



In-vitro Chemical Management of *Fusarium* Wilt of Tomato in Sindh, Pakistan

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ABSTRACT: *Fusarium* wilt disease of tomato (*Lycopersicon esculentum* L.) is a highly serious vascular disease worldwide. Chemicals can control fungal diseases by inhibiting or killing the fungi that cause destructive crop diseases. In this series of experiments, randomized complete block design (RCBD) was used and repeated 4 times. Different chemicals were tested in the lab and glasshouse against *Fusarium oxysporum* f.sp. *lycopersici* in septic conditions. The Nativo fungicide showed higher efficacy in the laboratory recording 95.55, 88.88 % inhibition of test fungus at 1000 and 500 ppm followed by Topsin-M 88.51% at 1000 ppm. The minimum inhibition percent was recorded by Curzate 11.11 % and Melody Duo 14.07% fungicides at 10 ppm as compared to other fungicides. The Topsin-M, Nativo and Carbendazim were discovered the most effective chemicals followed by Cabriotop, prevail and Antracol in a pot experiment. Significantly ($P < 0.05 = 0.0000$) the minimum plant mortality percent was recorded by Topsin-M 5 and 8% at 500 and 100 ppm followed by Nativo 9.66% at 500 ppm. The highest plant mortality was noted in untreated. The higher concentrations of fungicides were observed highly effective for encouraging the plant biomass as compare to lower and medium doses. The plant biomass (Shoot and root) was noted higher by Topsin-M, Cabriotop, Nativo at 500 and 100 ppm. The Prevail and Antracol were found to the less effective at 50 ppm as compare to other fungicides. Interestingly no root infection was observed in plants treated with higher and medium doses of fungicides. The highest root infection was noted in the untreated plants. Based on the findings, it is suggested that Topsin-M, Nativo, Cabriotop and Carbendazim., may be used as potential chemicals for the management of tomato wilt disease.

Keywords: *Fusarium oxysporum* f. sp. *lycopersici*, Bioagent, Eco-friendly management.

I. INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is one of the most extensively cultivated and consumable vegetable crops in the world and is supposed to be the second chief vegetable later potato in the world [1; 2]. It is used as fresh fruit and salad whereas many fruit items are also prepared from tomatoes such as tomato juice, tomato ketchup, soup and many other dishes [3]. It contains water 95.3%, niacin and calcium with the same quantity 0.07%, vitamin A, C, E and a rich source of nutrients like Na, K, Fe, and antioxidants especially lycopene and salicylate. All have great importance in the human metabolic system [4; 5].

Tomatoes are very much important for the health of human beings. It declines the threats of cancer, osteoporosis, and cardiovascular diseases. The daily uses of tomatoes lowered the danger of cancer diseases of lung, prostate, stomach, cervical, breast, oral, colorectal, esophageal, pancreatic and countless other categories of cancer. Lycopene and the newly detected bioflavonoids in tomatoes are accountable as cancer aggressive agents and are beneficial to liver strength. Tomato produces detoxification power in the human body. This is almost certainly due to the presence of chlorine and sulfur. In line with some

modifications, 51 mg of chlorine and 11 mg of sulfur in 100 g tomatoes play a key role in detoxification [6].

It is cultivated in 144 countries of the world. Among them, China, United States, Turkey, India and Egypt are the uppermost tomato-producing countries of the world. In Pakistan, it is grown on an area of 58.4 thousand hectares which produces 0.55 million tons with an average yield of 9.4 thousand tons per hectare [7]. Sindh province is a major tomato-producing region of Pakistan, where it is cultivated on 21 thousand hectares in an area with general production of 153.3 thousand tons and 7.3 thousand tons yield per hectare. Tandojam is one of the major tomato-producing city in Pakistan. It produces several tons of tomatoes a year [8]. This common yield is at a low level as compare to different tomato cultivating nations. In terms of tomato yield per hectare, the USA is on top producing 96.80 t/ha followed by China 59.25 t/ha, Egypt 40.96 t/ha, India 24.65t/ha and Pakistan 9.44 t/ha respectively [9].

The tomato plant is highly susceptible to various biotic and abiotic diseases at different critical growth stages from seedling stage to maturity [10]. Tomato is very sensitive to more than 200 diseases caused by pathogenic fungi, nematodes, bacteria and viruses during cultivation or postharvest storage [11; 12].

Despite our best efforts to deal with plant pathogens, they are still a huge challenge for crops. Early blight, anthracnose, bacterial wilt, bacterial canker, tomato spotted wilt, Verticillium wilt, *Fusarium* Wilt and bacterial wilt are considered as an important threat of tomato crop [13; 14].

