



Arbuscular mycorrhizal fungi improved the chlorophyll concentration and symbiotic root colonization in *Zea mays* (Tzers White)

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Abstract

Many plants species live in symbiotic associations with some fungal species of the glomeromycota which are popularly called as arbuscular mycorrhizal fungi (AMF). AMF helps plants to capture macro and micronutrients which result to better plant growth and performance. Investigations were carried out to ascertain the effect of bio-fertilizer on maize growth as compared to organic and inorganic fertilizers. Experiments were conducted in the field and treatments laid out in a complete randomized block design. The treatments were replicated four times each. The result of the investigations on chlorophyll a revealed that plant leaves treated with organic manure had the highest (0.955mg/g) chlorophyll a, followed by leaves of plant treated with arbuscular mycorrhizal fungi (0.84mg/g), control (0.83mg/g) leaves and lowest (0.63mg/g) value obtained from leaves fertilized with NPK fertilizer. Results revealed that leaves of maize inoculated with AMF, organic manure and untreated check were statistically ($P < 0.05$) the similar in the chlorophyll a and were significantly different ($P > 0.05$) from plant leaves that have been fertilized with NPK fertilizer. Although there were no significant differences ($P < 0.05$) between the AMF, cow dung and control treatments with regard to their level of chlorophyll b, there was significant difference between cow dung manure and NPK treated plants. Result of the study in respect to roots colonization affected by NPK, cow dung manure and control were statistically ($P < 0.05$) similar, and AMF treated plant had the highest percentage (70%). Findings from these experiments indicate AMF could be used as soil amendment since it improved crop leaves chlorophyll and root colonization respectively. Further studies on bio-fertilizer are recommended to characterize the active spores for crop developments.

Keywords: biofertilizer, crop, fertilizer, mycorrhiza, *Zea mays*

Introduction

Mycorrhizal fungi are naturally occurring soil organisms that create associations with most plants [1]. Mycorrhiza, which translates to "fungus-root," refers to the symbiotic relationships between fungi and the living cells in plant roots. Approximately 90% of plants are colonized by endomycorrhizal fungi, while 10% are colonized by ectomycorrhizal fungi. In this relationship, the fungus gets its carbohydrate needs from the plants and, in exchange, takes up minerals including phosphorus, zinc, copper, and potassium and transfers them to the plants via ramifying hyphae [2, 3]. Arbuscular mycorrhizal fungi promote water transport to plant roots by extending their hyphae into areas of deficient soil outside the root hair uptake zone [4]. In addition, the fungus may create a coating around the roots to shield them from infections, which promotes root growth. Arbuscular mycorrhizal and ectomycorrhizal fungi can colonize roots to protect against parasitic nematodes and fungi [5-9].

It has been investigated whether mycorrhizal relationships may be altered to boost crop productivity and forest establishment [10, 11]. It has been suggested that a network of mycorrhizal hyphae supported by trees like *Faidherbia albida* can hasten seedling establishment or promote the growth of shaded understory plants [12, 13]. Arbuscular mycorrhizal fungi are thought to help shape soil structure by connecting soil particles together through their networks of hyphae. However, their significance in the mechanical aggregation of soil particles has been disputed [14, 15].

Mycorrhizal fungi are thought to improve nitrogen fixation and lessen the effects of drought on plants, which will improve crop growth, development, and yields. Arbuscular mycorrhizal fungi have been shown to be more resilient to environmental stress than non-mycorrhizal plants [16, 17]. Many endomycorrhizal fungi (EMF) lack host specificity, which is well recognized [18, 19], despite the fact that their efficacy in identical environmental conditions might vary greatly [20]. The ability of mycorrhizal fungus to promote plant development has been used to measure their efficacy [21]. Between crops and soil, arbuscular mycorrhizal fungi create vital linkages [22].

Maize (*Zea mays* L.) crop is one of the principal cereal food crops in the tropics and sub tropics [23] and forms an essential component of the global food security as a major part of the diet of millions of people including Nigeria [24]. More than 1.2 billion people in Sub-Saharan Africa and Latin America depend heavily on maize as a staple diet, making it the most significant cereal crop in the regions [25]. The entire crop can be utilized to make both food and non-food goods. In developed nations, it is also commonly utilized for industrial raw materials and animal feed, whereas in developing nations, it is mostly used for food and feed [26]. There is an increasing interest in using maize to make ethanol as a replacement for fossil fuels. In Eastern and Southern Africa, 30–50% of low-income households' spending is on maize [26]. Depending on the type, it grows in a larger range of ecological conditions [27]. The crop is adaptable to its environment, has a wide range of uses, and

is eaten by both people and animals over the world [28]. High yielding, stress-tolerant cultivars of maize have been developed to increase productivity.

