Bangladesh J. Zool. ***53***(2): 209-218, 2025 ***Article***
ISSN: 0304-9027
eISSN: 2408-8455

MOLECULAR IDENTIFICATION AND PHENOTYPIC CHARACTERIZATION OF *ASPERGILLUS LENTULUS* FROM DRIED MOLA CARPLET, *AMBLYPHARYNGODON MOLA* (HAMILTON 1822)

Tamalika Kar[1], Tamanna[1], Ismot Ara[1*] and Nuhu Alam[2]

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

ABSTRACT: The mola carplet (*Amblypharyngodon mola*) is a well-adored small indigenous fish species (SIS) in Bangladesh. To evaluate the molecular and classical identification of the fungus associated with dried fish, the mola carplet was the subject of the current study. *Aspergillus lentulus*, a very common fungus in mola carplet was characterized molecularly using a 590 bp PCR product of its ITS (internal transcribed spacer) region. The molecular phylogenetic tree constructed therefrom showed 100% sequence similarity in *A. lentulus* species complex. The impact of cultural medium on mycelial growth and development of *A. lentulus* was pointed. The mycelial growth of *A. lentulus* was highest (71 mm) on the potato dextrose agar (PDA) medium, followed by (53 mm) in glucose peptone (GLP), (51 mm) in potato sucrose agar (PSA), (39 mm) in yeast extract agar (YEA), and (29 mm) Hoppkins (29 mm), respectively. *A. lentulus* grew and developed vegetatively best at 30°C and 7.0 pH, respectively. As far as we are aware, this experimental identification of the dry fish fungus *Aspergillus lentulus* from the mola carplet is the first report and confirmation of a fungus in Bangladesh using both traditional and molecular methods.

***Key words*:** *Amblypharyngodon mola*, *Aspergillus lentulus,* dry fish, molecular characterization, mycelial growth.

INTRODUCTION

Amblypharyngodon mola, the mola carplet, is found throughout Asia, including Bangladesh, India, Myanmar, and Pakistan (Talwar & Jhingran 1991). It is a popular food fish, particularly on the Indian subcontinent, with high protein, vitamin, and mineral content (Saha *et al.* 2009) and other nutritional advantages (Mazumder *et al.* 2008). Bangladesh's rivers, canals, beels, ponds, and flooded fields are all home to *A. mola*. This species is a significant source of micronutrients that rural Bangladeshis, particularly women and children, require to avoid malnutrition (Thilsted *et al.* 1997).

**Author for correspondence: <ismotzool@yahoo.com>; [2]Department of Botany, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh*
 DOI: *https://doi.org/10.3329/bjz.v53i2.85047*

One of the most popular techniques for preserving fish is sun drying in Bangladesh. The country makes a large amount of foreign exchange by exporting a large quantity of dried fish (Galib 2013). The protein content of the sun-dried SIS fish can reach 60–80%. However, fungus growth signals the beginning of product deterioration and spoiling (Pitt and Hocking 1999). The most common fungi found in dried fish included *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. glaucus*, *A. restrictus*, *Penicillium chalybeum*, and *P. expansum* (Akter *et al.* 2023).

When fungus grows on dried fish, it softens its flesh, diminishes tastes, and in some cases, can produce mycotoxins (Pitt and Hocking 1999). Several fungi from India, Pakistan, Thailand, Malaysia, and Hong Kong, including *Aspergillus* species, *Penicillium* species, *Fusarium* species, *Alternaria* species, *Rhizopus* species, *Mucor* species, *Acremonium* species, *Wallermia seba*, and *Sporodendron epizoum* were reported (Sivaraman *et al.* 2018). Numerous fungi, particularly *Aspergillus* species, are known to produce a variety of toxins, including aflatoxins, ochratoxins, and sterigmatocystine, which can cause illnesses in humans (Motalebi *et al.* 2008).

