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In vitro efficacy of systemic fungicides, bioagents and phytoextracts against *Colletotrichum truncatum* infecting soybean crop

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Abstract
Soybean pod blight, caused by *Colletotrichum truncatum*, is a major production constraint in India, leading to severe yield and quality losses. The present study was undertaken to evaluate the *in vitro* efficacy of systemic fungicides, biological control agents, and selected Phyto-extracts against the pathogen. Among the fungicides tested, tebuconazole (25.9% EC) completely inhibited mycelial growth, followed by carbendazim, propiconazole, and hexaconazole. The biocontrol agent *Trichoderma harzianum* exhibited the highest level of antagonistic activity compared to other antagonists evaluated. Among the Phyto-extracts, garlic extract showed the strongest antifungal effect, while ginger, mehandi, and onion extracts also displayed significant growth inhibition. The results highlight the potential of integrating chemical, biological, and botanical agents in developing sustainable management strategies for soybean pod blight under Indian conditions.

Keywords: Soybean, Phytoextracts, Trichoderma, Tebuconazole

Introduction
Soybean (*Glycine max* (L.) Merrill), a member of the family Leguminaceae and subfamily Papilionaceae, is a globally important oilseed and leguminous crop valued for both its nutritional and industrial applications. Its seeds contain approximately 18-20% oil and 38-42% protein, making it a vital source of food and feed for humans and livestock. In India, soybean contributes substantially to edible oil production, with Madhya Pradesh, Maharashtra, and Rajasthan representing the primary cultivation regions. Soybean crop is affected by many economic diseases worldwide (Khaire and Hake, 2018; Rhouma *et al*, 2024) [18, 15]. Among the biotic stresses limiting soybean productivity, pod blight (anthracnose) caused by *Colletotrichum truncatum* is one of the most destructive diseases. The pathogen predominantly affects soybean during reproductive stages, producing irregular lesions on pods, reducing plant vigor, and causing significant yield losses ranging from 16% to complete crop failure, while also deteriorating seed quality (Gurjar *et al*., 2021; Amrate *et al*., 2018) [6]. Disease severity is markedly influenced by agronomic practices, particularly sowing time, with warm and humid climatic conditions (20-25 °C) favoring pathogen development and disease outbreak. Although host resistance offers the most sustainable solution for disease management, its practical implementation remains constrained by breeding complexities and limited availability of resistant cultivars. Chemical fungicides have been widely used and are effective in disease suppression (Vyavhare *et al*, 2020; Naik *et al*, 2020; Chavan *et al*, 2023; Shinde *et al*, 2024) however, their indiscriminate application can lead to fungicide resistance and environmental hazards (Samanta *et al*, 2023). In recent years, alternative eco-friendly strategies such as the use of biological control agents (Naik *et al*, 2020; Sahane *et al*, 2021; Khaire *et al*, 2021; Rhouma *et al*, 2024; Thakare *et al*, 2024) [18, 15, 22] and plant-derived extracts with antifungal properties have gained prominence as safer and more sustainable options (Khaire *et al*, 2018; Misal *et al*, 2019; Matrood *et al*, 2021) [11, 10]. The present investigation aims to evaluate the *in vitro* efficacy of systemic fungicides, selected Phyto-extracts, and biological control agents against *C. truncatum*.

The study seeks to generate comparative data on their antifungal activity, thereby contributing to the development of effective, eco-friendly management strategies for pod blight in soybean. The findings will provide useful insights for integrated disease management and sustainable soybean cultivation under Indian agricultural conditions.

