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BIOCONTROL OF PEA ROOT ROT INCITED BY *FUSARIUM SOLANI* F. SP. *PISI*

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Abstract– Pea (*Pisum sativum* L.) root rot caused by *Fusarium solani* f.sp. *pisi* causes significant crop losses in northern India. The antagonistic potentials of four microbial inoculants (*Trichoderma harzianum, Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis*) were evaluated in a lab situation and pot conditions. Among the five treatments, Including the control. *Trichoderma harzianum* had the highest inhibition percentage (81.63%) followed by *T. viride* found 77.46 per cent growth inhibition in dual culture. In pot condition maximum pre-emergence disease reduction of (62.99%) was obtained from *T. harzianum* followed by *T. viride* (60.30%) and maximum post-emergence disease reduction of (71.41%) was obtained from *T. harzianum* followed by *T. viride* (65.69 %). *Trichoderma harzinaum* was shown to have a higher rate of germination, root and shoot length as compared to untreated control in earthen pots. This result shows that these four biocontrol agent are very effective.

INTRODUCTION

One of the most significant vegetable crops is the pea (*Pisum sativum* L.). It is a diploid species of the Leguminosae family. Root rot disease is considered the most destructive in pea as it affects their initial plant stand. Among the various constraints, the root rot of pea causes heavy loss of pea in IIIrd A zone of Rajasthan. The root rot disease incited by *Fusarium solani* (Mart.) Appel and Wolleweber f. sp. *pisi* (E. R. John) Snyder and Hansen is a serious disease of pea (*Pisum sativum* L) throughout the world.

Pea root rot is a severe issue that is on the rise and endangers pea farming in the state. The illness first manifests as a yellowing of the basal leaves caused by maceration and root necrosis, and it finally results in the death of the diseased plants.

Lesions form at the cotyledon-hypocotyl junction of infected seedlings. As the illness spreads, these lesions at the roots and lower stem turn dark black. Infected plants grow more slowly, have less chlorophyll, and have less relative water content. (Porter, 2015).

It has proven successful in controlling plant diseases with several fungicides. It has been realized that the use of chemicals in agriculture is not as beneficial as it was visualized. Chemicals present serious health risks to both the person applying them and the person using the treated material. Therefore, one of the most efficient, economical, and ecologically harmless methods for controlling plant diseases is biological control. Market-available *Trichoderma* is used to protect plants against diseases and promote the growth of plants. Many species are used to control plant diseases, including *T. asperellum, T. viride, T. harzianum, Pseudomonas fluorescens* and *Bacillus subtilis* (Hermosa *et al.,* 2000). Integrated plant disease control includes the application of beneficial microorganisms (Monte, 2001). Research is going on developing *Trichoderma* formulations utilizing different agricultural wastes for plant growth promotion, protection, and enhanced yield quality.

MATERIALS AND METHODS

Microbial biocontrol agents (*Trichoderma harzianum*, *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis*) were collected from the Plant Pathology Laboratory of Rajasthan agricultural research institute, Durgapura, Jaipur. Pathogen was isolated from root rot affected pea plants.

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 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 to record periodic symptom development.

Isolation of pathogen

A small Tissue bit transfer approach was used to isolate the causative agent **(**Nisa *et al*., 2021). With a sharp sterilized surgical blade, the symptomatic diseased roots were cut into small bits (2 mm) along with a healthy portion (Adnan *et al*., 2017). These parts were surface sterilized for 10 seconds with the chemical of 1 per cent 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 colour of mycelium or colony.

Purification

Pathogen was purified by the hyphal tip technique (Ahmed *et al.*, 2017). Microbial growth of tested organism on diseased tissue bits was aseptically transferred to Petri plates containing PDA kept in a BOD incubator for incubation at 28±2°C for seven days. The subcultured 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 an inverted position under the 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 (1987).

Identification of the culture

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

For confirmation of identity, the pure cultures of all collected isolates were sent to Indian type culture collection (ITCC), Division of Plant Pathology, IARI, New Delhi, (ID No. 11,832.23, Pea, 16.03.2023).

Pathogenicity test

The technique was employed under pot house conditions. To multiply the test fungus (*F. solani*), corn meal sand (2:1) medium was used and inoculated with the 7 day old culture of the fungus and incubated at 25+2°C for 7 days. The inoculum was applied in the upper 3" layer of sterilized soil filled in a pot by following the layering method and watered as and when the need arise for 10 days. Seeds of pea @10 seeds/pot were sown. An un-inoculated pot filled with sterilized soil served as a check. Observations were regularly made for the appearance and development of symptoms. After symptom development, re-isolation was done from the artificially infected plants. The symptoms observed in inoculated plants and the culture obtained were compared with the original symptoms and original culture for confirmation.

