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Harnessing Biocontrol and Fungicide Synergy for Efficacious Prophylaxis of Chilli Fusarium Wilt

Aabida*[1], Deepak Kumar[1], MD Thabrez[1], Ifra Altaf[2] and Ansab Manzoor[1]

[1]*Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, India*
[2]*Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology, Srinagar, India*

ABSTRACT

The study assessed the efficacy of several fungicides and bio-control agents against Fusarium oxysporum f. sp. capsici and the compatibility of fungicides with effective bio-control agents under in vitro conditions. Trichoderma harzianum exhibited the highest efficacy among bio-control agents, significantly reducing mycelial growth of the pathogen with 77.77% inhibition compared to the control, followed by Trichoderma asperellum and Pseudomonas fluorescens. Among fungicides, Carbendazim + mancozeb and tebuconazole + trifloxystrobin showed 100 % suppression of mycelial growth of the pathogen at both recommended and half of the recommended dose compared to the control. Propineb demonstrated moderate efficacy, allowing minimal mycelial inhibition of 79.26% and 72.92% of the pathogen at the recommended and half of the recommended dose, respectively. The compatible evaluation of fungicides with bio-control agents, found that Propineb was highly compatible with Trichoderma harzianum and Trichoderma asperellum, showing 100% growth at the half-recommended dose, while the other fungicides showed the least compatibility. Challenges of the study were compatibility issues between bio-agents and the most effective fungicides. The outcome of the study endorsed that amalgamating Trichoderma spp. with Propineb can be effective for management of the fusarium wilt in chili and encourages reducing the application dosage of propineb to a moiety, making it more eco-friendly.

Keywords: *Chilli, wilt, Fusarium, bio-agents, fungicides, management, compatibility, Trichoderma.*

Introduction

Chilli (*Capsicum annuum* L.), a vegetable and spice crop from the Solanaceae family, is very important to the world's economy. It is renowned for its diverse culinary applications, ranging from mild to intensely hot varieties, each contributing distinct Flavors to cuisines worldwide. The versatility of chilies extends beyond culinary delight. The chili fruits are a rich source of essential nutrients such as vitamins A, B, and C, providing vital dietary supplements, particularly in regions with nutritional deficiencies [10]. Moreover, the bioactive compounds found in chilies, including capsaicinoids, carotenoids, volatile oils, and fatty acids, not only impart their characteristic pungency but also possess significant antioxidant and anti-inflammatory properties [17]. These attributes underscore the nutritional and medicinal value of chili in promoting human health. Despite its nutritional and economic significance, chili production faces formidable challenges from both biotic and abiotic stresses. Among the biotic stresses, Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *capsici,* is notable as a significant biotic stressor that affects chili plants all over the world. Fusarium wilt poses a severe threat to chili cultivation, causing substantial yield losses under favourable environmental conditions. The disease manifests as vascular discoloration and wilting of plants, leading to necrosis and ultimately death in severe cases [9]. The pathogen persists in soil and spreads through infected plant debris, making it challenging to manage once established in agricultural fields [5].

Efforts to manage Fusarium wilt have predominantly relied on chemical fungicides, but these approaches often pose environmental and health risks while being economically burdensome for farmers [11]. Furthermore, the evolving nature of the pathogen and its ability to develop resistance against fungicides underscore the need for sustainable and integrated disease management strategies [15]. Despite extensive research on Fusarium wilt in chilies globally, including in India, there remains a significant research gap concerning the comprehensive understanding of compatibility between biocontrol agents and fungicides and the development of sustainable and locally adaptable management strategies. By leveraging both biocontrol and conventional techniques, the study seeks to explore environmentally friendly and economically viable strategies for controlling Fusarium wilt in chili crops. The findings are expected to contribute to the development of practical recommendations for farmers, extension workers, and policymakers to enhance chilli production sustainability while mitigating the impact of Fusarium wilt.

Methodology

Efficacy of Bio-control Agents against *Fusarium oxysporum f.sp. capsici*

Bio-agents such as *Trichoderma harzianum, Trichoderma asperellum,* and *Pseudomonas fluorescens* were evaluated for their antagonistic effects against *Fusarium oxysporum* f. sp. *capsici* using the dual culture technique [13].

