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Efficacy of *Trichoderma asperellum* against different pathogens of selected medicinal plants

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Trichoderma asperellum is one of the plant growth-promoting fungi (PGPF) presently marketed as biopesticide, biofertilizer and in soil amendments, due to its ability to protect plants, enhance vegetative growth and reduce pathogen populations. Keeping this in the view, here, we assessed its efficacy against pathogens of medicinal plants *in vitro* and in field conditions against the Leaf spot disease of Green leaf Tulsi (*Ocimum basilicum*). We selected 10 different plant pathogens of medicinal plants, namely Ashwagandha (*Withania sominifera*) pathogens (*Alternaria alternata* and *Fusarium solani*); Sarpgandha (*Rauwolfia serpentina*) pathogens (*Colletotrichum* sp. and *Alternaria alternata*); Tulsi (*Ocimum basilicum*) pathogens (*Colletotrichum gloeosporioides* and *Alternaria alternata*); Mint (*Mentha arvensis*) pathogens (*Curvularia* sp. and *Alternaria alternata*); and Mandukparni (*Centella asiatica*) pathogens (*Cochliobolus* sp. and *Alternaria* sp.) for evaluation by Dual culture plate method. After 144 h, maximum inhibition (56.36%) was recorded against *F. solani.* This was followed by inhibition of *Cochliobolus* sp. where the reduction was 55.38%. *T. asperellum* was also found to be effective against *Alternaria* sp. isolated from Ashwagandha (53.06%), *Alternaria* sp. from Sarpagandha (53.51%), and *Curvularia* sp. (52.12%). In field study, FYM enriched with *T. asperellum*, *Pseudomonas fluorescence*, neem oil and fungicide were tested against Leaf spot of Tulsi caused by *Alternaria alternata.* The treatment T5 [soil application of FYM (1.0 kg/m^2) enriched with *T. asperellum* + *P. fluorescence* talc based formulation each @ 2.0% at planting time+ 3 sprays of copper oxychlorde @ 0.3% with 15 days interval on the onset of disease symptoms] was found effective with per cent disease index recorded 10.37%. This was followed by treatment T6 [soil application of FYM (1.0 kg/m^2) enriched with *T. asperellum* + *P. fluorescence* talc based formulation each @ 2.0% at planting time + 3 sprays of with bordeaux mixture @ 0.5% onset of disease symptoms], and both the treatments were at par with each other. Maximum biomass of tulsi (3.97 g) observed in treatment T5. The result implies that FYM enriched with *T. asperellum* and *P. fluorescence* which was found to be most effective can be incorporated in IDM programs for ecofriendly leaf spot disease management of Green leaf Tulsi.

Keywords: Ashwagandha, Biocontrol, Bioagents**,** *Ocimum basilicum*, Leaf spot disease of Tulsi, Mint, *Mentha arvensis*, *Rauwolfia serpentine*, Sarpgandha, *Withania sominifera*

Trichoderma asperellum, one of the plant growth-promoting fungi (PGPF), is a well known antagonist, and one of the most documented biological control agents of various pathogens[1,2]. The bioagents exhibited different mechanism of action against phytopathogenic fungal strain including antibiosis, competition for nutrients and mycoparasitism[3,4]. Furthermore, *Trichoderma* spp. capable to produce a wide range of secondary metabolites that protect against pathogenic fungi[5]. Biological control of plant diseases is considered as ecofriendly approach to manage plant diseases. Use of pesticides pollute the environment, leave harmful residues, and can lead to the development of resistant strains of the pathogen. *Trichoderma asperellum* has been shown effective against many pathogens[6,7]. Biocontrol agents have been recommended for the management of different pathogens. However, with the increasing interest in biological control, owing to environmental and economic concerns, thousands of research experiments are going on for searching novel, potential, safe and have ability to inhibit wide range of phytopathogens.

