Progress Report

Determining the Status of Diseases of Natural Rubber Plant in Bangladesh

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This is an interim report on Morphological and molecular characterization of the causal agents of rubber leaf disease. The final report will be submitted at the end of the year, 2023.

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The main objective of the research project is to identify and determine the status of undiscovered diseases of natural rubber in Bangladesh so that further research can be undertaken to prevent those diseases

Background:

Rubber is an important industrial commodity in Bangladesh. Diseases are one of the limiting factors for rubber production. It reduces the quality of rubber and also can cause remarkable losses in the rubber plantation industry (Oktavia et al. 2021). Different types of diseases appear on rubber leaves, stems, fruits and roots (related to SDG 15: Life on Land). However, no researches have been conducted yet to identify the pathogens of rubber diseases in Bangladesh.

In order to address and determine the Status of Diseases of Natural Rubber in Bangladesh, the Bangladesh Rubber Development Board (BRDB) financed the above-mentioned research project for a period of 8 months (session 2022-2023). Prof. Dr. Md. Amin Uddin Mridha, Research Professor of Daffodil International University led the project as the principal investigator. A team of relevant experts and students worked on this project. The main objective of the research project is to identify and determine the status of undiscovered diseases of natural rubber in Bangladesh so that further research can be undertaken to prevent those diseases for improvement of sustainable rubber production and development of the rubber industry not only in Bangladesh but across the world.

Five isolates primarily identified as Pestaliopsis, Neopestaleopsis and Pseudopestaleopsis fungi based on conidial characters

Progress and Methodology:

In July 2023, a large number of leaf spot infected rubber leaf samples and one fruit sample were collected from Raozan rubber garden and Khagrachori rubber gardens, Chittagong. Among the leaf samples, five samples showed brown spots, which gradually expanded to large with irregular necrotic lesions. Rest of the two samples showed dark spots with lighter center with chlorotic halos whereas, one infected fruit sample showed circular shaped scattered spots. For pathogen isolation, small tissues (5x5 mm) from lesions of each category were sterilized by 0.3% sodium hypochlorite for 2 min and rinsed with sterile distilled water three times and air dried. The leaf and fruit tissues were then incubated on potato dextrose agar (PDA) plates at 28 °C for seven days. The colonies developed on five plates were whitish to creamy with concentric rings and waved edges. These five isolates primarily identified as *Pestaliopsis*, *Neopestaleopsis* and *Pseudopestaleopsis* fungi based on conidial characters (Maharachchikumbura et al. 2014). All of the isolates produced five celled conidia which were ellipsoid, straight on slightly curved with three colored median cells and two colorless terminal cells. Two isolates of *Pestaliopsis* with three median cells were versicolorous, light brown to olivious with clear end two cells. The sizes of conidia were ranged from 11.5-15.4 µm x 5.4-6.3 µm. Other two *Pseudopestalotia* isolates conidia were versicolorous, 16.7-21 µm x 3.2-5.1 µm with three median dark brown cells. Therefore, conidia of *Neopestaloptiosis* were also present with versicolorous, two upper cells dark brown and one lower cell light brown conidia, ranges from 14-18.7 µm x 3.2-5.1 µm.

The fungal plates of two other isolates were very fast growing. Dense, aerial whitish cottony mycelium grew which turned to dark black after 7 days of incubation. Immature conidia were unicellular and a septate formed around on 15 days old culture with the size of conidia were 15.4-20 µm x 5-8.5 µm. Mature conidia were oval in shape and brown in color. These morphological characters were similar to descriptions for

The fungi were re-isolated from inoculated leaves and confirmed as Pestaliopsis sp., Pseudopetaliopsis sp., Neopestaliopsis, Colletotrichum sp., and Lasiodiplodia sp. through morphological feature observation.

Lasiodiplodia sp. (Phillips et al. 2013). In addition, one leaf isolate colony on PDA was grey, with the leading edge of growth was white. Conidia were hyaline, septate, and cylindrical with obtuse ends, 12-18 µm x 3.2-5.8 µm. These morphological features confirmed the identity of the fungus as Colletotrichum (Yang et al. 2009).

