Plant Tissue Cult. & Biotech. **36**(1): 129-138, 2026 (June) **ISSN 1817-3721, E-ISSN 1818-8745**
DOI: https://doi.org/10.3329/ptcb.v36i1.91166

PTC&B

Mycelial Growth and Molecular Identification of *Curvularia moringae* Isolated from Dried Brackish Water Fish of *Coilia dussumieri* (Valenciennes, 1848)

Abdullah Al Masud[1], Rubama Amin, Farhana Rahman[2] and Ismot Ara*

Department of Zoology, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

Key words: Curvularia moringae, Dried fish fungus, Molecular characterization, Cultural characteristics

Abstract

The gold-spotted, brackish water fish, scientifically known as *Coilia dussumieri*, is widely consumed in Bangladesh, both fresh and dried. This study employed both traditional and molecular methods to detect the fungal species associated with dried *C. dussumieri*. The properties of fungal mycelial growth were also studied. *Curvularia moringae* was identified in the selected dried fish, which are often consumed in Bangladesh. It was molecularly identified using a 593 bp PCR product from the internal transcribed spacer (ITS) region. The molecular phylogenetic tree revealed that the examined fungus has 100% sequence similarity to the *C. moringae* species complex. The results of the study on the impact of culture media on the mycelial growth and development of *C. moringae* were evaluated. *C. moringae* mycelial growth was highest (81.33 mm) in the potato dextrose agar (PDA) medium, followed by (66.25 mm) in potato sucrose agar (PSA), (47.16 mm) in glucose peptone agar (GLP), (40.25 mm) in Hoppkins, and (35.12 mm) in yeast extract agar medium. *C. moringae* thrived and matured optimally at 25°C and pH 6.0. As far as we know, the experimental identification of the dry fish fungus *C. moringae* from *C. dussumieri* is the first to be reported in Bangladesh using both traditional and molecular approaches.

Introduction

Coilia dussumieri (Valenciennes, 1848) is known as the gold-spotted fish and called "Oluua" in coastal areas of Bangladesh (Amin et al. 2006). It is a widespread brackish water fish in Malaysia, Thailand, Myanmar, Bangladesh, India, Pakistan, and Sri Lanka. This fish has a systematic migration pattern between freshwater and the sea, engaging not only in reproduction but also in intensive feeding (Akhter et al. 2024).

---

*Author for correspondence: <ismotzool@juniv.edu>. [1]Department of Zoology, Jagannath University, Dhaka-1100, Bangladesh. [2]Laboratory of Mycology and Plant Pathology, Department of Botany, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

Chittagong, Moheshkhali, Sonadia, and St. Martin Island in Cox's Bazar, and Patuakhali's Kuakata are the most important places for dried fish production (Sultana et al. 2020). Dried fish and fish items are made from a variety of marine fish species as well as freshwater fishes. Bangladeshi dry fish has enormous commercial potential since it contains high-quality proteins, necessary vitamins, and important polyunsaturated fatty acids (Ara et al. 2020a).

Sun drying is the most common method of fish preservation worldwide. This method is also regarded as the least expensive method of preservation (Galib et al. 2013). Fungal development on dried fish shows that the food has begun to degrade and deteriorate (Kar et al. 2025). The global demand for processed and preserved fish products has increased drastically, with dried fish being used as an important source of low-cost protein in many developing countries. However, the typical drying technique frequently makes these items susceptible to microbial contamination, especially by filamentous fungi, which can reduce nutritional value and pose major health risks to consumers (Ara et al. 2020b).

