



Comparative *In vitro* Assessment of Fungicides for Managing *Fusarium oxysporum* f. sp. *capsici* in Chilli

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Chilli wilt disease, caused by *Fusarium oxysporum* f. sp. *capsici*, significantly threatens chili cultivation, impacting yield and quality. This study 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%+ Difenconazole 11.4% SC were tested at a concentration of 500 ppm and 1000 ppm using the

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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.

Keywords: *Fusarium oxysporum* 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 chili cultivation, affecting its marketability and export potential by reducing the quality and quantity of the produce. It involves various 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 significant states involved in green chili production are Bihar, Maharashtra, and Jharkhand [1].

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 [2], *Fusarium* wilt is prevalent in India, leading to around 25% yield losses [3]. 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 chili seedlings within 30 days following soil infection [4,5]. Globally, *Fusarium* wilt has been associated with yield reductions ranging from 10% to 80% [6]. 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 by using 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. MATERIALS AND METHODS

2.1 Isolation and Identification of *Fusarium oxysporum* 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 chili plant. 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 $27 \pm 1^\circ\text{C}$. 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 [7] standard descriptions of *Fusarium* spp.

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

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 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 *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%+ Difenconazole 11.4% SC were tested at concentrations of 500 ppm and 1000 ppm using the poison food technique [8]. 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 $27 \pm 1^\circ\text{C}$ for seven days. The inhibition percentage of the mycelial growth of the pathogen was calculated using the formula proposed by Vincent [9]. The data collected were subjected to statistical analysis following the procedures described by Panse and Sukhatme [10].

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

Where C = Radial growth of fungus on the control plate

T = Radial growth of fungus on treated plate

3. RESULTS 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 an increase in the concentration of fungicide.

At 500 ppm concentration, the growth inhibition rates of the *Fusarium oxysporum* 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 subsequent best 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% + Difenconazole 11.4% SC with 60.19 percent growth inhibition. The least growth inhibition was observed with 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 highest efficacy 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%+ Difenconazole 11.4% SC (74.81%), Pyraclostrobin 20% WG (64.45%) and Chlorothalonil 75% WP (61.67%). The slightest inhibition was found in Thiophenate Methyl 70% WP, with growth inhibition of 51.86 percent.

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%+ Difenconazole 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

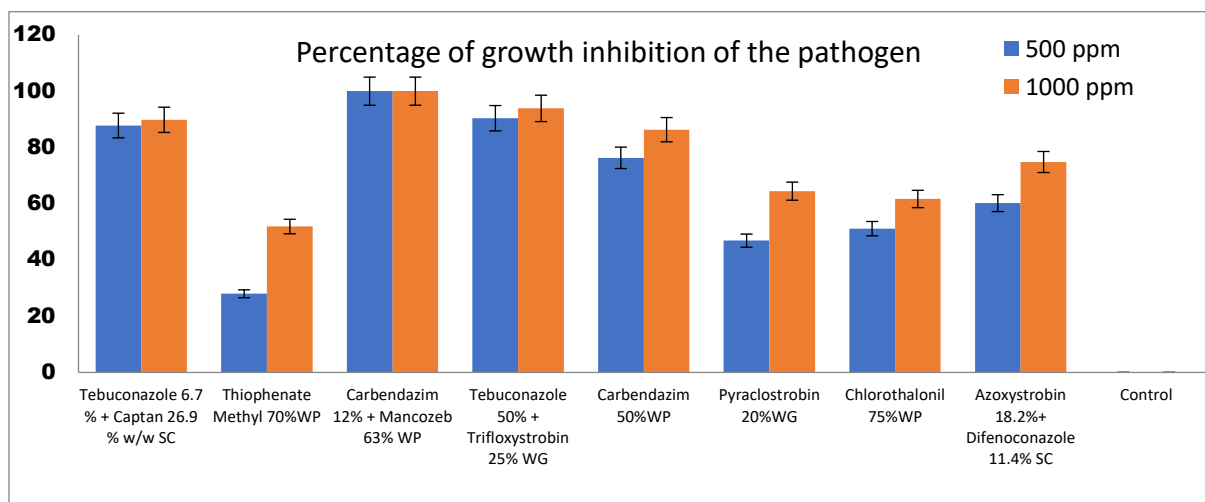


Fig. 1. Percentage of growth inhibition of *Fusarium oxysporum* f. sp. *capsici* under *in vitro* conditions