Methodology

Isolation of fungal pathogen responsible for pod blight of soybean diseases

Soybean pods showing typical symptoms of the disease were collected from different fields in Ahilyanagar district, Maharashtra. To isolate the fungi associated with the disease, the infected tissues were first sterilized by dipping them in 3% sodium hypochlorite solution for 2 to 3 minutes and then rinsed with sterile distilled water to remove any traces of the disinfectant. Small pieces about 5 by 5 millimeters were cut from these sterilized tissues and placed on Potato Dextrose Agar (PDA) plates. The plates were kept at room temperature (around 25 °C) for seven days to allow fungal growth. Once fungal colonies appeared, pure cultures were obtained by transferring a single growing hyphal tip from the colony to fresh PDA plates using a sterile technique under a dissecting microscope. The pathogenicity of the isolated fungi was tested by inoculating healthy soybean seedlings under controlled conditions. Seeds of the JS-335 variety were planted in sterilized soil in earthen pots. When the seedlings reached the flowering stage, they were gently wounded with a sterile needle and inoculated with a suspension of fungal spores. Control plants were treated with sterile water. The inoculated plants were then covered with polythene bags for 48 hours to maintain humidity and support disease development. The fungi were identified up to the genus level by observing their morphological features under a microscope and comparing them with descriptions from Dugan (2006) [4].

Evaluation of fungicides for pathogen control under *in vitro* conditions

The *in vitro* performance of seven fungicides at 0.1% concentration was assessed against each pathogen using the poisoned food technique on potato dextrose agar (PDA) medium. The required amount of fungicide was measured and thoroughly mixed with the medium, which was then poured aseptically into Petri plates and allowed to solidify. Control plates contained only the PDA medium without any fungicide. After solidification, a 5 mm diameter fungal disc was inoculated at the center of each plate, and the plates were incubated at 27±2 °C. Observations on mycelial growth (colony diameter) of the test pathogens were recorded until the colony in the untreated control plate was fully grown. The percentage inhibition of pathogen growth was calculated using the formula. Percent Inhibition (%) = [(C - T) / C] × 100. Where, C = Colony diameter in the control plate (mm), T = Colony diameter in the treatment plate (mm). This method, as described by Vincent (1927) [23], is widely used for evaluating the antifungal activity of substances.

Evaluation of bio-control agents against pathogen under *in vitro* conditions

Eight bio-control agents known to be antagonist were evaluated *In vitro* applying dual culture technique on PDA medium in petri dishes. Efficacy of eight bio-agents were evaluated by dual culture technique. Seven days old cultures of the test bio-agents and test isolate of the pathogen grown on respective culture media were used for the study. Two 5 mm culture discs, one each of the test pathogen and the test bio-agent were cut out with the help of sterilized cork borer and placed at equidistance (30 mm away from each other), exactly opposite to each other on sterilized PDA medium (fungal bio-control agent) and Nutrient Agar medium (bacterial bio-control agent) in Petri dishes having diameter of 90 mm and incubated at 27±20C. PDA Petri dishes inoculated alone with pure culture disc (5 mm) of the test pathogen were served as untreated control.

Observations on radial mycelial growth were recorded in all the replicated treatments at 24 hours interval and continued till the control (untreated) plate was fully covered with mycelial growth of the test pathogen. Per centage inhibition of the growth of the test pathogen was calculated by applying the formula given by Arora and Upaddhyay (1978). Per cent growth inhibition (I) =(C-T)/C×100. Where, I = Per cent inhibition, C = Growth of the test fungus in (mm) untreated / control plates and T = Growth of the test pathogen in (mm) treated plates.

Evaluation of phytoextracts agents against pathogen under *in vitro* conditions

To evaluate the antifungal potential of selected phytoextracts against *Colletotrichum truncatum*, plant extracts were incorporated into autoclaved potato dextrose agar (PDA) medium at a concentration of 10%. The amended medium was dispensed into sterile Petri dishes (20 ml per dish), with each treatment replicated three times. Following solidification, each plate was inoculated centrally with a 5 mm mycelial disc from actively growing *C. truncatum* cultures; untreated PDA plates served as controls. Plates were incubated at 27±2 °C until complete fungal growth on control plates was observed. Observations on radial mycelial growth were recorded in all the replicated treatments at 24 hours interval and continued till the control (untreated) plate was fully covered with mycelial growth of the test pathogen. Per cent inhibition of the growth of the test pathogen was calculated by applying the formula given by Vincent (1927) [23].