High ambient humidity, inappropriate handling, and residual moisture content make dried fish products susceptible to fungal infection (Chowdhury et al. 2025). Numerous fungal species that seriously deteriorate dried fish have been identified (Chanda et al. 2021). Common fungal isolates identified in dried fish included *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., and *Fusarium* sp. (Akter et al. 2023). *Curvularia moringae* is a dematiaceous fungus with curved, multiseptate conidia (Billah et al. 2021). It causes the breakdown of muscle tissues, resulting in discoloration and a rotten odor. *Curvularia* species have traditionally been identified primarily based on morphological and cultural traits such as colony color, growth rate, and conidial structure (Khatun et al. 2025). A recent molecular phylogenetic study has demonstrated that the ITS region of genomic DNA is extremely useful for analyzing phylogenetic relationships at lower taxonomic levels. Because of its short length, the ITS region can be amplified fast by PCR with single universal primer pairs (Sikder et al. 2019). According to Rahman et al. (2024), fungal mycelial development showed the highest (75 mm) growth in Potato Dextrose Agar (PDA) and the lowest (40 mm) growth in Yeast Extract Agar (YEA) media, with ideal conditions of pH 6.5 and temperature of 25°C. The cultural characteristics of *C. moringae* include optimal growth temperatures, moisture needs, pH tolerance, colony traits, and sporulation habits. Integrating mycelial growth dynamics with definitive molecular identification enables stakeholders to map out exactly how *C. moringae* adapts to dried seafood environments, providing the framework for highly optimized, species-specific food safety barriers.

The present research will provide comprehensive information on fungal contamination of brackish water dried fish caused by fungi. Thus, acquiring knowledge for the implementation of appropriate and effective measures against fungal contamination would play a vital role in our economy through quality dry fish production.

Thus, the goal of the current study was to identify possible fungal contaminants associated with Bangladesh's brackish water dried fish through morphological and molecular methods.

Materials and Methods

Dried brackish water fish *Coilia dussumieri* samples (Fig. 1A) were obtained from Kuakata, Potuakhali, a coastal landing and fish drying station of Bangladesh. Before being sun-dried for 15 days in their natural habitat, fresh fish were properly washed with tap water. After drying, the samples were placed in a sterile glass jar and sent to the JU Laboratory of Mycology and Plant Pathology in the Department of Botany for fungal inoculum isolation and identification of fungal contaminants.

Each of the small, 0.5 cm-long contaminated portions from both fungus-infested and uninfected dried fish parts was separated. Following several rinses with distilled water, these small particles were placed in a 90 mm Petri dish filled with PDA medium before being sterilized. The fungal colonies were subsequently monitored for ten days at room temperature under a 12 hrs light/12 hrs dark cycle. After numerous transfers of the hyphal tip, a pure culture was created. Standard procedures were used to identify the pure culture of the isolated fungus under a microscope (Ahmmed et al. 2022).

To extract genomic DNA from the fungus, we used the Maxwell Cell Kit (AS1030, Promega, USA). The primers used for the PCR reaction were ITS4 (5′-TCCTCCG CTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAA AGTCG TAACAAGG-3′) (Alam and Rahman 2020). 20 ng of genomic DNA was used as the PCR template in a 25-µl reaction mixture with a LA Taq (TAKARA BIO INC., Japan). After 35 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for five min each, with a ten-minute extension at 72°C, Taq polymerase was activated at 94°C for one min.

After the amplification PCR products were electrophoresed on a 1.5% agarose gel in 1 × TAE buffer for one hour at 100V using a 1kb DNA ladder as a size marker, they were stained while being swirled in an EtBr solution (0.5% µg/ml). The stained gels were observed and photographed using a UV transilluminator (Kodak Image Station 4000R; Molecular imaging equipment, Carestream Health Inc., 150 Verona Street, Rochester, NY 14608). The Maxwell® 16 DNA Purification Kits (Promega, USA) were used to purify the amplified products. First, the purified PCR products were subjected to bi-directional sequencing by BASE Laboratories Sdn Bhd (Malaysia).

DNA sequences were verified using MEGA 11 and BioEdit. The ITS sequences were used in a BLAST search to find the closest matched taxa. Multiple sequence alignments were performed using MEGA6. The data were converted from Fasta to MEGA format using Clustal W. The evolutionary models were defined using the Akaike Information Criterion (AIC). The Tamura-3 parameter served as the model for the analysis. Using 1000 bootstrap repetitions and max-trees set to 1000, the robustness of the branches was evaluated. Neighbor-joining analyses were performed. The number of replicates was calculated using the halting criterion. Bootstrap values greater than 60% were considered acceptable, according to Tamura et al. (2013).

