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The impact of bioagents cultural filtrate on the postharvest management of disease caused by *Asperisporium caricae* (SPEG.) Maubl

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Abstract
Bioagents, which are natural organisms used for pest and disease management, have gained significant attention as they offer a promising and environmentally friendly alternative to chemical controls. In the context of managing papaya black spot disease caused by *Asperisporium caricae* after harvesting, researchers evaluated cultural filtrates from two fungal and three bacterial bioagents in real-world conditions. Among the bioagent cultural filtrates tested at varied concentrations, *Trichoderma viride* demonstrated the highest efficacy. At 100 percent concentration, it achieved the lowest infection percentage (0.32-5.02%) compared to *Bacillus subtilis* (0.93-7.67%), *T. harzianum* (2.76-6.47%), *B. megaterium* (5.13-11.41%), and *Pseudomonas fluorescens* (5.96-9.70%) at both 7 and 10 days after treatment. These results were notably better than the infection rates observed in the control group, which ranged from 13.56% to 21.46%. This study underscores the potential of bioagents, particularly *Trichoderma viride*, in effectively managing papaya black spot disease post-harvest, highlighting their role as a viable and sustainable option for disease control in agriculture.

Keywords: *Asperisporium caricae*, bioagents, cultural filtrates, post-harvest

Introduction
Papaya (*Carica papaya* L.) is a very popular and essential table fruit in tropical and subtropical countries, belonging to the family *Caricaceae*. The fruit is not only healthy and delicious, but all parts of the plant such as the fruits, pulp, seeds, bark, peel, and roots - are also known to have significant medicinal properties (Saran and Choudhary, 2013) [15].
The top papaya producing countries in the world are India, Brazil, Indonesia, Nigeria, Mexico, Philippines and Thailand. In India, papaya is grown over an area of about 144000 Ha, with annual production of 5951000 MT. Major papaya growing states are Gujarat, Andhra Pradesh, Karnataka, Madhya Pradesh and Tamil Nadu. Karnataka stands third in the papaya production (NHB, 2020-21). In the world, India stands first in the production followed by Brazil and Indonesia
The low yield of papaya is mainly attributed to the occurrence of several diseases, including foot rot, powdery mildew, anthracnose, papaya ring spot, leaf curl and mosaic, brown spot, and black spot diseases. Among these, an emerging and highly lethal disease is black spot disease, caused by *Asperisporium caricae*. Leaf spots are visible on both surfaces of the leaf as black, circular, or sometimes angular spots, 1-4 mm in diameter, with yellow halo margins. Later, the spots become whitish and necrotic. Sometimes, these lesions or spots are enclosed by dark blackish masses of fungal spores, usually on the lower surface of the leaf. On the fruits, round spots with a watery appearance are observed initially; later, the lesions become brown and may reach up to 5 mm in diameter. These spots are generally epidermal and do not reach the pulp of the fruit, causing only a hardening of the skin in the affected area (Ventura, 2008) [17].
Therefore, an investigation was carried out to evaluate the effectiveness of bioagents in the post-harvest management of black spot disease. This approach is considered eco-friendly, cost-effective, and sustainable.

Materials and Methods

Preparation of cultural filtrate of bioagents

The fungal bioagents used in this study were *Trichoderma viride* and *Trichoderma harzianum*, which were cultured on potato dextrose broth for 15 days at 20–25 °C. The fungal biomass was then centrifuged at 10,000 rpm for 20 minutes, and the culture medium was discarded. The obtained supernatant was filtered through Whatman No. 1 filter paper. *Bacillus subtilis* and *Bacillus megaterium* were cultured on nutrient broth, and *Pseudomonas fluorescens* was cultured on King's B medium for 24 hours at 28–30 °C. After 24 hours, the bacterial cell suspension was pelleted by centrifugation at 7,000 rpm for 10 minutes. The obtained supernatant was then filtered using a glass filter to obtain cell-free culture filtrate (El-Boghdady, 1993) [8].

