Original Research Article
Comparative *In Vitro* Assessment of Fungicides for Managing *Fusarium oxysporum* f. sp. *capsici* in Chilli

ABSTRACT

Chilli wilt disease, caused by *Fusarium oxysporum* f. sp. *capsici*, ~~poses a significant threat to~~significantly threatens~~chilli~~ chilicultivation, impacting yield and quality. This study ~~was~~aimed to evaluate the fungi-toxic effects of various fungicides against *Fusarium oxysporum* f. sp. *capsici* under *in vitro* conditions. Eight different fungicides *viz.*, Tebuconazole 6.7 % + Captan 26.9 % w/w SC, Thiophenate Methyl 70% WP, Carbendazim 12% + Mancozeb 63% WP, Tebuconazole 50% + Trifloxystrobin 25% WG, Carbendazim 50% WP, Pyraclostrobin 20% WG, Chlorothalonil 75% WP and Azoxystrobin 18.2%+ Difenoconazole 11.4% SC were tested at aconcentration of 500 ppm and 1000 ppm using the Poison food technique.The experiment was designed using a Completely Randomized Design (CRD) and conducted at the Department of Plant Pathology, Dr. Sharadchandra Pawar College of Agriculture, Baramati, during the academic year 2023-24. All fungicides significantly ($P < 0.01$) inhibited the mycelial growth of *Fusarium oxysporum*f.sp. *capsici*, with Carbendazim 12% + Mancozeb 63% WP showing the highest efficacy, followed by Tebuconazole 50% + Trifloxystrobin 25% WG and Tebuconazole 6.7 % + Captan 26.9 % w/w SC across both concentrations. This study suggests that the inhibition of pathogen growth increased with higher fungicide concentrations.

Key words: *Fusarium oxysporum f.sp. capsici, fungicides, growth inhibition, ~~chilli~~chili*

1. INTRODUCTION

Chilli (*Capsicum annuum* L.) is a valuable vegetable and spice crop, recognized globally for its aroma, taste, ~~flavour~~flavor, and hotness. Chilli peppers are widespread in cultivation across tropical and subtropical areas around the globe. It is susceptible to numerous pathogens~~, that can impair its growth and reduce~~impairing its growth and reducing yields. *Fusarium* wilt is ~~some of~~ one of the severe biotic threats to ~~chilli~~ chilicultivation, affecting its marketability and export potential by reducing ~~both~~ the quality and quantity of the produce. It ~~affects~~ involvesvarious commercial varieties and species within the *Capsicum* genus across different regions.

India ~~stands as~~is the world's leading producer, consumer~~ and exporter of chilli~~, and exporter of chili peppers. India exports ~~chilli to numerous international markets, including the USA, Canada, UK, Saudi Arabia, Germany, Malaysia, Singapore~~chili to numerous international markets, including the USA, Canada, the UK, Saudi Arabia, Germany, Malaysia, Singapore, and ~~many~~ others. Major ~~chilli cultivation regions in India include Andhra Pradesh, Maharashtra, Karnataka, Uttar Pradesh, Punjab, Tamil Nadu, Rajasthan, Odisha, West Bengal~~chili cultivation regions in India include Andhra Pradesh, Maharashtra, Karnataka, Uttar Pradesh, Punjab, Tamil Nadu, Rajasthan, Odisha, West Bengal, and Madhya Pradesh. Andhra Pradesh leads in dry ~~chilli production with 700,000 tonnes, contributing 37.35 per cent~~chili production with 700,000 tonnes, contributing 37.35 percent of the total national production. Telangana follows with 433,120 tonnes (23.11%), while Madhya Pradesh is third, producing 296,690 tonnes (15.83%) of dry ~~chillies~~chilies. Other top dry ~~chilli producing regions consist of Karnataka, Orissa, Maharashtra, Gujarat, Tamil Nadu, Assam~~chili-

producing regions include Karnataka, Orissa, Maharashtra, Gujarat, Tamil Nadu, Assam, and Punjab. In the context of ~~production of green chillies in India, Karnataka stands as the top producer, contributing 1,006.96 thousand tonnes~~ the production of green chilies in India, Karnataka stands as the top producer, contributing 1,006.96 thousand tonnes of production. ~~Following this~~Then, Madhya Pradesh produces 726.90 thousand tonnes, while Andhra Pradesh ranks third with 506.87 thousand tonnes of production.Other ~~major~~ significantstates involved in green ~~chilli production are Bihar, Maharashtra~~chili production are Bihar, Maharashtra, and Jharkhand (Anonymous, 2021-22).

