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Assessment of Local Strain of *Trichoderma asperellum* against *Fusarium* Spp.

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ABSTRACT

Trichoderma is unique fungus and now being most popular among the farmers as growth promoter and biocontrol agent. In present study local *Trichoderma asperellum* strain was evaluated against different *Fusarium* species *in vitro* and greenhouse conditions. *In vitro* study, *Trichoderma asperellum* was evaluated against 7 *Fusarium* species i.e., *Fusarium oxysporum* f. sp. *ciceris*, *F. oxysporum* f. sp. *cubense (Tropical Race 1)*, *F. oxysporum* f. sp. *cubense (Tropical Race 2)*, *F. oxysporum* f. sp. *cubense (Tropical complex)*, *F. oxysporum* f. sp. *lycopersici* and *Fusarium solani* and percentage reduction in the growth of these pathogens was ranged between 40.38 and 46.02%. This strain showed strong antagonistic potential against *F. oxysporum* f. sp. *lycopersici* (73.91%) followed *F. oxysporum* f. sp. *cubense race* TR1 (64.49%), *Fusarium udum* (59.17), *F. oxysporum* f. sp. *ciceris* (58.33%), *Fusarium solani* (56.30%), *F. oxysporum* f. sp. *cubense* TR 2 (52.78%) and *F. oxysporum* f. sp. *cubense TR (complex)* 46.02%. In greenhouse study, *Trichoderma asperellum* was evaluated alongwith chitosan and botanicals against *Fusarium oxysporum* f. sp. *lycopersici* and minimum per cent disease index (PDI) was observed in treatment T4 (Seedling treatment with *Trichoderma asperellum* @ 5 g/lit + Chitosan @ 0.1% followed by its foliar spray) in which 44.66 per cent PDI recorded followed by T5 (Seedling treatment with *Trichoderma* isolate 8 @ 5 g/lit + Chitosan @0.1% followed by its foliar spray) in which 49.37 per cent PDI observed. The maximum per cent disease index was recorded in inoculated control (71.35%).

Keywords: *Trichoderma asperellum; Fusarium* spp; Biocontrol agent; Tomato wilt

INTRODUCTION

Plant diseases are major obstacle in enhancing the crop productivity and have direct role in the destruction of natural resources in agriculture. Crops are attacked by various pathogens; in particular, soil borne plant pathogens cause heavy crop losses all over the world. Control of soil borne plant pathogens are very difficult because the resting body of the fungus survive in soil for many years and whenever susceptible crops is grown in the field, fungus may cause the infection. They are often difficult to control, even with conventional strategies. The use of chemicals in controlling plant diseases has significant role in enhancing the crop productivity and quality over the past 100 years but chemical control of such plant pathogens disturbs the environment, subverts ecology, degrades soil productivity, and mismanages water resource [1,2]. Worldwide organic produce and pesticide free food demand is increasing day by day and scientific community searching new option for controlling the diseases. Bio control agents have proved potential in minimizing soil borne plant pathogens and considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods [3]. Biological control agents are cost-effective without having any adverse effects on animal, plant, human health or environment, self-sustaining, development of host resistance is unlikely and it is compatible with other crop disease control techniques.

Biocontrol techniques have gained momentum in plant disease control of crop plants in recent time. These technologies minimising the application of chemical pesticides and also safer to human and environment. Among biocontrol agents, *Trichoderma* spp. have been accepted a potent fungal biocontrol agent for the management of a wide range of destructive soil borne plant pathogens because of their antagonistic mechanisms and plant-growth promoter effects [4]. *Trichoderma* is a fungus that exists in almost all soils and a wide range of habitats [5]. The soil application of *Trichoderma* conidial preparations has been demonstrated experimentally to increase the crop growth and resistant to *Fusarium* diseases [6]. Lytic enzymes released by *Trichoderma* isolates are very important in the biocontrol of root rot fungi such as *Rhizoctonia, Sclerotium, Phytium* and *Fusarium* species [7-10]. *Fusarium* species are one of the yield limiting factors of crops in agriculture areas of the rhizosphere microbiome [11]. The incidence and destructive of *Fusarium* species is increasing in recent years due to climate change and changing cropping system. Hence, the present study carried to isolation,

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identification and evaluation of the antagonistic activities of local strain of *Trichoderma asperellum* against some *Fusarium* species.

