Available online at www.elixirpublishers.com (Elixir International Journal)

Agriculture

Elixir Agriculture 30 (2011) 1774-1777

ISSN: 2229-712X

# Antagonistic efficiency of pseudomonas strains against soil borne disease of chickpea crop under in vitro and in vivo

D.P Patel, H.B Singh, S.Shroff and J.Sahu

Department of Mycology and Plant Pathology, Institute of Agriculture Sciences, Banaras Hindu University Varanasi-221005(India).

**ARTICLE INFO**

**Article history:**

Received: 14 November 2010;

Received in revised form:

12 December 2010;

Accepted: 22 December 2010;

**Keywords**

Rhizoctonia bataticola,

Sclerotinia sclerotiorum.

**ABSTRACT**

The test on *in vitro* antagonistic acitivity in dual culture showed significance reduction in the radical growth of *Rhizoctonia bataticola* and *Sclerotinia sclerotiorum* over control (90 mm colony diameter) and in the field trial study seed treatment with strains of *Pseudomonas fluorescence* significantly reduced the disease incidence of dry root rot and stem rot in all treatments in comparison to uninoculated control, however *Pseudomonas fluorescence* strains $BHUPf_4$ was found more effective in mycelial growth reduction as well as disease reduction of both fungal pathogen namely *Rhizoctonia bataticola* and *Sclerotinia sclerotiorum* this is followed by strains $BHUP_5$, $BHUP_6$ and BHUPsb.



## Introduction

*Pseudomonas fluorescences* are widely used in agriculture as biopesticides, biporotectants, biostimulants and bio-fertilizers applied on wide variety of plants (Woo *et al.*, 2006). *Pseudomonas* are the most extensively studied biocontrol agents for management of soilborne and seed borne pathogen as they play an important role in managing the natural population of fungal pathogens. The chosen susceptible variety of chickpea, Radhey is a medium maturing variety (150 days) plant are tall, semispreading with light green foliage, flowers are pink in colour, seeds are smooth, light brown and bold. It is suitable for growing in Eastern and Central region of U.P. and it has yield potential of 25-30 q / ha. with this perspective the experiment is taken up to fulfill the objective of antagonistic efficacy of selected strains of *Pseudomonas fluorescence* against soilborne fungus *Sclerotinia sclerotiorum* and *Rhizotonia bataticola in vitro* as well as *in vivo*.

The PGPR, especially *Pseudomonas flouresences* assist in plant colonization and suppress various plant diseases caused by soil borne pathogenic microorganisms (Handelsman and Stabb, 1966). The disease suppressive effect of rhizospheric microorganism may involve mechanism such as production of antibiotics (Hebber *et al*., 1992), iron sequestrating compound siderophore (Loper and Bayer, 1991), extracellular hydrolytic enzyme (Fridlender *et al*., 1993), other secondary metabolites like Hydrocyanic acid (HCN) and induced systemic resistance. Soil *pseudomonas* is excellent biological control agents against disease caused by soilborne plant pathogenic fungi, they are efficient root colonizers on crop plants. The mechanisms of plant diseases control by soil pseudomonas includes production of antibiotics, siderophores, volatile compounds like HCN, ammonia, and induction of systemic resistant and competition for nutrients (O'Sallivan and O'Gara, 1992). Kumar *et al*. (1996) showed that a siderophore producing *Pseudomonas* strains, was inhibiting to *Fusarium oxysporum* f.sp. *ciceri*, *F. udum* under iron limiting condition. *Pseudomonas* colonizes on the root systems through seed bacterization and exhibit antagonism against soilborne phytopathogenic fungi (Nautiyal, 1997) and screened large number of *Pseudomonas* strains and found *Pseudomonas fluorescence* NBRI-1303R effective against *Fusarium oxysporum* f.sp. *ciceri*, *Rhizoctonia bataticola* and *Pythium* spp. Antagonistic effect of *Pseudomonas fluorescence* against *S. rolfsii* has been reported by Rangeshwaran and Prasad (2000) under dual culture as well as field conditions reduced infection 60 to 63%. Goel *et al*. (2002) conducted field study and demonstrated that *Pseudomonas fluorescence* as potential biocontrol agent against *Rhizoctonia solani*, *Pythium* and *Fusarium fsp. ciceri*. In a study, Vinod Kumar *et al*. (2007) revealed from field study that *Pseudomonas fluorescence* PF-4 was effective controlling dry root rot of chickpea caused by *Macrophomia phaseolina* and also reported increase in plant height by 25%, root length by 34.6% and shoot length by 16%.