Despite this production-level intervention, there is evidence of food insecurity brought on by insecurity, ecological and financial factors [24, 29]. With the ever-increasing population in Nigeria, new management techniques must be promoted especially in the semi-arid region in order to improve crop growth for food production while also protecting the land against the persistent desert encroachment. One of these technologies is the use of mycorrhizal fungi, an environmentally beneficial biological fertilizer, to promote crop vegetative development instead of artificial fertilizers, which are ruinous to the environment and are also becoming out of the reach of peasant farmers due to their expensiveness [30]. Under both sterile and non-sterile soil conditions, mycorrhizal inoculation considerably increased cereals' ability to absorb nutrients, especially nitrogen, phosphorus, potassium, calcium, magnesium, sodium, ferric, copper, magnesium, and zinc [3]. It is against this backdrop, the present study, examined the effect of AMF on the chlorophyll and fungal root colonization of maize (as indicators of plant growth) as compared to other fertilizer sources. This is with a view to harnessing its potentials for better maize productivity and ensuring food security.

Materials and Methods

Study Area

The experiment was carried out in the Teaching and Research Farm of the Faculty of Agriculture, University of Maiduguri (Latitude: 11.48° 16.29" North, Longitude: 13.11° 55.11", East and on an altitude 300 meters above the sea level) during a rainy season.

Sample Collection

The Lake Chad Research Institute Maiduguri provided maize seed and fertilizers, and the seeds were checked for viability using a simple floating method. To eradicate surface pollutants, all viable seeds examined were surface sterilized with 5% Sodium hypo-chloride and washed 3 to 4 times with distilled water [31]. The experiment employed decomposed cow dung (organic manure) obtained from the University of Maiduguri Livestock Farm in Dalori, Maiduguri.

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 [32].

Experimental Design and Treatments

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

Fertilizer application

To one group of the plots, 200 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 cereals) was added to the second group of the replicate plots [33]. For the inorganic fertilizer, 0.117Kg N, 0.117Kg P₂O₅, 0.117Kg K₂O/18m² combined with 46 kg/18 m² of urea to give one treatment combinations plus 60 Kgha⁻¹ N, 30 Kgha⁻¹ P₂O₅, 30 Kgha⁻¹ K₂O which is the recommended rate for maize crop was added to the replicate plots. Soil without AMF spores, cow dung and NPK was considered as control treatment [34].

Chlorophyll Concentrations (mg/ml) in Plant Leaves

Total chlorophyll concentrations (mg/ml) in fresh leaves of maize crops used in field experiments were calculated using the Lichtenthaler and Buschmann method [35]. A pestle and mortar was used to grind 100 mg of fresh leaves from the inter-venial area in 10ml of 85% acetone. The suspension was decanted and filtered into a Buchner funnel using a Whatman filter paper No. 1. Using a Spectro-photometer, the optical density (OD) of the solution was measured at 663nm and 665nm to estimate chlorophyll concentrations (UV-1700, Shimadzu, Japan). The concentrations of chlorophyll a, chlorophyll b, and total chlorophyll (a and b) (mg/ml) were estimated using the following formula [36]:

$$\text{Chlorophyll a} = 12.70 (A_{663}) - 2.69 (A_{645}) \times \frac{V}{W \times 1000}$$

$$\text{Chlorophyll b} = 22.90 (A_{645}) - 4.68 (A_{663}) \times \frac{V}{W \times 1000}$$

$$\text{Total chlorophyll level} = 20.20 (OD_{645}) + 8.02 (OD_{663}) \times \frac{V}{W \times 1000}$$

Where A= absorbance

OD = optical Density

V = final volume of the extract

AMF Colonization Percentage (%) in the Plants Roots

Root samples were collected from the maize crop according to the methods of [32]. The rate AMF infestation was determined by the examination of roots with compound microscope using different magnifications (x10 and x40). The magnified inter-section approach was used to determine the degree of root colonization by arbuscular mycorrhizal fungi [37]. Finely stained roots were put on a microscope slide in a drop of glycerol and covered with a cover slip. The microscope field was adjusted using the stage graticule to make complete passes across each slide perpendicular to its long axis. Intersections were scored for any field that cut the vertical eyepiece [38]. The inter-sections were scored positively (+) if they crossed arbuscules or vesicles and negatively (-) if they did not [4]. The percentage (%) of arbuscular mycorrhizal fungi root colonization was calculated using the formula [39]:

$$\text{Percentage of Root colonization} = \frac{\text{No of Colonized segments by AMF}}{\text{Total of Segments examined}} \times 100$$

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.