MATERIALS AND METHODS

The study was conducted in the Bio-Control Lab, Department of Plant Pathology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India.

Collection of soil simples

Sixty two soil samples were collected from rhizospheric soils of different crops at different location of Muzaffarpur and Samastipur districts, Bihar. At the time of soil sample collection the crops were Chickpea, Brinjal, Lentil, Tomato, Rajama, Carrot, Arhar, Bhindi, Yambean, Sweet Potato, Pea, Potato, Chilli, Brinjal, Papaya, Rice, Banana and Mustard (Table 1). Samples were placed in sterile polyethylene bags, transported to the laboratory and stored at 4°C for further studies. Soil samples were serially diluted and transferred on Trichoderma Selective Medium.

Isolation of *Trichoderma* from the soil

Soil samples were serially diluted and transferred on Trichoderma Selective Medium. One ml of 10^{-4} dilution was poured onto *Trichoderma harzianum* Selective Agar Base medium (Hi Media, M18360) and ingredients in medium were Magnesium sulphate heptahydrate: 0.20 g, Dipotasium hydrogen phosphate: 0.90 g, Ammonium nitrate: 1.00 g, Potassium nitrate: 0.15 g, Glucose:3.00 g, Rose Bengal: 0.15 g, Agar: 20.00 g and Distilled water: 1.00L. The appearance of the colonies of *Trichoderma* on Petri dishes purified by hyphal tip isolation techniques. *Trichoderma* spp. was identified, picked on the basis of their morphological, microscopic characteristics. The purified and identified cultures of *Trichoderma* spp. were maintained on Potato Dextrose Agar (PDA) medium and stored at 4°C for further experimentation. Pure cultures were kept in 20% (w/v) glycerol at -20°C.

Serial dilution technique

Isolation was done employing serial dilution technique [12]. 1 g soil sample was suspended in 10 ml of sterilized distilled water, stirred well and called 10^{-1} dilution. One ml of this suspension, well shaken, was added to 9 ml of sterilized distilled water to make 10^{-2} dilution. The procedure was repeated till the desired dilutions were obtained (10^{-4}, for rhizospheric soil).

Collection of pathogens

Seven *Fusarium* spp. namely *Fusarium oxysporum* f. sp. *ciceris, F. oxysporum* f. sp. *cubense (Tropical Race 1), F. oxysporum* f. sp. *cubense (Tropical Race 2), F. oxysporum* f. sp. *cubense (Tropical complex), F. oxysporum* f. sp. *lycopersici, Fusarium udum* and *Fusarium solani* were obtained from Department of Plant Pathology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India. The isolates were maintained on PDA medium and placed until use at 4°C.

Cultural and morphological characteristics of *Trichoderma* isolates

In the cultural characteristics studies including growth, growth rate, sporulation, colony colour and growth pattern were examined on potato dextrose agar. Cultural and morphological characters were compared with morphologically identified strains. *Trichoderma* species mycelia growth initially seem creamy white, uniform which latter appeared in sector (2 no.) and turned dark green in colour. Fungal hyphae of *Trichoderma* species are septet, hyaline and smooth-walled which produces numerous conidiophores are highly branched, the main branches were mostly in groups of 2-3 and stand at 90^0 angle. Conidia were one-celled, either ellipsoidal or globose and light to dark green, or sometimes colorless, greyish or brownish. Chlamydospores also observed under the stress conditions for the survival. They are normally found as thickwalled, enlarged

Table 1: Details of soil samples collected from different crops and locations.

