



Response of Pearl Millet (*Pennisetum typhoides* (Burm F.) to Different Fertilizer Applications under Field Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. Author GMN designed the study, performed the experiment and statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author IHY wrote the first draft of the manuscript, conduct the literature search and perfect the analysis and final version of the manuscript. Author MS conceived the idea, supervised the work, managed the analyses of the study and perfect the manuscript. All authors read and approved the final version of the manuscript.

Article Information

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Original Research Article

Received 10 February 2022
Accepted 16 April 2022
Published 18 May 2022

ABSTRACT

Aims: To study and compare the response of Pearl millet to organic manure, NPK fertilizer, and arbuscular mycorrhizal fungi as biofertilizer.

Study Design: The study employed a Complete Randomized Block Design (CRBD) with a split-split arrangement and the treatments were replicated four times each.

Place and Duration of Study: The experiment was conducted under field conditions during the rainy season of 2020 at the Agriculture Research and Demonstration Farm Land, University of Maiduguri, Nigeria.

Methodology: Pearl millet seeds were planted under three (inorganic, organic and biofertilizer) fertilizer applications and monitored for growth over a period of 60 days. Plant growth parameters were measured appropriately and statistically analyzed.

Results: Results indicated that NPK significantly ($P < 0.05$) improved the growth of millet, with mean shoot heights of 142.45 ± 10.0 cm and mean shoot diameter of 5.70 cm. Leaf area was negatively affected by fertilizer application with the smallest (885.53 ± 0.49) cm^2 and largest (1070.91 ± 6.0 cm^2) areas observed in plants of the control and biofertilizer treated soil respectively. The mean root lengths ranged from 34.75 cm to 58.50 cm and dry shoot biomass ranged from 2.16 g to 15.45 g with more biomass in plants treated with NPK. Plant root colonization was however greater (40%) in plants treated with biofertilizer than in all the remaining treatments (0.71%). The shoot lengths, stem diameters, leaf growth, and root biomass was also enhanced by biofertilizer application when compared to those treated with organic manure and the controls

Conclusion: A positive response was observed using arbuscular mycorrhizal fungi (AMF) as biofertilizer and its application could serve better in millet cultivation where inorganic and organic fertilizers are undesirable or inaccessible.

Keywords: Arbuscular; Biofertilizer; *Faidherbia albida*; fungi; millet; mycorrhizal.

1. INTRODUCTION

Pearl millet is among the leading crops produced in different parts of the world. It has been estimated that over one third of the world's Pearl millet production is in Africa [1]. In West Africa, the arable land put to Pearl millet production has been estimated to be 23.99 million hectares; in which about 5 million hectares occurs in Nigeria [2]. Millet in Nigeria is predominantly produced in Bauchi, Borno, Gombe, Kano, Katsina, Sokoto and Yobe States. Pearl millet is important in Nigeria as staple crop used in preparation of different kind of local and processed foods, feeds, pharmaceutical products and other essential products [3, 4]. It has also been fermented, processed, distilled and used in the manufacture of industrial alcohol [5].

Millet production in Nigeria is significantly affected by a myriad challenges. Millet is attacked by various diseases and pests in the field [6]. The most important of the fungal diseases of Pearl millet is downy mildew, caused by the fungus *Sclesopora, graminicola* [7]. Other diseases and pests of Pearl millet in Nigeria are smut and Ergot caused by *Moesziomyce pennicullariae* and *Claviceps fusikormis* respectively [8, 9]. Some important invertebrate pest includes stem borer (*Coniesta ignefusalis*), head miner (*Raghuva* sp.), the head beetle (*Pocnoda* sp.) and grasshoppers, while *Quelea quelea* is the major vertebrate pest [10]. Fragility of the soils arises from various factors such as desertification, drought, security challenges, bush burning, overgrazing and limited supply of plant nutrients, thus limiting the productivity of the soil which virtually limit agricultural and agro-forestry developments. Other predominant factors include drought, poor soil with low water holding capacity [9, 11] cultural practices such as

low crop density [12], cost of fertilizers, pest invasion and disease infestations [7].

Efforts to address the current challenges of millet production have been targeted towards improving soil fertility and controlling pests and diseases. Soils in semi-arid zones have moderate to poor fertility due to low content of nitrogen (N), available phosphorus (P) and sometimes potassium (K) [10]. This has made soil amendments with fertilizers necessary to enable adequate food and feed supply. However, most soils in dry land areas are very low in plant available water which makes artificial fertilizers rarely used by farmers [13]. The use of organic manure such as cow dung on farmlands is also essential for enhancing soil fertility and protecting agricultural soils from wind and water erosion, leaching and volatilization [14]. The effect of organic manure on soil is also noticed through improvement of soil physicochemical and biological properties [15]. In the recent past, the availability of organic manure remained a major challenge to the farmers especially that the rate of livestock production has significantly decreased. As a result, the search for a better alternative has remained imperative.