DNA barcoding is now an essential part of mycological research for fungal molecular identification. It has been officially acknowledged as the primary barcoding marker for fungi. ITS1-F, ITS, and ITS2 primers bias the amplification of ITS1, ITS2, and ITS4. Recent molecular phylogenetic studies have demonstrated the great utility of the ITS region of genomic DNA for assessing phylogenetic relationships at lower taxonomic levels (Alam and Rahman 2020). Recent molecular phylogenetic reserch showed that phylogenetic connectionat lower taxonomic levels are determinedby the ITS region genomic DNA (Alam *et al.* 2010).

F. oxysporum mycelial development showed highest on PDA media (75mm) and lowest on YEA media (40mm) where its best requirement was pH of 6.5 and temperature was 25°C (Rahman *et al.* 2024). According to our research, Bangladesh's dry fish fungus is the subject of surprisingly little research. Because of this, the current study has focused heavily on the isolation of the fungus, morphological and molecular identification of the causative organism, and the fungal cultural characteristics of fungi from dry mola carplet, *Amblypharyngodon mola* (Hamilton1822) in Bangladesh.

MATERIAL AND METHODS

Fresh and dried samples of the mola carplet (Fig. 1), a popular small indigenous fish, were collected from various locations in the Chalan Beel, South-East part of the Rajshahi district (latitudes 24˚12' and 24˚50' and longitudes

88˚21' and 88˚35'). The samples were subsequently sent to the Department of Zoology's Limnology and Fishery Sciences laboratory at Jahangirnagar University (JU) to be prepared as dry fish. The fresh samples were cleaned with tap water and allowed to dry in the sun before processing. After completion of the preparation process, the dry sample was kept in a plastic pot and brought to the Mycology Laboratory, Department of Botany, JU for fungi isolation and identification.

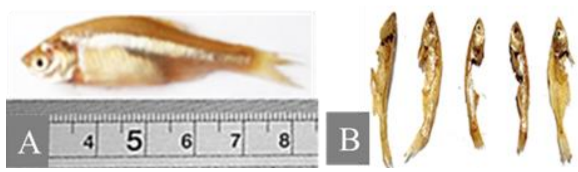

Fig. 1. Mola carplet (*Amblypharyngodon mola*) in fresh (A) and dried (B) condition.

Sundried fish samples of *A. mola* were infected by fungi. Cut into pieces of 0.5 cm in length, infected parts of *A. mola* were designed to contain fungal-infected tissues. After that, disinfection was carried out using a five per cent solution of NaOCl for 2-3 minutes before being washed numerous times with distilled water. The PDA culture medium was used for 10 days at 25±2°C with 12/12 hours of light and darkness. New PDA plates and PDA slants were used to transfer the mycelial development of a growing fungal colony in order to create a pure culture. Following many transfers of hyphal tips, a pure culture was produced.

Using conventional techniques, the isolated fungus's pure culture was detected under a microscope (Ara *et al.* 2020). Based on the color of the conidial masses, the shape, septation, and basal and apical cells of the macroconidia, the shape of the microconidia, the presence or absence of chlamydospores, and the conidiophores in the aerial mycelium, Seifert (1995) classified microconidia and macroconidia in cultures that were 10 days old.

The Maxwell Cell Kit (AS1030, Promega, USA) was used to extract genomic DNA samples from fungi. The primers ITS4R (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5F (5′-GGAAGTAAAAGTC GTAACA AGG-3′) were employed in the PCR reaction by White *et al.* (1990). A 25 µl reaction mixture, a LA Taq (TAKARA BIO INC., Japan), and 20 ng of genomic DNA were used as the template for the PCR reaction. To activate Taq polymerase, 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 5 minutes each were carried out following a minute of activation at 94°C. At 72°C, the final extension was a 10-minute step. After being electrophoresed for one hour at 100V on a 1.5% agarose gel in 1 × TAE buffer using a 1 kb DNA ladder as a size marker, the amplified PCR products were stained while being shaken in an EtBr solution (0.5%µg/mL). A UV transilluminator was used to view and take pictures of the stained gels (Kodak

Image Station 4000R; Molecular imaging equipment). The purification of the amplification products was done using the Maxwell® 16 DNA Purification Kits (Promega, USA). Bi-directional sequencing was initially performed on the purified PCR products by BASE Laboratories SdnBhd (Malaysia).

