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Invitro Evaluation of Bio-Agents for Controlling *Cercospora cruenta* Leaf Spot in Horse Gram

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A B S T R A C T

Macrotyloma uniflorum, commonly referred to as horse gram, is a resilient legume belonging to the Fabaceae family and is extensively cultivated in India, Africa, and Southeast Asia. The fungal pathogen *Cercospora cruenta* is responsible for leaf spot disease, which can lead to significant yield losses in horse gram by causing leaf spot formation. This study seeks to evaluate the *in vitro* antagonistic potential of six known biological agents against *C. cruenta* in order to identify promising candidates for integrated disease management programs. Three different techniques were employed for the evaluation: dual culture, pathogen-at-periphery, and pathogen-at-centre methods. The antagonists examined include *Trichoderma* spp., *Pseudomonas fluorescens*, and *Chaetomium globosum*. The results clearly indicate the efficacy of various fungal bioagents against the pathogen, with *Trichoderma harzianum* showing the highest inhibition rate. Additionally, *Pseudomonas fluorescens* demonstrated significant antifungal properties. These findings highlight the potential of *Trichoderma* as a highly promising candidate for biological control measures against this disease. Further studies are needed to evaluate and optimise application strategies for sustainable disease management.

Introduction

Macrotyloma uniflorum, commonly known as horse gram, is a hardy legume from the family Fabaceae, widely cultivated in India, Africa, and Southeast Asia. It includes several varieties such as *var. uniflorum*, *var. stenocarpum*, *var. verrucosum*, and *var. benadirianum*, differing mainly in pod and leaf characteristics. The plant is an annual twining herb (30–90 cm tall) with trifoliate leaves and small pale-yellow flowers producing linear pods with 5–10 seeds. Highly drought-tolerant and adapted to poor soils (pH 5–8), horse gram grows in regions receiving 300–1000 mm of rainfall, making it ideal for dryland farming. It is valued as a pulse, fodder, and green manure crop, rich in protein (22–25%), minerals, and fibre.

In India and other tropical countries, it is consumed as food and used in traditional medicine for treating kidney stones, obesity, and diabetes. With its climate resilience, soil-enriching ability, and nutritional importance, *M. uniflorum* plays a key role in sustainable agriculture and food security (Bhardwaj *et al.,* 2013; Kaundal *et al.,* 2019; Ojha *et al.,* 2020; Oli *et al.,* 2024).

Cercospora cruenta is a prominent fungal pathogen responsible for significant yield losses in horse gram through the induction of leaf spot disease. This pathogen can lead to substantial reductions in crop productivity, impacting both the quality and quantity of the harvest (Navinkumar *et al.,* 2020). Current disease management strategies often rely on chemical fungicides, which pose environmental risks and can lead to the development of resistant pathogen strains. Consequently, there is a growing imperative to explore eco-friendly and sustainable alternatives, such as biological control agents, for effective disease management (Kadam *et al.,* 2018). This study aims to evaluate the in vitro antagonistic potential of various bio-agents against *C. cruenta* to identify promising candidates for integrated disease management programs (Aravindasamy *et al.,* 2018; Hameed *et al.,* 2022).

Materials and Methods

In this study, six antagonistic organisms were evaluated in vitro for their effectiveness against the pathogen *Cercospora cruenta*, which was isolated from infected horsegram plants. The antagonists examined include *Trichoderma viride, T. hamatum, T. harzianum, T. virens, Pseudomonas fluorescens*, and *Chaetomium globosum* (Urdukhe and Mogle 2025). Culture discs (5 mm diameter) were aseptically excised from actively growing colonies of pure cultures maintained on potato dextrose agar medium and positioned according to the specific techniques described below. In the dual culture technique, one disc of the test antagonist and pathogen was placed opposite one another, 4 cm apart, on Petri plates containing 20 mL of PDA medium. This arrangement allowed for the direct observation of antagonistic interactions (Sinha, 2017). In the pathogen-at-periphery technique, a single disc of the test antagonist was positioned at the centre of the plate, with four pathogen discs placed radially 4 cm away at equidistant peripheral positions on 20 mL PDA medium (Rajput *et al.,* 2013). In the pathogen-at-centre technique, a single pathogen disc was placed at the centre, surrounded by four discs of the test antagonist positioned 4 cm away at the periphery on 20 mL PDA medium (Gajre and Chauhan, 2020).

Control treatments consisted of pathogen discs placed in the corresponding positions for each technique in the absence of antagonists. All treatments were incubated at room temperature (27 ± 2°C), and radial growth of both the antagonists and the pathogen was measured after 6 days. The experiments followed a completely randomised design with three replications per treatment. Percent growth inhibition was calculated using the formula of Vincent. This methodology allowed for a quantitative assessment of the bio-agents' efficacy in suppressing *C. cruenta* mycelial growth, thereby providing insights into their potential as biocontrol agents (Begum & Nath, 2015).

Results and Discussion

The antagonistic potential of six known bio-agents was assessed in vitro against *Cercospora cruenta*, the pathogen responsible for leaf spot in horse gram. This evaluation employed three different techniques: dual culture, pathogen-at-periphery, and pathogen-at-centre methods.

