



INFLUENCE OF SIMULATED ACID SOIL pH (4.0) ON PROXIMATE AND ELEMENTAL COMPOSITION OF *Phaseolus lunatus* (Linn.) (LIMA BEAN), AMENDED WITH AGRICULTURAL LIME AND ORGANIC MANURE

EFFA EFFA, ANOBEJA, MAURICE T. AKESA, NGELE BLESSING, ANN MOFUNANYA
AND GODWIN M. UBI

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ABSTRACT

This study examines the impact of acidic soil conditions, specifically at pH 4.0, on the proximate and elemental composition of *Phaseolus lunatus* (Lima bean) and the effectiveness of agricultural lime and organic manure as soil amendments. The experiment was laid in a 1×1×4 factorial and data for all parameters were analyzed using analysis of variance. Growth of the beans on the simulated acid soil was achieved by irrigating soil with acidified water, simulated by mixing sulphuric and nitric acids in a 2:1 ratio. The simulated acid soil was subsequently treated with agricultural lime and organic manure prior to planting, with 50 g of each amendment mixed with 5 kg of each soil. Proximate composition of *Phaseolus lunatus* such as moisture content, protein, fat, fiber, and ash, while macro and micronutrient contents in the harvested beans were carried out. Results of physico-chemical properties revealed that acidic soils amended with a combined treatment of agricultural lime and organic manure improved the soil pH towards neutrality (from 4.0 to 7.65). Mineral nutrients analysis revealed a significant ($P<0.05$) improvement in content for *P. lunatus* grown on soils amended with organic manure and a combination of agricultural lime and organic manure. The mineral composition was comparable, calcium was significantly ($P<0.05$) higher in Lima beans grown on unamended acidic soils (pH 4.0) with values ranging from 2022.3% to 5962.3% compared to amended soils values of 1326.3 % to 1381.3%. The application of agricultural lime effectively raised soil pH and improved the nutrient composition of the beans, while organic manure enhanced nutrient availability and improved overall plant health. The combined application of both amendments resulted in the highest nutritional values, indicating a synergistic effect in ameliorating the negative impacts of soil acidity. *P. lunatus* had higher carbohydrates and moisture content ($p<0.05$) under the different amended soils. The results of the elemental composition revealed that *P. lunatus* is rich in macro and micro nutrients. This study has shown the importance of appropriate soil management strategies for enhancing the nutritional quality of *Phaseolus lunatus* in acid induced soil. The study also revealed that these amendments not only enhanced the nutrients composition of *Phaseolus lunatus* but also positively impacted the soil physico-chemical properties and enhanced nutrients availability.

KEYWORDS: Acidic soil. *Phaseolus lunatus*, soil nutrient status, physio-chemical properties, lime fertilizers, organic manure.

INTRODUCTION

Background of the study

Lima beans (*Phaseolus lunatus*) is an important food and agricultural crop belonging to the family Fabaceae.

This family includes a large number of domesticated species used by humans as food and for horticultural purposes (Lewis *et al.*, 2005). It is the third largest family of angiosperms after Orchidaceae (orchids) and

Effa Effa, Anobeja, Department of Plant and Ecological Studies, Faculty of Biological Sciences, University of Calabar, P.M.B 1115 Calabar, Nigeria

Maurice T. Akesa, Department of Plant and Ecological Studies, Faculty of Biological Sciences, University of Calabar, P.M.B 1115 Calabar, Nigeria

Ngele Blessing, Department of Plant and Ecological Studies, Faculty of Biological Sciences, University of Calabar, P.M.B 1115 Calabar, Nigeria

Ann Mofunanya, Department of Plant and Ecological Studies, Faculty of Biological Sciences, University of Calabar, P.M.B 1115 Calabar, Nigeria

Godwin M. Ubi, Department of Genetics and Biotechnology, Faculty of Biological Sciences, University of Calabar, P.M.B 1115 Calabar, Nigeria.

Asteraceae, and second only to Poaceae (grasses) in terms of agricultural and economic importance (Garg *et al.*, 2014).

Beans are grown principally for their grain seeds called Pulse, and are rich in protein and relatively low in fiber making it a potentially valuable ingredient for all livestock species and humans.

The consumption of beans provides minerals especially iron and zinc which compliment cereals consumption. Most legumes possess symbiotic nitrogen- fixing bacteria in structures called root nodules, hence play a key role on nitrogen fixation in soil. They are considered important sources of dietary protein for the majority of the people who cannot afford expensive animal protein, hence play significant roles in eliminating human malnutrition. The leaves are also occasionally used as vegetable and the straw as fodder (Ndakidemi *et al.*, 1998).

Beans are high in proteins, carbohydrates, fibers, minerals and vitamins making them an excellent nutrient source (Batista *et al.*, 2010). The high level of potassium in beans help in proper functioning of the heart by regulating blood circulation and pressure; it also prevents cancer, especially breast cancer (Eboibi *et al.*, 2018).

Lima bean (*Phaseolus lunatus*) is a tropical and subtropical legume cultivated for its edible seeds. The perennial form is an indeterminate, vigorous climbing and trailing plant, up to 26 m tall with axillary flowering, swollen and fleshy roots up to 2 m long with thin roots (Baudoin, 2016). The stems may be up to 4.5-8 m long, the leaves are alternate and trifoliate with ovate leaflets, 3-19.5 cm long, and 1-11 cm broad. Inflorescences are 15 cm long and bear 24 white or violet bisexual flowers. The fruits are 5-12 cm long, dehiscent pods with 2 to 4 seeds. Seeds are variable in size, shape and colour. Cultivar have been developed and grouped according to seed differences (Baudoin, 2016).

In Nigeria, lima beans are cultivated mainly for the dry seeds. Like other grain legumes, it is an important source of vegetable protein. It is well adapted to the humid rainforest environment of Southern Nigeria. Despite the great potential of this crop, it is highly underutilized in the country and has not received much attention in terms of crop improvement. To this end, local cultivars are still being grown by the farmers. Lima bean has not received the benefit of intensive research program devoted to cowpea (Lyman *et al.*, 1985). It is cultivated in only about 40 % land area devoted to grain legumes, and is usually intercropped with cassava, maize, yam, cocoyam and pepper. It is mainly produced for consumption as only about 35 % of grain produced is sold (Sexa *et al.*, 2004). The leaves, young pods and green seeds (immature or dry) are edible and eaten as vegetables. The dry seeds are often boiled, fried, grounded into powder and baked, and used in soups and stews. The vines, leaves and empty pods left after the harvest can serve as fodder, made into hay or silage.

