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Evaluation of antifungal activity of *Trichoderma* spp. against the wilt disease of brinjal (*Solanum melongena* L.) caused by *Fusarium solani* f. sp. *melongenae*

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Abstract
Brinjal is one of the important economic vegetable crops which are attacked by several serious diseases such as wilt. The dominant pathogen, which causes Fusarium wilt of brinjal, was isolated and identified as *Fusarium solani* f. sp. *melongenae* (FSM). Due to the harmful effect of chemical pesticides on the environment, biological control is emerging as an alternative management method. The aim of the present study was to evaluate antagonistic activity of *Trichoderma* spp. against *Fusarium solani* by dual culture method under *in vitro* conditions. Ten native *Trichoderma* antagonists were isolated from healthy brinjal rhizosphere soil in different geographical regions. The results revealed that *Trichoderma asperellum* (TB3) isolate was found to effectively inhibit the radial mycelial growth of the pathogen (85.77%) when compared to all other isolates. However, their efficient interaction with the host needs to be accompanied by production of secondary metabolites and cell wall degrading enzymes. Most of the Trichoderma species produced toxic volatile metabolites, having significant effects on growth and development of the plant.

Keywords: Biocontrol, *Trichoderma* spp., fusarium wilt, *Fusarium solani*

Introduction
Vegetables play a vital role in human health by providing nutrients, vitamins and antioxidants (kumar *et al.,* 2016) [15]. Eggplant (*Solanum melongena* L.) popularly known as aubergine, baingan jhonga, guinea squash, garden egg and brinjal (especially in home town, India) (Gaur and Chaudhary, 2009) [8]. Which are the fifth most economically important consumed vegetable all around the world. The multifaceted use of brinjal in Indian food, for both every day and festive occasions (Gowda, 2016) [12]. The egg plant is widely cultivated in India, Bangladesh, Pakistan, China, Phillipines, Egypt, France, Italy and United States. India is recorded with the production of 12,779.54 thousand tonnes of brinjal. Correspondingly, Tamil Nadu occupies eleventh place with regard to production of brinjal in India (Apeda, 2018) [1]. Brinjal is cultivated under an area of 728.00 thousand hectares resulting for yearly yield of 12,660.00 thousand metric tonnes and productivity of 17.7 metric tonnes per hectare (Indiastat, 2019)

Several serious pathogens attack brinjal plant and cause significant reduction in brinjal production and productivity. One of the main pathogen of both greenhouse and field grown brinjal is the soil-borne and host specific pathogen *Fusarium solani* (Chakraborty *et al.,* 2009) [4]. *Fusarium solani* is a soil inhabiting fungus and can survive in soil for several years in the absence of host. Typical symptoms caused by the pathogen contain stunting of infected seedlings, yellowing of older leaves, and browning of vascular tissues. It is responsible for causing Fusarium wilt in eggplant causes wilting and early death of the plant (Virendra *et al*., 2017) [20].

Fusarium solani produced mycotoxin which is secondary metabolites that creates a serious threat to plants and animals. In case of plant, it causes wilt and rot disease on wide variety of crop at least 111 plants species (Bogale *et al.,* 2008) [3]. There are two main approaches to control the disease; chemical application and biocontrol agents. Chemical application is a widely applied method to control soil-borne diseases, however, it has a potential risk to human health and increases environmental pollution, such as afecting the benefcial functions of microorganisms living in the soil and root ecosystem (Akrami, 2015) [14]. In contrast, A biological control agent colonizes the rhizosphere, the site requiring protection and leaves no

toxic residues as opposed to chemicals. The first requirement of biological control is the identification and deployment of highly effective strains (Dubey *et al.*, 2007) [19]. The filamentous fungi, Trichoderma have attracted the attention because of their multiprong action against various plant pathogenic fungi, including *Fusarium* species (Saravanakumar *et al*., 2016) [11]. Isolates of several Trichoderma species have been reported to effectively reduce Fusarium wilt diseases (Singh *et al*., 2014) [18]. Biocontrol mechanisms of *Trichoderma* spp. include antibiosis, mycoparasitism, competition for nutrients and potential infection courts, and induced systemic resistance in plants (Segarra *et al*., 2013) [9]. Considering these points, the present study was undertaken to find out the most effective isolates of *Trichoderma asperellum* against Fusarium wilt of eggplant.

