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RESEARCH ARTICLE

Identification and Cultural Characteristics of *Aspergillus flavus* Associated with Dry Spotted Snakehead Fish (*Channa punctata* Bloch)

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Abstract

The spotted snakehead fish, scientifically known as *Channa punctata*, is highly popular in Bangladesh under both fresh and dry conditions. This work aimed to use both traditional and molecular methods to discover the fungus associated with dried *C. punctata*. The fungus's cultural characteristics were also examined. *Aspergillus flavus*, a very common fungus in dry *C. punctata,* was characterized molecularly using a 594 bp PCR product of its ITS (internal transcribed spacer) region. The sequence was submitted to NCBI under accession number PX122472.1 (JUF0125). The molecular phylogenetic tree derived from it revealed that *A. flavus* species complex had 100% sequence similarity. Mycelial growth characteristics analysis of the fungus revealed that Hennerberg (56 mm) and yeast extract mannitol (28 mm) culture media, respectively, showed the highest and lowest mycelial growth rates. The optimal pH and temperature for mycelial growth of *A. flavus* were 7 and 30°C, respectively. To the best of our knowledge, this experimental identification of the dry fish fungus *Aspergillus flavus* associated with *C. punctata* is the first report and confirmation of a fungus in Bangladesh using both classical and molecular approaches.

Keywords: *Channa punctata*, Dry fish fungus, *Aspergillus flavus,* Molecular identification, Cultural characteristics.

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Introduction

Spotted snakehead *Channa punctata* (Bloch), commonly known as 'taki' is one of the most important small indigenous fish species in Bangladesh. *C. punctata* is widely distributed in the country in all types of water bodies (Talwar and Jhingran 1991). Additionally, this species is highly regarded for its flavor, high protein content, lack of intramuscular pin bones, high nutritional value, and restorative and therapeutic capabilities, making it a recommended diet during convalescence. These fish have been considered as an excellent source of essential protein, macro and micronutrients, and minerals, which can play a significant role in the fulfillment of nutritional deficiencies in human beings (Ara and Nabi 2018).

Fish is an awfully perishable food item and needs preservation for future uses (Sultana et al. 2020). Dried fish is one of the popular food items in Bangladesh. Sun drying is the most widely used method of fish preservation throughout the world (Ara et al. 2020a). This method is also considered as the least expensive method of preservation. A significant amount of dried fishes was exported from Bangladesh and earned good amount of foreign currency (Galib et al. 2013). The sun-dried SIS fishes contain up to 60-80% protein. Fungal growth on dried fish indicates the onset of spoilage and deterioration of the product (Pitt and Hocking 2009). Atapattu and Samarajeewa (1990) reported that the most prevalent fungus such as *Aspergillus niger*, *A. flavus, A. fumigatus, A. glaucus, A. restrictus, Aureobasidium* spp. *Basipetospora halophila*, *Cladosporium herbarum, Gliomastix,* spp., *Penicillium chalybeum* and *P. expansum* were recorded in dried fish. According to Sivaraman et al. (2018), *Basipetospora halophila* and *Polypaecilum pisce* are two white halophilic moulds that are associated with the spoilage of salted fish.

Fungal growth on dried fish causes off flavors, softens the flesh, and some can produce mycotoxins under certain circumstances (Pitt and Hocking 2009). Several fungi, *viz.*, *Aspergillus* spp., *Penicillium* spp., *Fusarium* sp., *Alternaria* sp., *Rhizopus* sp., *Mucor* sp., *Acremonium* sp., *Wallermia seba,* and *Sporodendron epizoum* are associated with dry fish in Asian countries (Ara et al. 2020b). Many fungal species, especially *Aspergillus* sp., are known to produce many types of toxins such as aflatoxins, ochratoxins, and sterigmatocystine, which have mutagenic effects on human health. This causes a considerable decrease in the consumption of dried fish (Motalebi et al. 2008).

The ITS region of genomic DNA is highly helpful for evaluating phylogenetic relationships at lower taxonomic levels, as shown by recent molecular phylogenetic research (Alam et al. 2010). Due to its short length, the ITS region can be quickly amplified by PCR using single universal primer pairs (Sikder et al. 2019). According to Rahman et al. (2024), *F. oxysporum* mycelial development showed the highest (75 mm) growth on potato dextrose agar (PDA) and the lowest (40 mm) growth on yeast extract agar (YEA) media, with optimal requirements of pH 6.5 and a temperature of 25°C. Therefore, the present research has received extensive attention regarding the morphological and molecular identification, and cultural characteristics of the dry fish fungus associated with the native SIS spotted snakehead fish in Bangladesh.