## Material and Methods

Diseased chickpea plant showing typical symptoms of root rot and stem rot were collected from experimental field of Department of Mycology and Plant Pathology, I. Ag. Sc., B.H.U. The pathogens *Sclerotinia sclerotiorum* causing stem rot and *Rhizoctonia bataticola* causing dry root rot were isolated from the freshly infected stem and infected root respectively on PDA medium. Freshly infected chickpea stem and roots were washed thoroughly with distilled water.

A small portion of diseased tissues along with a portion of adjacent healthy tiss were cut into small pieces (3 to 5 mm in length) and then surface sterilized with 0.1% $HgCl_2$ for 30 seconds. The pieces then were rinsed thrice with sterilized distilled water. The surface sterilized and rinsed pieces were inoculated aseptically on sterilized petriplate containing PDA medium.

The inoculated petriplates were incubated at 20°C to 25°C for five to six days. When the fungal colony developed, a small cut of single mycelium is transferred on another petriplate containing PDA medium to obtain pure culture. The pure culture of the pathogens *Rhizoctonia bataticola* and *Sclerotinia sclerotiorum* were maintained in PDA slants and

Tele:

E-mail addresses: devprakashdev_2007@rediffmail.com

sharadshroff1984@gmail.com

after full growth the slants were stored and in a refrigerator at 4°C. The pure culture were maintained throughout the period of investigation by periodic sub culturing on fresh media and stored in refrigerator at 4°C.

**Pathogenicity Test**

*Sclerotinia sclerotiorum* causing stem rot and *Rhizoctonia bataticola* causing root rot of chickpea were confirmed on healthy chickpea plants grown on sterilized soil in pots. 25 cm diameter earthen pots containing 5 kg of sterilized soil were taken for the study. The soil was inoculated with inoculum of *Sclerotinia sclerotiorum* and *Rhizoctonia bataticola* grown on PDA medium. The inoculum containing mycelial propagules and sclerotia were applied one week before seed sowing. Five healthy chickpea seeds were sown per pot. Pots containing only sterilized soil were kept as a control the pathogenicity test for *Sclerotinia sclerotiorum* and *Rhizoctonia bataticola* were carried out separately.

**Source and maintenance of *pseudomonas* strains**

All four strains of *Pseudomonas fluorescence* (BHUPf-4, $BHUP_6$, $BHUP_5$ and BHUPsb) were obtained from Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, B.H.U., Varanasi. The purified strains of *Pseudomonas were* maintained in pure form in King's B medium by transferring in fresh medium periodically and storing in refrigerator at 4°C.

**Dual culture technique**

The strains of *Pseudomonas fluorescence* (BHUPf-4, $BHUP_6$, $BHUP_5$ and BHUPsb) were evaluated against *Rhizoctonia bataticola* and *Sclerotinia sclerotiorum* separately in laboratory by dual culture techniques as described by Haung and Hoes (1976) to screen out the most efficacious one. Petridishes (90 mm) containing nutrient agar medium were inoculated with *Pseudomonas* and *Rhizoctonia bataticola* and same for *Sclerotinia sclerotiorum* at equal distance from the periphery of the plate. Inoculated plates were incubated at 25±1°C in BOD indicator and the radial growth of pathogen (*Rhizoctonia* and *Sclerotinia*) was measured at interval of 24 hours upto7 days after incubation. Controls without *pseudomonas* were maintained and each treatment was replicated thrice.

Observations were recorded after 7 days of inoculation on area covered by the *pseudomonas* strains and pathogen Inhibition of mycelial growth of pathogenic fungi by each strains was recorded.

**Percentage growth inhibition was calculated as per formula by Arora and Upadhyay (1978) given below:**

$$\%\text{growth inhibition} = \frac{\text{Colony growth in control plate - colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

**Preparation of *pseudomonas* strains**

Four strains of *Pseudomonas fluorescence* were used in the present study. Pure culture of *Pseudomonas* were grown in King'S B broth medium and after 10 days the broth media were centrifuged at 10000 rpm to obtain pure bacterial pellets.

