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ORIGINAL RESEARCH ARTICLE

Comparative Study on the Effect of Biofertilizer, Organic and Inorganic Fertilizers on Chlorophyll and Moisture Contents of *Pennisetum typhoides*

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ABSTRACT

The most prevalent type of mycorrhiza are arbuscular mycorrhizal fungi (AMF). in plants and have been used as biofertilizer due to its ability to capture and supply water and nutrients to plants. In this study, investigations were carried out to ascertain the effect of biofertilizer (AMF), organic (cow dung) and inorganic fertilizers (NPK) on chlorophyll and moisture contents of pearl millet crop. Experiments were conducted in greenhouse and treatments arranged in a Complete Randomized Block Design. The treatments were replicated four times each and monitored for chlorophyll concentration and shoot moisture contents as it affects crop yield. The chlorophyll a concentration varied from 0.92 mg/g to 1.18 mg/g, while the plants treated with NPK fertilizer exhibited highest chlorophyll a (1.18 mg/g). It was followed by the control (1.15mg/g) and organic manure. The plants inoculated with biofertilizer had the infinitesimal chlorophyll a (0.92mg/g). However, for chlorophyll b, plants treated with biofertilizer (1.01mg/g), organic manure (1.09mg/g) and NPK (1.26mg/g) exhibited higher content than in control plants that had the lowest (0.53mg/g) value. Statistics revealed that there was no discernible difference. ($P<0.05$) between leaves chlorophyll contents in plants treated with organic manure and NPK fertilizer. Millet treated with organic manure, biofertilizer and the control did not significantly ($P<0.05$) differ from one another with respect to shoot moisture contents. Although NPK had more chlorophyll contents, biofertilizer promoted more grain yield (2030 grains /m^2) than all the treatments. Findings from this study indicated that biofertilizer can alternatively be used for better yield when organic and inorganic fertilizers are costly and unaffordable to peasant farmers. Further studies on biofertilizer are recommended to provide effective formulations for improved crop growth and better yield.

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INTRODUCTION

Pennisetum typhoides (Burm F.) originated from India and widely distributed in Africa between latitudes 10°N and 17°E (FAO, 2017), is grown in northern Guinea, Sudan and Sahel Savanna (FAO, 2016). Nigeria produces 3.4 million tons annually on average, ranking second in the world only to India (FAO, 2017). Pearl millet production in Nigeria was third only to maize and sorghum. Millet is the cereal crop with the second-highest importance in Nigeria, after sorghum (Pingali *et al.*, 2020). However, because of its resistance to drought and arid climate, its production volume outpaces that of sorghum. It yields moderately well in regions that are too hot and dry to support a good yield of sorghum and maize (Ajayi *et al.*, 1998). It is a staple food crop grown across the Sahel including a large part of Borno State, Nigeria (Ajayi *et al.*, 2007). The grain is highly nutritious and has a 5 – 7% oil content (Slama *et al.*, 2020). In many nations, including Sudan, Niger, Egypt, and Libya, pearl millet is a significant staple food and is consumed widely throughout the arid zone. Over 80% of millet is thought to be consumed as food, with the remaining 20% being used as seed and animal feed (Anon, 2007).

Among the different millet cultivars, *"Ex-Borno"* is the variety that is most widely grown in Nigeria (Odo and Gwary, 1994). When grown using modern methods, the annual mean yield of pearl millet is much higher (> 2000kg ha^{-1}) than 561kg ha^{-1} produced when grown using local cultural practices (Anon, 1999). A number of biotic and abiotic factors causes low crop yield. The cultivation

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of pearl millet in Nigeria is hampered by low and unpredictable rainfall, droughts of various lengths, poor soil with low natural fertility levels, and low water holding capacity. These conditions also increase water movement to the plant roots (Dalpe and Seguin, 2013; Basiru *et al*., 2020). When inorganic fertilizer is applied, pearl millet responds very well (Hamilton, 2009). As part of the Northeast Arid Zone Development Programme's (NEAZDP) research efforts, FAO (2017) conducted a multi-locational trial in Yobe State and the results showed that the application of NPK fertilizer resulted in a grain yield of 1603Kg ha^{-1} of millet as opposed to 818Kg ha^{-1} of millet produced by plots that were not fertilized. Farmers in Borno State have been using compound fertilizer in a 1:1:1 ratio for NPK fertilizer. Research have demonstrated that irregular and low rainfall does not have the same growth-limiting effects on plants as low availability of mineral nutrients, particularly nitrogen and phosphorus (Langham, 2007).