To verify pathogenicity, three leaves of rubber plants at the light green stage were taken for each isolate. All leaves were surface sterilized and wounded with sterile needles. 5 mm PDA plugs of 3-days- old culture were inoculated on the lower part of leaves. Other three leaves were treated with PDA plugs without mycelium as a control. The inoculation site was kept moist in a plastic box at 28 °C with 100% relative humidity. After 3 days, lesions appear on the inoculated sites similar to those observed with the spots in the infected leaves. The fungi were re-isolated from inoculated leaves and confirmed as *Pestaliopsis* sp., *Pseudopetaliopsis* sp., *Neopestaliopsis*, *Colletotrichum* sp., and *Lasiodiplodia* sp. through morphological feature observation.

To confirm the morphological identification, the internal transcribed spacer (ITS) region was amplified with the corresponding primer pairs ITS1 and ITS4 (White et al. 1990). More than 500-bp fragments were amplified and sequenced. Blast search comparison in GenBank database revealed that the isolates 3R, 4R, 9R and 13R had 99% sequence similarity with other submitted sequences of *Pestaliopsis* sp., and *Neopestaliopsis sp.* Isolate 5 R had 100% sequence similarity with *Pseudopetaliopsis* sp., whereas, isolate 11R had 100% sequence similarity with *Colletotrichum* sp., In addition, isolates 10R and 15 R showed 97% sequence similarity with *Lasiodiplodia* sp.

