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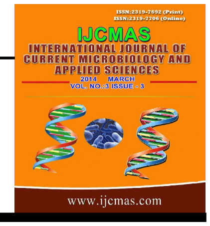

Original Research Article

Effect of AM fungi and Trichoderma species as stimulations of growth and morphological character of chilli (*Capsicum annuum* .L)

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A B S T R A C T

In the present investigation deals with the beneficial effect of *Glomus mosease*, with *Trichoderma harzianum* under pot experiment. In double inoculated chilli plant showed significant increase in plant growth parameters like number of shoot length, root length dry weight of shoot and root, number of leaves, number of branching and number of spores as compared to control. The overall plant growth of chilli plants cultivated in greenhouse conditions along with inoculation treatments of beneficial fungi species was observed. Mycorrhizal granules (consisting of both *Glomus. mosseae &Trichoderma harzianum*) and hydroponically grown on soils for six weeks. These findings are consistent with other studies displaying a synergistic effect between various species of fungi. Compatible combinations of various species of *AM* and *Trichoderma*, which result in cropping systems that fully utilize *AM* and *Trichoderma* symbiosis, are the most efficient, sustainable, and environmentally sound large scale methods for food production. Further study of synergistic affects of *AMF* and *Trichoderma* on plant growth, and should be conducted to ensure fungal species are compatible with each other and are compatible for a given a given crop. The results reveal that mixed Mycorrhizal and Trichoderma species inoculation contribute best growth and development of *chilli* (*Capsicum annuum L*) plant under pot experiment.

Keywords

Glomus mosseae; Trichoderma harzianum;, Capsicum annuum L.

Introduction

Arbusculars mycorrhizal fungi *(AMF)* improve plant growth profoundly through increased of phosphorus nitrogen and other nutrients. Arbuscular mycorrhizal fungi (*AMF*) play vital roles in plant product in agro ecosystem. Mycorrhizal colonization of roots results in an increase in root surface area for nutrient acquisition. The extrametrical fungal hyphae can extend several centimeters into the soil and absorb large amounts of nutrients for the host root (Khan *et al.*, 2000). The Arbuscular mycorrhizal (*AM)* symbiosis is the most common occurred underground symbiosis in plants. About 80% of plants are colonized by Arbuscular mycorrhizal fungi (*AMF)* (Hildebrandt et al., 2002) in the present study, there is well-documented evidence that AM fungi contribute to increasing availability and uptake of P and micronutrients (Krishna and Bagyaraj, 1991). The fine roots that perform most of the uptake process are symbiotically associated with fungi which improve nutrient uptake, drought and frost tolerance and protect higher plants against pathogens. The fungi withdraw glucose from plant roots and act as a significant sink for carbohydrates (Kottke, 2002). As a rule the fungus is strongly or wholly dependent on the higher plant, whereas the plant may or may not benefit (Marschner, 2002). *AMF* on their host plant is an increase in plant growth and nutrient uptake (Ortas *et al*., 2001).

Trichoderma harzianum is a saprophytic fungus which is used generally as a biological control agent against a wide range of economically important aerial and soil borne plant pathogens (Papavizas, 1985). The increased growth response of plants caused by *T.harizanum* depends on the ability of the fungus to survive and develop in the rhizosphere (Kleifield and Chet 1992). A possible mechanism for increased plant growth is an increase in nutrient transfer from soil to root, which is supported by the fact that *Trichoderma* can colonize the interior of roots (Kleifield and Chet 1992). Several mechanisms, by which *Trichoderma spp.* influences plant development were suggested, such as production of growth hormones (Windham et al., 1986), solubilization of insoluble minor nutrients in soil (Altomare et al., 1999) and increased uptake and translocation of less-available minerals (Baker, 1989; Growth stimulation is evidenced by increases in biomass, productivity, stress resistance and increased nutrient absorption (Hoyos-Carvajal,2009). The plant benefits by the presence of *Trichoderma spp.*, suggesting an interaction as a virulent Symbionts (Howell et al. 2000; Harman et al., 2004; Yedidia et al., 1999, 2000). *Trichoderma spp.* can also produce metabolites with activities analogous to plant hormones (Cutler et al., 1989, 1991).

