as recommended by Middleton (1930). The clay dispersion index for the soil under the PP land-use differed significantly (p=0.05) from the soil under the CF land-use. However, the clay dispersion index had no significant difference among OP, CF, and PO land-uses. The clay flocculation index for the soil under the CF land-use differed significantly (p=0.05) from that for the soil

under the PP land-use, but CFI had no significant difference among the soil under OP, PO, and PP land-uses. According to Agassi and Bradford (1999) and Igwe (2012), the variation among the erodibility indices under different land-use types could be associated to soil textures, aggregate stability, shear strength, soil structures, infiltration capacity, soil depth, bulk density, soil organic matter and chemical constituents.

Table 3. Erodibility indices of the studied land-uses.

Replicate	CDR	CDI%	CFI
OIL PALM PLANTATION (OP)			
1	79.38	23.60	76.46
2	64.41	41.20	58.80
3	56.83	64.00	36.10
4	38.00	28.00	72.20
5	42.60	25.40	74.60
Mean	56.24^{a}	36.44^{ab}	63.63^{ab}
CASSAVA FARM (CF)			
1	36.90	21.00	79.50
2	49.00	24.90	75.10
3	38.00	27.00	73.40
4	55.80	17.00	83.20
5	59.90	55.40	45.00
Mean	47.92^{a}	29.06^{bc}	71.24^{a}
PINEAPPLE ORCHARD (PO)			
1	56.83	38.00	62.30
2	46.00	51.60	48.40
3	45.40	45.10	54.90
4	49.70	33.00	67.20
5	59.53	37.10	62.90
Mean	51.49^{a}	40.96^{ab}	59.14^{ab}
PLANTAIN PLANTATION (PP)			
1	64.40	64.70	35.30
2	49.20	31.10	68.90
3	37.20	34.20	66.00
4	36.10	62.80	37.20
5	53.90	52.40	47.40
Mean	48.16^{a}	49.04^{a}	50.96^{bc}
$LSD_{(0.05)}$	15.99^{NS}	19.17	19.19

Rep= replicate; CDR= clay dispersion ratio; CDI= clay dispersion index; CFI= clay flocculation index; $LSD_{(0.05)}$= least significant difference at the 5% probability level.

The CFI index was greater than the CDR and CDI indices in all the studied land-uses as rated in percentage. However, the CDR was greater than the CDI in all the studied land-uses except for the PP land-use where the CDI (49.04%) was greater than the CDR (48.16%). Soils high in CFI are well aggregated and will not easily disperse in water. Igwe (2005) opined that CFI ranked highest among other

micro and macro aggregate indices in predicting potential soil loss in some soils of south-eastern Nigeria. Soils with high CDR and CDI are known to be structurally weak and can easily erode. Also, the high value of CDR with low CFI indicates low resistance of soil aggregates to the breakdown by water. This is in conformity with the findings of Bajracharya et al. (1992). Research findings showed that soil with a relatively low erodibility factor might indicate signs of severe erosion. However, the soil could be highly erodible and suffer little erosion because soil erosion is a function of many factors (Nyakatawa et al., 2001). However, Chakrabarti (1990) has reported that soils susceptibility to erosion is significantly related to the clay dispersion ratio. This result, therefore, indicates that the soils from the studied area are susceptible to erosion. Similarly, CDR, CDI and CFI depend on the aggregation of soil particles.

The relationship between erodibility indices and soil properties

The result, as indicated in Table 4, shows that CDI correlated positively (r=0.25, r=0.10, r=0.38) with silt, porosity and moisture content but had a negative correlation (r=-0.17, r=-0.21, r=-0.094, r=-0.155, r=-0.19) with sand, clay, bulk density, pH(H_2O) and OC. CFI had a positive correlation (r=0.17, r=0.21, r=0.095, r=0.19) with sand, clay, bulk density and OC while it had a negative relationship with silt, moisture content and pH (H_2O). CDR had a positive significant relationship (r=0.47, p=0.05) with OC and a negatively relationship (r=-0.286, r=-0.527) with bulk density and pH(H_2O) while it had a negative significant relationship (r=-0.56, p=0.05) with silt. Igwe and Udegbunam (2008) reported that CFI correlated positively with clay and OC. The sign of the coefficient indicates the direction of the relationship. If both variables tend to increase or decrease together, the coefficient is positive, whereas if one variable tends to increase as the other decreases, the coefficient is negative.

Table 4. The relationship between erodibility indices and selected soil properties.

Soil properties	CDI	CFI%	CDR
Sand (g/kg)	-0.173	0.174	0.366
Clay (g/kg)	-0.213	0.209	0.322
Silt (g/kg)	0.336	-0.333	-0.601*
BD (g/cm^3)	-0.094	0.095	-0.286
Po (%)	0.096	-0.097	0.307
MC (%)	0.381	-0.379	0.272
pH(H_2O)	-0.155	-0.283	-0.527
ECEC (cmol/kg)	-0.155	0.157	0.359
OC (%)	-0.190	0.192	0.469*

ECEC= effective cation exchange capacity; OC= organic carbon; Po= porosity; MC= moisture content; CDR= clay dispersion ratio; CDI= clay dispersion index; CFI= clay flocculation index.

Table 5 shows the relationship between the erodibility indices. The CDR had a positive relationship (r=0.143) with CDI, whereas it had a negative relationship (r=-0.146) with CFI. However, CDI had a highly significant negative relationship (r=-1.000, p=0.01) with CFI.

Table 5. The relationship between erodibility indices.

	CDR	CDI	CFI
		%	
CDR	1		
CDI	0.143	1	
CFI	-0.146	-1.000**	1

CDR= clay dispersion ratio; CDI= clay dispersion index; CFI= clay flocculation index.

Conclusion

The study assessed erodibility indices relationship with soil properties affected by land-use types. According to the evaluation made for three erodibility indices (CDR, CDI and CFI), it was found that soils of the study area are susceptible to erosion. The erodibility varied among the land-use types in an increasing order of CF<PP<PO<OP, which is an indication that soils of CF were the least erodible. However, preventive measures against erosion should be taken through modifications of the regular management procedures, which will ameliorate the rate of soil degradation. In order to prevent soil erosion, some preventive measures such as planting of cover crops, application of organic matter and mulching should be put in place as these will help ensure proper aggregate stability, required infiltration rate and soil productivity. Also, activities such as topsoil mining, indiscriminate bush burning, and deforestation should be avoided. The government should conduct enlightenment programmes for the public on sustainable environmental conservation practices.

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OCENA INDEKSA ERODIBILNOSTI I SVOJSTAVA ZEMLJIŠTA USLOVLJENIH RAZLIČITIM NAČINIMA KORIŠĆENJA ZEMLJIŠTA U MBANU, JUGOISTOČNA NIGERIJA

Donald N. Osujieke[1*], Bernardine N. Aririguzo[2] i Chioma M. Ahukaemere[2]

[1]Odsek za pedologiju i upravljanje zemljišnim resursima, Federalni univerzitet Vukari, PMB, 1020, Vukari, Država Taraba, Nigerija
[2]Odsek za pedologiju i tehnologiju zemljišta, Federalni tehnološki univerzitet Overi, PMB 1526 Overi, Država Imo, Nigerija

R e z i m e

Erozija zemljišta je najizraženiji vid degradacije zemljišta u okviru različitih načina korišćenja zemljišta na jugoistoku Nigerije. Stoga je cilj ovog istraživanja da se odredi erodibilnost zemljišta u okviru različitih načina korišćenja zemljišta u Mbanu. Odabrana su četiri različita načina korišćenja zemljišta: plantaža uljane palme (UP), zasad manioke (ZM), zasad ananasa (ZA) i plantaža banana (PB). Međutim, koeficijent disperznosti gline (KDG), indeks disperznosti gline (IDG) i indeks flokulacije gline (IFG) su ispitivani indeksi erodibilnosti. Ukupno 5 kompozitnih uzoraka je nasumično prikupljeno iz svakog od načina korišćenja zemljišta sa dubine od 0–20 cm. Uzorci su ispitivani u laboratoriji. Dobijeni podaci su analizirani statistički prema potpuno slučajnom planu korišćenjem analize varijanse (ANOVA) i korelacije. KDG je imao rastući redosled vrednosti od 47,92% <48,16% <51,49%, i <56,24% za zemljišta pod ZM, PB, ZA i UP, respektivno, dok je IDG imao vrednosti od 36,44%, 29,06%, 40,96% i 49,04% za zemljišta pod UP, ZM, ZA i PB. IFG je imao vrednosti od 63,62%, 71,24%, 59,14% i 50,96% za zemljišta pod UP, ZM, ZA odnosno PB, respektivno. ANOVA je ukazala da proučeni indeksi erodibilnosti nisu imali značajne razlike (p = 0,05) među različitim načinima korišćenja zemljišta. Istraživanje će udružiti poljoprivrednike i ostale korisnike zemljišta da usvoje samo dobre prakse konzervacije zemljišta, koje će pomoći održivosti celokupnog područja.

Ključne reči: koeficijent disperznosti gline, indeks disperznosti gline, indeks flokulacije gline, način korišćenja zemljišta, svojstva zemljišta, erozija.

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[*]Autor za kontakt: e-mail: bigdonax@yahoo.com

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GENETIC CHARACTERIZATION OF FARMED AND WILD POPULATIONS OF AFRICAN CATFISH (*CLARIAS GARIEPINUS* BURCHELL, 1822) USING THE RANDOM AMPLIFIED POLYMORPHIC MARKER

Sa'adu B. Suleiman*, Mohammed Y. Diyaware, Mohammed Aliyu and Zanna B. Mohammed

Department of Fisheries, University of Maiduguri, Faculty of Agriculture, Nigeria

Abstract: The genetic characterization of *Clarias gariepinus* was conducted in this study. Thirty (30) *C. gariepinus* specimens were collected, fifteen (15) each from the wild and farms in northeastern Nigeria for their genetic relatedness and diversity using the RAPD markers. DNA extraction from the blood sample was performed using the Gene Jet Genomic DNA Purification Kit. Five primers were used in employing PCR and amplified 402 RAPD bands from the four strains of *Clarias gariepinus*. Three hundred and six (76.12%) bands were polymorphic while 86 (21.39%) were monomorphic. The percentage of polymorphism obtained from farmed and wild populations ranged from 58 (47.3%) to 69 (75.9%), respectively. The polymorphic bands per loci within the populations ranged from 67.9% to 82.0%. The mean numbers of inbreeding coefficient (FIS) were 0.083 and 0.053 in the farmed and wild populations. Gene diversity values within farmed and wild populations (Ht) were 0.4522 and 0.4018. The mean genetic differentiation (FST) ranged between (0.203) in the farmed and 0.129 in the wild populations. The analysis of molecular variance revealed that there was 96% of genetic variance within the population and 4% among the population. The genetic identity and distance of four populations were 0.9490 and 0.1038, respectively. The phylogenetic measure has shown that the four strains were divided into two clusters at approximately 0.089 similarity levels. The result indicated a significant level of genetic variation and minimal dendrogram separation in *Clarias gariepinus* in northeastern Nigeria. Conclusively, this information will be a useful tool for the genetic and breeding program of *Clarias gariepinus*.

Key words: RAPD, genetic variation, *Clarias gariepinus*, polymorphism, Nigeria, markers.

