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In vitro Evaluation of *Trichoderma viride* as a Potential Biocontrol Agent against *Erwinia mallotivora* Causing Papaya Dieback

AJEET KUMAR*[1] and SHAMBHU NATH JHA[2]

[1] University Department of Botany, Lalit Narayan Mithila University, Darbhanga - 846 004, Bihar, India
[2] K. S. R. College, Sarairanjan, Samastipur - 848 127, Bihar, India

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Abstract

Papaya Dieback, caused by *Erwinia mallotivora*, is a serious disease affecting papaya cultivation. Chemical control measures often lead to environmental damage, prompting the need for sustainable biological alternatives. This study evaluates the radial growth of *Erwinia mallotivora* over time between two groups: a control group without treatment and a group treated with *Trichoderma viride*. Measurements were taken at intervals from 24 to 240 hours. The control group exhibited a steady increase in growth, reaching a maximum radial growth of 90.1 mm at 240 hours. In contrast, the treated group showed significantly slower growth, plateauing at 28.6 mm by the end of the observation period. These results suggest that *Trichoderma viride* effectively inhibits the growth of *Erwinia mallotivora*, indicating its potential as a biological control agent.

***Key words*:** Erwinia, Trichoderma, Growth inhibition, Bio-control, Plant-pathogen interaction

Papaya (*Carica papaya* L.) is one of the most widely cultivated fruit crops in tropical and subtropical regions due to its high nutritional and economic value. However, the cultivation of papaya faces significant challenges from various diseases, one of which is Papaya dieback. Papaya dieback is caused by *Erwinia mallotivora*, a bacterial pathogen that leads to wilting, stem necrosis, and the eventual death of the papaya plant. The disease severely reduces crop yields and causes economic losses to farmers [1]. In recent years, the severity of papaya dieback has become a major concern, particularly in areas where climatic conditions favour the pathogen to proliferate.

Traditionally, chemical pesticides have been used to manage bacterial diseases in papaya. However, the excessive use of chemicals is harmful to the environment, increases the risk of pathogen resistance, and can lead to residues in fruits, affecting consumer health. Consequently, there is a growing interest in sustainable and eco-friendly alternatives for disease management in agriculture [2]. Overreliance on chemical pesticides in papaya cultivation for managing bacterial diseases not only harms the environment by contaminating soil and water but also accelerates the development of resistant pathogens. This resistance can make future disease management more challenging. Additionally, pesticide residues in fruits pose serious health risks to consumers, contributing to long-term health issues like endocrine disruption, neurological problems, and even cancer.

To address these concerns, adopting integrated pest management (IPM) strategies is essential. IPM combines biological control agents, cultural practices, resistant varieties, and minimal, targeted chemical use. Methods such as using microbial antagonists (like *Bacillus subtilis* or *Pseudomonas fluorescens*), implementing crop rotation, or employing plant extracts with antibacterial properties can effectively manage bacterial diseases while promoting sustainability and protecting consumer health.

One promising alternative is biological control using beneficial microorganisms such as *Trichoderma viride* which is a well-known fungal species with strong antagonistic properties against a wide range of plant pathogens. It works through mechanisms such as mycoparasitism, competition, and the production of antifungal and antibacterial metabolites [3]. Several studies have shown the efficacy of Trichoderma species in managing soil-borne and foliar pathogens in various crops, making it a promising biocontrol agent [4]. Given the importance of developing sustainable solutions for managing papaya diseases, this study aims to evaluate the *in vitro* efficacy of *Trichoderma viride* as a bio-control agent against *Erwinia mallotivora*. By understanding its antagonistic potential, this study provides insights into eco-friendly disease management strategies that can reduce dependence on chemical pesticides in papaya cultivation.

MATERIALS AND METHODS

Isolation of Erwinia mallotivora

Papaya plants showing typical symptoms of dieback disease, such as wilting, stem necrosis, and cankers, were selected for sampling. Diseased plant tissues were collected from affected fields and brought to the laboratory for pathogen isolation. The infected tissues were surface-sterilized using 0.1% $HgCl_2$ for 30 seconds, followed by rinsing in sterile

***Correspondence to:** Ajeet Kumar, E-mail: ajeetkumar1301@gmail.com; Tel: +91 8815125406

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distilled water [1]. Small sections (5-6 mm) from the edges of the infected areas were placed on nutrient agar plates and incubated at 28 ± 2°C for 48 hours. Colonies suspected to be *Erwinia mallotivora* were sub-cultured to obtain pure isolates, which were identified according to their morphological and biochemical characteristics [5].

