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In vitro evaluation of different bioagents against *F. oxysporum* f. sp. *lycopersici* causing wilt in tomato

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Abstract

Tomato (*Lycopersicon esculentum* Miller) crop suffers from several diseases, among which wilting caused by *F. oxysporum* f. sp. *lycopersici* is one of the serious diseases observed regularly in tomato growing areas. Therefore, efforts were made to evaluate bio efficacy of the different bioagents *in vitro* condition against *F. oxysporum* f. sp. *lycopersici*. Among five fungal and two bacterial bio-agents tested *in vitro*, exhibited significant inhibition of mycelial growth of *F. oxysporum* f. sp. *lycopersici*. However, *Trichoderma koningii* recorded significantly highest inhibition of mycelial growth (92.22%) followed by *T. longibrachiatum* (90%), *T. harzianum* (87.60%), *T. asperellum* (67.22%), *T. hamatum* (60.93%), *Bacillus subtilis* (53.52%) and *Pseudomonas fluorescens* (42.41%) over control.

Keywords: Tomato, wilt, *F. oxysporum* f. sp. *lycopersici*, bioagents, dual culture technique and Potato dextrose agar

Introduction

Tomato (*Lycopersicon esculentum* Miller) is one of the important vegetable crops of solanaceous family originated in Peru and South America. It belongs to family *Lycopersicae*. Tomato is one of the most important vegetable crops cultivated for its fleshy fruit and also considered as important commercial and dietary vegetable crop. India is the second largest producer and consumer of tomato in the world after China. In India, tomato was grown in about 0.797 million ha with an annual production of 207.08 million tonnes and productivity of 25.98 tonnes per ha during 2017 (FAOSTAT, 2019) [3]. The major tomato growing states in India are Madhya Pradesh, Orissa, Karnataka, West Bengal, Chhattisgarh, Andhra Pradesh, Telangana, Gujarat, Bihar, Maharashtra and Tamil Nadu which accounted for 91 per cent of the total production of the country (NHB, 2017) [5]. In Maharashtra tomato was cultivated on an area of 0.43 million ha with 9.57 Mt of annual production and productivity of 21.93 Mt/ha. Among various factors responsible for low production and productivity of tomato, the diseases caused by biotic agents are major one. The crop is vulnerable to number of diseases such as Bacterial wilt (*Ralstonia solanacearum*), Fusarium wilt (*Fusarium oxysporum*), Early blight (*Alternaria solani*), Late blight (*Phytophthora infestans*), Damping off (*Pythium* and *Rhizoctonia*) and Yellow leaf curl. Among all these diseases, *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is the most devastating fungal disease. Joshi *et al.* (2013) [4] reported that the soil borne fungus *F. oxysporum* is the causal agent of vascular wilt, the disease that affects a large variety of economically important crops worldwide. Considering these issues, present study was planned and conducted with the aim to evaluate the different non- systemic fungicides *in vitro* condition against *F. oxysporum* f. sp. *lycopersici* causing wilt in tomato.

Material and Methods

Following five fungal and two bacterial antagonists were evaluated *in vitro* against *F. oxysporum* f. sp. *lycopersici* by applying Dual culture technique. Seven-day old culture of bioagents and the pathogen i.e. *F. oxysporum* f. sp. *lycopersici* were used for the study. The pathogen and bioagent were placed aseptically at equidistance exactly opposite with each other on solidified PDA medium in Petri plates and were incubated at 25± 20c. The plates inoculated with culture disc of the test pathogen were maintained as untreated control. Observation on radial mycelial growth / colony diameter of the *F. oxysporum* f. sp. *lycopersici* were recorded at an interval of 24 hours and continued till untreated control plates were fully covered with mycelial growth. Per cent mycelial growth inhibition of the pathogen with the bioagents over the untreated control were calculated by using the formula (Vincent, 1927) [8]

C - T

Per cent inhibition = ----- x 100

C

Where,

C = Growth of the test fungus in untreated control plates. T = Growth of the test fungus in treated plates.

Result and Discussion

The results obtained on mycelial growth and inhibition of *Fusarium oxysporum* f. sp. *lycopersici* with five fungal and two bacterial antagonists are presented in Table 1, PLATE I & Fig.1(a and b). Results revealed that all the bioagents evaluated exhibited fungistatic/antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici* and significantly inhibited its growth over untreated control.

Out of the five fungal antagonists tested, *Trichoderma koningii* was found to be most effective and recorded least mycelial growth (7.00 mm) with highest mycelial inhibition (92.22%) and second best antagonist was

T. longibrachiatum with mycelial growth of 9.00 mm and 90% mycelial growth of inhibition. The third best antagonist found was *T. harzianum* with mycelial growth of 11.16 mm and mycelial inhibition of 87.60%, which was followed by *T. asperellum* with mycelial growth of 29.50 mm and 67.22% mycelial inhibition and *T. hamatum* with mycelial growth of 35.16 mm and 60.93% mycelia inhibition. The least effective fungal bioagent was

T. hamatum with mycelia growth of 35.16 mm and 60.93% mycelial inhibition over untreated control. Among two bacterial bioagents *Bacillus subtilis* was found best with mycelial growth of 41.83 mm and 53.52% mycelial inhibition but was less effective than all the fungal bioagents. Both the bacterial bioagents were found significant over all the other bioagents. The *Pseudomonas fluorescens* was comparatively least effective than all the five fungal and one bacterial bioagent and recorded 51.83 mm mycelial growth with 42.41% mycelial inhibition of *Fusarium oxysporum* f. sp. *lycopersici* as compared to 90.00 mm growth of test pathogen in untreated control.

