Investigating the Antagonistic Effects of Novel Combination Fungicides Against *Fusarium oxysporum* f. sp. *ricini* Under *In-vitro* Conditions

ABSTRACT

Aim: The study aimed to assess the efficacy of nine novel fungicide combinations against *Fusarium oxysporum* f. sp. *ricini*, the causal agent of wilt disease in castor.

Methodology: The efficacy of fungicides was evaluated using the poisoned food technique under *in-vitro* conditions. Radial mycelial growth of the pathogen was measured across various concentrations (100 to 1000 ppm) of the fungicide combinations to determine their impact on mycelial inhibition.

Results: Results indicated a substantial reduction in radial mycelial growth with increased fungicide concentration. Among the tested fungicides, Tebuconazole + Trifloxystrobin and Prochloraz + Tebuconazole recorded complete mycelial inhibition (100 %) at all the tested concentrations, demonstrating significant fungicidal activity followed by Hexaconazole + Captan with a mean mycelial inhibition of 85.2 %, indicating strong potential for disease management. In contrast, the least mean mycelial inhibition (24.7 %) was observed with Thiophanate methyl + Pyraclostrobin.

Conclusion: These findings underscored the importance of selecting effective fungicide combinations to manage *F. oxysporum* f. sp. *ricini,* thereby enhancing crop protection and agricultural sustainability.

Keywords: Castor, Fungicide, F. oxysporum f. sp. ricini, Mycelial inhibition, Management

1. INTRODUCTION

Castor (*Ricinus communis* L.) is an economically important non-edible oilseed crop known for its versatile applications, including the production of oil used in various industries such as cosmetics, pharmaceuticals and biodiesel. However, the cultivation of castor crop is threatened by various challenges, particularly from biotic stresses such as wilt disease caused by *F. oxysporum* f. sp*. ricini* (Nanda and Prasad, 1974). This disease poses a serious threat to the castor crop throughout their growth stages. The disease is characterized by symptoms such as yellowing, wilting and subsequent death of the plant, significantly impacting crop yield and oil production. As a soil and seed-borne pathogen, *F. oxysporum* f. sp. *ricini* colonizes the xylem vessels, completely obstructing them and resulting in wilting of the plant (Dange, 2003; Santhalakshmi Prasad *et al.,* 2019).

The prevalence of wilt disease has become a major constraint on castor cultivation, resulting in substantial economic losses to the farmers. The extent of yield loss depends on the stage at which plant wilts 77 % at flowering, 63 % at 90 days and 39 % in later stages on secondary branches (Pushpavathi *et al.,* 1997), highlighting the urgent need for effective management strategies to combat this issue. Although resistance breeding programs have been initiated, their success has been limited primarily due to the wide diversity among the isolates of *F. oxysporum* f. sp*. ricini* and their ability to overcome host resistance (Anjani *et al.,* 2004).

To combat the adverse effects of wilt disease, various management practices have been proposed, with the application of fungicides emerging as a potentially effective strategy (Maitlo *et al.,* 2014). The chemical control of wilt disease through the use of fungicides can significantly mitigate pathogen spread and reduce disease incidence. Recent studies have demonstrated that certain

fungicides exhibit notable antagonistic activity against *F. oxysporum* f. sp. *ricini*, indicating that these chemicals may play a vital role in the integrated management of the wilt disease (Shankar *et al.* 2016; Yerukala *et al.,* 2021). Therefore, understanding the efficacy of fungicides against *F. oxysporum* f. sp. *ricini* is essential for developing sustainable agricultural practices to enhance castor production and prevent significant yield losses.

2. MATERIAL AND METHODS

The wilt affected root samples were collected from a wilt sick plot at the ICAR-Indian Institute of Oilseeds Research, Hyderabad. The pathogen was isolated from diseased root samples of castor following the methods described by Dhingra and Sinclair (1985). Diseased root samples were collected and 2 mm sections containing both infected and healthy tissues were thoroughly washed with sterilized distilled water. These sections were then surface sterilized using a 1 % sodium hypochlorite solution for 1 minute and rinsed thrice with sterile distilled water to remove any residual disinfectant. The sterilized pieces (4-5 per dish) were aseptically placed in sterilized Petri dishes containing Potato Dextrose Agar (PDA). The Petri dishes were then incubated at 27 ± 1°C and fungal growth was monitored after 2-3 days. The growing mycelium was subsequently transferred to fresh PDA for maintenance and storage. Pathogenicity tests were conducted on the susceptible castor variety JI-35. Re-isolation of the pathogen culture from the artificially inoculated plants was performed and the cultures obtained were compared to the original isolates to validate Koch's postulates. This isolated culture of *F. oxysporum* f. sp. *ricini* was utilized for fungicide antagonistic studies.

