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In vitro efficacy of systemic 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 systemic fungicides *in vitro* against *Fusarium oxysporum* f. sp. *lycopersici* by poisoned food technique. Among the seven systemic fungicides tested *in vitro*, against *Fusarium oxysporum* f. sp. *lycopersici*, carbendazim and hexaconazole at all three different concentrations (@ 500, 1000 and 1500 ppm) showed complete (100%) mycelial inhibition, followed by tebuconazole (95.06%), difenconazole (92.65%), propiconazole (89.94%) and thiophanate methyl (62.28%), respectively. The pyraclostrobin was found less effective with 44.93% mycelial inhibition over untreated control.

Keywords: Tomato, wilt, *Fusarium oxysporum* f. sp. *lycopersici*, systemic 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) [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 (Anonymous, 2017) [2]. 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) [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 systemic fungicides *in vitro* condition against *F. oxysporum* f. sp. *lycopersici* causing wilt in tomato.

Material and Methods
Seven systemic fungicides (each @ 500, 1000 and 1500 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. Seven systemic fungicides namely: Carbendazim 50 WP, Difenconazole 25 EC, Hexaconazole 5 EC, Propiconazole 25EC, Tebuconazole 25.9 EC, Pyraclostrobin 20 WG and Thiophante methyl 70WP were tested each at three concentrations (each @ 500, 1000 and 1500 ppm). Based on active ingredient, requisite quantity of the test fungicides was calculated, dispensed separately and mixed thoroughly with autoclaved and cooled (40^0C) 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 systemic 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 500, 1000 and 1500 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

Seven systemic fungicides belonging to different groups were tested for their efficacy against *Fusarium oxysporum* f. sp. *lycopersici* (@ 500, 1000 and 1500 ppm) by employing Poisoned food technique. Results (Table 1, PLATE I & Fig.1.a) revealed that all the systemic fungicides tested exhibited a wide range of radial mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* and the mycelial growth was found to be decreased drastically with increase in the concentrations of the fungicides tested.

At 500 ppm, radial mycelial growth of the test pathogen ranged from 0.00 mm (carbendazim and hexaconazole) to 51.83 mm (pyraclostrobin). Significantly least mycelial growth was recorded with the carbendazim and hexaconazole (0.00 mm) followed tebuconazole (7.83 mm), difenconazole (8.16 mm), propiconazole (11.16 mm), thiophanate methyle (37.16 mm) and pyraclostrobin (51.83 mm) as compared to highest growth (90 mm) in untreated control.

At 1000 ppm, all the systemic fungicides tested exhibited similar trend of mycelial growth as that of at 500 ppm concentration and ranged from 0.00 mm (carbendazim and hexaconazole) to 50.33 mm (pyraclostrobin). Significantly least mycelial growth was recorded with the Treatment carbendazim and hexaconazole (0.00 mm) followed by tebuconazole (5.50 mm), difenconazole (6.50 mm), propiconazole (9.00 mm), thiophanate methyl (35.16 mm) and pyraclostrobin (50.33 mm) as compared to highest growth (90 mm) in untreated control.

At 1500 ppm, all the systemic fungicides tested exhibited somewhat similar trend of mycelial growth as that of at 500 ppm and 1000 ppm and it ranged from 0.00 mm (carbendazim, hexaconazole and tebuconazole) to 48.33 mm (pyraclostrobin). Significantly least mycelial growth was recorded with the Treatment carbendazim, hexaconazole and tebuconazole (0.00 mm) followed by difenconazole (5.16 mm), propiconazole (7.00 mm), thiophanate methyl (29.50 mm) and pyraclostrobin (48.33 mm) as compared to highest growth (90 mm) in untreated control.

Average radial mycelial growth recorded with all the fungicides tested at 500, 1000, 1500 ppm ranged from 0.00 mm (carbendazim and hexaconazole) to 50.16 mm (pyraclostrobin). Highest average radial mycelial growth was recorded with pyraclostrobin (50.16 mm), followed by thiophanate methyl (33.94 mm), propiconazole (9.05 mm), difenconazole (6.60 mm) and tebuconazole (4.44 mm). The least mean radial mycelial growth was recorded with carbendazim and hexaconazole (0.00 mm).

Mycelial inhibition

Results (Table 1, PLATE 1 & Fig. 1) revealed that all the systemic fungicides tested (@ 500, 1000 and 1500 ppm each) inhibited mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* over untreated control (0.00%). Further, the per cent mycelial inhibition of the test pathogen increased with the increase in concentrations of the fungicides tested.

At 500 ppm, per cent mycelial growth inhibition was recorded in the range of 42.41 per cent (pyraclostrobin) to 100 per cent (carbendazim and hexaconazole). The highest mycelial inhibition was recorded with carbendazim and hexaconazole (100%), followed by tebuconazole (91.30%) difenconazole (90.93%), propiconazole (87.60%), thiophanate methyl (58.71%), and pyraclostrobin (42.41%). The fungicide pyraclostrobin was found less effective with 42.41 per cent inhibition of the test pathogen over untreated control

At 1000 and1500 ppm, similar trend of mycelial growth inhibition was observed and it was ranged from 44.07 (pyraclostrobin) per cent to 100 per cent (carbendazim and hexaconazole) and 46.30 per cent (pyraclostrobin) to 100 per cent (carbendazim and hexaconazole) over untreated control, respectively.

