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Potentiality of bioagents and botanicals against papaya black spot fungus: *Asperisporium caricae*

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Abstract
Among the seven bio agents evaluated in the experiment by following dual culture technique *T. viride* (72.59%) exhibited highest mycelial inhibition, followed by *T. asperellum* (70.37%), *T. harzianum* (64.81%), *Ampelomyces quisqualis* (63.33%), antagonistic bacteria *Bacillus subtilis* (9.63%), *P. fluorescens* (6.67%) whereas, least inhibition was observed by *Bacillus pumilis* (4.44%). Poison food technique was followed with seven plant extracts to test their efficacy against papaya black spot (*A. caricae*). Excellent mycelial growth inhibition was observed by *Allium sativum* (27.93%) which was significantly higher than all other treatments, which was followed by *Zingiber officinale* (24.15%). Next best inhibition was imparted by *Vinca rosea* (1.77%) but which was much less effective as compared to *Allium sativum* and *Zingiber officinalis.* Little inhibition was imposed by *Tinospora cordifolia* (1.47%), *Azardiracta indica* (1.12%), *Tagetes erecta* (1.07%) and Seaweed extract (0.67%) which were on par with each other.

Keywords: Black spot, bioagent, plant extracts, *in vitro*

Introduction
In tropical and subtropical countries papaya (*Carica papaya* L.) is widely grown and it is economically important fruit tree. Globally papaya is the third most cultivated tropical crop, India and Brazil are the largest producers of papaya. This is the fourth most traded tropical fruit. It has gained more importance due to its high palatability, early fruiting, maximum productivity per unit area, multifarious uses such as food, medicine, and as industrial input (Kumara and Rawal, 2009) [1].

Papaya fruit is very susceptible to diseases caused by many micro-organisms especially fungi, because this fruit has a very thin skin and high in moisture and nutrients (Evans and Ballen, 2012) [2]. Important diseases in the field and storage, affecting papaya are powdery mildew, *Phytophthora* root rot, anthracnose, stem end rot, black spot disease and virus diseases like papaya ring spot and papaya leaf curl (Rawal, 2010) [7].

Infection of *Asperisporium caricae* which causes papaya black spot disease is becoming one of the obstacle in commercial production of papaya. This disease has been reported in Asia, Africa, the Western Hemisphere, USA and Oceania. The widespread occurrence of this disease was observed in China, India, Philippines, Srilanka and Taiwan (Anonymous, 2005) [1]. Losses of 30 per cent in papaya fruit commercialization was recorded due to black-spot disease (Santos and Barreto, 2003) [8]. Henceforth efficient management tactics of this disease is an important prerequisite for papaya cultivation.

Few fungicides *viz.*, Difenconazole, Chlorothalonil, Propiconazole and Hexaconazole were found effective in managing this disease (Shanthamma *et al.*, 2018) [9]. But the indiscriminate and overzealous practice of inorganic pesticide application has imposed several kinds of environmental and toxicological hitches. In the present era, in different parts of the world, attention has been paid towards exploitation of organic approaches in plant protection. In line with this purpose study was conducted to evaluate the effect of various botanicals and bioagents against *A. caricae* under *in vitro* condition.

Material and Methods
In vitro study on effect of biological agents against papaya black spot fungus (*A. caricae*)
The effectiveness of seven bioagents *viz., Bacillus pumilis, Bacillus subtilis, Pseudomonas fluorescens, Trichoderma asperellum, Ampelomyces quisqualis, Trichoderma harzianum* and *Trichoderma viride* were evaluated against *A. caricae* to check per cent inhibition of radial