Isolates of Trichoderma	Crop ecosystem	Place of collection
Trichoderma isolate-1	Chickpea (*Cicer arietinum*)	TCA Dholi (Muzaffarpur)
Trichoderma isolate-2	Lentil (*Lens culinaris*)	TCA Dholi (Muzaffarpur)
Trichoderma isolate-3	Rajama (*Phaselous vulgaris*)	TCA Dholi (Muzaffarpur)
Trichoderma isolate-4	Potato (*Solanum tuberosum*)	Pusa (Samstipur)
Trichoderma isolate-5	Yambean (*Pachyrhizus erosus*)	TCA Dholi (Muzaffarpur)
Trichoderma isolate-6	Sweet potato (*Ipomoea batata*)	TCA Dholi (Muzaffarpur)
Trichoderma isolate-7	Brinjal (*Solanum melongena*)	Jahagirpur (Muzaffarpur)
Trichoderma isolate-8	Tomato (*Solanum lycopersicum*)	Mirapur (Muzaffarpur)
Trichoderma isolate-9	Potato (*Solanum tuberosum*)	Mirapur (Muzaffarpur)
Trichoderma isolate-10	Chilli (*Capsicum annuum*)	Dholi Bazar (Muzaffarpur)
Trichoderma isolate-11	Brinjal (*Solanum melongena*)	Harpur (Samastipur)
Trichoderma isolate-12	Tomato (*Solanum lycopersicum*)	Mahmada (Samastipur)
Trichoderma isolate-13	Carrot (*Daucus carota*)	Harpur (Samastipur)
Trichoderma isolate-14	Bhindi (*Abelmoschus esculentus*)	Dighra (Samastipur)
Trichoderma isolate-15	Bhindi (*Abelmoschus esculentus*)	Pusa (Samastipur)
Trichoderma isolate-16	Pea (*Pisum sativum*)	Garhia (Samastipur)
Trichoderma isolate-17	Bhindi (*Abelmoschus esculentus*)	Deopar, (Samastipur)
Trichoderma isolate-18	Brinjal (*Solanum melongena*)	Pusa (Samastipur)
Trichoderma isolate-19	Tomato (*Solanum lycopersicum*)	Pusa (Samastipur)
Trichoderma isolate-20	Brinjal (*Solanum melongena*)	Malinagar (Samastipur)

vegetative cells with condensed cytoplasm which are unicellular, globose to subglobose chlamydospores are either formed within hyphae or at the hyphal tips [13,14].

Molecular characterization and identification of *Trichoderma* isolate 4

The best *Trichoderma* isolate 4 was sent to National Fungal Cultural Collection of India (NFCCI), Agharkar Research Institute, pune for morphological, molecular characterization and identification of isolate. This potential isolate on molecular basis identified as *Trichoderma asperellum*, Samuels Lieckf. & Nirenberg and awarded Accession no. NFCCI 4586 by National Fungal Cultural Collection of India (NFCCI).

Evaluation of antagonistic potential of *Trichoderma asperellum* with *Fusarium* species *in vitro*

The *Trichoderma asperellum* was evaluated against *Fusarium* spp. namely *Fusarium oxysporum* f. sp. *ciceris*, *F. oxysporum* f. sp. *cubense (Tropical Race 1)*, *F. oxysporum* f. sp. *cubense (Tropical Race 2)*, *F. oxysporum* f. sp. *cubense (Tropical complex)*, *F. oxysporum* f. sp. *lycopersici*, *Fusarium udum* and *Fusarium solani* by dual culture technique. For this, seven mm disc from actively growing culture of each *Fusarium* sp. was placed on one side of the Petri dish and seven mm disc of *Trichoderma asperellum* was placed at opposite side in the same plate. Each treatment was replicated three times and appropriate control with alone inoculation of *Fusarium* species also maintained. The plates were incubated at room temperature (28 ± 1°C) for seven days and observations were made after 7 days of incubations. The colony diameter of *Fusarium* species in dual culture plates and control plates was recorded to work out the per cent inhibition of growth of *Fusarium* spp. by respective *Trichoderma asperellum*. The per cent inhibition of mycelial growth over control was calculated by following formula given by Vincent [15].

