In-vitro bioefficacy of fungal antagonists against root rot of Pea caused by *Rhizoctonia solani* (Kuhn)

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**ABSTRACT**

*In-vitro* effectiveness of various antagonistic fungi namely *Aspergillus niger*, *A. flavus*, *Trichoderma koningii*, *T. atroviride*, and *T. harzianum* were evaluated against *Rhizoctonia solani* by dual culture technique on potato dextrose agar. According to the observation recorded after 5 days, all the treatments were found to be superior over control (*R. solani*), but among all treatments *A. niger* was found to be the most effective antagonist, with highest radial growth inhibition of the pathogen (77.01 percent), followed by *A. flavus*, *T. harzianum* and *T. koningii* i.e., 66.23, 64.42 and 62.20 percent. While as, *T. atroviride* was found to be the least effective one with minimum growth inhibition i.e., 42.21 percent. Whereas, at the same time control (*R. solani*) showed 100 percent radial growth and covered the whole Petri Plate within 5 days. All the bio-control agents were significantly effective to inhibit the sclerotia formation and development, except *T. atroviride* in which formation of sclerotia was recorded, while in all other treatments complete inhibition of sclerotia formation was recorded after 10 days of incubation.

**KEYWORDS**

*R. solani*, Biocontrol, *Trichoderma* sp., *Aspergillus* sp., Radial Growth

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**In-vitro** bioefficacy of fungal antagonists against root rot of Pea caused by *Rhizoctonia solani* (Kuhn) is one of the most important and traditionally cultivated leguminous crop in Indian sub-continent. It can be utilized both as green vegetables (green pods and immature seeds) and pulse (mature dried seeds as dal). It is also an integral part of cropping pattern and utilized in crop rotation cereal based cropping system (Davies *et al.*, 1985, McCallum *et al.*, 2000) due to its enormous property of nitrogen fixation, which helps in improving soil fertility and thus, yield of succeeding crops (Postgate, 1998, Yusuf *et al.*, 2009). The crop is vulnerable to a number of abiotic and biotic stresses. Diseases are one of the main biotic constraints for successful pea cultivation. Among all the diseases, root rot caused by *Rhizoctonia solani* is one of the major limiting factors in terms of plant growth and 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, Ajayi-Oyetunde and Bradley, 2018). Managing *R. solani* is a challenging task due to its wide host range and soil-borne nature (Ogoshi, 1996, Mubarak, 2003). Once it is established in the field, it remains indefinitely there, due to hard and thick dormant structure known as sclerotia. This may allow it to tolerate a wide range of adverse conditions in absence of the suitable host for a long time (up to 6 years) (Papavizas *et al.*, 1975, Porter *et al.*, 2011). Hence various agronomic practices such as crop rotation, host resistance, proper drainage, green manuring, application of organic
amendments etc. may found to be effective in reducing the root rot severity, but serious losses are still occurring due to lack of knowledge among the farmers about the proper recommended practices or they are still adhering to their traditional crop husbandry practices. A number of fungicides are found to be significantly effective; however, persistent and injudicious use of chemicals is usually associated with some familiar problems such as environment pollution, toxic effects on non-target organisms, and development of fungicide-resistant pathogenic strains (Vinale et al., 2008, Khare et al., 2010). Consequently, the biological control of soil borne plant pathogens using antagonistic microorganisms has been viewed as a promising alternative to existing chemical and cultural practices, which offers an eco-friendly alternative to the use of chemicals and pesticides for suppressing plant pests and diseases. Yet growers continue to prefer the use of chemicals to biological agents because biological control agents have not attained efficiencies of available fungicides under all environmental conditions and are less efficient when applied alone. Hence, an integrated system employing biological agent(s) and fungicide(s) may hold promise as the most effective and economical method of disease control (Chakravarty et al., 1990, B Hale and Rajkonda, 2015).

Materials and Methods

The study was carried out under laboratory conditions in the Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, U. P. (India) during 2016-17.

Collection of Fungal Antagonists

The five fungal antagonists namely Aspergillus niger, A. flavus, Trichoderma harzianum, T. koningii and T. atroviride were collected from the stock cultures of Department of Plant Protection, Aligarh Muslim University, Aligarh, India.

Isolation and purification of R. solani

The infected plants of pea exhibiting characteristic symptoms of 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.