Materials and Methods

Sample collection and identification of dry fish fungus

Channa punctata were collected from Chalan Beel in Natore district (latitudes 24.35° and 24.70° N and longitudes 89.10° and 89.35° E), Bangladesh, and transported to the Limnology and Fishery Sciences laboratory, Department of Zoology, Jahangirnagar University (JU), where they were dried. Fresh fish samples (Fig. 1A) were cleaned with regular water and dried in the sun for 10-15 days. After completing the preparation process, the dry fish (Fig. 1B) sample was placed in a plastic pot and sent to the Laboratory of Mycology and Plant Pathology, Department of Botany, JU, for dry fish fungus isolation and identification.

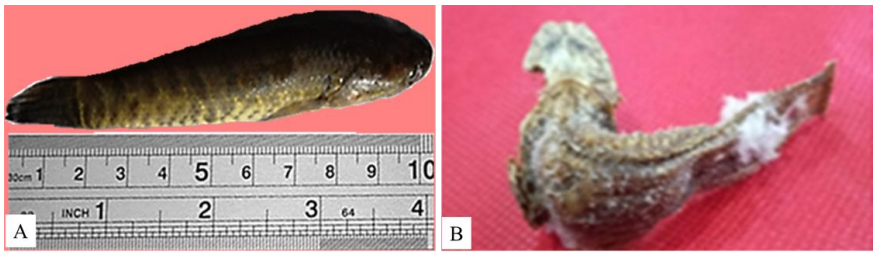

Fig. 1(A-B): Spotted snakehead fish (*Channa punctata*) in fresh (A) and sun-dried, (B) conditions.

Fungus was isolated from the dry fish of *Channa punctata* through the tissue planting method (Rahman et al. 2025). These were cut into pieces of 0.5 cm in length, infected parts of *Channa punctata* were designed to contain both fungal-infected and non-infected tissues. After that, sterilization was carried out using a NaOCl (5%) solution for 3 minutes before being rinsed several times with distilled water. Four samples were put into potato dextrose agar (PDA) medium and cultured for ten days at 27±2°C with 12/12 hours of darkness and light. To develop a pure culture, the mycelial growth of a developing fungal colony was transferred to new PDA plates and PDA slants. After several hyphal tip transfers, a pure culture was generated. The pure culture of the isolated fungus was identified microscopically using standard methods (Akter et al. 2023).

Molecular characterization was done with the help of commercial service provided by Invent Technology, Dhaka, Bangladesh. Fungus genomic DNA samples were extracted using the Maxwell Cell Kit (AS1030, Promega, USA). The primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAA AGTCG TAACAAGG-3′) were used for the PCR reaction (Alam and Rahman 2020). The polymerase chain reaction (PCR) was performed with 20 ng of genomic DNA as the template in a 25-µl reaction mixture using an LA *Taq* (TAKARA BIO INC, Japan). The thermal cycle was performed with activation of *Taq* polymerase at 94°C for 1 minute; 35 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 5 minutes each; finishing with a 10-minute step at 72°C for final extension. Amplified PCR products were electrophoresed on 1.50% agarose gel in 1 × TAE buffer for 1hr at 100V with a 1kb DNA ladder as a size marker and then stained while agitated in an EtBr solution (0.05% µg/mL). The stained gels were

visualized and photographed using a UV transilluminator (Kodak Image Station 4000R; Molecular imaging system, Carestream Health Inc., 150 Verona Street, Rochester, NY 14608). The Maxwell® 16 DNA Purification Kits were used to purify the amplification products (Promega, USA). The purified PCR products were sequenced bi-directionally in First BASE Laboratories SdnBhd (Malaysia).

BioEdit and MEGA 6 checked DNA sequences. A BLAST search with the ITS sequences was used to reveal the closest matching taxa. Multiple sequence alignments were done using MEGA6. Data was converted from fasta to MEGA format with Clustal W. The models of evolution were determined under the Akaike Information Criterion (AIC). The model selected was the Tamura-3 parameter for analysis. UPGMA method with 1000 bootstrapping along with max-trees set at 1000. Bootstrap values greater than 60% were accepted (Tamura et al. 2013).