Percent inhibition over check = C-T/C × 100

Where,

C= Growth of pathogen in check

T = Growth of pathogen in treatment

Evaluation of efficacy of *Trichoderma asperellum*, botanicals extracts and chitosan against *Fusarium oxysporum* f. sp. *lycopersici* in green house

The experiment was conducted under green conditions in plastic pots (5 kg capacity) filled with sterilized well pulverized sandy-loam soil mixed with vermicompost (200 g /pot). Each pot was inoculated with culture of *Fusarium oxysporum* f. sp. *lycopersici* @ 10 g/ pot. The extracts were prepared by crushing 100 g of Elephant foot yam (rhizome), Galic (bulb), and Yambean (seed) of each in 100 ml sterilized distilled water using mixer and grinder. The supernatant was filtered through double layered muslin cloth. The stock solution (100%) was further diluted with the help of double sterilized distilled water to prepare concentration of 30 per cent. The tomato seeds (Jagannath tomato -3) obtained from the Vegetable Department, RPCAU, for the study. Twenty one days old tomato seedlings were transplanted in each pots (5No.) after one week of pathogen inoculation while without any treatment served as check. Watering of pots was done when required to maintain optimum level of moisture. The experiment was laid out in a completely randomized design with three replications. The details of treatments are given in Table 2.

Mass multiplication of *Fusariun oxysporum f.* sp. *lycopersici*

The mass culture of the fungus was prepared on maize seed. The seeds were soaked overnight in 5% sucrose and chloramphanicol (30 mg/L) solution. The soaked seeds were then transferred to conical flasks (500 ml) and were autoclaved twice at 1.5 kg/cm^2 pressure at 121°C for 20 minutes. Thereafter, the flasks were inoculated with pure cultures of *F. oxysporum* f. sp. *lycopersici* and were incubated for 10 days in an incubator at 28°C. During incubation the flask were shaken periodically for 1-2 min. to attain the uniform growth of fungus and finally ground into fine powder.

Seedling root dipping treatment

For seedling root dipping treatment, 21 days old tomato seedlings were uprooted gently, the roots of seedling were washed with distilled water and then dipped in different treatments and transplanted in *Fusarium* inoculated pots.

Observations

Disease infection per cent was recorded after 75 days transplanting and per cent disease index was worked out by Silme and Cagirgan, [16] which was based on infection percent as follows:

1-10% = 1, 11-20% =2,21-30% = 3,31-50% = 4, 51-100% = 5

Table 2: Details of treatments evaluated under green house.

Sl. No	Treatment	Description
1.	T_1	Seedling treatment with Chitosan @ 0.1%
2.	T_2	Seedling treatment with Garlic @ 10%
3.	T_3	Seedling treatment with Garlic @ 10% followed by its foliar spray
4.	T_4	Seedling treatment with *Trichoderma asperellum* (5 g/lit) + Chitosan (0.1%) followed by its foliar spray
5.	T_5	Seedling treatment with *Trichoderma* isolate 8 (5 g/lit) + Chitosan (0.1%) followed by its foliar spray
6.	T_6	Seedling treatment with Yam bean seed extract @30%
7.	T_7	Seedling treatment with Yam bean seed extract @30% followed by its foliar spray
8.	T_8	Seedling treatment with Elephant foot yam @ 30%
9.	T_9	Seedling treatment with Elephant foot yam @ 30% followed by its foliar spray
10.	T_{10}	Seedling treatment with Carbendazim @ 0.1%
11.	T_{11}	Inoculated Control

$$Per\ cent\ Disease\ Index = \frac{Sum\ of\ all\ disease\ Rating}{Total\ number\ of\ leaf\ exam\ in\ ed\ X\ Maximum\ Rating} \times 100$$

Statistical analysis

Data were analysed using statistical package OPSTAT.