Medicinal plants are natural source of high value industrial raw material for pharmaceutical, agrochemical, food and cosmetic industries. The medicinal and aromatic plants area in the India during 2019-20 was 6.34 lakh hectare with the production 8.22 million ton and the global herbal medicine market is continuous expending and it is expected to touch from $165.66 billion in 2022 to $347.50 billion by 2029[8,9]. Medicinal plants have better economic opportunities as against the traditional field crops and price of these

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crops as raw-material to the pharmaceutical industries has increased substantially. All these have led to the emergence of medicinal plants as alternatives to some of the traditional crops. Medicinal plants are attacked by fungi, bacteria, viruses, nematodes and some are notably notorious and lead to epidemics under favourable environmental conditions. The quality of its leaves deteriorates due to the attack of various pathogens. Disease management in medicinal plants through chemical restricted. Considering these points and high demand for organic produce, in the present study, we explored the most effective native *Trichoderma* strain alone or in combination with organic amendments to contain the leaf spot disease in tulsi.

Materials and Methods

The study was conducted in the Biocontrol Lab and field of All India Coordinated Research Project on Medicinal Plants, Department of Plant Pathology, Dr. Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar, India.

Isolation of *Trichoderma* from soil

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 agar medium (Hi Media, M18360) and ingredients in medium were magnesium sulphate heptahydrate, 0.20 g; dipotasium hydrogen phosphate, 0.90 g; ammonium nitrate, 1.0 g; potassium nitrate, 0.15 g; glucose, 3.00 g; rose Bengal, 0.15 g; agar, 20 g; and distilled water, 1.00 L.

Soil samples were serially diluted and transferred on Trichoderma selective agar medium. One mL of 10^{-4} dilution was poured onto *Trichoderma* selective agar medium. From the appearance of the colonies of *Trichoderma* on Petri dish purified by hyphal tip isolation techniques, *Trichoderma* spp. was identifie and, 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.

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

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

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 nos.) 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 colourless, greyish or brownish. Chlamydospores also observed under the stress conditions for the survival. They are normally found as thickwalled, enlarged vegetative cells with condensed cytoplasm which are unicellular, globose to subglobose chlamydospores are either formed within hyphae or at the hyphal tips[10,11].

Molecular characterization and identification of *Trichoderma* isolate

The best *Trichoderma* isolate was selected after screening. The screening was done on the basis of fast growth, sporulation and antagonistic potential. The radial growth of each *Trichoderma* isolate was recorded after 48 h and sporulation observed visually. Antagonistic potential was done by dual test. In the dual cultural test, an agar disc (6 mm) was taken from 4th day PDA culture plates of each *Trichoderma* isolate and place at the periphery of PDA plates (9 mm). Another agar disc of the same size of *F. oxysporum* f.sp. *lycopersici* was also placed at the periphery but on the opposing end of the same Petri plate. Observation was continued on dual culture plates after four days incubation and Percent inhibition was calculated using formula given by Vincent[12].

$$\text{Percent inhibition over check} = C–T/C\times100$$

The screening was done on the basis of fast growth, sporulation and antagonistic potential against different pathogens. This isolate 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).

Isolation and purification of medicinal plant pathogens

The infected leaves of Ashwagandha (*Withania sominifera*), Sarpagandha (*Rauwolfia serpentina*), Mint (*Mentha arvensis*), Green leaf Tulsi (*Ocimum basilicum*) and Mandukparni (*Centella asiatica*) showing typical symptoms of fungal diseases were collected from MAP germplasm blocks at different places of RPCAU, Pusa and its neighbouring areas in Samastipur. The infected leaves were carried in sterilized polybags in the laboratory where the collected leaf samples were washed in sterile distilled water to remove the impurities on the surface. Small bits of infected tissues of 1-2 mm from advancing margin of spots, adjacent to healthy portion were cut with a blade. These pieces were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 30 seconds followed by washing in 2-3 changes of sterilized distilled water. These leaf cuttings were placed on blotter paper blotted and aseptically plated on potato dextrose agar (PDA). In each plate, two to three pieces were placed and incubated in BOD (28±2°C) for 7 days. The pure culture was obtained by culturing fungi on PDA and making fresh culture from "Hyphal Tip" selected from periphery of actively growing colony under aseptic conditions. The entire process of isolation and purification was carried out inside the laminar air flow.

