

**MANAGEMENT IN FUSARIUM WILT MANAGEMENT OF CASTOR:
EXPLORING THE POTENTIAL OF BIORATIONALS AND NEW FUNGICIDE
MOLECULES AGAINST *Fusarium oxysporum* f. sp. *ricini***

ABSTRACT

To mitigate the harmful effects of synthetic chemical pesticides, eight biorationals *i.e* ginger, onion, bougainvillea, garlic, chili, turmeric, neem, and aloe vera were tested at concentrations of 10%, 15%, and 20% against *Fusarium oxysporum* f. sp. *ricini*, the pathogen causing wilt in castor plants. Aloe vera exhibited the highest radial growth at all concentrations, while neem demonstrated the best mycelial inhibition at 10% and 15%. Chili was most effective at 20%. Overall, neem and chili were the most effective biorationals, whereas aloe vera showed the least pathogen suppression. The *In vitro* evaluations of new fungicide combinations *i.e* Picoxystrobin + Tricyclazole, Fluopyram + Tebuconazole, Boscalid + Pyraclostrobin, and Iprovalicarb + Propineb were conducted. Picoxystrobin + Tricyclazole and the standard fungicides Carbendazim and Thiophanate-methyl achieved 100% pathogen inhibition at all tested concentrations (500-2000 ppm). Fluopyram + Tebuconazole and Boscalid + Pyraclostrobin also showed high efficacy, while Iprovalicarb + Propineb was the least effective. The study revealed that neem and chili are recommended as effective biorationals against *F. oxysporum* f. sp. *ricini*. Among fungicides, Picoxystrobin + Tricyclazole proved most effective, suggesting its potential for managing castor wilt.

Keywords: Castor, *Fusarium* spp, Biorationals, Fungicides

INTRODUCTION

Castor (*Ricinus communis*), a versatile and resilient plant, has captivated the interest of mankind for a long time for its numerous applications. This hardy plant exhibits exceptional oil production, yielding 350 and 900 kg of oil per hectare, and demonstrated adaptability to marginal soils. The oil has numerous industrial applications, as evidenced by the recent surge in global demand, with over 700 documented applications [1]. India stands as a prominent global cultivator of castor, holding the foremost position in terms of both

cultivation (0.83 million ha) and production (1.57 million tons) [2]. At every stage of the growth castor is affected by several pathogens, depending on the seasonal conditions, and a small number of them emerge as economically significant for the crop [3]. *Fusarium* wilt, is one such important disease which is soil and seed-borne.

The fungus is 10–20 per cent internally as well as externally seed borne [4]. The disease affects all crop growth phases throughout the year, however it spikes during the flowering, spike production, and capsule maturity stage. The disease was initially reported in Morocco [5] and in India, at Udaipur and Sirohi of Rajasthan [6]. Description of the symptoms of castor wilt caused by *F. oxysporum* f. sp. *ricini* as yellowing, sickly appearance of infected plant and marginal leaf necrosis, which later covers the leaves entirely. Leaves shrivel and lower leaves drop leaving limited top leaves followed by irretrievable wilting of the plant leading to rapid death [6]. Transverse and longitudinal sections of the affected roots revealed the presence of fungus in vascular tissues and xylem parenchyma. The tyloses development is observed in xylem vessels of infected roots.

Recent advancements in integrated disease management have focused on the use of biorational products alongside novel fungicides to mitigate *F. oxysporum* infections. Biorationals, such as biocontrol agents and plant extracts, offer promising alternatives due to their eco-friendly nature and minimal impact on non-target organisms [7]. These products harness natural mechanisms to suppress fungal growth and enhance plant resistance. Moreover, the development and application of new fungicides tailored to combat *F. oxysporum* provide additional avenues for disease control. These fungicides are designed to be effective against specific fungal strains while minimizing environmental persistence and toxicity concerns.

