Global Journal of Current Research Vol. 2 No.2. 2014. Pp.30-35

Full Length Research Paper

Combined Effect of *Pseudomonas fluorescens* and *Glomus fasciculatum* with Zinc on Growth of Indian Basil (*Ocimum sanctum* Linn.)

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Abstract
The present investigation on VAM fungi have been undertaken with a view to study the effect of Glomus fasciculatum and fluorescent Pseudomonas on growth and productivity of Ocimum sanctum in pot culture to assess the impact of these two on biomass yield individually and in combination. Application of Pseudomonas fluorescens and Glomus fasciculatum in pot trials substantially increased the growth and yield of Ocimum sanctum applied with Zn amended soil. The inoculation of Pseudomonas fluorescens and Glomus fasciculatum in Ocimum sanctum enhanced seed germination over control under pot trial studies. Inoculation of Pseudomonas fluorescens and Glomus fasciculatum enhanced all the growth parameters of plant under pot trials.

Keywords: *Glomus fasciculatum, Ocimum sanctum, Pseudomonas fluorescens, and Zn*

Introduction

Ocimum sanctum (vern. Tulsi) belongs to *Lamiaceae,* grows throughout the Eastern World tropics and is a widespread and cultivated sacred plant of India. It is an aromatic one and well known for its medicinal properties. Its essentials oils bear several medicinal properties; hence it is used across south Asia as a medicinal plant and for herbal tea.

Fluorescent pseudomonads are ubiquitous soil microorganisms and common inhabitants of rhizosphere. Pseudomonads are well known potential bacteria, which enhances the plant growth and control soil-borne pathogens. Fluorescent *Pseudomonads* has been paid much attention in recent years (Schipper *et al*., 1987) as bio control agents for suppression of phytopathogenic fungi. The siderophores of fluorescent *Pseudomonas* and HCN control the growth of soil-borne pathogen (Gupta *et al*., 2002). Siderophore production has been reported by a number of workers in different groups of plant growth-promoting bacteria such as rhizobia (Deshwal *et al.* 2003a; Vargas *et al.* 2010), Pseudamonads (Gupta *et al.* 2002; Bhatia *et al.* 2003; Singh *et al.* 2010a; Khare *et al.* 2011), *Bacillus* spp. (Singh *et al.* 2008; Mehta *et al.* 2010), and *Burkholderia* spp. (Pandey *et al.* 2005a). PGPR that indirectly enhance plant growth via suppression of phytopathogens do so by a variety of mechanisms, such as the ability to produce siderophores that chelate iron making it unavailable to pathogens (Pandey *et al.* 2005b).

Fluorescent *Pseudomonas* has another merit in controlling several phytopathogenic fungi besides simultaneously enhancing the growth and yield of various vegetables and cereals (Iswandi *et al*., 1987). These rhizobacteria produce IAA and produce solubilized phosphates (Gupta *et al*., 2002). Beneficial *Pseudomonas* rapidly and aggressively colonizes the root system and suppresses pathogenic micro-organisms and enhances plant growth and development (Weller 1988).

Hariprasad and Niranjana (2009) reported that solubilization of P in the rhizosphere is the most common mode of action implicated in PGPR that increase nutrient availability to host plants. Zinc and phosphate solubilizing bacteria and their role as PGP have also been reported by Iqbal *et al*. (2010). The application of PGPR has also been extended to remediate contaminated soils in association with plants (Khan *et al.* 2009).

Vesicular arbuscular mycorrhiza (VAM) is formed in most medicinal plants, agronomic and vegetable crops. VAM association benefits the plants with inorganic minerals, growth substances and protect from abiotic stresses also. Spores of VAM under favorable environmental conditions germinate and undergo a sequence of steps that are based on structural morphogenesis. These stages have been categorized into the asymbiotic, presymbiotic and the symbiotic stages (Bago and Bécard 2002).

VAM fungi have been reported to enhance the plant productivity and biomass accumulation in plants. VAM hyphae penetrate the root and grow intercellular to the linear cortical layers, where it penetrates the individual cells and forms the arbuscules or hyphal coils. Mycorrhiza regulate not only uptake, but also the relative abundance of available and transportable nutrients in the tissue concentration of essential micronutrient like Cu and Zn (Swaminathan and Verma, 1979).

