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In-vitro Evaluation of Different Fungicides against Black Leaf Spot Causing Fungus *Asperisporium caricae* in Papaya

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Ten different contact, systemic and combination products of fungicides were evaluated for their effectiveness against black spot of papaya *Asperisporium caricae* under *in vitro* condition by following poisoned food technique. Among fungicides tested, trifloxystrobin 25% + tebuconazole 50% WG, propiconzole 25% EC and zineb 68% + hexaconazole 4% WP were successful in completely (100%) inhibiting the growth of *A. caricae* at all concentrations (50,100, 250, 500, 750, 1000 ppm) which is followed by hexaconazole 25% EC, difenconazole 25% EC, zineb 68% hexaconazole 4% WP completely inhibited the growth of the fungus at 500 ppm and above concentration. Least inhibition of fungal growth was recorded in copper oxychloride 50% WP at all concentrations. Trifloxystrobin 25% + tebuconazole 50% WG, propiconazole 25% EC and zineb 68% + hexaconzole 4% WP were most effective which completely inhibited the spore germination at all concentrations, while the copper oxychloride 50% WP was recorded the least per cent inhibition of fungal spores.

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Keywords: Papaya; black spot disease; contact fungicides; systemic fungicides; combi product fungicides.

1. INTRODUCTION

Papaya (*Caricae papaya*. L) commonly called as "Paw`paw" or "Common man's fruit" or "Tree melon" is cultivated both in commercial and kitchen gardens throughout India. The papaya belongs to the family "*Caricaceae*" genus Caricae and species papaya. Papaya (*Caricae papaya* L.) is one of the most important fruit crop cultivated in many parts of tropical and subtropical regions and rich in vitamins, minerals and digestive fibres required for human health [1]. Papaya is native of tropical America. Papaya was introduced to India during 16th century by portuguese and spanish sailors. Papaya mainly cultivated in India, Brazil, China, Congo, Indonesia, Mexico, Nigeria, Peru, Philippines, Taiwan and Thailand. India ranks first in papaya production which is cultivated over an area of 1.39 million hectares with production of 5831 Metric Tonnes (MT). Among the states, Andhra Pradesh occupied highest area under production (1288.58MT) followed by Gujarat (1241.25MT) and Karnataka (527.86MT) (Hort stat, 2017). In Tamil Nadu the crop is being cultivated with an area of 1.76 thousand hectares with the production of 403.19MT during 2018. Popular varieties widely cultivated are Red lady, Sinta and CO 8. Papaya crop is being affected by many fungal, bacterial and viral diseases. Among the various fungal diseases, Black spot disease of papaya is a minor disease which becomes major one and the recently emerging disease. This disease was first observed in the variety "Honey dew" at Chettali, Karnataka later it was observed in variety CO 1 in Palani hills, Tamil Nadu during cooler months was reported by Ullasa et al. [2]. The symptoms appear on the upper surface of leaves as small water-soaked spots, later these spots become greyish white in colour and the corresponding lower surface of the leaves develop black velvety conidial mass around the lesions. Severely affected leaves shrivel, develop larger necrotic lesions which become brittle and finally die. This leads to severe defoliation of the older leaves. In the fruits, the symptoms are small dot like spots appear initially, later they enlarge up to 2-6mm in diameter. The spots are confined to the outer rind of the fruit which reduces the market value of exported fruit [3]. The fungicides play a major role in the reduction of plant diseases. The present study was carried out with fungicides for the management of black spot of papaya, which showed good efficacy in plant disease reduction with improved fruit quality.

2. MATERIALS AND METHODS

2.1 Effect of Fungicides on the Growth of Black Spot Pathogen

Ten chemicals with six different concentration were tested against *A. caricae* under *In vitro* conditions for their inhibitory activity. The fungicides viz., trifloxystrobin 25% + tebuconazole 50% WG, zineb 68% + hexaconazole 4% WP, copper oxychloride 50% WP, chlorothalonil 75% WP, carbendazim 12% + mancozeb 63% WP , tebuconazole 25% EC, hexaconazole 25 % EC, difenconazole 25% EC , propiconazole 25 % EC and mancozeb 75% WP were used in this study (Table 1).

