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The Effect of Inoculation with *Glomus mosseae* on Early Growth and Pattern of Root Exudates in Potted Cowpea [*Vigna unguiculata* (L.) Walp]

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ABSTRACT

Due to the poor state and low fertility value of soil in Sub-Saharan African, this study assessed the effect of mycorrhization, using *Glomus mosseae* on the growth of cowpea. The experimental design was randomized, with four replicates per treatment in four blocks. The effect of treatment on plant growth response data (at p<0.05) was investigated by analysis of variance. There was a favourable and synergistic interaction between mycorrhiza inoculums and successfully colonized plant roots, and *G. mosseae* treatment resulted in enhanced plant development. The effect of mycorrhiza inoculation on the growth of cowpea was significant (p<0.05) when compared with cowpea growth without inoculation. Unsterilized soil inoculated with *G. mosseae* ($M^{+*}S^{-}$) had the highest mycorrhizal colonization. The cowpea grown in inoculated soil presented the best result with plant's height (38%). The growth response observed was in the order of unsterilized soil inoculated with *G. mosseae* ($M^{+*}S^{-}$) > sterilised soil inoculated with *G. mosseae* ($M^{+*}S^{+}$) (26%) > unsterilized soil without *G. mosseae* ($M^{-*}S^{-}$) (24%) > sterilized soil without inoculation with *G. mosseae* ($M^{-*}S^{+}$) (12%). The intensity of fresh weight of the cowpea planted after treatment was also in the order $M^{+}*S^{-}$(35%) > $M^{+}*S^{+}$ (28%) > $M^{-}*S^{-}$ (24%) > $M^{-}*S^{+}$ (20%) and the treatment was significantly different at (p< 0.05). The result showed that inoculation with *G. mosseae* improved growth performance of the plant. Hence, this study confirms the positive effect of mycorrhization using *G. mosseae* in the growth of cowpea.

Keywords: *Arbuscular mycorrhizal*, Green fertilizers, Green and sustainable agriculture, Plant growth response.

Introduction

Cowpea (*Vigna unguiculata* L Walp) is a versatile legume that is produced as human food, cattle fodder, and other uses in semi-arid areas on most continents and a source of revenue.[1] Cowpea is cultivated on roughly 11.3 million hectares across the globe 2013, with Sub-Saharan Africa (SSA) accounting for 70% of total global production.[1] In high-consumption areas like Nigeria, this crop provides a substantial amount of the daily protein needs of most people.[1,2]
Cowpea is a major leguminous crop in Nigeria and other Sub-Saharan African nations that feeds about 200 million people. Nigeria produces around 3.4 million metric tons of cowpea every year, accounting for more than 45% of global production.[3] Nigeria is the world's largest consumer of cowpea grain, with a population of over 200 million people. As a result, enormous quantities of cowpea are imported into Nigeria from surrounding countries, particularly the Republic of Niger, Chad, and Cameroon.
Because of the state of the soil, management techniques, low soil fertility becomes a major barrier for cowpea production, especially amongst Sub-Saharan African smallholder farmers.[4]

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Over the previous 30 years, this has resulted in an annual depletion rate of 22 kg of nitrogen (N), 2.5 kg of phosphorus (P), and 15 kg of potassium (K) per hectare of farmed land in 37 African countries.[5] The traditional method of overcoming nutrient depletion is the use of chemical fertilizers, which are unfortunately too costly for the majorly resource-poor smallholder farmers. This necessitates the use of sustainable agricultural practices such as the incorporation of beneficial soil biota that promotes the uptake of limited soil nutrients such as N and P by plants.
Moreover, the current agriculture that is moving towards ecological intensification relies on strategies like crop rotation, cultural association, and biological control to promote ecological mechanisms. Beneficial microorganisms, such as arbuscular mycorrhizal fungi (AMF), are becoming more widely recognized as one of the most sustainable organic farming approaches.[6] It is well established that the cowpea forms a symbiotic relationship with arbuscular mycorrhizal fungi while the beneficial effect of mycorrhizal symbiosis on plant growth and production has been the subject of several studies.[6-9] It would be possible to increase cowpea productivity by utilizing this relationship. AMF rely on the host plant for photosynthetic carbohydrates and, in exchange, provide a variety of agroecosystem functions such as soil aggregation, nutrient uptake, and carbon sequestration via an extraradical hyphal network that spreads from colonized roots into the soil.[10]
AMF also improves plant resilience to biotic and abiotic stressors, as well as the synthesis of essential plant secondary metabolites, all of which help to produce safe and high-quality food.[11] These symbiotic fungi have also been found to increase nodulation and atmospheric Nitrogen fixation potential in legumes such as cowpea[12] and because the fungus promotes plant P absorption, more energy is available for

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rhizobia to fix nitrogen. AMF may form a tripartite symbiosis with legumes and rhizobia to stimulate nodulation and plant growth.[13] Other extra consequences resulting from this interaction include a increased quantity and dry weight of nodules, improved symbiotic relationship of N fixation and higher N content.[14] The beneficial effect of N_2 fixation by AMF colonization has been thought to be caused by increased P supply to the nodules by the symbiotic fungal partner. The aim of this study is to assess the effect of mycorrhization, using *Glomus mosseae* on the growth of cowpea.

