



Research Article

Effect of Biofertilizer, Organic and Inorganic Fertilizers on the Growth Parameters and Root Colonization of *Pennisetum Typhoides* (Burm F.) under Greenhouse Conditions

¹Grema. M. N., ²Ismail, H. Y.* and ³Muhammad, S.

¹Department of Biological Sciences, Faculty of Science, University of Maiduguri, Nigeria

²Department of Microbiology, Faculty of Science, University of Maiduguri, Nigeria

³Department of Biological Sciences, Faculty of Science, Usmanu Danfodiyo University Sokoto, Nigeria

*Corresponding author: yismailh@gmail.com doi.org/10.55639/607flrx

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ABSTRACT

Soil fertility remains a major driving force in plant growth and performance. In recent times, there has been an increase in demand for fertilizers to augment the fertility of marginal soils. However, the high cost of inorganic fertilizer has made farmers search for a cost-effective alternative. This study was carried out to investigate the effects of Arbuscular mycorrhizal fungi (AMF) as an alternative biofertilizer, and its effect was compared with that of organic manure (OM) and inorganic fertilizer (NPK) on the growth parameters of Pearl millet. The experiment employed a randomized complete block design (RCBD), and the treatments were replicated four times each under experimental greenhouse conditions. The shoot heights of the plants ranged from 38.06 cm to 83.45 cm, which was significantly higher ($P < 0.05$) in OM-treated plants than those in the remaining treatments. The plants' number of leaves and leaf area (cm^2) differed significantly from the NPK treated plants, which out-competed the remaining treatments. The AMF significantly increased fungal root colonization by 30%, which was higher than in plants with NPK, OM, and the control (0.71% each). Although AMF did not significantly improve the plant's growth, it was better at supporting shoot girth, root elongation, and overall root biomass than unfertilized plants. Inoculation with AMF was more efficient as it was positively reflected in colonized roots and other plant growth traits than non-biofertilized plants. Although the AMF was unable to compete with conventional fertilizers favorably, its potential to support millet growth has been established. Therefore, further investigations channeled towards the search for better mycorrhizal species under different experimental conditions are necessary.

Corresponding author: Ismail, H. Y, Email: yismailh@gmail.com
Department of Microbiology, Faculty of Science, University of Maiduguri, Nigeria

INTRODUCTION

Pearl millet (*Pennisetum typhoides* (Burm F.) Stapf and Hubbard) is the first grain crop to be planted by farmers in the northern part of Borno State (Ikwele, 1998). Its early planting, coupled with its relatively short growth duration, ensures the availability of food grains for consumption at critical periods of food scarcity and when the prices of the basic foodstuffs like yams, rice, Irish potatoes, and other grains are at their peak.

In Nigeria, Pearl millet is second only to sorghum in importance among cereal crops. However, its production volume supersedes sorghum due to its ability to withstand drought and a drier climate. It yields relatively well in regions too hot and too dry to support a good yield of maize and sorghum (Ajayi *et al.*, 1998). Millet in Nigeria is predominantly produced in Bauchi, Borno, Gombe, Kano, Katsina, Sokoto, and the Yobe States.

The grain is nutritious and contains 5-7% oil. Pearl millet is widely eaten all over the arid zone and is an important staple food in many countries such as Sudan, Niger, Egypt, and Libya. It is estimated that over 80 % of millet is used as food; the remaining 20 % is utilized as animal feed and seed (Anon, 2007). In Zimbabwe, Pearl millet is also used for commercial brewing and opaque beer production (Nkama *et al.*, 1994). Food technologists process Pearl millet into composite flour, but the commercial application of this technology is limited (Anon, 1996). Pearl millet is also essential in Nigeria and particularly Borno State in the production of local foods such as 'tuwo', 'burabusko', 'Fura', 'Kamu', 'Kunu', 'Ndalaye', 'Kessire', 'Masa' and 'Couscous' as well as in the production of local beer called 'burukutu' and local snacks called 'mpursa' (Nkama *et al.*, 1994; Anon, 1999). Industrially, it is fermented, processed, distilled, and used to manufacture industrial alcohol (Hamilton, 2009).

