Bioefficacy of rhizospheric fungal isolates against wilt and root-rot pathogens of Pea

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ABSTRACT

Pea (*Pisum sativum*) is an important leguminous crop in many countries including India. Wilt and root rot of pea is an important and widespread disease that often causes significant reduction in the yield and quality of harvested peas throughout the production areas. It is the most important and widespread disease of pea grown in relatively dry and warm area. *In-vitro* effectiveness of various antagonistic fungal isolates namely *T. harzianum* (*Th1, Th2, Th3, Th4 and Th5*) was evaluated against *Fusarium oxysporum* f. sp. *pisi*, *Rhizoctonia solani* and *Pythium ultimum* by dual culture technique on potato dextrose agar. According to the observation recorded after 5 days, all the rhizospheric fungal isolates evaluated for their antagonistic potential against wilt and root-rot pathogens, exhibited significant effect on radial growth inhibition of pathogens in comparison to control. Among the fungal isolates, Th3 and Th5 of *T. harzianum* proved to be most effective in reducing the growth of *F. oxysporum* f. sp. *pisi*, *R. solani* and *P. ultimum*. It was worthy to note that all rhizospheric fungal isolates visualized an increase in their antagonistic potential over the period of time in subsequent hours of inoculation.

KEYWORDS

Pea, Wilt, Root Rot, *Fusarium oxysporum* f. sp. *pisi*, *Rhizoctonia solani*, *Pythium ultimum*

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production. The pathogen can attack any growth stage of the plant including germinating seeds, hypocotyl or epicotyl at the early stage and lead to pre-emergence damping off or may infect the root/collar region to cause post-emergence damping off (Melzer et al., 2016) These diseases can significantly decrease both yield and quality. The losses ultimately result in reduced food supplies, poor quality of agricultural produces besides hardship to growers and processors and ultimately higher prices. Hence, the management of plant diseases is an imperative need in the present scenario to meet the increasing demand for the continuous and healthy food supply for an ever-increasing human population. Several methods, like cultural, chemical, biological and genetic manipulations are being employed to minimize the losses caused by the plant pathogens.

However, biocontrol is the most cheapest, safer and eco-friendly. In past, a great deal of research has been conducted on the nature of suppressive soil (Lockwood, 1986) and these investigation have led to the identification of a number of microorganisms with biological activity, many of which have been developed further for inundative biological control of plant diseases (Deacon, 1991, Kluepfel et al., 1993, Pandey and Upadhyay, 2000). Utilization of the biological diversity in eco-system is the foundation for the development of most classic examples of biological control of plant diseases (Boland and Kuykendall, 1998, Pandey and Upadhyay, 2000). Therefore the present investigation was undertaken to screen efficacy of rhizospheric fungal isolates against wilt and root rot pathogens of pea.

**Materials and Methods**

The present investigation was carried out under laboratory conditions in the Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, U. P. (India)

**Source of Culture: Causal Pathogens**

Isolation of Pathogens *Fusarium oxysporum, Rhizoctonia solani* and *Pythium ultimum* done in culture media from diseased plant part collected from field. The infected plants of pea exhibiting characteristic symptoms of wilt and root rot were collected and brought to the laboratory for isolation. Such plants were washed thoroughly under the running tap water to remove the adhering soil. The roots of infected plants were cut into small pieces and then rinsed with tap water. Such pieces were then sterilized with sodium hypochlorite or mercuric chloride for 2 minutes followed by 2-3 washing with distilled water. Two to three surface sterilized pieces were placed on solidified potato dextrose agar medium poured in previously sterilized 90 mm diameter Petri plates, aseptically in laminar flow. The inoculated Petri plates were incubated in the BOD incubator at 27± 2°C. These plates were observed daily for fungal growth, if any, was repeatedly sub-cultured on PDA slants for obtaining pure culture. Thereafter, isolated fungus was identified and confirmed on the basis of their cultural and morphological characteristics, respectively.