Borno soils have moderate to low mineral fertility occasioned by the lack of adequate nitrogen (N), available phosphorus (P) and, in some cases, potassium (K) (FAO, 2017). Although farmers resort to the use of inorganic fertilizers, the cost of the product remains a barrier to achieving food security. Furthermore, most dryland soils are deficient in both plant available water and soil nutrients, which consequently affect crop yield. With the current ecological problems instigated by climate change, shift in conventional agricultural practices is imminent. Previously, intercropping with legumes have been suggested as an important means of improving soil nitrogen for millet growth. However, according to FAO (2007), future research should focus on creating fertilizer recommendations for millet-legumes mixtures. In the recent past, the use of biofertilizer has been advocated and previous works have demonstrated its effect on millet growth indices (Grema *et al*., 2022a). Consequently, the present study was aimed at understanding the effect of biofertilizer on the plant chlorophyll and moisture content as important determinants of crop yield.

MATERIALS 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 (coordinates: 11.8024° N, 13.1931° E, and alt.: 300 meters).

Sample collection

Pearl millet seed (*Pennisetum typhoides* (Burm F.) Stapf and Hubbard) were obtained from Lake Chad Research Institute Maiduguri and tested for viability using the simple floatation method. To eliminate surface contaminants, all viable seeds tested were surface sterilized with 5% sodium hypochloride and rinsed four times with distilled water (Ndirmbula, 1998). Inorganic fertilizer (NAFCON; NPK 15:15:15) was also obtained from Lake Chad Research Institute Maiduguri. The experiment used decomposed cow dung (organic manure) obtained from the University of Maiduguri Livestock Farm, Dalori, Maiduguri. The biofertilizer used was aburscular mycorrhizal fungi (AMF) obtained from the rhizosphere of *Faidherbia albida* (Grema *et al*., 2022b). The soil sample for the experiment was collected from the Department of Biological Sciences in the Botanical Garden.

Treatment and Experimental Design

The experimental sites and experimental conditions are similar to that of Grema *et al.* (2022a). The experiment employed a Complete Randomized Block Design (CRBD) made up of four sets of polythene bags containing 2.8Kg sterilized soil in triplicates. To one set of the soil samples (A), 100g (containing about 1000 spores) of the soil containing AMF (biofertilizer) was added to each polythene bag and mixed thoroughly. Cow dung and NPK were added to another two sets of the soil samples (B and C) as organic and inorganic fertilizers at the rate of 0.777 Kg/m² and 0.36 Kg/m² respectively (Ajayi *et al*., 2007). The remaining set (D) was left as a control treatment. All the treatments were irrigated with equal volume of water and allowed to stand for one week in order to acclimatize. Following that, viable millet seeds were sown in each treatment at the rate of 10 seeds per bag. After emergence, the seedlings were thinned and only 5 seedlings were left in each bag. The plantlets were sparingly irrigated after every other day until maturity.