**Mode of application of antagonists**

Chickpea seeds were surface sterilized with 70% ethanol for 5 minutes and rinsed thrice in sterilized distilled water. Seeds were first coated with few drops of 2% gum arabic as an adhesive and uniformly coated with the cell pellets of different strains of *pseudomonas* and kept for about 30 minutes. Seeds treated with only sterile distilled water with gum arabic served as the untreated control. Seeds treated with different strains were then sown in the field.

$T_3$ = $BHUP_6$
$T_4$ = BHUPsb
$T_1$ = $BHUTPf_4$
$T_2$ = $BHUP_5$
$T_5$ = Uninoculated control

**Field experiments**

A field trial was conducted to evaluate the biocontrol potential of different strains of *pseudomonas* in chickpea crop against *Sclerotinia sclerotiorum* and *Rhizoctonia bataticola* under field condition.

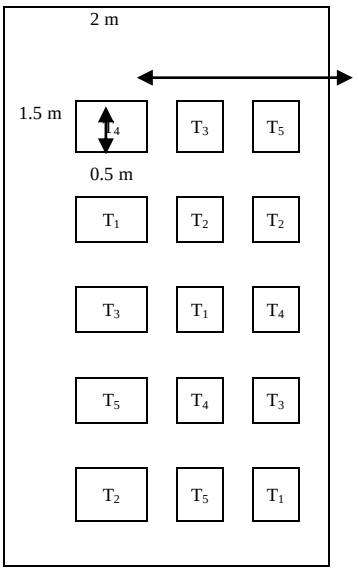
| Month | Year | Average Temperature | | Total Rainfall (mm) | Relative humidity(%) | |
|---|---|---|---|---|---|---|
| | | Max. | Min. | | Max. | Min. |
| November | 2007 | 28.05 | 13.05 | 0.00 | 81.75 | 39.25 |
| December | 2007 | 25.00 | 9.00 | 0.00 | 80.50 | 38.00 |
| January | 2008 | 22.64 | 9.28 | 2.36 | 79.00 | 43.20 |
| February | 2008 | 27.30 | 11.17 | 4.15 | 77.25 | 42.25 |
| March | 2008 | 34.10 | 17.50 | 0.00 | 61.25 | 30.25 |
| April | 2008 | 37.00 | 21.10 | 0.00 | 53.00 | 32.70 |

The selected field had a known history of sick plot with *Sclerotinia sclerotiorum* and *Rhizoctonia bataticola* for last several years.

The seeds of highly susceptible chickpea variety, Radhey were treated with different strains of *pseudomonas* and left for 30 mins. in shade for natural drying. Then the seeds were sown in randomized block design with three replication of each treatment. Sowing of chickpea was done on 28th October 2007 in plot size of 2 x 1.5 $m^2$ with spacing of 30 x 10 cm row to row and plant to plant respectively.

A control plot was maintained by treating the seeds of chickpea only with gum arabic. The observation for plant mortality and incidence of disease were recorded at 15 days interval from germination to maturity of crop.

**Table : Meteorological data during experiment (November, 2007- April, 2008)**




Source: Department of Agronomy, I.Ag.Sc., B.H.U., Varanasi

**Layout plan of the field Experiment:-**

**Data analysis**

The observation were recorded analyzed statistically in completely randomized design (factorial) for *in vitro* experiment and in randomized black design for field experiment Gomez and Gomez, (1984).

