

Original Research Article

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## Evaluation of Different Fungal and Bacterial Antagonists against *Fusarium* Wilt of Eggplant caused by *Fusarium oxysporum* f. sp. *melongenae*

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### ABSTRACT

#### Keywords

Eggplant, *Fusarium* wilt, Biological control, Duel and pot culture

#### Article Info

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Eggplant is one of the important economic vegetable crops which are attacked by several serious diseases such as wilt. *Fusarium oxysporum* f. sp. *melongenae* was isolated from a naturally occurring epidemic of wilt in eggplant plants grown in Maharashtra. Among six bioagents evaluated *in vitro* against *F. oxysporum* f.sp *melangene*, *Trichoderma harzianum* was found effective with maximum inhibition of mycelial growth of the pathogen (67.77 %), followed by *Aspergillus nizer* (65.92%). While, *B.subtilis* found to be most effective (45.55 %) among antagonistic bacteria under duel culture study. Similar efficacy observed during pot culture study. *Trichoderma harzianum* was found most effective with least pre-emergence seedling mortality (7.66 %) with 84.89 per cent reduction over control. The second and third best antagonists found were *A. nizer* and *T. hamatum* which recorded pre-emergence seedling mortality of 11.33 and 13.66 with 77.51 and 64.23 per cent reduction over control respectively.

### Introduction

Brinjal is commonly known as Eggplant (*Solanum melongena* Linn.) It belongs to the family Solanaceae. Brinjal is a widely grown vegetable crop in Asian countries, probably a native of South Asia. Brinjal is growing throughout the India covering an area of 668.72 thousand ha with production of 123.99 thousand tonnes and productivity of 18.53 M. tonnes / ha. In Maharashtra, the area, production and productivity of Brinjal were 221.40 thousand ha, 433.28 thousand tonnes and 19.68 M. tones / ha, respectively during 2016-17 (Horticultural statistics at a glance, 2017).

*Fusarium* species are the most important plant pathogens in the world and highly variable because of their genetic makeup and changes in environment in which they grow causing morphological changes (Nelson, 1983). *Fusarium* wilt of eggplant caused by *Fusarium oxysporum* f. sp. *melongenae* is an economically important soil borne disease limiting eggplant production worldwide. This pathogen was initially reported in Japan (Matuo and Ishigami, 1958), and next in China (Zhuang *et al.*, 2005).

It is extremely difficult to control soil-borne fungi through conventional strategies such as

the use of synthetic fungicides, etc. Since their spores are able to survive for many years in the soil, biological control strategies for this pathogen should, therefore, be carefully selected and handled in an eco-friendly way instead of using chemical fungicides.

The application of microorganisms as biocontrol agents is important, since they may increase beneficial microbial activity which extends for a long period of time. *Trichoderma* spp. are considered as potential biocontrol and growth promoting agents for many crop plants (Verma *et al.*, 2007; Savazzini *et al.*, 2009).

The competition with pathogens, parasitism and the production of antifungal compounds are the most important mechanisms in biocontrol activity (Verma *et al.*, 2007; Savazzini *et al.*, 2009). *Trichoderma* populations can be established relatively easily in different types of soil and can continue to persist at detectable levels for months.

In the above context, the present study was undertaken to isolate the *Fusarium* wilt pathogen from the economically important eggplant crop and evaluate the potential of an isolated indigenous strain of fungal and bacterial antagonists under *in vitro* and screen house conditions.

## Materials and Methods

### Biocontrol agents

Pure cultures and talc based formulations of biocontrol agents *viz.*, *Trichoderma viride*, *T. harzianum*, *T. hamatum*, *T. longibrachiatum*, *T. virens*, *T. koningii*, *Aspergillus niger*, *Pseudomonas fluorescens*, *Pseudomonas striata* and *Bacillus subtilis* were obtained from the Spawn Production-cum-Biocontrol Laboratory, Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani. These cultures were maintained and multiplied on appropriate culture media and used for present studies.

