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Evaluation of *in vitro* efficacy of fungicides against *Fusarium proliferatum* inciting root rot of cassava

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Abstract
Cassava (*Manihot esculenta* Crantz. Inst.) is one of the most important tropical tuber crops which play a vital role in sustainable food production and consumption in many countries. Root rot disease caused by *Fusarium proliferatum* causing a severe threat to cassava cultivation and yield loss may go upto 100%. *In vitro* evaluation was carried out for testing the efficacy of eight fungicides *viz.,* mancozeb 75% WP, copper oxy chloride 50% WP, propineb 70% WP, carbendazim 50% WP, propiconazole 25 % EC, cymoxanil 8% + mancozeb 64% WP, carbendazim 12% + mancozeb 63% WP and trifloxystrobin 25%+ tebuconazole 50 % 75 WG at 50, 100 and 200 ppm concentrations respectively against the *Fusarium proliferatum* by poisoned food technique. The results of the study revealed that trifloxystrobin 25% + tebuconazole 50% 75 WG at 200 ppm (100 percent inhibition) and carbendazim 50% WP at 200 ppm (87.87 percent inhibition) highly inhibited the mycelial growth of the pathogen.

Keywords: Fungicides, *Fusarium proliferatum*, root rot, cassava

Introduction
Cassava (*Manihot esculenta* Crantz. Inst.) is one of the most important tropical tuber crops which play a vital role in sustainable food production and consumption in many countries. This crop has the ability to produce more food per unit area, capacity to withstand adverse biotic and abiotic stresses and resilient to adverse conditions of drought and marginal lands. It is cultivated as a sole crop or as a mixed crop, mainly cultivated in homesteads and primarily used for household consumption (Kumar *et al.*, 2017) [4]. It is commonly used in textiles and cosmetics industries. It is also used as the raw material for industrial products. Cassava has an important role in mitigating nutritional deficiencies through diet diversification (Byju *et al.*, 2020) [1].

Since 2005, cassava tuber rot was observed in Kolli hills, Tamil Nadu and Kollam and Kottayam districts of Kerala. Cassava root rot can cause yield loss of 50–70 per cent (Sankar *et al.*, 2013) [6]. This disease mainly occurs in low land causing a major threat to cassava cultivation. It is characterised by browning and wilting of leaves followed by defoliation. Rotting of the roots result in lodging of stem and death of the plant. Root rot disease caused by *Fusarium proliferatum*. All local cultivars have been identified as susceptible in areas where the disease is found and 100 per cent yield loss has been observed from affected low land paddy fields of Kollam and Thiruvananthapuram district. Hence, the present investigation is envisaged to evaluate the efficacy of fungicides in managing the disease.

Materials and Methods
The efficacy of eight fungicides *viz.,* mancozeb 75% WP, copper oxy chloride 50% WP, propineb 70% WP, carbendazim 50% WP, propiconazole 25 % EC, cymoxanil 8% + mancozeb 64% WP, carbendazim 12% + mancozeb 63% WP and trifloxystrobin 25%+ tebuconazole 50 % 75 WG were evaluated at 50, 100 and 200 ppm concentrations against the .*F. proliferatum* using poisoned food technique (Nene and Thapliyal, 1993) [5].

The double strength potato dextrose agar medium is used for this evaluation. The 100 ml of double strength PDA medium and 100 ml distilled water was poured in separate conical flasks and sterilized them in an autoclave. The desired concentration of fungicides was weighed out, which was sufficient to prepare 50,100 and 200 ppm concentrations. The fungicide was mixed with 100 ml sterile water to double the field concentration. This fungicide solution was mixed in an equal (1:1 proportion) amount of sterile double-strength PDA (100 ml). The 20 ml of prepared media was poured into sterile Petri plates and allowed to solidify. It was the medium for culturing the pathogen by poison food technique.

A culture disc (5mm) from an actively growing five-day-old culture of pathogen was placed at the centre of the Petri plate and kept for incubation at room temperature (28 ± 2 °C) after wrapping properly. This technique was done for all the treatments with three replications each. A culture disc (5 mm) from a five-day-old pathogen placed in a normal PDA medium served as the control. The observations were made at 24 h intervals.

The radial growth of the pathogen in the control plate and treated plate were noted to find the percent inhibition by the fungicide using the following formula, as described by Vincent (1927) [9], to identify and select the effective fungicide.

Percent inhibition = (C-T) / C X 100,

where C-radial growth of the pathogen in control;
T - radial growth of the pathogen in the treatment with fungicides.

