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Biological Control of Damping Off of Okra caused by *Rhizoctonia solani*

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A B S T R A C T

Damping off of okra is known to occur worldwide and caused by different pathogens under variable climatic conditions.. In Himachal Pradesh *Rhizoctonia solani* was found major associated pathogen with damping off of okra in summer season grown crop. Twenty isolates of *Trichoderma* spp. isolated from different rhizosphere soil and five standard isolates of *Trichoderma* spp. already available were evaluated for their antagonistic activity *in vitro* against *R. solani*. Among all the isolates tested, *Trichoderma* sp. -2 was found best bioagents for inhibiting mycelial growth of *R. solani* (67.8%) in dual culture followed by *Trichoderma* sp.-9 (67.0%), *Trichoderma* sp. -6 (64.8%) and *Trichoderma* sp. -11(61.9%). Significantly, maximum mycelial growth inhibition (42.4%) of pathogen was with volatile compounds activity of *Trichoderma* sp.-2 while, filter sterilized culture filtrate of *Trichoderma* sp. -6 showed maximum pathogen mycelial inhibition. On the basis of morpho-cultural characteristics the potential bioagent isolates, *Trichoderma* sp.-6 and *Trichoderma* sp.-9 were identified as *T. harzianum* and *T. viride*, respectively. In mycoparasitic interaction, bioagents hyphae coiled around *R. solani* mycelium and after penetration lysed the pathogen hyphae. Under net house conditions, seed biopriming application method of bioagents was found superior for management of damping off of okra caused by *R. solani* than soil application, seed treatment and drenching.

Introduction

Okra (*Abelmoschus esculentus* L.) is an important vegetable crop belongs to Malvaceae family usually grown in summer as well as rainy season in almost all parts of India. Okra is affected by several diseases viz., cercospora leaf spot (*Cercospora malayensis*), wilt (*Fusarium oxysporum* f. sp. *vasinfectum*), powdery mildew (*Erysiphe cichoracearum*), anthracnose (*Colletotrichum gloeosporioides*), pod rot (*Botrytis* spp.), root rot (*Rhizoctonia solani*), damping off (*Pythium* spp.), viral disease like yellow vein mosaic and bacterial disease like leaf spot (*Xanthomonas esculenti*) but damping off is one of the major destructive diseases. Damping off of okra is known to occur worldwide and caused by *Pythium* spp. (Jukte *et al.*, 2016), *Phytophthora nicotianae* (Matny, 2013),

Rhizoctonia solani and *Macrophomina phaseolina* (Jiskani *et al.,* 2007) under variable climatic conditions. Damping off of okra caused by soil borne pathogens has presently emerged one of the most important and destructive diseases of okra responsible for heavy losses in initial plant stand in the field particularly in summer grown crop. The reported pathogens associated with damping off have ability to survive as saprophyte in soil, perpetuate with resting structure and having wide host range. These attributes of pathogens have enabled the disease very difficult to manage with cultural, host resistance and chemical means. Soil borne pathogens can be managed successfully with the use of bioagents. Thus, biological control of damping off of okra can be one of alternative approach. Biological control agents (BCAs) can be highly specific and have different modes of action such as competition for space, nutrients and antibiosis. Suppression of pathogens have been reported due to volatile, non-volatile compounds of BCAs, degradation of cell wall by degrading enzymes like chitinase, β-1,3 glucanase, lipase results in lyses of cell wall and activate induced systemic resistance (ISR) (Junaid *et al.,* 2013). So, in present investigation *Trichoderma* spp. were isolated from okra rhizosphere soil, screened *in vitro* against dominant pathogen *R. solani* and potential strains were *in vivo* evaluated for the management of damping off.

Materials and Methods

Isolation and identification of pathogen(s) associated with damping off of okra

Okra fields were visited during summer months (April –June) in different locations of Kangra district in 2018 and 2019. Diseased plants of okra showing typical symptoms of damping off at seedling stage were collected and brought to the laboratory for studying associated causal pathogen. Isolation was done from diseased samples. Later fungal colony arising from single hypha of each isolate was multiplied on PDA medium and used for further studies.

Pathogenicity test of isolated pathogen (*R. solani*) was done by mixing 12 days old inoculum prepared on sterilized sand-wheat meal (8:2) medium (Sharma, 2011) at the rate 100g/kg in sterilized soil. Ten apparent healthy and surface sterilized seeds of susceptible okra variety P-8 were sown in each sick pot. Seeds sown in a pot having only sterilized soil were served as check. The pathogen was re-isolated from the diseased plant and cultured by standard methods discussed previously. The characteristics of pathogen culture thus obtained were compared with that of corresponding inoculated isolate of the pathogen to prove the pathogenicity. The most aggressive *R. solani* culture form Kangra was used for further studies.

