International Journal of Life Sciences Vol. 3. No.2. 2014. Pp. 41-45

Full Length Research Paper

Effect of Microbial Inoculants of *Pseudomonas* and VAM on Indian Basil (*Ocimum sanctum* Linn.)

Pravita Pandey*, Ajai Swami and A. B Bhatt**
Department of Botany and Microbiology, HNB Garhwal University Srinagar- 246 174,
***Chinmaya PG College, Haridwar, Uttarakhand, India*

**Corresponding Author: Pravita Pandey*

Abstract
The present study was focused on the efficiency of 'effect of microbial inoculants of Pseudomonas and VAM on Indian Basil (Ocimum sanctum Linn.)' to provide indications of their effectiveness in improving growth and yield of Ocimum sanctum. Application of Pseudomonas fluorescens and Glomus fasciculatum in pot trials substantially increased the growth and yield of Ocimum sanctum. In order to understand the impact of various physico- chemical parameters of soil on the growth of Ocimum sanctum under various treatments, four soil types were mixed to get a compact soil sample and analysed for various physico-chemical characteristics.

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

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 plant 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.

Pseudomonas fluorescens strains are the plant growth promoters which express growth-stimulating hormones that affect growth and yield of plant. They are aerobic, Gram-negative rods, motile and generally chromogenic in nature. It also grows at different temperatures (Hendrie and Shewan 1966; Narayanasamy 1997), primarily characterized by their ability to produce water-soluble, yellow-green fluorescent pigment of unknown chemical nature. The pigment production by *P. fluorescens* was observed by inoculating into Pikovskaya's broth (Dave and Patel, 1999).

On the basis of relationship with plants, PGPR can be divided into two groups: symbiotic bacteria and free living rhizobacteria (Khan, 2005). Moreover, PGPR can also be divided into two groups based on their residing sites: intracellular PGPR (iPGPR or symbiotic bacteria) which live inside the plant cells, produce nodules, and localized inside the specialized structures (Vessey, 2003) and extracellular PGPR (ePGPR or free-living rhizobacteria) which live outside the plant cells and do not produce nodules, but still promote plant growth (Gray and Smith, 2005). The best-known iPGPR are rhizobia which produce nodules in leguminous plants.

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 vesicles. Mycorrhiza regulates 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). *Pseudomonas* and vesicular arbuscular mycorhizal fungi are well known potential biocontrol agents among the others. Pseudomonads produce siderophores and HCN, which inhibit the soil borne pathogens and improve plant growth. *Pseudomonas* also solubilises phosphate and produce IAA (Gupta *et al*., 2002). Siderophores/antibiotics suppress deleterious microbes, produce phytohormones, growth enhancing compounds that directly affect the plant growth. Exploitation of these bacteria to improve crop production has therefore, become important in sustainable agriculture.

It is suggested that VAM alone or in combination with rhizobacteria like *Pseudomonas* in inoculation are promising biofertilizers because they are cheap, eco-friendly, easy to handle and improve growth, yield and seed quality of the crops.

Materials and Methods

Doon valley lies in the foothills of the Himalaya and is located at 30° 20' N latitude and 78° 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 °C and minimum up to 4 ° 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 favourable for *Ocimum sanctum*.

Collection of soil samples: Soil was collected 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 Pseudomonas: 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 bacteria: Different types of bacterial colonies with characteristic colour were obtained by serial dilution and plating on Pikovskaya's agar medium. Each strain of bacterium was spot inoculated on Pikovskaya's TCP medium plate separately and incubated for 4-5 days at 28 $\pm$ 2 °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 medium (Alcamo, 2001).

Isolation of the VAM Spores from the Soil Samples: Wet sieving and decanting procedure of Gerdemann and Nicolson (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 %).

Seed bacterization: Before application, the prepared bioinoculant mixture was mixed in cool jiggery that 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) VAM + *Pseudomonas.* 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

Effect of soil types: The analysis revealed that variation among the four soil types is highly significant. The soil type D1 was found to show maximum colonization and soil type D2 displayed least colonization. Soil types D2 and H1 were statistically significant with respect to colonization (Fig 1).

Effect of soil type: The analysis revealed that variation among the four soil types was not significant. This indicated that all the four soil types are same in respect of *Pseudomonas* colonies. It may be asserted that variation in localities does not create any significant variation in *Pseudomonas* colonies. The trend of soil types resembled that of the VAM colonization (Fig 2).

