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In vitro assessment of bio-agents and fungicides for the management of cowpea wilt caused by *Fusarium oxysporum* f. sp. *tracheiphilum*

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Abstract

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important pulse crop cultivated for its nutritional value and adaptability to diverse agro-climatic conditions. Wilt caused by *Fusarium oxysporum* f. sp. *tracheiphilum* is one of the most destructive diseases limiting cowpea production in the Konkan region of Maharashtra, India. The present investigation was conducted to evaluate the efficacy of six bioagents (*Trichoderma viride*, *T. harzianum*, *T. longibrachiatum*, *T. koningii*, *Pseudomonas fluorescens*, and *Bacillus subtilis*) and ten fungicides against the pathogen under *in vitro* conditions. Bioagents were evaluated using the dual culture technique, whereas fungicides were tested by the poisoned food technique in a Completely Randomized Design (CRD) with three replications. Experimental data were subjected to analysis of variance (ANOVA), and treatment means were compared using the Critical Difference (CD) test at the 1% level of significance using OPSTAT software. Among the bioagents, *Trichoderma harzianum* recorded the highest inhibition of mycelial growth (58.52%), followed by *T. viride* (53.70%). Among the fungicides, Carbendazim 50% WP, Tebuconazole 25.9% EC, Captan 50% WP, Carbendazim 12% + Mancozeb 63% WP, and Carboxin 37.5% + Thiram 37.5% WS completely inhibited the mycelial growth of the pathogen (100% inhibition). The findings indicate that these bioagents and fungicides possess considerable antifungal activity against *F. oxysporum* f. sp. *tracheiphilum* under *in vitro* conditions. However, further greenhouse and field evaluations are required before recommending these treatments for practical disease management.

Keywords: Biological control, disease management, *Fusarium oxysporum* f. sp. *tracheiphilum*, *Trichoderma harzianum*, fungicides

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is an annual dicotyledonous legume belonging to the family Fabaceae and order Fabales. It is widely cultivated in tropical and subtropical regions and is valued for its edible dry seeds and green pods. The crop is also recognized for its potential use as a green manure, contributing to the improvement of soil fertility and properties (Langyintuo *et al.*, 2003) [7]. In India, cowpea is predominantly cultivated in the central and southern regions and is well adapted to warm climatic conditions. Its ability to withstand periods of drought and relatively heavy rainfall makes it an important pulse crop under variable environmental conditions. The crop performs well under temperatures ranging from approximately 27 to 35 °C and grows satisfactorily on a range of soil types, particularly well-drained loamy and moderately heavy soils (Kumar and Narain, 2005) [6]. From a nutritional perspective, cowpea is an important source of dietary protein, carbohydrates, minerals and vitamins. The crop provides substantial nutritional value while having relatively low energy and fat content (Goncalves *et al.*, 2016) [3]. Cowpea leaves and seeds contain several essential nutrients, with seeds generally containing about 23-25% protein, 50-67% carbohydrates and approximately 8-9% moisture, while the fat content remains comparatively low (Rangel *et al.*, 2003) [11]. These nutritional attributes contribute considerably to the importance of cowpea as a food and multipurpose crop. Despite its adaptability and nutritional significance, cowpea is affected by several fungal, bacterial and viral diseases. Among the major biotic constraints, wilt caused by the soil-borne pathogen *Fusarium oxysporum* f. sp. *tracheiphilum* is of considerable economic importance.

The pathogen invades the vascular tissues of infected plants, interfering with the movement of water and nutrients and resulting in symptoms such as yellowing, drooping and premature plant death. Disease development is particularly favoured by warm and humid environmental conditions, high soil moisture and the acidic lateritic soils prevalent in the Konkan region.

During the last two to three years, wilt incidence in cowpea Cv. Konkan Safed has resulted in considerable crop losses, estimated at approximately 30-40%, at the Research Farm, Botany field, College of Agriculture, Dapoli. The disease has also received limited attention under the specific conditions of this location. Therefore, identification of effective and environmentally compatible management measures is essential for reducing the impact of the disease. Management of Fusarium wilt generally involves the use of chemical fungicides and biological control agents. Bioagents such as *Trichoderma* spp., *Pseudomonas fluorescens* and *Bacillus subtilis* can restrict pathogen development through mechanisms including competition, antibiosis and mycoparasitism, thereby providing an alternative or complementary approach to chemical disease management. However, information regarding the comparative efficacy of different fungicides and bioagents against *F. oxysporum* f. sp. *tracheiphilum* under the agro-climatic conditions of the Konkan region is limited. Therefore, the present investigation was undertaken to evaluate selected fungicides and bioagents under *in vitro* conditions against *F. oxysporum* f. sp. *tracheiphilum*, with the objective of identifying effective treatments for further evaluation under greenhouse and field conditions and for developing an integrated approach to the management of cowpea wilt.