Results

Chlorophyll a concentration in the leaves of maize crop

The result of the investigations on chlorophyll a revealed that plant leaves treated with organic manure had the highest (0.955mg/g), followed by leaves of plant treated with arbuscular mycorrhizal fungi (0.84mg/g) and that of the control (0.83mg/g). The lowest value (0.63mg/g) obtained was from the leaves fertilized with NPK fertilizer. In compliance of the performance of the treatment's application for maize after flowering showed there was a significant difference ($P>0.05$) among the treatments means. Result revealed that leaves of the maize inoculated with AMF, organic manure and untreated check were statistically ($P<0.05$) the similar in the chlorophyll a and significantly differed ($P>0.05$) from plant leaves that have been fertilized with NPK fertilizer.

Effect on chlorophyll b concentration in the leaves of maize

The result of the effect of arbuscular mycorrhizal fungi, organic manure, NPK fertilizer and untreated check on the chlorophyll b level in the leaves of the field maize obtained in all the treatments showed great efficiency, and were positively reflected in chlorophyll b in the *Z. mays* leaves; although there were no significant statistical differences ($P<0.05$) between the AMF, cow dung and control treatments as shown in Table 1.

Total chlorophyll (a and b) concentrations in maize leaves

Data on the total chlorophyll a and b level in the leaves of maize crop ranged between 1.96 and 2.23mg/g, where leaves of plant fertilized with cow dung had more (2.23mg/g) chlorophyll, followed by leaves of plant inoculated with biofertilizer (2.02 mg/g) and control (2.01mg/g) treatment. Likewise, the lowest value of the total chlorophyll was obtained in the leaves of maize crop fertilized with NPK fertilizer.. There were a significant difference ($P>0.05$) statistically within the treatments in respect to total chlorophyll in the leaves of the maize plant.

Table 1: Effect of AMF, organic and inorganic fertilizers on chlorophyll a, b and total chlorophyll (a and b) concentrations (mg/g fresh leaves) of maize leaves

Maize crop Treatments	Chlorophyll a	Chlorophyll b	Total chlorophyll level
AMF	0.84 ^a	1.18 ^b	2.02 ^b
OM	0.96 ^a	1.28 ^{ab}	2.23 ^a
NPK	0.63 ^b	1.33 ^a	1.96 ^b
CNT	0.83 ^a	1.18 ^b	2.01 ^b
Mean	0.82	1.24	2.06
SE±	0.07	0.04	0.06
LSD	0.20	0.11	0.17

*Means of four replications.

Percentage (%) of Maize Roots Colonization by AMF

Maize plants inoculated with arbuscular mycorrhizal fungi (AMF) had the highest (70%) percentage of roots colonization which was observed in this study and was statistically more than the rest of the treatments as shown in Table 2. Maize plant inoculated with AMF had the highest

percentage of root colonization and was statistically significant ($P>0.05$) than NPK, cow dung and control plants in terms of roots colonization of field experiments (Table 2). Result of the study in respect to roots colonization affected by NPK, cow dung manure and control remained the same statistically ($P<0.05$) as shown in field experiment.

Table 2: Percentage (%) of roots colonization of maize as affected by AMF, NPK and cow dung for field experiment

Treatments	AMF	Cow dung	NPK	Control	Mean	SE ±	LSD _{0.05}
Colonization (%)	70 ^a	0.71 ^b	0.71 ^b	0.71 ^b	19.63	17.32	48.50

*Means of four replications.

Discussion

A complete description of the arbuscular mycorrhizal fungi (AMF) community of a soil would include the identities of fungi present as spores type and intraradical hyphae and vesicles as well as information on the relative abundance of each species in each component of different crops used for the experiments which revealed great interactions among the plants under the impact of bio-fertilizer (AMF), organic and inorganic fertilizers respectively. According to [38], an increase in propagule population can result to increase in the crop biomass (g) in infected mycorrhizal plant than non-mycorrhizal plants. These could have been due to either higher propagule density in the non-sterile soil which led to faster colonization and better nutrition uptake by the plants or that the sterilization process might have released more plant available nutrients (N, P and K) which are capable of

increasing biomass of crops inoculated with arbuscular mycorrhizal fungi than other treatments. It, therefore, suggests that arbuscular mycorrhizal fungi (AMF), which was a cheaper source of nutrient supply in comparison with the NPK fertilizer and cow dung, could serve as best option in enhancing maize chlorophyll contents. This finding agreed with the work of [22] that showed that inoculation of *Pinus vocarpa* and *P. caribea* with mycorrhizal fungi resulted in significant ($P>0.05$) increases in nutrient absorption, root colonization and green pigments over the un-inoculated ones.