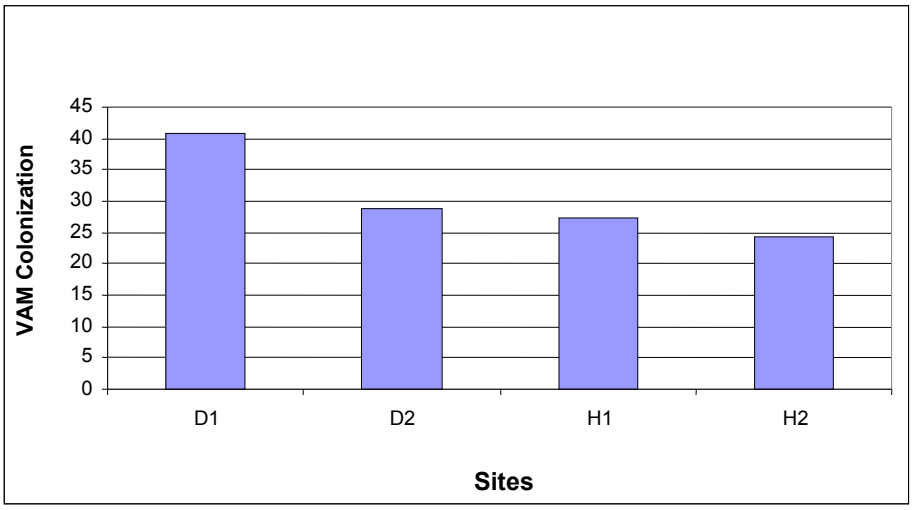

Fig 1: Variation in VAM colonization of four soil types.

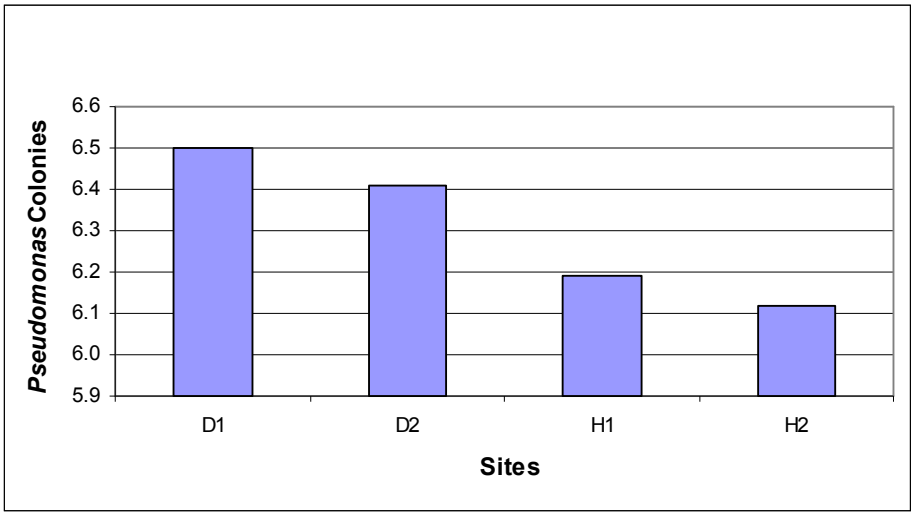

Fig 2: Variation in *Pseudomonas* colonies of four soil types.

Leaf Dry Weight (gm)

Effect of soil types: Leaf dry weight is an important growth characteristics as higher leaf dry weight is an indication of greater leaf area and good plant growth. It was observed that basil plants at D2 site had maximum foliar dry weight being 0.00621g while plants at H1 site had least foliar dry weight being 0.00323 g. Basil plants at D1 were found to gain dry weight. Soil types D2 follows D1 in leaf dry weight (0.00554). It may be asserted that H soil types were associated with lower leaf dry weight in comparison to D soil types (Fig 3).

Effect of soil types and treatments on leaf dry weight: The joint effect of soil types and months on leaf dry weight was observed to be minimal. In spite of significant variations with respect to treatments and season, their combined effect on leaf dry weight was found to be non-significant. Soil types D2 and D1 appeared to be most suitable for gain in leaf dry weight (Fig 4).