Five distinct culture media, including potato sucrose agar (PSA), potato dextrose agar (PDA), glucose peptone (GLP), yeast extract agar (YEA), and Hoppkins (HOP) medium, have been developed to assess the mycelial growth characteristics of the isolated fungi, according to Alam et al. (2023). To determine the ideal temperature for mycelial growth, inoculated PDA plates were incubated at 15, 20, 25, 30, and 35°C. The effect of pH on the pathogen's growth was examined using the PDA medium. A range of pH levels, including 5.0, 6.0, 7.0, 8.0, and 9.0, was employed. Before autoclaving, the medium pH was adjusted with 1 N NaOH or HCl. The medium was inoculated with the fungal strain and incubated at 30°C for ten days. The average colony diameter was calculated by measuring the radial mycelial development of each Petri plate in three opposing directions. Every experiment was carried out using the procedure described by Sikder et al. (2019).

Standard statistical analysis tools like Microsoft Excel, MEGA 11.0, and BLAST were used to examine the data produced during the research work. The data was examined using Duncan's post-hoc analysis.

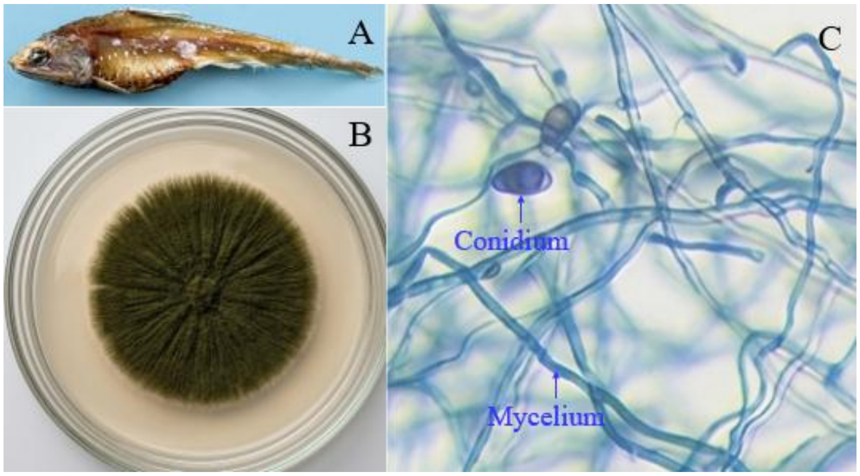

Fig. 1. Morphological characteristics of the fungus associated with dried *C. dussumieri*: (A) fungal contamination of the dried fish, (B) mycelial growth of *C. moringae* in PDA medium, and (C) microscopic view of conidia, conidiophore, and mycelium of *C. moringae* (10 × 40).

Results and Discussion

The mycelial colony of *C. moringae* begins as a dirty gray or olive color, eventually turning dark brown to coal-black as it matures and produces melanin. The reverse side of the plate is usually completely black. The surface is velvety, woolly, or floccos**e**. It is a fast-growing fungus, typically filling a standard Petri dish within 6 to 7 days. It often grows in a circular, spreading pattern, sometimes displaying concentric rings or a slightly raised center (Fig. 1B). Hyphae are septate, having cross-walls, and are branched. They are typically brown-pigmented. Conidiophores are simple or branched brown stalks that often grow sympodially. Conidia are smooth-walled, brown, and usually consist of 3 to 4 cells with transverse septa. *C. moringae*, the third cell from the base is typically larger and darker than the others. This unequal expansion causes the spore to curve. The cells at the tip and the base of the spore are generally paler in color compared to the middle cells (Fig. 1C). Typically, vesicles have a subglobose form. According to Van-Vuuren et al. (2024), the first report of *C. moringae* occurring in grasses and soil; it was previously only recovered from *Moringa ovalifolia* in the Namib Desert. The shapes and sizes of various structures, such as colony diameter of mycelium, conidia, and conidiophores of fungi, are taxonomical characters for morphological identification of *C. moringae* (Billah et al. 2021).

The PCR products of the internal transcribed spacer (ITS) region in *Curvularia moringae* was 593 bp (Fig. 2). Using ITS1 and ITS4 primers, the ITS region was amplified and sequenced. The ITS region of genomic DNA has recently been found to be extremely helpful for identifying fungi at the species level, according to molecular phylogenetic research. When it comes to variation between species and even strains, the internal transcribed spacer of rDNA is thought to be a key factor (Alam et al. 2010).