Results and Discussion

The growth of the pathogen was completely arrested in the media poisoned with trifloxystrobin 25% + tebuconazole 50% 75 WG at 200 ppm. The mycelial growth was observed in media poisoned with 50, 100 and 200 ppm concentrations of different fungicides tested except trifloxystrobin 25% + tebuconazole 50% 75 WG at 200 ppm. The 100% inhibition was exhibited by trifloxystrobin 25% + tebuconazole 50% 75 WG at 200 ppm, followed by carbendazim 50% WP at 200 ppm with an inhibition of 87.87%. The fungicides, trifloxystrobin 25% + tebuconazole 50% 75 WG at 200 ppm (100 percent inhibition) and carbendazim 50% WP at 200 ppm (87.87 percent inhibition) showed the highest percent inhibition of the mycelial growth. The inhibition of mycelial growth of the pathogen occurred by the toxic nature of fungicides (Table 1 and Plate 1).

Vatankhah *et al.* (2019) [8] evaluated five fungicides, with active ingredients azoxystrobin, imazalil, thiabendazole, azoxystrobin + difenoconazole and fludioxonil + difenoconazole against two isolates of *Fusarium solani* and two isolates of *F. oxysporum*, causing potato dry rot in Mashhad region. He found that Imazalil and Thiabendazole completely stopped the mycelial growth of all fungal isolates, even at their lower concentration (40 and 50ppm, respectively).

The post-harvest dry rot disease of potato tubers could be controlled by imazalil and thiabendazole fungicides which show a toxic effect on *F. solani* and *F. oxysporum.* The key mechanism of action of benzimidazole compounds, including carbendazim, is mainly binding to the β- tubulin and disturbing the microtubule dynamic (Thelingwani *et al*., 2009 and Jamieson *et al*., 2011) [7, 3]. This resulted in the blockage of nuclear division, which is responsible for the inhibition of the growth of *Fusarium* sp.

Gupta *et al.* (2020) [2] evaluated six fungicides, namely carbendazim 50% WP, Propiconazole 25% WP, Hexaconazole 75% WP, Mancozeb 80% WP, Copper Oxychloride 50% WP and Azoxystrobin 250 SC against *Fusarium solani* and found that Carbendazin 50% WP @150 ppm exhibited higher per cent inhibition of mycelial growth of *Fusarium solani* followed by propiconazole, hexaconazole, mancozeb, copper oxychloride and azoxystrobin.

Table 1: Effect of different fungicides on the radial growth of *F. proliferatum*

Treatments	Radial growth of the pathogen on 7 DAI (cm)*	Percent inhibition 7 DAI (%) *
Mancozeb 75% WP 50 ppm	2.30 ± 0.00^{b}	$30.30(33.40)^{n}$
Mancozeb 75% WP 100 ppm	1.40 ± 0.00^{e}	$57.57(49.35)^{k}$
Mancozeb 75% WP 200 ppm	2.10 ± 0.00^{d}	$36.36(37.08)^{l}$
Copper oxychloride 50% WP 50 ppm	0.60 ± 0.00^{i}	$81.81(64.76)^{c}$
Copper oxychloride 50% WP 100 ppm	0.60 ± 0.00^{i}	$81.81(64.76)^{c}$
Copper oxychloride 50% WP 200 ppm	0.60 ± 0.00^{i}	$81.81(64.76)^{c}$
Propineb 70% WP 50 ppm	2.10 ± 0.00^{d}	$36.36(37.08)^{l}$
Propineb 70% WP 100 ppm	1.10 ± 0.00^{h}	$66.66(54.73)^{h}$
Propineb 70% WP 200 ppm	2.10 ± 0.00^{d}	$36.36(37.08)^{l}$
Carbendazim 50% WP 50 ppm	0.60 ± 0.00^{l}	$81.81(64.76)^{d}$
Carbendazim 50% WP 100 ppm	0.50 ± 0.00^{m}	$84.84(67.09)^{c}$
Carbendazim 50% WP 200 ppm	0.40 ± 0.00^{n}	$87.87(69.92)^{b}$
Propiconazole 25% EC 50 ppm	0.80 ± 0.00^{j}	$75.75(60.50)^{f}$
Propiconazole 25% EC 100 ppm	0.70 ± 0.00^{k}	$78.78(62.57)^{e}$
Propiconazole 25% EC 200 ppm	0.60 ± 0.00^{l}	$81.81(64.76)^{d}$
Cymoxanil 8% + mancozeb 64% WP 50 ppm	2.10 ± 0.00^{d}	$36.36(37.08)^{l}$
Cymoxanil 8% + mancozeb 64% WP 100 ppm	1.10 ± 0.00^{h}	$66.66(54.73)^{h}$
Cymoxanil 8% + mancozeb 64% WP 200 ppm	2.13 ± 0.05^{c}	$35.35(36.47)^{m}$
Carbendazim 12% + mancozeb 63% WP 50 ppm	1.20 ± 0.00^{f}	$63.63(52.91)^{j}$
Carbendazim 12% + mancozeb 63% WP 100 ppm	1.13 ± 0.05^{g}	$65.65(54.12)^{I}$
Carbendazim 12% + mancozeb 63% WP 200 ppm	0.90 ± 0.00^{I}	$72.72(58.51)^{g}$
Trifloxystrobin 25% + tebuconazole 50% WG 50 ppm	0.50 ± 0.00^{m}	$84.84(67.09)^{c}$
Trifloxystrobin 25% + tebuconazole 50% WG 100 ppm	0.40 ± 0.00^{n}	$87.87(69.62)^{b}$
Trifloxystrobin 25% + tebuconazole 50% WG 200 ppm	0.00 ± 0.00^{o}	$100.00(88.72)^{a}$
Control	3.30^{a}	$0.00(1.28)^{o}$
CD (0.05)	0.08	0.28
SE(m)±	0.01	0.28

