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Management of Fusarium Wilt of Castor (*Ricinus communis* L.) Caused by *Fusarium oxysporum f.sp.* Ricini with Biorationals and Fungicides under *In vitro* Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. Author CPM conceptualized the research work. Author CPM did data curation. Authors CPM and Divyashree did formal analysis. Author CPM investigated the study. Author CPM did data visualization. Author CPM wrote original draft. Author CSR supervised the study. Authors CSR and Yamanura did data validation. Author CRJB wrote, reviewed and edited the manuscript. Authors Yamanura and Divyashree supervised the study. Author Yamanura did funding acquisition. All authors read and approved the final manuscript.

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Original Research Article

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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. Among all the tested biorationas, chilli found as most effective while aloe vera showed the least pathogen suppression. The study recommended neem and chili as effective biorationals against *F. oxysporum f. sp. ricini*. New fungicide combinations *i.e* Picoxystrobin + Tricyclazole, Fluopyram + Tebuconazole, Boscalid + Pyraclostrobin, and Iprovalicarb + Propineb were tested under *in vitro* condition out of which Picoxystrobin + Tricyclazole and the standard fungicides Carbendazim and Thiophanate-methyl showed cent percent 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.

Keywords: Castor; Fusarium spp; biorationals; fungicides.

1. 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 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]. at all crop growth stages depending on the seasonal conditions [3]. Among all diseases *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]. Describtion of the 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. Droping of lower leaves 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 and novel fungicides to mitigate *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 evaluate 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.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Inoculum

Plants showing characteristic wilt symptoms were collected from the AICRP Castor Research plot, K-block, ZARS, GKVK, UAS, Bengaluru. Tissue isolation technique was followed to isolate the pathogen on Potato Dextrose Agar (PDA) under *in vitro* condition.

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 1×10^6 conidia/ml.

2.2 Evaluation of Biorationals *In vitro*

Efficacy of biorationals against the test pathogen were tested by following Poison Food Technique as described by Nene and Thapliyal, 1973. Plant parts of 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 Complete Randomized Design (CRD). Plates were inoculated with 5 mm mycelium discs of seven days old fungal culture and incubated at 26±10 ºC for 5 days. The radial growth of the mycelium was measured after a day of incubation and percent growth inhibition was calculated [8]. The plates were incubated until the growth of the control plate was completely covered by test fungus. Each treatment was replicated thrice. The efficacy of different 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 in growth of pathogen
C = Radial growth of the test fungus in control (mm) and
T = Radial growth of the test fungus in treatment (mm)

2.3 Evaluation of New Fungicide Molecules *In vitro*

Poison food technique was followed to evaluate the efficacy of fungicide molecules under *in vitro* for their effect on fungus development [9]. 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. 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.

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

where,

I = Per cent inhibition in growth of pathogen
C = Radial growth of the test fungus in control (mm) and
T = Radial growth of the test fungus in treatment (mm)

3. RESULTS

3.1 In vitro Evaluation of Biorationals and New Fungicide Molecules against *Fusarium oxysporum* f. sp. *Ricini* wilt Pathogen of Castor

To reduce the harmful effect of synthetic chemical pesticides, the current study was planned with biorationals which 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 aloevera at three different concentrations of 10, 15, and 20 per cent against *F. oxysporum*. f. sp. *ricini* pathogen causing wilt in castor.

The data from the Table 1, Fig. 1A and Plate 1A clearly showed that Neem (T_6) showed a mean maximum inhibition of 46.30% followed by chilli (T_7) with the mean inhibition per centage of 46.05 however both are on par with one another. Lowest mean inhibition percentage was recorded in T_8 *i.e* Aloevera (28.02%). Among all the biorationals tested at three different concentrations i.e 10, 15 & 20%. Neem @ 10 & 15% concentration showed maximum inhibition of 54.07 & 53.70% respectively followed by ginger at 15% concentration (53.33%) and with inhibition percentage of 51.85 and 51.11% in chilli at 10 & 15% respectively.

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, Fig. 1B and Plate 1B revealed that among the tested combi products against the test pathogen, picoxytrsobin 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 their combi products 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 pof 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%.

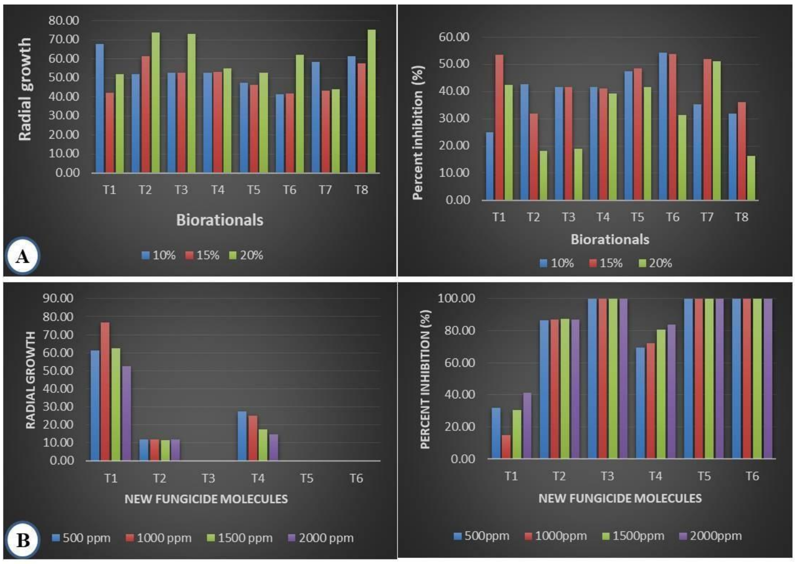

Fig. 1. 1A. *In vitro* evaluation of biorationals showing radial growth (mm) and mycelial inhibition (%)

