

# Plant Growth Promoting activity in Chickpea Crop by Using Various Combinations of Micro-Organism

<sup>[1]</sup> Prashant Kumar Dewangan, <sup>[2]</sup> Bhumika Koma, <sup>[3]</sup> Sanghmitra Baghel

Department of Plant Pathology, College of Agriculture (IGKV), Raipur, 492006 Chhattisgarh

**Abstract**— The present investigation was carried out in the Department of Plant Pathology, College of Agriculture, (IGKV), Raipur (CG.). In the recent times, there has been a reversed interest in the search of Plant Growth Promoting Rhizobacteria (PGPR) for sustainable crop production. Biological control using PGPR strains especially from the genus *Trichoderma* and *Pseudomonas* is an effective substitute for chemical pesticides to suppress plant diseases. The present investigation was evaluated to growth promoting activities in pulse crop such as chickpea by using different thirteen combination of *Trichoderma* sp., *P. fluorescens* and *Rhizobium* sp. five treatment used which were *P. fluorescens* (spray) + *Trichoderma* sp.(soil), *P. fluorescens*(soil)+ *Trichoderma* sp.(spray), *P. fluorescens* (seed)+*Rhizobium* sp.(seed)+ *Trichoderma* sp.(spray), *P. fluorescens* (spray) + *Rhizobium* sp. (seed)+ *Trichoderma* sp.(soil). After 14 days of uprooting of crop found that significantly high vigour index in T1 (2794.69) treatment of chickpea. All treatment combination increases root length, shoot length and overall vigour index as compared to control in all pulse crops.

**Keywords**— *Trichoderma* sp., *Pseudomonas fluorescens*, *Rhizobium* sp., Plant Growth Promotion, in vitro.

## I. INTRODUCTION

Biological control using antifungal rhizobacteria to suppress plant diseases offers a powerful alternative to the use of synthetic chemicals (Emmert and Handelsman, 1999). Different studies have demonstrated the ability of certain bacteria to suppress diseases caused by soil and seed-borne plant pathogens (Whipps, 2001; Dobbelaere et al., 2003). Rhizobacteria usually do not rely on single mechanism of promoting plant growth (Glick et al., 1999). The use of biological control agents as an alternative to fungicides is increasing rapidly in the present day agriculture due to the deleterious effects of chemical pesticides. Members of the genus *Pseudomonas* and *Trichoderma* have long been known for their potential to reduce the plant disease caused by fungal pathogens and they have gained considerable importance as potential antagonistic microorganisms (Pant and Mukhopadhyay, 2001). Among these the bacterial antagonists have the twin advantage of faster multiplication and higher rhizosphere competence hence, *P. fluorescens* have been successfully used for biological control of several plant pathogens (Ramamoorthy et al., 2002) and biological control using PGPR strains especially from the genus *Pseudomonas* is an effective substitute for chemical pesticides to suppress plant diseases (Compant et al., 2005). Co-inoculation studies with PGPR and *Rhizobium*, *bradyrhizobium* sp. have shown to increase root and shoot weight, plant vigour, nitrogen fixation and grain yield in various legumes (Valverde et al.,

2006; Yadegari et al., 2008). Chickpea (*Cicer arietinum* L.) is a major grain in India. It contributes to 38% of national pulse production in India respectively. Legume crop can obtain a significant portion of its N requirement through symbiotic N<sub>2</sub> – fixation to give high grain yield when grown in association with effective and competitive *Rhizobium* strain (Stephen et al., 2002). Interactions between these PGPR with *Rhizobium* may be antagonistic or synergistic and the beneficial effects of such interactions could be exploited for economic gain (Dubey, 1996). The objective of the present study was evaluation to growth promoting activities in pulse crop such as chickpea by using different thirteen combinations of *P. fluorescens*, *Trichoderma* sp. and *Rhizobium* sp.