### *In vitro* evaluation of bioagents (Duel culture)

Using dual culture technique (Dennis and Webster, 1971), seven fungal and three bacterial bioagents were evaluated *in vitro* against FOM. In this study seven days old bioagents cultures and test pathogen (FOM) culture were used. 5 mm culture discs of the test pathogen and test bioagents were cut with sterilized cork borer and placed on autoclaved and solidified PDA medium in Petri plates, exactly opposite to each other. PDA plates inoculated only with the test pathogen were maintained as untreated control. Three replications were incubated at  $27 \pm 2$  °C.

### Experimental details

Design	C.R.D. (Completely Randomized Design)
Replications	Three
Treatments	Eleven

### Bioagent Treatments

Tr.No.	Treatments	Tr. No.	Treatments
T <sub>1</sub>	<i>Trichoderma viride</i>	T <sub>6</sub>	<i>T. koningii</i>
T <sub>2</sub>	<i>T. harzianum</i>	T <sub>7</sub>	<i>Aspergillus niger</i>
T <sub>3</sub>	<i>T. hamatum</i>	T <sub>8</sub>	<i>Pseudomonas fluorescens</i>
T <sub>4</sub>	<i>T. longibrachiatum</i>	T <sub>9</sub>	<i>Pseudomonas striata</i>
T <sub>5</sub>	<i>T. (Gliocladium) virens</i>	T <sub>10</sub>	<i>Bacillus subtilis</i>
		T <sub>11</sub>	Control (Untreated)

Observations on linear mycelial growth of the test pathogen and test bioagent were recorded at a 24-hour interval and continued until untreated control plates were fully covered with mycelial growth of the test pathogen. By applying the following equation (Arora and Upadhyay, 1978), per cent inhibition of the test pathogen with the test bioagent was determined over untreated territory.

$$\text{Per cent growth Inhibition} = \frac{\text{Colony growth in control plate} - \text{Colony growth in intersecting plate}}{\text{Colony growth in control plate}} \times 100$$

### In vitro evaluation of bioagents (Pot culture)

Brinjal seeds were soaked in double the volume of sterile distilled water containing the talc based formulation (10 g kg<sup>-1</sup> of seed). After 24 h, the suspension was drained off and the seeds were dried under shade for 30 min and used for sowing (Nandakumar *et al.*, 2001). A pure culture of test pathogen was introduced into a Sand-Maize (19:1) medium and incubated for 15 days at room temperature for multiplication. The potting soil (red soil: sand: cow dung manure, 1:1:1 w: w: w) was incorporated with the fungus, and seeds of brinjal were surface-sterilized with 0.1% mercuric chloride for 30 seconds, rinsed three times with sterile distilled water and sown at 10 seeds per pot. Five grams of talc based formulation per kg of soil was added 30 days after sowing.

Observations on Pre-emergence mortality (PREM) after a week of sowing and post-emergence mortality (POEM) / wilting at 90 days after sowing were recorded. Percent of pre-emergence mortality (PREM) and post-emergence mortality (POEM)/wilting were calculated using the following formulae:

$$\text{PREM (\%)} = \frac{\text{No. of seeds ungerminated}}{\text{Total no. of seeds sown}} \times 100$$

$$\text{POEM (\%)} = \frac{\text{No. of seedlings died}}{\text{Total no. of seedlings}} \times 100$$

## Results and Discussion

### In vitro evaluation of bioagents (Duel culture)

The results obtained on mycelial growth and inhibition of *Fusarium oxysporum* f.sp. *melongenae* with seven fungal and three bacterial antagonists were presented in Table 1. All the bioagents exhibited fungistatic / antifungal activity against *Fusarium oxysporum* f.sp. *melongenae*. and significantly inhibited its growth, over untreated control (Fig. 1 and Plate I).