Nine novel combination fungicides were evaluated for their efficacy against *F. oxysporum* f. sp. *ricini* using the poisoned food technique under *in-vitro* conditions as described by Nene and Thapliyal (1973). The fungicides were tested at concentrations of 100, 250, 500, 750 and 1000 ppm. To prepare the fungicide poisoned medium, each fungicide was individually incorporated into sterilized molten Potato Dextrose Agar (PDA) to achieve the desired concentrations. Subsequently, 20 ml of the poisoned medium were dispensed into sterilized 90 mm Petri dishes. A 5 mm mycelial disc taken from the periphery of an actively growing fungal culture was placed at the center of each plate. Control plates were prepared without the addition of any fungicide. The plates were then incubated at 27 ± 1°C, with three replicates maintained for each fungicide. Radial mycelial growth of the *F. oxysporum* f. sp. *ricini* was measured once the control plates were fully colonized. The per cent inhibition of radial growth of mycelium was calculated using the formula provided by Vincent (1947).

$$I = \frac{(C-T)}{C} \mathbf{X} 100$$

Where, I = Per cent growth reduction of test pathogen; C = Radial growth of test pathogen in control (mm); T = Radial growth of test pathogen in treatment (mm).

3. RESULTS AND DISCUSSION

The efficacy of novel fungicide combinations against *F. oxysporum* f. sp. *ricini* was evaluated utilizing the poisoned food technique under *in vitro* conditions. The fungicide combinations tested included Azoxystrobin + Tebuconazole, Carboxin + Thiram, Hexaconazole + Captan, Hexaconazole + Zineb, Penflufen + Trifloxystrobin, Fluxapyroxad + Pyraclostrobin, Prochloraz + Tebuconazole,

Thiophanate methyl + Pyraclostrobin and Tebuconazole + Trifloxystrobin. These fungicides were evaluated at concentrations of 100 ppm, 250 ppm, 500 ppm, 750 ppm and 1000 ppm.

The results demonstrated a significant difference in the efficacy of fungicide combinations tested against mycelial inhibition of *F. oxysporum* f. sp. *ricini* (Table.1 and Fig.1). Although all the fungicides effectively suppressed the mycelial growth of *F. oxysporum* f. sp. *ricini,* significant reduction in mycelial growth of the pathogen was observed with the increase in concentration of fungicides. Notably, the combinations of Tebuconazole + Trifloxystrobin and Prochloraz + Tebuconazole recorded complete inhibition (100 %) of mycelial growth of *F. oxysporum* f. sp. *ricini* at all the tested concentrations, underscoring their superior fungicidal activity (Plate. 1 and Plate. 2). These fungicides exhibited the mean mycelial inhibition of 100 %. Following these, Hexaconazole + Captan showed mean mycelial inhibition of 85.2 %. The mycelial inhibition ranged from 83.0 % at 100 ppm to 88.1 % at 1000 ppm. Similarly, Carboxin + Thiram also displayed notable efficacy with a mean mycelial inhibition of 81.4 %, with inhibition ranged from 72.2 % at 100 ppm to 90.0 % at 1000 ppm (Table. 1 and Fig. 1).

Moreover, the combinations of Fluxapyroxad + Pyraclostrobin and Penflufen + Trifloxystrobin showed moderate efficacy in inhibiting the mycelial growth of *F. oxysporum* f. sp. *ricini,* with mean inhibition rates of 77.3 % and 73.4 %, respectively. For Fluxapyroxad + Pyraclostrobin, the inhibition ranged from 72.2 % at 100 ppm to 85.2 % at 1000 ppm. Meanwhile, Penflufen + Trifloxystrobin demonstrated a mycelial inhibition ranging from 65.2 % at 100 ppm to 78.5 % at 1000 ppm. In contrast, fungicide Thiophanate Methyl + Pyraclostrobin demonstrated the lowest efficacy by recording the mean inhibition of only 24.7 % with inhibition ranged from 16.3 % at 100 ppm and 33.3 % at 1000 ppm (Plate. 3). Following this, the combination of Azoxystrobin + Tebuconazole recorded a mean mycelial inhibition of 58.3 %, with inhibition varied from 50.4 % at 100 ppm and 73.3 % at 1000 ppm. Similarly, Hexaconazole + Zineb recorded a mean inhibition of 60.4 %, with inhibition ranging from 44.1 % at 100 ppm to 78.9 % at 1000 ppm.

The complete inhibition of mycelial growth of *F. oxysporum* f. sp. *ricini* observed with the combinations of Prochloraz + Tebuconazole and Tebuconazole + Trifloxystrobin may be attributed to the synergistic action of these fungicides. The superior efficacy of certain fungicides, particularly Tebuconazole, Prochloraz and Trifloxystrobin, can be attributed to multiple factors including their mechanism of action, concentration-dependent efficacy and chemical properties that enhance their interaction with fungal structures. Tebuconazole and Prochloraz, both Demethylation Inhibitors (DMIs), disrupt ergosterol biosynthesis in fungal cell membranes, disturbing the membrane integrity and causing cell death. Trifloxystrobin further enhances this effect by inhibiting mitochondrial electron transport, which reduces the energy production and impairs fungal growth. The solubility of Tebuconazole and Prochloraz allows better tissue penetration, in contrast to fungicides like Azoxystrobin, which have lower solubility and reduced efficacy.