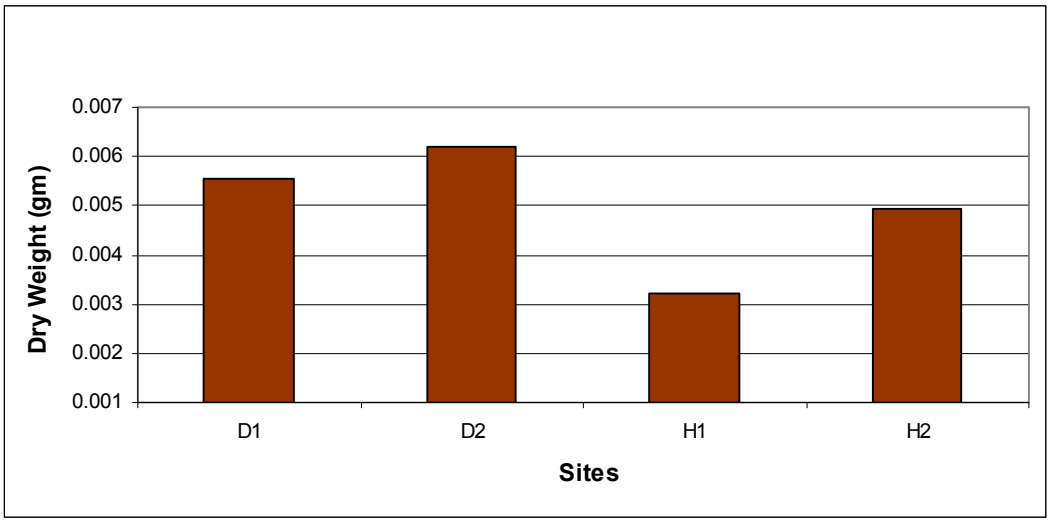

Fig 3: Variation in total Dry weight among different soil types.

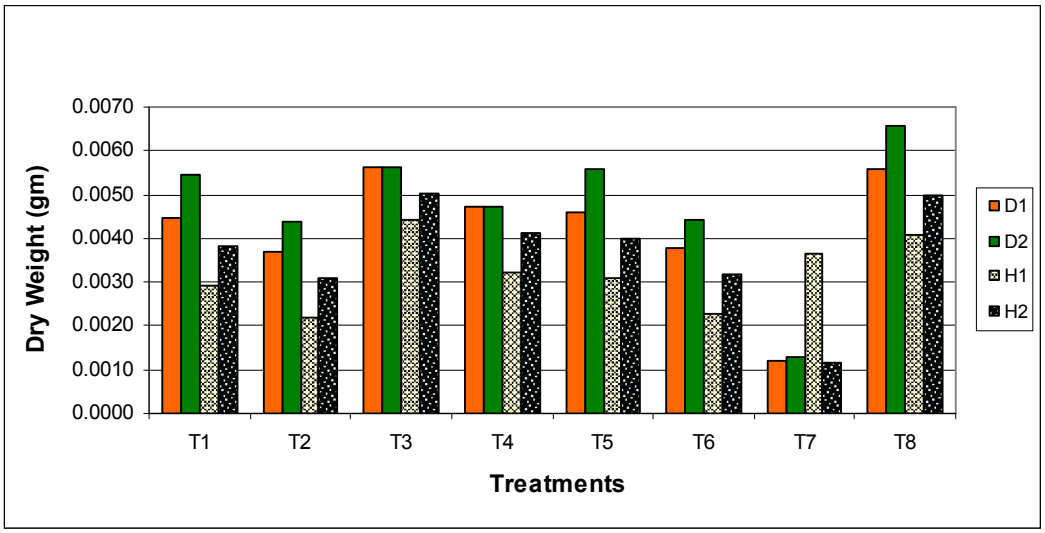

Fig 4: Variation in total Dry weight due to different treatments in different soil types

Effect of soil types and months on plant dry weight: Interactive effects of different soil type and months were not significant. Soil type –D2 was noted to be associated with highest leaf dry weight during all the months while H1 had minimum leaf dry weight even in best seasons. All soil types exhibited nearly equal leaf dry weights in all the seasons (Fig 5).

Conclusion

The present piece of work was aimed to the study the effect of VAM and *Pseudomonas* (PGPR) on growth and productivity of *Ocimum sanctum*. VAM and fluorescent *Pseudomonas* play a significant and complex role in plant health. Fluorescent *Pseudomonas* 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.

