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# Co-inoculation effect of *Glomus macrocarpum* and fluorescent pseudomonads on the population density of mycorrhiza in chilli rhizosphere

**Ramesha V and Dr. VP Savalgi**

**Abstract**
In plant mycorrhizal association, AMF has been shown to be beneficial to the host plant by increasing nutrient uptake particularly N, P, K. Certain plant growth promoting rhizomicroorganisms has been reported to enhance the activity of AM fungi and consequently plant growth. Hence an experiment was conducted in a green house study with plant growth promoting Pseudomonas and AM fungi by using byadagi dabbi variety of chilli. The enhanced activity by rhizosphere microbial population such as phosphate solubilizers and nitrogen fixers in rhizosphere soil was observed. The fluorescent pseudomonads and AM fungi are known to produce certain plant metabolites, plant growth promoting substances, vitamins. The mutual interactions of inoculated organisms was responsible for the increased activity of microflora and enzyme activity in rhizosphere. However, among the treatments the highest spore count was observed in the treatment which inoculated with *Glomus macrocarpum* and Fluorescent pseudomonads supplied with 50% rock phosphate at flowering and harvesting respectively.

**Keywords:** A.M Fungi: Arbuscular mycorrhizal fungi

## Introduction
Chilli (*Capsicum annum* L.) is the fruit plant from the genus *Capsicum*, family Solanaceae, originated in the Central America. It appears pink after ripening due to the pigment capsanthin. It is a rich source of ascorbic acid. Chilli extracts are used in a wide range of medicines against tonsillitis, diptheria, loss of appetite, flatulence, intermittent fever, rheumatism, sore throat, swelling and hardened tumors (De, 1992) [6].

India is the largest cultivator and producers of chillies in the world. In India, the area under chilli cultivation during 2001-02 was 9.4 lakh ha and the production was 10.30 lakh tonnes.

Capsaicin, the pungent principle in chilli is also used in several pain killers. Karnataka state stands second in area (1.90 lakh ha) and production (1.21 lakh tonnes) and eleventh in productivity (639 kg/ha) (Anonymous, 1999) [1]. Generally, solanaceous vegetables require a large quantity of major nutrients like nitrogen, phosphorus and potassium for better growth, fruit and seed yield. The cost of inorganic fertilizers is increasing enormously to an extent that they are out of reach of small and marginal farmers.

And by using inorganic fertilizers and insecticides, the population of beneficial organisms decrease and natural regeneration of nutrition in the soil cease. Later soil becomes barren and soil fertility decreases. Hence Use of fermented liquid manures in such situation is practically advantageous. In these liquid manures, beneficial organisms survive and are helpful in phosphate solubilization, nitrogen fixation etc.

Phosphorus (P) is one of the most important element for plant growth and metabolism. It plays key role in many plant processes such as energy metabolism, the synthesis of nucleic acids and membranes, photosynthesis, respiration, nitrogen fixation and enzyme regulation (Raj *et al.*, 1981) [11]. Adequate phosphorus nutrition enhances many aspects of plant development including flowering, fruiting and root growth.

Arbuscular mycorrhizal association is of great economic significance on growth of agricultural crops (Bagyaraj, 1984) [3]. which Improved plant growth was attributed to increased nutrient uptake especially phosphorus, tolerance to water stress and pathogens and adverse soil environments, production of growth promoting substances and synergetic interactions with other beneficial soil microorganisms (Azcon, 1989) [2].

Davies *et al.* (1992) [5] reported that the roots of chilli normally form a symbiotic association with AMF. In mycorrhizal association, AMF had been shown to be beneficial to the host plant by increasing nutrient uptake particularly N, P, K and micro nutrients (Perner *et al.*, 2007) [10].

