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Efficacy of fungicides and bio agents in managing the black leaf spot disease of papaya incited by *Asperisporium caricae* (Speg.) Maubl

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ABSTRACT

Black leaf spot of papaya caused by *Asperisporium caricae* is one of the new emerging destructive disease of papaya that cause considerable yield loss of 5 to 29 per cent .Hence, experiments were conducted to determine the efficacy of both bio agents and fungicides in *in vitro* and *in-vivo* against *Asperisporium caricae* causing black leaf spot of papaya. *In vitro* studies of fungicides were carried out by using poisoned food technique and dual culture technique for bio agents carried out at aseptic conditions. Results revealed that the fungicide trifloxystrobin 25% + tebuconazole 50% WG, propiconazole 25% EC and zineb 68% + hexaconazole 4% WP were most effective which inhibited growth of the pathogen at all concentrations. Among the biocontrol agents tested, *T. asperellum and B. subtilis* PG 12 was found to be most effective showing 84.07 and 78.88 per cent per cent reduction. The compatibility study revealed that the *B. subtilis* PG 12 were compatible with effective fungicides trifloxystrobin 25% + tebuconazole 50% WG and propiconazole 25% EC under *in vitro* conditions, whereas the fungal bio agents *T. asperellum* doesn't show any compatibility with the any of the fungicides tested. The treatments which were found effective under laboratory conditions were evaluated at glass and field conditions. The results revealed that the treatment with *B. subtilis* PG 12 (seed treatment @ 10 g/kg + soil application @ 20 g/plant Ist DAP) + foliar spray of trifloxystrobin 25 % + tebuconazole 50% WG @ 0.45 g/l recorded the least disease incidence under glass and field conditions.

Key words: *Black spot, Papaya, Bio agents, Fungicides, In vitro and In-vivo studies.*

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 crops cultivated in many parts of tropical and subtropical regions and rich in vitamins, minerals and digestive fibres required for human health. Green papayas are used for making salads, curries and soups. Green papaya releases a latex fluid rich in proteolytic enzymes, used as meat tenderizer. Papaya leaves have been used in traditional ayurvedic medicine for the treatment of malaria, asthma and dengue. The skin, pulp and seeds of papaya contains a variety of phytochemicals, carotenoids, polyphenols and cyanogenic substances (Asha *et al*., 2014). 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, 2021).

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.* (1978). 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 (Thiribhuvanamala *et al.,* 2016). The Black spot disease, which has been on the rise recently, was shown to be worrisome since it significantly reduced yield. 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. Since continues application of fungicides will develop resistance and also cause residual problem in field. In order to overcome these constrains the biological control has become as alternate strategy for the management of plant diseases now a days. With above considerations attempts were made to use potential bio control agents along with the compatible effective fungicides for the management of black spot of papaya. Therefore, developing an integrated disease management package for the black spot of papaya is being carried out.

Materials and Methods

Effect of bacterial and fungal antagonists on the growth of *A. caricae*

The fungal and bacterial antagonists were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. The antagonists were tested for their inhibitory effect on the growth of *A. caricae* by dual culture technique (Dennis and Webster, 1971). The bio-agents and test fungus *Asperisporium caricae* were inoculated leaving one centimeter from the corner of the single Petri dish containing solidified PDA medium. Three replications were maintained for each treatment. They were incubated at 23±1° C for 3 weeks. The colony diameter of both bio-agents and the pathogen was measured and average was recorded.

Percent inhibition of growth of the test pathogen *A. caricae* was calculated by using the formula given by Pandey and Upadhyay (2000).

$$PI = \frac{Dc - Dt}{Dc} \times 100$$

Where,

Dc = Average diameter of fungal growth (cm) in control

Dt = Average diameter of fungal growth (cm) in treatment

PI = Percent inhibition over control

Effect of fungicides on the growth of *A. caricae*

The poison food technique was followed to evaluate the efficacy of different fungicides in inhibiting the mycelial growth of *A. caricae*. Systemic and non-systemic fungicides were tested at 50, 100, 250, 500, 750 and 1000 ppm concentration against *A. caricae.* 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. Seven mm mycelial disc of one month old culture of *A. caricae* was placed at the centre of the fungicide amended Petri plates and incubated at 23±1° 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.*, 2012

$$PI = \frac{C - T}{C} \times 100$$

Where,

PI = Per cent inhibition

C = Radial colony growth of fungus in control
T = Radial colony growth of fungus in fungicide treatment.

