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Growth, Yield, Nodulation and Amino Acid Content of Bambara Groundnut (*Vigna subterranea*) under Inorganic and Organic Fertilizer Application

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ABSTRACT

Background: Bambara groundnut is an edible legume which is highly nutritious. This study was carried out to find the effect of inorganic and organic fertilizer on the growth, nodulation, nitrogen yield and nutritional composition of bambara groundnut (*Vigna subterranea*).

Methods: During the period of 2018, a glasshouse experiment was conducted in a randomized complete block design (RCBD) at Ladang 15, Faculty of Agriculture, Universiti Putra Malaysia. Different rates of compost, biofertilizer, gypsum were used along with the combination of different rates of nitrogen (N) and phosphorus (P) fertilizer. The amino acid content of the seed was measured by HPLC.

Result: N and P fertilizer were found to play a dominating role in increasing the vegetative growth and yield of the plant. Plant height (21.73 cm), leaf area (2802.9 cm^2) and the number of the pods per plant (41.75) increased with the application of N and P. Application of $N_{30}P_{60}$ kg/ha influenced nodule number (35.50) of the plant. Lysine content in seed was 6.03 mg/kg at T_1 ($N_{30}P_{60}$ kg/ha) whereas 5.13 mg/kg was recorded at T_7 (compost 10 t/ha). The application of $N_{30}P_{60}$ kg/ha has increased the growth, yield, nodulation and amino acid content of bambara groundnut.

Key words: Amino acids, Bambara groundnut, Compost, Growth, Inorganic fertilizer, Nodulation, Yield.

INTRODUCTION

Bambara groundnut (*Vigna subterranea*) plays a major role in the diet of West African people and positioned it as the third most significant legume crop following groundnut and cowpea. Ripe seeds of bambara groundnut contain protein 16 to 21%, fat 4.5 to 6.5% and carbohydrate 50 to 60% (Nyau *et al*. 2015).

There is an inadequate study concerning the effect of inorganic and organic fertilizer on the growth, nitrogen yield and nutritional component in the seed of bambara groundnut, especially in Malaysia. Organic material is broken down and decomposed in the natural and biological process and form compost. Although urea is the most suitable source of nitrogen (N), the plant does not use more than 50% (Uddin *et al*. 2017 and Hayat *et al*. 2012) and through volatilization, denitrification and leaching the remaining N is lost. Phosphorus (P) furnishes superior root and soil contact which outcome the advanced getting of phosphorus and other vital and low availability of nutrients and attractions of higher absorption of mineral nutrients (Gharpinde and Wankhade, 2017; Kamble *et al*. 2016).

Biofertilizer is cost-effective and also the renewable basis during which plant acquires essential nutrients to complement inorganic fertilizers (Hasan *et al*. 2019). Biofertilizers supply nutrients like nitrogen and phosphorus and their actions in the soil or rhizosphere and formulate them obtainable for plants in soil (Zi *et al*. 2019). A legume lives in association with rhizobium bacteria and helps to improve the soil status (Haque *et al*. 2020 and Uddin *et al*. 2020). Soil bacteria are one of the important biotic components that influence decomposition and nutrient mineralization in the terrestrial ecosystem (Khan *et al*. 2018).

Although some reports on the relationship between fertilizers and growth, yield and seed quality of bambara groundnut are available, relatively less attention has been paid on the application of chemical fertilizer and compost in Malaysia. Thus, the main objective of the present study was to find out the effect of inorganic and organic fertilizer on the growth, yield, nodulation and amino acid content of bambara groundnut.

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MATERIALS AND METHODS

Experimental site, design and treatments

A glasshouse experiment was conducted during the period of 2018, at the Faculty of Agriculture in Ladang 15, Universiti Putra Malaysia, Malaysia. The size of the pot was 65.94 cm^2. There were 40 experimental units (pot) and in each pot, one seed was sowed. Sandy clay loam soil was used to fill the pot and the amount was 10 kg/plot. Ten different levels of inorganic and organic fertilizers (T_1: N_{30} + P_{60} kg/ha, T_2: N_{20} + P_{60} kg/ha, T_3: N_{25} + P_{50} + K_{75} kg/ha, T_4: compost 2.5 t/ha, T_5: compost 5.00 t/ha, T_6: compost 7.5 t/ha, T_7: compost 10 t/ha, T_8: B-green biofertilizer 15 kg/ha, T_9: gypsum 400 kg/ha, T_{10}: B-green biofertilizer 15 kg/ha + gypsum 400 kg/ha) were applied. N, P and K were used in the form of urea, triple superphosphate (TSP) and MOP and the inorganic and organic fertilizer were mixed with the soil before sowing the seeds. The experiment was desingned with RCBD with four replication.