The pathogen was identified by studying mycelial colour, diameter and constriction at the branching point, sclerotia size and colour on PDA

Isolation of *Trichoderma* spp. from rhizosphere soil

Isolation of *Trichoderma* spp. from okra rhizosphere soil samples, a serial dilution plate technique was followed. In this method, 10 g of soil was transferred in 100 ml of sterile distilled water and contents were mixed thoroughly by shaking for five minutes. Ten ml of aliquot was drawn and transferred to 90 ml of sterile distilled water. The suspension was shaken for one minute before it was further diluted. Further, dilutions till 10^{-4} to 10^{-6} were obtained and 1 ml of suspension from respective dilutions were transferred aseptically into Petri plates containing PDA medium and incubated at 25±1°C for five days

for the development of the colonies. Three replications were maintained for each dilution. Mycelial growth from such colonies was subculture on agar slants.

Dual culture technique

Twenty fungal antagonists obtained from okra rhizosphere soil were evaluated by dual culture technique against *Rhizoctonia solani*. Five mm discs of antagonist and pathogen were co-inoculated 7 cm apart on PDA plates and incubated at 25±1°C for 5 days.

In control, only a disc of pathogen was inoculated. Whole of experiment was carried out in triplicates. Percent growth inhibition was determined by Vincent, (1947) formula:

$$\text{Per cent growth inhibition} = \frac{\text{Growth (mm) in control} - \text{Growth (mm) in treatment}}{\text{Growth (mm) in control}} \times 100$$

Four bioagent isolates which exhibited significantly high inhibition of *R. solani in vitro* were further studied.

Morphological identification of potential bioagents

Two per cent malt extract agar Petri plates were inoculated in the centre with a 5 mm mycelial disc taken from pure culture of the isolates and incubated at 21±1°C for 4 days.

Each isolate was examined daily and colony cultural characters such as aroma and pigmentation on media were recorded.

Examination of the size and shape of conidia and phialide provided a tentative identification of *Trichoderma* spp. under a light microscope and compared with keys given by (Kubicek and Harman, 1998).

Mycoparasitism of biocontrol agent(s) on pathogen

The interactions between different bioagents and *R. solani* were studied *in vitro* using slide culture method (Bhat, 2017) to avoid the disturbance caused to the interacting mycelium while removing from the interaction zone in dual culture Petri plates.

In this method, pathogen and antagonist were grown as dual culture on a sterilized glass slide kept in a sterilized Petri plate chamber on PDA by keeping 3 mm bits each of pathogen and the antagonist 3 cm apart and incubated for 3 days at 25±1°C. Three replicates were maintained for each bioagent. When contact zone developed, the slide was taken out from Petri plate and stain with lactophenol cotton blue and viewed directly under microscope for hyphal interaction between the bioagent and host fungus.

Effect of volatile and non volatile compounds of bioagents against *Rhizoctonia solani*

Growth inhibition of pathogens by non-volatile compounds released by *Trichoderma* species

The effect of volatile compounds released by *Trichoderma* species was evaluated by 'invert plate technique'(Dennis & Webster, 1971b).

The PDA plates were inoculated with 5 mm mycelial discs of 3 days old growing culture of *Trichoderma* isolates. The lid of each plate was replaced with the bottom of other plate inoculated with 5 mm mycelial discs of pathogen. Both plates were sealed together with adhesive tape (parafilm) and incubated at 25±1°C for 5 days. Control treatment did not contain *Trichoderma* isolate. Percent growth inhibition was determined by Vincent, (1947) formula.

Effect of non-volatile compounds of bioagents against *Rhizoctonia solani*

Growth inhibition of pathogens by non-volatile compounds released by *Trichoderma* species

Poisoned food technique (Nene and Thapliyal, 1993) was followed to evaluate the effect of non-volatile compounds released by the *Trichoderma* spp. on the growth of *R. solani*. The *Trichoderma* spp. were grown in potato dextrose broth (PDB) assuming that the antagonist will utilize the nutrients from broth and release some non-volatile metabolites in the medium, which may affect the growth of pathogen. The culture filtrate was passed through Millipore 0.42 µ filter paper using vacuum pump and the filtrate was collected in a sterilized flask.

Then half of the culture filtrate was sterilized by autoclaving and remaining half was kept un-sterilized for further studies. PDA amended with culture filtrate at final concentrations of 1:1 and 2:1 culture filtrate/ PDA (v/v). Petri plates were centrally inoculated with 5 mm discs of *R. solani* cut from 4 days old culture and incubated at 25±1°C for 5 days. PDA plates not amended with culture filtrate and inoculated with the test pathogen were maintained as check. Per cent inhibition of the mycelial growth was calculated by formula given by Vincent, (1947).

