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Comparing the use of a Dual-purpose Biofertilizer ('Njoku') and an Inorganic Fertilizer on Plantain (Musa sp.) Growth and Infestation in the Nursery

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ABSTRACT

Background: Plantain is an important staple in Cameroon as they help ensure food security and generate revenue. A major constraint faced by farmers is managing plantains in the nursery and obtaining high quality planting materials.

Methods: A study was carried out to compare the effects of a locally made biofertilizer – 'Njoku' with pesticide properties, foliar fertilizers and a synthetic inorganic fertilizer on plantain growth and infestation in the nursery. A randomized complete block design of six treatments with four replications was used. The treatments (T) per plant were as follows; T1 = Control (no application), T2 = 5g of biofertilizer (Njoku), T3 = Inorganic NPK (20-10-10), T4 = 2.5g of Counter® (terbufos) + 2.5g of NPK (20-10-10), T5 = 50ml of Radix Tim® solution + 2.5g of biofertilizer and T6 = 100ml of Radix Tim® solution.

Results: The vegetative growth parameters (Plant height, leaf number, leaf area, and collar girth) of the fertilized plants were significantly higher as compared to the control plants. The highest mean plant heights were recorded in T4 (21.88cm) and T5 (21.5cm) while the least mean plant height was from T1 (9.82cm). The highest mean leaf area of $289.12cm^2$ and $282.37cm^2$ were recorded respectively from T5 and T6. T4, T5 and T6 had the same mean number of leaves (4 leaves) while T2 and T3 had mean number of leaves of 3. The highest mean collar girth of 5.6cm and 5.5cm was recorded in T5 and T6 respectively and the mean collar girth for T2, T3, T4, T5 and T6 never differed significantly. T2 harboured many types of pests but recorded the least mean number of pests while T4 harboured the highest number of snails followed by T6 and T1.

Conclusions: Due to the high cost and potential environmental hazards posed by synthetic chemicals, T5 (a combination of reduced doses of the biofertilizer and Radix Tim®) is most suitable for the growth and maintenance of plantain plantlets in the nursery.

INTRODUCTION

Plantains and bananas (*Musa spp)* are of extraordinary significance to human societies (Thomas *et al*., 2012). They are of great economic, nutritional and medicinal importance in most regions of tropical and subtropical Africa and they play a major role in food and nutrition security in these regions (Olaoye et al., 2006; Adejoro *et al*., 2008; Hauser and Amougou, 2008; Okolle *et al*., 2009; Okolle *et al*., 2018). It has been reported that, these crops serve as a staple food for at least 400 million people in the world and therefore have been rated as the 4th most important or valuable food after rice, wheat and maize (Sahayaraj *et al*., 2009; Azam *et al*., 2010; Honfo et al., 2011; Thomas *et al*., 2012). In Africa, Cameroon has been reported to be one of the major banana and plantain producing, consuming and exporting countries (FAO, 2011). According to FAOSTAT (2018), the total production of plantain in Cameroon was estimated at 4,314,910 tons in 2016. Consumption of plantains in Cameroon can reach about 100kg/person/year (Hauser and Amougou, 2008), with seven regions (Centre, East, Littoral, South West, North West, West and South) being the major production areas (Okolle *et al*., 2009). Plantain is known to contribute about 73 billion FCFA to the income of small farm holders in the country (Temple *et al*., 1997).

In spite of the importance of plantains and bananas in Cameroon and the World at large, farmers are still faced with production constraints. Some major constraints are the inaccessibility to quality planting materials in large quantities and high costs of synthetic fertilizers needed for plantain production (El Moniem *et al.,* 2008). In addition, pests such as snails, mealy bugs, caterpillars and aphids are very common in the nursery (Hu *et al.,* 1996). In order to manage these pests and enhance growth of plantlets, most of the farmers (mainly smallholders) do not apply any technique whereas a few of them apply synthetic fertilizers (mainly NPK) and Counter® (Terbufos – an organophosphate nematicide with insecticidal properties) granules on the plants. Due to the potential negative effects of synthetic pesticides and fertilizers on human health (El Moniem *et al.,* 2008), there is a need to evaluate the efficacy of locally formulated organic pesticides and fertilizers as alternatives to synthetic agrochemicals in order to enhance growth of plantlets. Abuno (2014) and Nangonoa *et al.,* (2016) reported an aggregate referred to as Njoku otherwise known as a bioferticide which is a combination of biological powders obtained from local bioresources, with both fertilizing and insecticidal properties. Therefore, the present study seeks to compare the effects of the Bioferticide, foliar fertilizer and NPK (commonly used synthetic inorganic fertilizers) on plantain growth and infestation in the nursery.

