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EFFICACY OF BIOCONTROL AGENTS AND FUNGICIDES FOR THE MANAGEMENT OF FUSARIUM WILT IN CROSSANDRA

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ABSTRACT

Fusarium wilt severely limits the production of crossandra (*Crossandra infundibuliformis*), a commercially important loose flower crop. This study evaluated fungicides and biological control agents for wilt management. Four fungicides, viz., Carbendazim, Tebuconazole, Fluopyram + Tebuconazole, and Tribasic copper sulphate were tested *in vitro* using the poisoned food technique. Carbendazim, Tebuconazole and Fluopyram + Tebuconazole completely inhibited mycelial growth in all the concentrations, while Tribasic copper sulphate was less effective (33.73-47.38%). Among bioagents, *Trichoderma* isolate TRI I (95.75% inhibition) and *Bacillus* isolate 164 KB (79.39%) were most effective in dual culture assays. Field evaluation revealed that carbendazim significantly reduced wilt incidence to 2.78% compared with 27.77% in the untreated control, representing 90% reduction, and produced the highest flower yield (22.63 g plant^{-1}; 0.27 kg plot^{-1}). The consortium of *Trichoderma* + *Bacillus* sp.+ vesicular arbuscular mycorrhiza (VAM) reduced wilt incidence to 8.33% (70% reduction) and improved yield (20.49 g plant^{-1}; 0.24 kg plot^{-1}) compared with the control (17.91 g plant^{-1}; 0.21 kg plot^{-1}). Other fungicides did not differ significantly from the control under field conditions. Overall, carbendazim was most effective for wilt suppression and yield enhancement, while the *Trichoderma* + *Bacillus* sp. + VAM consortium has shown promise as an eco friendly alternative for integrated disease management in crossandra.

***Key words*:** Crossandra, Fusarium wilt, carbendazim, *Trichoderma*, *Bacillus* sp.

Introduction

Crossandra (*C. infundibuliformis* L.) is a major traditional loose flower crop. The crop is widely cultivated across tropical and subtropical regions of the world. It is an erect, evergreen shrub that grows as a perennial. The flowers can be used alone or in conjunction with jasmine flowers to create garlands. In India, 430 tonnes of crossandra are produced on an estimated 1700 hectares of land (Venkatesan *et al*., 2004). Leaf spot (*Alternaria amaranthi* var. crossandrae, *Cercospora crossandrae*), blight (*Colletotrichum crossandrae*), stem rot (*Rhizoctonia solani*), and collar rot and wilt (Fusarium sp.) are some of the fungal diseases that impact crossandra cultivation. Fusarium wilt is the most damaging of them, resulting in production losses of up to 92% (Mallaiah *et al*., 2020) and under favourable circumstances, total crop is under loss (Navas-Cortés *et al*., 2000). One of the most persistent and challenging soil-borne infections to control, the pathogen is a facultative saprophyte that can live for five to six years in soil and agricultural wastes as mycelium and chlamydospores (Haware *et al*., 1986).

Although chemical fungicides are an efficient way to manage the disease, frequent use of them can cause

fungicide resistance and environmental problems. Although bio-agents are safer substitutes, they can occasionally be less reliable under variable field and environmental conditions. Hence, evaluating both can help identify effective and sustainable management options. The present study was therefore undertaken to assess the efficacy of fungicides and bioagents against Fusarium wilt of crossandra.

Material and Methods

In vitro evaluation of fungicides against *Fusarium* sp.

The efficacy of selected fungicides (Table: 1) against *Fusarium* sp. was evaluated *in vitro* using the poisoned food technique (Nene and Thapliyal, 1993). Each fungicide was incorporated into potato dextrose agar (PDA) at 100, 250 and 500 ppm and poured into petri plates, with unamended PDA serving as the control. A 5 mm mycelial disc obtained from a 7-day-old culture of *Fusarium* sp. was placed at the centre of each plate. The experiment was conducted in three replications, and plates were incubated at 28 ± 2 °C. When the mycelial growth in the control plates reached the periphery, colony diameter was recorded and the percentage inhibition of mycelial growth over the control was calculated using Vincent's (1947) formula:

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

Where,

I= Percent inhibition,

C= Radial growth of test fungus in control plate, and

T= Radial growth of test fungus in treated plate

In vitro evaluation of biocontrol agents against *Fusarium* sp.

