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Original Article

Isolation and Characterization of Locally Isolated Jute Fungi and Determination of their Pathogenicity

Md Abul Kashem[1], Md Towhid Hossain[1*] and M N Anwar[2].

[1]Department of Microbiology, Faculty of Biological Sciences, University of Chittagong, Chittagong 4331, Bangladesh, [2]Vice Chancellor, Port City International University, South Khulshi, Chittagong, Bangladesh.

Currently Jute farmer faces a number of problems in jute sector such as higher labor cost, fungal diseases, low market price, natural disasters etc. As a result the production of jute is far below than the desired figure. The severe yield lost of jute depends on a number of factors of which fungal diseases play a dominant role. In the present investigation 56 samples of infected jute plants were collected from 8 (Eight) different area of Bangladesh and the samples were investigated by Agar plate and Blotter methods. Symptoms of diseases were recorded and associated fungal pathogens were purified and characterized. Based on cultural and morphological characterizations three fungal pathogens were identified as *Botryodiplodia theobromae* Pat. (Griffon & Maubl.), *Colletotrichum corchori* (Ikata and Yoshida) and *Macrophomina phaseolina* (Tassi) Goid and allthe isolates showed their pathogenicity on jute plants.

Key words:Isolation, Characterization, Jute, Fungi, Pathogenicity

Introduction

Jute is a natural fibers yielding annual herbaceous plant in the world [1, 2]. It is an attractive natural fiber for use as reinforcement, low cost renewable textile fibers and is obtaining from the bast/ phloem layer of the stem from mature jute plant.

Jute plays a vital role in the economy of Bangladesh. Now-a-days, Jute cultivation is alleged to be a losing concern because of fall of business in the International market signaling a great threat to the economy of Bangladesh[3]. Annually approximately 8 lakh metric tons of raw Jute fiber are produced from nearly 6 lakh hectares of land that offers cash income to 40 lakh farmers. About 8 lakh people are engaged in different phases of industry and trade of Jute[4]. At the present time raw Jute or green Jute is being used in paper mills. This is very good alternative use of Jute.

Presently in Bangladesh the production of Jute is greatly deceased. This is because of many constraints that reduced the yield of the crops and fungal disease is one of them. The pathogen may affect certain group of chemical substances/enzymes secreted in the host tissues during penetration and infection[5, 6]. The loss of production due to disease is about 8-20% with variation in severity from year to year[7]. The fungal diseases are not only responsible for yield lost but also deteriorate the quality of fiber and seeds.

Therefore, present work deals with the isolation and characterization of locally distributed fungi and their pathogenicity on jute plants.

Materials and Methods

Samples and sampling sites

In the present study 56 samples frominfected parts (stem, seeds etc.) of jute plant were collected (suspected for infection) from 8 (Eight) different regions of Bangladesh. After collection, samples were carefully preserved in the refrigerator at 4°C for further studies.

Isolation and purification

To facilitate the germination of fungal pathogens on plant parts, Blotter and water agar methods were used. During isolation the samples of infected plant parts (except seed) were cut into 1-2 cm long piece. All the samples (the pieces of individual plant parts/seeds) were treated separately into 1:1000 $HgCl_2$ solutions for 1 minute in aseptic condition and shake properly[9]. Then the samples were washed at least 6-7 times with sterile distilled water thenput on a sterile moist blotter or water agar mediawere as follows:

Detection of fungi by blotter method

Samples (seed/plant parts) were analyzed for the detection of associated fungi by the blotter method as recommended by the International Seed Testing Association (ISTA) with some modifications[9]. Three circles of blotter were soaked in distilled water and placed on the bottom of the sterilized petri dishes. At the rate of 25 seeds per dish, 400 seeds were studied in aseptic conditions. The petri dishes with the seeds were then incubated at room temperature (±27°C) on the laboratory desk under diffused day light and fluorescent tube light at night. The major

Corresponding author:
Dr. Md Towhid Hossain, Professor, Department of Microbiology, Faculty of Biological Sciences, University of Chittagong, Chittagong 4331, Bangladesh, Email: towhid.mbio@cu.ac.bd

modification of the international rule of seed testing was the use of fluorescent tube light at night and diffused day light[10]. After 5 to 12 day of incubation, the germination and the fungi associated with the seeds were recorded.