Plant growth promoting rhizobacteria (PGPR) like pseudomonads also assist the activities of VAM fungi and influence host. The present study is focused on the effect of microbial inoculants of *Pseudomonas* and VAM on Indian Basil *(Ocimum sanctum)* in laboratory as well as in field to provide preliminary indications of their effectiveness in improving growth and yield of *Ocimum sanctum.*

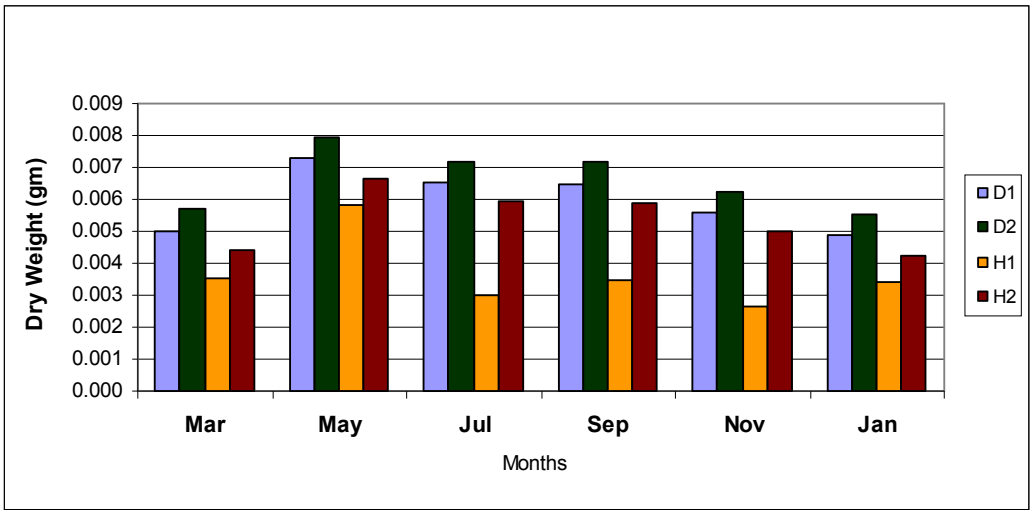

Fig 5: Variation in total Dry weight due to soil type and seasons.

Acknowledgements

The authors acknowledge the research facilities provided by the Department of Botany and Microbiology, HNB Garhwal University, Srinagar and Research assistance by Dr R.C Dubey Gurukul Kangri University Deptt of Microbiology, Haridwar. Authors are also thankful to Dr Alok Kumar Principal Chinmaya PG College, Haridwar.

References

Alcamo, I. E., (2001). *Laboratory fundamentals of Microbiology* (6th ed.), Jones & Barlett Publishers, Sudbury, London.

Dave, A. and Patel, H. H., (1999). Inorganic phosphate solubilizing soil pseudomonads. *Indian Journal of Microbiology,* **39**: 161-164.

Gerdemann, J., W. and Nicolson, T., H. (1963). Spore of mycorrhizal Endogone species extracted from soil by wet sieving and decanting Trans. *Br. Mycol. Soc.* **45**: 235-244.

Gray, E.J. and Smith, D.L. (2005). Intracellular and extracellular PGPR: commonalities and distinctions in the plant-becterium signaling processes. *Soil. Biol. Biochem.* **37**: 395-412.

Gupta, C.P., Dubey, R.C. and Maheshwari, D.K. (2002). Plant growth enhancement and suppression of *Macrophomina phaseolina* causing charcoal rot of peanut by fluorescent *Pseudomonas*. *Biol. Fertl.Soil* **35**: 295-301.

Hendrie, M. S. and Shewan, J. M., (1966). The identification of certain *Pseudomonas* species. In *Identification Methods for Microbiologyogists* (Part- A), Academic Press, London, pp. 1-7.

Johnson, T. R. and Case, C. L., (1946). *Laboratory Experiments in Microbiology*, Benjamin/Cummings publishing company, California.

Khan, A. G. (2005). Role of soil microbes in the rhizosphere of plants growing on trace metal contaminated soils in phytoremediation. *J. Trace. Elem. Med. Biol.* **18**: 355-364.

Narayanasamy, P., (1997). *Plant Pathogen Detection and Disease Diagnosis, Marcell Dekker, New York, USA, pp* 119.

Sigee, D. C., (1993). Taxonomy of plant pathogenic bacteria. In *Bacterial Plant Pathology* (Cellular and Molecular aspects), *Cambridge University Press,* London, pp 53.

Subba Rao, N. S., (1982a). Phosphate solubilization by microorganisms. *In Advances in Agricultural Microbiology,* Butterworth Scientific Publishers, London.

Subba Rao, N. S., (1982b). Phosphate solubilizing microorganisms. In *Biofertilizers in Agriculture* (3rd ed.), Omega Scientific Publishers, New Delhi.

Swaminathan, K. and Verma, B., C. (1979). Response of three crops species to vesicular arbuscular mycorrhizal infection on zinc deficient Indian soils. *New Phytol.* **82** : 481- 487.

Vessey, K.J. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil* **255**: 571-586.