Among the fungal wilt diseases, tomato wilt is caused by the fungus *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder and H.N. Hans, that almost affect the roots and causing production losses between 30% and 40% and it may even reach up to 90% [15; 16]. *Fusarium* spp. are the important soil-borne pathogens and infects wide variety of hosts [17].

The drooping, yellowing, wilting, and dying of the lower leaves, regularly on one part of the plant are the common symptoms of this disease [18]. *Fusarium oxysporum* produced circular, aerial mycelium initially white, later changed to light pink [19].

The managing of *Fusarium* wilt disease of tomato is very essential for maintaining fruit quality, quantity, and plant vigor. Although *Fusarium* wilt is a challenging disease to control [20]. Various control strategies are suggested for this fungus, the disease is mostly controlled by the uses of fungicides applications [21]. Recently Patiyal and Prasad [22] assessed six different fungicides against *Fusarium* wilt disease of tomato *In-vitro* conditions and recommended Custodia fungicide as a possible control measure. However, due to the development of new pathogenic races efforts to control the disease have limited success. The use of cultural, physical and chemical control including early methods to deal with this destructive plant disease. None of these methods were able to generate a clear impact, except for the cultural and resistance varieties approaches, which are the main prevention [23]. The application of fungicides can improve the genetic potential of crops and reduce the yield caused by diseases. Preventive fungicides inhibit spore germination and penetration, but the pathogen can develop resistance to the application

of fungicides. Therefore, fungicides must be applied repeatedly at appropriate doses and intervals [24; 25]. In addition to affecting the health of users and consumers, the unplanned use of fungicides often leads to serious environmental problems. Therefore, it is of great significance to select suitable and reliable chemicals to control the disease loss. This study aimed to screen effective fungicides against Tomato *Fusarium* wilt disease.

II. MATERIALS AND METHODS

A. Fungal isolation

The isolation of *Fusarium* wilt pathogen (*Fusarium oxysporum* f.sp. *lycopersici*) was carried out using Potato Dextrose Agar (PDA) medium through tissue isolation technique. The infected parts of the tomato plant including roots, stem and branches were cut into slight pieces of about 0.5-2.0 cm longitudinally. The pieces were cleaned in running tap water followed by surface sterilization in 0.1 percent mercuric chloride solution and rinsed frequently twice in sterilized distilled water to eliminate mercuric chloride solution. Five pieces were placed on Petri plates having sterilized PDA. These plates were incubated at $25 \pm 2^\circ\text{C}$ for 7 days for recovery of the target pathogen [26]. The isolated fungus was identified according to their morphological characteristics by using standard keys [27; 28].

***In-vitro* assay of different fungicides.** The fungicides viz., Nativo 75 WG, Topsin-M, Carbendazim, Alliate 80 WG, Copper oxychloride 50 WP, Cabriotop 70 WDG, Melody Duo, Antracol 70 WP, Curzate 72 WP and Prevail 40% were used by food poison method *in-vitro* conditions. The detail of fungicides is given in (Table 1) The concentrations 10, 20, 50, 100, 200, 300, 400 and 500 ppm were prepared in PDA during pouring in 90 mm sterilized Petri plates. PDA medium without fungicides was served as control.

Table 1: Detail of fungicides used in the experiments.

Trade name	Chemical name	Active ingredient	Chemical group
Topsin-M	Thiophanate-methyl	70% Thiophanate-methyl	Thiophanate-methyl
Nativo	Trifloxystrobin+Tebuconazol	25% Trifloxystrobin+50% Tebuconazol	Trifloxystrobin+Tebuconazol
Curzate	Cymoxanil+Mancozeb	8% Cymoxanil+64% Mancozeb	Acetamida-dithiocarbamate
Alliate	Fostyl-aluminium	80% Fostyl-aluminium	Phosphonate
Prevail	Carboxi+ Metalaxyl +Pentachloronitrobenzene	15% Carboxin and Pentachloronitrobenzene +3.12 % Metalaxyl + 66.88% others	Pentachloronitrobenzene
Antracol	Propineb	70% Propineb	Dithiocarbamate
Copper oxychloride	Copper oxychloride	50% Copper oxychloride	Copper compound
Cabriotop	Pyraclostrobin + Metiram	5 % Pyraclostrobin + 55% Metiram	Benzimidazole
Melody Duo	Iprovalicarb+ Propineb	5.5% Iprovalicarb + 61.25% Propineb	Carbamic Acid Isopropylesters
Carbendazim	Carbendazim	50% Carbendazim	Benzimidazole

Subsequently the solidification of the used medium, the five mm disc was taken from the pure culture of target fungus by using disinfected cork borer and inoculated in the middle of petri plates and kept in an incubator at 25°C for 7 days for its growth and multiplication. Each treatment was possessing four replications. The mycelial growth data was continuously taken in (ml) after every 24 hours till the control plates grow full of the tested fungus. The efficacy of all tested fungicides was calculated by using the following percent inhibition formula as described by [29].