This study aims to investigate the efficacy of selected biorational agents and new fungicides against *F. oxysporum* f. sp. *ricini* under controlled in vitro conditions. By assessing their impact on fungal growth inhibition, spore germination, and disease progression, this research seeks to contribute to the development of integrated disease management strategies for sustainable castor bean production. Through this investigation, we aim to provide valuable insights into the potential of biorationals and new fungicides as effective tools for managing *F. oxysporum* f. sp. *ricini* in agricultural settings. This research is crucial in advancing our understanding of alternative disease management practices that balance efficacy with environmental and economic sustainability.

MATERIALS AND METHODS

Collection and Preparation of inoculum

Plants displaying characteristic symptoms was selected from the AICRP Castor Research plot, K-block, ZARS, GKVK, UAS, Bengaluru. Tissue isolation technique was employed to isolate the pathogen on Potato Dextrose Agar (PDA).

Preparation of inoculum

Semi-cooked sorghum grains (100g in 250 ml conical flask) were autoclaved for approximately 20 minutes at 121°C and 15 psi pressure. Subsequently, the flasks were inoculated with actively growing fungal mycelial culture derived from purified culture on Potato Dextrose Agar (PDA). The inoculated flasks were then placed in an incubator and maintained for 15 days at $27 \pm 2^\circ\text{C}$. Once the sorghum grains were completely colonized by the fungus, they were regularly shaken by hand to achieve a concentration of 1×10^6 conidia/ml.

IN VITRO EVALUATION OF BIORATIONALS

The poison food technique was used to evaluate biorationals under *in vitro* for their influence on pathogenic fungus development (Nene and Thapliyal 1973). Plant parts of the eight selected medicinal crops (100 g) were first washed with tap water and then with sterilized distilled water and air dried, weighed plant materials were ground in pestle and mortar. The materials were homogenized for 5 minutes then filtered through double layered muslin cloth followed by Whatman No. 1 filter paper and filtrates were considered as standard extract (100%). The standard extracts solution was individually incorporated into sterilized medium in 250 ml conical flasks at required quantities of 10%, 15% and 20% concentration after Millipore filtration. The melted PDA was poured into 90 mm sterilized Petri plate and PDA without extracts was maintained as control. All plates were replicated three times and the data was analysed using CRD. Plates were inoculated with 5 mm mycelium disc of seven days old test fungus culture and incubated at $26 \pm 10^\circ\text{C}$ for 5 days. The radial growth of the mycelium was measured after day of incubation and percent inhibition of growth was calculated (Vincent, 1947). Then plate was incubated at room temperature until control plate was entirely covered by test fungus. Each treatment was

replicated thrice. The efficacy of various biorationals was expressed as per cent inhibition of mycelial growth over control and calculated by using the formula [8] as follows

$$I = (C-T)/C \times 100$$

where ,

I = Per cent inhibition of mycelial growth

C = Growth of mycelium in control

T = Growth of mycelium in treatment

EVALUATION OF NEW FUNGICIDE MOLECULES *IN VITRO*

The poison food technique was used to evaluate the fungicide effect under *in vitro* for their effect on fungus development [9]. The PDA medium was prepared and autoclaved, various test fungicides of concentrations 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm were prepared adding to the same medium. Initially the test fungicides were dissolved in 100 ml of sterile molten PDA medium and 15 ml of PDA was poured into sterile Petri plates under aseptic conditions and allowed to solidify. Mycelial discs of *Fusarium* (7 mm diameter) were cut from 7-day old culture plate and placed it in the centre of Petri plate containing PDA medium amended with fungicide. The PDA medium (without fungicide) inoculated with test fungus alone served as control. The plates were incubated at room temperature. Each treatment was replicated thrice. After the control plate was fully covered with the pathogen culture, the radial growth of fungus was measured. The efficacy of various new fungicide and radial growth of fungus was measured and calculated.

RESULTS

***In vitro* evaluation of biorationals and new fungicide molecules against *Fusarium* wilt pathogen of castor**

***In vitro* evaluation of biorationals**

To reduce the harmful effect of synthetic chemical pesticides, the use of biorationals was initiated. The biorationals are biological products extracted and used directly in the process of pathogen inhibition. A total of 8 biorationals were tested viz., ginger, onion,

bougainvillea, garlic, chilli, turmeric, neem and aloevera at three different concentrations of 10, 15, and 20 per cent against *F. oxysporum*. f. sp. *ricini* pathogen causing wilt in castor.