Antagonistic activity biocontrol agents against *Fusarium solani*

The efficacy of biocontrol agents, *i.e. Trichoderma harzianum, Trichoderma viride, Pseudomonas fluorescens* and *Bacillus subtilis* were tested by using the dual culture plate method on PDA medium. A 3mm diameter mycelium bit of seven day old culture of Fusarium was inoculated in the centre of 1st half of the Petri plate and *Trichoderma* spp. was in the centre of 2nd half of the Petri plate containing the sterilized PDA medium. In *Pseudomonas fluorescens* and *Bacillus subtilis* fungus was inoculated at one end of the PDA plate and at the other end bacteria are streaked. For each treatment, three replications were taken. Inoculated plates were incubated at 28±1°C temperature in the incubator. Observations on colony diameter were recorded up to the complete coverage of control plates, which were inoculated with only pathogens. The linear growth after seven days of incubation was recorded and per cent inhibition was calculated. 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 the control plate, T is mycelial growth of test organisms in an inoculated plate and I is inhibition of mycelial growth.

RESULTS AND DISCUSSION

Symptoms

Below ground symptoms: Roots affected with root rot showed reddish brown lesions as initial symptoms near the soil line and below it. As the disease progressed, these lesions became dark brown to black, collapsed together and spread continuously throughout the roots. Lateral roots were reduced with distorted root hairs. Diseased roots were sloughed and macerated. When the roots showing initial symptoms were cut longitudinally, no vascular discoloration was observed. However, in an advanced phase of the disease, when there was extensive tissue disintegration, the discolouration had advanced to the interior of the roots as well. Pathogenicity was assessed using soil inoculation techniques.

Similarly, Lestari *et al.* (2021) observed that different isolates of *F. solani* produced various levels of typical sudden death syndrome symptoms, such as interveinal chlorosis and necrosis on young leaves.

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 a little bit of epinasty.

Isolation and identification of the pathogen

In the current study, *F. solani* was isolated from the discolouration (black-brown), decaying infected roots of pea. Pure culture of the test fungus produced off-white (creamy) with fluffy cottony growth on PDA. The fungal cultures had produced macro (3-5 septa) and microconidia (0-1 septa) in mycelium. *Fusarium solani* isolates showed hyaline, branched and septate conidiophores. The macroconidia are sickle-shaped with a blunt end and the microconidia are round to oval shaped. Intercalary and terminal chlamydospores were observed in all the *Fusarium solani* isolates. The present results are also reported by Nazir *et al.*(2022) isolated *F. solani* from the black-brown, decaying infected roots of pea. The pathogenic isolate on pea plants was identified based on morphological characteristics of somatic and reproductive structures.

In vitro evaluation of bio agents

Results indicated that all the bio-control agents had antagonistic activity against the growth of *F. solaniin-vitro*. Severe antagonism and significant high per cent inhibition of *F. solani* growth with 81.63 per cent was recorded by *T. harzianum* in the dual culture method which was followed by *T. viride*, which showed moderate antagonism with 77.46 per cent growth inhibition. While the bacterial antagonist *Pseudomonas fluorescens* also expressed moderate antagonism with 70.29 per cent mycelial growth inhibition. The lowest mycelial growth inhibition and weak antagonism were shown by *Bacillus subtilis* which was 66.07 per cent. Whereas minimum mycelium growth was found at 16.53 mm in *T. harzianum* followed by 20.28 mm in *T.viride*, 26.73 mm in *P. fluorescens,* 30.53 mm in *Bacillus subtillus* and maximum mycelium growth was found 85.43 mm in control, respectively.

Similarly, Hamid *et al.* (2012) also found that *Trichoderma spp*. had strong antagonistic activity towards *F. solani* f. sp. *pisi* under in vitro conditions. The dual culture studies of four bio-control agents revealed that *Trichoderma harzianum* exhibited the

Table 1. Bio-efficacy of different bio-agents on the growth of *Fusarium solani* f. sp. *pisi in vitro* by dual culture method.

S. No.	Treatments (bio-agents)	Colony diameter of antagonist (mm)*	Colony diameter *Fusarium solani* f. sp. *pisi* *	Inhibition of growth of *Fusarium solani* f. sp. *pisi**
1	*T. harzianum*	80.30 (63.63)	16.53 (23.96)	81.63
2	*T. viride*	75.96 (60.62)	20.28 (26.74)	77.46
3	*P. fluresence*	66.36 (54.53)	26.73 (31.11)	70.29
4	*Bacillus subtillus*	56.98 (48.99)	30.53 (33.52)	66.07
5	Control	00.00	85.43 (71.53)	0.00
	S.Em.±	0.47	0.85	0.99
	C.D. at 5%	1.51	2.72	3.14
	C.V. %	1.47	4.00	2.88

*Mean of three replications

Figures in parentheses are angular transformed values**.**

highest inhibition percentage of (78.60 %) (Table 1, Fig. 1).