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

I= The inhibition % growth of test pathogen
 C= The control plate radial colony mycelial growth (mm)
 T= The treatments radial colony growth (mm)

B. Effect of Fungicides on Plant Growth and Disease Development

Pot experiment assay. The fungicides found highly effective *in-vitro* experiments were further selected under glasshouse at 60 to 70 % relative humidity with 23-28°C temperature. The six fungicides such as Nativo 75 WG, Topsin-M, Carbendazim, Cabriotop 70 WDG, Antracol 70 WP and Prevail 40% were used at a concentration of 50, 100 and 500 ppm. The seed of local commonly growing tomato variety “Desi local” was superficially sterilized with 5% commercial bleach for 2 minutes and washing with sterile water. Ten seeds per pot were grown in each earthen pot of 20 cm diameter, holding 2-kilogram steam-sterilized soil. The suspension of the test pathogen was adjusted at 10^5 conidia/g⁻¹ and incorporated artificially in the soil. These seeds were slightly sheltered with a tinny layer of soil. The pots were kept in the greenhouse. The prepared concentration of the fungicides 50, 100 and 500 ppm were drenched into the earthen pots after 7 days of sowing. The irrigation water was applied to earthen pots whenever required. The plants were uprooted after 40 days of sowing and data such as mortality %, root infection%, shoot and root length (cm) and shoot and root weight (g) were noted.

C. Experiment Design

The experiments were designed as RCBD with 4 replicates of each treatment. The experiments were repeated twice within two years. Statistical analysis of the data was carried out using software *Statistix*, version 8.1. To judge the treatment effect, ANOVA was used. For average separation, the least significant difference (LSD) test and $P < 0.05$ were used for significant differences between treatments.

III. RESULTS

A. *In-vitro* efficacy of different fungicides

According to results, the minimum colony mycelial growth of test fungus was recorded by Nativo followed by Topsin-M, Carbendazim and prevail fungicides respectively at 1000 ppm concentrations whereas the highest colony growth recorded by Curzate, Melody Duo and Alliate fungicides respectively at 10 ppm. The Nativo was found the most effective fungicides recording (95.55 and 88.88 %) inhibition at 1000 and

500 ppm followed by Topsin-M (88.51%) at 1000 ppm. These two fungicides were recorded as highly effective at 1000, 500 and 300 ppm concentrations whereas the minimum inhibition % was recorded by Curzate and Melody Duo fungicides and both were found less effective at 10 ppm as compare to others.

Table 2: Efficacy of different fungicides on the inhibition percent of *Fusarium oxysporum* f.sp. *lycopersici*.

Fungicides	% Inhibition (mm)					
	10 ppm	20 ppm	100 ppm	200 ppm	500 ppm	1000 ppm
Nativo 75 WG	63.7 04 l	71.11 1hi	77.4 07 f	84.4 44 d	88.8 89 b	95.55 6 a
Cabrioto p 70 WDG	27.0 37 wx	39.25 9 s	44.4 44 q	50.3 70 p	55.5 56 n	61.48 1m
Melody duo	14.0 74 b	22.22 2 z	27.7 78 w	33.3 33 v	41.1 11 r	44.81 5 q
Alliette 80 WG	17.0 37 a	25.92 6 x	38.8 89 st	44.0 74 q	50.0 00 p	55.92 6 n
Antracol 70 WP	27.0 37 wx	35.55 6 u	44.4 44 q	50.0 00 p	53.3 33 o	64.44 4 kl
Curzate 72 WP	11.1 11 c	21.85 2 z	27.0 37 wx	32.9 63 v	37.4 07 t	44.07 4 q
Copper oxychloride 50 WP	17.0 37 a	21.85 2 z	28.5 19 w	33.7 04 v	40.3 70 rs	44.81 5 q
Carbendazim	24.0 74 y	43.33 3 q	54.4 44 no	65.9 26 jk	73.7 04 g	86.29 6 c
Topsin-M	61.1 11 m	67.03 7 j	72.5 93 gh	76.2 96 f	84.0 74 d	88.51 9 b
Prevail 40%	50.7 41 p	61.85 2 m	67.0 37 j	70.7 41 i	77.4 07 f	81.85 2 e

The fungicides including prevail, Antracol, Cabriotop and Alliate were found as moderate effective with 50 % inhibition at 10, 200, 200 and 500 ppm as compare to other fungicides and were not significantly different from each other at 500, 200 and 10 ppm in falling the mycelial growth of target fungus (Table 2). The minimum doses of all tested fungicides were found moderately unsuccessful to check the mycelial growth of the test fungus (Table 2).