Using the dual culture method of Bell *et al*., (1982), the antagonistic activity of ten Trichoderma isolates from ICAR-Directorate of Floricultural Research (ICAR-DFR), Rajahmundry, was assessed against *Fusarium* sp. A 5 mm mycelial disc of *Fusarium* sp. and the respective *Trichoderma* isolate were placed at opposite ends of PDA plates. Eleven bacterial isolates obtained from ICAR-National Institute for Research on Commercial Agriculture (NIRCA), Rajahmundry, were similarly evaluated by streaking the bacterial culture approximately one cm from the plate margin and placing a 5 mm pathogen disc on the opposite side. Plates inoculated only with the pathogen served as controls. Both experiments were conducted in a Complete Randomized Design with three replications and incubated at 28 ± 2 °C. Percentage inhibition of mycelial growth was calculated using Vincent's (1947) formula:

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

Where,

I = is the percentage inhibition,

C=is the pathogen colony diameter in the control, and,

T= is the colony diameter in the treatment.

Preparation and mass multiplication of *Fusarium* sp. inoculum

Fusarium sp. inoculum was prepared following Geroche and Ugay (2019). The pathogen was multiplied in PDB at 25-28 °C for 7-10 days, and the spore suspension was standardized to 1 × 10v spores mL^{-1}. For mass multiplication, 1 L of the standardized suspension was mixed with 5 kg sterilized FYM and incubated in gunny bags under shade for 15 days under moist conditions. The colonized FYM was used for artificial inoculation at 10 g $plant^{-1}$.

Selection and preparation of fungicides and biocontrol agents

Fungicides with the greatest *in vitro* inhibition were selected for field evaluation at the recommended manufacturer's rates. Tri-1 and 164KB were selected for further evaluation since they exhibited the highest antagonistic activity. Talc formulations were prepared as described by Vidhyasekaran and Muthamilan (1995). Tri-1 was cultured in Potato dextrose broth (PDB) for 7 days and 164KB in Nutrient broth (NB) for 24 h and mixed with sterilized talc to obtain 1 × 10x spores g^{-1} and 1 × 10x CFU g^{-1}, respectively. 250 g of each formulation were mixed thoroughly with 250 g of FYM and kept moist for 15 days for field application Vimala *et al*., (2025). The consortium was prepared by mixing the formulations of *Trichoderma* and *Bacillus* sp. in 1:1 ratio with FYM. The enriched formulations were applied at 18 g $plant^{-1}$ around the root zone. A commercial formulation of VAM was procured from Tamil Nadu Agricultural University (TNAU) and utilized for the experiment.

Field Trial

A field trial was conducted at the ICAR-Directorate of Floricultural Research Regional Station, Vemagiri, during 2025-2026 with nine treatments and three replications (Table 2) in a Randomized Block Design. Each treatment comprised 12 plants per replication at 80 × 40 cm spacing. The treatments were applied prophylactically, followed by artificial inoculation with *Fusarium* sp. using colonized FYM at 10 g $plant^{-1}$ around the root zone. The FYM-enriched biocontrol formulations

were applied at 18 g plant^{-1}, and fungicides were applied as soil drenches at manufacturer-recommended dosages at an intervals of 15 days. Untreated plants served as the control.

Wilt incidence was recorded at the entire six-month crop period and calculated as:

$$\text{Wilt incidence (\%)} = \frac{\text{Number of wilted plants}}{\text{Total number of plants}} \times 100$$

Plant height and Plant spread were recorded at 180 days after planting (DAP). Flower yield per plant (g) and Flower yield per plot(kg) were recorded continuously for a duration of 180 days.