The present results were found in accordance with the findings of Patil *et al.* (2012), who reported that Tebuconazole completely inhibited the mycelial growth of *F. oxysporum* f. sp. *cepae* even at 0.025% concentration. Chennakesavulu *et al.* (2013) supported the effectiveness of Tebuconazole, Carbendazim and Propiconazole in recording the complete inhibition of *F. udum* at 50

ppm. Ravichandran and Hegde (2015) also revealed that Carbendazim and Tebuconazole were most effective in completely inhibiting the mycelial growth of *F. oxysporum* f. sp. *ciceris.* Shankar *et al.* (2016) further confirmed the efficacy of Tebuconazole + Trifloxystrobin against mycelial inhibition of *F. oxysporum* f. sp. *ricini*. Priya *et al.* (2019) found that both Carbendazim and the Tebuconazole + Trifloxystrobin were most effective against *Fusarium* species. Patel *et al.* (2021) further supported these findings by demonstrating the efficacy of Carbendazim (0.1%) and Tebuconazole (0.1%) in inhibiting the mycelial growth of *F. udum*.

4. CONCLUSION

The findings underscored the importance of selecting highly effective fungicide combinations, such as Tebuconazole + Trifloxystrobin and Prochloraz + Tebuconazole, to manage *F. oxysporum* f. sp. *ricini* effectively. This approach can significantly enhance castor crop protection and contribute to sustainable agricultural practices.

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Table 1. Efficacy of fungicides against the *F. oxysporum* f. sp. *ricini* (IIOR) by poisoned food technique under *in-vitro* conditions

S. No.	Fungicide combination	Mycelial inhibition % at ppm					Mean mycelial inhibition (%)
		100	250	500	750	1000	
1	Azoxystrobin + Tebuconazole	50.4 (45.2)*	54.1 (47.3)	54.1 (47.3)	59.6 (50.6)	73.3 (58.9)	58.3[g] (49.8)
2	Carboxin + Thiram	72.2 (58.2)	74.8 (59.9)	84.1 (66.5)	85.9 (68.0)	90.0 (71.6)	81.4[c] (64.5)
3	Hexaconazole + Captan	83.0 (65.6)	83.3 (65.9)	84.8 (67.1)	86.7 (68.6)	88.1 (69.9)	85.2[b] (67.4)
4	Hexaconazole + Zineb	44.1 (41.6)	51.1 (45.6)	62.2 (52.1)	65.6 (54.1)	78.9 (62.6)	60.4[f] (51.0)

5	Penflufen + Trifloxystrobin	65.2 (53.8)	72.2 (58.2)	74.4 (59.6)	76.7 (61.1)	78.5 (62.4)	73.4[e] (59.0)
6	Fluxapyroxad + Pyraclostrobin	72.2 (58.2)	74.8 (59.9)	76.7 (61.1)	77.4 (61.6)	85.2 (67.4)	77.3[d] (61.5)
7	Prochloraz + Tebuconazole	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0[a] (90.0)
8	Thiophanate Methyl + Pyraclostrobin	16.3 (23.8)	21.5 (27.6)	24.1 (29.4)	28.1 (32.0)	33.3 (35.3)	24.7[h] (29.8)
9	Tebuconazole + Trifloxystrobin	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0[a] (90.0)
10	Control	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0[i] (0.0)
SE(m)±	Fungicide	0.3					
	Concentration	0.2					
	Fungicide x Concentration	0.6					
CD	Fungicide	0.7					
	Concentration	0.5					
	Fungicide x Concentration	1.6					

* Values expressed are mean of three replications; Means with similar alphabet do not differ significantly

