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In vitro evaluation of different fungicides against *Fusarium oxysporum* f.sp. *lycopersici*

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Abstract
Tomato (*Lycopersicon esculentum* Mill) is one of the most widely cultivated food crops. *Fusarium* wilt of tomato is one of the most important disease, which affect all plant stages (seedling stage, flowering stage, and fruiting stage). The fungicides evaluated *in vitro* against *Fusarium oxysporum* f.sp. *lycopersici* were effective and reduced the mycelial growth significantly. Among systemic fungicides Tebuconazole 25%EC (91.96%) and Thiophanate methyl 70%WP (94.44%) were found most effective at concentration of 500 and 1000 ppm respectively. Among non systemic fungicides Copper oxychloride 50%WP (94.44%) were found most effective at both 1500 and 2000 ppm concentration. From combination fungicides Hexaconazole 4% + Zineb 68% WP (94.44%) and Picoxystrobin 7.05% + Propiconazole 11.7% EC (94.44%) were found most effective at 2500 and 3000 ppm respectively.

Keywords: *Fusarium oxysporum* f.sp. *lycopersici*, Fungicides, Tomato, Wilt

Introduction
Tomato (*Lycopersicon esculentum* Mill) is one of the most widely cultivated food crops. It belongs to family *Solanaceae.* Tomato is attacked by many fungal pathogens in the field resulting in losses. *Fusarium* wilt of tomato is one of the most important diseases, which affect all plant stages (seedling stage, flowering stage, and fruiting stage). Also, it can affect the whole plant parts, leaves and stems. It is recognized as a devastating disease in tomato growing areas all over the world (Beckman, 1987; Reantaso *et al*., 2005) [2, 8] and also in different regions of India from severe to moderate (50-60%) (Sherf and Macnab, 2009) [9]. *Fusarium oxysporum* f.sp. *lycopersici* is a soil borne born pathogen in the class Hyphomycetes that causes wilting in tomato as the only host of pathogen (Rai *et al*., 2011) [7]. *Fusarium oxysporum* infects the roots mainly through penetrating wounds and proceeds into and throughout the vascular system, leading to functional collapse, systemic wilting due to xylem clogging and often the death of the infected (wilted) plant (Bowers and Locke., 2000) [3]. *Fusarium* spp. are saprophytes and are able to grow on soil organic matter for a prolonged period. Most infections originate from the population associated with infected tomato debris. Healthy plants can become infected by *F. oxysporum* if the soil in which they are growing is infested with the pathogen (Farr *et al*., 1989) [4].
The first symptoms of the disease are clearing of the veinlets and chlorosis of the leaves. The younger leaves may die in succession and the entire may wilt and die in a course of few days. Browning of the vascular tissue is strong evidence of *Fusarium* wilt (Snyder and Hans, 2003) [10]. 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
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 ℃ for seven days. Observation on radial mycelial 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) [11] and the data obtained were averaged and analysed statistically

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

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

Table 1: *In vitro* evaluation of systemic fungicides

Treatment	Fungicide	Trade Name	Concentration (ppm)
T1	Propiconazole 25%EC	Tilt	500,1000ppm
T2	Hexaconazole 5%EC	Contaf	500,1000ppm
T3	Azoxystrobin 23%EC	Amistar top	500,1000ppm
T4	Tebuconazole 25%EC	Folicure	500,1000ppm
T5	Thiophanate methyl 70% WP	Topsin – M	500,1000ppm
T6	Fosetyl-Al 80%WP	Alliete	500,1000ppm
T7	Myclobutanil 10%WP	Systhane	500,1000ppm
T8	Control		

Table 2: *In vitro* evaluation of Non systemic fungicides

Treatment	Fungicide	Trade Name	Concentration (ppm)
T1	Chlorothaonil 75%WP	Kavach	1500, 2000 ppm
T2	Propineb 70%WP	Antracol	1500, 2000 ppm
T3	Copper oxychloride 50%WP	Biltox	1500, 2000 ppm
T4	Mandipropomide 23.4%SC	Revus	1500, 2000 ppm
T5	Dinocap 48%EC	Karathane	1500, 2000 ppm
T6	Prochloraz	Bekor	1500, 2000 ppm
T7	Fludioxonil	Kocide	1500, 2000 ppm
T8	Control		