Materials and Methods

Isolation, maintenance and identification of Fusarium wilt pathogen

The pathogenic fungal strain used in all the experiments was isolated from infected vascular tissues from stem and root regions of diseased brinjal plant showing wilt symptoms were collected separately from farmer's field. Tissue bits were surface sterilized with 10 per cent sodium hypochlorite for 5-10 min. and subsequently three washings with sterile distilled water.

Then, they were placed on Potato Dextrose Agar (PDA) medium separately and incubated at the laboratory conditions at 25 ± 3°C for five days.

The fungi were purified separately by transferring the tip of the mycelia into PDA slants and maintained as stock cultures for further studies (Rangaswamy,1972) [17]. The pathogen was identified as *Fusarium solani* based on the conidial character.

Isolation and identification of *Trichoderma* spp

Soil samples were collected from healthy brinjal rhizosphere at ten different brinjal growing tracts of Tamil Nadu, India. For rhizospheric soil, plant was gently and carefully uprooted, soil tightly adhering the roots was collected, randomly selected, mixed and one forth part was used as composite rhizospheric soil sample of the region. The pH of soil was determined in 1:2 (soil:water) ratio, keeping 30 min as equilibration time.

Collected soil samples were air dried for 4h and isolation was done by serial dilution technique. Trichoderma selective medium (TSM) was used for isolation of the isolates of Trichoderma 1 mL soil suspension was taken with the help of 5 mL sterilized pipette and poured on the Petri plate seeded with TSM. The plates were incubated at 28±2°C for 5 days. Observation on the appearance of colonies was recorded from 3rd to 5th day. Individual colonies were picked up and maintained in pure culture for further study. The Trichoderma species were identified and examined under compound microscope on the basis of their cultural and morphological characters and the cultures were maintained on PDA slants at 4°C for further study. Total of ten *Trichoderma* isolates were obtained and identified isolates were designated as TB1, TB2, TB3, TB4, TB5, TB6, TB7, TB8, TB9 and TB10.

Screening of *Trichoderma* spp. for their antagonistic activity against *Fusarium solani*

In this experiment, a total of 10 *Trichoderma* isolates (TAB1-TAB10) were chosen to assess the antagonistic activity against Brinjal fusarium wilt pathogen through dual culture technique. The Interactions between the isolates and FSM were determined by the method described by (Dennis and Webster,1971) [5]. In this assay, a 5 mm diameter mycelial disc from the growing edge of one week old Trichoderma and one week old Fusarium culture were placed on the opposite of the PDA petri dish (Size – 90 × 15 mm) and equal distance apart distance. A complete randomized experimental design was used with four Petri dishes for each antagonist. In control plates (without *Trichoderma*), a sterile agar disc was place at the opposite side of the pathogen inoculated disc. The plates were incubated at 28 ± 2 °C for 5 days in the dark. The experiments were repeated three times.

After the incubation period, the inhibition zone was measured and used to determine percentage of inhibition by using the formula

$$\text{Per cent inhibition (I)} = \frac{C-T}{C} \times 100$$

Where

C – Growth of pathogen in control plates

T – Growth of pathogen in dual culture plates

I – Per cent inhibition in mycelial growth

Statistical analysis

Complete Randomized Design (CRD) was used as an experimental design. Data were analyzed by using statistical analysis system AGRES statistics 21. Least significant difference (LSD) was used to compare the significant difference between means at P≤ 0.05.