Corresponding Author:* ***Aabida*

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The fungal antagonists were cultured on potato dextrose agar (PDA) plates, while the bacterial antagonist was cultured on nutrient agar (NA) plates and incubated at 25±2°C in a BOD incubator for 3-4 days. For evaluating the fungal biocontrol agents, a 5 mm mycelial disc of *Fusarium oxysporum* f. sp. *capsici* was placed at one end of the Petri dish, with the antagonistic fungi (*T. asperellum* and *T. harzianum*) placed at the opposite end. For the bacterial antagonist evaluation, *P. fluorescens* was streaked on one end of the Petri dish, with a 5 mm mycelial disc of *F. oxysporum* f. sp. *capsici* placed at the other end. Plates were incubated at 25±1°C in a BOD incubator with alternating 12-hour light and dark periods. Five replications were maintained for each antagonist interaction in a completely randomized design. Plates inoculated only with the test fungus served as control. Observations were recorded after contact between the pathogen and the antagonist. Mycelial growth was measured by drawing two perpendicular lines on the back of each Petri dish and colony diameter was measured in both directions. The average diameter of the colony in both directions was expressed in millimeters [8]. For irregular or wavy growth patterns, the average of the longest and shortest diameters was used [4]. The inhibition of mycelial growth by the antagonists was recorded, and the percentage inhibition of radial mycelial growth compared to the control was calculated using Vincent's (1947) formula [16].

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

Where,
I = Per cent inhibition
C = Colony diameter in control

Compatible efficacy of fungicides against *Fusarium oxysporum* f. sp. *capsici* and effective bio-agents

The effectiveness of six fungicides was tested *in vitro* against the pathogen and effective fungal bio-agents (*T. asperellum* and *T. harzianum*) using the poisoned food technique [14]. The fungicides included two systemic ones, Propiconazole (25 EC) and Flusilazole (40 EC); two non-systemic ones, Propineb (70 WP) and Captan (50 WP); and two combination products, Carbendazim 12% + Mancozeb 63% WP and Trifloxystrobin 25% + Tebuconazole 50% WG. Each fungicide was evaluated at two concentrations: the recommended dose and half of the recommended dose. To prepare the test media, the desired amount of each fungicide was added to 60 ml of sterilized and liquefied potato dextrose agar medium, which was then shaken to ensure homogeneity. Twenty ml of this amended medium was poured aseptically into three 90 mm Petri dishes for each treatment, allowing it to solidify. Control plates without fungicide were also prepared. Each Petri dish was inoculated with a 5 mm mycelial disc from a 4-day-old culture of the pathogen using a sterile cork borer and incubated at 25±2°C. The experiment was set up using a completely randomized design (CRD). Radial growth of the colonies was recorded once the control plates displayed complete fungal growth. The radial growth was measured using the methods described by Lilly and Barnett (1951) and Brown (1923). The per centage inhibition of mycelial growth compared to the control was calculated using Vincent's (1947) formula.

Results and Discussion

Efficacy of different bio-control agents against *F. oxysporum* f. sp. *Capsici*

Under *in vitro* conditions, all treatments were highly effective in reducing the growth of the pathogen compared to the untreated control. *Trichoderma harzianum* proved to be the most successful treatment in slowing the mycelial proliferation of *Fusarium oxysporum* f. sp. *capsici*. *Pseudomonas fluorescens* exhibited the maximum mycelial growth (30.60 mm) of *F. oxysporum* and minimum growth inhibition (58.33%), whereas *T. harzianum* exhibited the minimum mycelial growth (16.59 mm) and maximum growth inhibition (77.70%) followed by *Trichoderma asperellum* which exhibited the mycelial growth of 25.75 mm and growth inhibition of 65.27% (Table 1; Plate 1). The antagonistic effects of Trichoderma are attributed to the production of various toxic and antibiotic metabolites, such as trichodermin, trichodermol, harzianum-A, harzianolide, T39-butenolide, terpenes, and polypeptides [2], as well as extracellular hydrolytic enzymes [6]. These compounds contribute to the inhibition, competition, and mycoparasitism of Fusarium species. *T. harzianum* was also found most effective biocontrol agent against *F. oxysporum,* causing wilt disease in crops like safflower, tomato, onion, and chili [12].