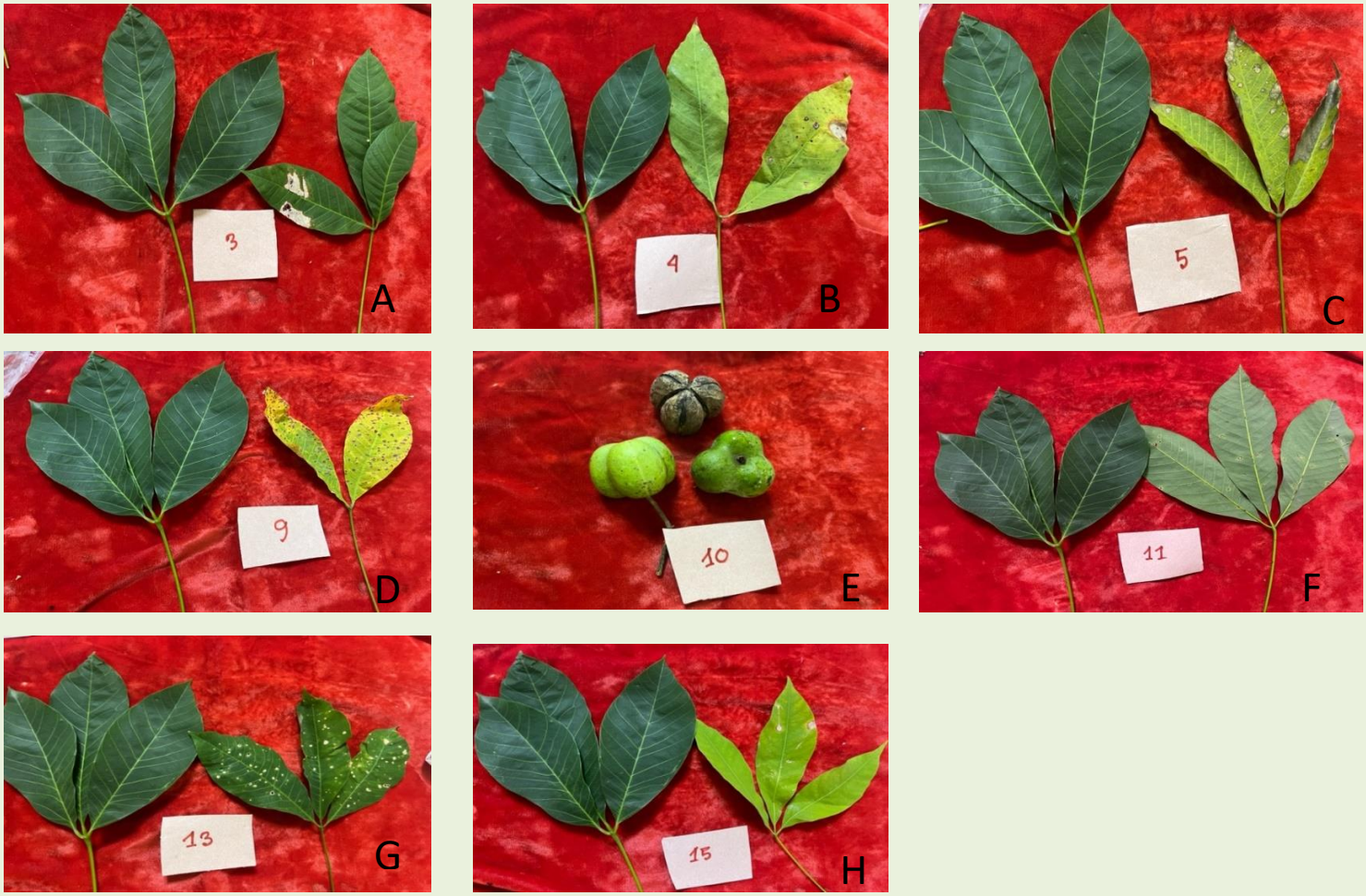

Fig.1. Symptoms of infection with fungi on rubber leaves

Fig.2. Morphology of fungi on PDA plates after 7 days of incubation

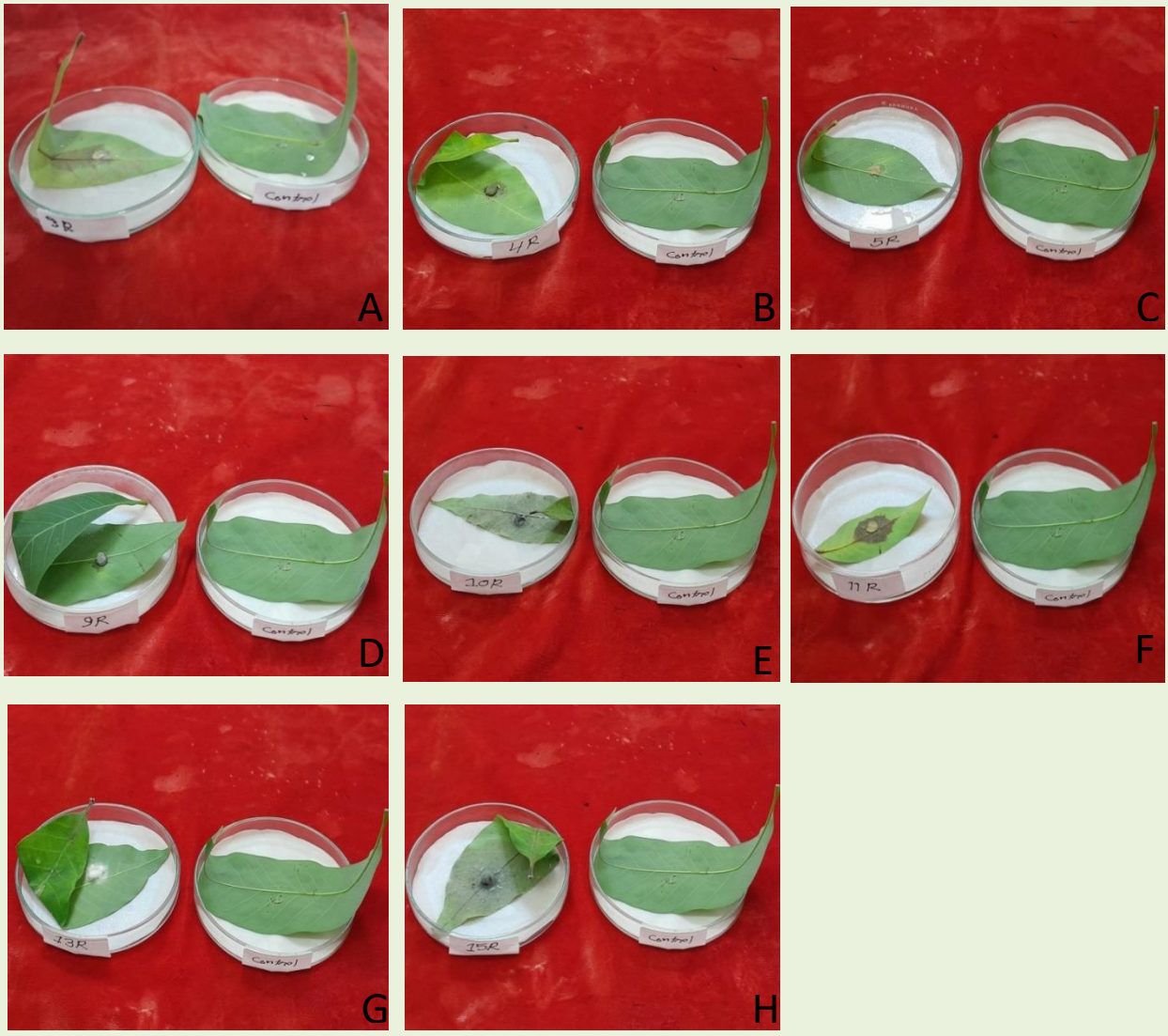

Fig.3. Rubber leaves showing typical symptoms of infection after artificial inoculation with fungi.