Micronutrients play major role in the production of healthy plants. Micronutrients are needed in minute amounts to produce healthy plants. Zinc is one of the essential micronutrients required for optimum crop growth. Phosphorus (P) occupies a critical position both in plants and in the biology of soil. It is the second most important plant nutrient required for biological growth (Alexander, 1961; Tilak, 1961; Kapoor *et al,*. 1989) and development (Fernandez *et al.,* 2007). High level of soil phosphorus is commonly associated with Zinc deficiency and absence of *Pseudomonas* and VAM fungi. *Pseudomonas* and VAM fungi haulage Zn from soil to plants root and transported in the xylem tissues from the roots to the shoots. However, high levels of zinc have been detected in the phloem tissues.

Zinc (Zn) is involved not only in plant metabolism but also in soil microbiological process as well as in other essential processes such as cell division and development, photosynthesis, nutrient transport, etc. (Subba Rao, 1982a). It stimulates growth of young plants, promotes vigorous start and hastens maturity.

It is generally recognized that the effectiveness of inorganic zinc fertilizers in soils, at least in the short term, is determined by their water solubility. Various authors have concluded that at least a 40 or 50% water soluble source of zinc is required. This is one of the reasons why highly soluble zinc sulphate is so widely used for treating deficiencies (as well as its relatively low cost and generally wide availability). Zinc sulphate (98% soluble), zinc lignosulphonate (91% soluble) and Zn EDTA (100% soluble) were all very efficient at supplying zinc to plants.

Materials and Methods

Doon valley lies in the foothills of Himalayas and is located at 30^{o} 20' N latitude and 78^{o} 04' E longitude. The valley possesses sub-tropical climate i.e. cold winter, warm and crispy springs, hot summers followed by strong monsoon. The maximum temperature during summers reaches up to 36 ^{o}C and minimum up to 4 ^{o} C during winters. The average rainfall is 420.85 mm and mostly occurs from June to September. Little rain is also observed during winters that makes Doon valley favorable for *Ocimum sanctum*.

The climate of this holy religious place (Haridwar) is temperate all through the year, but the best time is from October to April. Winter (October-February) is little chilly with minimum night temperature of about 6°C. Summer (March-May) has a temperature band of 18°C to 40°C. Monsoon (June-September) receives average rainfall is 266.25 mm and humidity increases, making day activities a challenge.

Preparation of soil samples: Soil was taken from two sites of Dehradun viz., Archadia (D_1) and Good Rich (D_2) and two sites of Haridwar viz., Jwalapur (H_1) and Shivalik Nagar (H_2) for pot culture experiments. The soil samples from these four agriculture fields were collected for isolation.

Isolation of fluorescent bacteria: The fluorescent *Pseudomonas* strains were isolated from the soil collected from selected localities of two sites individually. Enrichment culture technique (in liquid medium) was used for isolation following Subba Rao (1982).

After purification of bacterial colonies on King's B medium, the bacterial colonies were examined on the basis of morphology, physiology and biochemical tests (Sigee, 1993).

Phosphate solubilizing fluorescent bacteria: Different types of bacterial colonies with characteristic colour were obtained by serial dilution and plating on Pikovskaya's media. Each strain of bacterium was spot inoculated on Pikovskaya's TCP media plate separately and incubated for 4-5 days at 28 $\pm$ 2 ^{o}C to observe the clear or halo zone around the colony. The strains showing zone of solubilization were presumed to be phosphate solubilizers which were then subjected to culture on King's B media (Alcamo, 2001).

Isolation of the VAM Spores from the Soil Samples: Wet sieving and decanting procedure of Gerdemann and Nicolson's (1963) was adopted for isolation of VAM spores.

Field trial: To study the effect of inoculation of isolated bacteria on growth and yield response of basil (*Ocimum sanctum)*, field experiments were conducted during March to January. Soil was sandy loam having 81% sand, 9% silt and 10% clay with a pH of 6.8. The elemental composition includes carbon (0.26%), nitrogen (0.07%), available P (1.2 %) and micronutrient zinc was estimated to be 58.63 mg/kg at D_1, 58.30 mg/kg D_2, 62.73 mg/kg $H_1$and 54.48 mg/kg H_2, respectively.