In Nigeria, 'Ex-Borno' is the most widely grown, accounting for 95 % of the total cultivated area (Odo and Gwary, 1994). The annual mean yield of Pearl millet grown under local cultural practices is much lower (561kg ha⁻¹) than under improved methods (> 2000 kg ha⁻¹) (Anon, 1999). Several biotic and abiotic factors are responsible for low crop yield. Abiotic factors militating against Pearl millet production in Nigeria include low and erratic rainfall with the drought of varying lengths, poor soil with a low natural fertility level, and low water holding capacity

(Udaiyan and Muthukumar, 2006; Sharma *et al.*, 2014). Cultural practices such as low crop density have been recognized as important factors affecting Pearl millet production (Ajayi *et al.*, 1998). Various diseases and pests attack millet on the field (Azcon-Aguilar and Barea, 1996). The most important of the fungal infections of Pearl millet is downy mildew, caused by the fungus *Sclesopora and graminicola* (Sacc.) Schroet (Anon, 2007). Other factors such as none or low application of fertilizers and poor tillage were also implicated in the low millet production.

Soils in Borno have moderate to poor mineral fertility. The real causes are the low content of nitrogen (N), available phosphorus (P), and sometimes potassium (K) (FAO, 2017). Although inorganic fertilizers are within farmers' reach, the cost of the product remains a challenge to achieving food security. In addition, most soils in dryland areas are very low in both plant available water and soil nutrients, and inorganic fertilizers are rarely used by farmers in the arid zone (Helen and Ardon, 2009). On many occasions, farmers opt for organic manures such as cow dung which supply balanced nutrients to the soil (El-kramany, 2001; Alhassan *et al.*, 2021). Proper management of organic manure on farmlands is also essential for protecting agricultural soils from wind, water erosion through increased water infiltration, water holding capacity, water content, and aeration, soil aggregation, decreasing soil bulk density and increasing rooting depth (Akande *et al.*, 2011; Yadav *et al.*, 2020). Despite their benefits, organic manure, especially cow dung, has its shortfalls in that it carries along with many weed seeds that pose a serious problem to the farmer.

The potential use of biofertilizers in agriculture plays a significant role in providing an economically viable option for achieving the ultimate goal of enhancing plant growth and better yield. Arbuscular mycorrhizal fungi (AMF) are important components of agricultural ecosystems and can directly influence the productivity of crops. Mycorrhiza is the symbiotic association between fungi and the living root cells of plants. In this association, the fungus obtains its carbohydrate requirements from the plants. In return, it absorbs mineral elements mainly, phosphorus, zinc, copper, potassium (Babajide and Fagbola, 2014) and potassium (Bhale, 2013), and transports them to the plants through its ramifying hyphae. The hyphae of arbuscular mycorrhizal fungi extend beyond the root hair-uptake zone into undepleted soil zones and this

increases water movement to the plant roots (Dalpe and Seguin, 2013; Basiru *et al.*, 2020). Mycorrhizal fungi as biofertilizers, form a bridge between the roots and the soil and also gather nutrients from the soil and supply it to the roots (Peters, 2002). The association helps the plant obtain water, which is critical to crop growth and development under dry conditions. Crop growth and productivity in the arid zone region depend on the magnitude, photorespiration length, and level of the plant that is affected by moisture stress (Havargi, 2007). In view of the above, the present study investigated the effect of AMF on millet production as compared to the use of organic or inorganic fertilizer. This is to provide recent information on the subject matter to enable the proper harnessing of the AMF for better crop yield in Borno State, Nigeria.

MATERIAL AND METHODS

Study Area

The experiment was conducted in a restricted environment (in a greenhouse pot experiment) in the Faculty of Agriculture, University of Maiduguri (Lat.: 11.48° 16.29" N; Long.: 13° 11' 55.11" E., and alt.: 300 meters) during July to October 2019 (Buba, 2006).