*Corresponding author: e-mail: saadbala@unimaid.edu.ng

Introduction

Clarias gariepinus is the most important and suitable catfish species for tropical aquaculture in Africa (Barasa et al., 2017). The *C. gariepinus* production in Nigeria exceeded that of Nile tilapia (*Oreochromis niloticus*), due to its high fecundity and fast growth, which contributes for about 70–80% of the total freshwater fish production (Ikpeme et al., 2015). It is found all over the country because it is the major culturable fish species in Nigeria. *Clarias gariepinus* has been introduced into most African countries, as well as several countries in Europe, Asia and South America (FAO, 2013). It is also generally considered to be one of the economically important freshwater fish species for rearing, whose aquaculture potential has been documented (Dada and Wanah, 2003). Species identification and its genetic structure are a crucial issue for the economic importance of *C. gariepinus*. The traditional method of the characterization of *Clarias gariepinus* has been used to evaluate this species, and it has not been found to be a reliable method for species identification (Asagbra et al., 2014). Molecular markers such as Random Amplified Polymorphic DNA are used for the genetic identification of fish species.

The Random Amplified Polymorphic DNA (RAPD) PCR method is a technique for the assessment of genetic markers that are capable of differentiating between species and subspecies of organisms. Soufy et al. (2009) reported that the identification of species was possible using the Random Amplified Polymorphic DNA (RAPD) method as a molecular marker. Various authors have seen RAPD analysis as the basis for determining polymorphisms based on allele frequency and amplification of DNA segments with a single primer of the arbitrary nucleotide sequence. The levels of DNA polymorphisms can be detected using this method by the presence or absence of amplification products when two strains or individuals are compared (Williams et al., 1990; Ali et al., 2004; Soufy et al., 2009; Asagbra et al., 2014).

Several studies have been carried out to determine the genetic diversities and characterization of fish species using RAPD such as *Clarias gariepinus* (Saad et al., 2009; Popoola et al., 2014); *Ompok bimaculatus* (Rashid et al., 2012); Tilapia species (Ahmed et al., 2004; Soufy et al., 2009; Usman et al.,2013; Asagbra et al., 2014). Other molecular markers used for the genetic evaluation of fish species are microsatellite markers (Galbusera et al., 1996; Agnèse et al., 1997; Agbebi et al., 2013; Barasa et al., 2017); restriction fragment length polymorphism (RFLP) (Hallerman and Beckmann, 1988); Mitochondrial DNA markers (Barasa et al., 2017). Genetic characterization is required for species improvement, viability and fishery management for the conservation of genetic resources.

Therefore, the main objective of this study was to analyze the genetic characterization among and between the farmed and wild populations of *Clarias*

gariepinus through the Random Amplified Polymorphic DNA (RAPD) PCR method.

Materials and Methods

Samples collection and DNA extraction

Thirty (30) brood fish of *C. gariepinus* (15 each from the wild and farmed populations) were used for the study. Wild fish were obtained from Lake Alau, Borno State (Wild Maiduguri Strain) which is located between latitudes $11^0$39". 84'N and 11^0 40". 02'N, and longitudes $13^0$39". 92'E and 13^0 40".12'E (Figure 1), and Gubi, dam Bauchi State (Wild Bauchi Strain) located between latitudes 10^0 19' 3. 31"N and longitudes 9^0 48' 25.99"E (Figure 2). Farmed fish were procured from the Garbati fish farm (Farmed Maiduguri Strain) in Borno State and the Nafisat fish farm (Farmed Bauchi Strain) in Bauchi State. Blood samples were collected through the caudal vein of the fish using a 5-ml syringe fitted with 23G needles and immediately emptied into an EDTA bottle containing anticoagulants and stored at -80^0C for the subsequent DNA extraction and amplification. Genomic DNA was extracted from blood samples using Gene Jet Genomic DNA Purification Kit protocols (Thermo Scientific, USA) following the manufacturer protocol with some minor modifications. The purity and concentration of genomic DNA were determined by recording the ratio of the optical density measured at 260/280 nm by using a UV spectrophotometer.

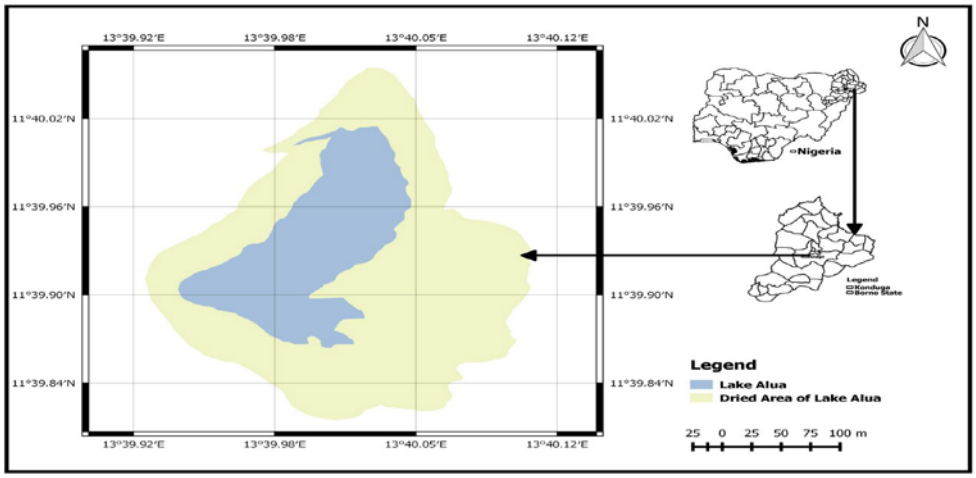

Figure 1. The map of Lake Alau showing the sampling site.
Source: GEONETcast software.

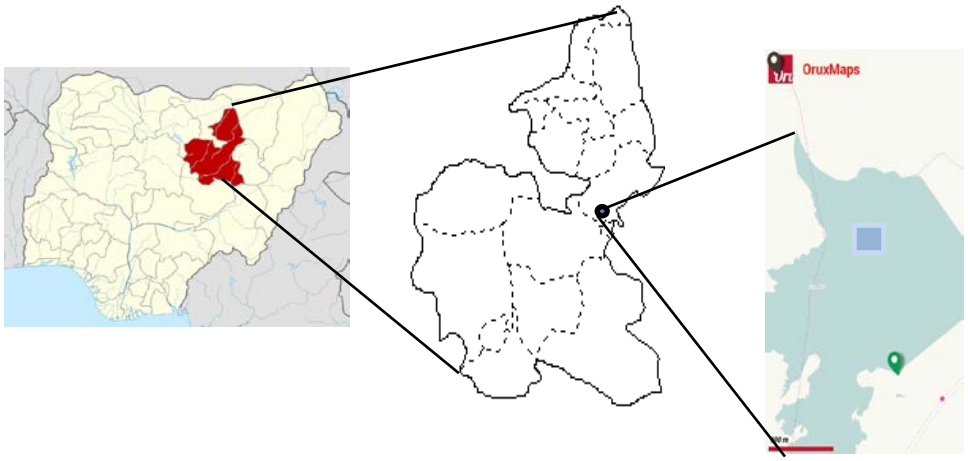

Figure 2. The map of Gubi Dam showing the sampling site.
Source: OruxMaps software.

Polymerase chain reaction (PCR) conditions and electrophoresis

The reaction mix was carried out in the 20-µl final volume containing 60ng–80ng genomic DNA, 0.1 µM of the primers, 2 mM of $MgCl_2$, and 125 µM of each dNTP and 1unit of Taq DNA polymerase. The initial denaturation temperature was set for 3 mins at 94^0C, followed by 45 cycles of denaturation temperature at 94^0C for 20 seconds, the annealing temperature of 37^0C for 40 seconds and the primer extension temperature of 72^0C for 40 seconds, then the final extension temperature at 72^0C for 5 mins was added. Polymerase chain reaction products were separated on 1.4% agarose gel and scored by comparison to a 100 bp standard DNA ladder digest (Thermo scientific, USA) and the molecular weight corresponding to the expected band size was estimated using the Pyelp software version 1.3.

Molecular analysis

The RAPD fragments (bands) generated from the five primers were scored as binary data; 1 = presence and 0 = absence from the bottom to the top of the gels. The total number of alleles, monomorphic alleles and polymorphic alleles were determined using the Pyelp software version 1.3. The percentage of polymorphic loci, polymorphic information content, gene diversity, total number of segregating fragments (alleles), mean number of fragments per individual, inbreeding coefficient (FIS) and the total gene diversity among the populations (HT) were estimated using the GenAlex software version 6.1 (Peakall and Smouse, 2006). The mean Nei's gene diversity within the populations (HW), genetic differentiation

among the population (HB) and the proportion of the total gene diversity that occur among the population (F_{ST}) were also evaluated using the GenAlex software version 6.1. Pairwise indices of genetic distance and identity were computed between the populations based on Nei's identity (1972) using the FSTAT version 2.9.3.2. The unweighted pair-group method, with the average (UPGMA) dendrogram, was constructed based on Nei's identity (1972), using the tool for the population genetic analysis (TFPGA) software version 1.3 (Miller, 1997; MEGA 5.0 Tamura *et al.*, 2007).

The analysis of molecular variance (AMOVA) was used to partition the genetic variance into components due to differences between populations, among populations and within populations of each strain using the GenAlex software version 6.1 (Peakall and Smouse, 2006).

Results and Discussion

Five (5) RAPD primers (OPB-10, OPB-12, OPB-20, OPE-08 and OPE-18) synthesized from Operon Technology (Operon Technology Inc. Alamed, USA) were obtained and used in this study as presented in Table 1. The mean numbers of estimated polymorphic loci and genetic variation are shown in Table 2. The number of fragment bands observed per primer ranged from 4 to 11. This range of fragment bands per primer (4–11) recorded in this study was close to 6–12, as observed by Saad et al. (2009) in the African catfish *Clarias gariepinus* in Egypt. However, it is lower (8–28) than reported in the same species by Asagbra et al. (2014). The differences in the numbers of fragment bands might be attributed to the numbers and sources of species. In the present study, high-quality fragment bands with good reproducibility were obtained in the size range from 150 to 4100 base pairs. This range of fragment bands is comparable from 150 to 5500 bp reported by Popoola et al. (2014) in Nigeria, and 183-1627 bp in Butter catfish (*Ompok bimaculatus*) as reported by Rashid et al. (2012). Gyan-chandra et *al*. (2010) used five RAPD primers and obtained a range of bands of 250–2,000 bp of amplicons in *Eutropiichthys vacha*. Also, Abdelkader et al. (2013) employed 15 random primers, and 215 loci were amplified, ranging from 118 to 2,556 bp among three species of Tilapia in Egypt. The large number of fragment bands recorded in this study might be attributed to the use of more numbers of primers or the presence of more priming sites at the template DNA with the particular series of primers used. Gyan-chandra et al. (2010) reported that the use of more numbers of random primers from different Operon series in more numbers of samples could lead to more reliable results in the genetic studies. In this study, 402 fragment bands were generated (Table 2). This result was found to be similar to that observed in *Clarias gariepinus* – 425 generated bands, as noted by Popoola et al. (2014). However, it is higher than 215 amplified fragments among three species of Tilapia in Egypt, as

reported by Abdelkader et al. (2013), using RAPD analysis. These variations in the number of amplified fragments may be due to the differences in species.

Table 1. Primer codes, sequences and GC percentage used for the genetic characterization of *Clarias gariepinus.*

S/NO	Primer code	Sequences	GC%
1	OPB-10	CTGCTGGGAC	70
2	OPB-12	CCTTGACGCA	60
3	OPB-20	GGACCCTTAC	60
4	OPE-08	TCACCACGGT	60
5	OPE-18	GGACTGCAGA	60

The percentage of polymorphic loci for each primer ranged from 67.9% to 82.0% (Table 2). The range of percentage polymorphic loci recorded in this study varied between 25% and 35.5% in three populations of *Clarias batrachus* as reported by Khedkar et al. (2010) in India. The variation in these values of polymorphic loci might be due to the differences in species. Three hundred and six (76.12%) fragment bands were polymorphic while the remaining 96 (21.39%) were found to be monomorphic (Table 2). The percentage of polymorphic loci in the two populations coincided with 70.59% reported in *O. bimaculatus* by Rashid et al. (2012) and also similar to the result (69.5%) obtained in the African catfish *Clarias gariepinus* by Saad et al. (2009). However, in the present study, the percentage of polymorphic loci recorded was higher than of those observed in catfish (*C. gariepinus*) (22%) by Asagbra et al. (2014) and in two populations of *Heteropneustes fossilis* (18.75%) reported by Garg et al. (2009). The high level of polymorphism recorded in this study is an indication of a relatively higher level of genetic variation.

