ISSN Print: 2664-844X
ISSN Online: 2664-8458
NAAS Rating (2025): 4.97
IJAFS 2025; 7(12): 60-64
www.agriculturaljournals.com
Received: 02-09-2025
Accepted: 06-10-2025

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Evaluation of different fungicides against *Colletotrichum capsici*

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DOI: https://www.doi.org/10.33545/2664844X.2025.v7.i12a.1026

Abstract
Colletotrichum capsici is an important fungal pathogen that causes anthracnose in chilli (*Capsicum annuum*), leading to considerable yield losses. This study examined the in-vitro effectiveness of nine fungicides against *C. capsici* using the poisoned food technique. Four fungicides-Propiconazole (0.1%), Carbendazim + Mancozeb (0.2%), Captan + Hexaconazole (0.1%), and Azoxystrobin + Difenoconazole (0.05%)-completely inhibited the fungus's mycelial growth. These findings indicate that selective systemic and combination fungicides are highly effective for managing *C. capsici* and may be successfully integrated into disease management programs.

Keywords: *Colletotrichum capsici*, anthracnose, chilli, fungicides, poisoned food technique, *in-vitro* evaluation

Introduction
Chilli (*Capsicum annuum*), commonly known as Mirchi, is an important solanaceous spice crop cultivated widely as both a commercial and vegetable crop. It is considered an exotic species, originating from Brazil in South America, and was introduced to India by the Portuguese in the 17th century. Today, it is grown across the entire country, making India the largest producer, consumer, and exporter of chillies. Besides India, major chilli-growing countries include Pakistan, Bangladesh, China, Sri Lanka, Egypt, Italy, and the U.S.A. Globally, India leads chilli production, followed by China, Thailand, Ethiopia, and Indonesia. In India, the main chilli-producing states are Andhra Pradesh, Telangana, Madhya Pradesh, Karnataka, and West Bengal (Source: ANGRAU Chilli Outlook Report, January-December 2021). Indian chillies are world renowned for their distinctive colour and pungency.

Chilli crops are vulnerable to many diseases caused by fungi, bacteria, viruses, nematodes, and physiological disorders, which can severely affect both yield and quality. Among fungal diseases, anthracnose or die-back is one of the most destructive, caused primarily by three species of *Colletotrichum*: *C. capsici*, *C. acutatum*, and *C. gloeosporioides*. *Colletotrichum capsici* (Syd.) Butler & Bisby is particularly damaging, with severe losses reported especially in ripe fruits. It is considered one of the most important plant pathogens globally, widely distributed across chilli-producing regions, and responsible for the economically significant disease anthracnose-which may cause up to 50% yield loss. Apart from chilli, *C. capsici* infects cereals, legumes, vegetables, plantation crops, and various tree fruits as well.

Anthracnose caused by *C. capsici* was first reported in India by Sydow in 1913. The disease develops rapidly under warm, humid conditions and can affect any aerial part of the plant at any stage. It causes necrosis of young twigs, progressing from the tip downward, often resulting in the dieback of branches or even the entire plant. Numerous black fungal fruiting bodies (acervuli) appear on the necrotic tissues. Infected plants produce fewer fruits, and the fruits are often of poor quality. Symptoms on fruits begin as small black circular spots, which enlarge along the fruit length to form sunken, dark-margined lesions. Severely infected fruits turn straw coloured, develop pinkish spore masses, and may drop prematurely, leading to substantial yield losses. Seeds may also become infected.

Farmers frequently rely on chemical fungicides for managing chilli anthracnose. Commonly used fungicides include Carbendazim and copper-based compounds such as dithiocarbamates, benzimidazoles, and triazoles.

However, excessive chemical use poses environmental risks, highlighting the need to identify fungicides that are both effective and safer.

Given the severity of anthracnose caused by *C. capsici*-which can reduce yields by more than 50%-this study was conducted to evaluate the in-vitro efficacy of various fungicides. The goal is to determine the most effective treatments for managing this pathogen and to support integrated disease management strategies in chilli cultivation.