**Figs in parenthesis are arc sine transformed values

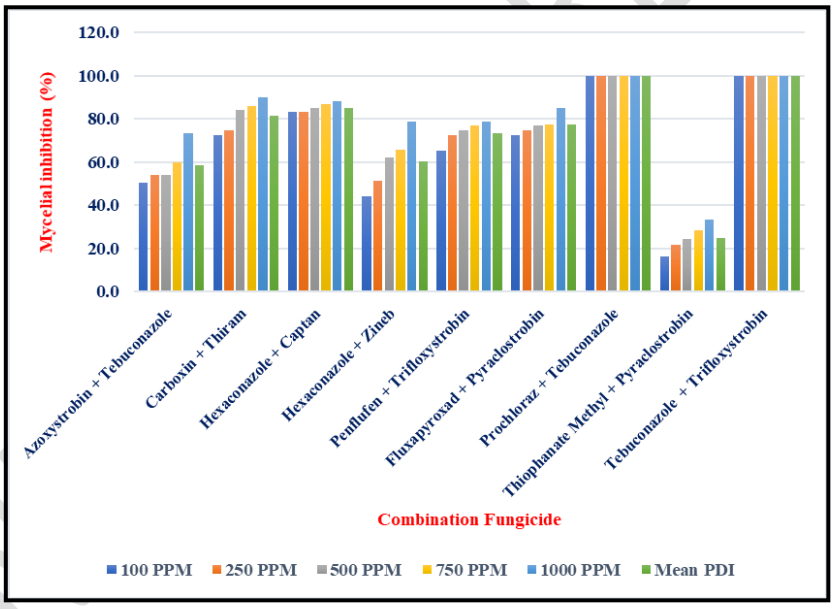

Fig 1. Efficacy combination fungicides on mycelial inhibition of *F. oxysporum* f. sp. *ricini* under *in-vitro* conditions

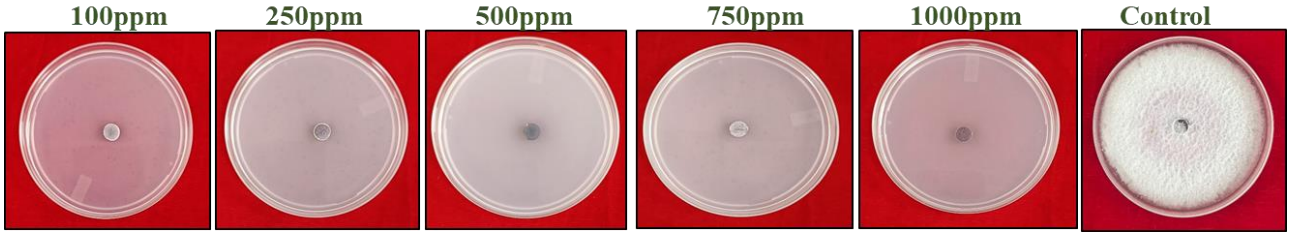

Plate 1. Efficacy of Tebuconazole + Trifloxystrobin on mycelium inhibition of *F. oxysporum* f. sp. *ricini under in-vitro conditions*

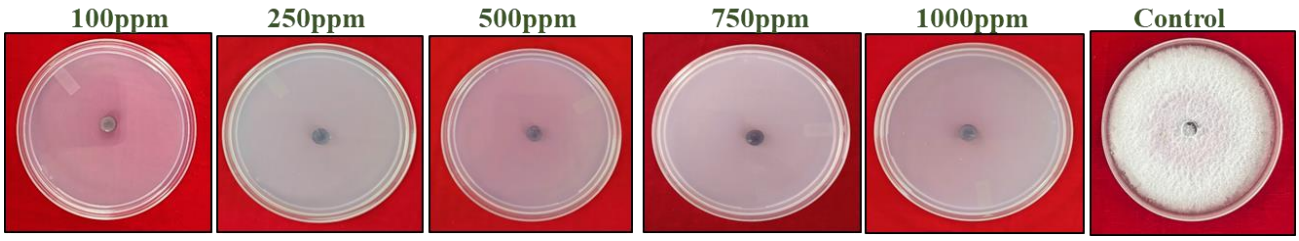

Plate 2. Efficacy of Prochloraz + Tebuconazole on mycelium inhibition of *F. oxysporum* f. sp. *ricini under in-vitro conditions*

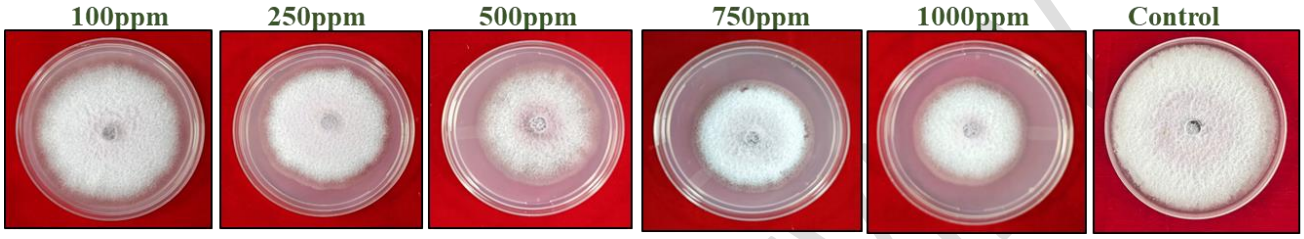

Plate 3. Efficacy of Thiophanate Methyl + Pyraclostrobin on mycelium inhibition of *F. oxysporum* f. sp. *ricini under in-vitro conditions*