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

ABSTRACT

Chilli wilt disease, caused by *Fusariumoxysporum* f. sp. *capsici*, significantly threatenschilicultivation, impacting yield and quality. This study aimed to evaluate the fungi-toxic effects of various fungicides against *Fusariumoxysporum* 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 *Fusariumoxysporum*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: *Fusariumoxysporum f.sp. capsici, fungicides, growth inhibition, chili*

1. INTRODUCTION

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

India is the world's leading producer, consumer, and exporter of chili peppers. India exports chili to numerous international markets, including the USA, Canada, the UK, Saudi Arabia, Germany, Malaysia, Singapore, and others. Major 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 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 chilies. Other top dry chili-producing regions include Karnataka, Orissa, Maharashtra, Gujarat, Tamil Nadu, Assam, and Punjab. In the context of the production of green chilies in India, Karnataka stands as the top producer, contributing 1,006.96 thousand tonnes of production. Then, Madhya Pradesh produces 726.90 thousand tonnes, while Andhra Pradesh ranks third with 506.87 thousand tonnes of production.Other significantstates involved in green 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 crop yield and quality. The agricultural sector, which depends heavily on fruit and vegetable cultivation, is particularly vulnerable. Chilli peppers are prone to pathogens that hinder their growth and reduce yields. Initially identified in New Mexico (Leonian, 1919), Fusarium wilt is prevalent in India, leading to around 25% yield losses (Najar *et al*., 2006). The disease is observed in about 35% of states in the United States. It has been reported that *Fusariumoxysporum* can cause the death of up to 56% of chiliseedlings within 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 chili depends on implementing cultural, biological, and chemical methods. In many cases, chemical treatments offer the most practical means for managing plant diseases, being cost-effective and more efficient than other options. Therefore, this research was conducted to manage wilt disease byusing various fungicides under *in vitro*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 *Fusariumoxysporum*f. sp.*capsici*

The diseased plant samples were collected from wilt-infected chili plots in Malegaon, Baramati. The diseased 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 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 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 *Fusariumoxysporum*f. sp.*capsici*

Following Koch's postulates, the pathogenicity of Fusariumoxysporum f. sp. capsici was confirmed using the sick soil method. Pots were inoculated with the 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 and cultured, and its characteristics matched those of the naturally wilted plants. This confirmed *Fusariumoxysporum* f. sp. *capsici* as the cause of chilli wilt, fulfilling Koch's postulates.

2.3 Evaluation of fungicides against *Fusariumoxysporum*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, a control treatment, and three replications for each treatment. Potato 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 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-treated PDA. Three PDA plates per treatment were prepared with fungicide and inoculated accordingly. 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 27 ± 1°C for 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 *Fusariumoxysporum* f. sp. *capsici* compared to the control group. Inhibition levels increased with 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 percent to 100 percent. Among the eight fungicides tested, Carbendazim 12% + Mancozeb 63% WP showed 100 percent mycelial growth inhibition of the test pathogen. This treatment was superior compared to other treatments. The subsequentbest treatment was Tebuconazole 50% + Trifloxystrobin 25% WG with growth inhibition of 90.36 percent. Following this, Tebuconazole 6.7% + Captan 26.9% w/w SC showed 87.78 percent growth inhibition. Carbendazim 50% WP showed moderate efficacy with mycelial growth inhibition of 76.30 percent, 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%), 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 percent to 100 percent. Among the fungicides tested, Carbendazim 12% + Mancozeb 63% WP again showed 100 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 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 slightestinhibition was found in Thiophenate Methyl 70% WP, with growth inhibition of 51.86 percent.

The results of the current study showed that 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 evaluating different fungicides at different concentrations against *Fusariumoxysporum* 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, 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 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 *Fusariumoxysporum* f. sp. *capsici*, suggesting its potential for further *in vivo* testing. Additionally, Pimpale*et al*. (2023) assessed various fungicides against *Fusariumoxysporum* f. sp. *capsici*. They found that Carbendazim 12% + Mancozeb 63% WP had the highest efficacy, achieving a maximum growth reduction of 93.72%. 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