Table 3: *In vitro* evaluation of combi fungicides

Treatment	Fungicide	Trade Name	Concentration (ppm)
T1	Azoxystrobin + Difeneconazole 11.4% SC	Amistar	2500, 3000 ppm
T2	Tebuconazole 50% + Trifloxystrobin 25% WG	Natio	2500, 3000 ppm
T3	Hexaconazole 4% + Zineb 68% WP	Avtar	2500, 3000 ppm
T4	Metalaxyl M 4% + Mancozeb 64% WP	Ridomil	2500, 3000 ppm
T5	Pyrochlostrobin 13.3% + Epoxyconazole 5% EC	Opera	2500, 3000 ppm
T6	Tebuconazole 10% + Sulphur 65% SC	Cultio	2500, 3000 ppm
T7	Picoxystrobin 7.05% + Propiconazole 11.7% SC	Galolio	2500, 3000 ppm
T8	Control		

Result and Discussion

In vitro evaluation of systemic fungicides

Evaluation of systemic fungicides against *F. oxysporum* f. sp. *lycopersici* all of the seven systemic fungicides (Table 4) evaluated *in vitro* (each at 500 and 1000 ppm) were found fungistatic and significantly inhibited mycelial growth of *F. oxysporum* f. sp. *lycopersici*, at all two test concentrations, over untreated control. At 500 ppm, per cent mycelial growth inhibition of *F. oxysporum* f.sp. *lycopersici* was ranged from 57.03 per cent (Myclobutanil 10% WP) to 91.96 per cent (Tebuconazole 25%EC). However, fungicide Tebuconazole 25% EC was found best which inhibited 91.96 per cent mycelial growth followed by Propiconazole 25%EC and Thiophanate methyl 70% WP (90.77%) which were at par with each other. These treatments were followed by the fungicides *viz*., Hexaconazole 5% EC (89.44%), Fosetyl – Al 80% WP (59.62%) and Azoxystrobin 23%EC (58.99%). Amongst all tested fungicides Myclobutanil 10%WP was found comparatively less effective with minimum mycelial inhibition of pathogen *i.e.* 57.03 per cent.

At 1000 ppm, per cent mycelial growth inhibition of *Fusarium oxysporum* f.sp. *lycopersici* was ranged from 62.51 per cent (Fosetyl – Al 80%WP) to 94.44 per cent (Thiophanate methyl 70%WP). However, fungicide Thiophanate methyl 70%WP was found best which inhibited 94.44 per cent mycelial growth. These treatments were followed by Tebuconazole 25%EC (93.40%), Propiconazole 25%EC (91.22%), Hexaconazole 5%EC (90.33%), Myclobutanil 10%WP (75.14%) and Azoxystrobin 23%EC (63.88%). Amongst all the fungicides tested, Fosetyl-Al 80%WP was found comparatively less effective with minimum mycelial inhibition of pathogen *i.e.* 62.15 per cent. Thus, all the fungicides tested were found fungistatic against *F. oxysporum* f.sp. *lycopersici* and significantly inhibited its mycelial growth over untreated control.

In vitro evaluation of non systemic fungicides

Evaluation of non systemic fungicides against *F. oxysporum* f. sp. *lycopersici* all of the seven non systemic fungicides (Table 5) evaluated *in vitro* (each at 1500 and 2000 ppm) were found fungistatic and significantly inhibited mycelial growth of *F. oxysporum* f. sp. *lycopersici*, at all two test concentrations, over untreated control. At 1500 ppm, per cent mycelial growth inhibition of *F. oxysporum* f.sp. *lycopersici* was ranged from 55.47 per cent (Fludioxonil) to 94.44 per cent (Copper oxychloride 50%WP). However, fungicide Copper oxychloride 50%WP was found best which inhibited 94.44 per cent mycelial growth followed by Chlorothaonil 75%WP (77.58%). These treatments were followed by the fungicides *viz*., Propineb 70%WP (76.32%), Mandipropomide 23.4%SC (73.88%), Dinocap 48%EC (70.44%) and Prochloraz (66.14%). Amongst all the fungicides tested, Fludioxonil was found comparatively less effective with minimum mycelial inhibition of pathogen *i.e.* 55.47 per cent.