The emergence of aggressive and invasive plant pathogens poses a serious risk to sustainable crop production and food security, significantly affecting ~~both~~ crop yield and quality. The agricultural sector, which depends heavily on fruit and vegetable cultivation, is particularly vulnerable. Chilli peppers are prone to ~~various~~ pathogens that hinder their growth and reduce yields. Initially identified in New Mexico (Leonian, 1919), Fusarium wilt is ~~now~~ prevalent in India, ~~where it leads~~leading to around 25% yield losses (Najar *et al*., 2006). ~~In the United States, the disease is observed in about 35% of states~~The disease is observed in about 35% of states in the United States. It has been reported that *Fusarium oxysporum* can cause the death of up to 56% of ~~chilli~~ chiliseedlings within ~~a 30 days period~~30 days following soil infection (Roberts *et al*., 2007; Ragab *et al*., 2012). Globally, Fusarium wilt has been associated with yield reductions ranging from 10% to 80% (Loganathan *et al*., 2013). Disease management for wilt in ~~chilli depends on the implementation of cultural, biological~~chili depends on implementing cultural, biological, and chemical methods. In many cases, chemical treatments offer the most practical means for managing plant diseases, being ~~both cost effective~~cost-effective and more efficient than other ~~available~~ options. Therefore, this research was conducted to manage wilt disease by using various fungicides under *in vitro*~~condition~~ conditions at the Plant Pathology Department, Dr. Sharadchandra Pawar College of Agriculture, Baramati, during the 2023-24 academic year.

2. MATERIAL AND METHODS

2.1 Isolation and identification of *Fusarium oxysporum* f. sp. *capsici*

The diseased plant samples were collected from ~~wilt infected chilli plot,~~ wilt-infected chili plots in Malegaon, Baramati. The diseased ~~chilli plants exhibiting symptoms of wilt, including yellowing and drying of leaves followed by~~chili plants exhibit wilt symptoms, including yellowing and drying of leaves, followed by the browning of the vascular tissues. The pathogen was isolated from infected roots/stems of ~~chilli~~ the chiliplant. The isolation was performed using the tissue isolation method on Potato dextrose agar. After 3 to 4 days, the growth of the pathogen was observed in Petri dishes. A pure culture was ~~subsequently~~ obtained using the hyphal tip culture method under aseptic conditions and maintained through PDA slants in test tubes.

The pathogen was identified by its distinct cultural and morphological characteristics. The culture achieved maximum growth after incubating for 7 days at 27 ± 1°C. The culture showed circular fluffy growth with a whitish and pinkish colour of the colony. The pigmentation of the colony was pinkish to brownish. Microscopic observations like mycelium, size and shape of conidia, and chlamydospores were examined and correlated with Booth's (1971) standard descriptions of *Fusarium* spp.

2.2 Pathogenicity of *Fusarium oxysporum* f. sp. *capsici*

~~The pathogenicity of *Fusarium oxysporum* f. sp. *capsici* was confirmed using the sick soil method, following Koch's postulates~~Following Koch's postulates, the pathogenicity of Fusarium oxysporum f. sp. capsici was confirmed using the sick soil method. Pots were inoculated with the ~~mass multiplied pathogen and chilli~~mass-multiplied pathogen, and chili seedlings (Pusa Jwala) were transplanted. After 10–12 days, inoculated plants showed characteristic symptoms such as leaf yellowing, browning, stem base browning and wilting. These symptoms matched those of naturally diseased plants, while uninoculated plants remained healthy. The pathogen was reisolated from diseased plants~~, cultured, and its~~

characteristics matched those from and cultured, and its characteristics matched those of the naturally wilted plants. This confirmed *Fusarium oxysporum* f. sp. *capsici* as the cause of chilli wilt, fulfilling Koch's postulates.

