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SYNERGISTIC EFFECT OF AM FUNGUS AND *RHIZOBIUM* INOCULATION ON *ARACHIS HYPOGEAE L.* IN UNSTERILE SOIL

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ABSTRACT

Arachis hypogeae L. groundnut is a second largest source of edible vegetable oil. Medicinally the oil is used as a laxative and emollient. *Arachis hypogeae* L. seedlings were grown in earthen pots containing unsterilized soil inoculated with *Glomus fasiculatum* , *Rhizobium* or a combination of symbionts. After 90 days growth, plant height, dry weight, root dry weight,mycorrhizal colonization, spore number, percent of yield, nitrogen and phosphorous content were quantified. Plant with either a combination of mycorrhizal fungi and R*hizobium* grew taller and produced higher dry matter of root and shoot than infected with *Rhizobium* species alone or control plants. The presence of *Rhizobium* increased the nitrogen content of above the ground. Foliage number increased with inoculation of mycorrhizal fungi and phosphorus content was increased. In the present study mycorrhizal fungi and *Rhizobium* were found to be synergistic with respect to nitrogen fixation and percent of root colonization.

KEYWORDS:Arachis hypogeae L., Arbuscular mycorrhizal fungi(AMF),Rhizobium, *Glomus fasciculatum,* unsterile soil, percent root colonization, Biomass production.

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INTRODUCTION

The groundnut (*Arachis hypogeae* L.) is belongs to legume "bean" family (Fabaceae). India is one of the largest producers of oilseeds in the world and occupies an important position in the Indian agricultural economy. It is also called 'King' of oil seeds or poor men's cashew nut. It contain 26 percent of protein and 45 percent of oil, used as vegetable oil, medicinally the soil is used a laxative and emollient. Cold pressed ground nut is golden yellow in colour and has a faint agreeable odour. The principal fatty acids are oleic acids 56%, Linolenic and 25% palmitic acid 6-12% and little of each of stearic and Arachidic acids. In recent years there has been great interest in the production mycotoxin from *Aspergillus flavus* link which grows rapidly on most peanut cake.Although man and higher plants live immersed in atmosphere which has 78% nitrogen, they ironically are unable to use that element in its gaseous form. Instead they must rely on other sources to provide them with fixed nitrogen in either in oxidized or reduced form which is suitable for further metabolism. From an agronomic point of view the most important of these organisms are bacteria of the genus *Rhizobium* which live symbiotically within root nodules of leguminous plants (Phillips *etal.*, 1978). Leguminous herbs and shrubs are currently being suggested as nodulation and the resources of vegetable oil (Kochhar, 1998; Lakshman, 2001). Reports dealing with the interaction of AM fungi with *Rhizobium*, N_2 fixers present in the soil are increasing nutrient cycle (Tilak, 1985).Most of the land plants require Arbuscular mycorrhizal fungi(AMF) to achieve maximum growth in nutrient deficient soils by an increased uptake of soil phosphorus (P) (Barea and Azcon-Aguilar, 1983). These fungi traverse soils with their extra radical mycelium (Khanna *et al.,* 1993). This helps in the transport of phosphorus to the host root. As the absorbed phosphorus helps in the energy spending process of N_2 Fixation *Rhizobium*, this association benefits the plants with both P and N Fixation. Beneficial responses to AM fungi inoculation in sterilized in natural soils have also been obtained on French bean (Daft and El-Giahmi, 1976), on Peanut (Krishna and Bagyraj : 1984)This paper reports the growth response of *Arachis hypogeae L.* to AM Fungal inoculation with *Rhizobium* in poly house pot experiments in unsterilized soil.

MATERIALS AND METHODS

Experiments were conducted in earthen pots with unsterile soil, placed in randomized complete design with four replicate per each treatment. All the posts were maintained at Department of Botany, Karnatak University, Dharwad. The used sandy loam with pH 6.9 , Phosphorus deficient (2.6 ppm) available P extracted with NH_4 and HCL) and practically devoid of *rhizobium* nodulation. *Archis hypogeae* L. rhizosphere but had an indigenous AMF spore population 239/ 50g soil. Earthen pots measuring (25×30 cm) in diameter. *Arachis hypogeae* L. seeds were soaked in water for 12 hrs and they were surface sterilized with 2% hypochlorite and germinated in pots containing unsterile soil. The following treatments were adapted.