Compatible efficacy of fungicides against *Fusarium oxysporum* f. sp. *capsici* and effective bio-agents

Examining the results (Table 2 and plate 2) made it abundantly evident that, every fungicide considerably impeded the radial growth of *Fusarium oxysporum* f. sp*. capsici.* When evaluated at half of the recommended dose, Carbendazim + Mancozeb and Tebuconazole + Triflustrobin exhibited 100 per cent mycelial growth inhibition of *Fusarium oxysporum* f. sp*. capsici*. Flusilazole was the next most effective fungicide which exhibited a radial mycelial growth of 8.50 mm and mycelial growth inhibition of 89.63 percent. It was followed by Propiconazole which exhibited the radial mycelial growth of 10.30 mm and mycelial growth inhibition of 87.43 percent. Propineb was the least effective which exhibited highest radial growth (13.63 mm) and lowest mycelial growth inhibition (72.92%). The evaluation of fungicides at the recommended dose revealed a similar pattern of mycelial growth inhibition. Carbendazim + mancozeb and tebuconazole + trifloxystrobin were again found most effective, which completely inhibited (100%) mycelial growth of *Fusarium oxysporum* f. sp. *capsici*. Flusilazole was next best in inhibiting the growth of the pathogen, with a radial growth of 6.1 mm followed by Propiconazole with the radial growth of 7.4 mm and mycelial growth inhibition of 86.38 percent. Propineb proved to be the least efficient fungicide, exhibiting the highest radial growth (16.87 mm) and lowest mycelial growth inhibition (79.26 %). Tebuconazole 50% + Trifloxystrobin 25% WG was also found most effective fungicide in suppressing the mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* [1][7].

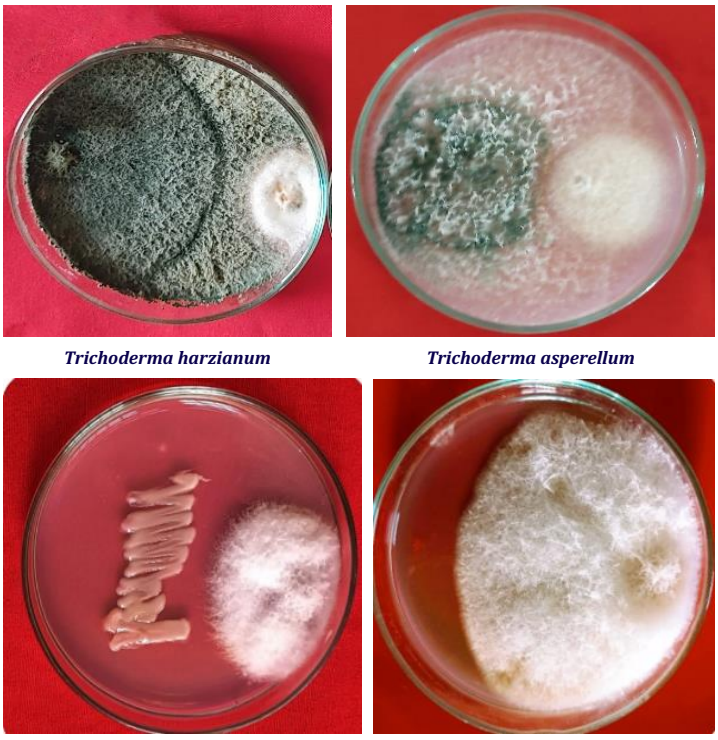
Tebuconazole + Trifloxystrobin and Carbendazim + Mancozeb also completely inhibited the growth of *Trichoderma harzianum*, attaining 100% inhibition, at half the recommended dose. Flusilazole showed radial growth of 7.33 mm with inhibition of 91.85%. Propineb demonstrated radial growth of 90.00 mm and growth inhibition of 0.00 per cent respectively, indicating that it was highly compatible for the growth of *Trichoderma harzianum*. Tebuconazole + Trifloxystrobin, Carbendazim + Mancozeb, Propiconazole, and Flusilazole, on the other hand, totally inhibited the growth of *Trichoderma harzianum* at the recommended dosage, resulting in 0.00 mm mycelial growth and 100 percent inhibition. Radial growth measurement in the Captan treatment was 7.67 mm, with mycelial growth inhibition of 91.48 per cent. Conversely, Propineb demonstrated the maximum radial growth

(82.33mm) and minimum mycelial growth inhibition of 8.52 per cent. (Table 2, plate 4).

Tebuconazole + Trifloxystrobin and Carbendazim + Mancozeb were also incompatible with *Trichoderma asperellum* at half of the prescribed dose, causing 100 per cent suppression of mycelial growth. Flusilazole exhibited radial growth of 6.67 mm with mycelial growth inhibition of 87.77 per cent. Propineb exhibited compatible interaction with radial growth of 90.00 mm and growth inhibition of 0.00 per cent. A similar pattern of results was obtained at the prescribed dose also, with propineb being the most compatible for *Trichoderma asperellum* growth (Table 2, Plate 3). Similar findings were reported by [3], who discovered that even at lower concentrations, propiconazole had a 100% inhibitory effect on the mycelial growth of Trichoderma.

