Research Article

In vitro efficacy of fungicides against *Fusarium oxysporum* f.sp. *melongenae* causing eggplant wilt

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Abstract

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. In the present research *in vitro* efficacy of eight systemic fungicides evaluated @ 500, 1000 and 1500 ppm against *Fusarium oxysporum* f.sp.*melongenae* by using poisoned food technique. Mean radial mycelial growth of the test pathogen was ranged from 1.00 mm (Carbendazim) to 58.22 mm (Fosetyl-AL). However, the order of mycelial growth was observed with Difenconazole, Propiconazole, Azoxystrobin, Tebuconazole, Hexaconazole, Thiophanate methyl and Fosetyle AL. Similarly,*In vitro* efficacy of eight contact/combi-fungicides @ 1500 to 2500 ppm. The fungicides Tebuconazole +Trifloxystrobin recorded no mycelial growth. However the order of efficacy was Carboxin + Thiram followed by Carbendazim + Mancozeb, Captan + Hexaconazole, Pyraclostrobin + metiram,Mancozeb ,Propineb and Metalaxil + Mancozeb.

Keywords: *Fusarium oxysporum*, fungicides, *In vitro* efficacy

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Introduction

Brinjal is commonly known as Eggplant (*Solanum melongena* Linn.) It is 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 [17]. *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 [15] and next in China [30]. *F.oxysporum* f.sp. *melongenae* is a soil borne fungal wilt pathogen which induces vascular disease in eggplant causing heavy yield losses in Asian countries [11, 12]. This disease also occurs both in greenhouse and open field cultivations [2, 25, 28]. *Fusarium oxysporum* and its various formae speciales characterized by the following symptoms: vascular wilt, yellows, corm rot, root rot, and damping-off. [1]; [24]. Due to prolonged survival in soil as a saprophyte and as resistant structures, *Fusarium oxysporum* was difficult to control [15] ; [7] ; [10].The main objective of presented study was to evaluate the possibility of controlling Fusarium wilt of eggplant with the use of fungicides under *in vitro* conditions.

Materials and Methods

In vitro efficacy of fungicides

Eight systemic fungicides (each @ 500, 1000, 1500 ppm) and eight combi fungicides (each @ 1500, 2000 and 2500 ppm) were separately tested *in vitro* against test pathogen (*F.oxysporum* f.sp. *melongenae*), using poisoned food technique [18] and Potato dextrose agar (PDA) as a basic culture media. Based on the active ingredient, the necessary quantity of test fungicides were determined and thoroughly mixed with autoclaved and cooled (40^{o}C) PDA medium to obtain desired concentrations in conical flasks separately. This PDA medium was then poured aseptically in Petri plates (90 mm dia.) with the sample fungicides separately (20 ml / plate) and allowed to solidify at room temperature. Three plates / treatment / replication were maintained for each of the sample fungicide and its target concentrations.

Experimental details : Systemic Fungicides
Design : C.R.D. (Completely Randomized Design)
Replications : Three
Treatments : Nine

Systemic Fungicides Treatments

Tr. No.	Treatments	Tr. No.	Treatments
T_1	Azoxystrobin 23 %EC	T_6	Tebuconazole 10% EC
T_2	Carbendazim 50% WP	T_7	Propiconazole 25 % EC
T_3	Difenconazole 25% EC	T_8	Thiophanate methyl 70 % WP
T_4	Fosetyl-AL 80 %WP	T_9	Control (Untreated)
T_5	Hexaconazole 5 % EC		

After solidification of the PDA medium, all the plates were aseptically inoculated by placing a 5 mm culture disk of test pathogen in the center obtained from the 7 days old pure culture of test pathogen and incubated in an inverted position at 28±2 ^{o}C. Petri plates filled with plain PDA (without any fungicide) and inoculated with pure culture disc of test pathogen were maintained as untreated control.

