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## Isolation and *in vitro* control of a fungus infecting common beans in the Darjeeling hills

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**Abstract** From the results of the agar cup bioassay, it was evident that among six plant materials tested, only four extracts were effective against the pathogen, *Aspergillus* sp. The four potential plants were *Bergenian ciliate*, *Artemisia vulgaris*, *Centella asiatica*, and *Urtica dioica*. Among these four effective plant materials, *Bergenian ciliate* had the most inhibitory effect, which developed an inhibition zone with a diameter of 12.28 mm. Next to *Bergenian ciliate*, there was inhibitory activity in *Artemisia vulgaris* leaf extract, which showed an inhibition zone of 10 mm in diameter. The inhibitory effect of these botanicals against the fungus was verified by the poisoned food technique. The medium of growth was supplemented with aqueous extracts of the plant materials. Final concentrations of the plant extracts in the medium were maintained at 5 %, 10 % and 20 %. The inhibition of growth was computed in per cent. Data were recorded after 5 and 10 days. The inhibitory effect of *Bergenian ciliate* and *Artemisia vulgaris* was significantly high and 100% inhibition was by 10 % and 20% concentrations of the aqueous extracts after 10 days of incubation. Thus, the present study significantly sheds light on the management of the disease of common bean plants caused by *Aspergillus* sp.

**Keywords:** Common bean, Fruit rot, *Aspergillus* sp., Botanicals

### Introduction

The common bean (*Phaseolus vulgaris* L.) is an essential legume recognized for its delicious flavour, high protein levels, and as a significant source of antioxidants, minerals, starch, dietary fibre, and vitamins. Within the five domesticated and cultivated species of the genus *Phaseolus*, one encounters a genus that encompasses approximately 70 species (Freitag and Debouck, 2002; Singh *et al.*, 1991, 2001).

Many groups of organisms, like fungi, bacteria, viruses, mycoplasma, and nematodes, infect bean plants. Bean rust, powdery mildew, seed rot, damping-

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off, and angular leaf rot are prevalent in common bean (Meena, 2022; Taboada *et al.*, 2022; Gabrekiristos and Wondimu, 2022).

Establishment of successful disease control requires a good understanding of plant-pathogen interaction, along with an understanding of the environmental conditions required for disease establishment.

A survey was conducted on the bean growing field of Darjeeling hills, mainly the Lower Baktey Busty area of Darjeeling district, covering more than 1000 sq. meters, where symptoms were mainly found on the leaves and fruits of the plants. Symptoms like rot condition of various intensities and fungal growth were observed on fruits and leaves of infected plants. The research presented here aims towards the appropriate isolation and identification of the pathogens from infected plants. In addition, control of the pathogen by botanicals has also been taken into consideration. The key objectives of the current study were isolation of major pathogens responsible for foliar and fruit diseases of common bean, morphological characterization of the isolated pathogenic fungi, identification of isolated pathogens, and *in vitro* control of the isolated pathogens using ethnobotanically important botanicals of this area.

## **Materials and methods**

### ***Plant materials collection and maintenance***

This study utilized plant materials for two main purposes: initially, as experimental host plants to explore plant-pathogen interactions, and subsequently, as sources for extracting botanicals employed in pathogen control trials. The specifics regarding the plant materials acquired for these purposes are detailed below.

Healthy common bean fruits were collected from plants growing in uninfected fields of villages of the Darjeeling hills. The selection criteria for these fruits were based on their source from resilient plants that flourish in the unique agro-climatic conditions of the research region, as well as their commonality in local agricultural techniques. Additionally, healthy common bean fruits were obtained from local farmers to facilitate disease induction studies.

Common bean (*Phaseolus vulgaris*) specimens were cultivated under regulated circumstances in both pots and field plots in the experimental garden of the Department of Botany, Darjeeling Government College. Round clay pots, each with a diameter of 30 cm, were filled with a soil mixture consisting of 3.0 kilograms, which included 0.5 kg of sun-dried cow dung manure and 2.5 kg of fine dry soil. The identical substrate was employed in the creation of experimental field plots for common bean growing. A 0.1% mercuric chloride solution was used to sterilize the seeds' surfaces before sowing, and any

impurities were then removed with three rounds of sterile distilled water washings. In both pot and field settings, sterilized fruits were sown one inch deep in the soil. A single plant was grown in each pot, making sure that there was a 30-cm gap between each plant in the row as well as between the rows in the field arrangement. To maintain ideal soil moisture levels during the research period, irrigation was carried out every other day. To avoid competition for resources, weeds were also methodically eradicated 15 days after planting as part of the weed control process.

From the naturally occurring flora of the Darjeeling hill region, botanical materials—more especially, different plant parts including leaves, bark, roots, and rhizomes—were methodically collected for extraction purposes. Some plants were also collected from the villages of Darjeeling Hill. Voucher specimens of the obtained plant species were taxonomically confirmed and subsequently deposited in Darjeeling Government College's Department of Botany herbarium. These species were selected for their ethnobotanical value. A list of plants has been presented in Table 1.

