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EFFECT OF *GLOMUS FASCICULATUM* ON SEEDLING GROWTH AND POD PRODUCTION OF COWPEA (*Vigna unguiculata L. Walp.)*

[1]Ajiboye A.A, [2]Ajiboye M.D, [1]Iwuala E.N [2]Aigbevbole I.S and [1]Awoyemi B.S
[1]Department of Plant Science and Biotechnology, Federal University OyeEkiti, Ekiti State.
[2]Department of Biological Sciences, Osun State University, Osogbo, Osun State.

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Corresponding author:
abiodun.ajiboye@fuoye.edu.ng

Abstract

Investigation on the effect of Glomus fasciculatum on seedling growth and pod production of cowpea crop was carried out. . The effect of the fungal organisms greatly influenced the fresh shoot, root and dry shoot and root. The G. fasciculatum was also found to greatly improve the seed and pod production of the cowpea, when compared with the untreated seedlings. The use of G. fasciculutum was found to be another alternative source of biofertilizer for yield improvements in cowpea.

1.0 Introduction

Cowpea is an annual herbaceous legume that can reach more than 80 cm in height. Some varieties grow upright, while others have procumbent stems, often tinged with purple, that trail along ground. Large dark green trifoliate leaves provide a good ground cover that helps conserve soil moisture. The plant has a deep taproot with numerous spreading lateral roots that help stabilize the soil [3]. Cowpea is a dicotyledonous plant that belongs to the order *Fabaceae*; the crop is of major importance to the livelihood of people in the tropics. It provides food, animal feed and cash for the rural populace. It is beneficial to farmlands through *in situ* decay of roots, residues and ground cover from its spreading habits [1]. In the humid tropics of some western Nigeria, it is cultivated for its grains, leaves, green pods, Stover and as an anti-erosion crop. Cowpea grain is consumed directly after cooking or as a component of meals made from cereals or root crops.

Cowpea can sufficiently satisfy the tripartite need of providing (i) food for the farmer(ii) fodder for livestock especially during the critical period of the year (dry season) and (iii)fertility replenishment for the soil (through nitrogen fixation) which will ensure sustainable use of the farmer's limited land (for a longer period without much depletion of its nutrient (authority).The protein in cowpea seed is rich in the amino-acids, lysine and tryptophan, compared to cereals grains however, the seed is deficient in methionine and cysteine when compared to animal proteins. Therefore cowpea seed is valued as a nutritional supplement to cereals and an extender of animal proteins [3]. The crop can be used at all stages of growth as a vegetable crop; the tender green leaves are important food source in Africa and prepared as a pot herb, like spinach, immature snapped pods are used in the same way as snap beans, often being mixed with other foods, green cowpea seeds are boiled as a fresh vegetable, or maybe carried or frozen and dry mature seeds are also suitable for boiling and canning. [4]. In many areas of the world, cowpea is the only available high quality legume hay for livestock feed. Digestibility and yield of certain cultivators have been shown to be comparable to alfalfa. Cowpea may be used green or as dry fodder. It is also used as a green manure crop, a nitrogen fixing crop, or for erosion control. Similar to other grain legumes, cowpea contains trypsin inhibitors which limit protein utilization.

2.0 Materials and Methods

2.1 Experimental site: The experiment was carried out beside the general biology laboratory, College of Science, Engineering and Technology of the Osun State University Osogbo, Osun State. This experiment was conducted in the screen house of the Department of Biological Sciences, Osun State University, Osogbo, Osun state.

2.2 Collection of Cowpea seeds: The variety of cowpea named IT99k-377-1 was sourced from the

International Institute of Tropical Agriculture (IITA) Ibadan.

2.3 Collection of Crude Inoculum and soil: Crude inoculums of *Glomusfasciculatum* was collected from IITA, Ibadan. The loamy soil samples were collected in the Tanishe area of Oke-Baale, Osogbo.
The soil was characterized by its blackish color and fine texture. The soil was sterilized by oven drying in a temperature preset to about 90-100 ^{o}C for 12 – 24 hrs. The soil was thereafter allowed to cool at 45 ^{o}C.

2.4 Seed Treatment: About 100 seeds were put in one gram of fungicide and was weighed and dispensed into a conical flask containing the number of seeds to be treated. The conical flask was corked and mixed vigorously until the fungicide coarse with the seeds. The work was carried out in a fume cupboard in order to avoid contamination while face was masked to avoid inhalation of the chemicals.