Experimental Details: Combi- fungicides
Design : C.R.D. (Completely Randomized Design)
Replications : Three
Treatments : Nine

Combi- fungicides treatments

Tr. No.	Treatments	Tr. No.	Treatments
T_1	Mancozeb 75% WP	T_6	Pyraclostrobin 5% + Metiram 55% WG
T_2	Propineb 70% WP	T_7	Metalaxyl M 4% + Mancozeb 64 % WP
T_3	Carbendazim 12 % + Mancozeb 63 % WP	T_8	Captan 70 % + Hexaconazole 5 % WP
T_4	Carboxin 37.5 %+ Thiram 37.5 % DS	T_9	Control (Untreated)
T_5	Tebuconazole 50% + Trifloxystrobin 25% WG		

Radial mycelial colony growth measurements were recorded at a 24 hour interval and continued until untreated control plates were fully covered with mycelial growth of test pathogen. By applying the following equation [29], per cent inhibition of the test pathogen with the test fungicides over untreated control was calculated.

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

Where, C = growth of the test fungus in untreated control plate, T = growth of the test fungus in treated plate

Results and Discussion

In vitro evaluation of systemic fungicides

Mycelial growth

Results of *in vitro* evaluationp of systemic fungicides revealed that all the tested eight systemic fungicides exhibited significant effect on radial mycelial growth of *Fusarium oxysporum* f.sp. *melongenae* where the radial growth was decreased with increase in concentrations(500 to 1500 ppm) of test fungicides.

At 500 ppm, radial mycelial growth of *Fusarium oxysporum* f.sp. *melongenae* was ranged from 3.00 mm to 73.33 mm as against 90.00 mm in untreated control (**Table 1**, **Figure 1** and **2**). However, minimum mycelial growth was observed with the fungicide Carbendazim (3.00). It was followed by the fungicides *viz,* Difenconazole (6.33 mm), Propiconazole (10.67 mm), Azoxystrobin (11.00 mm), Tebuconazole (13.00 mm), Hexaconazole (14.33), Thiophanate methyl (55.33 mm) and Fostyl-Al (73.33). Comparatively maximum mycelial growth was recorded with the fungicide Thiophanate methyl (73.33 mm).

At 1000 ppm, radial mycelial growth of *Fusarium oxysporum* f.sp. *melongenae* was found to be decreased as compared to that of at 500 ppm and it was ranged from 0.00 mm to 56.00 mm, as against 90.00 mm in untreated control. However, with the fungicide Carbendazim no mycelial growth was observed. It was followed by the fungicides *viz,* Difenconazole (3.00 mm), Propiconazole (6.00 mm), Azoxystrobin (8.33 mm), Tebuconazole (8.67

mm), Hexaconazole (9.33 mm), Thiophanate methyl (46.67 mm) and Fosetyl-AL (56.00). Comparatively maximum mycelial growth was recorded with the fungicides Fosetyl- AL(56.00).

Table 1 In *vitro* efficacy of systemic fungicides against mycelial growth and inhibition of *Fusarium oxysporum* f.sp.*melongenae* in Eggplant

Tr. No	Treatments	Colony Diameter (mm)				Per cent inhibition of mycelia growth			
		500 ppm	1000 ppm	1500 ppm	Mean *(mm)	500 ppm	1000 ppm	1500 ppm	Mean*
T_1	Azoxystrobin 23 % SC	11.00	8.33	5.33	8.22	87.77 (69.52)	90.74 (72.26)	94.07 (75.89)	90.86
T_2	Carbendazim 50% WP	3.00	0.00	0.00	1.00	96.66 (79.54)	100.00 (90.00)	100.00 (90.00)	98.89
T_3	Difenconazole 25 % SC	6.33	3.00	0.00	3.11	92.96 (74.59)	96.51 (79.34)	100.00 (90.00)	96.49
T_4	Propiconazole 25% EC	10.67	6.00	2.67	6.44	88.14 (69.83)	93.33 (75.04)	97.03 (80.09)	92.83
T_5	Thiophanate methyle 70 % WP	55.33	46.67	33.33	45.11	37.40 (38.34)	48.14 (43.92)	62.96 (52.49)	49.50
T_6	Tebuconazole 25% EC	13.00	8.67	0.00	7.22	85.55 (67.64)	90.37 (71.90)	100.00 (90.00)	91.97
T_7	Fosetyl-AL 80% WP	73.33	56.00	45.33	58.22	18.51 (25.46)	37.77 (37.90)	49.63 (44.77)	35.30
T_8	Hexaconazole 5% EC	14.33	9.33	4.00	9.22	84.07 (66.45)	89.63 (71.19)	95.55 (77.85)	89.75
T_9	Control	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
SE(m) ±		0.50	0.48	0.40		0.54	0.56	0.45	
C.D (P=0.01)		1.49	1.45	1.20		1.61	1.69	1.34	

