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IN VITRO MYCOPARASITIC EFFICACY OF NATIVE ISOLATES OF *TRICHODERMA* SPP. AGAINST PHOMOPSIS BLIGHT (*PHOMOPSIS VEXANS*) OF BRINJAL (*SOLANUM MELONGENA* L.)

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ABSTRACT

The eight isolates of *Trichoderma* spp. are isolated from the different rhizosphere of the tree and compare all eight isolates in *in vitro* mycoparasitic efficacy against *Phomopsis vexans* that cause disease Phomopsis blight or fruit rot of brinjal, ability to form hyphal coiling around the hyphae of the pathogen (*Phomopsis vexans*) in dual culture on PDA medium and their effectiveness in reducing on *Phomopsis vexans* of brinjal. The laboratory assays of mycoparasitic efficacy of eight isolates of *Trichoderma* spp. revealed that *Trichoderma* S1 and S2 seemed to be most effective with 85.05% and 82.96% respectively coiling and reducing the hyphal growth of the *Phomopsis vexans*. Trichoderma S7 and S8 formed fewer hyphal coiling compared to all eight isolates of *Trichoderma* spp. The effectiveness of Trichoderma S7 and S8 is 68.62% and 64.93% to inhibit the hyphal growth of the pathogen. The study analyses the effective biocontrol of *Phomopsis vexans* by using different isolates of *Trichoderma* spp.

Key words : *Trichoderma* spp., *Phomopsis vexans*, Mycoparasitism, Biological control.

Introduction

Eggplant (*Solanum melongena* L.) is one of the most important solanaceous vegetable crops and is grown worldwide. India is considered to be the center of origin of cultivated brinjal by Thompson and Kelly (1957). Eggplant tissues and extract have known ayurvedic medicinal properties and are used in the treatment good for diabetes, asthma, cholera, bronchitis, and diarrhea patients while have also been recommended as an excellent remedy for those who suffer from liver complaints (Shukla and Naik, 1993). The crop is susceptible to various biotic and abiotic stresses at different stages of growth and development, among them the most significant being *Phomopsis blight* and fruit rot disease. Many fungal pathogens especially species of *Fusarium Colletotrichum*, *Phytophthora* and *Phomopsis* are associated with fruit rot disease of brinjal. But the rot incited by *Phomopsis vexans*. Phomopsis blight and fruit rot caused by *Phomopsis vexans* is a destructive fungal disease that has gained national importance and is considered a major constraint for limited production and productivity. It has been treated as one of the major constraints to eggplant cultivation in India by Das *et al*. (2014) and yield losses to 30- 50% due to flower drop and fruit rot (Mahadevakumar and Janardhana, 2016). The diseases cause serious loss in brinjal production in terms of fruit yield by affecting seed germination, seedling mortality, killing the plants rotting of fruits and spoiling the fruit quality. Phomopsis blight diseases are responsible for the spoilage of the fruit, leaves and other parts of the plants. *Phomopsis vexans* viable for about 14 months in soil debris and in the seed from infected fruits. The pathogen is reported externally and internally as seed-borne (Hossain *et al*., 2013). The pathogen attacks leaves, but older ones are more susceptible. The leaves are more prominent during the early stages of the plant growth. Lesions are small, more or less circular, buff to olive in colour and later becoming cinnamon bark-like, with an irregular blackish margin

(Panwar and Patel, 1957). Irregular spots result from coalescence. After transplanting, lover leaves in contact with the soil may become infected directly or develop leaf spots due to infection by conidia. It was reported that *Phomopsis vexans* reduces the yield and marketable value of the crop by nearly 20-30% (Jain and Bhatnagar, 1980; Kaur *et al*., 1985). Certain preventive fungicides although hazardous to the environment are still used for the control of fungal diseases (Nwankiti *et al*., 1990; Vaish and Sinha, 2003). Therefore, the experiment was undertaken to find out the different isolates of Trichoderma in controlling Phomopsis fruit rot of eggplant.

Materials and Methods

The mycoparasitic potential of different *Trichoderma* spp. was examined, *in vitro* conditions in the Laboratory of Plant Pathology Department, Bundelkhand University, Jhansi, U.P., India.

