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In vitro evaluation of the chemical fungicides against *Fusarium oxysporum* f. sp. *lycopersici* causal organism of fusarium wilt of tomato

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Abstract
Tomato is the third important vegetable crop grown in India, after potato and onion with an area of 0.78 M ha and production and productivity of 19,759 Mt and 25.04 Mt ha^{-1} respectively. Tomato crop is very often affected by several diseases incited by pathogens such as fungi, bacteria, viruses and nematodes. Among all the fungal diseases that infect tomato, Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (*Fol)*, is one of the most serious and destructive diseases across the world causing severe economic losses, wherever tomato is grown. Due to prolonged survival of *Fol* in the soil as saprophyte and overcoming the challenging conditions by producing resistant structures, it has become very difficult to manage the disease. The most feasible method for managing Fusarium wilt disease of tomato is by usage of effective fungicides. Ten fungicides *viz*., Copper oxychloride 50%WP, Mancozeb 75% WP, Carbendazim 12% + Mancozeb 63% WP, Metalaxyl 4% + Mancozeb 64% WP, Hexaconzole 5% + Captan 70% WP, Thiophanate Methyl 70% WP, Thiophanate Methyl 45% + Pyraclostrobin 5% FS, Tebuconazole 250 E.C, Tebuconazole 50% + Trifloxystrobin 25% WP and Azoxystrobin 23% SC were evaluated against *Fol* under *in vitro* conditions at three concentrations *i.e.* 500, 1000 and 1500 ppm. Three replications were maintained for each treatment with control. Tebuconazole 50% + Trifloxystrobin 25% WP was found to be the most effective fungicide followed by Tebuconazole 250 E.C with high fungitoxicity against *Fol*. Azoxystrobin 23% SC was the least effective. The results of this present investigation will be helpful for future research on fungicide management for Fusarium wilt of tomato.

Keywords: Tomato, fusarium wilt, chemical fungicides

Introduction
Tomato is cultivated extensively in many countries under both protected and open field conditions. India ranks second to China with an area 0.789 M ha, with production 21.24 Mt and productivity of 25 Mt ha^{-1} respectively followed by USA, Turkey and Egypt (Horticultural statistics at a Glance, 2018). Tomato crop is very often affected by several diseases incited by pathogens such as fungi, bacteria, viruses and nematodes (Mardi *et al*., 2002) [4]. Among all the fungal diseases that infect tomato, Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* is one of the most serious and destructive diseases across the world (Sheu and Wang, 2006) [8] causing severe economic losses, wherever tomato is grown (Sudhamoy *et. al*., 2009) [9]. Due to prolonged survival of *Fol* in the soil as saprophyte and overcoming the challenging conditions by producing resistant structures, it has become very difficult to manage the disease. Some workers were success with using different chemicals and fungicides against this Fusarium wilt. *In vitro* studies conducted reveals that some fungicides restrict or prevent the growth of *Fol*. Fungicide carbendazim is being recommended and there is still a need to identify a effective fungicide to control *Fol* of tomato. The aim of the study was to determine under *in vitro* effects of chemical fungicides on the mycelial growth of *Fol*, the causal organism of fusarium wilt of tomato.

Materials and Methods
Isolation and maintenance of the pathogen
Diseased tomato plant samples were brought to laboratory, washed under running tap water to

remove adhered soil particles, surface sterilized with 1 per cent NaOCl (sodium hypochlorite) solution for 1 to 2 minutes followed by rinsing twice with sterile distilled water, then dried between sterile filter papers for 10 to 15 minutes. Infected and discolored stem portions were cut into small pieces with sterilized knife and again washed with distilled water followed by disinfection for 2 minutes with 2.5 per cent sodium hypo chlorite solution. They were again washed thrice with distilled water to remove residues of sodium hypo chlorite and then transferred aseptically under laminar air flow system on to sterilized Petri plates containing PDA medium. The plates were incubated at room temperature at 27 + 2^0 C for 10 days for development of typical mycelial growth of *Fol*. The cultures were further purified by single spore isolation method (Rangaswami, 1958) [7].

Identification of the pathogen

The isolated fungus cultures associated with wilt diseased specimens were identified based on cultural and morphological characters (micro and macro conidial characters and mycelia colors) with the help of monograph: The *Fusarium* (Booth, 1971; Nelson *et al*., 1983) [2, 6].

