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In vitro efficacy of non-systemic and combi fungicides against wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*

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Abstract
Tomato (*Lycopersicon esculentum* Miller) crop suffers from several diseases, among which wilting caused by *Fusarium oxysporum* f. sp. *lycopersici* is one of the serious diseases observed regularly in tomato growing areas. Therefore, efforts were made to evaluate the efficacy of different non-systemic and combi fungicides *in vitro* against *Fusarium oxysporum* f. sp. *lycopersici* by poisoned food technique. Among the Five non-systemic and four combi fungicides tested *in vitro*, against *Fusarium oxysporum* f. sp. *lycopersici*, carbendazim 25% + mancozeb 50% at all three different concentrations (@ 1500, 2000 and 2500 ppm) showed complete (100%) mycelial inhibition, followed by tebuconazole 50% + trifloxystrobin 25% (90.55%), carboxin 37.5% + thiram 37.5% (89.32%), copper hydroxide (50.93%), propineb (33.71%), copper oxychloride (31.61%), metalaxyl 4% + mancozeb 64% (31.36%) and mancozeb (25.68%). The fungicide chlorothalonil was found comparatively less effective with 18.90 per cent inhibition of the test pathogen over untreated control.

Keywords: Tomato, Wilt, *Fusarium oxysporum* f. sp. *lycopersici*, Non-systemic & combi fungicides and Poisoned food technique

Introduction
Tomato (*Lycopersicon esculentum* M.) 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) [4]. 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 (Anonymous, 2017) [1]. 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. *lycopersic*i is the most devastating fungal disease. Joshi *et al.* (2013) [5] 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 and combi fungicides *in vitro* condition against *Fusarium oxysporum* f. sp. *lycopersici* causing wilt in tomato.

Material and Methods
Five non-systemic and four combi fungicides (each @ 1500, 2000 and 2500 ppm) were evaluated *in vitro* against *F. oxysporum* f. sp. *lycopersici*, using Potato dextrose agar as basal culture medium and applying Poisoned food technique (Nene and Thapliyal, 1993). The experiment was conducted in Completely Randomized Design (CRD) with three replications of each treatment. Five non-systemic fungicides namely: Mancozeb 75 WP, Copper oxychloride 50 WP, Propineb 70 WP, Chlorothalonil 75 WP, Copper hydroxide 77 WP and Four combi fungicides namely: Carboxin 37.5% + thiram 37.5%, Metalaxyl 4% + mancozeb 64%, Tebuconazole 50% + trifloxystrobin 25% and Carbendazim 25% + mancozeb 50% were tested each at three concentrations (each @ 1500, 2000 and 2500 ppm).
Based on active ingredient, requisite quantity of the test fungicides was calculated, dispensed separately and mixed thoroughly with autoclaved and cooled (40 ^{0}C) PDA medium in glass

conical flasks (250 ml capacity) to obtain desired concentrations. This PDA medium amended separately with the test fungicides was then poured (20 ml/plate) aseptically in sterile glass Petri-plates (90 mm dia.) and allowed to solidify at room temperature. For each of the test fungicide and its test concentration, three plates / treatment / replication were maintained. After solidification of the PDA medium, all these plates were inoculated aseptically by placing in the centre a 5 mm culture disc obtained from actively growing 7 days old pure culture of *F. oxysporum* f. sp. *lycopersici* test isolate and incubated in an inverted position at 28±2^{0}C. Petri-plates filled with plain PDA (without any fungicide) and inoculated with pure culture disc of the test isolate was maintained as untreated control. Observations on radial mycelial growth / colony diameter of the test pathogen was recorded at 24 hrs interval and continued till growth of the test pathogen in untreated control plate was fully covered. Per cent inhibition of the test pathogen was calculated by applying following formula (Vincent, 1927).

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

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

Result and Discussion

In vitro evaluation of non-systemic and combi fungicides against *F. oxysporum* f. sp. *lycopersici* was done as described in material and methods by Poisoned food technique. The systemic fungicides were tested at 1500, 2000 and 2500 ppm concentrations each and the observations on colony diameter and per cent inhibition of colony growth over control are presented in Table 1.

Radial mycelial growth

Five non-systemic and four combi fungicides belonging to different groups were tested for their efficacy against *Fusarium oxysporum* f. sp. *lycopersici* (@ 1500, 2000 and 2500 ppm) by employing Poisoned food technique. Results (Table 1 Plate I & Fig. 1.a) revealed that all the Non- systemic fungicides tested exhibited a wide range of radial mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* and it was found to be decreased drastically with increase in the concentration of the fungicides tested.