Statistical Analysis

All the observations recorded from the *in vitro* and field experiments were statistically analyzed using OPSTAT, a statistical package for agricultural research Sheoran *et al*., (1998). The data were subjected to analysis of variance (ANOVA) appropriate to the experimental design, and the significance of treatment effects was tested at the 5% level ($P \leq 0.05$). Wherever treatment differences were significant, means were separated and compared using the critical difference (CD at 5%) and Duncan's multiple range test (DMRT).

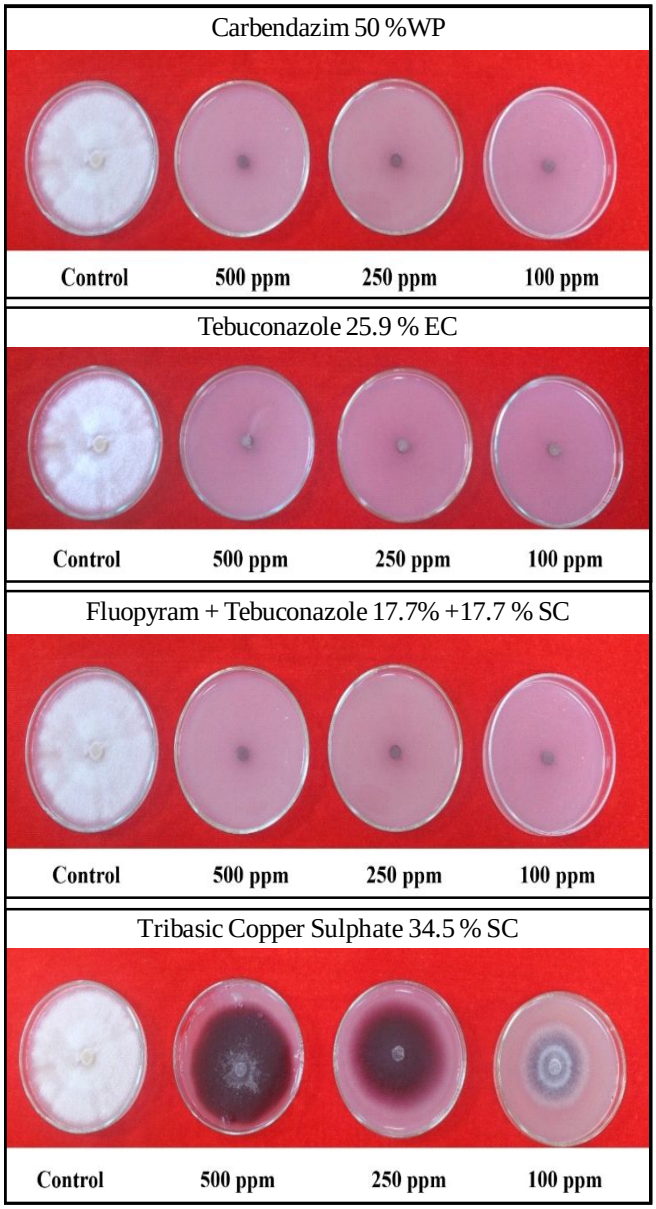


Plate 1: *In-vitro* evaluation of Fungicides against *Fusarium* sp.at different concentrations.

Table 1: Fungicides evaluated against Fusarium wilt of Crosssandra.

Common name	Chemical name	Trade name
Carbendazim	Methyl 1H-benzimidazole-2-ylcarbamate	Rodazim 50 % WP
Tebuconazole	(RS)-1-(4-chlorophenyl)-4, 4-dimethyl-3-(1H-1,2, 4-triazol-1-ylmethyl) pentan-3-ol	Santeb 25.9 % EC
Fluopyram + Tebuconazole	N-[2-[3-chloro-5-(trifluoromethyl) pyridin-2-yl] ethyl]-2-(trifluoromethyl) benzamide +(RS)-1-(4-chlorophenyl)-4, 4-dimethyl-3-(1H-1,2,4-triazol -1-ylmethyl) pentan-3-ol	Luna Experience 400 SC
Tri basic copper sulphate	Tetra copper hexa hydroxide sulphate	Cuflow 34.5%SC

Results and Discussion

In vitro evaluation of fungicides, *Trichoderma* isolates, Bacterial isolates against *Fusarium* sp.

