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# FUNGI ASSOCIATED WITH ANTHRACNOSE OF CHILLI FRUITS AND *IN VITRO* CONTROL OF *COLLETOTRICHUM CAPSICI* USING PLANT EXTRACTS

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**ABSTRACT**

The study was conducted at Sher-e-Bangla Agricultural University to identify the fungi responsible for anthracnose of Chilli and to evaluate the effectiveness of native plant extracts against *Colletotrichum capsici*. Six fungal genera—*Aspergillus flavus*, *Alternaria alternata*, *Colletotrichum capsici*, *Fusarium oxysporum*, *F. moniliforme and Rhizoctonia solani*— were isolated and identified from infected fruits using moistened blotter paper and PDA medium procedures, as recommended by the ISTA. In the *in vitro* study, five selected plant extracts (Neem seed extract, Neem leaf extract, Ginger rhizome extract, Garlic clove extract, and Mehndi leaf extract) were tested at a 5% concentration, with one control, using a Completely Randomized Design (CRD) model with three replications. Among the isolated fungi, *Colletotrichum capsici* exhibited the highest frequency of occurrence compared to the other fungi. In *in vitro* evaluation, all native plant extracts significantly suppressed *Colletotrichum capsici* compared to the control. The efficacy of Garlic clove extract (52.22%) and Neem seed extract (48.89%) was found to be particularly effective in controlling anthracnose in chilli. Overall, the results indicated that *C. capsici* was the most virulent fungal pathogen responsible for anthracnose in chilli, with Garlic clove extract showing the greatest effectiveness, followed by Neem seed extract.

**Keywords**: Fungal characteristic, percent inhibition, fungal growth, *Colletotrichum capsici*

## INTRODUCTION

Chilli, scientifically known as *Capsicum annuum* L., is a member of the Solanaceae family. Green chillies are a very nutritious food, containing a significant number of vitamins (Saimbhi *et al.* 1977, Sayed and Bagvandas 1980). Chilli has been domesticated and commercially farmed in Bangladesh mostly during the Kharif and Rabi seasons. Bangladesh is a prominent global producer of chilli. In Bangladesh, the cultivation of this crop an area of around 192.70 thousand acres in winter and 50.29 thousand acres in summer, resulting in a production of 0.496 million tons and 0.128 million tonnes respectively during the 2021-2022 season (BBS 2022). Chilli anthracnose disease can cause up to 50% reductions in chilli production (Pakdeevaraporn *et al*. 2005). Thus, similar to other places in Bangladesh, the occurrence of chilli anthracnose diminishes the yearly crop yield, resulting in a significant impact on the overall demand. Consequently, a substantial amount of chilli has to be imported annually. *C. capsici* induces chilli anthracnose, occurring when the plant is infected by the pathogen's spores, located on and inside the seeds as acervuli and microsclerotia (Montri *et al.*, 2009*).* The spores disperse, settle, and proliferate on the epidermis of the chilli plants. Appressoria are formations created by germinating spores that penetrate the cuticular layer of plants. This penetration leads to the production of infectious hyphae, which in turn causes the formation of lesions. The characteristic signs of anthracnose, produced by the fungus *C. capsici*, manifest as depressed necrotic tissues on chilli fruit, accompanied by concentric rings of acervuli. Anthracnose inflicts significant pre-harvest and post-harvest effects on chilli fruits, resulting in the formation of anthracnose lesions. The marketability of chillies is negatively impacted when they have little anthracnose lesions on their fruits (Manandhar *et al*., 1995).

