



RESEARCH ARTICLE

Emerging incidence of pod rot caused by *Choanephora cucurbitarum* (Berk. & Rav.) Thaxter in dolichos bean (*Dolichos lablab* L.) and its management in Odisha, India

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Abstract

Dolichos bean (*Dolichos lablab* L.) is an important legume vegetable crop widely grown in India. The research farm of All India Coordinated Research Project (AICRP) on Vegetable Crops, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar, Odisha, situated in Eastern India, first recorded the incidence of *Choanephora* blight during the months of August–October 2023–24. During the subsequent kharif season of 2023–24 a survey was conducted in key dolichos bean farming areas in Odisha, such as Khordha, Cuttack and Ganjam. According to the results of the survey, which were collected from all areas, the disease incidence ranged from 17–33 %. Therefore, research into the etiology and thorough symptomatology was undertaken. Based on the morphological features, molecular characterisation and pathogenicity test, *Choanephora cucurbitarum* (Berk. & Rav.) Thaxter was identified as the causal agent of pod rot in dolichos bean. The *in vitro* evaluation of fungicides indicated potential for disease management. Specifically, hexaconazole 5 % Emulsifiable Concentrate (EC) and azoxystrobin 12.5 % + tebuconazole 12.5 % Suspension Concentrate (SC), were shown to be effective against the test pathogen.

Keywords: *Choanephora cucurbitarum*; disease management; dolichos bean; fruit rot; pod rot

Introduction

Dolichos bean (*Dolichos lablab* L.) is one of the major pulse-cum vegetables in India. It is also called as Australian pea, Egyptian bean, Indian bean, hyacinth bean, poor man's bean or field bean and is a predominantly self-pollinated crop. In Oriya, it is referred to as “Simba”. The crop originated from India and spread over to Nigeria, West Indies, South Central America and China (1, 2). It is economically important vegetable crop and widely grown in tropical and subtropical regions and is well-adopted to drought and low fertility soils (3). A total of 50000 metric tonnes of pods is produced annually on an area of about 12000 hectares under cultivation in India, with an average yield of 4.17 t ha⁻¹ (4).

Dolichos bean is known by several vernacular names in India, these include, Assamese (*urahi*, *urchi* and *uri*); Bengali (*rajashimbi*); Hindi (*bhatvas*, *shimi* and *sem*); Telugu (*chikkudu*, *alsanda*), Kannada (*capparada-avare*, *avare* and *avare baele*), Malayalam (*amara* and *avara*), Manipuri (*huawai uri*); Sanskrit (*nispavah*); Tamil (*avare*, *motta* and *asanda*); Gujarati (*oliya*, and *val*) and Punjabi (*kalalobia* and *katjang*)(5).

The dolichos bean production environment is not without challenges. Though India did not have any known cases of dolichos bean disease, these may be triggered by changing agricultural techniques, unpredictable weather and the introduction of new germplasm. Many problems affect the dolichos bean harvest in India. There are several different types of mildews and rots that can affect fruit trees, including white mold, rust, anthracnose, powdery mildew, charcoal rot and *Choanephora* blight. All of these have been described by various researchers (6–10).

Choanephora blight is an emerging disease that affects dolichos beans; it has a massive economic effect and a broad host range, making it a possible concern. Species of *Choanephora*, including *C. infundibulifera* and *C. cucurbitarum*, are now the only ones known to exist (11). Separation according to Kirk's taxonomy criteria is dependent on sporangium form and striation presence. Inducing leaf blights and fruit rots, both species infect a diverse range of hosts, including angiosperms (monocotyledons and dicotyledons) and gymnosperms. It has been recognized that *Candida cucurbitarum* affects a wider

variety of hosts and is a more widespread plant pathogen than *Candida infundibulifera* (12, 13).

As a result of our conditions in Eastern India, *Choanephora* blight has recently become a major issue for several crops (7, 14). Dolichos bean, okra, squash, chilies, pumpkin, cotton, cowpea and maize were among the crops for which it was recorded (7, 15–21).

A rising number of people in the coastal humid belt of Odisha are worried about the devastating effects of *Choanephora* blight on their dolichos bean crops. This research aims to address that worry. Even though this disease has a major effect on crop quality and production, very little thorough study has been carried out on it. The disease was first noticed at the study field of the All India coordinated research project (AICRP) on Vegetable Crops between July and October 2023–24, the disease was found to be prevalent in regions of Odisha where dolichos bean is cultivated on a larger scale. However, a comprehensive analysis of the disease, its origin and treatment were lacking. In this context, the current study aimed to describe the prevalence and occurrence of the disease in some districts of Odisha, identify and characterize the associated pathogen and evaluate its management under *in vitro* conditions.

Materials and Methods

Survey, isolation and purification of the fungi

To document the frequency and severity of disease infection in dolichos bean, a survey was conducted in several places within the major dolichos bean producing districts of Odisha, namely Cuttack, Khordha and Ganjam, during the kharif season of 2023–2024 (Table 1). A 1 m² rectangle was demarcated in the center of the field and four corners adjacent to the rows that left the boundary during the survey. The number of *Choanephora* blight infected plants were counted and finally, the percent disease incidence was calculated using the following formula:

$$\text{Percent disease incidence} = \frac{\text{No. of infected plants}}{\text{Total no. of plants observed}} \times 100$$

To ensure that all disease samples were free of contaminants, they were rinsed with distilled water. Both the good and diseased portions of the dolichos bean were chopped into pieces 2–3 mm in size. After being exposed to 0.1 % mercuric chloride for 30 sec, the pieces were thoroughly rinsed with 3 changes of sterile water to eliminate any remaining residues of the chemical. Petri dishes with potato dextrose agar (PDA) medium were used to transfer these chopped pieces under aseptic conditions. For 7–10 days, the Petri plates that had been infected were kept at room temperature (27 ± 1 °C). After inoculation for 5 days, the Petri plates showed signs of fungal growth. We used the single spore and hyphal tip approach to get a pure culture of the fungus. Each isolated fungus was maintained on PDA in Petri plates. Hyphae from the periphery of young colonies were examined carefully. This process was repeated 2–3 times till the pure fungus was found.