B. Pot Experiment Effect

The fungicides found the best in results during the *in-vitro* trial such as Nativo 75 WG, Topsin-M, Carbendazim, prevail 40%, Cabriotop 70 WDG and Antracol 70 WP were selected for the glasshouse experiment. The plants treated artificially with fungicides performed extremely operative in decreasing the mortality % of the plant as compare to the control plant. The Topsin-M, Nativo and Carbendazim fungicides were

recorded as most effective followed by Cabriotop, prevail and Antracol in pot experiments in reducing plant mortality. The plant mortality ranging from (5.00-27.66 %) was noted in plants treated with fungicides except an untreated plant. The minimum plant mortality was recorded by Topsin-M (5 and 8 %) at 500 and 100 ppm followed by Nativo (9.66 %) at 500 ppm. The maximum plant mortality% was noted in untreated (control) plants (Fig. 1). Generally, the higher concentrations of fungicides were observed highly effective for encouraging the plant biomass as compare to the lowest and moderate doses. The maximum shoot length was noted by Topsin-M (27.66 and 25.66 cm) at 500 and 100 ppm followed by Cabriotop (24.66), Nativo (24.33 cm) at 500 ppm whereas the prevail and Antracol

were found as the less effective at 50 ppm as compare to other fungicides (Fig. 2). The minimum shoot length was recorded in untreated plants (7 cm). The maximum shoot weight was observed in plants treated with Topsin-M (2.2, 1.83 and 1.53 cm) at 500, 100 and 50 ppm. The lowest shoot weight was noted in plants treated with Antracol and Prevail at 50 ppm as compare to other fungicides (Fig. 3). Similarly, the maximum root length was observed in plants treated with Topsin-M (13.66 and 12.33 cm) 500 and 100 ppm followed by and Cabriotop (11.66 cm), Carbendazim (11.33 cm) and Nativo (11 cm) 500 ppm, respectively.

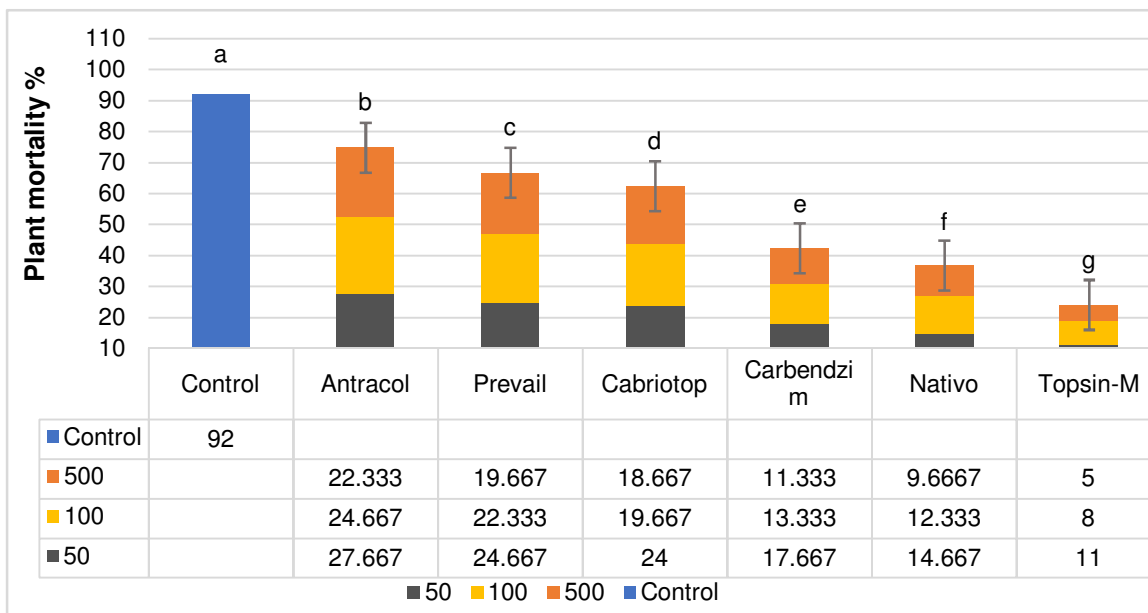


Fig. 1. Effect of different fungicides on Plant mortality inoculated with *Fusarium oxysporum* f.sp. *lycopersici* in a pot experiment.