Anthracnose disease can be effectively managed with plant extracts like neem (*Azadirachta indica*), mahogany (*Swietenia mahagoni*), kromcha (*Carissa carandas*), garlic (*Allium sativum*), ginger (*Zingiber*

*officinale*), marigold (*Tagetes erecta),* and allamonda (*Allamonda cathertica*) (Ashrafuzzaman and Khan 1992; Freeman *et al.* 2001; Rashid *et al.* 2007; Masuduzzaman *et al.* 2008, Amin *et al.* 2009; Perello *et al.* 2013; Kabir *et al.* 2014; Moretto *et al.* 2001; Fitsum *et al.* 2014). According to Harbant *et al.* (1999), garlic extract is an efficient method of controlling chilli fruit rot/anthracnose disease. The use of botanical treatments, particularly crude extracts made from the leaves, rhizomes, stolon fruits, and cloves of ginger, neem and garlic, has been shown in numerous laboratory and field studies to effectively reduce the fungi (Khan and Fakir 1995). Neem seed is typically employed as an insecticide, but it also possesses antifungal properties. It can prevent soil-borne diseases (Pervaiz *et al*., 2003). This study aims to evaluate the causal fungi responsible for chilli anthracnose and the efficacy of five native extracts of different plant parts against *C. capsici* in *in vitro*.

## MATERIALS AND METHODS

The study was carried out between 25 October 2022 and 28 January 2023, at the Department of Plant Pathology, Sher-e-Bangla Agricultural University, located in Dhaka.

The Poisoned Food Technique, as outlined by Nene and Thapliyal (1993), was used to examine the effectiveness of plant extracts in PDA medium with 3 replications. The treatments were distributed in 18 petri plates and organized randomly within each replication. After being cleaned, all glassware and media were autoclaved for 45–60 minutes at 121°C and 15 psi of pressure. Before being used, the metallic instruments were heated to red hot over a flame after being sanitized by soaking them in 90% alcohol.

**Collection and identification of fungi**

Three different locations in Dhaka city (Farmgate, Town Hall Bazar and Krishi Market) were selected for collecting anthracnose disease samples. These infected samples (fruits) were collected in sterile polythene bags from numerous locations just after the initiation of the disease. The infected portion was taken to the plant pathology laboratory to examine the symptoms with a stereomicroscope and compound microscope.

**Isolation, identification of causative fungi**

Infected tissue fragments of 2-3 mm in length were removed at the interface of damaged and healthy areas using a sterile blade after surface sterilization of the sample with alcohol. These pieces were placed in Petri dishes in a laminar airflow cabinet and surface was sterilized by NaOCl. Subsequently, they were rinsed thrice in sterile distilled water. The specimens were positioned on sterilized blotting paper and PDA medium (Plate 1). Moist blotter paper was used to avoid bacteria and allow the proper fungal growth. After 3 or 4 days, the fungal structures were visible in both the moist chamber and PDA media. Identify a pure culture of the organism based on characteristics such as colony appearance, growth of the mycelium, color, and sporulation of the fungi.

**Frequency of occurrence**

The frequency of occurrence of a fungus was determined using the following formula(Sajad *et al*. 2017):

Frequency of occurrence =

$$\frac{\text{Total No. of fungal colonies on chilli fruit}}{\text{Total No. of all fungus colonies}} \times 100$$

**Pure culture preparation of the pathogen**

Pure culture of fungi was generated using the hyphal tip culture method, followed by repeated sub-culturing into PDA for a minimum of 3-5 times. Fungi were identified by examining their colony characteristics, mycelial growth, color, and sporulation (Akter *et al*. 2021).

