Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(3): 1670-1673
Received: 05-03-2020
Accepted: 08-04-2020

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In vitro evaluation of fungicides against *Fusarium oxysporum* f. sp. Wilt of tomato

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Abstract
The present study permitted "*In vitro* Evaluation of Fungicides against *Fusarium oxysporum* f. sp. *lycopersici*. (Wilt of Tomato)." Were conducted at the laboratory. Tomato (*Lycopersicon esculentum* Mill) is most important crop grown in India wilt disease is caused by *Fusarium oxysporum* were serious threat to crop production. The fungus *Fusarium oxysporum* f. sp. *Lycopersici* were evaluated with the six fungicides to check the *in vitro* evaluation against the pathogen. The six fungicides (systemic + non systemic + combi fungicides) were Cabriotop, Chlorothalonil, Custodia, Difenoconazole, Azoxistrobin and Azoxistrobin + Difenoconazole. Three replications were conductedfor each fungicide concentration (50, 100, 150ppm). Desired concentrations obtained by adding appropriate amount of oil to PDA in petri plates by Poison food technique. Average mycelial growth was reported with the evaluation of fungicides at 50, 100, 150 ppm ranged between 29.11mm to (Custodia) 47.33mm (Azoxistrobin + difenoconazole). Maximum average radial mycelial growth was recorded with the treatment, Chlorothalonil (37.88mm), Difenoconazole (40.66mm), Cabriotop (42.55mm), Azoxystrobin (43.88mm), Azoxistrobin + difenoconazole (47.33mm).
Thus, all the fungicides against *Fusarium oxysporum* f. sp. *lycopersici* and inhibited its mycelial growth over untreated control. Fungicides found most effective in the order of merit were Custodia, Chlorothalonil, Difenoconazole, Cabriotop, Azoxystrobin, Azoxystrobin + difenoconazole.

Keywords: Fungicide, disease, *Fusarium*, concentration

Introduction
Tomato (*Lycopersicon esulentum Mill.*) is most crucial solanaceous vegetable crops, belongs to the family *Solanaceae* & said to the native of South America. Tomato is considered as "poor man's Orange" in India while "love of Apple" in England. Second largest producer in India and After the China, India is second consumer of tomato in the world. After potato, it ranked second largest consumed vegetable in India, along with onion (NHB, 2018). In India, tomato was grown in 0.797mha and production and productivity 207.08million tonnes and 25.98 tonnes per ha during 2018 (FAOSTAT, 2019). In India tomato crop grown in Orrisa, Bihar, U.P, A.P, Karnataka, M.P, Maharastra, Punjab, Haryana, Himachal Pradesh, Telangana, Gujarat, Tamil Nadu and Assam which accounted for 91% total production of India (NHB2018). Annual production of tomato in India is 19759MT/789ha (NHB-2018). In Uttrakhand the annual production of tomato is 94.95 tonnes (NHB-2018).
Tomato is sensitive vegetable crop and growing conditions are not good then fails despairing, it is lukewarm season crop and extremly over senstive to frost. Tomato grows very well on mineral soils and its favour is deep well drained sandy soil. The soil depth is 15-20cm is used to prove the good and robust crop. Tomato crop is grown in ph 5 to 7 is preferred.
Tomato is affected by many plant diseases among them *Fusarium* wilt on tomato is caused by fungus *F. oxysporum* f. sp. *lycopersici* is one of the most destructive crop disease of the world that cause losses on almost vegetables and different other field crops, plantation crops (banana and sugarcane) and some shade trees. *Fusarium oxysporum* enter to the plant by roots and raise to the water vessels of roots and stem. Then the water vessels connected & breakdown, then the water supply to the leaves are stopped. Wilting symptoms are shown on the crop plants and in lower part of the plant symptoms of lower leaves are shown. After that this procedure going on to the plant show wilting symptoms and died.
Haware (1993) reported the pathogen moving through the xylem vessel and envading the vascular system, noticing yellowing and wilting symptoms. The pathogen can live up to six years in the absence of a host plant.
Singh *et al.* (2007) found that the pathogen is confined mainly to the xylem vessels where the mycelium branches contain microconidia. The microconidia is removed and carried up in the vessel system until motion becomes halted, at which point they germinate and the mycelium penetrates the adjacent vessels surface.

