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The effect of coinoculation of pea plants with arbuscular mycorrhizal fungi and *rhizobium* on the nodulation, growth and productivity

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Abstract: Soil microorganisms can be used to decrease the input of fertilizers, pesticides and other chemicals. Among soil microorganisms, arbuscular mycorrhizal fungi (AMF) and *Rhizobium* spp. can promote plant growth. Integration of arbuscular mycorrhizal fungus with *Rhizobium* spp. thus appears to be a promising approach for sustainable agriculture. The study evaluated the response of pea (*Pisum sativum*) to AMF species *Glomus fasciculatum* and *Glomus intraradix* and *Rhizobium leguminosarum* bv. *viceae*, regarding the growth, nodulation and yield. Pea plants were grown in pots until the flowering stage (35 days). Five replicates of control, with *Rhizobium* and mycorrhiza alone and the dual inoculation of *Rhizobium* and AMF were maintained during present studies. The obtained results demonstrated that the dual inoculation of pea plants significantly increased the plant growth, nodule biomass and nodule number in comparison with single inoculation with AMF and *Rhizobium leguminosarum* bv. *viceae*.

Key words: Pisum sativum; Glomus fasciculatum; Glomus intraradix; Rhizobium leguminosarum

Introduction

The biotic community of the hyphosphere and rhizosphere of all plants generally comprises bacteria, fungi, actinomycetes and various macroorganisms such as protozoa, nematodes, earthworms, etc. (Edwards *et al.* 1988). These organisms may directly or indirectly influence the growth of plants. However, the interaction of most microorgmisms with plants is restricted to the rhizosphere or the rhizoplane. A few groups of microorganisms Co-inhabit the endorhizosphere and therefore, may have a direct effect on the growth and productivity of associated plant species (Brundett, 1991; Baldani *et al.* 1997; Nehl *et al.* 1996). However, relationships between plants and these strictly endophytic microorganisms are often determined by the interaction effects on the individuals involved in the association (Burkholder, 1952 and Bronstein, 1994). Two groups of microorganisms which are very intimately associated with and influence the growth and nutrition of legume plants are rhizobia and arbuscular mycorrhizal fungi.

Members of the Rhizobiaceae are among a special group of soil bacteria which have the unique ability to convert atmospheric nitrogen into ammonia through the biological nitrogen fixation (BNF) process (Michiels and Vanderleyden, 1994). Rhizobia are mostly associated with legume roots (Geurts and Franssen, 1996: Freiberg *et al.*, 1997). But occasionally found within the endorhizosphere of non-leguminous hosts (Yanni *et al.* 1997). Pacovsky and co-workers (1986) inoculated soybean with *G. fasciculatum* and *Bradyrhizobium japonicum* and found that co-inoculated plants produced significantly higher shoot biomass than uninoculated plats or those inoculated with only *Rhizobium*.

As the information presented in the studies in this area is insufficient, the objective of our study was to evaluate the effectiveness of triple symbioses – pea plants, AM fungi (*Glomus fasciculatum* and *Glomus intraradix*) and *Rhizobium leguminosarum* bv. *viceae* on the growth parameters, nodulation and yield.

Materials and Methods

Isolation of *Rhizobium*:

For the isolation of *Rhizobium*, pink coloured nodules were selected and removed from the root by keeping on either side a short portion of the root attached to it. Pea roots were washed thoroughly with distilled water and dried with paper towel. Nodules from pea plant collected at each field site were selected at random, separated from the roots and placed in a sterilized glass petridish. Nodules were surface-sterilized by immersion in 95% ethanol for 10 seconds, then soaked in a 0.1% mercuric chloride solution for three minutes and washed thoroughly in sterile distilled water. The surface-sterilized nodules were transferred to a clean, sterilized glass petridish and crushed in three millilitres of sterile distilled tap water with a flame-sterilized glass rod. One millilitre of the solution was pipetted out and inoculated on yeast extract mannitol (YEM) agar medium and incubated at 28±°C for 72 hrs. Large

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white and opaque colonies were selected for the inoculation on pea plant. These colonies were used to inoculate yeast extract mannitol (YEM) broth.
Pea plant was used as the test plant for this experiment. The following scheme was used:

1. Control plants (autoclaved soil + pea seeds alone)
2. Plants inoculated with *Rhizobium leguminosarum*; (Autoclaved soil+ pea seeds + *Rhizobium Leguminosarum*)
3. Plants inoculated with AM fungi (Autoclaved soil + pea seeds + AM fungi)
4. Plants inoculated with *Rhizobium leguminosarum* +AM fungi in normal soil

For the control treatment, four pea seedlings were placed at 5-cm depth from the soil surface. For the *Rhizobium* treatment, two millilitres of the *Rhizobium* culture was added on the seedlings placed at 5-cm depth from the soil surface.