Cultural and morphological characterization

Agar media was used to understand the effect of different media on the growth and sporulation of *C. cucurbitarum* (i.e. PDA, carrot agar, host extract agar, V8 juice agar, Czapek dox agar and potato carrot infusion). A 7 mm disc of a test fungal culture whose age was 3 days old was inoculated into the center of the 20 mL of media filled Petri plates. The cultural characterization of pathogen was done with various media, such as PDA, carrot agar, host extract agar, V8 juice agar, Czapek dox agar and potato carrot infusion agar (Table 2). The sample itself was analysed regularly and characterised with the help of morphological features like mycelium, sporangiospore and sporangiophores structure.

Molecular characterization

Additional proof was sought by doing molecular characterisation on the causative fungus isolate DB2. For 7 days, the fungus culture was incubated in PDA at a temperature of 28 ± 2 °C. The supplied

Table 1. Survey on incidence of *Choanephora* blight of dolichos bean in different major dolichos bean growing districts of Odisha

SI. No.	Name of Districts	Name of block	Area/village	Sample number	Incidence <i>Choanephora</i> blight (%)
			AICRP on Vegetable Crops Central Horticulture	1	17.0
1	Khordha	Bhubaneswar	Experimental Station (IIHR-ICAR)	2	20.0
			Gelapur	3	33.0
			Saradeipur	4	30.0
		Cuttack Sadar	Urali	5	19.0
2	Cuttack		Nachhipur	6	24.0
		Tangi choudwar	Chintamanipur	7	32.0
			Naranpur	8	20.0
3	Ganjam	Berhampur sadar	Ambagada	9	23.0
			Ankusapur	10	28.0

Table 2. Cultural characteristics of *Choanephora* blight in different nutritional media

SI. No	Media	Colony colour	Colour of hyphae	Sporulation	Growth pattern
1	Potato dextrose Agar	Initially white later turns into yellow.	Hyaline	Present	As the white growth reaches maturity, it produces aerial mycelium that sporulates at edges of colony.
2	Carrot agar	White	Hyaline	Present	White growth of mycelium and produces sporulation at edges of colony when its matured.
3	Host extract agar	White	Hyaline	Present	White growth mycelium producing spores at middle of the colony.
4	V8 juice agar	Initially white later turns into yellow.	Hyaline	Present	White growth was first observed and as it matured, it got denser & yellowish. At colony boundaries, sporulation was observed upon maturity.
5	Czapek Dox agar	White	Hyaline	Absent	There is no sporulation visible & puffy mycelial development was noted.
6	Potato carrot infusion agar	White	Hyaline	Absent	White layer with irregular mycelium development was noted. Absence of sporulation.

DB2 culture was used to extract DNA. A single band of high-molecular weight DNA was identified during the quality evaluation on 1.8 % agarose gel. The Veriti® 96-well Thermal Cycler was used to amplify the isolated DNA with the 18Sr DNA specific primers ITS1 and ITS4. There was just one distinct PCR amplicon band, measuring around 600 bp. After undergoing bead purification, the PCR amplicon was sent on to Sanger sequencing. ITS1 and ITS4 primers were used in a bidirectional DNA sequencing reaction of the PCR amplicon (22). A 25 µL reaction mixture was used for PCR amplification, which included yeast genomic DNA, PCR buffer, magnesium chloride, deoxyribonucleotide primers, Taq DNA polymerase and water that did not include nuclease. A total of 35 denaturation, annealing and extension cycles made up the amplification procedure. Gel documentation software was used to observe the electrophoresis of PCR products on agarose gels after staining with ethidium bromide. Following amplicon purification, bidirectional sequencing was performed using ITS1 and ITS4 primers. We got accession numbers and submitted the resulting sequences in GenBank. Nucleotide sequence identity was analyzed using the Basic Local Alignment Search Tool (BLAST) program on the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov).

Primers used:

ITS1- TCC GTA GGT GAA CCT GC GG

ITS4- TCC TCC GCT TAT TGA TAT GC

MEGA version X was used for phylogenetic analysis (23). The nucleotide sequence of the ITS-rDNA region of the fungus was compared to previously published ITS sequences from other *Choanephora* species that infected different hosts. Referring to the closest type species within the same genus, the MEGA program clustal W method aligned the reference sequence. The nucleotide sequence identity matrices for *Choanephora* sp. were created using Bio Edit Sequence scanner 2v2.0. To construct the phylogenetic tree and evaluate evolutionary distances, the Neighbor-Joining technique was used and the bootstrap consensus tree was inferred from 500 repetitions.

In-vitro bio-efficacy of latest fungicide molecules against the test pathogen

The relative bio-efficacy of fungicides in preventing mycelial development at certain doses was evaluated using the poisoned food approach (24). The following seven fungicides were administered *in vitro*: mancozeb 75 % WP (AGASTYA M-45), copper oxychloride 50 % WP (ALL COP), chlorothalonil 75 % WP (Kavach), carbendazim 50 % WP (Bavistin), hexaconazole 5 % EC (Contaf), azoxystrobin 12.5 % +t ebuconazole 12.5 % SC (DELICY) and cymoxanil 8 % + mancozeb 64 % WP (DuPont™ Curzato®) at concentrations of 0.1 %, 0.15 %, 0.2 % and 0.25 %.

Before adding the necessary number of fungicides to the PDA medium and shaking it thoroughly to ensure that all the fungicides were evenly mixed, the desired concentration of each fungicide's active ingredient was obtained. For every fungicide, 3 replications were kept for every concentration in CRD. Before plating, a small amount of streptomycin was added to each flask to prevent bacterial contamination. A sterile cork borer was used to extract a 7 mm disc from a 3-day-old culture of the test fungus. The disc was then placed in the middle of the medium in reverse to ensure that the pathogen was constantly exposed to the poisoned media. As a control, fungicide-free PDA plates were used. The radial

development of the colony was measured when the control plates' growth approached the edge of the Petri dish. To calculate the percentage of pathogen growth inhibition induced by different fungicides, the pathogen's growth in the control plate was compared.