Chilli (*Capsicum annuum L.)* which belongs to Solananceae is known as a vegetable and consumed both as fresh and dehydrated species (Bosland and Vostava, 2000).Pepper is good source of vitamins A, C, E, B_1 and B_2 potassium, phosphors and calcium. They can increase plant uptake of nutrients especially relatively immobile elements such as P, Zn and Cu (Ryan & Angus, 2003) and consequently, increase root and shoot biomass and improve plant growth. Vegetable crops that require a nursery stage can benefit from *AMF* inoculation, thus its use has been incorporated into horticultural practices (Evans, 1997). In the life of chilli plant, there are many challenges within the environment to overcome; these include infection us diseases from bacteria, fungus, and viruses, or other physical damage from insects that continuously feed on the foliage. Another major problem for tomato plants is the availability of essential nutrients necessary for proper plant growth and development of reproductive organs such as the flowers and fruit. These deficiencies can not only be caused by the lack of nutrients in the media, but also if the nutrients needed for plant growth exceeds the roots ability to uptake nutrients that are available when rapid growth is occurring.

It has been known for a long time that beneficial microorganisms within the soil form symbiotic relationships with the roots of plants, which in turn, enhance the uptake of essential nutrients by plants. This enhancement allows the nutrients already in the soil to become more available for plant use, reducing the need to add extra fertilizers or chemicals, and also assist in preventing, not correcting, deficiency problems. These microorganisms also have the ability to uptake water, as they act as extensions of the roots. Currently, there are a few companies that manufacture beneficial microorganisms, so any homeowner or commercial grower has easy access to these products. The main species of beneficial microorganisms produced are species of *Trichoderma* and Mycorrhizal fungi, and both can be used in soil or soilless media. This is important, as the tomato industry not only grows in the field, but also in greenhouses and using soilless media such as Rockwool. Taken as a whole, the benefits of improved nutrient uptake can be observed by comparing overall height, fresh weight and dry weight of plants of various treatments. The purpose of this experiment is to evaluate the overall growth of *chilli var*. (Indira F_1 Hybrid) plants inoculated with various species of *Trichoderma spp* and *AM fungi.*

Materials and Methods

Chilli seed preparations

Chilli from *var*.Indira F_1 Hybrid (*capsicum annuum L)* was supplied by *vegetable and fruit research station, Periyakulam, Tamil nadu, India*. Chilly seeds were surface sterilized with 0.05% sodium hypo chloride for 45 min before.

Mycorrhizal Inoculation

Arbuscular mycorrhizal fungi (AMF) (*Glomus mosseae)* (Thaxter Gerdemann and Trappe emend. Sterilized earthen pots of 30cm diameter maintained with sterilized sand and soil borne fungi like; *Trichoderma harzianum* were procured from Department of Agricultural Microbiology, Annamalai University. Double sterilized soil was used for experiments and seeds of (*Capsicum annuum L)* plants were shown in the other soil fungi in different combinations. The experiment was conducted of five treatments with sixth replicates. Treatments were as follows.

1. No mycorrhizal fungi (control)
2. Mycorrhizal fungi with (*G. mosseae*)
3. Mycorrhizal fungi with (*T. harzianum*)
4. Mycorrhizal fungi with (*G.mosseae +T.harizanum*)