Determination of Chlorophyll Concentrations in Plant Leaves

Using the Lichtenthaler and Buschmann (2001) method, the total chlorophyll concentrations (mg/ml) in fresh leaves of mature Pearl millet were calculated. Using a pestle and mortar, 100 milligram (mg) of fresh leaves from the inter-venial area were ground in 10 ml of 85% acetone. The suspension was decanted onto a Buchner funnel and put through a Whatman filter paper No. 1 filter. Using a spectro-photometer, the optical density (OD) of the solution was measured at 645 nm and 663 nm to estimate the chlorophyll contents (UV-1700, Shimadzu, Japan). The following equations were used to compute the concentrations of chlorophyll a, chlorophyll b, and total chlorophyll (a and b) (mg/ml) (Udin, 2003):

$$\text{Chlorophyll a} = 12.70\,(A_{663}) - 2.69\,(A_{645}) \times \frac{V}{W*1000} \quad \textbf{eqn. (1)}$$

$$\text{Chlorophyll b} = 22.90\,(A_{645}) - 4.68\,(A_{663}) \times \frac{V}{W*1000} \quad \textbf{eqn. (2)}$$

$$\text{Total chlorophyll level} = 20.20\,(OD_{645}) + 8.02\,(OD_{663}) \times \frac{V}{W*1000} \quad \textbf{eqn. (3)}$$

Where v = final volume of extract

OD = optical density
W = wavelength

Moisture Contents in the Plant Shoots and Roots
The shoots moisture contents of the plant were determined using Digital electronic balance by measuring the fresh weight (g) minus dry-biomass (g) of the shoots and divided by the weight (g) of the fresh sample multiplied by 100. The percentage (%) of moisture contents of the plant shoots were calculated using the formula below (Akinoso *et al.,* 2010):

$$\frac{\textit{Fresh weight of the plant shoots} - \textit{Dry weight of the plant shoots}}{\textit{Fresh weight of the plant shoots}} \times 100 \quad \textbf{eqn. (4)}$$

Statistical Analysis
Using the analytical statistical software Statistix Version 8.0 (SX), analysis of variance (ANOVA) was performed on the acquired data (Microsoft, 2013). Where "F" test showed significant difference, treatments means were separated using the Least Significant Difference (LSD) at 5% Probability level.

RESULTS AND DISCUSSION

In the present study, the soil used in the experiment had a neutral pH (7.05-7.07) and was poor in nitrogen and phosphorus contents (data not shown), typical of arid and semi-arid soils as earlier reported (Grema *et al.*, 2022a). The soil has limited water and moisture retention capacity since it is categorized as sandy loam based on particle size distribution.

Table 1 shows the pearl millet chlorophyll contents as affected by arbuscular mycorrhizal fungi, organic manure and inorganic fertilizer. The results obtained from leaves chlorophyll a contents ranged from 0.92 mg/g to 1.18mg/g, where leaves treated with NPK fertilizer exhibited highest chlorophyll a (1.18 mg/g) followed by the control (1.15 mg/g). The high chlorophyll a concentration observed in this study might be linked to the provision of adequate nutrient required for plant growth by NPK fertilizer. Earlier study by Amujoyegbe *et al.* (2007) indicated that abundant nutrient supplementation increases chlorophyll a concentration in *Sorghum bicolor* and *Zea mays*. Additionally, they observed that S. bicolor had increased chlorophyll a content when inorganic fertilizer and organic manure were combined, as opposed to Z. mays, where the effects were more pronounced on chlorophyll b. Results revealed that plant leaves fertilized with cow dung, NPK and control did not showed significant difference ($P<0.05$). The chlorophyll a concentration in the leaves of pearl millet of the NPK treatments was statistically greater ($P>0.05$) than leaves of millet treated with biofertilizer (AMF) at 5% level of significance. Lower chlorophyll a concentration observed in plants treated with biofertilizer might have resulted from the use of high biofertilizer concentration. Recent reports indicated that lower biofertilizer concentration triggers more leave chlorophyll contents than higher amounts of biofertilizer. Siswanti and Umah (2021) indicated that while applying 10l/ha of biofertilizer improved the chlorophyll content, applying 20l/ha of biofertilizer boosted plant height and the number of leaves. Chlorophyll a remains the dominant pigment used by plants to absorb light energy for photosynthesis. Previous studies have shown that chlorophyll a concentration was directly correlated with plants' primary productivity (Richard and Christopher, 1994).