Isolation of *Trichoderma*

Eight isolates of *Trichoderma* spp. from the rhizosphere of Mahua, Pipal, Arjun, Aonla, Pakar, Barged, Mango and Ashoka trees from Jhansi district UP were collected. The soil of 100 gm was collected from each sample (5–15cm depth) from the first soil samples, 5gm of soil from the sample was dissolved in 100 ml of distilled water and shaken well to homogenize the soil sample thoroughly and prepare stock solution, dilution of four concentrations (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}), each concentration (about 100 µl) suspension poured on the sterilized 90 mm petri dish containing PDA (250 gm Potato, 20 gm agar, 20 gm dextrose, 1000 ml distilled water) each plate pour 20 ml of PDA and inoculated plates incubated at 28^{0}C for 24 to 96 hours. Plates were observed at 24-hour intervals and the *Trichoderma* colony was transferred to a new PDA plate by sterilized inoculation needle in an aseptic condition by Zhou *et al*. (2020) and Iqbal *et al*. (2017).

Isolation of Pathogen

Leaves and fruits of brinjal show that symptoms of *Phomopsis* blight were collected from the field and washed thoroughly with water. Removes excess moisture and Place between blotting paper. The infected plant parts were cut aseptically into small pieces and surface sterilized with mercuric chloride 0.1% for one minute followed by two to three washes with sterilized distilled water and then placed aseptically on solidified potato dextrose agar (PDA) in a Petri dish were incubated at 25±1^{0}C. Mycelial growth appeared on PDA after 2 days of incubation. A single unit of mycelial growth is transferred on a fresh PDA plate and sub-cultured to maintain pure culture. The pure culture was maintained in the deep freezer at 5^{0}C for future studies by Thesiya *et al*. (2020).

Cultural Identification of *Trichoderma*

The cultural characteristics of the 8 isolates of Trichoderma were determined on Potato dextrose agar (PDA). A 5 mm diameter disc cut from the edge of the new colony growth (before the start of conidial production) with help of a sterile cork borer and placed on a Petri dish containing 15 to 20 ml fresh medium (PDA for cultural) Disk was placed on the centre of the Petri dish and make sure that surface of the colony disc is downwards. Maintained the three replication was each isolate. For cultural study, Petri dishes were incubated at 25^{0}C. The colonies were examined at 24 h intervals for a pattern of conidiation, first appearance of green conidia, formation of conidial pustules, presence of any odour or yellow pigmentation and colony radius was measured from the edge of the inoculum block after 72 h at 25^{0}C. Species identification was based on the colony character's key identification provided by Rahman *et al*. (2011) and Bissett (1991).

Pathogenicity test

Stem inoculation : The pathogenicity test was carried out in pots with sterilized soil. The seedling of a susceptible variety of brinjal is brought to the local market of Jhansi and was used for this purpose. 2 weeks old plants after transplanting are well established on the pots and ready to use for the experiment by Kanematsu *et al*. (1999). Stem inoculation of the pathogen was made by brush in which the first injury is made by carborundum powder on the stem and then sterilized toothbrush deep

Table 1 : Location and other details of Trichoderma isolates from different parts of Jhansi.

S. no.	Trichoderma spp. isolates	Locations	Isolation source
1.	Trichoderma S1	BU, Organic Field	Rhizosphere of Mahua
2.	Trichoderma S2	BU, Campus	Rhizosphere of Pipal
3.	Trichoderma S3	BU, Chemical Field	Rhizosphere of Arjun
4.	Trichoderma S4	Chirgaon	Rhizosphere of Aonla
5.	Trichoderma S5	Narayan bag	Rhizosphere of Pakar
6.	Trichoderma S6	Karguanji	Rhizosphere of Barged
7.	Trichoderma S7	Bharari	Rhizosphere of Mango
8.	Trichoderma S8	Medical College	Rhizosphere of Ashoka

into the pathogen spore suspension and rubbing the spore deep brush on the stem in various places of the stem. Spore inoculated place of the plant covered with non-absorbing cotton to prevent other contamination and proper moisture for growing pathogens. The observation was recorded after 8 – 9 days by Thesiya *et al*. (2020).

Dual culture of pathogen with different isolates of *Trichoderma* spp.