Seed bacterization: Before application, the prepared bioinoculant mixture was mixed in cool jaggery. It works as sticky material. *Ocimum sanctum* seeds were surface sterilized by dipping in 2% Na_2OCl_2 for 30 min. and with distilled water for at least 5 times to remove the traces of Na_2OCl_2 (Johnson and Case 1946). After air drying, seeds were mixed in jaggery containing bacterial inoculants. Before sowing, seeds with bacterial inoculation were dried in shade for 2 h so that they get separated.

Pot experiment

Pot experiment was conducted in 3 kg capacity pots and polyethylene bags for two year. The experiments consisted of eight treatments with four replicates each for different times at four sites separately. Treatment 1 (T1) Control condition, Treatment 2 (T2) VAM, Treatment 3 (T3) *Pseudomonas,* Treatment 4 (T4) Zn, Treatment 5 (T5) VAM + Zn, Treatment 6 (T6) *Pseudomonas* + Zn, Treatment 7 (T7) VAM + *Pseudomonas,* Treatment 8 (T8) VAM + *Pseudomonas* + Zn. Pots were marked with treatments and times for finding out the objectives of the proposed study. Four treatments involved in the study under sterilized soil conditions.

Bacterized *Ocimum* seed (10) were sown on each pot. The pots were irrigated at regular intervals. For seedling growth analysis, number of leaves, leaf area, leaf area index, leaf chlorophyll and dry weight were recorded. Eight plants from each site were randomly selected for recording the data. The data were analyzed statistically using analysis of variance (ANOVA).

Results and Discussion

P. fluorescens colonies appeared pink in colour with Gram's stain and found to be Gram negative. Morphologically, they are rod shaped and possess more than one flagella**.** Production of fluorescent pigment by strains of pseudomonads has been reported by King's *et al*., (1954). Production of siderophores, hydrocyanic acid, indole acetic acid and phosphate solubilization was exhibited by *Pseudomonas.* The observations are compiled in Table 1.

Table 1. Morphological and biochemical observations on *Pseudomonas fluorescens*

Characters	*P. fluorescens*
Gram's staining	Negative
Flagella	>1
Pigment production	Green blue
Catalase activity	Positive
Gelatin hydrolysis	Positive
Urea hydrolysis	Negative
Fermentation of	
Sucrose	Positive
Mannitol	Highly positive
Lactose	Negative
Indole production	Positive
MR-VP test	Negative
Citrate utilization	Positive
Oxidase reaction	Positive
Starch hydrolysis	Positive
Hydrogen sulphide production	Negative

Analysis of Pseudomonas Colonies

Pseudomonas colonies were also studied in all the four soil types during the six months to study the effect of varied soil types as well as that of the seasons.

The combined effect of seasons and soil types*:* The combined effects of seasons and soil types displayed significant variation in *Pseudomonas* colonies. It is seen that, in July and September the number of *Pseudomonas* colonies remained higher in all the soil types (Fig 1).

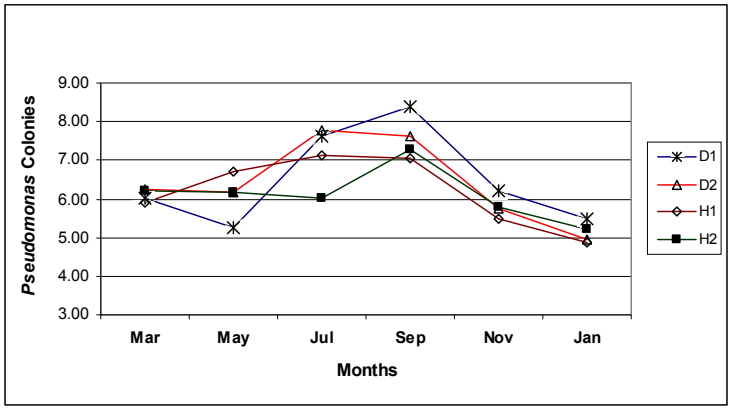

Fig 1: Variation in *Pseudomonas* colonies with months and four soil types.

VAM Colonization:

The combined effect of seasons and soil types*:* The combined effect of seasons and soil types varied significantly. It was seen that in soil types D_1 the number of VAM spores remained maximum during all the seasons. In the contrary in the soil types H_2 the numbers of VAM spores were least during all the seasons (Fig 2).