Detection of fungi by Agar plate method

In this method three samples were selected from the same lot which used during blotter method to observe the differences in the rate of germination and the rateof individual fungi on the agar plate and blotter. The seeds were placed in petri dishes containing approximately 20 ml of water agar medium. Seeds (taken randomly) were placed in 16 replicate petri dishes at the rate of 25 seeds per dish. The petri dishes with the seeds were then incubated aseptically at room temperature (±27°C). After 5 days of incubation the occurrence of fungi were recorded and observation was continued up to twelve days of incubation[11].

For the isolation of target fungal pathogenspour plate and streak plate methods on potato dextrose agar (PDA)were usedand the temperature was maintained 27ºC for 4 to 5 days as per requirements.From these plates, some morphologically distinct single colonies were selected aspure culture of fungal isolates. The purifiedisolates were designated as Mac_1 to Mac_7, Col_1 to Col_7, Bot_1 to Bot_7, A. nig_1 to A. nig_7, A. $flav_1$ to A. $flav_7$, Cur_1 to Cur_7, Alt_1 to Alt_7 and Pen_1 to Pen_7.

Maintenance and storage of the isolates

The purified isolates were maintained on PDA slants and preserved at 4ºC in a refrigerator as stock culture. Periodically sub-culturing was maintained to keep the culture in active condition with characters unimpaired.

Cultural and morphological characteristics of the fungal isolates

The selected fungal isolates were grown on potato dextrose agar (PDA) mediumand they were then characterized on the basis of their cultural and morphological properties.During microscopic study, the size and shapes of vegetative cells, mycelia color, spore size, spore shape etc. were considered.

Determination of Pathogenicity

In the present investigation, susceptible varieties of Jute seeds (O-9897) were collected from Mymensingh Jute Research Centre, Bangladesh. The pathogenicity test of fungi was done by artificial inoculation of the pathogens inoculum applied on3 days old jute seedling surface. After inoculation, the inoculated plants were incubated at room temperature for infection.Non inoculated control seedlings were similarly treated with distilled water. The results were recorded after 5days of incubation[12].

Results and Discussion

Selection ofsamplesbased ontheir symptoms

Based on symptoms of diseases, fifty six samples were collectedfrom jute cultivated field.During sampling different symptoms ofdiseases on plant parts werecarefully observed and recorded and at the same time photographic feature were also taken (Figure 1 to 3).Three symptoms (black band, Anthracnose and stem rot) were observed in most of the cases. Finally, three jute samples with different symptoms were randomly selected for detail study. Symptoms of samples were as followed:

Disease symptoms of sample -1

Usually infection wasstarted on leaf then the lesion gradually reachednode through petiole and became dark-brown in colour. The spot encircled the stem which later on became internally rotten and breaks causing death of the plant. Several brown to blackish dotsappeared in the lesion (Figure 1).

Figure 1. *Symptoms (Stem rot) on jute stem of sample -1*

Disease symptomsof sample -3

At early stage black spot occurred on the stem. It grew and encircled the stem. Dense-black bands developed around the stem in the matured plants. On rubbing hand on diseases stems, finger became black due to dark spore. The fungi attacked all parts of the plants and such affected plantsultimately loosen all leaves and remained in the field, as dry black stem (Figure 2).

Figure 2. *Symptoms (Black band) on jute stemof sample -3*

Disease symptomsof sample -7

The symptomfirst appeared as yellowish brown lesions of variable shape with size, water-soaked, depressed spots on the stem, which soon developed into characteristics irregular spots. The color of the spot might became dark brown and finally black (Figure 3). The entire stem became covered with numerous black acervuli, with sparse, black setae.