2.2 *In vitro* Evaluation of Fungicides on the Mycelial Growth

The poison food technique was followed to evaluate the efficacy of both the systemic and contact fungicides in inhibiting the spore production and mycelial growth of *A. caricae* at 50, 100, 250, 500, 750 and 1000 ppm concentration. The fungus *A. caricae* was grown on potato dextrose agar medium for one month prior to setting up of an experiment. The potato dextrose agar medium was prepared and to the melted medium fungicidal suspension was added to obtain the desired concentration of the chemical. The fungicide amended medium was poured in each sterilized Petri plates. Control was maintained without adding any test chemical. The mycelial disc of *A. caricae* was placed at the center of the fungicide amended Petri plates and incubated at 23±1^{o} C. Each treatment was replicated thrice. The radial growth of colony was recorded when the control plate was fully covered by the fungus. The per cent inhibition was calculated by using the formula given by Hajano et al. [4].

$$PI = C – T / C X 100$$

Where, PI-Per cent inhibition, C-Radial colony growth of fungus in control, T-Radial colony growth of fungus in fungicide treatment.

2.3 Effect of Fungicides on the Growth of *A. caricae* on Liquid Media

The ten fungicides mentioned were evaluated against *A. caricae* under *in vitro* condition in

liquid medium. The potato dextrose broth was prepared and mixed at the test fungicide with 50, 100, 250, 500, 750 and 1000 ppm concentration and control was maintained without adding test chemical. The mycelial disc of pathogen was aseptically transferred to the fungicide amended conical flask and incubated at 23±1^{o}C. Each treatment was replicated thrice. When the control was fully covered with fungus the fresh weight and dry weight of the mycelium was recorded.

2.4 Effect of Fungicides on Spore Germination of *A. caricae*

The per cent spore germination inhibition studies was carried by following the methods adapted by Peterson [5]. Spore suspension of 1x10^{3} was prepared from thirty days old culture and the required concentration of test chemical was prepared by using sterile distilled water. In a sterilized cavity slide twenty micro litre of test chemical was placed at the center and allowed to dry at room temperature. Spore suspension of twenty micro litre was prepared and placed where the test chemical was placed. Then, the slides were placed in Petri plates lined with sterilized moist cotton and incubated at moist chambers at 23±1^{o}C. After incubation the slides were examined on the high power (40X objective) image analyser. The number of spores germinated and the total number of spores present were counted under the microscope. For each chemical treatment three replication was maintained. Percent spore inhibition was calculated by using the formula given by Vincent (1927).

$$PI = C – T / T \times 100$$

Where, PI = Per cent inhibition, C = Number of spores germinated in control, T = Number of spores germinated in fungicide treatment.

2.5 Statistical Analysis

In the data analysis, computing packages like AGRES, Microsoft excel (XLSTAT, 2010) were used. All the datas during the study were subjected to analysis of varience (ANOVA) as suggested by (Gomez and Gomez, 1984). Critical difference (CD) at 0.05 level of probability was calculated for the treatments wherever statistical significance was observed using inferential statistics (t-test) and the treatment means were compared by Duncan's Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