The pots were watered early in the morning every alternative day. Fresh and dry weight, number and size of leaf, root and shoot length, size number of branching were recorded on 46 days of showing. The roots were cleared and stained using Phillips and Hayman (1970) technique. Root colonization was measured according to the Giovannetti and Mosse (1980) method. Hindered gram rhizosphere soils samples were harvest (shoot and root) oven dried at 70°c for 48 hours. After the completion of data recording, the dried shoot parts were kept in paper bag covered with aluminum foil and stored for nitrogen and phosphorus estimation in shoot which was done by following the same method as described for the soil nutrient analysis. Until a constant weight was obtained to determine the dry weight. Roots were washed carefully in tap water and placed in a test-tube containing 2.5% KOH solution (w/v). Roots were then heated at 90°C in a water bath for 30 min (Koske and Gemma, 1989).Cooled root samples were washed several times with tap water and were acidified in 1% HCl solution for 30 min and stained in an acidic glycerol solution (500 ml H2O, 50 ml 1% HCL) containing 0.05% tryphan blue (Phillips and Hayman, 1970).

Statistical analysis

The data were subjected to analysis of variance (ANOVA) the statistical systems (SAS 6.12) package. Comparison of multiple means was performed using the least significant difference (LSD) test at the 5%.

Results and Discussion

Arbuscular mycorrhizal Fungi are the most widespread root fungal symbiotic and are associated with the vast majority of higher plants. *AMF* have been shown to improve soil structure (Miller and Jastrow, 2000) and have great importance due to their great capability to increase the plant growth and yield through efficient nutrient uptake (Smith and Read, 1997).The result of plant growth parameters are given in table-1 and the plate. *Capsicum annuum L.* plants inoculated with Arbuscular mycorrhizal fungi of single inoculation *G. mosseae, T.harizanum,* or double inoculation *((T.h+G.m*) increased growth parameters viz. number of leaf, size of leaf d, shoot length, number of branching, root length, fresh root weight, dry root weight, fresh shoot weight, dry shoot weight, and spores number as compared to control. The percent of root colonization were maximum in treatment of plants with inoculation *G.mosseae + T.harizanum (*19.42%) followed by single inoculation. G.mosseae (15.92%) and *T.harizanum* (16.26%) and was followed by other treatments and control (12.58%).Evidently, there was no positive correlation between spore numbers and the level of percent root colonization. Uptake of certain minerals, such as P and N, is of key importance considering their role in plant growth (Johansen, 1999; Kim et al., 1997). Promotion of growth and yield by *Trichoderma* spp. may also be a result of increased root area allowing the roots to explore larger volumes of soil to access nutrients, and increased solubility of insoluble compounds as well as increased availability of micronutrients (Altomare et al., 1999; Yedidia et al., 2001). However, initially *Trichoderma* must be able to establish an interaction with the root system. The ability of a *Trichoderma* species to colonize the root system of a plant depends also on the plant species. The lower percent root colonization might be associated with reduction of growth in control treatment as observed in the present study. Number of spores was maximum in treatment of plants with double inoculums *T.harzinum* + *G.mosseae* (30.173) spores /50 gm of rhizosphere soil followed by *G. mosease* 27.11spores/50gm,T.harzinum (25.99) spores/50gm of rhizosphere soil respectively. Showed maximum root colonization with growth parameters viz. Number of leaf (15.630**)** size of leaf (2.950 mm),shoot length (4.190 cm), number of branching (5.133),root length (4.656 cm), fresh shoot weight (5.166gm) fresh root weight (5.313 gm), dry shoot weight(4.690gm) dry root weight (4.490gm) as compared to control. In the present study, root colonization was maximum in treatment of *Capsicum annuum L.* plants with *T.harizanum G. mosseae.* Evidently, this also positive correlation between *AM fungi* spore numbers and the level of root colonization.