For AM treatment, AM spores were inoculated on the pea plants which were placed at 5-cm depth from the soil surface. In a sterile Petri dish, surface seeds of pea were placed on a moist filter paper and seeds were allowed to germinate. When the radicles are about two to three cm, the picked up spores were surface sterilized by 1% streptomycin. Radicles of pea were sterilized by using alcohol. Later with the help of needle, surface sterilized spores were inoculated on the radicle of pea seedling. After three days, the seedlings of pea plants were transferred in a pots containing sterilized soil, these pots were kept in greenhouse.

For AMF-*Rhizobium* treatments, AM spores were inoculated on the four pea seeds and two millilitres of the *Rhizobium* culture was added on the seeds. After 35 days all the growth parameters, nodulation and yield pea plants had been recorded.

Results

It has been reported that the pea plants which were coinoculated with *Rhizobium* and AMF resulted in the statistically highest levels of plant dry mass, nodule number, nodule fresh weight, more seed weight, maximum number of seeds per pod, high seed yield, more number of pods per plant, maximum pod length and pod weight as compared to the those which were inoculated with *Rhizobium* alone. Those which were inoculated with AMF and control plants had shown no nodules in the roots.

Plant dry mass was 0.61±0.12 g in control plants, pea plants inoculated with AM fungi has shown 0.83±1.10 g, with *Rhizobium* alone, it was 1.13±0.08 g and coinoculation showed 1.36±0.86 g of plant dry mass. As nodules were absent in both control and those inoculated with AM fungi alone, no fresh weight was recorded. Number of seeds per pod was less in control plants (3.3±0.32), with AM fungi alone, the number of seeds per pod was 4.0±0.51, with *Rhizobium* alone, it was 4.3±0.87 and combined effect of both *Rhizobium* and AM fungi had shown a higher number seeds per pod i.e. 6.3±0.68 (Table No.1). Seed weight per plant was quite low in control plants (0.20±0.46 g), those inoculated with AM fungi alone, seed weight was relatively higher than control plants (0.32±0.19 g) but lower than those inoculated with *Rhizobium* alone (0.37±0.38 g). However, combined effect of both AM fungi and *Rhizobium* has shown a seed weight of 0.50±0.14 g which was highest among the all. Seed yield per plant was 20 g in control, 38 g in those inoculated with AM fungi alone, 45 g in those inoculated with *Rhizobium* alone and 80 g in those which were coinoculated with both AM fungi and *Rhizobium* (Table No.1).

Table 1: Effect of *Rhizobium* and AMF on growth parameters and yield of pea

Treatment	Plant Dry Mass	Nodule number/plant	Nodule fresh weight(g/plant)	No. of Seeds/pod	Seed weight/plant	Seed yield/plant (g)
Control	0.61±0.12	-	-	3.3±0.32	0.20±0.46	20
AM fungi	0.83±1.10	-	-	4.0±0.51	0.32±0.19	38
Rh.leguminosarum	1.13±0.08	21±0.06	0.81±0.03	4.3±0.87	0.37±0.38	45
Rh.leguminosarum +AM fungi	1.36±0.86	50±0.19	2.43±0.07	6.3±0.68	0.50±0.14	80

It has been found that the plant height was recorded 43±0.36 cm in control plants, with AM fungi, it was 47±0.33 cm, with *Rhizobium* alone it was 53±0.31 cm, and co inoculation of both *Rhizobium* and AM fungi showed highest growth of 61±0.67 cm (Table No. 2).

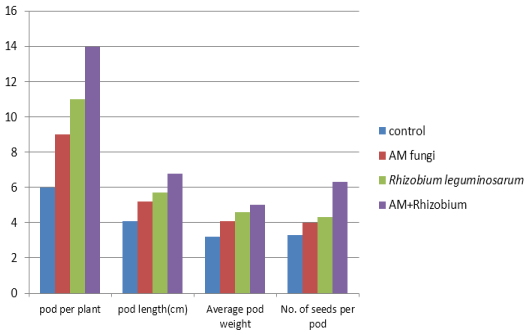
The control plants and pea plants inoculated with AM fungi alone, root nodules were absent, however, those inoculated with *Rhizobium* alone showed root nodules (21±0.06) and coinoculation showed highest number of root nodules (50±0.19). In control, number of pods per plant was very less (6±0.23), with AM fungi alone, it was 9±0.65, with *Rhizobium* alone it was recorded 11±0.67 and co-inoculation showed 14±0.43 pods per plant. Average pod weight was 3.2±0.69 g in control plants, those inoculated with AM fungi alone showed average pod weight of 4.1±0.04 g, with *Rhizobium* alone, the average pod weight was 4.6±0.90 g and those inoculated with both AM fungi and *Rhizobium* showed an average pod weight of 5.0±0.21g. Pod length in control pea plants was 4.1±0.28 cm, those inoculated with AM fungi alone had a pod length of 5.2±0.09 cm, those inoculated with *Rhizobium* alone had a pod length

of 5.7±0.18 cm and coinoculation showed pod length of 6.8±0.39 cm (Table No. 2). All results differ significantly at P≤0.05.