Isolation and purification

The isolation of fungal pathogen from infected jute sampleswas carried out by Blotterand water agar methods. The purification of the fungi was done by streak and pour plate methods.During isolation, three fungal isolates in sample 1, 3, and 7 showed vigorously growth on blotter/ agar media which were isolated as pure form and designated as Mac_1, Bot_3 and Col_7 respectively. These three isolates were finally selected for further study.

Characterization and Identification of the isolates

Isolate ID Mac_1:

Cultural characteristics

Colour of the colonies of Mac_1 in culture ranged from white to brown or gray and dark with age, broadly spreading, largely submerged with little aerial hyphae (Figure 4A).

Microscopic characteristics

Hyphae were brown, septate, usually containing numerous vesicles. Hyphal branch generally formed right angles to parent hyphae. Microsclerotiawere formed, aggregated of hypal cells joined by many individual cells. The pycnidiawere100-200ìm in diameter, dark to grayish; became black with age, globose or flattened globose, membranous to subcarbonaceous with an inconspicuous or definite truncate ostiole (4B). Conidiophores rod shaped, 12-16ìm long. Conidia were single celled, hyaline, thin walled and elliptic or oval, ranged of 15-30ìmx7-10 ìm.

Figure 3. *Symptoms (Antracnose)on jute stem of sample -7*

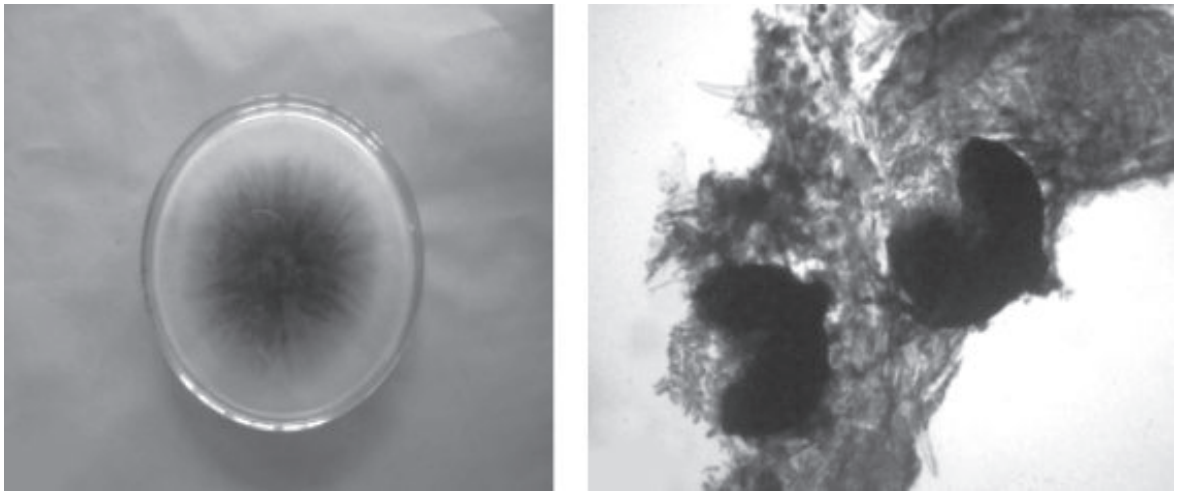


Figure 4 (A-B). ***C****ultural and microscopic features of isolate Mac_1*

Identification

The isolate Mac_1 were provisionally identified as *Macrophominaphaseolina.* (Tassi) Goid.

Isolate ID Bot_3:

Cultural characteristics

The mycelium of the isolated fungus Bot_3, were found to grow vigorously on PDA media. The aerial mycelia grew uniformly in all direction and fully covered the media surface with in 3 and 4 days (Figure 5A). Mycelia growth pattern was aggregated with fluffy appearance (wooly/velvety).The colour of the mycelia colony changed gradually from light grey to grayish black within 2 weeks of incubation. The back ground colour of the media only became darker after 3 weeks of incubation. Sometimes, exudations in the form of hyaline drops were observed on velvety mycelial mat. The fungus produced stromata or pycnidia. The pycnidia were initially soft but hardened when the culture matured after 4 weeks of incubation. The culture sporulated only after 4 weeks of incubation.