2. Materials and Methods

2.1 *In vitro* exploration of bio-agents

Six bioagents, namely *Trichoderma viride*, *T. harzianum*, *T. longibrachiatum*, *T. koningii*, *Pseudomonas fluorescens* and *Bacillus subtilis*, were assessed for their antagonistic activity against *Fusarium oxysporum* f. sp. *tracheiphilum* (Table 1). The experiment was conducted using the dual culture technique described by Dennis and Webster (1971) under a Completely Randomized Design (CRD) with three replications. For the assay, 5 mm diameter mycelial discs of the test pathogen and individual bioagents were obtained from seven-day-old cultures using a sterilized cork borer. The discs were placed at equal distances and opposite to each other on Petri plates containing Potato Dextrose Agar (PDA) medium. Plates inoculated only with the pathogen were maintained as the untreated control. All inoculated plates were incubated at 28±2 °C. The radial growth of *F. oxysporum* f. sp. *tracheiphilum* was recorded after seven days of incubation, and the percentage inhibition of mycelial growth over the control was calculated according to the formula proposed by Vincent (1947) [14].

$$\text{Per cent Growth Inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

C = Growth (mm) of the test fungus in control plate

T = Growth (mm) of the test fungus in treatment plates.

Table 1: Bioagents evaluated for their antagonistic activity against *F. oxysporum* f. sp. *tracheiphilum*

Tr. No.	Bio-agents used
T ₁	<i>Trichoderma viride</i>
T ₂	<i>T. harzianum</i>
T ₃	<i>T. longibrachiatum</i>
T ₄	<i>T. koningii</i>
T ₅	<i>Pseudomonas fluorescens</i>
T ₆	<i>Bacillus subtilis</i>
T ₇	Control

2.2 *In vitro* exploration of fungicides

Ten fungicides belonging to systemic, contact and combination groups were tested against *F. oxysporum* f. sp. *tracheiphilum* using the poisoned food technique described by Nene and Thapliyal (1993) [8]. The experiment was conducted in a Completely Randomized Design (CRD) with three replications. The required quantity of each fungicide was incorporated into molten PDA medium to obtain the respective test concentration. Approximately 20 ml of the fungicide-amended medium was dispensed into each sterilized Petri plate. A 5 mm diameter mycelial disc taken from a seven-day-old actively growing culture of the pathogen was placed at the centre of each plate. PDA medium without fungicide was maintained as the untreated control. The inoculated plates were incubated at 28±2 °C for seven days. Following incubation, radial colony growth was measured and the percentage inhibition of mycelial growth over the control was calculated using the formula of Vincent (1947) [14] given above.

Table 2: Fungicides evaluated for their efficacy against *F. oxysporum* f. sp. *Tracheiphilum*

Tr. No.	Name of fungicides	Concentration
T ₁	Tebuconazole 25.9% EC	0.1%
T ₂	Carbendazim 50% WP	0.2%
T ₃	Captan 50% WP	0.2%
T ₄	Mancozeb 75% WP	0.2%
T ₅	Copper oxychloride 50% WP	0.3%
T ₆	Bordeaux mixture	0.2%
T ₇	Zineb 68% + Hexaconazole 4% WP	0.2%
T ₈	Carbendazim 12% + Mancozeb 63% WP	0.2%
T ₉	Carboxin 37.5% + Thiram 37.5% WS	0.2%
T ₁₀	Tebuconazole 50% + Trifloxystobin 25% WG	0.1%
T ₁₁	Control	

2.3 Statistical analysis

The data obtained from both *in vitro* experiments were statistically analyzed according to the Completely Randomized Design. Standard error of the mean (S.E.m.±) and critical difference (C.D.) were calculated at the 1% level of significance. The analysis was performed using OPSTAT software, following the statistical procedures described by Panse and Sukhatme (1978) [9].