**Table 1.** List of plants used for the extraction of botanicals

| Serial no. | Name of the plant             | Local name | Ethnobotanical importance                                         | Parts used                           | References                    |
|------------|-------------------------------|------------|-------------------------------------------------------------------|--------------------------------------|-------------------------------|
| 1.         | <i>Bergenia ciliate</i>       | Pakhenbet  | Used in intestinal infection<br>And dysentery                     | Young leaves                         | Khan and Kumar, 2016          |
| 2.         | <i>Centella asiatica</i>      | Golpata    | Used in cold and throat ailments                                  | Leaves with a petiole                | Jahan <i>et al.</i> , 2012    |
| 3.         | <i>Urtica dioica</i>          | Sisnu      | Used as a remedy to lower blood pressure.                         | Young leaves with a petiole          | Jan <i>et al.</i> , 2017      |
| 4.         | <i>Eupatorium adenophorum</i> | Banmara    | Used as a blood-clotting agent and antiseptic for cuts and wounds | Leaves                               | Tripathi <i>et al.</i> , 2018 |
| 5.         | <i>Artemisia vulgaris</i>     | Tetaypati  | Highly recommended as an antiseptic and disinfectant              | Apical portion                       | Pandey <i>et al.</i> , 2017   |
| 6.         | <i>Macropanax sp.</i>         | Chindey    | Used as a healthy option during summer to keep the stomach cool.  | Young leaves with the apical portion | Afrin <i>et al.</i> , 2021    |

### ***Field survey and collection of disease samples***

An inspection was conducted on the prevalence of diseases in common bean fruits and leaves in different common bean growing areas of the Darjeeling hills. Four surveys totalling at least 10,000 square feet were carried out in expansive open agricultural areas between July and September of 2022. To determine the prevalence of illnesses, 50 fruits were randomly selected for each survey. The number of impacted fruits was measured, and the proportion of infected specimens was computed (Figure 1). A thorough analysis was then conducted by averaging the responses from the four questionnaires. Furthermore, common bean fruits and leaves with varying levels of rot were methodically gathered from many common bean growing locations. After being properly labeled and packed in separate, sterile zip-lock bags, these samples were delivered to the Mycopathology Laboratory at the Darjeeling Government College's Department of Botany. After being collected, the samples were provided within two hours and kept at 4°C for further analysis.

### ***Isolation of fungi***

Using a sterile scalpel, the diseased tissues were carefully removed from common bean fruits and leaves that showed signs of the illness. To remove any remaining HgCl<sub>2</sub>, the separated sick segments were surface sterilized for one minute using 0.1% mercuric chloride (HgCl<sub>2</sub>) and then washed four times with sterile distilled water. The damaged fruit tissue was then surface-sterilized and cut into 2 mm<sup>2</sup> pieces under aseptic conditions using a heat-sterilized blade. Following that, these segments were inoculated in triplicate into potato dextrose agar (PDA) slants. As controls, healthy fruit samples were also prepared in the same way: they were chopped into tiny pieces, surface sterilized, and then put into PDA tubes. For five days, all inoculation tubes were incubated at 28°C (Thiyam and Sharma, 2013).

### ***Maintenance of stock cultures***

Potato dextrose agar (PDA) slants were used to maintain the isolated fungal cultures. Either 4°C or room temperature was used to preserve the cultures. These standard cultures were used to create subcultures for every experimental use.

### ***Pathogenicity test of isolated fungi***

#### **Inoculum preparation**

Conidial suspensions derived from fungal isolates served as the inocula for this study. Each isolate was cultivated on potato dextrose agar (PDA) plates under controlled conditions at 28°C for 10-12 days to promote optimal sporulation. Following this incubation period, sterile distilled water was aseptically introduced to the culture plates. Conidia were then harvested by gently brushing the surface of the mycelial mat using an inoculation needle to detach the spores. The resulting suspension was subsequently filtered through three layers of muslin cloth to eliminate residual mycelial fragments. The concentration of the conidial suspension was quantified using a haemocytometer, and it was standardized to a final concentration of  $1 \times 10^6$  spores  $\text{ml}^{-1}$ , following the methodology outlined by Saha *et al.* (2005).

#### **Disease induction in fruits**

Freshly gathered from cultivation areas, healthy fruits weighing about 0.1 kg each were properly cleaned with sterile distilled water (SDW). They were placed on damp blotting paper in sizable glass chambers after being pat dried using sterile blotting paper. Using a sterile scalpel, six superficial scratches (between 2 and 5 mm) were created on the fruit surfaces to inoculate them. After that, conidial suspensions of every fungal isolate were added to these scratches. The inoculation wounds were covered with sterile cotton plugs that had been gently moistened with the conidial solution.

Three fruits received each fungal isolate, while three more fruits received sterile distilled water treatment as a control. After that, the glass chambers were sealed with perforated polythene sheets and kept in a plant development room with a 12-hour photoperiod at 28°C. Every day, the infected fruits were checked for the emergence of disease signs; evaluations were carried out every other day, and pertinent information was carefully documented.

#### **Disease induction in leaves of the whole plant**

The Dickens and Cook (1989) methodology was used to induce disease in whole common bean plants. Well-established one-month-old plantlets from a local variety grown in pots were used in the study. Each experimental set had three plants, with independent inoculation using a pure conidial slurry (0.05% Tween 20) produced from unique fungal strains. Control plants were treated with sterile distilled water.

Post-inoculation, the seedlings were moved to perforated, clear polythene containers that had been misted with sterile distilled water beforehand to guarantee high humidity. Following two days of incubation under these

circumstances, the plants were transferred to a greenhouse setting with ideal natural light and temperature, where they were given regular soil surface watering. By frequently spraying with sterile distilled water, the humidity was meticulously maintained at 46%. To guarantee the accuracy of the findings, the full experimental process was carried out three times.

### **Verification of Koch's postulates**

The fungal isolates were found to be the cause of fruit rot disease in common beans (*Phaseolus vulgaris*) after a thorough application of Koch's postulates. The pathogenicity of each fungal isolate was evaluated by inoculating healthy bean fruits with a conidial solution made from 10-day-old fungal cultures cultivated on potato dextrose agar (PDA) at a concentration of  $1 \times 10^6$  conidia/ml. A sterile knife was used to remove parts of the afflicted fruit after it had been incubated for 10 days, during which time indications of the disease appeared. The contaminated fruit sections were thoroughly rinsed with sterile distilled water (at least three times) after being surface sterilized for one minute in 0.1% HgCl<sub>2</sub> to remove any remaining surface pollutants. Following sterilization, the tissue was cut into tiny pieces and aseptically moved on sterile PDA slants. Throughout 10 to 12 days, these slants were incubated at 28°C and checked for fungal sporulation. After sporulation became apparent, the cultures were stained with cotton blue in lactophenol and examined under a microscope. By contrasting their morphological characteristics with those of the original fungal isolates, the re-isolated fungi's identification was confirmed. A fungal isolate that was repeatedly isolated from diseased fruits was considered a pathogen. Similarly, conidial suspension spraying at the same dose was used to produce leaf infections in common bean seedlings to support Koch's postulates. After ten days, contaminated leaf segments were collected, surface sterilized, and then moved to sterile PDA slants for further culture to identify the pathogen. The spore structures and morphological traits of the isolated fungus were analyzed under a microscope, and their growth parameters were noted on PDA. If a fungal organism showed similarities to the original characterisation, it was considered a pathogen based on a comparison of the data with the original isolation.