Among the biorationals tested during the investigation, radial growth was reported to be high in Aloe vera with 61.33, 57.67 and 75.33mm at 10, 15 and 20 per cent concentrations, respectively. Onion extract showed second highest radial growth with 51.67, 61.33 and 73.67mm radial growth in 10, 15 and 20 per cent concentrations, respectively. The mycelial inhibition at 10 per cent of biorational extraction was best in neem (54.07 %), followed by turmeric (47.41%) and least effect in aloe vera (31.85%). Whereas at 15 per cent concentration, neem and ginger proved to be the best with 53.70 and 53.33 per cent, respectively and least inhibition of 31.85 mm was obtained in onion extract. At 20 per cent concentration chilli extract (51.11%) inhibited best followed by ginger (42.22%) and turmeric (41.48%) and least results were shown in aloe vera (16.30%). Overall, among the treatments tested the best was neem at 10 and 15 per cent followed by chilli (15 and 20%). Aloe vera was not that much effective when compared to the effectiveness shown by other treatments in suppressing the pathogen causing wilt (**Table 1, Figure 1A and Figure 2A**).

***In vitro* evaluation of new fungicide molecules**

Various molecules of fungicides are being used for the management of wilt in castor fields. So, for the updation of chemical fungicides under field conditions, such as Picoxystrobin 6.78% + Tricyclazole 20.33%; Fluopyram 17.7% + Tebuconazole 17.7%; Boscalid 25.2% + Pyraclostrobin 12.8% and Iprovalicarb 5.5% + Propineb 61.25% along with two standard fungicides, new molecules were assayed under in vitro. Checks Carbendazim and Thiophanate-methyl were tested against the pathogen *F. o. f. sp. ricini* in castor. Different concentrations such as 500, 1000, 1500 and 2000 ppm were taken to study the pathogen growth inhibition.

Among the tested combi-products against the pathogen, Picoxystrobin 6.78% + Tricyclazole 20.33% proved to be best with 100 per cent inhibition at all the concentrations (500, 1000, 1500 and 2000 ppm) followed by Fluopyram 17.7% + Tebuconazole 17.7% with 87.04, 87.41 and 87.04 per cent at 1000, 1500 and 2000 ppm. Boscalid 25.2% + Pyraclostrobin 12.8% proved to be best at 1500 and 2000 ppm with 80.74 and 83.70 per cent mycelial inhibition respectively. The least response was observed in Iprovalicarb 5.5% + Propineb 61.25% with 31.85, 22.63, 30.74 and 41.48 per cent at the four different

concentrations mentioned, respectively. Carbendazim 50% and Thiophanate-methyl 70% also recorded 100 percent inhibition rate at 500, 1000, 1500 and 2000 ppm. Highest mean radial growth of pathogen Iprovalicarb 5.5% + Propineb 61.25% was 63.25 mm mean radial growth when compared to Picoxystrobin 6.78% + Tricyclazole 20.33% with a zero mm radial growth during the study (**Table 2, Figure 2B and Supplementary figure 2B**).

DISSCUSSION

The *in vitro* evaluation of biorationals and new fungicide molecules against *Fusarium oxysporum* f. sp. *ricini* has provided insightful data on their efficacy in controlling the castor wilt pathogen. This investigation aimed to identify environmentally friendly and effective alternatives to synthetic chemical pesticides, addressing both pathogen suppression and sustainability concerns. Among the eight biorationals tested (ginger, onion, bougainvillea, garlic, chili, turmeric, neem, and aloe vera), the results demonstrated variable effectiveness. Aloe vera exhibited the highest radial growth across all concentrations (10%, 15%, and 20%), indicating its limited inhibitory effect on the pathogen. Onion extract also showed high radial growth, further suggesting its lower efficacy.

Conversely, neem extract showed the highest mycelial inhibition at 10% and 15% concentrations, followed by turmeric. Neem extract's effectiveness at lower concentrations underscores its potential as a biocontrol agent. Chili extract proved most effective at 20% concentration, followed by ginger and turmeric, highlighting the concentration-dependent efficacy of biorationals. Overall, neem and chili extracts emerged as promising candidates for further exploration and application in integrated disease management strategies for castor wilt.

