



Effect of Nitrogen and Phosphorus Fertilizers on Growth, Yield, Nodulation and Nutritional Composition of Bambara Groundnut [*Vigna subterranea* (L.) Verdc.]

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ABSTRACT

Background: Bambara groundnut is an edible legume that is highly nutritious. This study was carried out to find the effect of nitrogen (N) and phosphorus (P) fertilizers on the growth, yield, nodulation, the proximate and nutritional composition of bambara groundnut [*Vigna subterranea* (L.) Verdc.].

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 N and P fertilizer were used. Kjeldahl method and HPLC, respectively, determined the protein and amino acid content in seed.

Result: N and P fertilizer were found to play a dominating role in increasing the vegetative growth and yield of the plant. Plant height (20.65a), pod number (45.75a) and harvest index (41.61a) increased significantly with the application of N₃₀ + P₆₀ kg ha⁻¹. Application of N₃₀P₆₀ kg ha⁻¹ significantly influenced nodulation and nitrogen yield. Protein, fibre, Mg and amino acid content increased with N₃₀P₆₀ kg ha⁻¹. The application of N₃₀P₆₀ mg kg⁻¹ has increased the growth, yield, nodulation, proximate and nutritional composition of bambara groundnut.

Key words: Amino acids, Bambara groundnut, Growth, Nitrogen, Nodulation, Nutritional composition, Phosphorus, Yield.

INTRODUCTION

Bambara groundnut [*Vigna subterranea* (L.) Verdc.] is cultivated in the west and central Africa, but now its cultivation is spread worldwide. It can be grown in all kinds of soil, especially in marginal soil. It played a significant role in the diet of West African people and positioned it as the third most significant legume crop, following groundnut and cowpea (Lo *et al.*, 2019). Bambara groundnut is measured as a balanced diet because of the high percentage of carbohydrates (65%), protein (18%) and fat (6.5%) contain in the seed (Mazahib *et al.*, 2013).

Nitrogen plays a beneficial role in legume growth and promotes extensive root development, thereby ensuring a good yield. Although urea is the most suitable source of N, the plant does not use more than 50% (Hasan *et al.*, 2018) and through volatilization, denitrification and leaching, the remaining N is lost. Nitrogen plays a vital role in cell division and the formation of active photosynthetic pigments. Vegetative growth of the plant tended to enlarge with an increase in different levels of N fertilizer (Uddin *et al.*, 2017). The application of N fertilizer enhanced the agronomic parameters of bambara groundnut and showed a remarkable impact on plant growth. When N is released into the soil, it helps in the root development of bambara groundnut while also assisting in N₂ fixation (Hasan *et al.*, 2021).

Following N, P is considered a crucial mineral fertilizer for the flourishing production of the crop. Physiological parameters such as photosynthesis, transpiration rate, chlorophyll content and root growth *etc.*, are considered as critical factors for plant growth and development (Motmainna

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et al., 2021). Insufficient phosphorus restrictions directly or indirectly manipulate them. In the soil, P is remaining as a critical constitute and produced an insufficiently resolved phosphate mineral (Hasan *et al.*, 2018, Haque *et al.*, 2020). The physiology of the plant is affected to a great extent with the application of P fertilizer and it also helps to get better yield (Hasan *et al.*, 2019, Uddin *et al.*, 2020). Plants' ability to produce seeds is enhanced by phosphorus to the soil, which helps to accelerate meristem tip activity, root formation and early growth of the plant. Phosphorus fertilizer is required for nitrogen fixation as well as nutrient and water intake from the soil (Hasan *et al.*, 2019). This is accomplished through increased root number

and the formation of lateral and fibrous roots. Phosphorus fertilizer also promotes the development of lateral, fibrous and healthy roots.

Although some reports on the relationship between fertilizers and growth, yield and seed quality of bambara groundnut are available, relatively little attention has been paid to N and P fertilizer application in Malaysia. Thus, the main objective of the present study was to find out the effect of N and P fertilizer on the growth, yield, nodulation and nutritional composition of bambara groundnut.