Edwards *et al.* (1998) [7] reported that the presence of *G. mosseae* increased the population of

*P. fluorescens* in the rhizosphere of tomato *(L. esculentum*) and leek (*A. porrum),* although the bacterium had no effect on the AM fungus. *P. fluorescens* performed better in terms of improving growth of tomato and leek when the bacterium was co-inoculated with *Glomus mosseae.* However, another plant growth-promoting bacterium, *P.putida,* which is taxonomically closely related to *P. fluorescens,* increased colonisation of roots by AM fungi (Meyer and Linderman 1986; Gryndler and Vosátka 1996) [9].

The plant-growth-promoting rhizobacteria (PGPR) can influence growth of hyphae from germinating Arbuscular mycorrhizal spores, colonisation of plant roots by AM fungi and growth of external AM hyphae and dehydrogenase activity of the AM fungus (Burla *et al.,* 1996).

Certain plant growth promoting rhizomicroorganisms had been reported to enhance the activity of AM fungi and consequently plant growth. Present investigations was undertaken to study the coinoculation effect of AM fungi and fluroscent pseudomonas on rhizosphere spore count and percent root colonization of mycorrgiza and growth of chilli.

## Materials and Methods

### Arbuscular Mycorrhizal fungal population assessment in soils

### Spore extraction and enumeration

The soil samples collected from each treatment pot from were sieved through a 2 mm sieve to remove large debris. The air dried sub sample (100 g) was taken from each sample and placed in a 500 ml beaker containing 200 ml 0.08 M sodium hexametaphosphate solution to break up clay clumps. The suspension was agitated for 5 mins and left to settle for 15 secs (Smith and Dickson, 1997) [12]. The supernatant was decanted through sieves with reducing mesh sizes from 425 µm, 250 µm, 125 µm to 45 µm. This step was repeated with water twice and the debris from the 425 µm was discarded (Smith and Dickson, 1997) [12].

### Spore enumeration

The Spores from lower sieve were then washed onto a 9 cm grided filter paper disc. The filter paper was transferred to clean Petri dish lids and enumerated. AM fungal spore enumeration included both dead and viable spores, although every attempt was made to count only healthy looking spores. Spores were recorded as representatives of AM fungal species present in 100g of sample (Smith and Dickson, 1997) [12]. This was done using a dissecting microscope (Leica S4E).

### Root colonisation

The Root samples from pot trials, were carefully washed and cut into 1- 3cm sections. Sections were covered with 5% KOH solution and incubated at 90° C for 45 mins, to remove the cytoplasm and all coloured material from the plant cells. The KOH solution was discarded and the roots were rinsed well with distilled water. The roots were covered with a freshly prepared alkaline $H_2O_2$ solution to bleach for 60 mins. The bleaching solution was discarded and the roots were rinsed with water. The roots were acidified in a 0.1M HCl solution overnight to ensure adequate binding of stain to fungal structures. The HCl solution was discarded and roots were covered with Lactoglycerol Trypan Blue (0.05%) stain and incubated for 45 mins at 90°C. The stain was poured off and roots were covered with lactoglycerol destain. The roots were allowed to destain overnight before microscopic examination (Smith and Dickson, 1997) [12]. Finally, roots were mounted on microscopic slides and using a compound microscope examined. The percentage root colonization was calculated using a modified Line Intersect Method (McGonigle *et al.,* 1990). This method involved squashing segments of stained roots on a microscope slide after it is covered with a cover slip. The roots were selected and examined for their entire length, a field of view at a time. One field of view using 40 x magnifications was scored at intersects between an eyepiece micrometer with or without mycorrhizal structures (arbuscules, vesicles or hyphae). The number of mycorrhizal structures in a 100 fields of view was equal to the percentage colonisation.

## Results and Discussion

### Spore count and per cent root colonization

The results pertaining to Spore count of chilli recorded at flowering and harvesting are presented in table However, among the treatments, the highest spore count of 103.06 and 107.40 /50 g of soil was observed in the treatment that received 50 per cent RP + *Glomus macrocarpum* + Fluorescent pseudomonads ($T_3$), at flowering and harvesting respectively. and among the inoculated treatments, The Treatments of 75 per cent RP + *Glomus macrocarpum* + Fluorescent pseudomonads ($T_4$), and 50 per cent SSP + *Glomus macrocarpum* + Fluorescent pseudomonads ($T_6$), recording the of 84.31 and 83.70 /50 g of soil respectively, they are on par at flowering.