Sample procurement

Seed of Pearl millet (*Pennisetum typhoides* (Burm F.) Stapf and Hubbard) were obtained from the germplasm of Lake Chad Research Institute Maiduguri and tested for viability by the simple floatation method. One hundred percent (100%) viable seeds tested were surface sterilized with 5% sodium hypochloride to eliminate surface contaminants and rinsed 4 times with distilled water (Ndirmbula, 1998). NPK 15:15:15 kg/ha of National Fertilizer Company of Nigeria (NAFCON) was also obtained from Lake Chad Research Institute Maiduguri. While decomposed cow dung (organic manure) was procured from the University of Maiduguri Livestock Farm, Dalori, Maiduguri was used for the experiment.

Description of the Experimental Sites and Conditions

The Experiment was conducted in a greenhouse at 27±3°C in polythene bags in the Faculty of Agriculture, the University of Maiduguri during the winter season from July 2019 to October 2019 (31.33 ± 2.46°C; 40.75 ± 17.54% RH). The soil sample for the experiment was collected from the Department of Biological Sciences in the Botanical Garden,

University of Maiduguri. The texture of the soil is sandy loam with 70% sand, 17.0% silt and 10.0% clay with a pH of 7.05 (Shi *et al.*, 2011).

Collection and Extraction of Arbuscular Mycorrhizal Fungi Spores from Soil

Samples of rhizosphere soils with feeder roots were collected at the depth of 5 - 15 cm of the soil from a matured *Faidherbia albida* tree growing on a farm behind Ramat Library, University of Maiduguri Campus, using a hand trowel. The sample was placed in sterile polythene bags and taken to the laboratory for further analysis as described by Grema *et al.* (2022).

Collection and Preparation of Soil for Greenhouse pot Experiment

A sample of topsoil at depth of 5 – 15 cm was collected from the Botanical Garden of the Department of Biological Sciences, Faculty of Science, University of Maiduguri. The soil was steam-sterilized in a mini sterilizer (Simplex of Cambridge Limited England) at 121°C for 45 min and allowed to cool for 2 days. The sterilized soil was used for filling up 64 standard sizes (22 x 15 cm) black polythene bags with drainage holes (Horst *et al.*, 2005).

Experimental Design and Treatments

Randomized Complete Block Design (RCBD) was used to assess the effects of Arbuscular mycorrhizal fungi (AMF), organic manure (cow dung), and inorganic fertilizer (NPK) on the Pearl millet crop. A control treatment was also formed which contains no form of fertilizer application. A different form of the applied fertilizers is described below:

Application of biofertilizer

About 2.8 kg of the sterilized soil was weighed using Metra Model TL- 5000 digital electronic balance with a capacity of 5000g (D-0.1g) and used in filling each polythene bag. To each soil in the bags, 100 g (containing about 1000 spores) of the soil containing AMF was added to each polythene bag, while soil without Arbuscular mycorrhizal fungi (AMF) spores were considered as a control treatment. This was also done for millet under cow dung and chemical fertilizer treatments. The treatments were replicated four times (Ndirmbula, 1998).

