



ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(10): 2090-2093
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www.thepharmajournal.com
Received: 01-07-2023
Accepted: 08-08-2023

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Efficacy of biocontrol agents and plant extracts against Phomopsis blight of brinjal caused by *Phomopsis vexans* (Sacc. & Syd.) *in vitro*

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Abstract

Eggplant (*Solanum melongena* L.) is a vital vegetable crop in tropical and subtropical regions, with South Asia contributing nearly 50 percent of the global cultivation. However, the crop faces a significant threat from Phomopsis blight, caused by *Phomopsis vexans*, which can result in yield losses of up to 70 percent. In this study, we explored biocontrol agents and plant extracts as potential strategies to manage Phomopsis blight in eggplants. Our results revealed promising outcomes in combatting this disease. Among the biocontrol agents evaluated, *Trichoderma viride* stood out, demonstrating a remarkable 70.57 percent inhibition of *Phomopsis vexans* growth. Furthermore, neem leaf extract exhibited significant antifungal activity, with a 39.61 percent reduction in mycelial growth. These findings highlight the effectiveness of these natural alternatives in suppressing Phomopsis blight. Biological control, particularly through *Trichoderma* species, presents an eco-friendly and sustainable approach, offering potential for reducing the reliance on chemical fungicides. Moreover, neem leaf extract's antimicrobial properties against the pathogen underscore its potential for integrated disease management.

Keywords: Biocontrol, eggplant, Phomopsis blight, *Phomopsis vexans*, *Trichoderma* spp, neem leaf extract.

Introduction

Eggplant, scientifically known as *Solanum melongena* L., is a significant vegetable crop that falls within the Solanaceae family. Eggplant is among the most widely cultivated, popular, and essential vegetable crops in tropical and subtropical regions. It is highly productive and often serves as a vital crop for those with limited resources. The cultivation of eggplant is widespread in countries like India, Pakistan, Bangladesh, China, and the Philippines, with South Asia contributing to nearly 50 percent of the global eggplant cultivation (Harish *et al.*, 2011) [4]. This resilient crop is cultivated year-round, including during the hot and wet monsoon season when other vegetables are in short supply. In India, eggplant is primarily grown in states such as West Bengal, Orissa, Bihar, Gujarat, Maharashtra, Andhra Pradesh, and Uttar Pradesh. The total cultivation area covers 7.22 lakh hectares, resulting in a production of 135.58 metric tonnes and a productivity of 19.10 tonnes per hectare (Anon., 2014) [1]. Eggplant contributes approximately 12.47 percent of India's total vegetable production. Phomopsis blight caused by *Phomopsis vexans* is a major disease of brinjal that can cause significant yield losses. According to a study, yield losses of up to 70% have been reported due to this disease. Fruit rot, which is induced by the fungus *Phomopsis vexans*, has emerged as a severe disease in the arid regions of the north. The first recorded instance of this disease dates back to 1914 in the Gujarat state, and since then, it has been reported in various regions of India. In general, this disease results in crop losses ranging from 15-20% (Hossain *et al.*, 2013) [5]. Studies have indicated that *Phomopsis vexans* can cause a reduction in both yield and the market value of the crop by as much as 20-30% (Jain and Bhatnagar, 1985) [7].

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The fruit becomes susceptible to infection in the final days of fruit formation, just a few days before the crop is due for harvesting. This infection intensifies significantly during the harvest and subsequent marketing of the crop. Several reports have suggested the utilization of plant extracts, bioagents, and hot water treatments as potential solutions to control this disease (Srinivas *et al.*, 2005; Islam and Meah, 2011; Das *et al.*, 2014) [17, 6, 2]. Furthermore, the effectiveness of seed treatment with fungicides against *P. vexans* was evaluated (Srinivas *et al.*, 2005; Islam and Meah, 2011; Das *et al.*, 2014) [17, 6, 2]. Furthermore, the effectiveness of seed treatment with fungicides against *P. vexans* was evaluated (Srinivas *et al.*, 2005; Das *et al.*, 2014) [17, 2]. In this current study, experiments were conducted to assess the efficacy of biocontrol agents and plant extracts in combatting Phomopsis leaf blight disease in eggplants under controlled laboratory conditions.