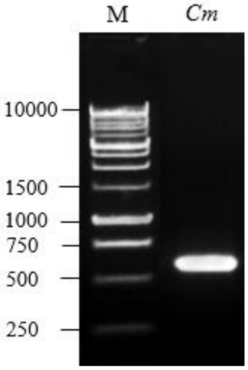

Fig. 2. PCR products of the ITS region of *Curvularia moringae*. Lane M, molecular size marker (1kb DNA ladder); Lane *Cm*, *C. moringae*.

The ribosomal DNA sequence of the studied fungus (JUF0134, the NCBI accession number, PZ245849.1) was included in the phylogenetic tree constructed by MEGA version 11. Our studied organism showed a 100% identity with other C. *moringae* (ON332841.1, OR992648.1, and MW175363.1), according to the BLAST search analysis. The phylogenetic tree based on the nucleotide sequence of the ITS region comprises twenty-six taxa of representative species of *Curvularia*, using *Phytophthora brassicae* as an outgroup to root the tree. There were five different clades found in the neighbor-joining tree (Fig. 3). The tree is organized into distinct clusters (clades) corresponding to different species within the genus *Curvularia*. The branching pattern indicates the evolutionary relationships and genetic similarities between these isolates. A bootstrap value of 99 supports the internal nodes of this subgroup, showing very high confidence.

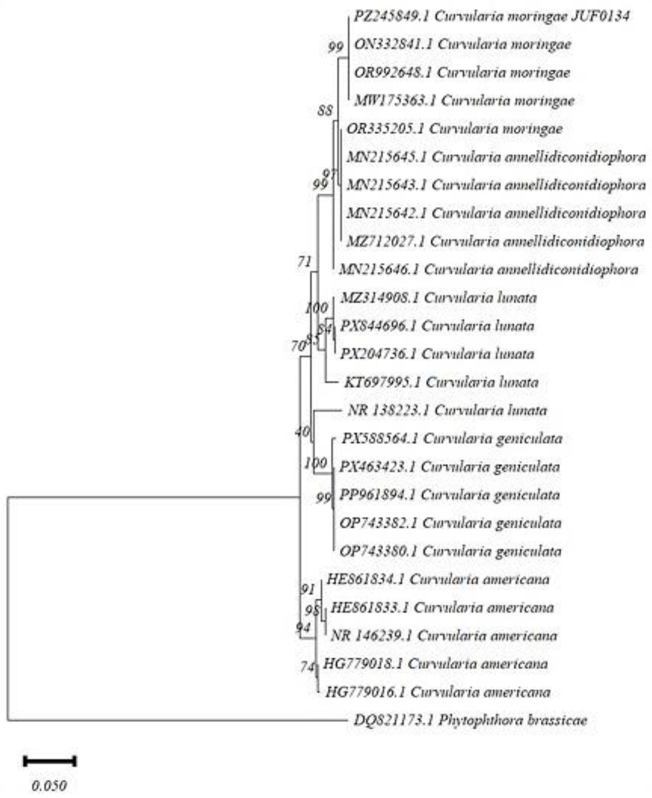

Fig. 3. Phylogenetic tree obtained by analysis of the ITS sequence of the studied *C. moringae* (accession number PZ245849.1, *JUF0134*) associated with dried fish *Coilia dussumieri* using the neighbor-joining tree method.

According to Lee et al. (2010), ITS sequences are genetically stable within species but vary between species within a genus. DNA sequences are necessary for reliable identification at the species level of fungi (Cho et al. 2010). The genus currently lacks a complete, validated reference database. To improve the list's taxonomic value, we additionally provide the MycoBank number of each accepted species, live ex-type strains, and GenBank accession numbers for ITS sequences. This generates a confirmed set of sequences for each species in the genus (Rahman et al. 2025).

Five distinct culture media, including PSA, PDA, glucose peptone (GLP), YEA, and Hoppkins medium, were employed to evaluate the optimal conditions for the growth and development of *C. moringae*. The results indicated that PDA shows the maximum mycelial growth (81.33 mm) diameter, followed by PSA (66.25 mm), while the lowest mycelial growth (31.12 mm) was observed on YMA medium (Fig. 4). Similar results were also supported by Sultana et al. (2023). Chanda et al. (2021) reported that the mycelial growth pattern of *Aspergillus elegans* was the best on PDA medium. PDA is a standard culture medium for fungi, which derived from potato infusion and dextrose, provides an ideal carbon source for *C. moringae* growth.