Screening of chemical fungicides against *F.o* f.sp. *lycopersici* under *in vitro* conditions

The laboratory experiment was conducted at Horticultural Research Station, Kovvur during 2018-19. The study was conducted in a Complete Randomized Design (CRD) with ten treatments and three replications. Ten different fungicides (Table 1) such as Copper oxychloride 50% WP, Mancozeb 75% WP, Carbendazim 12% + Mancozeb 63% WP, Metalaxyl 4% + Mancozeb 64% WP, Hexaconzole 5% + Captan 70% WP, Thiophanate Methyl 70% WP, Thiophanate Methyl 45% + Pyraclostrobin 5% FS, Tebuconazole 250 E.C, Tebuconazole 50% + Trifloxystrobin 25% WP and Azoxystrobin 23% SC were screened against the *Fol* under laboratory conditions to find out their efficacy in inhibiting the mycelial growth of the pathogen in culture media by the "Poisoned food technique" (Grover and Moore, 1962) [3] at 500,1000 and 1500 ppm concentrations respectively. PDA medium was prepared and amended with different concentrations of the fungicides. About 20 ml of sterilized culture medium was poured in each 9 cm petri dish. After solidification, the plates were inoculated with a 5 mm disk of seven days-old *Fol* culture. Three replications were maintained for each concentration and radial growth was recorded. The Petri plates were incubated at 27± 2^0C. One set of control was maintained without adding any fungicide to the medium. The observations were recorded until the control plate was full of growth of the pathogen. The data of radial growth of fungal colony was measured in millimeters. The per cent mycelia inhibition over control was calculated by the following formula (Vincent, 1972) [11].

$$I = \frac{C - T}{C} \times 100$$

Where

I = Per cent reduction in growth of the test pathogen
C = Radial growth of test pathogen (mm) in control
T = Radial growth of test pathogen (mm) in treatment

Table 1: Details of fungicides employed in the present investigation

S. No	Name of the fungicide	Trade name (Company Name)	Mode of action
1	Copper oxychloride 50%WP	Blitox (Tata Rallies)	Contact
2	Mancozeb 75% WP	Dithane M-45 (UPL)	Contact
3	Carbendazim 12% + Mancozeb 63% WP	Saaf (UPL)	Systemic + contact
4	Metalaxyl 4% + Mancozeb 64% WP	Ridomilgold (KrushiKendra)	Systemic + contact
5	Hexaconzole 5% + Captan 70% WP	Taqat (Tata Rallis)	Systemic + contact
6	Thiophanate Methyl 70% WP	Topsin M WSB (UPL)	Systemic
7	Thiophanate Methyl 45% + Pyraclostrobin 5% FS	Xelero (BASF)	Systemic
8	Tebuconazole 250 E.C	Folicur (Bayer)	Systemic
9	Tebuconazole 50% + Trifloxystrobin 25% WP	Nativo (Bayer)	Systemic
10	Azoxystrobin 23% SC	Amistar (Syngenta)	Systemic

*WP - Wettable Powder; EC- Emulsifiable Concentrate; SC- Suspension Concentrate; WG- Wettable Granules

Results and Discussion

The efficacy of ten fungicides was tested under *in vitro* by poisoned food technique and the results are presented in Table 2 and 3. Most of the fungicides were effective in inhibiting the mycelia growth of the *Fol* pathogen to varying degrees. Significant difference in inhibiting the mycelial growth of the pathogen among the fungicides was observed.

Table 2: Radial colony growth along with % inhibition of *F.* of. sp. *lycopersici* with fungicides

S. No	Fungicide	*500 ppm		* 1000 ppm		* 1500 ppm	
		Radial growth (mm)	Growth inhibition (%)	Radial growth (mm)	Growth inhibition (%)	Radial growth (mm)	Growth inhibition (%)
1	Copper oxychloride 50%WP	35.00 (36.75)	61.10	27.20 (31.59)	69.77	19.00 (25.71)	78.80
2	Mancozeb 75% WP	27.50 (31.78)	69.44	20.00 (26.50)	77.70	15.90 (23.46)	82.33
3	carbendazim 12% + mancozeb 63% WP	24.80 (29.87)	72.44	15.10 (22.80)	83.22	00.00 (0.00)	100.00
4	metalaxyl 4% + mancozeb 64% WP	29.20 (32.80)	67.50	26.00 (30.62)	71.10	18.20 (25.48)	79.77
5	hexaconzole 5% + captan 70% WP	27.00 (31.33)	69.70	19.10 (26.00)	78.77	13.00 (21.01)	85.55
6	thiophanate methyl 70% WP	29.70 (33.07)	67.00	23.10 (28.87)	74.33	18.90 (25.80)	79.00
7	thiophanate methyl 45% + pyraclostrobin 5%	28.10 (32.16)	68.77	26.90 (31.22)	70.11	24.10 (29.30)	73.22
8	tebuconzole 250 E.C	18.60 (25.63)	79.33	12.00 (20.28)	86.66	00.00 (0.00)	100.00
9	tebuconzole 50% + trifloxystrobin 25% WP	12.00 (20.11)	86.60	00.00 (00.00)	100.00	00.00 (0.00)	100.00
10	azoxystrobin 23% SC	37.00 (37.21)	58.80	27.50 (31.70)	69.44	18.5 (25.53)	79.44
11	Control	90.00 (71.53)	-	90.00 (71.53)	-	90.00 (71.53)	-