MATERIALS AND METHODS

Study site:

The study was conducted at the plantain nursery of IRAD-Ekona Regional Research Centre in the South West Region of Cameroon. Ekona is located between latitude: 4°12.504’ and longitude: 9°19.383’ with an altitudinal range of 404-443m above sea level. It falls within the agro-ecological zone IV (characterized by humid tropical forest) and a monomodal rainfall with an average annual range from 2000-4000 m. The rainy season which lasts from mid-march to the end of October and the dry season which begins from early November and ends in mid-march are the only two common seasons observed in the area. Mean daily temperature ranges from 20°C- 23°C (minimal) and from 27°C - 32°C (maximal).

Nursery site preparation

A plot of 12 x 10m was weeded manually and raked so as to ensure uniform experimental conditions. It was fenced with a wire mesh so as to prevent stray animals, and then shaded with palm branches, to minimize the effect of direct sunlight penetration and heavy rain.

Preparation of materials for the nursery

Plantlets used in the nursery experiment were of the French Claire cultivar obtained from macro-propagation chambers located in the research centre. The source of the plant material was of prime importance in order to ensure their reliability. The plantlets were transferred from the propagator to polythene bags filled with black soil that has been sterilized by heating in a 200L metallic drum for 3 hours with fuel wood. A total of 240 well sorted plantlets, each with an average height of 8cm and having about 3 leaves were used for this study.

Experimental Design

The experiment was arranged in a completely randomized block design (CRBD) with six treatments, each replicated four times. The treatments were as follows;

T1: No chemical application
T2: 5g of the bioferticide (‘Njoku’) per plant
T3: 5g of NPK (20:10:10 inorganic fertilizer) per plant
T4: 2.5g of Counter® (terbufos) + 2.5g of NPK per plant (Common application by farmers)
T5: 50ml of Radix Tim® solution (100ml of Radix Tim® in 1L of water) + 2.5g of bioferticide (‘Njoku’) per plant
T6: 100ml of Radix Tim® solution per plant

Chemical composition of experimental products

- **Bioferticide ('Njoku')**

The Bioferticide ('Njoku') is composed of a powdery mix of dry leaves of the wild sun flower (*Tithonia diversifolia)*, oil palm bunch residue ash (*Elaeis guineensis*) and ground dried bush pepper seeds (*Piper nigrum*). According to Abuno (2014), matured leaves of *T. diversifolia* were harvested and sun dried for 10 days. Thereafter, they were ground to powder form using an electric grinding machine (Molinex -type ME2B). The oil palm bunch residue ash was collected from a local smallholder's oil palm mill while the ground bush pepper seed was obtained from a local super market. Table 1 shows the chemical composition of the components of the bioferticide according to Nanganoa *et al*., 2016. These three components (oil palm bunch residue ash, powder of the dry leaves of wild sun flower and powder of dry bush pepper seeds) were measured in grams in the ratio 6: 3: 1 (v/v) respectively and thoroughly mixed manually.

Table 1: Chemical composition of bioferticide components (leaves of *T. diversifolia*, oil palm bunch residue ash, and dry seeds of *P. nigrum* – Bush pepper)

Chemical composition	Tithonia leaves	Oil palm ash	Bush pepper
Nitrogen (%)	5.46 ±0.12	0.35 ±0.02	2.67±0.64
Phosphorus (%)	0.29 ±0.01	1.07±0 .12	0.23±0.01
Potassium (%)	2.63 0.18	3.68±0.21	2.10±0.24
Organic carbon (%)	50.5 ±50.82	6.74±1.64	54.64±2.13

Values shown are means ±standard errors

- **Radix Tim®**

It is a biostimulant which is derived from amino acid. It consists of nitrogen (3.36%), phosphorus (11.3%) potassium oxide (4.06%), zinc (0.23%), Essential amino acid (5.80%) and organic compounds (negligible amount).

- **NPK**

It is a synthetic inorganic granular fertilizer commonly used by farmers and the variant used consisted of Nitrogen (20%), Phosphorus (10%) and Potassium (10%).