## II. MATERIALS AND METHODS

### Seed treatment by *Pseudomonas fluorescens*:

The seeds of chickpea were surface sterilized with sodium hypochlorite solution (1%) for 10 seconds, followed by 2-3 washings with sterilized water. These seeds were then dipped into bacterial suspension having known cfu population for 6 hours and subsequently the excess suspension was decanted. After this, these seeds were air dried on plastic sheets under shade and cool condition. The seeds similarly treated with only sterilized water were kept to serve as control (Astrom and Gerhardson, 1988). Then the seeds were sown in different pots containing soil with sand and compost in the ratio of 3:1:1. After sowing and watering, germination percentage was recorded. The plants

were uprooted after 14 days of sowing and care was taken to avoid root damage. Plants were then washed with tap water, stretched on clean transparent surface and shoot length and root length of the plants were measured.

Vigour index was also calculated as per Abual Baki and Anderson (1971)

Vigour index = Germination % X (Root length + Shoot length)

**Soil treatment by Pseudomonas fluorescens:**

The Pseudomonas fluorescens isolate was applied as soil treatment (Twenty ml of bacterial suspension with a 108 cfu/ml) and mixed with pot soil. Five seeds each of chickpea were planted in different pots. After sowing, germination percentage was recorded. The plants were uprooted after 14 days of sowing and care was taken to avoid root damage. Plants were then washed with tap water, stretched on clean transplant surface and shoot length and root length of the plants were measured. Vigour index was also calculated as described earlier.

**Foliar spray of Pseudomonas fluorescens:**

Bacterial suspension at the concentration level of approximately 108 cfu/ml was thoroughly sprayed on the foliage of the seedlings with the help of automizer. The treatment was continued until fine droplets appeared on the foliage (Mew and Rosales, 1986). The plants were uprooted after 14 days of sowing and care was taken to avoid root damage. Plants were then washed with tap water, stretched on clean surface and shoot length and root length of the plants were measured. Vigour index was also calculated as described earlier.

**Seed treatment by Rhizobium culture:**

Seeds were dipped into bacterial suspension having 107 to 108 cfu population for 6 hours and subsequently the excess suspension was decanted. After this, these seeds were air dried on plastic sheets under shade and cool condition. Then the seeds were planted in different pots containing soil with sand and compost in the ratio of 3:1:1.

**Soil treatment by Trichoderma spp.:**

Twenty ml of metabolites/ culture filtrates (as mentioned earlier) were mixed with pot soil. Five seeds each of chickpea were planted in different pots. After sowing, germination percentage was recorded.

**Foliar spray of Trichoderma spp.:**

For foliar application of Trichoderma spp. culture 5 gm per liter of water was thoroughly sprayed on the foliage of the seedlings with the help of automizer. The treatment was continued until fine droplets appeared on the foliage.

**Treatments details**

- T1 : Seed treatment of Pseudomonas fluorescens
- T2 : Soil treatment of P. fluorescens

- T3 : Foliar Spray treatment of P. fluorescens
- T4 : Seed + soil treatment of P. fluorescens
- T5 : Seed + one foliar application of P. fluorescens
- T6 : Soil + one foliar application of P. fluorescens
- T7 : Seed + Soil + one foliar application of P. fluorescens
- T8 : One foliar application of P. fluorescens + soil treatment of *Trichoderma spp.*
- T9 : Soil treatment of P. fluorescens + one foliar application of *Trichoderma spp.*
- T10 : Seed treatment of Pseudomonas fluorescens + Rhizobium culture
- T11 : Seed treatment of Pseudomonas fluorescens + Seed treatment of *Rhizobium culture* + one foliar application of *Trichoderma spp.*
- T12 : One foliar application of P. fluorescens + Seed treatment of *Rhizobium culture* + soil treatment of *Trichoderma spp.*
- T13 : Control (Without any thing)

**III. RESULTS AND DISCUSSIONS**

In general, it was evident from the data presented in the table that all treatment combination increases the root and shoot length and overall vigour index as compared to that of control. Among thirteen treatments, T10 and T1 were showing highest on par shoot length (19.1cm and 18.7cm) and root length was significantly highest in T10 (15.2 cm) followed by T3, T8 and T9 (14.2 cm).