The evaluation of new fungicide molecules revealed significant variations in pathogen growth inhibition. Picoxystrobin 6.78% + Tricyclazole 20.33% achieved 100% inhibition at all tested concentrations (500, 1000, 1500, and 2000 ppm), demonstrating its superior efficacy. Similarly, Carbendazim and Thiophanate-methyl also achieved 100% inhibition across all concentrations, reaffirming their reliability as standard fungicides. Fluopyram 17.7% + Tebuconazole 17.7% and Boscalid 25.2% + Pyraclostrobin 12.8% showed high inhibition rates at higher concentrations, though not as consistently effective as Picoxystrobin + Tricyclazole. Iprovalicarb 5.5% + Propineb 61.25% exhibited the least inhibition, with the highest mean radial growth of the pathogen, indicating its relatively lower effectiveness.

Studies conducted by [10-12] on castor wilt, revealed that the extract of turmeric rhizome showed inhibition of 42.22 per cent followed by marigold leaves extract (36.67%) and least by datura (18.89%). Singh *et al.* (2021) evaluated the botanicals in the management of *F. o. f. sp. ciceri* of chickpea and found that neem leaves was most effective at all three concentrations (5, 10 and 15%) with 86.61, 89.66 and 94.29 per cent of inhibition or growth, respectively followed by guava by 87.94 and 92.41 per cent at 10 and 15 per cent concentrations. [12] conducted studies against *F. o. f. sp. phaseoli* using five aqueous plant extracts where he found out cumin aqueous extract (85.80, 91.80 and 94.81%) was showing the best results compared to others.

The findings of [13] obtained best treatment at the concentration of 1500 ppm for Mancozeb 50% + Thiophanate-methyl 25% followed by Propineb 86.6% at 2000 ppm and least inhibition was observed in Azoxystrobin 55.8% at 1000 ppm.

[14] found similar results in castor wilt using combi fungicides at 4 different concentrations (250, 500, 1000, 1500 ppm). Carbendazim 12% + Mancozeb 63% recorded highest growth inhibition with 66.39, 69.02, 69.88, 75.12 percent, respectively at various concentrations. The least was recorded in Zineb 68% + Hexaconazole 4% with the inhibition rate of 37.07, 43.66, 45.23 and 48.16 per cent concentrations used, respectively.

CONCLUSION

The *in vitro* evaluation of biorationals and new fungicide molecules against *Fusarium oxysporum* f. sp. *ricini* provided valuable insights into their potential for managing castor wilt. Among the biorationals, neem extract demonstrated the highest efficacy in inhibiting mycelial growth at 10% and 15% concentrations, followed by chili extract at 20%. In contrast, aloe vera and onion extracts exhibited the least inhibitory effects. These findings highlight the potential of neem and chili extracts as promising biocontrol agents for sustainable castor wilt management. Regarding fungicides, Picoxystrobin 6.78% + Tricyclazole 20.33% and the standard fungicides Carbendazim 50% and Thiophanate-methyl 70% achieved 100% inhibition across all tested concentrations. Fluopyram 17.7% + Tebuconazole 17.7% and Boscalid 25.2% + Pyraclostrobin 12.8% also showed high inhibition rates at higher concentrations. Iprovalicarb 5.5% + Propineb 61.25% was the least effective fungicide, indicating a need for further optimization or replacement with more potent alternatives.

Future research should focus on field trials to validate the efficacy of the most promising biorationals and fungicide combinations under natural conditions. Additionally, exploring the synergistic effects of combining biorationals with fungicides could enhance overall disease management strategies. Investigations into the modes of action, optimal application methods, and long-term environmental impacts of these treatments will be crucial for developing integrated and sustainable approaches to managing *Fusarium* wilt in castor.

ETHICAL STATEMENT

All the experimental procedures involving only on plant species which were conducted in accordance with the University of Agricultural Science, Bangalore institutional guidelines. There are no human and animal subject/ trails conducted in this article and informed consent is not applicable.