Eight isolates of *Trichoderma* spp. were cultured with *Phomopsis vaxans*. Three weeks old Pathogen culture of *Trichoderma* spp. was used for dual culture. The *Phomopsis vexans* mycelium and *Trichoderma mycelium* disks 5 mm in diameter were placed in the fresh PDA plates, disks were placed on opposite sides of each other and 2cm away from the edge of the Petri plates. Control was cultured without treatment. All cultures were placed in the BOD incubator at 25^{0}C ± 1 for seven days. After 7 days radial growth of the test pathogen was measured. Growth inhibition of the pathogen was calculated using the following formula given by Vincent (1947).

$$\text{Percent growth inhibition (PGI)} = \frac{C - T}{C} \times 100$$

Where; I = Inhibition percent, C = Colony diameter in control (mm), T = Colony diameter in treatment (mm).

Results and Discussion

Cultural characterization of *Trichoderma* spp.

The cultural characteristics and growth rates of the 8 *Trichoderma* isolates were determined on Potato dextrose agar medium (PDA). All eight native isolates of *Trichoderma* spp. are fast-growing and their radial growth at 25^{0}C up to 41.6 to 50.1mm after 72 ha by Rahman *et al*. (2011).

Characterization of Pathogen (*Phomopsis vexans*)

The cultural characters of Phomopsis isolate : The colonies on the PDA plates showed that the isolated fungus bore white aerial hyphae and scattered, relatively large pycnidial stroma with irregular pycnidial locules. When the colony seen through the underside of the petri dish was whitish and occasionally had pale pink, brown and or grey zones. The reverse side of the colony was usually grey or brownish. After four days colony incubation on PDA at 25^{0}C was 27.2mm. This isolate produces Alfa (á) conidia at 25^{0}C but does not produce Beta (β) conidia at 25^{0}C. Conidia of the isolated pathogen are oblong to oval in shape and have one to two nuclei (Sundaresan *et al*., 1986; Islam *et al*., 2010) (Fig. 1).

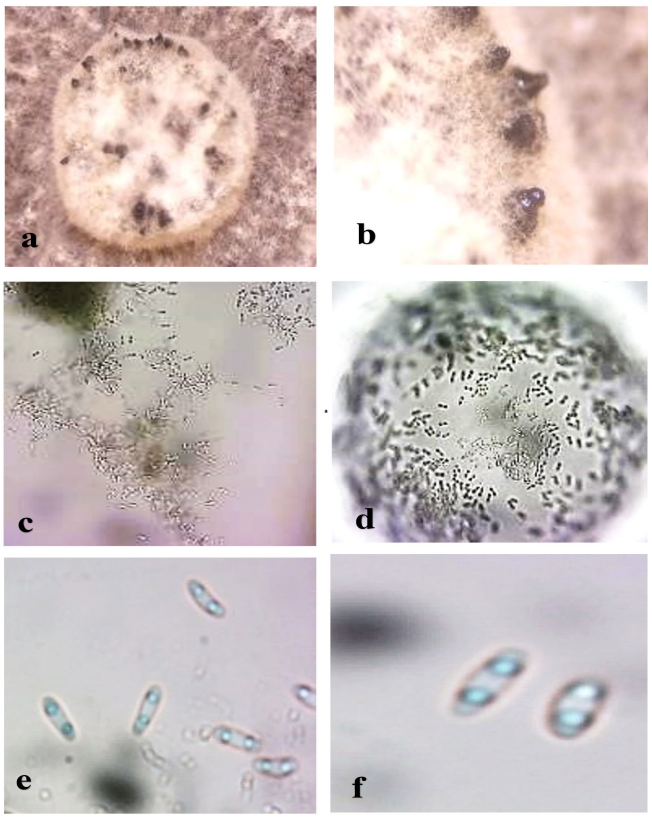

Fig. 1 : a) Colony of the *Phomopsis vexans*, **b)** Pycnidia of *P. vexans*, **c, d)** Alfa (α) conidia of *P. vexans*, **e, f)** Alfa (α) conidia with two nuclei.