RESULTS AND DISCUSSION

Isolation of *Trichoderma* from collected soil samples

Twenty *Trichoderma* isolates were isolated from sixty two soil samples collected from rhizospheric soils of different crops at different location of Muzaffarpur and Samastipur districts. At the time of soil sample collection the crop were Chickpea, Brinjal, Lentil, Tomato, Rajama, Carrot, Arhar, Bhindi, Yambean, Sweet Potato, Pea, Potato, Chilli, Brinjal, Papaya, Rice, Banana and Mustard.

Cultural and morphological characteristics of *Trichoderma* isolates

Cultural characteristics including growth, growth rate, sporulation, colony colour and growth pattern were examined on potato dextrose and presented in Table 3. All the isolates grew rapidly on PDA and showed different characteristics on conidial masses. Initially mycelial growth was creamy white, uniform, fluffy which latter appeared in sector (2 no.) and turned dark green in colour. Most of the isolates conidiophores were much branched and form loose tuft, the main branches were mostly in groups of 2-3 and stand at 90^0 angle. Phialides arised in whorls of 3-5 and branched at 45°-50° angle in divergent verticillate fashion. Phialospores (conidia) were small, subglobose, smooth walled, pale green in colour. The confirmation of species level identification of *Trichoderma* isolates was carried out according to an interactive key provided by Samuels [17] at http://nt.ars-grin.gov/ taxadescriptions/ keys/ Frame Key. cfm? gen -*Trichoderma*. Rifai [18] reported that *Trichoderma* is a septate fungus and produces highly branched conidiophores with a conical or pyramidal outline. He further described that *Trichoderma* species form floccose or tufted colonies of various colors (white, yellow, green), which can be used to identify and differentiate about various species of the genus. Similar results were reported by Gams and Bissett [19].

The best *Trichoderma* isolate 4 was sent to National Fungal Cultural Collection of India (NFCCI), Agharkar Research Institute, pune for morphological, molecular characterization and identified as *Trichoderma asperellum*, Samuels Lieckf. & Nirenberg and further this potential strain tested against *Fuarium* spp. *in vitro* and *in vivo*. Details of brief description of the *Trichoderma asperellum*, Samuels Lieckf. & Nirenberg is given below.

Brief description of fungal identification: Macroarphology: Colondy on PDA at 25+2, fast growing, dull green, fasciculate, floccose, reverse buff. Micromorphology: Chlamydospored produce abundantly, intercalary, globose to to subglobose, hyaline, smooth walled, wall thick and darkened, up to 11.13 × 12.45 um *Phialides ampulliform*, 2-3 in groups 7.42-11.45 × 2.5 × 3.67 um. Phialospores various shape and size, hyaline smooth walled, globose to oval to cylindrical upto 3.9-8.15 × 2.15 – 3.64 um. Hyphae branched, smooth walled, hyaline up to 11.7 um wide (Figure 1).

Results on molecular identification: The tested fungal strain T1 showed 100% sequence similarity with *Trichoderma asperellum*. Sequence analyses with NCBI accession number KR86829, *Trichoderma asperellum* strain ZWPBG2 resulted in following statistics.

Table 3: Cultural characteristics and growth of different *Trichoderma* isolates.

Trichoderma isolates	Colony growth (mm) After 72hrs at 28^0C	Culture colour	Sporulation initiate after (hrs)
Trichoderma isolate-1	61.33	Whitish green	48-72
Trichoderma isolate-2	63.33	Dark green	48
Trichoderma isolate-3	62.00	Whitish green	48-72
Trichoderma isolate-4	**71.00**	Dark green	48-72
Trichoderma isolate-5	68.33	Dark green	48
Trichoderma isolate-6	**70.00**	Dark green	48
Trichoderma isolate-7	62.33	Whitish green	48
Trichoderma isolate-8	**69.00**	Light green	48
Trichoderma isolate-9	62.33	Dark green	48
Trichoderma isolate-10	58.00	Whitish green	48-72
Trichoderma isolate-11	61.00	Whitish green	48
Trichoderma isolate-12	62.00	Whitish green	48
Trichoderma isolate-13	66.67	Light green	48-72
Trichoderma isolate-14	65.33	Whitish green	48-72
Trichoderma isolate-15	66.33	Light green	48-72
Trichoderma isolate-16	68.00	Whitish green	48
Trichoderma isolate-17	66.33	Dark green	48
Trichoderma isolate-18	63.00	Whitish green	48-72
Trichoderma isolate-19	64.00	Dark green	48
Trichoderma isolate-20	64.00	Dark green	48
CD at 5%	2.63		