- **Counter®**

It is a synthetic pesticide with the active ingredient being terbufos. It serves as a nematicide that also has insecticidal effects. This organophosphate pesticide is registered for use in banana plantations in Cameroon and it is one of the pesticides most commonly used by farmers (Mongyieh *et al.,* 2015; Okolle *et al.,* 2018)

Treatment application and frequency

Treatments were applied twice in 3 months (once in 6 weeks) (Table 2). Equal distances of 30cm were maintained between treatments, 15cm between replicates and 10cm between plantlets. A total of 40 plantlets were used for each treatment.

Table 2: Treatments and frequency of application.

Treatment	Composition /application per plant	Number of application	Interval between applications
T1	No chemical application	0	6 weeks
T2	5g of the bioferticide ('Njoku')	2	6 weeks
T3	5g of NPK (20:10:10 inorganic fertilizer)	2	6 weeks
T4	2.5g of Counter® (terbufos) + 2.5g of NPK	2	6 weeks
T5	50ml of Radix Tim® solution (100ml of Radix Tim® in 1L of water) + 2.5g of bioferticide ('Njoku')	2	6 weeks
T6	100ml of Radix Tim® solution	2	6 weeks

Data Collection

Growth parameters (height, girth, leaf length and width, leaf surface area, number of healthy leaves, number of dry leaves, number of roots, root length and girth) and pests found on plants, were recorded every three weeks beginning from the first three weeks after treatment application. Data were collected early in the mornings where a graduated ruler was used to measure the height of the plant from soil level to the last 2 leaf curvatures, the leaf length (L) from petiole to tip and leaf width at its widest part using a graduated metal ruler while a Vernier caliper was used to measure the diameter of the plants at 2cm from the substrate level (soil level). The leaf surface area was calculated as: length (L) x width (W) x 0.8 (Summerville, 1944 and Obiefuna and Ndubizu, 1979). The collar girth of the plant was calculated from the diameter as; stem girth (SG)= πd, where d is the diameter and $\pi = 3.14$.

Data were collected from five (5) randomly selected plantlets per treatment, giving a total of 120 plants sampled within the 4 blocks.

Statistical Analysis

Data collected were keyed into Microsoft Excel spread sheet 2010, after which they were transferred into a compatible software (GraphPad Instat of version 3. 10, 32 bit 2009) where descriptive statistics and ANOVA were carried out while correlations/regressions analysis were performed on growth parameters and indices of pests using XLSTAT 2007. 8.4 statistical software. Also, in cases where ANOVA showed that treatment means were different, appropriate statistical tests (Tukey's test) were used to separate means that were significantly different. All data were tested at 0.05 significant levels.

RESULTS AND DISCUSSION

Effect of treatment on growth parameters

Plant height

Plant height showed significant difference ($P<0.05$) across plantlets treated with bioferticide and inorganic fertilizer (Table 3). This implies that chemical application had a significant effect on plant height than plantlets with no chemical application, with T4 (Counter® (terbufos) + NPK) having the highest plant height (21.88cm) while T1 (No chemical application) had the least (9.8cm). Plant height increased from T2 (Njoku) to T5 (Radix Tim® + Njoku) and decreased to T6 (Radix Tim®). This could be attributed to the fact that T3 (NPK), T4 (Counter® + NPK) and T5 (Radix Tim® + Njoku) contain high level of nitrogen and phosphorus that improved on crop production and the high carbon enhanced some of the physical properties of the soil. This is in conformity with the fact that nitrogen and potassium are the key nutrient elements for optimum growth and yield in *Musa spp* (Twyford and Walmsley, 1974; Lahav, 1995) and promote starch accumulation in fruits.

Table 3: Variation of plant mean (±) height (PH), leaf area (LA) and collar girth (CG) for the different treatments.

Treatment	PH (cm)	LA (cm^2)	CG (cm)
T1	9.82^{a} ±0.58	56.92^{a} ±9.90	3.00^{a} ±0.23
T2	15.61^{a} ±0.44	145.92^{a} ±16.86	4.60^{b} ±0.25
T3	18.98^{ba} ±0.92	210.79^{ba} ±28.22	4.60^{b} ±0.29
T4	21.88^{b} ±0.64	253.73^{b} ±14.13	5.40^{b} ±0.27
T5	21.50^{b} ±0.85	289.12^{b} ±35.29	5.60^{b} ±0.30
T6	20.30^{b} ±0.83	282.37^{b} ±14.06	5.50^{b} ±0.21

*Means in the same column with same superscript are not significantly different at 5% level of significance (Tukey's test). Where T1 = Control (no chemical application). T2 = 5g of Bioferticide ('Njoku'). T3 = 5g of NPK (20:10:10) inorganic fertilizer. T4 = 2.5g of Counter® (Terbufos) + 2.5g NPK. T5 = 50mL of Radix Tim® Solution + 2.5g of Bioferticide. T6 = 100mL of Radix Tim® Solution PH = plant height, LA = Leaf area, CG = Collar girth

Leaf area

The effect of treatment on leaf area differed significantly ($p< 0.05$), with T5 having the highest value (282.12cm^2) and T1 (control) being the primary source of variation. Though leaf area did not differ significantly between organic and inorganic fertilizers, inorganic fertilizer showed more increase in leaf area than organic fertilizer.