For chlorophyll b, millet leaves treated with biofertilizer (1.01mg/g), organic manure (1.09mg/g) and NPK (1.26mg/g) had higher chlorophyll b contents when compared with what was recorded in control plants (0.53mg/g). Statistical analysis indicated that there were significant ($P<0.05$) variations between plants in the control and all other treatments concerning leaf chlorophyll b contents. However, the results revealed that there were no significant ($P<0.05$) variations among plants treated with manure, NPK and biofertilizer. Statistically, addition of the fertilizers increased the concentration of chlorophyll b in the leaves. It has been reported recently that biofertilizer in form of AMF enhanced the growth characteristics, seed yield, chlorophyll content, stomatal movement, antioxidant enzyme activities, and radical scavenging activity in *Lallemantia iberica* and *L. royleana* compared to control plants (Paravar *et al.*, 2021).

In addition, results of the study with respect to total chlorophyll level in the leaves of the Pearl millet fertilized with organic manure and that of inorganic fertilizer showed highest (2.44 and 2.16mg/g respectively) total chlorophyll contents. Plants leaves treated with biofertilizer (1.93 mg/g) followed it, while the plant leaves in the control treatment had the lowest (1.68mg/g) level of total chlorophyll. But there was no appreciable distinction ($P<0.05$) between plant leaves treated with organic manure and NPK fertilizer. The result also revealed that plant leaves treated with biofertilizer (AMF) and control were statistically similar ($P<0.05$). This observation signifies that nutrient addition is important in influencing the pigment of the crop under the same conditions. This was however, not unexpected since chlorophyll concentrations are directly correlated with the availability of nitrogen elements and are crucial to the process of photosynthesis. Because there was more nitrogen available to the NPK-treated plants, there was more chlorophyll in the leaves, which speeds up photosynthesis (Setiawati *et al.*, 2019). More so, water and light intensity are vital components of physiological activities of the plant especially photosynthesis with the result that the more available it was, the higher the chlorophyll concentration (Liu *et al.*, 2012). While accessory pigments, which include chlorophyll b, absorb energy that chlorophyll a does not absorb, such as green-yellow-orange wavelengths, chlorophyll a absorbs energy from the violet-blue and reddish orange-red wavelengths and little from the intermediate wavelengths (Claereboudt *et al*., 2010).

Table 1: Effect of biofertilizer, organic and inorganic fertilizers on leaf chlorophyll contents

Treatments	**Chlorophyll Contents (mg/g fresh leaves)**		
	Chlorophyll a	Chlorophyll b	Total chlorophyll (a and b)
Biofertilizer	0.92^{b}	1.01^{a}	1.93^{b}
Organic Manure	1.07^{ab}	1.09^{a}	2.16^{ab}
NPK	1.18^{a}	1.26^{a}	2.44^{a}
Control	1.15^{a}	0.53^{b}	1.68^{b}
Mean*	1.08	0.97	2.05
SE ±	0.06	0.16	0.16
LSD	0.17	0.45	0.45

*Means of four replications. Values with the same superscript along column are statistically not significant at $P<0.05$ using LSD

Results of shoot moisture contents varied from 66.47% to 74.79%. Pearl millet treated with organic manure had the highest (74.79%) moisture contents, whereas the lowest moisture content (66.47%) was observed in shoots of plant treated with NPK fertilizer which showed significant difference ($P>0.05$). Millet treated with organic manure, biofertilizer and the control did not significantly ($P<0.05$) differ from one another as shown in Table 2. Organic fertilizers have been described as an important source of plant nutrients because it increases water uptake and nutrients from the soil (Alhassan *et al*., 2021; Bastami *et al*., 2021). Generally, organic manure provides nutrient at the later stage of plant development because it takes some time for mineralization to take place. High moisture observed in biofertilizer-treated plants might be related with extensive growth of fungal mycelia in the plant-fungal association, which helps in nutrient mobilization and water conservation. Püschel *et al*. (2020) revealed that AMF facilitated plant water-uptake in *Medicago truncatula*. Leventis *et al*. (2021) also reported that AMF enhanced the growth of tomato under drought conditions through metabolic water use efficiency. Yang *et al*. (2022) have also reported similar findings recently. The increase in yield of sweet maize (Zea mays) utilizing arbuscular mycorrhiza fungi (AMF) and cow manure fertilizer (CMF) on an ultisol was also reported by Sabaruddin *et al*., (2021).