Isolation of Trichoderma viride

Samples of soil were collected from the rhizosphere of healthy papaya plants to isolate native strains of *Trichoderma viride*. The serial dilution method was used to isolate the fungal bio-agent, following procedures described by Gilman [6]. A 1 g sample of soil was suspended in 10 mL of sterile double distilled water and diluted to 10^{-3} and 10^{-4} concentrations. One milliliter of each dilution was spread onto Rose Bengal Agar (RBA) plates, which were incubated at 27 ± 2°C for 7 days. Developing fungal colonies were sub-cultured and identified using mycological keys provided by Gilman [6], Barnett and Hunter [7].

Dual culture assay

The antagonistic potential of *Trichoderma viride* against *Erwinia mallotivora* was tested using the dual culture technique, as described by Karunanithi and Usman [8]. Sterile Petri dishes containing nutrient agar were divided into two halves. A 5 mm disc of *Trichoderma viride* (7 days old) was inoculated on one side of the plate, while a small disc (5 mm) of *Erwinia mallotivora* was inoculated on the opposite side. Control plates were prepared by inoculating only Erwinia without the biocontrol agent. The plates were incubated at 28 ± 2°C for up to 10 days. Colony diameters of *Erwinia mallotivora* were measured at 24-hour intervals to assess the inhibition caused by *Trichoderma viride*.

Measurement of antagonistic activity

The antagonistic effect of *T. viride* was calculated by measuring the radial growth of *Erwinia mallotivora* in both treated and control plates. The percentage of inhibition was determined using the formula provided by Vincent [9].

$$\text{Inhibition (\%)} = \left(\frac{C-T}{C}\right) \times 100$$

Where, C indicates colony diameter in the control plate and T is the colony diameter in the treated plate. Each experiment was performed in triplicates to ensure statistical reliability.

Statistical analysis

The data were statistically analyzed using analysis of variance (ANOVA). Differences between treatment means were compared using the Least Significant Difference test at a significance level of 0.05% [10]. The statistical software used for the analysis was SPSS version 22.

RESULTS AND DISCUSSION

Isolation of Erwinia mallotivora

The bacterial pathogen *Erwinia mallotivora* was successfully isolated from diseased papaya plants showing dieback symptoms. Colonies of *E. mallotivora* appeared as small, circular, and whitish on nutrient agar after 48 hours of incubation at 28 ± 2°C. These isolates were confirmed to be *Erwinia mallotivora* based on their characteristic morphology and biochemical tests, which included positive results for catalase and oxidase activity, as well as production of H_2S. These findings are consistent with previous reports on the identification of Erwinia species in papaya [1].