It is valuable in intercropping systems, though only few cultivars are suitable for this purpose (Baudoin, 2006). Like other grain legumes, lima beans are relatively rich in protein as it contains about 25 % dry matter making it a crop of commercial importance and offer various optimum nutritional and/ or health benefits (Tharanathan and Mahadevamma, 2003). The protein content of Lima beans ranges from 17 to 40/100 g much higher than that in cereals (7-11.8 g/100 g and approximately equal to the protein of meat 18-25 g/100 g (De Oliveira, 2006).

In Southern Nigeria and other developing countries, legumes are the second largest sources of human food after cereals; lima beans are the fourth important legume food in Nigeria after cowpea, groundnut and Bambara nut (De Olivera, 2006). The high protein content as well as the presence of the amino acid lysine makes them a suitable cheap source for fortification than cereal diets in terms of dietary value (Kaur *et al.*, 2009). Consumption of plant proteins, including lima beans, reduces heart and kidney diseases, lowers the sugar indices of diabetic patients and reduces the occurrence of cancer (Mathres, 2002).

As with most legumes, some undesirable constituent of Lima beans includes anti-nutrients (trypsin inhibitors, phytic acid, saponins, haematoglutins) that interfere with absorption and utilization of important minerals such as calcium, iron, zinc and magnesium. It is known that processing methods such as de-husking, soaking, cooking and roasting inactivate the anti-nutrients and can be used to improve the nutritional quality of lima beans (Akinmoladun *et al.*, 2007).

The oil from Lima bean is a good source of potassium, iron, copper, and manganese. Its low sodium content is recommended for reduction in blood pressure. The soluble fibre of Lima bean contributes to cholesterol-lowering effect (Singh *et al.*, 2007; Bazzano *et al.*, 2003; McIntosh and Miller, 2007).

Acidic soils cause significant losses in bean production and where the choice of crops is restricted to acid tolerant species and varieties, profitable market opportunities may be reduced (Liu *et al.*, 2011). The associated chemical changes in the soil can restrict the availability of essential plant nutrients especially macro nutrients, that is; nitrogen, phosphorus and potassium. Essential plant nutrients can also be leached below the rooting zone in acid soil (Lambridge and Godwin, 2007). Biological processes favourable to plant growth may be affected adversely by acidity (Lambridge and Godwin, 2007). Soil acidity have a major impact on beans productivity both on surface soil and subsoil layers posing serious problems for plant root development and remedial action. Acid deposition also has adverse effect on plants, as it reduces plant growth and yield due to foliar injury and low nutrients availability (Liu *et al.*, 2011).

Application of compost materials of plants and animals, finely crushed limestone and other liming materials are some of the practical steps to neutralizing the acidity and alkalinity of soil (Alleoni *et al.*, 2010).

This study aimed to investigate the effects of soil amendment on proximate and elemental composition of *Phaseolus lunatus* (Lima beans) grown on simulated soil pH.

MATERIALS AND METHODS

Study area/experimental site

This study was carried out in the Department of Botany, University of Calabar. Calabar is located between latitude $4^{\circ}78'1$ and $5^{\circ}09'1$ and longitudes $8^{\circ}15'1$ and $8^{\circ}26'1$ E and lies between the valleys of two rivers. The great Qua river on the Eastern side and the Calabar River on the West. The total annual rainfall for

the area is 2109.5 mm and 4168.7 mm. The experiment was carried out in a screen house in the Department of Botany, Faculty of Biological Sciences, University of Calabar, Calabar.

Seed collection and planting materials

Planting materials used for the study were obtained from International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria. Polythene bags (Planting bags) were obtained from the Ministry of Agriculture, Calabar. Agricultural lime was obtained from Cross River Agricultural Development Project and Organic manure was obtained from the Department of Soil science, Faculty of Agriculture, University of Calabar, Calabar.

Field investigations

Soil sampling, collection and preparation

Topsoil (0-20 cm depth) was collected from Esuk-Atu Community in Calabar Municipality, Calabar. Soil sample was air-dried for 3 days, sieved through 2 mm mesh to remove debris, and then analyzed for physico-chemical properties (Figure 1).

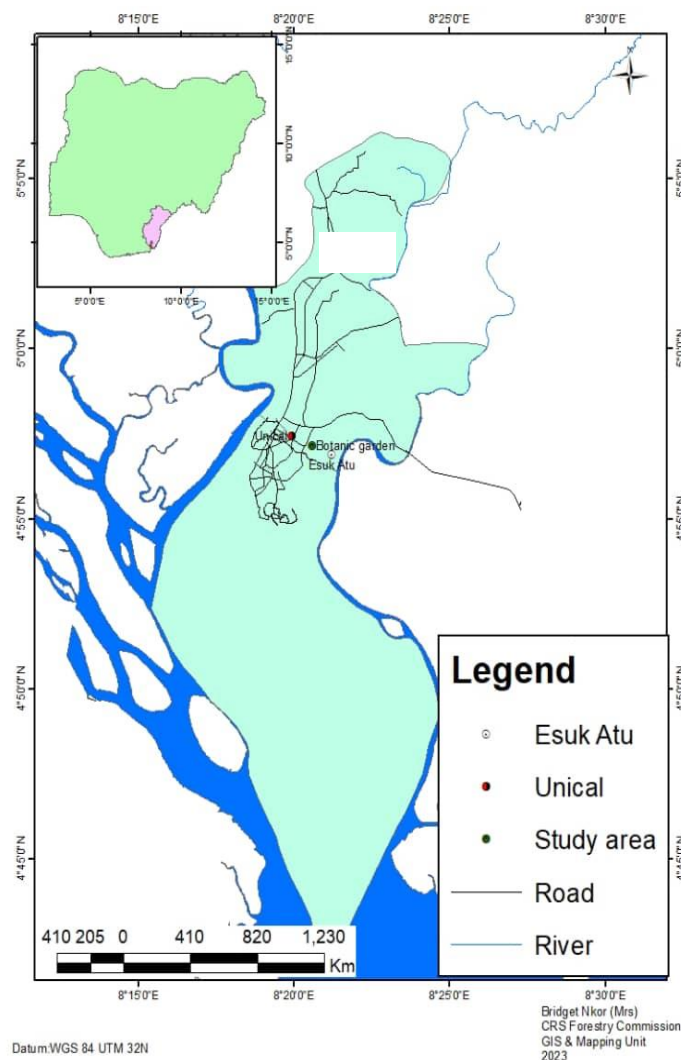


FIG. 1: Geolocation of soil sample and the study areas
Source: Culled from Nigeria Data (2023).