Similar trend in inhibition of the pathogen was observed at concentration of 2000 ppm, where per cent mycelial growth inhibition of *Fusarium oxysporum* f.sp. *lycopersici* was ranged from 68.66 per cent (Fludioxonil) to 94.44 per cent (Copper oxychloride 50%WP). However, fungicide Copper oxychloride 50%WP was found best which inhibited 94.44 per cent mycelial growth followed by Chlorothaonil 75%WP (91.29%). These treatments were followed by the fungicides *viz*., Propineb 70%WP (81.33%), Mandipropomide 23.4%SC (79.25%), Dinocap 48% EC (73.59%) and Prochloraz (68.32%). Amongst all the fungicides tested, Fludioxonil was found comparatively less effective with minimum mycelial inhibition of pathogen *i.e.* 68.66 per cent.

***In vitro* evaluation of combi fungicides**

Evaluation of combi fungicides against *F. oxysporum* f. sp. *lycopersici* all of the seven combi fungicides (Table 6) evaluated *in vitro* (each at 2500 and 3000 ppm) were found fungistatic and significantly inhibited mycelial growth of *F. oxysporum* f. sp. *lycopersici*, at all two test concentrations, over untreated control. At 2500 ppm, per cent mycelial growth inhibition of *F. oxysporum* f.sp. *lycopersici* was ranged from 77.84 per cent (Tebuconazole 10% + Sulphur 65% EC) to 94.44 per cent (Hexaconazole + zineb 68% WP). However, fungicide Hexaconazole + zineb 68% WP was found best which inhibited 94.44 per cent mycelial growth followed by fungicide Pyrochlostrobin 13.3% + Epoxyconazole 5% EC (92.36%). These treatments were followed by the fungicides *viz*., Picoxystrobin 7.05% + Propiconazole 11.7% (91.96%), Azoxystrobin + Difenconazole 11.4% SC (91.44%), Tebuconazole 50% + Trifloxystrobin 25% WG (89.70%) and Metalaxyl M 4% + Mancozeb 64% WP (80.88%). Among all tested fungicides, Tebuconazole 10% + Sulphur 65% EC was found comparatively less effective with minimum mycelial inhibition of pathogen *i.e.* 77.84 per cent.

At 3000 ppm, per cent mycelial growth inhibition of *F. oxysporum* f.sp. *lycopersici* was ranged from 83.18 per cent (Metalaxyl M 4% + Mancozeb 64% WP) to 94.44 per cent (Hexaconazole + Zineb 68% WP and Picoxystrobin 7.05% + Propiconazole 11.7% EC). However, fungicide Hexaconazole + Zineb 68% WP and Picoxystrobin 7.05% + Propiconazole 11.7% were found best which inhibited 94.44 per cent mycelial growth followed by Azoxystrobin + Difenconazole 11.4% SC (94.14%). These treatments were followed by the fungicides *viz*., Tebuconazole 10% + Sulphur 65% EC (92.66%), Tebuconazole 50% + Trifloxystrobin 25% WG (91.76%) and Pyrochlostrobin 13.3% + Epoxyconazole 5% EC (91.29%).

These results are in conformity with earlier findings of Kanuri *et al*., (2019) [6] who reported that copper oxychloride was most effective against *F. oxysporum* f.sp. *lycopersici* causing wilt in tomato. Similarly, Bashir *et al.*, (2017) [1] and Ghante *et al.*, (2019) [5].