**Pathogenicity test**

Four distinct inoculation techniques were used to investigate the pathogenicity of *C. capsici* in vitro (Parey et al. 2013). The used techniques included pin-prick (PP), spore suspension spray (SSS), spore suspension injection (SSI), and spore suspension dip (SSD). The spore suspension isolate was generated by adding 10 ml of sterilized distilled water to a 15-day-old culture grown on PDA in a 30 ml test tube, achieving a spore concentration of $10^7$ spores/ml. The chilli fruits collected from the field were subjected to surface sterilization with a 0.1 percent mercuric chloride solution for 30 seconds, followed by three rinses with sterile distilled water to remove any remaining mercuric chloride. The chilli fruits were air-dried on sterilized blotting paper and then inoculated with a spore suspension of the recovered fungus. The pinprick technique included the use of a sterile needle to create three punctures on the fruit before inoculation with a spore suspension spray. The spore suspension spray (SSS) included the application of the spore suspension via a sprayer, ensuring comprehensive coverage of the fruit's surface with the spray. The inoculation was also conducted by soaking the fruit in spore suspension (SSD) for 5 minutes. The spore suspension injection (SSI) technique entails the introduction of spore suspension into the fruit using a sterile syringe, executed under aseptic circumstances. The infected fruits were placed on moist chambers, covered with glass jars containing wet cotton swabs, and incubated at a temperature of 25±2°C. The disease's progression was recorded by measuring the lesion length in the affected region.

**Plant extracts preparation**

The collected plant materials were chopped and subsequently soaked overnight in cold distilled water to facilitate tissue softening. The soaked leaves were subsequently ground with a pestle and mortar, using a

water ratio of 1:2 (one part botanical to two parts water). The extraction was conducted by the method outlined by Jacob and Sivaprakasam (1994), including a few modifications. The homogenates were filtered through double-layered sterilized muslin fabric and then through Whatman filter paper No. 1. A 100% stock solution was prepared, followed by the preparation of 5 percent concentrations.

The antifungal efficacy of several indigenous plant extracts was examined *in vitro* using the poisoned food method (Nene and Thapliyal 1971). Each extract was separately mixed with liquid PDA medium in a conical flask to reach the desired concentration of 5%. A trace amount of streptomycin sulfate was incorporated to inhibit bacterial contamination and subsequently transferred into 90mm Petri plates. The standard PDA plates functioned as the control group. A 5mm fungus culture disc was positioned at the center of the medium and incubated at 28±2˚C. Three replications were conducted for each treatment, and measurements of radial growth were taken starting on the second day at two-day intervals until the seventh day. Data collected on the seventh day were utilized for analysis.

The PDA medium, modified with plant extract, was dispensed at 20 ml per Petri dish. Following the solidification of the media, the plates were inoculated with a 0.5 mm mycelium disc of *C. capsici* obtained from fungal culture. Plates with unaltered media functioned as the control. The inoculation plates were incubated at 26±2˚C in an incubator. The colony diameter was measured when the control plates were completely covered. The effectiveness of native plant extracts was quantified as the percentage suppression of the mycelial growth compared to the control, computed using the method provided by Vincent (1947).

$PI = C – T/C \times 100$

Where,

PI = Percent mycelial growth inhibition over control

C = Diameter of fungal mycelial growth in control plate

T = Diameter of fungal mycelial growth in treatment plate.

**Data analysis**

Statistix 10 software was used for proper interpretation. The mean value was compared according to Tukey's Test at a 5% level of probability. Bar diagram and graphs was used to interpret the data as and when required.

## RESULTS AND DISCUSSION

Infected fruits were sectioned and placed into the moist chamber and PDA media for isolation and making a pure culture of associated fungi (Plate 1). That pure culture was then observed under a microscope for identification. Six fungi (*Colletotrichum capsici, Fusarium oxysporum, Fusarium moniliforme, Rhizoctonia solani, Aspergillus flavus* and *Alternaria alternata*) were isolated. These fungi were recognized and compared to the standard literature by producing slides and seeing them using a compound microscope. The highest percentage frequency was found by *C. capsici* (70.58 %). *Aspergillus flavus, A. alternata* and *R. solani* had the lowest frequency compared to all isolates. *F. moniliforme* was mostly found fungal isolate next to *C. capsici* (Figure 1).

**Characteristics of identified fungi**

Cultural, morphological, and microscopic characteristics of identified fungi were observed (Plate 2). The following characteristics were found:

***F. oxysporum***

Initially, produced aerial mycelium that transitioned from white to light pink and finally to dark pink when it matured. The hyphae were septate and hyaline, as well as macro and microconidia were observed during microscopic examination.