Compatibility of effective bio agents with fungicides

The compatibility test was carried for the promising bio agents under *in vitro* with the identified two effective fungicides of *viz.*, propiconazole 25% EC and trifloxystobin 25% + tebuconazole 50% WG at one ppm and four ppm respectively. The potato dextrose agar medium was amended with desired fungicides of one and four ppm concentration. The PDA medium was poured to the sterilized Petri plates and allowed to solidify. The medium without fungicides were served as control. The two effective bio agents were placed/streaked at the centre of the Petri plates. Each treatment was replicated thrice and kept for incubation. After incubation the compatibility of bio agents with the fungicide was observed and recorded.

Evaluation of effective bio agents and fungicides under glass house and field conditions

The best performing bio-agent and fungicides which were effective under *in vitro* condition were evaluated under glass and field condition. A experiment was conducted in order to estimate the disease severity due to black spot of papaya and to find out suitable management strategies in controlling disease at field level. Field experiment and glass house was conducted with seven treatments in three replications. The untreated plants were used as a control. Three sprays of fungicides and bio agents were (PDI) was calculated by using the formula

The Per cent Disease Index for black spot disease was recorded by using the formula.

$$PDI = \frac{\text{Sum of individual ratings}}{\text{Total number of ratings}} \times \frac{100}{\text{Maximum disease grade}}$$

Statistical analysis

In the data analysis, computing packages like AGRES, Microsoft excel (XLSTAT, 2010) were used. All the data's during the study were subjected to analysis of variance (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).

Results and Discussion

Efficacy of different bioagents against the pathogen:

Among the biocontrol agents tested, *T. asperellum* was found to be most effective showing 84.07 per cent reduction over control followed by *B. subtilis* PG 12 and *S. fabae* indicating 78.88 per cent and 75.54 per cent reduction over control respectively. *Streptomyces spiroverticillatus* was found to be least effective with 27.03 per cent reduction over control (Table 1). The work was supported by Siddaramaiah (1986) who reported that *T. harzianum* was effective against *Cercospora moricola* pathogen which hyperparasite the pathogen and also induces the systemic resistance in sugarbeet plants which increases the defensive response against *Cercospora beticola*.

Table 1. Effect of antagonists on the mycelial growth of *A. caricae* (Dual culture technique)

T. No.	Antagonists	Mycelial growth of the pathogen (cm)	Per cent growth reduction (%)
1	*Bacillus subtilis* EPC 5	4.50(2.24)^{e}	49.99
2	*Bacillus subtilis* PG12	1.90(1.55)^{ab}	78.88
3	*Trichoderma asperellum*	1.43(1.39)^{a}	84.07
4	*Pseudomonas fluroscenes*	2.97(1.86)^{d}	75.54
5	*Streptomyces fabae*	2.20(1.64)^{b}	67.77
6	*Streptomyces reticuli*	2.45(1.58)^{c}	67.03
7	*Streptomyces globosus*	4.73(2.29)^{f}	47.40
8	*Streptomyces spiroverticillatus*	6.57(2.66)^{e}	27.03
9	Control	9.00(3.00)^{f}	-
	SEd	0.29	
	CD	0.60	

*Mean of three replications. Values in parentheses are square root transformed values.

Krishna et al., 2001 also stated that the prophylactic spray of *B. subtilis* reduced the leaf spot of groundnut *Cercospora arachidicola.*

Efficacy of fungicides against the pathogen:

The fungicides of both contact and systemic were tested at concentrations of 50, 100, 250, 500, 750 and 1000 ppm were evaluated against *A. caricae* under *in vitro*. Results revealed that the fungicide trifloxystrobin 25% + tebuconazole 50% WG, propiconazole 25% EC and zineb 68% + hexaconazole 4% WP were most effective which inhibited growth of the pathogen at all concentrations. This was followed by carbendazim 12% + mancozeb 63% WP, difenconazole 25% EC and tebuconazole 25% EC which exhibited complete inhibition of growth at concentration of 500 ppm and above (Table 2). The present work was supported by Shantamma *et al.* (2018) 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.* Reddi Kumar *et al*. (2015) 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.