**Result and discussion**

Antagonistic effect of four strains of *Pseudomonas fluorescence viz*., $BHUPf_4$, $BHUP_6$, $BHUP_5$ and BHUPsb were studied *in vitro* against *Rhizoctonia bataticola* and *Sclerotinia sclerotiorum* in PDA medium by dual culture methods. The result showed (Table:-1) that all the antagonistic strains of *Pseudomonas* used in the present study, restricted the mycelial growth of *Rhizoctonia bataticola* and *Sclerotinia sclerotiorum* significantly. Out of all these antagonistics strain $BHUPf_4$ found significantly highest mycelial growth inhibition against both fungus this is followed by *strains* $BHUP_5$, $BHUP_6$ and BHUPsb . This is on conformity of the finding made by (Krishnamurthy and Gnananamanikam, 1998) they reported that antagonists of *Pseudomonas* spp. against several fungus both *in vivo* and *in vitro* condition. Goel *et al*. (2002) also reported from field study that *Pseudomonas* strains as potential biocontrol agents against *Rhizoctonia solani*, *Pythium* spp. and *Fusarium* f.sp *ciceri* under culture condition as well as field experiment. Inhibition of chickpea root rot pathogen by *Pseudomonas* was also reported by Selvarajan and Jeyarajan (1996).. It was further demonstrated that all the strains of *Pseudomonas* exhibited antagonistic effect leading to reduced radial growth of the test pathogens namely *Rhizoctonia bataticola* ranged from (16 to 47 mm) and *Sclerotinia sclerotiorum* ranged from (23 to 45 mm). Similar results were also obtained by Samanta *et al*. (2004) by *Pseudomonas fluorescence* against *Macrophomina phaseolina*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*. Successful control of stem rot and root rot *by Pseudomonas* was demonstrated by several workers (Sharma, 1994; Sharma *et al*., 1999). In *in vivo* experiment (Table:-2) reduction in incidence of disease by *Pseudomonas* strains were 33 to 44% against *Rhizoctonia bataticola* and 16 to 32% against *Sclerotinia sclerotiorum*. However *pseudomonas* stains $BHUPf_4$ showed more effective against both the fungal diseases and followed by *strains* $BHUP_5$, $BHUP_6$ and BHUPsb. Kumar *et al*. (2007) suggested the extracellular secretion of antifungal by *Pseudomonas fluorescence* and also suggested a significant role of secondary metabolites such as antibiotics siderophore in suppression of fungal pathogens. Biological mechanism of *Pseudomonas* may involve mechanism such as production of antibiotics (Hebbar *et al*., 1992), iron sequestering compounds, siderophores (Loper and Buyer, 1993), extracellular hydrolytic enzymes (Fridlender *et al*., 1993), other secondary metabolites like HCN.

**Table 1: Antagonistic activity of *Pseudomonas* strains in dual culture**

| Treatments | *Rhizoctonia bataticola* | | *Sclerotinia sclerotiorum* | |
|---|---|---|---|---|
| | Colony diameter (mm) | % inhibition | Colony diameter (mm) | % inhibition |
| *Pseudomonas* strains | | | | |
| $BHUPf_4$ | 29 | 67 | 30 | 66 |
| $BHUP_5$ | 38 | 58 | 35 | 61 |
| $BHUP_6$ | 35 | 61 | 38 | 55 |
| BHUPsb | 47 | 48 | 45 | 52 |
| Control | 90 | - | 90 | - |

**Table 2: Effect of *Pseudomonas* strains on plant mortality of chickpea crop due to *Rhizoctonia bataticola* and *Sclerotinia sclerotiorum***

| Treatments | *Rhizoctonia bataticola* | | *Sclerotinia sclerotiorum* | |
|---|---|---|---|---|
| | % plant mortality | % disease reduction | % plant mortality | % disease reduction |
| *Pseudomonas* strains | | | | |
| $BHUPf_4$ | 14.0 | 44.0 | 13.3 | 31.6 |
| $BHUP_5$ | 14.0 | 44.0 | 14.0 | 22.2 |
| $BHUP_6$ | 14.4 | 42.4 | 14.0 | 22.2 |
| BHUPsb | 16.6 | 33.6 | 15.0 | 16.6 |
| Control | 25.0 | - | 18.0 | - |

**Summary**

Chickpea suffers from various fungal diseases but in the present study deals with the dry rot caused by *Rhizoctonia bataticola* and stem rot caused by *Sclerotinia sclerotiorum*. In the present study biocontrol potential of four strains of *Pseudomonas viz*. $BHUPf_4$, $BHUP_5$, $BHUP_6$, BHUPsb were studied both *in vitro* and in vivo condition. The test on the *in vitro* antagonistic acitivity in dual culture showed significance reduction in the radial growth of *Rhizoctonia bataticola* and *Sclerotinia sclerotiorum*. However, *Pseudomonas* strains $BHUPf_4$ was found more effective in reduction of mycelial growth of both fungal pathogen viz; *Rhizoctonia bataticola* and *Sclerotinia sclerotiorum* over control (90 mm colony diameter). In the field experiment study seed treatment with strains of *Pseudomonas fluorescence* reduced the disease incidence of dry root rot and stem rot significantly in all treatments in comparison to uninoculated control. *Pseudomonas* strains $BHUPf_4$ found maximum reduction in disease and followed by strains $BHUP_5$, $BHUP_6$, and BHUPsb. Results obtain from all *in vitro* and *in vivo* studies suggested that all strains of *Pseudomonas* used in experiment can be applied to control dry root rot and stem rot of chickpea.