Sunita J. Magar *et al* (2019) All the fungicides as maximum inhibition of mycelium was reported with Tebuconazole, carboxin=thiram and minimum inhibition by captan and azoxystrobin with untreated control in 90mm petriplates.
Muhammad Nasir Subhani *et al*. (2011) Six fungicides with four concentrations 5, 10, 20 and 50ppm with three replications and the fungicides are ridomil, cabriotop, vitavax, benomyl, derosal were tested by Poisoned Food Technique. The maximum inhibition of pathogen are Derosal, benomyl and vitavax. The minimum inhibition of pathogen were found by ridomil.

Materials and method

In vitro evaluation of fungicides

The total six fungicides *viz*. Cabriotop, Chlorothalonil, Custodia, Difenoconazole, Azoxistrobin, Azoxistrobin + Difenoconazole were obtained from the Department of Plant Pathology, school of Agriculture, Deharadun for *in vitro* experiments conducted during present studies. A quality of PDA medium adjusted at the required fungicide concentration was poured onto sterilized petri plates. Three replications were conducted for each fungicide concentration (50, 100, 150ppm). Desired concentrations obtained by adding appropriate amount of oil to PDA in petri plates by Poison food technique. After medium solidification, the plates were centrally inoculated, using a sterilized cork borer, with 4 mm disks of fungus taken from the edge of a fully grown five-day-old culture. Unamended (fungicide-free) PDA plates, inoculated with the test pathogen, acted as regulation. In incubator all of the inoculated plates were incubated at 25±2 ºC.

Table 1: List of fungicides used against Fusarium *oxysporum* f. sp. *Lycopersici*

Treatment	Common Name	Trade Name
T8	Cabriotop	Basf
T9	Chlorothalonil	Kavach
T10	Custodia	Adama
T11	Difenoconazole	Score
T12	Azoxistrobin	Amistar
T13	Azoxistrobin + Difenoconazole	Amistar top

Radial mycelial growth observation/ colony diameter of the *F. oxysporum* f. sp. *lycopersici*. Was recorded at 3, 5, and 7 day intervals and continued untill untreated control plates were completely covered with mycelial growth.
Poison Food Technique Formula given under,

$[v_1 = c_2v_2/c_1]$

Whereas
c_1 = Concentration of stock solution (1000ppm)
c_2 = Desired concentration of fungicides
v_1 = Available volume of fungicides
v_2 = Desired volume of growth media

Calculation of percent inhibition by applying the following formula (Vincent 1927)

$$I = [(C- T)/C] \times 100$$

Where
I = % of growth inhibition
C = Colony diameter in control (mm)
T = Colony diameter in treatment (mm)