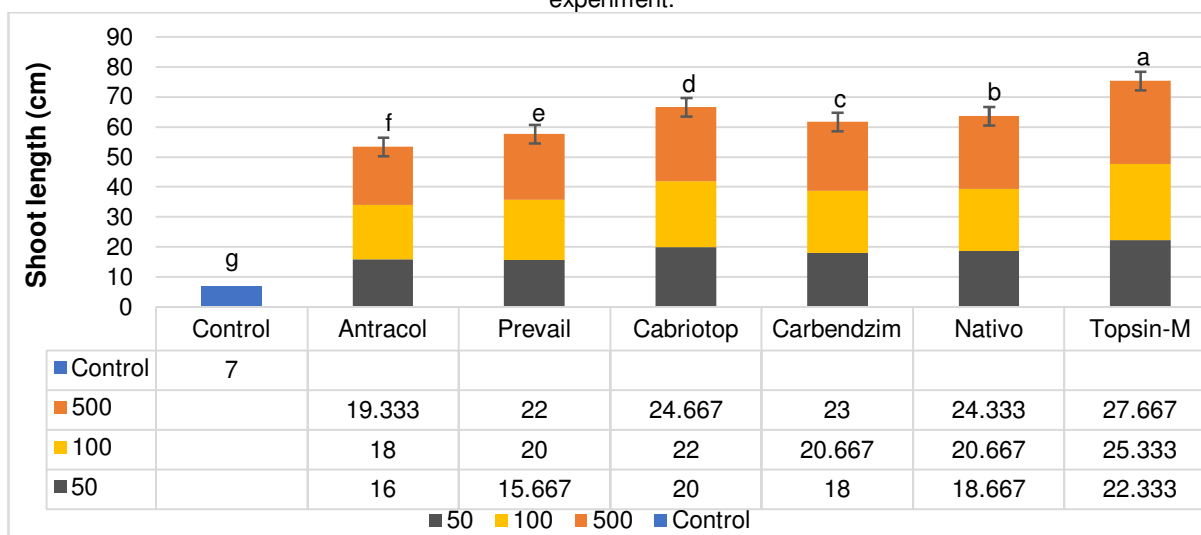


Fig. 2. Effect of different fungicides on shoot length inoculated with *Fusarium oxysporum* f.sp. *lycopersici* in a pot experiment.

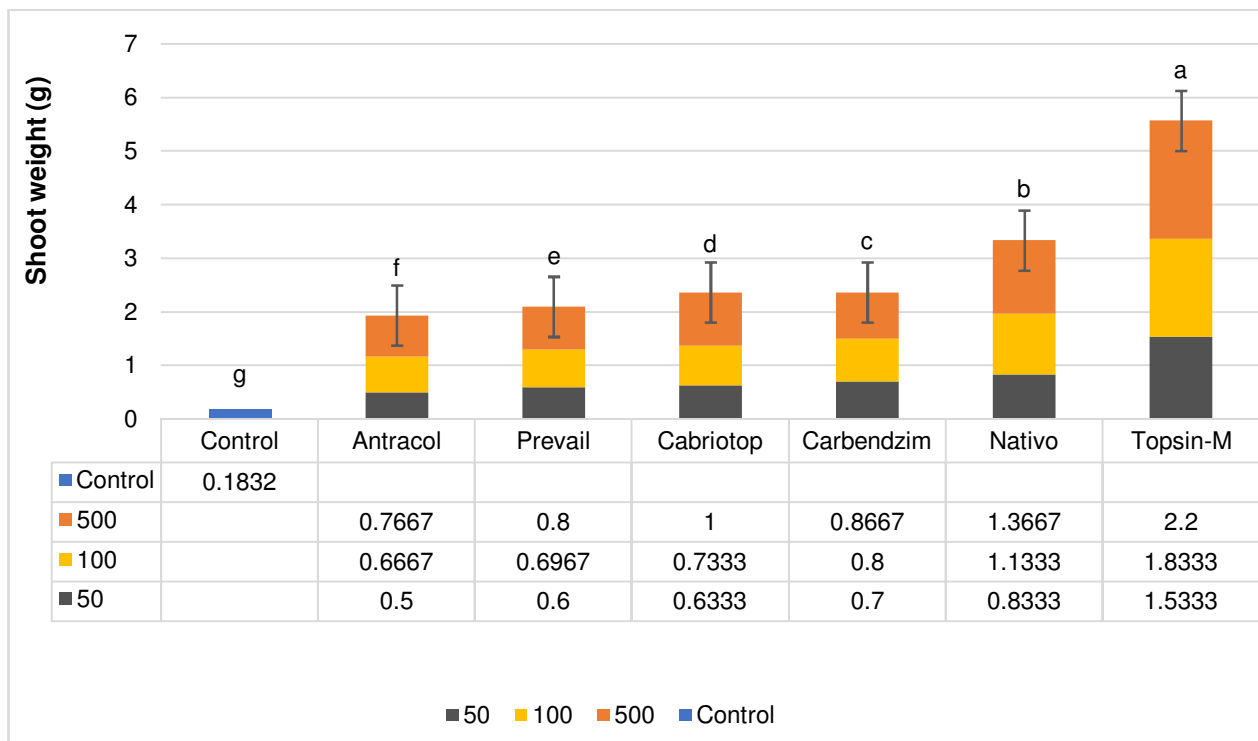


Fig. 3. Effect of different fungicides on shoot weight inoculated with *Fusarium oxysporum* f.sp. *lycopersici* in the pot experiment.