***F. moniliforme***

The colony morphology of *F. moniliforme* was characterized by round, fluffy mycelium, initially white then transitioning to a subtle blue hue with a purple reverse. Microscopic analysis indicated that the hyphae were septate and hyaline, accompanied by the presence of macroconidia and microconidia. Macroconidia were few, somewhat sickle-shaped to virtually linear, and had 2-5 septa.

***C. capsici***

White-to-black brown, irregular colony. Aseptate unbranched conidiophores adorned septate mycelium. Hyaline, unicellular, fusiform, narrow-ended conidia.

***A. alternata***

The greenish-white aerial mycelium generated by *A. alternata*. Pyriform, multicellular conidia often have long, golden brown beaks or small, conical or cylindrical beaks.

***R. solani***

White and cream were the colony's colors. White colonies were the most colorful. Young colonies are often white, whereas older isolates become brown.

***A. flavus***

*A. flavus* exhibited a cottony, granular flat mycelium in shades ranging from olive to lime green, with a cream-colored reverse characteristic. Microscopic examination revealed that the hyphae exhibited septate and hyaline characteristics. Conidiophores generally emerge from submerged hyphae, which are distinguished by their rough walls that expand upward, ultimately forming globose vesicles. Sterigmata were organized closely adjacent to one another, thoroughly encompassing the entire surface of the vesicle.

To assess the pathogenicity of isolated *C. capsici*, four distinctive inoculation techniques were utilized: pinprick (PP), spore suspension spray (SSS), spore suspension injection (SSI), and spore suspension dip (SSD), all performed *in vitro* on detached semi-ripe chilli fruits. The pathogenicity test utilizing only the