Pathogenicity test of pathogens

Pathogenicity test was carried out using the Koch's postulates. Sterilized soil was taken in earthen pots and seedlings of medicinal plants were grown in these pots. 30 days old plants were sprayed with sterile distilled water before inoculation. Mycelial and spore suspension (2×10^6 spores/mL) was prepared in sterile water from a 15 days old culture. This suspension was sprayed and swabbed with moist cotton on the leaves. Controls were maintained by spraying the plants with sterile distilled water. Observations were made for development of symptoms periodically. Re-isolation of pathogens was done from artificially inoculated and infected plants and pure cultures thus obtained were compared to the original cultures to confirm the identity and pathogenicity of the test pathogens.

Identification and characterization of pathogens

Pathogens were identified by studying the morphological and cultural characteristics and were confirmed using the literature given by different researcher[13,14]. The morphology is presented in Fig. 1.

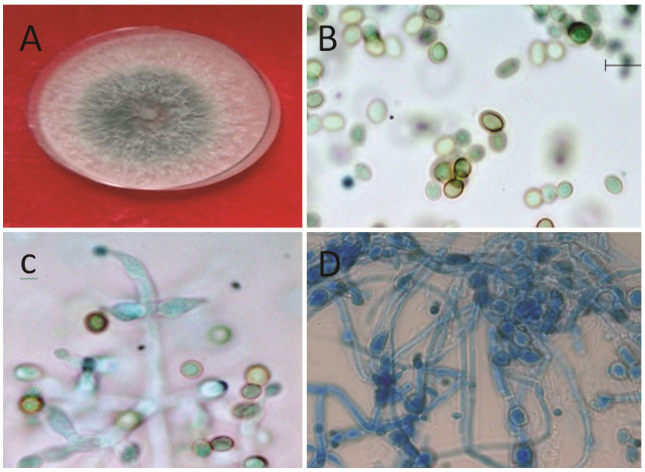

Fig. 1 — (A) *Trichoderma asperellum* culture; (B) Conidia; (C) Conidiophore & Philalides; and (D) Clamydospores

Evaluation of *Trichoderma asperellum* against isolated pathogens of medicinal plants *in vitro*

The local strain *T. asperellum* evaluated against different isolated pathogens of medicinal plants by Dual Culture technique. For this process, 7 mm disc from actively growing cultures of isolated pathogens was placed on one side of the Petri plate and 7 mm disc of *Trichoderma asperellum* was placed at opposite side in the same plate. Each treatment was replicated 3 times and control plates for each pathogen were also maintained. These plates were incubated at 28±1ºC for 5 days. The colony diameter of different pathogens in dual culture plates and control plates were recorded to calculate the percentage inhibition of growth of each fungus by the *Trichoderma* strain. The per cent inhibition of mycelial growth over control was calculated by following formula given by Vincent[12].

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

where, C = growth of pathogen in check; and T = growth of pathogen in treatment

Evaluation of *Trichoderma asperellum* for management of leaf spot of green leaf tulsi (*Ocimum basilicum*)

The FYM enriched with *T. asperellum* and *Pseudomonas fluorescence*, neem oil copper oxychloride and bordeaux mixture were tested in field against Leaf spot of Tulsi caused by *Alternaria alternata.* Details of treatments were as follows: T1, soil application of FYM (1.0 kg/m2) enriched with *Trichoderma asperellum* + *Pseudomonas fluorescence* talc based formulation each @ 2.0% at planting time; T2, T1+ 3 sprays of P. fluorescence @ 2.0% on the onset of disease symptoms; T3, T1 + 3 sprays of neem oil @ 300 ppm on the onset of disease symptoms; T4, T1+3 spray of *P. fluorescence* @ 2% and neem oil @ 300 ppm on the onset of disease symptoms; T5, T1+ 3 sprays of copper oxychlorde @ 0.3% with 15 days interval on the onset of disease symptoms; T6, T1 + 3 sprays of with bordeaux mixture@ 0.5% at 15 days interval on the onset of disease symptoms; and T7, Control (without treatment).

Per cent disease severity was calculated as per the standard area diagram and 0 to 9 disease rating scale developed by Mayee & Datar[15]. For this purpose, two leaves located at the bottom, two middle and two top of the plant were chosen and scored as per scale i.e. for leaf area infected: 0, Immune; 1, <1%; 3, 1 to 10%; 5, 11 to 25%; 7, 26 to 50 % and more than 9, >50%.