*: Mean of three replications, Dia: Diameter, Av.: Average Figures in parentheses are angular transformed values

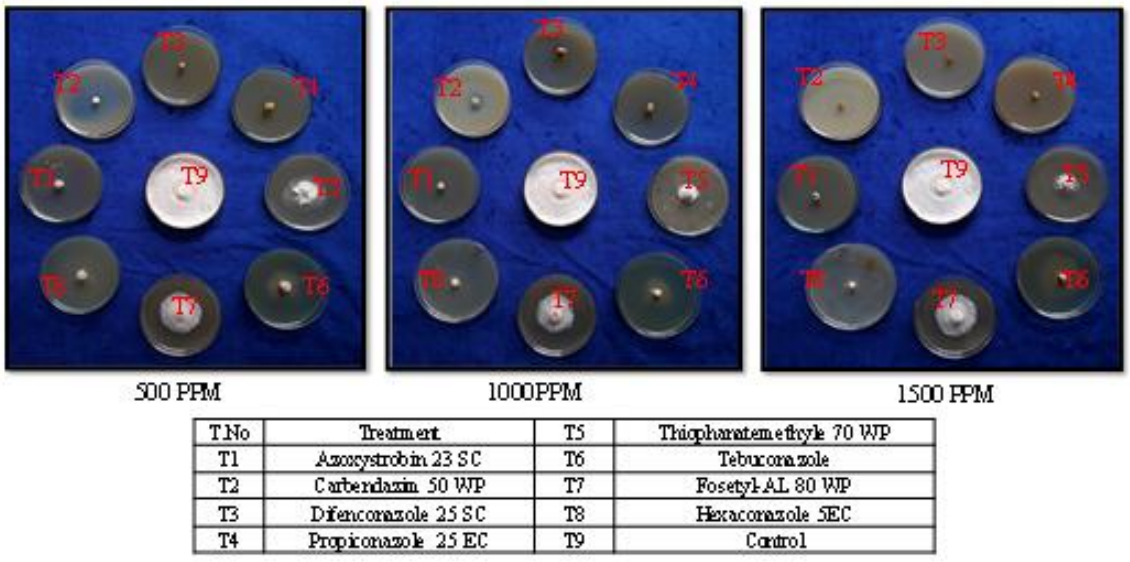

Figure 1 In *vitro* efficacy of systemic fungicides against mycelial growth and inhibition of *Fusarium oxysporum* f.sp.*melongenae*

At 1500 ppm, radial mycelial growth of *F.oxysporum* f.sp. *melongenae* was found to be decreased as compared to 500 and 1000 ppm and it was ranged from 0.00 mm to 45.33 mm, as against 90.00 mm in untreated control. However, with the fungicides *viz.*, Carbendazim, Difenconazole and Tebuconazole, no mycelial growth was observed. It was followed by the fungicides *viz.*, Propiconazole (2.67) followed by Hexaconazole (4.00), Azoxystrobin (5.33), Thiophanate methyl (33.33), Fosetyl-AL (45.33 mm). Comparatively maximum mycelial growth was recorded with the fungicide Fosetyl-AL (45.33 mm).

