Effect of Microbial Antagonist and Fungicide against *Fusarium Solani* Causing Root Rot of Pea

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Abstract

Root rot is a one of the major disease in pea caused by *Fusarium* spp. Fungal pathogen of the disease was isolated from naturally infected pea crop and was confirmed as *Fusarium solani* f. sp. *pisi*. Infected plants exhibited poor growth, yellowing and drying of foliage with partially or fully damaged root system. Assessment of five microbial inoculants and one fungicide carbendazim were tested in lab and in pot condition. Among the seven treatments including the control, *Trichoderma harzianum* exhibited highest inhibition percentage of 81.11% in dual culture studies. In case of percentage of germination, treated seeds with bio control agentsin earthen pots, *Trichoderma harzinaum* was found superior. Soil treatment with bio control agents and fungicide,T. harzianum was found superior in reducing young seedling mortality and increasing yield percentage in pea. Therefore, the current studies could be helpful for pea growers by using microbial antagonist to achieve suitable management of seedling mortality by *Fusarium solani*in Pea crop.

Keywords: Trichoderma, pea, bio control agents

Introduction:

Root rot complex is a serious pea disease that causes yield losses of 15–60 percent all around the world **(Bruce A. Williamson-Benavides, 2020)**. *Fusarium* species, particularly *Fusarium solani* and *F. oxysporum*, are the principal causes of the disease. The cotyledon-hypocotyl junction of infected seedlings develops lesions. These lesions occur at the roots and lower stem and turn dark black as infection progresses. Infected plants have slowed growth, lower chlorophyll levels, and lower relative water content **(Lyndon D. Porter, 2014).**

Several fungicides have been used successfully to control of plant diseases. However, the emergence of fungicide resistance and negative side effect on soil, environment and health, due to this reason, plant

pathologist have been focused to search eco friendly methods for controlling plant diseases (**Chattopadhyay et al. 2002**). Control of Soil borne diseases by using fungicide is very challenging task. Therefore biological control isone of the most effective, low-cost and environmentally friendly techniques for controlling plant diseases brought against soil-borne pathogens including Fusarium, Rhizoctonia, and Pythium **(Stirling, 1991).***Trichoderma* are available in the market and used to save Plants from diseases or to enhance plant growth. There are many species such as T. asperellum, T. viride ,T. *harzianum*and T. virens**(Hermosa et al. 2000;**) are applied for control of Plant diseases. Application of beneficial microbes has become a component of Integrated Plant disease management **(Monte, 2001).**Research are going on developing *Trichoderma* formulation utilizing different agricultural wastes for plant growth promotion, protection, and enhanced yield quality (**Kancelista et al. 2013)**

Therefore,*Trichoderma* spp. have gained the most attention among the many biocontrol agents (BCAs) tested against plant pathogenic fungi for their capacity to exert biocontrol **(Abd El-Khair et al., 2010)**

Material and method

Microbial bio control agents (T. harzianum and *Pseudomonas fluorescence*) and fungicides were collected from the Plant Pathology Laboratory of Lovely Professional University. Pathogen was isolated from pea.

Symptomatology. Symptomatology was carried out on pea plants showing typical symptoms of fusarium root rot. The diseased plants showing above ground symptoms in the fled during the course of survey were bought to the Plant pathology laboratory. The roots were washed using fresh tap water before making observations for root rot symptoms. Symptomatic plants were maintained under natural conditions in fled to record periodic symptom development.

Isolation of pathogen. Small Tissue bit transfer approach was used to isolate the causative agent **(Nisa et al., 2021).** With a sharp sterilizedsurgical blade, the symptomatic diseased roots were cuts into small bits (2 mm) along with healthy portion**(Adnan S et al., 2017).** These parts were surface sterilized for 10 seconds with chemical of 1% sodium hypochlorite and rinsed two times with distilled sterilized water to eliminate any remaining chemical solution. The bits were dried on tissue paper before being aseptically transferred to Potato Dextrose Agar (PDA) media in sterile Petri-plates and incubated at 28±2 °C and examined periodically the color of mycelium or colony.

Purification ofpathogen.