Preparation of soil at different pH levels

The three pH levels of soil used in this study were prepared by irrigating the soil with acidified water, simulated by mixing sulphuric and nitric acid in a ratio of 2:1. The acidic solution was then calibrated using distilled water with a deluxe pH meter to get the desired of pH of 4.0 as control.

Application of soil amendments

The growth medium (pH 4.0) were amended with agricultural lime, organic manure and a combine treatment comprising agricultural lime and organic manure (1:1); a control without amendments. 50 g of each amendment was added to 5 kg of each soil sample and labeled appropriately.

Planting procedure and treatment application

Thirty six perforated polyethylene bags (16 cm internal diameter) were used in planting (Plate 1a & b). The bags were divided into three groups of 12 bags each. The subgroup of 12 bags represented triplicates of each soil pH and amendments as follows: Control, agricultural lime, organic manure, and a combination of agricultural lime and organic manure (1:1). The polyethylene bags were labeled appropriately. Five kilograms (5 kg) of each soil sample was weighed into each polyethylene pot accordingly and placed in a screen house allowed to stay for two weeks before planting was carried out. This time lapse before planting was to allow for microbial activities and interaction within treatment combinations. Each planting bag was sown with three seeds of *Phaseolus lunatus* accordingly. Plants were observed for germination, and established seedlings were allowed to grow for ten weeks.

Determination of physico-chemical properties of soil

The Physico-chemical properties of the different soil samples were determined before and after treatment. This include soil pH, exchangeable cation, moisture content, base saturation, clay, silt, organic matter and effective cation exchange capacity (ECEC). Also, soil sample was analyzed for mineral contents including organic carbon, total nitrogen, available phosphorus, calcium, magnesium, potassium, sodium, aluminum and hydrogen.

Determination of soil pH

The pH of the various soil samples, amended and unamend, was determined using a deluxe pH meter. The different soil samples were divided into three portions each. Ten (10) grams each of soil sample was placed into 100 ml beaker. To this, was added 50 ml of distilled water and stirred vigorously with a glass rod for 20 minutes. The mixture was allowed to stand for 30 minutes. The pH was read by immersing the electrode of the pH meter into the upper part of the suspension (AOAC, 1996).

Determination of soil organic carbon

Organic carbon content of soil samples was determined using dichromate wet oxidation method as described by Eno *et al.* (2009).

One gram of each soil sample was dispersed with 10 ml of 1 N Potassium dichromate ($K_2Cr_2O_4$).

The organic matter in the mixture was oxidized using 20 ml of sulphuric acid (H_2SO_4). The resulting solution was allowed to stand for 30 minutes and diluted with 200 ml of distilled water. Also 1 ml of diphenylamine indicator was added before titrating with 1 N ferrous sulfate ($FeSO_4$).

Determination of total nitrogen

The total nitrogen of each soil sample was determined using the Kjeldahl method as described by Bremner and Mulvancy, (1982). Ten grams of each soil sample was weighed into a Kjeldahl digestion flask. To this, was added 10 ml of distilled water. After 30 minutes, sulphuric acid and selenium mixture were added, carefully mixed and digested for 3 hours until a colourless or clear solution was observed. The digest was then diluted with 50 ml of distilled water and allowed to cool. The digest was made up to 100 ml with distilled water and mixed very well. An aliquot of 10 ml of the digest was transferred to the reaction chamber and 20 ml of 40 % NaOH solution was added followed by distillation. The distillate obtained after distillation was collected over 4 % boric acid. The distillate was titrated with 0.02 N HCl solution using bromocresol green as an indicator. A blank distillation and titration were also set to take care of traces of nitrogen in the reaction as well as the water used. Nitrogen in the samples was calculated using the formula:

$$\text{Weight of N in the soil} = \frac{14 \times (A-B) \times N}{1000}$$

Where;

A= Volume of standard HCl used in the sample titration

B= Volume of standard HCl used in blank titration

C= Normality of Standard HCl

Available phosphorus

The acid-soluble forms of phosphorus from the different soil samples were extracted with Bray No. 1 solution as described by Olsen and Sommer (1982). Phosphorus was determined using a spectrophotometer (210 VGP Back Scientific) by the blue ammonium molybdenum with ascorbic acid as a reducing agent. Five gram of soil sample was weighed into 100 ml extraction bottle and 35 ml of Bray 1 solution (0.03 M NH_4F and 0.025 M HCl) was added. The extraction bottle was placed on a rotary shaker and agitated for 10 minutes and then filtered through a Whatman No.42 filter paper. A 5 ml aliquot of the filtrate was pipette into a 25 ml flask and 10 ml of ammonium paramolybdate (colouring reagent) was added followed by a small amount of ascorbic acid. After mixing, the mixture was allowed to stand for 15 minutes to develop a blue colour. The colour was measured spectrophotometrically at 660 nm wavelength.

A standard series of 0, 1.2, 2.4, 3.6, 4.8 and 6.0 mg/L were prepared by pipetting 0, 10, 20, 30, 40 and 50 mg P/L respectively in 100 ml volumetric flask and made up to volume with distilled water. The initial available phosphorus was extrapolated from the standard curve which gave 6.91 mg/kg.

Phosphorus content in soil samples was calculated by the formula;

$$P \text{ (mg/kg)} = \frac{(a-b) \times 35 \times 15 \text{ mcf}}{g}$$

Where;

a = mg P/l in the sample extract

b = mg P/l in the blank

g = Sample weight in grams

mcf = moisture correction factor

35 = volume of extraction solution

15 = Final volume of the sample solution

Determination of exchangeable cations (bases)

Exchangeable bases were extracted by leaching 5 g of each soil sample into density bottles using NH_4OAC (ammonium acetate) solution at pH 4.0 for an hour (Benson *et al.*, 2010). The filtrate obtained after 30 minutes centrifugation at 200 rpm was used for calcium and magnesium content determination by titration with 0.1 EDTA solution using NH_4Cl buffer, and Calcium and KOH in the first and second titrations (Benson *et al.*, 2010).