### **Identification of isolated fungal pathogens**

Comprehensive research concentrating on the phenotypic characteristics of the isolated fungal pathogens was carried out, including microscopic analysis and in-depth evaluations of colony shape. The identification process was then completed at the Fungal Biotechnology and Molecular Plant Pathology Laboratory within the Department of Botany, NBU.

### ***Studies on phenotype***

Both the hyphal and conidial spore morphology, as well as the fungal colony features, were included in the phenotypic analysis. Microscopy was used to evaluate the cellular morphology. By analyzing growth traits on Potato Dextrose Agar (PDA) plates, culture morphology was examined.

### ***Colony characteristics***

Each fungus was centrally injected onto sterile potato dextrose agar (PDA) plates, which were 9 cm in diameter. The fungi were then cultured for 3–4 days at 28°C to allow for the formation of mycelial mats to examine the colony features of the isolated fungal pathogens. A sterile cork borer was then used to remove mycelial plugs (4 mm in diameter) from the hyphal growth's advancing border and move them to the middle of brand-new, sterile PDA plates. To ensure proper sporulation, these plates were then incubated for 10 days at 28°C. Growth rate, mycelial coloring and texture, radial expansion patterns, and spore production features (color, texture, and quantity) were among the cultural factors that were carefully documented during this time.

### ***Microscopy***

Cotton blue in lactophenol was used to stain a little portion of mycelium from sporulated pure fungal cultures on a sanitized, grease-free glass slide. After that, the slides were sealed and coated with a cover slip so that they could be examined using an Olympus microscope (India) at 40X and 100X magnifications (oil immersion) using a compound microscope. The hyphal septation and the kind and shape of the spores were among the many morphological traits of the fungus that were evaluated. We also measured the diameter of the mycelia and the length and width of the spores (Amadi *et al.*, 2014).

### ***Evaluation of botanicals for inhibition of pathogen growth***

#### **Extraction of botanicals**

With a few adjustments, plant extracts were made using the technique described by Mahadevan and Sridhar (1982). After being completely cleaned with sterile distilled water, freshly harvested plant materials were allowed to air dry. After being weighed and finely powdered in liquid nitrogen, the samples were extracted separately using sterile distilled water. After passing through a four-layer muslin cloth, the resulting mixtures were centrifuged for 15 minutes at 5,000 g. To get rid of any possible impurities, the supernatants were then

sterilized using Millipore filtration (0.2 µm). All extracts were kept for later examination at 4°C. Several bioassays were used to evaluate their antifungal qualities.

#### **Agar cup bioassay**

For screening of the inhibitory effect of botanicals against the test pathogen, the Agar Cup Bioassay technique of Suleman *et al.* (2002) and Saha *et al.* (2005) was followed. At first, 2 ml of mycelia suspension was poured into a Petri dish, and subsequently, 18 ml of molten PDA medium was poured into the Petri dish. The medium and the mycelia suspension were mixed by rotating the Petri dish carefully. After solidification, using a cork borer (5mm in diameter), 4 to 5 cups or wells were made, keeping a sufficient distance between the wells and also from the end of the petri dish. Plant extracts prepared from one gram of tissue in one millilitre of distilled water were poured into the cups. Each cup contained fifty microliters of plant extracts. Finally, the plates were incubated at 28±1°C for 48 hours or as desired. In the control set, sterile distilled water was poured into the well. In the first phase agar cup method was followed for screening of antimicrobial activity of the plant extracts. In the second phase poison food technique was followed.

#### **Bioassay by poisoned food technique**

One millilitre of plant extract was added to 9 ml of the molten sterile PDA medium, mixed well and poured into a sterile Petri dish (90 mm diameter). The plates were allowed to solidify. In control plates, 1 mL of sterile distilled water was added instead of plant extracts. Both experimental and control plates were inoculated with the pathogen and incubated for the required period. Radial growth of the pathogen was measured after 7 days of inoculation. Per cent inhibition of fungal growth was calculated as  $[(\text{radial growth in control} - \text{radial growth in extract-amended plate}) / (\text{radial growth in control})] \times 100$ . Each extract was tested thrice against each pathogen, and the data were averaged.

### **Results**

#### ***Disease occurrence in leaves and fruits in the common bean***

At the onset of the present study, different common bean-growing areas of Darjeeling hill were surveyed for disease occurrence on fruits and leaves. It was observed that fruit rot is a major problem in the cultivation of common bean in the present study area. Fruits at the stage of 10-15 cm in length were generally attacked by some fungal pathogens. In addition, nearby leaves were also found to contain fungal infections. Severe rots were observed in the fruits. Necrotic

symptoms were also found in the leaves. In the most severe conditions, fruits as well as leaves turned into black necrotic conditions. Disease incidence varied from month to month and place to place. An increase in disease incidence was noted during August, which is the monsoon season. Severity of the disease was found at an alarming percentage of around 60% in August, in Darjeeling Hill. Symptoms of the disease are given in the figure (Figure 1). Lesions on fruits began as irregular, black and white spots that enlarged, coalesced, and spread around the fruit within 4-5 days. Fungal spores appeared outside the fruit, and finally, the whole fruit was covered with fungus. As the disease progressed, portions of the fruits were distorted and dried, and growth was reduced.

