Biocontrol Potential of Plant Growth Promoting Rhizobacterium (PGPR)-
*Pseudomonas* against Plant Pathogenic Fungi

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**Abstract**

Soil borne plant pathogenic fungi are of major concern in agriculture. The plant diseases need to be controlled to maintain the level of yield both quantitatively and qualitatively. Biocontrol methods are safe, cost effective and eco-friendly. Understanding the mechanism by which the biocontrol of plant diseases occurs is critical to the eventual improvement and wider use biocontrol method. Many microbial antagonists such as *Pseudomonas*, *Bacillus* and *Trichoderma* have been reported to possess antagonistic activities against plant fungal pathogens. The present study was aimed to screen the antagonistic activity of *Pseudomonas* against *Fusarium oxysporum*, *Fusarium solani*, *Pythium aphanidermatum* and *Sclerotina sclerotiorum*. *Pseudomonas* exhibited the maximum inhibition of growth of *Fusarium oxysporum* which is responsible for the wilt disease in pea.

**Keywords**

Biocontrol, *Pseudomonas*, Antagonist, *Fusarium*

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**Introduction**

Agriculture is heavily dependent on the use of chemical fertilizers and pesticides to achieve higher yields. This dependence is associated with problems such as environmental pollution, interruption of natural ecological nutrient cycling and destruction of agriculturally important biological communities. The use of bio resource to replace chemical fertilizers and pesticides is growing. In this context, plant growth promoting rhizobacteria (PGPR) are novel and potential tools to provide substantial benefits to agriculture (Sivasakthi et al., 2013).

Plant growth is influenced by many abiotic and biotic factors. Rhizosphere is a thin layer of soil immediately surrounding plant roots that is extremely important and active area for root activity and metabolism. A large number of organisms such as bacteria, fungi, protozoa co-exist in the rhizosphere, bacteria being the
most abundant among them. However, there is a vast array of pathogenic microorganisms in the rhizosphere that cause diseases to the plants affecting their growth and yield. Fusarium has been recognized for a long time as being important plant pathogen (Booth, 1971; Nelson et al., 1983). Fusarium solani exists in the soil and attacks more than 2000 species of plants (Parmeter, 1970). Sudana et al., (1999) reported that ten millions of banana trees died because of wilt disease caused by fungus, Fusarium oxysporum within two years (2007-2009). Wilt caused by Fusarium oxysporum is one of the most devastating disease of pea. Pea wilt alone is responsible for 93% disease total yield in India (Sharma et al., 2006). Sclerotina sclerotiorum is another important plant pathogenic fungi that has been reported as a pathogen of more than 400 plant species around the world (Boland and Hall, 1994). White mold is a very serious problem in crops, especially when they are cultivated in contaminated wet soils and the weather is cool and wet (Reis and Lopes, 2007).

Plant growth promoting rhizobacteria (PGPR) are free living soil borne bacteria which enhance the growth of plant either directly or indirectly (kloeper et al., 1980). The direct mechanisms involve nitrogen fixation, phosphorus solubilization, HCN production of phytohormones such as auxins, cytokinins, and gibberellins (Glick, 1995). Indirect mechanism includes combating various soil borne diseases that helps in better plant growth.

PGPR are indigenous to soil and the plant rhizosphere and play a major role in the biocontrol of the plant pathogens. They can suppress broad spectrum of bacterial, fungal and nematode diseases. The use of PGPR has become a common practice in many regions of the world, owing to significant control of the plant pathogens demonstrated by PGPR in the laboratory, greenhouse and field experiments. Recent progress in our understanding of their diversity, colonizing ability and mechanism of action, formulation and application should facilitate their development as reliable biocontrol agents against plant pathogens (Siddiqui, 2006).

Pseudomonas is the principal rhizobacterium with at tremendous potential for biological control against a wide range of plant pathogen (Kremer and Kennedy, 1996). Pseudomonas sp.is a ubiquitous bacterium in agricultural soils and has many traits that qualify it as PGPR. Many microbial antagonists have been reported to possess antagonistic activities against plant fungal pathogens, such as Pseudomonas fluorescens, Bacillus subtilis, Bacillus cereus, Trichoderma viride and Trichoderma harzianum. The successful control by these antagonists mainly against the diseases caused by different pathogenic genera viz. Fusarium, Pythium, Aspergillus, Alternaria, Rhizoctonai and Botrytis displays an important role in stimulating growth and enhancing yield traits of various crops(Gardener, 2006). This paper describes briefly the biocontrol potential of microbial antagonists particularly against plant fungal pathogens.