Percent inhibition of mycelial growth =

$$\frac{\text{Colony diameter in control (mm)} - \text{Colony diameter in treatment (mm)}}{\text{Colony diameter in control (mm)}} \times 100$$

Results

Effect of different nutritional media on mycelial growth of the pathogen

Six different media were tested for the cultivation of *Choanephora cucurbitarum*, namely carrot agar, potato carrot infusion agar, Czapek Dox agar, V8 juice agar and host extract agar. Among these, PDA showed the highest radial growth of the fungus (90.0 mm), decisively surpassing all other media (Fig. 1). The lowest growth was observed on carrot agar (47.70 mm). Czapek Dox agar (80.35 mm) and host extract agar (83.23 mm) showed excellent radial fungal growth five days after inoculation. Rapid pathogen growth on PDA enabled complete colonization of the 90-mm Petri plate within 5 days.

The fungal colonies appeared white on the upper surface and yellowish on the reverse side of the Petri dish (Fig. 2). Upon maturity, white mycelium formed black pin heads, indicating sporangiola formation.

Identification and phenotypic characterization of pathogen

Several morphological characteristics were used to identify the pathogen, such as sporulation at the edges of the colony (Fig. 3), the colour and shape of the mycelium (Fig. 4), monosporous sporangiola (Fig. 5), the size and shape of the sporangiospores (Fig. 6) and sporangiophore bearing sporangium with columella (Fig. 7).

The following observations were made on morphological



Fig. 1. Pure culture of *Choanephora cucurbitarum* on potato dextrose agar media.

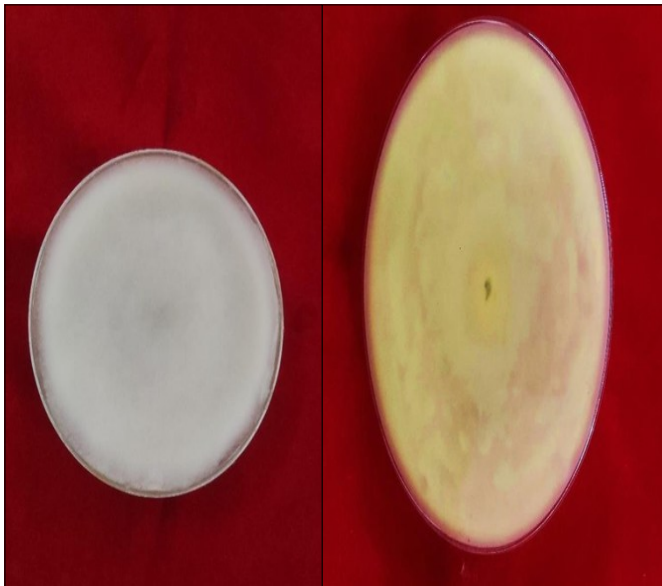


Fig. 2. Mycelial growth upper and lower view.



Fig. 3. Sporulation at edges of the colony.

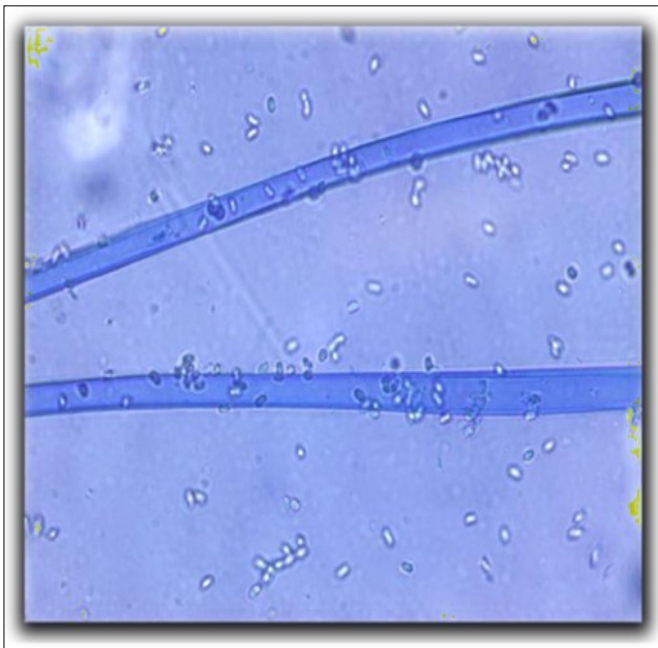


Fig. 4. Mycelium of the fungus (40x).

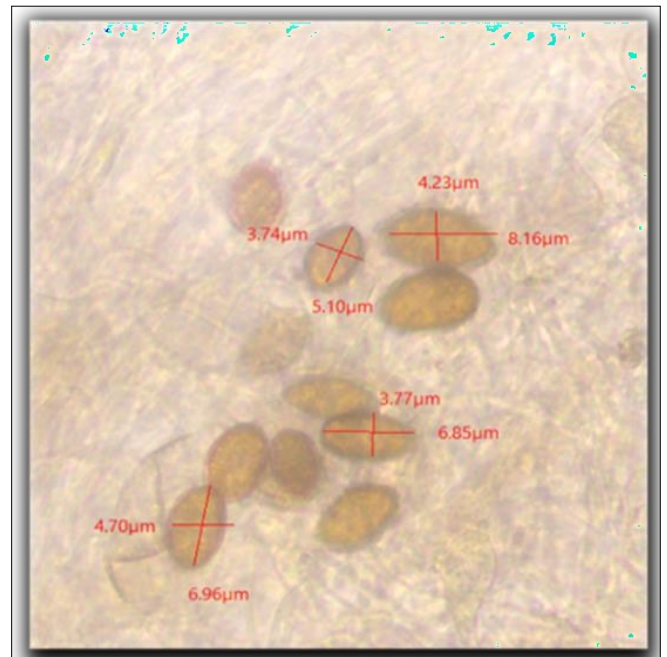


Fig. 5. Monosporous sporangia.