Materials and Methods

Glassware plastic wares and requirments

Glass Petri dishes (90 mm), test tube (15×150 mm), conical flasks (250 to 500 ml), borosil measuring cylinder (50ml) and blotter paper were used. The laboratory equipment used included a hot air oven and an autoclave for sterilizing glassware and media respectively, a stereo-binocular microscope and a research binocular microscope for observing the seed borne fungi, a laminar air flow cabinet for isolation, a BOD incubator for incubation, a seed germinator and a weighing balance.

Colletotrichum capsici was isolated from infected chilli fruits and cultured on Potato Dextrose Agar (PDA). A 5 mm disc from a 7-day-old culture was used as inoculum. Nine fungicides were tested using the poisoned food technique, with treatments arranged in a Completely Randomized Design (CRD) with three replications. Mycelial growth was measured at 3rd, 5th, and 7th day after inoculation (DAI). Inhibition percentage was calculated using the standard formula.

Preparation of culture media

The common laboratory medium i.e., Potato dextrose agar (PDA) was used for isolation of fungal pathogens associated with chilli, for sub culturing, maintenance of pure cultures, dual culture technique and poison food technique.

The medium was prepared with following ingredients.

1. **Potato (peeled):** 200.00 g
2. **Dextrose:** 20.00 g
3. **Agar:** 20.00 g
4. **Distilled water:** 1000 ml

Potato dextrose agar (PDA) medium was made by slicing 200 g of peeled potatoes into pieces and boiling them in 800 ml distilled water until they were thoroughly cooked. The starch extract was strained through muslin cloth. Dextrose and agar (20 g each) were added into it and the volume was adjusted to one litre. Autoclave the media for 15 min at 121°C and 15 lbs pressure. Final pH of the medium should be 5-6 ± 0.5.

Preparation of Slants

For preparing the slants, PDA medium with streptomycin sulphate waspoured into the culture tubes to avoid the bacterial infection. Approximately, ¼ part of the tube was filled in and plugged with non-absorbent cotton. After sterilization that tubes were tilted to 45 0 -60 0 angle till the medium solidified properly.

Sterilization of laboratory materials

To detect fungus on seeds, plastic plates with a diameter of 90 mm were used. The plates were washed with cleaning powder under running water, dried and then disinfected with denatured spirit. However, glasswares were sterilized in hot air oven at 180°C for 1 hour before use. Distilled water, blotter paper discs 9 cm in diameter and plastic plates wrapped with blotter paper were sterilized in an autoclave at 1.04 kg/cm 2 for 15 minutes.

Incubation room

The incubation room was used to keep the petriplates and the temperature in the incubation room was 27 ± 2°C, which was automatically controlled with an alternate cycle of 12 hours of light and 12 hours of darkness.

Isolation and Detection of pathogen through following method

Standard blotter paper method

Three layers of sterilized blotter paper disc of 90 mm diameter were placed in sterilized Petri plates (90 mm diameter) moistened with sterile distilled water. The excess water was drained out from the plates. Chilli seeds were transferred to the plates containing three layers of moist blotter papers. 25 seeds were placed in each plate (16 in outer layer, 8 in middle and 1 in centre) and the plates were incubated at 27 ± 20C under alternate cycles of 12 hour near light and darkness for 7 days. After seventh day of incubation, the seeds were examined under stereoscopic binocular microscope for associated fungi and they were identified based on habit and colony characters.

Agar plate method

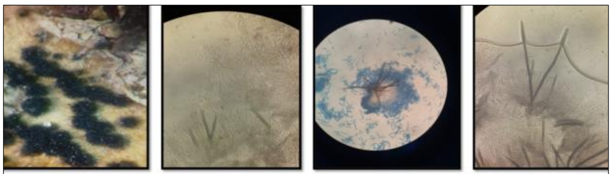
In Northern Ireland, agar plate method was first used for seed health testing. In this method, presterilized Petriplates were poured with 15 ml of autoclaved potato dextrose agar. On cooling the medium, 25 seeds were placed in each plate (16 in outer layer, 8 in middle and 1 in centre) and the the infected fruit tissue were placed in each and the plates were incubated at 27 ± 20C under diurnal conditions. Four hundred seeds of each variety were tested. On seventh day of incubation, plates were examined under stereoscopic microscope for observing the fungal growth. The identification and further confirmation of seed borne fungi was made by preparing slides of the fungi.