Materials and Methods

Plant, Soil and mycorrhizal sample preparation

The experiment was carried out at the botanical garden of the Ladoke Akintola University of Technology, Ogbomoso, Nigeria (longitude 4°0'11"E and latitude 8°0'05"N). Seeds of the cowpea (*Vigna unguiculata* L. Walp), voucher number TGx-1448 used were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria in May, 2017. The seeds were authenticated by Prof A. T. J. Ogunkunle of Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso. Sandy loam forest sub-soil was collected within the campus of the Ladoke Akintola University of Technology, Ogbomoso with a coordinate (longitude 4°0'05" `E and latitude 8°0'08"N). The soil samples were sieved through 0.5 × 0.5 cm wire mesh to remove unwanted materials. The soil was moistened to its maximum water holding capacity and placed in the oven at a temperature of 140 °C for 1 hour in a metal container. The temperature was reduced to 80 °C after an hour and left for 24 hours to ensure that all resistant nematodes spores and other microbial contaminants were destroyed. The mycorrhizal inoculum used consisted of spores, mycelium and root segments of *G. mosseae.* The inoculum of the AM Fungus *G. mosseae* used was prepared as described by Liasu.[15]

Experimental design

The experiment was a completely randomized design. Arrangement of the treatment was in four blocks randomized design with four replicates per treatment. There were four soil treatments; inoculated (M^+), un-inoculated (M^-), sterilized soil (S^+) and non-sterilized soil (S^-). The experiment was arranged in a factorial combination to give four treatments (i.e. M^+*S^-, M^+*S^+, M^-*S^-, M^-*S^+). *G. mosseae* was applied equally (20 g) at 7-8 cm soil depth in a sterilized inoculated soil and 4 kg unsterilized inoculated soil, respectively. Five seeds were sown in each pot at a depth of 4 cm of soil and trimmed to four after seed germination; these treatments were repeated four times. The crop was harvested 4weeks after planting.

Determination of AMF colonization and plant growth response

The roots of the seedlings were rinsed several times with distilled water after being washed with running tap water. Samples were cut into tiny root segments (about 1 cm) at random and soaked in a 10% KOH solution before being placed in a water bath at 90°C for 30 minutes. After the root segments became transparent, any residual KOH was washed off with distilled water, and the root segments were dyed with 0.05% trypan blue staining solution at 90°C for 30 minutes. Samples were then decolourized with a mixture of lactic acid and glycerine (v/v = 1:1) three times.[16] Samples were observed under a 400× optical microscope (Olympus Bx43, Tokyo, Japan), and the AMF colonization rate was calculated according to the magnifying cross method.[17] Seedling heights were measured using a tape measure and a vernier calliper before harvest. Root, stem, and leaf fresh weights were measured immediately after the harvest, whereas dry weights were determined after drying the biomass at 65°C, until constant weight.[18]

Statistical analysis

The differences between treatments were compared using one-way analysis of variance (ANOVA) and the independent-sample T-test, using the SPSS 21.0 program. Differences among the treatments were examined using the Duncan test at $p < 0.05$. All data are reported as mean of four replicates.

Results and Discussion

Cowpea planted in unsterilized soil with *G. mosseae* ($M^{+*}S^-$) recorded 38% of the total cowpea height, while those grown on sterilized soil with *G. mosseae* ($M^{+*}S^+$) recorded 26% of the total cowpea height. Unsterilized soil without *G. mosseae* ($M^{-*}S^-$) gave 24% of the total cowpea height, while those planted in sterilized soil without *G. mosseae* ($M^{-*}S^+$) gave 12% of the total cowpea height. Cowpea height appreciated better in unsterilized soil with *G. mosseae* than other treatments, but the difference was not significant (P<0.05) until the fourth week after planting (Figure 1). The number of cowpea leaves observed for unsterilized soil with *G. mosseae* ($M^{+*}S^-$) was 33% of the total number of cowpea leaves, while those observed for sterilized soil with *G. mosseae* ($M^{+*}S^+$) was 27% of the total cowpea leaf. The leaf of unsterilized soil without *G. mosseae* ($M^{-*}S^-$) was 24%, while the leaf of sterilized soil without *G. mossea* (M^-*S^+) was 20% of the total number of the leaf (Figure 2). Cowpea fresh weight in unsterilized soils without *G. mosseae* ($M^{+*}S_-$) was 35% of the total cowpea fresh weight, whereas cowpea fresh weight on sterilized soil inoculated with *G. mosseae* ($M^{+*}S^+$) was 28% of total cowpea fresh weight. Fresh weight of unsterilized soil without *G. mosseae* ($M^{-*}S^-$) was 24% of the total fresh weight of the cowpeas, while the fresh weight of sterilized soil without *G. mosseae* ($M^{-*}S^+$) was 20% of the total fresh weight. The difference was significant (p<0.05) only at the 4th week after planting (Figure 3).