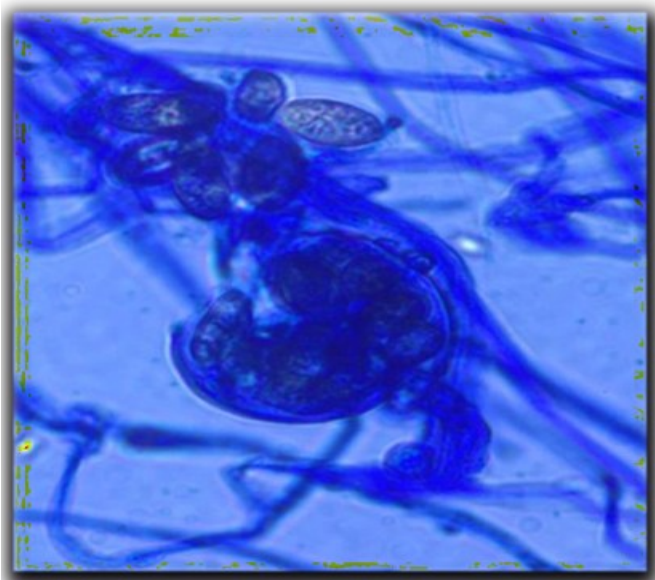


Fig. 6. Sporangiospores.

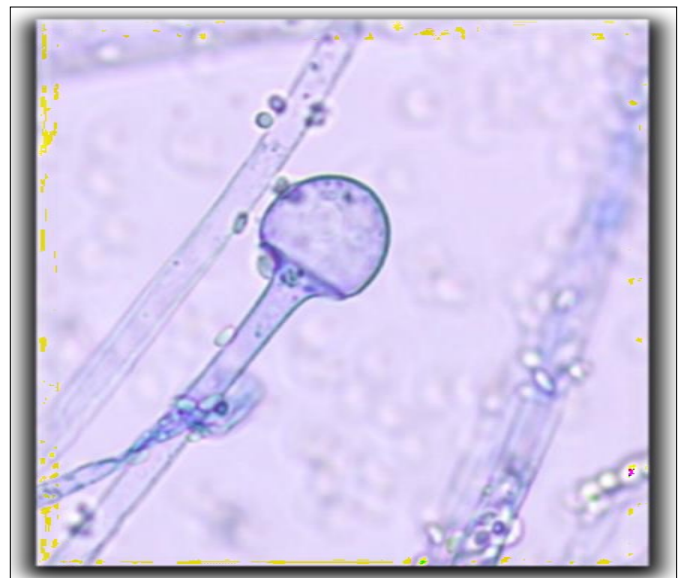


Fig. 7. Sporangiophore bearing sporangium with columella.

characters which are summarized below.

The mycelium was unbranched, without any septation and hyaline. Non-septate sporangiophores were observed. Spores were formed at the edges of the colony. Sporangiospores measured $6.19 \mu\text{m} \times 4.02\text{--}4.08 \mu\text{m}$ and had a light brown or dark brown colour. They might be elliptic, fusiform or ovoid in shape. The multispored, spherical sporangia were white or yellow when young and light brown or dark brown when fully grown. The sporangiola from monosporous spores were elliptical, fusiform or ovoid in shape with striations and dimensions of $5.10\text{--}8.16 \mu\text{m} \times 3.74\text{--}4.70 \mu\text{m}$.

Studies on pathogenicity

To determine the pathogenicity of the DB2 isolate fungus, detached fruit tests were carried out in conjunction with suitable controls (Fig. 8). After rinsing with three changes of sterile water, the dolichos bean fruit was surface sterilized with 0.1 % HgCl_2 for 30 sec. Every fruit in the circle was punctured using a sterilized stainless-steel needle. Fungal mycelial growth was then placed onto the fruit surface. Pods were placed in Petri dishes and incubated under dark conditions at 25 °C with 100 % relative humidity.

The onset and progression of symptoms were monitored periodically. After 5 days, both infected fruits and controls showed no symptoms of wet rot. The diseased tissues of artificially infected fruits were used for re-isolation once symptoms appeared. To establish the pathogenicity of the fungus, the re-isolated pure culture was compared with the original culture.

Studies on symptomology

Brown to black, initially water-soaked lesions formed on flowers and the affected tissue quickly rotted. Later, young pods became infected and dried, showing wet rot symptoms. Infected leaves became dried, showed upward curling and subsequently fell off. Whisker-like growths were observed on affected areas, crowned with black ball-like or pin-headed sporangial fructifications of the pathogen (Fig. 9). Severe infections caused early flower and fruit drop, reduced plant vigour and sometimes complete plant mortality. High relative humidity (> 90 %) increased the risk of infection. The effect of different nutritional media on mycelial growth and cultural characteristics is presented in Fig. 10, while agarose gel electrophoresis of genomic DNA and PCR products is shown in Fig. 11.

Molecular characterization of the pathogen

PCR amplification of the ITS-rDNA region of fungal isolate DB2 produced the expected amplicon size of approximately 600 bp as observed on 1.8 % agarose gel (Fig. 11). The ITS sequence-based phylogenetic analysis showed that dolichos bean *C. cucurbitarum* isolates clustered with reference *C. cucurbitarum* isolates from GenBank, forming a distinct clade (Fig. 12). The DB2 isolate (GenBank accession no. JQ776458.1) showed 99.83 % similarity with *C. cucurbitarum* based on BLASTn analysis (Table 3). Based on these results, the isolate was confirmed as *Choanephora cucurbitarum*.

In vitro efficacy of different fungicides against the test pathogen

All tested fungicides significantly reduced mycelial growth of *C. cucurbitarum* compared to the control. Hexaconazole (5 % EC) and



Fig. 8. Fruit detached method on dolichos bean fruit, showing typical wet rot symptoms on inoculated plates and control plates are symptomless.