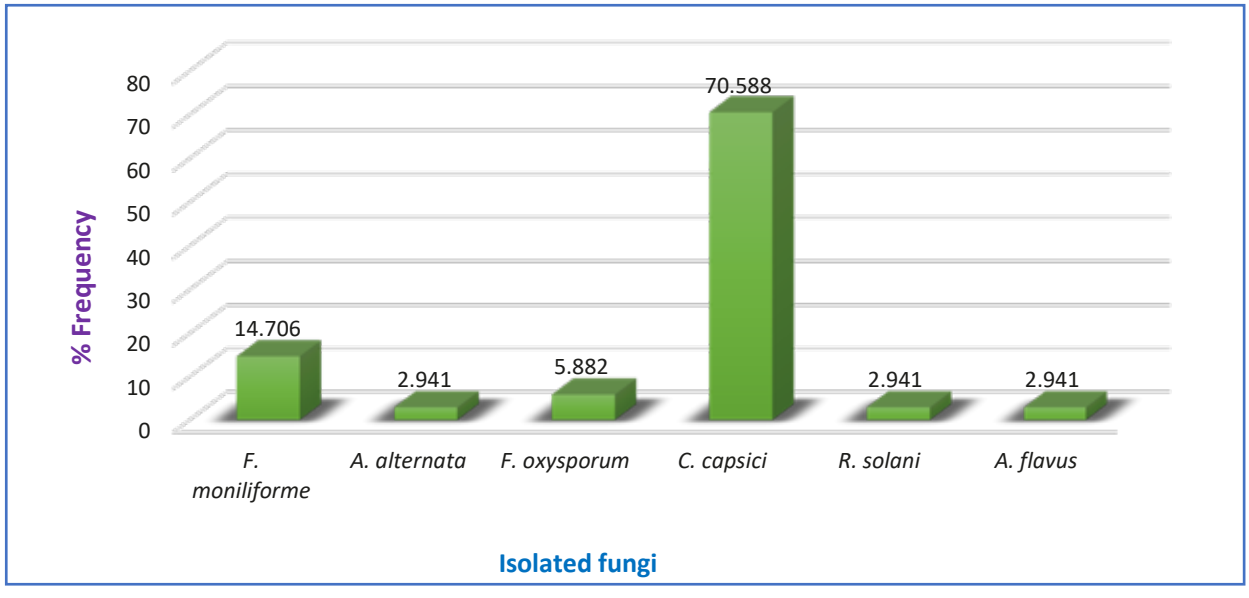



Figure 1. Frequency percentage of isolated fungi from infected chilli fruits

pinprick method demonstrated the characteristic symptoms of an infection; however, the presence of the pathogen was noted in all inoculated chilli fruit through Stereomicroscopic examination. The results demonstrated that *Colletotrichum capsici* was the most pathogenic isolate, producing lesion diameters between 0.29 and 1.32 mm in length and width, respectively (Plate 3).

The five treatments were evaluated against *C. capsici*. Different interventions did not result in a complete growth inhibition of the fungus *C. capsici*. The experiment demonstrated that the control group exhibited the highest mycelial growth (2.7 cm), while the garlic application exhibited the lowest mycelial growth (1.30 cm). These results are statistically similar to the results of the neem seed (1.35 cm) at 4 days after inoculation. The control exhibited the highest mycelial growth (5.53 cm), while the Garlic application exhibited the lowest mycelial growth (2.9 cm). These results were statistically comparable to those of the other treatments at 6 DAI. The control exhibited the highest mycelial growth (9 cm), while the Garlic application exhibited the lowest mycelial growth (4.3 cm), which is statistically equivalent to the Neem seed (4.6 cm) during 9 DAI. At 9 DAI the highest restricted growth over control was observed in Garlic application up to 52.22% and the lowest restricted growth over control was observed in neem leaf extract (34.44%) (Table 1, Plate 4).

The findings from the current experiment indicate the identification of six fungal species*: Alternaria alternata, Aspergillus flavus, Colletotrichum capsici, Fusarium oxysporum, F. moniliforme*, and *Rhizoctonia solani* associated with anthracnose disease in chilli fruit. Notably, *Colletotrichum capsici* exhibited the highest frequency percentage at 78.58% and was determined to be the most virulent isolate compared to the other fungi isolated. Other researchers have documented similar results. Rony et al. (2024) reported that nine fungal species were identified from anthracnose-infected chili fruits. The fungi responsible for infected chilli fruits included *Alternaria alternata, A. tenuissima, Aspergillus flavus, A. niger, Colletotrichum dematium, C. gloeosporioides, Curvularia clavata, Fusarium semitectum,* and *Rhizopus stolonifer*. Ali et al. (2016) identified *Colletotrichum* sp. as a significant contributor to post-harvest decay in chilli.

Another result of this study found that, in case of reduction in the growth over control at 9 days, all the five native plant extracts show the effects and among them, garlic shows the

the highest reduction in growth (52.22 %) over control. Many investigations have shown that Neem extract minimizes the occurrence of fruit rot in mature chili (Yadav *et al*. 2017). According to Harbant *et al.* (1999), garlic extract is an efficient method of controlling chilli fruit rot disease.

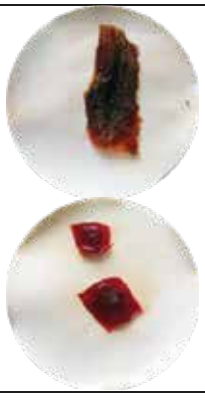
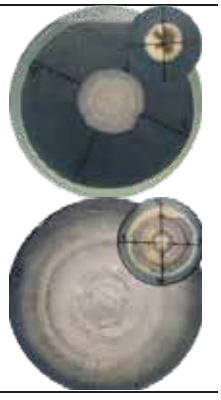
| Infected fruits | Moist chamber | Microscopic view (seta and conidia) | Sample set on PDA media | Pure culture |
|---|---|---|---|---|
|  |  |  |  |  |