Experimental Results

Efficacy of fungicides against *Fusarium oxysporum* f. sp. *Lycopersici*

Radial mycelial growth

Six fungicide (systemic + non – systemic + combi fungicides) belonging to different groups were tested against *Fusarium oxysporum* f.sp. *Lycopersici* (@ 50, 100 and 150 ppm) with Poisoned food technique.
The result, shows that a different range of radial growth of *Fusarium oxysporum* f. sp. *Lycopersici* was tested for systemic fungicides. Mycelial growth has been shown to decrease with an increase in the concentration of fungicides.
In 50ppm, growth of pathogen mycelium was ranged from 35.00mm (Custodia) to 56.00mm (Azoxistrobin + difenoconazole). Least mycelial growth was reported with the treatment, Custodia (35.00mm), Chlorothalonil (43.66mm), Difenoconazole (47.33mm), Cabriotop (53.33mm), Azoxystrobin (54.00mm) and Azoxistrobin + Difenoconazole (56.00mm) compared with fully grown (80mm) in untreated control.
At 100ppm, all the fungicides were tested same way of mycelial growth as that of 50 ppm 31.00mm (Custodia) to 47.33mm (Azoxystrobin + difenoconazole). Least mycelial growth was reported with the treatment, Custodia (31.00mm), Chlorothalonil (38.00mm), Difenoconazole (40.66 mm), Cabriotop (40.33 mm), Azoxystrobin (41.66 mm), Azoxistrobin + difenoconazole (47.33mm) as compared with fully grown (80mm) in untreated control.
At 150ppm, all the fungicides were tested exhibits somewhat same way of mycelial growth as that of 50ppm, 100ppm were in between from 21.33mm (Custodia) to 38.66mm (Azoxistrobin + difenoconazole). Least mycelial growth was reported with the treatment, Custodia (21.33mm), Chlorothalonil (32.00 mm), Difenoconazole (34.00mm), Cabriotop (34.00mm), Azoxystrobin (36mm), Azoxistrobin + difenoconazole (38.66mm) as compared to fully grown (80mm) in untreated control.
Average mycelial growth was reported with the evaluation of fungicides at 50, 100, 150 ppm ranged between 29.11mm to (Custodia) 47.33mm (Azoxistrobin + difenoconazole). Maximum average radial mycelial growth was recorded with the treatment, Chlorothalonil (37.88mm), Difenoconazole (40.66mm), Cabriotop (42.55mm), Azoxystrobin (43.88mm), Azoxistrobin + difenoconazole (47.33mm). The minimum mean radial mycelial growth was reported with Custodia (29.11).

Mycelial inhibition

As a result all the systemic fungicides tested (@ 50, 100, 150ppm each) inhibited mycelial development of *Fusarium oxysporum* f. sp. *Lycopersici* for untreated control. In addition, the percentage of mycelial inhibition of the test pathogen increased with an increase in the concentration of fungicides tested.
In 50 ppm, mycelial development inhibition percentage was recorded in the range of (30.00%) Azoxistrobin + difenoconazole to (56.25%) Custodia. The maximum mycelial inhibition were reported with, Custodia (56.25%), Chlorothalonil (45.41%), Difenoconazole (40.83%), Cabriotop (33.33%), Azoxystrobin (32.50%). The fungicide Azoxistrobin + Difenoconazole was found less effective with 30.00% inhibition of the pathogen over untreated control.
In 100ppm, per cent development of mycelial inhibition were recorded in the range of (40.83%) Azoxistrobin + difenoconazole to (61.25%) Custodia. The maxi. mycelial

inhibition was recorded with, Custodia (61.25%), Chlorothalonil (52.50%), Difenoconazole (49.16%), Cabriotop (49.58%), Azoxystrobin (47.91%). The fungicide Azoxistrobin + Difenoconazole was found less effective with 40.83% inhibition of the pathogen over untreated control.

In 150ppm, per cent development of mycelial inhibition were recorded in the range of (51.66%) Azoxistrobin + difenoconazole to (73.33%) Custodia. The highest mycelial inhibition was recorded with, Custodia (73.33%), Chlorothalonil (60.00%), Difenoconazole (57.50%), Cabriotop (57.50%), Azoxystrobin (55.00%). The fungicide Azoxistrobin + Difenoconazole was found less effective with 51.66% inhibition of test pathogen over untreated control.

Average mycelial inhibition of all the fungicides tested in between from (40.83%) Azoxistrobin + difenoconazole to (63.61%) Custodia. The maximum mycelial inhibition was recorded with, Custodia (63.61%), Chlorothalonil (52.63%), Difenoconazole (49.16%), Cabriotop (46.83%), Azoxystrobin (45.13%). The fungicide Azoxistrobin + Difenoconazole was found less effective with 40.83 per cent inhibition of the test pathogen over untreated control.

Thus, all the fungicides against *Fusarium oxysporum* f. sp. *lycopersici* and inhibited its mycelial growth over untreated control. Fungicides found most effective in the order of merit were Custodia, Chlorothalonil, Difenoconazole, Cabriotop, Azoxystrobin, Azoxystrobin + difenoconazole.