Alignment statistics: Query Length 5654, Score- 1018 bits (1128), Expect-0.0, Identities- 564/564(100%), Gaps-0/564 (0%), Strand-Plus/Minus.

Several workers isolated the *Trichoderma* spp from different crop rhizosphere. Neelamegam [20] isolated eight isolates of *Trichoderma viride* from rhizosphere soil of healthy tomato seedlings, collected at different locations and three were identified as antagonists. Joshi [21] isolated 62 isolates of *Trichoderma* sp. from different rhizospheric soil samples collected from different places located in western Himalayas region.

Evaluation of antagonistic potential of *Trichoderma asperellum* with *Fusarium* spp. *in vitro*

The *Trichoderma asperellum* was evaluated against *Fusarium* spp. namely *Fusarium oxysporum* f. sp. *ciceris*, *F. oxysporum* f. sp. *cubense (Tropical Race 1)*, *F. oxysporum* f. sp. *cubense (Tropical Race 2)*, *F. oxysporum* f. sp. *cubense (Tropical complex)*, *F. oxysporum* f. sp. *lycopersici*, *Fusarium udum* and *Fusarium solani* (Figure 2) by dual culture technique. *Trichoderma asperellum* had the ability to inhibit the mycelial growth of the 7 pathogens. The percentage reduction in the growth of these pathogens was ranged between 46.02% to 73.91% (Table 4). This strain showed strong antagonistic potential against *F. oxysporum* f. sp. *lycopersici* (73.71%) followed by *F. oxysporum* f. sp. *cubense race* TR1 (64.49%), *Fusarium udum* (59.17), *F. oxysporum* f. sp. *ciceris* (58.33%), *Fusarium solani* (56.30%), *F. oxysporum* f. sp. *cubense TR 2*(52.78%) and *F. oxysporum* f. sp. *cubense TR (complex)* 46.02%. Fungi in the genus *Trichoderma* have been known since at least 1920's for their ability to act as biocontrol agents against plant pathogens [22]. Earlier some worker had studied the *Trichoderma* antagonistic potential *in vitro Fusarium* spp. This result was in agreement with the results found by Segrra [23] who reported an efficient biological control agent in controlling *Fusarium* wilt in tomato and Ommati and Zaker [24] reported highest inhibition of the growth of *F. oxysporum* by *T. asperellum*. Padmodaya and Reddy [25] also studied the efficacy of ten isolates of *Trichoderma* against *Fusarium oxysporum* f. sp. *lycopersici* by dual culture method and found that *Trichoderma viride* was most effective