Growth and yield components

The observations on the growth and yield related data were recorded manually from each pot of each replication separately as well as harvest index was determined as the weight of pod divided by total plant weight with pod and then multiplied by 100. Leaf area and chlorophyll content of the seed was measured by Leaf Area meter (LI-3100C) and Spad meter respectively.

Estimation of nitrogen yield

Bambara groundnut plants were dug carefully around the plants to a depth of 50 cm without disturbing the roots for nodule number, nodule weight, root and shoot dry weight. Nitrogen content in the root, shoot and pods with seed was determined by dry combustion technique using a LECO CR-412 carbon analyzer (LECO, Corporation, St. joseph, USA).

Amino acids content in seed

Concentrations of amino acids in grains was measured by HPLC described by (Strydom and Cohen, 1994) following pre-column derivatization with the 6-aminoquinolyl-N-hydroxysuccinimdly carbonate (Waters, USA).

Statistical analysis

All data were subjected to statistical analysis of variance (ANOVA) using SAS version 9.4 at the 5% significance level and the least significant difference was employed for mean separation (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Vegetative growth and yield

From the results presented, it appears that inorganic and organic fertilizer greatly increased the vegetative growth of the plant (Table 1). Plant height ranges from 20.43 to 21.73 cm in this experiment. The less plant height was recorded at treatment T_4 (compost 2.5 t/ha). At harvesting time, use of T_3 (N_{25} + P_{50} + K_{75} kg/ha) and T_7 (compost 10 t/ha) treatment gave 1.61% and 2.76% respectively less advanced in plant height compare to T_1 (N_{30} + P_{60} kg/ha) (control). There was a decrease in plant height by decreasing the N and P levels. Similar result was also reported by Hasan *et al*. (2019) where plant height decreased with decreasing N and P fertilizer.

The leaf area of the plant influences CO_2 uptake by determining active radiation absorbed by the canopy and stomatal area. Leaf area ranges from 1899.0 to 2802.9 cm^2 and T_5 (compost 5.0 t/ha) gave 47.60% decrese in leaf area compare to T_1 (N_{30} + P_{60} kg/ha) (control). Application of N and P fertilizer leaf area, as well as accumulation of dry matter, was increased. The finding of this experiment is similar to Hossain *et al*. (2007), where N (0, 20, 40, 60 kg/ha and P (0, 30, 60, 90 kg/ha) dose were applied and found the better growth and yield at N_{60} + P_{60} kg/ha. Increase the

Table 1: Effect of inorganic and organic fertilizer on plant height (cm) (PH), leaves number (LN), branch number (BN), leaf area (cm^2) (LA), chlorophyll content (spade value) (CHL), Plant dry weight (g) (PDW), number of pod per plant (NPPP), pod dry weight (Pod DW), harvest index (HI) (%) of bambara groundnut.

Treatments	PH	LN	BN	LA	CHL	PDW	NPPP	Pod DW	HI
T_1	21.7a	265.5a	88.5a	2802.9a	44.7ab	20.6a	41.8a	19.2a	48.5ab
T_2	21.3abc	254.3bc	84.8bc	2256.2ab	42.4abc	19.9a	34.8bcd	16.4ab	45.03b
T_3	21.4ab	258.8ab	86.3ab	22.43.6ab	42.1abc	19.5ab	29.5de	18.6a	49.2ab
T_4	20.4e	238.5e	79.5e	2049.6ab	37.3bc	13.1d	25.75	13.3b	49.8ab
T_5	20.8cde	246.8cde	82.3cde	1899.0b	37.9bc	14.9cd	28.5de	16.2ab	52.8a
T_6	20.7de	240.8de	80.3de	2138.8ab	39.0abc	13.9cd	26.3e	14.6b	50.9ab
T_7	21.1bcd	245.3de	81.8de	2018.0ab	43.0abc	15.7b-d	33.8bcd	16.1ab	50.5ab
T_8	21.1bcd	247.5cd	82.5cd	2330.0ab	46.6a	13.8cd	31.5cde	14.2b	50.7ab
T_9	20.0bcde	243.8de	81.3de	2425.1ab	39.3abc	16.9abcd	37.5abc	14.1b	45.3ab
T_{10}	21.3abc	247.5cd	82.5cd	2253.3ab	36.1c	17.5abc	38.0ab	18.4a	51.0ab
LSD	**	***	***	ns	ns	**	***	**	ns