Of the bioagents / antagonists tested, *Trichoderma harzianum* was found most effective with least linear mycelial growth (29.00 mm). The second and third best antagonists found were *A.nizer.* and *T. hamatum*, which recorded least mycelial growth of 30.67 mm and 34.00 mm, respectively. These were followed by *T.longibrachiatum* (43.67), *Bacillus subtilis* (49.00), *T.koningi* (54.00), *Pseudomonas striata* (63.33), *Pseudomonas fluorescens* (65.33), *T. viride* (67.67) and *T. virens* (84.00). All treatments were significantly superior over untreated control (Table 1, Fig. 1 and Plate I).

Out of the ten antagonists tested, *Trichoderma harzianum* was found most effective with highest mycelial growth inhibition (67.77 %) of the test pathogen. The second and third inhibitor antagonists found were *A. nizer* and *T. hamatum* with and inhibition of 65.92 and 62.22 per cent, respectively. These were followed by *T. longibrachiatum* (51.48), *Bacillus subtilis* (45.55), *T. koningi* (40.00), *Pseudomonas.striata* (29.63), *Pseudomonas fluorescens* (27.40), *T. viride* (24.81) and *T. virens* (6.66). Thus, in order of merit the

bioagents viz., *Trichoderma harzianum*, *A.nizer*, *T. hamatum*, *T. longibrachiatum*, *Bacillus subtilis*, *T.koningi*, *Pseudomonas striata*, *Pseudomonas fluorescens*, *T. viride* and *T. virens* were found most potential antagonists against *Fusarium oxysporum* f.sp. *melongenae*.

These results were in agreement with Bashar and Chakma (2014) who demonstrated that the maximum inhibition (70.58%) of the pathogen was recorded with *T. harzianum*, followed by *T. viride*. Rini and Sulochana (2006) as well as Kapoor (2008) reported that *Trichoderma* spp., i.e., *T. harzianum* was more effective antagonist than *P. fluorescens* against *F. solani* and *F. oxysporum*. The

present findings were in conformity with the work of Devi and Singh (2012) who found similar *in vitro* results, in which the inhibition of the pathogen was maximum with *T. harzianum* followed by *A.nizer* and very less effect of *P. fluorescens* and *B.subtilis* were found in inhibition of the pathogen. However, Srideepthi and Krishna (2015) reported the efficacy of *T. viride* and *T. harzianum* against *Fusarium oxysporum* f.sp. *capsici*, which showed 68.4 and 34.2 per cent of mycelial inhibition, respectively. This variation in effectiveness might be due to difference in nature, quality and quantity of the inhibitory substances produced by these antagonistic soil fungi (Kexiang *et al.*, 2002).

**Table.1** *In vitro* efficacy of bioagents against *Fusarium oxysporum* f. sp.*melongenae* (duel culture)

Tr. No.	Treatments	Colony Dia.(mm)	Per cent growth inhibition
T <sub>1</sub>	<i>Trichoderma viride</i>	67.67	24.81 (29.84)*
T <sub>2</sub>	<i>T. harzianum</i>	29.00	67.77 (55.39)
T <sub>3</sub>	<i>T. hamatum</i>	34.00	62.22 (52.05)
T <sub>4</sub>	<i>T. virens</i>	84.00	6.66 (14.84)
T <sub>5</sub>	<i>T. longibrachiatum</i>	43.67	51.48 (45.83)
T <sub>6</sub>	<i>Aspergillus niger</i>	30.67	65.92 (54.26)
T <sub>7</sub>	<i>T. koningii</i>	54.00	40.00 (39.21)
T <sub>8</sub>	<i>Pseudomonas fluorescens</i>	65.33	27.40 (31.55)
T <sub>9</sub>	<i>Pseudomonas striata</i>	63.33	29.63 (32.96)
T <sub>10</sub>	<i>Bacillus subtilis</i>	49.00	45.55 (42.43)
T <sub>11</sub>	Control (untreated)	90.00	0.00 (0.00)
SE(m) ±		0.69	0.59
C.D (P=0.01)		2.03	1.75

