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

KEYWORDS
Antagonism, Biological control, Colletotrichum spp., Fusarium spp., Pseudomonas alcaligenes, Rhizoctonia spp.

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 greenhouse 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 Colletotrichum spp. to 0.73, 1.54 and 2.01 cm with 91.20%, 78.90% and 77.67% inhibition. In vivo, PKJ25 had the least disease incidence in tomato and highest 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 has 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 - 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. fluorescens (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. fluorescens 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 Colletotrichum spp.
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).

\[ I = \frac{C - T}{C} \times 100 \]

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 1x10^7 cfu/g of soil (Nandkumar et al., 2001). After 3 days of inoculation of antagonist *Pseudomonas* isolates, 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 *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 comparison 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 7.3 respectively for *Rhizoctonia* spp., 2.99, 3.17, 1.54, 2.3, 2.1 and 9.0 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

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Isolates</th>
<th>Fungal pathogen</th>
<th>Inhibition%</th>
<th>Fusarium Colony diameter (cm)</th>
<th>Inhibition%</th>
<th>Colletotrichum Colony diameter (cm)</th>
<th>Inhibition%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>LIPKS10</td>
<td><em>Rhizoctonia</em></td>
<td>78.07</td>
<td>2.99</td>
<td>59.04</td>
<td>3.24</td>
<td>64.00</td>
</tr>
<tr>
<td>2.</td>
<td>LIPKM11</td>
<td><em>Rhizoctonia</em></td>
<td>79.79</td>
<td>3.17</td>
<td>56.57</td>
<td>3.11</td>
<td>65.44</td>
</tr>
<tr>
<td>3.</td>
<td>LIPKJ25</td>
<td><em>Rhizoctonia</em></td>
<td>91.20</td>
<td>1.54</td>
<td>78.90</td>
<td>2.01</td>
<td>77.67</td>
</tr>
<tr>
<td>4.</td>
<td>LIPKB27</td>
<td><em>Rhizoctonia</em></td>
<td>83.01</td>
<td>2.30</td>
<td>68.49</td>
<td>2.65</td>
<td>70.56</td>
</tr>
<tr>
<td>5.</td>
<td>LIPKM11</td>
<td><em>Fusarium</em></td>
<td>84.93</td>
<td>2.10</td>
<td>71.23</td>
<td>2.31</td>
<td>74.33</td>
</tr>
<tr>
<td>6.</td>
<td>Control</td>
<td><em>Fusarium</em></td>
<td>0.00</td>
<td>7.30</td>
<td>0.00</td>
<td>9.00</td>
<td>0.00</td>
</tr>
<tr>
<td>7.</td>
<td>CD</td>
<td><em>Fusarium</em></td>
<td>1.66620</td>
<td>0.538992</td>
<td>7.379785</td>
<td>0.558001</td>
<td>6.500835</td>
</tr>
</tbody>
</table>
BIOCONTROL POTENTIALITIES OF NATIVE PSEUDOMONAS ISOLATES

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* *fluorescens* strains (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* *fluorescens* 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 *Pseudomonas* isolates 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

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

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Pseudomonas isolates</th>
<th>Survival % of tomato seedlings</th>
<th>Fusarium</th>
<th>Colletotrichum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. PKS10</td>
<td>46.67</td>
<td>40.00</td>
<td>60.00</td>
<td></td>
</tr>
<tr>
<td>2. PKM11</td>
<td>53.33</td>
<td>60.00</td>
<td>53.30</td>
<td></td>
</tr>
<tr>
<td>3. PKJ25</td>
<td>80.00</td>
<td>73.33</td>
<td>66.60</td>
<td></td>
</tr>
<tr>
<td>4. PKB27</td>
<td>86.67</td>
<td>80</td>
<td>80.00</td>
<td></td>
</tr>
<tr>
<td>5. Pmtcc</td>
<td>13.33</td>
<td>26.67</td>
<td>20.00</td>
<td></td>
</tr>
<tr>
<td>6. Control</td>
<td>23.71</td>
<td>20.54</td>
<td>20.54</td>
<td></td>
</tr>
</tbody>
</table>

![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.](image-url)
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 100 ppm 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. isolated showed antifungal activity against Rhizoctonia spp. in the range of 7.27-53.84% inhibition. Also P. fluorescens isolate 3 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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