T_1: N_{30} + P_{60} kg ha^{-1}, T_2: N_{20}+P_{60} kg ha^{-1}, T_3: N_{25} + P_{50} + K_{75} kg ha^{-1}, T_4: compost 2.5 t ha^{-1}, T_5: compost 5.00 t ha^{-1}, T_6: compost 7.5 t ha^{-1}, T_7: compost 10 t ha^{-1}, T_8: B-green bio-fertilizer 15 kg ha^{-1}, T_9: gypsum 400 kg ha^{-1}, T_{10}: B-green bio-fertilizer 15 kg ha^{-1} + gypsum 400 kg ha^{-1}. Means with the same letter have no significant difference.

vegetative growth of bambara groundnut with the application of N and P with the rate of 0, 100, 150 and 200 kg/ha was reported by Wamba *et al*. (2012). A similar result was also reported by Hasan *et al*. (2019). The plant containing more leaves is not helpful for more economic yield and also if the vegetative growth of the plant is more it is undesirable for seed production.

The result showed that there was a significant difference in the pod yield and yield-related traits indicating a high variation of fertilizer among these traits (Table 1). As soon as the vegetative stage finish pod are started to form until the harvest. The results suggested that the highest number of the pod recorded in the treatment of T_1. The highest number of the pod was obtained at T_1 (41) which was treated with N_{30} + P_{60} kg/ha. Treatment T_1 gave a 64% increased pod number compare to T_4. Treatment T_1 (N_{30} + P_{60} kg/ha) (49.67 g) also produced the highest pod weight and T_4 (compost 2.5 t/ha) (34.95 g) was the lowest. These result was quite dissimilar with Ikenganyia *et al*. (2017) because they used the dose of single super phosphate (0, 25, 50, 75 kg/ha). The difference observed between the treatments was because, in the organic residues, nutrient availability depended on nutrient concentration and release with synchrony with crop needs. In the current study, the highest plant growth and yield reported at $N_{30}P_{60}$ kg/ha could be attributed to the nutrients being readily available from the source. Organic sources alone could not improve the growth and yield of this crop but Ram and Dhaliwal (2012) advocated the use of intergrated nutrient management through chemical and organic sources for getting maximum crop yields.

Correlation among agronomic and yield characteristics

Correlation studies have great significance between the agronomic and yield character of bambara groundnut (Table 2). The parameter analysis showed that plant height recorded positive correlation coefficient with leaf number (r = 0.908**), branch number (r = 0.908**), plant fresh weight (r = 0.833**), revealing that the more plant height the higher leaf number, leaf area and plant fresh weight. The table also showed that the weight of hundred seed weight recorded significant correlation coefficient with pod number (r = 0.794**), pod fresh weight (r = 0.894**), pod dry weight (r = 0.938**) days of flowering (r = 0.932**). From the result it clearly showed that the more weight of 100 seed the higher pod number, pod fresh weight, pod dry weight and days of flowering.

Nodule and nitrogen yield

The effects of different levels of organic fertilizer on nodule number, nodule weight and nitrogen yield were varied significantly (Table 3). The number and weight of nodules were increased with the application of N and P fertilizer (N_{30} + P_{60} kg/ha) compare to other organic fertilizers. The highest nodule number of 35.50 was recorded at T_1 (N_{30} + P_{60} kg/ha) followed by 32.50 at T_2 (N_{20} + P_{60} kg/ha) and 27.00 at T_3 (N_{25} + P_{50} + K_{75} kg/ha). Treatment T_4 (compost 2.5 t/ha)

Table 2: Pearson correlation coefficient among agronomic and yield characteristics of bambara groundnut.

	PH	LN	BN	CS	CH	DF	PFW	PDW	PN	Pod FW	Pod DW	100SW	HI%
PH	1												
LN	0.908**	1											
BN	0.908**	1**	1										
CS	0.963**	0.846**	0.846**	1									
CH	0.555	0.500	0.500	0.548	1								
DF	-0.959**	-0.855**	-0.855**	-0.904**	-0.3264	1							
PFW	0.833**	0.759*	0.759*	0.817**	0.060	-0.910**	1						
PDW	0.868**	0.873**	0.873**	0.867**	0.238	-0.898**	0.933**	1					
PN	0.782**	0.572	0.572	0.854**	0.243	-0.794**	0.802**	0.695*	1				
Pod FW	0.877**	0.79**	0.791**	0.830**	0.159	-0.931**	0.919**	0.854**	0.780**	1			
Pod DW	0.800**	0.822**	0.822**	0.720*	0.113	-0.870**	0.800**	0.800**	0.534	0.865**	1		
100SW	0.919**	0.901**	0.901**	0.896**	0.316	-0.932**	0.868**	0.923**	0.671*	0.894**	0.938**	1	
HI%	-0.324	-0.264	-0.264	-0.437	-0.167	0.298	-0.473	-0.553	-0.44893	-0.269	0.052	-0.233	1