Determination of exchangeable Potassium

Potassium (K) in the percolate in each soil sample was determined using the flame photometry as described by Helmke and Sparks, (1996). Series of potassium standard were prepared by diluting 1000 mg/l potassium solution to 100 mg/l. Different portions (0, 5, 10, 15 and 20 ml) of the 100 mg/l standard solutions were put into 200 ml volumetric flasks. One hundred milliliters of 0.1 M NH_4OAC solution was added to the flask and made to 200 ml with distilled water. The standard series of 0, 2.5, 5.0, 7.5, 10.0 mg/l were obtained for potassium. Potassium in percolate was measured directly by flame photometry at wavelength of 766.5 nm.

Exchangeable potassium was computed using the formula:

$$\text{Exchangeable k (cmol (+)/ kg soil)} = \frac{(A-B) \times 250 \times \text{mcf}}{10 \times 39.1 \times g}$$

Where;

A = mg/l K in the diluted sample

B = mg/l K in the blank sample

G = air-dried sample weight of soil in grams

Mcf = moisture correction factor

Exchangeable calcium and magnesium determination

Twenty-five milliliter of extract was measured into a conical flask and the volume constituted to 50 ml with distilled water. Potassium ferrocyanide (1 ml) at 2 % hydroxylamine hydrochloride (1 ml), potassium cyanide (1 ml) at 25 (from a burette), ethanolamine buffer (10 ml) and 0.2ml Eri-chrome Black T solution were added. The mixture was titrated with 0.01 M ethylene diamine tetra acetic acid (EDTA) to a clear turquoise blue colour. Twenty mils (20 ml) of 0.01 M EDTA with the aid of 23 ml of 1.0 M ammonia acetate solution was added to provide a standard blue colour for titration and the titre value recorded. The titre value of calcium was subtracted from this value to get the titre value for magnesium.

Calculation;

$$\text{Ca + Mg (cmol(+)/ kg)} = \frac{0.01 \times (V_1 - V_2) \times 1000}{0.1 \times w}$$

Where;

V_1 = ml of 0.01 M EDTA used in the sample

V_2 = ml of 0.01 M EDTA used in the blank titration

W = weight in grams of soil extracted

0.01 = Concentration of EDTA used

Experimental design and layout

The experiment was conducted using 1x3x4 factorial experimental unit laid out in a complete randomized design with three replicates. The first factor was the plant – *Phaseolus lunatus*, the second factor was soil pH at level 4.0, while the third factor was the treatments - control (0 g), AL (50 g), OM (50 g) and AL+OM (50 g).

Determination of nutrient composition

The nutrient contents (moisture, crude protein, carbohydrates, ash, lipid and fibre) of the samples were determined by the method of Association of Official Analytical Chemists (AOAC, 2000).



PLATE 1a: Pods/Seeds of *Phaseolus lunatus*



PLATE 1b: Screenhouse layout of *Phaseolus lunatus* plant.

Determination of moisture content

Three grams (3 g) of seed samples from each treatment group were weighed to obtain their fresh weight. Weighed leaves were then dried in an oven at 100 °C to a constant dry weight, allowed to cool and then weighed again to obtain the dry weight. This was used to compute the percentage moisture content (MC) of leaves as:

$$MC (\%) = \frac{\text{fresh weight} - \text{dry weight}}{\text{Fresh weight}} \times \frac{100}{1}$$

Determination of crude protein content

The crude protein content was determined by Kjeldahl method. Dried seed samples (1.0g) were weighed into a digestion flask. To this was added 5g of Kjeldahl catalyst and 200ml of conc. H₂SO₄. The flask was placed in a stand and heated over a gentle flame until the initial charring subsided after which 2 Kjeldahl catalyst (5g K₂SO₄ and 0.1g CUSO₄.5H₂O) were added to the flask by sliding them carefully down the neck of the inclined flask. The content of the flask was further heated, gently at first and then more fiercely until the solution turned green.

This was allowed to cool to room temperature and 200ml of distilled water was added and the flask swirled carefully to mix the content. The flask was connected to the distillation apparatus, ensuring that the delivery tube dipped below the surface of 50 ml boric acid solution (prepared by dissolving 40g of boric acid in 800ml of distilled water and made up to 1 litre by adding water). Two to three drops of silicon antifoam reagent followed by 80 ml of NaOH solution (1g of NaOH in 1 litre of water) was added and the distillation apparatus gently agitated to ensure thorough mixing. The flask was then heated. About 160ml of distillate was obtained and this was titrated using 0.2N hydrochloric acid, to a blue-grey end point of indicator solution (pH 4.5); prepared by dissolving 0.16 g methyl and 0.083 g bromocresol green in 100ml industrial methylated spirit. A blank experiment (with the sample) was carried out in a similar manner and the titre from it was subtracted from that containing the samples to obtain true titre.

Percentage nitrogen in the sample was obtained using the formula:

$$\text{Percentage (\%)} = \frac{(A-B) \times N \times 1.4007 \times 6.25}{W}$$

Where;

A = Volume (ml of 0.2N HCl used sample titre)

B = Volume (ml of 0.2N HCl used in blank titre)

N = Normality of HCl

W = Weight (g) of sample

1.4007 = atomic weight of nitrogen

6.25 = the protein nitrogen conversion factor

Determination of ash content

Ash content was determined by weighing 5g of dried samples into dry pre-weighed porcelain crucibles. The crucibles with the samples were put in a muffle furnace (Gallenkamp, Muffle furnace Scie 2, UK) and ignited for 24hr at 550°C to eliminate the organic components (ashed to greyish-white). The crucible and their contents were cooled in desiccators and later re-weighed. The difference in weights before and after incineration was used to compute ash content in percentage as:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of samples}} \times 100$$

