

Original Research Article

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

Commented [D1]: Management of Fusarium wilt of Castor (*Ricinus communis* L.) caused by *Fusarium oxysporum* f.sp. *ricini* with biorationals and fungicides under *in vitro* conditions

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 **different test** concentrations of 10%, 15%, and 20% against *Fusarium oxysporum* f. sp. *ricini*, the pathogen causing wilt in castor **plants**. **Among all the tested biorationals, chilli founs as most effective while** aloe vera showed the least pathogen suppression. 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, 1000, 1500 and 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.

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

INTRODUCTION

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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 **the** 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]. Castor is affected by

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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]. Symptoms of castor wilt caused by *F. oxysporum* f. sp. *ricini* seen 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 of castor plants.

Recent advancements in integrated disease management have focused on the use of biorational products alongside novel fungicides to mitigate the *Fusarium 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 the natural mechanisms to suppress the fungal growth and enhance plant resistance.

The current study aims to investigate the efficacy of selected biorationals and new fungicide molecules 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 plays a crucial role in advancing the understanding of alternative disease management practices that balance the efficacy with both environmental and economic sustainability.

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2. MATERIALS AND METHODS

2.1 COLLECTION AND PREPARATION OF INOCULUM

Plants displaying characteristic wilt 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) under *in vitro* conditions.

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2.1.1 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 ± 2°C. Once the sorghum grains were completely colonized by the fungus, they were regularly shaken by hand to achieve a concentration of 1 × 10⁶ conidia/ml.

2.2 EVALUATION OF BIORATIONALS IN VITRO

(Nene and Thapliyal 1973). Plant parts of the eight selected medicinal crops (100 g) were first washed thoroughly 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 and 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 statistically using Completely Randomized Design (CRD). Plates were inoculated with 5 mm mycelium discs of seven days old test fungus culture and incubated at 26±10 °C for 5 days. The radial growth of the mycelium was measured after a day of incubation and percent growth inhibition of growth was calculated (Vincent, 1947). Then plate was incubated at room temperature until the growth of the control plate was entirely covered by test fungus. Each treatment was replicated thrice. The efficacy of various biorationals was

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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 = Radial growth of mycelium in control

T = Radial growth of the test fungus in treatment (mm)

2.3 EVALUATION OF NEW FUNGICIDE MOLECULES *IN VITRO*

The Poison food technique was followed to evaluate the fungicide effect under in vitro [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 (check). 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.

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

where ,

I = Per cent inhibition of mycelial growth

C = Radial growth of mycelium in control

T = Radial growth of the test fungus in treatment (mm)

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Commented [D35]: The diameter of the fungal colonies in the treatments was measured when the growth in the control plate was full. The colony diameter was measured, compared with control and reduction in the growth was taken as a measure of fungi toxicity. Per cent inhibition of the pathogen in different chemical treatments over control was calculated by the following formula.

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3. RESULTS AND DISCUSSION

3.1 *In vitro* evaluation of biorationals and new fungicide molecules against

Fusarium oxysporum f.sp. *ricini*

3.1.1 *In vitro* evaluation of biorationals against *Fusarium oxysporum* f.sp. *ricini*

To reduce the harmful effect of synthetic chemical pesticides, the use of biorationals was initiated. The biorationals are biological products extracted from the plant source and are used directly in the process of pathogen inhibition. In the current investigation, a total of eight biorationals were tested viz., ginger, onion, bougainvillea, garlic, chilli, turmeric, neem and aloe vera at three different concentrations of 10, 15 and 20 per cent against *F. oxysporum* f. sp. *ricini*, causing wilt in castor.

(Table 1, Figure 1A and Figure 2A).

3.1.2 *In vitro* evaluation of fungicides on the mycelial growth *Fusarium oxysporum* f.sp. *ricini*

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

Evaluation of biorationals and new fungicide molecules *in vitro* against *F. oxysporum* f. sp. *ricini* has provided insightful data on their efficacy in controlling the wilt disease in castor. The current investigation aimed to identify the environmentally friendly and effective treatments 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 percentage at 10% and 15% concentrations, followed by Ginger and chilli extracts turmeric. Neem extract's effectiveness at lower concentrations underscores its potential as a biocontrol agent. Chilli extract proved most effective at 20% concentration, followed by ginger and turmeric, highlighting the concentration-dependent efficacy of biorationals. Overall, neem and chili

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Commented [D41]: Efficacy of four new fungicides along with two standard fungicides as checks (Thiophante methyl 70 % WP and Carbendazim 50 % WP) were evaluated against *Fusarium oxysporum* f.sp. *ricini* at four different concentrations viz., 500, 1000, 1500 and 2000 ppm by following Poisoned Food Technique as described in Materials and Methods (Section 2.3)

The data from the Table 2, Figure 1B and Plate 1B revealed that among the tested combi products against the test pathogen, picoxytrobin 6.78%+Tricyclazole 20.33% was found highly effective and completely inhibited the mycelia growth of the test pathogen at all four different concentrations tested and it was superior over the combiproducts treatments tested in reducing the radial growth of the pathogen whereas, Fluopyram 17.7% + Tebuconazole 17.7% was found to be the next best with per cent inhibitions of 87.41 (1500 ppm); 87.04 (1000 & 2000 ppm) and 86.67 (500 ppm) followed by Boscalid 25.2 % + Pyraclostrobin 12.8 % with the inhibition of 83.70% (2000 ppm) and 80.74 % (1500 ppm).

Lowest inhibition per cent of 31.85 (500 ppm), 14.81 (1000 ppm), 30.74 (1500 ppm) and 41.48 (2000 ppm) were recorded with Iprovalicarb 5.5 % + propineb 61.25% . It is evident from the results that the inhibition in the growth of the test pathogen increased with the increase in the concentration of the fungicides. Two standard check fungicides (Thiophante Methyl and carbendazim) also recorded cent per cent inhibition at 500, 1000, 1500 and 2000 ppm.