PH: Plant height, LN: Leaf number, BN: Branch number, , CS: Canopy spread, CH: Chlorophyll content, DF: Days of flowering, PFW: Plant fresh weight, PDW: Plant dry weight, PN: Pod number, Pod FW: Pod fresh weight, Pod DW: Pod dry weight, 100SW: 100 seed weight, HI: Harvest index.

Table 3: Effect of inorganic and organic fertilizer on nodule number, nodule weight, shoot nitrogen, root nitrogen and nitrogen in a pod with seed in bambara groundnut.

Treatments	Nodule plant^{-1}	Nodule weight plant^{-1} (g)	Shoot nitrogen (%)	Root nitrogen (%)	Nitrogenin a pod with seed (%)
T_1	35.50a	0.26a	2.43a	2.33a	3.29a
T_2	32.50a	0.25a	2.40ab	2.32ab	3.26ab
T_3	27.00b	0.24ab	2.34abc	2.28abc	3.21abc
T_4	20.75d	0.21c	2.22de	2.14de	2.98cd
T_5	22.25d	0.22c	2.26cde	2.18cde	2.99bcd
T_6	22.75cd	0.23bc	2.19e	2.11e	2.85d
T_7	24.50bcd	0.23bc	2.32abcd	2.21bcde	3.10abcd
T_8	26.25bc	0.24ab	2.27cde	2.18cde	3.14abc
T_9	24.50bcd	0.23bc	2.36abc	2.23abcd	3.06abcd
T_{10}	24.50bcd	0.23bc	2.30bcde	2.20cde	3.10abcd
$LSD_{0.05}$	**	**	**	**	ns

T_1: N_{30} + P_{60} kg ha^{-1}, T_2: N_{20}+P_{60} kg ha^{-1}, T_3: N_{25} + P_{50} + K_{75} kg ha^{-1}, T_4: compost 2.5 t ha^{-1}, T_5: compost 5.00 t ha^{-1}, T_6: compost 7.5 t ha^{-1}, T_7: compost 10 t ha^{-1}, T_8: B-green biofertilizer 15 kg ha^{-1}, T_9: gypsum 400 kg ha^{-1}, T_{10}: B-green biofertilizer 15 kg ha^{-1} + gypsum 400 kg ha^{-1}. LSD = least significant difference. Means with the same letter have no significant difference, P≤0.05 = significant (*), P≤0.01 = significant (**), P≤0.001=significant (***). ns=Non significant**.**

Table 4: Effect of inorganic and organic fertilizer on amino acid content (mg kg^{-1}) of bambara groundnut.

Treatments	Lys	Ala	Arg	Asp	Cys	Glu	Gly	His	Ile	Leu	Met	Phe	Ser	Val
	mg/kg													
T_1	6.03a	4.50a	4.75a	10.40a	1.28a	20.05a	3.28a	3.08a	4.00a	6.83a	1.05a	5.45a	3.65ab	4.58a
T_2	5.73a	4.35a	4.30a	10.05a	1.05abcd	19.70a	3.15a	2.83a	3.93a	6.40ab	1.20a	5.45a	3.78a	4.48a
T_3	5.63a	4.33a	4.58a	10.08a	1.15abc	19.03a	3.08a	2.85a	3.98a	6.18ab	1.20a	5.48a	3.78a	4.38a
T_4	5.65a	4.18a	4.68a	9.78a	1.08abcd	19.80a	3.28a	2.90a	3.95a	6.48ab	1.00a	5.40a	3.38abc	4.30a
T_5	5.38a	4.43a	3.93a	10.18a	0.78d	19.60a	3.28a	2.78a	3.80a	6.20ab	1.20a	5.40a	3.53abc	4.43a
T_6	5.18a	4.13a	4.00a	9.98a	0.88bcd	19.63a	3.18a	2.80a	3.95a	5.90ab	1.35a	5.40a	3.05bc	4.60a
T_7	5.13a	4.15a	3.90a	9.90a	0.85cd	19.08a	3.05a	2.78a	3.95a	6.68a	1.40a	5.40a	2.95c	4.58a
T_8	5.45a	4.10a	4.65a	10.20a	1.18abc	19.68a	3.23a	2.85a	3.85a	6.95a	1.48a	5.48a	3.28abc	4.35a
T_9	5.70a	4.40a	4.48a	10.08a	1.28a	19.78a	3.18a	2.80a	4.00a	5.53b	1.25a	5.40a	3.38abc	4.33a
T_{10}	5.73a	4.20a	4.60a	10.35a	1.23ab	19.43a	3.25a	2.88a	3.88a	6.55ab	1.28a	5.35a	3.63ab	4.48a
$LSD_{0.05}$	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