The results obtained from these three in vitro methods are detailed in Tables 1–3. The results of the study clearly demonstrate the efficacy of various fungal bioagents against Cercospora cruenta under controlled *in vitro* conditions, with *Trichoderma harzianum* emerging as the most effective antagonist, achieving an impressive inhibition rate ranging from 80% to 82%. This significant inhibitory action suggests its potential for application in biological control strategies. In comparison, *Trichoderma viride* also displayed substantial antifungal properties, with inhibition rates between 72% and 77%. *T. hamatum* followed closely, exhibiting a moderate level of inhibition (67% to 70%). The bacterium *Pseudomonas fluorescens* showed moderate effectiveness, inhibiting fungal growth within a range of 53% to 63%. In contrast, *T. virens* and *Chaetomium globosum* demonstrated comparatively lower inhibition, underscoring the varying degrees of efficacy among these microbial agents. Collectively, these findings underscore the potential of *T. harzianum* as a promising candidate for biological control measures against *Cercospora cruenta*, warranting further exploration into its application in sustainable agriculture practices.

Similar findings from *in vitro* assays, significant variation was observed in the inhibitory effects of the tested bioagents against *C. cruenta*, reflecting their diverse mechanisms of action and varying degrees of antagonism. Notably, the dual culture technique revealed distinct zones of inhibition, with certain *Trichoderma* species demonstrating superior suppression of mycelial growth compared to bacterial antagonists (Saideekshith *et al.,* 2020).

Table.1 Antagonistic effect of different microorganisms against C. cruenta (Dual culture method)

Sr. No.	Test organism	Av. diameter of pathogen (mm)	Growth inhibition (%)
1	*Trichoderma harzianum*	4.25 (17.50)	**80.10**
2	*Trichoderma viride*	4.96 (24.00)	**72.95**
3	*Trichoderma hamatum*	5.38 (28.50)	**68.25**
4	*Trichoderma virens*	7.08 (50.00)	**43.20**
5	*Pseudomonas fluorescens*	5.72 (32.00)	**63.55**
6	*Chaetomium globosum*	7.76 (60.00)	**31.50**
7	Control	9.38 (88.00)	—
	S.Em. ±	0.054	
	C.D. at 5%	0.15	
	C.V. %	1.58	

Table.2 Antagonistic effect of different microorganisms against *C. cruenta* (Pathogen at periphery)

Sr. No.	Test organism	Av. diameter of pathogen (mm)	Growth inhibition (%)
1	*Trichoderma harzianum*	4.20 (17.00)	**80.45**
2	*Trichoderma viride*	5.01 (24.50)	**72.30**
3	*Trichoderma hamatum*	5.42 (28.80)	**67.65**
4	*Trichoderma virens*	7.26 (52.00)	**41.10**
5	*Pseudomonas fluorescens*	5.89 (33.00)	**62.65**
6	*Chaetomium globosum*	8.03 (64.00)	**27.90**
7	Control	9.40 (88.30)	—
	S.Em. ±	0.060	
	C.D. at 5%	0.17	
	C.V. %	1.63	

Table.3 Antagonistic effect of different microorganisms against *C. cruenta* (Pathogen at centre)

Sr. No.	Test organism	Av. diameter of pathogen (mm)	Growth inhibition (%)
1	*Trichoderma harzianum*	4.00 (15.80)	**82.40**
2	*Trichoderma viride*	4.55 (20.50)	**76.80**
3	*Trichoderma hamatum*	5.18 (26.20)	**70.10**
4	*Trichoderma virens*	7.21 (51.00)	**42.10**
5	*Pseudomonas fluorescens*	6.54 (40.00)	**53.50**
6	*Chaetomium globosum*	7.40 (62.00)	**36.30**
7	Control	9.42 (88.00)	—
	S.Em. ±	0.046	
	C.D. at 5%	0.14	
	C.V. %	1.27	

Trichoderma harzianum consistently formed clear inhibition zones, indicating that direct mycoparasitism or antibiosis may serve as a primary mode of action (Muthukumar *et al.,* 2023). *Pseudomonas fluorescens* exhibited a less pronounced yet still significant inhibitory effect, likely through the production of secondary metabolites or competition for nutrients (ElWakeil *et al.,* 2023; Teixeira *et al.,* 2014). These findings align with previous research that emphasises the multifaceted antagonistic capabilities of *Trichoderma* species against fungal pathogens, along with the indirect suppressive effects of *Pseudomonas fluorescens* (Avozani *et al.,* 2023; Mane, 2017).

Further analysis using the pathogen-at-periphery and pathogen-at-centre techniques clarified the spatial dynamics of these interactions, offering insights into whether the bioagents primarily act through pre-emptive colonisation or confrontation (Khursheed *et al.,* 2021; Spada *et al.,* 2020). Quantitative assessments of percent growth inhibition further supported these observations, providing a comparative measure of efficacy across all tested bioagents and experimental setups (Biswas, 2017). The most effective bioagents, particularly *Trichoderma harzianum,* exhibited a high percentage of mycelial growth inhibition, highlighting their potential for practical application in integrated disease management strategies (Badgujar *et al.,* 2021; Hussain *et al.,* 2024). Additional investigation into the specific mechanisms underpinning these inhibitory effects, such as enzyme production or antibiotic synthesis, would yield a more comprehensive understanding of their biocontrol potential (Biswas, 2017).

Based on these findings, *Trichoderma harzianum* emerges as a highly promising biocontrol agent for managing *C. cruenta* in horse gram, demonstrating superior inhibitory effects in vitro. This efficacy can be attributed to its capacity for mycoparasitism, antibiosis, and competition for nutrients, leading to substantial mycelial growth inhibition. Further studies are warranted to evaluate the in vivo performance of *T. harzianum* under field conditions and to optimise its application strategies for sustainable disease management.

Author Contributions

Sadashiv N. Shinde: Investigation, formal analysis, writing—original draft. Venkat S. Maske: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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