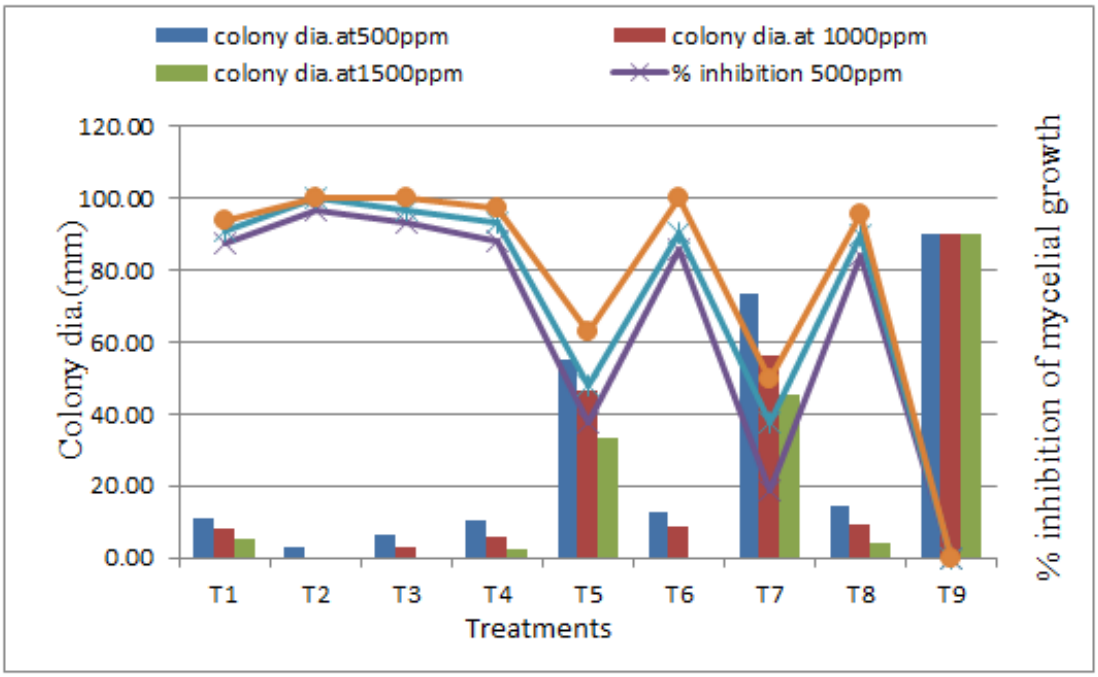

Figure 2 *In vitro* efficacy of systemic fungicides against mycelial growth and inhibition of *Fusarium oxysporum* f.sp.*melongenae* in eggplant

Mean radial mycelial growth of the test pathogen was ranged from 1.00 mm (Carbendazim) to 58.22 mm (Fosetyl-AL). However, there was substantial amount of mycelial growth was observed with Difenconazole (5.67 mm), Propiconazole (6.44 mm), Azoxystrobin (8.22),Tebuconazole(7.22),Hexaconazole (9.22), Thiophanate methyl (45.11 mm)and Fosetyle AL(58.22 mm). Comparatively maximum mycelial growth was recorded with the fungicides Fosetyle-AL (58.22 mm).

Mycelial inhibition

Results (Table 1, Figure 1 and 2) revealed that all the systemic fungicides tested (each @ 500, 1000 and 1500 ppm) significantly inhibited mycelial growth of *Fusarium oxysporum* f.sp. *melongenae*, over untreated control. Further, per cent mycelial inhibition was increased with increase in concentrations of the fungicides tested.

Among the systemic fungicides evaluated, Carbendazim recorded maximum per cent inhibition of mycelial growth from 96.66 to 100 per cent at all the concentrations tested, followed by Difenconazole with 92.96,96.51 and 100 per cent at 500, 1000 and 1500 ppm concentration respectively with a mean of 98.89 per cent. Propiconazole recorded 88.14, 93.33 and 97.03 per cent inhibition at 500, 1000 and 1500 ppm concentration respectively with a mean of 92.83 per cent. Azoxystrobin recorded 87.77, 90.74 and 94.07 per cent at 500, 1000 and 1500 ppm concentration respectively with a mean of 90.86 per cent. Tebucinazole showed 85.55, 90.37 and100 per cent at 500, 1000 and 1500 ppm concentration respectively with a mean of 91.97 per cent. Hexaconazole recorded 84.07, 89.03 and 95.55 per cent at 500, 1000 and 1500 ppm concentration respectively with a mean of 89.75 per cent and Thiophanatemetyle recorded 37.40, 48.14 and 62.96 per cent at 500, 1000 and 1500 ppm concentration respectively with a mean of 49.50 per cent.

