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Short communication

Efficacy of carbendazim and other fungicides against *Fusarium oxysporum* f. sp. *lycopersici* causing wilt of tomato

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Abstract

Wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* is becoming the cause of concern for tomato growing farmers as it has devastated around 80% crop in some instances. Farmers usually prefer chemical treatment over other methods applying various fungicides. In the present study, eleven *Fusarium* isolates were collected from tomato fields across various tomato-growing regions of Maharashtra.The efficacy of fungicide like carbendazim is evaluated against the collected *Fusarium* isolates. A minimum inhibitory concentration of carbendazim against all the isolates was measured out for all the eleven isolates. The sensitivity of the isolates ranged from 7µg/ml to 750µg/ml. Isolate Fol-1 showed lowest minimum inhibitory concentration while isolate Fol-7 showed highest minimum inhibitory concentration. Then the isolates with more MIC values were subjected to successive passages on plates containing carbendazim. Additionally, the passages are alternated between carbendazim and other fungicides. The results demonstrated that continuous exposure to carbendazim led to reduced fungal responsiveness to fungicide while inhibition of fungal growth was observed on the alternating fungicides.

Keywords: Carbendazim, *Fusarium oxysporum*, MIC, Passage, Tomato

Introduction

Tomatoes are widely recognized for their high nutritional value and are one of the freshest and most nutritious vegetables around the world (Poussio et al., 2018). They are also very affordable compared to other vegetables. Tomatoes are a crucial component of the daily diet, consumed both fresh and in various processed forms (Brookie et al., 2018). While several factors, including bacteria,viruses, nematodes and fungi can cause tomato diseases, *Fusarium* wilt remains a major threat to tomato production, causing significant losses that can range from 30% to 80% under favourable climatic conditions for the fungus (Kumar et al., 2021).

F.oxysporum is a soil borne fungus belonging to class Hyphomycetes that has a wide plant host range including both dicots and monocots. It causes wilt and root rot diseases in a wide variety of plants (Sonkar et al., 2014). Due to its economic impact, it reached fifth rank among all the economically important plant pathogens (Dean et al., 2012). Of the two *Forma specialis* that infect tomato plant, *Fusarium oxysporum* f. sp. *lycopersici* is found to cause tomato wilt (Edel-Hermann and Lecomte, 2019). Severe infections of this fungus lead to plant death, while mild cases result in stunted growth and reduced productivity (Hanan Aref, 2020; Joshi, 2018; McGovern, 2015; Srinivas et al., 2019; Takken and Rep, 2010). Many attempts were made to develop effective fungicide, but occurrences of resistance thwarted all the efforts (Zhao et al., 2021). The present study aimed to determine the optimal concentration of the widely preferred fungicide for effective disease management. The research also investigated the impact of prolonged exposure to the fungicide on the pathogen's sensitivity. Additionally, the study explored effective application strategies utilizing the same fungicides to mitigate disease occurrence while preventing the development of resistance. The findings revealed that a combination of three fungicides - carbendazim, mancozeb, and thiophanate methyl - effectively controlled *Fusarium* wilt at lower concentrations. These results provide valuable insights for recommending appropriate fungicide concentrations, either individually or in combination, for the effective management of *Fusarium* wilt in tomatoes.

Materials and Methods

Isolation of pathogens

Tomato plants exhibiting symptoms of wilt were

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systematically collected from 11 tomato-growing districts across Maharashtra during August and September 2023. A total of 25 symptomatic samples were obtained, out of which 11 were selected for detailed investigation. The diseased plant material was carefully dissected into smaller fragments, followed by surface sterilization using 70% alcohol for 2-3 minutes. Subsequently, the samples were thoroughly rinsed with sterile distilled water three times. The roots were then aseptically inoculated onto Czapek Dox Agar (CDA) plates supplemented with 30 mg/L streptomycin to suppress any bacterial contamination. Incubation was carried out at a controlled temperature of 28±2°C for seven days. The fungi were identified using relevant mycological literature (Kharbanda and Stevens,1988., Subramanian, 1971., Barnett and Hunter, 1998). Eleven isolates of *F. oxysporum* were obtained from the collected samples. It was then cultured on CDA slants and stored at 4°C for further study.