The percent disease index (%) was calculated by the following formula:

$$\text{PDI (\%)} = \frac{\text{Sum of all the diseases ratings}}{\text{Total no. of diseased branches observed} \times \text{Maximum rating}} \times 100$$

Statistical analysis

Data were analyzed using statistical package OPSTAT.

Result and Discussion

Isolation, cultural and morphological characteristics of *Trichoderma* isolates from collected soil samples

Twenty *Trichoderma* isolates were isolated from rhizospheric soils of different crops at different location of Muzaffarpur and Samastipur districts. Cultural characteristics including growth, growth rate, sporulation, colony colour and growth pattern were examined on potato dextrose. 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 45^0-50^0 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[16] at http://nt.ars-grin.gov/ taxadescriptions/ keys/ Frame Key.cfm? gen -*Trichoderma*. Rifai[17] 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 & Bissett[18].

The best *Trichoderma* isolate 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 the molecular basis identified as *Trichoderma asperellum* Samuels Lieckf. & Nirenberg, and awarded Accession no. NFCCI 4586 by National Fungal Cultural Collection of India (NFCCI). Brief description of the above isolate is given below.

Brief description of *Trichoderma asperellum*

Macroarphology: Colony on PDA at 25+2, fast growing, dull green, fasciculate, floccose, reverse buff. Micromorphology: Chlamydospores produce abundantly, intercalary, globose 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 up to 3.9-8.15 × 2.15 – 3.64 um. Hyphae branched, smooth walled, hyaline up to 11.7 um wide (Fig. 1).

Results on molecular identification

The tested fungal strain T1 showed 100% sequence similarity with *T. asperellum*. Sequence analyses with NCBI accession number KR86829, *T. asperellum* strain ZWPBG2 resulted in following statistics.

Alignment statistics

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

Isolation of *Trichoderma* from soil

Several workers isolated the *Trichoderma* spp. from different crop rhizosphere and identified as potential antagonist. Neelamegam[19] isolated 8 isolates of *Trichoderma viride* from rhizosphere soil of healthy tomato seedlings, collected at different locations, 62 isolates of *Trichoderma* spp. from different rhizospheric soil samples collected from different places located in Western Himalayas region[20]. *Trcichoderma asperellum* isolated from salinity soil found most effective against different pathogens of cowpea[21] and isolated from banana rhizosphere found to be most effective against *Fusarium* spp.

Evaluation of *T. asperellum* against isolated pathogens of medicinal plants *in vitro*

The efficacy of local *Trichoderma* strain was evaluated against the growth of different isolated pathogens from important medicinal plants by dual culture technique. Data on radial growth and percent growth inhibition of all fungi were taken after 96 h (4th day), 120 h (5th day) and 144 h (6th day) of inoculation. The radial growth of pathogens (G in mm) and growth inhibition percent (I) were recorded (Table 2 and Fig. 2).

Data presented in Table 2 show that *Trichoderma asperellum* showed inhibitory effect and was effective in controlling the growth of pathogens significantly. After 96 h (3rd day) and 120 h (4th day) of inoculation, it showed remarkable inhibition against *Fusarium solani* where the radial growth of the pathogen was

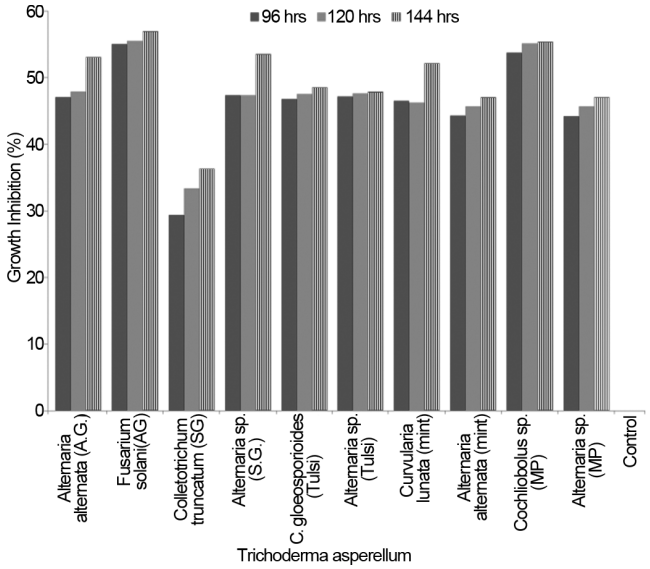

Fig. 2 — *In vitro* effect of *Trichoderma asperellum* on isolated pathogens

Table 2 — *In vitro* evaluation of *Trichoderma asperellum* (local strain) against isolated pathogens