The result of the investigations on chlorophyll a revealed that plant leaves treated with organic manure had the highest (0.955mg/g), followed by leaves of plant treated with arbuscular mycorrhizal fungi (0.84mg/g), control (0.83mg/g) leaves and lowest (0.63mg/g) value obtained

from leaves fertilized with NPK fertilizer. High nutrient concentration create some level of decreased soil water level. This might lead to an induced-drought especially that it is in the semi-arid region. It has been shown that drought stress in arid and semi-arid zone significantly decrease chlorophyll concentrations. Reduction of chlorophyll concentrations in drought stress conditions has been reported in maize plant [40]. Dehydration through chlorophyllase and peroxidase enzymes activities in plants lead to chloroplast destruction and chlorophyll reduction [41]. The plants water conditions have important effects on leaves chlorophyll concentrations [42]. Reduction in chlorophyll contents due to drought stress is related to the increase of oxygen radicals in the plant cells [43].

Although there were no clear statistical differences ($P < 0.05$) between the AMF, cow dung and control treatments with regard to their chlorophyll b concentrations, reduction of leaves chlorophyll concentrations in this study was consistent with the outcome of other researchers [44, 45]. The highest rate of chlorophyll contents and percentage of root colonization in maize plant was obtained when inoculated with bio-fertilizer [46]. There were a significant difference ($P > 0.05$) within the treatments in respect to total chlorophyll in the leaves of the maize plant. The outcome of the experiment revealed that there was no significant difference ($P < 0.05$) between leaves treated with bio-fertilizer, NPK fertilizer and those of untreated control.

The findings of this study are contrary to the report of [47] where he indicated that less chlorophyll a are produced by mycorrhizal plants than NPK ones because most of the carbohydrates released by the plants for the purpose of pigment formation, are taken up by the mycorrhizal fungi in the association. This was not surprising given that water and light intensity were vital components of the plant's physiological activities, such as photosynthesis, with the result that the more available it was, the higher the chlorophyll concentration enhancement brought about by this process, in line with work done by [48]. Chlorophyll a absorbs energy mostly from the violet-blue and reddish orange-red wavelengths, with limited absorption from the intermediate (green-yellow-orange) wavelengths, whereas accessory pigments (including chlorophyll b) absorb energy that chlorophyll a does not absorb. Based on my research and the findings of other researchers, plants require more than just chlorophyll a and b [49].

AMF-inoculated maize plants had the highest proportion of root colonization and were statistically significant ($P > 0.05$) compared to NPK, cow dung, and control plants. The study's findings revealed that root colonization was statistically ($P > 0.05$) less impacted by NPK, cow dung manure, and control. According to [50], the majority of the carbohydrate (approximately 1-17%) supplied by plants for the purpose of root production (chlorophyll concentrations and root colonization) was absorbed by the mycorrhizal fungi in the association as absorbing agents (there by taking up the function of root hairs). Bonnie *et al.* [1] discovered that the presence of arbuscular mycorrhizal fungus mycelia greatly inhibits the production of root expansions, branching, and root hairs. As a result, research suggests that arbuscular mycorrhizal fungi (AMF), which were a less expensive source of nutrient supply than NPK fertilizer and cow manure, could be the best alternative for increasing maize development. This discovery was consistent with the findings of [22], who found that inoculating *Pinus vocarpa*

and *P. caribea* with mycorrhizal fungus resulted in significant ($P > 0.05$) increases in nutrient absorption, root colonization, and green pigments compared to un-inoculated plants.

Irfan *et al.* [51] detected AMF or VAMF colonization in roots and proposed that mycorrhizal colonization aids plant growth, root production, root colonization, chlorophyll content, and development. Songachan and Kayang [52] found mycorrhizal colonization in *Solanum khasianum* (38.80%), *Solanum sisymbriifolium* (41.74%), and *Solanum torvum* (36.10%), with differences in arbuscules, vesicles, and hyphae. According to [53], the difference may possibly be related to the formation of additional roots, which enhances absorption of water and minerals (N, P, and K+). Thus, the increases can be attributed to higher mineral nutrition in the infected plants, which clearly resulted in greater physiological processes including photosynthesis and photorespiration. Bababe *et al.* [54] have earlier showed that organic manure application to soils have longer residual effect in terms of nutrient availability on soil fertility.

Conclusion

Arbuscular mycorrhizal fungi absorbs and transports water and nutrients, as well as providing adequate sources for boosting plant growth and performance. In general, the findings of this study demonstrated that AMF can be employed to boost chlorophyll in plant leaves and root colonization thereby reducing stress, nitrogen, and phosphate fertilizer input. However, the manufacturing of inoculants is currently preventing the widespread application of this symbiosis, although a system of inoculation using multi-seed pellets containing *Rhizobium* and mycorrhizal fungi has been advocated. The implementation of this knowledge in large scale application especially in the arid zones becomes critical in order to boost its industrial production and improve their dissemination in order to ensure food security in Borno and Nigeria at large.

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