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BIOCONTROL POTENTIALITIES OF NATIVE *PSEUDOMONAS* ISOLATES AGAINST PLANT PATHOGENIC FUNGI *RHIZOCTONIA* SPP., *FUSARIUM* SPP. AND *COLLETOTRICUM* SPP. IN TOMATO RHIZOSPHERE UNDER GREEN HOUSE CONDITION.

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ABSTRACT

A total of four *Pseudomonas* isolates were obtained from rhizospheric soil of Korea district of Chhattisgarh, India. Two were identified as *Pseudomonas syringae*, another two were as *Pseudomonas alcaligenes* from IARI Delhi. Soil *Pseudomonas* spp. were examined for their antagonistic effect on a fungal pathogen *Fusarium* spp., *Rhizoctonia* spp. and *Colletotrichum* spp. in *in-vitro* plate assay and *in-vivo* green house condition. The result from *in-vitro* analysis showed that PKJ25 was the most active isolate and significantly suppressed the vegetative growth of all the test fungi by restricting the hyphal growth of *Rhizoctonia* spp., *Fusarium* spp. *and Colletotricum* spp. to 0.73, 1.54 and 2.01cm with 91.20%, 78.90% and 77.67% inhibition. *In vivo*, PKJ25 had the least disease incidence in tomato and highest percent disease control. The survival % of tomato plantlets was significantly higher with isolate PKJ25 in comparison to other isolates and was recorded 93% in case of damping off due to *Rhizoctonia* spp. , 86% in case of wilt due to *Fusarium* spp., 86% in case of fruit anthracnose due to *Colletotrichum* spp. This result shows that *Pseudomonas* isolate PKJ25 *(Pseudomonas alcaligenes)* was very effective biocontrol agents and should be exploited for further biocontrol applications.

INTRODUCTION

Tomato is one of the economically important vegetable crops in most regions of the world. The productivity of tomatoes have declined due to various infections and diseases that includes Bacterial wilt, root knot nematodes disease, early blight, late blight, Fusarium wilt, damping - off and anthracnose. The causal agent of damping - off of tomato is *Rhizoctonia* spp., the fungus that causes Fusarium wilt in tomato is *Fusarium* spp. and *Colletotrichum* spp. is the causal agent of Anthracnose in tomato. Several microorganisms are being used in the biocontrol of tomato pests and diseases.

In biological control, microorganisms isolated from plants and soils are employed to protect crop plants from disease. Applications of these specific biocontrol agents can suppress the growth and number of phytopathogens by manipulating their physicochemical and microbiological environment. *Pseudomonas* spp. is one of the most promising groups rhizobacteria which are known to impart an important role in plant growth promotion and disease suppression (Kloepper *et al.,* 1980; Jayaswal *et al.,* 1993) and are able to control pathogenic soil- borne microorganisms (O'Sullivan and O'Gara, 1992). They show antagonistic activity against diverse phytopathogens such as *Rhizoctonia* spp. (Howell and Stipanovic, 1979), *Fusarium* spp. (Olivain *et al.,* 2004) *and Colletotrichum falcatum (*Viswanathan and Samiyappan, 1996*)*. Various species of *Pseudomonas* such as *P.fluorescence* (Howell and Stipanovic, 1980; Weller and Cook, 1983) and *P.cepacia* (Jayaswal *et al.,* 1993) have been considered as potential biological control agents. *P. fluorescens* has been used as a biocontrol agent to manage bacterial wilt of tobacco and cucumber (Liu *et al.,* 1999), *Xanthomonas oryzae pv. oryzae* in rice (Vidhyasekaran *et al.,* 2001). Shalini and Srivastava (2008) screened out antifungal activities of *P. fluorescence* against phytopathogenic fungi and have an excellent potential to be used as biocontrol agents of fusarium oxysporum in tomato greenhouses at the field level (Asha *et al.,* 2011). Mina *et al.* (2013), Sharma *et al.* and Mezeal, (2014) reported antifungal activity of fluorescent Pseudomonads against *R. solani* by production Secondary metabolites. The mycelial growth of *R. solani* was inhibited up to 1.9 (cm) by rhizobacterial isolates PTR-3 and were found to exhibit antagonism of over 68.9% which is followed by PCF-3(65.6%) as revealed by Kamei *et al.* (2014).