Methods and Materials

To evaluate the antifungal properties of plant extracts, fresh samples were rinsed in tap water and then further cleansed by washing them three times with distilled water. Subsequently, the samples were crushed in a sterilized mortar and pestle, with a small amount of alcohol (in a 1:1 w/v ratio) added just enough to facilitate the crushing process. The resulting extracts were strained through double layers of muslin cloth. These filtrates obtained from the leaves served as the stock solution. To investigate the antifungal mechanism of these plant extracts, we followed the damaged food method, as proposed by (Nene and Thapliyal 1982) [10]. For this, 5 and 10 ml of the stock solutions were mixed with 95 and 90 ml of sterile potato dextrose agar medium, respectively, to achieve concentrations of 5 and 10 percent. The medium was thoroughly shaken to ensure a uniform distribution of the plant extract.

Approximately 20 ml of this medium was poured into each of the 90 mm sterilized Petri plates. Every plate was then inoculated with 5 mm mycelial discs, aseptically obtained from the periphery of a 7-day-old culture. The plates were then incubated at 25±2 °C until the fungal colony growth reached its maximum, as observed in the control plates. Three replications were maintained for each treatment, and appropriate control plates were also set up. The mean colony diameter was recorded in each case, and the effectiveness of

the plant extracts was expressed as the percentage of mycelial growth inhibition over the control, which was calculated using the formula provided by Vincent in 1927 [18].

The effectiveness of bio-agents was evaluated using a double-culture method. In sterile Petri plates, 20 ml of sterilized and cooled potato dextrose agar medium was poured. To assess fungal adversaries, a pathogen was inoculated on one side of the Petri plate, while the adversary was placed on the exact opposite side, with approximately 4 cm of space in between. Cultures that were actively growing were used for this purpose. In the case of bacterial adversary assessment, two mycelial discs of the pathogen were inoculated at the periphery of the Petri plate, and the bacterial adversary was streaked in the center of the same plate. After the necessary incubation period, which was determined when the control plate exhibited a 90 mm diameter of growth, the radial growth of the pathogens was measured. The percentage of inhibition compared to the control was calculated using the formula provided by (Vincent, 1927) [18]. Various antagonistic organisms used against the pathogens causing eggplant fruit spoilage included *Trichoderma harzianum*, *T. viride*, *Pseudomonas fluorescens*, and *Bacillus subtilis*, which were maintained in the biocontrol laboratory of the department.

Effect bio agents and botanicals against *Phomopsis vexans* after 7 days of inoculation:

Biocontrol agents and botanical extracts were assessed for their inhibitory effects against *Phomopsis vexans* using the dual culture method. All treatments displayed inhibitory effects on hyphal growth with varying degrees of efficacy in (Table 1 and Fig.1). The efficacy of biocontrol agents, including *T. viride*, *T. harzianum*, *Bacillus subtilis*, and *P. fluorescens*, was evaluated. Among these bioagents, *T. viride* exhibited the greatest reduction in radial mycelial growth, achieving a maximum of 26.48 mm with a 70.57 percent inhibition against the pathogen.

On the other hand, among the various plant extracts, neem leaf extract showed the highest percentage of inhibition in mycelial growth at 39.61. The table demonstrates that all tested biocontrol agents and plant extracts exhibited statistically significant reductions in the mycelial growth of the pathogen, compared to the control, for up to 7 days following inoculation.

Table 1: Effect bio agents and plant extracts against *Phomopsis vexans* after 7 days of inoculation

Treatments Symbol	Radial mycelial growth in (mm)	Inhibition percent over control
<i>Trichoderma. harzianum</i>	33.56	62.71
<i>T. viride</i>	26.48	70.57
<i>P. fluorescens</i>	39.17	56.47
<i>Bacillus subtilis</i>	44.67	54.81
neem leaf extract	54.35	39.61
Datura leaf extract	56.78	36.91
Control	90.00	00.00
CD at 5%	2.175	-
SE m±	0.698	-
CV	2.517	-