The use of fungi in agriculture as an alternative biofertilizer play a significant role of providing an economically viable level for achieving ultimate goal to enhance plant growth and developments. Arbuscular mycorrhizal fungi (AMF) are important components of agricultural ecosystems, and can directly influence the productivity of crops. Mycorrhiza is the symbiotic associations between fungi and the living root cells of plants. In this association, the fungus obtains its carbohydrate requirements from the plants, and in return it absorbs mineral elements particularly, phosphorus, zinc, copper [16] and potassium [17]

and transport them to the plants through its ramifying hyphae. The hyphae of AMF extend beyond the root hair uptake zone into undepleted soil zones and this increases water and nutrients movement to the plant roots [18]. Mycorrhizal fungi as a biofertilizer, form a bridge between the roots and the soil and also gather nutrients from the soil and give it to the roots [19]. In our previous study Grema et al. [20], the role of AMF in enhancing millet growth have been established under greenhouse conditions. However, crop growth and productivity in the arid zone depends on photo-respiration length and level of the plant that is affected by moisture [21]. It is against this backdrop, the present study investigated the effect of AMF on millet growth under field conditions as compared to organic and inorganic fertilizer application.

2. MATERIALS AND METHODS

2.1 Study Area

The experiment was conducted during the rainy season of 2020 at the Agriculture Research and Demonstration Farm Land in the Faculty of Agriculture, University of Maiduguri, Maiduguri, Borno State, North Eastern Nigeria (Latitude: 11.48° 16.29' North, Longitude: 13° 11' 55.11", East and on an altitude 300 meters above sea level and covering an area of 54.2 m²).

2.2 Sample Collection

Pearl millet and fertilizers were obtained from the Lake Chad Research Institute Maiduguri, and the seeds were tested for viability by simple floatation method. One hundred percent (100%) viable seeds tested was surface sterilized with 5% Sodium hypo-chloride to eliminate surface contaminants and rinsed 3 to 4 times with distilled water [22]. While decomposed cow dung (organic manure) procured from University of Maiduguri Livestock Farm, Dalori, Maiduguri was used for the experiment. Biofertilizers in the form of AMF spores consisting of *Glomus mosseae* and *Glomus intraradices* was extracted as described by Grema et al. [20].

2.3 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 growth and root colonization of Pearl millet crop. The gross land area was disc-harrowed, leveled

and marked out into plots. The apportioned area was divided into four groups each plot measured 19 m x 4.5 m, with spacing of 1.0 m between groups. Each of the plots was divided into four replicates in which the four treatments (AMF, cow dung, NPK and control) were apportioned. Each plot measuring 4 m x 4.5 m was fertilized with either AVM, cow dung or NPK. A set of control plots remained without applying any treatment (zero) with spacing of 1.0 m between them [23].

2.3.1 Fertilizer application

To one group of the plots, 100 g (containing about 1000 spores) of soil was added on each replicate as biofertilizer-inoculum. Similarly, 67.5 g /18 m² cow dung (equivalent to 7.5 tonnes / ha⁻¹, field rate for Pearl millet) was added to the second group of the replicate plots [24]. For the inorganic fertilizer, 0.117 Kg N, 0.117 Kg P₂O₅, 0.117 Kg K₂O/18 m² combined with 46 kg/18 m² of urea to give one treatment combinations plus 60 Kg ha⁻¹ N, 30 Kg ha⁻¹ P₂O₅, 30 Kg ha⁻¹ K₂O which is the recommended rate for Pearl millet crop was added to the replicate plots. Soil without AMF spores, cow dung and NPK was considered as control treatment [22].

2.3.2 Sowing and experimental management practices

The seed of Pearl millet was surface sterilized with 5 % Sodium hypo-chloride and about 10 seeds of millet was sown in the center of each of the respective field plots. The millet seedling was thinned to one per plot under rain fed to encourage root system formation [24].

2.4 Data Collection

2.4.1 Percentage of root colonization by AMF

This was carried out by collecting, staining and microscopic observations as described by Grema et al. [20]. The percentage (%) Arbuscular mycorrhizal fungi root colonization was calculated using the formula (1) below [25]:

$$\% \text{ AMF colonization} = \frac{\text{Number of inter-section with AMF structures}}{\text{Total Number of intersections viewed}} \times 100 \quad (1)$$

2.4.2 Shoot heights

Shoot heights were measured in centimeters from cotyledonary scar to shoot tips of all the crops using a Laboratory wooden meter ruler

from seedling plants in each replicate for the cereal (millet) and the mean was computed [26].

2.4.3 Stem diameter

Stem girths of the plants were measured using a macro Vernier caliper for the field experiment and was expressed in cm [26].