The present work is aimed at the study of biocontrol efficacy of *Pseudomonas* spp against *Rhizoctonia* spp., *Fusarium* spp. *and Colletotricum* spp.

MATERIALS AND METHODS

Isolation and characterization on of *Pseudomonas*

In the present investigation soil samples were collected from randomly selected locations in the field region from Korea district of C.G. by composite sampling method (Walworth, 2004). Total of 28 bacterial cultures were isolated from 25 soil samples of 5 blocks of Korea district of Chhattisgarh. Isolation of rhizospheric bacteria was carried out by serial 10-fold dilutions technique (Pandey *et al.,* 2006) on Nutrient agar and Pseudomonas agar base (all from Hi Media). All total 28 bacterial isolates were characterized by various microscopic and cultural examinations. Four out of 28 bacterial cultures were identified as *Pseudomonas* spp. by IARI Delhi.

Isolation of the pathogen

Fungal pathogen *Rhizoctonia spp., Colletotrichum spp.* was isolated from stem of infected tomato plant of local field and *Fusarium spp.* was isolated from infected groundnut seed on PDA. All of them were characterized by microscopic examination (Ganesan and Gnanamanickam, 1987) and their pure cultures was maintained on PDA for further use. (Devi et al., 1989).

Assay for in vitro antibiosis

A loopful of bacterial culture was placed (5mm in diameter) at one edge on the periphery of PDA plate and mycelial discs (5mm in diameter) were cut from actively growing fungal culture and placed opposite to the bacterial inoculation on PDA plate (Ganesan and Gnanamanickam, 1987; Podile *et al.,* 1988; Babu *et al.,* 2000). Zone of inhibition was recorded after 1 week of incubation, by measuring the restricted growth zone between the edges of fungal and bacterial colonies. Plate with pure *Pseudomonas* inoculum corresponding to pure fungal inoculum was taken as control. Inoculated Petri plates were incubated at 25 + 1°C for 07 days. The assays of dual culture interaction were conducted in triplicates in Completely Randomized Design and repeated twice. The per cent inhibition of mycelial growth of the pathogens was calculated using following formula: (Perveen and Bokhari, 2012).

$I = (C - T/C) \times 100$

Where, I = Inhibition (%) or Antagonistic effect, C = Colony diameter of test fungus in control plate and T = Colony diameter of the same test fungus in dual culture against *Pseudomonas* as Antagonist.

Green House Experiment

Pot experiment was designed under green house condition using earthen pots containing sterilized soil. Tomato seedlings were raised in autoclaved soil, in wooden tray. The pot experiment was conducted in triplicates in Completely Randomized Design with 06 treatments for each fungus. Treatments taken were T1- PKS10 + Test fungus, T2- PKM11 + Test fungus, T3- PKJ25 + Test fungus, T4- PKB27 + Test fungus, T5- Pmtcc + Test fungus, T6- Test fungus pathogen only.

Pots were inoculated by test fungus pathogen (*Rhizoctonia* spp., *Fusarium* spp., *and Colletotrichum* spp.) @ 1 gm fresh mycelium/ 200g autoclaved soil separately and at the time of application, the population of bacteria (*Pseudomonas*) in the soil was 1x 10^9 cfu/g of soil (Nandkumar *et al.,* 2001). After 3 days of inoculation of antagonist *Pseudomonas* isolate, total 05 seeds per pot were sown in each pot along with fertilizer to raise good crop. Survival of tomato seedlings, tomato plantlets and population of fungal pathogen and antagonist *Pseudomonas* was observed in soil after 30th day of sowing seeds. Data analysis was made through anova as per CRD for each test fungal pathogen and antagonist *Pseudomonas* isolates (Jayaswal *et al.,* 1990; Khanna *et al.,* 1990).