However, the least inhibition was shown by Fosetyle-AL with 18.51, 37.77 and 49.63 with a mean of 35.30. It was observed that in most of the cases effect of concentration level (ppm) of fungicides on mycelial growth was inversely proportionate among the three concentrations tested.Thus, all the systemic fungicides tested were found fungistatic against *Fusarium oxysporum* f.sp. *melongenae* and significantly inhibited its mycelial growth, over untreated control. However, the systemic fungicides found most effective in the order of merit were Carbendazim, Difenconazole, Propiconazole, Azoxystrobin, Tebuconazole, Hexaconazole, Thiophante methyl and Fosetyle-AL.

In vitro, evaluation of combi-fungicides

Radial mycelial growth

Results (**Table 2**, **Figure 3** and **4**) revealed that all of the eight tested combi- fungicides were exhibited a wide range of radial mycelial growth of *Fusarium oxysporum* f.sp. *melongenae* and decreased drastically with increase in concentrations of the test fungicides from 1500 to 2500 ppm.

Table 2 *In vitro* efficacy of Contact / Combi fungicides against mycelial growth and inhibition of *Fusarium oxysporum* f.sp. *melongenae* in Eggplant

Tr. No.	Treatments	Colony Diameter(mm)				Per cent inhibition of mycelial growth			
		1500 ppm	2000 ppm	2500 ppm	Mean	1500 ppm	2000 ppm	2500 ppm	Mean
T_1	Mancozeb 50% WP	40.26	35.00	21.67	32.31	55.27 * (48.01)	61.11 (51.42)	75.92 (60.62)	64.10
T_2	Propineb 70 % WP	49.89	42.00	37.67	43.19	44.57 (41.81)	53.33 (46.89)	58.14 (49.67)	52.02
T_3	Carbendazim 12% + Mancozeb 50 WP	13.56	10.00	9.67	11.07	84.93 (67.03)	88.88 (70.56)	89.25 (70.90)	87.70
T_4	Carboxin 37.5% + Thiram 37.5% WP	5.40	0.00	0.00	1.80	94.00 (75.80)	100.00 (90.00)	100.00 (90.00)	98.00
T_5	Tebuconazole 50% + Trifloxystrobin 25 % WG	0.00	0.00	0.00	0.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00
T_6	Pyraclostrobin 5% + Metiram 55 % WG	18.89	13.33	7.00	13.07	79.01 (62.57)	85.18 (67.39)	92.22 (73.88)	85.47
T_7	Metalaxyl 8% + Mancozeb 64% WP	56.63	53.33	47.00	52.32	37.08 (37.41)	40.74 (39.60)	47.77 (43.70)	41.87
T8	Captan 70% + Hexaconazole 5% WP	16.55	11.33	7.80	11.89	81.61 (64.44)	87.40 (69.26)	91.33 (72.90)	86.78
T9	Control	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00
	SE(m)±	2.18	1.85	1.29		1.44	1.32	1.03	
	C.D.(P=0.01)	6.52	5.55	3.88		4.31	3.96	3.09	