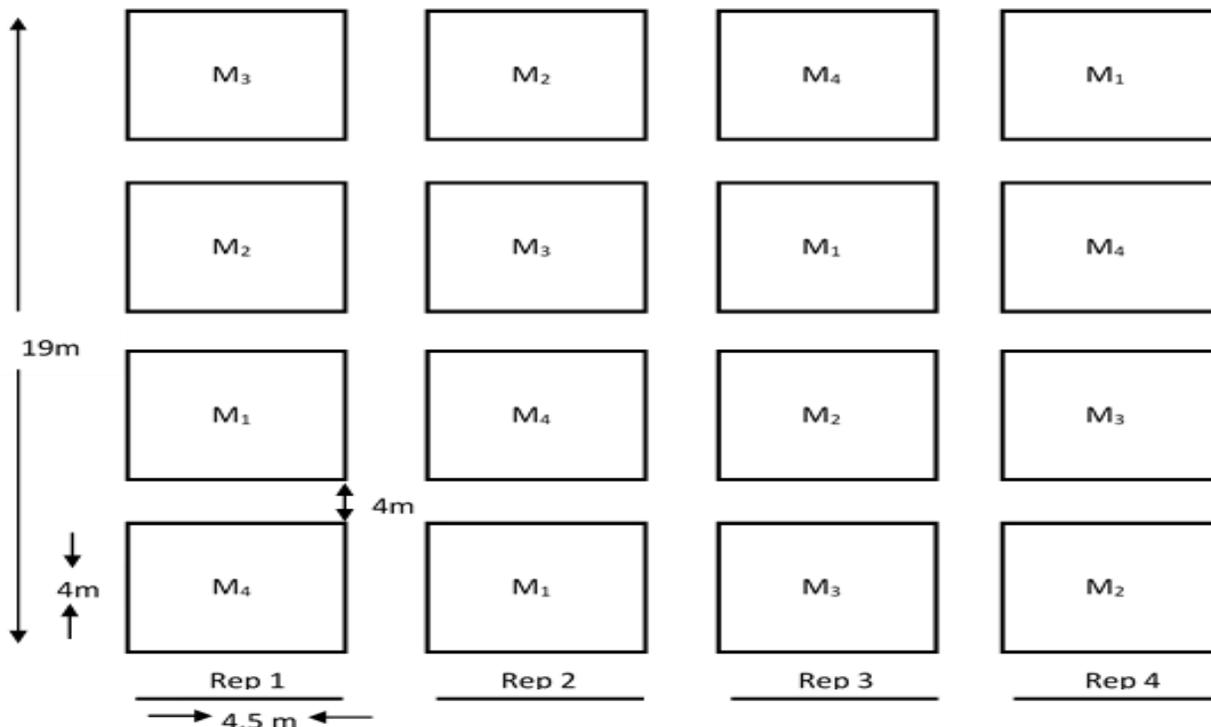


Figure 1: Experimental layout. M₁: Arbuscular mycorrhizal fungi, M₂: Cow dung, M₃: NPK fertilizer, M₄: control

Application of Organic Manure (cow dung)

A mixture of 0.777 Kg/1.036 m² cow dung (equivalent to 7.5 tonnes / ha⁻¹, field rate) and 2.8 Kg of the sterilized soil was placed in polythene bags for Pearl millet. The treatments were replicated four times (Odo and Gwary, 1994).

Application of Inorganic Fertilizer (NPK)

For the Pearl millet crop, 0.36 Kg/1.036 m² of the compound fertilizer (15 kg N., 15 kg P₂O₅, 15 kg K₂O) to give one treatment combination plus 60 Kg N: 30 Kg P₂O₅:30 Kg K₂O which is the recommended rate of NPK for Pearl millet crop, as a check was mixed with 2.8 Kg of the sterilized soil and polythene the bags were filled with the mixture. Control plants were maintained without any form of fertilizer. All treatments were replicated four times (Ajayi *et al.*, 2007).

Sowing and Experimental Management Practices

The seed of Pearl millet (Ex-Borno) was surfaced sterilized with 5 % sodium hypochloride and about 10 seeds of millet were sown in the center of each of the respective treatments. The setup was watered each with 500 ml distilled water regularly to encourage root

system formation. The millet seedlings were thinned to one per bag and the watering regime continued after two days intervals (Odo and Gwary, 1994).

Data Collection and Analysis of plant growth parameters

Percentage of Root Colonization by AMF

The percentage of root colonization by Arbuscular mycorrhizal fungi was determined by the magnified inter-section method (McGonigle and Miller, 1999). Feeder roots from the crop roots were collected using cork borers. The root samples were collected at 5 - 15 cm depth with a cork borer and placed on a 37 µm sieve and washed under running tap water to wash out all adhering soil particles. The feeder roots were cut to about 2 cm segments and placed in McCartney bottles. A 10% solution of potassium hydroxide (KOH) was added to the plant roots in the bottles and steamed in a water bath at 90°C for 45 minutes to increase their transparency. The roots were washed with tap water and finally rinsed with distilled water. About 10 ml of 10% HCl was added to the cleared roots, which are highly alkaline, and have to be neutralized, so that stain can bind well to the fungal structures. The roots in 10%