MATERIALS AND METHODS

Experimental site, design and treatments

A glasshouse experiment was conducted in 2018 at the Faculty of Agriculture in Ladang 15, Universiti Putra Malaysia, Malaysia. The temperature and humidity were 31.27°C and 84%, respectively. The volume of the pot was 70.65 m³ and one seed was sowed in each pot. Sandy clay loam soil was used to fill the pot and the amount was 10 kg pot⁻¹. The amount of calcium, magnesium, potassium and sodium was 1.60, 2.30, 0.36 and 4.55, 140.6 and 147.3 cmol_c kg⁻¹, respectively, in the soil with pH 6.4. Sixteen treatments of this experiment were the combination of different rates of N and P (T₁: N₀P₀, T₂: N₀P₂₀, T₃: N₀P₄₀, T₄: N₀P₆₀, T₅: N₁₀P₀, T₆: N₁₀P₂₀, T₇: N₁₀P₄₀, T₈: N₁₀P₆₀, T₉: N₂₀P₀, T₁₀: N₂₀P₂₀, T₁₁: N₂₀P₄₀, T₁₂: N₂₀P₆₀, T₁₃: N₃₀P₀, T₁₄: N₃₀P₂₀, T₁₅: N₃₀P₄₀ and T₁₆: N₃₀P₆₀ kg ha⁻¹). T₁ (N₀P₀ kg ha⁻¹) was considered as the experimental control. Nitrogen and P were used in the form of urea and triple superphosphate (TSP), respectively. The fertilizer treatments were mixed with the soil before sowing the seeds. The experiment was performed randomized complete block design (RCBD) with four replications.

Measurement of growth and yield parameters

The observations on the growth and yield-related data were recorded manually from each pot of each replication separately. The harvest index was calculated as described by Hasan *et al.* (2021). Leaf area was measured by leaf area meter (LI-3100C) (Motmainna *et al.*, 2021).

Nodulation and nitrogen yield estimation

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 (Hasan *et al.*, 2021). Nitrogen content in the root, shoot and pods with seed were determined by dry combustion technique using a LECO CR-412 carbon analyzer (LECO, Corporation, St. Joseph, USA) (Hasan *et al.* 2021).

Determination of proximate composition in seed

The most standard method for determining protein is the Kjeldahl method (Kjeldahl, 1883). Fiber determination was carried out by utilizing Fibertec System (Fenton *et al.*, 1979). The moisture content of the seed was determined following the method developed by Willits, 1951. The mineral composition was measured in the dry ash method (Cotteine, 1980).

Amino acids content in seed

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

Statistical analysis

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

RESULTS AND DISCUSSION

Impact of N and P on growth, yield and nodulation of Bambara groundnut

The plant height, leaf area, nodule number, nodule weight, nitrogen yield, pod number and harvest index were investigated at harvesting time were significantly increased with different levels of applied N and P fertilizer (Table 1 and Fig 1). The mean highest plant height of 20.65 cm was recorded at T₁₆ (N₃₀P₆₀ kg ha⁻¹) and gave 5.46% more advanced than T₁ (control). The result also revealed that the growth of plants increased with the increasing N and P levels. Hasan *et al.* (2019) also reported a similar effect where plant height decreased with decreasing N and P fertilizer. Highest increase in pod number and pod dry weight at N₃₀P₆₀ kg ha⁻¹ was reported by Hasan *et al.* (2021). The number and weight of nodules were increased with N₃₀P₆₀ kg ha⁻¹ compared to other N and P fertilizer combinations. The highest nodule number of 38.00 was recorded at T₁₆ (N₃₀P₆₀ kg ha⁻¹) followed by 34.75 at T₁₅ (N₃₀P₄₀ kg ha⁻¹) and 33.75 at T₈ (N₁₀P₆₀ kg ha⁻¹). These findings are similar to other studies (Egbe and Egbo, 2011). The most significant increase in the pod number was 74.28% at T₁₆ (N₃₀P₆₀ kg ha⁻¹), followed by 65.71% at T₁₂ (N₂₀P₆₀ kg ha⁻¹) and 53.33% at T₄ (N₀P₄₀ kg ha⁻¹) compared to T₁ control (control). There exists an increasing trend with increasing levels of N and P fertilizer. The finding of this experiment is similar to Hossain *et al.* (2007), where N (0, 20, 40, 60 kg ha⁻¹ and P (0, 30, 60, 90 kg ha⁻¹) doses were applied and found the better growth and yield at N₆₀P₆₀ kg ha⁻¹.