Treatments	96 h (4th day)			120 h (5th day)			144 h (6th day)		
	G	C	I	G	C	I	G	C	I
Alternaria alternata (A.G.)	9.16	17.30	47.05	10.10	19.57	47.87	10.20	21.73	53.06
Fusarium solani (A.G.)	13.10	29.17	55.01	14.50	32.03	55.47	15.10	35.10	56.36
Colletotrichum truncatum (S.G.)	7.13	10.10	29.40	8.13	12.20	33.36	9.13	14.33	36.36
Alternaria sp. (S.G.)	9.13	17.33	47.31	10.00	19.00	47.36	10.00	21.52	53.51
C. gloeosporioides (Tulsi)	9.15	17.20	46.80	10.12	19.33	47.56	11.12	21.60	48.52
Alternaria sp. (Tulsi)	9.16	17.35	47.20	10.21	19.41	47.58	11.2	21.50	47.91
*Curvularia lunata(*mint)	8.20	15.13	46.51	9.00	17.11	46.26	9.16	19.13	52.12
Alternaria alternate (Mint)	10.18	18.27	44.25	11.30	20.50	45.70	11.43	21.60	47.08
Cochliobolus sp .(MP)	12.16	26.27	53.71	13.30	29.30	55.11	14.13	31.67	55.38
Alternaria sp. (M.P.)	10.18	18.23	44.15	11.30	20.50	45.70	11.43	21.60	47.08
SE (m)±		0.08			0.09			0.07	
C.D. AT 5%		0.25			0.27			0.22	
C.V.		1.07			1.02			0.78	

13.10 mm while in control it grew 29.17 mm which means the growth was inhibited by 55.01% followed by *Cochliobolus* sp. where the radial growth recorded was 12.16 mm while in control it was 26.27 mm which indicated that the growth was inhibited by 53.71%.

After 144 h (6th day) of inoculation, maximum inhibition was shown against *Fusarium solani* where the radial growth was recorded to be 15.10 mm with control having 35.10 mm growth indicating the reduction in percent growth by 56.36%. This was followed by inhibition of *Cochliobolus* sp. where the radial growth of pathogen was recorded to be 14.13 mm with control having 31.67 mm growth indicating the reduction in percent growth by 55.38%. *Trichoderma asperellum* was also found effective against *Alternaria alternata* isolated from Ashwagandha (53.06%), *Alternaria* sp. from Sarpagandha (53.51%) and *Curvularia lunata* (52.12%).

The growth inhibition percent for *Alternaria alternata* isolated from Mint and *Alternaria* sp. isolated from Mandukparni were at par with each other *i.e.* the growth of pathogens was inhibited by 47.08%.

Minimum growth inhibition was shown against *Colletotrichum truncatum* where the radial growth of pathogen was recorded to be 9.13 mm while growth in control was recorded to be 14.33 mm which means the growth was inhibited by 36.36%.

Overall, the *in vitro* study indicates that *Trichoderma asperellum* has different level of inhibitory effect against different pathogens. The findings of present investigation are in accordance with the reports of several workers. The *T. asperellum* showed strong antagonistic against *Fusarium verticillioides*[22] and *F. oxysporum* f.sp.*lycopersici*[23-26]. Rahman *et al*.[27] also evaluated five strains of *Trichoderma* against *Alternaria alternata* causing leaf blight in Ashwagandha (*Withania somnifera*) and observed that *Trichoderma harzianum* IMI- 392433 showed maximum inhibition percentage (54.89%) followed by *T. harzianum* IMI-392432 (53.83%), *T. harzianum* IMI- 392434 (48.94%) and *T. virens* IMI- 392430 (43.62%). Panchalingam *et al.*[28] assessed various antagonistic mechanism of *Trichoderma* strain against the brown root rot pathogen *Pyrrhoderma noxium* and reported that *Trichoderma* strains has the ability to more quickly utilized space and nutrients with pathogen, and hence this biocontrol agent gets some competitive advantage.