Determination of Minimum Inhibitory Concentration (MIC) of carbendazim against collected isolates of F. oxysporum

The sensitivity of *Fusarium oxysporum* f. sp. *lycopersici* (Fol) to the fungicide carbendazim was assessed using the food poisoning technique (Dekker and Gielink, 1979). CDA medium plates were prepared, each containing varying concentrations of carbendazim. After solidification of the medium, 8 mm agar discs were excised from actively growing Fol mycelium and placed inverted on the agar surface. Incubation was carried out at a constant temperature of 28 ± 2°C, with a 12-hour cycle of alternating light and dark periods. Linear mycelial growth was monitored at regular intervals. Plates without carbendazim served as 'Control'. Each isolate was performed in triplicate. Initially the isolates were grown on plates containing broad range of carbendazim concentration. Then it was narrowed down to the range, where the growth of mycelium was reduced, to arrive at the specific minimum inhibitory concentration of the fungicide. The concentration of carbendazim for an isolate which showed minimum growth of the mycelium and beyond which no growth was observed was selected as the MIC for that isolate. From the obtained results two isolates were selected, one that showed lowest MIC and the one that showed highest MIC. Both these isolates were subjected to further study.

Effect of exposure of isolates to fungicides continuously and alternately

To study the impact of prolonged exposure to fungicides on pathogen, the approach of continuous, alternate and mixed passage was applied. Continuous and alternate treatment of two fungicides with different modes of action and mixture of all on the development of carbendazim resistance in both Fol-1 and Fol-7 was studied. To study the effect of continuous passage on mycelial growth, the sensitive Fol-1 isolate was cultured on plates containing carbendazim at its MIC level. After seven days the mycelium from this initial plate was cultured on another plate having the same concentration of carbendazim as the previous one. These process was repeated eight times. The same procedure was followed for the resistant Fol-7 isolate.

In another instance, the alternative approach was followed where plates amended with other fungicide altered with carbendazim. The sensitive Fol-1isolate was cultured on plates containing carbendazim at its MIC level. After seven days the mycelium from this plate was inoculated on a plate containing another fungicide with same concentration as that of carbendazim. These process was continued upto eight passages. The other fungicides used were mancozeb and thiophanate methyl. The same procedure was followed for the resistant Fol-7 isolate.

In the mixed approach, all three fungicides viz. carbendazim, mancozeb and thiophanate methyl were mixed at the same concentration in CDA plates. The sensitive isolate was cultured on these plates for eight successive passages. The same procedure was followed for the resistant Fol-7 isolate. In all three approaches, the percent inhibition of mycelial growth was calculated as follows(Rini and Sulochana, 2007)-

$$PI = \frac{C - T}{C} \text{ X } 100$$

Where, PI = Percent inhibition of mycelial growth
C = Radial growth of pathogen in control plates
T = Radial growth of pathogen in test plates

Results

Determination of MIC of Fol isolates

A comparative data of the minimum inhibitory concentration of all 11 Fol isolates demonstrates that three isolates Fol-1, Fol-2 and Fol-11 were found to be sensitive to the fungicide carbendazim. In comparison, two isolates Fol-6 and Fol-7 were shown to be resistant to the fungicide. The remaining six isolates Fol-3, Fol-4, Fol-5, Fol-8, Fol-9 and Fol-10 have shown moderate response to the fungicide. Table 1 and Table 2 show the effect of the concentration of carbendazim on the growth of fungal isolates Fol-1 and Fol-7 respectively. From this data, the MIC values of carbendazim for that particular isolate were obtained. Table 3 shows the MIC values of all the isolates. It is evident from Table 1 that there was incremental growth of mycelium in the plates from day 1 to day 7. When the concentration of carbendazim increased above 7 µg/ml there was no growth of Fol-1 isolate. At 7 µg/ml, the growth started to appear at around the third and fourth day. However, it was very low compared to the lower

Table 1. In vitro sensitivity of Fol-1 to carbendazim.