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T1	Ginger	67.67	42.00	52.00	53.89	24.81 (29.87)	53.33 (46.89)	42.22 (40.51)	40.12 (39.29)
T2	Onion	51.67	61.33	73.67	62.22	42.59 (40.72)	31.85 (34.35)	18.15 (25.20)	30.86 (33.74)
T3	Bougainville	52.67	52.67	73.00	59.44	41.48 (40.08)	41.48 (40.08)	18.89 (25.75)	33.95 (35.62)
T4	Garlic	52.67	53.00	54.67	53.44	41.48 (40.08)	41.11 (39.86)	39.26 (38.78)	40.62 (39.58)
T5	Turmeric	47.33	46.33	52.67	48.78	47.41 (43.50)	48.52 (44.13)	41.48 (40.08)	45.80 (42.58)
T6	Neem	41.33	41.67	62.00	48.33	54.07 (47.32)	53.70 (47.11)	31.11 (33.89)	46.30 (42.86)
T7	Chilli	58.33	43.33	44.00	48.56	35.19 (36.37)	51.85 (46.04)	51.11 (45.62)	46.05 (42.72)
T8	Aloe vera	61.33	57.67	75.33	64.78	31.85 (34.35)	35.93 (36.81)	16.30 (23.80)	28.02 (31.95)
	Mean	54.13	49.75	60.92		43.33 (41.15)	44.72 (41.95)	32.31 (34.63)	
	Source	SE(d)		C. D. (p 0.01)		SE(d)		C. D. (p 0.01)	
	Biorational (B)	0.56		1.51		0.39		1.05	
	Concentration (C)	0.34		0.93		0.24		0.65	
	B × C	0.97		2.62		0.68		1.83	

Table 2: *In vitro* evaluation of new fungicide molecules against *Fusarium* wilt pathogen of castor

Tr No.	Fungicide	Radial growth (mm)*				Mean	Per cent inhibition over control *				Mean
		Concentration (ppm)					Concentration (ppm)				
		500	1000	1500	2000		500	1000	1500	2000	
T1	Iprovalicarb 5.5% + Propineb 61.25% WP	61.33	76.66	62.33	52.66	63.25	31.85 (34.35)	14.81 (22.63)	30.74 (33.66)	41.48 (40.08)	29.72 (33.02)
T2	Fluopyram 17.7% + Tebuconazole 17.7% SC	12.00	11.667	11.33	11.66	11.67	86.67 (68.56)	87.04 (68.87)	87.41 (69.19)	87.04 (68.87)	87.04 (68.87)
T3	Picoxystrobin 6.78% + Tricyclozole 20.33% SC	0.00	0.00	0.00	0.00	0.00	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
T4	Boscalid 25.2 % + Pyraclostrobin 12.8 % WG	27.33	25.00	17.33	14.66	21.08	69.63 (56.54)	72.22 (58.17)	80.74 (63.94)	83.70 (89.96)	76.57 (61.03)
T5	Thiophanate-methyl 70% WP	0.00	0.00	0.00	0.00	0.00	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
T6	Carbendazim 50% WP	0.00	0.00	0.00	0.00	0.00	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)	100.00 (89.96)
Mean		16.78	18.89	15.17	13.17		81.36 (64.39)	79.01 (62.71)	83.15 (65.74)	85.37 (63.57)	
Source		SE(d)		C. D. (p 0.01)			SE(d)		C. D. (p 0.01)		
Fungicide (F)		0.39		1.06			0.30		0.82		
Concentration (C)		0.32		0.86			0.25		0.67		

F × C	0.79	2.11	0.61	1.63
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UNDER PEER REVIEW