Impact of N and P on mineral and proximate composition of seed

The results showed that calcium (Ca), magnesium (Mg) and manganese (Mn) content in bambara groundnut differed significantly due to the different levels of N and P application but have no significant difference was recorded on protein, fiber, ash, moisture, potassium (K), copper (Cu), iron (Fe) and sodium (Na) (Table 2). The highest value of Ca was illustrated at 660.00 mg kg⁻¹ at T₁₃ (N₃₀P₀ kg ha⁻¹) and the lowest was 592.75 mg kg⁻¹ at T₁ (control) (Table 2). Manganese content increased 70.45% at T₁₆ (N₃₀P₆₀ kg ha⁻¹), followed by 61.36% at T₁₂ (N₂₀P₄₀ kg ha⁻¹) and 56.06% at T₄ (N₀P₄₀ kg ha⁻¹). The result mentioned that the mineral

composition increased with increasing at a different level of N and P fertilizer but decreased where no fertilizer was applied. A similar finding was reported by Hasan *et al.* (2019) that the mineral composition was increased at N₃₀P₆₀ kg ha⁻¹ compare to other fertilizer treatments. The amount of protein contains in seed at different levels of N

and P was varied among 191.30 to 203.00 mg kg⁻¹. On the other hand, in this study bambara groundnut seeds contain 51.50 mg kg⁻¹ fibre, which was the same as the result obtained from Abdulsalami and Sheriff (2010) but higher than that of the result by Mazahib *et al.* (2013). Nitrogen and P contribute significantly to the synthesis of

Table 1: Effect of N and P fertilizer on nodule plant⁻¹, nodule weight (g), leaf N (%), shoot N (%), root N (%) and seed N (%) with seed in Bambara groundnut.

Treatments (mg kg ⁻¹)	PH	LA	Leaf N	Shoot N	Root N	Seed N
T ₁ (N ₀ P ₀)	19.58c	1492.70d	1.71i	1.05g	1.21g	2.84f
T ₂ (N ₀ P ₂₀)	20.15ab	1926.30bcd	1.78hi	1.20gf	1.33efg	2.95ef
T ₃ (N ₀ P ₄₀)	19.95bc	1806.40cd	2.13fg	1.20efg	1.45def	2.99ef
T ₄ (N ₀ P ₆₀)	20.65a	2461.50ab	2.76bc	1.64b	1.75bc	3.19abcd
T ₅ (N ₁₀ P ₀)	20.10bc	2244.50abc	1.10gh	1.21def	1.29fg	3.02def
T ₆ (N ₁₀ P ₂₀)	20.23ab	1876.30cd	2.34ef	1.23def	1.51de	3.10cde
T ₇ (N ₁₀ P ₄₀)	20.00bc	1985.80bcd	2.41e	1.36cd	1.64cd	3.19abcd
T ₈ (N ₁₀ P ₆₀)	20.43ab	2464.00ab	2.90abc	1.76b	1.82bc	3.26abc
T ₉ (N ₂₀ P ₀)	20.03bc	2117.40abc	2.31ef	1.26cde	1.38efg	3.04de
T ₁₀ (N ₂₀ P ₂₀)	20.18ab	2284.40abc	2.40e	1.30cde	1.51de	3.13bcde
T ₁₁ (N ₂₀ P ₄₀)	20.15ab	1501.70d	2.46ed	1.40c	1.65cd	2.95ef
T ₁₂ (N ₂₀ P ₆₀)	20.45ab	2265.90abc	2.99ab	1.78b	1.95ab	3.31ab
T ₁₃ (N ₃₀ P ₀)	20.08bc	2100.80bc	2.24efg	1.29cde	1.47def	3.07cde
T ₁₄ (N ₃₀ P ₂₀)	20.20ab	2103.80bc	2.48ed	1.36cd	1.62cd	3.21abcd
T ₁₅ (N ₃₀ P ₄₀)	20.25ab	2231.90abc	2.69cd	1.42c	1.74bc	3.24abc
T ₁₆ (N ₃₀ P ₆₀)	20.65a	2654.90a	3.11a	2.00a	2.11a	3.35a
LSD	**	*	***	***	***	***

Means with the same letter in a column have no significant difference $p>0.05$.