At 1500 ppm, radial mycelial growth of the test pathogen ranged from 0.00 mm carbendazim 25% + mancozeb 50% to 80.83 mm chlorothalonil. Significantly least radial mycelial growth was recorded with carbendazim 25% + mancozeb 50% (0.00 mm), which was followed by tebuconazole 50% + trifloxystrobin 25% (10.33 mm), carboxin 37.5% + thiram 37.5% (11.83 mm), copper hydroxide (46.50 mm), propineb (65.33 mm), copper oxychloride (66.33 mm), metalaxyl 4% + mancozeb 64% (71.33 mm), mancozeb (72.83 mm) and chlorothalonil (80.83 mm) as compared to highest growth (90 mm) in untreated control.

At 2000 ppm, all the non-systemic and combi fungicides tested exhibited similar trend of mycelial growth as that of at 1500 ppm and ranged from 0.00 mm carbendazim 25% + mancozeb 50% to 71.50 mm (chlorothalonil). Significantly least radial mycelial growth was recorded with carbendazim 25% + mancozeb 50% (0.00 mm), which was followed by tebuconazole 25% + trifloxystrobin 50% (8.50 mm), carboxin 37.5% + thiram 37.5% (9.50 mm), copper hydroxide (44.16 mm), propineb (59.50 mm), copper oxychloride (61.33 mm), metalaxyl 4% + mancozeb 64% (62.16 mm), mancozeb (67.66 mm) and chlorothalonil (71.50 mm) as compared to highest growth (90 mm) in untreated control.

At 2500 ppm, all the non-systemic and combi fungicides tested exhibited similar trend of mycelial growth as that of at 1500 ppm and 2000 ppm and ranged from 0.00 mm (carbendazim 25% + mancozeb 50%) to 66.66 mm (chlorothalonil). Significantly least radial mycelial growth was recorded with carbendazim 25% + mancozeb 50% (0.00 mm) which was followed by tebuconazole 50% + trifloxystrobin 25% (7.00 mm), carboxin 37.5%+ thiram 37.5% (7.50 mm), copper hydroxide (41.83 mm), propineb (54.16 mm), copper oxychloride (57.00 mm), metalaxyl 4% + mancozeb 64% (51.83 mm), mancozeb (60.16 mm) and chlorothalonil (66.66 mm) as compared to highest growth (90 mm) in untreated control.

Average radial mycelial growth recorded with all the fungicides tested was ranged from 0.00 mm carbendazim 25% + mancozeb 50% to 72.99 mm (chlorothalonil). Highest average radial mycelial growth was recorded with chlorothalonil (72.99 mm), which was followed by mancozeb (66.88 mm), metalaxyl 4% + mancozeb 64% (61.77 mm), copper oxychloride (61.55 mm), propineb (59.16 mm), copper hydroxide (44.16 mm), carboxin 37.5% + thiram 37.5% (9.61 mm), tebuconazole 50% + trifloxystrobin 25% (8.61 mm), and carbendazim 25% + mancozeb 50% (0.00 mm). The average radial mycelial growth in control was 90.00 mm.

Mycelial inhibition

Results (Table 1, Plate 1 & Fig. 1) revealed that all the non-systemic fungicides tested (@ 1500, 2000 and 2500 ppm each) inhibited mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* over untreated control. The per cent mycelial inhibition of the test pathogen was increased with the increase in concentration of the fungicides tested.

At 1500 ppm, percent mycelial growth inhibition ranged from 10.18 per cent (chlorothalonil) to 100 per cent (carbendazim 25% + mancozeb 50%). Significantly highest mycelial inhibition was recorded with the carbendazim 25% + mancozeb 50% (100%), which was followed by tebuconazole 50% + trifloxystrobin 25% (88.52%), carboxin 37.5% + thiram 37.5% (86.85%), copper hydroxide (48.33%), propineb (27.41%), copper oxychloride (26.30%), metalaxyl 4% + mancozeb 64% (20.74%), mancozeb (19.07%). The fungicide chlorothalonil was found comparatively less effective with 10.18 per cent inhibition of the test pathogen over untreated control.

At 2000 and 2500 ppm, similar trend of mycelial growth inhibition as that of at 1500 ppm was observed and it was ranged from 20.55 per cent (chlorothalonil) to 100 per cent (carbendazim 25% + mancozeb 50%) and 25.93 per cent (chlorothalonil) to 100 per cent (carbendazim25% + mancozeb 50%), respectively. Treatment 6 (carboxin 37.5% + thiram 37.5%) and Treatment 8 (tebuconazole 50% + trifloxystrobin 25%) were found statistically at par at both 2000 ppm and 2500 ppm concentration, respectively.