The DNA arrangements have been verified using MEGA11 and BioEdit. The closest matched taxa were found by running a BLAST search with the ITS sequences. Multiple sequences were aligned using MEGA11. Clustal W was used to convert the data from Fasta to MEGA format. The Akaike Information Criterion (AIC) was used to define the evolutionary models. For analysis, the Tamura-3 parameter model was chosen. Using 1000 bootstrap replicates and max-trees set at 1000, maximum likelihood (ML), neighbor-joining (NJ), and maximum parsimony (MP) analyses were used to assess the branches' robustness. The halting condition was used during replications but Tamura *et al.* (2013) considered above 60% bootstrap values.

To determine the mycelial growth characteristics of the isolated fungi five distinct culture media (potato sucrose agar, PSA; potato dextrose agar, PDA; glucose peptone, GLP; yeast extract agar, YEA; and Hoppkins, HOP) were prepared following Ahmmed *et al.* (2022). Different levels of temperature conditions (15°C, 20°C, 25°C, 30°C, and 35°C) were set up at pH of 6.5 after autoclaving the instruments. In a laminar airflow cabinet, prepared PDA medium was poured aseptically into the Petri plates and the specific fungus were inoculated using 2 mm diameter agar plugs from a 7-day cultured PDA media in the center of the solidified PDA medium Petri plates. To adjust the pH (pH 5, 6, 7, 8, and 9) on the PDA media using digital pH meter and adding 1N NaOH or lactic acid were done to observe the influences of the development and growth of the tested fungi. Afer that each agar plate was labeled, and sealed with parafilm. Before autoclaving, the medium was incubated at 30°C for 7 days with the desired pH and 1 N NaOH or HCl, Sikder *et al.* 2010 assessed the radial development of mycelia on each Petri dish in three directions.

Statistical analysis: MS Excel, SPSS 16.0, MEGA 11.0, and BLAST were used to explain the produced data. The data was analyzed in SPSS-16 using Duncan's post-hoc analysis and one-way ANOVA.

RESULT AND DISCUSSION

Conidia of *A. lentulus* were scattered throughout the petriplate with grey-green patches and their colonies surfaces were white, velvety to fluffy like wool (suede-like to floccose). Usually hyaline and septate, its mycelia form a network of branching filaments. Within the conidial heads, the conidia are typically smooth, globose to subglobose, and grouped in chains on the ends of phialides.

Conidial heads are uniseriate, short, and columnar. Smooth-walled, hyaline, occasionally sinuous, and frequently constricted at the neck are characteristics of conidiophore stipes (Fig. 2). Typically, vesicles have a subglobose form. Balajee *et al.* (2005) reported that 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 *A. lentulus*.

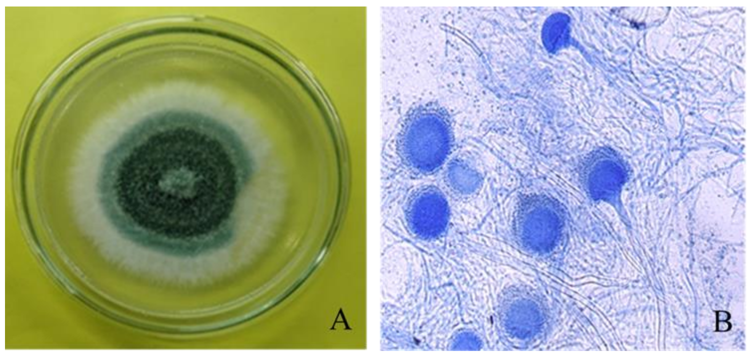

Fig. 2. Morphological traits of *Aspergillus lentulus*. A. Mycelial growth of *A. lentulus* in PDA medium; B. Microscopic view of *A. lentulus* conidia, conidiophore, and mycelium (10x40).

A. lentulus's ITS region revealed 590 bp result from PCR (Fig. 3). The ITS region was amplified and sequenced using the ITS1 and ITS2 primers. Molecular phylogenetic studies have recently shown that the ITS region of genomic DNA is very useful for species-level fungal identification. The internal transcribed spacer of rDNA is considered to be a crucial component in species and even strain variation (Cho *et al.* 2010).