Lesions on leaves were less severe. It also began as small brown spots near the margins, which enlarged and progressed inward. In the advanced stages, the lesions turned dark brown, and more than 50% of the leaf was affected.

From the disease survey results, it was evident that some portions of the Darjeeling hills were most severely affected. Several infected samples from this region were collected and brought to the laboratory for isolation of the pathogen.



**Figure 1.** Naturally infected common bean plant(A) and infected fruits (B & C) collected from bean growing field

### ***Isolation of fungi and pathogenicity test***

Altogether 05 fungal cultures (BF1, BF2, BF3, BF4 and BF5) were obtained in PDA. Each of these was tested for its pathogenicity in common bean fruits and leaves. Of these, BF2 and BF4, which were later identified as *Aspergillus* sp., were found to induce disease symptoms.

### ***Pathogenicity test on common bean fruits and leaves of the whole plant***

Pathogenicity of fungal isolates was tested on healthy common bean fruits and leaves of whole plants (Figure 2). The symptoms appeared in the area of inoculation as brownish necrotic spots, which gradually increased in size and turned dark. The spots coalesced as the disease progressed, and brown patches developed around the site of inoculation after 10 days. Control fruits did not show any disease symptoms during the entire period of observation. Lesions appeared on leaves after inoculation on leaves of whole plants (Figure 3). After 10 days of inoculation, leaves turned yellow and fell off. The pathogens were reisolated from the infected fruits and found to be similar to the original isolates by microscopic studies. Koch's postulations were thereby verified.



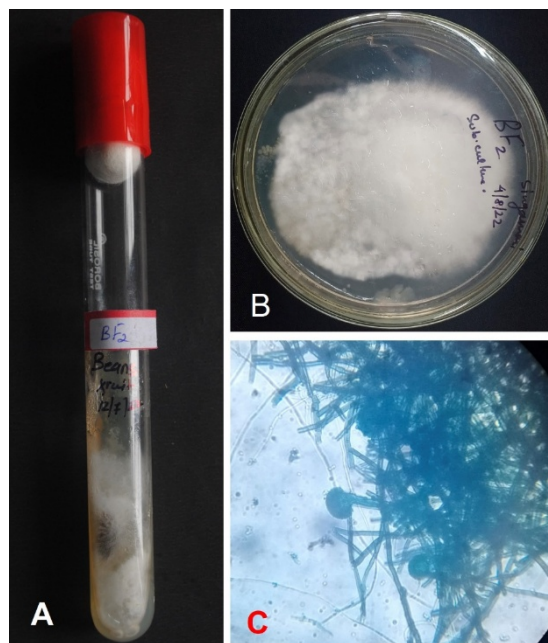
**Figure 2.** Fruit inoculated by isolated pathogens. Brown lines started coalescing after 10 days of inoculation by isolated pathogens BF2 and BF4 (A & B), respectively; control (C)



**Figure 3.** Whole-plant inoculation of common bean: healthy plant (A); after inoculation (B) and plant after 10 days of inoculation (C)

### ***Morphological characterizations of the fungal isolates***

The development and sporulation of the two pathogenic isolates were observed after growing in PDA, and morphology was observed under a microscope. The colony of the isolates was white with cottony mycelium enclosed with a compact layer of dark brown-to-black conidial heads (Fig. 4), while the reverse side of the colony was colourless to pale. The growth was circular with the radial advancements. Sporulation was observed from the 8th day onwards. In light microscopy, the fungus's conidial heads are rather massive, up to 3 mm by 15-20  $\mu\text{m}$  in diameter. They are dark brown and globose at first, but as they age, they take on a radiating shape and frequently break into several loose columns. The smooth, usually hyaline walls of the conidiophores change color to a deeper shade in the vicinity of the vesicle. Phialides are positioned above brown, often septate metulae in the conidial heads, which exhibit a biserial arrangement. The conidia themselves are globose to sub-globose, with a diameter of 3.5 to 5.0  $\mu\text{m}$ . They are distinguished by their rough-walled structure and dark brown to black coloring. From the above-mentioned morphological features, BF2 and BF4 were identified as *Aspergillus* sp.



**Figure 4.** Pure culture of pathogen (A & B); Microscopic field showing the pathogen (C)

### ***Screening of potential antifungal phytoextracts against fungal isolates***

#### **Agar cup bioassay**

In the present study, 06 ethnobotanically important plant extracts were evaluated for their antifungal efficacy against the isolated pathogen *in vitro* by agar cup bioassay. The detailed methods of the agar cup bioassay technique have been described in the Materials and Methods.

From the results of the agar cup bioassay (Table 2), it was evident that among 06 plant materials tested, only 04 extracts of 06 plant materials were effective against the pathogen. The 04 potential plants were *Bergenia ciliate*, *Artemisia vulgaris*, *Centella asiatica*, and *Urtica dioica*. Among these 03 effective plant materials, *Bergenia ciliate* had the most inhibitory effect, which developed an inhibition zone with a diameter of 12.28 mm. Next to *Bergenia ciliate*, there was inhibitory activity in *Artemisia vulgaris* leaf extract, which showed an inhibition zone of 10 mm in diameter. The diameter of the inhibitory zones has been presented in Table 2.

#### **Poisoned food technique**

Among the 06 plant species tested in the agar cup assay, only 04 plant species exhibited inhibitory effect against the fungal pathogen. The inhibitory

effect of these botanicals against the fungus was verified by the poisoned food technique. The medium of growth was supplemented with aqueous extracts of the plant materials. Final concentrations of the plant extracts in the medium were maintained at 5 %, 10 % and 20 %. The inhibition of growth was computed in per cent and has been presented in Table 4.20. Data were recorded after 5 and 10 days. The inhibitory effect of *Bergenia ciliate* and *Artemisia vulgaris* was significantly high and 100% inhibition was possible by 10 % and 20% concentrations of the aqueous extracts after 10 days of incubation (Table 3).