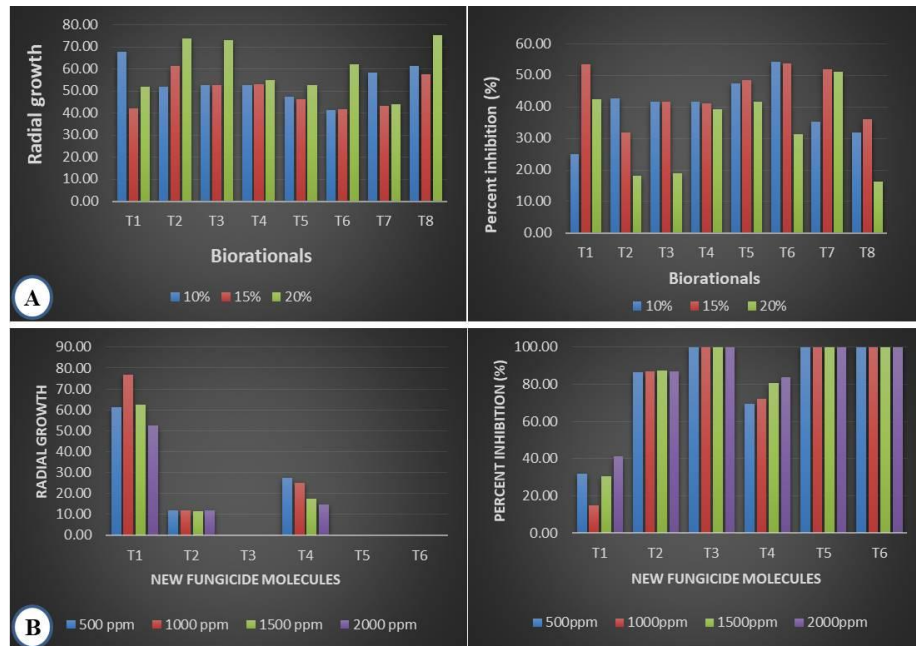


Figure 1: 1A. *In vitro* evaluation of biorationals showing radial growth and mycelial inhibition
1B. *In vitro* evaluation of new fungicide molecules against *Fusarium* wilt pathogen of castor showing inhibition

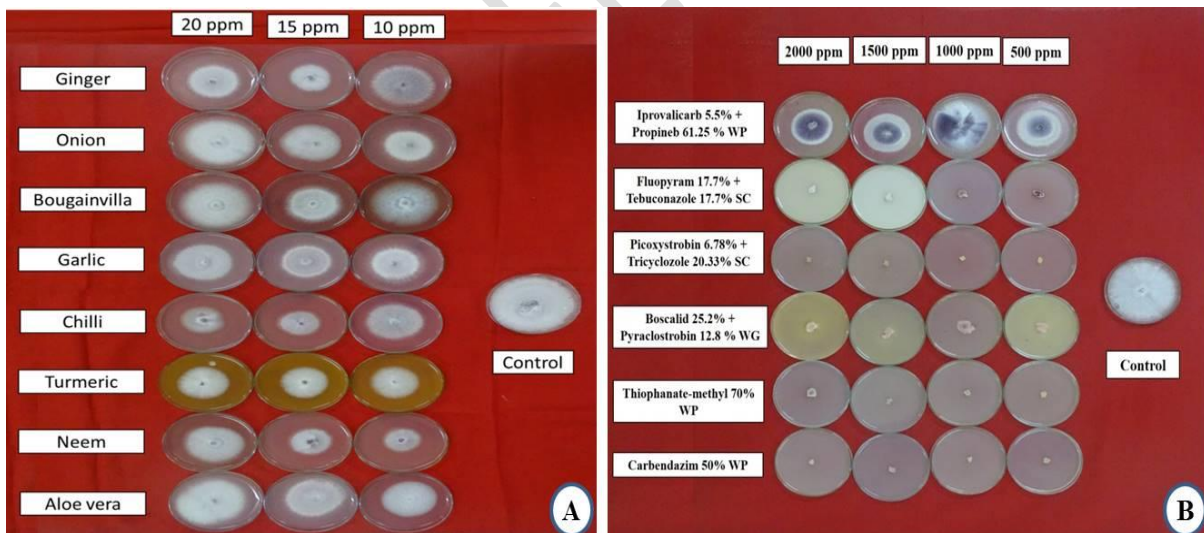


Figure 2. 2A. *In vitro* evaluation of biorationals against *Fusarium* wilt pathogen of castor showing inhibition **2B.** *In vitro* evaluation of new fungicide molecules against *Fusarium* wilt pathogen of castor showing inhibition.

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