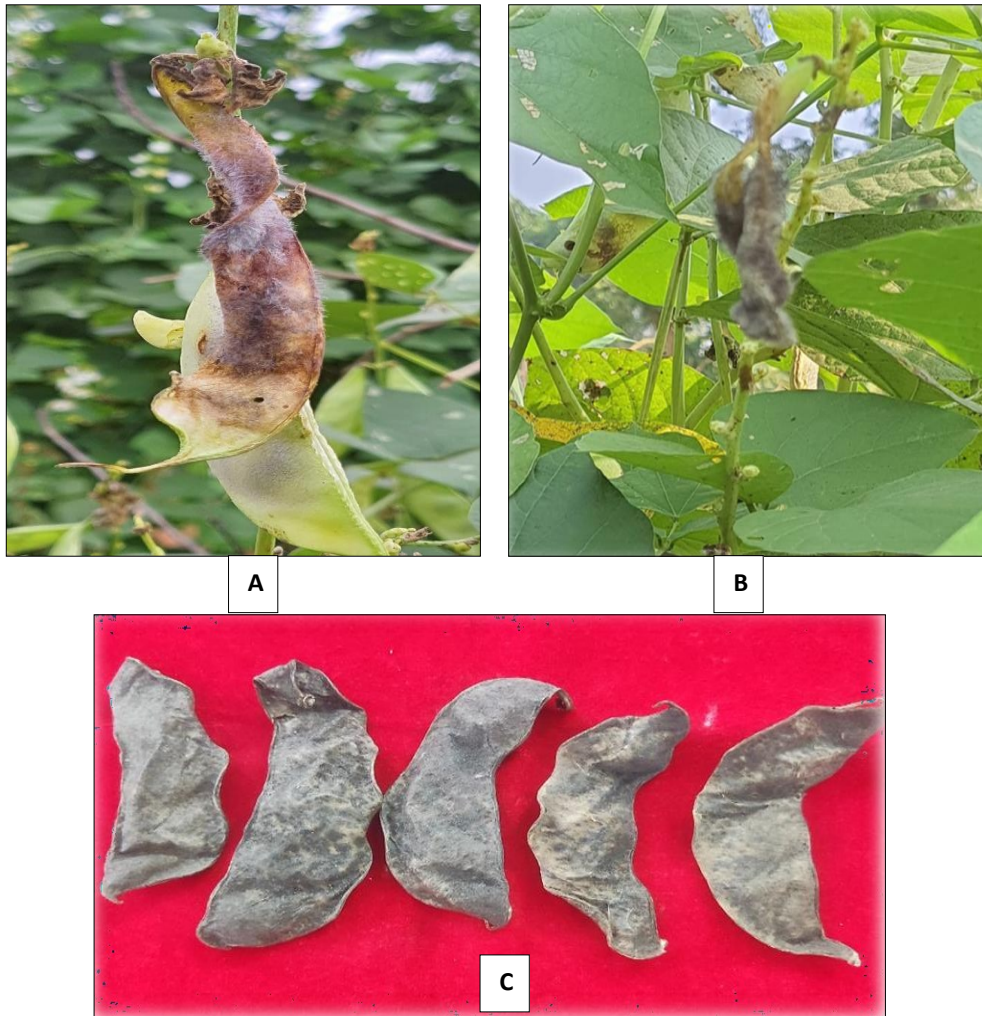


Fig. 9. Symptoms of *Choanephora* blight in dolichos bean. (A) Fruit rot or pod blight; (B) Flower rot or blossom blight; (C) *Choanephora* blight causing wet rot

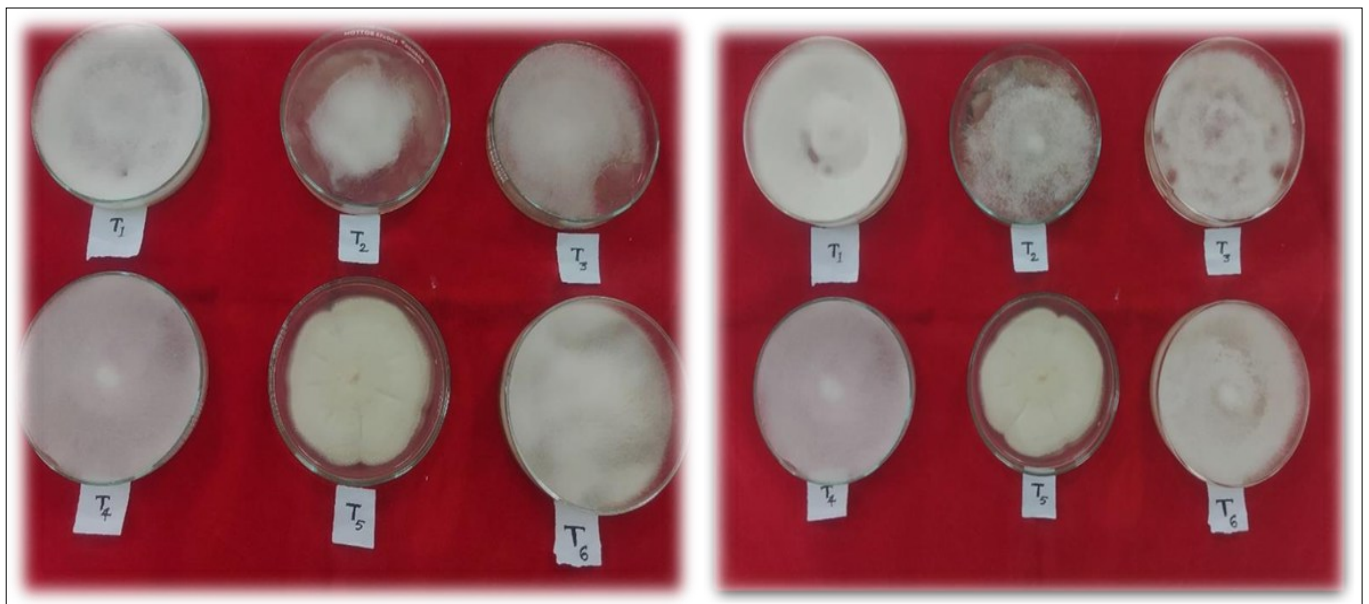


Fig. 10. Effect of different nutritional media on the growth and cultural characteristics of *Choanephora cucurbitarum*. (A) Effect of different nutritional media on mycelial growth; (B) Cultural characteristics of *Choanephora cucurbitarum* on different nutritional media

The ITS sequence of pathogen (Sanger sequencing).