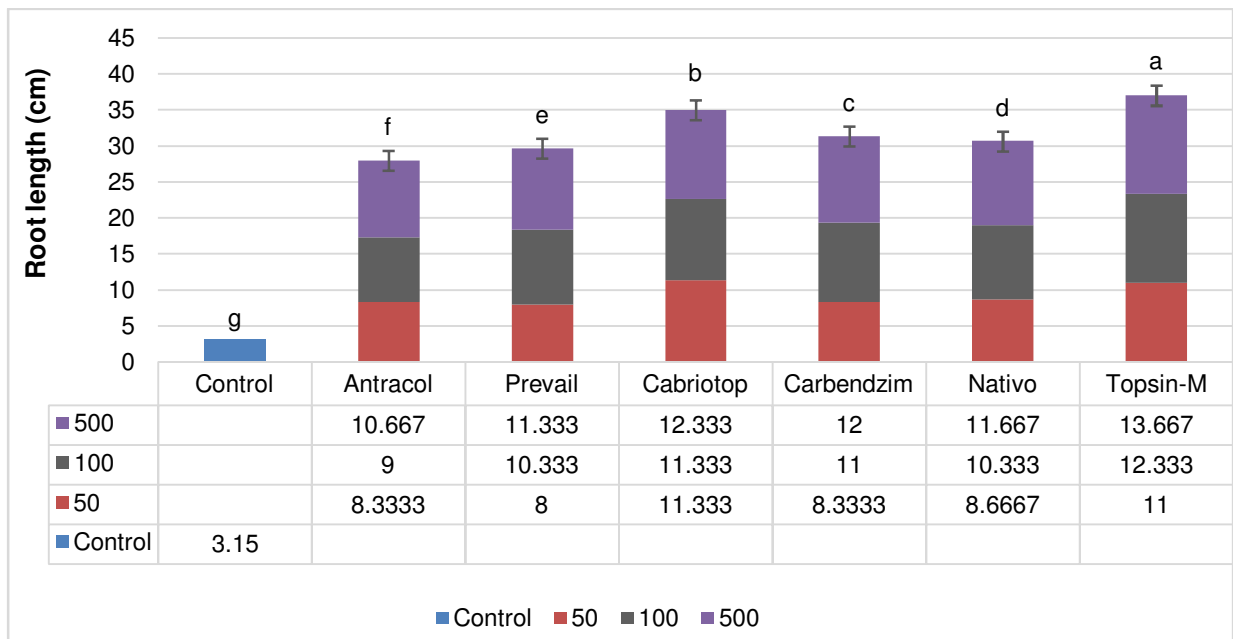


Fig. 4. Effect of different fungicides on root length inoculated with *Fusarium oxysporum* f.sp. *lycopersici* in a pot experiment.