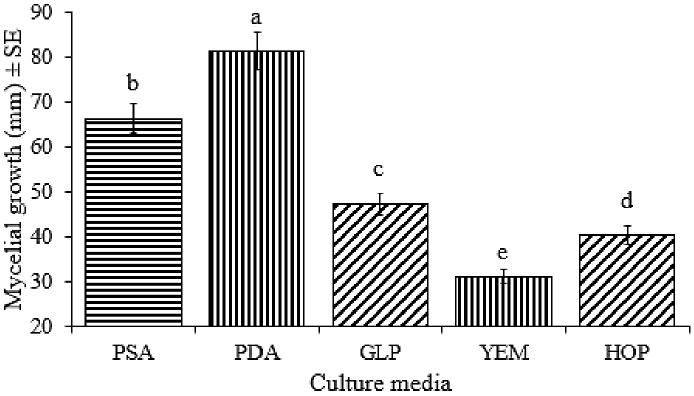

Fig. 4. The effect of culture media on the mycelial growth (mm) of *C. moringae JUF 0134* at 7dpi. The bars in the figure show the means ± standard errors (n = 3). Significant differences between cultural conditions ($p < 0.05$) are shown by different letters. PSA, potato sucrose agar; PDA, potato dextrose agar; GLP, glucose peptone; YEA, yeast extract agar; HOP, Hoppkins.

The experimental findings of the present study, which examined the impact of temperature on *C. moringae* radial mycelial growth on PDA medium incubated at five different temperatures i.e. 15, 20, 25, 30, and 35°C, are displayed in Fig. 5. According to the findings, the maximum radial growth (83.33 mm) of *C. moringae* JUF0134 was found at 25°C and then (71.66 mm) at 30°C. A similar result was observed by Masud et al. (2023), which reported a notable decline in mycelial development and sporulation between 30 and 35°C, the maximum levels of *C. blakesleeana* mycelial growth and sporulation were observed at 30°C. The results also demonstrate that 40-45°C temperature has no effect on the mycelial growth of *C. blakesleeana*, and that the proper combination of these variables can be used to stop or delay the mold's growth, reducing product losses and the financial consequences of fungal contamination.

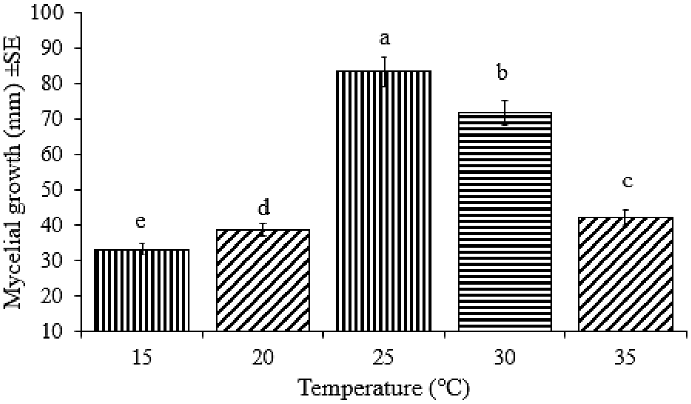

Fig. 5. Effects of different temperatures on the mycelial growth of *C. moringae JUF 0134* at 7dpi. The bars in the figure show the means ± standard errors (n=3). Significant differences between treatments ($p < 0.05$) are shown by different letters.

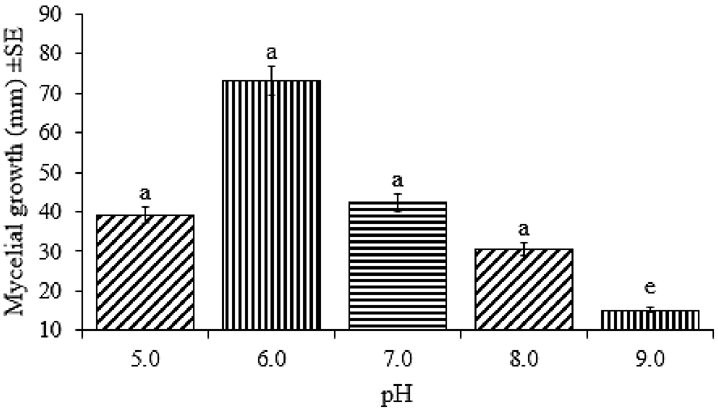

Fig. 6. Effects of different pH on the mycelial growth of *C. moringae JUF 0134* at 7dpi. The bars in the figure show the means ± standard errors (n=3). Significant differences between treatments ($p < 0.05$) are shown by different letters.

