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HARNESSING OF BOTANICAL, BIOLOGICAL AND CHEMICAL APPROACHES FOR *IN-VITRO* MANAGEMENT OF CHILLI ANTHRACNOSE CAUSED BY *Colletotrichum capsici*

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ABSTRACT

One of the most economically significant vegetable crops in the world is the chilli (*Capsicum annum* L.). The most common uses of chilli are as a spice, flavoring, or coloring in cooking. However, there are many uses for chillies, including in paint production, cosmetics, medications, farm protection, and self-defense weapons (pepper spray). Chilli is susceptible to a wide range of illnesses caused by fungi, bacteria, viruses, nematodes, and abiotic stressors. One of the main biotic restrictions impacting the productivity and yield of the chilli crop is anthracnose disease. *In vitro* testing of botanicals against *Colletotrichum capsici* revealed that the highest percentage of radial growth inhibition was exhibited by onion extract at 40.22% (10%) concentration, which was followed by Madar, Lantana, and Crow foot grass. Among bioagents and fungicides, *Pseudomonas fluorescens* had the minimum radial growth (17.49 mm) and highest per cent inhibition (68.72%), followed by *Trichoderma asperellum* (24.96 mm and 64.52%). Compared to other treatments, hexaconazole produced 100% suppression and zero radial growth.

Key words: Chilli, *Colletotrichum capsici*, Bio-agents, Botanicals, Fungicides, *In vitro* evaluation. PDA (Potato Dextrose Agar medium).

Introduction

Chilli (*Capsicum annum*) belongs to the family Solanaceae. The global cultivation area for chilli is 17,76,334 ha, with a total production of 41,64,594 tonnes (FAOSTAT, 2020). Although it is grown year-round, it is particularly popular in the Indian subcontinent during the Kharif and Rabi seasons. It flourishes at temperatures ranging from 20 to 30 degrees Celsius; however, temperatures above 30 degrees Celsius or consistently below 15 degrees Celsius impede chilli growth and harvest. It thrives in warm, humid environments, including tropical and subtropical zones. It is regarded as the 4th important vegetable cum spice crop in tropical and subtropical areas.

Chilli is affected by many diseases, one of which is anthracnose (dieback/fruit rot) disease caused by *Colletotrichum capsici* (Nishanthi *et al.*, 2020). This fungus excessively damages both mature and immature fruits, reducing the market value and nutritive value (Saxena *et al.*, 2016).

Anthraco, a Greek word meaning “coal,” refers to plant diseases with dark, sunken lesions that carry spores. Halsted (1890) first reported chilli anthracnose in New Jersey, USA, and identified the causative agents as *Gloeosporium piperatum* and *Colletotrichum nigrum*. Anthracnose disease can affect leaves, stems, and both pre- and post-harvest fruits. (Isaac, 1992).

Anthrachnose of chilli (*Capsicum annuum* L.) caused by *Colletotrichum capsici* (Syd.) (Butler and Bisby) is one of the primary and damaging diseases of chilli, causing substantial losses (10-60%) in both yield and quality of the chilli depending on the types (Bansal and Grover, 1969). On the leaves, tiny dots develop differently. Severely sickly leaves fall off, resulting in defoliation. Infection from growing tips causes necrosis of branches, which spreads backwards on sick branches (Dieback Stage). The dieback may kill the whole plant (Mehrotra, 1995).

Materials and methods

Isolation, purification, and identification of the pathogen

Chilli fruits with anthracnose symptoms were collected from the fields of the Students Instructional Farm of ANDUAT, Kumarganj, Ayodhya, U.P. to evaluate the Botanicals, Bio-agents and Fungicide against the pathogens. For isolation and purification of pathogens, Potato Dextrose Agar medium was prepared following the method outlined (Johnston Booth 1983). The medium was prepared by using 200 g of peeled potato, 20 g of dextrose, 20 g of agar and 1000 ml of distilled water. Isolation was done by cutting small pieces from the margin of lesions, which were then surface sterilised by immersing in 1 per cent Sodium Hypochlorite solution for 30 seconds and washed with sterilised distilled water. To remove excess moisture from the samples, the pieces were transferred onto sterilised blotter paper. These pieces were then transferred to Petri plates containing PDA medium under aseptic conditions, followed by incubation at $26 \pm 2^\circ\text{C}$. Similarly, Lokhande *et al.*, (2019) isolated *Colletotrichum capsici* from infected chilli leaves. (According to Rajamanickam *et al.*,).