Identification of fungi

Identification of the fungal species were done by observing the growth on media, colony characteristics and microscopic observations made from the slides prepared directly from the active cultures. The morphological features like colour, branching and septation of hyphae, conidiophores and conidia of the isolates were studied under compound microscope. The growth characters and morphology were compared with available descriptions from mycological books such as Morphology and taxonomy of fungi, Manual of soil fungi (Gilman, 1957) and Manual on identification of Plant Pathogen and Biocontrol fungi of Agriculture importance.

Purification and maintenances of culture

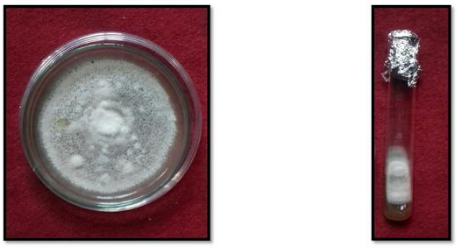
Following the identification of the fungal culture, it was further purified using the single spore isolation technique or purification of fungal isolates were done by transferring a small bit of hyphae to PDA slants in aseptic condition. The obtained pure culture was maintained on PDA slant and the culture was stored in a refrigerator at 5°C and renewed once a month for further research.



Acervuli and setae on chilli fruit observed under stereoscopic

Acervuli and setae at 40X

Conidiophore and Conidia with oil globule



(b) Petri plate of pure culture (c) slant of pure culture

Plates 1: (a) Disease sample of Anthracnose of chilli; (b) and (c) Pure culture of Colletotrichum capsici

Table 1: Fungicide Treatments and Their Concentrations

Treatment No.	Fungicide	Concentration (%)
T_1	Carbendazim 50% WP	0.1
T_2	Propiconazole 25% EC	0.1
T_3	Carbendazim + Mancozeb	0.2
T_4	Captan + Hexaconazole	0.1
T_5	Azoxystrobin + Difenoconazole	0.05
T_6	Tebuconazole + Trifloxystrobin	0.05
T_7	Penflufen + Trifloxystrobin	0.1
T_8	Azoxystrobin 23% SC	0.1
T_9	Flupicolide + Promocarb HCl	0.1
T_{10}	Control	-

Results and Discussion

Efficacy of nine different fungicides *viz*., Carbendazim (0.1%), Propiconazole (0.1%), Carbendazim + Mancozeb (0.2%), Captan + Hexaconazole (0.1%), Azoxystrobin + Difenoconazole (0.05%), Tebuconazole + Trifloxystrobin (0.05%), Penflufen + Trifloxystrobin (0.1%), Azoxystrobin (0.1%) and Flupicolide + Promocarb hydrochloride (0.1%) were tested against Colletotrichum capsici in-vitro. Results obtained were tabulated in table 3. The data clearly indicates that all the treatments were significantly superior over control in inhibiting the growth of Colletotrichum capsici. Among the nine fungicides, the most promising fungicide which restricted 100% growth of the test pathogen was Propiconazole (0.1%), Carbendazim + Mancozeb (0.2%), Captan + Hexaconazole (0.1%), Azoxystrobin + Difenoconazole (0.05%) followed by Tebuconazole + Trifloxystrobin (0.05%) and Cabendazim (0.1%) which recorded 83.61 and 81.46 percent inhibition respectively. The next best treatments were Penflufen + Trifloxystrobin (0.1%) recorded 65.95 percent inhibition of Colletotrichum capsici. Whereas the least control of the pathogen or minimum percent inhibition was observed in Azoxystrobin (0.1%) treatment followed by Flupicolide + Promocarb hydrochloride (0.1%) having 40.86 and 43.04 per cent inhibition respectively.

All fungicides significantly reduced the growth of *C. capsici* compared to control. The most effective fungicides with 100% inhibition were Propiconazole (0.1%), Carbendazim + Mancozeb (0.2%), Captan + Hexaconazole (0.1%), and Azoxystrobin + Difenoconazole (0.05%). Moderately effective fungicides included Tebuconazole + Trifloxystrobin (0.05%) and Carbendazim (0.1%). Less effective fungicides were Azoxystrobin (0.1%), Flupicolide + Promocarb hydrochloride (0.1%), and Penflufen + Trifloxystrobin (0.1%).