The polymorphic information content (PIC) per primer ranged from 0.548±0.018 to 0.572±0.016 (Table 2). The result of this study is within the threshold of 0.5 that is considered the value for genetic markers to be informative (Botstein et al., 1980). These results are similar to the findings of Laloei et al. (2013), who reported a PIC of 0.53, but slightly lower than those reported in the *Clarias macrocephalus* – 0.765 (Nazia et al., 2014) and 0.785, as reported by Wang et al. (2009) in *Pelteobagrus fulvidraco*, using microsatellite markers. The differences might be due to the difference in molecular markers used. Gene diversity per primer was between 0.93 and 0.95 (Table 2). In the present study, the gene diversity among the two populations was higher than 0.21–0.30 as obtained by Abdelkader et al. (2013). This is an indication of high proportions of heterozygous genotypes.

Table 2. The estimates of polymorphic loci and genetic variation.

Primer code	Total number of loci	Monomorphic loci	Polymorphic loci	Polymorphic loci (%)	Polymorphic information content (±PIC)	Gene diversity
OPB-10	80	18	62	77.5	0.548±0.018	0.942
OPB-12	68	17	51	75.0	0.564±0.018	0.952
OPB-20	87	19	68	78.16	0.561±0.017	0.940
OPE-08	88	16	72	82.0	0.570±0.020	0.946
OPE-18	79	26	53	67.9	0.572±0.016	0.930
Total	402	96	306			

Genetic variability

In the present study, the percentage of polymorphisms obtained by each population was 58 (47.3%) for the farmed and 69 (75.9%) for the wild populations (Table 3). This result showed that the wild population had a higher level of heterozygosity than the farmed population, which was found to be consistent with the result of Rahid et al. (2012) in *O. bimaculatus*. The relatively low percentage of polymorphic loci (47.3%) recorded in a farmed population of *C. gariepinus* showed the level of inbreeding in the hatchery population. The lowest percentage of polymorphic loci (64.52%) was also reported in the hatchery population of *Heteropneustes fossilis* (Sultana et al., 2010) through RAPD marker techniques. Simonsen *et al.* (2005) have reported that inbreeding is a common scenario in fish hatcheries and the offspring produced are genetically inferior. Popoola et al. (2014) reported polymorphisms of 89.9% for the wild and 74.7% for the farmed populations in *Clarias gariepinus*. However, the result for the wild population (75.9%) in this study was higher than (55.56%) – the result obtained by Gyan-chandra *et al.* (2010) in two populations of *Eutropiichthys vacha* in India. The mean number of segregating fragments per individual within the population was 41.6 for the farmed and 38.7 for the wild populations (Table 3).

The inbreeding coefficient (FIS), total gene diversity among the populations (HT), mean Nei's gene diversity within the populations (HW), the proportion of the total gene diversity that occur among the population (HB) and genetic differentiation among the population (FST) are presented in Table 3. The mean numbers of the inbreeding coefficient (FIS) found (0.083) in the farmed population were much higher than 0.053, as observed in the wild population. Barasa et al. (2017) also obtained a high range (0.069–0.250) of the mean value of the inbreeding coefficient (FIS) in the farmed population of *C. gariepinus*. Abdul-Muneer et al. (2009) found a high inbreeding coefficient (0.211) in *Horabagrus brachysoma*. Barasa et al. (2017) posited that a high value of the inbreeding coefficient could be obtained in fish farms due to the loss of heterozygosity. The average number of total gene diversity among the populations (HT) recorded

(0.452) in the farmed population was higher than the one (0.402) observed in the wild population. Closer to the present study, Ikpeme et al. (2015) also found high gene diversity (0.385 and 0.365) in two fish farms of *Clarias gariepinus* in southwestern Nigeria. However, Popoola et al. (2014) obtained a relatively lower (0.30) gene diversity in the cultured population of *C. gariepinus.* Gene diversity is a parameter used to determine the expected heterozygosity in a given population of plants and animals. The proportion of the total gene diversity that occurs among the population (HB) is significantly higher (0.075) in the farmed population compared to 0.042 obtained in the wild population ($p \leq 0.05$). Gyan-chandra et al. (2010) reported high (0.197) gene diversity among the population of *Eutropiichthys vacha* in India. The mean genetic differentiation (FST) values in the farmed population were relatively higher (0.203) than 0.129 in the wild population when compared in the present study. Wachirachaikarn et al. (2009) reported a similar average value of (0.250) within populations of four hatchery strains of *Clarias gariepinus* in Thailand. However, Barasa et al. (2017) reported a much higher value of FST: 0.290 among the four farmed populations of *Clarias gariepinus* in Kenya. Meanwhile, Na-Nakorn et al. (2004) obtained the lower genetic differentiation (FST = 0.099) in walking catfish *Clarias batrachus*. This result of the present study shows little genetic differentiation among the population, which is an indication of the small gene flow. It could also be due to the small number of samples. A high value of FST is evidence of little gene exchange in each generation of the populations (Abdul-Muneer et al., 2009; Gyan-chandra et al., 2010).

Table 3. The estimation of genetic variation in wild and cultured populations of the sample.

Populations	Total no. of segregating alleles	Mean no. of alleles per individual	F_{IS}	H_T	H_W	H_B	F_{ST}
farmed	58 (47.3%)	41.6	0.083	0.452	0.434	0.075	0.203
wild	69 (75.9%)	38.7	0.053	0.402	0.312	0.042	0.129

H_T = (Total gene diversity among the populations), H_W = (Mean Nei's gene diversity within the populations), H_B = (The proportion of the total gene diversity occurring among populations), F_{ST} = (Genetic differentiation among population), F_{IS} = (Inbreeding coefficient).

The analysis of molecular variance (AMOVA) is presented in Table 4. AMOVA revealed that there was 4% of genetic variability among the population, which is an indication of 96% of the genetic molecular variance within the population. This result was similar to the study reported by Barroso et al. (2005), who observed 4.32% of the variance among the populations and 95.68% within the population in *Brycon opalim.* Li et al. (2007) have suggested that the stability of the habitat has a significant influence on the level of either low or high genetic variation.

Table 4. The analysis of molecular variance (AMOVA) of wild and cultured *Clarias gariepinus* populations from the study areas.

Source of variation	DF	SS	MS	Est. Var	%
among populations	3	11.285	3.706	0.013	4%
within populations	27	69.537	3.624	3.624	96%
Total	30	80.822		3.637	100%

Pairwise indices of genetic distance and identity

The genetic identity of the four populations of *C. gariepinus* is shown in Table 5. The highest level of genetic identity (0.9490) was found between the farmed Bauchi strain and the wild Bauchi strain of *C. gariepinus*, which shows that they are genetically related compared to the other strains. This could be attributed to the source of brood fish in the study areas. This result is higher than the one (0.939) recorded between two wild populations of *Heteropneustes fossilis* reported by Sultana et al. (2010). The lowest (0.9014) value of genetic identity was obtained between the farmed Bauchi strain and the farmed Maiduguri strain. The highest (0.1038) and lowest (0.0524) measurements of genetic distance among the four populations of *C. gariepinus* were recorded between the farmed Maiduguri strain and the farmed Bauchi strain and between the wild Bauchi strain and the farmed Bauchi strain, respectively (Table 5). The results of this study indicate that the farmed Maiduguri strain and the farmed Bauchi strain are more genetically similar than the wild population from Bauchi and Maiduguri, respectively. A similar result (0.157) was obtained by Rashid et al. (2012) for wild and cultured *O. bimaculatus.* There was no significant correlation between genetic identity and geographical distance in the present study.

Table 5. The genetic distance (below) and identity (above diagonal) between farmed and wild populations of *Clarias gariepinus* of the RAPD profile.

Populations	FBS	WBS	FMS	WMS
FBS	-	0.9490	0.9014	0.9055
WBS	0.0524	-	0.9168	0.9284
FMS	0.1038	0.0869	-	0.9416
WMS	0.0992	0.0743	0.0601	-

FBS = Farmed Bauchi strain, WBS = Wild Bauchi strain, FMS = Farmed Maiduguri strain, WMS = Wild Maiduguri strain.

The unweighted pair-group method with average (UPGMA) dendrogram shows the segregation of the four *Clarias gariepinus* populations sampled into two clusters (Figure 3). The phylogenetic dendrogram indicates that cultured Bauchi

strain (CBS) and wild Bauchi strain (WBS) are in cluster-1 while cluster-2 contained cultured Maiduguri strain (CMS) and wild Maiduguri strain (WMS). The dendrogram revealed that CBS and WBS were genetically close to a similarity value of 0.0525. The dendrogram of cluster-2 clearly shows that CMS and WMS were closely related to a similarity value of 0.0601. This result demonstrates a separation of the hatchery strains from the wild strains, which agrees with the geographical distance. The genetic variation observed in the study could be as a result of hybridization among and between species in the wild. Similar results were obtained by Saad et al. (2009), who studied the phylogenetic relation of the same species India through RAPD-PCR analysis.

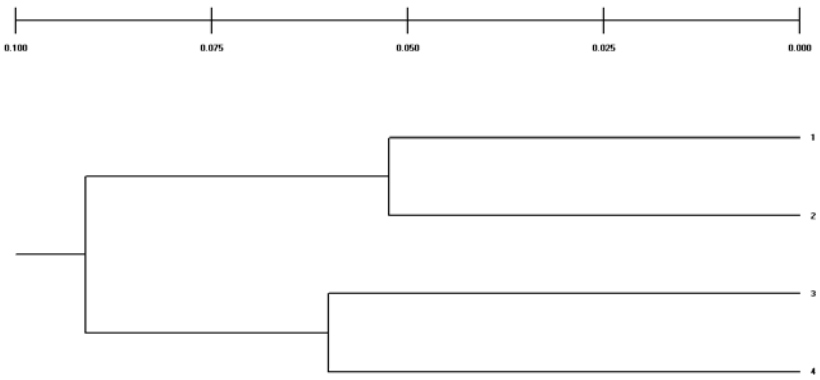

Figure 3. Clustering of four strains of *Clarias gariepinus* populations plotted using the unweighted pair-group method with average (UPGMA) dendrogram method based on Nei's genetic distance value (Nei's 1972) from RAPD-PCR analysis. Keys: (1 = Cultured Bauchi strain (CBS), 2 = Wild Bauchi strain (WBS), 3 = Cultured Maiduguri strain (CMS) and 4 = Wild Maiduguri strain (WMS)).

The relationship among the individual genotypes revealed by the UPGMA dendrogram based on Nei's D value is shown in Figure 4. The phylogenetic dendrogram trees separated the individual genotypes of four populations of *Clarias gariepinus* into nine major clusters at the co-efficient of 0.05, 0.15 and 0.10 percent of similarity to each other. The nine major clusters consist of minor sub-clusters at various degrees of genetic similarity. A similar observation was reported by Usman et al. (2013) in two Cichlid populations of *Tilapia guineensis* and *Sarotherodon melantheron*. This result can explain the possibility of hybridization between the closely related species to improve the genetic characters.