1. Unsterile soil infested without any inoculation (control)
2. Unsterile soil infested with AM Fungi (*Glomus fasciculatum*)
3. Unsterile soil infested with *Rhizobium species*
4. Unsterile soil infested AM Fungi(*Glomus fasciculatum* and *Rhizobium*).

Rhizobium inoculation was done by treating the seeds with a peat based culture before sowing. Mycorrhizal inoculation was done by placing the seeds over a thin layer of mycorrhizal inoculum (10g mixed inoculum) at the time of sowing. The mycorrhizal inoculation consists of roots and soil from a pot of culture of *Sorghum bicolor*, *Moench. var sudnese* (Sudan grass) which was infected with *Glomus fasciculatum* and grown for four months. The inoculum contained

hyphae, vesicles, chlymadospores of *Glomus fasciculatum*. There were four replicates for each treatment. At two intervals that is 60 and 120 days after sowing, observation on plant height, dry weight of shoot and root, number of leaves, number of nodules per cent root colonization, spore, number per 50g soil, phosphorus content of shoot was determined calorimetrically by the Vanadomolybolate / phosphoric yellow colour method outlined by Jackson (1973). Total nitrogen was determined by the microkjeldahl method (Bremner,1960). Percent of mycorrhizal colonization of root was determined by the root slide technique (Nicolson, 1974) , after clearing the roots with 10% KOH and stained with 0.05% trypan blue in lactophenol (Phillips and Hayman, 1970). Number of *Glomus fasciculatum* spores in the soil roots were determined by the wet sieving and decanting technique of (Gerdemannad Nicolson,1963).

Per cent of root colonization was calculated according to the following formula.

$$\text{Per cent root colonization} = \frac{\text{No of root bits infected with AM fungi}}{\text{No of root bits examined}}$$

RESULTS AND DISCUSSION

The aboveground dry weight produced by *Arachis hypogaea* L. plants was greatest when *Glomus fasciculatum* and *Rhizobium* were present (Table 1). Plants infected with *Glomus fasiculatum* favorable increased plants height compared with plants inoculated with *Rhizobium* and that uninoculated control plants. The presence of both mycorrhizal fungi and N_2 fixing bacteria stimulated the greatest aboveground mean growth however, this growth was not significantly greater than AM fungus inoculated plants. The below ground portion of the plants responded differently to the various treatments tested. The control plants had limited root development, compared with the other treatments. In general, plant height was increased and proportional to the number of leaves. Dual inoculation with both symbionts resulted in significantly P *Rhizobium* but response was not significantly different from plants infected with *Glomus fasciculatum.* Separate inoculations with *Rhizobium* and *Glomus fasciculatum* produced similar responses in plant height and both treatments produced taller than control (Tabled.1). In the present study, the AM fungal colonization was steadly improved with the inoculation are bioinoculants. The dual inoculation brought improved per cent root colonization of Arachis hypogaea L. (Fig.1). It was clearly evident from the data incrased number of nodules were meagar those in plants received only AM fungal inoculation. However the number of nodules significantly increased when both inoculation are given compare to control plants. Inoculation with *Glomus, Rhizobium,* or a combination of *Glomus fasciculatum* and *Rhizobium* produced significantly (P = 0.05) more leaves than uninoculated control plants. Dual inoculation with *Glomus* and *Rhizobium* produced more leaves than the control and *Rhizobium* treatments, but there were no differences between plants inoculated with *Glomus* when compared with plants inoculated just with *Rhizobium.* This response may be a result of very low plant-available P levels in the soil. Phosphorus has been reported by Jacobson (1985) to stimulate root development to a greater extent than shoot. The magnitude of root production by plants inoculated with *Rhizobium*. These plants produced significantly more root biomass than all other treatments (Table-2). This result is supported with other above ground measurements and confirms similar findings (Hayman, 1986). This may be explained by the increased level of plant available nitrogen supplied by the symbiotic bacteria present or the formation of nodules.

