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*In-vitro E*valuation of Different Fungicides and Bioagents against *Fusarium oxysporum* f. sp. *lycopersici*

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A B S T R A C T

Fusarium wilt of tomato incited by *Fusarium oxysporum* f. sp. *lycopersici* is one of the biotic threats in profitable cultivation of tomato crop worldwide. Utilization of biocontrol agents along with fungicides is best suited for integrated disease management. The fungicides evaluated *in vitro* against *Fusarium oxysporum* f. sp. *lycopersici* were effective and reduced the mycelial growth significantly. Among that Carbendazim 50% WP, Copper oxychloride 50% WP and Carbendazim 25% + Mancozeb 50 % WS were found most effective with maximum growth inhibition (100%), (65.22%) and (100%) respectivly. Antagonist tested against *Fusarium oxysporum* f. sp. *lycopersici in vitro* significantly reduced the growth of test pathogen. Among that *Trichoderma harzianum* inhibited (52.33%) and *Trichoderma virens* (49.41%) found to be most effective with highest mycelial growth.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetable crops belonging to family Solanaceae. It is supposed to be originated from Peru, (South-America). Tomato is intensively cultivated in India.

It requires moderately cool weather and is grown in both *Kharif* as well as *Rabi* seasons. It is grown round the year on variety of soils with moderate summer temperatures, well drained sandy loam soils with neutral reactions are most suitable.

Annual tomato production of India was 22337.29MT during 2017-18 with an area of about 801 thousand ha, and productivity of 27.8 MT/ha (Anonymous, 2017), It is grown in Maharashtra on an area of about 43.64 thousand ha with production of 976.58.MT, and productivity of 22.07 MT/ha (Anonymous, 2017)

Tomato crop is succumbed to *Fusarium* wilt at all the stages of crop growth. Pathogen incites root, stem, leaves and fruit under

favorable condition cause drying off of the twigs and complete wilting of plants thereby resulting heavy losses. Keeping in view the economic importance of tomato, as a vegetable crop and losses incurred by *Fusarium* wilt in tomato, present investigations were carried out.

Materials and Methods

Evaluation of fungicides

Fungicides reported in Table 1, Table 2 and Table 3 were effective against *Fusarium oxysporum* causing wilt in tomato were evaluated *in vitro* by applying poisoned food technique using Potato dextrose agar as basal medium. An appropriate quantity of the fungicides was added in previously sterilized 100 ml PDA separately in 250 ml conical flasks. The flasks were shaken well to ensure uniform distribution of fungicides in the basal medium.

Twenty ml of the medium containing fungicides was poured into sterilized petri dishes. After solidification, the plates were inoculated by the fungal disc of 5 mm diameter cut out from seven days old culture and incubated at 27 ± 2 ^{o}C for seven days. Observation on radial mycelia growth was recorded in all the replicated treatments. Per cent inhibition of the growth of the test pathogen was calculated by applying the formula given by Vincent (1927) and the data obtained were averaged and analyzed statistically.

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

Where,

C= growth (mm) of test fungus in untreated control plate.
T= growth (mm) of test fungus in treated plate.

Evaluation of bio-control agents

Six antagonist's listed in Table 4 evaluated *in vitro* against *Fusarium oxysporum* f. sp. *lycopersici* by dual culture method (Dennis and Webster, 1971). All antagonist's and the pathogen were multiplied in PDA. Twenty ml of PDA was poured aseptically in each petri plates and allowed to solidify. Mycelial disc of 5 mm diameter of each antagonist and test fungus was placed on opposite ends of PDA containing petri plates. Each treatment was replicated three times. The plates were incubated at 27 ± 2 ºC for seven days. Observation on radial mycelia growth was recorded in all the replicated treatments. Per cent inhibition of the growth of the test pathogen was calculated by applying the formula. The data obtained were averaged and analyzed statistically.

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

Where,

C= growth (mm) of test fungus in untreated control plate.
T= growth (mm) of test fungus in treated control plate.

Results and Discussion

Evaluation of fungicides

Evaluation of systemic fungicides against *F. oxysporum* f. sp. *lycopersici*

All of the six systemic fungicides (Table 4) evaluated *in vitro* (each at 500, 750 and 1000 ppm) were found fungistatic and significantly inhibited mycelial growth of *F. oxysporum* f. sp. *lycopersici*, at all three test concentrations, over untreated control.