Pathogen was purified by hyphal tip technique**(Ahmed F et al., 2017)**.Microbial growth of tested organism on diseased tissue bits was aseptically transferred to Petri plates containing PDA kept in BOD incubator for incubation at 28±2 °C for seven days. The sub cultured plates were then observed for sporulation. Dilute spore suspension in sterile distilled water, prepared out of a sporulating colony was poured on Petri plates containing water agar and incubated for one day at 28±2 °C. The water agar plates were then observed in inverted position under microscope and the isolated germinated spores were transferred to fresh plates containing PDA and incubated at 28±2 °C. Pure obtained cultures were stored at 5 °C for further use. Identification of the isolated fungal pathogen was carried out according to their cultural, morphological and microscopic characteristics as described by **(Barnett and Hunter et al., 1987).**

Identification of pathogen. The pathogenic isolate on pea plants was identified on the basis of morphological characters of somatic and reproductive structures and compared with the monograph on Fusarium spp by (**Aksoy et al., 2021).**

Pathogenicity test: One-third portion of root system old seedlings was clipped of from distal end and dipped for 5 min in conidial suspension prepared from the spores of purified fungal culture isolated from root rot diseases pea plants. The inoculated plants were transplanted autoclaved soli containing earthen pots. Clipped plants were dipped in sterile distilled water for the same period of time in case of control. Observations on development of typical symptoms on the inoculated plants were made seven days after inoculation (**El-Dawy, et al., 2021)**.

Antagonistic activity Trichoderma against *Fusarium solani* : Antagonistic activity of bio control agents and efficacy of fungicide were tested against causal agent of root rot of pea (*Fusarium solani)*by using dual culture technique proposed by **(Astorga Quirós et al., 2014b),**which was the part of microbial collection in our research study. Two different isolate were placed towards to each other on same culture medium PDA plate with three replication. Inoculated Petri plates were kept in BOD incubator at28 ± 2^0C for seven days. The percentage inhibition of radial growth was determined employing the formula presented by **(Ezziyyani et al., 2004b).** The experiment was replicated thrice and percent growth inhibition was calculated by the formula of I = (C-T)/C X100, where C is mycelial growth in control plate, T is mycelial growth of test organisms in inoculated plate and I is inhibition of mycelial growth.

The bacterial isolate (*Pseudomonas fluorescence*) was streaked at the opposite side of the test pathogen; approximately 35 mm away from the center of Petri plate followed by (**Islam A, et al., 2018)**. Culture medium inoculated only with *Fusarium solani* was used as a control and triplicates were maintained. The plates were incubated at 28 ± 2 °C until full growth of the control. After the period of incubation, the percentage of inhibition of the test fungal pathogen was calculated as described by **(Kota et al., 2017).**

In vitro evaluation of fungicides. Onechemical fungicide carbendazim was tested against the tested pathogen by poisoned food technique. Potato Dextrose Agar Medium (PDA) was prepared and sterilized at 121 °C for 20 min. Before pouring in Petri plates, appropriate volumes of fungicide solution were added separately to equal quantities of PDA medium in an aseptic manner. Afer that, the plates were inoculated with a seven -day-old fresh culture of pathogen. In a control plate only PDA medium was poured without addition of fungicide . Each treatment was repeated three times, and inoculation plates were incubated in a Bio-Oxygen Demand (BOD) incubator at 25±1 °C. Using the formula above, the percentage in inhibition of mycelial growth at different test concentrations in comparison with control treatment.

Management of the disease in field. The field trail was conducted during Rabi season. The experiment was laid in Randomized Complete Block Design. The fungicide (Carbendazim) and Microbial inoculants were proved most effective in lab condition were used as seed treatments under vivo conditions against disease root rot of pea. Before sowing, seeds were primed withfungicide and bio control agents such as *Trichoderma* and *Pseudomonas*. During, seed treatment with fungicide, the seeds was slightly moistened with sterilized water and chemical fungicide was used @ 5 g kg−1 seeds. After treatments, seed were dried in shade before sowing in field. In case of seed treatment with *Trichoderma*, a spore suspension of the bioagent was standardized with the help of Hemocytometer and diluted to 10^7 colony forming unit (CFU). The seeds were immersed in the *Trichoderma*suspension for one 30 minutes and later dried in shade. In case of control plots, seeds were treated with CMC only

Results and discussions:

Symptoms . Detailed symptomatology of infected plant (underground and above ground) wasexamined and the observations are described as under:

Below ground symptoms: Roots affected with root rot showed reddish brown lesions as initial symptoms near the soil line and below it.. Lateral roots were condensed with distorted root hairs. Diseased roots were sloughed and macerated. When the roots showing primary symptoms were cut longitudinally, no vascular discoloration was found. On the other hand, in advanced phase of disease, the discoloration had advanced to the interior of the roots as well. **Porter et al., 2015** were found the similar results.