**Table 2.** Agar cup bioassay of plant extracts against the fungal pathogen

| Serial No. | Name of the plants            | Plant parts used                     | Diameter of inhibition zone (in mm) |
|------------|-------------------------------|--------------------------------------|-------------------------------------|
| 01         | <i>Bergenia ciliate</i>       | Young leaves                         | 12.28                               |
| 02         | <i>Centella asiatica</i>      | Leaves with petiole                  | 8.6                                 |
| 03         | <i>Urtica dioica</i>          | Young leaves with a petiole          | 8.8                                 |
| 04         | <i>Eupatorium adenophorum</i> | Leaves                               | 00                                  |
| 05         | <i>Artemisia vulgaris</i>     | Apical portion                       | 10.0                                |
| 06         | <i>Macropanax sp.</i>         | Young leaves with the apical portion | 00                                  |

**Table 3.** Per cent inhibition of fungal pathogen by aqueous extracts of plant species after different time intervals (poisoned food technique)

| Name of the plants        | Concentration (%) | *Percent inhibition of fungi by aqueous plant extracts after different time intervals |        |
|---------------------------|-------------------|---------------------------------------------------------------------------------------|--------|
|                           |                   | 5 Day                                                                                 | 10 Day |
| <i>Bergenia ciliate</i>   | 5                 | 90.90                                                                                 | 94.44  |
|                           | 10                | 93.02                                                                                 | 100.0  |
|                           | 20                | 95.79                                                                                 | 100.0  |
| <i>Centella asiatica</i>  | 5                 | 86.90                                                                                 | 80.44  |
|                           | 10                | 89.89                                                                                 | 84.06  |
|                           | 20                | 91.02                                                                                 | 86.99  |
| <i>Urtica dioica</i>      | 5                 | 85.90                                                                                 | 81.44  |
|                           | 10                | 88.89                                                                                 | 85.06  |
|                           | 20                | 90.02                                                                                 | 87.99  |
| <i>Artemisia vulgaris</i> | 5                 | 90.90                                                                                 | 94.44  |
|                           | 10                | 92.99                                                                                 | 100.0  |
|                           | 20                | 95.66                                                                                 | 100.0  |

Data are average of three replications. \*Percent inhibition was calculated in comparison to growth of the pathogen in control set.

## Discussion

Vegetables are important sources of proteins, vitamins, minerals, and vital micronutrients. They are also vulnerable to fungal infections due to their high water content. Vegetable production across the world is fraught with difficulties, chiefly because of the high incidence of disease. Fruits and vegetables that are susceptible to fungal diseases include cucumbers, tomatoes, bottle gourds, watermelons, grapes, and beans. Vigilant management techniques are necessary for these crops to reduce disease pressures and guarantee sustainable output.

In the present study, common bean (*Phaseolus vulgaris* L.) growing fields in the Darjeeling hills were surveyed. Disease incidence was maximum during August. The severity of the disease was found at an alarming percentage of around 60%. Lesions on fruits began as irregular, brown water-soaked spots that enlarged, coalesced, and spread around the fruit within 4-5 days. Fungal mycelia appeared outside the fruit, and finally, the whole fruit was covered with fungus. As the disease progressed, portions of the fruits were distorted and dried, and growth was reduced. Lesions on leaves were less severe. It began as small brown spots near the margins, which enlarged and progressed inward. In the advanced stage, the lesions turned dark brown, and more than 50% of the leaves were affected. Altogether 05 fungal cultures (BF1, BF2, BF3, BF4 and BF5) were obtained in PDA. But pathogenicity tests further confirmed that one pathogen, viz., *Aspergillus* sp., was consistent throughout the present study area.

Anthraxnose disease of common bean, caused by the fungus *Colletotrichum lindemuthianum*, is almost worldwide in distribution (Mudawi *et al.*, 2008). White mold, also known as sclerotinia rot or sclerotinia wilt, is caused by *Sclerotinia sclerotiorum*. (Souza *et al.*, 2013) observed that bean rust caused by the *Uromyces appendiculatus* limits bean growth and drastically affects grain yields, and is mostly distributed in humid tropical and subtropical regions. Singh *et al.* (2016) stated that Powdery mildew disease, instigated by *Erysiphe polygoni* of beans in India, is responsible for major economic losses in agriculture. Liebenberg and Pretorius (1997) reported that in tropical and subtropical production areas, Angular leaf spot caused by *Phaeoisariopsis griseola* is a damaging disease of beans.

In the present study, *Aspergillus* sp. showed the presence of an unbranched conidiophore with bulged vesicles from which conidia are produced in chains on sterigmata. A thick covering of conidial heads, ranging in color from dark brown to black, coated the fungal colonies. Conidia were rough-walled, dark brown to black, and globose to sub globose (diameter 3.5 to 5.0 µm). Dake (1995) isolated and identified *Aspergillus niger* from *Zingiber officinale*, causing

necrotic leaf spot with globose to subglobose conidia, 3.5 to 5 µm in diameter and dark brown to black. Sharma *et al.* (2012) also reported *A. niger* with 3.5-5.0 µm, globose to subglobose and dark brown to black and rough wall conidia from India. Sahab *et al.* (2016) isolated six *Aspergillus* species namely *A. flavus*, *A. ochraceus*, *A. niger*, *A. parasiticus*, *A. ruber* and *A. terreus* from Egypt. *Alternaria* spp., *Cladosporium* spp, *Penicillium* spp., *Aspergillus* spp., *Fusarium* spp., *Botrytis* spp., *Colletotrichum lindemuthianum*, *Rhizopus* spp., and *Rhizoctonia solani* are The main seed borne pathogenic fungi that cause losses of yield and quality of common bean [Marcenaro and Valkonen (2016), Hussain *et al.* (2020), Singh and Singh (1986), Kator *et al.* (2016), Al-Jaradi *et al.* (2018) and Diaz *et al.* (2022)]