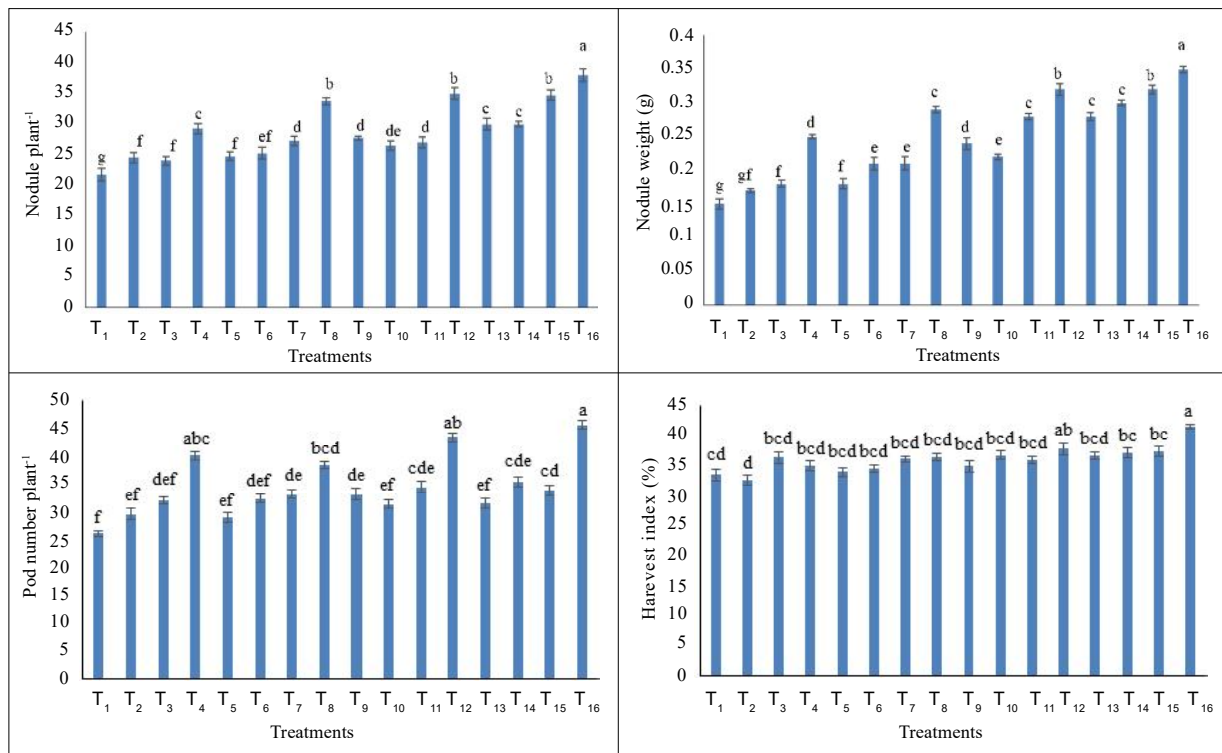


Fig 1: Effect of N and P fertilizer on nodule number, nodule weight, pod number and harvest index of Bambara groundnut.

Table 2: Effect of N and P fertilizer on proximate and mineral composition (mg kg⁻¹) of Bambara groundnut.