Average mycelial inhibition with all the systemic fungicides tested ranged from 44.93 per cent (pyraclostrobin) to 100 per cent (carbendazim and hexaconazole) over untreated control. The fungicide pyraclostrobin was found comparatively less effective with 44.93 per cent mycelial growth inhibition of the test pathogen over untreated control.

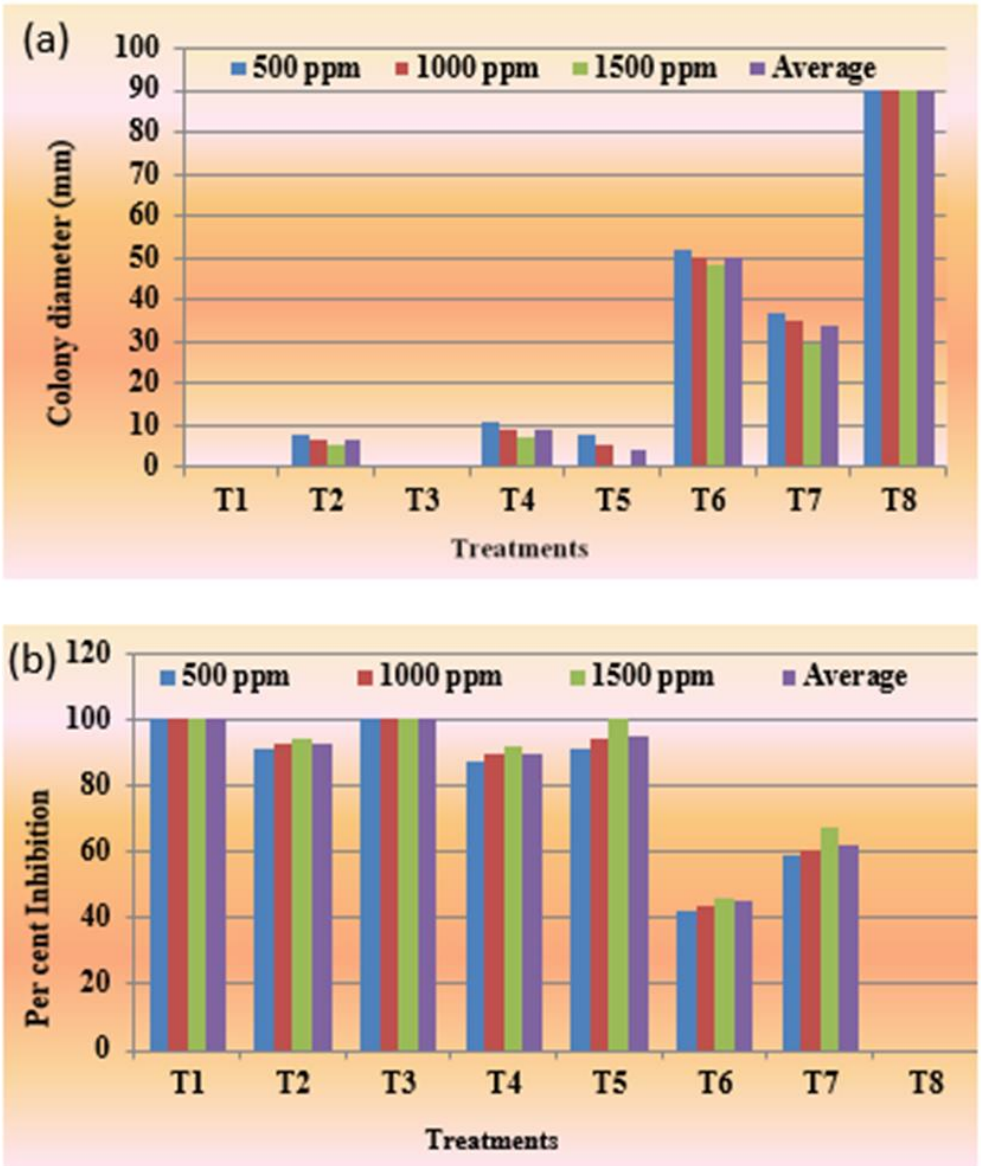
Thus, all the systemic fungicides tested were found fungistatic against *Fusarium oxysporum* f. sp. *lycopersici* and inhibited its mycelial growth over untreated control. Systemic fungicides found most effective in the order of merit were carbendazim at par with hexaconazole, followed by tebuconazole, difenconazole propiconazole, thiophanate methyl and pyraclostrobin.