At 500 ppm, mycelial growth inhibition of *F. oxysporum* f. sp. *lycopersici* ranged from 31.29 to 100.00 per cent. However,

significantly highest and cent per cent mycelial growth inhibition (100%) was recorded with the fungicides *viz.*, Carbendazim 50% WP, followed by Carboxin 75% WP (88.44%), Thiophanate methyl 70% WP (88.14%), Benomyl 50% WP (88.14%), Difenconazole 25% EC (72.59%), The fungicide Azoxystrobin 23% EC was found ineffective with (31.29%) mycelial growth inhibition.

At 750 ppm, mycelial growth inhibition of *F. oxysporum* f. sp. *lycopersici* ranged from 45 to 100.00 per cent. However, significantly highest and cent per cent mycelial inhibition (100%) was recorded with the fungicides *viz.*, Carbendazim 50% WP, followed by Carboxin 75% WP (88.70%), Thiophanate methyl 70% WP (88.29%), Benomyl 50%WP (88.22%), Difenconazole 25% EC (82.37%) and Azoxystrobin 23% EC (45%) which was least effective.

At 1000 ppm, fungicides tested exhibited similar trend but with increased mycelial growth inhibition as compared to that of at 500ppm and 750ppm and it was ranged from 52.77 to 100.00 per cent, However, significantly highest and cent per cent mycelial growth inhibition (100%) was recorded with the fungicides *viz.*, Carbendazim 50% WP, followed by Carboxin 75% WP (88.81), Thiophanate methyl 70% WP (88.70), Benomyl 50%WP (88.51), Difenconazole 25% EC (83.33), and Azoxystrobin 23% EC (52.77%), which was least effective (Fig. 1–3).

Table.1 List of fungicides used to check their efficacy against *F. oxysporum* f. sp. *Lycopersici* Systemic fungicides (each @ 500,750, and 1000 ppm)

Tr. No.	Treatments	Tr. No.	Treatments
T_1	Carbendazim 50% WP	T_5	Penconazole 10% EC
T_2	Thiophinate methyl 70% WP	T_6	Benomyl 50%WP
T_3	Difenconazole 25% EC	T_7	Carboxin 75% WP
T_4	Azoxystrobin 23% EC	T_8	Control (untreated)

Table.2 List of fungicides used to check their efficacy against *F. oxysporum* f. sp. *lycopersici.* Contact and combi – fungicides (each @ 1500, 2000 and 2500 ppm)

Tr. No.	Treatments	Tr. No.	Treatments
T_1	Captan 75% WP	T_5	Carbendazim 25% + Mancozeb 50 % WS
T_2	Thiram 75% WS	T_6	Carboxin 37.5 % +Thiram 37.5% WS
T_3	Copper oxychloride 50% WP	T_7	Metalaxyl 8% + Mancozeb 64% WP
T_4	Mancozeb 75% WP	T_8	Control (untreated)

Table.3 List of bioagents used to check their efficacy against *F. oxysporum* f. sp. *Lycopersici*

Tr. No.	Treatments	Tr. No.	Treatments
T_1	*T. harzianum*	T_5	*T. hamatum*
T_2	*T. viride*	T_6	*Pseudomonas fluorescens*
T_3	*T. virens*	T_7	Control (Untreated)
T_4	*T. koningii*		

Table.4 *In vitro* efficacy of various systemic fungicides against *F.oxysporum* f. sp. *Lycopersici*