Thus, all the fungal and bacterial bioagents tested were found fungistatic against *Fusarium oxysporum* f. sp. *lycopersici* and significantly inhibited its mycelial growth over untreated control. However, fungal and bacterial bioagents found most effective for mycelial inhibition of the pathogen in the order of merit were *T. koningii* (92.22%), *T. longibrachiatum* (90.00%), *T. harzianum* (87.60%), *T. asperellum* (67.22%), *T. hamatum* (60.93%) and the bacterial antagonist *Bacillus subtilis* (53.52%) and *Pseudomonas fluorescens* (42.41%).

Similar results were earlier reported by Basco *et al.* (2017) [2]; Taghdi *et al.* (2015) and Barhate *et al.* (2015) who reported that maximum inhibition zone of radial growth of *F. oxysporum* f. sp. *lycopersici* was observed with *Trichoderma viride* followed by *T. koningii*, *T. harzianum* and *P. fluorescens*. Shaikh and Nassreen (2013) evaluated *T. viride* and *T. harzianum* for their antagonistic activity against 5 different pathogens *in vitro* viz, *C. lindemutheanum*, *F. oxysporum*, *R. solani*, *A. solani* and *F. solani* in dual culture assay. *T. viridae* gave maximum inhibition of mycelial growth to all pathogens (67.45%), whereas, *T. harzianum* showed 63.89% inhibition of mycelial growth.

Table 1: *In vitro* bio-efficacy of bioagents against *Fusarium oxysporum* f. sp. *lycopersici* by dual culture technique

Treatments	Growth of test pathogen Treatments details(%)	Inhibition	
		(mm)*	
T1	<i>Trichoderma asperellum</i>	67 29.50	.22
		(55.07)	
T2	<i>T. harzianum</i>	87 11.16	.60
		(6 9.38)	
T3	<i>T. hamatum</i>	60 35.16	.93
		(51.31)	
T4	<i>T. longibrachiatum</i>	90 9.00	.00
		(7 1.56)	
T5	<i>T. koningii</i>	92 7.00	.22
		(73.80)	
T6	<i>Pseudomonas fluorescens</i>	42 51.83	.41
		(40.63)	
T7	<i>Bacillus subtilis</i>	53.52 41.83	(47.01)
T8	Control	90.00	0.00
		(0.00)	
	S.E.±	0.46	0.53
	C.D (P=0.01)	1.36	

* = Mean of three replications, Figures in parenthesis are arc sine transformed values

(a)

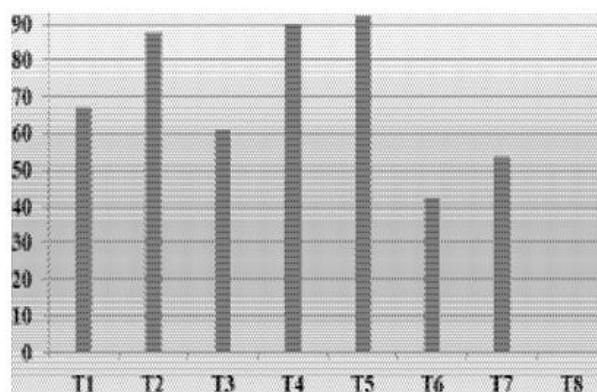
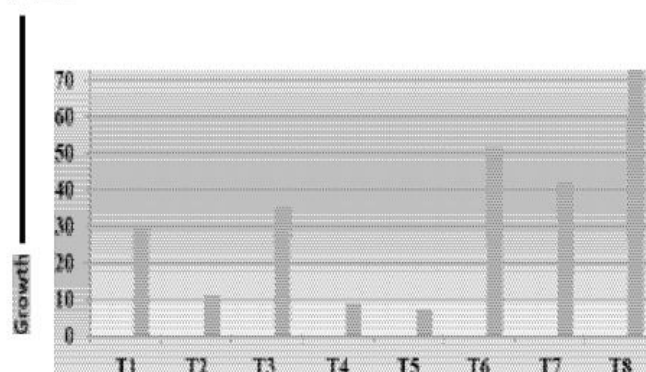


Table 2: *In vitro* bioefficacy of bioagents on mycelial growth and inhibition of *F. oxysporum* f. sp. *lycopersici*

Tr. No.	Treatments	Tr. No.	Treatments
T ₁	<i>T. asperellum</i>	T ₅	<i>T.koningii</i>
T ₂	<i>T. harzianum</i>	T ₆	<i>P. fluorescens</i>
T ₃	<i>T. hamatum</i>	T ₇	<i>B. subtilis</i>
T ₄	<i>T. longibrachiatum</i>	T ₈	Control (untreated)

**Plate 1****Table:** *In vitro* effect of bio-agents on growth and inhibition of *F. oxysporum* f. sp. *Lycopersici*

Tr. No.	Treatments	Tr. No.	Treatments
T ₁	<i>T. asperellum</i>	T ₅	<i>T.koningii</i>
T ₂	<i>T. harzianum</i>	T ₆	<i>P. fluorescens</i>
T ₃	<i>T. hamatum</i>	T ₇	<i>B. subtilis</i>
T ₄	<i>T. longibrachiatum</i>	T ₈	Control (untreated)

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