The effectiveness of fungicides against *Fusarium* sp. was assessed at three concentrations (100, 250 and 500 ppm) using the poisoned food technique (Table 3, Plate 1). Carbendazim, Tebuconazole and Fluopyram + Tebuconazole completely inhibited mycelial growth (100% inhibition) in all the three concentrations. Tribasic copper

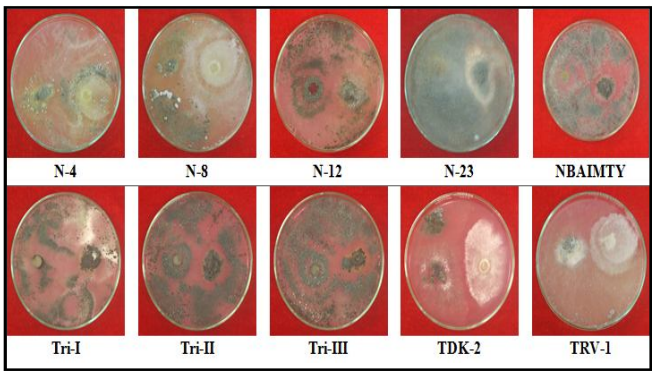


Plate 2: *In vitro* evaluation of *Trichoderma* isolates against *Fusarium* sp. by dual culture technique.

Table 2: Treatment details.

Treatment No.	Treatment details	Dosage / Plant
T_1	*Trichoderma*	18 g (FYM-enriched)
T_2	*Bacillus* sp.	18 g (FYM-enriched)
T_3	VAM	18 g
T_4	Tebuconazole 25.9 % EC	50 ml (1ml/L)
T_5	Carbendazim 50 % WP	50ml (1g / L)
T_6	Fluopyram + Tebuconazole 17.7%+17.7 % SC	50ml (1ml/L)
T_7	Tri Basic Copper Sulphate 34.5 % SC	50ml (1.5 ml/L)
T_8	*Trichoderma* + *Bacillus* sp. + VAM	18 g mixture
T_9	Control	-

sulphate was comparatively less effective, recording 47.38% inhibition at 100 ppm, 33.73% at 250 ppm and 36.54% at 500 ppm. The response of tribasic copper sulphate was not consistently dose-dependent.

Dual culture assays revealed considerable variation among the *Trichoderma* (Table 4, Plate 2) and bacterial isolates (Table 5, Plate 3) TRI-I recorded the highest inhibition among the *Trichoderma* isolates (95.75%), being statistically comparable with TRI-III (89.69%) and NBAIMTY (91.51%). Among the bacterial isolates, 164-KB recorded the highest inhibition (79.39%), comparable with 191-B1 (74.54%) and 214-B2 (74.27%), whereas isolate 162KB recorded the lowest inhibition (61.20%).

The complete inhibition of *Fusarium* sp. by Carbendazim, Tebuconazole and Fluopyram +

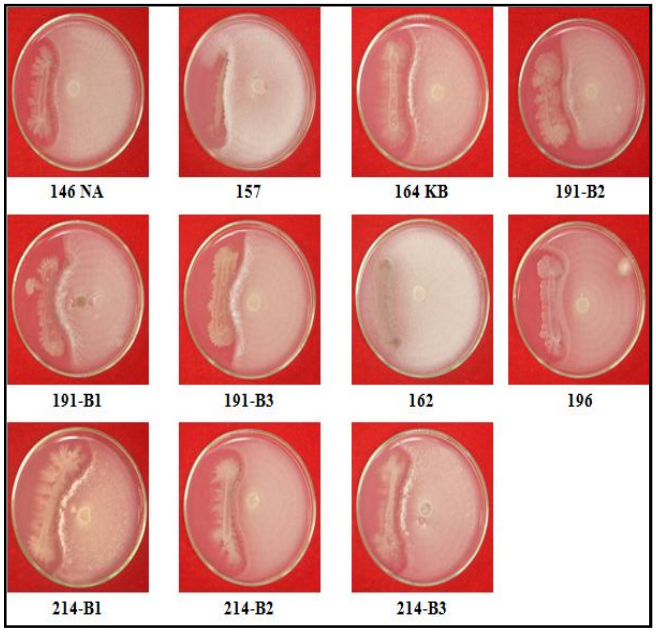


Plate 3: *In vitro* evaluation of bacterial isolates against *Fusarium* sp. by dual culture technique.