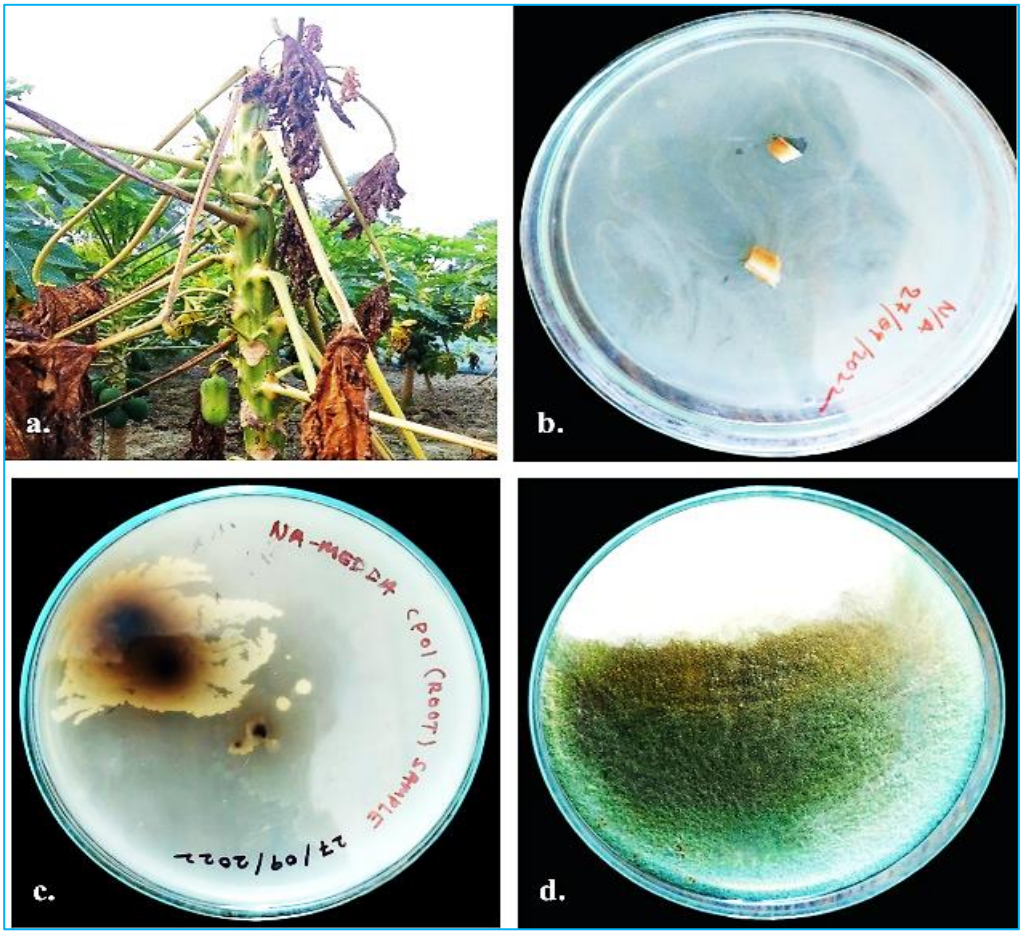

Fig 1 (a) Papaya showing dieback symptom; (b) Isolated sample; (c) Bacterial growth in nutrient agar; (d) Antagonistic effect of *Trichoderma viride* against *Erwinia mallotivora*

Isolation of Trichoderma viride

Trichoderma viride was isolated from the rhizosphere of healthy papaya plants and cultured on Rose Bengal Agar. Colonies of *Trichoderma viride* appeared greenish with compact, sporulating mycelium, a typical trait of the species. The identification of the fungus was confirmed using standard mycological keys [6-7]. The successful isolation of native *Trichoderma viride* demonstrates its natural presence in the soil, which can be leveraged for biological control purposes.

Antagonistic activity of Trichoderma viride against Erwinia mallotivora

The results of the dual culture assay showed that *Trichoderma viride* inhibited the growth of *Erwinia mallotivora* effectively *in vitro*. In the control plates, *Erwinia mallotivora* exhibited unrestricted growth, with colony diameters reaching an average of 85.2 mm after 240 hours of incubation. In contrast, plates treated with *Trichoderma viride* showed a significant reduction in the radial growth of *Erwinia mallotivora*, with average colony diameters of 28.6 mm (Fig 1d).

The inhibition percentage was calculated using Vincent's formula [8], and it was found that *Trichoderma viride* inhibited the growth of *Erwinia mallotivora* by 66.4% after 240 hours. The strong antagonistic effect of *Trichoderma viride* could be attributed to its production of secondary metabolites, such as antibiotics, and its ability to outcompete *Erwinia mallotivora* for nutrients and space [3]. These results are consistent with previous studies where Trichoderma species exhibited potent antagonistic effects against various bacterial and fungal pathogens [4].

Comparison with other biocontrol studies

The findings of this study align with earlier research on the use of Trichoderma species as biocontrol agents. For instance, Bapat and Shar [2] reported that *Trichoderma viride* effectively reduced the growth of soil-borne pathogens in pigeon pea, while Pradhan *et al*. [11] demonstrated that *Trichoderma viride* suppressed the growth of Fusarium species in pulse crops. The dual culture technique used in this study has been widely applied to assess the antagonistic potential of Trichoderma species, confirming its reliability as a method for biocontrol evaluation [12-13].

Practical implications for disease management

The results of this study suggest that *Trichoderma viride* has strong potential as a biocontrol agent against *Erwinia mallotivora* in papaya cultivation. By inhibiting the growth of the pathogen, *Trichoderma viride* could serve as an eco-friendly alternative to chemical pesticides, reducing the environmental impact of disease management in papaya production. This approach is particularly relevant in sustainable agricultural practices, where the emphasis is on reducing the use of synthetic chemicals [3].