2.5 Seed viability test: the viability test was carried out to ascertain its germinability. About 9ml of distilled water was placed in a sterile test tube; 1ml of hypochloride was dispensed into the test tube. The solution was autoclave at 121 ^{o}C at 15min.

2.6 Preparation of Potato dextrose agar (PDA):
Exactly 19.5 g of PDA was weighed into a conical flask and 500 ml of distilled water was added while the solution was stirred. The magnetic stirrer was placed into the conical flask and onto the hot plate for vigorous and proper mixing. About 0.4 % of ampiclox was dispensed into the agar; the conical flask was corked and sterilized using autoclave at 121 ^{o}C for 15 mins. The medium was allowed to cool and poured into the petridishes. Preparation of Normal Saline: This was prepared by weighing 4.5 g of NaCl into a beaker of which 500 ml of distilled water was added. The solution was allowed to mix before dispensing into the test tubes.

2.7 Serial Dilution:
This was carried out on the soil samples containing the desired organism. The soil sample was obtained from IITA, Ibadan. Exactly 9 ml of normal saline was dispensed into 3 test tubes, cooked and sterilized by autoclaving at 121 ^{0}C for 15 minutes. A gram of the soil sample was weighed and dispensed into the test tube containing normal saline, thereby making the original dilution. Exactly 1ml of the original dilution was dispensed through 5 ml syringe into another test tube. Another syringe was used to take a ml of the dilution to separate test tube while the soil sample was mixed vigorously.

2.8 Fungi Isolation:
Exactly 1 ml of the diluents was taken using a sterile syringe and placed in a sterile petridish containing the prepared PDA using pour plate method. Plates were observed after 72 hours.
Shortly after, identification of isolate was carried out by colony characterization, plates

2.9 Pure culture of isolate
containing isolate were sub-cultured in PDA in order to get a pure culture by using streak method.

2.10 Experimental design
The work was laid out in 3x3 factorial!
While the untreated seed served as control.

2.11 Planting method:
After six weeks of planting the seeds, the seedlings were inoculated with pure culture of *Glomus fasiculatum* using 5 ml sterile syringe for each pot. The G. *fasiculatum* was placed in the potted seedlings to ensure multiplication of the fresh spores. The plants were harvested after 90 days. The fresh and dry weight of the seedlings was determined by oven dry method pre-set at 80 ^{o}C for 24 hrs.

The overall fresh weight of the untreated seed sample showed slight increase over the treated seeds. Also, the shoot weight of the seedlings was not enhanced by the inoculation when compared with the control of the seedling growth. However, the fresh root, overall dry shoot and root weights were apparently influenced by the versicular arbuscular mychorrhizal (VAM) infestation at the initial stage of the first 30 days of the seedling growth evaluation (Table 1).

3.0 RESULTS
Table 1: Evaluation of seedling growth after 30 days

Vignaunguculata	Overall fresh weight (g)	Fresh shoot weight(g)	Fresh root weight(g)	Overall dry weight(g)	Dry shoot weight(g)	Dry root weight(g)
Treated seeds	11.9^{a}	10.9^{c}	0.8^{b}	1.9^{c}	1.6^{d}	0.3^{c}
Untreated seeds (Control)	12.5^{b}	11.5^{b}	0.6^{c}	1.6^{a}	1.4^{d}	0.2^{b}

Table 2: Evaluation of seedling growth after 60 days

Vignaunguculata	Overall fresh weight(g)	Fresh shoot weight(g)	Fresh root weight(g)	Overall dry weight(g)	Dry shoot weight(g)	Dry root weight(g)
Treated seeds	25.3^{a}	20.2^{b}	5.0^{c}	4.5^{d}	3.0^{a}	1.5^{d}
Untreated seeds (Control)	17.6^{a}	15.0^{b}	2.6^{d}	2.7^{c}	1.8^{d}	0.9^{d}

The inoculation of the *Glomus fasciculatum* greatly influenced the seedling growth of some of the growth parameters within 60 days of the experiment. This is evident in the overall fresh weight, fresh shoot and root weights. The overall dry, shoot and root weights were significantly ($P<0.05$) increased by the inoculation of the VAM when compared with the control (Table 2).