**Antagonistic Fungal Isolates**

Antagonistic fungi isolated from rhizosphere of pea field which was collected from Aligarh region and further isolation carried out by serial dilution. Isolation of *Trichoderma harzianum isolates* was isolated on *Trichoderma* specific media (TSM). The antagonistic potential of five hizospheric fungal isolates of *T. harzianum* against wilt and root rot pathogens of pea viz., *Fusarium oxysporum f. sp. pisi, Rhizoctonia solani* and *Pythium ultimum* was evaluated *in vitro* by dual culture technique.

**Dual Culture Technique**

Efficacy of antagonistic fungal isolates of *Trichoderma harzianum* on radial growth inhibition of test pathogens i.e. *Fusarium oxysporum f. sp. pisi, Rhizoctonia solani* and *Pythium ultimum* was studied *in vitro* by dual culture technique. Twenty ml sterilized melted PDA was poured in 90 mm diameter Petri plate. After solidification mycelial discs having diameter of 5mm were cut from the young culture of fungal bio-agents and test fungus with the help of sterilized cork borer. These discs were placed in the Petri plate containing PDA, maintaining the distance of 4cm between the discs of the test fungus. All the Petri plates were incubated for five days at 28 ±1°C. Each treatment had three replications. Radial growth inhibition of test pathogens i.e. *F. oxysporum, R. solani* and *P. ultimum* was measured at an interval of 24 hrs for five days to record different stages of antagonism.
Table 1. Efficacy of rhizospheric fungal antagonists against wilt and root rot pathogens of pea

<table>
<thead>
<tr>
<th>Fungal Antagonists</th>
<th>Isolate No.</th>
<th>F. oxyspsorum f. sp. pisi</th>
<th></th>
<th>Rhizoctonia solani</th>
<th></th>
<th>P. ultimum</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Radial Growth* (cm)</td>
<td>Inhibition (%)</td>
<td>Radial Growth* (cm)</td>
<td>Inhibition (%)</td>
<td>Radial Growth* (cm)</td>
<td>Inhibition (%)</td>
</tr>
<tr>
<td>Trichoderma harzianum</td>
<td>Th1</td>
<td>2.1</td>
<td>67.5</td>
<td>2.3</td>
<td>66.3</td>
<td>2.3</td>
<td>62.7</td>
</tr>
<tr>
<td></td>
<td>Th2</td>
<td>2.4</td>
<td>64.0</td>
<td>2.6</td>
<td>61.9</td>
<td>2.1</td>
<td>65.4</td>
</tr>
<tr>
<td></td>
<td>Th3</td>
<td>6.6</td>
<td>75.0</td>
<td>1.7</td>
<td>75.1</td>
<td>1.6</td>
<td>73.5</td>
</tr>
<tr>
<td></td>
<td>Th4</td>
<td>2.4</td>
<td>64.0</td>
<td>2.7</td>
<td>60.5</td>
<td>2.3</td>
<td>62.2</td>
</tr>
<tr>
<td></td>
<td>Th5</td>
<td>1.7</td>
<td>73.5</td>
<td>1.8</td>
<td>73.6</td>
<td>1.7</td>
<td>72.4</td>
</tr>
<tr>
<td>Control</td>
<td>C</td>
<td>6.6</td>
<td>00.0</td>
<td>6.8</td>
<td>00.0</td>
<td>6.2</td>
<td>00.0</td>
</tr>
<tr>
<td>CD at 5%</td>
<td></td>
<td>0.34</td>
<td>0.32</td>
<td>0.49</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mean of three replicate

Plate 1. Antagonistic effect of *Trichoderma harzianum* isolates against wilt and root rot pathogens

Fig 1. *In-vitro* efficacy of *T. harzianum* isolates, against wilt and root-rot pathogens in subsequent hour
The observations were recorded after 24, 48, 72, 96 and 120 hrs in the same way as described earlier. The percent inhibition over check, noted after 5 days of incubation was calculated by the formula given by (Vincet, 1947):