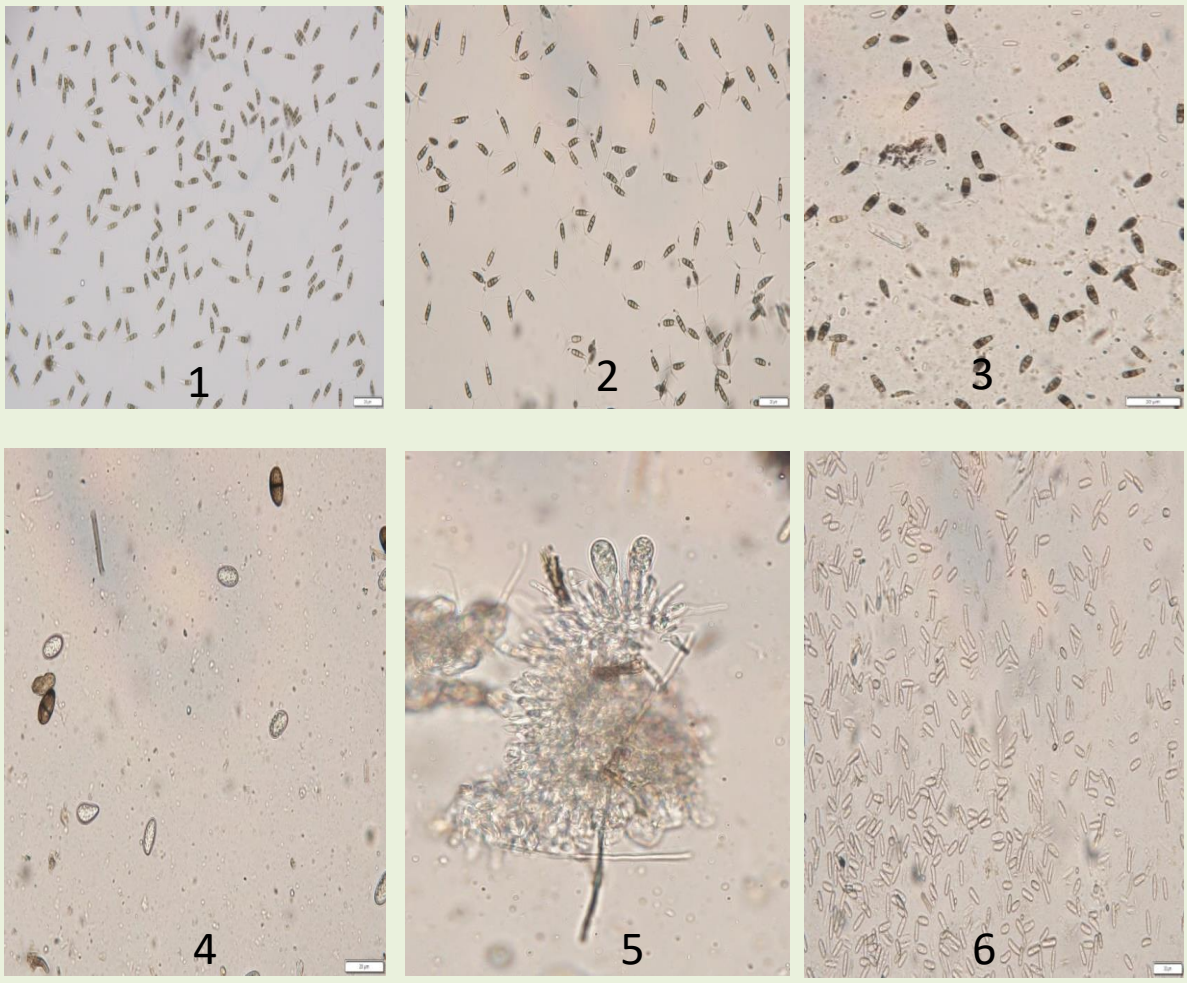

Fig.4. Fungal conidia of different pathogens 1. *Pestaliopsis* 2. *Neopestaliopsis* 3. *Pseudopestaliopsis* 4. *Lasiodiplodia* 5. Conidigenous cells and conidia of *Lasiodiplodia* 6. *Colletotrichum*.