Compatibility of effective bio agents with fungicides

The compatibility study revealed that the *B. subtilis* PG 12, *P. fluroscenes* Pf1, *S. fabae* were compatible with effective fungicides trifloxystrobin 25% + tebuconazole 50% WG and propiconazole 25% EC under *in vitro* conditions, whereas the fungal bioagents *T. asperellum* doesn't show any compatibility with the any of the fungicides tested (Table 3). Basamma and Kulkarni (2017) also stated that the *B. subtilis* was highly compatible with the fungicides *viz.*, carbendazim, difenconazole, hexaconazole and kresoxim methyl thus they can be effectively used for plant disease management along with fungicides.

Influence of antagonists and chemicals on disease incidence in a pot culture experiment

The pot culture results revealed that the treatment T6 comprising *B. subtilis* PG 12 (seed treatment @ 10

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

SL. No	Treatments	Per cent inhibition of mycelial growth(cm)					
		50 ppm	100 ppm	250 ppm	500 ppm	750ppm	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.

g/kg + soil application @ 20 g/plant I[st] DAP) + foliar spray of trifloxystrobin 25 % + tebuconazole 50% WG @ 0.45 g/lit recorded the least disease incidence of 1.33 PDI with 94.75 percent disease reduction over control which was followed by the treatments T5 – *B. subtilis* PG 12 (seed treatment @ 10 g/kg + soil application @ 20 g/plant I[st] DAP) + foliar spray of propiconazole 25 % EC @ 1 ml/lit recorded 3.23 PDI which accounted for 87.25 per cent disease reduction. The lowest disease reduction percentage of 69.29% was observed in the treatment T2 - foliar spray of *S. fabae* @ 0.5%. Control without any treatment shows the maximum per cent disease incidence of 25.34 PDI(Table 4). Similar results was supported by Jagtap *et al*. (2013) who stated that the results from the pot culture experiment revealed that the propiconazole (0.05%) was effective against leaf spot of turmeric caused by *Colletotrichum capsici*, followed by hexaconazole (0.10%) and chlorothalanil (0.15%). However, Pandey *et al*. (2019) also reported that fungicide indofil M-45 was found to be most effective against leaf Spot and fruit rot of chilli caused by *Alternaria alternata* in pot culture experiment followed by indofil Z-78 and chlorothalonil.

Evaluating the Impact of treatments in Field conditions

Under field conditions the results revealed that the treatment T6 comprising *B. subtilis* PG 12 (seed treatment @ 10 g/kg + soil application @ 20 g/plant I[st] DAP) + foliar spray of trifloxystrobin 25 % + tebuconazole 50% WG @ 0.45 g/ml recorded the least disease incidence of 4.67 PDI with 87.64 percent disease reduction over control which was followed by the treatments T7 – *B. subtilis* PG 12 (seed treatment @ 10 g/kg + soil application @ 20 g/plant I[st] DAP) + foliar spray of propiconazole 25 % EC @ 1 ml/lit, T4 - foliar spray of trifloxystrobin 25% + tebuconazole 50% WG @ 0.45 g/lit and T5- foliar spray of propiconazole 25 % EC @ 1 ml/lit by re-

Table 3. Compatibility of effective bio agents with fungicides

Sl. No	Fungicides	Bioagents	100 ppm	500 ppm
1	Trifloxystrobin 25% + Tebuconazole 50% WG	*Bacillus subtilis* PG 12	+	+
		Trichoderma asperellum	-	-
		Pseudomonas fluorescens Pf1	+	+
		Sreptomyces fabae	+	+
2.	Propiconzole 25% EC	*Bacillus subtilis* PG1 2	+	+
		Trichoderma asperellum	-	-
		Pseudomonas fluorescens Pf1	+	+
		Streptomyces fabae	+	+

+ Compactibility with fungicides - Not compatible with fungicide

Table 4. Effect of antagonists and chemicals on the management of black spot of papaya under pot culture experiment