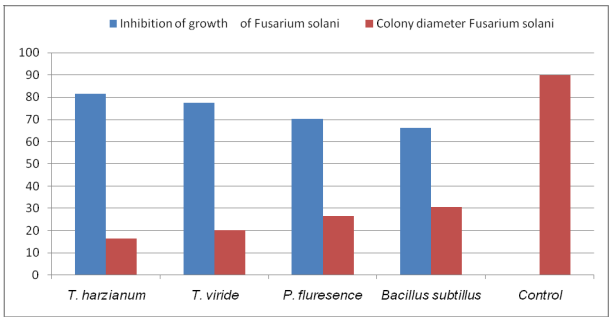

Fig. 1. Bio-efficacy of different bio-agents on the growth of *Fusarium solani* f. sp. *pisi invitro* by dual culture method.

Pot culture experiment

Results indicated that all types of seed treatments suppressed root rot incidence at pre-emergence after 35 days and post-emergence stage after 60 days compared with control treatments. Data in Table 4.21 indicated that the maximum pre-emergence disease reduction of (62.99 %) was obtained with *T. harzianum* followed by *T. viride* (60.30 %), *P. fluorescens* (45.05 %) and *Bacillus subtillus* (29.99 %). When applied as soil application whereas maximum post-emergence disease reduction of (71.41 %) was obtained with *T. harzianum* followed by *T. viride* (65.69 %), *P. fluorescens* (57.70 %) and *Bacillus subtillus* (49.69 %).

Shoot length

Data of all the treatments presented in Table 2 had significant higher shoot length as compared with control. Among the treatments maximum shoot length was observed in *T. harzianum* (69.00cm) followed by *T. viride* (59.77cm), *P. fluorescens* (55.02cm), *Bacillus subtillus* (50.59cm) and the lowest shoot length was observed in control (19.85cm).

Root length

Data of all the treatments presented in Table 2 had significant higher root length as compared with control. Among the treatments maximum root length was observed in *T. harzianum* (19.56 cm) followed by *T. viride* (16.49 cm), *P. fluorescens* (13.26 cm), *Bacillus subtillus* (11.19 cm) and the lowest root length was observed in control (9.58 cm).

Verma and Dohroo (2005) also reported the efficacy of bio-control agents as seed treatment against *F. oxysporum* f.sp *pisi* pathogen causing wilt of pea. The results showed that apart from inhibiting the disease the bio-control treatments used

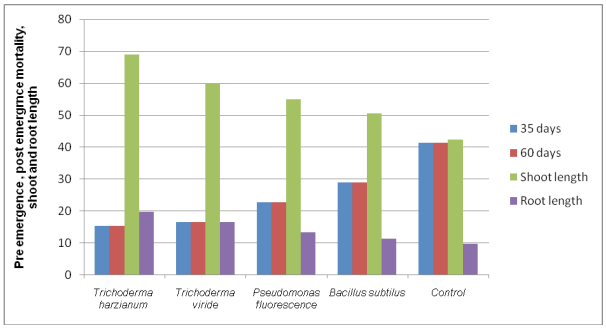

Fig. 2. Effect of different bio-agents against root rot incidence under polyhouse condition.

Table 2. Effect of different bio-agents against root rot incidence under greenhouse condition.

S. No.	Treatments (bio-agents)	Pre-emergence (35 days)	Reduction (%)	Post-emergence (60 days)	Reduction (%)	Shoot length (cm)	Root length (cm)
1	*T. harzianum*	15.27 (22.99)	62.99	26.81 (31.17)	71.41	69.00 (56.15)	19.56 (26.24)
2	*T. viride*	16.38 (23.86)	60.30	32.17 (34.54)	65.69	59.77 (50.62)	16.49 (23.95)
3	*P. florescens*	22.67 (28.42)	45.05	39.66 (39.02)	57.70	55.02 (47.87)	13.26 (21.34)
4	*Bacillus subtillus*	28.88 (32.49)	29.99	47.17 (43.36)	49.69	50.59 (45.32)	11.19 (19.53)
5	Control	41.25 (39.95)	0.00	93.75 (75.50)	0.00	42.37 (40.59)	9.58 (18.01)
	S.Em.±	0.66		0.49		0.92	0.38
	C.D. at 5%	2.12		1.55		2.93	1.20
	C.V. %	4.63		1.76		2.89	4.65

Average of three replications.
Figures in parentheses are angular transformed values

increased the yield by 83.6 per cent compared to control (Table 1, Fig. 1).

CONCLUSION

The current studies could be helpful for pea growers by using microbial antagonist to achieve suitable management of seedling mortality by *Fusarium solani* pea crop. Bio-priming seed treatments can provide a high level of protection against root rot disease of pea plants.

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Conflict of interest

There is no conflict of interest.

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