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extracts emerged as promising candidates for further exploration and application in integrated disease management strategies for castor wilt under field conditions also.

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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.

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The current results are in conformity with Vahunia *et al*, 2017; Yelmame *et al*, 2010 and Elhelaly and Ammar, 2022 who reported 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 per cent inhibition of 86.61, 89.66 and 94.29 respectively. 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.

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[14] found similar results in castor wilt using combi fungicides tested at four different concentrations (250, 500, 1000, 1500 ppm), carbendazim 12% + Mancozeb 63% recorded highest growth inhibitions 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.

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

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4. CONCLUSION

The *in vitro* Evaluation of biorationals and new fungicide molecules against *F. oxysporum* f.sp. *ricini* provided significant valuable insights into their potential for managing wilt disease in castor. Among the biorationals, neem extract demonstrated the highest efficacy in inhibiting mycelial growth at 10% and 15% concentrations, followed by chilli extract at 20%. In contrast, aloe vera and onion extracts exhibited the least inhibitory effects. These findings highlight the potential use of neem and chili extracts as promising biocontrol agents for sustainable castor wilt disease under field conditions too. Regarding fungicides, Picoxystrobin 6.78% + Tricyclazole 20.33% and the two standard check fungicides, carbendazim 50% and Thiophanate-methyl 70% achieved 100% inhibition across all tested concentrations. Fluopyram 17.7% + Tebuconazole 17.7% served as next best alternative in reducing the mycelia growth of *F.oxysporum* f.sp. *ricini* and maximum inhibition percentage.

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5. FUTURE LINE OF WORK

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 the *Fusarium* wilt in castor.

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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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Table 1. Observation of *in vitro* evaluation of biorationals against *Fusarium* wilt pathogen of castor

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Tr. No.	Biorational Extracts	Radial growth (mm) of pathogen at different Biorational concentration (%)			Radial growth Mean** (mm)	Per cent inhibition of pathogen over control (mm) Concentrations (%) of biorationals			Per cent Inhibition Mean** (%)
		10	15	20		10	15	20	
T ₁	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)
T ₂	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)
T ₃	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)
T ₄	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)
T ₅	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)
T ₆	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)
T ₇	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)
T ₈	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	

** Mean of three replications

Table 2: *In vitro* evaluation of new fungicides against *Fusarium oxysporum* f.sp. *ricini*

Tr.No.	Name of the Treatment	Radial growth (mm)				Radial growth Mean** (mm)	Per cent inhibition over control				Per cent Inhibition 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 x C		0.79		2.11		0.61		1.63			

** Mean of three replications

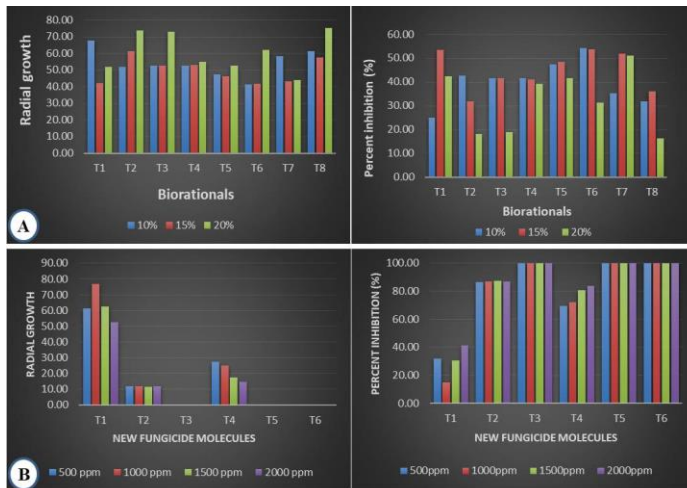


Figure 1A. *In vitro* evaluation of biorationals showing radial growth (mm) and mycelial inhibition (%)

Figure 1B. *In vitro* evaluation of new fungicide molecules against *Fusarium oxysporum f.sp. ricini*

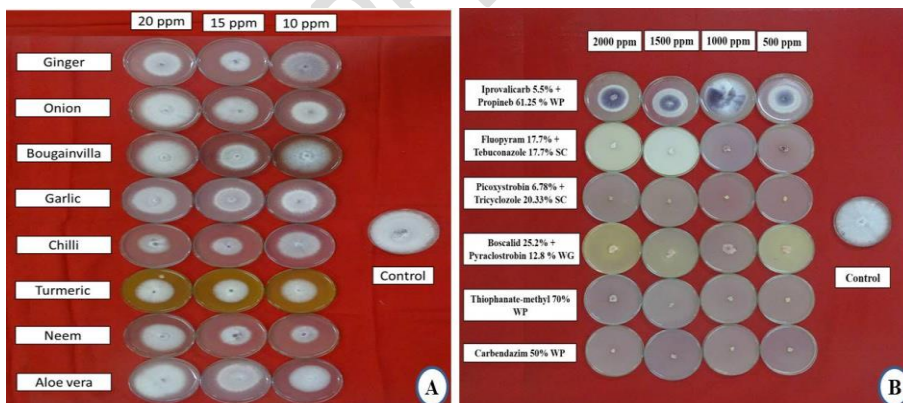


Plate 1A. *In vitro* evaluation of biorationals against showing inhibition against *Fusarium oxysporum f.sp. ricini*

Plate 1 B. *In vitro* evaluation of new fungicide molecules against *Fusarium oxysporum f.sp. ricini*

UNDER PEER REVIEW