T. No.	Treatments	Overall disease incidence	Disease reduction (%)
T1	Foliar spray of *Pseudomonas fluorescens* Pf1 @ 0.5%	7.01(2.74)[c]	72.33
T2	Foliar spray of *Streptomyces fabae* @ 0.5%	7.78(2.88)[de]	69.29
T3	Foliar spray of *Bacillus subtilis* PG 12 @ 0.5%	7.57(2.84)[e]	70.12
T4	Foliar spray of trifloxystrobin 25% + tebuconazole 50% WG @ 0.45g/lit	4.45(2.22)[b]	82.43
T5	Foliar spray of propiconazole 25% EC @ 1ml/lit	5.64(2.48)[b]	77.74
T6	*Bacillus subtilis* PG 12 (seed treatment @ 10 g/kg + soil application @ 20 g/plant I[st] DAP) + foliar spray of trifloxystrobin 25 % + tebuconazole 50% WG @ 0.45 g/ml	1.33(1.35)[a]	94.75
T7	*Bacillus subtilis* PG 12 (seed treatment @ 10 g/kg + soil application @ 20 g/plant I[st] DAP) + foliar spray of propiconazole @ 1 ml/lit	3.23(1.93)[a]	87.25
T8	Control	25.34(5.08)[f]	—
	SEd	1.28	
	CD	2.62	

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

Table 5.. Effect of antagonists and chemicals on the management of black spot of papaya under field conditions

T. No.	Treatments	Overall disease incidence (PDI)*	Disease reduction over control (%)
T1	Foliar spray of *Pseudomonas fluorescens* Pf1 @ 0.5%	18.45(4.35)^{de}	51.19
T2	Foliar spray of *Streptomyces fabae* @ 0.5%	22.12(4.75)^{f}	41.48
T3	Foliar spray of *Bacillus subtilis* PG 12 @ 0.5%	19.31(4.45)^{g}	48.91
T4	Foliar spray of trifloxystrobin 25% + tebuconazole 50% WG @ 0.45g/lit	13.45(3.75)^{c}	64.41
T5	Foliar spray of propiconazole 25% EC @ 1ml/lit	15.67(4.02)^{d}	58.54
T6	*Bacillus subtilis* PG 12 (seed treatment @ 10 g/kg + soil application @ 20 g/plant Ist DAP) + foliar spray of trifloxystrobin 25 % + tebuconazole 50% WG @ 0.45 g/ml	4.67(2.27)^{a}	87.64
T7	*Bacillus subtilis* PG 12 (seed treatment with @ 10 g/kg + soil application @ 20 g/plant Ist DAP) + foliar spray of propiconazole 25% EC @ 1 ml/lit	7.9(2.89)^{b}	79.10
T8	Control	31.80(5.64)^{h}	—
	SEd	1.73	-
	CD	3.57	-

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

cording 7.9 , 13.45 and 15.67 PDI which accounted for , 79.10, 64.41 and 58.54 per cent disease reduction over control respectively. The lowest disease reduction percentage of 48.91 per cent was observed in the treatment T2 – foliar spray of *S. fabae* @ 0.5% with 22.12 PDI (Table 5). Control without any treatment showed the maximum per cent disease incidence of 31.80 per cent. Similar results were supported by Rakesh and Rathi (2018) who recorded that the foliar spray of chemicals were effective under field conditions. He stated that spraying of mancozeb (0.2%) at 45 DAS followed by hexaconazole (0.05%) at 60 DAS was found to be most effective in controlling the *Alternaria* leaf blight severity upto 78 per cent in Indian mustard. Laxman *et al.* (2017) who stated that the synergistic effects of simultaneous application of *Bacillus* and fungicides can enhance disease control efficacy compared to their individual applications. This synergistic interaction is attributed to multiple factors, including complementary modes of action, enhanced bioavailability of active ingredients, and induction of plant defense responses.the compatibility between Bacillus spp. and fungicides is crucial for ensuring effective disease management without adverse effects on beneficial microorganisms or plant health.

There is no significant difference between the means of same superscript letters as per DMRT.

Conclusion

The synergistic interaction between *Bacillus* spp. and fungicides holds significant implications for developing sustainable and integrated disease management approaches in agriculture. Future research should focus on elucidating the underlying mechanisms driving the synergistic effects and exploring novel formulations or application methods to maximize the efficacy of these combined treatments while minimizing environmental impacts. The combination of bio control agents and fungicides reduced the disease severity with improved growth promotion and it also promotes the integrated disease package for reducing yield loss which is economically affordable that stimulate farmers to change from fungicide-based agriculture to an integrated approach. These combinations provide an answer to the consumer's demand for a more sustainable agriculture and could be a tool for achieving reduced or even zero-residue produce.

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