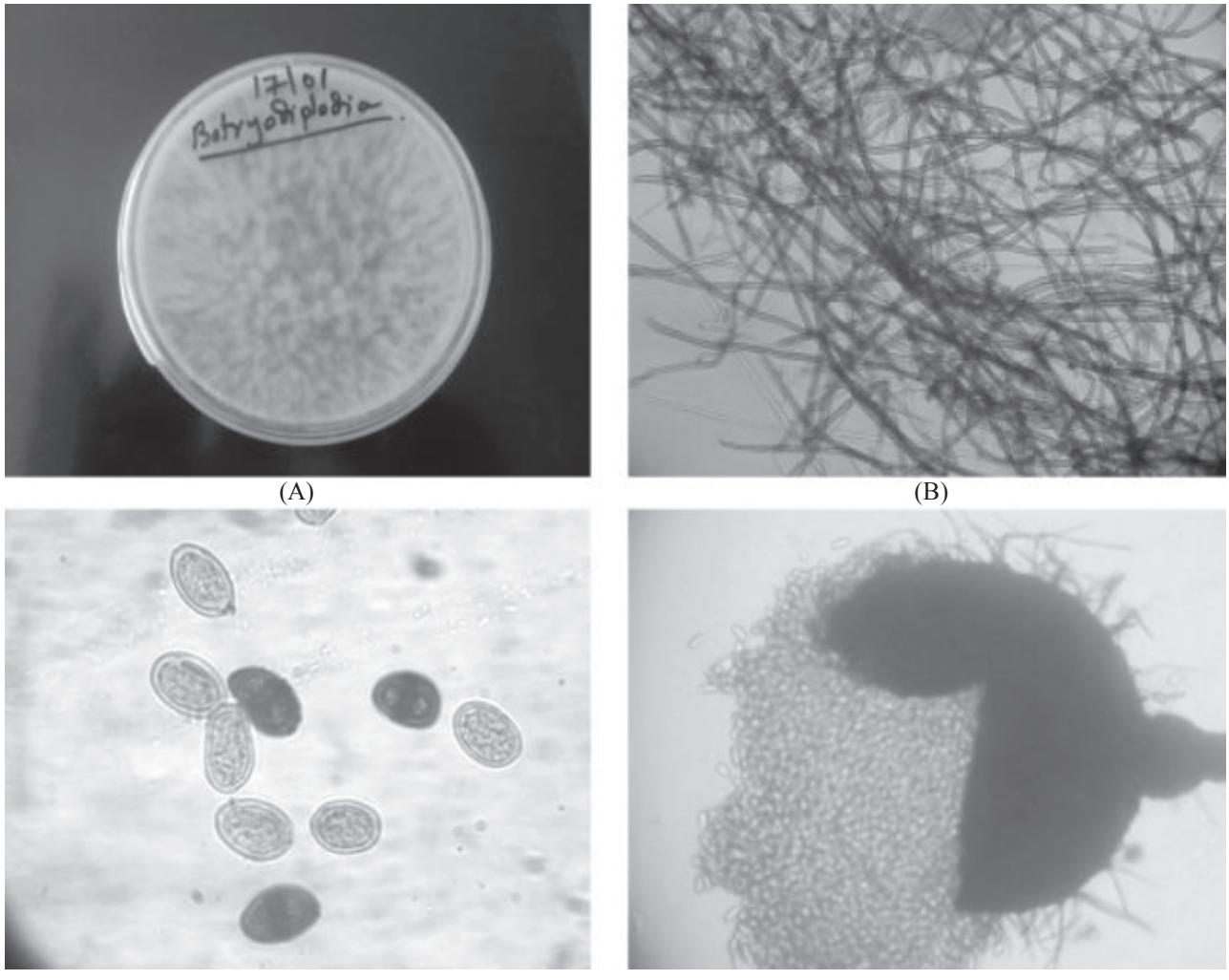
Microscopic characteristics

Matted hyphae observed to be forming stroma, which contained several pycnidia. The presence of pycnidia was regular, round, flask shaped, situated superficially or partially immersed in the substrate. The mycelia were brown, septet (Figure 5B) with big and numerous stroma. Pycnidia black, 180-220ìm in diameter, more or less hairy, on a hairy stroma. Conidiophores were hyaline, 40-50ìm long.