The increased level of available N may have caused a proliferation of roots as a result of N concentrating in the root cells and hastening cell division and elongation. Total root production was significantly (P < 05) less when *Glomus* us was present, as compared with *Rhizobium*. Perhaps this response is a result of mycorrhizal fungi increasing the surface area and thus the efficiency of roots to absorb nutrients, therefore reducing the amount of root material required the plant. There is much evidence in the literature that the presence of mycorrhizae deceases the root/shoot ratio by increasing aboveground production and possibly by reducing the heed for below ground production. Daft and El-Giahm(1975) and Crush (1974), reported reduced root: shoot ratios when *Glomus* was present, as compared with *Rhizobium*-inoculated plants. The root: shoot ratios calculated in the present study indicate that ratios decrease with the presence of mycorrhizal fungi (Table.2).The presence of *Glomus* or *Rhizobium* has been shown to have a positive influence on the and N content of infected plants: Mosse1977 and Garbaya, 1991; In the present study, plants *Arachis hypogeae* L. show that *Rhizobium* significantly increased N content and *Glomus influenced* significantly increased P content.

Nitrogen levels in shoot materials were significantly (P > 0.05) higher in *Rhizobium* inoculated plants when compared with plants that wit non infected (control) or infected with *Glomus* The presence of both symbionts increased the N level in the aboveground plant material only slightly over the single inoculation with *Rhizobium*. The response of inoculated plants in accumulating P in shoot material is also recorded. *Rhizobium*-inoculated plants represent, respectively, 21 and 22 increase in P content in above ground foliage. Plants infected with *Glomus* had higher levels of P in than *Rhizobium* inoculated plants. Dual inoculation resulted in the highest P level. It is possible that the response of *Arachis hypogeae* L. in accumulating may be directly related to the mycorrhizal infection level. The mean level of plants inoculated *Glomus* was 52% and dual inoculated plants had 57.2%, a significantly greater infection level (Table 2). Effect of mycorrhiza and Rhizobium on growth of different crop plants have been shown tgo cause an enhaqncement in various growth parameters (barea and Azcon-Angular, 1985; Harris *etal.,*1985). In the present study in Archis hypogaea the effect of AMF and Rhizobium inoculation on number of root nodules, biomass production N and P content as an appreciable increase. However, there was consistent increase in root nodules number in only AMF inoculation plants or Rhizobium alone inoculated plants at 60 to 90 Days. In unsterilized soils, the positive growth responses could in part be due to the low inoculum potential or effectiveness of the introduced mycorrhizal fungi (Laksman and Ratageri, 2005). Dual inoculation of Arbuscular mycorrhizae and *Rhizobium* beneficial to *Vigna umbellata*. has also shown that *Glomus fasiculatum* was very efficient strain at stimulating uptake, plant growth and nodulation of several legumes in many unsterile, of tropical acid soils (Bali and Mukerji, 1991; Joshi 2005). While the principal effect of mycorrhiza may have other secondary effects, possibly of hormonal nature (Mosse, 1977b; Jacobson, 1985).

Table 1

Showing the effect of AM fungus and Rhizobium interaction on Arachis hypogeae L. with regard to Plant height, Dry weight of root and shoot, Nodule number and Nitrogen and Phosphorus of nodules for 90 days.

Treatment	Plant height (cm)	Dry weight root (g)	Dry weight shoot (g)	Nodule number/ plant	% nitrogen in shoot(dry)	Yield % phosphorus in shoot(dry)
Control	17.8 a	5.1 b	0.78 a	_	1.23 a	0.10 a
Rhizobium	36.3 b	7.8 b	1.6 b	2.3 a	1.42 a	0.14 a
Glomus fasciculatum	41.2 c	9.4 c	2.2 b	2.4c	1.33 b	0.215
Glomus +Rhizobium	49.7 c	11.6 b	4.3 c	5.4 b	1.69 c	0.27 c