Dolezal *et al.* (2014) reported *A. flavus* infection in maize kernels. Onion bulbs suffer significant losses in the field and during storage due to black rot, which is linked to *Aspergillus niger*. *Aspergillus niger* clusters of black spores typically developed on or between the outer papery scales of onion bulbs and along veins (Rao and Rajasab 1992). Mangos (Prakash and Raoof, 1989), grapes (Sharma and Vir, 1986), and tomatoes (Sinha and Saxena, 1987) have also been reported to deteriorate due to *Aspergillus niger*. Most people agree that *Aspergillus niger* is a strict saprophyte (Farr *et al.*, 1989). *A. niger* infects different parts of flower and induces the typical black mould symptoms (Cohen *et al.*, 2021). *Aspergillus niger* produces secondary metabolites in plant tissue and served as a decaying agent for sweet oranges (Yasmeen and Mazhar, 2019). *A. fumigatus* causes leaf spot on loquat (*E. japonica*) trees in Iraq and worldwide (Al-Ghazali *et al.*, 2023).

In the present study, six ethnobotanically important plant extracts were evaluated for their antifungal efficacy against the isolated pathogen *in vitro* by agar cup bioassay and poisoned food technique.

From the results of agar cup bioassay, it was evident that among 06 plant materials tested, only 04 extracts of 06 plant materials were effective against the pathogen. The 04 potential plants were *Bergenia ciliate*, *Artemisia vulgaris*, *Centella asiatica*, and *Urtica dioica*. Among these 03 effective plant materials, *Bergenia ciliate* had the most inhibitory effect, developing an inhibition zone with a diameter of 12.28 mm. Next to *Bergenia ciliate*, there was inhibitory activity in *Artemisia vulgaris* leaf extract, which showed an inhibition zone of 10 mm in diameter. The following observations were also confirmed by the poisoned food technique.

The effect of different phytoextracts on different fungi and bacteria was reported by several workers. The inhibitory effect of neem (*Azadirachta indica*) extracts on *Aspergillus* and *Rhizopus* (Mondall *et al.*, 2009), *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Trichophyton rubrum*, and *Microsporum canis* (Ospina *et al.*, 2015) was reported. *Datura metel* extract was

effective against *Trichoderma harzianum*, *Fusarium oxysporum* f. sp. *melonis*, *Trichoderma viride*, *Fusarium oxysporum* f. sp. *lycopersici*, and *Fusarium oxysporum* f. sp. *tuberosi* (Rinez *et al.*, 2013). Piper betle extract showed effective activity on *Colletotrichum gloeosporioides*, *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *cubense*, *Sphaceloma ampelinum*, *Colletotrichum capsici*, *Alternaria brassicicola*, and *Pyricularia oryzae* (Singburadom, 2016). *Ocimum sanctum* extract was proved to be effective against *Trichophyton mentagrophytes*, *Microsporum gypseum*, *Epidermophyton floccosum*, *Trichophyton rubrum*, and *Microsporum nanum* (Balakumar *et al.*, 2011). Phytoextract of *Holarrhena antidysenterica* significantly inhibited the growth of *Penicillium expansum* and *Aspergillus fumigatus* (Baviskar and Dekate, 2016). *Trichophyton mentagrophytes* and *Microsporum gypseum* were reported to be inhibited by *Murraya koenigii* extract (Jayaprakash and Ebenezer, 2012). Extract of *Leucas aspera* checked the growth of *Penicillium* sp. and *Candida tropicalis* (Babu *et al.*, 2016). The inhibitory effect of *Mangifera indica* extract on *Candida glabrata* was reported by Jain and Nafis (2011). *Lagerstroemia speciosa* extract was active against 12 bacterial and 3 fungal isolates viz., *Bacillus subtilis*, *Sarcina lutea*, *Staphylococcus aureus*, *Bacillus megaterium*, *Bacillus cereus*, *Salmonella paratyphi*, *Vibrio mimicus*, *Vibrio parahemolyticus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella dysenteriae*, *Aspergillus niger*, *Sacharomyces cerevaceae* and *Candida albicans* (Nasrin *et al.*, 2012). *Boerhavia diffusa* extract was proved significantly effective against *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* (Das, 2012).

This study revealed some new evidence of essential importance. The survey made in the Darjeeling hills of West Bengal revealed that the fungal pathogens *Aspergillus* sp. attack common bean fields and severely damage fruits and leaves of the plants. To the best of our understanding, this study is the first report on *Aspergillus* sp. as a pathogen of common bean in this area. Additionally, the growth of the pathogen, as mentioned earlier, was also found to be inhibited by some botanical in *vitro* studies. Some of them have the potential to control the pathogens. Thus, the present study significantly sheds light on significant management of the disease of common bean plants caused by *Aspergillus* sp.

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## Conflict of interest

The authors declare that there is no conflict of interest.