Table 1: Cultural filtrates of bioagents and their concentrations used for postharvest management of papaya black spot fungus

Sl. No	Treatments	Concentrations (%)
1.	*Trichoderma viride*	25, 50, 75&100
2.	*Trichoderma harzianum*	25, 50, 75&100
3.	*Bacillus subtilis*	25, 50, 75&100
4.	*Bacillus megatherium*	25, 50, 75&100
5.	*Pseudomonas fluorescence*	25, 50, 75&100

Evaluation of cultural filtrates of bioagents against post - harvest management of papaya black spot disease

Diseased papaya fruits, pale green or yellowish in color with minimal spots, were collected from the farmer's field. These fruits belonged to the variety Taiwan Red Lady and were uniform in shape, size, and age. Upon collection, the fruits were brought to the laboratory to cool down and reduce field heat. Subsequently, the fruit surfaces were cleaned to remove mud, filth, and latex adhering to them. For the experiment, three fruits (replications) were used for each concentration of plant extracts and arranged in a completely randomized design (CRD) factorial setup. The cultural filtrates of bioagents were applied to the fruit surfaces using non-absorbent cotton at four different concentrations. Papaya fruits dipped in water (untreated) served as the control. Afterwards, the fruits were wrapped with paper.

Seven and ten days after treatment, the extent of rot caused by *A. caricae* infection was measured to assess disease severity. Infection percentage was measured according to Corikidi *et al*. (2006).

$$\text{Infection (\%)} = \frac{\text{Area covered by infection}}{\text{Total surface area of the papaya}} \text{X100}$$

Before treating the papaya fruits, surface area (S_a) of fruits were computed (Topuz *et al*., 2005) [16].

$$S_a = \pi D_e^2$$

Where, S_a = Surface area, m^2

D_e = Geometric mean diameter, cm

The geometric mean dimension (D_e) of the fruits was found using the formula (Mohsenin, 1980) [14] as given below (Fig. 1):