This decrease for organic fertilizer might be as a result of slow decomposition rate of the organic material. Generally, fertilized plantlets showed an increase in leaf area as compared to the control (unfertilized). This finding is in line with that of Guantilleke *et al.,* (1997) working with *Shorea* species. Pooter (1990) has reported that proper combinations of nitrogen and phosphorus, and other organic compounds may result in increases in leaf area.

Collar girth

There was no significant difference (p>0.05) in stem girth among treatments (Table 3). This indicates that both organic and inorganic fertilizers had a positive impact on collar girth. By implication, different fertilizer application had almost the same significant effect on the collar girth. Hegde *et al*. (1991) had similar observations where increasing fertilization had no effect on collar girth.

Application of fertilizer (organic and inorganic) significantly increased plant height, leaf area and collar girth than the control (Table 3). These findings are in accordance with those reported by other researchers working with other plant species in different locations **(**Hussein, 1992). Also, the growth response of plantain plants was more significant with fertilizers which contained relatively higher levels of nitrogen since it is very important in growth and development of plants. In close agreement, Tingwa (1970), working with banana plants presented superior increases in plant height, pseudostem girth and number of leaves produced in treatments containing the highest level of nitrogen. Plant height, collar girth, leaf area and number of leaves were similar and superior in all cases where fertilizers were applied, whereas the poorest growth was observed in the control plants.

Number of leaves

There was no significant difference (p>0.05) in the mean number of leaves among treatment (Table 4). However; T4, T5 and T6 had the highest number of healthy leaves (4 leaves). The least number of leaves was observed in T1 (2 leaves) while T2 and T3 had the same number of leaves (3 leaves). The number of leaves of a plant could reflect the healthy nature of the plant and even its productivity. Fertilizer application (organic and inorganic fertilizer) influenced the number of healthy leaves. Other studies demonstrated that the number of active leaves is an important parameter for banana bunch development because it reflects the potential yield since leaves are directly related to the plant photosynthetic rate (Soto-Ballestero, 2008) and promote starch accumulation in fruits.

Table 4: Effect of treatments on mean number of leaves

Treatment	Number of leaves	Standard deviation (SD)	Standard Error (±)
T1	2.15[a]	0.62	0.15
T2	3.20[b]	0.81	0.18
T3	3.18[c]	0.92	0.21
T4	4.23[d]	0.99	0.22
T5	4.30[a]	0.77	0.18
T6	4.12[ae]	0.96	0.24

. *Means in the same column with same superscript are not significantly different at 5% level of significance (Tukey test). Where T1 = Control (no chemical application). T2 = 5g of Bioferticide (‘Njoku’). T3 = 5g of NPK (20:10:10) inorganic fertilizer. T4 = 2.5g of Counter® (Terbufos) + 2.5g NPK. T5 = 50mL of Radix Tim® Solution + 2.5g of Bioferticide. T6 = 100mL of Radix Tim® Solution PH = plant height, LA = Leaf area, CG = Collar girth

Relationships between growth parameters

In order to understand the relationship between the different growth parameters, Pearson correlation analysis was carried out. Mean plant height was taken as a reference and correlated with other growth parameters as shown in Table 5.

A better understanding of plant height in relation to its girth, number of healthy leaves and leaf area gives us a better knowhow on its strength and performance under normal environmental conditions. Plant height showed a strong positive correlation with leaf area, number of healthy leaves and stem girth (Table 5). The results indicated that an increase in the number of functional leaves and collar girth increased plant height (R^2 = 0.90 and 0.95 respectively). This might be because the aerial stem is a pseudostem (false stem) consisting of concentric layers of leaf sheaths tightly rolled into a cylinder where the leaves of *Musa spp.* emerge, tightly rolled, from the center of the pseudostem in an anti-clockwise spiral manner (Pillary and Tripathi, 2007). There was also a positive correlation (R^2 = 0.94) between plant height and leaf area. This indicates that plant height can be influenced by large leaf area, translating into more leaf surface per unit weight available for light interception and photosynthesis accumulation (Baiyeri and Tenkouano, 2007). This will increase the net assimilation rate, leading to early shooting in plantain. According to Saeed *et al*., 2001, plant height is positively correlated with productivity of plants. Other studies demonstrated that the number of active leaves is an important parameter for banana bunch development where the leaves are the source of photosynthetic rate and the bunch is the sink. Hence, it reflects the potential yield (Soto-Ballestero, 2008).