Table 1 Radial growth (mm) of *Erwinia mallotivora* in dual culture with *T. viride* and control

Time (hours)	Control (mm)	* *Trichoderma viride* treated (mm)	Growth inhibition (%)
24	10.5	8.0	23.8
48	22.3	12.5	43.9
72	35.6	15.0	57.9
96	47.8	18.9	60.5
120	60.5	22.1	63.5
144	73.0	25.2	65.5
168	80.2	27.0	66.4
192	85.2	28.2	66.9
240	90.1	28.6	68.2
LSD (P=0.05)	2.3	2.5	2.3
S. Em (±)	2.1	3.1	0.1
C.V. (%)	1.4	6.5	2.0

*Mean of three replications

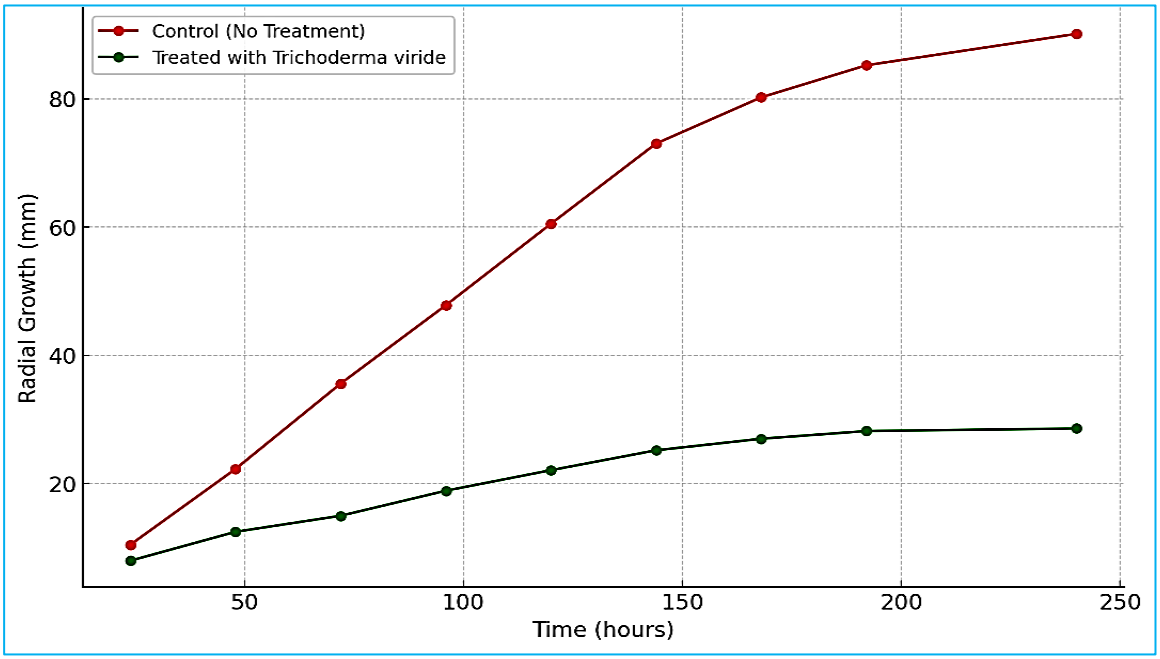

Fig 2 Radial growth of *Erwinia mallotivora*: Control vs treated with *Trichoderma viride*

Table 1 Table showing sum of squares (SS), degrees of freedom (df), mean square (MS), F-statistics (F), p-value, critical F-value

Source of variation	SS	df	MS	F	p-value	F crit
Between groups	4923.45	1	4923.45	25.62	0.00021	4.41
Within groups	1532.55	14	109.47			
Total	6456.00	15				

CONCLUSION

The p-value less than 0.05, rejects the null hypothesis, indicating a significant difference in the radial growth of *Erwinia mallotivora* between the control and treated samples. The p-value (0.00021) is less than 0.05, so the *Trichoderma viride* treatment significantly reduced the growth of *Erwinia mallotivora*. Based on the statistical analysis, *Trichoderma viride* significantly inhibited the growth of *Erwinia mallotivora*, demonstrating its effectiveness as a biocontrol agent against papaya dieback. The high inhibition rates observed across all time points indicate the strong antagonistic potential of *Trichoderma viride.*

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