Determination of fat content

Fat content was determined by extraction with petroleum ether (AOAC, 2000). Five grams (5g) of sample was weighed into a fat extraction thimble and 250ml petroleum ether was added. The thimble with it content was put into a Soxhlet apparatus (Quick fit, England), with a pre-weighed round bottom flask and was heated. The extraction process lasted for 8 hours. The flask was disconnected and the solvent distilled off over steam bath at 50°C, the remaining traces of the solvent were removed with a current of air. The flask was dried to constant weight at 100°C. After drying, the bottle with partially covered lid was transferred to the desiccator to cool. The bottle and its dried content were reweighed (AOAC, 2000).

$$\text{Fat (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

Determination of crude fibre

Five grams (5g) defatted samples were weighed into 500 ml conical flasks, 50ml of 1.25 % sulphuric acid was added and the mixture made up of 200ml with distilled water; this boiled for 30 minutes. The contents of the conical flasks were filtered using a Buchner funnel with the aid of suction pump. The residue was washed with hot water until it was acid free. The residue obtained after the digestion was transferred into a 500ml beaker to which 50ml of 1.25% NaOH was added and made up to 200ml with distilled water. The mixture was again heated for 30 minutes with constant stirring. The content of the beaker was filtered through the Buchner funnel and washed five times with water until freed from NaOH. The residue obtained was washed twice with 95% methanol, transferred into a porcelain crucible and dried at

100°C. The weight of the oven dried residue was noted and later ignited in a furnace at 550°C. The weight of the ash left after ignition was also noted. The crude fibre content was determined from the weight loss of crucible and its content after the ignition.

Mineral contents determination

Calcium (Ca), iron (Fe), magnesium (Mg) and zinc (Zn)

The contents of Ca, Fe, Mg, and Zn were determined using Atomic Absorption Spectrophotometry (Pye Unicam SP8-190, Spec. UK) as outlined in AOAC (1995). To 2 g of each sample in a digestion flask was added 20 ml of acid mixture (65 ml conc. HNO₃, 8 ml of perchloric acid and 2ml conc. H₂SO₄) and heated gently (50-70°C) on a Stuart hot plate until clear digest was obtained. This was made up to 100 ml with deionized water. Appropriate dilutions (2.0 ppm, 4.0ppm, 6.0 ppm, 8.0 ppm, and 10.0ppm) were made for each element. For Ca and Mg determination, strontium Chloride (SrCl₂) was added to yield a 1500 mg/ml of Sr²⁺ in the final solution. Internal standards were prepared for each element. The concentration of the elements was determined using the internal standards obtained spectrophotometrically using the appropriate wavelengths (as specified in the instruction manual of the instrument) for each the elements.

Sodium (Na) and potassium (K)

Sodium and potassium were obtained by flame photometry (Jahway Flame Analyzer P. F7, UK). The digestion procedure was as described for atomic absorption spectrophotometry. The stock solutions for Na (1000 ppm Na⁺) and K (1000 ppm K⁺) were prepared by dissolving 2.542 g of NaCl and 1.907 g of KCl in 1000ml deionized water. Working standards (2.0 ppm, 4.0 ppm, 6.0 ppm, 8.0 ppm and 10.0 ppm) from the stock solutions were prepared. The absorbance for Na (at 589 nm) and K (at 767 nm) were obtained using the flame photometer. Sodium and potassium concentration in the samples were obtained by reference to standards the results were expressed in milligram per 100g sample.

Phosphorus

Phosphorus content was determined as described by AOAC (1995). To 0.5 ml aliquot of the mineral digest was added 9.5ml of 10% trichloroacetic acid in a test tube. The mixture was agitated, centrifuged (Censaur 2 MSE, UK) for 5 min and then filtered through a Whatman filter paper. Five ml (5ml) of the filtrate and 5ml of the working standards prepared as previously described from phosphorus stock solution (4.324g KH₂PO₄ in 250ml of deionized water and made up to 1 liter) were measured into two cuvettes to which 0.5 ml of molybdate reagent (prepared by adding 200 ml distilled water to 83 ml of conc. H₂SO₄ and 25.0 g ammonium molybdate tetrahydrate made up to a litre by the addition of deionized water) was added.

The cuvettes were shaken and 0.2 ml sulphuric acid reagent (0.125g 1, 2, 4-aminoaphthosulphuric acid, 7.28g sodium bisulphate and 0.29 g sodium sulphate in 50 ml of distilled water) was added. This was stoppered, shaken and allowed to stand for 10min. The absorbance of the test solutions and standards were read at 660 nm using a spectrophotometer. Phosphorus concentration was obtained from calibration curve of the standards

Statistical analysis

Mean values were taken from measurements of three replicates. All data were statistically analyzed using the statistical package for social sciences (SPSS) for windows (version 20.0). Analysis of variance (ANOVA) was used to analyze the data and significant means were separated using least significant differences (LSD) at $p \leq 0.05$.

RESULTS

Physico-chemical properties of growth media

The physico-chemical properties of the growth media (pH 4.0), amended (Agricultural lime (AL), organic manure (OM), and a combination of both (AL+OM)) and unamended, used in this study is presented in Table 1. The pH of acidic media shifted towards neutrality with amendment, particularly when amended with a combination of agricultural lime and organic manure. Electrical conductivity was lower in media amended with organic manure. Also, effective cation exchange capacity (ECEC) and organic matter was higher in soil amended with the combined treatment of agricultural lime and organic manure.

Mineral content of growth media

Organic carbon was higher in acidic soils amended with organic manure. Nitrogen, phosphorus and calcium were also higher in soils amended with organic manure, and a combination of agricultural lime and organic manure (Table 2). However, magnesium was higher in unamended soils.