Table 5: Summary of correlation analysis between mean plant height and other growth parameters (mean number of healthy leaves, mean collar girth and mean leaf area).

Other growth parameters	Mean plant height	
	Coefficient of determination (R^2)	P-value
Mean HL	0.900	0.004
Mean CG	0.953	0.001
Mean LA	0.942	0.001

Pearson correlation matrix, Alpha = 0.05. Where: HL = Number of healthy leaves, CG = Collar girth and LA = Leaf area

Treatment Effects on Root Parameters: The mean number of roots and roots lengths varied with treatment. The highest mean number of roots was from T4 (18cm), followed by T6 (16.2) (Table 6) while the highest mean root length was from T3 (30), followed by T5 (29.1). Surprisingly, the lowest mean number of roots and mean root length was from the bioferticide alone (T2). This shows that the treatment has a negative effect or delayed effects on roots initiation and growth.

The mean root length was correlated with number of roots and root girth. The correlation between mean root length and mean number of roots was a weak positive correlation (R^2 = 0.097) (Table 7). This indicates that, root length slightly increases with the number of roots. In a similar manner, the correlation between mean root length and mean roots girth was a very weak positive correlation (R^2 = 0.015) (Table 7)**.** This is an indication that, increase in root length does not significantly lead to an increase in root girth. Other studies have demonstrated that the application or addition of fertilizer (bio-organic and chemical fertilizer) did not significantly increase root growth in terms of length and number (Emilia and Lilia, 2013).

Table 6: Effect of treatments on the mean number (±SE) of roots, root length and root girth

Treatments	Number of Roots	Root Length	Root Girth
T1 = Control (no chemical application).	13.1 ± 0.77	29.2 ± 3.51	0.41 ± 0.02
T2 = 5g of Bioferticide ('Njoku').	13 ± 0.65	21.2 ± 1.93 2	0.47 ±0.02
T3 = 5g of NPK (20:10:10) inorganic fertilizer	15 ± 0.83	30 ± 2.42	0.46 ± 0.02
T4 = 2.5g of Counter® (Terbufos) + 2.5g NPK	18 ± 0.51	27.1 ± 3.5	0.46 ± 0.04
T5 = 50mL of Radix Tim® Solution + 2.5g of Bioferticide.	16 ± 0.91	29.1± 2.78	0.5 ± 0.02
T6 = 100mL of Radix Tim® Solution	16.2 ± 1.46	27.4 ± 2.31	0.41 ± 0.02

Table 7: A Summary of correlation analysis between mean root length and other root parameters (mean number of roots and mean root girth).

Other root parameters	Mean root length	
	Coefficient of determination (R^2)	P- value
Mean nR	0.097	0.548
Mean RG	0.015	0.814

Pearson correlation metrix, Alpha = 0.05. Key: nR = number of roots and RG = root girth

Pest incidence

Pest incidence indicates the percentage of pests that affects the growth and development of plantlets. In this study, four different pests were identified namely; Snails (*Limacolaria spp*), Caterpillars, Millipedes and Termites (Fig. 1). Debonnaire (2010) also recorded *Limicolaria spp*. and millipedes as pests infesting plantains in the nursery. At least one type of pest was present in each treatment. However, for Plants treated with fertilizer, T2 harbored all the four identified pests though in very small numbers while T4 and T1 harbored the largest quantity.
T1 had the highest percentage incidence of millipedes and termites whereas plantlets treated with T3 and T6 were infested only with snails. Also, plantlets treated with T4 had the highest percentage incidence of caterpillar. All five treatments were infested with snails (Fig. 1). Plantlets treated with T2 harbored the least percentage of snails (12.5%), caterpillars (16.67%), millipedes (14.29%) and termites (28.57%) while plantlets treated with T4 harbored the highest percentage of snails (20%) and caterpillars (50%) (Fig. 1). The effect of treatment on the mean number of pest did not differ significantly ($P \geq 0.8247$). This lower number of pest in plantlets treated with T2 could be attributed to the fact that the bioferticide (Njoku) has pesticidal properties due to the presence of bush pepper. This study is in line with other studies, Abuno (2014) and Nanganoa *et al.,* (2016) reported a combination of a biological powder (Njoku) that has both fertilizing and insecticidal properties (bioferticide).