HCl were left for 15 minutes after which the 10% HCl was decanted. A 10% trypan blue, stain was added to the roots after which the latter was steamed in a water bath at 90°C for 1 hour (Lekberg and Koid, 2008). The roots were removed from the staining solution and left at room temperature (27°C) in glycerol for 24 hours or until required for microscopy (McGonigle and Miller, 2000). Fine-stained roots were mounted on a microscope slide in a drop of glycerol and covered with a coverslip. The field of the microscope was

moved with the help of the stage graticule to make complete passes across each slide, perpendicular to its long axis. Any field that cuts the vertical eyepiece was scored as an intersection (Bella *et al.*, 2006). The inter-sections were scored as positive (+), if they cut across arbuscules and or vesicles and scored negative (-) if they lacked those structures (Dalpe and Seguine, 2013). The percentage (%) Arbuscular mycorrhizal fungi root colonization was calculated using the formula below: (McGonigle and Miller, 2000).

$$\% \text{ AMF colonization} = \frac{\text{Number of inter – section with AMF structures}}{\text{Total Number of intersections viewed}} \times 100$$

Shoot Heights

Shoot height (cm) was measured from cotyledonary scar to shoot tips of all the plantlets using a laboratory wooden meter rule in each replicate and the mean was computed (Statistical Analysis System (SAS) Institute (2008).

Stem Diameter

The stem girth (cm) of the tagged plants was measured using a macro vernier caliper (Statistical Analysis System (SAS) Institute. (2008).

Number of Leaves

The number of leaves on each plant of Pearl millet leaf was counted per replicate and the means were computed (Statistical Analysis System (SAS) Institute (2008).

Leaf Area

The leaf area (cm²) of the crops was measured by using a laboratory wooden meter ruler. The diameter of the leaf and the length (cm) of the leaf were taken and then multiplied by the leaf weight (g) of the plants (Ajayi *et al.*, 1998).

Root Lengths

The root length (cm) of the plants was also measured using a meter ruler after harvest. The length (cm) was determined by placing the ruler at the cotyledonary scar (base) of the shoot to the tip of the longest root (Food and Agriculture Organisation, 2016).

Shoot and Roots DryBiomass

The fresh weight (g) of the shoot was taken as well as the shoots and roots dry biomass (g) of each plant treatment. The plants were harvested, oven-dried at 70°C for 24 hours to constant weight (g) and their dry weights (g) were determined using Digital electric balance (Akande *et al.*, 2011).

Statistical Analysis

The data collected were subjected to a two-way analysis of variance (ANOVA) based on Randomized Complete Block Design (RCBD) using the Analytical Statistical Software, Statistix Version 8.0 (SX) (Microsoft, 2013). Differences between means were determined using the Duncan's Multiple Range Test (DMRT) at 5% probability level (Statistical Analysis System (SAS) Institute. (2008).

RESULTS

Root colonization

The percentage of root colonization observed in Pearl millet grown under the impact of AMF exhibited greater mycorrhizal colonization of 30% than Pearl millet inoculated with the NPK, OM, and the control plants which had 0.71% each. The results also indicated that plants fertilized AMF were significantly (P<0.05) different from all the plants in the other treatments as shown in Table 1.

Table 1: Mean % of AMF colonization of potted millet crop

Treatment	AMF	NPK	Cowdung	Control	Mean	SE±	LSD _{0.05}
Colonization (%)*	30	0.71	0.71	0.71	8.03	15.85	33.76

*Mean obtained from 4 replications

Plant growth parameters

The results of the mean shoot height (cm) of millet during the experiment are also shown in Table 2. The shoot height of the treatments ranged from 38.06 cm to 83.45 cm. The outcome indicates that mean shoot height (cm) was significantly higher ($P < 0.05$) in OM plants than those obtained from the remaining treatments. The analysis also reveals that NPK fertilized plants were significantly taller ($P < 0.05$) than AMF plants and were more than the control plants. The mean difference is significant among the four treatments at the 95% probability level.

Table 2 shows the results for Pearl millet stem diameter obtained from different treatments in the bag experiment. The stem diameters were between 1.98 cm and 3.33 cm. The highest and the lowest value were from NPK fertilized plants and AMF inoculated plants respectively. The stem diameter of NPK fertilized plant was significantly higher ($P < 0.05$) than those obtained from all the rest of the treatments. The result also shows that there was no significant difference ($P > 0.05$) between the plant fertilized with AMF and that of control plants.