RESULTS AND DISCUSSION

***In-vitro* and *in-vivo* Antagonistic effect of different *Pseudomonas* isolates.**

All four isolates of *Pseudomonas* (PKS10, PKM11, PKJ25 & PKB27) along with standard check Pmtcc were tested *in vitro* and *in vivo* for their antagonistic activity against test plant pathogens, *Rhizoctonia* spp. (causal agent of damping - off of tomato), *Fusarium* spp.the fungus that causes Fusarium wilt in tomato, *Colletotrichum* spp. causal agent of Anthracnose in tomato, in comparision to control (pure cultures of antagonist and fungal pathogen taken as control for in vitro assay and untreated soil was taken as control for pot assay).

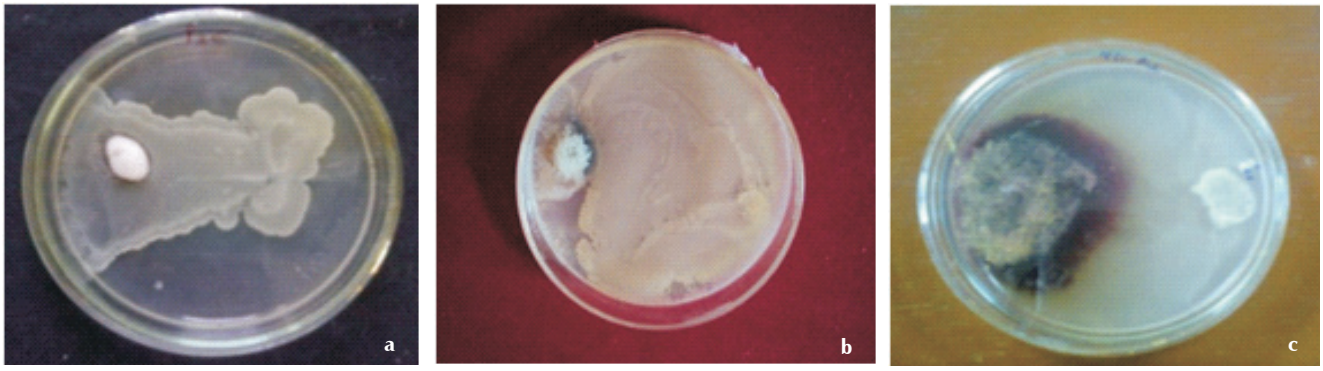
Result of *in vitro* Antagonistic effect of different *Pseudomonas* isolates against plant pathogenic fungi under dual culture technique is depicted in Table 1. All isolates were able to restrict the hyphal growth of *Rhizoctonia* spp., *Fusarium* spp. and *Colletotrichum* spp. on PDA over control. After 5days of incubation, restricted growth of fungal hyphae was clearly visible. The hyphal growth (in cm) of the fungi with isolates PKS10, PKM11, PKJ25, PKB27, Pmtcc and control were 1.82, 1.68, 0.73, 1.41, 1.25 and 8.3 respectively for *Rhizoctonia spp.,* 2.99, 3.17, 1.54, 2.3, 2.1 and 7.3 respectively for *Fusarium spp.* and 3.24, 3.11, 2.01, 2.65, 2.31 and 9.00 respectively for *Colletotrichum spp.* All the four *Pseudomonas* isolates PKS10, PKM11, PKJ25, PKB27 and Pmtcc inhibited

Table 1: in vitro Antagonistic effect of different *Pseudomonas* isolates against plant pathogenic Fungi under dual culture technique

S.No.	Isolates	Fungal pathogen *Rhizoctonia* Colony diameter (cm)	Inhibition%	*Fusarium* Colony diameter (cm)	Inhibition%	*Colletotricum* Colony diameter (cm)	Inhibition%
1.	LIPKS10	1.82	78.07	2.99	59.04	3.24	64.00
2.	LIPKM11	1.68	79.79	3.17	56.57	3.11	65.44
3.	LIPKJ25	0.73	91.20	1.54	78.90	2.01	77.67
4.	LIPKB27	1.41	83.01	2.30	68.49	2.65	70.56
5.	P-MTCC	1.25	84.93	2.10	71.23	2.31	74.33
6.	Control	8.30	00.00	7.30	00.00	9.00	00.00
7.	CD	0.139575	1.666208	0.538992	7.379785	0.558001	6.500835