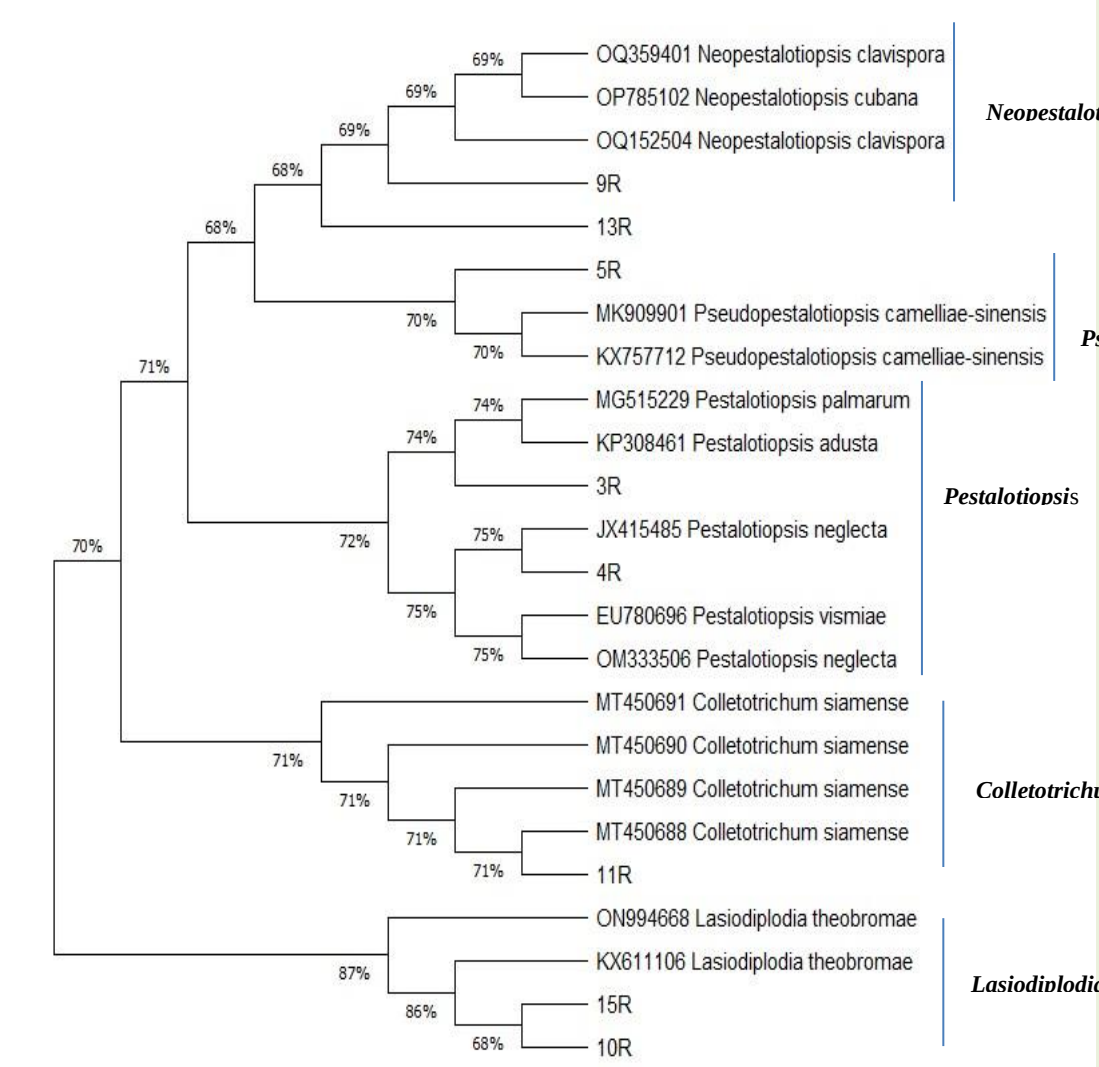

Fig.5. Phylogenetic tree obtained from ITS sequences of different fungal species infected rubber leaf. The numbers above the nodes are bootstrap values obtained from 1000 replicates.

References

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