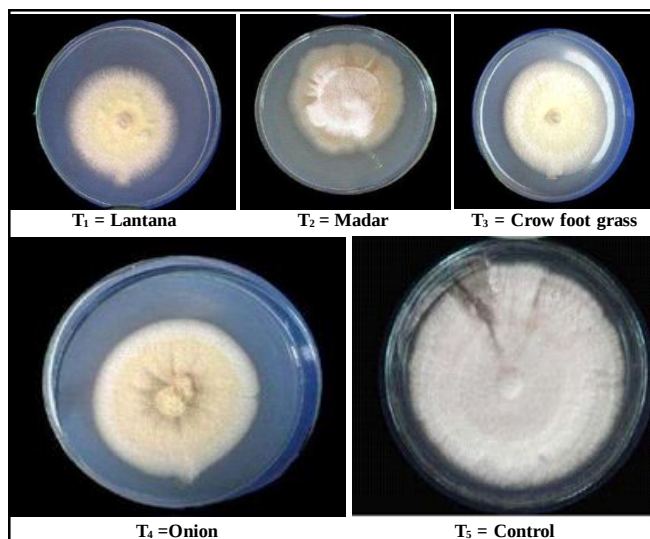


Plate 1: Effect of Botanical extracts on radial growth (mm) and per cent inhibition of mycelium growth.

Identification of the pathogen was confirmed based on symptomatology (visual and microscopic), cultural and morphological characteristics, and its Pathogenicity test. Similarly, Gautam *et al.*, (2014) reviewed various plant diseases in India caused by *Colletotrichum* species. Bagri *et al.*, (2004) isolated *C. capsici* from diseased chillies and proved its pathogenicity. Similarly, Parey *et al.*, (2013) and Saikia *et al.*, (2004) isolated *C. capsici* from anthracnose-affected fruit of chilli and proved its pathogenicity by the spore suspension spray method.

The fungus was further purified by the single hyphal tip method. They are grown by inoculating in the centre of a plain agar plate. The fungus spreads out with its hyphal strands in search of nutrients. These hyphal strands could be located under low power of the microscope, and the isolated hyphal tips marked. These tips were carefully transferred to potato dextrose agar slants to obtain the pure cultures of *Colletotrichum capsici*. The culture was maintained by sub-culturing on potato dextrose agar medium at room temperature (Butler and Bisby, 1931).

The infected portions (leaves and fruits) showing the typical symptoms of Anthracnose of chilli were collected from the diseased fields and brought to the laboratory, and then the isolation of the fungus was done on PDA.

Preparation of aqueous extract, and Bio-agents

Plant species that were locally available and have antifungal and therapeutic properties against fungal pathogens, such as lantana, madar, crow foot grass and onion, were collected then botanical extracts were prepared in the Department of Plant Pathology, ANDUA&T (Ayodhya).

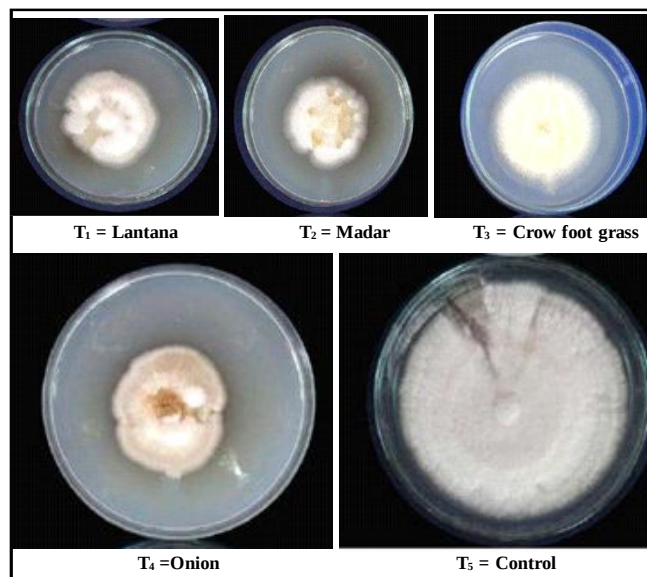


Plate 2: Effect of Botanical extracts on % inhibition of mycelium growth.