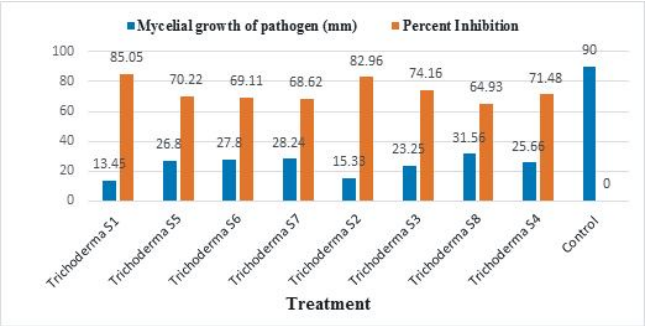

Fig. 2 : Effect of different bioagents on mycelia growth of *Phomopsis vexans*.

Pathogenicity study in pot conditions

Pathogenicity study was carried out on one most old seedlings of brinjal. The pathogenicity test the pathogen causes brown to black spots on the upper surface leaves and fruits shows mummification. Similar results have already been reported by Thippeswamy *et al*. (2006).

Jayaramaiah *et al*. (2013) reported that the Pathogenicity test (Koch's postulates) association of fungal pathogen with the leaf blight and fruit rot disease of brinjal, Koch postulates were conducted. Brinjal seed samples collected from healthy fruits from farmer's field were used. Each tray containing soil: sand: compost (2:1:1) was sown with seeds of brinjal. Seedlings of 30 day's old were

Table 2 : Characterization of *Trichoderma* spp. isolates of different sources from Jhansi.

S. no.	Isolates	Isolation source	Colony Characterization
1.	Trichoderma S1	Rhizosphere of Mahua	After inoculation colony was shown whitish with cottony growth and after turning dark green, initially light green after become turn dark green. The colony looked like velvet.
2.	Trichoderma S2	Rhizosphere of pipal	Initially colony of the fungus whitish then turn pel green with margin then turn olive green. The fungus grew irregular in shape.
3.	Trichoderma S3	Rhizosphere of Arjun	Fungus was firstly white in colure then it became turn pale green with a yellowish touch. The old culture of fungus dark green with a white cottony centre.
4.	Trichoderma S4	Rhizosphere of Aonla	In PDA initially colony grew transparent. After 2 days colony turned massy white and full growth of the plate then after 4th day become light to dark green with a Concentric ring.
5.	Trichoderma S5	Rhizosphere of Pakar	The beginning growth of the fungus on PDA was slow with the irregular shape of the colony, lathery dark green growth with a feathery white centre.
6.	Trichoderma S6	Rhizosphere of Barged	The colony was noticed that watery mycelium and turn after some days, yellow green then after become converted in olive colour.
7.	Trichoderma S7	Rhizosphere of Mango	Initially, the colony was grown very slowly with transparent mycelium on the PDA. Then turned dark green after 3-4 days. Full grow colony appeared white centre with dark green margin.
8.	Trichoderma S8	Rhizosphere of Ashoka	Whitish to pale green with thread-like flappy growth, after full growth colony becomes olive green to dark green forming a thick Concentric ring on the PDA.

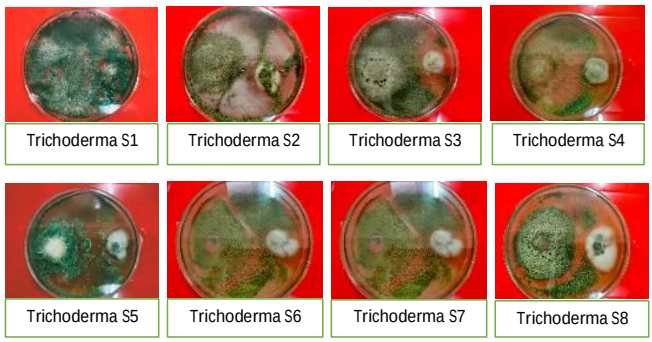

Fig. 3 : Interaction of different isolates of *Trichoderma* spp. with *Phomopsis vexans.*