Table 3: *In vitro* evaluation of fungicides against *Fusarium* sp. at different concentrations.

Fungicides	Inhibition of mycelial growth (%)
T_1: Carbendazim 50 % WP @ 100 ppm	100.00^a (90.00)
T_2: Carbendazim 50 % WP @ 250 ppm	100.00^a (90.00)
T_3: Carbendazim 50 % WP @ 500 ppm	100.00^a (90.00)
T_4: Tebuconazole 25.9 % EC @100 ppm	100.00^a (90.00)
T_5: Tebuconazole 25.9 % EC @ 250 ppm	100.00^a (90.00)
T_6: Tebuconazole 25.9 % EC @ 500 ppm	100.00^a (90.00)
T_7: Fluopyram+Tebuconazole 17.7% + 17.7 % SC @ 100 ppm	100.00^a (90.00)
T_8: Fluopyram+ Tebuconazole 17.7% + 17.7 % SC @ 250 ppm	100.00^a (90.00)
T_9: Fluopyram+ Tebuconazole 17.7% + 17.7 % SC @ 500 ppm	100.00^a (90.00)
T_{10}: Tri basic copper sulphate 34.5 % SC @ 100 ppm	47.38^b (43.50)
T_{11}: Tri basic copper sulphate 34.5 % SC @ 250 ppm	33.73^c (35.23)
T_{12}: Tri basic copper sulphate 34.5 % SC @ 500 ppm	36.54^c (36.77)
SE(m)	2.17
CD at 5%	6.32

Note: Values in parentheses are arcsine-transformed values. Means followed by the same superscript letter within a column are not significantly different (DMRT, P=0.05)

Tebuconazole is consistent with previous reports of strong fungicidal activity of carbendazim and triazole-based fungicides against *Fusarium* sp. (Taskeen-Un-Nisa *et al.*, (2011); Rana *et al.*, (2023); Patra and Biswas (2016). The present antagonistic activity of *Trichoderma* and *Bacillus* isolates is also in agreement with earlier studies reporting suppression of *Fusarium* spp. by *Trichoderma*

Table 4: *In vitro* evaluation of *Trichoderma* isolates against *Fusarium* sp. by dual culture technique.

Trichoderma isolate	Mean colony diameter of test pathogen (cm)	Per cent inhibition (%)
TRI-I	0.23	95.75^a (78.53)
TRI-II	0.77	86.05^b (68.23)
TRI-III	0.57	89.69^{ab} (71.39)
TDK-2	0.83	84.84^b (67.48)
TRV-1	2.17	60.60^c (51.27)
N-4	0.70	87.27^b (69.13)
N-8	0.70	87.27^b (69.13)
N-12	0.90	83.63^b (66.40)
N-23	0.63	88.48^b (70.52)
NBAIMTY	0.47	91.51^{ab} (73.21)
SE(m)		2.60
CD at 5%		7.68

Table 5: *In vitro* evaluation of bacterial isolates against *Fusarium* sp. by dual culture technique.

Bacterial isolate	Mean colony diameter of test pathogen (cm)	Per cent inhibition (%)
191-B1	1.23	74.54^{ab} (59.70)
191-B2	1.53	72.11^{bc} (58.20)
191-B3	1.43	73.67^{abc} (59.15)
162	2.13	61.20^{d} (51.48)
164 KB	1.13	79.39^{a} (63.01)
157	1.80	67.27^{cd} (55.10)
146NA	1.43	73.67^{abc} (59.17)
214 B2	1.40	74.27^{ab} (59.56)
214 B4	1.77	67.60^{cd} (55.31)
196	1.53	71.84^{bc} (57.99)
214-B1	1.57	71.24^{abc} (57.59)
SE(m)		1.25
CD at 5%		3.70

and *Bacillus* in ornamental and vegetable crops Chandel (2007); Vavre *et al*., (2021); Wang *et al*., (2021).