Four botanicals, i.e. *Lantana camara*, *Calotropis procera*, *Dactyloctenium aegyptium*, and *Alium cepa*, were used against the test pathogen for evaluating their efficacy. Leaves and bulbs of these four botanicals were collected from the local area, and two concentrations, 5 and 10 per cent, were used for their evaluation against *C. capsici* *in vitro*. Healthy and fresh leaves and bulbs of selected botanicals were taken and washed with tap water, followed by sterile distilled water and then dried at room temperature. After removal of water, these are chopped into small bits with a sterilised, sharp knife. Then each leaf sample was separately grinded and homogenised in a mechanical grinder with an equal quantity of sterile distilled water (1:1 w/v). The obtained homogenate was strained through double-layered sterilised muslin cloth, followed by filtration through Whatman filter paper No. 1 using volumetric flasks (50 ml capacity). The obtained clear leaf and bulb extracts serve as the standard/stock solution of 100 per cent, and subsequently, 5 and 10 per cent were formed (Senthil *et al.*, 2003).

Trichoderma asperellum and *Pseudomonas fluorescence* were obtained from the Department of Plant Pathology, A.N.D.U.A. & T., Kumarganj, Ayodhya. These biocontrol agents were screened in for their efficacy in suppressing the growth of *Colletotrichum capsici* by the dual culture technique, and the Fungicide purchase from the local market.

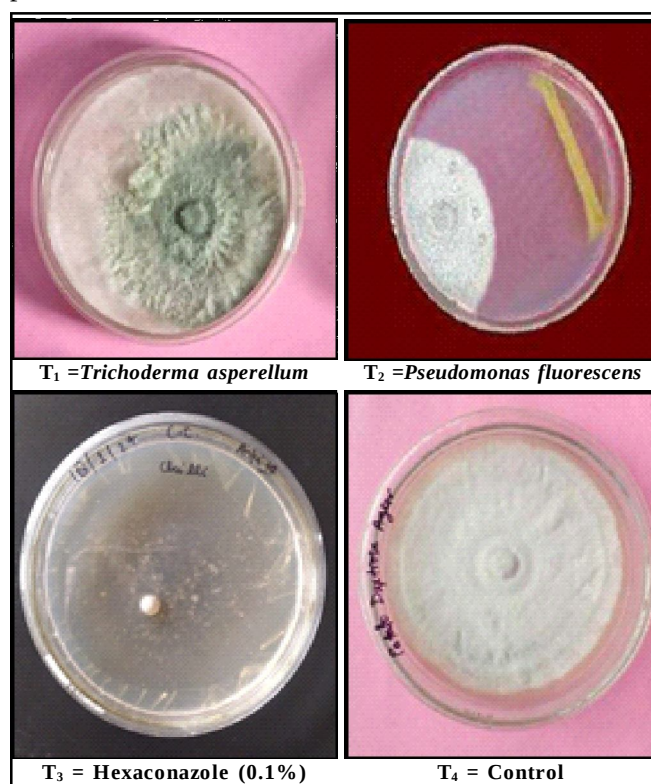


Plate 2: Effect of Botanical extracts on % inhibition of

Table 1: Effect of Botanical extracts on radial growth (mm) and per cent inhibition of mycelium growth at 5% concentration.

T	Botanicals	RG	MG
T ₁	Lantana (<i>Lantana camara</i>)	36.75	18.44
T ₂	Madar (<i>Calotropis procera</i>)	34.50	22.90
T ₃	Crow foot grass (<i>Dactyloctenium aegyptium</i>)	37.12	17.03
T ₄	Onion (<i>Alium cepa</i>)	32.25	27.04
T ₅	Control	44.75	-
	C.D.	1.43	1.009
	SE(m)	0.47	0.33
T: Treatments; RG: Radial growth (mm); MG: % Inhibition of Mycelium growth over control			

In vitro evaluation of botanicals, bio-agents and fungicides against *C. capsici*

Fungicides, botanicals, and bioagents were evaluated in relation to *Colletotrichum capsici*. Using two bioagents—*Trichoderma asperellum* and *Pseudomonas fluorescens*—as well as one fungicide, the efficacy of plant extracts of *Lantana camara*, *Calotropis procera*, *Dactyloctenium aegyptium*, and *Alium cepa* were assessed *in vitro* at 5 and 10% concentration. For example, 0.1% hexaconazole was employed in this investigation. The regular observations were noted for a full day. Using Vincent's formula (1927), the radial growth of the colony and the % inhibition of each treatment were determined.