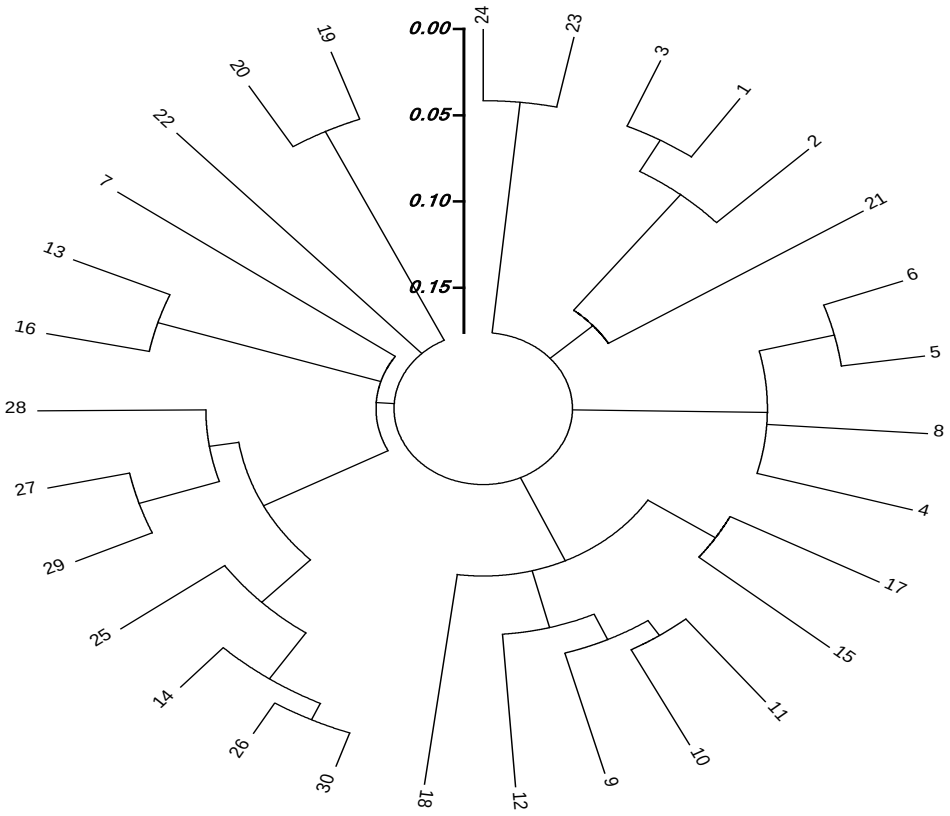

Figure 4. The individual unweighted pair-group method with average (UPGMA) dendrogram based on Nei's D value (Nei, 1972), original measures of genetic distance, summarizing the data on the differentiation between the four strains of *Clarias gariepinus.*

Conclusion

In conclusion, the study was aimed to characterize the four populations of *Clarias gariepinus* through RAPD techniques in northeastern Nigeria. This study revealed a relatively high level of genetic variation within and between the wild and cultured populations of *C. gariepinus* based on the phylogenetic dendrogram drawn from Nei's genetic distance. Studying genetic diversity is a prerequisite in understanding how populations of *C. gariepinus* will adapt to environmental changes. However, we suggest that a further study should be carried out with a larger number of RAPD primers and populations.

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GENETIČKA KARAKTERIZACIJA GAJENIH I DIVLJIH POPULACIJA AFRIČKOG SOMA (*CLARIAS GARIEPINUS* BURCHELL, 1822) PRIMENOM RAPD METODA

Sa'adu B. Suleiman*, Mohammed Y. Diyaware, Mohammed Aliyu i Zanna B. Mohammed

Odsek za ribarstvo, Univerziet u Maiduguriju, Poljoprivredni fakultet, Nigerija

R e z i m e

U ovom istraživanju sprovedena je genetička karakterizacija afričkog soma *Clarias gariepinus*. Prikupljeno je trideset (30) uzoraka afričkog soma *C. gariepinus*, po petnaest (15) iz divljine i sa ribnjaka u severnoistočnoj Nigeriji zbog njihove genetičke srodnosti i divergentnosti uz pomoć markera RAPD metode. Ekstrakcija DNK iz uzorka krvi izvršena je pomoću kompleta hemikalija Gene Jet Genomic DNA Purification Kit. Pet prajmera je korišćeno za PCR reakciju i amplifikovane su 402 RAPD trake poreklom iz četiri soja *Clarias gariepinus*. Trista šest (76,12%) traka bilo je polimorfno, dok je 86 (21,39%) bilo monomorfno. Procenat polimorfizma dobijen kod uzgajanih, odnosno divljih populacija, kretao se od 58 (47,3%) do 69 (75,9%). Polimorfne trake po lokusima unutar populacija kretale su se od 67,9% do 82,0%. Prosečne vrednosti koeficijenta inbridinga (FIS) iznosili su 0,083 kod uzgajane odnosno 0,053 kod divlje populacije. Vrednosti divergentnosti gena unutar uzgajanih i divljih populacija (Ht) bile su 0,4522 odnosno 0,4018. Srednja genetička diferencijacija (FST) kretala se između (0,203) kod uzgajanih i 0,129 kod divljih populacija. Analiza molekularne varijanse pokazala je da je unutar populacije bilo 96% genetičke varijanse, a među populacijom 4%. Genetički identitet i genetička distanca četiri populacije iznosili su 0,9490 odnosno 0,1038. Filogenetska mera pokazala je da su četiri soja podeljena u dva klastera na nivou sličnosti od približno 0,089. Rezultati su ukazali na značajan nivo genetičkih varijacija i minimalno odvajanje dendrograma kod *Clarias gariepinus* na severoistoku Nigerije. U zaključku, ove informacije će biti korisno sredstvo za program selekcije afričkog soma *Clarias gariepinus*.

Ključne reči: RAPD, genetička varijacija, *Clarias gariepinus*, polimorfizam, Nigerija, marker.

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*Autor za kontakt: e-mail: saadbala@unimaid.edu.ng

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EFFECTS OF DIFFERENT SOURCES OF DIETARY FIBRE ON THE LENGTH-WEIGHT RELATIONSHIP OF LEMON FIN BARB HYBRID (*BARBONYMUS GONIONOTUS* ♀ (BLEEKER, 1850) ×*HYPSIBARBUS WETMOREI* ♂ (H.M. Smith, 1931)) FINGERLINGS

Wasiu A. Jimoh[1,2*], Mohd S. Kamarudin[1], Akeem B. Dauda[1,3] and Oster F. Nwachi[1,4]

[1]Department of Aquaculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Darul Ehsan, Malaysia
[2]Department of Aquaculture and Fisheries, University of Ilorin, PMB 1515, Ilorin, Nigeria
[3]Department of Fisheries and Aquaculture, Federal University, Dutsin-ma, PMB 5001, Dutsin-ma Katsina State, Nigeria
[4]Department of Fisheries and Aquaculture, Delta State University, Abraka Asaba Campus, Delta State, Nigeria

Abstract: The effect of different sources of dietary fibre on the length-weight relationship of lemon fin barb hybrid (*Barbonymus gonionotus* ♀ × *Hypsibarbus wetmorei* ♂) was examined in a 56-day study using five dietary treatments; control and test dietary treatments containing different leaf meals of high dietary fibre added at the 10% inclusion level to a basal diet containing fish meal, soybean meal and rice bran. The weight of fish in each treatment was measured using a sensitive weighing balance while the total length was measured with a 15-cm-long ruler following standard procedures. The logarithmic transformation of the values obtained from the weight and length measurements was depicted using scatter diagrams. SPSS version 17.0 was used in regressing length against the weight to obtain the various components of the regression equations. The Levene test of homogeneity and interaction between covariate LogL and independent variable (treatments) was not significant – $F(4.85)=0.838$ and $F(4.84)=1.345$ respectively indicating that our data have not violated the assumption of homogeneity of variance. The various regression models developed for fish across various dietary treatments were statistically significant ($p<0.01$). The t-values for components of the regression equation such as intercept/constant (a) and slopes were all statistically significant except the t-value for the intercept/constant (a) of control. There was no significant effect of dietary treatments on LogW after controlling for logL, $F(4.84)=1.296$, revealing that the regression lines were not significantly

*Corresponding author: e-mail: jimoh.wa@unilorin.edu.ng

different from each other. This shows that the regression models could be used interchangeably.

Key words: ANCOVA, dietary fibre, regression model, lemon fin barb hybrid.

Introduction

Dietary fiber in fish feed has been identified to have not only the growth-promoting effect but also the health-enhancing effect on fish (Haidar et al., 2016). Including different sources of dietary fibre in fish feed is an attempt to maximize their potentiality to bring about the desired objectives of growth improvement and health enhancement (Buttriss and Stokes, 2008). The health and growth benefits of fiber consumption in animals are well recorded (Cummings and Stephen, 2007; Elia and Cummings, 2007). Though fibers are not digested in the small intestine, they are fermented in the large intestine producing short-chain fatty acids (SCFAs) which lower the pH of the colon preventing the growth of undesirable bacteria that could produce toxic substances. Short-chain fatty acids have a beneficial effect on lipid and glucose metabolism (Gray, 2006; Scott et al., 2008). They stimulate the growth of beneficial gut bacteria such as bifidobacteria and lactic acid bacteria (Gibson et al., 2004; Nugent, 2005) which enhance host health (Gibson and Roberfroid, 1995). Napier grass (*Pennisetum purpureum*), gliricidia (*Gliricidia sepium*) leafmeal, alfalfa (*Medicago sativa*) leafmeal and ipomea (*Ipomea aquatica*) were chosen as the high fiber feed ingredients to be used in this study because they constitute parts of the supplements being used by Malaysian Fish Farmers (Kamarudin Personal Communication, 2017). What is common to these ingredients used by Malaysian farmers is the high dietary fiber each of them contains (Jimoh et al., 2019). Foschia et al. (2013) reported that an ingredient as a dietary fibre source should contain at least 3% of dietary fibre.

The development of a regression model for the purpose of prediction or estimation is very important in the aquaculture industry wherein nutrition alone, which plays a veritable role in the growth of individual fish species, could be 60% of the variable cost of production (Jimoh et al., 2015). A comparative study has shown that the use of the length-weight relationship for the purpose of prediction or estimation in cultured fisheries is very low relative to captured fisheries. Datta et al. (2013) developed a model for *Channa punctata* under different feeding regimes. Similarly, Kumar et al. (2013) established the length-weight relationship for *Anabas testudineus* and *Channa species* under different culture systems. The length-weight relationship elucidates the possibility of predicting the weight of fish given its length (Giarrizzo et al., 2011). This study, therefore, attempts to develop regression models for lemon fin barb hybrid (*Barbonymus gonionotus* ♀ × *Hypsibarbus wetmorei* ♂) fed diets containing different dietary fibre sources. Developing a tool for prediction or estimation and establishing whether significant

variations exist among the various models developed that could imply interchangeability of model usage is still very new in aquaculture research.

Lemon fin barb hybrid, which is enjoyed by Malaysians for its nutritional value, is a new aquaculture species with many of its nutritional requirements yet to be determined (Suharmili et al., 2015). The present research, as far as our knowledge is concerned, represents the first study undertaken to examine the length and weight relationship under different high dietary fibre sources as dietary treatments for lemon fin barb hybrid (*Barbonymus gonionotus* ♀ × *Hypsibarbus wetmorei* ♂) and also establish whether significant variations exist among the various models developed.

Materials and Methods

A 56-day feeding trial was conducted using five dietary treatments; control and test dietary treatments containing different leaf meals of high dietary fibre (Table 1); Napier grass (*Pennisetum purpureum*); gliricidia (*Gliricidia sepium*), ipomea (*Ipomea aquatica*) and alfalfa (*Medicago sativa*) leaf meals were added at the 10% inclusion level to a basal diet containing fish meal, soybean meal and rice bran. Lemon fin barb hybrid (*Barbonymus gonionotus* ♀ × *Hypsibarbus wetmorei* ♂) was obtained from the Perlok Aquaculture Extension Centre, Jerantut, Pahang, Malaysia and acclimated for 15 days in a 1000L circular tanks of the wet laboratory of the Department of Aquaculture, Universiti Putra Malaysia. Two hundred twenty-five fingerlings were randomly distributed to 15 rectangular tanks at the beginning of the experiment. Each of the five dietary treatments was randomly allocated to different tanks with three replications per treatment. The fingerling fish were fed 4% body weight/day divided into equal proportion in the morning and evening. At the end of the feeding trial, the length and weight of the fish were measured to the nearest cm for length and to 0.1g for weight using a ruler and a sensitive weighing balance respectively.