## References

- Afrin, R. S., Islam, R. M., Khanam, H., Proma, M. N., Didari, S. S., Jannat, W. S. and Hossain, K. M. (2021). Phytochemical and pharmacological investigation of extracts of leaves and barks of *Macropanax dispermus*: a promising ethnomedicinal plant. *Future journal of pharmaceutical science*, 7:1-13.
- Al-Ghazali, N. A., Hameed, Z. L. and Abu-Duka, A. B. (2023). First report of *Aspergillus* leaf spot on loquat (*Eriobotrya japonica*) caused by *Aspergillus fumigatus* in Iraq. *OP Conference Series: Earth and Environmental Science*, 1158: 042030.
- Al-Jaradi, A., Al-Mahmooli, I., Janke, R., Maharachchikumbura, S., Al-Saady, N. and Al-Sadi, A. M. (2018). Isolation and identification of pathogenic fungi and oomycetes associated with beans and cowpea root diseases in Oman. *Peer J*, 13: e6064.
- Amadi, J. E., Nwaokike, P., Olahan, G. S. and Garuba, T. (2014). Isolation and identification of fungi involved in the post-harvest spoilage of guava (*Psidium guajava*) in Awka metropolis. *International Journal of Engineering*, 4:8269-8269.
- Babu, A., Mohamed, M. S. N., Jaikumar, K., Anand, D. and Saravanan, P. (2016). In-vitro antifungal activity of leaf extracts of *Leucas aspera* and *Leucas zeylanica*. *International Journal of Pharmaceutical Sciences and Research*, 7:752-756.
- Balakumar, S., Rajan, S., Thirunalasundari, T. and Jeeva, S. (2011). Antifungal activity of *Ocimum sanctum* Linn. (Lamiaceae) on clinically isolated dermatophytic fungi. *Asian Pacific Journal of Tropical Medicine*, 4:654-657.
- Baviskar, R. N. and Dekate, H. M. (2016). Screening of antifungal activity of *Holarrhena antidysenterica* and *Maduca longifolia* against fungal pathogens in apple fruit. *International Journal of Life Sciences*, 4:306-309.
- Cohen, Y., Shulhani, R., Rot, Y., Zemeah, H., Belausov, E., Grinberg-Baran, M., Borenstein, M., Povinia, S., Ezrad, D. and Shtienberg, D. (2021). *Aspergillus niger*, the causal agent of black mould disease in date fruits, infects and colonizes flowers and young fruitlets. *Plant Pathology*, 70:1195-1208.
- Dake, G. N. (1995). Diseases of ginger (*Zingiber officinale* Rosc.) and their management. *Journal of Spices and Aromatic Crops*, 4:40-48.
- Das, S. (2012). Antimicrobial activity study of ethanolic extract of *Boerhaavia diffusa* whole plant. *The International Journal of Pharmacy and Life Sciences*, 3:2006-2009.
- Diaz, S., Polania, J., Ariza-Suarez, D., Cajiao, C., Grajales, M., Raatz, B. and Beebe, S. E. (2022). Genetic correlation between Fe and Zn biofortification and yield components in a common bean (*Phaseolus vulgaris* L.). *Frontiers in Plant Science*, 12:739033. doi: 10.3389/fpls.2021.739033
- Dickens, J. S. W. and Cook, R. T. A. (1989). *Glomerella cingulata* on Camellia. *Plant pathology*, 38:75-85

- Dolezal, A. L., Shu, X., Obrian, G. R., Nielsen, D. M., Woloshuk, C. P., Boston, R. S. and Payne, G. A. (2014). *Aspergillus flavus* infection induces transcriptional and physical changes in developing maize kernels. *Frontiers in Microbiology*, 5:384. doi: 10.3389/fmicb.2014.00384.
- Farr, D. F., Bills, G. F., Chamuris, G. P. and Rossman, A. Y. (1989). *Fungi on Plants and Plant Products in the United States*. APS Press, St. Paul, Minnesota.
- Freytag, G. F. and Debouck, D. G. (2002). Taxonomy, distribution, and ecology of the genus *Phaseolus* in North America, Mexico, and Central America. Botanical Research Institute of Texas, Fort Worth, TX, USA.
- Gabrekiristos, E. and Wondimu, M. (2022). Emerging and reemerging diseases of common bean (*Phaseolus vulgaris* L.) in Ethiopia. *Journal of Plant Pathology & Microbiology*, 13:619.
- Hussain, S., Ajaib, M., Asghar, R., Ali, I. and Siddiqui, M. F. (2020). Mycoflora associated with *Phaseolus vulgaris* L. seeds and its impact on seed germination in Azad Jammu & Kashmir. *Pakistan Journal of Botany*, 52:1455-1463.
- Jahan, R., Hossain, S., Seraj, S., Nasir, D., Khatun, Z., Das, R. P., Islam, T. Md., Ahmed, I. and Rahmatullah, M. (2012). *Centella asiatica*: Ethnobotanical uses and their scientific validations. *American-Eurasian Journal of Sustainable Agriculture*, 6:261-270.
- Jain, P. and Nafis, G. (2011). Antifungal activity of crude aqueous and methanolic amchur (*Mangifera indica*) extracts against *Candida* species. *International Journal of Pharmaceutical Sciences Review and Research*, 9:85-87.
- Jan, K. N., zarafshan, K. and Singh, S. (2017). Stinging nettle (*Urtica dioica* L.): a reservoir of nutrition and bioactive components with great functional potential. *Food Measure*, 11: 423-433.
- Jayaprakash, A. and Ebenezer, P. (2012). Antifungal activity of curry leaf (*Murraya koenigii*) extract and an imidazole fungicide on two dermatophyte taxa. *Journal of Academia and Industrial Research*, 1:124-126.
- Kator, L., Ogo-Oluwa, A. T. and Kemi, A. B. (2016). Isolation and identification of seed-borne fungi of common bean (*Phaseolus vulgaris* L.) from selected markets in Makurdi. *International Journal of Applied Agricultural Sciences*, 2:75-78.
- Khan, Y. M. and Kumar, V. (2016). Phytopharmacological and chemical profile of *Berginia ciliate*. *International journal of phytoparmacy*, 6:90-98.
- Liebenberg, M. M. and Pretorius, Z. A. (1997). A review of angular leaf spot of common bean (*Phaseolus vulgaris* L.). *African Plant Protection*, 3:81-106.
- Mahadevan, A. and Sridhar, R. (1982). *Methods in Physiological Plant Pathology* (2nd ed.). Sivakami Publication, Chennai, India, pp.157-159.