growth of fungi on the potato dextrose agar media by following dual culture technique under *in vitro* condition. 20 mL of sterilized potato dextrose agar medium was melted and cooled at 45 ^{0}C and it was poured aseptically into 9 cm diameter sterilized Petri dishes. With the aid of a sterilized cork borer, mycelial disks of 5 mm diameter was cut from the edge of the actively developing ten days old pathogen culture and kept on the periphery of the Petri dish at about 1cm from edge. In the same manner 5mm mycelial disc of bioagents were also placed on Petri dish opposite to pathogen. In case of bacterial antagonist's evaluation, the bacterium was streaked on periphery of (at about one cm from the edge) the Petri plate and on the opposite side 5 mm diameter mycelial disc of the pathogen was placed. The Petri dish containing potato dextrose agar medium inoculated with the pathogen alone served as control. All the treatments were replicated thrice and were incubated at room temperature (28 ± 1 ^{0}C). After incubation when the growth of the pathogen was completed in the control, the colony diameter of bioagents and that of the pathogen were measured in each treatment and the per cent inhibition of the pathogen over control was calculated by adopting the formula given by Vincent (1947) [11]. The percentage values were converted to transformations of the arc sin, the data were statistically analyzed.

$$I = \frac{C - T}{C} X100$$

Where;
I = Per cent inhibition
C = Radial growth of fungus in control
T = Radial growth of fungus in treatment

Effect of aqueous plant extracts on mycelial growth of *Asperisporium caricae* under *in vitro* condition

Preparation of cold aqueous extract

Fresh plant materials (table 1) were collected and washed in tap water and then in distilled water. Hundred grams of fresh sample were chopped and crushed by adding 100 mL sterile water (1: 1 w / v) into a surface sterilized pestle and mortar. Two layers of muslin fabric was used to filter the sample. Subsequently extract collected was used as stock solution.

Table 1: Plant extracts *in vitro* evaluation against *A. caricae*

Sl.no.	Botanical name	Common name	Family	Plant Parts used	Concentration
1	*Azardiracta indica*	Neem	*Meliaceae*	Leaves	5, 10,15 & 20
2	*Allium sativum*	Garlic	*Amaryllidaceae*	Bulb	5, 10,15 & 20
3.	*Zingiber officinalis*	Ginger	*Zingiberaceae*	Rhizomes	5, 10,15 & 20
4	*Tagetes erecta*	Marigold	*Asteraceae*	Leaves	5, 10,15 & 20
5	*Vinca rosea*	Periwinkle	*Apocynaceae*	Leaves	5, 10,15 & 20
6	*Tinospora cordifolia*	Amritaballi	*Menispermaceae*	Leaves	5, 10,15 & 20
7	-	Seaweed extract	-	-	5, 10,15 & 20

To study the antifungal mechanism of plant extracts, the poisoned food technique was used (Nene and Thapliyal, 1973) [4]. Five, ten, fifteen, and twenty mL of stock solution was mixed with 95, 90, 85 and 80 mL of sterilized molten PDA medium respectively to achieve concentration of 5, 10, 15 and 20 per cent. The medium was thoroughly shaken for uniform mixing of extract. Twenty mL of medium was poured into sterile Petri dishes, mycelial discs of five mm size from periphery of actively growing culture were cut out by sterile cork borer and one such disc was placed at the center of each plate. Control was maintained by growing the pathogen on PDA plates. The plates were incubated at a temperature of 28 ± 1°C and radial development was taken when the maximum growth in the control plate was detected. The effectiveness of plant products or botanicals was expressed as a percentage inhibition of radial growth over control calculated using the formula of Vincent (1947) [11]. The percentage values were converted to transformations of the arc sin, the data were statistically analyzed.

Results and discussion

***In vitro* evaluation for the effect bioagents against papaya black spot fungi**

Results indicated significant difference between the seven different bioagents tested. Highly significant difference was noticed between antagonistic ability of fungal and bacterial bio control agents (table 2 & fig 1). Among the bioagents tested *T. viride* (72.59%) exhibited highest mycelial inhibition, followed by *T. asperellum* (70.37%), *T. harzianum* (64.81%) and *A. quisqualis* (63.33%). Among the antagonistic bacteria *B. subtilis* (9.63%) showed maximum inhibition of the mycelia, followed by *P. fluorescens* (6.67%). Least inhibition was observed by *B. pumilis* (4.44%).