2.3 Evaluation of fungicides against *Fusarium oxysporum*f. sp.*capsici*

Eight different fungicides *viz.*, Tebuconazole 6.7 % + Captan 26.9 % w/w SC, Thiophenate Methyl 70% WP, Carbendazim 12% + Mancozeb 63% WP, Tebuconazole 50% + Trifloxystrobin 25% WG, Carbendazim 50% WP, Pyraclostrobin 20% WG, Chlorothalonil 75% WP and Azoxystrobin 18.2%+ Difenoconazole 11.4% SC were tested at concentrations of 500 ppm and 1000 ppm using the poison food technique (Nene and Thapliyal, 1993). The experiment was designed in a Complete Randomized Design (CRD), which included eight treatments along with a control treatment, a control treatment, and three replications for each treatment. For this experiment, Potato dextrose agar was used as the basal culture mediumPotato dextrose agar was used as the basal culture medium for this experiment.

2.3.1 Experimental procedure:

Stock solutions of each fungicide were prepared by dissolving the appropriate amount of active ingredient in a conical flask.The required quantities of each fungicide were aseptically incorporated into molten PDA (Potato Dextrose Agar)to achieve concentrations of 500 and 1000 ppm. To prevent bacterial contamination, Streptomycin was added to each flask prior to pouring the medium into sterilized Petri plates. A medium without any fungicide served as the control. This mixture was then aseptically dispensed at a volume of 20 ml per plate into sterile glass Petri dishes with a diameter of 90 mm and allowed to solidify at room temperature. Using sterilized cork borer, 5 mm diameter culture discs were cut from 7 days old a sterilized cork borer, 5 mm diameter culture discs were cut from 7-day actively growing culture of the test pathogen. These discs were then inoculated in the center of the PDA medium in the Petri dishes, positioned in reverse to ensure close contact between the test pathogen and the fungicide-treatedfungicide-treated PDA. Three PDA plates per treatment were prepared with fungicide and inoculated accordingly, while, In contrast, an equal number of PDA plates without fungicide were inoculated separately with the test pathogen maintained as untreated control. All plates were then incubated at a temperature of 27 ± 1°C for 7 sevendays. The inhibition percentage of the mycelial growth of the pathogen was calculated using the formula proposed by Vincent (1927). The data collected were subjected to statistical analysis following the procedures described by Panse and Sukhatme (1967).

$$\text{Percent Inhibition} = \frac{C - T}{C} \times 100$$

Where, C = Radial growth of fungus on the control plate

T = Radial growth of fungus on treated plate

3. RESULT AND DISCUSSION

The study confirmed that all fungicides tested at a concentration of 500 ppm and 1000 ppm effectively restricted the mycelial growth of *Fusarium oxysporum* f. sp. *capsici* compared to the control group. Inhibition levels increased with increase in an increase in the concentration of fungicide.

At 500 ppm concentration, the growth inhibition rates of the *Fusariumoxysporum* f. sp. *capsici* vary from 27.97 per cent to 100 per centpercent to 100 percent. Among the eight fungicides tested, Carbendazim 12% + Mancozeb 63% WP showed 100 per centpercent mycelial growth inhibition of the test pathogen. This treatment was superior compared to other treatments. The next subsequentbest treatment was Tebuconazole 50% + Trifloxystrobin 25% WG with growth inhibition of 90.36 per centpercent. Following this, Tebuconazole 6.7% + Captan 26.9% w/w SC showed 87.78 per centpercent growth inhibition. Carbendazim 50% WP showed moderate efficacy with a mycelial growth inhibition of 76.30 per cent, which was followed by Azoxystrobin 18.2% + Difenoconazole 11.4% SC with 60.19 per centpercent, followed by Azoxystrobin 18.2% + Difenoconazole 11.4% SC

with 60.19 percent growth inhibition. The least growth inhibition was observedwith Chlorothalonil 75% WP (51.11%) ~~and Pyraclostrobin 20% WG (46.86%),~~, Pyraclostrobin 20% WG (46.86%), and Thiophenate Methyl 70% WP (27.97%) among all tested fungicides. (Table 1, Fig.1, Plate 1)

At 1000 ppm concentration, the test pathogen showed inhibition percentages ranging from 51.86 ~~per cent to 100 per cent~~percent to 100 percent. Among the fungicides tested, Carbendazim 12% + Mancozeb 63% WP again showed 100 ~~per cent mycelial growth inhibition of~~ percent mycelial growth inhibition of the test pathogen and was the treatment with the highestefficacy compared to others. Again, the second most effective treatment was Tebuconazole 50% + Trifloxystrobin 25% WG with 93.88 ~~per cent~~percent growth inhibition. This was followed Tebuconazole 6.7% + Captan 26.9% w/w SC with 89.81 per cent growth inhibition. Carbendazim 50% WP also showed moderate effectiveness at 1000 ppm concentration with an 86.30 per cent growth inhibition. Other fungicides also found effective at this concentration include Azoxystrobin 18.2%+ Difenoconazole 11.4% SC (74.81%), Pyraclostrobin 20% WG (64.45%) and Chlorothalonil 75% WP (61.67%). The ~~least~~ slightestinhibition was found in Thiophenate Methyl 70% WP, with ~~a~~ growth inhibition of 51.86 ~~per cent~~percent.