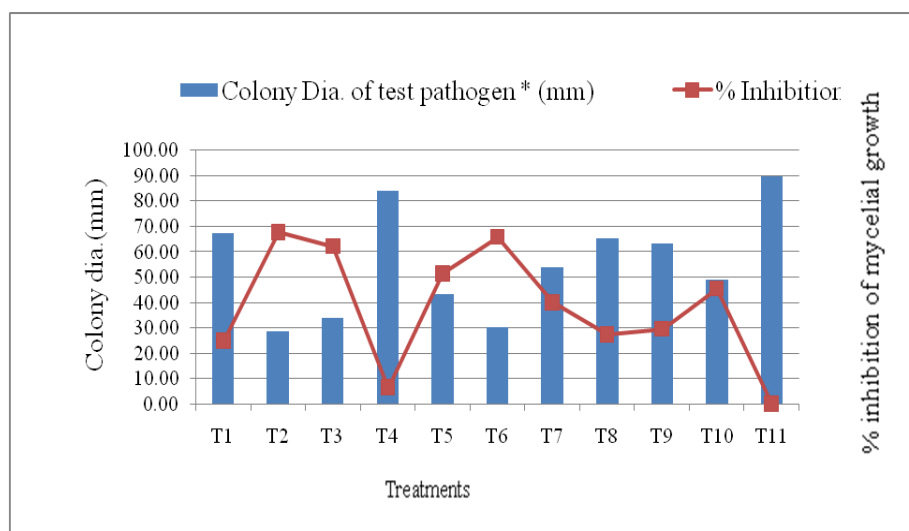
Dia: Diameter \*Figures in parentheses are angular transformed values

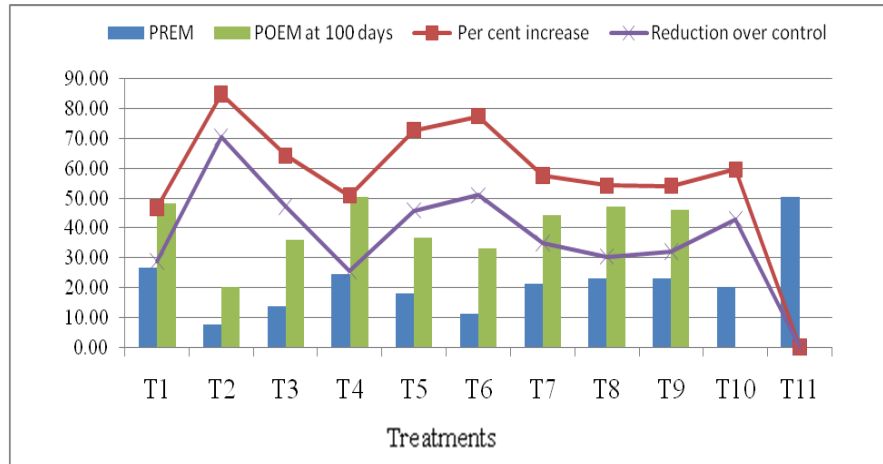
**Table.2** *In vitro* efficacy of bio control agents against *Fusarium oxysporum* f.sp. *melongenae* in pot culture