The results reveled that all the test fungicides significantly inhibited mycelial growth of *F. oxysporum* f.sp. *lycopersici* over untreated control. However, Tebuconazole 25%EC, Copper oxychloride 50%WP, Hexaconazole 4% + Zineb 68%WP and Picoxystrobin 7.05% + Propiconazole 11.7% EC were found most effective with highest inhibition percentage.

Table 4: *In vitro* evaluation of systemic fungicides against *F. oxysporum* f.sp. *lycopersici*

Tr. No	Treatment	Colony Dia.*(mm)		% Inhibition #	
		500 ppm	1000 ppm	500 ppm	1000 ppm
T1	Propiconazole 25%EC	08.30	07.90	90.77(72.32)	91.22(72.76)
T2	Hexaconazole 5% EC	09.30	08.70	89.44(71.03)	90.33(71.88)
T3	Azoxystrobin 23%EC	36.90	32.50	58.99(50.18)	63.88(53.06)
T4	Tebuconazole 25%EC	70.20	05.90	91.96(73.52)	93.40(75.11)
T5	Thiophanate methyl 70%WP	08.30	05.00	90.77(72.32)	94.44(76.36)
T6	Fosetyl-Al 80%WP	36.33	33.76	59.62(50.55)	62.51(52.24)
T7	Myclobutanil 10%WP	38.66	22.36	57.03(49.04)	75.14(60.09)
T8	Control	90.00	90.00	-	-
SE(m) ±		0.42	0.26	0.46	0.29
C.D (P=0.01)		1.27	0.81	1.41	0.90

Table 5: *In vitro* evaluation of non systemic fungicides against *F. oxysporum* f.sp. *lycopersici*

Tr. No	Treatment	Colony Dia. *(mm)		% Inhibition #	
		1500 ppm	2000 ppm	1500 ppm	2000 ppm
T1	Chlorothaonil 75% WP	20.16	07.80	77.58(61.74)	91.29(72.83)
T2	Propineb 70% WP	21.30	16.80	76.32(60.88)	81.33(64.39)
T3	Copper oxychloride 50% WP	05.00	05.00	94.44(76.36)	94.44(76.36)
T4	Mandipropomide 23.4% SC	23.30	18.66	73.88(59.26)	79.25(62.90)
T5	Dinocap 48% EC	26.63	23.76	70.44(57.04)	73.59(59.07)
T6	Prochloraz	30.46	28.50	66.14(54.41)	68.32(55.75)
T7	Fludioxonil	40.06	28.53	55.47(48.14)	68.66(55.95)
T8	Control	90.00	90.00	-	-
SE(m) ±		1.57	1.38	0.47	0.46
C.D (P=0.01)		4.76	4.19	1.45	1.41

Table 6: *In vitro* evaluation of combi fungicides against *F. oxysporum* f.sp. *lycopersici*

Tr. No	Treatment	Colony Dia. *(mm)		% Inhibition #	
		2500 ppm	3000 ppm	2500 ppm	3000 ppm
T1	Azoxystrobin + Difeneconazole 11.4% SC	07.70	05.26	91.44(72.98)	94.14(75.99)
T2	Tebuconazole 50% + Trifloxystrobin 25% WG	09.20	07.43	89.70(71.28)	91.76(73.29)
T3	Hexaconazole 4% + Zineb 68% WP	05.00	05.00	94.44(76.36)	94.44(76.36)

T4	Metalaxyl M 4% + Mancozeb 64% WP	17.20	15.13	80.88(64.07)	83.18(65.78)
T5	Pyrochlostrobin 13.3% + Epoxyconazole 5% EC	06.80	07.83	92.36(73.96)	91.29(72.83)
T6	Tebuconazole 10% + Sulphur 65% EC	19.93	06.60	77.84(61.92)	92.66(74.28)
T7	Picoxystrobin 7.05% + Propiconazole 11.7% EC	07.23	05.00	91.96(73.52)	94.44(76.36)
T8	Control	90.00	90.00	-	-
SE(m) ±		0.28	0.25	0.31	0.28
C.D (P=0.01)		0.86	0.77	0.96	0.86

*Mean of three replications, Dia. = Diameter

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