The result showed that the mean numbers of leaves varied between the treatments. The mean number of leaves produced in the NPK fertilized plant was significantly more ($P < 0.05$) than in AMF and control plants. Among these treatments OM treated plants produced more leaves number than AMF and control plants, but control plants were highly significant ($P < 0.05$) than AMF plants (Table 2).

The number of the leaves area also varied between 61.14 cm and 611.10 cm². The leaves obtained from the four treatments also showed significant differences among themselves. The plant treated with NPK fertilizer shows significantly higher leaves area than OM, AMF plants, and those in the control. Leaves area produced by the plant without treatment significantly outnumbered those in AMF treatment ($P < 0.05$) as shown in Table 2.

The data presented in Table 2 showed that plants fertilized with NPK fertilizer had the longest (58.50 cm) roots, followed by OM (39.25 cm), AMF (35.00 cm), and the control plant which have the lowest (34.75cm) root length. The AMF-treated plants were also significantly longer ($P < 0.05$) than untreated check (control) plants.

The results of the dry weight (g) of the Pearl millet shoots revealed that there were significant differences ($P < 0.05$) between the treatments, where NPK fertilized plant shoots were significantly greater ($P < 0.05$) than the plant shoots fertilized with OM which was significantly heavier ($P < 0.05$) than AMF treated shoots. A similar trend was also observed between the control plant shoots (4.00 g) and those of the AMF (2.16 g) as shown in Table 2.

Results show that plant roots fertilized with NPK fertilizer exceeded (8.64 g) of the remaining treatments. Organic manure fertilized plant roots were significantly greater ($P < 0.05$) than those of AMF plant roots, which were significantly greater ($P < 0.05$) than those of the control plant roots as shown in Table 2.

Table 2: The growth parameters of Pearl millet under four treatments in the experiment

Treatment	Mean* shoot height (cm)	Mean* stem diameter (cm)	Mean* number of leaves	Mean* root length (cm)	Mean* leaf area(cm ²)	Mean* dry biomass (g) shoot	Mean* dry biomass (g) Root
AMF	38.06	2.00	7.43	35.00	61.13	2.16	2.10
OM	83.45	2.58	8.57	39.25	130.96	5.84	3.62
NPK	74.64	3.33	9.29	58.50	611.10	15.45	8.64
Control	47.64	1.98	7.89	34.75	69.77	4.00	1.96
Mean	60.99	2.47	8.30	41.88	218.24	6.86	4.08
SE ±	5.53	0.55	0.14	3.93	93.48	1.58	0.89
LSD _{0.05}	0.01	1.00	0.30	0.66	0.17	0.22	0.38

*Mean obtained from 4 replications

DISCUSSION

In the present study, spores of *Glomus mossea*, *Glomus intraradices*, *Gigaspora magariata*, *Sclerocystis sinuosa* and *Acaurospora* spp were discovered from the rhizosphere soils collected under the *Faidherbia albida*. These fungal species were considered some of the suitable species among AMF. The soils in the experimental plots were slightly acidic and their nitrogen, phosphorus, and cation contents were typical of arid and semi-arid soils (Verinumbe, 1997).

In this study, a higher rate of mycorrhizal colonization was observed in AMF-treated soil than in the other plant treatments. This could be attributed to environmental factors and soil nutrients, such as the high quantity of phosphorus since some researchers have reported the negative effect of high P levels on AMF colonization (Blanke *et al.*, 2005, Kuila and Gosh, 2022). The variation in the level of root colonization of crops in the field conditions could also be due to uneven distributions of AMF spores in the soil. It could, therefore, be stated here that one factor which can affect the growth improvement of the crops under the effect of AMF in the soil is the availability of nutrients, particularly, phosphorous which promotes root and shoot elongation.

Cereal crops require basic nutrients for optimum growth and development that are mostly supplied from inorganic and organic sources. However, in addition to the organic and inorganic sources of nutrients, arbuscular mycorrhizal fungi could be exploited as sources of nutrients to enhance crop performance.