The mycelial growth and its inhibition per cent was also calculated as per the formula which is given below.

$$PI = \frac{C-T}{C} \times 100$$

or

$$\% \text{ Growth inhibition} = \frac{\text{Radial growth in control} - \text{Radial growth in treatment}}{\text{Radial growth in control}} \times 100$$

Where,

PI=Percent inhibition

C =Radial growth of the pathogen in the control plate

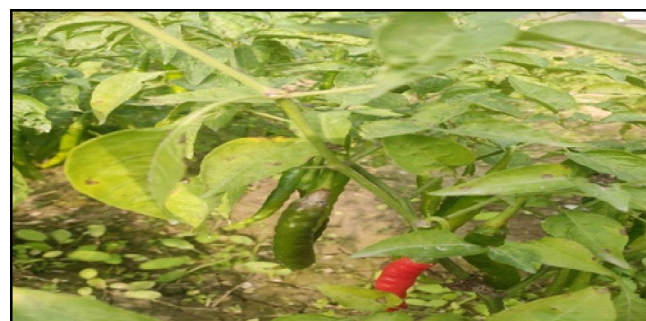


Plate 4: Infected plant.

Table 2: Effect of Botanical extracts on Radial growth (mm) and %inhibition of mycelium growth at 10 % concentration.

T	Botanicals	RG	MG
T ₁	Lantana (<i>Lantana camara</i>)	32.50	27.45
T ₂	Madar (<i>Calotropis procera</i>)	27.50	38.55
T ₃	Crow foot grass (<i>Dactyloctenium aegyptium</i>)	34.00	24.03
T ₄	Onion (<i>Alium cepa</i>)	26.75	40.22
T ₅	Control	44.75	-
	C.D.	2.55	5.48
	SE(m)	0.84	1.80

T: Treatments; RG: Radial growth (mm);
MG: % Inhibition of Mycelium growth over control

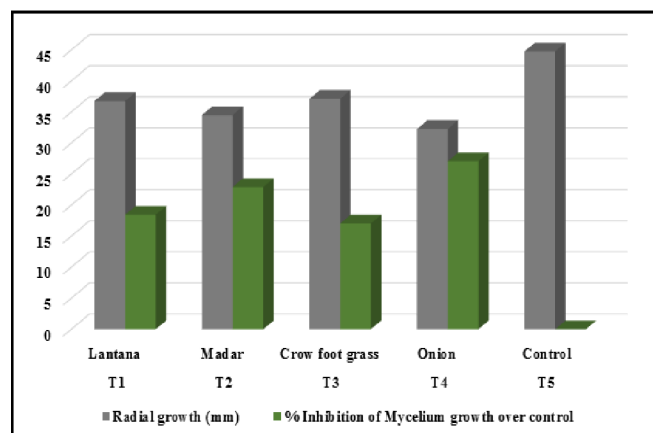
(mm)

T = Radial growth of the pathogen in the treated plate (mm)

Result and Discussion

In vitro Evaluation of Botanical extracts against *Colletotrichum capsici* at 5% and 10% concentration (9 days after inoculation)

The study was performed (Table 1 and Fig. 1) under laboratory conditions to know the efficacy of Four Botanical extracts at 5% conc. It was observed that Crow foot grass 37.12 mm, Lantana 36.75 mm, Madar 34.50 mm, and Onion 32.25 mm were significantly parasitic on the mycelial growth of the *Colletotrichum capsici*. Botanical extracts inhibited the growth of *Colletotrichum capsici* up to 18.44%, 22.90%, 17.03% and 27.94%, respectively, which was statistically significant over control. Maximum per cent inhibition of mycelium growth over control of onion, which was 27.94% inhibition of mycelium growth and minimum per cent inhibition of mycelium growth over control of Crow foot grass, which was 17.03% inhibition of mycelium growth at 5%

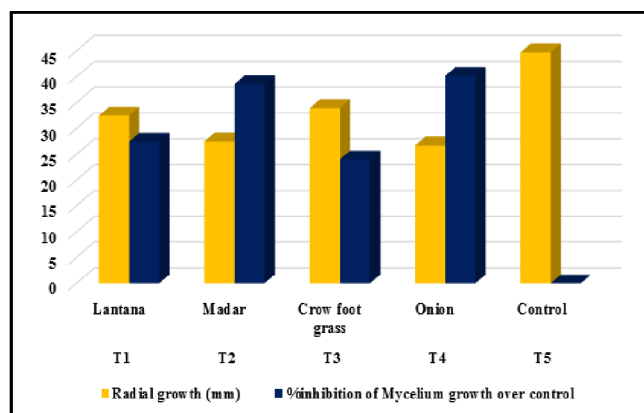
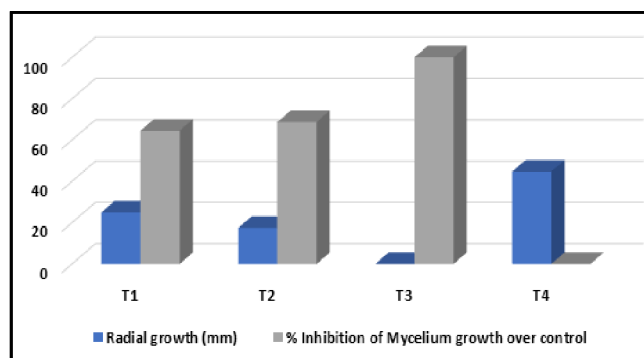
**Fig. 1:** Effect of Botanical extracts on Radial growth (mm) and per cent inhibition of mycelial growth.**Table 3:** Effect of Bioagents and Fungicide on Radial growth (mm) and %inhibition of mycelium growth over control.