Tr. No.	Treatment	Colony Dia.*(mm) at ppm			Av. Colony (mm)	% Inhibition* at ppm			Av. Inhibition (%)
		500	750	1000		500	750	1000	
T1	Carbendazim 50% WP	00.00	00.00	00.00	00.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T2	Thiophinate methyl 70% WP	10.66	10.53	10.16	10.45	88.14 (69.85)	88.29 (69.98)	88.70 (70.35)	88.37 (70.06)
T3	Difenconazole 25% EC	24.66	15.86	15	18.50	72.59 (58.42)	82.37 (65.17)	83.33 (65.90)	79.43 (63.02)
T4	Azoxystrobin 23% EC	61.83	49.5	42.5	51.27	31.29 (34.01)	45.00 (58.42)	52.77 (46.58)	43.02 (40.98)
T5	Benomyl 50%WP	10.66	10.6	10.33	10.53	88.14 (69.85)	88.22 (34.01)	88.51 (70.18)	88.29 (69.98)
T6	Carboxin 75% WP	10.4	10.16	10.33	10.29	88.44 (70.12)	88.70 (69.85)	88.81 (70.45)	88.65 (70.31)
T7	Control (untreated)	90.00	90.00	90.00	90.00	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)
	S. E. m ±	**0.27**	**0.23**	**0.16**	**-**				**-**
	C. D. at (p 0.01)	**1.16**	**0.99**	**0.70**	**-**				**-**

* Mean of three replications. Figures in parentheses are Arcsine values

Table.5 *In vitro* efficacy of contact and combi fungicides against *F. oxysporum* f. sp. *Lycopersici*

Tr. No.	Treatment	Colony Dia.*(mm) at ppm			Av. Colony (mm)	% Inhibition* at ppm			Av. Inhibition (%)
		1500	2000	2500		1500	2000	2500	
T1	Captan 75% WP	39.70	33.37	32.38	35.15	55.88 (48.37)	62.92 (52.48)	64.01 (53.13)	60.93 (51.31)
T2	Thiram 75% WS	37.06	34.71	32.70	34.82	58.82 (50.08)	61.48 (51.63)	63.66 (52.92)	61.32 (51.54)
T3	Copper oxychloride 50% WP	34.36	30.7	29.36	31.47	61.82 (51.83)	66.48 (54.62)	67.37 (55.16)	65.22 (53.86)
T4	Mancozeb 75% WP	66.02	47.70	47.05	53.59	26.64 (31.07)	47.00 (43.28)	47.72 (43.69)	40.45 (39.49)
T5	Carbendazim 25% + Mancozeb 50 % WS	0.00	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T6	Carboxin 37.5 % +Thiram 37.5% WS	15.00	12.00	10.33	12.44	83.33 (65.90)	86.66 (68.57)	88.51 (70.18)	86.16 (68.15)
T7	Metalaxyl 8% + Mancozeb 64% WP	65.03	55.03	35.02	51.69	27.74 (31.78)	38.85 (35.55)	61.09 (51.40)	42.56 (40.72)
T8	Control (untreated)	90.00	90.00	90.00	90.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
	S. E. m ±	**0.16**	**0.20**	**0.24**	**-**				**-**
	C. D. at (p 0.01)	**0.68**	**0.83**	**0.98**	**-**				**-**

* Mean of three replications. Figures in parentheses are Arcsine values

Table.6 *In vitro* efficacy of different bioagents against *F. oxysporum* f. sp. *lycopersici*

Tr. No.	Treatments	Mean Colony Diameter (mm)*	Percent Inhibition Over control
T1	*Trichoderma harzianum*	42.90	52.33 (46.33)
T2	*T. viride*	60.83	32.41 (34.70)
T3	*T. virens*	45.53	49.41 (44.66)
T4	*T. koningii*	53.50	40.55 (39.55)
T5	*T. hamatum*	64.67	28.14 (32.03)
T6	*Pseudomonas fluorescens*	70.50	21.66 (27.73)
T7	Control (Untreated)	90.00	00.00 (00.00)
S. E. m ±		**0.22**	
C.D (P = 0.01)		**0.96**	