	SE(m)+	0.16		0.08		0.08	
	C.D	0.48		0.24		0.23	
	C.V	0.81		0.49		0.62	

At 500 ppm concentration of tested fungicides, the results (Table 2) revealed that per cent growth inhibition of *Fol* varied from 61.10% with copper oxychloride to 86.60% under tebuconzole + trifloxystrobin and radial growth of *Fol* from lowest 12.00 mm under tebuconzole + trifloxystrobin to maximum 37.00 mm under azoxystrobin. It is observed that at 500ppm concentration, tebuconzole + trifloxustrobin was significantly superior in inhibiting the radial growth of *Fol* with growth inhibition of 86.00% followed by tebuconzole with growth inhibition of 79.33%. Azoxystrobin was found to the least effective fungicide in inhibiting the radial growth with recording maximum growth of 37.00% with growth inhibition of only 58.80%.

At 1000 ppm concentration, the results (Table 2) revealed that maximum per cent growth inhibition of *Fol* varied from cent per cent with tebuconazole + trifloxystrobin to 69.44 per cent with azoxystrobin. It is found that tebuconazole + trifloxystrobin was significantly superior with maximum inhibiting the radial growth of zero per cent (00.00%). It is found that azoxystrobin was least effective in inhibiting the radial growth with maximum growth of 27.50mm. Azoxystrobin and copper oxychloride fungicides were found on par in inhibiting the radial growth and per cent growth inhibition of the *Fol* at 1000ppm concentration. The next significantly superior fungicide followed by tebuconzole + trifloxystrobin in inhibiting the radial growth and per growth inhibition of *Fol* was tebuconzole with recording only 12.00mm growth and 86.66% growth inhibition respectively.

In continuation of efficacy evaluation of fungicides at 1500 ppm concentration, the results (Table 2) revealed that per cent growth inhibition of *Fol* varied from maximum of cent per cent (100.00%) with carbendazim + Mancozeb, Tebuconzole and Tebuconzole + Trifloxystrobin to least of 73.22 per cent with thiophanate methyl + pyraclostrobin. At this 1500 ppm concentration all the tested fungicides were found to be significantly superior compared to control in inhibiting the growth of the tested pathogen.

Similar results were reported by Tazeem Akhtar *et al*. (2017) [10] that Tebuconzole 50% + Trifloxystrobin 25% WP (nativo) was found most effective by reducing mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici* with 0.53cm at 750 ppm over control followed by Ridomil Gold, Antracol and Cordate with 1.44cm, 2.37cm and 2.73c respectively and under *invivo* disease incidence was recorded with 32.75 per cent reduction over control @ 0. 1 per cent.

Aroosa Khan *et al*. (2012) [1] also observed similarly that Fosetyl aluminium (Wisdom) and tebuconazole (Treety) were found significantly effective by suppressing radial growth and with reduction of fungal biomass from 20 to 90 per over control of *Fol*. Similar results were obtained by Yamuna (2015) [12] that fungicides such as Bavistin (carbendazim), Folicur (tebuconazole), Tilt (propiconazole) and Nativo (trifloxystrobin + tebuconazole) showed 100% mycelial inhibition of the *B. ricini* pathogen at 250 and 500 ppm concentrations by poison food technique.

Conclusion

Nativo (trifloxystrobin + tebuconazole) showed complete mycelial inhibition of *Fol* at 1000 ppm using poison food technique. So, may be suggested to use nativo (trifloxystrobin + tebuconazole) by tomato growers for the management of Fusarium wilt disease.

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