2.4.4 Number of leaves

Number of leaves on each plant of Pearl millet was counted on the seedling plants per replicate for each plant per field trial and the means was computed using [26].

2.4.5 Leaf area

The leaf area of the field crops was measured by using laboratory wooden meter ruler. The diameter of the leaf and the length of the leaf was taken and then multiplied by the leaf weight of the seedling plants under field study and expressed in cm^2 [12].

2.4.6 Root length

A root length of the plants was also measured using meter ruler after harvest. The length was determined by placing the ruler at the cotyledonary scar (base) of the shoot to the tip of the longest root [27].

2.4.7 Shoot and roots dry biomass

The fresh weight of the shoot was taken as well as the shoots and roots dry biomass of each field trial millet plants harvested, oven dried at 70°C for 24 hours to constant weight and their dry weights was determined using Digital electric balance with capacity of 5000 g and expressed in grams [15].

2.5 Statistical Analysis

The data collected was subjected to two-way Analysis of Variance (ANOVA) based on Randomized Complete Block Design (RCBD) with Split - Split arrangement using the Analytical Statistical Software, Statistix Version 8.0 (SX). Differences between means were determined using the Duncan's Multiple Range Test (DMRT) at 5% probability level.

3. RESULTS

The response of millet to the application of organic, inorganic and biofertilizer was investigated. Results in Table 1 showed that the shoot height of Pearl millet was significantly taller ($p < 0.05$) in the treatments that received NPK fertilizer. It was followed by millet in the control (138.78 cm) and biofertilizer (120.75 cm) treated millet. Shoots of the plants that received AMF as fertilizer were significantly longer ($p < 0.05$) than the plants treated with organic manure. The results of millet stem diameter showed that plants treated with biofertilizer, NPK and the control had significantly greater ($p < 0.05$) stem diameter than plant treated with organic manure (cow dung). However, statistical analysis showed no significant difference between the biofertilizer, NPK fertilized plant and that of the control plants respectively (Table 1). The millet leaves observed in this study ranged between 50 - 73 leaves, where plant treated with NPK fertilizer exceeded (73.04) the other treatments and the least (50.83) number of leaves occurred in millet grown without fertilizer. Analysis of variance indicated that millet that received NPK has significantly ($P < 0.05$) more leaves than organic manure and also those of the plant inoculated with AMF was significantly more ($P < 0.05$) than the control plants.



Fig. 1. Map of Nigeria showing the study area Borno State

Table 1. The Pearl millet growth parameters under field conditions

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	120.75 ± 0.25	5.17 ± 0.50	56.41 ± 0.67	35.00 ± 0.50	885.53 ± 0.49	2.16 ± 0.25	2.10 ± 0.50
OM	107.67 ± 0.50	4.98 ± 0.75	64.95 ± 0.83	39.25 ± 0.66	945.39 ± 6.0	5.84 ± 0.46	3.62 ± 0.23
NPK	142.45 ± 10.0	5.69 ± 0.58	73.04 ± 1.67	58.50 ± 2.20	951.53 ± 10.17	15.45 ± 1.20	8.64 ± 1.40
Control	138.78 ± 3.33	5.58 ± 0.75	50.83 ± 0.33	34.75 ± 2.40	1070.90 ± 6.80	4.00 ± 0.12	1.96 ± 0.20
Mean*	127.41	5.36	61.31	41.88	963.34	6.86	4.08
SE ±	9.14	0.93	9.24	3.93	101.35	1.58	0.89
LSD _{0.05}	0.689	0.502	0.153	0.086	0.098	0.002	0.007

*Mean obtained from 4 replications

Table 2. Rate of millet crop root colonization

Treatment	AMF	NPK	Cowdung	Control	Mean	SE±	LSD _{0.05}
Colonization (%)	40 ± 0.50	0.71 ± 0.02	0.71 ± 0.10	0.71 ± 0.2	10.53	19.64	41.84

*Mean obtained from 4 replications

The result showed that mean root length of the field planted millet under the four treatments ranged from 34.75 - 58.50 cm, where NPK fertilizer treated plants had the longest (58.50 cm) root length and the shorter was observed in the control plants (Table 1). The outcome of the study also shows that plants treated with organic manure had significantly ($P < 0.05$) taller roots than that of the plant treated with biofertilizer whereas; millet treated with biofertilizer had significantly longer roots than that of the control.

Results in Table 1 showed that fertilizer application reduced the leaf area of millet crop with the lowest area observed in the plants treated with biofertilizer (885.53 cm²). The highest leaf area (1070.90 ± 0.68 cm²) were observed in the control plants which were significantly ($P < 0.05$) larger than plants in the remaining treatments.