TABLE 1: Physico-chemical parameters of growth media

Soil pH Amendments	4.0			
	CTRL	AL	OM	AL:OM
pH	4.00	8.01	6.34	7.62
Moisture (%)	1.70	1.43	3.28	2.64
EC (%)	158.40	195.00	160.00	189.90
ECEC (%)	6.46	28.70	5.19	25.04
BS (%)	87.62	99.02	87.67	98.40
Sand (%)	69.00	70.00	71.00	67.00
Silt (%)	23.00	27.00	26.00	23.00
Clay (%)	8.00	5.00	6.00	6.00
OM (%)	2.03	2.17	2.79	2.35

CTRL: Control; AL: Agricultural lime; OM: Organic manure; AL+OM: Agricultural lime: Organic manure; EC: Electrical conductivity; ECEC: Effective cation exchange capacity; BS: Base saturation; OM: Organic matter

TABLE 2: Mineral content of growth media

Soil pH Amendments	4.0			
	CTRL	AL	OM	AL:OM
OC (%)	1.18	1.26	1.62	1.36
TN (%)	2.24	1.68	2.24	3.92
AP (mg/ kg)	691.7	98.90	389.62	206.18
Ca (cmol/ kg)	3.80	27.00	3.00	23.60
Mg (cmol/ kg)	1.60	1.20	1.40	0.80
Na (cmol/ kg)	0.12	0.11	0.14	0.12
K (cmol/ kg)	0.14	0.11	0.01	0.12
Al (cmol/ kg)	0.40	Trace	0.20	Trace
H (cmol/ kg)	0.40	0.28	0.44	0.40

CTRL: Control; AL: Agricultural lime; OM: Organic manure; AL+OM: Agricultural lime: Organic manure; EC: Electrical conductivity; ECEC: Effective cation exchange capacity; BS: Base saturation; OC: Organic carbon; TN: Total nitrogen; AP: Available phosphorus; Ca: calcium; Mg: Magnesium; Na: Sodium; K: Potassium; Al: Aluminum; H: Hydrogen.

Influence of soil amendment on mineral content of lima beans grown using acidic soil media

Calcium content in seedlings of lima beans was significantly ($P \leq 0.05$) improved following amendment with agricultural lime with AL+OM significantly ($P \leq 0.05$) improved calcium in pH 4.0 soil (Figure 2). Magnesium, phosphorus, sodium and potassium

content of the studied species were mostly comparable both in amended and unamend soils.

Influence of soil amendment on nutrient content of lima beans and soybeans grown using acidic soil media

Protein, ash, fiber, fat and moisture content of lima beans (Figure 3) seedlings grown using acidic and neutral soil media was comparable, both in the amended and unamend soils.

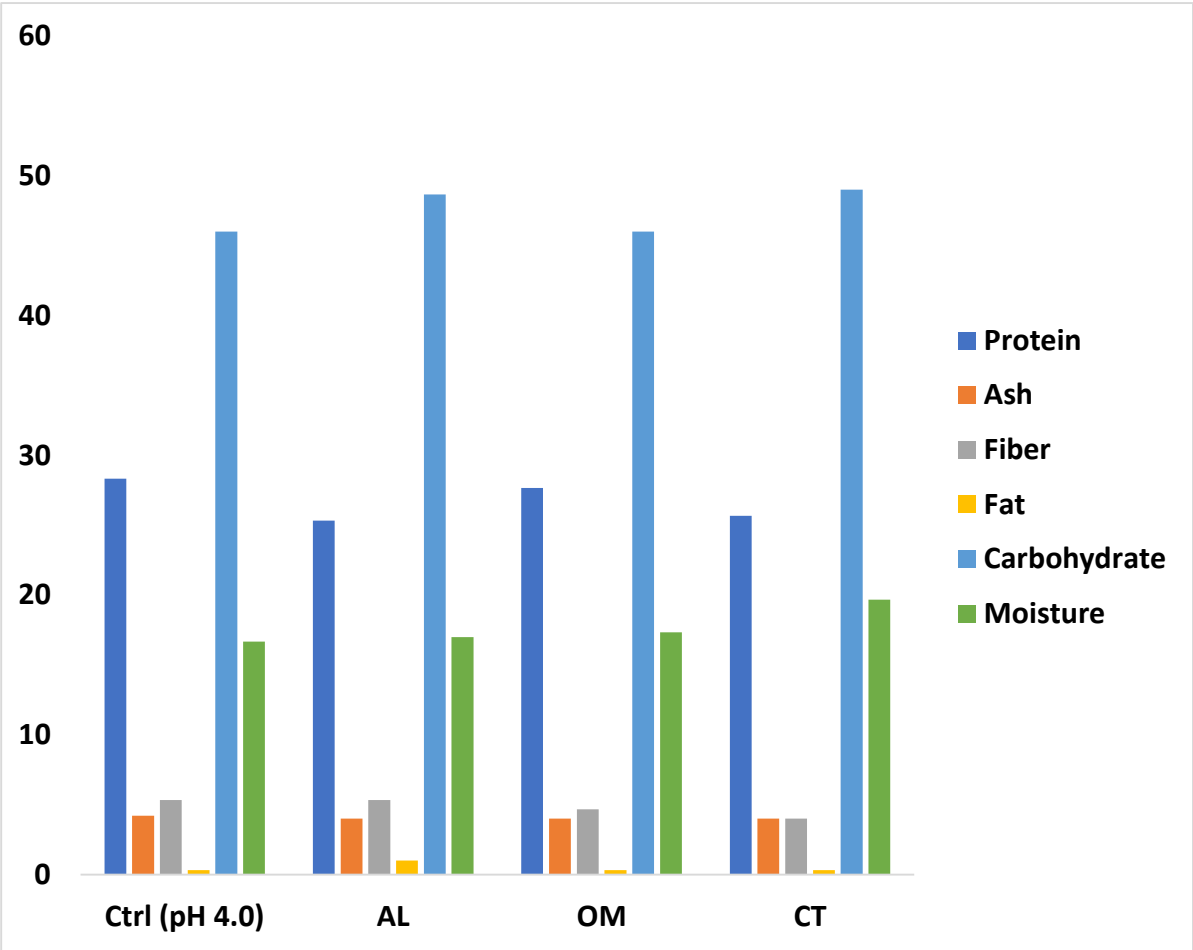


FIG. 2: Effect of amendment on nutrient content (%) of lima beans grown using acidic soils
Ctrl: Control; AL: Agricultural lime; OM: Organic manure; CT: Composite treatment