In vivo management of damping off of okra

Effect of different methods of application of potential antagonists on disease development in pots

Soil was inoculated simultaneously with the mass cultured *R. solani* @ 100g/kg. Regular irrigation was provided to maintain adequate moisture necessary for development of antagonists and pathogen.

Preparation of spore suspension

Spore suspension was prepared by adopting the procedure of Singh *et al.*, (2016). The culture of *Trichoderma* strains was grown separately on PDA plates for 7 days at 25±1°C. The spores were harvested individually by scraping the surface of the colonies with a spatula in sterilized saline water (NaCl 0.85%) and filtered with sterile muslin cloth. The spore suspension of each strain was centrifuged at 10,000 rpm for 10 minutes. The pellet was resuspended in same volume of autoclaved 1.5 per cent CMC (Carboxymethyl cellulose).

Seed treatment with potential bioagents

Fifteen seeds of okra variety P-8 were immersed in spore suspension of each *Trichoderma* isolates for 30 minutes and sown in sick soil. Seeds soaked in distilled water kept as a control.

Seed biopriming with potential bioagents

The okra seeds were surface sterilized with 1.5 per cent sodium hypochlorite for 5 minutes, rinsed thrice with sterilized water and dried under laminar air flow on blotting paper.

Then, seeds soaked in the spore suspension for 4 hrs and treated with CMC. Seeds treated with CMC only were used as control. The seeds were dried in laminar air flow for 2 hours and placed in germination sheets saturated with water and kept at 25±1°C, maintained up to radicle emergence and then after sown in pots containing sick soil.

Soil application with potential bioagents

Trichoderma spp. were multiplied on FYM. For this purpose, FYM filled in flasks and autoclaved consecutively for 2 days. These flasks were then inoculated separately with

each antagonist culture aseptically and kept in incubator at 25±1°C for 15 days.

Drenching with potential bioagents

The culture of *Trichoderma* strains was grown separately on PDA plates for 7 days at 25±1°C. The spores were harvested individually by scraping the surface of the colonies with a spatula in distilled water. The spore suspension of each strain was centrifuged at 10,000 rpm for 10 minutes. Seedling roots were treated with bioagents by drenching into soil in each pot.

Results and Discussion

Pathogen(s) associated with damping off of okra

Analysis for associated pathogens of the infected plants from different locations (Baijnath, Bhawarna, Palampur, Kangra and Nagrota) in Kangra district showed that *Rhizoctonia solani* was dominant pathogen and associated with damping off of okra. Out of total 404 samples evaluated, the frequency of *R. solani* associated with the disease was 71 per cent while *F. solani* was associated with 11 per cent samples from different locations. During summer season, prevalence of warm and dry conditions may be attributed to the dominance of *R. solani* as damping off causing agent (Plate 1). Diwakar *et al.,* (1986) who also reported in India that damping off of okra caused by *R. solani* at early growth stage in the month of June-July from Konkan region of Maharashtra. Jandaik *et al.,* (2015) observed that *F. oxysporum, R. solani, Sclerotium rolfsii* were associated pathogens with damping off and wilt affected plants of okra from Solan area of Himachal Pradesh. In Iraq recently, Jalal *et al.,* (2019) reported that *R. solani, F. solani* and *Macrophomina phaseolina* were associated with damping off of okra.

Identification of pathogen(s)

The pathogen was identified as *R. solani*. A characteristic mycelium was observed under microscopic examination which had a small constriction at the base of the branch and the branches arise frequently at right angle from the parent mycelium. *R. solani* developed the light brownish to dark brown colour mycelium on PDA with small (less than 1 mm) and brown to dark brown coloured sclerotia. It covered the 90 mm Petri plates in 3-4 days when kept at 25 ±1°C. In microscopic study of fungal isolates which slowed colony growth with white mycelia revealed two types of macro and micro conidia. Macro conidia were slightly curved 3-5 septate and measured 30-40 X 4.5-6.0 µm while micro conidia were spherical to oval and measured 8-15 x 2.0-4.0 µm. It covered the 90 mm Petri plate in 7-8 days when kept at 25 ±1°C.

