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Prevalence and pathogenesis of black gill disease in wild caught black tiger shrimp, *Penaeus monodon* (Fabricius, 1798)

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ABSTRACT

The black tiger shrimp (Penaeus monodon) is a key species in global aquaculture, particularly in the Indo-Pacific region, where it is prized for its large size, rapid growth, and high market value. However, the species is increasingly affected by diseases, particularly black gill disease, a fungal infection that impairs respiratory function, leading to mortality. This study aims to investigate the prevalence and pathogenesis of black gill disease in wild-caught Penaeus monodon from the coastal waters of Mudasalodai, Tamil Nadu, India. Shrimp were collected seasonally from July 2023 to June 2024, with affected specimens showing signs of blackened gills. Histopathological examinations revealed fungal hyphae in the gill tissues, accompanied by immune responses and tissue necrosis. Microbiological investigations identified Aspergillus sp. as the causative agent, with fungal colonies forming characteristic conidiophores and conidia. Scanning electron microscopy (SEM) provided detailed imagery of fungal structures, confirming the identification of Aspergillus sp. Prevalence data indicated varying levels of infection across different seasons. These findings highlight the importance of monitoring fungal infections in shrimp aquaculture and suggest that environmental factors may contribute to the spread of black gill disease.

KEYWORDS: Penaeus monodon, black gill disease, Aspergillus sp., prevalence.

1.INTRODUCTION

Penaeus monodon, the black tiger shrimp, is a crucial species in the world's aquaculture and fisheries industry, particularly in the Indo-Pacific area. The species has been preferred for shrimp farming because of its large size and fast growth rates. However, the

business has been dealing with issues like disease outbreaks and competition from other species. According to the Food and Agricultural Organization (2014), 42% of the 158 million tons of fish produced by capture and culture fisheries are farmed fish, and the aquaculture sector is growing to be worth millions of dollars.

Throughout the 1980s and 1990s, *P. monodon* continued to be the species of choice in shrimp aquaculture, particularly in Asia, while Thailand, Vietnam, and the Philippines appear to be the top producers. The species' popularity has increased due to its high market value and ability to adapt to varying salinities. Throughout the 1980s and 1990s, *P. monodon* continued to be the species of choice in shrimp aquaculture, particularly in Asia, while Thailand, Vietnam, and the Philippines appear to be the top producers.

Shrimp farming has a long history in India and was expanded in the 1990s. *P. monodon* was the most widely grown species, mostly in coastal states like Tamil Nadu, Gujarat, Kerala, West Bengal, and Andhra Pradesh. The species was selected because of its distinctive flavor, quick pace of development, and steady demand in the international market. However, the introduction of the whiteleg shrimp, *Litopenaeus vannamei*, and disease outbreaks caused *P. monodon* production to diminish, just like in other nations (Salunke et al., 2020). In recent years, the cultivation of *P. monodon* has increased in India. Because of its higher market value and sustainability, farmers are again turning back to this species. The Indian government has been helpful by offering incentives and technical aid to promote sustainable methods and restore traditional shrimp farming areas. Research institutes are working hard to create high-yield, disease-resistant cultivars to support this industry even more. In the aspect of fisheries sector and Indian marine ecosystems, (*Penaeus monodon*) black tiger shrimp, is an important species. Mostly found on India's east coast, this shrimp mostly inhabits depths of 30 to 40 meters. Mature species are most successful while fishing during the post-monsoon season, which runs from October to April. Once the waters stabilize, the salinity reaches its high. The capture of wild *P. monodon* is an important supply of broodstock for Indian hatcheries, which are essential to India's shrimp aquaculture sector. It is difficult since it decreases wild stock levels and can cause diseases to spread from wild broodstock to cultivated populations. A complex relationship between the infection, environment, and animal prompts disease outbreak (Lightner and Redman 1998). However, in the shell fish, fungal infection is one of the most common disease issues (Khoa et al., 2005). Among all fungal diseases in shrimp, black gill disease is the most prevalent in wild as well as cultured facilities. It impairs the shrimp's ability to breathe, causes various other illnesses, and ultimately leads to death. In shrimps, the gills are the most vital organs involved in breathing. Since fungus can obstruct breathing, raise chronic mortality, and make shrimp more susceptible to other illnesses, a fungal infection in the gills may be fatal for the shrimp. Gill color change is the initial clinical sign of a fungal infection. Initially, the gills are opaque white, then become yellow or brown, and finally their turn black (Rhoobunjongde et al., 1991; Khoa et al., 2004; Le et al., 2005).