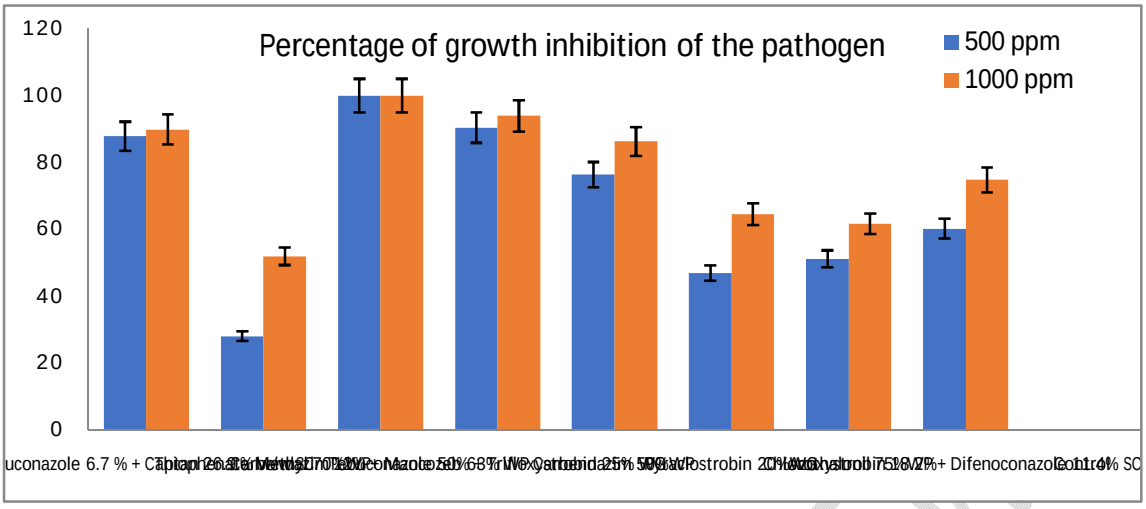

Fig. 1 Percentage ofgrowth inhibition of*Fusariumoxysporum*f. sp*. capsici*under *in vitro* conditions

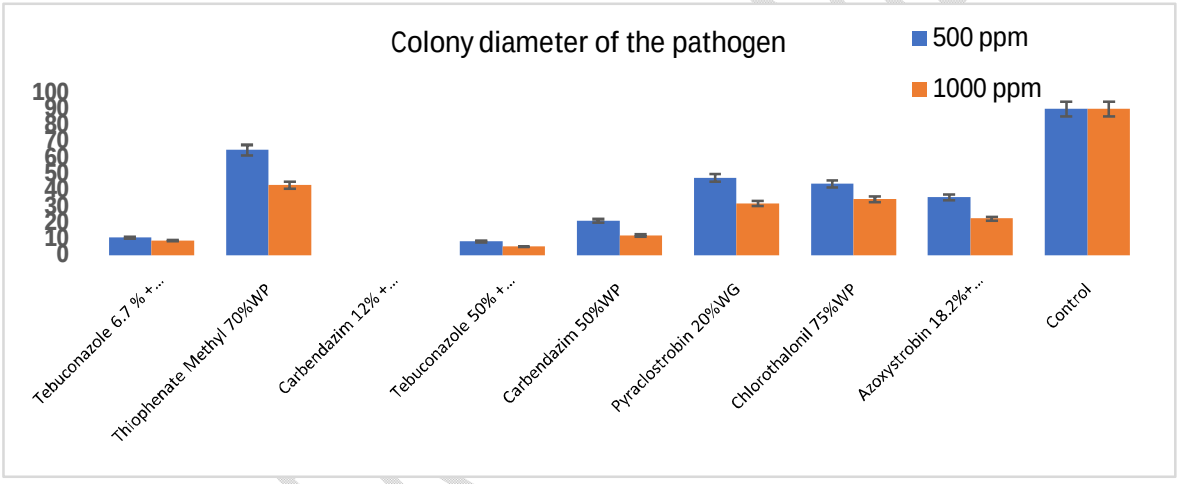

Fig.2 Colony diameter of*Fusariumoxysporum*f. sp*. capsici*

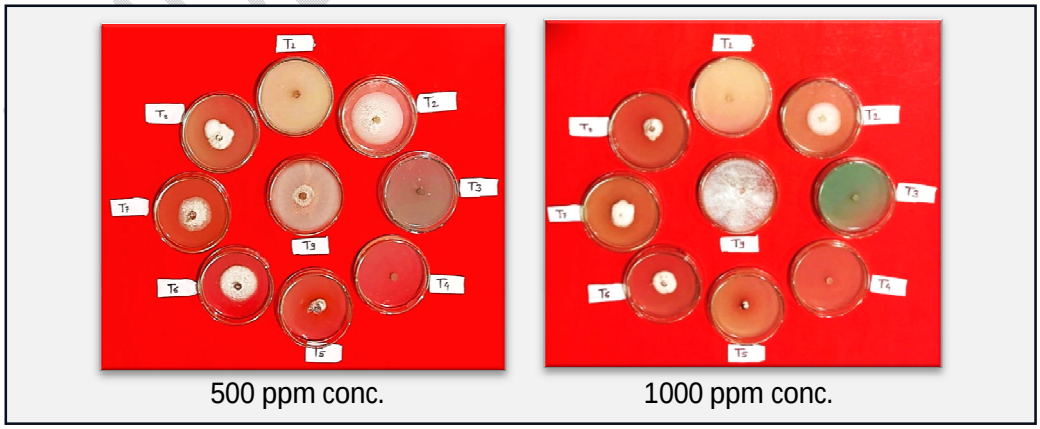

Plate 1: Evaluation of various fungicides against *Fusariumoxysporum*f. sp. *capsici*under*in vitro* conditions

4. CONCLUSION

The study demonstrated that all tested fungicides significantly inhibited the mycelial growth of *Fusariumoxysporum* 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 manuscript writing or editing. COMPETING INTERESTS: The authors have declared that no competing interests exist.

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