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ATTCAGATCAAGTTTAAAAAATGATTTATTTGGGAGGCCCCCAAGATAGTCTTTTAACTAGAGCATTCTTTATATTAATAAAAAATGTTCCAGGCTAATAGAACAAATAGT
TCAGGCCTAATAGTTTTAAAGAGTTCAATTTTTACATCGATACTCTCACTTCCATTCAACAACAAATTTGGGATGTGGGTTGTTGTTGATACTGAAACAGGCGTGCTC
AATGGAATACCATTGAGCGCAAGATGCGTTCAAAGACTCGATGATTCACTGAATATGCAATTCACACTAGTTATCGCAGTTTGTCTACGTTCTTCATCGATGCGAGAA
CCAAGAGATCCGTTGTTAAAAAGTTGTTTTATAAGTTTTTACGCTTATGTTACAATATTAATTCTGAATTCCTTTGGTAAATAATGAATTAGGATACCAGGCCTAAGCCT
GACTTTAGCTAGGTTAACATCCTAAAGACCTATCCCTATAATCTTTAGGCAATCCCTCAAACGCTAAACAGTAAAACAGTTCACAGTAAATTAGAAATATGCATAAGC
ATACTCAAATTATTTAATGATCCTTCCGCAGGTTACCTACGGAACGATCT

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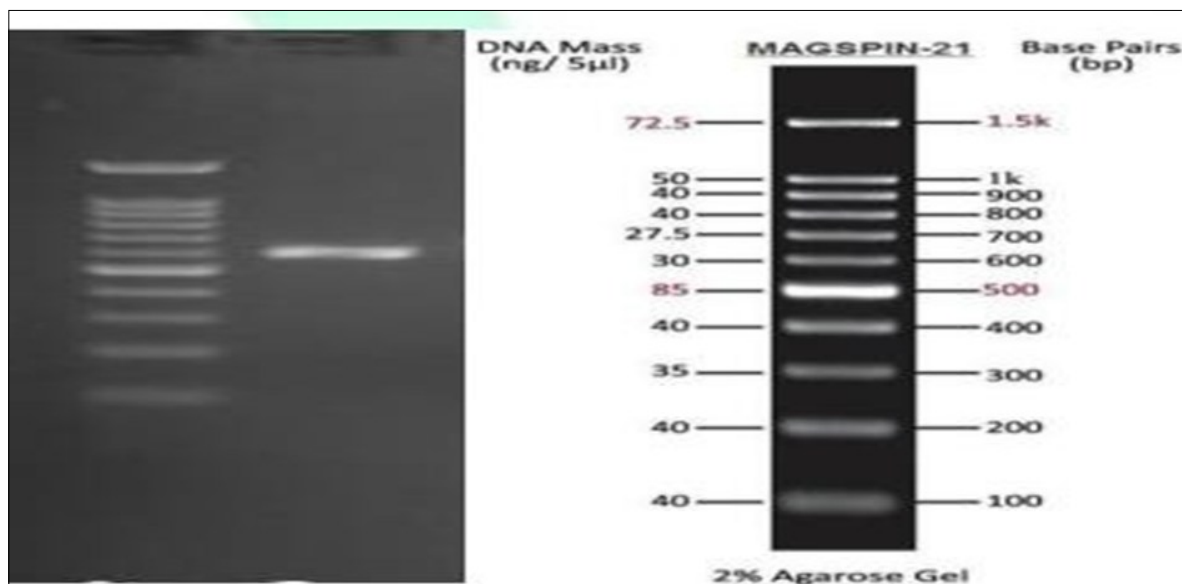


Fig. 11. Agarose gel electrophoresis of genomic DNA and PCR product.

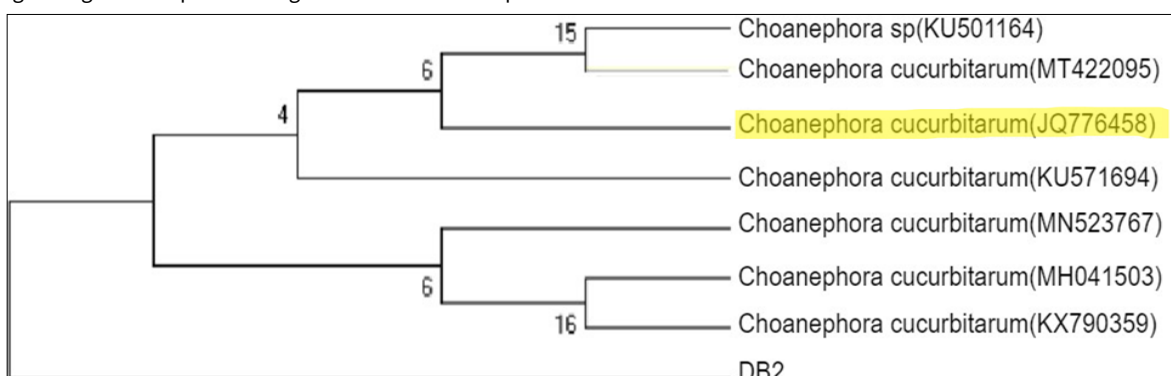


Fig. 12. Phylogenetic tree generated from ITS-rDNA sequence of *Choanephora cucurbitarum* obtained from DB2 isolate.

Table 3. Sequence homology observed for *Choanephora* sp. in BLASTn analysis as per BLAST results

Sl. No	Description	Max.score	Query coverage (%)	E Value	Identity (%)	Accession No.
1	<i>Choanephora cucurbitarum</i> strain SGF 39	1094	99%	0.0	99.83%	JQ776458.1
2	<i>Choanephora cucurbitarum</i> strain LY 57	1086	98%	0.0	99.74%	MN523767.1
3	<i>Choanephora cucurbitarum</i> strain BAB 4605	1077	98%	0.0	99.61%	KU571694.1
4	<i>Choanephora cucurbitarum</i> strain WYSFB- T3	1074	98%	0.0	99.49%	MT422095.1