Table 2: Efficacy of bioagents against *A. caricae* under *in vitro* condition

Sl.no	Bio agents	Percent inhibition of the pathogen over control
1.	*A. quisqualis*	63.33 (52.75)
2.	*B. pumilis*	4.44 (12.17)
3.	*B. subtilis*	9.63(18.08)
4.	*P. fluorescence*	6.67 (14.96)
5.	*T. asperellum*	70.37 (57.04)
6.	*T. harzianum*	64.81 (53.64)
7.	*T. viride*	72.59 (58.45)
F		**
SEm±		0.54
CD @ 1%		2.34

Note: values in parenthesis are arcsine transformed values

Results were in confirmation with findings of Taj and Kumar (2013) [10] wherein among the different bioagents tested against *A. caricae* under *in vitro* condition the maximum reduction in colony growth was observed in *T. viride* (53.33 mm) which was significantly superior over all. Prashanth (2004) [6] also reported that *T. viride* showed the maximum inhibition of *C. kikuchii*. They observed *B. subtilis* as best mycelial growth inhibitor among bacterial bioagents used.

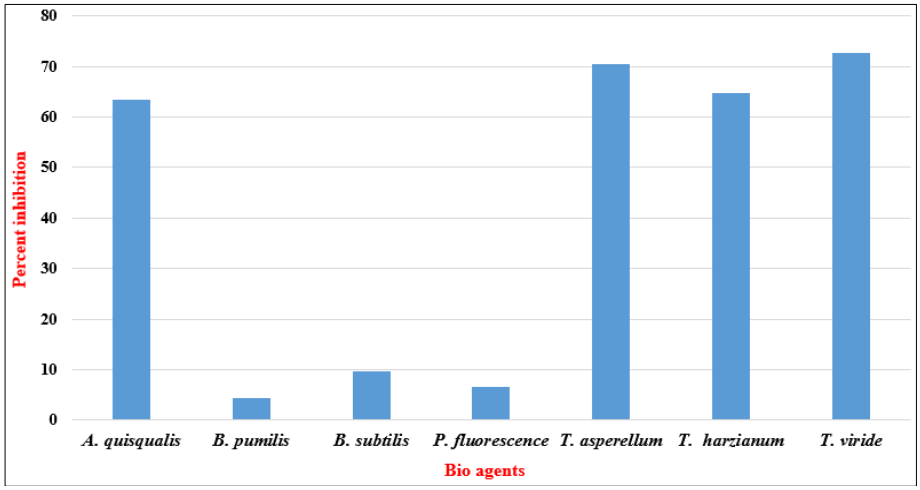

Fig 1: Effect of bioagents on mycelial growth inhibition of *A. caricae*

In vitro* evaluation for the effect plant extracts against *A. caricae

Excellent mycelial growth inhibition was observed by *Allium sativum* (27.93%) which was significantly higher than all other treatments, which was followed by *Zingiber officinale* (24.15%). Next best inhibition was imparted by *Vinca rosea* (1.77%) but which was much less effective as compared to *Allium sativum* and *Zingiber officinalis.* This was followed by *Tinospora cordifolia* (1.47%), *Azardiracta indica* (1.12%), *Tagetes erecta* (1.07%) and Seaweed extract (0.67%) on par with each other. Results concerned to efficacy of plant extract to inhibit the mycelial growth of fungus is presented in table 3 and fig. 2. Results obtained are in line with the findings of Patel (2019) [11], she reported highest inhibition of *A. caricae* by aqueous extracts of *Allium sativum* and least inhibition was obtained by aqueous extracts treatment by *Azardiracta indica* under *in vitro* condition.