* Mean of three replications
Figures in parentheses are Arcsine values

Fig.1

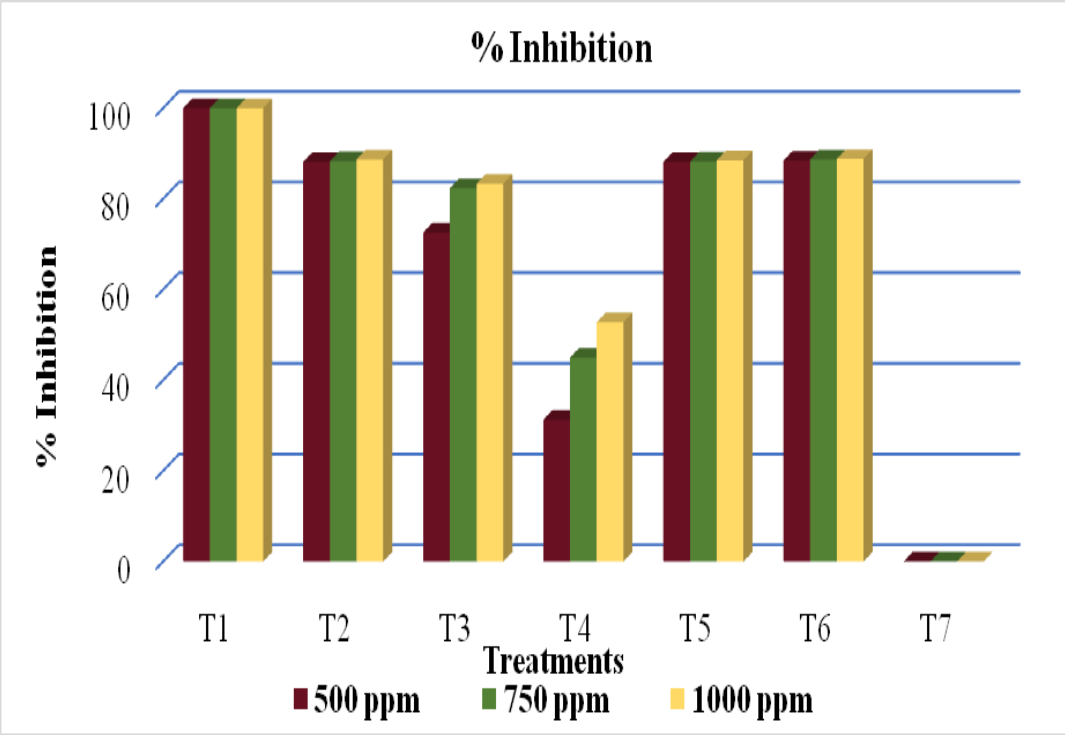

Fig.2

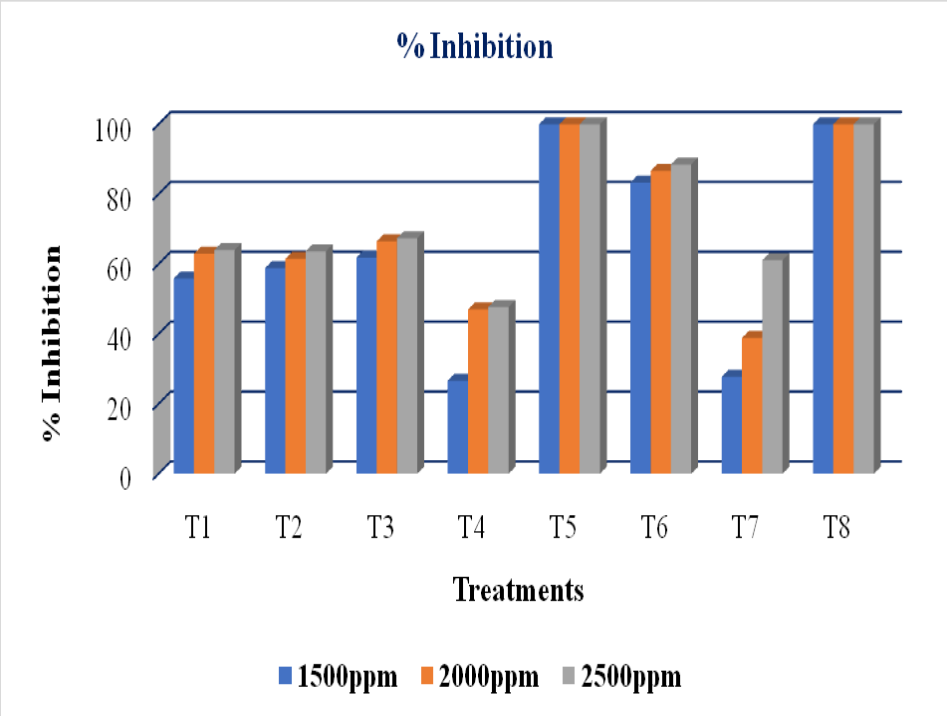
% Inhibition
100
80
60
40
20
0
% Inhibition
T1
T2
T3
T4
T5
T6
T7
T8
Treatments
1500ppm
2000ppm
2500ppm