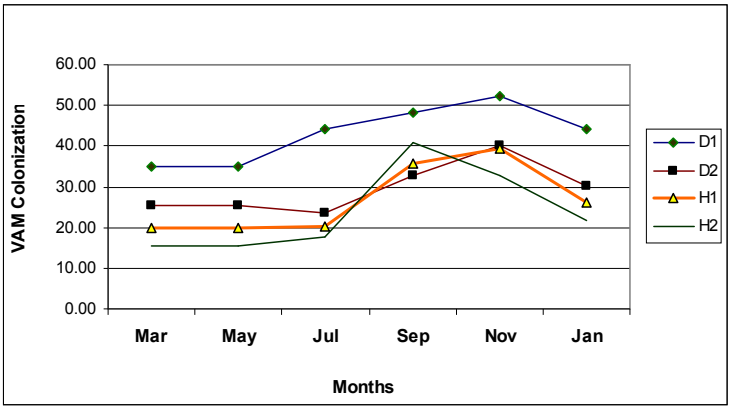

Fig 2: Variation in VAM colonization of four soil types in different seasons.

The present investigation is an attempt to determine the diversity of VAM fungi by counting the number of VAM spore on alternate month basis and observing the effect of environmental factors on growth of VAM fungi.

The numbers of VAM spores were more in monsoon and late summer seasons. Results of the present investigation were found in agreement with Haymand (1978) who observed that spore density of *Glomus* was found to increase during mid-summer and rainy season. VAM propagates varied in selected soil sample and among these *G. fasciculatum* and *G. mosseae* were found to be the dominant. Rainy months have shown significantly higher growth patterns in comparison to the normal months. This study has shown a relatively high level of spore population during rainy season The low levels of spores were observed in winter season as apparent in Fig 3.

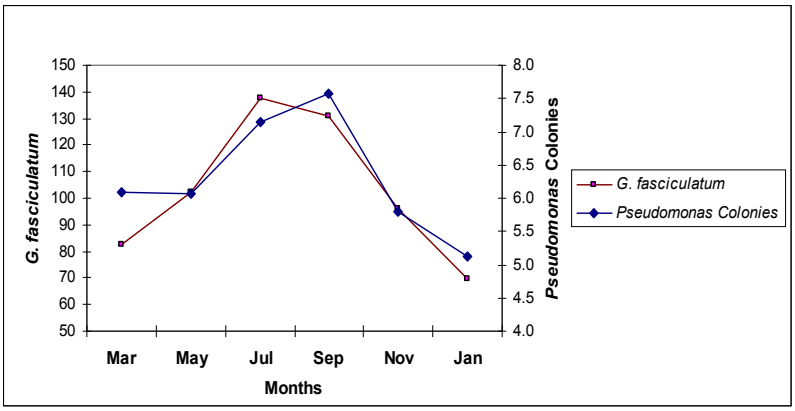

Fig 3: Similarity of seasonal trends between VAM spores & *Pseudomonas* colonies of four soil types

The study demonstrated that application of *Pseudomonas fluorescens*, *Glomus fasciculatum* and Zn in field substantially increases the growth and yield of *Ocimum sanctum* applied with Zn organically amended soil. It revealed that inoculation of *P. fluorescens, Glomus fasciculatum* in basil (*Ocimum sanctum*) enhances the seed germination over control in pots. Our observations support the findings of Gholami *et al.* (2009) who assessed the effect of inoculation of different strains of PGPR *Pseudomonas* and VAM on growth of maize.

It may be concluded that *Glomus fasciculatum* and fluorescent *Pseudomonas* play a significant and complex role in plant health. Fluorescent Pseudomonads have emerged as the largest and most promising group of PGPR as well as PSM and *Pseudomonas fluorescens* have received more attention than other Pseudomonads.

Conclusion

It may be concluded that the results of interaction study varied in all the eight treatments. It was found that the leaf area, leaf area index, dry weight, chlorophyll content and number of leaves, etc., increased with the increase in plant age in both control and VAM inoculated *Ocimum* plants. The result indicated clearly that VAM enhanced the essential nutrients needed for healthy growth and development of *Ocimum* plants from the soil by converting the complex forms of nutrients into the simpler forms by their system.

Thus, mycorrhizal fungi and Pseudomonad have the potential for maintaining plant vigor, while reducing the need for chemical and fertilizer inputs, by mediating nutrient flux between plant and soil. These fungi influence both the plant growth and health and the development of communities of soil organisms.

Acknowledgement
The authors acknowledge the research facilities provided by the Department of Botany and Microbiology, HNB Garhwal University, Srinagar and Chinmaya PG College, Haridwar.

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