Isolation of bioagents from rhizosphere and *in vitro* screening of antagonistic activity against *Rhizoctonia solani*

The antagonistic activity of different isolates of *Trichoderma* spp. viz., *Trichoderma koningii* (DMA-8), *T. harzianum* (SMA-5), *T. koningii* (JMA-11), *T. viride, T. harzianum* (TH-11) available from the Department of Plant Pathology and 20 isolates of *Trichoderma* sp. (I 1 to 20) isolated from okra rhizosphere soil were evaluated against *R. solani in vitro* by dual culture method and results on mycelial growth inhibition are presented in table 1. The data revealed that all the isolates of *Trichoderma* spp. significantly inhibited the mycelial growth of *R. solani*. The mycelial growth inhibition of *R. solani* ranged from 22.2 to 67.8 per cent. Among all the isolates, *Trichoderma* sp. -2 proved to be the most effective strain that inhibited 67.8 per cent mycelial growth of *R. solani* followed by *Trichoderma* sp. -9 (67.0%), *Trichoderma* sp. -6 (64.8%) and *Trichoderma* sp. -11 (61.9%).

Trichoderma sp.-15 gave 22.2 per cent mycelial growth inhibition and proved to be least effective. Hence, four potential isolates were further studied for morpho-cultural identification, mechanisms of antagonism and management against *R. solani* causing damping off of okra. Kamala and Devi, (2012) also reported that 114 *Trichoderma* isolates inhibited growth of *R. solani* by dual culture method. Recently, Erayya Kumar *et al.*, (2018) evaluated 25 *Trichoderma* isolates which inhibited growth of *R. solani* and other pathogens by dual culture method.

Mycoparasitism between potential *Trichoderma* spp. and *Rhizoctonia solani*

Mode of antagonism between potential *Trichoderma* isolates and *R. solani* was studied by slide culture technique. The microscopic examination revealed that hyphae of *Trichoderma* sp.-2, *Trichoderma* sp.-6, *Trichoderma* sp.-9 and *Trichoderma* sp.-11 coiled with the hyphae of *R. solani* and penetrated hyphae of test pathogen (Plate 2). Earlier Dennis and Webster, (1971c) reported that most of *Trichoderma* isolates coiled around different test fungi (*Rhizoctonia solani*, *Fomes annosus*, *Fusarium oxysporum*, *Pyronema domesticum*, *Mucor hiemalis* and *Pythium ultimum*) on 2 per cent malt extract agar in dual culture. Elad *et al.,* (1983) reported mycoparasitic activity of *T. harzianum* and *T. hamatum* against *Sclerotium rolfsii* and *R. solani* under scanning electron microscopy. *Trichoderma* attach to host either by hyphal coils, hooks or appressoria.

Morpho- cultural identification of potential bioagents

On the basis of cultural characters, *Trichoderma* sp.-2 and *Trichoderma* sp.-9 was found to produce a typical smell resembling coconut and *Trichoderma* sp.-6 produce a smell resembling earthy. Colonies of *Trichoderma* sp.-6 produced diffusible yellow pigments in the medium thus PDA turn to yellowish in colour. The production of diffusible pigments was not found in other *Trichoderma* isolates. The microscopic features of *Trichoderma* isolates were observed under light microscope. light green and dark green coloured mature conidia observed in *Trichoderma* sp.-6 and *Trichoderma* sp.-9 on 2 per cent malt extract agar medium, respectively. Both *Trichoderma* sp.-6 and *Trichoderma* sp.-9 produce sub-globose to obovoid shape conidia. Phialide shape in *Trichoderma* sp.-9 and *Trichoderma* sp.-6 was observed verticillate and lageniform and ampulliform to lageniform, respectively.

On the basis of morphological characters *Trichoderma* sp. isolate - 6 was identified as *T. harzianum* while, *Trichoderma* sp. isolate - 9 was identified as *T. viride* (Table 2).

Effect of volatile an dnon volatile metabolites of bioagents on *Rhizoctonia solani*

The volatile compounds released by *Trichoderma* spp. have also exerted inhibitory effect on the growth of the *R. solani* (Table 3). Perusal of data revealed that the potential isolates of *Trichoderma* spp. significantly affected the mycelial growth of *R. solani*. Among the tested isolates, *Trichoderma* sp. -2 had shown maximum inhibition of mycelial growth (42.4%) followed by *Trichoderma* sp. -9 (39.3%), *Trichoderma* sp. -6 (30.7%) and *Trichoderma* sp. -11 (12.0%). Many workers reported that *Pseudomonas* and different *Trichoderma* species inhibited the normal mycelial growth by production of volatile substances such as alkyl pyrones and several heteroaromatic compounds (Claydon *et al.*, 1987; Michrina *et al.*, 1995; Schalchli *et al.*, 2011).