Table 2: *In-vitro* efficacy of fungicides against *Colletotrichum capsici* on 3rd DAI, 5th DAI and 7th DAI

Treatment		Conc (%)	***Colletotrichum capsici***					
			Mean Colony diameter (mm)			**Per cent growth inhibition**		
			3 DAI	**5 DAI**	**7 DAI**	**3 DAI**	**5 DAI**	**7 DAI**
T_1	Carbendazim 50% WP	0.1	0.00	0.00	15.33	100.00	100.00	80.00
T_2	Propiconazole 25% EC	0.1	0.00	0.00	0.00	100.00	100.00	100.00
T_3	Carbendazim 12% + Mancozeb 63% WP	0.2	0.00	0.00	0.00	100.00	100.00	100.00
T_4	Captan 70% + Hexaconazole 5% WS	0.1	0.00	0.00	0.00	100.00	100.00	100.00
T_5	Azoxystrobin 18.2% + Difenoconazole 11.4% SC	0.05	0.00	0.00	0.00	100.00	100.00	100.00
T_6	Tebuconazole 50% + Trifloxystrobin 25% WG	0.05	0.00	0.00	13.33	100.00	100.00	82.61
T_7	Penflufen13.28 + Trifloxystrobin 13.28 w/w FS	0.1	0.00	23.33	25.66	100.00	53.02	66.52
T_8	Azoxystrobin 23% SC	0.1	23.33	33.66	45.33	26.31	32.21	40.86
T_9	Flupicolide 5.56% w/w +promocarb hydrochloride55.5%w/w SC	0.1	24.33	30.66	43.66	23.15	38.26	43.04
T_{10}	Control	--	31.66	49.66	76.66			
F test			Sig	Sig	Sig			
S.E(±m)			0.18	0.20	0.25			
C.D (P=O.O1)			0.73	0.84	1.03			

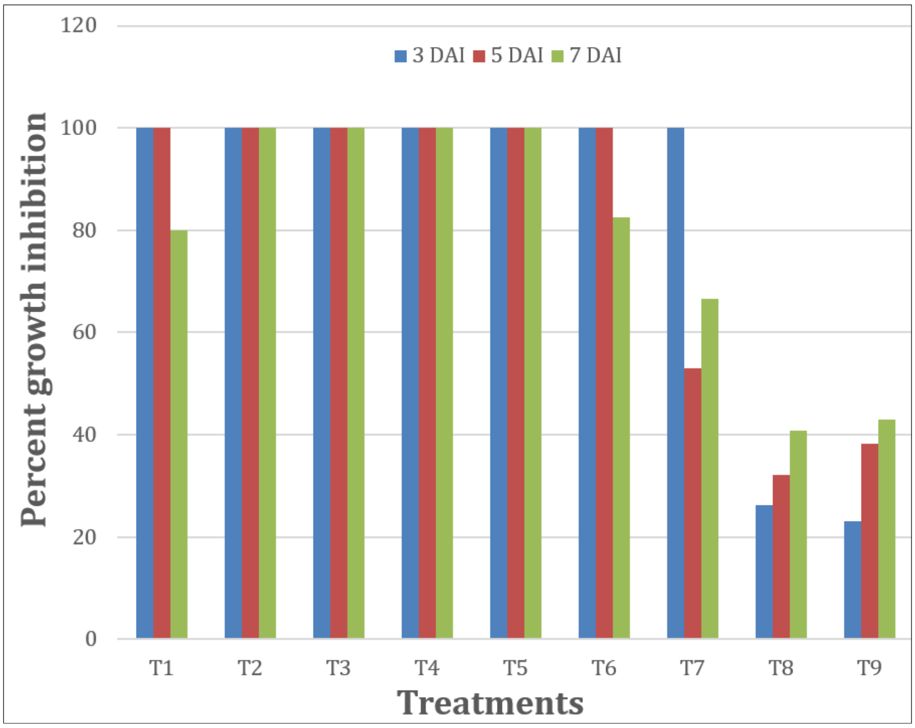


Fig 1: Effect of fungicides on per cent growth inhibition of *Colletotrichum capsici*

Conclusion

This study demonstrates that certain fungicides, especially Propiconazole and combination formulations like Carbendazim + Mancozeb and Captan + Hexaconazole, are highly effective in inhibiting *Colletotrichum capsici in vitro*. These findings support their recommendation in integrated disease management programs for anthracnose in chilli cultivation.

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