Moreover, *AMF* were shown to have beneficial effects on the CO_2 assimilation rate (Amerian*et al.* 2001), which results in

Table.1 The effect of Arbusculars mycorrhizal fungi (*AMF)* with *Trichoderma spp*. Growth and biomass of (*Capsicum annuum L.)* Plant

Treatments	**Control**	***G.mosseae***	***T.harizanum***	***G.mossease+T.harizanum***
Shoot Length (cm)	1.713±0.15	1.95±0.14	1.93±0.04	1.99±0.20
Root Length (cm)	1.53±0.10	1.64±0.08	1.60±0.01	1.68±0.08
Fresh shoot weight (gm)	2.65±0.20	2.73±0.08	2.72±0.20	2.83±0.21
Fresh root weight (gm)	2.63±0.21	2.69±0.30	2.68±0.32	2.71±0.31
Dry shoot weight (gm)	1.42±0.02	1.46±0.10	1.46±0.09	1.55±0.32
Dry root weight (gm)	1.36±0.12	1.44±.023	1.42±0.23	1.47±0.25
No. of Leaf	7.33±0.03	8.60±0.04	8.57±0.12	9.24±0.32
Size of Leaf	1.86±0.12	1.90±0.12	1.87±0.21	2.12±0.23
No.of Branching	1.66±0.04	2.66±0.30	2.56±0.12	2.60±0.24
No. of spores.	12.50±0.60	18.00±0.10	16.23±0.04	20.17±0.26

Figure.A,B Showing morphological character of Chilli (Capsicum annuum L.)

Fig. A

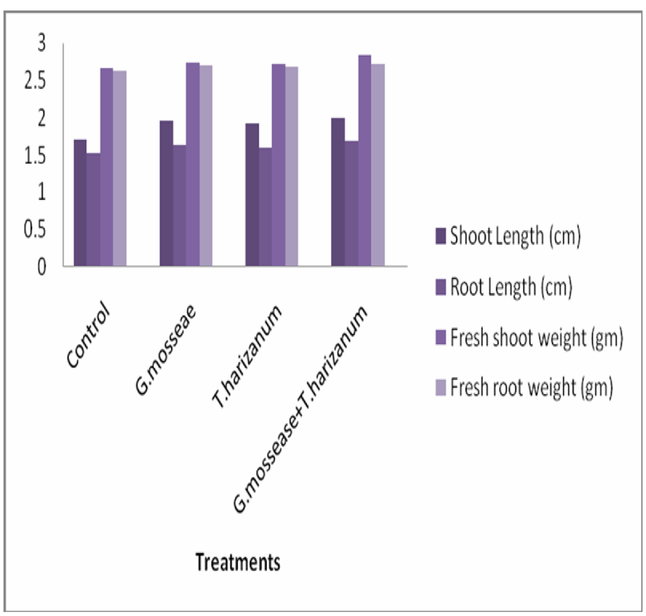
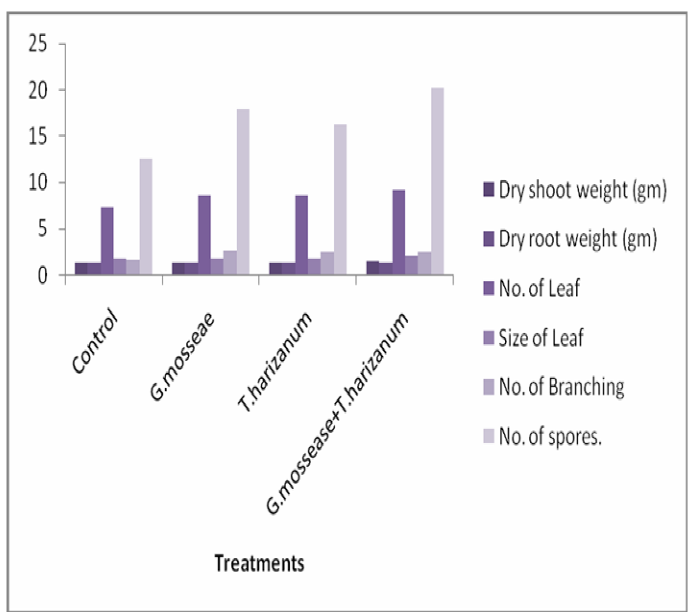