Black gill disease has also been linked to chemical contaminants such as oil, cadmium, copper, zinc, potassium permanganate, ozone, ammonia, nitrate, the lack of ascorbic acid, excessive siltation, and a high organic load from leftover feed, discharge of trash, and feces (Lavilla et al., 2000). However, there are several factors that are thought to contribute to black gill disease in shrimp, which can induce gill damage and breathing difficulties by blocking gas exchange across the gill lamellae (Lightner et al., 1975). Previously fungal disease in Penaeus monodon has been reported by Pramanik and Mohanty 2015; Chavda and Sujata; Karthikeyan and Gopalakrishnan 2014. From the coastal farms. In contrast to research on fungi in freshwater and terrestrial environments, the distribution of fungi in diseased shrimp has not been thoroughly examined, despite the fact that fungi are extensively found in marine environments (Velmurugan and Gopalakrishnan 2014). Therefore, the present study aimed to investigate the Prevalence and pathogenesis of black gill disease in wild caught black tiger shrimp, Penaeus monodon

2. MATERIALS AND METHODS

2. 1. Study area

Mudasalodai is a coastal region (Lat: 11°29'15"N Long: 79°46'28"E) in the Cuddalore district of Tamil

Nadu, India. It is known for its fishing port, which plays a crucial role in the local economy and the fishing industry of the region. The Mudasalodai fishing port is located on the Bay of Bengal and serves as a hub for both traditional and mechanized fishing activities.

2. 2. Sample Collection

Black tiger shrimp *(Penaeus monodon)* were collected from the Mudasalodai coastal waters using a commercial shrimp trawler during the period from July 2023 to June 2024. These shrimp were found to be The light microscope was used to examine the tissue at various magnifications.

2. 4. Microbiological investigations

Shrimp gills were separated and then cleaned two to three times in a 0.85% NaCl solution. After cleaning, the gills were inoculated in potato dextrose agar (PDA) media that had been treated with streptomycin and ampicillin to prevent the growth of bacteria. They were then incubated at 25 °C. Following gill isolation, fungi affected by the black gill illness. After their collection, the affected shrimp were transported to the laboratory for further analysis.

2. 3. Histopathological study

To preserve them, black and normal gills were fixed independently in Davidson's fixative and then transferred in 50% ethanol. Gill tissues were cut into sections of about 3 µm using a microtome, and the tissues were then stained with Eosin and Haematoxylin. were observed under a microscope using lactophenol cotton blue. Additionally, normal gills were injected using the same medium as the control. The experiment was performed in triplicate.

2. 5. Scanning Electron Microscopy (SEM)

Using a sterile blade, *Penaeus monodon* black gill-caused Aspergillus sp. scraped onto an SDA plate, dried, and sputter-coated with gold before being seen under a JEOL JSM-IT200 scanning electron microscopy.

3. RESULTS

3. 1. Gross Pathology

The *Penaeus monodon* weighing approximately 20 grams were observed with prominent signs of blackened gills. This condition, characterized by a noticeable dark discoloration throughout the gill structure Fig 1B and Fig 1A (normal gill), raises concerns about a potential fungal infection as the underlying cause. Further investigation, including microbiological and histopathological analyses, is essential to confirm the presence of fungal pathogens and to determine the specific causative agent.