Table.1 *In vitro* efficacy of bioagents against mycelial growth of *Rhizoctonia Solani*

Bioagent isolates	Mycelial growth (mm) of *Rhizoctonia solani* in dual culture	Per cent inhibition
***T. harzianum* (TH-11)**	36.67	59.3 (7.76) *
***T. koningii* (JMA-11)**	37.67	58.2 (7.69)
***T. koningii* (DMA-8)**	37.00	58.9 (7.74)
T. viride	34.33	61.9 (7.93)
***T. harzianum* (SMA-5)**	35.33	60.7 (7.86)
***Trichoderma* sp.- 1**	39.33	56.3 (7.57)
***Trichoderma* sp.- 2**	29.00	67.8 (8.30)
***Trichoderma* sp.- 3**	43.33	51.9 (7.27)
***Trichoderma* sp.- 4**	43.33	51.9 (7.27)
***Trichoderma* sp.- 5**	45.00	50.0 (7.14)
***Trichoderma* sp.- 6**	31.67	64.8 (8.11)
***Trichoderma* sp.- 7**	43.33	51.9 (7.23)
***Trichoderma* sp.- 8**	42.33	53.0 (7.34)
***Trichoderma* sp.- 9**	29.67	67.0 (8.25)
***Trichoderma* sp.-10**	42.33	53.0 (7.35)
***Trichoderma* sp.-11**	34.33	61.9 (7.93)
***Trichoderma* sp.-12**	44.67	50.4 (7.17)
***Trichoderma* sp.-13**	58.00	35.7 (6.04)
***Trichoderma* sp.-14**	46.67	48.2 (7.01)
***Trichoderma* sp.-15**	70.00	22.2 (4.81)
***Trichoderma* sp.-16**	48.33	46.3 (6.89)
***Trichoderma* sp.-17**	63.33	29.6 (5.53)
***Trichoderma* sp.-18**	49.33	45.2 (6.80)
***Trichoderma* sp.-19**	61.33	31.9 (5.73)
***Trichoderma* sp.-20**	45.00	50.0 (7.14)
Control	90.00	0.0 (1.00)
CD (p=0.05)	3.693	0.293

* The figures in parentheses are square root transformed values

Table.2 Morpho-cultural identification of potential bioagents

Isolates	Aroma	Reverse colour on Petri plate	Conidia size (um) and shape	Phialide size (um) and shape	Identification
Trichoderma sp.-6	Earthy	Yellowish	**Size**- 2.7-3.5 ×2.1-2.6 **Shape**-Sub globose to obovoid, smooth walled and sub-hyaline to pale green	**Size**- 3.5-7.5 ×2.5-3.8 **Shape**- Ampulliform to lageniform	*Trichoderma harzianum*
Trichoderma sp.- 9	Coconut	No colour	**Size**- 2.6-3.8 ×2.2-3.4 **Shape**- dark green colour, smooth walled and Sub globose at maturity	**Size**- 6.0-12.0×2.4-3.0 **Shape**-Verticillate and more or less lageniform	*Trichoderma viride*
Trichoderma sp.-2	Coconut	No colour	**Size**-4.8×4.8 **Shape**- globose, dark green and smooth walled	**Size**-3.5-7.27×2.5-3.03 **Shape**-Verticillate, narrow ampulliform or lageniform	-
Trichoderma sp.-11	Absent	No colour	**Size**-2.7 ×2.7 **Shape**-globose, smooth walled and green to dull green	**Size**- 6.0-10.0×2.5-3.5 **Shape**- Ampulliform to lageniform	-

Table.3 *In vitro* efficacy of volatile metabolites of bioagents against mycelial growth of *Rhizoctonia solani*

Biocontrol agent	Mycelial growth (mm)	Per cent inhibition
***Trichoderma* sp. – 2**	51.8	42.4 (6.60) *
***Trichoderma* sp. – 6**	62.4	30.7(5.63)
***Trichoderma* sp. – 9**	54.6	39.3(6.35)
***Trichoderma* sp. -11**	79.2	12.0(3.60)
Control	90.0	0.0(1.00)
CD (p=0.05)	1.188	0.125

* The figures in parentheses are square root transformed value

Table.4 *In vitro* efficacy of non-volatile metabolites in autoclaved sterilized and filter sterilized culture filtrates of bioagents against mycelial growth of *Rhizoctonia solani*

Biocontrol agent	Autoclaved sterilized culture filtrate				Filter sterilized culture filtrate			
	Mycelial growth (mm)		Per cent inhibition		Mycelial growth (mm)		Per cent inhibition	
	1:1	2:1	1:1	2:1	1:1	2:1	1:1	2:1
***Trichoderma* sp.-2**	90.0	84.8	0.0	5.8 (2.60) *	79.4	65.6	11.8 (3.57) *	27.1 (5.30) *
***Trichoderma* sp.- 6**	90.0	80.0	0.0	11.1 (3.48)	41.2	34.0	54.2 (7.43)	62.2 (7.95)
***Trichoderma* sp.- 9**	90.0	81.0	0.0	10.0 (3.31)	52.0	51.0	42.2 (6.57)	43.3 (6.66)
***Trichoderma* sp.-11**	90.0	81.2	0.0	9.8 (3.28)	64.0	51.6	28.9 (5.47)	42.7 (6.61)
Control	90.0	90.0	-	-	90.0	90.0	0.0 (1.00)	0.0 (1.00)
CD (p=0.05)		1.246		0.217	1.260	1.792	0.138	0.156