3.1 Efficacy of Fungicides against the Growth *A. caricae*

The results revealed that among the ten fungicides tested against the growth of *A. caricae* trifloxystrobin 25% + tebuconazole 50% WG, propiconazole 25% EC and zineb 68% + hexaconzole 4% WP were the most effective fungicides which completely inhibited growth of the pathogen at all concentrations, while the copper oxychloride 50% WP was found to be recorded the least per cent of inhibition of fungal growth (Table 2, Table 3). The present work was supported by Sood and Kapoor [6] stated that trifloxystrobin 25% + tebuconazole 50% WG was effective against *Magnaporthe oryzae* which acts as a respiration inhibitor by blocking electron transfer at fungal mitochondria and Tebuconazole acts as demethylation inhibitor of fungal sterol biosynthesis. Reddi Kumar et al. [7] supported that the combination fungicide hexaconazole + zineb shows 100 per cent inhibition against *A. caricae* since due to presence of more than one active ingredient and more than one target site of the pathogen is disrupted. The chance of development of resistance was less when the combination products were used. Shantamma et al. [8] stated that difenoconazole was effective against *A. caricae* which inhibited the sterol biosynthesis of the pathogen, followed by chlorothalonil and propiconazole whereas copper oxychloride had less effect on inhibition of *A. caricae*.

3.2 Efficacy of Different Fungicides on Spore Germination

Trifloxystrobin 25% + tebuconazole50% WG, propiconazole 25% EC and zineb 68% + hexaconzole 4% WP were effective which completely inhibited the spore germination at all concentrations, while the Copper oxychloride was recorded the least per cent of inhibition of fungal spores (Table 4). Similar results was supported by Vawdrey et al. [9] who observed the sensitivity levels of *A. caricae* to tebuconazole and difenconazole using a germination tube elongation test. From the spore germination studies they noted that the difenconazole (EC50 of 2 ppm) was more effective than the tebuconazole (EC50 of 14 ppm) that completely inhibited the germination of conidia by decreasing germ tube elongation and germ tube branching.

Table 1. *In vitro* evaluation of fungicides on the mycelial growth

Sl. No.	Chemical name	Trade name	Nature
1	Trifloxystrobin 25% + Tebuconazole 50% WG	Nativo	Systemic
2	Zineb 68%+ hexaconazole 4% WP	Avtar	Contact +Systemic
3	Copper oxychloride 50%WP	Blue copper	Contact
4	Chlorothanonil 75% WP	Kavach	Contact
5	Carbendazim 12% + Mancozeb 63% WP	Saaf	Contact +Systemic
6	Tebuconazole 25.9% EC	Folicur	Systemic
7	Hexaconazole 25% EC	Sitara	Systemic
8	Difenconazole 25% EC	Score	Systemic
9	Propiconazole 25% EC	Tilt	Systemic
10	Mancozeb 75% WP	Dithane M-45	Contact

Table 2. Per cent inhibition of mycelial growth of *A. caricae* by different fungicides on solid media

Sl. No	Treatments	Per cent inhibition of mycelial growth (cm)					
		50 ppm	100 ppm	250 ppm	500 ppm	750 ppm	1000 ppm
1	Trifloxystrobin 25% + Tebuconazole 50% WG	100(10.02)^{a}	100(10.02)^{a}	100(10.02)^{a}	100(10.02)^{a}	100(10.02)^{a}	100(10.02)^{a}
2	Zineb 68%+ hexaconazole 4% WP	99.33(9.99)^{a}	100(10.02)^{a}	100(10.02)^{a}	100(10.02)^{a}	100 (10.02)^{a}	100(10.02)^{a}
3	Copper oxychloride 50%WP	53.33 (7.34)^{h}	57.78 (7.63)^{f}	63.33(7.99)^{e}	67.78(8.26)^{f}	90.78(9.55)^{c}	100(10.02)^{a}
4	Chlorothanonil 75% WP	70.00(8.40)^{d}	76.33(8.77)^{d}	80.44(9.00)^{b}	85.22(9.26)^{f}	89.33(9.48)^{e}	94.11(9.73)^{b}
5	Carbendazim 12% + Mancozeb 63% WP	55.56(7.49)^{g}	66.66(8.20)^{e}	73.33(8.59)^{d}	96 (9.86)^{b}	100(10.02)^{a}	100(10.02)^{a}
6	Tebuconazole 25% EC	64.45(8.06)^{f}	66.66(8.20)^{e}	74.89(8.68)^{c}	93(9.67)^{c}	100(10.02)^{a}	100(10.02)^{a}
7	Hexaconazole 25% EC	86.67(9.34)^{c}	87.56(9.36)^{a}	89.78 (9.47)^{d}	90.33 (9.50)^{e}	91.78(9.58)^{b}	93.33 (9.66)^{c}
8	Difenconazole 25% EC	97.78(9.91)^{b}	96.89(9.84)^{b}	91.78(9,58)^{b}	92.78 (9.63)^{d}	91.33 (9.54)^{b}	100(10.02)^{a}
9	Propiconazole 25% EC	100 (10.02)^{a}	100(10.02)^{a}	100(10.02)^{a}	100(10.02)^{a}	100(10.02)^{a}	100 (10.02)^{a}
10	Mancozeb 75% WP	66.67(8.20)^{e}	68.89(8.33)^{d}	80.78 (9.02)^{b}	83.67(9.17)^{g}	91.11(9.57)^{c}	94.44(9.74)^{b}
11	Control	9.00(3.08)^{i}	9.00 (3.08)^{g}	9.00(3.08)^{f}	9.00 (3.08)^{h}	9.00(3.08)^{f}	9.00(3.08)^{c}
	SEd	0.79	0.64	0.409	0.407	0.28	0.17
	CD	1.61	1.31	0.837	0.832	0.59	0.36