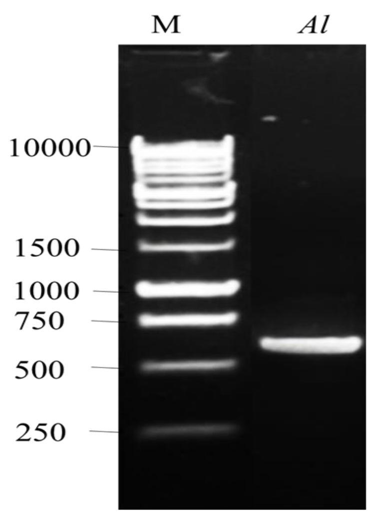

Fig. 3. PCR results for the *Aspergillus lentulus* ITS region. M is the molecular size marker (1 kb DNA ladder); *A. lentulus* is located in lane *Al*.

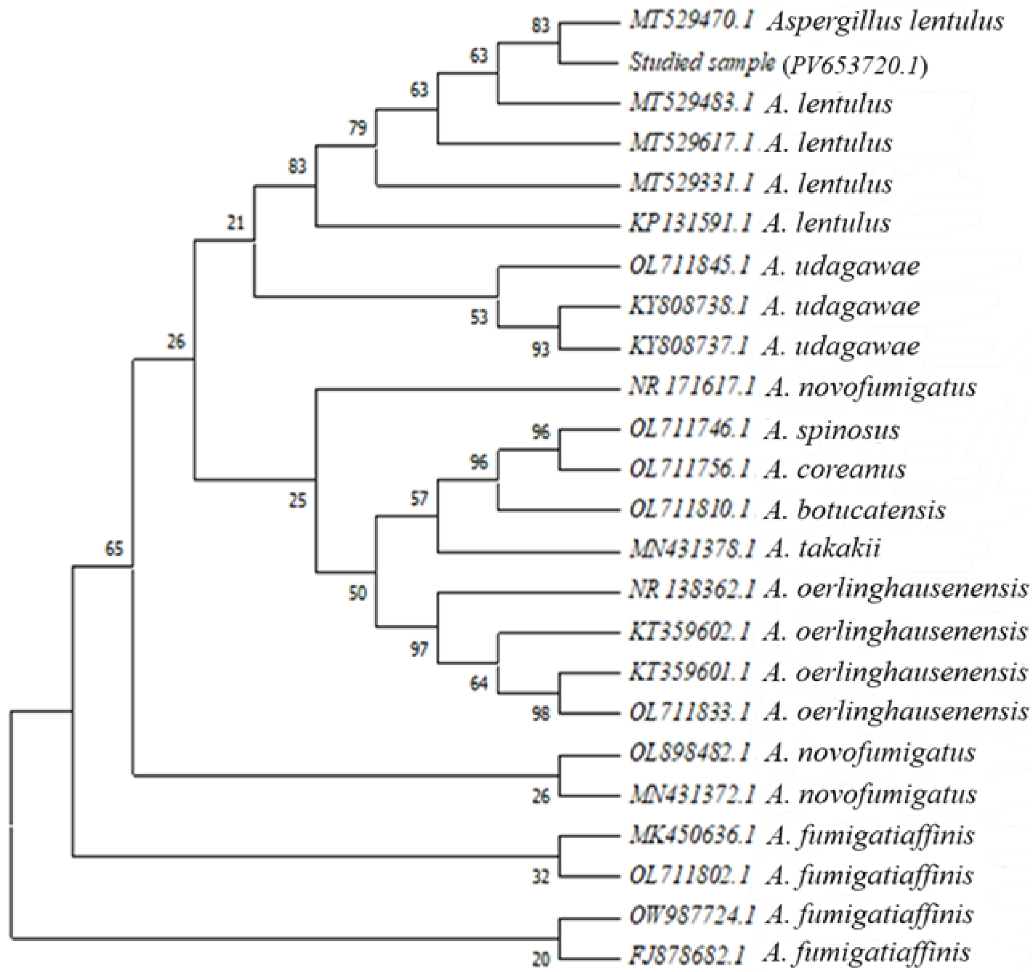

Fig. 4. Phylogenetic tree obtained by analysis of the ITS sequence of *Aspergillus lentulus* (studied sample) associated with dried *Amblypharyngodon mola* using the Neighbor joining method with 1000 bootstrapping.