* The figures in parentheses are square root transformed value

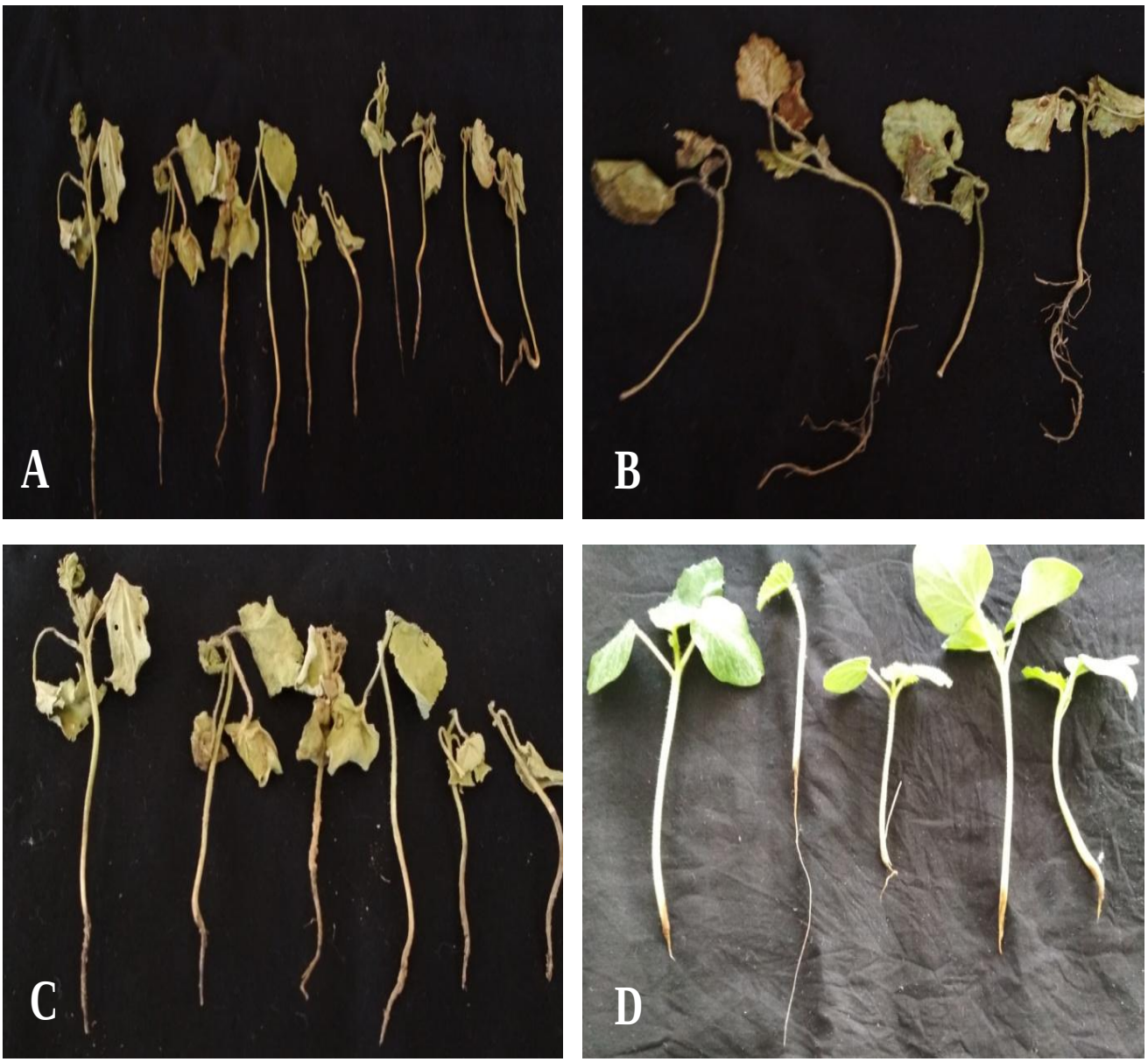
Table.5 Biological control of damping off of okra with seed treatment and biopriming of potential bioagents