Table 2: Effect of Pseudomonas spp. on survival % of Tomato seedlings infected with plant pathogenic fungi under pot experiment

S.No.	*Pseudomonas* isolates	Survival % of tomato seedlings *Rhizoctonia*	*Fusarium*	*Colletotricum*
1.	PKS10	46.67	40.00	60.00
2.	PKM11	53.33	60.00	53.30
3.	PKJ25	93.33	86.67	86.67
4.	PKB27	80.00	73.33	66.60
5.	Pmtcc	86.67	80	80.00
6.	Control	13.33	26.67	20.00
7.	CD	23.71991	20.54204	20.54204



Picture 1: Biological control of plant pathogenic fungi *Rhizoctonia* spp., *Fusarium* spp and *Colletotrichum* spp. by soil *Pseudomonas* spp. isolate P25 in picture a, b and c respectively.

the hyphal growth of *Rhizoctonia* by 78.07, 79.79, 91.20, 83.01, 84.93 %, *Fusarium* by 59.04, 56.57, 78.90, 68.49, 71.23% and *Colletotrichum* by 64.00, 65.44, 77.67, 70.56, 74.33 % respectively.

Table 2, indicates that isolate PKJ25 was the most effective antagonist against all the three test pathogens. All the isolates of *Pseudomonas* spp. reduced mortility of test plant, due to damping off, wilt and anthracnose incidence caused by *Rhizoctonia* spp., *Fusarium* spp., *Colletotrichum* spp. respectively over control. The survival % of tomato plantlets was significantly higher with isolate PKJ25 in comparison to other isolates and was recorded 93% in case of damping off due to *Rhizoctonia* spp. , 86% in case of wilt due to *Fusarium* spp., 86% in case of fruit anthracnose due to *Colletotrichum* spp. followed by isolate Pmtcc reference strain 86%, 80%,80% survival respectively and PKB27 80%, 73.33%, 66.60% survival respectively. Isolate PKS10 and PKM11 (*Pseudomonas syringae*) is tomato plant pathogen causes bacterial speck but no lesions were observed in tomato fruits in green house experiment and survival % of tomato plantlets was recorded as 46.67%,40%,60% and 53.33%, 60%, 53.30% respectively in case of *Rhizoctonia* spp., *Fusarium* spp. and *Colletotrichum* spp.

Our results suggest that among all isolates, *Pseudomonas* isolate PKJ25 (*Pseudomonas alcaligenes*) was the most effective against *Rhizoctonia* closely followed by *Fusarium and Colletotrichum* both in *in vitro* and *in vivo* condition. Work of Devi *et al.,* (1989) suggested that antagonistic bacteria *Pseudomonas fluorescent* isolates (Pfr1-14) obtained from rice rhizosphere suppressed the rice ShB pathogen, *R. soloni in-vitro* by inhibiting mycelial growth and sclerotial germination.

Similar reports of Jeyalakshmi *et al.* (1998) on various fluorescent *Pseudomonas* isolates revealed that bacteria belonging to genus *Pseudomonas* inhibited the mycelial growth of *Colletotrichum capsici in vitro* on PDA, maximum inhibition was exhibited by *Pseudomonas fluorescent* isolates 27, followed by 13,7,4 and 10 from Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore with inhibition percent 44.8% (4.8cm), 42.5%(5.0cm), 39.9%(5.2cm), 36.8% (5.5cm) and 33.3% (5.8cm). While studying about the factors influencing the germination of pathogenic and weakly pathogenic isolates of *Colletotrichum gloeosporioides* on leaf surfaces of *Stylosanthes guianensis* Lenne and Browm, (1991) reported that the two non-fluorescent *Pseudomonas* spp.(I10 and I12) and *Pseudomonas strutzeri* 3708 inhibited the mycelial growth of *C.gloeosporioides* isolates I1966+, I12245+, I12251* and I12590* by 70.3%, 76.5%, 28.3%, 38.5%; 70.9%, 81.3%, 27.0%, 37.6% and 75.4%, 80.85%, 10.0%, 46.7% respectively. Antagonistic effect of Fluorescent *Pseudomonas* was reported by Khan and Zaidi, (2002) for *Rhizoctonia soloni* and *Fusarium oxysporium*. Findings of Akhtar and Siddiqui, (2009) suggested the use of plant growth promoting rhizobacteria for the biocontrol of root-rot disease complex of chickpea and their studies showed that the three *Pseudomonas* spp. had inhibitory effect on *Macrophomina phaseolina*, *Pseudomonas alcaligenes was* one of the biocontrol agent.