Treatments (mg kg ⁻¹)	Protein	Fibre	Ash	Ca	Mg	Moisture	K	Cu	Fe	Mn	Na
T ₁ (N ₀ P ₀)	192.3ab	43.8a	36.0a	592.75c	594.5bcd	30.3a	370.3c	1.4c	44.4a	13.2d	78.0c
T ₂ (N ₀ P ₂₀)	194.0ab	47.3a	35.8a	648.53abc	619.3abcd	30.3a	417.3ab	1.7bc	46.7a	18.3abc	88.3abc
T ₃ (N ₀ P ₄₀)	195.8ab	45.8a	37.5a	611.75abc	632.0abcd	30.0a	403.8abc	1.9abc	46.0a	18.4abc	82.0bc
T ₄ (N ₀ P ₆₀)	196.8ab	47.0a	38.3a	658.00a	641.8abc	30.3a	426.3ab	2.9abc	52.3a	20.6ab	98.8a
T ₅ (N ₁₀ P ₀)	195.5ab	51.5a	37.5a	637.50abc	590.0cd	32.5a	402.5abc	2.4abc	49.3a	15.2cd	89.8abc
T ₆ (N ₁₀ P ₂₀)	193.0ab	47.3a	38.3a	597.00bc	615.0abcd	31.5a	412.5ab	2.4abc	47.8a	16.7bcd	88.5abc
T ₇ (N ₁₀ P ₄₀)	193.80ab	49.8a	36.3a	617.75abc	604.5abcd	30.5a	411.5abc	3.3ab	47.7a	18.1abc	84.3bc
T ₈ (N ₁₀ P ₆₀)	195.3ab	52.0a	37.0a	645.25abc	650.5a	31.5a	420.8abc	3.5a	53.2a	20.8ab	88.8abc
T ₉ (N ₂₀ P ₀)	192.5ab	47.3a	37.5a	610.00abc	589.3d	30.3a	401.5abc	2.4abc	49.8a	17.0bcd	85.3bc
T ₁₀ (N ₂₀ P ₂₀)	191.3ab	49.3a	37.0a	649.25abc	630.3abcd	30.3a	407.5abc	2.4abc	49.8a	15.8cd	87.5abc
T ₁₁ (N ₂₀ P ₄₀)	196.8ab	49.0a	37.3a	627.75abc	609.8abcd	30.8a	421.3ab	2.9abc	49.6a	18.0abc	89.5abc
T ₁₂ (N ₂₀ P ₆₀)	203.0a	51.3a	36.5a	657.00ab	633.3abcd	32.5a	421.8ab	3.3ab	56.2a	21.3ab	93.0ab
T ₁₃ (N ₃₀ P ₀)	191.5ab	50.8a	36.3a	660.00a	590.0cd	33.3a	409.0abc	2.8abc	49.5a	19.4abc	83.8bc
T ₁₄ (N ₃₀ P ₂₀)	192.5ab	48.8a	38.8a	638.25abc	609.0abcd	31.0a	409.8abc	3.1abc	49.6a	17.0bcd	89.0abc
T ₁₅ (N ₃₀ P ₄₀)	188.0b	4.95a	36.5a	630.50abc	639.8abcd	29.8a	397.8bc	3.1abc	53.4a	18.9abc	90.3abc
T ₁₆ (N ₃₀ P ₆₀)	198.5ab	4.90a	37.3a	656.00ab	642.8ab	30.5a	440.8a	3.60a	44.3a	22.5a	89.8abc
LSD	ns	ns	ns	**	*	ns	ns	ns	ns	*	ns

Means with the same letter in a column have no significant difference at p>0.05.

Table 3: Effect of N and P fertilizer on amino acid content (mg kg⁻¹) of Bambara groundnut.