Sr. No.	Concentration in µg/ml	Days and Radial growth in mm						
		1	2	3	4	5	6	7
1	Control	16.33 (±0.67)	27.33 (±0.88)	37.67 (±0.33)	50 (±0.58)	64.67 (±0.33)	72.67 (±0.33)	81 (±0.58)
2	4	12.33 (±0.33)	26.33 (±0.88)	36 (±0.58)	48 (±1.00)	61 (±1.15)	67.67 (±1.45)	72.33 (±0.67)
3	5	10.67 (±0.67)	18 (±1.15)	28.33 (±0.88)	38 (±1.00)	46.67 (±0.88)	52.67 (±0.67)	61.67 (±0.33)
4	6	8.33 (±0.88)	13.67 (±0.67)	17.33 (±0.88)	18.33 (±0.33)	20.67 (±0.33)	22.67 (±0.33)	24 (±0.00)
5	7	0 (±0.00)	0 (±0.00)	8 (±0.00)	8(±0.00)	9 (±0.00)	9(±0.00)	10.33 (±0.00)
6	8	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)
7	9	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)

Values of radial growth are means of three replicate determinations (± Standard Error of a mean).

Table 2. In vitro sensitivity of Fol-7 to carbendazim.

Sr. No.	Concentration in µg/ml	Days and Radial growth in mm						
		1	2	3	4	5	6	7
1	Control	18.67 (±0.88)	33.67 (±0.88)	45.67 (±1.45)	65.33 (±1.67)	73.33 (±1.67)	79.67 (±0.88)	84.33 (±1.67)
2	710	15.33 (±0.33)	21.67 (±0.33)	29 (±0.58)	34 (±0.58)	37.67 (±0.33)	41.67 (±0.88)	50.67 (±0.33)
3	720	11.67 (±0.33)	15.33 (±0.33)	22.67 (±0.67)	29.00 (±0.58)	31.67 (±0.67)	36.33 (±0.88)	44.33 (±0.88)
4	730	9.33 (±0.67)	14.33 (±0.67)	21 (±1.00)	24.67 (±0.67)	29.67 (±0.88)	35.67 (±0.88)	41.67 (±0.33)
5	740	8.67 (±0.33)	11 (±0.00)	16 (±0.58)	23.33 (±0.88)	25.33 (±1.45)	30 (±1.15)	38 (±0.58)
6	750	0 (±0.00)	0 (±0.00)	8 (±0.00)	8.33 (±0.33)	9.33 (±0.33)	10 (±0.00)	10.33 (±0.33)
7	760	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0(±0.00)

Values of radial growth are means of three replicate determinations (± Standard Error of a mean).

Table 3. Minimum Inhibitory Concentration (MIC) of carbendazim against *Fusarium oxysporum* f. sp. *lycopersici* isolates causing tomato wilt

Sr. No.	Isolate	MIC (µg/ml)
1	Fol-1	7
2	Fol-2	9
3	Fol-3	60
4	Fol-4	75
5	Fol-5	50
6	Fol-6	560
7	Fol-7	750
8	Fol-8	60
9	Fol-9	50
10	Fol-10	80
11	Fol-11	9

concentrations of fungicide. Likewise, in the case of Fol-7 isolate, the growth of mycelium was nil beyond 750 µg/ml as shown in Table 2. So, for isolates Fol-1 and Fol-7, the MIC was determined at 7 µg/ml and 750 µg/ml respectively.

Effect of different fungicides on Fol isolates: individual and in combination

For Fol-1 isolate, the CDA plates were prepared with the same concentration of carbendazim at its MIC level of 7µg/ml. In continuous passage, the successive culturing of the fungus from one plate to the other eight times was done keeping the concentration of the carbendazim constant at 7µg/ml in each plate. Table 4 shows the gradual increase in the radial growth of a fungus from plate 1 to plate 8. In the second case, the plates were prepared with mancozeb and culturing was done alternately. Initially, the Fol-1 was cultured on the plate containing carbendazim (7µg/ml). Then fungus from this plate was cultured on plate containing mancozeb at 7µg/ml concentration. This process was repeated eight times. For three successive passages the growth of a fungus was observed but after that the growth reduced to nil. The same process was followed with the fungicide thiophanate methyl where growth was reduced to nil after fourth passage.The growth of the same isolate was completely inhibited in a mixed passage, where the mixture of all three fungicides was used.