Table 3: Efficacy of plant extracts to inhibit mycelial growth of *A. caricae* under *in vitro* condition

Sl.no	Plant extracts	Percent inhibition of the pathogen over control				Mean
		Concentration				
		5%	10%	15%	20%	
1.	*Allium sativum*	21.11 (27.36)	25.19 (30.14)	29.63 (32.99)	35.55 (36.62)	27.93 (31.91)
2.	*Zingiber officinalis*	15.93 (23.53)	21.11 (27.36)	28.15 (32.06)	31.48 (34.15)	24.15 (29.45)
3.	*Azardiracta indica*	0.74 (4.94)	0.93 (5.52)	1.30 (6.54)	1.48 (6.99)	1.12 (6.08)
4.	*Vinca rosea*	1.48 (6.99)	1.67 (7.42)	1.85 (7.82)	2.04 (8.21)	1.77 (7.65)
5.	*Tinospora cordifolia*	1.11 (6.05)	1.30 (6.54)	1.67 (7.42))	1.74 (7.58)	1.47 (6.97)
6.	*Tagetes erecta*	0.93 (5.52)	1.04 (5.85)	1.11 (6.05)	1.19 (6.25)	1.07 (5.94)
7.	Sea weed extract	0.37 (3.49)	0.56 (4.28)	0.81 (5.18)	0.93 (5.52)	0.67 (4.70)
Mean		5.99 (14.17)	7.42 (15.81)	9.21 (17.67)	10.62 (19.03)	8.31 (16.76)
		Plant extract (P)		Concentration (C)		P×C
SEm±		0.02		0.016		0.042
CD @ 1%		0.08		0.06		0.16

Note: values in parenthesis are arcsine transformed values

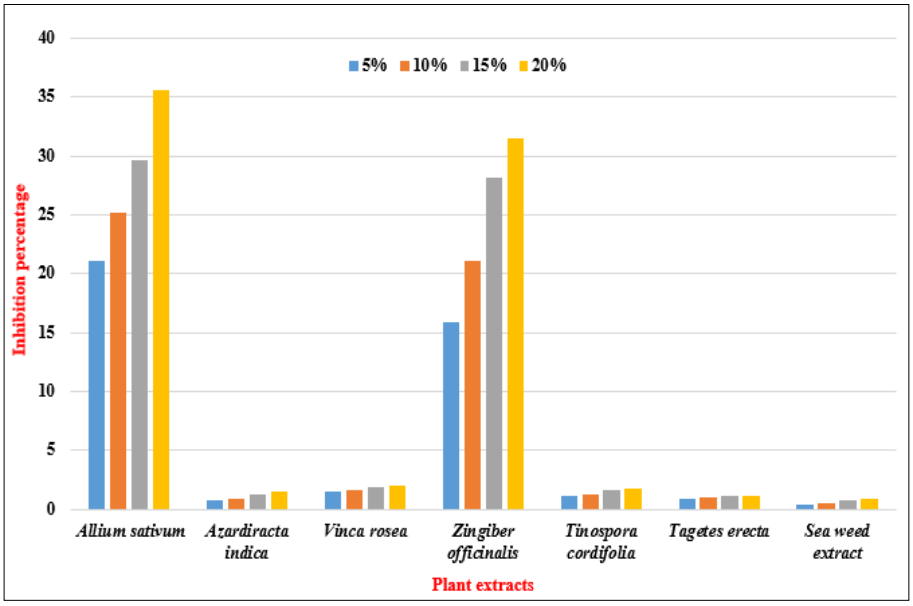

Fig 2: Effect of different plant extracts on mycelial inhibition of *A. caricae*

Conclusion

This study could be concluded that *T. viride*, T. *asperellum*, *T. harzianum and A. quisqualis* were effective in suppressing the mycelial growth of *A. caricae* under *in vitro* condition and these should be evaluated in field condition to determine their efficacy in suppressing the disease. In case of plant extracts evaluated against *A. caricae* under *in vitro* condition *A. sativum* imparted highest inhibition of mycelial growth followed by *Z. officinalis.* Future study on effectiveness of these materials under greenhouse condition and field study are required.

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