Cowpea planted in unsterilized soil supplemented with *G. mosseae* ($M^{+*}S^-$) recorded dry weight which was 32% of the total dry weight, whereas cowpea grown on sterilized soil inoculated with *G. mosseae* ($M^{+*}S^+$) recorded dry weight that was 27% of the total dry weight. Furthermore, the dry weight of cowpea planted in unsterilized soil without *G. mosseae* ($M^{-*}S^-$) was 23% of total dry weight, while those of cowpeas grown on sterilized soil without *G. mosseae* ($M^{-*}S^+$) was 18% of total dry weight. The difference became significant (p<0.05) only at the 4th week after planting (Figure 4). The production of AMF-based inoculants in agriculture is recent, with just a few experiments reporting the elucidation of the effect on the productivity of large crops.[19] The majority of the studies were conducted with AMF propagules that were collected directly from the soil and preserved in pots with host plants.[20,21]

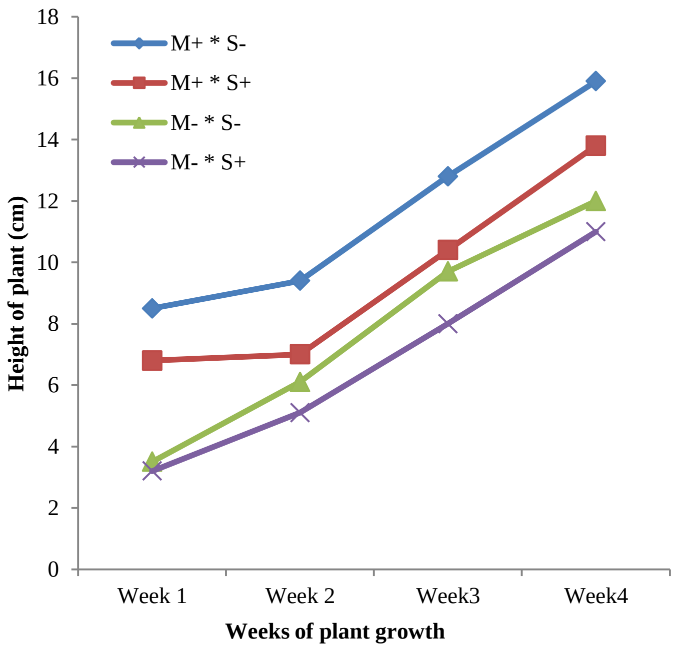

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Figure 1: Effect of *G. mosseae* and soil sterilization on the height of cowpea plant

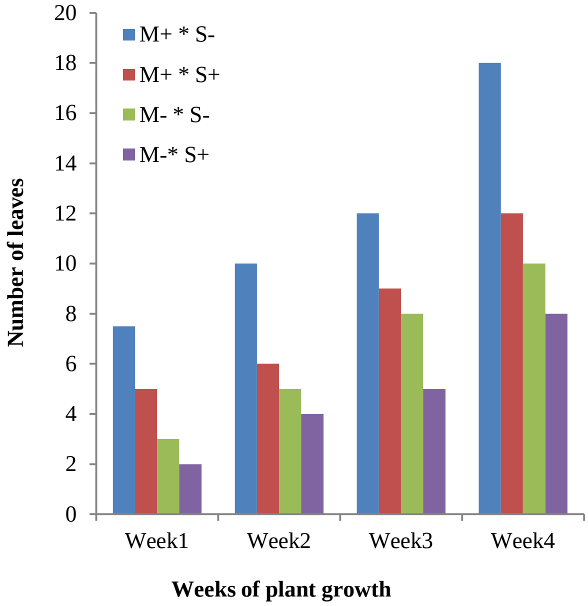

Figure 2: Effect of *G. mosseae* and soil sterilization on the leaf number of Cowpea