Treatment-T1 (2982.41) recorded the highest plant vigor index followed by T10 (2919.04), T2 (2729.56), T4 (2684.33), T8 (2400.42), T6 (2393.88), T7 (2241.64), T5 (2234.29), T12 (2230.50), T11 (1892.30), T3 (1603.31), T9 (1601.58) and T13 (1469.59) in the decreasing order and also change in overall vigour index in T1 (102.94) followed by T10 (98.63), T2 (85.74), T4 (82.66), T8 (63.34), T6 (62.89), T7 (52.53), T5 (52.03), T12 (51.78), T11 (28.76), T3 (9.10) and T9 (8.98) in the decreasing order. The results of present study are in agreement with those of other scientists (Vidyasekaran and Muthamilan, 1995; Rao et. al., 1996; Nanda Kumar 2001; Raji and Lekha, 2003), who also found that seed treatment of PGPR as one of the promising methods of inoculation. The growth promoting substance produced by P. fluorescens might have exerted a synergistic action and enhanced the growth promotion. Pseudomonas spp. was reported to produce amino acids, salicylic acid and IAA (Sivamani and Gnanamanickam, 1988; O'Sullivan and O'Gara, 1992) which might have improved the plant growth and seedling vigour. Production of indole acetic acid (IAA) by the strains of Pseudomonas spp. responsible for increasing root elongation was also reported (O' Dowling

**International Journal of Science, Engineering and Management (IJSEM)**  
**Vol 3, Issue 3, March 2018**

and O' Gara, 1994). Ramesh and Korikanthimath (2003) were reported that growth parameters and vigour index recorded in nursery were higher in *P. fluorescens* treatments.

Almost similar results were recorded in the present study conforming the findings of earlier researchers.

**Table : Effect of different combination of *Pseudomonas fluorescens*, *Rhizobium sp.* and *Trichoderma sp.* on various plant growth parameters of chickpea.**

S.N.	Treatment	Root Length (cm)	Shoot Length (cm)	Germination (%)	Vigour Index	% Increase in Root length	% Increase in Shoot length	% Increase in Vigour index
1	<i>P. fluorescens</i> (Seed) - T1	12.7	18.7	95.0	2982.41	16.70	37.28	102.94
2	<i>P. fluorescens</i> (Soil) - T2	13.7	18.4	85.0	2729.56	26.39	34.86	85.74
3	<i>P. fluorescens</i> (Spray) - T3	14.2	17.9	50.0	1603.31	30.76	31.04	9.10
4	<i>P. fluorescens</i> (Seed + soil) - T4	13.9	16.0	90.0	2684.33	27.81	16.98	82.66
5	<i>P. fluorescens</i> (Seed + Spray) - T5	13.9	18.1	70.0	2234.29	27.81	32.31	52.03
6	<i>P. fluorescens</i> (Spray + Soil) - T6	13.9	18.1	75.0	2393.88	27.81	32.31	62.89
7	<i>P. fluorescens</i> (Seed + Soil + Spray) - T7	14.1	17.9	70.0	2241.64	30.03	31.31	52.53
8	<i>P. fluorescens</i> (spray) + <i>Trichoderma sp.</i> (soil) - T8	14.2	17.8	75.0	2400.42	30.76	30.60	63.34
9	<i>P. fluorescens</i> (soil) + <i>Trichoderma sp.</i> (spray) - T9	14.2	17.9	50.0	1601.58	30.76	30.79	8.98
10	<i>P. fluorescens</i> (seed) + <i>Rhizobium sp.</i> (seed) - T10	15.2	19.1	85.0	2919.04	40.34	40.11	98.63
11	<i>P. fluorescens</i> (seed) + <i>Rhizobium sp.</i> (seed) + <i>Trichoderma</i> (spray) - T11	14.0	17.6	60.0	1892.30	28.85	28.70	28.76
12	<i>P. fluorescens</i> (spray) + <i>Rhizobium sp.</i> (seed) + <i>Trichoderma sp.</i> (soil) - T12	14.1	17.8	70.0	2230.50	29.60	30.49	51.78
13	Control - T13	10.8	13.7	60.0	1469.59	-	-	-
SE <sub>(m)</sub> ±		1.22	1.52					
CD (5%)		3.49	4.34					

Appl. Environ. Microbiol. 71 : 1685–1693.