Results and Discussion

Identification of Isolated Pathogens associated with pod blight disease: Following extensive field surveys, isolations, and purifications, it was determined that the pod blight disease affecting soybean plants was caused by *C. truncatum*. The most prominent symptoms appeared on foliage were brown-coloured patches with gray coloured centre on upper surface and scorched appearance on the lower surface. In advance stage of infection, necrosis of leaf vein, leaf rolling, petiole canker and defoliation occurred (Plate 2). The colonies of *C. truncatum* were whitish to creamy in color. On PDA, the mycelium was hyaline, which was septate and branched. The acervuli were oval to conical and appeared single. Conidia were falcate and slender in shape and slightly curved (Plate 1).

Evaluation of fungicides against pathogen under *in vitro* conditions

The present study evaluated the efficacy of eight systemic fungicides against *Colletotrichum truncatum* under *in vitro* conditions to identify effective chemical control options for soybean pod blight. The results, summarized in Table 1, demonstrate the varying degrees of mycelial growth inhibition achieved by these fungicides at a 0.1% concentration.

Table 1: *In vitro* efficacy of systemic fungicides against *C. truncatum*

Tr. No.	Treatments	Concentration%	*Colony diameter (mm)	% Inhibition
T_1	Hexaconazole 5% EC	0.1	12.50	86.10
T_2	Difenconazole 25% EC	0.1	26.00	71.10
T_3	Propiconazole 25% EC	0.1	7.50	91.60
T_4	Azoxystrobin 23% SC	0.1	43.00	52.20
T_5	Carbendazim 50% WP	0.1	3.00	96.66
T_6	Tebuconazole 25.9% EC	0.1	0.00	100
T_7	Thiophanate Methyl 70% WP	0.1	42.30	53.00
T_8	Control (untreated)		90	-
	SE±(m)		0.36	
	C.D @ 1%		1.51	

*Colony diameter = Average of three replications

Among the fungicides tested, tebuconazole 25.9% EC emerged as the most potent, completely inhibiting mycelial growth (100%), followed closely by carbendazim 50% WP and propiconazole 25% EC with over 90% inhibition. Hexaconazole 5% EC also showed strong antifungal activity, whereas difenoconazole, thiophanate methyl, and azoxystrobin were moderately effective (Plate 3). These findings are in agreement with previous investigations by Gurjar *et al*. (2021) [6], Gavhare *et al*. (2025) [5], and Misal *et al*. (2019) which reported similar levels of efficacy for tebuconazole, propiconazole, and hexaconazole against *C. truncatum* and other fungal pathogens. Such consistency underscores the reliability of these fungicides in managing fungal diseases under controlled conditions.

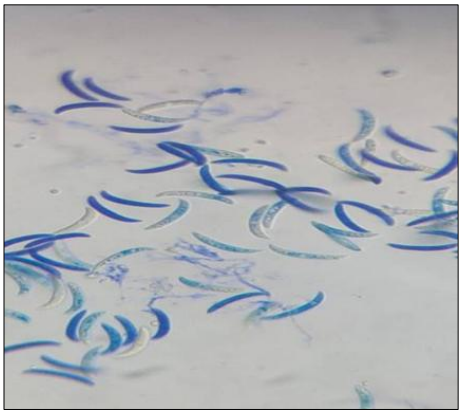

Plate 1: Conidia of *Colletotrichum truncatum*

Plate 2: Symptoms of pod blight of soybean

Plate 3: *In vitro* efficacy of systemic fungicides against *Colletotrichum truncatum*

Plate 4: *In vitro* efficacy of bioagents against *Colletotrichum truncatum*

Plate 5: *In vitro* efficacy of phytoextracts against *Colletotrichum truncatum*

Evaluation of bioagents against pathogen under *in vitro* conditions

The *in vitro* evaluation of eight bioagents against *Colletotrichum truncatum*, the causative agent of soybean pod blight, revealed significant variations in their antagonistic efficacy. Data presented in Table 2, along with supporting plates and figures, show that all bioagents tested were effective in suppressing the mycelial growth of the pathogen compared to the untreated control.