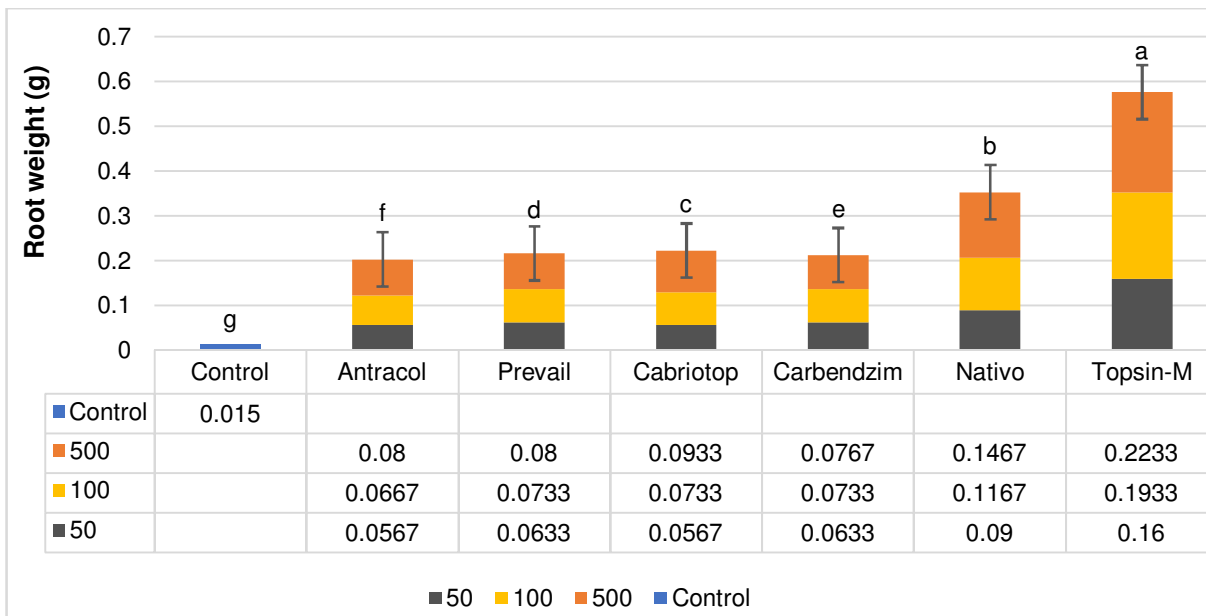


Fig. 5. Effect of different fungicides on root weight inoculated with *Fusarium oxysporum* f.sp. *lycopersici* in a pot experiment.

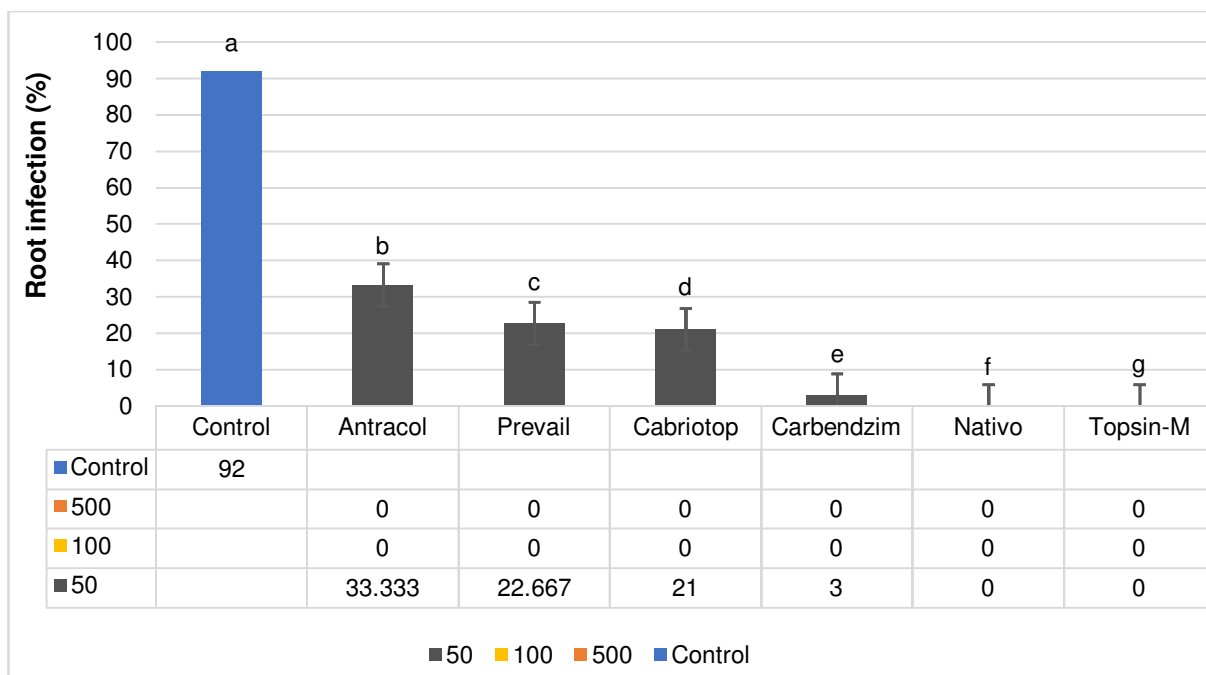


Fig. 6. Effect of different fungicides on root infection inoculated with *Fusarium oxysporum* f.sp. *lycopersici* in a pot experiment.

The minimum root length was observed in plants treated with Prevail and Antracol at 50 ppm (Fig. 3). The root weight ranging from (0.05 to 0.22 grams) was recorded in plants treated with fungicides. The highest shoot weight was observed in plants when treated with Topsin-M, Nativo and Cabriotop fungicides at 1000 ppm. The Cabriotop, Prevail and Antracol were not found effective at 50 ppm (Fig. 4). Moreover, the infection % was not observed in plants treated with higher and medium doses of fungicides. The maximum

(93 %) root infection was noted in un-treated plant followed by (33.33, 22.667, 21 and 3 %) by Antracol, Prevail, Cabriotop and Carbendazim at 50 ppm (Fig. 5).