Table 2: Efficacy of fungicides against *Fusarium oxysporum* f. sp. *lycopersici.* (Mycelial Growth Colony Diameter in mm)*

Treatment	50 ppm	100 ppm	150 ppm	Average	Control	S.Em±	C.D at 5%
T_8 Cabrio top	53.33	40.33	34.00	42.55	80	1.08	3.84
T_9 Chlorothalonil	43.66	38.00	32.00	37.88	80	0.69	2.44
T_{10} Custodia	35.00	31.00	21.33	29.11	80	0.90	3.18
T_{11} Difenoconazole	47.33	40.66	34.00	40.66	80	0.98	3.46
T_{12} Azoxistrobin	54.00	41.66	36.00	43.88	80	1.30	4.60
T_{13} Azoxy + difeno	56.00	47.33	38.66	47.33	80	0.79	2.79

* = Mean of three replications.
C.D = colony diameter mean
S.E (m) = standard error mean

Table 3: Mycelial % inhibition of *Fusarium oxysporum* f. sp. *lycopersici.*

Treatment	50 ppm	100 Ppm	150 Ppm	Avg% inhibi.	Control	S.Em±	C.D at 5%
T_8 Cabrio top	33.33	49.58	57.50	46.80	100	1.36	4.80
T_9 Chlorothalonil	49.58	52.50	60.00	54.02	100	0.86	3.06
T_{10} Custodia	56.25	61.25	73.33	63.61	100	1.12	3.09
T_{11} Difenoconazole	40.83	49.16	57.50	49.16	100	1.12	4.03
T_{12} Azoxistrobin	32.50	47.91	55.00	45.13	100	1.63	5.75
T_{13} Azoxy + difeno	30.00	40.83	51.66	40.83	100	0.99	3.49

* = Mean of three replications.
C.D = colony diameter
S.E (m) = standard error mean

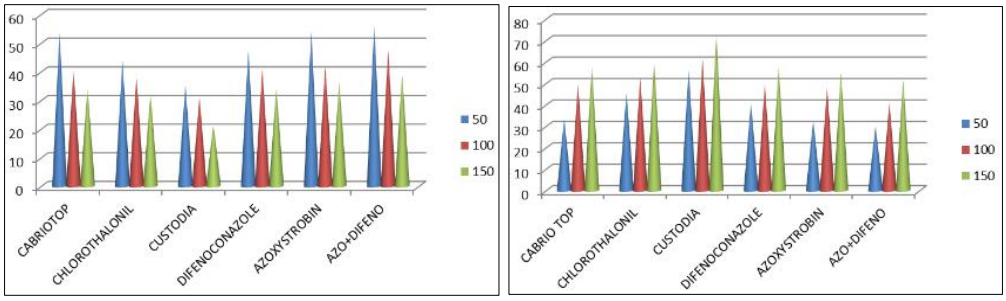

Fig 1: *In vitro* evaluation of fungicides againts *F. oxysporum* f. sp. *lycopersici.* (Mycelial growth)

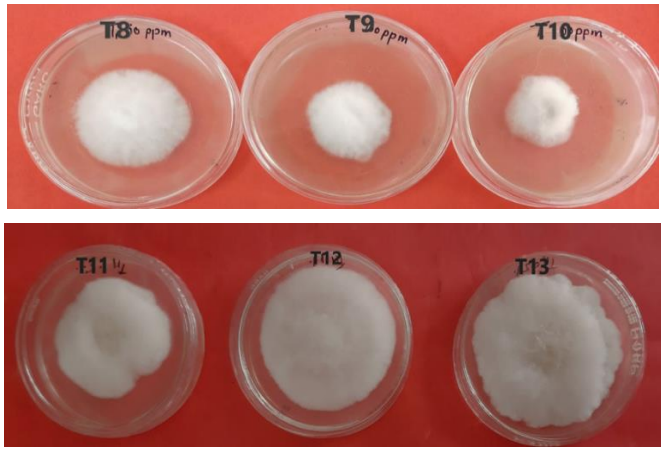

Plate 1: Evaluation of fungicides against *Fusarium oxysporum* f. sp. *Lycopersici*

Conclusion

In this study we revealed that Custodia fungicide gave best effective against mycelial growth inhibition of *Fusarium oxysporum* f. sp. and it may be used for the control of Tomato wilt disease.

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