Fig.3

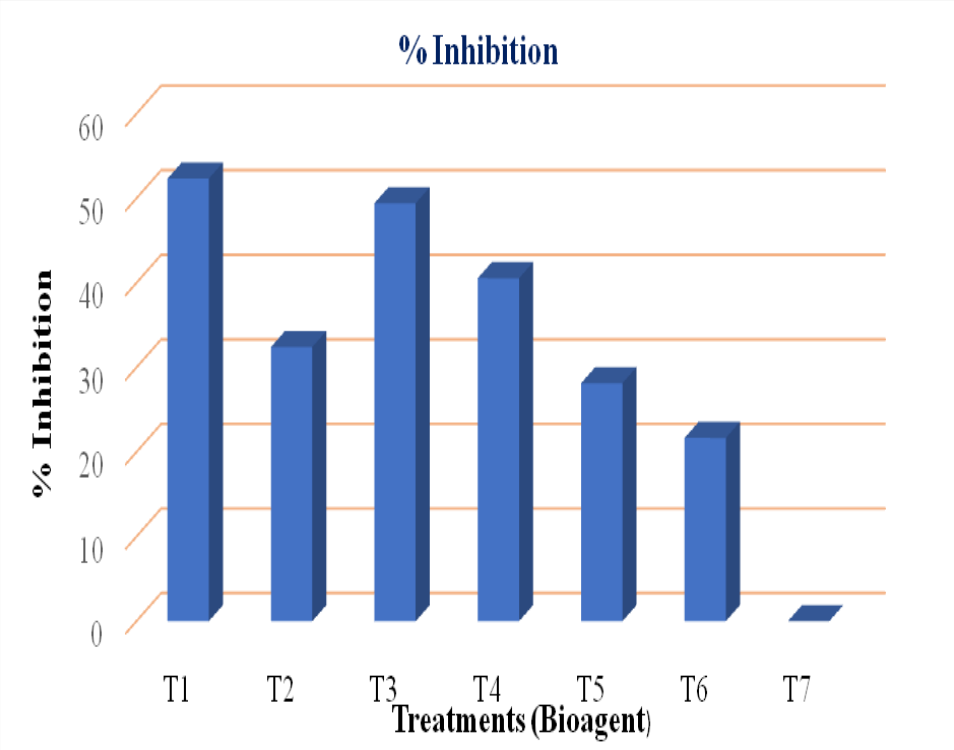
% Inhibition
60
50
40
30
20
10
0
% Inhibition
T1
T2
T3
T4
T5
T6
T7
Treatments (Bioagent)

Fig.4 *In vitro* efficacy different bioagents against *Fusarium oxysporum* f. sp. *lycopersici*

Evaluation of contact and combi fungicides against *F. oxysporum* f. sp. *lycopersici*

All of the four contact and three combi fungicides (Table 6) evaluated *in vitro* (each @ 1500, 2000 and 2500 ppm) were found fungistatic and significantly inhibited mycelial growth of *F. oxysporum* f. sp. *lycopersici* at all three test concentrations, over untreated control.

At 1500 ppm, mycelial growth inhibition of *F. oxysporum* f. sp. *lycopersici* ranged from 26.64 to 61.82 per cent. However, the contact fungicides *viz.*, significantly highest mycelial inhibition (61.82%) was recorded with the fungicide Copper oxychloride 50% WP, followed by Thiram 75% WS (58.82%), Captan 75% WP (55.88%) and Mancozeb 75% WP (26.64%), which was least effective.

At 1500 ppm, mycelial growth inhibition of *F. oxysporum* f. sp. *lycopersici* ranged from 27.74 to 100 per cent. However, the combi fungicides *viz.*, significantly highest mycelial inhibition (100%) was recorded with the fungicide Carbendazim 25% + Mancozeb 50 % WS, followed by Carboxin 37.5 % +Thiram 37.5% WS(83.33%) and Metalaxyl 8% + Mancozeb 64% WP (27.74%), which was least effective.