Average mycelial inhibition with all the non-systemic and combi fungicides tested ranged from 18.90 per cent (chlorothalonil) to 100 per cent (carbendazim 25% + mancozeb 50%). Highest average mycelial inhibition was recorded with the carbendazim 25% + mancozeb 50% (100%) followed by tebuconazole 50%+ trifloxystrobin 25% (90.43%), carboxin 37.5% + thiram 37.5% (89.32%), copper hydroxide (50.93%), propineb (33.71%), copper oxychloride

(31.61%), metalaxyl 4% + mancozeb 64% (31.36%) and mancozeb (25.68%). The fungicide chlorothalonil was found comparatively less effective with 18.90 per cent inhibition of the test pathogen over untreated control.

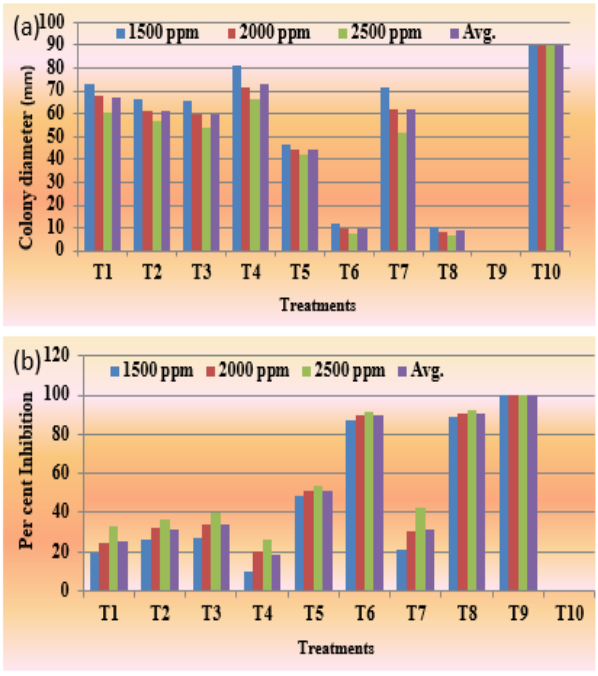
Similar fungistatic effects of non-systemic and combi fungicides against *F. oxysporum* f. sp. *lycopersici* were reported earlier by several workers.

Barhate *et al*. (2015) [2] studied eight fungicides against *F. oxysporum* f. sp. *lycopersici.* Among the eight fungicides mancozeb + carbendazim had completely checked the growth of the pathogen which inhibited 100 per cent growth of *F. oxysporum* f. sp. *lycopersici.* Bashir *et al*. (2017) [3] studied five non- systemic fungicides *viz*., captan 50 WP, copper oxychloride 50 WP, dodine 65 WP, mancozeb 75 WP, antracol 70 WP and five systemic fungicides *viz*., bitertanol 25 WP, carbendazim 50 WP, difenconozole 25 EC, hexaconozole 5 EC, and myclobutanil 10 WP against chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceris*. The *in-vitro* evaluation of non-systemic fungicides through poisoned food technique at five different concentrations *viz*., 50, 100, 250, 500 and 1000 µg ml-[1] indicated that two fungicides *viz*., dodine and captan proved the most effective. Singh and Jha (2003) [6] reported thiram and bavistin as the most suitable fungicides inhibiting the mycelial growth of *F. oxysporum* f. sp. *ciceri*.

Table 1: *In vitro* efficacy of non-systemic and combi fungicides against *Fusarium oxysporum* f. sp. *Lycopersici*