Above ground symptoms. Root rot affected plants showed yellowing of the lower leaves which later progressed towards the top. As the disease became more aggressive, the lower leaves appeared wilted. Root rot affected plants also showed downward curling.

Isolation of pathogen. In the current study F. solani was isolated from the discoloration (black-brown), decaying infected roots of pea. The pathogen produced scanty aerial mycelium having pink cottony growth with 30–36 mm diameter in three days.

Identifcation ofpathogen. The morphological characteristics of the pathogen on Potato dextrose agar medium (PDA) were tested which are given in table 1. The study exposed that the mycelium was septate and branched and the colonies appeared white to creamy. Macro conidia were septate, fusiform and curved whereas Micro conidia were abundantly present, oval to ellipsoid in shape. On the basis of morphological features and colony characters and the monograph referred by **Aksoy et al.2021,** the fungus was confirmed as *Fusarium solani*. Similar morphological characters were observed by **Kumari et al., 2016 and Haddoudi et al.,2017).**

Table no 1. Morpho-cultural characteristics of isolated Pathogen (*Fusarium solani*).

Features	Shape and morphological appearance	Colour
Colony	Fast growing and circular	Pink colour
Mycelium	Septate, branched	Transparent
Macro conidia	Fusiform	Brown
Micro conidia	Oval	Brown
Chylamydospore	Single celled	Brown

Table 2*In vitro* assessment of *Trichoderma*, Fungicide and *Pseudomonas fluorescence* against *Fusarium solani*

S.No.	***Trichoderma* species**	**% growth inhibition**
1	*Trichoderma koningii*	64.44
2	*Trichoderma virens*	68.00
3	*Pseudomonas fluorescence*	73.33
4	*Trichoderma viride*	78.88
5	*Trichoderma harzianum*	81.11
6	Carbendazim	73.00
Control	-	00
Cv	13.49	-
CD	1.21	-
SE(m)±	0.40	-

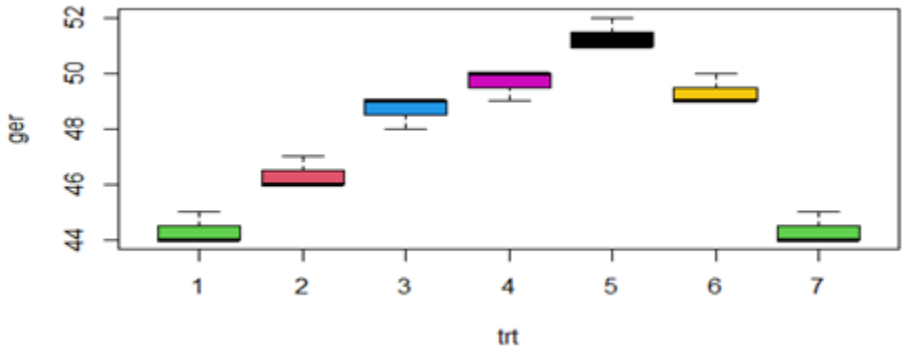
In vitro evaluation of bio agents. In this research all the treatments gave significant results.. Antagonistic assessment of four different species of *Trichoderma*, One bacterial bio agent and fungicide (carbendazim) were confirmed against tested pathogenunder *in vitro* conditions and it is clear showing from the data obtained that all the antagonist showed antagonistic against *Fusarium solani* (Table 2).Highest mycelium growth inhibition was found in *Trichoderma harzianum*(81.1) whereas Minimum percent growth inhibition was found in dual culture with *Trichoderma koningii* (64.44%) followed by *Trichoderma viride* (78.8%) *Pseudomonas fluorescence*73.33%).Similar finding were observed by **Ahmed et al.**.