Table 1: In vitro efficacy of various bio-agents against mycelial growth of F. oxysporum f. sp. Capsica

S. No	Treatment	Mycelial growth of *F. oxysporum* f. sp. *Capsici* (mm)*	Per cent inhibition over control
1	*Trichoderma harzianum*	16 .00	77.77
2	*Trichoderma asperellum*	25.00	65.27
3	*Pseudomonas fluorescence*	30.60	58.33
4	Control	72.00	-
	SE (m) ±	0.16	-
	CD	0.48	-



Trichoderma harzianum ***Trichoderma asperellum***

Pseudomonas fluorescens ***Control***

Fig 1: In vitro efficacy of various bio-agents against mycelial growth of F. oxysporum f. sp. capsici

Table 2. Efficacy of fungicides against pathogen and fungal bioagents

Tr. No	Treatments	Dose (ppm)	*Fusarium oxysporum f.* sp. *capsica*				*Trichoderma asperellum*				*Trichoderma harzianum*			
			Radial Growth (mm)*		Per cent inhibition over control		Radial Growth (mm)*		Per cent inhibition over control		Radial Growth (mm)*		Per cent inhibition over control	
			4th Day	8th Day	4th Day	8th Day	4th Day	8th Day	4th Day	8th Day	4th Day	8th Day	4th Day	8th Day
Half Dose														
1	Propineb 70 WP	1000	13.63	22.20	66.20	72.92	39.67	90.00	17.37	0.00	39.33	90	18.06	0
2	Captan 50 WP	1000	10.00	15.30	75.20	81.34	7.33	15.12	84.72	83.33	7.67	12.33	84.04	75.18
3	Propiconazole 25 EC	500	7.50	10.30	81.40	87.43	6.49	11.02	88.89	87.77	6.67	9.67	86.12	89.26
4	Flusilazole 40 EC	500	6.00	8.50	85.12	89.63	5.33	6.67	88.89	92.58	5.00	7.33	89.58	91.85
5	Carbendazim (12%) + Mancozeb (63%)	500	0.00	0.00	100	100	0.00	00.00	100	100	0.00	0.00	100	100
6	Tebuconazole (50%) + Trifloxystrobin (25%) WG	500	0.00	0.00	100	100	0.00	00.00	100	100	0.00	0.00	100	100
7	Control	-	40.33	82.00			48.00	90.00			48.27	90.00		
	SE (m) ±		**0.33**	**0.85**			**0.50**	**0.33**			**0.54**	**0.22**		
	C.D		**1.01**	**2.62**			**1.54**	**1.02**			**1.64**	**1.24**		
Recommended Dose														
1	Propineb 70 WP	2000	9.6	16.87	76.23	79.26	41.00	83.00	15.16	7.00	39.04	82.33	20.40	8.52
2	Captan 50 WP	2000	6.7	9.1	83.41	88.81	5.00	8.66	89.67	90.37	5.33	7.67	89.19	91.48
3	Propiconazole 25 EC	1000	5.5	7.4	86.38	90.9	0.00	0.00	100	100	0.00	0.00	100	100
4	Flusilazole 40 EC	1000	5.00	6.1	87.50	92.49	0.00	0.00	100	100	0.00	0.00	100	100
5	Carbendazim (12%) + Mancozeb (63%)	1000	0.00	0.00	100	100	0.00	0.00	100	100	0.00	0.00	100	100
6	Tebuconazole (50%) + Trifloxystrobin (25%) WG	1000	0.00	0.00	100	100	0.00	0.00	100	100	0.00	0.00	100	100
7	Control	-	40.40	81.33			48.33	90.00			49.32	90.00		
	SE(m) ±		**0.19**	**0.49**			**0.42**	**0.59**			**0.33**	**0.56**		
	C.D		**0.58**	**1.50**			**1.28**	**1.81**			**1.02**	**1.73**		