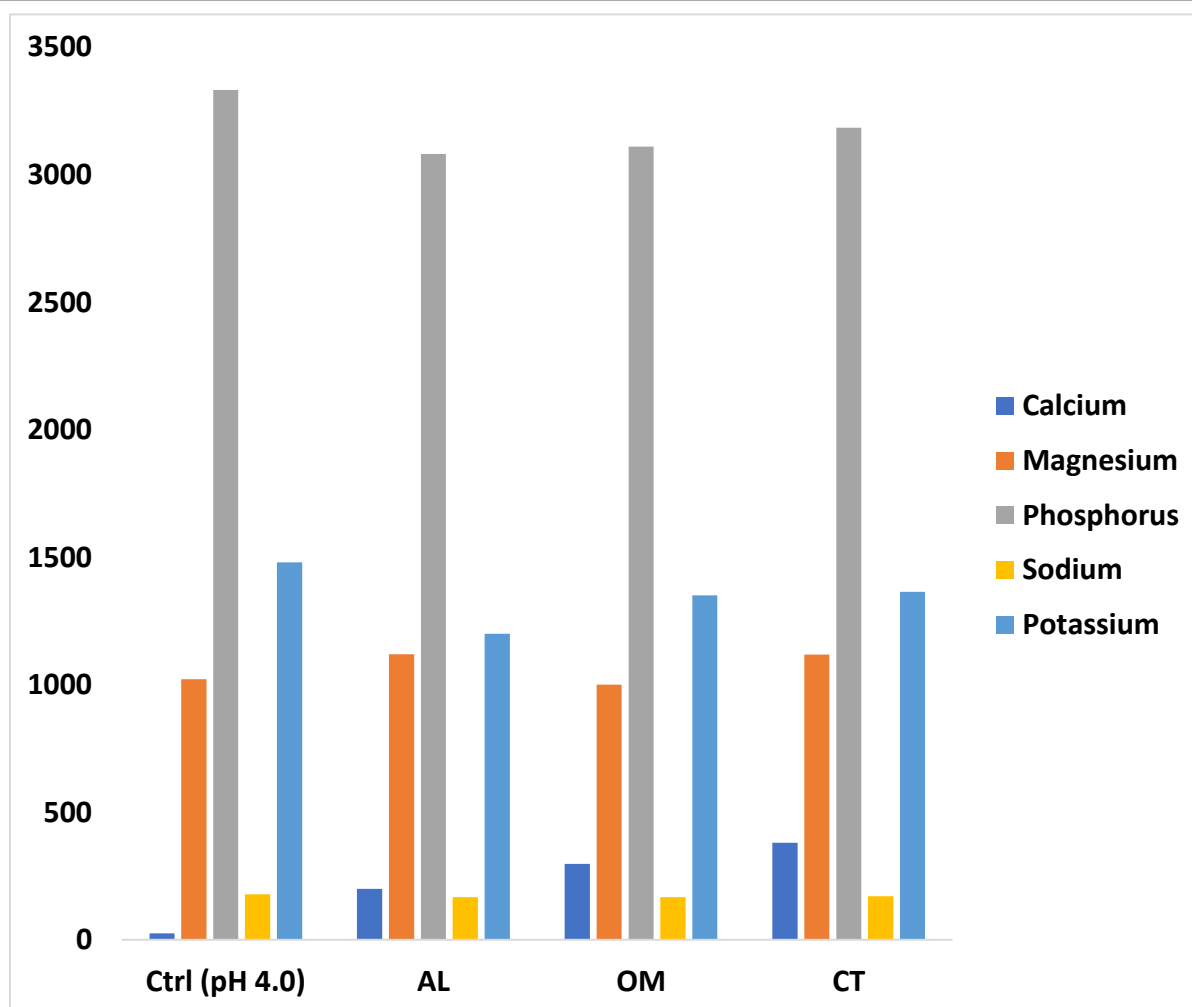


FIG. 3: Effect of soil amendment on mineral content (%) of lima beans grown using acidic soils
Ctrl: Control; AL: Agricultural lime; OM: Organic manure; CT: Composite treatment.

DISCUSSION,

Effects of acidic soil and substrate amendments on proximate and elemental composition of *Phaseolus lunatus* was studied. Results of the soil physico-chemical properties of acidic soils investigated at pH 4.0 level before and after substrate amendment (agricultural lime and organic manure singly and in combination) revealed that the soils were generally low in organic carbon content before application of the different treatments which was similar to low organic matter earlier reported by Bello and Ukut (2015). However, the application of organic manure and agricultural lime successfully improved organic carbon and organic matter of the soils indicating the effectiveness of the manures in improving the soil fertility.

The nitrogen content rating of the different soils according to Landon (1991) ranged from 1.68 to 3.92. Nitrogen is a major component of chlorophyll as well as helping to bulk amino acid of protein in plant.

It can be said that the amount of nitrogen content in the soils can assist the plant in growth and subsequent yield production. Exchangeable cation including calcium, potassium, magnesium, sodium, aluminum and hydrogen ions ranged from medium to high. According to Bello and Ukut, (2015), this is an important reaction in soil fertility in correcting soil acidity or basicity, in changes altering soil physical properties and as a mechanism of purifying or altering percolating waters. Phosphorus, magnesium, potassium are all major elements in the formation of plant nutrients. The present study indicated that these elements were in adequate quantity in soils studied following amendment with organic manure singly and in combination with agricultural lime.

The result of the present study on lima beans revealed that the plant is a reservoir of essential nutrients and minerals. In early research, Yellavila *et al.* (2015) reported crude protein in the range of 20.69-23.08 %, crude fat (0.59-1.14 %), crude fibre (4.06-5.64 %), moisture (9.19-11.83 %) and carbohydrate (54.31-59.64 %).

The variation here may be due to differences in soil content between the two study location, variation in protein content and other nutrient content of the plant can also be attributed to different environmental conditions, genotype and method of analysis (Yellavila *et al.*, 2015). Additionally, nutrient compositions of the plant is sensitive to rainfall, light intensity, length of growing season, day duration, temperature and agronomic practices (Bampidis *et al.*, 2011). Thus, these factors must have contributed to the differences observed in the nutrient composition of the plant. In terms of the treatments, it was generally observed that OM and combination of AL+OM enhanced the nutrient composition of the seeds than the control. This is a clear suggestion that the application of these biofertilizers had contributed immensely to nutrients availability in the soil for the plant uptake and utilization in food production.