Tr. No.	Treatments	PREM	Per cent reduction over control	POEM at 100 days	Per cent reduction over control
T <sub>1</sub>	<i>Trichoderma viride</i>	26.66 (30.98)	46.74	48.16 (43.92)	28.82
T <sub>2</sub>	<i>T. harzianum</i>	7.66 (15.92)	84.89	20.00 (26.44)	70.44
T <sub>3</sub>	<i>T. hamatum</i>	13.66 (25.04)	64.23	35.83 (34.92)	47.04
T <sub>4</sub>	<i>T. virens</i>	24.66 (29.76)	50.86	50.52 (45.28)	25.34
T <sub>5</sub>	<i>T. longibrachiatum</i>	18.00 (21.59)	72.79	36.66 (37.21)	45.81
T <sub>6</sub>	<i>Aspergillus niger</i>	11.33 (19.63)	77.51	33.23 (36.73)	50.89
T <sub>7</sub>	<i>T. koningii</i>	21.33 (27.43)	57.59	44.11 (41.57)	34.81
T <sub>8</sub>	<i>Pseudomonas fluorescens</i>	23.00 (28.63)	54.14	47.14 (43.33)	30.33
T <sub>9</sub>	<i>Pseudomonas striata</i>	23.00 (28.60)	54.05	46.00 (42.66)	32.02
T <sub>10</sub>	<i>Bacillus subtilis</i>	20.33 (26.76)	59.54	38.66 (38.40)	42.86
T <sub>11</sub>	Control (untreated)	50.33 (45.17)	0.00	67.66 (55.33)	0.00
	<b>SE(m)<sub>±</sub></b>	<b>1.33</b>	<b>2.35</b>	<b>2.57</b>	<b>4.22</b>
	<b>C.D.(P=0.01)</b>	<b>3.93</b>	<b>6.94</b>	<b>7.6</b>	<b>12.46</b>

\*Figures in the parenthesis are angular transformed values

**Fig.1** *In vitro* evaluation of bioagents against mycelial growth and sporulation of *Fusarium oxysporum* f. sp. *melongenae*



**Fig.2** *In vitro* efficacy of bioagents against *Fusarium oxysporum* f.sp.melonae in pot culture

Species of *Trichoderma* viz., *T. harzianum* (T2) and *A.nizer* (T6) showed higher mycelial inhibition of the test fungus as compared to the bacterial antagonists (T8, T9 & T10). This can be attributed to the higher competitive ability of the *Trichoderma* species as these antagonists adopt different mechanisms viz., competition, lysis, antibiosis, siderophore production and hyperparasitism. In comparison to *T. viride*, the *T. harzianum* showed faster growth with higher inhibition of the fungus by producing extracellular lytic enzymes, which arrested the growth of *F. oxysporum*. High antagonistic activity of the *Trichoderma* species observed against the test fungus might be due to their fast growing nature, rapid sporulation and toxic metabolite producing capacity. Similar findings of higher antagonistic effect of *Trichoderma* species against *Fusarium* species recorded previously by Vidyasekaran (1999) and Barakat *et al.*, (2013).

#### ***In vitro* efficacy of bioagents (Pot culture)**

The results obtained on efficacy of ten bioagents against pre-emergence mortality and post emergence mortality of eggplant seedlings caused by *Fusarium oxysporum* f.sp. *melonae* are presented in Table 2.

Results revealed that all the bioagents exhibited fungistatic / antifungal activity against *Fusarium oxysporum* f.sp. *melonae* and significantly inhibited its pre-emergence seedling mortality over untreated control (Fig.2 and Plate II).

Among the bioagents / antagonists tested, *Trichoderma harzianum* was found most effective with least pre-emergence seedling mortality (7.66 %) with 84.89 per cent reduction over control.

The second and third best antagonists found were *A. nizer.* and *T. hamatum* which recorded pre-emergence seedling mortality of 11.33 and 13.66 with 77.51 and 64.23 per cent reduction over control respectively. These were followed by, *T. longibrachiatum* (18.00), *T. koningi* (21.33), *T. (Gliocladium) virens* (24.66), *Bacillus subtilis* (20.33), *Pseudomonas fluorescens* (23.00), *Pseudomonas striata* (23.66) and *T. viride* (26.66) with 72.79, 59.54, 57.59, 54.14, 54.05, 50.86 and 46.74 per cent reduction over control respectively. All treatments were significantly superior over untreated control.

Out of the ten antagonists tested, *Trichoderma harzianum* was found most effective with lowest Post-emergence seedling mortality

(20.00 %) caused by the test pathogen with 70.44 per cent reduction over control.