**Plate 1. Preparation of pure culture from anthracnose-infected chilli fruits**

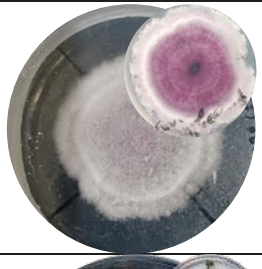
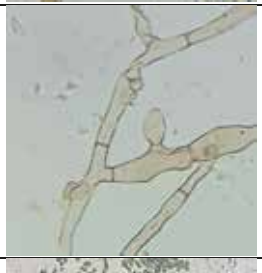
| | Pure culture | Microscopic view | | Pure culture | Microscopic view |
|---|---|---|---|---|---|
| *F. oxysporum* |  |  | *A. alternata* |  |  |
| *F. moniliforme* |  |  | *R. solani* |  |  |
| *C. capsici* |  |  | *A. flavus* |  |  |

**Plate 2. Different fungal (fungal colony and microscopic structure) isolates from anthracnose-infected chilli fruits**


**Plate 3. Pathogenicity test of *C. capsici* causing anthracnose of chilli (4 methods with 3 replications)**

**Table 1.** *In in vitro* management of *Colletotrichum capsici* with native plant extracts

| Treatments | Mycelial growth (cm) | | | Reduction in growth (cm) (9 days) | % reduction in the growth over control (9 days) |
|---|---|---|---|---|---|
| | 3 days | 6 days | 9 days | | |
| Control | 2.7 A | 5.53A | 9.0 A | --- | --- |
| Neem leaf extract | 2.0 B | 4.0 B | 5.9 B | 3.1 | 34.44 |
| Neem seed extract | 1.35 C | 3.4 B | 4.6 CD | 4.4 | 48.89 |
| Garlic clove extract | 1.30 C | 2.9 B | 4.3 D | 4.7 | 52.22 |
| Zinger rhizome extract | 1.70 B | 3.6 B | 4.9 C | 4.1 | 45.55 |
| Mahendi leaf extract | 1.90 B | 3.7 B | 5.0 C | 4.0 | 44.44 |
| CV (0.05) | 10.73 | 21.52 | 4.13 | -- | -- |
| Std Error | 0.159 | 0.677 | 0.189 | | |

*Means followed by the same letters do not differ at 5% level of significance

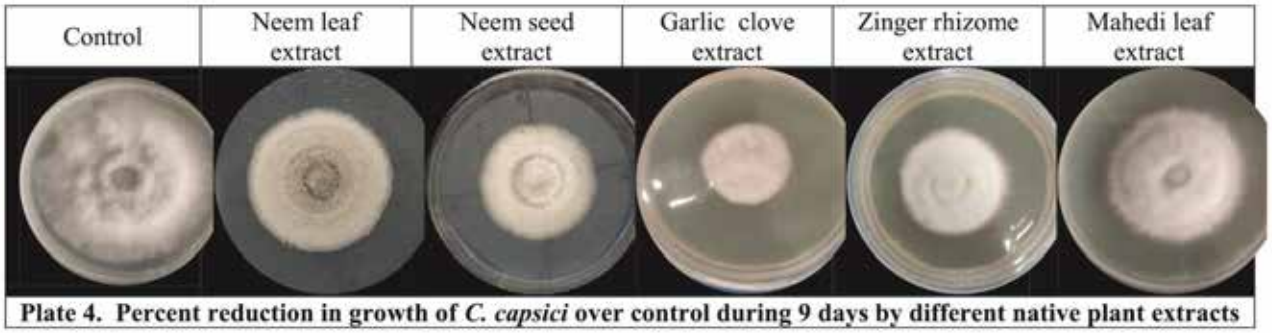



**Plate 4. Percent reduction in growth of *C. capsici* over control during 9 days by different native plant extracts**

## CONCLUSION

This research concludes that *Colletotrichum capsici* is the major anthracnose-causing fungus of chilli and 5% Garlic extract may be suggested for eco-friendly management of chilli anthracnose disease.

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