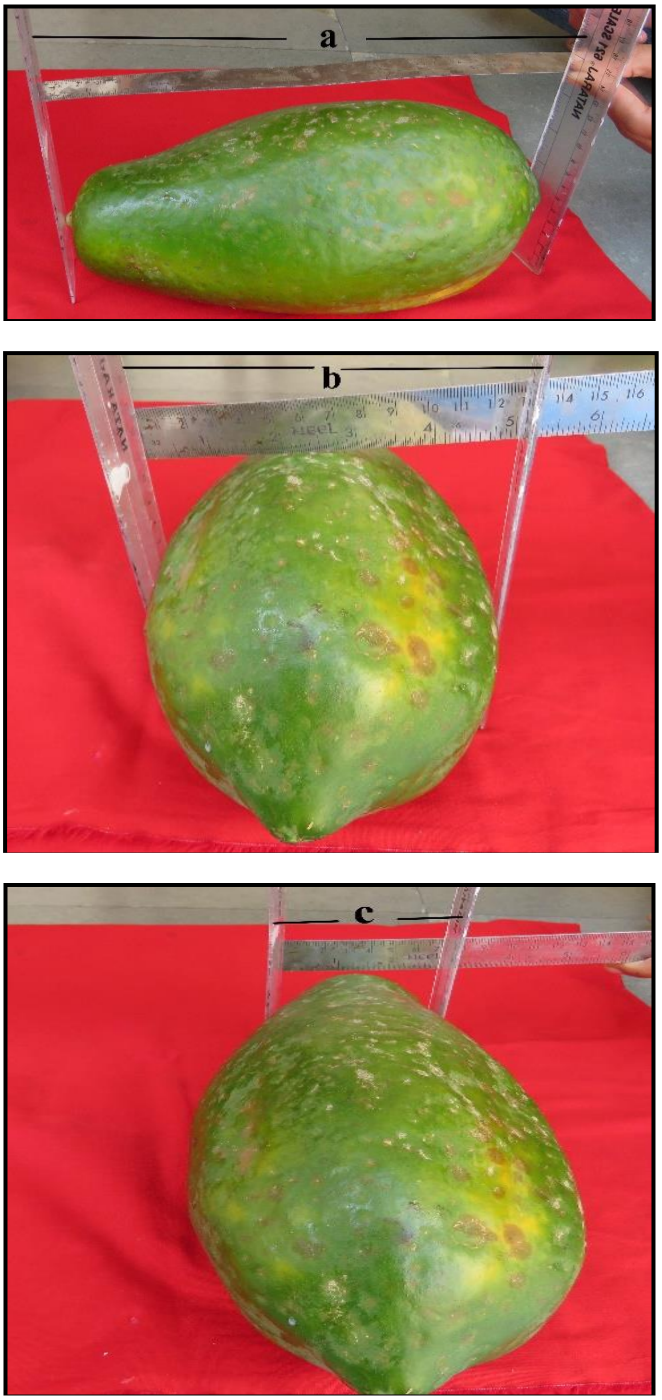
$$D_e = (abc)^{1/3}$$

Where, De = Geometric mean diameter

a = Length of the fruit, cm

b = Width of the fruit, cm (longest diameter)

c = Thickness of the fruit, cm (shorter diameter)



a = Length of the fruit (cm), b = Width of the fruit, (cm) (longest diameter), c = Thickness of the fruit (cm) (shorter diameter)

Fig 1: Measurements of surface area (S_a) of papaya fruits

Results and Discussion

Elucidating non-chemical methods for reducing postharvest decay has become increasingly important globally. In this context, microbial antagonists have shown significant potential as substitutes for synthetic fungicides in controlling postharvest decay of fruits (Janisiewicz and Korsten, 2002) [11]. This shift towards bioagents has garnered considerable attention and is emerging as a promising and viable alternative to chemical control methods.

The study presented results focusing on the impact of cultural filtrates of bioagents on the infection percentage of *A. caricae*, seven and ten days after treatment, as detailed in Table 2 and 3. The findings revealed significant differences among the various cultural filtrates of biocontrol agents, their concentrations, and their interactions. These differences were observed in terms of the infection percentage relative to the control, and they were statistically significant at the 1% level. This research underscores the effectiveness of cultural filtrates of bioagents in managing papaya black spot disease postharvest. By demonstrating significant control over infection rates compared to traditional methods involving synthetic fungicides, the study highlights the potential of bioagents in sustainable agriculture practices. Moving forward, further exploration and adoption of these natural alternatives could lead to reduced reliance on chemical pesticides while promoting environmentally friendly approaches to disease management in agriculture.

Table 2: Efficacy of bioagents against *A. caricae* on infection percentage of fruits at seven days after treatment

Sl. No.	Bioagents	Infection percentage				
		Concentration (%)				Mean
		25	50	75	100	
1.	*Trichoderma harzianum*	5.70 (13.82)	3.51 (10.81)	2.96 (9.91)	2.76 (9.56)	3.73 (11.14)
2.	*Trichoderma viride*	1.48 (7.00)	1.06 (5.92)	0.86 (5.31)	0.32 (3.23)	0.93 (5.53)
3.	*Pseudomonas fluorescence*	10.06 (18.50)	5.72 (13.84)	4.29 (11.96)	3.79 (11.22)	5.96 (14.14)
4.	*Bacillus subtilis*	7.41 (15.80)	1.62 (7.31)	1.23 (6.37)	0.93 (5.54)	2.80 (9.63)
5.	*Bacillus megaterium*	9.35 (17.82)	9.11 (17.57)	8.12 (16.56)	5.13 (13.10)	7.93 (16.36)
6.	Control (Water)	13.56 (21.62)	13.56 (21.62)	13.56 (21.62)	13.56 (21.62)	13.56 (21.62)
Mean		7.92 (16.37)	5.76 (13.89)	5.16 (13.15)	4.41 (12.11)	5.78 (13.84)
		Bioagents		Concentrations		B xC
SEm±		0.31		0.40		0.15
CD @ P=0.01		0.29		0.22		0.57