Moisture content estimates directly the water content and indirectly the dry matter content of the sample while crude fibre accounts for indigestible plant material (Yellavila *et al.*, 2015). The present study reported a moisture content of 15-47% which was higher than 9.19-11.83 reported by (Yellavila *et al.*, 2015) in lima bean. However, the crude fibre of 3.33 to 6.67% was similar to the range of 4.06-8.86% by Yellavila *et al.* (2015). It was also in agreement with many other earlier submissions (Fasoyiro *et al.*, 2006; Iheanacho, 2010; Moses *et al.*, 2012). The ash content of both plants was higher than 2.57 % reported in lima bean by Seidu *et al.* (2014). Whereas, the carbohydrate content in *P. lunatus* under the different soil amendment were similar to the report of 47.52 % reported by Seidu *et al.* (2014). The amount of carbohydrate revealed in the plant indicated that the plant is a good protein source of energy (Nwadike *et al.*, 2018). Assessing the nutrient content of the plant material is fundamental in understanding their abundance in relevant nutrients that can be used to manage and mitigate nutrient related illness. The protein content of legume grains ranges from 17 to 40 g/100g which is much higher than that of cereals (7-11.8 g/100g) and approximately equal to the protein content of meat which is 18-25 g/100g (De Oliveira, 2006).

In this study, different elemental contents of *P. lunatus* was studied. It was revealed that the calcium content was significantly higher in all the control plants of *P. lunatus* and application of AL+OM and OM alone significantly increased Ca content in both plants under different pH levels. It has been earlier reported that calcium intake is essential in prevention of several chronic diseases including osteoporosis, hypertension, colon cancer (Nwadike *et al.*, 2018) as well as playing a key role in tooth and bone development. The results of the present study suggest that lima bean is a good source of calcium and its consumption could serve as a cheaper source of calcium to meet daily body requirement for bone development and disease prevention. Notably,

planting this beans with OM or combination of AL+OM will significantly improve calcium content of the plant. This is a suggestion that *P. lunatus* is generally a good source of iron and may not necessarily require soil amendment with AL and OM for its production. It can be suggested that consumption of lima beans could provide the body with iron requirements. Therefore, the consumption of lima beans could help tackle iron deficiency, which is a common disorder worldwide affecting a large proportion of women and children in developing countries (Nwadike *et al.*, 2018; Yellavila *et al.*, 2015). Iron contents in the plant under the different soil simulations were higher than the range of 2.45 to 2.67 mg/100m⁻¹ reported by Yellavila *et al.* (2015) and Kathirvel and Kumudha (2011).

Magnesium plays a key role to improve insulin sensitivity, protect the body against diabetes complications and reduces blood pressure (Haque and Ullah, 2011). It is also involved in many enzymatic reactions of oxidative metabolism of nutrients and cell constituents' synthesis, transmission of nerves impulses, production and the formation of healthy bones and teeth (Kartika *et al.*, 2011). The amount of magnesium deposited in the seeds of lima bean plants suggesting the effectiveness of the plant in providing the body with adequate quantity of Mg after consumption. The amount of Mg in all the treated plants were higher than 0.71 mg/g reported in seeds of *Cassia sophera* by Aziza *et al.* (2019). The results of the present research on Mg were similar to that reported by Adnan *et al.* (2010) in five medicinal plants.

Zinc as a trace element is necessary for the growth and multiplication of enzymes responsible for DNA and RNA synthesis, for skin integrity, bone metabolism and functioning of taste and eyesight (Saracoglu *et al.*, 2009) as well as important constituent of viable sperm especially human sperm (Aziza *et al.*, 2019). Zinc deficiency may contribute to delay sexual maturation, growth retardation and hair loss (Khan *et al.*, 2019). This was especially higher following the soil amendment with combined treatment (AL+OM). Zinc content of 0.071 mg/g reported in seeds of *Cassia sophera* by (Aziza *et al.*, 2019) was lower than the average zinc composition in the plant used in this study.

Phosphorus helps build and protect the bones and teeth, as part of DNA and RNA, helps convert food into energy (Yellavila *et al.*, 2015).

Sodium is concerned with the conduction of nervous impulses, muscles contractility and control of heart muscle condition (Aziza *et al.*, 2019). It also maintains the osmotic equilibrium between extracellular fluid and the tissue cells and maintains the pH of blood within normal limit (Aziza *et al.*, 2019; Dahl, 1992). Following the results obtain in this study, it is submitted that *P. lunatus* has a higher sodium content especially when grown on soil amended with AL+OM treatment.

By implication, the consumption of *P. lunatus* provides the body with all the health benefits associated with the plant material (Yellavila *et al.*, 2015) reported a range of 19.99 to 21.33 mg/100 g in lima accession which was lower than values obtained in the present study. It was also lower than those reported by many previous researchers (Fasoyiro *et al.*, 2006; Ikechukwu *et al.*, 2010; Kathivel and Kumudha, 2010). Potassium was higher in *P. lunatus* treated soil at pH 4.0. It was therefore revealed that the combined treatment of AL and OM was the best in enhancing potassium in *P. lunatus*. Potassium plays vital role in reducing hypertension and maintaining cardiac rhythm. In the human body, K is helpful in many physiological reactions and its deficiency can result in adverse effect in human health. The present findings suggest adequate consumption of *P. lunatus* for its nutritional values especially potassium. The potassium content of the the plant in this study were higher than 50.04 to 52.08 mg/100 g reported in lima beans. It was also different from 55.85 mg/g reported by Aziza *et al.* (2019).

Macro and micro elements are nutrient source for growth, life cycle and biological functions in plants. These in turn facilitate food production in plant for direct and indirect use by heterotrophs in the environment. Therefore, measures to improve these elements in plants are of utmost importance in plant agronomy.

CONCLUSION

Based on the findings of this study, it can be deduced that *P. lunatus* plant possesses abundant nutrients and minerals that hold significant potential for utilization by humans. Furthermore, the enhancement of acidic soils through the application of organic manures, as well as a combination of organic manures and agricultural lime, resulted in improved nutrients and minerals of the studied bean specie.

RECOMMENDATIONS

Based on the findings of this study, the following recommendations are made:

- 1) Farmers are encouraged to employ a blend of agricultural lime and organic manures when cultivating *P. lunatus* on acidic soils.
- 2) The use of organic fertilizers is recommended as a safer and environmentally friendly substitute for inorganic fertilizers to enhance the growth and yield of the studied plants.
- 3) Future research should explore the varying effects of different concentrations of agricultural lime and organic manures on leguminous crops.

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