**Table 1:** Effect of *Glomus macrocarpum* and Fluorescent pseudomonads with different source and levels of phosphorus on rhizosphere spore count and percent root colonization of Mycorrhiza in chilli at flowering and harvesting

| Treatments | Mycorrhizal spore count/50 g of soil | | Per cent root colonization of mycorrhiza | |
|---|---|---|---|---|
| | Flowering (75 DAT) | Harvesting (135 DAT) | Flowering (75 DAT) | Harvesting (135 DAT) |
| $T_1$ - Uninoculated control | 6.30 | 8.30 | 2.43 | 4.83 |
| $T_2$ - GM + FP B25 | 95.13 | 99.13 | 50.33 | 58.13 |
| $T_3$ - GM + FP B25 + RP 50% | 103.06 | 107.04 | 52.16 | 62.10 |
| $T_4$ - GM + FP B25 + RP 75% | 84.31 | 94.21 | 42.13 | 53.33 |
| $T_5$ - GM + FP B25 + RP 100% | 75.36 | 77.13 | 41.06 | 50.16 |
| $T_6$ - GM + FP B25 + SSP 50% | 83.70 | 81.36 | 42.73 | 51.20 |
| $T_7$ - GM + FP B25 + SSP 75% | 54.36 | 56.06 | 34.10 | 45.26 |
| $T_8$ - GM + FP B25 + SSP 100% | 50.66 | 55.70 | 29.10 | 40.16 |
| S.E.m± | 0.39 | 0.34 | 0.33 | 0.30 |
| CD(0.01) | 1.17 | 1.00 | 0.98 | 0.87 |

GM : *Glomus macrocarpum*
FP : Fluorescent pseudomonads
RP : Rock phopshate
SSP : Single super phosphate

The lowest Spore count of 6.30 and 8.30 /50 g of soil was recorded at flowering and harvesting in the treatments that maintained as uninoculated control, and the rest of the treatments were significantly comparable to each other.

Colonization results pertaining to per cent root colonization of chilli recorded at flowering and harvesting are presented in table However, among the treatments, the highest per cent root colonization of 52.16 and 62.10 per cent was observed in the treatment that received 50 per cent RP + *Glomus macrocarpum* + Fluorescent pseudomonads ($T_3$), at flowering and harvesting respectively. among the inoculated treatments, the treatments of 75 per cent RP + *Glomus macrocarpum* + Fluorescent pseudomonads ($T_4$), and 50 per cent SSP + *Glomus macrocarpum* + Fluorescent pseudomonads ($T_6$), recording the of 42.13 and 42.73 per cent respectively, they are on par at flowering. The lowest per cent root colonization of 2.43 and 4.83 was recorded at flowering and harvesting in the treatments that maintained as uninoculated control, and the rest of the treatments were significantly comparable to each other.

**Discussion**

Inoculation of both the AMF and *P. fluorescens* resulted in the highest mycorrhizal colonization that may have been due to a synergistic interaction between the AM fungi and *P. Fluorescens*. Higher sporulation and root colonization helps increase fungal host contact and the exchange of nutrients.

It is thus probable that the stimulatory effects on the mycorrhizal symbiont may be caused by some low-molecular weight compound present in cells of *P. putida. G. mosseae* However, another plant growth-promoting bacterium, *P. putida,* which is taxonomically closely related to *P. fluorescens,* increased colonisation of roots by AM fungi (Meyer and Linderman, 1986) [9]. Their results indicate that the stimulating effect of the bacterium on the AM fungus was due to release of biologically active molecules from the bacteria. Hence investigating the bacterial stimulating compounds will be the future study of interest.

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