Table 5 shows the effect of exposure of Fol-7 isolate to carbendazim and other fungicides. In continuous passage, the radial growth of a fungus appears to increase when

Table 4. Effect of exposure of Fol-1isolate to carbendazim continuously, alternating with other fungicides and combination of all in eight successive passages (*In vitro*)

Sr. No.	Fungicides	Passage Number (Radial growth in mm)							
		1	2	3	4	5	6	7	8
1	Carbendazim Continuous	12 (±0.58)	17.00 (±0.58)	19.50 (±0.29)	20.67 (±0.33)	23.00 (±0.58)	23.17 (±0.73)	24.00 (±0.29)	27.17 (±0.73)
2	Carbendazim alters Mancozeb	12 (±0.58)	14.67 (±0.33)	8 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)
3	Carbendazim alters Thiophanate methyl	12 (±0.58)	15 (±0.33)	10 (±0.00)	8 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)
4	Carbendazim + Mancozeb + Thiophanate methyl	12(±0.00)	0(±0.00)	0(±0.00)	0(±0.00)	0(±0.00)	0(±0.00)	0(±0.00)	0(±0.00)

Values of radial growth are means of three replicate determinations (± Standard Error of a mean).

Table 5. Effect of exposure of Fol-7 to carbendazim continuously and alternating with other fungicides in eight successive passages (*In vitro*)

Sr. No.	Fungicides	Passage Number (Radial growth in mm)							
		1	2	3	4	5	6	7	8
1	Carbendazim Continuous	11.17 (±0.60)	12.50 (±0.29)	13.50 (±0.50)	16.83 (±0.44)	20.83 (±0.44)	21.17 (±0.17)	23.50 (±0.29)	24.33 (±0.17)
2	Carbendazim alters Mancozeb	11.17 (±0.60)	14 (±0.58)	10.33 (±0.17)	8 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)
3	Carbendazim alters Thiophanate methyl	11.17 (±0.60)	15.33 (±0.44)	12 (±0.00)	12 (±0.00)	8 (±0.00)	0 (±0.00)	0 (±0.00)	0 (±0.00)
4	Carbendazim + Mancozeb + Thiophanate methyl	11.17(±0.00)	8(±0.00)	0(±0.00)	0(±0.00)	0(±0.00)	0(±0.00)	0(±0.00)	0(±0.00)

Values of radial growth are means of three replicate determinations (± Standard Error of a mean).

continuously sub cultured on successive CDA plates amended with 750µg/ml carbendazim, the MIC value of isolate Fol-7. When carbendazim plates altered with mancozeb, the growth of a fungus was nil after fourth passage. While when altered with thiophanate methyl the growth was seem to reduce to nil after the fifth passage. In a mixed approach, the growth of fungus completely inhibited in the third passage.

Discussion

The management of *Fusarium* wilt in tomato cultivation has been extensively studied, with various fungicidal treatments demonstrating significant efficacy. Fungicides tested at varying concentrations of 50, 100, and 150 ppm showed effective inhibition of mycelial growth compared to the untreated control (Dholu et al., 2022). Further, Prochloraz and Bromuconazole emerged as the most effective fungicides against *F. oxysporum*, significantly reducing disease infestation in both *in vitro* and *in vivo* conditions, followed by Benomyl and Carbendazim (Amini and Sidovich., 2010). In the present study, similar results with MIC at 7 and 750 ppm was observed with the total inhibition of the mycelial growth. However, Akram highlighted concerns regarding resistance development due to the overuse of fungicides with resistance being influenced by the evolutionary adaptability

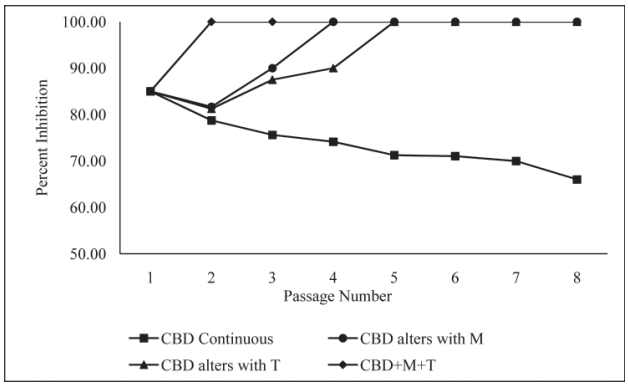

Figure 1. Percent inhibition of Fol-1 isolate with passages on carbendazim alone, carbendazim altered with mancozeb, carbendazim altered with thiophanate methyl and combination of all three fungicides.