The fungus produces conidia with some distinct features. The immature conidia were non septet, thick cell wall, oval in shape and hyaline (Figure 5C). However, the mature conidia showed slightly different feature. Mature conidia were observed to be septate, oval-shaped or elongated and slightly brownish in color with the presence of irregular longitudinal striations (Figure 5D). The conidia ranged of 25-35ìm x10-15ìm.

Identification

The isolate Bot_3 were provisionally identified as *Botryodiplodiatheobromae*Pat.(Griffon &Maubl.)



(A) (B)

(C) (D)

Figure 5. *(A). CulturalGrowth of Isolate Bot_3onPDA. (B). Mycelial mat of isolate Bot_3. (C). Young and mature conidia of isolate Bot_3. (D). Pycnidia with pycnidiospre of isolate Bot_3*

Isolate ID Col_7:

Cultural characteristics

The colony colour of Col_7varied from white to gray or pink gray, while the reverse side the colonies was white, dark gray, orange or a mixture and with regular colony margin. The growth pattern were either circular with mycelia showing a uniform growth pattern and radial (Figure 6A)

Microscopic characteristics

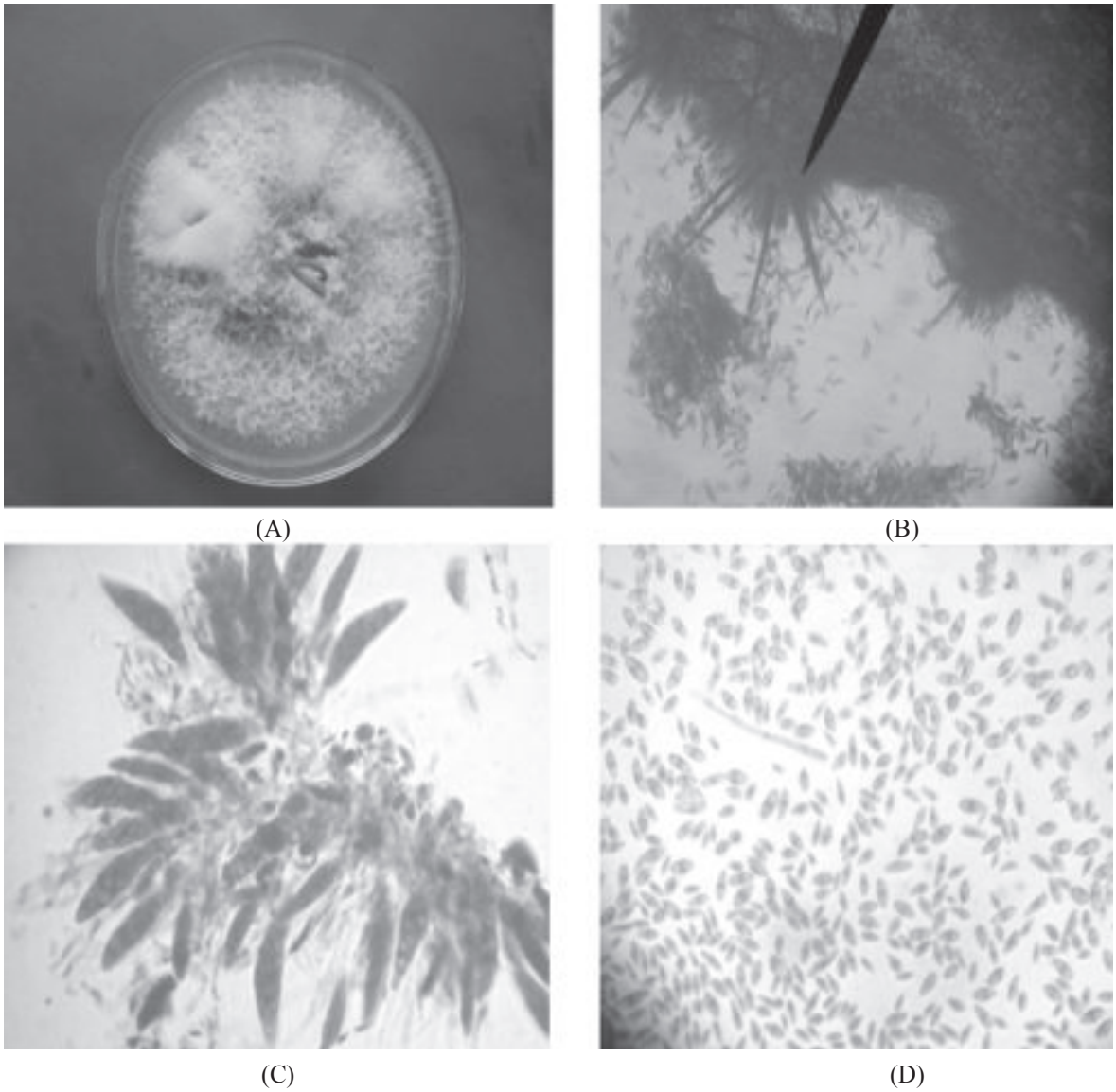
The mycelium was hyaline, branched and septate, at times spares with floccose, loose or compact growth.Conidium produce in cushion like acervulus developed on the cankrous tissues. Black setae occur sparingly (6B). The conidia were hyaline, thin-walled, and cylindrical with both apices rounded or with one apex rounded and the other end pointed, slightly curved, one-celled. Typically elongated with rounded ends, characteristically are slightly narrower in the middle than at the ends (Figure 6D). Conidia with length to width ratio of about 3:1. The conidia sizes varied from 10-20ìm x 4.5-9ìm. Interesting and lucky event during characterization of Col_7 was to observe the perfect stage of infected seedlings of jute plant (Figure 6C).