The second and third inhibitor antagonists found were *A.nizer* and *T. hamatum* with least post-emergence seedling mortality of 33.23.00 and 35.83 per cent with 50.89 and 47.04 per cent reduction over control respectively. They were followed by *T. longibrachiatum* (36.66%), *Bacillus subtilis* (38.66%), *T. koningi* (44.11%), *Pseudomonas striata* (46.00%), *Pseudomonas fluorescens* (47.14%), *T. viride* (48.16%) and *T. virens* (50.52%). However, in view of per cent reduction over control performed by bioagents against Post-emergence seedling mortality were *Trichoderma harzianum* (70.44%), *A.nizer* (50.89%) *T. longibrachiatum* (45.81%), *Bacillus subtilis* (42.86%), *T. koningi* (34.81%), *Pseudomonas.striata* (32.02%), *Pseudomonas fluorescens* (30.33%), *T. viride* (28.82%) and *T. virens* (25.34%).

The variation among the different isolates of *Trichoderma* species may be due to their genetic makeup for the antagonistic activity, production of virulence factor such as metabolites, trichodene (Lorito *et al.*, 1994). Moreover, a variety of extracellular lytic enzymes such as high chitinase and  $\beta$ -(1,3)-glucanase reported to be produced by *T. harzianum*, and there might be relationship between the production of these enzymes and the ability to inhibit the pathogen. In order to survive and compete, *Trichoderma* produces a wide variety of toxic and antibiotic metabolites that are active against large number of plant pathogens, such as trichodermin, trichodermol, harzianum-A, harzianolide, T39-butenolide, terpenes and polypeptides and extracellular hydrolytic enzymes (Thrane *et al.*, 2000), which were involved in the inhibition, competition, and mycoparasitism of *Fusarium* species.

The *B. subtilis*, a bacterial antagonists produces a wide range of antifungal compounds, such as subtilin, TasA, subtilosin, bacilysin, mycobacillin and some enzymes, which can degrade fungal cell wall (Berg *et al.*, 2001). Antagonistic activity of three isolates of *Trichoderma* species against *F. solani* and *F. oxysporum* f.sp. *lycopersici* due to parasitism, competition and antibiosis, coiling and penetration of antagonistic hyphae of *T. virens* and *T. harzianum* around the hyphae of *F. solani* and their lysis, production of organic metabolites and induction of plant defense system (Thangavelu and Musataffa, 2010). In addition, some antagonistic mechanisms of these *Bacillus* species involves in the competition for nutrients and space, the induction of plant resistance, increased activities of polyphenol oxidase (PPO) and peroxidase (POD), whereas, *P. fluorescens* produce an array of antifungal metabolites like siderophores and different antibiotics like phenazine-1 carboxylic acid, and 2,4-diacetyl-phloroglucinol (2,4-DAPG) preventing further advancement of the fungus by inducing severe cell disturbances in pathogenic fungi (Beckman *et al.*, 1982). *T. harzianum* is a non pathogenic fungus that captures the root zone for its profuse growth and competes with the pathogenic microorganisms for space and nutrition. Sometimes *T. harzianum* secretes some toxins and enzymes injurious to pathogenic organisms. Moreover, it can directly parasitize other soil borne pathogens. This myco-parasitism might be the reason of controlling wilt pathogens (Faruq *et al.*, 2014).

In conclusions among six bioagents evaluated *in vitro* against *F. oxysporum* f.sp *melangene*, *Trichoderma harzianum* was found effective with maximum inhibition of mycelial growth of the pathogen (67.77 %), followed by *Aspergillus nizer* (65.92%). While, *B.subtilis* found to be most effective (45.55 %) among

antagonistic bacteria under duel culture study. Similar efficacy observed during pot culture study. *Trichoderma harzianum* was found most effective with least pre-emergence seedling mortality (7.66 %) with 84.89 per cent reduction over control.

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