pH is an important parameter to understand fungal ecology (Alam et al. 2025). Five different pH levels, viz., 5.0, 6.0, 7.0, 8.0, and 9.0, were used to find the optimum pH level for the isolated fungal species. Maximum mycelial growth (73.33 mm) of *C. moringae* JUF 0134 was recorded at pH 6.0 and followed by pH 7.0 and pH 5.0, while minimum mycelial growth (15.25 mm) was found at pH 9 (Fig. 6). The fungal isolates in this investigation grew best in slide acetic conditions. Colony diameter, pigmentation, and sporulation were most prominent at these pH levels. Masud et al. (2023) found that *C. blakesleeana* showed maximum mycelial growth (88.25 mm) at pH 7, followed by pH 8 and pH 6, and minimal mycelial growth at pH 9. So, the result suggested that neutral pH conditions are favorable for *C. moringae*.

This study represents the first comprehensive report on the isolation, morphological features, cultural characteristics, and molecular identification of the dematiaceous fungus *Curvularia moringae* from dried gold-spotted brackish water fish in Bangladesh.

Acknowledgements

The Department of Botany at Jahangirnagar University provided laboratory facilities for this study, for which the authors are grateful.

References

Ahmmed SM, Sikder MM, Sultana A and **Alam N** (2022) Molecular characterization of endophytic fungi-*Daldinia eschscholtzii* from *Aloe vera* plants in Bangladesh. Jahangirnagar Univ. J. Biol. Sci. **11**(1&2): 1-11.

Akhter M, Hasan MM, Khan ABS, Ullah MR, Bosu A, Yasmin F, Haque MA, Islam MA and **Mahmud Y** (2024) Reproductive biology and feeding habit of *Coilia dussumieri* Valenciennes, 1848 (Actinopteri: Engraulidae): a review. Int. J. Sci. Technol. Res. Arch. **6**(1): 51-56.

Akter H, Ara I, Alam NB and **Alam N** (2023) The first report of *Aspergillus ochraceopetaliformis* Bat. & Maia from small indigenous dry fish *Setipinna phasa* (Hamilton 1822) in Bangladesh. Jahangirnagar Univ. J. Biol. Sci. **12**(1&2): 87-95.

Alam N, Lee JS and Lee TS (2010) Mycelial growth conditions and molecular phylogenetic relationships of *Pleurotus ostreatus*. World Appl. Sci. J. **9**(8): 928-937.

Alam NB, Rahman F and **Alam N** (2023) Molecular identification of *Alternaria alternata* (Fr.) Keissl. from the leaf blight disease of *Centella asiatica* L. Nat. Sci. **15**(8): 223-232.

Alam NB, Rahman F, Bhowmik DD, Alam N and **Alam N** (2025) *In silico* approaches for effective and potential biocontrol agents against targeting chitin synthase protein of pathogenic fungus *Alternaria alternata* (Fr.) Keissl. of onion. Plant Tiss. Cult. Biotech. **35**(2): 347-359.

Amin SMN, Ara R and **Zafar M** (2006) Conservation of marine and coastal shrimp resources and sustainable aquaculture. Res. J. Fish. Hydro. **1**(1): 18-22.

Ara I, Sultana R, Chanda IJ and **Alam N** (2020a) The first report of fungus *Scopulariopsis brevicaulis* from fresh water dry shrimp in Bangladesh. Bangladesh J. Zool. **48**(1): 203-209.

Ara I, Sultana R, Chanda IJ and **Alam N** (2020b) First report on *Ovatospora brasiliensis* from fresh water dried shrimp and prawn in Bangladesh. Int. J. Fauna Biol. Stud. **7**(3): 43-47.

Billah MB, Sikder MM, Mallik MRI, Hossain MK and **Alam N** (2021) Molecular characterization and vegetative growth of pathogenic seed-borne fungus *Curvularia moringae* of tomato and it's *in vitro* control measures. Int. J. Agril. Res. Innov. Technol. **11**(2): 124-132.