The low moisture contents observed in NPK-treated plants might be linked to induced water stress caused by abundant soil nutrients. Qi and Pan (2022) reported that moisture content in maize treated with inorganic fertilizer gradually reduced to that of the control. Amico *et al*. (2002) confirmed that mycorrhizal fungi increase water use efficiency in several crops more than non-mycorrhizal fungi. The mycorrhizal plants and non-mycorrhizal (cow dung manure and control) plants were found to have the same ($P<0.05$) percentage of maize shoots moisture contents in this study. Liu *et al*. (2021) reported that in *Nicotiana tabacum*, biofertilizer (AMF) inoculation alone boosted root function (root biomass, root/shoot ratio, and root system architecture), aided in solar energy absorption and conversion (photosynthetic rate), and increased nutrient intake. Benaffari *et al*. (2022) reported that the growth parameters of *Chenopodium quinoa* were significantly enhanced by biofertilizer application in the form of AMF. Additionally, it has been demonstrated that using organic manures increases fenugreek trigonelline content, biological yield, leaf area index, and seed production (Baghbani-Arani *et al*., 2017).

Table 2: Effect of biofertilizer, organic manure and inorganic fertilizers on moisture contents in millet shoots

Treatments	**AMF**	**OM**	**NPK**	**Control**	**Mean**	**SE ±**	**$LSD_{0.05}$**
Moisture (%)	74.04^{a}	74.79^{a}	66.47^{b}	72.17^{a}	71.87	1.88	5.27

*Means of four replications. Values with the same superscript are statistically not significant at $P<0.05$ using LSD, AMF =arbuscular mycorrhizal fungi OM = organic manura, NPK = Nitrogen-Phosphorus-Potassium

The result of the yield (number of seeds per m^2) ranged between 470 and 2030. The highest (2030) yield was obtained in plant treated with biofertilizer (AMF) followed by the control (1965) and those treated with organic cow manure (1095). Significant yield differences ($P>0.05$) existed between the treatments. The highest yield observed in this study was due to biofertilizer and organic manure application. AMF fungi and organic fertilizer from cow manure significantly partake in nutrient absorption and increasing uptake of water from the soil, which led to the overall increase in yield. This is in accordance with the findings of Sabaruddin *et al*. (2021) who made similar observation in maize plantation. Al-Zubade *et al*. (2021) have shown the impact of biofertilizer on hard red winter wheat growth, yield, and baking quality and encouraged further research to determine the best way to use biofertilizer in crop production.

Table 3: Yield of pearl millet grown under different fertilizer application

Treatments	AMF	OM	NPK	Control	Mean	SE ±	$LSD_{0.05}$
Moisture (%)	2030[a]	1095[b]	470[c]	1965[a]	71.87	1.88	5.27

*Means of four replications. Values with the same superscript are statistically not significant at P<0.05 using LSD, AMF =arbuscular mycorrhizal fungi OM = organic manura, NPK = Nitrogen-Phosphorus-Potassium

CONCLUSION

The findings of this study indicated that application of the three fertilizers increased the millet chlorophyll concentration with NPK fertilizer exerting more effect on both chlorophyll a and b. Although biofertilizer did not differ significantly from the control, its effect was reasonably higher. As organic manure treatment resulted to the millet's high chlorophyll and moisture contents, the plants treated with recorded the least moisture contents in its shoots. It is evident that the low moisture contents in the latter probably resulted to poor yield as opposed to organic manure and biofertilizer. With the performance of biofertilizer in this study, it is recommended that optimization of biofertilizer application should be prioritized.

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