Results

Isolation and identification of *Trichoderma* species from rhizospheric region

Ten isolates of *Trichoderma* sp were isolated from the rhizospheric regions of brinjal grown in different areas of Tamil Nadu

Table 1: *Trichoderma* spp. isolated from brinjal rhizosphere soil of southern districts of Tamil Nadu

S. No	Place of Collection	Isolates Code	District
1.	Saruvarayapatty	TB1	Madurai
2.	Usilampatti	TB2	Madurai
3.	Palamedu	TB3	Madurai
4.	Ottanchatram	TB4	Dindugal
5.	Palani	TB5	Dindugal
6	Koppuchitampatti	TB6	Virudhunagar
7.	Muthalnaickenpatti	TB7	Virudhunagar
8.	Andipatti	TB8	Theni
9.	Uttamapalaiyam	TB9	Theni
10.	Singalpadi	TB10	Vellore

Antifungal activity of different isolates of *Trichoderma* spp. against the mycelial growth of *Fusarium solani* f. sp. *Melongenae*: Ten native isolates of *Trichoderma* spp. were screened for their *in vitro* antagonism against the *Fusarium solani* f. sp. *melongenae* by dual cultural technique. The results of antagonistic activity of ten *Trichoderma* isolates against *F. solani* soilborne pathogen of brinjal are shown in Table 1. All isolates of *Trichoderma* had significant antagonistic effect on the pathogens causing wilting diseases in brinjal. Among ten isolates of *T. asperellum*, isolate TB3 (85.77%) showed highest antagonistic activity followed by the isolates TB8, TB2, TB1, TB5, TB4 and TB9 while least antagonistic activity was reported for isolateTB10 (54.55%) respectively (Table 2)

Table 2: Antifungal activity of different isolates of *Trichoderma* spp. against the mycelial growth of *Fusarium solani* f. sp. *melongenae*

S. NO	Isolates	Mycelial growth of the pathogen (cm)	Per cent reduction over control
1.	TB1	3.15	65.00(53.73)
2.	TB2	2.79	69.00(56.17)
3.	TB5	3.31	63.22(52.67)
4.	TB9	3.62	59.77(50.84)
5.	TB3	1.28	85.77(67.84)
6.	TB8	2.40	73.33(58.91)
7.	TB10	4.09	54.55(47.61)
8.	TB7	2.86	68.22(55.69)
9.	TB6	3.97	55.88(47.61)
10.	TB4	3.54	60.66(51.15)
	Control	9.00	00.90
	CD (P=0.5)	0.11	-