3. Results and Discussion

3.1 *In vitro* antagonistic activity of bio-agents

The six bioagents tested in the present study exhibited significant inhibitory effects on the mycelial development of *Fusarium oxysporum* f. sp. *tracheiphilum* when compared with the untreated control, which recorded a colony diameter of 90.00 mm (Table 3, Plate I and Fig. 1). Among the treatments, *Trichoderma harzianum* demonstrated the strongest antagonistic activity, restricting the pathogen

colony to 37.33 mm and resulting in 58.52 percent inhibition of mycelial growth. The next highest inhibitory effect was observed with *T. viride*, which recorded a colony diameter of 41.67 mm and 53.70 percent inhibition. *Trichoderma longibrachiatum* and *T. koningii* produced 47.67 and 49.67 mm colony diameters, corresponding to 47.03 and 44.81 percent inhibition, respectively. Among the bacterial bioagents, *Bacillus subtilis* exhibited moderate antagonistic activity, with 54.17 mm colony growth and 39.81 percent inhibition. In contrast, *Pseudomonas fluorescens* was comparatively less effective, recording the highest pathogen growth (59.83 mm) and the lowest inhibition (33.52%) among the bioagents evaluated.

Table 3: *In vitro* assessment of bio-agent efficacy against *F. oxysporum* f. sp. *tracheiphilum*.

Tr. No.	Bio agents evaluated	Mean colony diameter (mm)*	Percent inhibition
T ₁	<i>Trichoderma viride</i>	41.67	53.70
T ₂	<i>T. harzianum</i>	37.33	58.52
T ₃	<i>T. longibrachiatum</i>	47.67	47.03
T ₄	<i>T. koningii</i>	49.67	44.81
T ₅	<i>Pseudomonas fluorescens</i>	59.83	33.52
T ₆	<i>Bacillus subtilis</i>	54.17	39.81
T ₇	Control	90	-
	S.E.m±	0.31	
	C.D. @ 1%	1.29	



*Mean of three replications

Plate I: *In vitro* assessment of bio-agent efficacy against *F. oxysporum* f. sp. *Tracheiphilum*

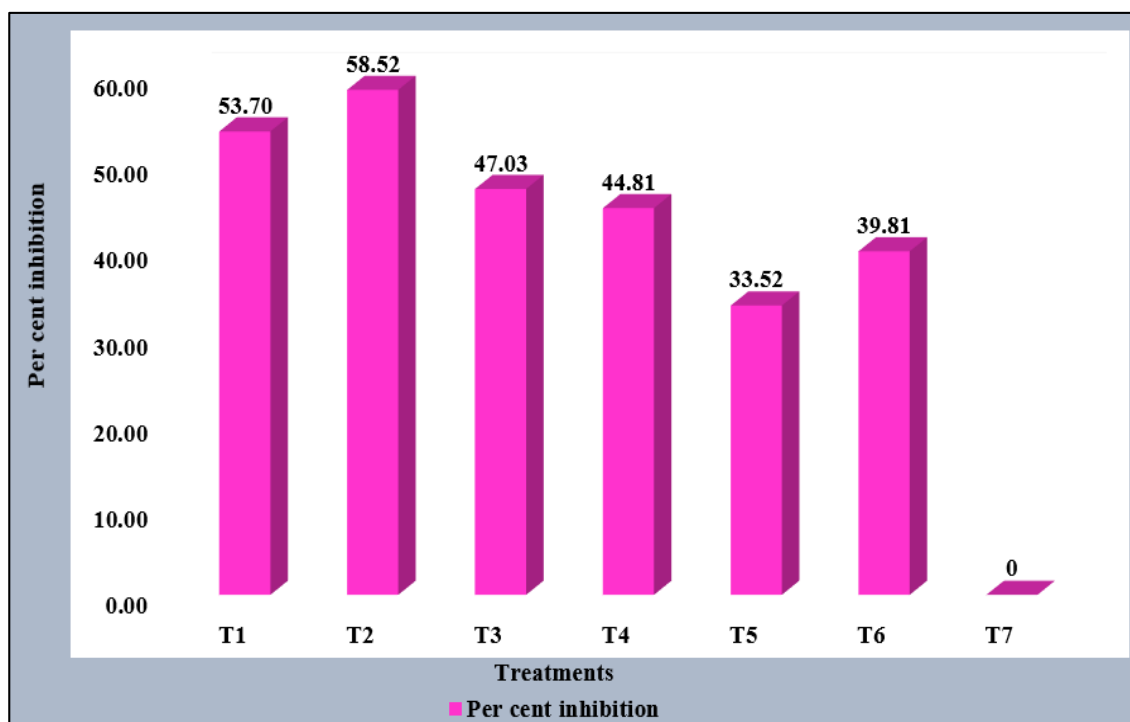


Fig 1: *In vitro* exploration of bio-agents

The superiority of *T. harzianum* over other *Trichoderma* species and bacterial antagonists corroborates several previous studies. Gadhave (2020) ^[2] reported *T. harzianum*