**** Values are not followedby identical letters in each vertical column are significantly different at P= 0.05 by DMRT***

Table 2

Showing the effect of AM fungus and Rhizobium interaction on Arachis hypogeae L. with regard to No. of leaves, root, shoot ratio, percent of root colonization spore number for 90 days

Treatment	Number of leaves / plant	Root / shoot ratio (%)	Per cent root of colonisation	Spore number 50g soil
Control	**17.8 a**	**10.1 b**	**0.78 a**	**_**
Rhizobium	**36.3 b**	**7.8 b**	**11.6 b**	**12.0a**
Glomus fasciculatum	**41.2 c**	**9.4 c**	**52.2 b**	**98.7c**
Glomus +Rhizobium	**49.7 c**	**9.6 b**	**57.2c**	**103b**

Values are not followed by identical letters in each vertical column are significantly different at P= 0.05 by DMRT

Figure 1

Showing the effect of AM fungus and Rhizobium interaction on Arachis hypogeae L. with regard to Plant height, Nodule number and Nitrogen and Phosphorus of nodules for 90 days.

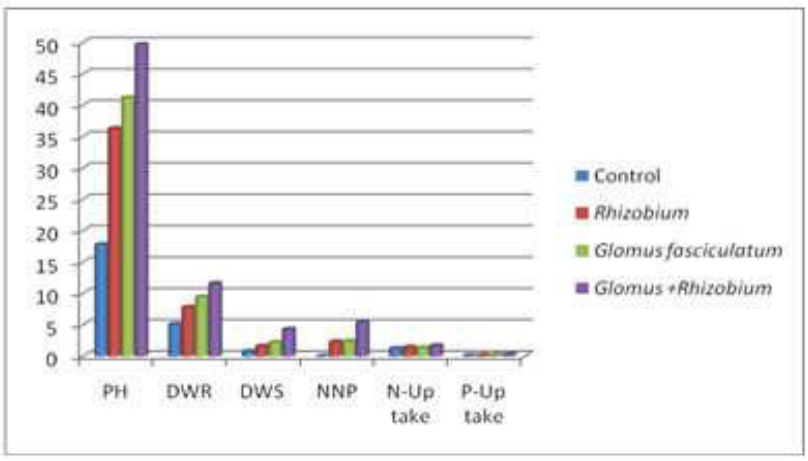
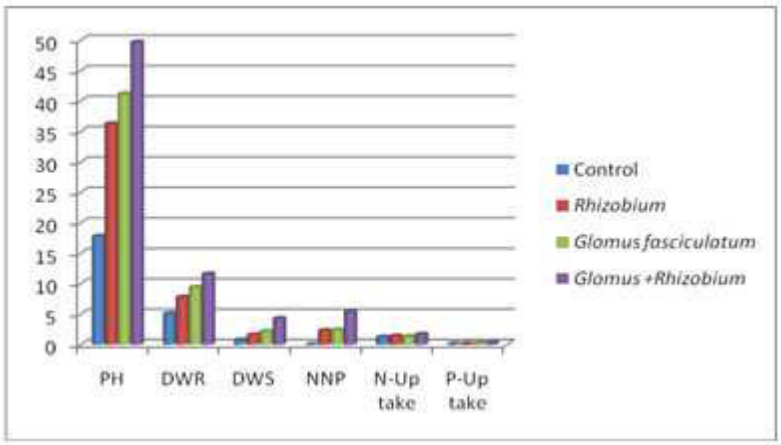


Figure 2
Showing the effect of AM fungus and Rhizobium interaction on Arachis hypogeae L. with regard to No. of leaves, root, shoot ratio, percent of root colonization spore number for 90 days



CONCLUSION

In conclusion the present study brings out that an effective AM fungus *Glomus fasiculatum* with *Rhizobium* inoculation could contribute to the efficiency of such a system on many legumes especially in phosphorous deficient soils, even though native endophytes may be present. Therefore, plants with dual symbiotic associations possess with *Archis hypogaea* L. mutualistic partners Rhizobium and mycorrhizal fungi.

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