Table 2: Effect of *Rhizobium* and AMF on plant height, number of root nodules, number of pods per plant, average pod weight, pod length and seeds per pod

Plant Part	Control	AM fungi	*Rhizobium leguminosarum*	*Rhizobium* and AM fungi
Plant height(cm)	43±0.36	47±0.33	53±0.31	61±0.67
Root nodules(no.)	-	-	21±0.06	50±0.19
Pod per plant	6±0.23	9±0.65	11±0.67	14±0.43
Average pod weight(g)	3.2±0.69	4.1±0.04	4.6±0.90	5.0±0.21
Pod length(cm)	4.1±0.28	5.2±0.09	5.7±0.18	6.8±0.39

Data are expressed as means ± SE where $n = 4$. Comparison of means was performed by the Fisher LSD test ($P \leq 0.05$) after performing multifactor ANOVA analysis.



Discussion

When *Rhizobium* bacteria are present in the soil, mycorrhizal colonization can also increase the nodulation and symbiotic nitrogen fixation in mycorrhizal legumes (Hamel, 2005). Manjunath *et al.* (1984) inoculated *Leucaena leucocephala* with *G. fasciculatum* and a *Rhizobium* spp. and found that plants co-inoculated with AMF and rhizobia had increased root nodulation, mycorrhizal colonization, dry weight and N and P content compared to plants inoculated with AMF or rhizobia alone. Nodules from root cores of pea plants were picked, counted, washed and weighed to record the following data: (1) Total number of nodules per plant (2) Fresh weight of nodules per plant. The number of nodules on the main and lateral roots was counted, and nodulation was categorized as follows: low 0-20; medium 21-40; and high 40 nodules. Concomitant development of mature functional nodules and effective AM infection depended on microsymbiont species and strains (Saxena *et al.* 1997).

Species of the genus Rhizobium have been found in different crops, such as cotton (McInroy and Kloepper, 1995), maize (Gutie´rrez-Zamora and Martı´nez-Romero, 2001), wheat (Sharma *et al.*, 2005), rape (Chaintreuil *et al.*, 2000), sugar cane (Bellone and Bellone, 2006), carrot (Antoun *et al.*, 1998) and rice (Gutie´rrez-Zamora and Martı´nez-Romero, 2001; Peng *et al.*, 2008; Yanni *et al.,* 1997; Zhang *et al.,* 2011).

The AM fungi associated with legumes are responsible for adequate P nutrition. Increased P assimilation influence positively nitrogenase activity that in turn promotes root and mycorrhizal growth. The conducive effect of dual inoculation of roots with AM fungi and *Rhizobium* on growth, nutrient uptake and N2 fixation in soybean (Bethlenfalvay *et al.,* 1990), cowpea (Islam *et al.,* 1990), and pea (Xavier and Germida, 2003) has been established. Olivera *et al.,* (2004) reported that P application increased leaf area, plant dry biomass, nodule biomass, and shoot and root P content in common bean plants.

Jia and Gray (2004) reported that inoculation with AM fungi promoted biomass production and influenced positively photosynthetic rate in *Vicia faba* due to the enhanced P supply resulting from AM inoculation. Studies with several legumes have consistently shown a positive response to P application plant dry weight and N content were found to increase (Pereira and Bliss, 1987).

Walley *et al.,* (2013) studied that the mixed species AMF inoculants performed better than the single species. *G. irregulare* alone by promoting mycorrhizal colonization, field pea biomass, N and P uptake, and N2 fixation and did not result in a significant compositional change of the AMF community that subsequently assembled in field pea roots. In contrast, the single species *G. irregulare* inoculants did not significantly enhance field pea biomass, N and P uptake, and N2 fixation; although a significant compositional change of the subsequent AMF community was observed.

From the above discussion, it has been found that pea plants coinoculated with *Rhizobium leguminosarum* and AMF has shown best results regarding plant height, plant dry mass, nodule fresh weight, number of seeds, seed weight, seed yield, number of root nodules, number of pods per plant, average pod weight and pod length over single inoculation with AMF and *Rhizobium leguminosarum*. However, control plants have shown least variation in all the growth parameters and yield.

Conclusions

Our data indicates that those pea plants which were grown under the combined effect of AMF and *Rhizobium* had shown marked increase in number of pods per plant, pod length, average pod weight and number of seeds. Control plants and the plants inoculated with AM fungi did not show any nodules in the roots. However, plants inoculated with *Rhizobium leguminosarum* alone and those inoculated with both AM fungi and *Rhizobium leguminosarum* had shown root nodules but the number of nodules and nodule weight was more in those which were coinoculated with both AM fungi and *Rhizobium.*

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