**Mean of three replications. Figures in parentheses are square root transformed values. There is no significant difference between the means of same superscript letters as per DMRT*

Table 3. Per cent inhibition of mycelial growth of *A. caricae* by different fungicides on liquid media

Sl. No	Treatments	Per cent inhibition of mycelial growth (cm)					
		50 ppm	**100 ppm**	**250 ppm**	**500 ppm**	**750 ppm**	**1000 ppm**
1	Trifloxystrobin 25% +Tebuconazole 50% WG	100(10.02)^{a}	100(10.20)^{a}	100(10.20)^{a}	100(10.20)^{a}	100(10.20)^{a}	100(10.20)^{a}
2	Zineb 68%+Hexaconazole 4% WP	100(10.20)^{a}	100(10.20)^{a}	100(10.20)^{a}	100(10.20)^{a}	100(10.20)^{a}	100 (10.20)^{a}
3	Copper oxychloride 50% WP	26.59(5.20)^{g}	29.06 (5.44)^{g}	36.53 (6.08)^{f}	50.78(7.16)^{f}	65.23(1.84)^{c}	100(10.20)^{a}
4	Chlorothanonil 75% WP	60.24(7.79)^{e}	72.46 (8.54)^{d}	81.32(9.05)^{b}	89.82(9.50)^{b}	100 (10.20)^{a}	100(10.20)^{a}
5	Carbendazim 12%+Mancozeb 63% WP	21.56(4.70)^{h}	34.49(5.92)^{f}	48.06 (6.97)^{e}	60 (7.78)^{e}	100(10.20)^{a}	100(10.20)^{a}
6	Tebuconazole 25% EC	35.69(6.02)^{f}	47.66(6.94)^{e}	60.96 (7.84)^{c}	73.73(8.62)^{d}	100(10.20)^{a}	100 (10.20)^{a}
7	Hexaconazole 25% EC	85.87(9.29)^{c}	92.14(9.62)^{c}	100 (10.20)^{a}	100(10.20)^{a}	100(10.20)^{a}	100(10.20)^{a}
8	Difenconazole 25% EC	86.27(9.31)^{c}	96.61(9.85)^{b}	100(10.20)^{a}	100(10.20)^{a}	100 (10.20)^{a}	100 (10.20)^{a}
9	Propiconazole 25% EC	100(10.20)^{a}	100(10.20)^{a}	100(10.20)^{a}	100(10.20)^{a}	100(10.20)^{a}	100(10.20)^{a}
10	Mancozeb 75% WP	24.55(5.01)^{g}	34.69(5.93)^{f}	50.66 (7.15)^{d}	78.84(8.91)^{c}	93.17(9.67)^{b}	100 (10.20)^{a}
11	Control	8.35(2.97)^{i}	8.35(2.97)^{h}	8.35(2.97)^{g}	8.35(2.97)^{g}	8.35(2.97)^{d}	8.35(2.97)^{c}
	SEd	0.13	0.12	0.11	0.09	0.08	0.08
	CD	0.26	0.25	0.24	0.19	0.18	0.16