**References**

1. Friedlender, M., Inbar, J., Chet, I. (1993). Biological control of soilborne plant pathogens by a B-1, 3-gluconase-producing *Pseudomonas cepacia*. *Soil Biol Biochem* 25: 1211-1221.
2. Goel, A.K., Sindhu, S.S., Dadarwal, K.R. (2002). Stimulation of nodulation and plant growth of chickpea by *Pseudomonas* spp. antagonistic to fungal pathogens. *Biol. Fertil Soils*. 36: 391-396.
3. Gomez, K.A. and Gomez, A.A. (1984). Statistical procedures for Agricultural Research, John Wiley and Sons, Singapore, pp. 139-153.
4. Handelsman, J., Stabb, E.V. (1996). Biocontrol of soilborne plant pathogens. *Plant Cell* 8: 1855-1869.
5. Hebber, K.P., Davey, A.G., Dart, P.J. (1992). Rhizobacteria of maize antagonistic to *Fusarium moniliformae*, a soilborne fungal pathogen: Colonization of rhizosphere and roots. *Soil Biol Biochem* 24: 989-997.
6. Krishnamurthy, K. and Gnanamanickam, S.S. (1998). Bicontrol of rice sheath blight with formulated *Pseudomonas putida*. *Indian Phytopath.* 51(3): 233-236.
7. Kumar, B., Chahal, S.S. and Aulakh, K.S. (1996). Seed health impairment caused by *Rhizopus* head rot in sunflower. *Pl. Dis. Res*. 11: 9-11.
8. Kumar, V., Kumar, A., Verma, V.C., Gund, S.K. and

Kharwar, R.N. (2007). Induction of defence enzymes in *Pseudomonas fluorescence* treated chickpea roots against *Macrophomina phaseolina*. *Indian Phytopath*. 60(3): 289-295.
9. Loper, J.E. and Buyer, J.S. (1991). Siderophore in microbial interaction on plant surfaces. *Mol. Plant-Microbe Interact*. 4: 5-13.
10. Nautiyal, C.S. (1997). Selection of chickpea Rhizosphere – competent *Pseudomonas fluorescens* NBRI 1303 antagonistic to *Fusarium oxysporum* f.sp. *ciceri*, *Rhizoctonia bataticola* and *Pythium* sp. *Current Microbiology*. 35: 52-58.
11. O'Sullivan, D.J. and O'Gara, F. (1992). Trials of fluorescens *Pseudomonas* spp. involved in suppression of plant root pathogens. *Microbiological Review* 56: 662-667
12. Rameshwaran, R., Prasad, R.D. (2000). Biological control of *Sclerotium rolfsii* rot of sunflower. *Indian Phytopath*. 53: 444-449.
13. Samanta, S.K. and Dutta, S. (2004). Potential of native plant growth promoting rhizobacteria in the management of *Sclerotinia* stem rot of mustard. *J.Mycol. Pl. Pathol*. 34(3): 761-768.
14. Selvarajan, R. and Jeyarajan, R. (1996). Inhibition of chickpea root rot pathogens (*Fusarium solani* and *Macrophomina phaseolina*) by antagonists. *Indian J. Mycol. Pl. Pathol*. 26: 248-251.
15. Sharma, B.K. (1994). Efficacy of biocontrol agents for the control of chickpea stem rot. *J. Biol. Contol*. 8: 1 Sharma, S.K., Verma, B.R. and Sharma, B.K. (1999). Biocontrol of *Sclerotinia sclerotiorum* causing stem rot of chickpea. *Indian Phytopath*. 50: 316.15-117.
16. Woo, S.L., Scala, F., Ruoco, M. and Lorito, M. (2006). The molecular biology of the interactions between *Trichoderma* spp., Phytopathogenic fungi and plants. *Phytopathology* 96(2): 181-185.