Effect of culture media, temperature, and pH on the mycelial growth of *Aspergillus flavus*

Five distinct culture media, including Hennerberg (HEN), potato dextrose agar (PDA), glucose peptone (GLP), yeast extract mannitol (YEM), and Hoppkins (HOP) media, were prepared according to Ahmmed et al. (2022) to assess the mycelial growth characteristics of the isolated fungus. Before autoclaving, the medium was adjusted to a pH of 6.5. To determine the optimal temperature for the mycelial development of an isolated fungus, different levels of temperature (15°C, 20°C, 25°C, 30°C, and 35°C) were tested. To the effect of pH, the PDA medium was accustomed to pH 5, 6, 7, 8, and 9 with the help of a digital pH meter and the addition of 1N NaOH or lactic acid to adjust the pH, and then autoclaved. The radial growth of mycelia on each Petri dish was assessed in three directions accordingly (Masud et al. 2023).

Data analysis

The research activity was analyzed using standard statistical analysis programs like MS Excel, SPSS 16.0, MEGA 11.0, and BLAST were used to explain the produced data. In SPSS-16, one-way ANOVA and Duncan's post-hoc analysis were used to analyze the data.

Results and Discussion

Identification of *Aspergillus flavus*

Aspergillus flavus was isolated from the selected dry SIS fish of *Channa punctata*. The *A. flavus* colonies expand quickly (45 to 55 mm in 7 days). While the conidial heads are normally distributed in zones on an agar plate, the vegetative mycelium is mainly immersed in the agar. The colony's distinctive color is yellow. On a petri dish, the reverse side appears pale to brownish. The conidiophores of *A. flavus* resemble a powdery mass to the unaided eye. Phialides are placed on the conidial heads in a biseriate pattern under a microscope, either smooth or finely roughened. Initially globose in culture, the conidial heads eventually acquire two or three divergent columns as the conidial chains stick to them over time (Fig. 2). Typically, vesicles have a subglobose form. Kar et al. (2025) reported that the shapes and sizes of various structures, such as colony diameter of mycelium, conidia, and conidiophores of fungus, are taxonomical characters for morphological identification of the species *Aspergillus*.

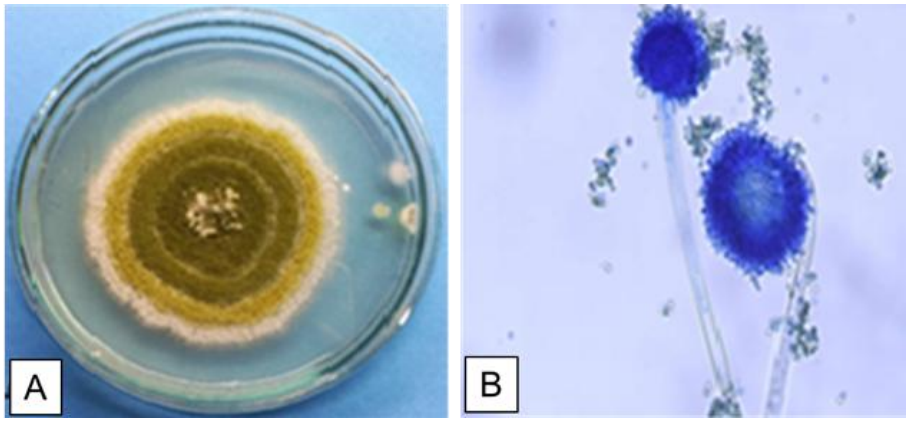

Fig. 2 (A-B): Morphological characteristics of *Aspergillus flavus*. A, mycelial growth of *A. flavus* in PDA medium; B, Microscopic view of conidia, conidiophore of *A. flavus* (10 × 40).

The PCR products of the ITS region in *A. flavus* was 594 bp (Fig. 3). Using ITS4 and ITS5 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 levels, according to molecular phylogenetic research. When it comes to variation between species and even strains, the ITS of rDNA is thought to be a key factor (Cho et al. 2010).

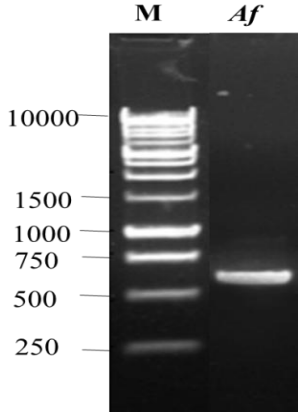

Fig. 3: PCR products of the ITS region of *Aspergillus flavus*. Lane M, molecular marker (1 kb DNA ladder); Lane *Af*, *A. flavus*.