Values in parenthesis are arcsine transformed values

The study's results underscore the significant effectiveness of cultural filtrates in reducing the infection percentage of papaya black spot disease compared to control treatments. Among the six bioagents evaluated, treatments using cultural filtrates of *T. viride* exhibited the highest efficacy, achieving a substantial reduction in infection percentage (0.93%) relative to the control. This was followed by treatments with cultural filtrates from *Bacillus subtilis* (2.80%), *T. harzianum* (3.73%), Pseudomonas fluorescence (5.96%), *Trichoderma spp*. (6.22%), and *B. megaterium* (7.93%), which also demonstrated varying degrees of effectiveness in reducing infection rates.

Furthermore, the study investigated the impact of different concentrations of cultural filtrates on infection percentage, revealing a significant concentration-dependent effect. Swabbing fruits with 100% concentrations of cultural filtrates proved to be highly effective, achieving a notably low infection percentage (4.41%) compared to lower concentrations. Specifically, the lowest infection percentages were observed at 100% concentration of *T. viride* (0.32%), followed by *B. subtilis* (0.93%), *T. harzianum* (2.76%), *Pseudomonas fluorescence* (3.79%), and *B. megaterium* (5.13%). These findings highlight the importance of using higher concentrations of cultural filtrates to maximize their efficacy in controlling papaya black spot disease (Fig. 2).

Table 3: Efficacy of bioagents against *A. caricae* on infection percentage of fruits at ten days after treatment

Sl. No.	Bioagents	Percent infection				
		Concentration (%)				Mean
		25	50	75	100	
1.	*Trichoderma harzianum*	12.76 (20.92)	7.40 (15.78)	6.79 (15.10)	6.47 (14.73)	8.36 (16.63)
2.	*Trichoderma viride*	7.65 (16.05)	7.03 (15.37)	6.89 (15.21)	5.02 (12.94)	6.64 (14.89)
4.	*Pseudomonas fluorescence*	17.59 (24.79)	14.71 (22.55)	12.78 (20.94)	9.70 (18.14)	13.69 (21.60)
5.	*Bacillus subtilis*	17.74 (24.91)	11.76 (20.05)	10.22 (18.64)	7.67 (16.08)	11.85 (19.92)
6.	*Bacillus megaterium*	15.12 (22.88)	14.58 (22.45)	11.52 (19.84)	11.41 (19.73)	13.16 (21.22)
7.	Control (Water)	21.46 (27.59)	21.46 (27.59)	21.46 (27.59)	21.46 (27.59)	21.46 (27.59)
Mean		14.90 (22.48)	13.27 (20.98)	11.63 (19.58)	10.28 (18.20)	12.53 (20.30)
		Bioagents		Concentrations		B x C
SE m±		0.22		0.29		0.11
CD @ P=0.01		0.20		0.15		0.40

Values in parenthesis are arcsine transformed values

On the 10th day after treatment, significant results were observed in the study of post-harvest management of papaya black spot disease, specifically concerning the infection percentage relative to the control. As the study progressed, the infection percentage increased steadily due to the development of rot caused by black spots on the papaya surface.

The study evaluated various cultural filtrates of bioagents, each showing distinct effectiveness in reducing the infection percentage. Among these, *Trichoderma viride* demonstrated exceptional efficacy with an infection percentage of 6.64%, followed closely by *Trichoderma harzianum* (8.36%), *Bacillus subtilis* (11.85%), *Bacillus megaterium* (13.16%), and *Pseudomonas fluorescence* (13.69%). These results were notably lower compared to the control group, which had an infection percentage of 21.46%.

Additionally, different concentrations of the bioagents' cultural filtrates were tested, revealing varying levels of effectiveness. The lowest infection percentage was observed at the highest concentration of 100% (10.28%), followed by 75% (11.63%), 50% (13.27%), and 25% (14.90%) concentrations. Specifically, *Trichoderma viride* exhibited significant efficacy at 100% concentration, resulting in an infection percentage of 5.02%, substantially lower than at lower concentrations (75%, 50%, 25%). *Trichoderma harzianum* also showed notable efficacy at 100% concentration with an infection percentage of 6.47% after 10 days of treatment.