Treatments (mg kg ⁻¹)	Ala	Arg	Asp	Cys	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Ser	Val
T ₁ (N ₀ P ₀)	4.13a	4.85ab	9.90a	0.88a	18.65a	3.15a	2.73a	3.88a	5.93a	4.80c	1.05ab	5.33ab	3.30a	4.28bc
T ₂ (N ₀ P ₂₀)	4.03a	4.93ab	10.35a	1.08a	18.88a	3.20a	2.93a	4.00a	5.70a	5.30abc	1.10ab	5.05b	3.33a	4.40abc
T ₃ (N ₀ P ₄₀)	4.23a	5.35a	10.13a	1.03a	18.80a	3.05a	2.90a	3.95a	5.85a	5.30abc	0.93b	5.65ab	3.60a	4.60ab
T ₄ (N ₀ P ₆₀)	4.05a	5.43a	9.80a	1.03a	18.28a	3.15a	3.05a	4.03a	6.33a	5.53abc	1.25ab	5.43ab	3.58a	4.70a
T ₅ (N ₁₀ P ₀)	4.10a	5.23ab	9.85a	1.15a	18.68a	3.23a	3.08a	3.85a	5.70a	5.70ab	1.40a	5.73a	3.75a	4.23c
T ₆ (N ₁₀ P ₂₀)	4.15a	4.85ab	9.98a	1.05a	18.75a	3.18a	2.78a	4.08a	5.90a	5.43abc	1.05ab	5.25ab	3.30a	4.40abc
T ₇ (N ₁₀ P ₄₀)	4.18a	4.30b	10.03a	0.98a	18.68a	3.13a	2.98a	3.93a	6.35a	5.40abc	1.08ab	5.58ab	3.78a	4.60ab
T ₈ (N ₁₀ P ₆₀)	4.18a	5.28ab	10.28a	0.95a	18.35a	3.30a	2.95a	3.95a	5.93a	5.48abc	1.15ab	5.28ab	3.43a	4.55abc
T ₉ (N ₂₀ P ₀)	4.10a	5.05ab	7.73b	0.88a	18.38a	3.05a	3.03a	3.88a	5.90a	5.18bc	1.05ab	5.40ab	3.53a	4.65a
T ₁₀ (N ₂₀ P ₂₀)	4.10a	4.98ab	10.03a	1.20a	18.90a	3.15a	2.85a	3.98a	6.35a	5.73ab	1.23ab	5.30ab	3.38a	4.50abc
T ₁₁ (N ₂₀ P ₄₀)	4.08a	5.10ab	9.95a	1.03a	18.78a	3.25a	2.98a	3.90a	6.18a	5.73ab	1.00ab	5.45ab	3.58a	4.40abc
T ₁₂ (N ₂₀ P ₆₀)	4.20a	5.28ab	10.00a	1.03a	19.43a	3.28a	3.10a	4.08a	6.23a	5.80ab	1.40a	5.68ab	3.45a	4.48abc
T ₁₃ (N ₃₀ P ₀)	4.30a	4.68ab	10.08a	1.13a	19.48a	3.28a	2.90a	3.88a	6.45a	5.50abc	1.40a	5.53ab	3.33a	4.43abc
T ₁₄ (N ₃₀ P ₂₀)	4.30a	4.85ab	10.00a	1.13a	19.03a	3.23a	2.80a	4.10a	5.88a	5.65abc	1.30ab	5.45ab	3.25a	4.43abc
T ₁₅ (N ₃₀ P ₄₀)	4.25a	5.03ab	10.08a	1.08a	18.45a	3.15a	2.95a	3.95a	5.95a	5.43abc	0.95ab	5.68ab	3.58a	4.60ab
T ₁₆ (N ₃₀ P ₆₀)	4.15a	5.18ab	10.13a	1.00a	19.03a	3.10a	2.90a	3.95a	5.90a	6.08a	1.05ab	5.75a	3.70a	4.60ab
LSD _{0.05}	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Ala: Alanine, Arg: Arginine, Asp: Aspartic acid, Cys: Cysteine, Glu: Glutamic acid, Gly: Glycine, His: Histidine, Ile: Isoleucine, Leu: Leucine, Lys: Lysine, Met: Methionine, Phe: Phenylalanine, Ser: Serine, Val: Valine. Means with the same letter in a column have no significant difference at p>0.05.

fibre and protein and the higher N and P available to the crop, the higher protein can be synthesized.

Impact of N and P on amino acid content of seed

Application of N and P fertilizer at different levels had no significant impact on all amino acid content on the seed of bambara groundnut plant (Table 3). The seeds contain sufficient quantities of lysine, cysteine and methionine, such kind of essential amino acids. Amino acids content in seeds were decreased at T₁₆ (N₃₀P₆₀ kg ha⁻¹) such as aspartic acid (2.32%), arginine (6.80%), cystine (13.64%), glutamic acid (2.03%) and histidine (6.23%) in comparison to T₁ (control). It is seen that aspartic acid, glutamic 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 is an edible legume that serves as one of the primary sources of income for smallholder farmers. Our findings indicated that N and P fertilizer significantly influenced the growth, yield, nitrogen content and nutritional composition of bambara groundnut. This is a clear indication that N₃₀P₆₀ kg ha⁻¹ increases the growth, yield, nitrogen content and nutritional composition of bambara groundnut rather than other fertilizers.

CONCLUSION

Bambara groundnut is an edible legume that serves as one of the primary sources of income for smallholder farmers. It has numerous agronomic and nutritional attributes, which make it an excellent crop to develop. The results indicate that the inorganic N and P applications strongly influence the growth, yield, nitrogen content and seed quality of bambara groundnut. This is a clear indication that 30 kg ha⁻¹ nitrogen and 60 kg ha⁻¹ phosphorus fertilizer increase the growth, yield, nitrogen content and nutritional composition of bambara groundnut. The application of N₃₀ + P₆₀ kg ha⁻¹ could be recommended for getting maximum growth, yield, nodulation and nutritional composition of bambara groundnut.

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