Table: 3 Efficacy of Microbial biocontrol agents and fungicide as seed treatment against Fusarium root rot incidence in pea

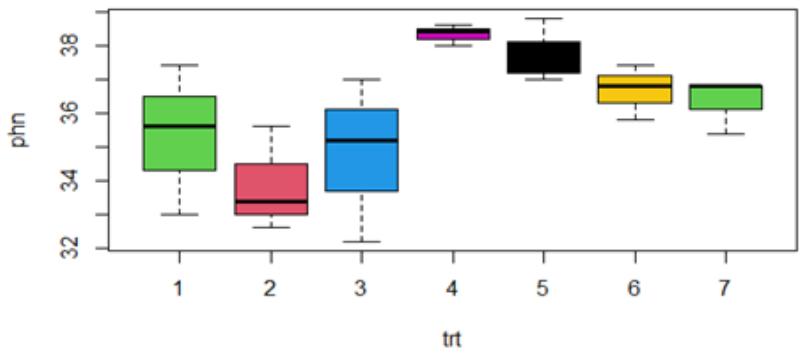
Treatments	**Germination %**	**Plant height 60days**	**Plant height 90 days**	**Disease incidence (%)**
Trichoderma	44.33± 0.57b	17.66 ±2.30ab	35.33±2.21abc	10.00± 3.30abc

koningii				
Trichoderma virens	46.33 ±0.57ab	19.33± 2.88ab	33.86± 1.55c	7.76 ±5.08abc
Pseudomonas fluorescence	48.66± 0.57ab	20.66 ±5.77ab	34.80±2.42bc	13.33± 3.35ab
Trichoderma viride	49.66± 0.57a	22.66 ±2.30ab	38.33 ±0.30a	14.43 ±1.96a
Trichoderma harzianum	51.33± 0.57a	24.00 ±1.00a	37.73±0.94ab	5.56 ±1.96c
Carbendazim	49.33± 0.57ab	22.66 ±2.30ab	36.66±0.80abc	6.66 ±3.35bc
control	44.33± 0.57	21.00 ±2.64ab	36.33 ±0.80abc	12.23 ±5.08abc

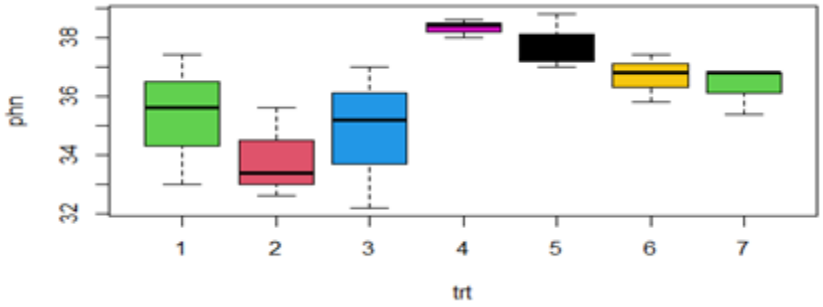
Pot experiment. In our study treated seeds with bio control agent *Trichoderma harzanium* gave maximum percentage of germination (51.33%) followed by carbendazim (49.33) and the lowest germination was found in Control (44.33%) The similar results were observed by **Kumar and Dubey, 2001, 15 Xue, 2003)** who have reported significant increase in percentage of seed germination, reduce Pea root rot caused by *Fusarium solani* f.sp.pisi after treatment with bio-control agents.The present findings are also in conformity with the findings of **(Lacicowa and Pieta, 1994, Della et al.,1998)**who reported the effectiveness of various isolates of *Trichoderma* in controlling the root rot of pea caused by *Fusarium solani* f.sp.pisi. The present results are also in agreement with those of (**Jha and Jalali, 2006).** The current study has demonstrated that *Trichoderma* species can be used for controlling root rot disease complexes of pea in organic farming or in low-input sustainable agriculture.



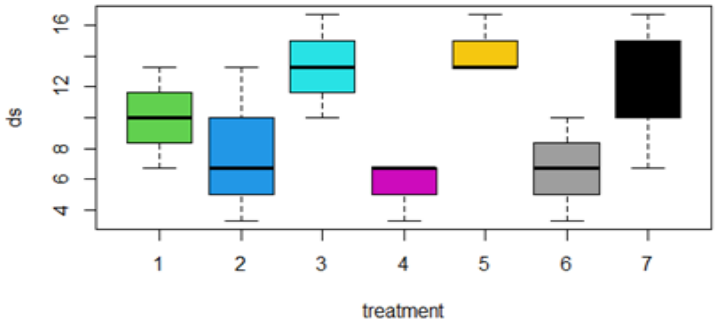
Graph 1: Germination percentage



Graph 2: Plant heights in 60 days



Graph 3 : Plant heights in 90 days



Graph 4 : Disease severity

Conclusion: The current studies could be helpful for pea growers by using microbial antagonist to achieve suitable management of seedling mortality by *Fusarium solani*in Pea crop.

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