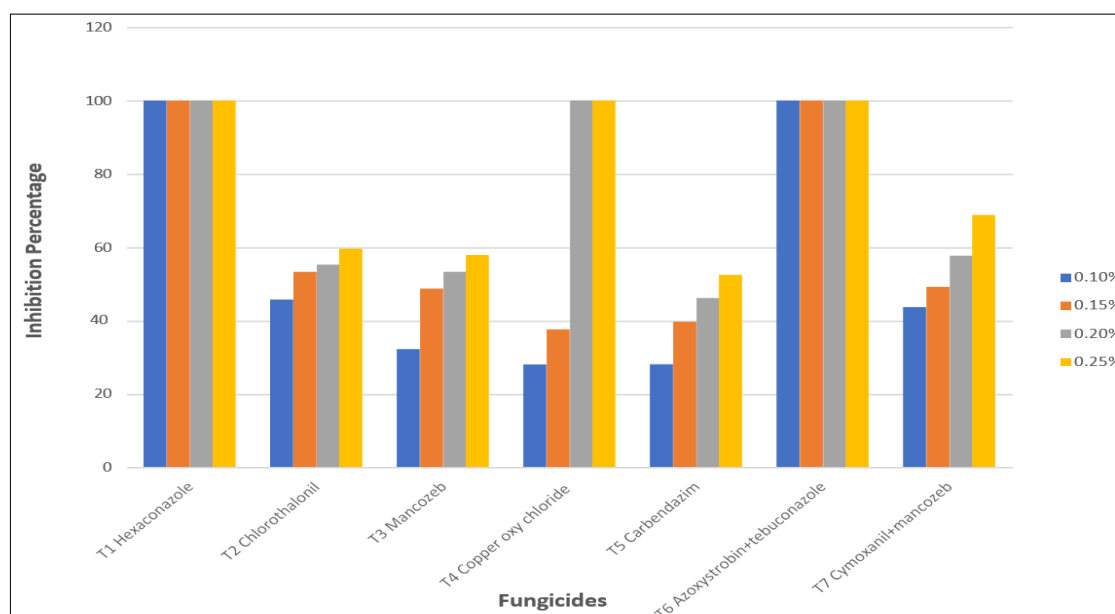


Fig. 13. Histogram showing *in vitro* efficacy of different fungicides against *Choanephora cucurbitarum* at different concentrations.

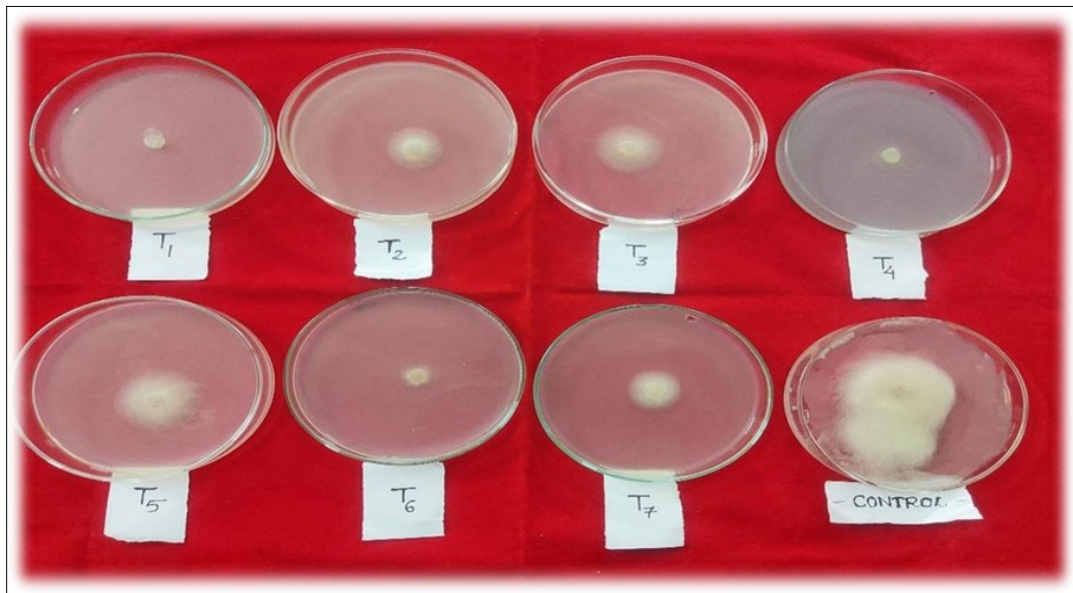


Fig. 14. Effect of different fungicides against on *Choanephora cucurbitarum*.

azoxystrobin (12.5 %) + tebuconazole (12.5 % SC) completely inhibited mycelial growth (100 %) at all tested concentrations. Copper oxychloride (50 % WP) also showed complete inhibition at 0.2 %. Mycelial inhibition by cymoxanil + mancozeb, chlorothalonil and mancozeb increased with concentration, reaching 68.88 %, 59.64 % and 57.33 %, respectively, at 0.25 %. Carbendazim showed the lowest efficacy against the pathogen. The comparative efficacy of fungicides is presented in Fig. 13 & Fig. 14.

Discussion

Incidence rates of disease range from 17–33 %, according to the results of a nationwide study. Disease incidence was lowest at 17 % in AICRP on Vegetable Crops, OUAT, Bhubaneswar and highest at 33 % in Gelapur village, Khordha District. Cuttack (19.0 & 32.0 % of the total) and Ganjam (23.1 & 28.0 % of the total) are two additional districts. Localized weather patterns and farming techniques are responsible for the difference in infection rates among locales.

Isolating the sick samples in PDA medium was a part of the survey process. Hyphal tip and single spore procedures were used to purify the fungus. Aerial mycelial growth originally white, eventually became a light yellow as the fungus progressed. Aerial mycelial growth originally white, eventually became a light yellow as the fungus progressed. The mycelium lacked branches, septa and was transparent. Sporangiospores measured $6.19 \mu\text{m} \times 4.02\text{--}4.08 \mu\text{m}$ and had a light brown or dark brown color. They might be elliptic, fusiform or ovoid in shape. The multispored, spherical sporangia were white or yellow when young and light brown or dark brown when fully grown. The sporangia that reproduced monosporously were elliptical, fusiform or oval in shape, striate and were $5.10\text{--}8.16 \mu\text{m} \times 3.74\text{--}4.70 \mu\text{m}$. The pathogen was determined to be *C. cucurbitarum* using cultural and morphological characteristics, as well as descriptions provided in previous study (33). The results of our investigation agree with previous reports of *C. cucurbitarum*, as the sporangia observed were indehiscent, striated longitudinally and ellipsoid to broadly elliptical, as described in other studies (11, 25).