At 2000 ppm, mycelial growth inhibition of *F. oxysporum* f. sp. *lycopersici* ranged from 47.00 to 66.48 per cent. However, the contact fungicides *viz.*, significantly highest mycelial inhibition (66.48%) was recorded with the fungicide, Copper oxychloride 50% WP, followed by Captan 75% WP (62.92%), Thiram 75% WS (61.48%) and Mancozeb 75% WP (47.00%) which was less effective.

At 2000 ppm, mycelial growth inhibition of *F. oxysporum* f. sp. *lycopersici* ranged from 38.85 to 100 per cent. However, the combi fungicides *viz.*, significantly highest mycelial inhibition (100%) was recorded with the fungicide Carbendazim 25% + Mancozeb 50 % WS, followed by Carboxin 37.5 % +Thiram 37.5% WS(86.66%) and Metalaxyl 8% + Mancozeb 64% WP (38.85%), which was least effective.

At 2500 ppm, test contact fungicides exhibited similar trend but with increased mycelial growth inhibition as compared to

that of at 1500 ppm and 2000 ppm and it was ranged from 47.72 to 67.37 per cent, However, the contact fungicides *viz.*, significantly highest mycelial growth inhibition (67.37%) was recorded with the fungicide Copper oxychloride 50% WP, followed by Captan 75% WP (64.01%), Thiram 75% WS (63.66%) and Mancozeb 75% WP (47.72%) which was less effective.

At 2500 ppm, test combi fungicides exhibited similar trend but with increased mycelial growth inhibition as compared to that of at 1500 ppm and 2000 ppm and it was ranged from 61.09 to 100 per cent, However, the combi fungicides *viz.*, significantly highest mycelial growth inhibition (100%) was recorded with the fungicide Carbendazim 25% + Mancozeb 50 % WS, followed by Carboxin 37.5 % +Thiram 37.5% WS (88.51%) and Metalaxyl 8% + Mancozeb 64% WP (61.09%), which was least effective.

Evaluation of bioagents

The test biocontrol agents significantly inhibited mycelial growth of *F. oxysporum*f. sp. *lycopersici*, over untreated control. However, *T. harzianum* was found most effective with significantly least mycelial growth (42.90 mm) and it's highest inhibition (52.33%), followed by *T. virens* (45.53 mm and 49.41%, respectively), *T. koningii* (53.50 mm and 40.55%, respectively.), *T. viride* (60.83 mm and 32.41%, respectively), *T. hamatum* (64.67 mm and 28.14% respectively), *P. fluorescens* (70.50 mm and 21.66%, respectively) (Fig. 4).

These results are in conformity with the earlier findings of Barari H. (2015) who reported *Trichoderma harzianum* as most effective against *F. oxysporum* f. sp. *lycopersici* causing wilt disease in tomato crop. Similarly, Hegd, *et al.,* (2017), Malathi (2015), and Mishra *et al.,* (2017) reported the efficacy of *T. harzianum* against *F. oxysporum*, causing wilt disease in safflower, tomato, fir, tomato, onion and chilli crops. These results are in conformity with the earlier findings of Rudresh *et al.,* (2005), who reported *Trichoderma virens* as most effective against *F. oxysporum* f. sp. *ciceris* causing wilt disease in Chick pea crop. Similarly, Govindappa *et al.,* (2010), Gupta (2016).

In conclusion the various fungicides and bioagents are evaluated *in vitro* by applying poisoned food technique by using Potato Dextrose Agar as basal medium. In case of fungicide they were found effective in reduction of mycelial growth.

All the treatments used in this were significantly highest and cent per cent mycelial growth inhibition (100%) was recorded with the fungicides viz., Carbendazim 50% WP resulted with (00.00 mm) mycelial growth followed by Copper oxychloride 50% WP resulted with (31.47 mm) mycelial growth and Carbendazim 25% + Mancozeb 50 % WS resulted with (00.00 mm) mycelial growth.

In case of bio-agent the results revealed that all of the test biocontrol agents significantly inhibited mycelial growth of *F. oxysporum* f. sp. *lycopersici*, over untreated control. However, *T. harzianum* was found most effective with significantly least mycelial growth (42.90 mm) and it's highest inhibition (52.33%), followed by *T. virens* (45.53 mm and 49.41%, respectively).

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