T	Botanicals	RG	MG
T ₁	<i>Trichoderma asperellum</i>	24.96	64.52
T ₂	<i>Pseudomonas fluorescens</i>	17.49	68.72
T ₃	Hexaconazole (0.1%) 5% EC	00	100
T ₄	Control	44.75	-
	C.D.	2.64	2.473
	SE(m)	0.85	0.79

T: Treatments; RG: Radial growth (mm);
MG: % Inhibition of Mycelium growth over control

concentration (9 days after inoculation). Similarly, Lokhande *et al.*, (2019).

The study was observed that Crow foot grass 34.00 mm, Lantana 32.50 mm, Madar 27.50 mm and Onion 26.75 mm were significantly parasitic on the mycelial growth of the *Colletotrichum capsici*. Botanical extracts inhibited the growth of *Colletotrichum capsici* up to 27.45%, 38.55%, 24.03% and 40.22%, respectively, which was statistically significant over control. Maximum per cent inhibition of mycelium growth over control of onion, which was 40.22% inhibition of mycelium growth, and minimum per cent inhibition of mycelium growth over

**Fig. 2:** Effect of Botanical extracts on Radial growth (mm) and per cent inhibition of mycelium growth.**Fig. 3:** Effect of Bioagents and Fungicide on Radial growth (mm) and per cent inhibition of mycelium growth.

control of Crow foot grass, which was 24.03% inhibition of mycelium growth at 10% concentration (9 days after inoculation). Similarly, Chauhan and Yadav (2022).

***In vitro* Evaluation of Bioagents and Fungicide against *Colletotrichum capsici* at 9 days after inoculation**

In the study was observed that *Trichoderma asperellum* 24.96 mm, *Pseudomonas fluorescens* 17.49 mm and Hexaconazole (0.1%), 0.0 mm were significantly parasitic on the mycelial growth of the *Colletotrichum capsici*. Bioagents and Fungicide inhibited the growth of *Colletotrichum capsici* up to (64.52%, 68.72% and 100%, respectively), which was statistically significant over control. Maximum per cent inhibition of mycelium growth over control of Hexaconazole (0.1%), which was 100% inhibition of mycelium growth and minimum per cent inhibition of mycelium growth over control of (*Trichoderma asperellum*) which was 64.52% inhibition of mycelium growth. (9 days after inoculation). Barhate *et al.*, (2012) evaluated six bioagents against *C. capsici*.

Efficacy of Botanicals against *Colletotrichum capsici* in-vitro.

After 9 days of inoculation, the minimum radial growth and maximum per cent inhibition were observed in Onion (32.25 mm, 27.04% at 5% concentration and 26.75 mm, 40.22% at 10% concentration). This was followed by Madar (34.50 mm, 22.90% and 27.50 mm, 38.55%), Lantana (36.75 mm, 18.44% and 32.50 mm, 27.45%), and Crow foot grass (37.12 mm, 17.03% and 34.00 mm, 24.03%) at 5% and 10% concentrations, respectively.

Efficacy of Bioagent and fungicide against *Colletotrichum capsici* in-vitro.

After 9 days of inoculation using the dual culture technique, the minimum radial growth and maximum percent inhibition were observed in *Pseudomonas fluorescens* (17.49 mm, 68.72%), followed by *Trichoderma asperellum* (24.96 mm, 64.52%).

After 9 days of inoculation, Hexaconazole resulted in zero radial growth and 100% inhibition, outperforming all other treatments.

Conclusion

In this case study effectively demonstrated the roles of botanical extracts, bio-agents, and fungicide in managing anthracnose disease in chilli crops under lab conditions. Based on the results, it is recommended that farmers use *T. asperellum* and onion extract at 5% and 10% concentrations to achieve maximum yield while avoiding the environmental and health risks associated

with chemical applications. These treatments provided superior control of anthracnose caused by *Colletotrichum capsici* compared to other methods. Instead of Hexaconazole, onion extract and *T. asperellum* can be recommended as effective and safer alternatives for managing anthracnose in chilli crops.

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