Evaluation of *Trichoderma asperellum* for management of leaf spot of green leaf tulsi (*Ocimum basilicum*)

The FYM enriched with *Trichoderma*, seedling treatment with *Trichoderma* followed by foliar spray of neemoil and copper oxychloride were tested in field against Leaf spot of Tulsi caused by *Alternaria alternata.* Among different organic based product the treatment FYM enriched with *T. asperellum*, *Pseudomonas fluorescence*, neemoil and fungicide were tested in the field against Leaf spot of Tulsi caused by *Alternaria alternata*. Table 3 shows that treatment T5 [soil application of FYM (1.0 kg/m^2) enriched with *Trichoderma asperellum* (2%)+ *Pseudomonas fluorescence* (2%) talc based formulation at planting time followed by 3 sprays of copper oxychlorde @ 0.3% with 15 days interval on the onset of disease symptoms] was effective in minimising the per cent disease index (10.37%) followed by treatment T6 [soil application of FYM (1.0 kg/m^2) enriched with *Trichoderma asperellum* (2%) + *Pseudomonas fluorescence* (2%) talc based formulation at planting time followed by three foliar sprays of bordeaux mixture@ 0.5% onset of disease symptoms] recorded 12.59% severity and both the treatments were at par with each other. Maximum per cent disease index recorded (41.48%) in control. Maximum fresh weight (3.97 g) also observed in treatment T5. Among ecofriendly treatments, T2 [soil application of FYM (1.0 kg/m^2) enriched with *Trichoderma asperellum* + *Pseudomonas fluorescence* talc based formulation each @ 2.0% at planting time+3 sprays of *P. fluorescence* @ 2.0% on the onset of disease symptoms] found effective and severity recorded 22.22%. Wang *et al*.[29] studied the control effect on the synergistic application of *Trichoderma harzianum* SH2303 and difenoconazole-propiconazole (DP) against leaf blight of maize and

Table 3 — Evaluation of bioagents and organics based products for management of diseases of Green leaf tulsi (*Ocimum basilicum*)

Treatments	Per cent Disease Index (%)	Fresh wt. of 10 plants (in kg)
T1	28.85	2.99
T2	22.22	3.57
T3	27.40	2.92
T4	22.89	3.49
T5	10.37	3.97
T6	12.59	3.83
T7	41.48	2.19
S.E. m(±)	1.31	0.14
CD (0.05)	4.04	0.47
CV (%)	9.61	8.10

reported that treatment induced the synthesis of defense-related enzymes (phenylalanine ammonia lyase (PAL), catalase (CAT), and superoxide dismutase (SOD)) and the defence-related gene expression of SA pathway (*PR1*). Under the natural field condition, maize treated with *Trichoderma harzianum* SH2303 and difenoconazole-propiconazole (DP) showed 60% control. Previously, in our research *T. asperellum* has been found effective against several leaf blight pathogens of Ashwagandha, Sarpagandha, Tulsi, and Mint *in vitro* conditions[30]. Biocontrol potential of *Trichoderma* species in bicontrol of plant diseases were also documented by researchers[31,32].

Conclusion

The present findings have demonstrated that the local plant growth promoting fungal strain *Trichoderma asperellum* enriched with FYM effectively minimize the leaf spot disease of green leaf tulsi and could be exploited in IDM program for ecofriendly disease management of green leaf tulsi. Among different treatments, T5 i.e., soil application of FYM (1.0 kg/m^2) enriched with *T. asperellum* (2%) + *Pseudomonas fluorescence* (2%) talc based formulation at planting time followed by 3 sprays of copper oxychlorde @ 0.3% at 15 days interval on the onset of disease can be recommended for effective ecofriendly management of tulsi leaf spot disease.

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Conflict of Interest

Authors declare no competing interests.

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