In phylogenetic tree, the ribosomal DNA sequence of studied fungus was homologically compared in percentage with previously acknowledged fungi *A. flavus*. There were five different clades founded in maximum parsimony tree (Fig. 4). Above 99% identity was showed in other formerly identified fungi *A. flavus* under the accession number such as KY689188.1, KR296888.1, MF9809141.1 and MT680400.1, respectively. The phylogenetic tree based on the nucleotide sequence of ITS region comprises of twenty taxa of representative strain of *A. flavus*, including our taxa and *Penicillium expansum* (OW987737.1) as an out group. Phylogenetic tree was generated by UPGMA methods with the studied fungus. The BLAST search revealed that our organism JUF0125 (accession number PX122472.1) was 100% similar to *A. flavus* (KY689188.1). ITS sequences change within a genus, although having genetically stable or indicating little variation within the species (Lee et al. 2010).

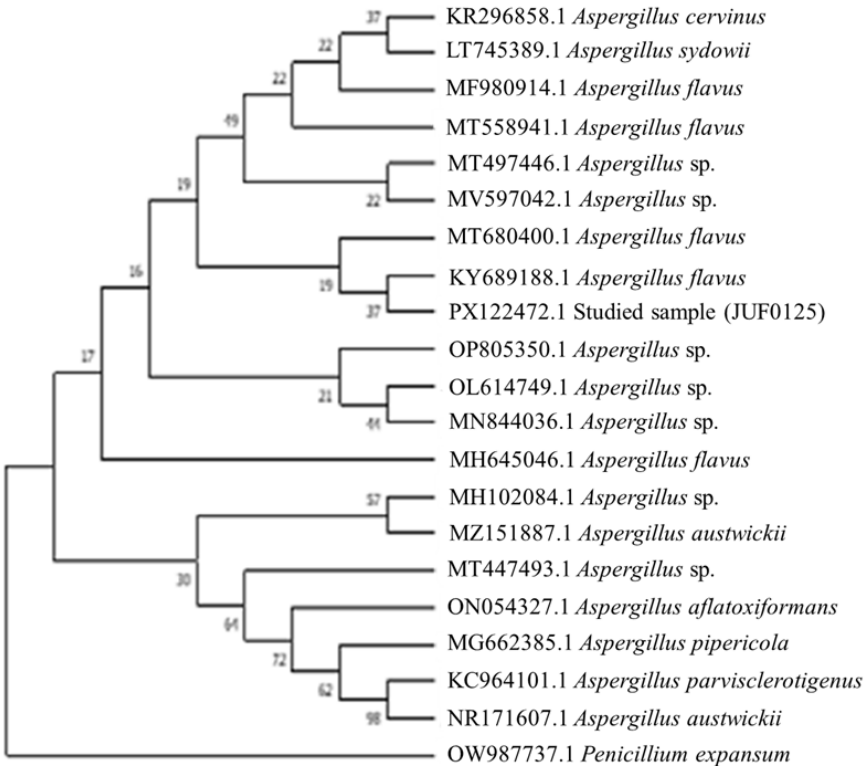

Fig. 4: Phylogenetic tree produced by analysis of the studied fungus associated with dry spotted snakehead fish using the UPGMA method with 1000 bootstrapping.

Cultural characteristics of *Aspergillus flavus*

A. flavus was grown and developed under ideal conditions using a selection of five culture mediums, including Hennerberg (HEN), potato dextrose agar (PDA), glucose peptone (GLP), yeast extract mannitol (YEM), and Hoppkins (HOP). The experimental findings suggested that HEN and GLP culture media showed the significantly maximum, while YEM medium was minimum mycelial growth of *A. flavus* (Fig. 5). Similar results also supported by Sultana et al. (2023). Chanda et al. (2021) reported that mycelial growth pattern of *Aspergillus elegans* was the best on PDA medium.