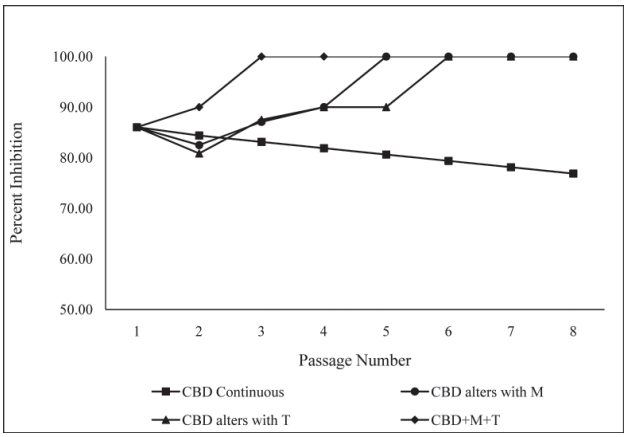

Figure 2. Percent inhibition of Fol-7 isolate with passages on carbendazim alone, carbendazim altered with mancozeb, carbendazim altered with thiophanate methyl and combination of all three fungicides.

and fitness of resistant genotypes (Akram et al., 2010). The efficacy of chemical fungicides is increasingly challenged by the development of resistance in *Fusarium* populations, particularly to fungicides such as phenamacril (Poznanski et al., 2025; Joe et al., 2025). As evident from Figure 1, when subjected to continuous passage, Fol-1 isolate showed continuous reduction in percent inhibition from day 1 to day 8. This indicates the possible development of resistance in the pathogen. The same type of response was shown by the resistant isolate Fol-7 as revealed by figure 2. The continuous passage on carbendazim show gradual reduction in the percent inhibition, indication of possible resistance development.

The effectiveness of fungicides can be influenced by their mode of action and the mechanisms of resistance that pathogens may develop. Continuous use of one fungicide can lead to increased fungal resistance, thereby compromising disease management strategies (Hassan 2020; Poznanski et al., 2025). To mitigate this issue, it is recommended to use a combination of fungicides with different modes of action or to rotate fungicides to prevent resistance development. Research indicates that mixing fungicides can slow the development of resistance compared