The results of the current study showed that~~, at both the concentrations (500 ppm and 1000 ppm)~~ at both the concentrations (500 ppm and 1000 ppm), the mycelial growth was completely inhibited by Carbendazim 12% + Mancozeb 63% WP. Sanap*et al*. (2020) conducted a similar experiment ~~of evaluation of~~evaluating different fungicides at different concentrations against *Fusarium oxysporum* under *in vitro* conditions. They reported that, at all three concentrations (@ 1500, 2000 ~~and 2500 ppm), Carbendazim 25% + Mancozeb 50% showed complete inhibition of mycelial growth~~, and 2500 ppm), Carbendazim 25% + Mancozeb 50% showed complete inhibition of mycelial growth, followed by Tebuconazole 50% + Trifloxystrobin 25%. Ghimire *et al.* (2022) also reported that Saaf (Carbendazim 25% + Mancozeb 50%) inhibits 100% mycelial growth of *Fusarium* spp. followed by Nativo (Tebuconazole 50% + Trifloxystrobin 25%) with ~~inhibition rate of 87.04 per cent~~an inhibition rate of 87.04 percent. Bhujbal *et al.* (2021) conducted an *in vitro* study to evaluate fungicides against *Fusariumoxysporum*, finding that the combination of Carbendazim 12% + Mancozeb 63% WP completely inhibited the mycelial growth of the pathogen. Gabrekiristos*et al*. (2020) reported that URGI 75% WP, which contains Carbendazim and Mancozeb, effectively suppressed the mycelial development of *Fusarium oxysporum* f. sp. *capsici*, suggesting its potential for further *in vivo* testing. Additionally, Pimpale*et al*. (2023) assessed various fungicides against *Fusarium oxysporum* f. sp. *capsici* ~~and found~~. They found that Carbendazim 12% + Mancozeb 63% WP had the highest efficacy, achieving a maximum growth reduction of 93.72 ~~per cent~~%. These findings are consistent with Golakiya*et al.* (2018), Rao *et al.* (2020), Sahane*et al.* (2021), Chand and Singh (2021), Yadav *et al.* (2022), Kartha *et al.* (2023) and Buttar *et al.* (2023).

Table 1. Efficacy of various fungicides against *F. oxysporum*f. sp*. capsici*under *in vitro* conditions

Tr. No.	Treatments	Colony Diameter* (mm)		% Growth Inhibition	
		500 ppm	1000 ppm	500 ppm	1000 ppm
T_1	Tebuconazole 6.7 % + Captan 26.9 % w/w SC	11.00	9.17	87.78	89.81
T_2	Thiophenate Methyl 70%WP	64.83	43.33	27.97	51.86
T_3	Carbendazim 12% + Mancozeb 63% WP	0.00	0.00	100	100

T_4	Tebuconazole 50% + Trifloxystrobin 25% WG	8.67	5.50	90.36	93.88
T_5	Carbendazim 50%WP	21.33	12.33	76.30	86.30
T_6	Pyraclostrobin 20%WG	47.83	32.00	46.86	64.45
T_7	Chlorothalonil 75%WP	44.00	34.50	51.11	61.67
T_8	Azoxystrobin 18.2%+ Difenoconazole 11.4% SC	35.83	22.67	60.19	74.81
T_9	Control	90.00	90.00		
	S.E.(m)±	**0.25**	**0.38**		
	C.D. (0.01)	**0.74**	**1.12**		