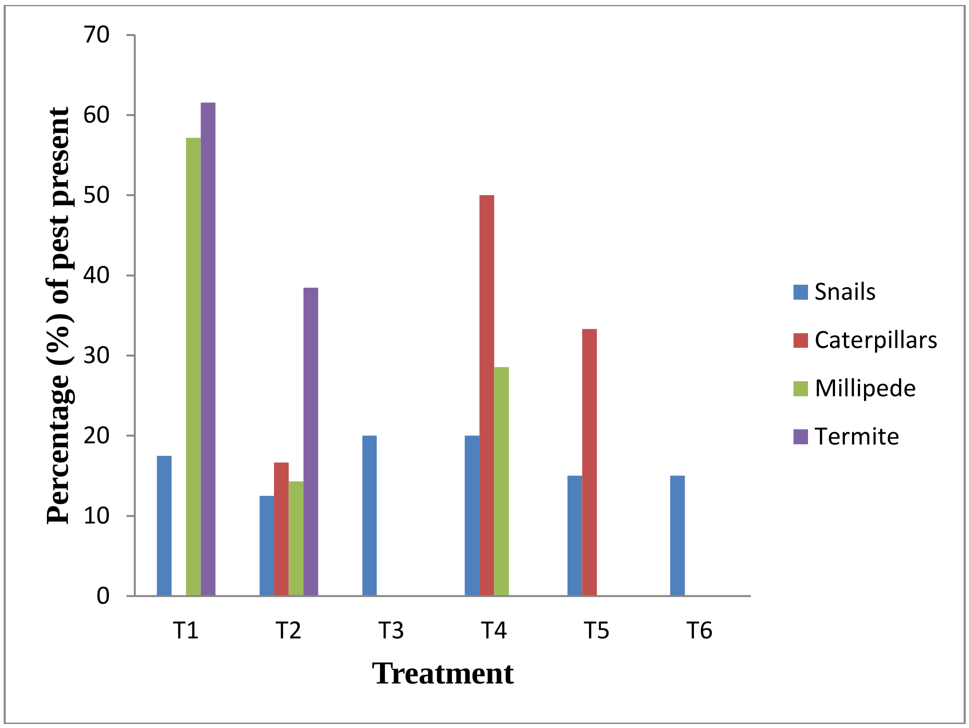

Figure 1: Effect of treatment on the percentage of pest present {T1 (No chemical application), T2 (5g of the mixture (Njoku)), T3 (5g of NPK), T4 (2.5g of Counter® (terbufos) + 2.5g of NPK), T5 (50ml of Radix Tim® + 2.5g of Njoku) and T6 (100ml of Radix Tim®)}

Effects of pest on mean plant height

The linear regression for mean plant height and mean number of pests was a positive one ($R^2 = 0.530$). There was no significant difference between the relationship ($P = 0.101$). This implies plant height is not a major factor affecting the number of pests since pests can live on all plant parts. In line with the present study, Hu *et al*., (1996) demonstrated that pests such as snails, caterpillars and aphids are very common in the nursery and the number of pest does not rely on plant height.

CONCLUSION

Conclusively, fertilizer application (organic and inorganic) generally has positively effect on the growth of plantain plantlets. Plantlets treated with T4 and T5 showed much improvement in growth parameters assessed. Plantlets treated with T2 harbored many types of pests but recorded the lowest number of pests. Due to the environmental hazards posed by chemical fertilizer, T5 is most suitable for the growth and maintenance of plantain plantlets in the nursery. Njoku (T2) gave a positive response to plant growth in the nursery since plant height and stem girth varied progressively. It is therefore important to test the different doses of the bioferticide (Njoku).

COMPETING INTEREST

Authors declare that there is no conflict of interest

AUTHORS CONTRIBUTIONS

APN: Collected data, performed statistical analysis, reviewed literature and wrote first draft of the manuscript
OAN: Collected data, reviewed literature and wrote the first draft
SAS: Reviewed literature, read and edited first draft of the manuscript
NJN: Reviewed literature, read and edited first draft of the manuscript
LTN: Read and edited first draft of the manuscript as well as produced the bioferticide
NJO: Designed the experiment, produced the bioferticide, read and edited first draft of the manuscript

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