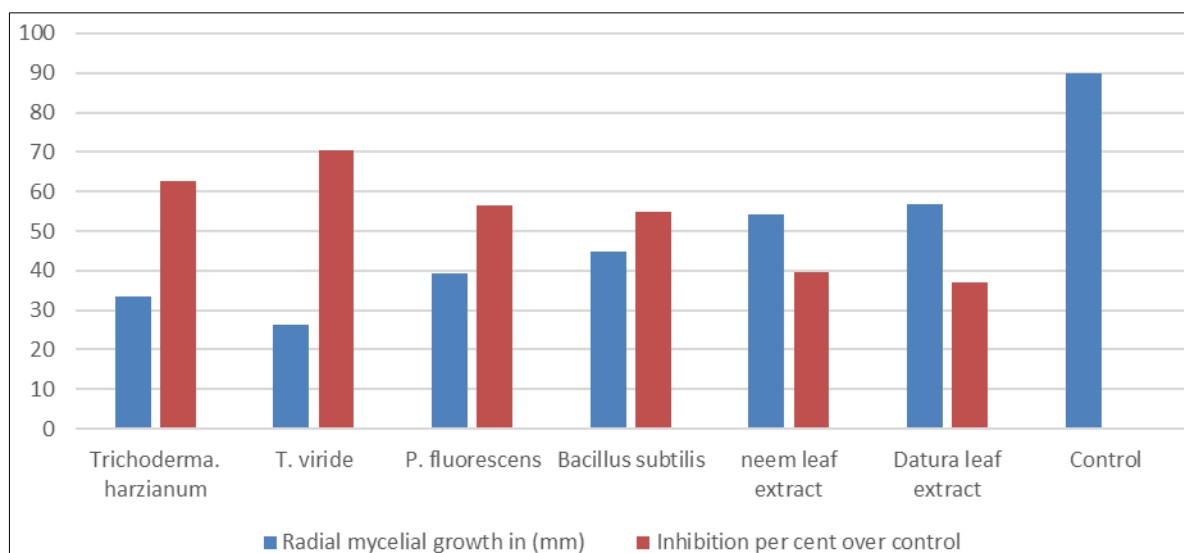


Fig 1: Effect bio agents and plant extracts against *Phomopsis vexans* after 7 days of inoculation

Discussion

Biological control presents an appealing alternative strategy for managing plant diseases, involving the utilization of living organisms to reduce disease incidence. In the current study, among the biocontrol agents evaluated against *P. vexans*, the highest percentage of growth inhibition was observed in *Trichoderma harzianum* (62.71%), closely followed by *T. viride* (70.57%). *Trichoderma*, acknowledged as a potent biocontrol agent, has been previously documented by several researchers. The observations in the current study align with the discoveries made by (Muneshwar *et al.* 2012) [9]. The antagonistic effects of *Trichoderma* spp. against numerous fungi primarily stem from the production of acetaldehyde compounds, as outlined by (Dennis and Webster 1971) [3].

Natural plant products serve as vital sources for developing new agrochemicals aimed at controlling plant diseases. *In vitro* assessments of plant extracts have demonstrated antimicrobial activity against fungal pathogens. A Poison Food Technique revealed that neem leaf extracts and datura leaf extracts, both at a 5 percent concentration, were highly effective in inhibiting the growth of *P. vexans*. Regarding the utilization of plant-based products, research conducted by (Ram *et al.* 2012) [12] demonstrated the antimicrobial properties of plant extracts. Furthermore, these plant extracts were found to effectively inhibit fungal growth in artificial media, as observed by (Sneha *et al.* 2016) [14].

One significant issue associated with chemical fungicides is their tendency to accumulate in various plant parts, including the fruit. Moreover, the prolonged or indiscriminate use of chemicals can lead to the buildup and retention of these substances in the soil, posing risks of groundwater contamination, harm to non-targeted wildlife, development of fungicide resistance, adverse effects on beneficial soil microorganisms, and potential health risks for consumers. Hence, in the context of environmental concerns, it is imperative to explore alternatives. Therefore, alternative biological agents are gaining recognition as eco-friendly, sustainable, and cost-effective methods of disease management.

Conclusion

The study demonstrates the potential of biocontrol agents and plant extracts in managing *Phomopsis* blight, a severe threat to

eggplant crops. *Trichoderma viride* and neem leaf extract proved highly effective, with significant inhibition of *Phomopsis vexans* fungal growth. These findings signify the viability of eco-friendly, sustainable alternatives, reducing reliance on chemical fungicides while preserving environmental and health integrity. By leveraging these natural solutions, we can enhance eggplant yield and quality, safeguarding this vital crop in the face of disease challenges. In addressing *Phomopsis* blight, our study offers valuable insights for sustainable eggplant cultivation.

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