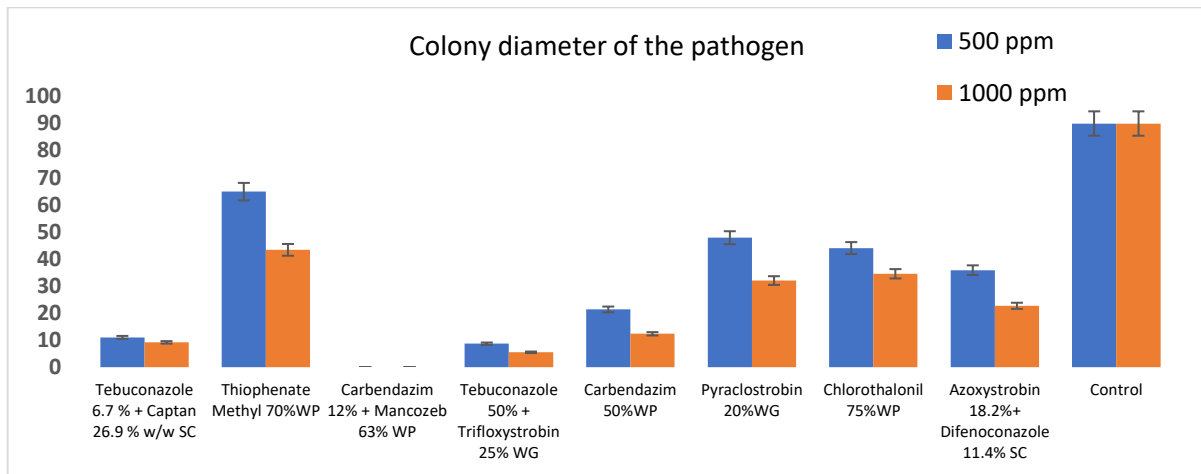


Fig. 2. Colony diameter of *Fusarium oxysporum* f. sp. *capsici*

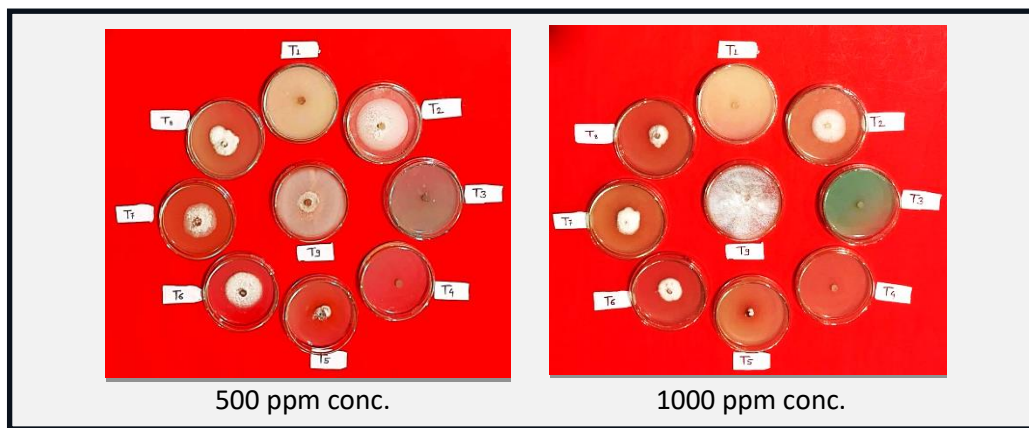


Plate 1. Evaluation of various fungicides against *Fusarium oxysporum* f. sp. *capsici* under *in vitro* conditions

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. [11] conducted a similar experiment 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, followed by Tebuconazole 50% + Trifloxystrobin 25%. Ghimire et al. [12] 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. [13] conducted an *in vitro* study to evaluate fungicides against *Fusarium oxysporum*, finding that the combination of Carbendazim 12% + Mancozeb

63% WP completely inhibited the mycelial growth of the pathogen. Gabrekiristos et al. [14] 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. [15] assessed various fungicides against *Fusarium oxysporum* 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. [16], Rao et al. [17], Sahane et al. [18], Chand and Singh [19], Yadav et al. [20], Kartha et al. [21] and Buttar et al. [22].

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 (ARTIFICIAL INTELLIGENCE)

Author(s) 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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