Field Trial

Under field conditions, carbendazim (T_5) recorded the lowest wilt incidence (2.78%), significantly lower than the other treatments (Table 6) whereas the untreated control recorded the highest incidence (27.77%). The *Trichoderma* + *Bacillus* sp. + VAM consortium (T_8) was next in performance, with 8.33% wilt incidence and significantly lower disease incidence than the control.

Carbendazim also recorded the highest plant height (74.11 cm), plant spread (44.73 cm), flower yield per plant (22.63 g) and flower yield per plot (0.27 kg). The consortium (T_8) recorded 72.97 cm plant height, 37.48 cm plant spread, 20.49 g flower yield per plant and 0.24 kg per plot. These results indicate that treatments associated with lower wilt incidence maintained better growth and floral productivity under the conditions of the present experiment.

The field performance of carbendazim agrees with reports of effective carbendazim-based management of Fusarium wilt in crossandra and other crops (Mallaiah *et al*., (2020); Ravichandra (2018); Yasmin *et al*., (2018). Sumana *et al*., (2012) also reported effective wilt suppression with carbendazim applied as a soil drench. The higher flower yield recorded in the present study is consistent with earlier reports of improved floral traits and yield following wilt management (Mallaiah *et al*., (2020); Yasmin *et al*., (2018).

The biological treatments also exhibited potential for disease suppression and improved productivity. Earlier studies have reported beneficial effects of *Trichoderma* and integrated fungicide-bioagent approaches on growth and floral traits in ornamental crops (Raj *et al*., (2016); Chandel (2007). The lower wilt incidence and higher flower yield obtained with the *Trichoderma* + *Bacillus* sp.+ VAM consortium in the present study support its potential as a biological component of integrated wilt management.

Overall, carbendazim recorded the lowest Fusarium wilt incidence, the highest growth and flower yield under the field conditions tested. The *Trichoderma* + *Bacillus* sp. + VAM consortium also reduced wilt incidence and increased flower yield compared with the untreated control, indicating its potential as an eco-friendly component of integrated disease management in crossandra.

Acknowledgement

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Table 6: Plant growth, yield parameters and wilt incidence as influenced by different bioagents and fungicides in crossandra.

Treatment	Plant height at 180 DAP	Plant spread at 180 DAP	Flower yield per plant (g)	Flower yield per plot (Kg)	Percentage of wilt incidence (%)
T_1: *Trichoderma*	67.89^{abc}	36.33^{bc}	18.72^{c}	0.22^{c}	16.66^{ab} (23.62) *
T_2: *Bacillus sp.*	59.50^{d}	37.17^{b}	16.94^{d}	0.20^{d}	22.22^{a} (28.03) *
T_3: VAM	67.55^{abc}	35.70^{bc}	18.52^{c}	0.22^{c}	16.67^{ab} (24.09) *
T_4: Tebuconazole 25.9 % EC	66.44^{bcd}	32.17^{c}	17.60^{cd}	0.21^{cd}	16.67^{ab} (23.62) *
T_5: Carbendazim 50 % WP	74.11^{a}	44.73^{a}	22.63^{a}	0.27^{a}	2.78^{c}(5.59) *
T_6: Fluopyram+Tebuconazole 17.7%+17.7 % SC	64.67^{cd}	35.11^{bc}	17.91^{cd}	0.21^{cd}	16.67^{ab} (24.09) *
T_7: Tri basic copper Sulphate 34.5 % SC	62.44^{cd}	32.33^{c}	17.02^{d}	0.20^{d}	19.44^{a} (26.06) *
T_8: *Trichoderma* + *Bacillus sp.* + VAM	72.97^{ab}	37.48^{b}	20.49^{b}	0.24^{b}	8.33^{b}(16.78) *
T_9: Control	73.56^{ab}	36.99^{bc}	17.91^{cd}	0.21^{cd}	27.77^{a}(31.75) *
SE(m)	2.23	1.43	0.42	0.00	2.67
CD at 5%	6.77	4.34	1.28	0.01	8.07

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