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

Where,
- \( I \) = Percent Inhibition
- \( C \) = Colony diameter in check
- \( T \) = Colony diameter in treated petriplate

**Results and Discussion**

Bioefficacy of fungal isolates namely isolates of *T. harzianum* (Th1, Th2, Th3, Th4, Th5,) were evaluated *in vitro* against *Fusarium oxysporum* f. sp. pisi *Rhizoctonia solani* and *Pythium ultimum* using dual culture technique on PDA. The observations, thus, recorded on radial growth of antagonists and test fungus is represented in table 1, fig. 1 and plate 1. It is evident from table 1 that all 5 isolates of *T. harzianum* (Th1, Th2, Th3, Th4, Th5,) significantly inhibited the radial growth of *Fusarium oxysporum* f. sp. pisi *Rhizoctonia solani* and *Pythium ultimum* in comparison to control.

However, isolated Th3 was found to be significantly most effective in inhibiting the growth of *Fusarium oxysporum* f. sp. pisi. *Rhizoctonia solani* (1.7) and *Pythium ultimum* (1.6), thereby resulting into 75.00, 75.12, 73.51, percent inhibition, respectively over control. Other isolate, Th5 was found to be statistically at par with Th3 in reducing the radial growth of pathogens. On the other hand, isolates Th1 and Th2 also did not visualise any significant difference in their antagonism when compared from each other. Though isolate T4, was found the least effective in this study (table 1).

A glance over the data plotted in fig. 1 reveals that all isolates of *T. harzianum* exhibited an increasing trend in their antagonistic potential against the pathogens in successive hours of inoculations. However, isolates Th3 and Th5 resulted in maximum growth inhibition of *F. oxysporum* f. sp. pisi, *R. solani* and *P. ultimum*, visualizing a close pace over the period of time (fig. 1 a, b, c,). It was worthy to note that all rhizospheric fungal isolates visualised an increasing rate of percent inhibition in growth of *F. oxysporum* f. sp. pisi, *R. solani* and *P. ultimum* in subsequent hours of inoculation 9fig. 1). This trend leads to infer that antagonists primarily require a period of time to establish them in substantiating their effect on targeted pathogens thereby getting involve themselves in one or more of the mechanisms of parasitism to suppress the pathogens. It is well documented in the literature that when two or more organisms are grown in close proximity, the interactions could be stimulating, inhibiting or antagonistic. The similar antagonistic effect of these rhizospheric fungal isolates has also been observed on test pathogens by several workers (Elad et al., 1980, Khara and Hadwan, 1989, Melo and Faull, 2000). Bell et al. (1982) observed that the hyphae of *Trichoderma* spp. penetrated the mycelium of pathogen and grew luxuriantly with it. The pathogenic hyphae were severely ruptured at the points of contact by *Trichoderma hyphae*, ultimately lead to hyphalysis. However (Elad et al., 1982, 1983 and Sharma et al., 2005) concluded that hyphalisis is caused by the action of cell wall degrading enzymes produced by the antagonists. Chitinase and β-1, 3-glucanase plays important role in the suppression of plant pathogens.

These enzymes are responsible in breaking down the polysaccharides, chitin and glucans which are responsible for the rigidity of fungal cell wall. Contrary to results obtained in the present study, many workers have suggested that the ability of isolates of *Trichoderma* spp. to control wilt and root rot pathogens is correlated to the level of metabolites produced by these antagonists and the main mechanism involved in biocontrol in antibiosis (Shanmugan and Varma, 1999, Hazarika et al., 2000). But, our results indicate that apart from antibiosis other mechanisms, like competition and mycoparasitism are also involved in the inhibition *Pythium ultimum, R. solani* and *F. oxysporum*, by *Trichoderma* in culture as well as in soil.

**References**


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