Length-weight relationship

The weight (g) of fish in each treatment was measured using a sensitive weighing balance while the total length (cm) was measured with a 15-cm-long ruler following the procedures explained in Datta et al. (2013). The logarithmic transformation of the values obtained from the weight and length measurements was done and depicted using scatter diagrams.

The formula $W = aL^b$ of Le Cren (1951) on the fish length-weight relationship was linearized by taking the common log of both sides:

$$\text{Log } W = \text{Log}a + b\text{Log}L$$

where a = intercept; b = slope.

Table 1. The feed and proximate composition, and fibre differentials (% as fed basis) of the experimental diets containing different sources of high fibre feed ingredients.

Feed ingredients	Diet				
	Control	Ipomea	Alfalfa	Napier	Glyricidium
Fishmeal	11.00	7.74	10.21	12.91	9.15
Rice bran	15.40	8.25	5.4	2.97	7.24
Soybean meal	50.00	50.00	50.00	50.00	50.00
Tapioca starch	20.00	20.00	20.00	20.00	20.00
Ipomea aquatica meal	0.00	10.00	0.00	0.00	0.00
Alfalfa meal	0.00	0.00	10.00	0.00	0.00
Napier grass meal	0.00	0.00	0.00	10.00	0.00
Gliricidia meal	0.00	0.00	0.00	0.00	10.00
[a]Vitamin premix	1.00	1.00	1.00	1.00	1.00
[b]Mineral premix§	1.00	1.00	1.00	1.00	1.00
Crude palm oil	1.60	1.60	1.60	1.60	1.60
	100	100	100	100	100
Proximate composition					
Moisture	4.74±0.87	5.29±0.14	4.99±0.28	5.31±0.06	5.51±0.26
Crude protein	31.55±0.22	31.55±0.22	32.34±0.18	33.72±0.01	31.64±0.20
Crude lipid	5.38±0.96	6.33±0.25	4.35±0.18	5.15±0.05	4.71±0.12
Ash	9.89±0.09	10.58±0.04	9.74±0.01	10.32±0.03	9.03±0.02
Crude fiber	5.61±0.71[b]	7.03±0.19[a]	4.93±0.12[b]	7.59±0.14[a]	5.17±0.36[b]
NFE	42.83±20.32	39.21±0.41	43.63±0.19	37.90±0.21	43.92±0.17
Fibre differential					
Hemicellulose	24.64±1.29	18.28±0.13	21.07±5.10	18.22±2.89	19.61±0.12
Cellulose	11.48±0.54	13.49±0.81	11.30±2.60	16.12±1.80	9.12±0.01
Lignin	1.90±0.06	3.10±0.47	1.64±0.06	1.76±0.13	2.95±0.40
NFC[ϕ]	10.42±0.05	11.38±0.36	14.57±0.04	9.4±0.14	17.43±0.03
Gross energy (kJ/g)	17.70±0.02	17.70±0.00	17.70±0.02	17.70±0.00	17.74±0.01

Vitamin premix (g kg-1 premix): ascorbic acid, 45; myo-inositol, 5; choline chloride, 75; niacin, 4.5; riboflavin, 1; pyridoxine, 1; thiamin mononitrate, 0.9; Ca-pantothenate, 3; retinyl acetate, 0.6; cholecalciferol, 0.08; vitamin K menadione, 1.7; α-tocopheryl acetate (500 IU g-1), 8; biotin, 0.02; folic acid, 0.1; vitamin B12, 0.001;cellulose, 845.1.

§. Mineral premix (g kg-1 premix): KCl, 90; KI, 0.04; $Ca(H_2PO_4).H_2O$,500; NaCl, 40; $CuSO_4.5H_2O$, 3; $ZnSO_4.7H2O$, 4; $CoSO_4$,0.02; $FeSO_47H_2O$, 20; $MnSO_4.H_2O$, 3; $CaCO_3$, 215; MgOH, 124; Na_2SeO_3, 0.03; NaF, 1.

ϕ NFC Non-fibre carbohydrates

$$\text{NFC} = \text{Total Carbohydrates(NFE + CF)} - \text{Hemicellulose} - \text{Cellulose} - \text{lignin}$$

Statistical analysis

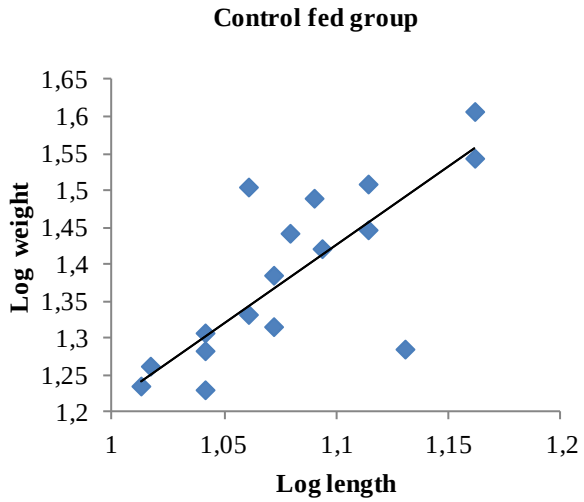
SPSS version 17.0 was used in regressing the length against the weight to obtain the various components of the regression equations. A one-way analysis of covariance (ANCOVA) was used to compare variations among the treatment regression lines of the length-weight relationship while controlling for LogL after

the data had been subjected to and found to have passed the Levene test of homogeneity of variance indicating that our data did not violate the assumption of homogeneity of variance for the ANCOVA test. The partial eta-squared results were compared with Cohen's guidelines (0.2 – small effect, 0.5 – moderate effect, 0.8 – large effect).

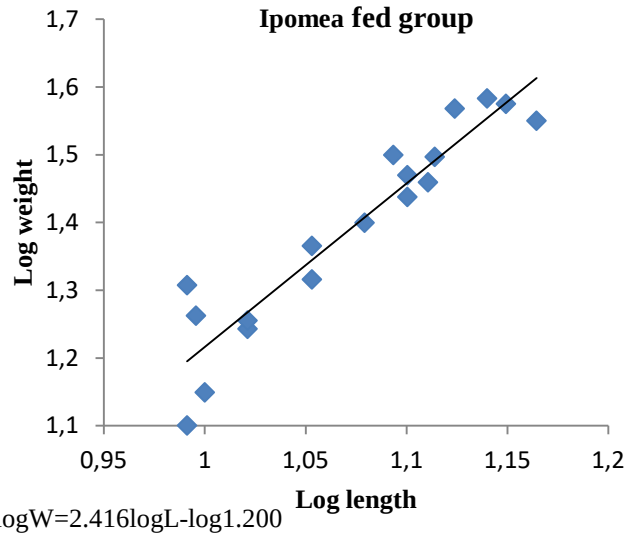
Results and Discussion

Length-weight relationship

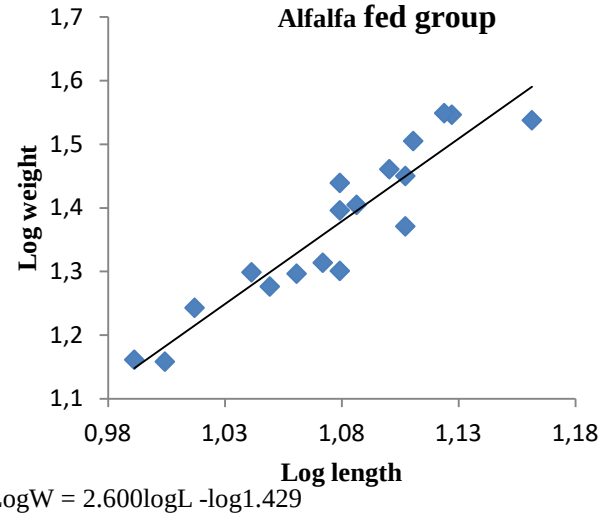
Figure 1 shows the graphs representing a logarithmic regression of the final length and weight of lemon fin barb hybrid (*Barbonymus gonionotus* ♀ × *Hypsibarbus wetmorei* ♂) fed diets containing various sources of high fibre leaf meals.



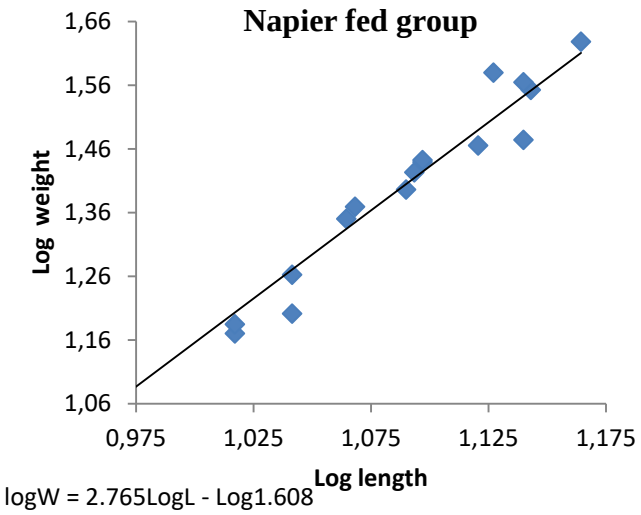
logW = 2.116logL - log0.901



logW=2.416logL-log1.200



LogW = 2.600logL -log1.429



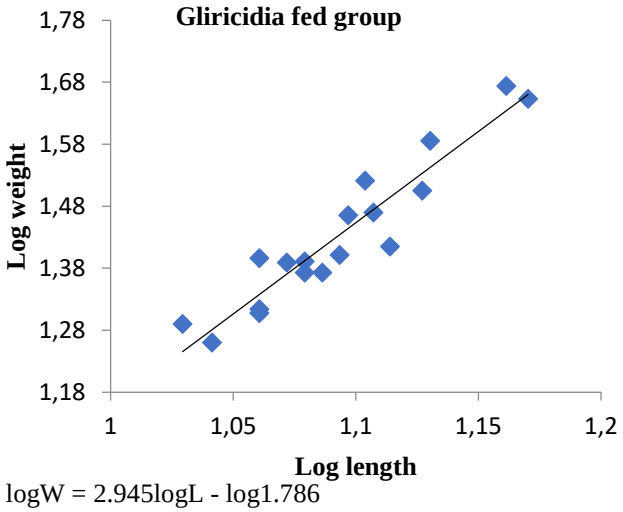


Figure 1. Graphs showing the logarithmic regression of the final length and weight of lemon fin barb hybrid (*Barbonymus gonionotus* ♀ × *Hypsibarbus wetmorei* ♂) fed diets containing various sources of high fibre leaf meals.

Table 2 presents an analysis of the length-weight relationship of lemon fin barb hybrid (*Barbonymus gonionotus* ♀ × *Hypsibarbus wetmorei* ♂) fed diets containing various sources of high fibre leaf meals. The various regression models developed for fish across various dietary treatments were statistically significant

($p<0.01$). The t-values for components of the regression equation such as intercept/constant (a) and slopes were all statistically significant except the t-value for intercept/constant (a) of CTR. The slope (b) ranged from 2.116 in the regression model for control to 2.945 in the regression model for the Gliricidium fed group. The 'r' values (0.788–0.958) indicate a positive correlation meaning that as the Log of length increases, the Log of weight also increases.