- Marcenaro, D. and Valkonen, J. P. T. (2016). Seedborne pathogenic fungi in common bean (*Phaseolus vulgaris* cv. INTA Rojo) in Nicaragua. PLoS ONE, 11:1-18.
- Meena, N. K. (2022). Important diseases of common bean and their management. Just Agriculture, 2:1-4.
- Mondall, K. N., Mojumdar, A., Chatterjee, K. S., Arnab, B., Datta, J. K. and Gupta, S. (2009). Antifungal activities and chemical characterization of neem leaf extracts on the growth of selected fungal species in vitro. Journal of Applied Sciences and Environmental Management, 13:1-13.
- Mudawi, H. I., Idris, M. O. and Balla, M. M. A. E. (2008). Anthracnose diseases in common bean (*Phaseolus vulgaris* L.) in Shambat, Sudan. I. Identity of the causal agent. University of Khartoum Journal of Agricultural Sciences, 16:477-489.
- Nasrin, F., Ahmad, S. and Kamrunnahar (2012). Evaluation of antimicrobial, antioxidant, and cytotoxic activities of methanolic extracts of *Lagerstroemia speciosa* leaves and barks. Journal of Applied Pharmaceutical Science, 2:142-147.
- Ospina salazar, d. I., Hoyos Sánchez, R. A., Orozco Sánchez, F., Arango Arteaga, M. and Gómez Londoño, L. F. (2015). Antifungal activity of neem (*azadirachta indica*: meliaceae) extracts against dermatophytes. Acta Biológica Colombiana, 20:201-207.
- Pandey, P. B., Thapa, R. and Upreti, A. (2017). Chemical composition, antioxidant and antibacterial activities of essential oils and methanol extract of *Artimesia vulgaris* collected from Nepal. Asian pacific journal of tropical medicine, 10:952-959.
- Prakash, O. and Raoof, M. A. (1989). Control of mango fruit decay with post-harvest application of chemicals against black rot, stem end rot, and anthracnose disease. International Journal of Tropical Plant Diseases, 6: 99-106 .
- Rao, C. V. and Rajasab, A. H. (1992). Investigation on black mould (*Aspergillus niger*) of onion. Onion Newsletter for the Tropics, 4:66-67.
- Rinez, A., Daami-Remadi, M., Ladhari, A., Omezzine, F., Rinez, I. and Haouala, R. (2013). Antifungal activity of *Datura metel* L. organic and aqueous extracts on some pathogenic and antagonistic fungi. African Journal of. Microbiology Research, 7:1605-1612.
- Saha, D., Dasgupta, S. and Saha, A. (2005). Antifungal activity of some plant extracts against fungal pathogens of tea (*Camellia sinensis*). Pharmaceutical Biology, 43:87-91.
- Sahab, A. F., Amin, H. and Ziedan, S. H. (2016). Seed-borne fungal pathogens associated with common Egyptian seeds and their efficiency to produce saponin hydrolase enzyme. International Journal of ChemTech Research, 9:299-306.
- Sharma, R. (2012). Pathogenicity of *Aspergillus niger* in plants. CIBTech Journal of Microbiology, 1:47-51.

- Sharma, R. C. and Vir, D. (1986). Post-harvest diseases of grapes and studies on their control with benzimidazole derivatives and other fungicides. *Pesticides*, 20:1415-1415.
- Singburadom, N. (2016). Hydroxychavicol from *Piper betel* leaves is an antifungal activity against plant pathogenic fungi. *Journal Of Biopesticides*, 8:82-92.
- Singh, K., Kumar, S. and Kaur, P. (2016). Detection of powdery mildew disease of beans in India – A review. *Oriental Journal of Computer Science and Technology*, 9:226-234.
- Singh, S., Sidhu, J. S., Huang, N., Vikal, Y., Li, Z., Brar, D. S., Dhaliwal, H. S. and Khush, G. S. (2001). Pyramiding three bacterial blight resistance genes (*xa5*, *xa13*, and *Xa21*) using marker-assisted selection into indica rice cultivar PR106. *Theoretical and Applied Genetics*, 102:1011-1015.
- Singh, S. P., Gepts, P. and Debouck, D. G. (1991). Races of common bean (*Phaseolus vulgaris*, Fabaceae). *Economic Botany*, 45:379-396.
- Singh, S. R. and Singh, N. I. (1986). Seed mycoflora of broad bean and its control. *Indian Phytopathology*, 39:541-543.
- Sinha, P. and Saxena, S. K. (1987). Effect of treating tomatoes with leaf extract of *Lantana camara* on development of fruit rot caused by *A. niger* in presence of *Drosophila busckii*. *Indian Journal of Experimental Biology*, 25:143-144.
- Souza, T. M. L., Faleiro, F. G., Dessaune, S. N., Pavla-Junior, T. J., Moreira, M. A. and de Barros, E. G. (2013). Breeding for common bean (*Phaseolus vulgaris* L.) rust resistance in Brazil. *Tropical Plant Pathology*, 38:361-374.
- Suleman., Al-Musallam, A. and Menezes, C. A. (2002). The effect of biofungicide *Mycostop* on *Ceratostyxis radicola*, the causal agent of black scorch on date palm. *Biocontrol*, 47:207-216.
- Taboada, G., Abán, C. L., Mercado Cárdenas, G., Spedaletti, Y., Aparicio González, M., Maita, E., Ortega-Baes, P. and Galván, M. (2022). Characterization of fungal pathogens and germplasm screening for disease resistance in the main production area of the common bean in Argentina. *Frontiers in Plant Science*, 13:986247.
- Thiyam, B. and Sharma, G. D. (2013). Isolation and identification of fungi associated with local fruits of Barak Valley, Assam. *Current World Environment*, 8:319-322.
- Tripathi, Y. C., Saini, N., Anjum, N. and Verma, K. P. (2018). A Review of ethno medicinal, Phytochemical, Pharmacological and toxicological aspects of *Eupatorium adenophorum* Spreng. *Asian Journal of Pharmaceutical Science*, 8:1-11.
- Yasmeen, R. and Mazhar, S. (2019). Isolation of *Aspergillus niger* from deteriorating sweet orange (*Citrus sinensis*) and their effect on fresh orange. *LGU journal of life sciences*, 3:66-71.

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