Nucleotide sequences of the ITS region of 23 fungal species were taken from the NCBI database and our studied sample (*PV653720.1*) were used to create a phylogenetic tree. Using accession number MT529470.1, the ITS region rDNA sequence of the investigated sample was compared to that of *A. lentulus*, a previously identified fungus, to calculate the percentage of homology. Eight distinct clades were found in the phylogenetic tree using the neighbor-joining approach (Fig. 4). The reciprocal homologies between the ITS region sequences ranged from 99 to 100%. According to the results of the BLAST search, our organism (*PV653720.1*) was 100% comparable to *Aspergillus lentulus* (MT529470.1). Although ITS sequences are genetically stable or exhibit minimal variation within the species, they do fluctuate within a genus (Lee *et al.* 2010).

Selected five culture media, such as potato sucrose agar (PSA). potato dextrose agar (PDA), glucose peptone (GLP), yeast extract agar (YEA), and Hoppkins (HOP) were employed to evaluate the optimal conditions for the growth and development of *A. lentulus*. The results indicated that yeast extract agar (YEA) medium was the least favorable, while potato dextrose agar (PDA) medium was significantly favorable for the mycelial growth and development *A. lentulus* (Fig. 5). Chanda *et al.* (2021) determined the same results for the mycelial growth pattern of *A. elegans* based on PDA medium. PDA media is the source of carbon and derived from potato infusion and dextrose and best for *A. lentulus* growth.

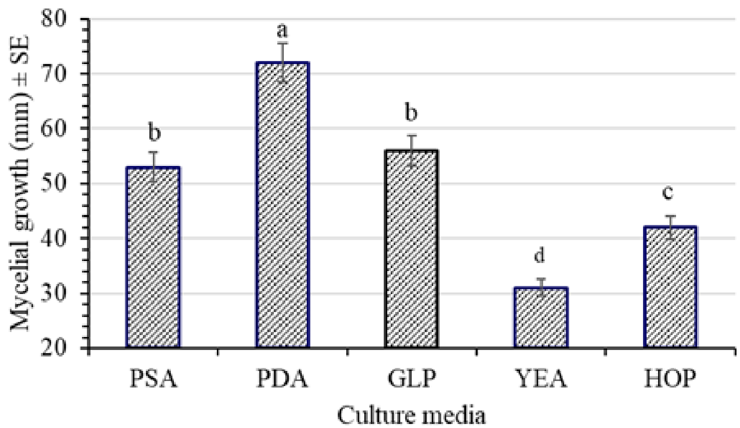

Fig. 5. Mycelial Growth of *Aspergillus lentulus* at 7dpi on different culture media.

PSA, potato sucrose agar; PDA, potato dextrose agar; GLP, glucose peptone; YEA, yeast extract agar; HOP, Hoppkins.

Radial mycelial growth of *A. lentulus* on PDA medium was incubated at 15°C, 20°C, 25°C, 30°C, and 35°C temperature during this study for investigation. The highest mycelial growth and sporulation of *A. lentulus* was found at 30°C and then at 25°C (Fig. 6). According to Masud *et al.* (2023), *C. blakesleeana* showed their maximum mycelial growth at 30°C which is closely similar to our recent research findings. The results also demonstrate that the proper combination of these variables can be utilized to restrict the mold's growth, thus we can reduce product losses and the financial consequences of fungal contamination.