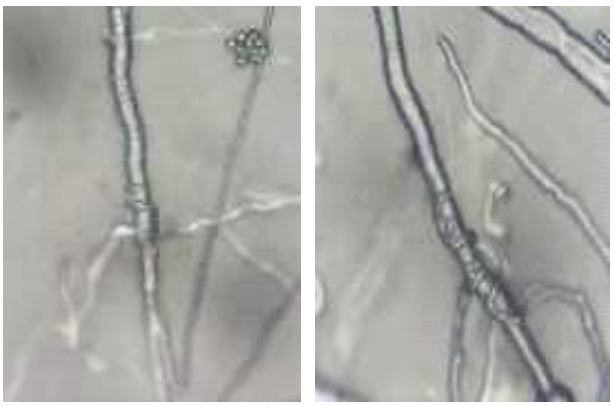

Fig. 4 : Coiling of *Trichoderma* spp. on mycelium of *Phomopsis vexans*.

sprayed with conidial suspension of *P. vexans* (1×10^5 conidia/ml) using a sprayer. High relative humidity condition was maintained for each inoculated brinjal plant for 24 h by covering it with plastic bag. The appearance of leaf blight symptoms was assessed after 15 days of post inoculation. The symptoms of the disease brown spots changing in the black colour in the stem, leaves, and fruits mummified.

In vitro evaluation of biocontrol agents against *Phomopsis vexans*

Laboratory experiments were conducted for testing the inhibitory effect of the bioagents against the pathogen (*Phomopsis vexans*). The study under *in vitro* conditions revealed that all the treatments gave significantly lower diameter of *Phomopsis vexans* as compared to control. Minimum radial growth of *Phomopsis vexans* was recorded with *Trichoderma* S1 (13.45 mm), followed by *Trichoderma* S2 (15.33 mm), and maximum radial growth of test pathogen was recorded in *Trichoderma* S8 (31.56 mm). All treatments were found significant compared with the control (Table 2). Data presented in Table 2 shows that maximum percent growth inhibition was recorded in the case of *Trichoderma* S1 (85.05 %), followed by *Trichoderma* S2 (82.96%). The minimum percent growth inhibition was recorded in the case of

Trichoderma S8 (64.93%). Similar finding was obtained by Das *et al*. (2014) in which they reported that all the antagonistic fungal cultures were found to have inhibitory effect on the mycelial growth of the pathogen and data showed that degree of inhibition was maximum with *T. viride* (84%) followed by *T. harzianum* (78.22%) after 7th days of incubation. *T. viride* was found to be highly effective in comparison to *T. harzianum*.

Dar *et al.* (2013) reported that *in vitro* evolution of isolated antagonists under dual culture. The bioagents *T. harzianum* gave maximum percent growth inhibition of 92.5% followed by *T. viride* (82.2%) against *F. oxysporum* f sp. *pini*. Rajput *et al.* (2013) reported that effect of fungal and bacterial bioagents against *Alternaria alternata in vitro* condition. The investigation on leaf spot disease (*Alternaria alternata*) of brinjal under south Gujarat condition was carried out to find out suitable management strategies. The results revealed that out of all the eight bioagents used, three bioagents *viz*., *Trichoderma viride* (IARI isolate) (74.77%), *Trichoderma viride* (Navsari isolate) (74.14%), *T. harzianum* (Junagadh isolate) (71.25%) maximum percent growth inhibition in dual culture method showed strong antagonistic effect to inhibit the mycelia growth of the pathogen significantly. Verma and Bhale (2010) evaluated the microorganisms for the management of Phomopsis blight and fruit rot of eggplant caused by *Phomopsis vexans*. Antimicrobial action of *Bacillus subtilis*, *Streptomyces griseus*, *Aspergillus* spp., *Penicillium* spp., *Trichoderma* spp. and *Periconia* spp. against *Phomopsis vexans*, the causal organism of eggplant blight and fruit rot, revealed the efficacy in terms of overgrowth, formation of vacuoles in mycelium, die back and mycelia – fruiting body damage, in veriable proportion. *Trichoderma harzianum* and *Bacillus subtilius* were found most effective.

Conclusion

Trichoderma is found in various soils like agricultural and industrial, this hidden fungus naturally controls plant diseases by itself. In this study, we found that the *Trichoderma* S1 and *Trichoderma* S2 were the most effective bio-control agents for management of Phomopsis blight of brinjal *in vitro* conditions. This fungistatic effect of *Trichoderma* is make more potential biocontrol agent in nature. *Trichoderma* is gifted by nature for us. The present study suggested that, biocontrol agents are the future of the management of the diseases of agricultural crops.

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