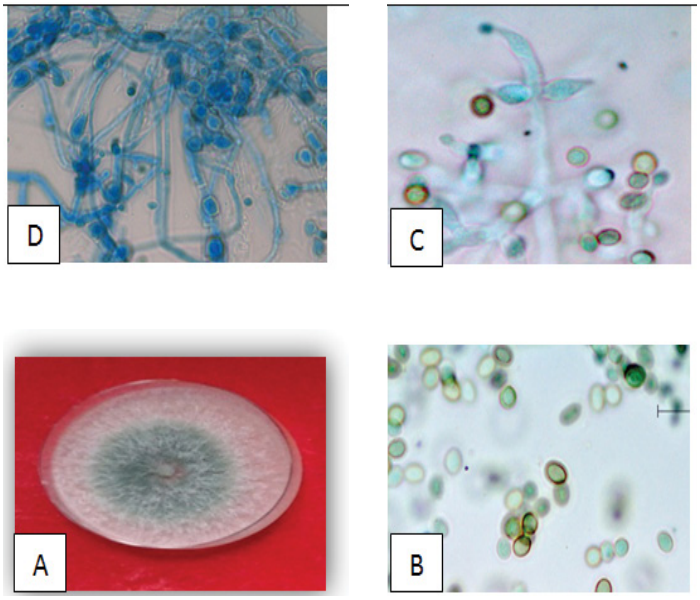

Figure 1: Micromorphology of *Trichoderma asperellum*. Isolate 4 *(Trichoderma asperellum)*. A. Culture, B Conidia, C. Conidiophore & Philalides D. Clamydospores,.

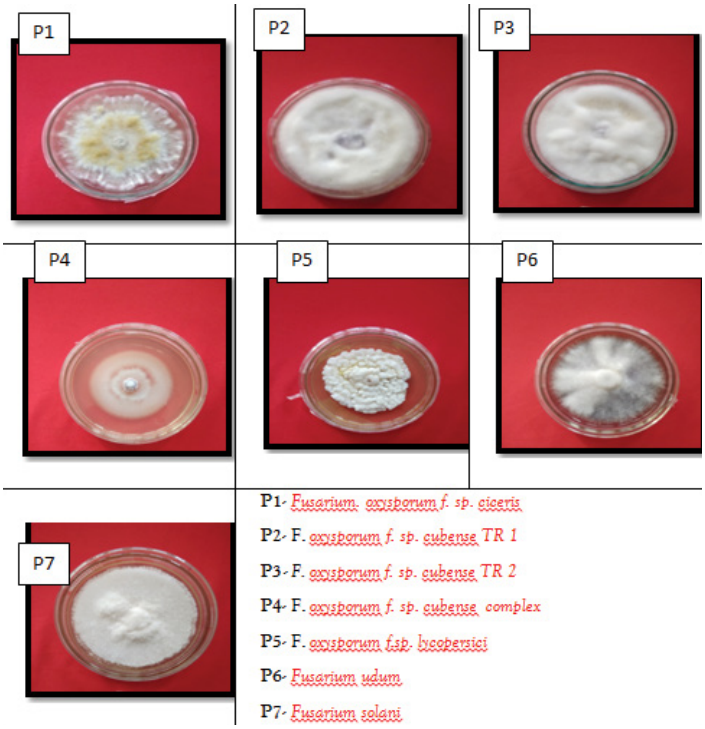

Figure 2: Different *Fusarium* species.

Table 4: Evaluation of antagonistic potential of *Trichoderma asperellum* with *Fusarium* spp. *in vitro*.

Soilborne plant pathogens	Percent reduction on colony diameter over control
Pathogens	***Trichoderma asperellum***
F. oxysporum f. sp. ciceris	58.33 (49.78)
F. oxysporum f. sp. cubense TR 1	64.49 (53.41)
F. oxysporum f. sp. cubense TR 2	52.78 (46.58)
F. oxysporum f. sp. cubense TR complex	46.02 (42.70)
F. oxysporum f.sp. lycopersici	73.91 (59.27)
Fusarium udum	59.17 (50.26)
Fusarium solani	56.30 (48.64)
CD at 5%	5.41 (3.16)
SE (m)	1.77 (1.03)
CV	5.21 (3.57)

and it inhibited the growth of pathogen by 44.80 per cent. Midhun Babychan and Sobita Simon [26] reported 58.4 per cent inhibition of radial mycelial growth of the pathogen (*Fusarium oxysporum* f. sp *lycopersici*) by *Trichoderma* isolate MiT-4.