Mycelial growth and development of *A. lentulus* showed an increasing tendency up to a pH of 7.0, after which it started to drop. The highest mycelial expansion, measuring 72 mm was recorded at the pH of 7.0 in *A. lentulus*. On the other hand, the fungus grew the least at pH 9.0 measuring 35 mm (Fig. 7). The fungal isolates in this investigation grew best in neutral conditions. Colony diameter, pigmentation, and sporulation were most prominent at these pH levels. Masud *et al.* (2023) stated that *C. blakesleeana* showed maximum mycelial growth

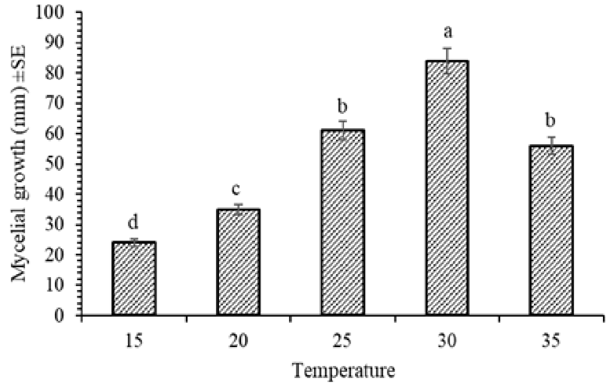

Fig. 6. Mycelial growth of *Aspergillus lentulus* at 7dpi on PDA medium in different temerature.

(88.25 mm) at pH 7, followed by pH 8 and pH 6, and minimal mycelial growth at pH 9. Nevertheless, pH plays a significant role in understanding the ecology of spoilage fungi.

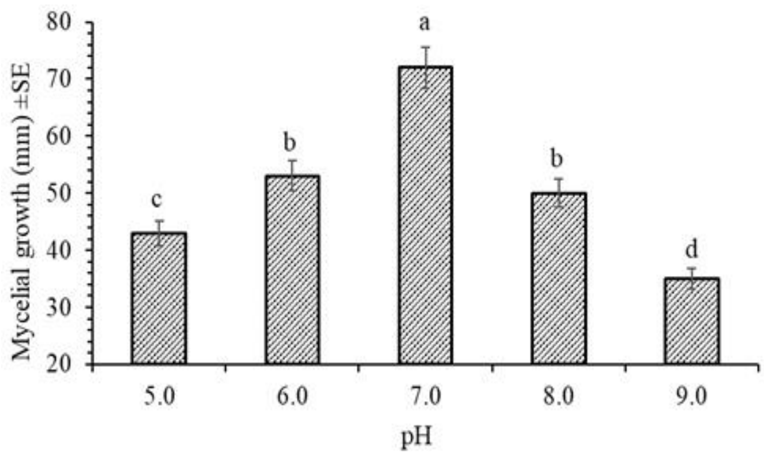

Fig. 7. Mycelial growth of *Aspergillus lentulus* at 7dpi on PDA medium in different pH levels.

Acknowledgements: This research was reinforced by the Jahangirnagar University research grant for the fiscal year 2024-2025.

LITERATURE CITED

AHMMED, S. M., SIKDER, M. M., SULTANA, A. and ALAM, N**.** 2022. Molecular characterization of endophytic fungi- *Daldinia eschscholtzii* from *Aloe vera* plants in Bangladesh. *Jahangirnagar University J. Biol. Sci.* **11**(1 & 2): 1-11.

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

ALAM, N. and RAHMAN, F. 2020. Vegetative growth and genetic diversity in different strains of *pleurotus salmoneastramineus* based on PCR polymorphism. *Bangladesh J. Bot.* **49**: 125-134.

ALAM, N., CHA, Y. J., SHIM, M. J., LEE, T. S. and LEE, U. Y. 2010. Cultural conditions for mycelial growth and molecular phylogenetic relationship in different wild strains of *Schizophyllum commune*. *Mycobiology*. **38**(1): 17-25.

ARA, I., SULTANA, R., CHANDA, I. J. and ALAM, N. 2020. The first report of fungus *Scopulariopsis brevicaulis* from fresh water dry shrimp in Bangladesh. *Bangladesh J. Zool.* **48**(1): 203-209.

BALAJEE, S. A., GRIBSKOV, J. L., HANLEY, E., NICKLE, D. and MARR, K. A. 2005. *Aspergillus lentulus* sp. nov., a new sibling species of *A.* fumigatus. *Eukaryotic Cell.* **4**(3): 625-632.