For the plants dry matter, the results showed that plants treated with NPK fertilizer had the highest (15.45 g) shoot dry weight and the lowest (2.16 g) was observed in the plants inoculated with biofertilizer which were significantly lighter than those in the control. Similarly, the plant root dry weight followed the same trend; except that biofertilizer treated millet was significantly heavier than that of the control plants. The mean values ranged between 8.64 g - 1.96 g, in which plants treated with inorganic fertilizer had the greater (8.64 g) dry root biomass. The percentage (%) root colonization observed in Pearl millet showed that biofertilizer had the highest percentage (40%) of root colonization and was significantly ($P < 0.05$) different from all other treatments.

4. DISCUSSION

Arbuscular mycorrhizal fungi could be exploited to source for nutrients to enhance crop performance as biofertilizer. Thus, in the present study, the inorganic fertilizer (NPK) was found to enhance millet shoot length, stem diameter, root length, leaf production, shoot and root biomass which the results indicated that treatment in this group had significantly ($P < 0.05$) improved growth. However, the root colonization was very much (40%) enhanced in plants treated with

biofertilizer than the remaining treatments. A positive response from such investigation has demonstrated a higher percentage of root colonization in plants treated with arbuscular mycorrhizal fungi spores than other treatments which were negative in the other experimental sites.

The lower response of millet to biofertilizer in the field experiment could be due to the routine tillage of the field soils, which could have resulted in poor root system development compared to the plants grown under the influence of inorganic fertilizer. The higher rate of growth observed in the latter could be attributed to the higher N and P contents in the field as reported by Kwari et al, [28], which resulted in similar crop growth. Adequate supply of N and P induce faster root development which would lead to better nutrients absorption and improved plant growth. According to Shinde [29], plant growth may be attributed to the production of more roots which increases absorption of water and minerals (N, P and K⁺). Thus, the increases may be attributed to the improved mineral nutrition of the inoculated plants which evidently resulted in increased physiological process such as photosynthesis and photorespiration [30].

Organic fertilizer (cow dung) was less effective in field conditions as observed in this study compared to inorganic fertilizer. This agrees with the observations of [31] who showed that organic manure application to soils have longer residual effect in terms of nutrient availability on soil fertility. Thus, the slow release of nutrients by organic manure as a fertilizer affects their capacity to elicit significant growth and yield responses.

The result of the present study revealed that lower and shorter roots were produced by crops treated with biofertilizer than non-biofertilized ones except the controls. Previously, arbuscular mycorrhizal fungi has been reported to have negative effect on root length and root biomass of plants [15]. According to [27], most of the carbohydrate (about 1 - 17 %) released by the plants for the purpose of root formation (root length and root biomass), is taken up by the mycorrhizal fungi in the associations. As a result,

root development is grossly jeopardized. In addition, mycorrhizal fungi may take up the function of root hairs as absorbing-agents which could further decrease the rate of root elongation. As such, it could be expected that mycorrhizal plants should contain less and shorter root length than their non-mycorrhizal counterparts. Bonnie et al. [32] have indicated that the production of root extensions, branching and root hairs are strongly inhibited in the presence of arbuscular mycorrhizal fungi mycelia.

There was a higher percentage (40%) of Pearl millet root colonization in plants treated with biofertilizer than in all other treatments (0.71% each). 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 arbuscular mycorrhizal fungi (AMF) colonization [33]. The variation in the level of root colonization of crops in the field conditions could also be due to uneven distributions of arbuscular mycorrhizal fungi (AMF) spores in the soil or due to the heavy rain. Comparatively, the millet plants treated with biofertilizer had longer shoots and stem diameters compared to those treated with organic manure, which contrastingly had better leaf properties and dry biomass. Similarly, biofertilizer application promoted the plants' shoot development, leaves area and shoots dry biomass as compared to the plants used as control. This indicated that, biofertilizer application could enhance food and feed production.

5. CONCLUSION

Fertilizer application has become a cornerstone for sustainable crop production. Due to the obvious challenges associated with inorganic fertilizers, alternative sources have been sought for. It has been established in this study that inorganic fertilizer promote millet growth better than other sources of fertilizer used in the study. Biofertilizer application enhanced shoots lengths and stem diameters when compared to those treated with organic manure. In addition to shoot diameter, biofertilizer application supported better leaf growth and root biomass. The present study has therefore highlighted the fact that Arbuscular mycorrhizal fungi (AMF) could serve better in millet cultivation than cow dung under field conditions and thus, its suitability for use as alternative for inorganic fertilizer for improved millet production.

ACKNOWLEDGEMENTS

The Authors acknowledged the contributions of staff in the Agriculture Research and Demonstration Farm, Faculty of Agriculture, University of Maiduguri, Maiduguri, Borno State, North Eastern Nigeria for their support throughout the experimental period.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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