Thus, in the present study, the NPK was found to enhance shoot, stem diameter, root growth, leaf production, shoot, and root biomass of the millet than the OM and AMF under greenhouse conditions. The lower significant response to AMF in the experiment could be due to the restricted nature of the bags, which could have resulted in poor root system development as compared to the field. This agrees with earlier work by Ndirmbula (1998) which showed that growth responses of millet treated with NPK fertilizers were higher than AMF in terms of plant heights. Recently, the work of Thomsen *et al.* (2021) using commercial AMF biofertilizer inoculants did not significantly improve the growth of *Vitis vitifera* (L.).

However, these parameters were significantly higher ($P < 0.05$) for NPK and AMF, a mean number of leaves, and shoot and roots dry biomass. This could be attributed to the higher N and P contents in the soil, as reported by Kwari *et al.* (1995), which resulted in similar crop growth. The difference may also be attributed to the production of more roots which increases the absorption of water and minerals (Shinde and Shinde, 2014). Thus, the increases may be attributed to the improved mineral nutrition of the inoculated plants, resulting in decreased physiological processes such as photosynthesis and photorespiration (Auge, 2000). Cow dung (OM) was less effective under the greenhouse settings. This agrees with the observations of Bababe *et al.* (1998), who showed that organic manure application to soils has a longer residual effect in terms of nutrient availability on soil

fertility. Thus, organic manure's slow release of nutrients as a fertilizer affects its capacity to elicit significant growth and yield responses.

Even though AMF has been reported to have a positive effect on root length (cm) and root biomass (g) production by crops (Akande *et al.*, 2011; Liu *et al.*, 2020), the result of the present study, revealed that AMF crops produced less and shorter roots than non-mycorrhizal ones. According to Food and Agriculture Organization (2016), most of the carbohydrate (about 1 - 17 %) released by the plants for the purpose of root formation is taken up by the mycorrhizal fungi in the associations. Also, regarding the function of mycorrhizal fungi in the association as absorbing agents, (thereby taking up the function of root hairs), it could be expected that mycorrhizal plants should contain less and shorter roots length (cm) than their non-mycorrhizal counterparts. Bonnie *et al* (2001) have indicated that the production of root extensions, branching, and root hairs are strongly inhibited in the presence of AMF mycelia.

The essential nutrients, nitrogen, phosphorous, and potassium had been reported to contribute significantly to crop growth (Bolan, 2001; Bhale, 2013, Kwari *et al.*, 1995). Therefore, millet fertilized with NPK, which contains some of the major essential nutrients was more productive under the prevailing conditions. Although NPK fertilizer proved more effective, its comparable effects under field conditions could be due to the high mobility of N due to leaching. This condition does not exist under restricted (bag) conditions; hence the greater effectiveness of NPK fertilizer (Abdullahi *et al.*, 2013). Similar results had been reported by Haruna and Abimiku (2012) who observed lower effects of cow dung in relation to NPK fertilizer. In addition to previous reports, the present study recorded better millet performance with AMF than with OM plants.

CONCLUSION

Soil fertility has been a major determinant of crop production and food security. In this study, the use of arbuscular mycorrhizal fungi (AMF) as biofertilizers was assessed and compared with conventional fertilizers used in millet production. Three fungal species were isolated and identified as the AMF from the rhizosphere of *Faidherbia albida*. Millet growth and performance were enhanced by the biofertilizer but its effect was less than that of NPK fertilizer and organic manure. Although shoot height, leaves number

and area were better in millet supplied with NPK, fungal root colonization and shoot thickness were best promoted by the biofertilizer. The present findings suggest that AMF could enhance plant performance and the use of rhizosphere soil of *Faidherbia albida* could facilitate millet performance, especially where inorganic fertilizer is inadequate. The use of different fungal species under different experimental conditions could provide more information on the use of AMF as a biofertilizer for crop production. Although AMF has been known to stimulate plant growth, this study is likely the first to report the use of the identified fungal isolates from the rhizosphere soil of *Faidherbia albida* as biofertilizers for millet production in Nigeria.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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