Table 3: Evaluation of seedling growth after 90 days

Vignaunguculata	Overall fresh weight(g)	Fresh shoot weight(g)	Fresh root weight(g)	Overall dry weight(g)	Dry shoot weight(g)	Dry root weight(g)
Treated seeds	28.3^{a}	22.2^{a}	7.0^{d}	4.5^{d}	4.0^{b}	1.6^{d}
Untreated seeds (Control)	19.6^{a}	17.0^{c}	4.6^{d}	3.7^{c}	2.9^{c}	1.9^{d}

The manifestation of the *Glomus fasciculatum* was strongly shown in the seedling evaluation within the 90 days of the experiment. It was observed that the overall fresh weight was greatly influenced by the fungus while the control of the same growth parameter was fairly influenced.

There was significant increase in the evaluation of the seedling growth within the 90 days of the seedling growth on the mycorrhizal treated seedlings than that of the untreated seedlings. Apparently, the determination of the fungus manifestation on the seedlings was evident in the overall dry, shoot and root weights, when compared with that of the un-inoculated seedlings within the time frame of 90 days.

Table 4: Evaluation of Pod production

Vignauguculata	No. of Pods	Pod length (cm)	Pod girth (cm)	Fresh Pod weight(g)	Dry Pod weight(g)	No. of seed per pod	Fresh seed weight(g)	Dry seed weight (g)
Treated Pod	5.0^{a}	13.3^{a}	0.7^{a}	4.0^{d}	0.5^{a}	32.0^{a}	1.3^{d}	0.6^{a}
Untreated Pod (Control)	2.0^{b}	7.6^{a}	0.2^{c}	2.2^{c}	0.1^{d}	20.0^{a}	0.9^{c}	0.2^{d}

The overall assessments of the pod production of the plant growth were also determined. It was observed that the number of pods produced was far higher than that which was produced by the untreated plant. Also, the length, girth, fresh and dry pod weights were greatly increased by the inoculation of the *Glomusfasciculatum*. The number of seeds per pod ranges from 32-50 while the fresh and dry weight seeds were significantly increased in the treated seeds when compared to control.

4.0 Discussion

Cowpea is a major important crop to livelihoods of the people living in the tropics. Subsistence farmers derive food, animal feed and cash along with spill over benefits to their farm lands resulting from the in situ decay of the root leaves and stems. Aside food and animal feed production effects of cowpea, it is also capable of fixing atmospheric nitrogen to the soil through symbiosis with the nodule bacteria such as the *Brady rhizobiumspp*, thereby, increasing the nitrogen content levels in the soil [6].

The research work has shown the significant improvement that the Fungal inoculation had brought to the seedling growth of the cowpea seedlings when compared with the control experiment. The findings revealed the need to use *Glomus* as an alternative growth enhancer for the large production of some important crop like cowpea that is of great importance to the food production of the Nigerian populace. The use of fungal organisms as biotechnological tools to raised bumper harvest for seedling growth of cowpea will further lift the burden of spending too much tax- payers' money on

importation of inorganic fertilizers for farmers; an approach that is not environmentally friendly to the ecosystem of the nation.

These fungal organism include mycorrhizal, binucleate *Rizoctoniaspp*, *Trichodermaspp*, *Piriforma sporakindica*. Many of these organisms have been known for decades as agents of biocontrol of plant diseases; but recent studies by other researchers have demonstrated that they have many other useful attributes. The first step for any of these microorganisms is for them to colonize roots. A fungal invertase is the key to initiation of the mechanism of root colonization. The fungal genome include plant-like sucrose transporters. The presence of sucrose-independent network in fungal cells regulates the symbiotic association.

The research has revealed that the fungal inoculation of *Glomus faciculatum* also greatly enchanced the pod production and also improve the number of seeds produced per pods per plant. Mycorrhizal symbiosis increases the plants uptake of inorganic compounds, such as nitrate and phosphate from soils having low concentrations of these key plant nutrients. The fungal partners may also mediate plant to plant [5].

The process of flowering and fruiting was first observed in the inoculated plants. Mycorrhizal plants reduce cropping time due to earlier flowering and fruiting.

5.0 Conclusion

From the research investigations, it could be deduced that the *Glomus-faciculatum* greatly increased the seedling growth of the cowpea under well watered conditions. The research has further established the use of fungal organisms as alternative source of obtaining the necessary nutrient that will promote cowpea seedling growth and pod production. This development will encourage farmers or the extension officers to use fungal organisms in Nigeria.

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