**Mean of three replications. *Figures in parentheses are square root transformed values. There is no significant difference between the means of same superscript letters as per DMRT*

Table 4. Per cent inhibition of spore germination of *A. caricae* on different fungicides

Sl. No	Treatments	Per cent inhibition of mycelial growth (cm)					
		50 ppm	**100 ppm**	**250 ppm**	**500 ppm**	**750 ppm**	**1000 Ppm**
1	Trifloxystrobin 25% + Tebuconazole 50% WG	100(10.02)^{a}	100(10.02)^{a}	100 (10.02)^{a}	100(10.02)^{a}	100 (10.02)^{a}	100(10.02)^{a}
2	Zineb 68% + Hexaconazole 4% WP	100(10.02)^{a}	100(10.02)^{a}	100(10.02)^{a}	100(10.02)^{a}	100(10.02)^{a}	100(10.02)^{a}
3	Copper oxychloride 50% WP	26.43(5.18)^{g}	35.08(5.96)^{e}	52.60(7.28)^{d}	68.40(8.30)^{e}	80.70(9.01)^{c}	98.2(9.90)^{b}
4	Chlorothanonil 75% WP	48.88(7.02)^{d}	66.66(8.20)^{b}	77.8(8.84)^{b}	84.4(9.21)^{d}	93.3(9.68)^{b}	100(10.2)^{a}
5	Carbendazim 12% + Mancozeb 63% WP	31.25(5.63)^{f}	52.08 (7.25)^{d}	77.10(8.80)^{b}	97.90(9.92)^{b}	100(10.02)^{a}	100(10.02)^{a}
6	Tebuconazole 25% EC	45.3(6.76)^{e}	63.63(8,01)^{c}	78.8(8.90)^{b}	90.9 (9.56)^{c}	100(10.02)^{a}	100(10.02)^{a}
7	Hexaconazole 25% EC	83.33(9.15)^{c}	90.19(9.52)^{b}	100(10.2)^{a}	100(10.02)^{a}	100(10.02)^{a}	100(10.02)^{a}
8	Difenconazole 25% EC	97.22(9.15)^{b}	100(10.2)^{a}	100(10.2)^{a}	100(10.02)^{a}	100(10.02)^{a}	100(10.02)^{a}
9	Propiconazole 25% EC	100(10.2)^{a}	100(10.02)^{a}	100(10.2)^{a}	100(10.02)^{a}	100(10.02)^{a}	100(10.02)^{a}
10	Mancozeb 75% WP	14.28(3.84)^{h}	29.65 (5.49)^{f}	59.50(7.74)^{c}	100(10.02)^{a}	100(10.02)^{a}	100(10.02)^{a}
11	Control	0	0	0	0	0	0
	SEd	0.32	0.23	0.13	0.04	0.03	0.02
	CD	0.66	0.48	0.27	0.10	0.07	0.05

**Mean of three replications. *Figures in parentheses are square root transformed values. There is no significant difference between the means of same superscript letters as per DMRT*

4. CONCLUSION

The emerging new diseases black spot of papaya are the main constraints which affects the production and productivity of the papaya crop both in terms of quality and quantity. The new emerging diseases leads to sudden epidemic over an area and hence an efficient management practices needs to be evolved to contain the disease. Since the fungicides play a major role for the management of black spot of papaya, which showed good efficacy in plant disease reduction with improved fruit quality. This article opens up for the further research on black spot of papaya and its management.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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