	Seed treatment						Biopriming					
Bioagents	Seed germination (%)	Pre-emergence (%)	Disease control (%)	Post-emergence (%)	Disease control (%)	Seedling stand (%)	Seed germination (%)	Pre-emergence (%)	Disease control (%)	Post-emergence (%)	Disease control (%)	Seedling stand (%)
***Trichoderma* sp.- 2**	66.7	33.3	37.5	39.1	36.2	41.7	81.7	18.3	64.5	22.7	61.6	63.3
***Trichoderma* sp.- 6**	61.7	38.3	28.1	54.7	10.7	28.3	73.3	26.7	48.4	21.0	64.5	58.3
***Trichoderma* sp.- 9**	65.0	35.0	34.4	52.7	14.0	31.7	78.3	21.7	58.1	18.9	68.1	63.3
***Trichoderma* sp.-11**	55.0	45.0	15.6	58.3	4.9	21.7	66.7	33.3	35.5	29.3	50.4	48.3
Control (with pathogen)	46.7	53.3	-	61.3	-	18.3	48.3	51.7		59.1		20.0
Control (without pathogen)	91.7	8.3	-	-	-	91.7	91.7	8.3		-		91.7
CD (p=0.05)	14.00	2.21		2.45			10.01	2.31		2.29		

Table.6 Biological control of damping off of okra with soil and drenching application of potential bioagents

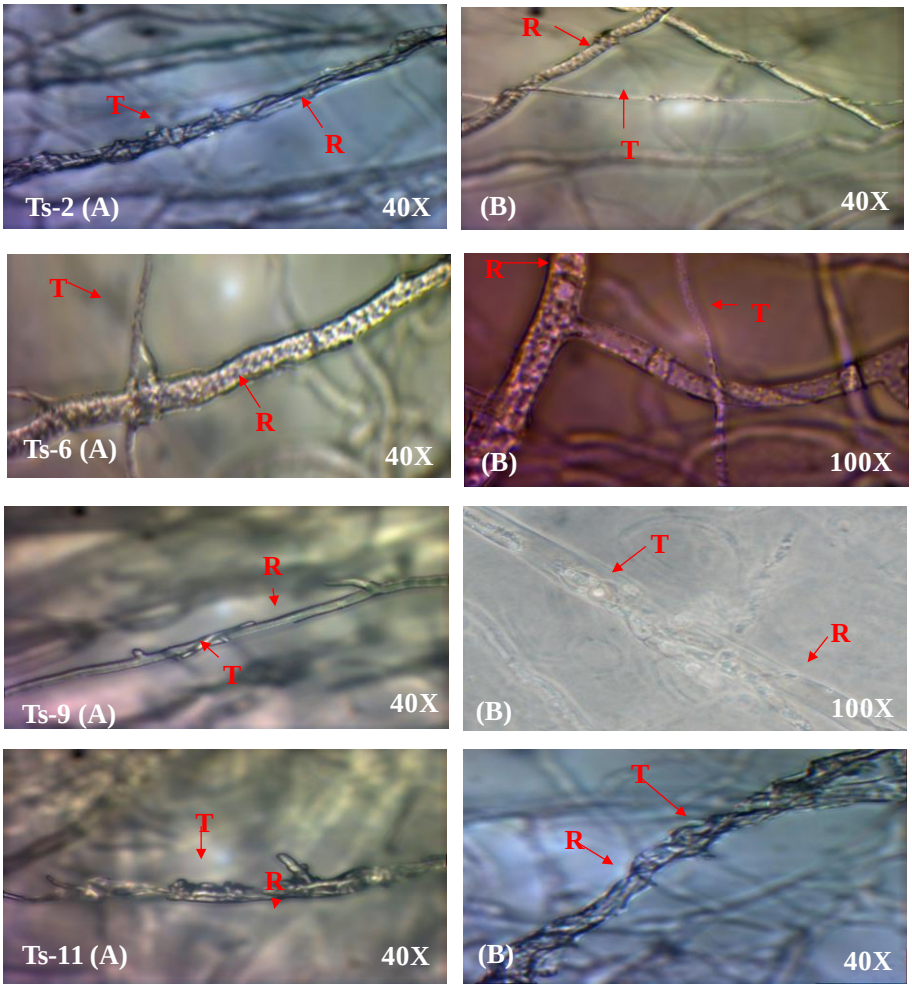
	Soil application						Drenching					
Bioagents	Seed germination (%)	Pre-emergence (%)	Disease control (%)	Post-emergence (%)	Disease control (%)	Seedling stand (%)	Seed germination (%)	Pre-emergence (%)	Disease control (%)	Post-emergence (%)	Disease control (%)	Seedling stand (%)
***Trichoderma* sp.- 2**	76.7	23.3	56.3	29.2	52.4	55.0	46.7	53.3	0.0	53.0	13.6	25.0
***Trichoderma* sp.- 6**	71.7	28.3	46.9	33.5	45.4	46.7	46.7	53.3	0.0	57.7	5.8	26.7
***Trichoderma* sp.- 9**	65.0	35.0	34.4	52.6	14.1	31.7	46.7	53.3	0.0	60.3	1.7	26.7
***Trichoderma* sp.-11**	56.7	41.7	21.9	58.0	5.4	20.0	46.7	53.3	0.0	53.6	12.6	20.0
Control (with pathogen)	46.7	53.3	-	61.3	-	18.3	46.7	53.3		61.3		28.3
Control (without pathogen)	91.7	8.3	-	-	-	91.7	91.7	8.3				91.7
CD (p=0.05)	9.45	2.26		2.29			5.80	0.01				