*Figures in parentheses are angular transformed values

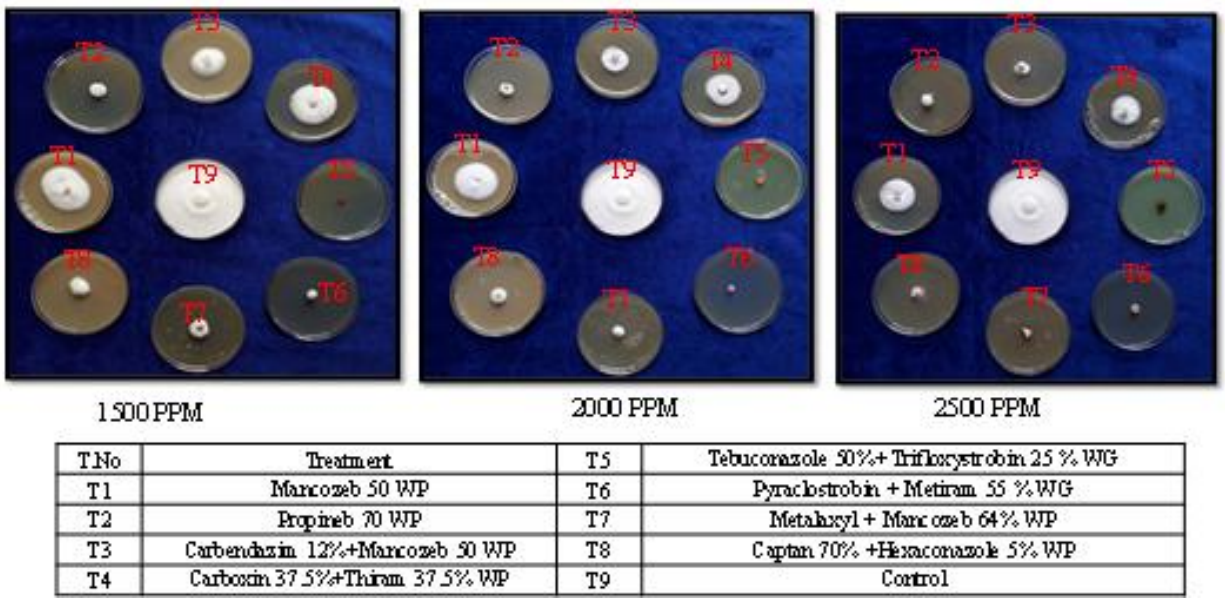

Figure 3 *In vitro* efficacy of Contact / Combi fungicides against mycelial growth and inhibition of *Fusarium oxysporum* f.sp. *melongenae*

At 1500 ppm, radial mycelial growth of *Fusarium oxysporum* f.sp. *melongenae* was ranged from 0.00 to 56.63 mm, as against 90.00 mm in untreated control. However, no mycelial growth was observed with the fungicide Tebuconazole +Trifloxystrobin. It was followed by the fungicides *viz*, Carboxyn + Thiram (5.40), Carbendazim + Mancozeb (13.56), Pyraclostrobin + Metiram (18.89), Captan + Hexaconazole (16.55) , Mancozeb (40.26 mm), Propineb (49.89 mm) and Metalaxyl + Mancozeb (56.63).However, maximum mycelial growth was recorded with Metalaxyl + Mancozeb (56.63).

At 2000 ppm, radial mycelial growth of *Fusarium oxysporum* f.sp. *melongenae* was ranged from 0.00 to 53.33 mm, as against 90.00 mm in untreated control. However, no mycelial growth was observed with the fungicides

Tebuconazole + Trifloxystrobin and Carboxin + Thiram. It was followed by the fungicides *viz*., Carbendazim + Mancozeb (10.00 mm), Captan + Hexaconazole (11.33 mm), Pyraclostrobin + Metiram (13.33 mm), Mancozeb (35.00 mm), Propineb(42.00 mm) and Metalaxil + Mancozeb (53.33 mm).However , maximum mycelial growth was recorded with the fungicide Metalaxil + Mancozeb (53.33 mm).

At 2500 ppm, radial mycelial growth of *Fusarium oxysporum* f.sp. *melongenae* was ranged from 0.00 to 47.00 mm, as against 90.00 mm in untreated control (Table 2, Figures 3 and 4). However, no mycelial growth was observed with the fungicides Tebuconazole + Trifloxystrobin and Carboxin + Thiram. It was followed by the fungicides *viz*, Pyraclostrobin + metiram(7.00 mm), Captan + Hexaconazole (7.80 mm), Carbendazim + Mancozeb (9.67 mm), Mancozeb (21.67 mm), Propineb (37.67 mm) and Metalaxil + Mancozeb (47.00 mm).However ,maximum mycelial growth was recorded with the fungicides Metalaxil + Mancozeb (47.00 mm).

Mean radial mycelial growth of *Fusarium oxysporum* f.sp. *melongenae* was ranged from 0.00 to 52.32 mm, as against 90.00 mm in untreated control (Table 2, Figures 3 and 4). However, no mycelial growth was observed with the fungicides Tebuconazole + Trifloxystrobin. This was followed by the fungicides *viz*, Carboxin + Thiram (1.80 mm), Carbendazim + Mancozeb (11.07 mm), Captan + Hexaconazole (11.89 mm), Pyraclostrobin + Metiram (13.07 mm), Mancozeb (32.31 mm), Propineb (43.19 mm) and Metalaxil + Mancozeb (52.32 mm). However, maximum mycelial growth was recorded with the fungicides Metalaxil + Mancozeb (52.32 mm). All treatments were significantly superior over untreated control.