Table 2. The analysis of the length-weight relationship of lemon fin barb hybrid (*Barbonymus gonionotus* ♀ × *Hypsibarbus wetmorei* ♂) fed diets containing various sources of high fibre feed ingredients.

Dietary treatment	Regression equation	R^2	F-statistics for the equation	t-statistics for the constant(a)	t-statistics for LogL	ANCOVA probability
Control	Log W = 2.116LogL – log0.901	0.621	F(1,16)=26.10**	t=-2.03^{ns}	t=2.12**	F(4.84)=1.296, P>0.05
Ipomea	Log W = 2.416LogL – log1.200	0.885	F(1,16)=122.63**	t=-0.51**	t=11.07**	
Alfalfa	Log W = 2.600LogL – log1.429	0.876	F(1,16)=112.56**	t=-5.407**	t=10.61**	
Napier	Log W = 2.765LogL – log1.608	0.958	F(1,16)= 365.92**	t=-10.33**	t=19.13**	
Glyricidium	Log W = 2.945LogL – log1.786	0.896	F(1,16)=137.26**	t=-6.498**	t=11.72**	

** = ($p<0.01$); R^2 = Coefficient of determination; Ns = Not significant.

The r-values were statistically significant from the Pearson's correlation output (Table 3). The coefficients of determination (R^2) which show how much of the variance in the dependent variable (weight) is explained by the independent variable (length) were all very high (0.621–0.958). The Levene test of homogeneity and interaction between the covariate LogL and the independent variable (treatments) was not significant $F(4.85)=0.838$, $p>0.05$; $F(4.84)=1.345$, $p>0.05$ respectively indicating that our data have not violated the assumption of homogeneity of variance. Similarly, there was no significant difference ($p>0.05$) among the five regression lines.

Table 3. Estimated marginal means of dietary treatments.

Dietary treatment	Estimated marginal means	(r)	Adjusted R^2	F-Statistics
Control	1.38±0.12	0.788	0.856	F(4.84)=1.296 (P>0.05)
Ipomea	1.39±0.14	0.941		
Alfalfa	1.37±0.12	0.936		
Napier	1.36±0.18	0.979		
Glyricidium	1.43±0.11	0.946		

There was no significant effect ($p>0.05$) of dietary treatments on LogW after controlling for logL, $F(4.84)=1.296$ $p>0.05$, revealing that the regression lines were not significantly different from each other. Partial eta squared of 0.058 means that the effect size of the dietary treatment was very small explaining 5.8% of the

variation in weight and partial eta squared of 0.86 was recorded for LogL meaning its effect size was very high and that 86% of the variation in LogW was explained by LogL. In contrast to results obtained from treatments, the covariate, LogL, had a significant effect ($P<0.05$) on the dependent variable, logW, ($F(1,84)=515.15$, $p<0.001$), showing that LogL had a significant impact on the outcome of dependent variables (logW).

Comparing the estimated marginal means (Table 3) for each dietary treatment group based on the influence of the covariance, revealed that the Napier fed group recorded the lowest value of LogW while the highest value of LogW was recorded for the Glyricidia fed group. A logarithmic regression of the final length and weight of lemon fin barb hybrid (*Barbonymus gonionotus* ♀ × *Hypsibarbus wetmorei* ♂) fed diets containing various sources of high fibre feed ingredients to elucidate the interchangeability of the various selected leaf meals was presented in Figure 2.

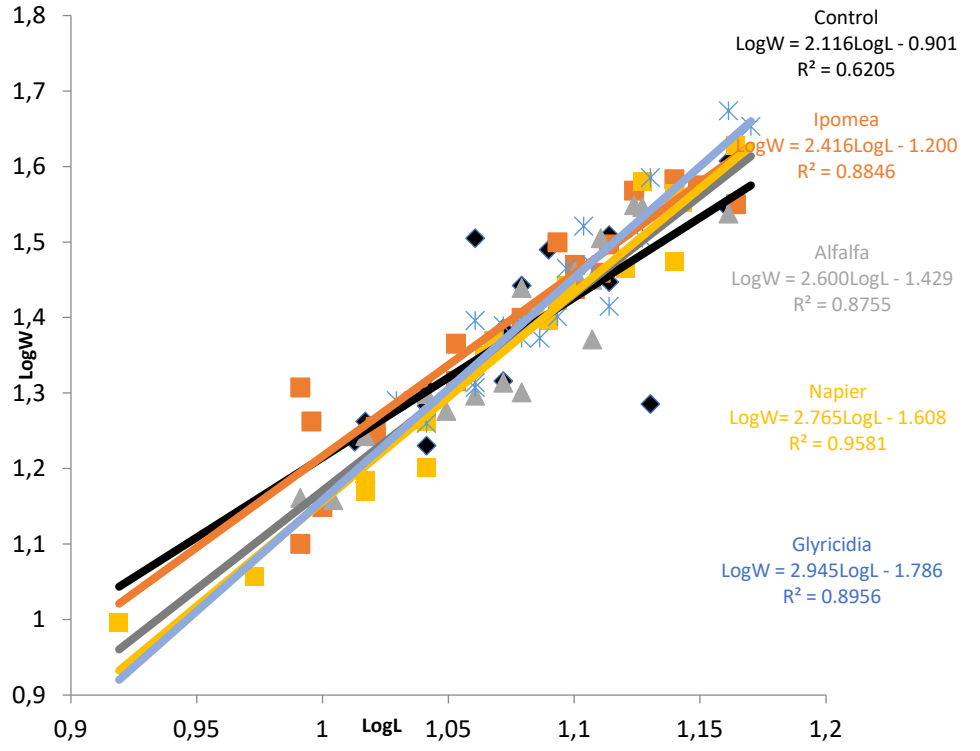


Figure 2. The graph showing a logarithmic regression of the final length and weight of lemon fin barb hybrid (*Hypsibarbus wetmorei* x *Barbonymus gonionotus*) fed diets containing various sources of high fibre feed ingredients.

The regression model provides reliable information about the growth pattern and the estimation of the fish weight (W) using a known length (L) (Froese, 2006; Schneider et al., 2000) and could be applied to studies on nutrition (Beyer, 1987). The various regression models developed in this study across various dietary treatment groups that were significant show that each of the models could be used for the purpose of the prediction and estimation of the weight of fish once its length is known. The r-values that were high in this study indicated that a strong positive correlation existed between the length and weight of fish exposed to various dietary fibre treatments. The high R^2-value recorded in this study revealed that a significant proportion of variance in weight could be explained by the variation in length. Jisr et al. (2018) have reported that a high coefficient of determination recorded in the assessment of length-weight relationships implies a good quality of the prediction of linear regression for the analyzed fish species. The t-value for the intercept (a) showed that the intercepts were significantly different from zero. The interpretation of regression parameters (regression coefficient and regression constant) of the various models developed in this study is consistent with what was reported in Hinton et al. (2014). The trend of the slope recorded in this study showed that the higher the value of slope (b) in the regression model, the faster the growth in terms of length and weight. Datta et al. (2013) made a similar observation when estimating the length-weight relationship of spotted snakehead *Channa punctata* (Bloch) under different feeding regimes. In this study, the slope of regression models of the dietary fibre sources was higher than that of the control. This implies a better growth performance among the fish fed dietary fibre sources. This is in consonance with the earlier report by Jimoh et al. (2019) that dietary treatments containing high fibre feed ingredients improve growth. The negative allometric growth pattern recorded in this study is in tandem with the report of Ali et al. (2002), Haniffa et al. (2006), Dua and Kumar (2006), Khan et al. (2011) and Datta et al. (2013). Wootton (1991) has reported that the slope value less than the critical value (3) is regarded as a negative allometric growth pattern. Negative allometry implies that the fish becomes slimmer as the length increases (Jisr et al., 2018). This might be attributed to morphological features specific to this species as they are laterally compressed fish species. Phenotypic features such as body forms and shapes could affect the allometric growth pattern (Karachle and Stergiou, 2012; Tsoumani et al., 2006). The regression lines developed in this study were not significantly different from one another, meaning the regression models could be used interchangeably.

Conclusion

This study serves as the first information report recorded on the development of predictive models for the lemon fin barb hybrid fed high fibre feed ingredients.

The negative allometric growth pattern was recorded plausibly owing to the body shapes and forms of the fish. Each of the regression models developed was statistically significant, indicating their suitability for weight prediction or estimation once the length of fish is known. The statistical similarity was recorded for the various regression models depicting their usage interchangeability.

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UTICAJI RAZLIČITIH IZVORA VLAKANA U OBROKU NA DUŽINU I TEŽINU MLAĐI HIBRIDA (*BARBONYMUS GONIONOTUS* ♀ (BLEEKER, 1850) × *HYPSIBARBUS WETMOREI* ♂ (H.M. SMITH, 1931))

Wasiu A. Jimoh[1,2*], Mohd S. Kamarudin[1], Akeem B. Dauda[1,3] i Oster F. Nwachi[1,4]

[1]Odsek za akvakulturu, Univerzitet Putra Malaysia,
43400 UPM Serdang, Selangor, Darul Ehsan, Malezija.
[2]Odsek za akvakulturu i ribarstvo, Univerzitet u Ilorinu, PMB 1515, Ilorin, Nigerija
[3]Odsek za ribarstvo i akvakulturu, Federalni univerzitet, Dutsin-ma,
PMB 5001, Država Dutsin-ma Katsina, Nigerija
[4] Odsek za ribarstvo i akvakulturu, Univerzitet u Državi Delta,
Kampus Abraka Asaba, Država Delta, Nigerija

R e z i m e

Uticaj različitih izvora vlakana u hrani na povezanost dužine i težine hibrida (*Barbonymus gonionotus* ♀ × *Hypsibarbus wetmorei* ♂) ispitivan je u 56-dnevnom istraživanju korišćenjem pet hranidbenih tretmana; kontrolni i ogledni tretmani su sadržali različita lisna brašna sa visokim procentom vlakana, koja su dodavana na nivou od 10% osnovnom obroku koji je sadržao riblje brašno, sojinu sačmu i pirinčane mekinje. Težina ribe u svakom tretmanu merena je osetljivom vagom, dok je ukupna dužina merena lenjirom dužine 15 cm prema standardnim procedurama. Logaritamska transformacija vrednosti dobijenih merenjima težine i dužine prikazana je pomoću scater dijagrama. SPSS verzija 17.0 korišćena je za izračunavanje regresije dužine prema težini, kako bi se dobile različite komponente regresionih jednačina. Leveneov test homogenosti i interakcije između kovarijantnog LogL i nezavisne promenljive (tretmani) nije bio značajan – F (4,85) = 0,838 odnosno F (4,84) = 1,345, ukazujući da naši podaci nisu prekršili pretpostavku homogenosti varijanse. Različiti regresioni modeli razvijeni za ribe u različitim dijetetskim tretmanima bili su statistički značajni ($p<0,01$). Vrednosti *t* za komponente regresione jednačine, kao što su konstanta (a) i nagibi, bile su sve statistički značajne, osim vrednosti *t* za konstantu (a) kontrole. Nije bilo značajnog uticaja dijetetskih tretmana na LogV nakon kontrole logL, F (4,84) = 1,296, što pokazuje da se regresione linije međusobno nisu značajno razlikovale. To pokazuje da bi se regresioni modeli mogli međusobno zamenjivati.

Ključne reči: ANCOVA, vlakna, regresioni model, hibrid (*Barbonymus gonionotus* ♀ × *Hypsibarbus wetmorei* ♂).