*Mean of three replications

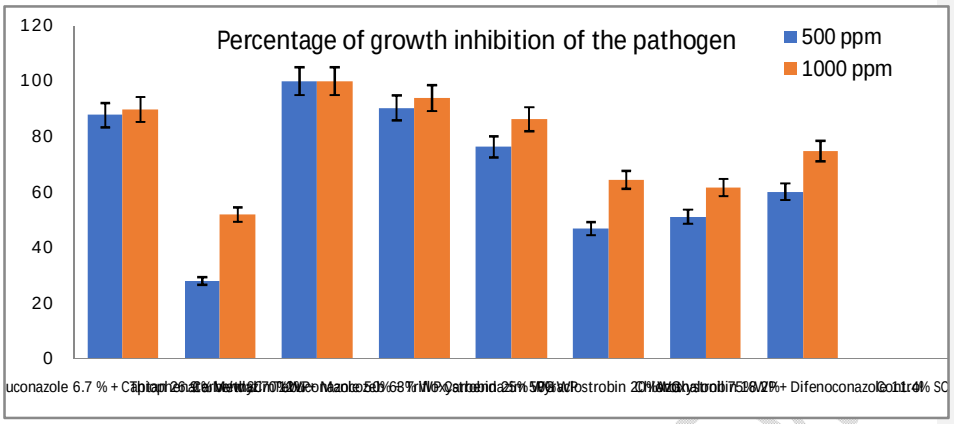

Fig. 1 ~~Per cent~~Percentage of growth inhibition of*Fusarium oxysporum*f. sp. *capsici*under *in vitro* conditions

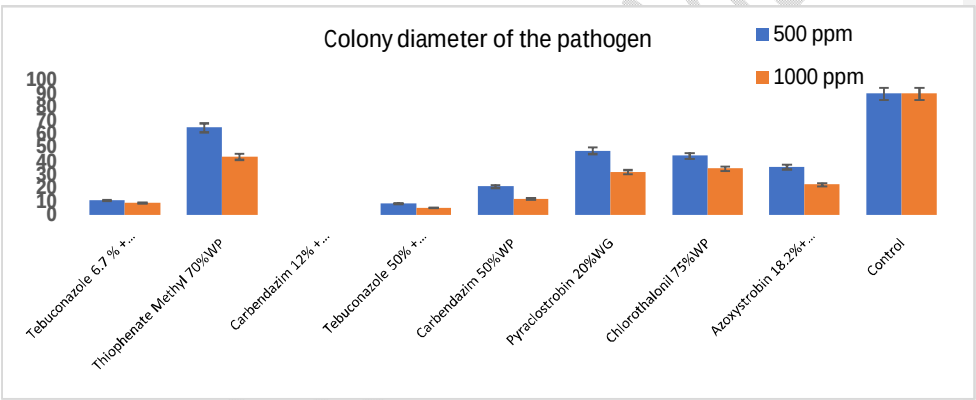

Fig.2 Colony diameter of*Fusarium oxysporum*f. sp. *capsici*

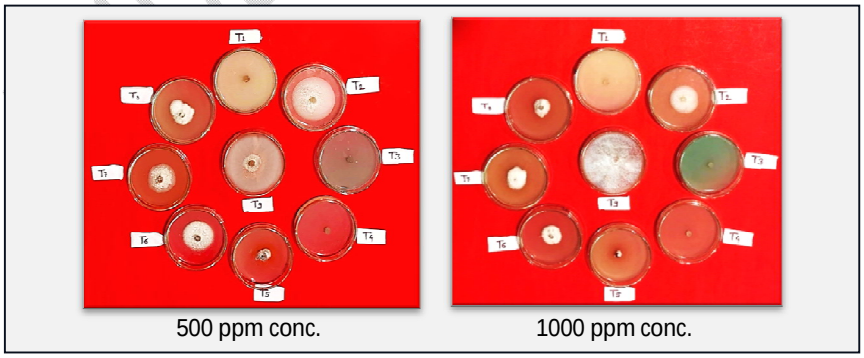

Plate 1: Evaluation of various fungicides against *Fusariumoxysporum*f. sp. *capsici*under*in vitro* conditions

4. CONCLUSION

The study demonstrated that all tested fungicides significantly inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *capsici*, with higher concentrations yielding greater inhibition. Among the fungicides, Carbendazim 12% + Mancozeb 63% WP was the most effective, achieving 100% inhibition at both 500 ppm and 1000 ppm. Tebuconazole 50% + Trifloxystrobin 25% WG and Tebuconazole 6.7% + Captan 26.9% w/w SC also exhibited high efficacy. Thiophanate Methyl 70% WP was the least effective in both concentrations.

DISCLAIMER

I hereby declare that ~~NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts. COMPETING INTERESTS: Authors~~no generative AI technologies such as large language models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during manuscript writing or editing. COMPETING INTERESTS: The authors have declared that no competing interests exist.

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