Jayaraj *et al.* (2007) tested 08 fluorescent Pseudomonads isolated from tomato rhizosphere and observed highest growth inhibition (15.5mm) of *Pythium aphanidermatum* and controlled damping off of tomato by 68.5%. Purohit *et al.,* (2013) demonstrated the possible role of T. harzianum and *P. fluorescens* in the induction of antagonistic compounds

against G. sorghi in vitro and under in vivo conditions. Th-43 and Psf-28 isolates achieved maximum inhibition of radial growth of the test pathogen by (77.77%) and (56.66%) respectively under in vitro study. In glasshouse conditions, maximum reduction in disease severity was obtained with Th-43 (57%) followed by Th-39 (53.63%) with three foliar sprays. Similarly, Th-39 (36.62%) showed maximum reduction in disease severity with three foliar sprays under field conditions. Combinations of these two microbes were applied as seed and seedlings treatment in tomato for plant growth promotion and management of *S. rolfsii*. The lowest mean disease rating (MDR) 1.96 and maximum percent disease reduction (PDR), 53.23% recorded in consortium treatment (Singh *et al.,* 2013). Choudhary *et al*. (2013) studied the efficacy of various chemical and biocidal agents on the germination and seedling vigour of *C. capsici* infected chilli seeds. Among different bioagents used for the experiment, maximum per cent seed germination (82.35%) was recorded in seed treated with T. viride. Pre and post emergence mortality was minimum in case of T. polysporum (2.65% and 6.10%) followed by T. viride (6.00% and 6.80%). Among all treatments maximum root/shoot length and vigour index was found by T. viride, Safeda, Bavistin and Thiram treated seeds and found superior to others. Adhikary et al.(2013)the efficacy of azoxystrobin,was evaluated both under *in vitro* and *in vivo* conditions. In *in vitro* tests, azoxystrobin significantly reduced both mycelial growth and conidial germination of *Colletotrichum gloeosporioides* in PDA media.The optimum rate of azoxystrobin was fixed to be at 100ppm for the control of anthracnose disease.The antagonistic nature of rhizobacterial isolates PTR-3 and PCF-3 against *R. solani* was also reported by Kamei *et al.,* (2014). Their finding suggested that rhizobacterial isolates PTR-3 restricted mycelial growth of *R. solani* up to 1.9 (cm) and were found to exhibit antagonism of over 68.9%. Sharma *et al.,* (2014) reported that *Pseudomonas* spp. isolates showed antifungal activity against *Rhizoctonia* spp. in the range of 7.27-53.84% inhibition. Also *P. fluorescens isolate* 5 restricted the linear growth of *R solani* by 81.3 % was reported by Mezeal (2014). Dewangan *et al.,* (2014) recorded maximum inhibition in *Sclerotium rolfsii* (63.15%) followed by Fusarium oxysporum (61.85%) *Rhizoctonia bataticola* (55.56%) and *R. solani* (53.15 %) in in vitro co-inoculation of P.fluorescens with fungal pathogens dual culture techniques.

The present study receives strong support from the above observations and the information generated through this study will help for future studies on the biocontrol efficacy of soil *Pseudomonas alcaligenes* on soil or air borne plant pathogens, in Chhattisgarh (India) and consequently for the maintenance of native microorganisms as microbial antagonists for enhancement of crop production.

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