Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(6): 152-154
Received: 15-09-2017
Accepted: 16-10-2017

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Effect of *Glomus macrocarpum* and fluorescent pseudomonads on Enzyme activity in chilli rhizosphere

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Abstract
The fluorescent pseudomonads and AM fungi are known to produce certain plant metabolites, plant growth promoting substances, vitamins etc. The mutual interactions of inoculated organisms is responsible for the increased enzyme activity in rhizosphere.
A green house study was carried out at university of agricultural sciences Dharwad where co-inoculation of plant growth promoting rhizo-microorganisms and AM fungi have enhanced enzyme activity in rhizosphere with increased growth and yield of chilli. The treatment incoculated with *Glomus macrocarpum* and fluorescent pseudomonads with 50% rock phosphate had shown increased phosphatase and dehydrogenase enzyme activity which was recorded at flowering and harvesting stages respectively.

Keywords: phosphatase, dehydrogenase, 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].
Today, India is the largest cultivator of chillies in the world and also one of the largest producers of chilli. 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 from small and marginal farmers. using inorganic fertilizers and insecticides, the population of beneficial organisms decrease and natural regeneration of nutrition in the soil cease and soil becomes barren and soil fertility decreases. Use of fermented liquid manures in such situation is, practically advantageous. In these liquid manures, beneficial microorganisms survive and are helpful in promoting plant growth by providing available phosphorus through phosphate solubilization by releasing enzymes like phosphatase and dehydrogenase in the soil.
Phosphorus (P) is one of the most important element for plant growth and metabolism. It plays a 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) [10].
Tarafdar and Jung (1987) [12] reported that application of phosphatic fertilizer in combination with vesicular arbuscular mycorrhizal fungi showed higher availability and P uptake, activity of phosphatase and dehydrogenase in the rhizosphere soil. The best results however, were obtained by application of SSP with VAM and TRP in combination with VAM performed on par with SSP alone as for as the dry mater yield, P uptake and availability was concerned.
The plant-growth-promoting rhizobacteria (PGPR) can influence on the 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) [4].
An experiment conducted by application of Single Super Phosphate in combination with vesicular Arbuscular mycorrhizal fungi showed highest P uptake, dry mater yield, activity of

phosphatase and dehydrogenase in the rhizosphere soil (Tarafdar and Jung 1987) [12].

According to Sumana (1998) [11] and Kumar *et al.* (2008), the acid phosphatase activity actually increases with increased root colonization by AM fungi. And The treatments which decrease the available phosphate cause an overall increase in the phosphatase activity (Azcón and Barea, 1997) [2].

Increase in the total P content might be due to the enhanced activities of the acid and alkaline phosphatases (Feng *et al.*, 2002) [8]. Gryndler and Vosátka (1996) [9] found a stimulating effect of *P. putida* not only on hyphal growth but also on dehydrogenase activity of *G. fistulosum*.

With this background we conducted an experiment at the university of agricultural sciences dharwad to know the coinoculation effect of *glomus macrocrpum* and *Pseudomonas* on the growth of chilli and enzyme activity in chilli rhizosphere.

Materials and Methods

Enzyme Activity

The rhizosphere soil samples were collected from each replication as per treatment schedule at flowering stage and used for determination of enzyme activities.

Dehydrogenase Activity

The enzyme assay involves colorimetric determination of 2, 3, 5- triphenyl farmazone (TPF) produced by reduction of 2, 3, 5-triphenyl tetrazolium chloride by soil microorganisms. Ten gram of soil samples was used to estimate this enzyme activity. The reduced product, TPF was extracted by methanol and its concentration was measured using a Spectrophotometer at 485 nm. The enzyme activity was expressed as µg of TPF produced per gram of soil when incubated for 24 hours at 37^0C (Casida *et al.*, 1964) [5].

The standard graph of different concentrations of TTC was prepared in methanol to include 0, 5, 10, 20, 30 and 40 µg TPF per ml.

Phosphatase Activity

The alkaline phosphatase activity was measured by estimating concentration of P-nitrophenol as hydrolyzed product of the substrate P-nitrophenyl phosphate (PNP). One gram of soil sample was used to estimate the activity of alkaline phosphatase. The enzyme activity was expressed as µg of P-nitrophenyl phosphate hydrolyzed per gram of soil per hour at 37 + 8^0C (Evazi and Tabatabai, 1979) [7].

The standard graph of different concentrations of P-nitrophenol solution was prepared to include 0, 10, 20, 30, 40 and 50 µg per ml. The intensity of colour was read in a spectrophotometer at 420 nm against a blank.

Results and Discussion

Dehydrogenase and Phosphatase activity

The results pertaining to Dehydrogenase activity of chilli recorded at flowering and harvesting are presented in table However, among the treatments the highest Dehydrogenase activity of 7.13 and 6.13 µg TFP B25/g soil/day 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), recorded the activity of 6.04, 6.05 and 5.06, 5.04 µg TFP B25/g soil/day respectively, they are on par at flowering and harvesting. The lowest Dehydrogenase activity of 5.21 and 3.51 µg TFP B25/g soil/day was recorded at flowering and harvesting in the treatments that maintained as uninoculated control and the rest of the treatments are significantly comparable to each other.