Overall, the results suggest that cultural filtrates of bioagents, particularly *T. viride*, can serve as effective alternatives to synthetic fungicides for managing postharvest diseases in papaya. By optimizing the concentration and application methods of these biocontrol agents, agricultural practices can potentially reduce reliance on chemical pesticides while promoting sustainable and environmentally friendly disease management strategies. Future research could further explore the practical application and economic feasibility of integrating cultural filtrates into agricultural practices on a larger scale.

Research conducted by Alstrom (2001) [2], Wheatley (2002) [3], Koitabashi (2005) [13], Fernando *et al.* (2005) [9], and Kai *et al.* (2006) [12] has consistently shown that compounds produced by these microbial antagonists exhibit potent antifungal properties against plant pathogens. For example, powerful antifungal peptides such as iturins from *Bacillus subtilis*, viridin from *Trichoderma viride*, and 2,4DAPG from *Pseudomonas fluorescence* have been highlighted for their significant contributions to disease control (Algam *et al.*, 2004; Hou *et al.*, 2006) [6, 10].

(a) Before treating papaya fruits with cultural filtrates of *T. viride*

(b) After treating papaya fruits with cultural filtrates of *T. viride*

Fig 2: Efficacy of cultural filtrates of *T. viride* at seven days after treatment

The active mechanisms through which these bioagents operate include antibiosis, where they directly inhibit the growth of pathogens, as well as the production of toxins that disrupt pathogen development. This dual approach effectively reduces infection percentages on fruits like papayas, as evidenced by the study's findings.

Similar results have been reported by Alka *et al.* (2017) [1] and El-Katatny and Emam (2012) [4], who found *Trichoderma* spp. to be effective in managing disease severity, such as tomato Rhizopus rot.

These bioactive compounds work by inhibiting the growth and development of fungal pathogens, thereby reducing infection rates in crops. Their effectiveness has been documented in numerous studies, reinforcing the potential of microbial antagonists as sustainable alternatives to synthetic fungicides in agricultural practices. By harnessing these natural compounds, farmers can potentially mitigate the adverse effects of chemical pesticides on human health and the environment, while promoting eco-friendly approaches to disease management in agriculture.

Conclusion

The current study has underscored the increasing interest in utilizing cultural filtrates of bioagents as a promising and viable alternative to chemical control methods. Specifically, the focus has been on managing post-harvest diseases, where the application of fungicides may inadvertently result in the accumulation of residue on fruits. This accumulation poses potential risks to consumer health and the environment.

Therefore, leveraging cultural filtrates of bioagents represents a sustainable approach for mitigating post-harvest papaya black spot disease. By utilizing these natural agents, farmers can potentially reduce their dependence on chemical pesticides while maintaining effective disease management. This shift not only addresses concerns related to fungicide residue but also aligns with growing global trends towards eco-friendly agricultural practices.

In conclusion, the findings suggest that cultural filtrates of bioagents hold significant promise as an alternative management strategy for post-harvest papaya black spot disease. By further exploring and implementing these natural solutions, agriculture can move towards more sustainable and environmentally conscious practices.

Conflict of interest

The authors declare no competing interests

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Authors Contributions

Pooja S Patel: Conducted the experiment, data analysis and drafted the article

V. B. Sanath Kumar: Conception of experiment, supervised and critical revision of the article

Kiran Kumar N: Design of the experiment, supervised and critical revision of the article

Chandrappa: Design of the experiment, supervised and critical revision of the article

K. B. Palanna: Design of the experiment, supervised and critical revision of the article

Data Availability

Not applicable

Research involving human and/or animals: Not involved

Consent for publications

All authors agree to publish this article in International Journal of Advanced Biochemistry Research

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