T_1: N_{30} + P_{60} kg ha^{-1}, T_2: N_{20}+P_{60} kg ha^{-1}, T_3: N_{25} + P_{50} + K_{75} kg ha^{-1}, T_4: compost 2.5 t ha^{-1}, T_5: compost 5.00 t ha^{-1}, T_6: compost 7.5 t ha^{-1}, T_7: compost 10 t ha^{-1}, T_8: B-green biofertilizer 15 kg ha^{-1}, T_9: gypsum 400 kg ha^{-1}, T_{10}: B-green biofertilizer 15 kg ha^{-1} + gypsum 400 kg ha^{-1}. $LSD_{0.05}$ = least significant difference. Means with the same letter have no significant difference, P≤0.05 = significant (*), P≤0.01 = significant (**), P≤0.001=significant (***). ns=Non significant.

(20.75) contained the lowest nodule number. These findings are similar to other studies (Egbe and Egbo, 2011). Nodulation plays a critical role in N fixation by leguminous crops. Nodule growth and function require proper light photosynthate and fertilizer. In the treatment of T_1 (0.26 g), the nodule weight was higher than any other treatment. Nodule weight was illustrated 0.21 g with T_4 (compost 2.5 t/ ha) which was the lowest among all the treatments. Nodule weight increase of 6.07% at T_{10} (biofertilizer 15 kg/ha+ gypsum 400 kg/ha) and 5.90% at T_8 (biofertilizer 15 kg/ha) compared to T_1 (N_{30} + P_{60} kg/ha) (control) was also recorded. There was an increasing trend of nodule number, the weight of nodule and nitrogen yield with increasing N and P fertilizer (N_{30} + P_{60} kg/ha). Significant differences in number and biomass of nodulation were also reported by Egbe and Bar-Nyam, (2011). The N content of roots shoots and pods with the seed of bambara groundnut seemed to have affected by the inorganic and organic fertilizer. The nitrogen yield of pods with the seed of bambara groundnuts was consistently higher than that of the shoot, which in turn was higher than root in all the treatments. A similar result was also reported by Egbe *et al*. (2013).

Amino acids content in seed

Bambara groundnut is an edible legume which serves as one of the main sources of income for smallholder farmers. The seeds contain sufficient quantities of lysine, cysteine and methionine such kind of essential amino acids. Effect of inorganic and organic fertilizer on amino acid content of bambara groundnut is presented in (Table 4). Application of

different levels of fertilizer and compost has non significant differences among the content of the amino acid of bambara groundnut seed. In seed, amino acid content was decreased at T_7 (compost 10 t ha^{-1}) such as lysine (14.92%), argenine (17.89%), cystine (33.59%), glumatic acid (4.83%), glysine (7.01%) and histidine (9.74%) in compare to T_1 (N_{30} + P_{60} kg/ha) (control). On the other hand alanine (4.50 mg/kg), arginine (4.75 mg/kg) and aspartic acid (10.40 mg/kg) was the highest from the T_1 (N_{30} + P_{60} kg/ha) treatment (control). It is seen that aspartic acid, glumatic acid and leucine are the most abundant amino acids in all the samples. (Olaleye *et al*. 2013) reported the same result. However, the amino acids contained in the seed were lower than the result reported by Mazahib *et al*. (2013).

Bambara groundnut has numerous agronomic and nutritional attributes which make it an excellent crop to develop. The results indicate that the inorganic N and P application exerts a strong influence on the bambara groundnut on growth, yield, nitrogen content and seed quality. This is the clear indication that N_{30} + P_{60} kg/ha increase the growth, yield, nitrogen content and yield of bambara groundnut rather than other fertilizer.

CONCLUSION

Bambara groundnut is a low- priced commodity but a valuable source of all the nutrients. The level of nutrients present in organic fertilizer is often low and usaully complexed in organic chemical structure. The growth, yield, nitrogen yield and amino acid content of bambara groundnut was increased with the combination 30 kg nitrogen and 60 kg phosphorus fertilizer. The application of 30 N and 60 P kg/ha could be recommended for getting maximum growth, yield, nitrogen yield and amino acid content of bambara groundnut.

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