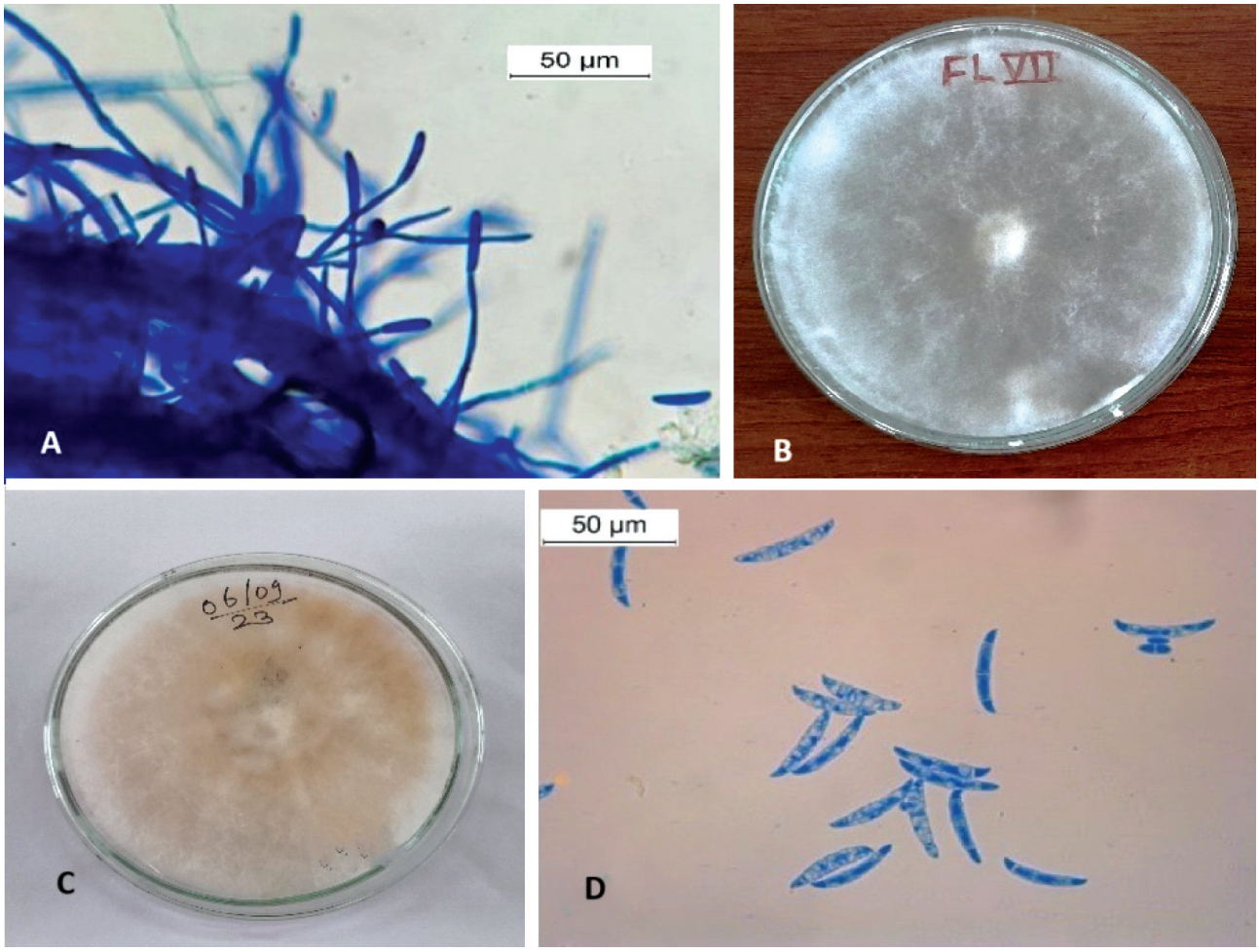

Figure 3. **A** - Conidia with Conidiophore, **B** and **C** – Colony Morphology of Fol-7 and Fol-2, **D** - Macroconidia

to using a single type (Poznanski et al., 2025). The combination of Mancozeb and Thiophanate Methyl proved to be the most effective, resulting in the lowest disease incidence of 5.3% (Gulya et al., 2023). Similarly, Azoxystrobin, both alone and in combination with Difenoconazole, showed strong antifungal activity, with the combination achieving an 82.79% inhibition rate and Azoxystrobin alone reaching 61.04% inhibition at 250 ppm after 10 days (Poudel and Osti, 2024). The use of copper oxychloride and Metalaxyl + Mancozeb demonstrated minimal negative effects on plant height and yield, with the tallest plants measuring 10.96 feet and 9.38 feet (Baloch et al., 2021). Among the tested fungicides, Carbendazim 50% WP and the combination of Carbendazim 25% + Mancozeb 50% WS exhibited complete growth inhibition (100%) (Gadhave et al., 2020). Carbendazim 12% + Mancozeb 63% WP and Tebuconazole 50% + Trifloxystrobin 25% WG demonstrated significant efficacy at 200 ppm (Sahoo et al., 2023). Among systemic fungicides, Carbendazim was the most effective, achieving a 99% inhibition rate at higher concentrations, while Propiconazole showed 98% inhibition at 500 ppm. In non-systemic fungicides, Propineb exhibited the highest growth inhibition of 99.50% at higher concentrations, followed by Chlorothalonil at 73.83% inhibition at 2000 ppm. Additionally, the combined fungicide treatment of Carboxin + Thiram achieved a remarkable 99.50% inhibition at 1000 ppm (Chaudhari and Patel, 2024). When the isolates Fol-1 and Fol-7 subjected to alternate treatment of carbendazim with mancozeb and thiophanate methyl separately, the percent inhibition appears to increase within first few passages. While the combination of all three fungicides effectively inhibited the growth of pathogen in the first passage itself. As shown in figure 2, the alternate passages of Fol-7 isolate on carbendazim with mancozeb and thiophanate methyl have shown the inhibition of 100 % after fifth passage. The mixed passage where all three fungicides were used have shown to completely inhibit the growth of pathogen in the third passage.

Conclusion

This study arrived at two MIC levels of carbendazim fungicide, one for the most sensitive pathogen and one for the most resistant. These values can be directive in chemical

control of the *Fusarium* wilt of tomato in the most extreme scenarios of the disease.The sensitive Fol-1 isolate was relatively more responsive to the fungicides than the resistant Fol-7 isolate. Moreover, it was found that the commonly used fungicide carbendazim, when used alone as a foliar spray, could not completely eradicate the pathogen from the crop. But when used alternately with other fungicides can effectively control the disease. This approach can eliminate the potential development of carbendazim resistance in the *Fusarium*. However, using a mixture of carbendazim, mancozeb and thiophanate methyl could be used in controlling the pathogen effectively.

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