Mycelial inhibition

Results (Table 2, Figures 3 and 4) revealed that all combi-fungicides tested (each @ 1500, 2000 and 2500 ppm) significantly inhibited mycelial growth of *Fusarium oxysporum* f.sp. *melongenae*, over untreated control. Further, per cent mycelial inhibition was increased with increase in concentrations of the fungicides tested.

Among the contact and combi-fungicides evaluated, Tebuconazole + Trifloxystrobin recorded maximum per cent inhibition of mycelial growth of 100 per cent at all the concentrations tested, followed by Carboxin + Thiram with 94.00, 100.00 and 100 per cent at 1500, 2000 and 2500 ppm concentration respectively with a mean of 98.00 per cent. Carbendazim + Mancozeb showed 84.93,88.88 and 89.25 per cent at 1500, 2000 and 2500 ppm concentration respectively with a mean of 87.70 per cent. Captan + Hexaconazole 81.61, 87.40 and 91.33 per cent at 1500, 2000 and 2500 ppm concentration respectively with a mean of 86.78 per cent. Pyraclostrobin + metiram recorded 79.01, 85.18 and 92.22 per cent at 1500, 2000 and 2500 ppm concentration respectively with a mean of 85.47 per cent. Mancozeb noted 55.27, 61.11 and 75.92 per cent at 1500, 2000 and 2500 ppm concentration respectively with a mean of 64.10 per cent. Propineb showed 44.57, 53.33 and 58.14 per cent at 1500, 2000 and 2500 ppm concentration respectively with a mean of 52.02 per cent. Metalaxil + Mancozeb 37.08, 40.74 and 47.77 per cent at 1500, 2000 and 2500 ppm concentration respectively with a mean of 41.87 per cent. Regarding effect of concentration level (ppm) of fungicides on mycelial growth, it was observed that mycelial growth decreases with increase in concentration level of fungicides.

Thus, all the combi-fungicides tested were found fungistatic against *Fusarium oxysporum* f.sp. *melongenae*. However, on the basis of order of merit combi fungicides Tebuconazole + Trifloxystrobin, Carboxin + Thiram, Carbendazim + Mancozeb, Captan + Hexaconazole, Pyraclostrobin + metiram, Mancozeb, Propineb and Metalaxil + Mancozeb were found effective against test pathogen. The results of systemic fungicides were in agreement with several previous reports of [9] ; [4] ; [13] and [23].Out of all these reports, it was important to understand the activity of a chemical fungicides against the pathogen at various life cycle stages especially on mycelia growth and conidial germination. Carbendazim interferes with energy production and cell wall synthesis of fungi by inducing nuclear instability thereby disturbing the mitosis and meiosis [18] ; [8].The azole group of fungicides (Difenconazole, Hexaconazole Propiconazole and Tebuconazole,) also registered relatively better efficacy against pathogen inhibition by sterol biosynthesis in membrane as specific mode of action [22]. The another effective fungicide Azoxystrobin was structurally a strobilurin group of fungicide also known for its mode of action as a Qo inhibitor(QoI).It inhibit the mitochondrial respiration by binding to the Qo site of the cytochrome enzyme complex [22].

Present findings of combi/contact fungicides were in conformity with [3] who reported that this benzamidazole group of fungicides significantly reduces sporulation in *F. oxysporum*. Similar finding were reported by[5] , [27] and [14] who reported a reduction in sporulation of the *F. oxysporum* isolates with the use of Carbendazim 50%WP and Carbendazim 12% + Mancozeb 63 %WP.The fungitoxic effect of Carbendazim 50% WP and Carbendazim 12%+ Mancozeb 63 % WP was provided by interfering with a number of cellular processes such as mitosis, meiosis, intracellular transport of molecules and interfering in spindle formation in mitosis where as Mancozeb react and inactivates the sulphydal groups of amino acids and enzymes with fungal cells resulting in disruption of lipid metabolism and ATP production [20].