* Mean of three replications ± SD
* Values in parenthesis are angular transformed data
* Means followed by similar superscripts are not significantly different at 5% level

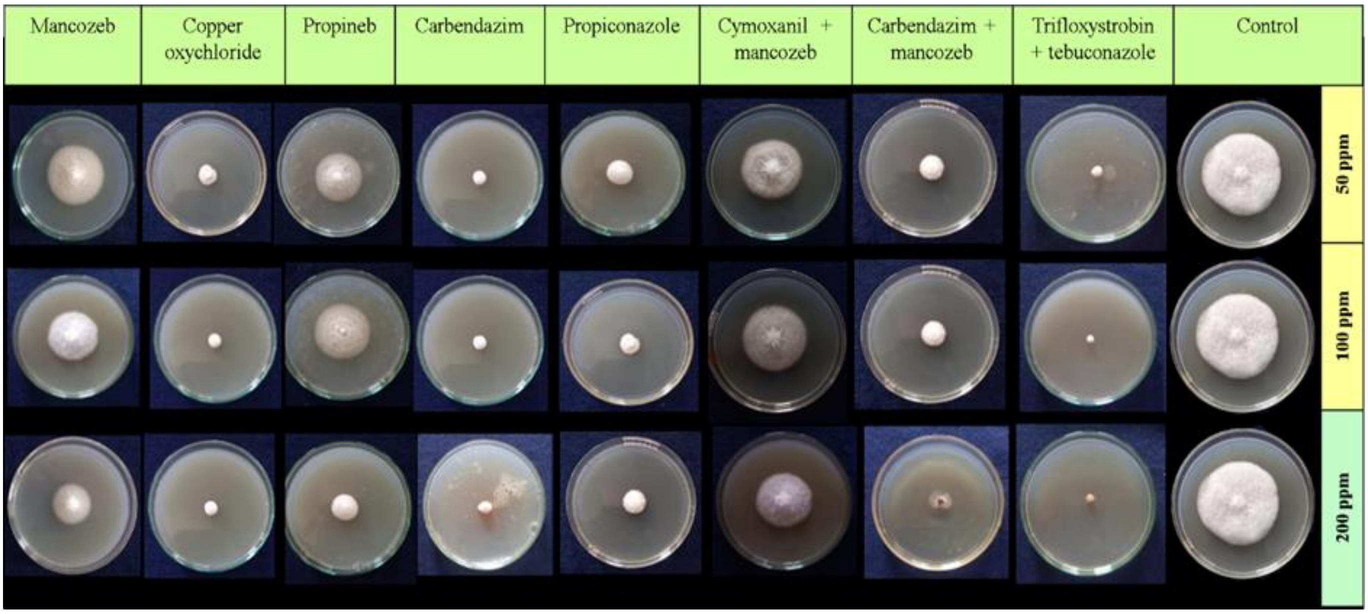

Plate 1: Effect of different fungicides on *F. proliferatum* at different concentrations

Conclusion

Cassava (*Manihot esculenta* Crantz. Inst.) is one of the most important tropical tuber crops which play a vital role in sustainable food production Nowadays Root rot caused by Fusarium proliteratum emerging as a severe threat to cassava cultivation. Cassava plants are quite resilient and have an extensive root system that enables the plant to remain to stand and is asymptomatic even when heavily infected which make the early detection difficult. Hence the prophilatic application of an effective fungicide is inevitable. Based on the above results it was clear that fungicides, trifloxystrobin 25% + tebuconazole 50% 75 WG at 200 ppm (100 percent inhibition) and carbendazim 50% WP at 200 ppm (87.87 percent inhibition) were effective against root rot disease of cassava.

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