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

Table 1. Efficacy of different biorationals on mycelial growth of the test pathogen, *Fusarium oxysporum* f. sp. *ricini In vitro*

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 the three replications*

Table 2. *In vitro* evaluation of new fungicide molecules against *Fusarium* oxysporum f. sp. ricini

Tr No.	Fungicide	Radial growth (mm)* Concentration (ppm)				Radial growth Mean** (mm)	Per cent inhibition over control * Concentration (ppm)				Per cent Inhibition Mean** (%)
		500	1000	1500	2000		500	1000	1500	2000	
T_1	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)
T_2	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)
T_3	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)
T_4	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)
T_5	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)
T_6	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		

***Mean of the three replications*

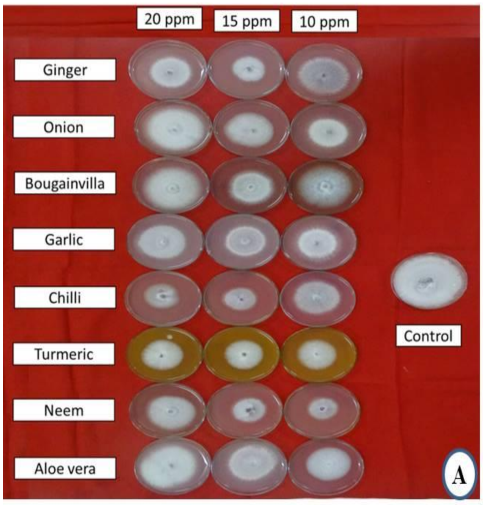

Plate 1A. *In vitro* evaluation of biorationals against *Fusarium* oxysporum f. sp. ricini

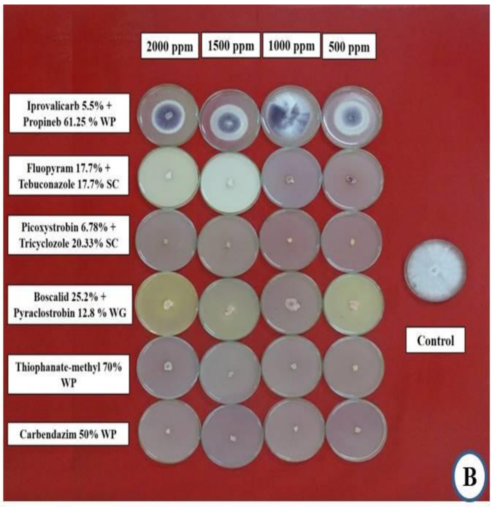

Plate 1B. *In vitro* evaluation of new fungicide molecules against *Fusarium oxysporum* f. sp. *ricini*

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.

4. DISCUSSION

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 chemicals pesticides, addressing both pathogen suppression and sustainability concerns. Among the eight biorationals tested (ginger, onion, bougainvillea, garlic, chili, turmeric, neem, and aloe vera).

Neem extract showed the highest inhibition at 10% and 15% concentrations, followed by Ginger and chilli extracts, neem and chili extracts emerged as promising treatments for further exploration and application in integrated disease management strategies for castor wilt under field conditions also.

The current results are in conformity with Vahunia et al. [10], Yelmane et al. [11] and Elhelaly and Ammar, [12] 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 of inhibition of 86.61, 89.66 and 94.29 respectively.
Among combi fungicides tested at four different concentrations (250, 500, 1000, 1500 ppm). Carbendazim 12% + Mancozeb 63% recorded highest growth inhibitions of 66.39, 69.02, 69.88, 75.12 percent respectively. The least was recorded in Zineb 68% + Hexaconazole 4% with the inhibition per cent of 37.07, 43.66, 45.23 and 48.16 per cent at 500, 1000, 1500 and 2000 ppm, respectively.

Vani et al. [13] reported that Mancozeb 50% + Thiophanate-methyl 25% found as best treatment at the concentration of 1500 ppm followed by Propineb (86.6%) at 2000 ppm and least inhibition was observed in Azoxystrobin (55.8%) at 1000 ppm [14].

5. CONCLUSION

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 chili extract at 20%. These findings highlight the potential use of neem and chili extracts as promising biocontrol agents for sustainable castor wilt disease under field conditions too. Picoxystrobin 6.78% + Tricyclazole 20.33% and the two standard check fungicides, Carbendazim 50% and Thiophanate-methyl 70% achieved cent per cent 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.

6. FUTURE PROSPECT

Future research should focus on field trials to validate the efficacy of the most promising biorationals and fungicide combinations which were proved as effective under *in vitro* 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 for managing the *Fusarium* wilt in castor.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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