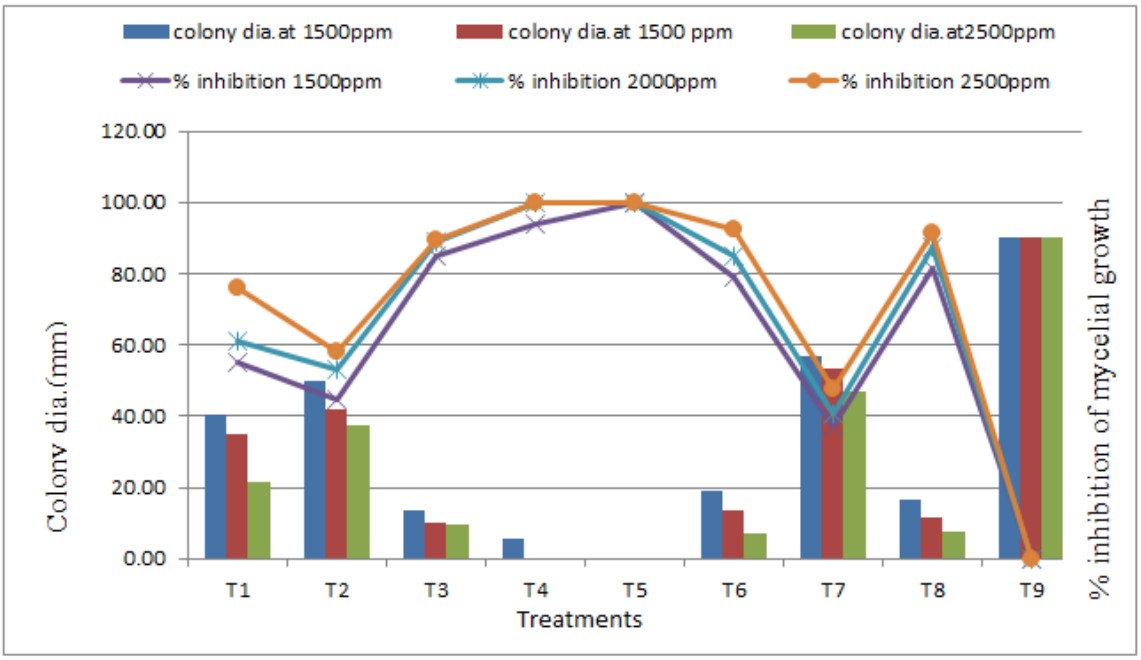

Figure 4 *In vitro* efficacy of contact/combi fungicides against mycelial growth and inhibition of *Fusarium oxysporum* f.sp.*melongenae* in eggplant

Similar results also recorded by [21] who reported complete inhibition of the mycelial growth of *Fusarium solani* with carbendazim, whereas, Captan was least effective against *F. solani* under *in vitro* condition. It was [19] also reported that Carbendazim (1000 ppm) alone and in combination with Thiram inhibited the growth of *Fusarium oxysporum* f.sp. *ciceris* to maximum extent. These two fungicides *viz*., Carbendazim 50% WP and Carbendazim 12% + Mancozeb 63% WP in the present study also caused complete inhibition in sporulation of the test fungi. Suman and Mohan (2016) reported that Tebuconazole + Trifloxystrobin (10.12 %) during *in vitro* studies against *Fusarium* wilt of chickpea. It was [6] also reported that Tebuconazole 50% + Trifloxystobin 25% WG was significantly inhibited fenugreek wilt caused by *Fusarium oxysporum* Schlecht.Whereas, in pot culture carbendazim 12 % + mancozeb 63% WP showed minimum PDI followed by carbendazim 50% WP and Tebuconazole 25.9% EC.

Conclusions

In vitro efficacy of eight systemic fungicides against *F. oxysporum* f.sp.*melongenae* revealed that mean radial mycelial growth of the test pathogen was ranged from 1.00 mm (Carbendazim) to 58.22 mm (Fosetyl-AL) at all the three concentrations (500, 1000 and 1500 ppm) tested. Similarly, among the eight contact/combi-fungicides tested, Tebuconazole +Trifloxystrobin recorded no mycelial growth at 1500 to 2500 ppm.

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