The fruit detached approach was used to artificially inoculate dolichos bean fruits with the pathogen. Five days following inoculation, fruits exhibiting the classic symptoms of wet rot lesions

saturated in water and indicative of sporangia emerged on the inoculated plate, but the untreated control plate remained unaffected. Supporting the current conclusion, an observation was made in a previous study comparable to the pathogenicity test (26). Lesions that were originally drenched in water and became dark to black appeared on flowers; the afflicted tissue decayed away rapidly. Wet rot symptoms manifested in dried pods that had previously been affected in immature pods. The diseased leaves eventually dry up, curl upward and eventually fall to the ground. The pathogen's sporangial fructifications appeared as whisker-like growths on the afflicted regions, topped with a black ball or little black-headed pin-like structures. Early fruit and blossom drop, reduced plant vigor and plant mortality were all symptoms of severe infections. The appearance of a white-stalked sporangiophore on the surface of infected tissues, topped with a black head like a mulberry, like a black-headed pin, was a common indicator of this fungal invasion. *Amaranthus*, eggplant, dolichos bean, yard long bean and *Hibiscus syriacus* are among the plants that have been shown to exhibit similar symptoms (7, 13, 27, 28).

The development and sporulation of fungus are affected by its nutritional requirements. The fungus was cultured in 6 different conditions to find out which one was ideal for growing the pathogen. After evaluating 6 different media, including carrot agar, potato carrot infusion agar, Czapek Dox Agar, V8 Juice Agar and host extract agar, the fungus showed the highest radial growth on PDA (90.0 mm), which made it the best medium for growing *C. cucurbitarum*. The next best was potato carrot infusion agar (89.08 mm) and V8 Juice Agar (84.46 mm). Among the media tested, PDA supported the maximum mycelial growth. On the other hand, carrot agar showed the slowest development (47.70 mm). However, after 5 days post-inoculation, Czapek Dox Agar (80.35 mm) and host extract agar (83.23 mm) both showed excellent radial fungal growth. Results from fungus studies are consistent with the present investigation and previous arguments suggesting that PDA provided ideal circumstances for the fungus's development (29, 30). The cultural similarities and differences were shown by the investigation of several culture mediums with different carbon and nitrogen supplies. In contrast to the white mycelial growth seen on host extract agar, the fungus displayed rosette-like structures on V8 Juice Agar, dense mycelial growth on potato carrot infusion agar and puffy

mycelial growth on Czapek Dox Agar. Over time, the color of the colony went from white to a dirty white and then to yellowish hue. The findings of the present study agree with earlier reports (7, 31, 32).

Pathogens were grown on PDA media to examine their morphological features. It was noted that the mycelium lacked branches, septa and was transparent. Sporangiospores measured 6.19 μm x 4.02–4.08 μm and had a light brown or dark brown color. They might be elliptic, fusiform or ovoid in shape. The multispored, spherical sporangia were white or yellow when young and light brown or dark brown when fully grown. The sporangia that reproduced monosporously were elliptical, fusiform or oval in shape, striate and were 5.10–8.16 μm x 3.74–4.70 μm . These results agree with previous studies (7, 32).

Heredity biosciences lab in Bhubaneswar performed the molecular characterisation of the infections. Based on the results shown on the 1.8% agarose gel, the anticipated amplicon size of 600 bp was produced by PCR amplification of the ITS-rDNA region of fungal isolate DB2. This DB2 isolate (Gene bank accession no. JQ776458.1) showed 99.83% similarity to *C. cucurbitarum* in the nucleotide BLASTn examination. Dolichos bean *C. cucurbitarum* isolates and reference *C. cucurbitarum* isolates from Genbank formed a separate clade in a phylogenetic tree built using the neighbour-joining technique. Therefore, the results showed that the isolate used in this research was indeed *C. cucurbitarum*.

To guarantee agricultural output and food security, plant disease control is essential. One of the most important ways to prevent fungal infections is to use fungicides. The effectiveness of commonly used fungicides in India was tested in a controlled environment. Hexaconazole (5% EC) and azoxystrobin (12.5%) + tebuconazole (12.5% SC) were the only 2 of the 7 drugs that completely inhibited mycelial growth (100.0%) at all dosages tested. On the other hand, mycelial development was completely inhibited (100.0%) by copper oxychloride (50% WP) beginning at a concentration of 0.2%. Concurrently, at a concentration of 0.25%, the mycelial inhibition of cymoxanil + mancozeb (WP), chlorothalonil (75% WP) and mancozeb (75% WP) rose with increasing concentration, reaching peaks of 68.88%, 59.64% and 57.33%, respectively. Carbendazim (50% WP), on the other hand, was the least effective against the test pathogen across the board. In line with our present results, prior research has shown that triazole-based fungicides such as hexaconazole, propiconazole, triadimenol and bitertanol are effective against pod rot of cowpea and *Choanephora* blight of winged bean flowers (33, 34).

Conclusion

The study investigated *Choanephora* blight, a new and emerging threat to dolichos bean cultivation in Odisha, India. With disease incidence rates ranging from 17–33% across surveyed regions, Khordha district showed the highest infection rate. Pathogen isolation confirmed *Choanephora cucurbitarum* as the causative agent, identified based on morphological traits and molecular analysis. Artificial inoculation trials on dolichos bean fruits reproduced wet rot symptoms, corroborating previous studies. Nutritional evaluations revealed PDA as the most conducive medium for fungal growth. The study highlights the necessity for management of the disease through safer fungicides to mitigate the economic impact of *Choanephora* blight on dolichos bean

cultivation. Fungicides evaluation demonstrated complete mycelial growth inhibition with hexaconazole (5% EC) and azoxystrobin (12.5%) + tebuconazole (12.5% SC) and copper oxychloride, whereas mancozeb and carbendazim showed lower efficacy. Findings align with previous research indicating triazole-based fungicides' efficacy in managing similar fungal pathogens. Field evaluations of promising fungicides are recommended to develop practical, sustainable disease management practices.

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Authors' contributions

BA conducted the field disease assessment, collected samples, assisted in pathogen isolation and contributed to drafting the manuscript. SS supervised the study, verified pathogen identification, designed the experimental layout and critically revised the manuscript. AM supported pathogen biology studies and reviewed the manuscript. PB assisted with microscopy work and documentation, while SSP helped in sample processing and culture maintenance. UB supported data recording and analysis. AB and DB contributed to the literature review and manuscript preparation and KKM assisted in field coordination and disease scoring. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

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