CHANDA, I. J., 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, H. J., LEE. J. S., YOON, K. N., ALAM, N., LEE, K. L., SHIM, M. J., LEE, M. W., CHEONG, J. C., SHIN, P. G., YOO, Y. B., LEE, U. Y. and LEE, T.S. 2010. Comparative molecular phylogenetic relationships in different strains of *Pleurotus* spp. *Korean J. Mycol.* **38**(2): 112-119.

GALIB, S. M., MOHSIN, A. B. M., CHAKI, N., FAHAD, M. F. H. and HAQUE, S. M. M. 2013. An overview of the traditional rice, prawn, fish farming in Kalia of Narail district, Bangladesh. *J. Fish.* **1**(1): 1-6.

LEE, J. S., CHO, H. J., YOON, K. N., ALAM, N., LEE, K. L., SHIM, M. J., LEE, M. W., LEE, Y. H., JANG, M. J., JU, Y. C., CHEONG, J. C., SHIN, P. G., YOO, Y. B., LEE, U. Y. and LEE, T. S. 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.

MAZUMDER, M. S. A., RAHMAN, M. M., AHMED, A. T. A., BEGUM, M. and HOSSAIN, M. A. 2008. Proximate composition of some small indigenous fish species (SIS) in Bangladesh. *Int. J. sustain. crop produc.* **3**(4): 18-23.

MOTALEBI, A. A., ARDALANI, K. and JAMILI, S. 2008. Effect of temperature on the produced aflatoxins in the rainbow trout feed in West Azerbaijan. *J. Fish Aquatic Sci.* **3**: 392-397.

PITT, J. I. and HOCKING, A. D. 1999. Fungi and food spoilage (2nd ed.). Gaithersburg, Md.: Aspen Publications. pp. 1306.

RAHMAN, F., SUMITRA, D. and ALAM, N. 2024. Pathogenic fungi associated with coriander wilt disease: cultural condition, molecular identification and biological control. *Plant Tissue Cult. & Biotech.* **34**(2): 193-204.

SAHA, B. K., ISLAM, M. R., SAHA, A. and HOSSAIN, M. A. 2009. Reproductive biology of the mola carplet *Amblypharyngodon mola* (Hamilton) (Cypriniformes: Cyprinidae) from Netrakona Water. *Bangladesh J. Sci. Indust. Res.* **44**(3): 377-379.

SEIFERT, K. A., WINGFIELD, B. D., AND WINGFIELD, M. J. 1995. A critique of DNA sequence analysis in the taxonomy of filamentous ascomycetes and ascomycetous anamorphs. *Canadian J. Bot.* **73**(1):760-767.

SIKDER, M. M., MALLIK, M. R. I. and ALAM, N. 2019. Identification and *in vitro* growth characteristics of entomopathogenic fungus-*Aschersonia sp.* in Bangladesh. *Advan. Zool. Bot.* **7**(1): 11-18.

SIVARAMAN, G.K., VISNUVINAYAGAM, S., JHA, A.K., REMYA, S., RENUKA, V., AJEESH, K. and VANIK, D. 2018. Molecular divergence and identification of *Aspergillus* species in dry fishes of Gujarat, India. Proceedings of the National Academy of Sciences. India Section B: *Biol. Sci.* **88**(2): 505-515.

TALWAR, P.K. and JHINGRAN, A.G. 1991. Inland fishes of India and adjacent countries (Vol. 2). CRC Press.

TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A. and KUMAR, S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Boil. Evol.*, **30** (12): 2725-2729.

THILSTED, S. H., ROOS, N. and HASSAN, N. 1997. The role of small indigenous fish species in food and nutrition security in Bangladesh. Naga, *ICLARM*, 20(3): 82-84.

WHITE, T. J., BRUNS, T., LEE, S. and TAYLOR, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. PCR protocol: a guide to methods and applications. Academic Press. San Diego, CA. pp. 315-322.

(Manuscript received on 5 June 2025 revised on 14 August 2025)