IV. DISCUSSIONS

Several approaches are to be used to struggle against this pathogenic fungus, from chemical methods such as the usage of fungicides from *Benzimidazoles* and *Triazoles* family. For this reason, sustained research against this fungus is vital to identify the management

practices [30]. In our study, the effect of fungicides Cabriotop, Melody duo, Nativo, Topsin-M, Curzate, Carbendazim, Copper oxychloride, Antracol, Prevail and Aliette were demonstrated experimentally *in-vitro* conditions. The results obtained that Nativo and Topsin-M produced the lowest mycelial growth in the laboratory and were found to be highly effective for managing the *Fusarium oxysporum f.sp lycopersici*. Our findings are in line with Akhtar *et al.* [31] and Sultana and Ghaffar [32]. All tested fungicides were recorded highest, moderate and less effective against the target fungus. Aroosa *et al.* [33] tested fungicides against tomato *Fusarium* wilt pathogen and found in their research that fungicides reduced fungal growth. Curzate and Melody duo fungicides were found less effective as compare to other tested fungicides. The fungicides including prevail, Antracol, Cabriotop and Aliette were found as moderately effective with 50 % inhibition at 10, 200, 200 and 500 ppm in falling the mycelial growth of target fungus under *in-vitro* conditions. Our studies are very much nearby the findings of Fareed *et al.* [34]. Sultana and Ghaffar [32] reported that infestation of bottle gourd and cucumber seeds along with fungicides and infested with *F. oxysporum* fungus. The results showed a reduction of root infection and mortality in seedlings. The tested fungicides such as Topsin-M, Benlate and Carbendazim were found highly imperative and reducing the mortality in the seedling of bottle gourd. In our results, the Topsin-M, Nativo and Carbendazim fungicides were recorded as most effective followed by Cabriotop, prevail and Antracol in pot experiment in reducing the plant mortality. Generally, the higher concentration of fungicides was observed as most effective for encouraging the plant biomass as compare to low and medium doses. The highest plant growth with lowest root infection % was noted by Topsin-M, Cabriotop and Nativo whereas the prevail and Antracol were found fewer effective fungicides. A similar type of results obtained by Dwivedi *et al.* [35] stated that Topsin-M and Thiram fungicides are highly efficient at 800 mg/g soil, against *Fol* in reducing 83.4% populations after 45 days of inoculations. Amini and Sidovich [36] assessed six fungicides against *Fol* in the laboratory and in the field. The result of glasshouse tests discovered a diverse degree of efficiency of all test fungicides in decreasing disease attack. Prochloraz and bromuconazole were the greatest operative fungicides both in the laboratory and in field conditions next to benomyl and Carbendazim. In our studies, the Topsin-M and Nativo fungicides were recorded as the most effective with lowest mortality and root infection % followed by Carbendazim between the treatments. The Prevail and Cabriotop fungicides were appeared as less effective as compare to other tested fungicides. All tested fungicides showed their effect against the test pathogen. The significantly lowermost infection of the pathogen was recorded in plants in which fungicides were incorporated as compare to control plants. Our results are very much like Dwivedi *et al.* [35] who proved that Topsin-M and Thiram are the most efficient against *Fol* populations. Similarly, Akhtar *et al.* [31] stated that Nativo reduces the disease up to 32.75 % at 1% concentration. Although Poly-beta-hydroxyl- butyric-acid successfully supported the growth

of tomato. The highest decline disease was noted by Nativo (30.14 %) and Teagro (25.06 %) during field trials. Sultana and Ghaffar [32] also observed that Topsin-M fungicide completely inhibits the colony growth at 1000 ppm in laboratory and field conditions against *Fusarium oxysporum*. Similarly, Patiyal and Prasad [22] also evaluated different fungicides against *Fusarium oxysporum f.sp. lycopersici* and found that Difenconazole, Cabriotop, Azoxystrobin, Azoxystrobin, Custodia, Chlorothalonil can reduce the tomato *Fusarium* wilt disease intensity.

V. CONCLUSION

The use of chemicals has been believed to be a rapid and potential control measure against fungal diseases. In the current study, the different chemical fungicides have been tested against *Fusarium oxysporum f.sp. lycopersici*. The Nativo and Topsin-M fungicides were observed as the most effective chemicals providing the highest inhibition of test fungus in the food poison method. Interestingly in pot experiments Topsin-M. was observed most effective than Nativo, Cabriotop and Carbendazim providing the lowest mortality and root infection percent with the highest plant biomass. The Prevail and Antracol chemicals were found to be less effective in all experiments. Based on the present findings the Topsin-M, Nativo, Cabriotop and Carbendazim fungicides are recognized as potential chemicals against *Fusarium* wilt disease of tomato moreover it is also suggested that these chemicals must be tested in field conditions against fungal wilt associated fungi of tomato and other crops.

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CONFLICT OF INTEREST

The authors of this manuscript confirm that there is no any conflicts of interest associated with the research article.

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