Identification

The isolate Col_7 were provisionally identified as *Colletotrichumcorchori* (Ikata and Yoshida[13])

Determination of Pathogenicity of the isolated fungi on jute plants

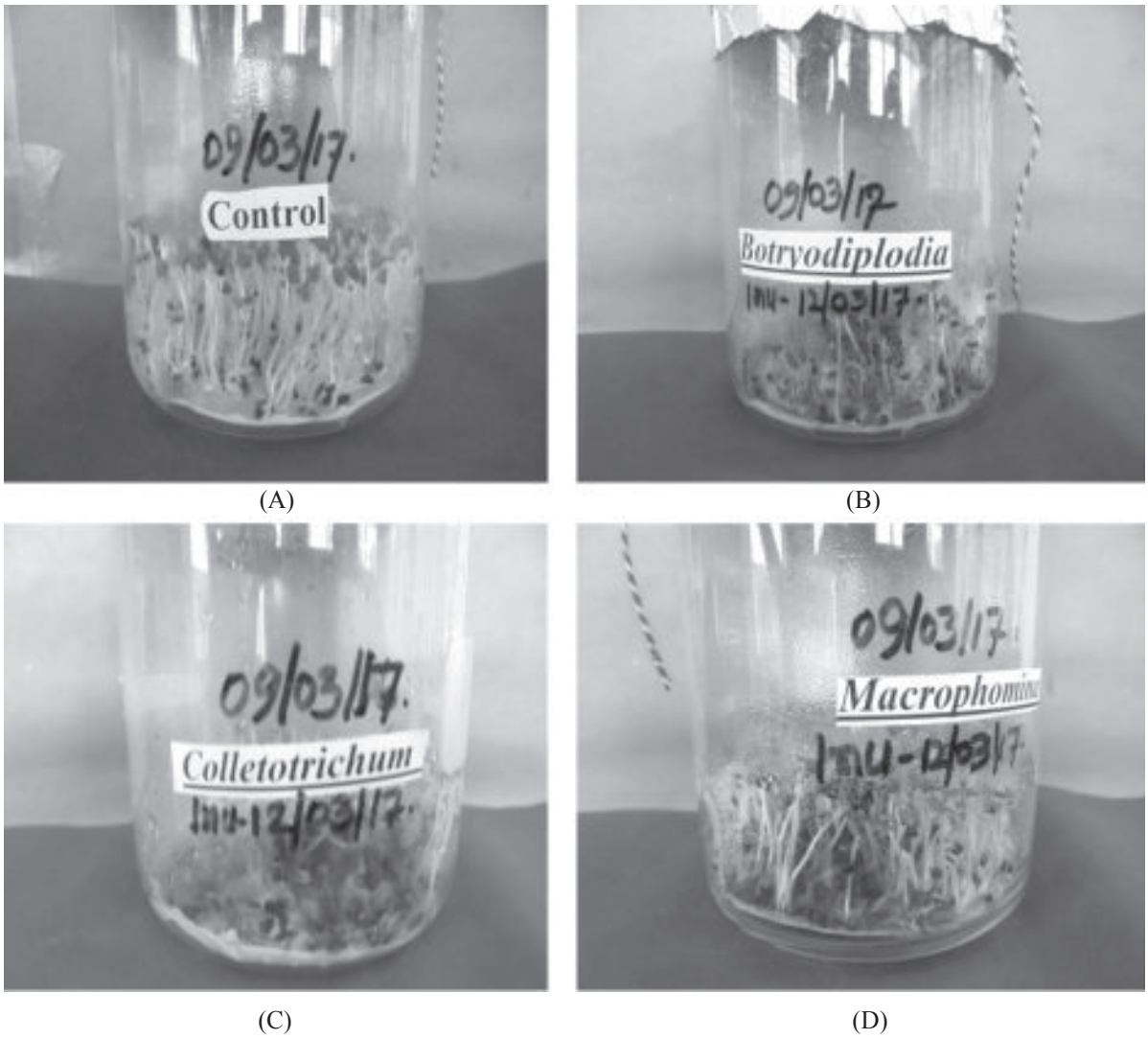
The result of pathogenicity test of three fungal isolates showed that the isolate *Botryodiplodiatheobromae* (Bot_3), *Colletotrichumcorchori*(Col_7), *Macrophomina phaseolina* (Mac_1) were found to bepathogenic on jute seedlings.Maceration of tissues of the susceptible seedlings occurred after infection (Figure7). All the isolates re-isolated from infected seedlings. The re-isolation of allthe pathogens from infected jute seedlings



(A) (B)

(C) (D)

Figure 6(A-D)**.** *Microscopic features of isolate Col 7.*



(A) (B)

(C) (D)

Figure 7. *Determination ofPathogenicity after 5days of incubation.*

revealed that all the fungi played significant role in the development of disease.A number of researchers worked on pathogenicity of different plant pathogenic fungi and found that *Macrophomina phaseolina*[12, 14], *Colletotricum sp*. [15] and *Botryodiplodia theobromae*[16] showed their potentiality in the development of disease in plant. Our results are in concurrence with their report.

Conclusion

In the present investigation the isolate *Botryodiplodiatheobromae* (Bot_3), *Colletotrichumcorchori* (Col_7) and *Macrophominaphaseolina* (Mac_1) were responsible for stem rot, black band and anthracnose disease in jute plants respectively and caused considerable damage. Fungal infection usually results in drastic changes in biochemical and physiological process of the host. Pathogens establish a close structural and nutritional relationship with jute plants.Therefore, isolation of locally distributed pathogens will be helpful during further studies to observe therole of enzymesof these fungi duringhost-parasite interactionin jute plants.

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