Table 1: Effect of *Glomus macrocarpum* and Fluorescent pseudomonads with different source and levels of phosphorus on rhizosphere Dehydrogenase and Phosphatase enzyme activities in chilli crop at flowering and harvesting

Treatments	Dehydrogenase activity (µg TFP B25/g soil/day)		Phosphatase activity (µM PNP/g soil/hr)	
	Flowering (75 DAT)	Harvesting (135DAT)	Flowering (75 DAT)	Harvesting (135 DAT)
T_1 - Uninoculated control	5.01	3.51	12.87	12.93
T_2 - GM + FP B25	5.76	4.76	14.72	18.5
T_3 - GM + FP B25 + RP 50%	7.13	6.13	22.43	21.64
T_4 - GM + FP B25 + RP 75%	6.04	5.06	20.21	19.86
T_5 - GM + FP B25 + RP 100%	5.18	4.78	17.81	16.46
T_6 - GM + FP B25 + SSP 50%	6.05	5.04	20.49	19.46
T_7 - GM + FP B25 + SSP 75%	4.36	4.06	19.17	18.19
T_8 - GM + FP B25 + SSP 100%	4.06	4.01	18.42	17.61
S.E.m±	0.07	0.09	0.11	0.20
CD(0.01)	0.21	0.25	0.32	0.59

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

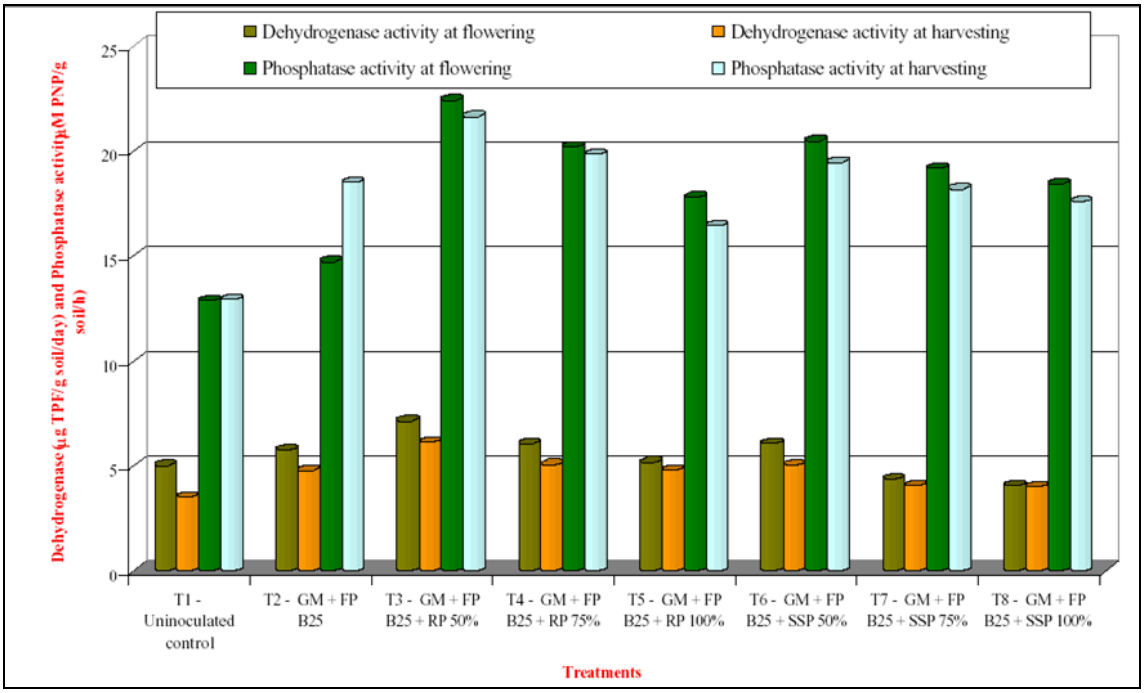

Fig 1: Effect of *Glomus macrocarpum* and Fluorescent pseudomonads with different source and levels of phosphorus on rhizosphere Dehydrogenase and Phosphatase enzyme activity in chilli crop at flowering and harvesting

The results pertaining to Phosphatase activity of chilli recorded at flowering and harvesting are presented in table However, among the treatments, the highest Phosphatase activity of 22.43 and 21.64 □M/g soil/h 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 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 20.21, 20.49 and 19.86, 19.46 □M/g soil/h respectively, they were on par at flowering and harvesting. The lowest Phosphatase activity of 18.87 and 12.93 □M/g soil/hr was recorded at flowering and harvesting in the treatments that maintained as uninoculated control, and the rest of the treatments are significantly comparable to each other.

Discussion

The Dehydrogenase and Phosphatase activity at flowering recorded significantly higher due to coinoculation of efficient FP + GM along with RP application compared to absolute control. Tarafdar and Jung (1981) reported that application of phosphatic fertilizer in combination with vesicular arbuscular mycorrhizal fungi showed higher availability of P, activity of phosphatase and dehydrogenase in the rhizosphere soil. Similarly, The inoculation with *P. fluorescens* along with AM fungi significantly increased the phosphatase activity. It shows coinoculation of glomus macrocarpum and pseudomonas with rock phosphate results into increased availability of phosphorous to the plants.

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