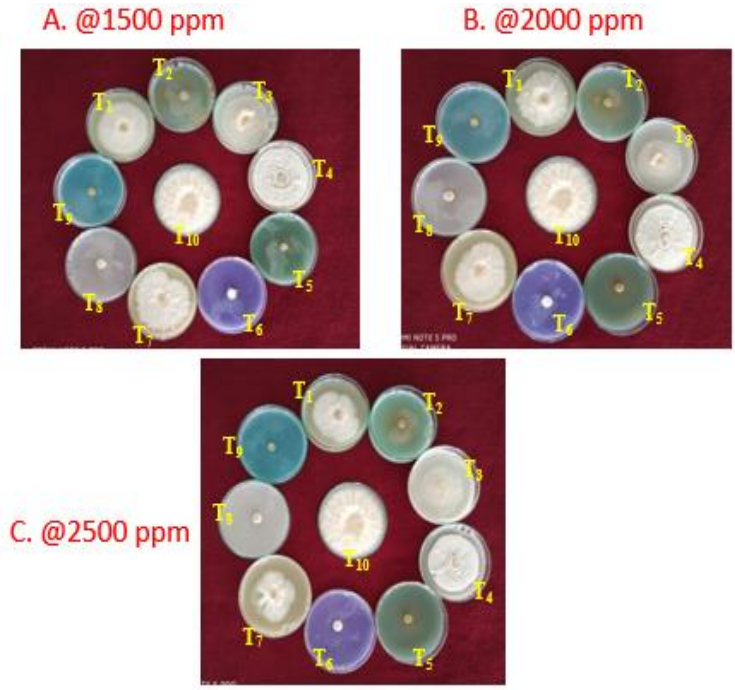
Treatment	Colony diameter (mm)*			Avg.	Per cent Inhibition *			Avg. % Inhibi.
	1500 ppm	2000 ppm	2500 ppm		1500 ppm	2000 ppm	2500 ppm	
Non-systemic fungicides								
T1 Mancozeb 75% WP	72.83	67.66	60.16	66.88	19.07 (25.89)	24.82 (29.88)	33.15 (35.15)	25.68 (30.44)
T2 Copper oxychloride 50% WP	66.33	61.33	57.00	61.55	26.30 (30.85)	31.85 (34.35)	36.66 (37.26)	31.61 (34.20)
T3 Propineb 70% WP	65.33	59.50	54.16	59.66	27.41 (31.57)	33.88 (35.59)	39.82 (39.12	33.71 (35.49)
T4 Chlorothalonil 75% WP	80.83	71.50	66.66	72.99	10.18 (18.60)	20.55 (26.95)	25.93 (30.61)	18.90 (25.76)
T5 Copper hydroxide 77 WP	46.50	44.16	41.83	44.16	48.33 (44.04)	50.93 (45.53)	53.52 (47.01)	50.93 (45.30)
Combi- fungicides								
T6 Carboxin 37.5% + thiram 37.5%	11.83	9.50	7.50	9.61	86.85 (68.73)	89.44 (71.03)	91.66 (73.21)	89.32 (70.92)
T7 Metalaxyl 4% + mancozeb 64%	71.33	62.16	51.83	61.77	20.74 (27.09)	30.93 (33.78)	42.41 (40.63)	31.36 (34.05)
T8 Tebuconazole 50% + trifloxystrobin 25%	10.33	8.50	7.00	8.61	88.52 (70.19)	90.55 (72.09)	92.22 (73.80)	90.43 (71.97)
T9 Carbendazim 25% + mancozeb 50%	0.00	0.00	0.00	0.00	100 (90)	100 (90)	100 (90)	100 (90)
T10 Control	90.00	90.00	90.00	90	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.E.+	0.60	0.59	0.38		0.59	0.54	0.22	
C.D (P=0.01)	1.98.	1.94	1.27		1.95	1.80	0.73	

* = Mean of three replications. Figures in parenthesis are arcsine transformed values.



Tr. No	Treatments	Tr. No.	Treatments
T_1	Mancozeb 75% WP	T_6	Carboxin 37.5% + thiram 37.5% (75% WP)
T_2	Copperoxy chloride 50%WP	T_7	Metalaxyl 4 % + mancozeb 64% (68% WG)
T_3	Propineb 70% WP	T_8	Tebuconazole 50% +trifloxystrobin 25% (75%WG)
T_4	Chlorothalonil 75% WP	T_9	Carbendazim 25% + mancozeb 50% (75%WP)
T_5	Copper hydroxide 77%WP	T_{10}	Control (Untreated)

Fig 1: *In vitro* efficacy of non-systemic and combi fungicides on mycelial growth and inhibition *of F. oxysporum* f. sp. *lycopersici*



Tr. No	Treatments	Tr. No.	Treatments
T_1	Mancozeb 75% WP	T_6	Carboxin 37.5% + Thiram 37.5% (75% WP)
T_2	Copperoxy chloride 50% WP	T_7	Metalaxyl 4 % + Mancozeb 64% (68% WG)
T_3	Propineb 70% WP	T_8	Tebuconazole 50% + Trifloxystrobin 25% (75% WG)
T_4	Chlorothalonil 75% WP	T_9	Carbendazim 25% + Mancozeb 50% (75% WP)
T_5	Copper hydroxide 77% WP	T_{10}	Control (Untreated)

Plate 1: *In vitro* effect of non-systemic and combi fungicides at various concentrations against *F. oxysporum* f. sp. *lycopersici*

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