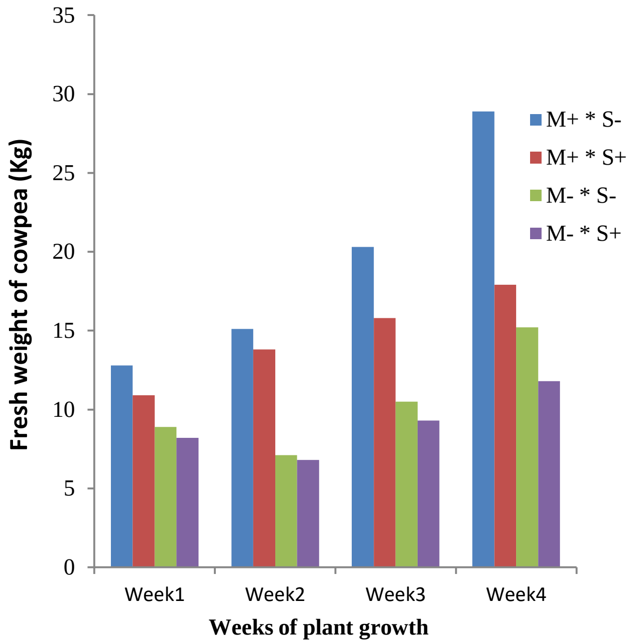

Figure 3: Effect of *G. mosseae* and soil sterilization on the fresh weight of cowpea

Previous research has shown that soil sterilization may reduce mycorrhizal colonization by inhibiting the growth of hyphae in soil and hyphal spreading after initial infection had occurred,[22] reducing the number of arbuscules.[23] This study confirmed the importance of G. mosseae inoculation on cowpea. This is similar to other reports of favourable legume response to AM fungi inoculation both in sterilized and unsterilized soil,[24,25] although mycorrhizal colonization was reduced with soil sterilization, the dependency of cowpea on mycorrhizal fungi was increased, which support symbiosis association between mycorrhizal fungi and cowpea.

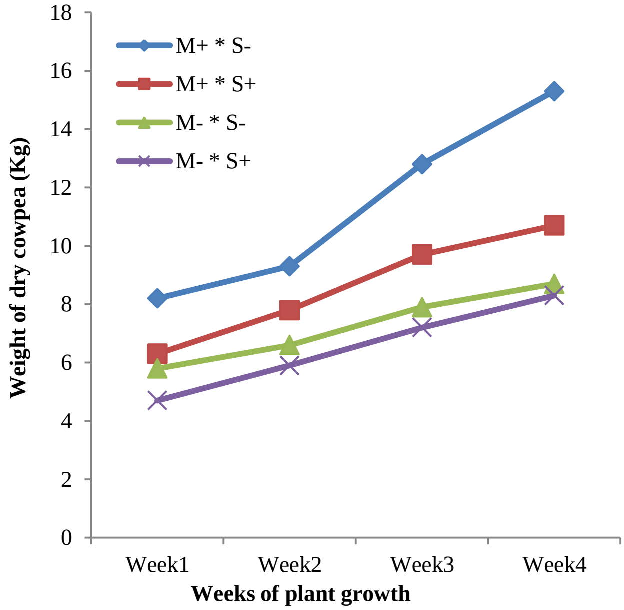

Figure 4: Effect of *G. mosseae* and soil sterilization on cowpea dry weight

In this study, the growth of cowpea was affected positively by mycorrhiza inoculation with G. mosseae which showed the response of plants to mycorrhizal inoculation with AMF ranges from very beneficial to harmful. The greater height recorded in cowpea grown in unsterilized inoculated soil ($M^{+*}S^{-}$) than other treatments ($M^{+*}S^{+}$, $M^{-*}S^{-}$, and $M^{-*}S^{-}$) conforms with the results of Yang et al.[26]
The highest number of leaves per cowpea in the soil inoculated with mycorrhiza corroborates the results of Ortas,[20] on cowpea and cucumber. Other studies have shown the effectiveness of G. mosseae inoculation on cowpea and cucumber.[27] The significantly high fresh weight of cowpea ($M^{+*}S^{-}$), grown on non-sterilized soil shows that Mycorrhiza increases colonization efficiency and these enrich the soil nutrients. The report of Yang et al. also supports the greatest dry weight of cowpea cultivated on non-sterilized soil with G. mosseae ($M^{+*}S^{-}$).

Conclusion

In this study, Arbuscular Mychorrizal Fungi (AMF) improves cowpea production enhancing the uptake of nutrients. AMF inoculation had a positive effect on different cowpea growth parameters including height, leaf number, fresh and dry weight. However, in the non-sterilized soils of this study, a positive relationship has been established between the growth performance of cowpea with the mycorrhizal promotion of plant growth and enables plants to actively regulate the rhizosphere microbial communities.

Conflict of Interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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