Chanda IJ, Ara I, Sultana R and **Alam N** (2021) Morphological and molecular characterization of *Aspergillus elegans* from small indigenous dry fish 'Jat Puti' *Puntius sophore* (Hamilton 1822) in Bangladesh. Jahangirnagar University J. Biol. Sci. **10**(1&2): 39-47.

Cho HJ, Lee JS, Yoon KN, Alam N, Lee KL, Shim MJ, Lee MW, Cheong JC, Shin PG, Yoo YB, Lee UY and **Lee TS** (2010) Comparative molecular phylogenetic relationships in different strains of *Pleurotus* spp. Korean J. Mycol. **38**(2): 112-119.

Chowdhury S, Masud AA, Ara I and **Alam N** (2025) Identification and cultural characteristics of *Aspergillus flavus* associated with dry spotted snakehead fish (*Channa punctata* bloch). J. Bio-Sci. **33**(2): 21-28.

Galib SM, Mohsin ABM, Chaki N, Fahad MFH and **Haque SMM** (2013) An overview of the traditional rice, prawn, fish farming in Kalia of Narail district, Bangladesh. J. Fisher. **1**(1): 1-6.

Kar T, Tamanna T, Ara I and **Alam N** (2025) Molecular identification and phenotypic characterization of *Aspergillus lentulus* from dried mola carplet, *Amblypharyngodon mola* (Hamilton 1822). Bangladesh J. Zool. **53**(2): 209-218.

Khatun MS, Rayhan MA, Akter P, Sikder MM and **Alam N** (2025) *Curvularia geniculatus* associated with garlic leaf blight disease: molecular identification, mycelial growth, and biological control. Plant Tiss. Cult. Biotech. **35**(2): 335-346.

Lee JS, Cho HJ, Yoon KN, Alam N, Lee KL, Shim MJ, Lee MW, Lee YH, Jang MJ, Ju YC, Cheong JC, Shin PG, Yoo YB, Lee UY and **Lee TS** (2010) Genetic diversity and phylogenetic relationship in Korean strains of *Lentinus lepideus* based on PCR polymorphism. Korean J. Mycol. **38**(2): 105-111.

Masud A, Ara I and **Alam N** (2023) Morphology and molecular identification of dry fish fungus *Cunninghamella blakesleeana* from small indigenous fish 'Kachki' *Corica soborna* (Hamilton 1822) in Bangladesh. American J. Plant Sci. **14**(11): 1316-1326.

Rahman F Dey S, Alam NB, Bhowmik DD, Alam N and **Alam N** (2025) Mycelial growth and identification of pathogenic fungus isolated from basal rot disease of onion and their biological control. Plant Tiss. Cult. Biotech. **35**(1): 93-104.

Rahman F, Sumitra D and **Alam N** (2024) Pathogenic fungi associated with coriander wilt disease: cultural condition, molecular identification and biological control. Plant Tiss. Cult. Biotech. **34**(2): 193-204.

Sikder MM, **Mallik MRI** and **Alam N** (2019) Identification and *in vitro* growth characteristics of entomopathogenic fungus-*Aschersonia* sp. in Bangladesh. Adv. Zool. Bot. **7**(1): 11-18**.**

Sultana A, Sikder MM and **Alam N** (2023) Identification and fungal biology of post-harvest fungi associated with amla fruits and it's *in vitro* control measures. Adv. Zool. Bot. **11**(1): 1-11.

Sultana S, Sikder MM, Ahmmed MS, Sultana A and **Alam N** (2020) Mycelial growth and biological control measures of *Botrytis cinerea* isolated from strawberry fruit rot disease in Bangladesh. Univ. J. Microbiol. Res. **8**(2): 13-18.

Tamura K, Stecher G, Peterson D, Filipski A and **Kumar S** (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. **30**: 2725-2729.

Van-Vuuren N, Yilmaz N, Wingfield MJ and **Visagie CM** (2024) Five novel *Curvularia* species (Pleosporaceae, Pleosporales) isolated from fairy circles in the Namib Desert. Mycol. Progr. **23**(1): 39.

(Manuscript received on 10 May, 2026; revised on 8 June, 2026)