Fig. B



improved plant growth. *Glomus mosseae* achieved greater root colonization than the other AM fungus we used, an important feature for a fungus to be introduced for the control of root parasites (Suslow 1982) and presumably the reason why *G. mosseae* improved growth more than *G. margarita*. Amerian et al. (2001) also reported that inoculation with *G. mosseae* resulted in higher CO_2 assimilation rate in plant.Trotta et al. (1996) reported that the *AM fungus, Glomus mosseae,* reduced adventitious root necrosis and necrotic root apices caused by Phytophthora nicotianae var. Parasitica by 63–89%.. Root colonization by AM fungi was directly related to nutrients uptake by plants (Parkash, 2004). The results on increase in growth parameters due to synergistic interactions among, microbial inoculants have been reported by Jayanthi et al., (2003) Vanitha et al., (2005) reported significant increase in plant height, number of leaves and number of branches, fresh weight and dry weight in Osmium kilimandscharicum on inoculation with *Glomus fasciculatum* compared to non mycorrhizal plants. It has been found that higher root colonization at the time of sampling may be attributed to high number of secondary *AMF* roots and its density which favor's active mycorrhizal colonization which resulted in better growth of plant in terms of height and biomass (Mosse*et al*., 1976). Larger pots were used for the mass production of and perhaps larger pots provide better soil aeration and root oxygen and influence *AM* sporulation. Cordiki *et al.* (2004) found that levels of colonization varied depending upon the inoculums as well as the composition of growth medium. Tanwar et al., (2010) reported levels in triple combinations of *G.mosseae + A. laevis + T. harzianum* were tested for their ability increase yield biomass and establishment of the tomato (*Lycopersican esculentum mill*) seedlings in pot cultures. This beneficial effect of endomychorriza on horticultural crops was also reported by Aguilera-Gómez *et al*. (1999) in peppers inoculated with *G. intraradices*, obtaining a higher number of leaves, foliar area, shoot and yield. The role of *AMF* inoculation in agriculture may be expressed not only by the acquisition of nutrients by host plant, but also by reducing the use of applied fertilizers and pesticides.

AMF have the ability to naturally increase water uptake, which in turn can lead to increased plant growth, yield, and still have moderate biocontrol affects on pathogens. While *Trichoderma* fungi greatly reduces infections from pathogens, but only has moderate increase in plant water uptake, growth, and yield, for the best results, a compatible combination of various species of *AMF* and *Trichoderma* fungi should be the considered. *Trichoderma* spp. can be a determining factor impacting on the microbial community in the rhizosphere to enhance or even inhibit plant growth, and occasionally establishing a positive interaction within plant roots as an entophytes. The latter association may be the most predictive for the selection of specific strains that can be used as bio inoculants to improve crop health and productivity. Direct plant-fungi interactions induce changes in both the fungus and plant transcriptomes affecting genes that regulate plant physiology including growth and plant *AMF (Glomus. mosseae, Glomus fasculatum,* and *Trichoderma harzianum*) are an essential component of natural soil-plant. The presence of mycorrhizal fungi had different influence on the population of *G. mosseae G. fasciculatum* and *was* able to stimulate the introduced beneficial bacterial growth and mycorrhizal dependency in the rhizosphere soil. Thus, the results in the present experiment showed that not all the combinations of hosts and entophytes have similar growth stimulating effects. Our result clearly suggests that in Indira F_1 Hybrid. var. Single and double inoculation of *AMF* showed better vegetative growth of all the plants while triple inoculation of both G. mosseae species *G. fasciculatum*, with *Trichoderma harzianum* showed the most promising and synergistic effects on vegetative growth of chilli plants. Advances in molecular biology techniques have made it possible to develop diagnostic tools to estimate potential contribution of microorganisms in a soil to a given crop. This routine testing of microorganisms' potential will lead to more accurate fertilizer recommendations and the safe reduction of fertilizer inputs, which will translate into reduced chemical seepage into the environment. Further study of synergistic affects of *AMF* and *Trichoderma* on plant growth, and should be conducted to ensure fungal species are compatible with each other and are compatible for a given a given crop.

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