as the most effective bio-agent against *F. oxysporum* f. sp. *lycopersici* (52.33% inhibition), followed by *T. koningii*, *T. viride* and *P. fluorescens*. Khillare *et al.* (2020) ^[5] similarly

found *T. harzianum* most inhibitory to *F. oxysporum* f. sp. *udum* (85.62%), followed by *T. hamatum*, *T. asperellum*, *T. koningii*, *T. longibrachiatum* and *P. fluorescens* in decreasing order of efficacy. Khatun (2018) reported complete inhibition of *F. oxysporum* f. sp. *sesami* by *T. harzianum*, followed by *T. viride*, and Patra and Biswas (2017) ^[10] similarly recorded the highest inhibition of *F. oxysporum* f. sp. *ciceri* by *T. harzianum*, followed by *T. koningii*, *T. viride* and *P. fluorescens*. Although absolute inhibition percentages vary with the host plant and *forma specialis* of the pathogen across these studies, the consistent ranking of *T. harzianum* as the most antagonistic species, and of *P. fluorescens* as comparatively weaker, suggests a genuinely superior and broadly applicable antagonistic capability of *T. harzianum* against *Fusarium oxysporum*, likely mediated through a combination of mycoparasitism, antibiosis and competition for space and nutrients.

3.2 In vitro evaluation of fungicides against the pathogen

All ten fungicides included in the experiment substantially restricted the mycelial growth of *F. oxysporum* f. sp. *tracheiphilum* compared with the untreated control, which showed 90.00 mm colony growth (Table 4, Plate II and Fig. 2). Complete inhibition of pathogen growth (100%) was obtained with tebuconazole 25.9% EC at 0.1%, carbendazim 50% WP at 0.2%, captan 50% WP at 0.2%, carbendazim 12% + mancozeb 63% WP at 0.2%, and carboxin 37.5% + thiram 37.5% WS at 0.2%. Tebuconazole 50% + trifloxystrobin 25% WG at 0.1% was the next most effective treatment, producing 88.44 percent inhibition. This was followed by hexaconazole 4% + zineb 68% WP at 0.2%, which resulted in 70.38 percent inhibition. Comparatively lower activity was observed with copper oxychloride 50% WP, mancozeb 75% WP and Bordeaux mixture, which recorded 37.66, 22.16 and 10.77 percent inhibition, respectively.

Table 4: In vitro evaluation of fungicidal activity against *F. oxysporum* f. sp. *tracheiphilum*.

Tr. No.	Fungicides	Conc. (%)	Mean colony diameter (mm)*	Per cent inhibition
T ₁	Tebuconazole 25.9% EC	0.1	00.00	100.00
T ₂	Carbendazim 50% WP	0.2	00.00	100.00
T ₃	Captan 50% WP	0.2	00.00	100.00
T ₄	Mancozeb 75% WP	0.2	70.05	22.16
T ₅	Copper oxychloride 50% WP	0.3	56.17	37.66
T ₆	Bordeaux mixture	0.2	80.33	10.77
T ₇	Hexaconazole 4% + Zineb 68% (72% WP)	0.2	26.65	70.38
T ₈	Carbendazim 12% + Mancozeb 63% (75% WP)	0.2	00.00	100.00
T ₉	Carboxin 37.5% + Thiram 37.5% (75% WS)	0.2	00.00	100.00
T ₁₀	Tebuconazole 50% + Trifloxystrobin 25% WG	0.1	10.40	88.44
T ₁₁	Control	-	90.00	-
	S.E.m±		0.43	
	C.D. @ 1%		1.72	



*Mean of three replications

Plate II: In vitro evaluation of fungicidal activity against *F. oxysporum* f. sp. *tracheiphilum*