In Vitro Evaluation of T. harzianum Isolates for their Antagonistic Effect on the Radial Growth of R. solani

Bio-efficacy of five different biocontrol agents was evaluated in-vitro, against Rhizoctonia solani. In this experiment dual culture technique was used to note the antagonistic activity of biocontrol agents against R. solani (Cherif and Benhamou, 1990). A mycelial disk of 5 mm diameter of pathogen was placed on the one side of the petri dish, thereafter; a disk of same diameter of biocontrol agent strains was placed on the other side of petri dish at equal distance. The inoculated Petri plates were incubated at 27±2°C until the growth of control treatment (R. solani), covered full diameter of Petri dish. The percent inhibition of radial growth was calculated by following formulae:

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

where,
C = radial growth of the pathogen in absence of antagonist, i.e. control,
T = radial growth of the pathogen in the presence of the antagonist.

In all experiments, test and control plates were set up in three replicates and an average thereof used for analysis.

Results and Discussion

Bio-efficacy of 5 Bio-control agents namely Aspergillus niger, A. flavus, Trichoderma harzianum, T. koningii and T. atroviride were evaluated in vitro against Rhizoctonia solani, using dual culture technique on PDA.
The observations, thus, recorded on radial growth of antagonists and test fungus is represented in table 1 and fig 1. It is evident from the observations that all bio-control agents significantly inhibited radial growth of \textit{R. solani} compared to control. However, \textit{A. niger} was noted to be most effective antagonist followed by \textit{A. flavus} and \textit{T. harzianum} resulting 77.01, 66.23 and 64.42 percent growth inhibition of test pathogen, respectively (table 1, fig 1), whereas \textit{T. atroviride} shows least i.e. 42 percent inhibition of the pathogen (Plate 1).

**Table 1. Inhibition of radial growth of \textit{R. solani} by biocontrol agents**

<table>
<thead>
<tr>
<th>Bio agent</th>
<th>Radial Growth</th>
<th>Percent Growth Inhibition</th>
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<tbody>
<tr>
<td></td>
<td>Biocontrol Agents (mm)</td>
<td>\textit{R. solani} (mm)</td>
</tr>
<tr>
<td>\textit{A. niger}</td>
<td>69.33 (56.35)</td>
<td>20.67 (27.02)</td>
</tr>
<tr>
<td>\textit{A. Flavus}</td>
<td>59.66 (50.55)</td>
<td>30.33 (33.40)</td>
</tr>
<tr>
<td>\textit{T. koningii}</td>
<td>56.00 (48.42)</td>
<td>34.00 (35.65)</td>
</tr>
<tr>
<td>\textit{T. atroviride}</td>
<td>38.00 (38.03)</td>
<td>52.00 (46.12)</td>
</tr>
<tr>
<td>\textit{T. harzianum}</td>
<td>58.00 (49.58)</td>
<td>32.00 (34.42)</td>
</tr>
<tr>
<td>Control</td>
<td>0.00 (0.00)</td>
<td>90.00 (71.53)</td>
</tr>
<tr>
<td>CD</td>
<td>2.81 (1.65)</td>
<td>2.81 (1.72)</td>
</tr>
</tbody>
</table>

*Figures in parentheses are the arcsin √percentage transformed values. **Each value is an average of 3 replicates.*

**Fig 1.** Bio- efficacy of Bio-control agents against \textit{Rhizoctonia solani}

All the biocontrol agents except *T. atroviride* were found to be successfully inhibiting sclerotial development (10 days after incubation). Similar inhibitory effect in terms of radial growth and sclerotia formation of bio control agents using *T. harzianum, T. atroviride and A. niger* against *R. solani* were also reported by several other workers (Dohroo *et al.*, 1990, Barbosa *et al.*, 2001, Seema and Devaki, 2012.). The inhibitory effect may be a result of mycoparasitism (Anees *et al.*, 2010, Asad *et al.*, 2014) competitive inhibition for space or nutrient (Benitez *et al.*, 2004) or may be due to production of antibiotics (Gliotoxin, gliovirin, viridian, trichoviridin), enzyme (chitinase) or secondary metabolite etc. (Vinale *et al.*, 2008, Schuster and Schmoll, 2010).

**References**


Melzer, M. S., Yu, H., Labun, T., Dickson, A., and Boland, G. J. (2016) Characterization and


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