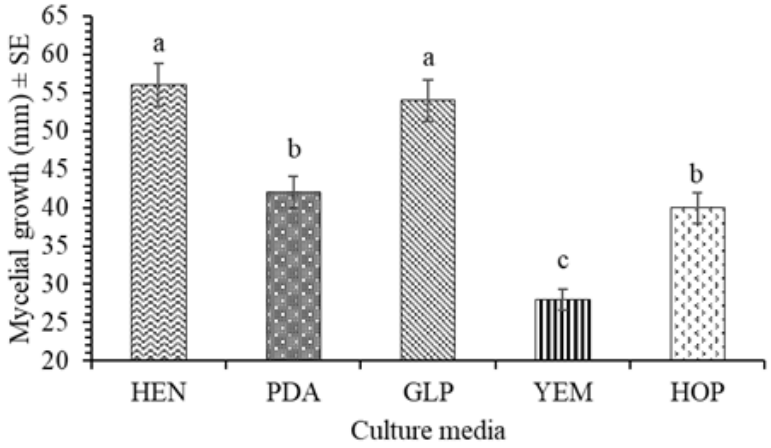

Fig. 5: Effect of culture media on the mycelial growth of *Aspergillus flavus* after 10 days of incubation at 25°C. HEN, Hennerberg; PDA, potato dextrose agar; GLP, glucose peptone; YEM, yeast extract mannitol; HOP, Hoppkins. Different letters in each treatment indicate a significant difference at $p<0.05$.

Temperature effects on the mycelial growth of *A. flavus* on PDA medium were evaluated at five different temperatures, such as 15°C, 20°C, 25°C, 30°C, and 35°C. The data showed that significantly the highest mycelial growth of *A. flavus* was recorded at 30°C, followed by 25°C (Fig. 6). In our experiment, *A. flavus* grew maximum at 30°C, which is consistent with the previous findings of Iwen et al. (2007), who cited that the highest mycelia growth and sporulation of *A. elegans* were registered at 30°C. Masud et al. (2023) reported that *C. blakesleeana* had the highest mycelial growth at 30°C, which is in close agreement with the results of our most recent study. The findings also show that by using the right combination of these factors, we may limit the growth of the mold and lessen product losses as well as the financial costs associated with fungal contamination.

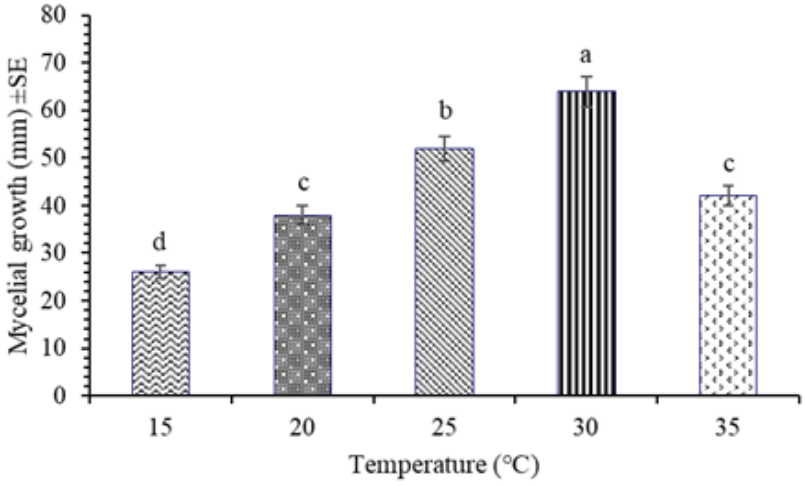

Fig. 6: Effect of temperatures on the mycelial growth of *Aspergillus flavus* after 10 days of incubation on PDA medium. Different letters in each treatment indicate a significant difference at $p<0.05$.