Table 2: *In vitro* efficacy of bioagents against *Colletotrichum truncatum*

Tr. No.	Treatments	*Colony diameter (mm)	% Inhibition
T_1	*Trichoderma asperellum*	18.00	80.00
T_2	*Trichoderma harzianum*	12.50	86.11
T_3	*Trichoderma hamatum*	23.50	73.88
T_4	*Lecanicillium lecanii*	39.50	56.11
T_5	*Metarhizium anisopliae*	30.50	66.11
T_6	*Pseudomonas fluorescens*	57.00	36.66
T_7	*Trichoderma koningii*	31.50	65.00
T_8	*Bacillus subtilis*	58.50	35.00
T_9	Control (untreated)	90	-
	S.E (m)±	0.34	
	C.D.@ 1%	1.42	

*Colony diameter = Average of three replications

Among the bioagents (Plate 4), *Trichoderma harzianum* demonstrated the highest antagonistic activity, resulting in 86.11% inhibition of mycelial growth. This superior efficacy establishes *T. harzianum* as the most promising bioagent for potential biological control of soybean pod blight. Other Trichoderma species such as *T. asperellum* and *T. hamatum* also showed strong inhibitory effects with 80.00% and 73.88% inhibition, respectively. *Metarhizium anisopliae* and *Trichoderma koningii* exhibited moderate activity with inhibition percentages above 65%, whereas *Lecanicillium lecanii*, *Pseudomonas fluorescens* and *Bacillus subtilis* showed relatively lower but significant antagonism ranging from 35% to 56%. These findings are consistent with earlier studies, such as the work by Shashikumara *et al*. (2020) [20], who reported high mycelial growth inhibition by *T. harzianum* (80.22%) against *C. truncatum*, confirming its strong biocontrol potential.

Evaluation of Phyto extracts against pathogen under *in vitro* conditions

The *in vitro* evaluation of various phytoextracts against *Colletotrichum truncatum*, the causal agent of soybean pod blight, revealed significant differences in their antifungal efficacy. The results presented in Table 3 clearly indicate that all tested plant extracts at 10% concentration effectively inhibited mycelial growth of the pathogen compared to the untreated control.

Table 3: *In vitro* efficacy of phytoextracts against *C. truncatum*

Tr. No.	Treatments	Concentration%	Colony diameter (mm)*	Inhibition%
T_1	Nilgiri (*Eucalyptus globulus*)	10	70.00	22.22
T_2	Mehandi (*Lawsonia innermis*)	10	46.00	48.88
T_3	Neem (*Azadirachta indica*)	10	61.00	32.22
T_4	Ghaneri (*Lantana camara*)	10	70.00	22.22
T_5	Nirgudi (*Vitex negundo*)	10	70.50	21.66
T_6	Garlic (*Allium sativum*)	10	15.50	82.77
T_7	Ginger (*Zingiber officinale*)	10	39.50	56.11
T_8	Onion (*Allium cepa*)	10	50.00	44.44
T_9	Control (untreated)		90	-
	SE± (m)		0.39	
	C.D @ 1%		1.62	

*Colony diameter = Average of three replications

Among the phytoextracts, garlic (*Allium sativum*) extract exhibited the highest antifungal activity with an 82.77% inhibition of mycelial growth, followed by ginger (*Zingiber officinale*), mehandi (*Lawsonia inermis*), and onion (*Allium cepa*), which showed considerable inhibitory effects. Other extracts such as neem (*Azadirachta indica*), nilgiri (*Eucalyptus globulus*), ghaneri (*Lantana camara*), and nirgudi (*Vitex negundo*) demonstrated moderate but significant suppression of fungal growth (Plate 5). These findings are consistent with previous research, notably by Gurjar *et al*. (2021) [6] and Khaire *et al.* (2018) [7], who also reported maximum inhibition by garlic bulb extract.

Conclusion

This study clearly showed that tebuconazole 25.9% EC was the most effective fungicide against the fungi causing soybean pod blight, with carbendazim, propiconazole, and hexaconazole also providing strong disease control. Among the biological agents tested, *Trichoderma harzianum* stood out as the most powerful antagonist, performing better than other promising bioagents like *T. asperellum* and *Metarhizium anisopliae*. Furthermore, among the natural plant extracts evaluated, garlic extract demonstrated the greatest antifungal activity, followed closely by ginger, mehandi, and onion extracts. These findings suggest that combining these chemical, biological, and botanical options

could form a practical and sustainable approach to managing soybean pod blight effectively.

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