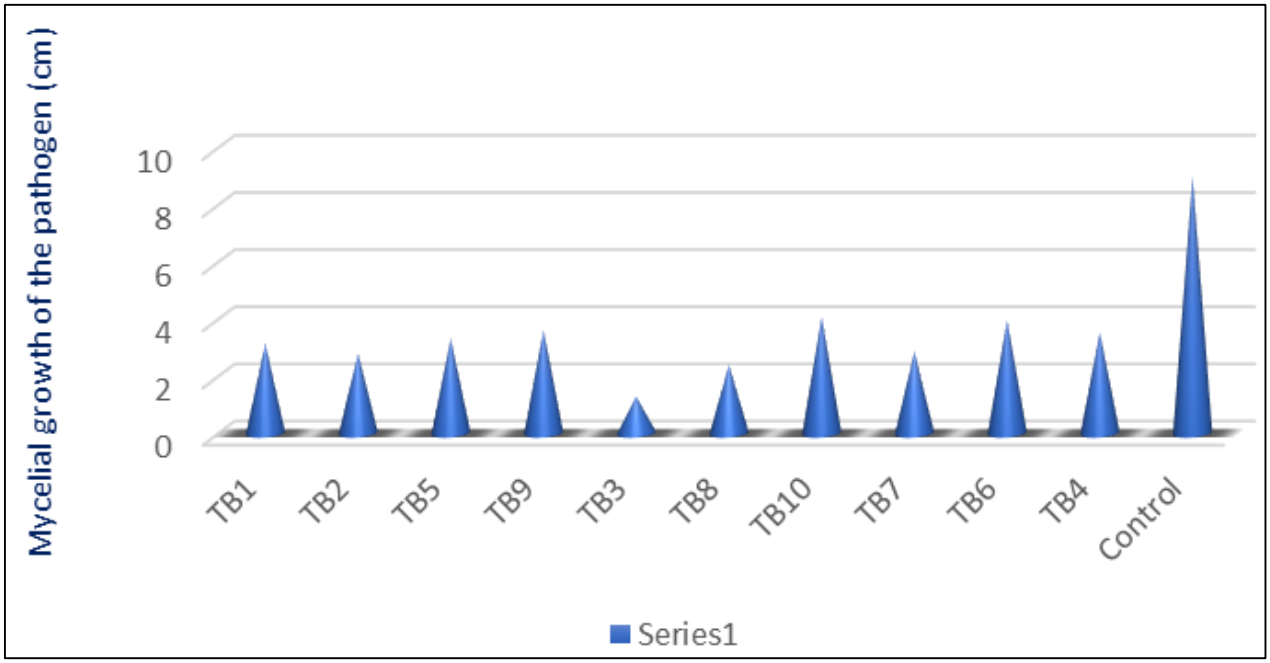

Fig 1: Antifungal activity of different isolates of Trichoderma spp, against the mycelial growth of *fusarium solani* f. sp melongenae

Discussion

Biological management, especially against soil-borne diseases, is the best alternative. The lack of biocontrol agents, on the other hand, has hampered the use of biocontrol strategies (Dubey *et al.,* 2008) [6]. Trichoderma is a well-known biocontrol fungus for its ability to control a wide variety of plant pathogens as well as promote plant growth (Martnez-Medina *et al.,* 2017) [13]. Ten isolates of *Trichoderma* spp. were obtained from various agricultural soils in different brinjal growing regions of Tamil Nadu, India, and physiologically, biochemically, and genetically studied in order to discover and choose the most effective antagonists.

The antagonistic impact of *T. asperellum* isolates was tested against isolates of *F. solani* in dual culture assays. The *T. asperellum* isolates developed more quicker than the *F. solani* isolates, and the pathogen was quickly overwhelmed. In the struggle for space and nutrients with pathogens, antagonists have a significant advantage due to their ability to develop quickly (Benitez *et al.,* 2004) [2]. The mycelial development of FSM strains was severely decreased by all of the *T. asperellum* isolates tested. Isolates TB3 and TB8 had the highest inhibition values. These isolates developed out of control and sporulated on pathogen colonies. The FSM mycelia at the interaction zone displayed aberrant morphology and were lysing, indicating the presence of severe mycoparasitism. Previously, some researchers looked into the *Trichoderma* antagonistic potential in *Fusarium* spp. *in vitro*. This finding was consistent with (Rajani *et al*., 2021) [16] who observed that the isolates *T. asperellum* against *F. solani* had the greatest ability to inhibit fungal growth. In another investigation, the inhibitory effect of *T. asperellum* against *F. solani*, the primary agents of potato wilt, was shown to be 81 percent in laboratory circumstances (Ommati, 2012) [7]. *T. harzianum* and *T. viride* were shown to have reduced the disease incidence of Fusarium wilt of eggplant caused by *Fusarium solani* by 86 percent and 83 percent, respectively, according to (Chakraborty *et al*.,2009) [4]. Aside from this mechanism, numerous significant mechanisms that contribute to the antagonism of these fungi have already been identified, including mycoparasitism, antibiosis by lytic enzymes, and the release of secondary metabolites (Vinale *et al.,* 2008) [20]

Conclusion

Based on the present study, we conclude that the *T. asperellum* strains TS-12 and TS-39 can be used as effective biocontrol agents to control Fusarium wilt disease of Brinjal, offering an alternative strategy for disease management. Further studies are recommended to test the effectiveness of

using the two *T. asperellum* strains either separately or in combination in open fields to develop long-term Fusarium wilt disease management strategies for Brinjal.

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