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[*]Autor za kontakt: e-mail: jimoh.wa@unilorin.edu.ng

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EFFECTS OF NATURAL ANTIOXIDANT EXTRACT SUPPLEMENTATION ON THE GROWTH PERFORMANCE AND MEAT QUALITY OF BROILER CHICKENS

Hakeem Ishola[1*], Elisha Z. Jiya[2], John Y. Adama[2] and Olushola J. Alabi[2]

[1]Department of Animal Production, Kwara State University, Malete, Nigeria
[2]Department of Animal Production, Federal University of Technology, Minna, Nigeria

Abstract: The study examined the effect of dietary antioxidant supplementation on the performance and meat quality of broiler chickens. Namely, 300 one-day-old Arbor Acres broiler chicks were fed a starter diet from 1 to 4 weeks and a finisher diet for the last 4 weeks. Birds were randomly assigned to treatments based on antioxidant supplementation in drinking water at 0.02% butylated hydroxyanisole (BHA), ordinary water (OW), 0.02% sweet orange peel extract (SOPE), 0.02% shaddock peel extract (SHPE) and 0.02% lemon peel extract (LMPE) per litre of water in a completely randomized design experiment. Feed intake and body weight gain were recorded on a weekly basis. Three birds were selected in each treatment and slaughtered for meat quality determination. BHA and LMPE treatments had the best weight gain and feed conversion efficiency at the significance level ($p<0.05$). There were significant differences ($p<0.05$) in the shear force (force peak and yield) in the dietary antioxidants fed to the birds. Sensory parameters (taste, aroma and overall acceptability) show significant differences ($p<0.05$) amongst the treatments. However, there were no significant differences ($p>0.05$) in appearance and texture perception amongst treatments. Finally, significant differences ($p<0.05$) in the lightness (L*) and redness (a*) of the meat samples were observed amongst the treatments. There were no significant differences ($p>0.05$) in the yellowness (b*) amongst the treatments. It can be concluded that broiler birds fed SOPE, SHPE and LMPE treatments performed better and that these treatments enhanced the meat quality of the birds when compared to BHA and OW treatments.

Key words: synthetic antioxidant, natural antioxidants, feed intake, weight gain, sensory parameters.

*Corresponding author: e-mail: abdulhakeemadisa5@gmail.com

Introduction

Broiler meat has several desirable nutritional characteristics such as high protein, low lipid contents and high polyunsaturated fatty acids (Mothershaw et al., 2009). These make it preferable, health-wise when compared to red meat. Broiler meat is, however, highly susceptible to lipid oxidation during storage (Sahin et al., 2010). Lipid oxidation is reported to have adverse effects on meat quality parameters such as colour, juiciness, tenderness and flavour, thus leading to the reduction of the meat shelf-life (Min et al., 2008). Wood and Enser (1997) recommended the use of dietary antioxidant in preserving animal products by reducing lipid peroxidation. In recent years, the use of synthetic substances like antibiotics as feed additives has been discouraged. In response to this, there is an increased interest in the use of natural antioxidants from plants and these antioxidants have gained popularity because they are believed to be safer than synthetic antioxidants (Moyo et al., 2011).

Synthetic antioxidants have been widely used as food and feed preservatives, because of their effectiveness in delaying lipid oxidation. With relatively low cost, there is an increase in consumers' preference for natural ingredients over synthetic compounds (Ahn et al., 2002). This has resulted in a need for more information on the efficacy of different natural additives that can reduce lipid oxidation in meat and meat products. Some natural sources of antioxidants like rosemary, sage, ginger and grape seed are shown to contain large amounts of phytochemicals such as phenolic acids and flavonoids, glycosides and alkaloids (Abeysinghe et al., 2007), which, if used as dietary supplementation in feed and water, can reduce the lipid peroxidation and extend the shelf life of broiler meats under different storage conditions (Botsoglou et al., 2002; Jang et al., 2007; Rababah et al., 2004; Sahin et al., 2010). Fruits are an important source of many bioactive compounds such as phenolic acids, flavonoids and glycosides. Peels are the primary by-products during citrus juice processing, and if unused, they turn to be waste and represent a source of environmental pollution. However, research conducted on several fruits (citrus, apples and grapes) has shown that peels are the major source of natural antioxidants (Lucia et al., 2008, Rababah et al., 2004, Sallam et al., 2004).

Phenolic compounds in peels and fruit can be used in food products as a natural antioxidant and can serve as substitutes to synthetic preservatives (Ignat et al., 2011). Phenolic compounds are important components of many fruits (Miguel et al., 2004). As Robards et al. (1999) reported, there are approximately 5000 known plant phenolics and model studies have demonstrated that many of them have antioxidant activities. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, and metal chelators (Rice-Evans et al., 1995). The aim of this research work is to evaluate the dietary supplementation of natural

antioxidant extracts and the effect it has on the growth performance and the meat quality of broiler chickens.

Materials and Methods

Broiler chicken managements and diets

The field experiment was conducted at the Kwara State University Teaching and Research Farm, Malete, on a geographical location of latitude 08° 71′ N and longitude 04° 44′ E at 365 m above sea level. The climate of Malete is characterised by distinct wet and dry seasons with the annual mean rainfall of about 1,150 mm and a mean annual temperature that ranges from 25 to 28.9° C (Olaniyan, 2003). Three hundred (300) one-day-old Arbor Acres broilers were used in this study. The chicks were weighed and randomly allotted to five treatments in a completely randomised design and were replicated in three units with twenty birds per replicate. Birds were housed in a metabolic battery cage for birds. The treatments were based on the natural antioxidant supplementation in drinking water, as stated below (USFDA, 2009):

Extraction procedure

Fifty g of dried powdered samples of sweet orange, shaddock and lemon peels were extracted with ethanol organic solvents using the procedure described by VanAcker et al. (2011):

1. Treatment one – Positive control (drinking water with 200 ppm BHA/litre),
2. Treatment two – Negative control (drinking water with no antioxidant supplement),
3. Treatment three – Drinking water with 200 ppm of sweet orange peel extract (SOPE)/litre,
4. Treatment four – Drinking water with 200 ppm of shaddock peel extract (SHPE)/litre,
5. Treatment five – Drinking water with 200 ppm of lemon peel extract (LMPE)/litre.

Routine management and vaccination were followed (Ishola and Atteh 2018). Feed and water were given *ad libitum* for the 8-week trial. The birds were placed on a formulated broiler starter diet containing 23% CP and 2879Kcal/kg ME for the first four weeks and a broiler finisher diet containing 17% CP and 2700 kcal/kg ME for the second four weeks (Table 1).

Table 1. The composition of the diets (% DM).

Ingredients	Starter (%)	Finisher (%)
Maize	57.50	56.50
Wheat offal	0.00	8.00
Soybean meal	20.00	15.00
Groundnut cake	16.00	8.50
Fish meal	2.00	1.00
Bone meal	2.50	4.00
Limestone	1.00	6.00
Vitamin premix	0.25	0.25
Methionine	0.25	0.25
Lysine	0.25	0.25
Salt	0.25	0.25
Total	100.00	100.00
Calculated analysis		
Crude protein %	23	17
ME Kcal/kg	2879	2700
Crude fibre %	3.91	2.94
Ether extracts %	4.63	2.44
Methionine + Cysteine %	0.92	0.46
Lysine %	1.31	0.85
Calcium %	1.18	3.63
Phosphorus %	0.50	0.83

DM = Dry matter, kg = Kilogramme, CP = Crude protein, ME = Metabolisable energy, Kcal = Kilocalorie.

Data collection

Performance and physicochemical determination

Feed intake and body weight gain were recorded weekly and used to determine the feed to gain ratio. At the end of the feeding trial, three birds were selected per replicate in each treatment, fasted overnight and slaughtered by severing the jugular vein, defeathered and eviscerated. Breast meat parts were collected for meat quality (shear force, sensory evaluation and colour coordinates) determination using procedures described by Sazili et al. (2005), Meilgaard et al. (2006) and Sabow et al. (2015) respectively.

Shear force

The textural assessment of the broiler breast meat fed dietary antioxidant supplementation was conducted using the HD plus® texture analyser (Stable Micro System, Surrey, UK) equipped with a Volodkevitch bite jaw. The equipment was

calibrated at 5 kg for weight, 10mm return distance for height and the blade speed was set at 10 mm/sec. The sample preparation was analysed following the procedure described by Sazili et al. (2005). From each sample, three replicate blocks (1 cm height × 1 cm width × 2 cm length) were cut parallel to the direction of the muscle fibres and each block was sheared in the centre perpendicular to the longitudinal direction of the fibres. Shear force was reported as the average peak positive force values for all blocks of each treatment sample.

Sensory evaluation

A consumer type sensory evaluation was conducted as described by Meilgaard et al. (2006). Twenty g of the breast meat for each treatment was trimmed free of fat, labelled and cooked in a water bath at 80°C for 10 mins. The meat samples were wrapped in aluminium foil and coded with numbers. A consumer type of sensory evaluation was conducted by thirty (30) assessors consisting of staff and students of the Kwara State University, Malete, Nigeria. Assessors were trained on the sensory protocol and instructed on the parameters (tenderness, juiciness, flavour, cooked colour and overall acceptability) to judge using a 9-point hedonic scale (Meilgaard et al., 2006). A value of nine indicated 'like extremely' and one indicated 'dislike extremely'.

Colour coordinates

Meat colour coordinates of the breast meat samples from each treatment (BHA, OW, SOPE, SHPE and LMPE) were determined according to the method of AMSA (2012) as described by Sabow et al. (2015). A dimension of 1 x 1 x 2 cm^3 breast meat part of bird per replicate were cut and placed at the base of the colour flex cup of a Colour Flex spectrophotometer (Hunter Lab, Reston, VA, USA). Readings for lightness (L*), redness (a*) and yellowness (b*) were taken based on the International Commission on Illumination (CIE), with D65 illuminant and 10° standard observer, tristimulus values (X,Y,Z) and reflectance at specific wavelength (400–700 nm). For each sample, triplicate readings for L*, a* and b* values were recorded and then averaged.

Statistical analysis

Data obtained from the experimental trial were analysed and subjected to analysis of variance (ANOVA) using the PROC MIXED procedure of SAS (2014). Means were separated at the 5% significance level.

Results and Discussion

Performance

There were significant differences (P<0.05) in the weekly feed intake, weight gain and feed conversion ratio of broilers fed with the different treatments (Table 2). Birds on dietary ordinary water treatment (OW) had the highest feed intake (p<0.05) while birds on butylated hydroxyanisole (BHA) treatment had the lowest. The weight gain ranged from 354.17g to 418.75g in dietary treatments OW and LMPE. Birds fed BHA and LMPE treatments were not significantly different from each other (p>0.05) but were significantly different (p<0.05) from those on other diets. Birds fed on BHA and LMPE diets had the best feed to gain ratio.

Table 2. The effect of dietary antioxidant supplementation on the performance of broiler chicken.

Parameter	Treatments					SEM	P value
	BHA (0.02%)	OW	SOPE (0.02%)	SHPE (0.02%)	LMPE (0.02%)		
Initial body weight (g/b)	37.10^{e}	45.10^{a}	38.50^{d}	43.50^{b}	41.50^{c}	0.07	<0.0001
Final body weight (g/b)	3333.00^{ba}	2878.5^{c}	2888.5^{c}	3093.5^{bc}	3390.00^{a}	62.29	<.0003
Feed intake (g/b/w)	629.03^{e}	652.87^{a}	646.41^{b}	636.53^{c}	633.33^{d}	0.08	<0.0001
Weight gain (g/b/w)	412.50^{a}	354.17^{c}	356.25^{c}	381.25^{b}	418.75^{a}	7.79	0.0003
FCR	1.53^{c}	1.85^{a}	1.81^{a}	1.67^{b}	1.51^{c}	0.03	0.0001

$^{a, b, c, d, e}$ means having different superscripts along the same row are significantly different (p < 0.05), g = Gramme, b = Bird, w = Week; FCR = Feed conversion ratio, BHA = Butylated hydroxyanisole, OW = Ordinary water, SOPE = Sweet orange peel extract, SHPE = Shaddock peel extract, LMPE = Lemon peel extract.