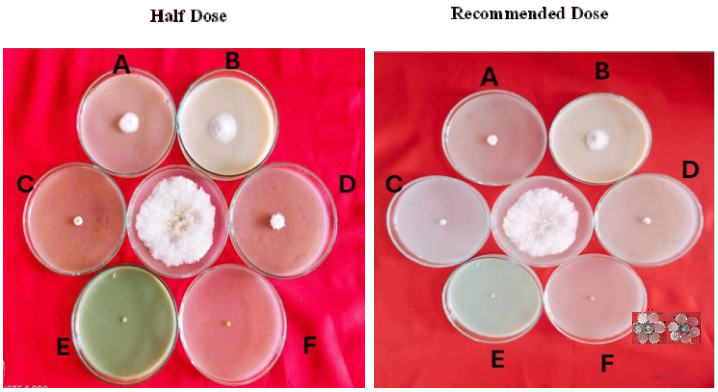


Plate 2. Efficacy of different fungicides against Fusarium oxysporum f. sp. capsici

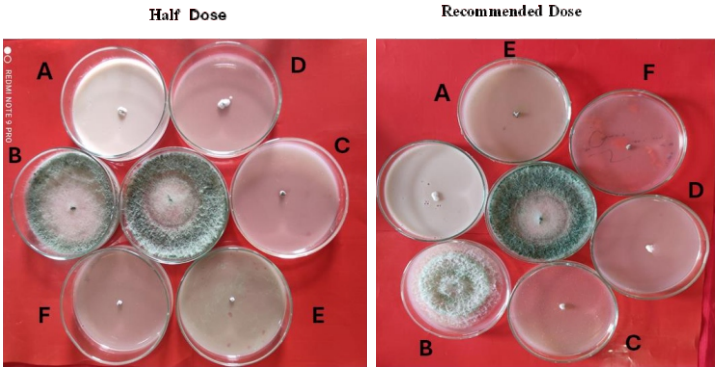


Plate 3. Efficacy of fungicides against Trichoderma asperellum

A	Captan 50 WP	D	Propiconazole 25 EC
B	Propineb 70 WP	E	Carbendazim (12%) + Mancozeb (63%)
C	Flusilazole 40 EC	F	Tebuconazole (50%) + Trifloxystrobin (25%) WG

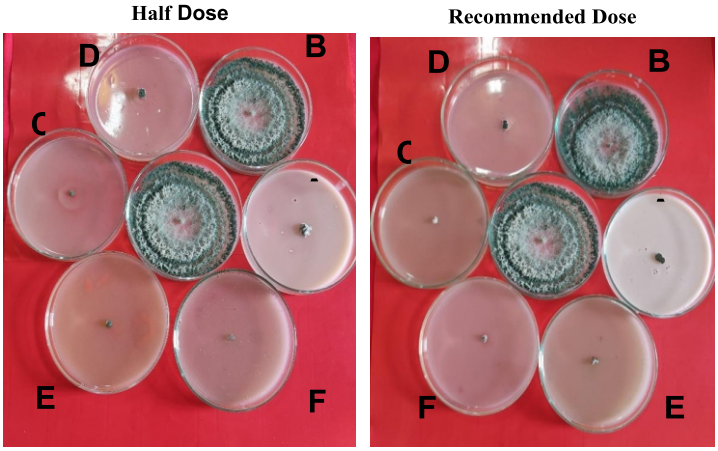


Plate. 4. Efficacy of fungicides against Trichoderma harzianum

Conclusion

The studies revealed that among bio-agents *Trichoderma harzianum* achieved the maximum mycelial growth inhibition of *F. oxysporum* f. sp. *capsici*. The combinations of Carbendazim + Mancozeb and Tebuconazole + Trifloxystrobin were found most effective fungicides against the mycelial growth of *F. oxysporum* f. sp. *capsici*, achieving complete inhibition (100%) at half of the recommended dose and the recommended dose. The fungicides, Carbendazim + Mancozeb, Tebuconazole + Trifloxystrobin, Propiconazole, and Flusilazole were found incompatible with *Trichoderma harzianum* and *Trichoderma asperellum*. Propineb was highly compatible with both the fungal bioagents. Therefore, amalgamation of Trichoderma *harzianum* and *Trichoderma asperellum* with propineb at half of the recommended dose can be promoted for the management of fusarium wilt of chilli. This will help to reduce the application dose of fungicides, thereby monitoring the fungicide resistance development in causal pathogen due to its overdose. Additionally, *Trichoderma* spp and effective fungicides could be used for integrated disease management of fusarium wilt of chili.

Future scope

This breakthrough could spark a surge in exploring the impact of changing environment on the disease-causing pathogen and the effective control measures. Further studies could focus on screening the additional antagonistic microbes against more potential fungicides for searching of more compatible interactions and effective management of the disease.

Conflicts of Interest: The authors declare no conflict of interest.

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