Materials and Methods

Antagonist activity of isolates of Pseudomonas against four fungi viz, Fusarium solani, Fusarium oxysporum, Pythium aphanidermatum and Sclerotinia sclerotiorum on potato dextrose agar (PDA) plates was examined by dual inoculation technique (Sakthivel et al., 1986). The fungal pathogen was inoculated on the plates containing potato dextrose agar medium and incubated at 28 ± 2°C for 72 hours. With the help of sterile cork borer, the disc of fungal growth from this plate was taken out and placed at the center of the fresh potato
Dextrose agar medium containing plate 24 hours old growth of bacterial isolate was then streaked on either side of the disc and kept for incubation at 28 ± 2°C for 72 hours. After the incubation for 72 hours, the plates were visually observed for the inhibition of fungal pathogen by comparing with the control PDA plate inoculated with only fungal pathogen. Radial growth of the fungus was measured and inhibition percentage was calculated by the formula:

\[
\% \text{ inhibition} = \left( \frac{C - T}{C} \right) \times 100
\]

Where,

C = Colony diameter (mm) of the control
T = Colony diameter (mm) of the test plate

**Results and Discussion**

Isolates of *pseudomonas* were evaluated for antifungal activity against four fungal plant pathogens i.e. *Fusarium solani*, *Fusarium oxysporum*, *Pythiumphanidermatum* and *Sclerotina sclerotiorum*. All tested fungal strains showed significant reduction in terms of radial growth after the treatment with *Pseudomonas* cultures, in comparison with the controls (table1).

The growth behaviour of test fungi with the culture of antagonist bacterium *Pseudomonas* sp. varied considerably. The culture of *Fusarium oxysporum* showed minimum colony diameter (0.78 mm) followed with *Fusarium solani* (0.84 mm), *Pythium aphanidermatum* (0.86 mm) and *Sclerotina sclerotiorum* (0.98 mm). The maximum inhibition in colony diameter was observed in *Fusarium oxysporum* (56.72%), followed by *Fusarium solani* (51.28%), *Pythium aphanidermatum* (40.16 %) and *Sclerotina sclerotiorum* (32.23 %) over control. *Pseudomonas* sp was found more effective for the control of *Fusarium oxysporum* which causes more and more loss in the productivity of pea crops in these areas. Our results corroborate with those of Singh *et al.*, (2011).

| S.No | Name of the fungi         | Colony diameter (in mm) | % inhibition (in mm) |
|------|---------------------------|-------------------------|----------------------|
| 1    | Control                   | 60                      | 00                   |
| 2    | *Fusarium oxysporum*      | 0.78                    | 56.72                |
| 3    | *Fusarium solani*         | 0.84                    | 51.28                |
| 4    | *Pythium aphanidermatum*  | 0.86                    | 40.16                |
| 5    | *Sclerotina sclerotiorum* | 0.98                    | 32.23                |

In general, the potential antagonistic microorganisms selected from *in vitro* tests often fail to effectively control plant disease in green house or field trails, particularly to soil borne pathogens. Several factors such as the type of content of organic matter, pH, nutrients, and moisture level of the soil influence the efficacy of biocontrol agents. Due to the variations in the environment factors from one place to other places, sometimes, a good biocontrol agent under *in vitro* conditions, fails under *in vivo* conditions. To achieve the success, the environmental factors should be similar to those from which the biocontrol agents were isolated. Likewise, the method of the application can influence the success of the field trials. In general, there are three means of applying the antagonists for biocontrol, namely seed inoculation, vegetative part inoculation and soil inoculation. Field trail showed that the control efficiency of
antagonist *Burkholderia cepacia* strain N9523 against *Phytophthora capsici* was higher by soil-drenching them by wounded stem inoculation (Lee et al., 1999; Dawar et al., 2008) tested the biocontrol potential of different microbial antagonists, *Bacillus, thuringiensis, Rhizobium meliloti, Aspergillus niger* and *Trichoderma harzianum* by coating the seeds with gumarabic, glucose, sucrose and molasses. This method reduced successfully the infection of root rot fungi on okra and Sunflower, i.e. *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium spp.* The highest suppression capacity was shown by seed treatment with *T. harzianum* using 2% of glucose.

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