Evaluation of efficacy of *Trichoderma asperellum*, chitosan and botanicals extract against *Fusarium oxysporum* f. sp. *lycopersici* in green house

In the study, *Trichoderma asperellum* was evaluated along with chitosan and botanicals against *Fusarium oxysporum* f. sp. *lycopersici* in green house conditions. The results obtained with seedling treatment alone and seedling treatment followed by foliar spray of treatments significantly superior over inoculated control in reducing per cent disease index (Table 5 and Figure 3). Among the ecofriendly treatments, minimum PDI was observed in treatment T4 Seedling treatment with *Trichoderma asperellum* @ 5 g/lit + Chitosan @ 0.1% followed by its foliar spray) in which 44.66 per cent PDI recorded followed by T5 (Seedling treatment with Trichoderma isolate 8 @

Table 5: Evaluation of efficacy of *Trichoderma asperellum*, chitosan and botanicals extract, against *Fusarium oxysporum* f.sp. *lycopersici* in greenhouse.

No.	Treatments	Description	PDI (%)
1.	T1	Seedling treatment with Chitosan @ 0.1%	52.95 (46.67)
2.	T2	Seedling treatment with Garlic @ 10%	53.09 (46.75)
3	T3	Seedling treatment with Garlic @ 10% followed by its foliar spray	51.42 (45.79)
4	T4	Seedling treatment with *Trichoderma asperellum* (5 g/lit) + Chitosan (0.1%) followed by its foliar spray	44.66 (41.91)
5	T5	Seedling treatment with *Trichoderma* isolate-8 (5 g/lit) + Chitosan (0.1%) followed by its foliar spray	49.37 (44.62)
6	T6	Seedling treatment with Yam beam seed extract @30%	56.28 (48.59)
7	T7	Seedling treatment with Yam beam seed extract @30% followed by its foliar spray	57.09 (49.05)
8	T8	Seedling treatment with Elephant foot yam @ 30%	62.70 (52.35
9	T9	Seedling treatment with Elephant foot yam @ 30% followed by its foliar spray	58.33 (49.78)
10	T10	Seedling treatment with Carbendazim @ 0.1%	32.39 (34.66)
11	T11	Inoculated Control	71.35 (57.63)
	C.D @ 5%		6.02 (3.50)
	C.V		6.69 (4.37)

Figure 3: Biological suppression of *Fusarium oxysporum* f.sp. *lycopersici* by *Trichoderma asperellum* under Green house.

5 g/lit + Chitosan @0.1% followed by its foliar spray) in which 49.37 per cent PDI observed. However, Treatment 4 was at par with Treatment 5 but was significantly better than treatments T3, T1 and T2. Treatment 5 was also at par with T3, T1 and T2 and PDI were recorded 51.42%, 52.95% and 53.09% respectively. The minimum per cent disease index (32.39%) was recorded in T10 Seedling treatment with Carbendazim @ 0.1%). The maximum per cent disease index was recorded in inoculated control (71.35%). Several *Trichoderma* spp. are being used in biological control of soil-borne plant pathogens, and identifying efficient species in different ecosystems seems to be useful for their further evaluations. The efficiency of *Trichoderma* spp. on Fusarium wilts is quite variable in different regions of the world; in contrary, it could be said that a species which is highly antagonistic against a particular pathogen in a region may act poorly against the same pathogen in another region may be due to differences in various agro-climatic conditions. In this study local strain of *Trichoderma* was tested and significantly minimised the severity of disease. The result of present study is similar to the finding of Christopher [27] who evaluated various isolates of *T. virens* under *in vitro* and greenhouse conditions and observed minimum incidence of *Fusarium* wilt disease and promoted plant growth in tomato. Margaret [28] investigated ability of isolate the *Trichoderma harzianum* (P52) and arbuscularmycorrhizal fungi (AMF) under Green-house condition and recorded the enhancing growth and control of a wilt pathogen. Barari [29] evaluated 28 native *Trichoderma* spp. against tomato wilt *in vitro* and *in vivo*. *Trichoderma harzianum*, isolate N-8, was most effective in reducing radial mycelial growth of the pathogen (by 68.22%) and least disease incidence (by 14.75%).

CONCLUSION

In conclusion, local identified potential *Trichoderma asperellum* strain provided better disease control with greater crop health. The present research may encourage farmers to integrate biofungicides in to tomato cultivation.

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