Shear force analysis

The current study shows that there were significant differences (p<0.05) in the shear force (force peak and yield) and strain peak of the meat samples of birds fed dietary antioxidants. The synthetic antioxidant (BHA) treatment force peak was significantly different (p<0.05) from OW, SOPE, SHPE and LMPE treatment force peaks (Table 3). Moreover, more force was expended in cutting through the meat of birds fed dietary treatment (OW) than other treatments (BHA, SOPE, SHPE and LMPE). There were no significant differences (p>0.05) in the stress (yield and peak) of the meat samples when subjected to texture analysing across the treatments. Also, there were significant differences in the strain (peak and yield

percentage) of the meat samples across the treatments, with treatment (SHPE) having the highest percentage of strain peak and treatment (OW) having the lowest percentage of strain yield.

Table 3. The effect of dietary antioxidant supplementation on meat quality (shear force) of broiler meat.

Parameters	Treatments					SEM	P value
	BHA (0.02%)	OW	SOPE (0.02%)	SHPE (0.02%)	LMPE (0.02%)		
Force peak (N)	15.90^{a}	12.00^{b}	7.20^{c}	3.50^{d}	10.40^{b}	0.469	<0.0001
Force yield (N)	3.20^{b}	11.60^{a}	1.50^{c}	1.20^{c}	3.90^{b}	0.184	<0.0001
Stress peak(N/mm^2)	0.06	0.05	0.03	0.01	0.04	0.017	0.4029
Stress yield (N/mm^2)	0.01	0.05	0.01	0.01	0.02	0.01	0.1297
Strain peak (%)	71.91^{b}	10.18^{e}	52.31^{d}	95.09^{a}	59.22^{c}	0.013	<0.0001
Strain yield (%)	11.97^{a}	0.04^{e}	3.83^{c}	5.49^{b}	0.92^{d}	0.019	<0.0001
Young's modulus (N/mm^2)	0.1^{c}	2.13^{a}	0.05^{dc}	0.01^{d}	0.76^{b}	0.015	<0.0001
Width (mm)	25.00	25.00	25.00	25.00	25.00	1.0	1.0000
Thickness (mm)	10.00	10.00	10.00	10.00	10.00	1.0	1.0000

$^{a, b, c, d, e, dc}$ means having different superscripts along the same row are significantly different ($p < 0.05$); BHA = Butylated hydroxyanisole, OW = Ordinary water, SOPE = Sweet orange peel extract, SHPE = Shaddock peel extract, LMPE = Lemon peel extract.

Sensory evaluation

The effects of dietary antioxidant supplementation on meat sensory scores were presented in Table 4. There were no significant differences ($p>0.05$) in the assessor's perception of the appearance and texture of the meat samples across the treatments (Table 4).

Table 4. The effect of dietary antioxidant supplementation on meat quality (sensory evaluation) of broiler meat.

Parameter	Treatments					SEM	P value
	BHA (0.02%)	OW	SOPE (0.02%)	SHPE (0.02%)	LMPE (0.02%)		
Appearance	7.50	7.95	7.05	7.20	7.55	0.30	0.27
Taste/flavour	7.40^{ab}	8.00^{a}	7.00^{ab}	6.50^{b}	7.75^{a}	0.30	0.01
Texture	7.80	7.25	6.50	6.55	7.60	0.35	0.30
Aroma	7.35^{abc}	7.80^{a}	6.50^{bc}	6.30^{c}	7.45^{ab}	0.29	0.001
Overall acceptability	7.95^{a}	7.95^{a}	7.15^{ab}	6.70^{b}	7.90^{a}	0.25	0.001

$^{a, b, c, ab, bc, abc}$ means having different superscripts along the same row are significantly different ($p < 0.05$);BHA = Butylated hydroxyanisole, OW = Ordinary water, SOPE = Sweet orange peel extract, SHPE = Shaddock peel extract, LMPE = Lemon peel extract.

The taste perception of broiler meat in BHA, SOPE and SHPE treatments was not significantly different ($p>0.05$), but different significantly ($p<0.05$) from LMPE and OW treatments. The aroma perception of the broiler meat in OW treatment was significantly different ($p<0.05$) from BHA, SOPE, SHPE and LMPE treatments. The overall acceptability perception of the broiler meat in BHA, OW and LMPE treatments was significantly different ($p<0.05$) from SOPE and SHPE treatments.

Colour coordinates

As shown in Table 5, post mortem dietary antioxidant supplementation had a significant effect on broiler meat colour. The lightness (L*) and redness (a*) of the broiler chicken breast colour were significantly different across the treatments used. On the other hand, yellowness (b*) of the pectoralis muscles of the broiler chicken as a result of the dietary antioxidants administered showed no significant differences ($p>0.05$) among the treatments.

Table 5. The effect of dietary antioxidant supplementation on meat quality (colour coordinates) of broiler meat.

Parameters	Treatments					SEM	P value
	BHA (0.02%)	OW	SOPE (0.02%)	SHPE (0.02%)	LMPE (0.02%)		
Lightness (L*)	20.03^{c}	38.23^{a}	18.47^{c}	34.62^{ba}	22.52^{bc}	2.733	0.0011
Redness (a*)	2.82^{b}	1.22^{b}	1.97^{b}	8.19^{a}	1.95^{b}	0.904	0.0017
Yellowness (b*)	5.33	4.67	3.12	5.11	2.33	0.827	0.1051

$^{a, b, c, ba, bc}$ means having different superscripts along the same row are significantly different ($p < 0.05$); BHA = Butylated hydroxyanisole, OW = Ordinary water, SOPE = Sweet orange peel extract, SHPE = Shaddock peel extract, LMPE = Lemon peel extract.

Performance

The feed intake observed in this study suggests that the dietary intake under OW diet may have aided nutrient digestion, especially energy. Weight gain was reported as a function of the appropriate nutrient intake by the bird (Ishola and Atteh, 2018). NRC (2004) has observed that the feed intake in birds is inversely proportional to the energy content of the diet. Through a feedback mechanism, energy requirement satisfaction can reduce voluntary feed intake by the birds.

Shear force analysis

Tenderness is the most significant component of meat quality that influences consumers' eating satisfaction (Hildrum et al., 2009). The different values of the

shear force mean values obtained in this work correspond to the study conducted by Harris et al. (2001) who have affirmed that an increase in phenolic compounds and vitamin E has an impact on the tenderness of the broiler meat. Table 3 shows the effect of dietary antioxidant extract supplementation on shear force values of pectoralis muscles of the broiler chicken. The shear force values (force peak and yield) reduced significantly in SHPE treatment as compared to other treatments. Similar findings were found in other studies where post mortem ageing increased meat tenderness (Lomiwes et al., 2014; Marino et al., 2013; Sabow et al., 2015).

Sensory evaluation

Within the assessment of sensory parameters, assessors ranked the broiler meat samples based on the hedonic scale. The assessors were with a view that the taste, aroma and overall acceptability perceptions of treatment (OW) were the best, while treatment (SHPE) had a slightly liked taste and aroma perception. This could be due to the absence of phytochemicals in (OW) as compared with other treatments (BHA, SOPE, SHPE and LMPE) that contained some of the phytochemicals which could impact on or affect postmortem weakening of myofibrillar proteins in the course of ageing (Lawne and Ledward, 2006). Since the treatment (OW) did not contain any phytochemical compounds as compared to other treatments (BHA, SOPE, SHPE and LMPE), this could be the reason why the flavour and aroma perception of treatment (OW) was liked very much by the assessors. This agrees with the findings of Elmore et al., (2004a), who postulated that phytochemicals like phenolic compounds could impact negatively on the volatile compounds like butyric acid and 2-Ethylbenzaldehyde found in meat, which contributes to its flavour and aroma. The overall acceptability of the broiler meat fed dietary treatments (BHA, OW and LMPE) was rated higher by the assessors as compared to treatment (SHPE).

Colour coordinates

Redness of meat is a very important colour parameter for the assessment of meat oxidation (Traore et al., 2012). The customer first appraisal of meat quality is based on its colour and this could be linked to both perceived and actual values (Holman et al., 2015). The colour assessment of the meat is related to the oxidation of myoglobin and the decreased metmyoglobin reducing activity (MRA) which eventually leads to metmyoglobin accumulation in the meat (Xue et al., 2012). This change reduces the redness and makes the meat unpleasant for consumers (Filgueras et al., 2010). This current study suggests that broilers fed with SHPE treatment had greater accumulation of metmyoglobin at the pectoralis muscle part of the bird than other treatments (BHA, OW, SOPE and LMPE). This agrees with

the results of Seydim et al., (2006), who have stated that a reduction in redness is due to myoglobin oxidation, especially when meat pH is above 6.

Conclusion

This study indicates that the growth performance and meat quality of broiler chickens subjected to dietary treatments (SOPE, SHPE and LMPE) are comparable and show better results than BHA and OW treatments. Hence, the results obtained from this study affirm that dietary treatments (SOPE, SHPE and LMPE) neither resulted in the poor growth performance nor negatively impacted on the meat quality of the broiler chickens.

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Ethical standards

All human and animal studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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UTICAJI DODATAKA EKSTRAKTA PRIRODNIH ANTIOKSIDANASA NA PERFORMANSE PORASTA I KVALITET MESA BROJLERA

Hakeem Ishola[1*], Elisha Z. Jiya[2], John Y. Adama[2] i Olushola J. Alabi[2]

[1]Odsek za stočarsku proizvodnju, Univerzitet u Državi Kvari, Malete, Nigerija
[2]Odsek za stočarsku proizvodnju, Federalni tehnološki univerzitet, Mina, Nigerija

R e z i m e

U istraživanju je ispitivan uticaj dodavanja antioksidanata u obroke, na performanse i kvalitet mesa brojlera. Naime, 300 jednodnevnih brojlera Arbor Acres hranjeni su početnom smešom od 1. do 4. nedelje i završnom smešom tokom poslednje 4 nedelje. Ptice su nasumično raspoređivane u tretmane zasnovane na dodavanju antioksidanata u vodi za piće u količini od 0,02% butiliranog hidroksianisola (BHA), obične vode (OV), 0,02% ekstrakta kore slatke pomorandže (EKSP), 0,02% ekstrakta kore pomela (EKS) i 0,02% ekstrakta kore limuna (EKL) po litru vode u ogledu sa potpuno slučajnim rasporedom. Konzumiranje hrane i prirast telesne mase beleženi su nedeljno. U svakom tretmanu po tri ptice su odabrane i zaklane radi utvrđivanja kvaliteta mesa. Tretmani sa BHA i EKL imali su najbolji prirast i efikasnost iskorišćavanja hrane na nivou značajnosti ($p<0,05$). Postojale su značajne razlike ($p<0,05$) u mekoći mesa (maksimalna snaga i prinos) pri korišćenju različitih antioksidanasa u ishrani brojlera. Senzorni parametri (ukus, aroma i ukupna prihvatljivost) pokazuju značajne razlike ($p<0,05$) među tretmanima. Međutim, nije bilo značajnih razlika ($p>0,05$) u percepciji izgleda i teksture među tretmanima. Konačno, među tretmanima su uočene značajne razlike ($p<0,05$) u svetlini (L*) i crveno-zelenoj komponenti (a*) boje mesa. Nije bilo značajnih razlika ($p>0,05$) u žuto-plavoj (b*) komponenti boje mesa među tretmanima. Može se zaključiti da su brojleri hranjeni u tretmanima sa EKSP, EKP i EKL imali bolje performanse i da su ovi tretmani poboljšali kvalitet mesa brojlera u poređenju sa tretmanima sa BHA i OV.

Ključne reči: sintetički antioksidans, prirodni antioksidansi, konzumiranje, prirast, senzorni parametri.

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[*]Autor za kontakt: e-mail: abdulhakeemadisa5@gmail.com

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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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