Similar fungistatic effect of systemic fungicides against *F. oxysporum* f. sp. *lycopersici* was reported earlier by several workers. Amini and Sidovich (2010) and Sreenu and Zacharia (2017) [1, 6] revealed that the least growth of pathogen was recorded in carbendazim (treated control) (00 mm). There was no growth in carbendazim treatment in poisoned food techniques. Rather *et al*. (2012) [5] evaluated four different fungicides *in vitro* namely capton 0.3%, carbendazim 0.2%, metalaxil 0.2% and carboxin 0.2% (each @ 50,100,150, and 250 ppm) against three wilt pathogens of bell paper that is *F. oxysporum* f. sp. *capsici*, *R. solani* and *S. rolfsii* carbedazim and capton (each @ 50 and 500 ppm) completely inhibited the growth *F. oxysporum.*

Table 1: *In vitro* efficacy of systemic fungicides against *Fusarium oxysporum* f. sp. *lycopersici.*

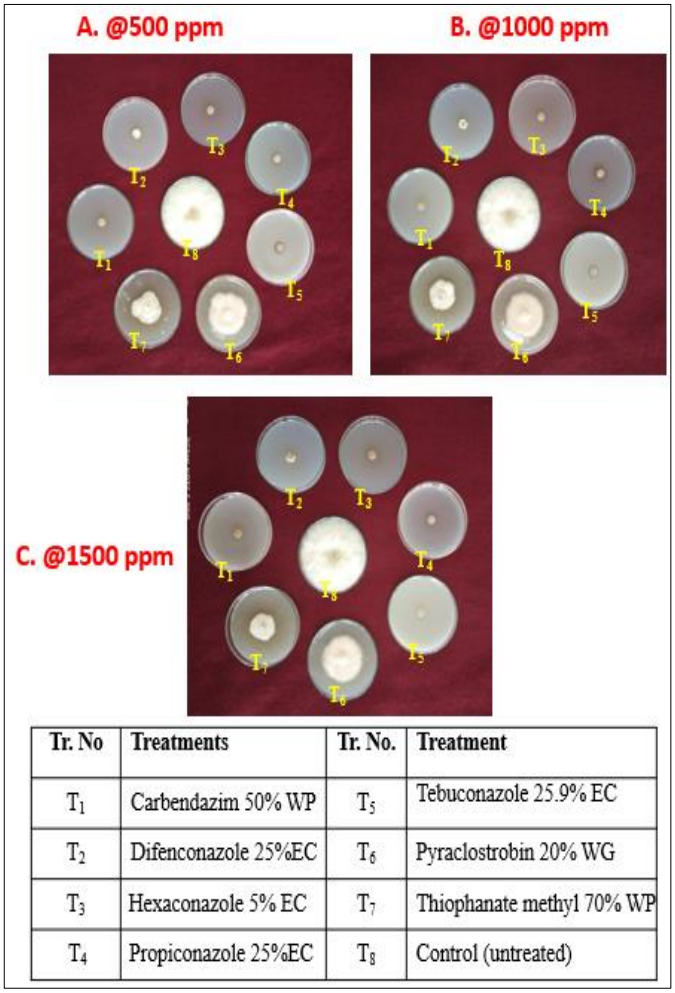
Treatment	Colony diameter (mm)*			Avg. Col. Dia.	Per cent Inhibition *			Avg. % Inhibi.
	500 ppm	1000 ppm	1500 ppm		500 ppm	1000 ppm	1500 ppm	
T1 Carbendazim 50% WP	0.00	0.00	0.00	0.00	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T2 Difenconazole 25% EC	8.16	6.50	5.16	6.60	90.93 (72.47)	92.77 (74.40)	94.26 (76.13)	92.65 (74.26)
T3 Hexaconazole 5% EC	0.00	0.00	0.00	0.00	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T4 Propiconazole 25%EC	11.16	9.00	7.00	9.05	87.60 (69.38)	90.00 (71.56)	92.22 (73.80)	89.94 (71.50)
T5 Tebuconazole 25.9 EC	7.83	5.50	0.00	4.44	91.30 (72.84)	93.88 (75.67)	100 (90.00)	95.06 (77.15)
T6 Pyraclostrobin 20WG	51.83	50.33	48.33	50.16	42.41 (40.63)	44.07 (41.59)	46.30 (42.87)	44.93 (42.09)
T7 Thiophanate Methyl70WP	37.16	35.16	29.50	33.94	58.71 (50.01)	60.93 (51.31)	67.22 (55.07)	62.28 (52.10)
T8 Control	90	90	90	90	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
S.E.+	0.28	0.50	0.41		0.40	0.57	0.59	
C.D (P=0.01)	0.84	1.46	1.20		1.19	1.68	1.73	

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



Tr. No	Treatments	Tr. No.	Treatment
T_1	Carbendazim 50% WP	T_5	Tebuconazole 25.9% EC
T_2	Difenconazole 25%EC	T_6	Pyraclostrobin 20% WG
T_3	Hexaconazole 5% EC	T_7	Thiophanate methyl 70% WP
T_4	Propiconazole 25%EC	T_8	Control (untreated)

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



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T_1	Carbendazim 50% WP	T_5	Tebuconazole 25.9% EC
T_2	Difenconazole 25%EC	T_6	Pyraclostrobin 20% WG
T_3	Hexaconazole 5% EC	T_7	Thiophanate methyl 70% WP
T_4	Propiconazole 25%EC	T_8	Control (untreated)

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

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