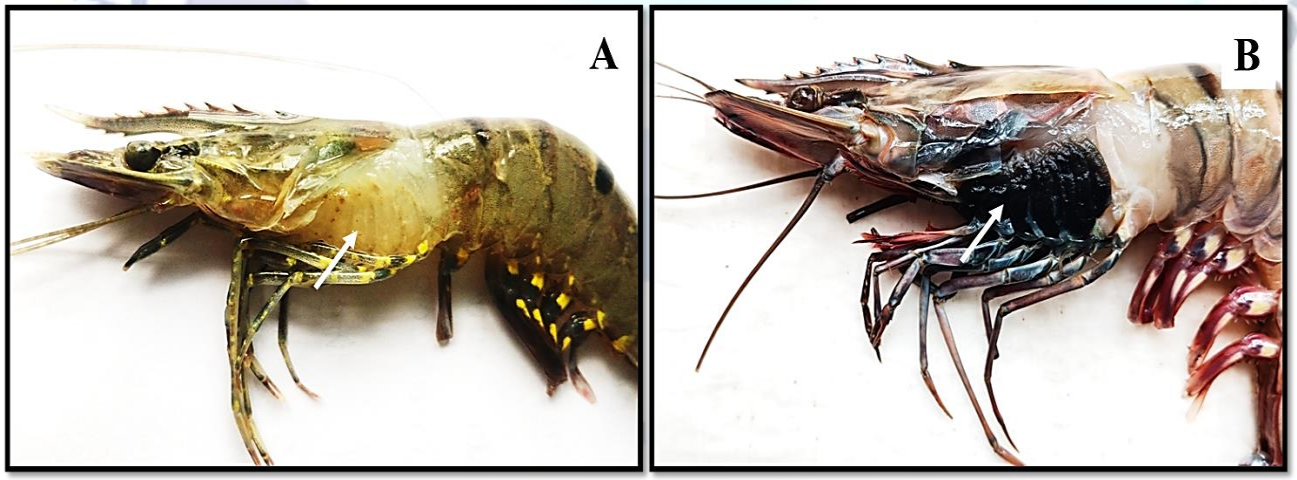

Figure 1: Images of *Penaeus monodon* gills. A: Normal gill of *Penaeus monodon*; B: Black gill of diseased *Penaeus monodon*.

3. 2. Histopathological study

In The histological examination revealed in the case of normal gills, the gill physiology remained unchanged and there were no fungi in the gill lamella (Fig.2A). In the diseased shrimp the presence of encapsulated hyphae within the gill tissues, indicating a fungal infection. These hyphae were prominently observed not only within the gills but also at their base, where significant pathological changes were noted. Surrounding the hyphae, there was extensive

haemocytic infiltration (arrow), suggesting an active immune response and highly vacuolized (doted arrow) Fig 2B. This infiltration was accompanied by large deposits of substances characteristic of coagulation necrosis, indicating severe tissue damage Fig 2B. The findings highlight the progression of infection and its destructive impact on gill integrity and function.