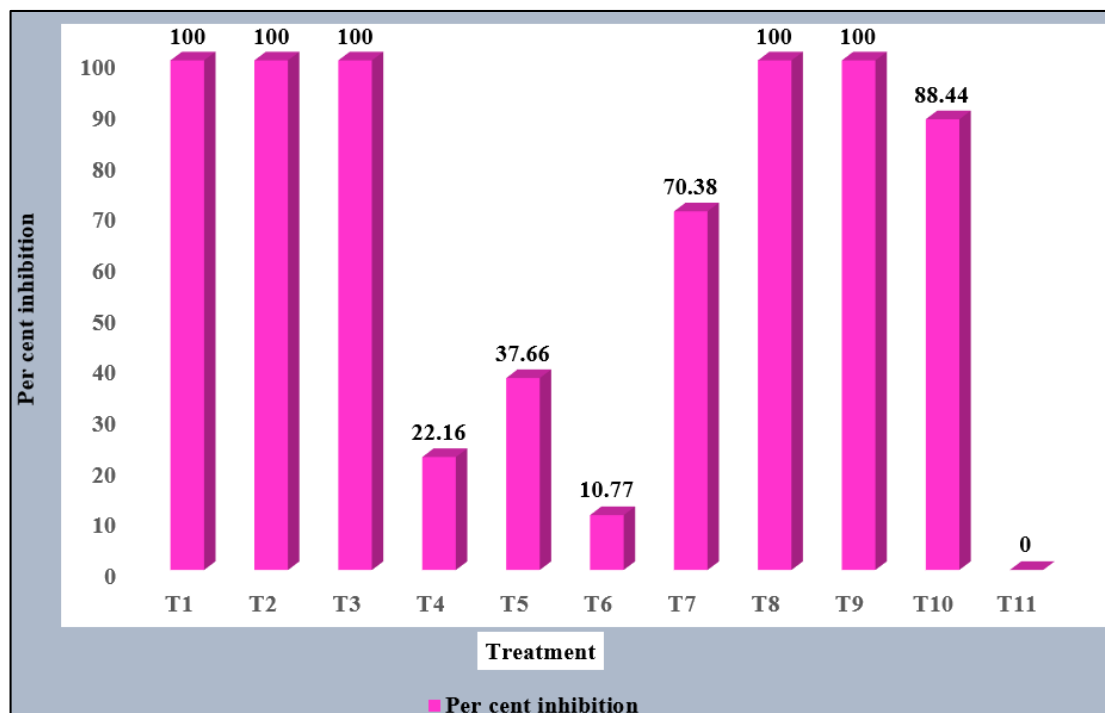


Fig 2: *In vitro* exploration of Fungicide

These results are in close agreement with several earlier reports. Shinde (2025) ^[13] similarly found carbendazim 50% WP, tebuconazole 25.9% EC, hexaconazole 4% + zineb 68% WP, carbendazim 12% + mancozeb 63% WP and carboxin 37.5% + thiram 37.5% WS gave complete inhibition of *Fusarium oxysporum* f. sp. *sesami*, the sesame wilt pathogen. Khillare *et al.* (2020) ^[5] reported that tebuconazole 25% WP, carboxin + thiram and carbendazim + mancozeb combinations gave 100 percent inhibition of *F. oxysporum* f. sp. *udum*, the pigeon pea wilt pathogen, followed by carbendazim 50% WP and thiophanate methyl. Ravichandran and Hegde (2015) ^[12] reported complete inhibition of *F. oxysporum* f. sp. *ciceri* by tebuconazole 25.9% EC and carbendazim + mancozeb. The consistently superior performance of systemic and combination fungicides over contact fungicides such as mancozeb and Bordeaux mixture across these studies, and in the present investigation, likely reflects the deeper penetration and broader mode of action of systemic and combi-product active ingredients against *Fusarium oxysporum* relative to the surface protective action of straight contact fungicides.

4. Conclusion

From the results of present *in vitro* study, it is concluded that *Fusarium oxysporum* f. sp. *tracheiphilum* causal agent of wilt disease of cowpea can be effectively controlled by fungicides screened *in vitro*, tebuconazole 25.9% EC, carbendazim 50% WP, captan 50% WP, carbendazim 12% + mancozeb 63% WP and carboxin 37.5% + thiram 37.5% WS gave complete inhibition of the pathogen and are recommended as priority candidates for field evaluation. Among the bio-agents, *Trichoderma harzianum* was clearly the most promising, offering a viable biological alternative or complement to chemical management. These findings provide a sound basis for subsequent field-level testing of the most effective fungicides and bio-agents identified here, towards developing an integrated management strategy for cowpea wilt in the Konkan region.

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