Plate.1 Damping off symptoms of infected okra seedlings collected from different locations in Kangra district



A- Kangra; B-Baijnath; C- Bhawarna; D- Palampur

Plate.2 Mycoparasitic interaction between *Rhizoctonia solani* and potential bioagents



Ts-2 – *Trichoderma* sp.-2; Ts-6 – *Trichoderma* sp.-6
Ts-9 – *Trichoderma* sp.-9; Ts-11– *Trichoderma* sp.-11
A- Coiling ; B-Penetration

Effect of non-volatile metabolites of bioagents on *Rhizoctonia solani*

The growth inhibition of *R. solani* by autoclaved and filter sterilized culture filtrate of *Trichoderma* spp. is presented in table 4. The data on inhibition of mycelial growth of *R. solani* obtained at two ratios (1:1 and 2:1) of autoclaved sterilized and filter sterilized filtrate revealed that all the tested isolates of *Trichoderma* spp. though significantly inhibited the mycelial growth of *R. solani* but comparatively less than filter sterilized. Autoclaved sterilized culture filtrates at 1:1 ratio did not have any impact on the radial growth of fungus however, at 2:1 ratio *Trichoderma* sp. -6 exhibited maximum mycelial inhibition (11.1%) followed by *Trichoderma* sp. -9 (10.0%). Filter sterilized culture filtrate of *Trichoderma* sp. -6 resulted in maximum mycelial inhibition of 54.2 and 62.2 followed by *Trichoderma* sp. -9 inhibited 42.2 and 43.3 per cent at 1:1 and 2:1 ratio, respectively. Our results were in accordance with several workers who have also found inhibition of mycelial growth by cultural

filtrates of various bioagents (Kucuk and Kivanc, 2003; Raut *et al.*, 2014 and Tapwal *et al.*, 2015).

Evaluation of potential bioagents for management of damping off of okra

The *Trichoderma* spp. isolates found as most potential antagonist against *R. solani* under *in vivo* screening were evaluated for the management of damping off through the application of bioagents as, seed treatment, soil application, seed biopriming and drenching under net house conditions and had shown significant reduction in pre and post-emergence damping off of okra (Table 5 and 6).

Among all the treatments, *Trichoderma* sp.-2 was significantly superior for controlled pre-emergence damping off, 64.5, 56.3 and 37.5 per cent when applied as biopriming, soil application, seed treatment, respectively followed by *Trichoderma* sp.-9 controlled 58.1 and 34.4 per cent disease when applied as biopriming and seed treatment, respectively and *Trichoderma* sp.-6 controlled 46.9 per cent disease when applied as soil application.

Post- emergence damping off highest controlled (68.1%) by *Trichoderma* sp.-9 when applied as biopriming followed by *Trichoderma* sp.-2 controlled disease upto 52.4, 36.2 and 13.6 per cent when applied as soil application, seed treatment and drenching followed by *Trichoderma* sp.-6 controlled disease upto 64.5 and 45.4 per cent when applied as biopriming and soil application and *Trichoderma* sp.-9 controlled disease upto 14 per cent when applied as seed treatment and *Trichoderma* sp.-11 controlled disease upto 12.6 per cent when applied as drenching.

In general, highest pre-emergence disease control (64.5%) obtained when *Trichoderma* sp.-2 and post-emergence disease control (68.1%) obtained when *Trichoderma* sp.-9 applied as biopriming. Similarly, the *Trichoderma* sp.-2 isolate was also most effective in *in vitro* screening as it inhibited 67.8 per cent mycelial growth of *R. solani* in dual culture and its volatile metabolites inhibited pathogen growth up to 42.4 per cent. Similarly, in summer season, Rai and Basu (2014) conducted a field experiment with *T. viride* and *P. fluorescens* bio-primed okra seeds and observed that *T. viride* improved plant length, pod length, pod diameter and seed yield.

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