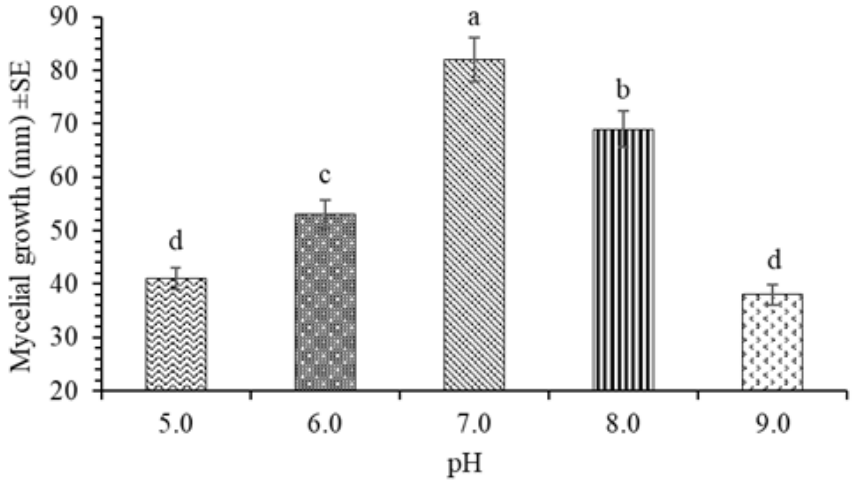

Fig. 7: Effect of different pH on the mycelial growth of *Aspergillus flavus* after 10 days of incubation on PDA medium. Different letters in each treatment indicate a significant difference at $p<0.05$.

A. flavus mycelial growth and development exhibited an increasing trend until pH 7.0, at which point it began to decline. The maximum mycelial growth (82 mm) was observed in *A. flavus* at a pH of 7.0. However, at pH 9.0, the fungus grew with the lowest (38 mm) (Fig. 7). The best growing conditions for the fungal isolates in this study were neutral. These pH values were the most noticeable for colony diameter, color, and sporulation. Our findings were supported by Sikder et al. (2019) stated that *Aschersonia* sp. fungus's ideal pH for vegetative growth was found to be pH 7.0.

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Author's contribution: IA and NA designed the experiment, supervised the study, and finally corrected the article. SC and AAM conducted experiments, collected data statistical analysis procedure and completed the draft of the article.

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Data availability: All data generated in the study are reported in the article, and unprocessed data is with the corresponding author and available upon request.

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 University Journal of Biological Sciences 11(1&2): 1-11.

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 University Journal of Biological Sciences 12(1&2): 55-51.

Alam N and Rahman F (2020). Vegetative growth and genetic diversity in different strains of *Pleurotus salmoneastramineus* based on PCR polymorphism. Bangladesh Journal of Botany 49(1): 125-134.

Alam N, Cha YJ, Shim MJ, Lee TS and Lee UY (2010). Cultural conditions for mycelial growth and molecular phylogenetic relationship in different wild strains of *Schizophyllum commune*. Mycobiology 38(1): 17-25.

Ara I and Nabi MR (2018). Morphometric and meristic comparison of local and Thai *Anabas testudineus*. Journal of Fish 6(2): 599-604.

Ara I, Sultana R, Chanda IJ and Alam N (2020a). First report on *Ovatospora brasiliensis* from fresh water dried shrimp and prawn in Bangladesh. International Journal of Fauna and Biological Studies 7(3): 43-47.

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

Atapattu R and Samarajeewa U (1990). Fungi associated with dried fish in Sri Lanka. Mycopathologia 111(1): 55-59.

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 Journal of Biological Sciences 10(1&2): 25-30.

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. Journal of Fisheries 1(1): 1-6.

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 Journal of Mycology 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 Journal of Plant Sciences 14(11): 1316-1326.

Motalebi AA, Ardalani K and Jamili S (2008). Effect of temperature on the produced aflatoxins in the rainbow trout feed in West Azerbaijan. Journal of Fish and Aquatic Science 3(2): 392-397.

Pitt JI and Hocking AD (2009). Fungi and food spoilage. New York: Springer, pp. 205.

Rahman F, Dey S, Alam NB, Bhowmik DD, Alam N and Alam N (2025). Mycelial growth and molecular identification of pathogenic fungi isolated from basal rot disease of onion. Plant Tissue Culture and Biotechnology 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 Tissue Culture and Biotechnology 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. Advances in Zoology and Botany 7(1): 11-18.

Sivaraman GK, Visnuvinayagam S, Jha AK, 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: Biological Sciences 88(2): 505-515.

Sultana A, Sikder MM**,** Alam N (2023). Identification and fungal biology of post-harvest fungi associated with amla fruits and its *in vitro* control measures. Advances in Zoology and Botany 11(1): 1-11.

Sultana R, Ara I, Chanda IJ and Alam N (2020). First report of *Lichtheimia hyalospora* from fresh water dried shrimp in Bangladesh. International Journal of Fisheries and Aquatic Studies 8(3): 474-477.

Talwar PK and Jhingran AG (1991). Inland Fishes of India and Adjacent Countries. Oxford-IBH Publishing Co. Pvt. Ltd., New Delhi, India, pp. 1158.