### REFERENCES

- Abdul Baki, A.A. and Anderson, J.D. 1973. Vigour determination in soybean seeds by multiple criteria. *Crop Sci.*, 13: 630-633.
- Astom, B. and Gerhardoson, B. 1988. Differential reactions of wheat and pea genotypes to root inoculation with growth affecting rhizosphere bacteria. *Plant and Soil*, 109: 263-269.
- Compant, S., Reiter, B., Sessitsch, A., Nowak, J., Clément, C. and Barka, E. A. 2005. Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia sp.* strain PsJN;
- Dobbelaere, S., Vanderleyden, J., and Okon, Y. 2003. Plant growth-promoting effects of diazotrophs in the rhizosphere. *Crit. Rev. Plant Sci.* 22:107-149.
- Dubey, S.K. 1996. Combined effect of *Bradyrhizobium japonicum* and phosphate-solubilizing *Pseudomonas striata* on nodulation, yield attributes and yield of rainfed soyabean (*Glycine max*) under different sources of phosphorus in Vertisols. *Ind. J. Microbiol.*, 33: 61-65.
- Emmert, E.A.B, and Handelsman, J. 1999. Biocontrol of plant disease: a (Gram-) positive

- perspective. *FEMS Microbiol. Lett.*, 171:1-9.
7. Glick, B.R., Patten, C.L., Holguin, G., and Penrose, D.M. 1999. *Biochemical and Genetic Mechanism Used by Plant Growth Promoting Bacteria*. Imperial College Press, London.
  8. Mew, T.W. and Rosales, A.M. 1986. Bacterization of rice plants for control of sheath blight caused by *Rhizoctonia solani*. *Phytopathology*, 76: 1260-1264.
  9. Nandakumar, R., Babu, S., Viswanathan, T., Raghuchander, T. and Samiyappan, R. 2001. Induction of systemic resistance in rice against sheath blight diseases by plant growth promoting rhizobacteria. *Soil Biol. Biochem.*, 33:603-612.
  10. O'callaghan, M., Swaminathan, J., Lottmann, J., Wright, D.A. and Jackson, T.A. 2006. Seed coating with biocontrol strain *Pseudomonas fluorescens* f113. *New. Zeal. Plant Protect.*, 59: 80-85.
  11. Raji, P. and Lekha, B.N. 2003. *Pseudomonas fluorescens* for enhancing plant growth and suppressing sheath blight of rice. 6th International PGPR Workshop 5-10 October, Calicut, India.
  12. Ramesh, R. and Korikanthimath. 2003. Use of rhizosphere bacteria and soil fungi for the management of damping off and wilt in brinjal. 6th International PGPR Workshop 5-10, October, Calicut, India.
  13. Ramamoorthy, V., Raghuchander, T. and Samiyappan, R. 2002. Enhancing resistance of tomato and hot pepper to pythium diseases by seed treatment with fluorescent *Pseudomonads*. *Eur. J. Plant Pathol.*, 108: 429-441.
  14. Rao, C.H. and Johri, B.N. 1999. Seed and root extracts in chemotaxis, agglutination, adherence and root colonization of soybean (*Glycine max*) by fluorescent *pseudomonads*. *Indian J. Microbiol.*, 39: 31-38.
  15. Stephen, K.B., Slinkard, A.E. and Walley, F.L. 2002. Evaluation of rhizobial inoculation methods for chickpea. *Agron. J.*, 94: 851-859.
  16. Valverde, A., Burgos, A., Fiscella, T., Rivas, R. and Quez et al., 2006. Differential effects of coinoculations with *Pseudomonas jessenii* PS06 (a phosphate-solubilizing bacteria) and *Mesorhizobium ciceri* C-2/2 strains on the growth and seed yield of chickpea under greenhouse and field conditions. *Plant Soil*, 287: 43-50.
  17. Vidyasekaran, P. and Muthamilan, M. 1995. Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Dis.*, 79: 782-786.
  18. Whipps, J.M. 2001. Microbial interactions and biocontrol in the rhizosphere. *J. Exp. Bot.* 52:487-511.
  19. Yadegari, M., Rahmani, H.A., Noormojammadi, G. and Ayneband, A. 2008. Evaluation of bean (*Phaseolus vulgaris*) seeds inoculation with *Rhizobium phaseoli* and plant growth promoting rhizobacteria on yield and yield components. *Pak. J. Biol. Sci.*, 11:1935-1939.