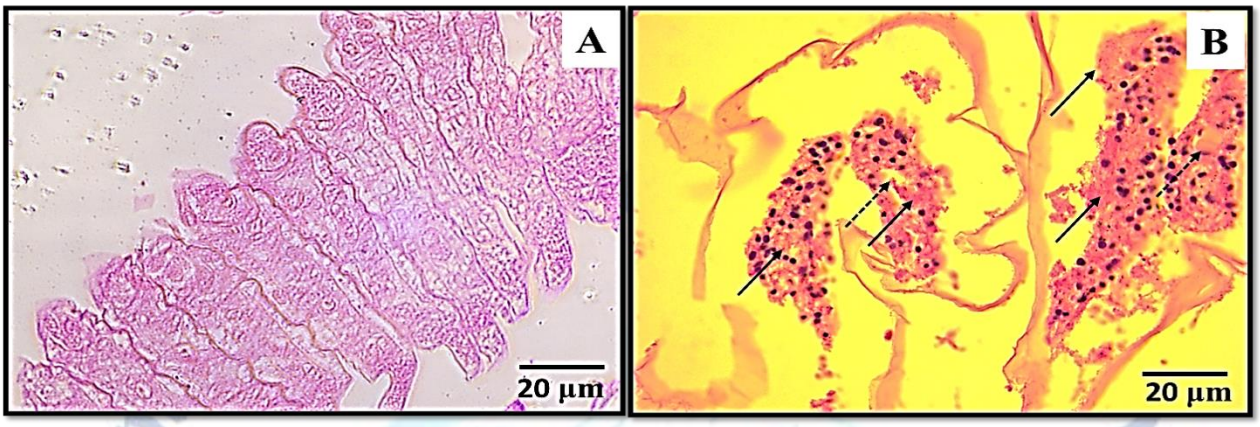

Figure 2: Normal Gill (A); black gill histological examination revealed encapsulated hyphae in the gill tissues and their base, accompanied by haemocytic infiltration (arrow) and vacuolization (dotted arrow) Coagulation necrosis deposits indicate severe tissue damage and infection progression (B).

3. 3. Microbiological investigations

The colonies grown on PDA plates initially appeared grey but quickly transitioned to a bright to light greenish-black coloration, characteristic of fungal growth. No growth was observed on the control plate, confirming the sterility of the experimental setup. The photomicrograph from the culture, as shown in (Fig 3A), illustrates the structural components of *Aspergillus* sp. under an LPCB mount. The image distinctly highlights the presence of conidiophores, specialized hyphal structures that bear conidia, along with the conidiospores and conidia, which are the spore-producing units. (Fig 3B) provides a magnified view of mature conidiophores, showcasing their unique morphology specific to *Aspergillus sp.* These photomicrographs collectively emphasize the diagnostic features of the fungal species, aiding in accurate identification.

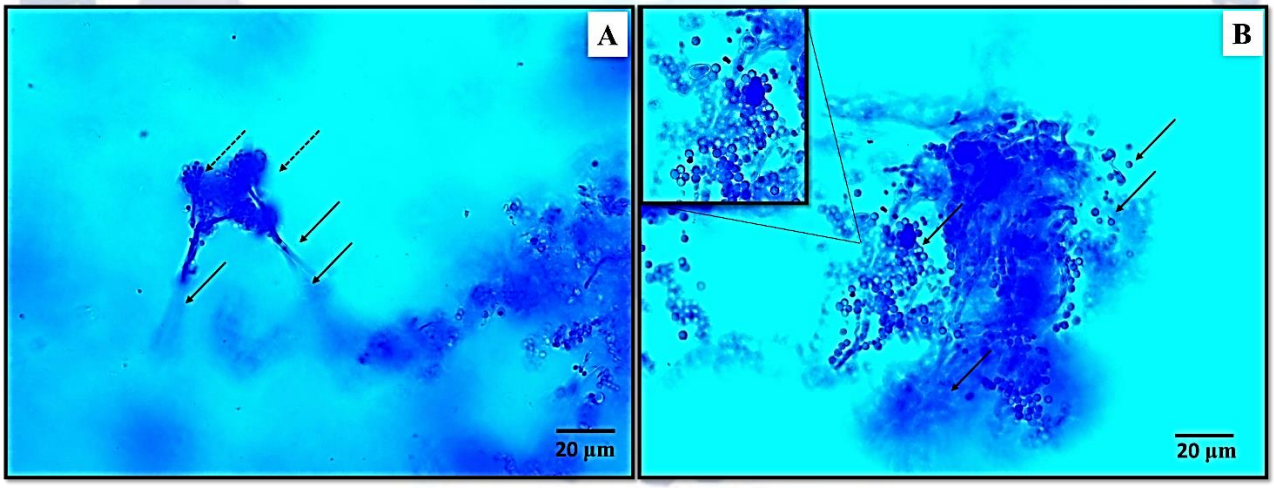

Figure 3: shows the photomicrograph (Fig. 3A) highlights *Aspergillus* sp. structures under an LPCB mount, showing conidiophores, conidiospores, and conidia. A closer view in (square) Fig. 3B reveals mature conidiophores with distinctive morphology of *Aspergillus sp.*

3.4. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) analysis provides a detailed visualization of Aspergillus sp., revealing numerous hyphae intertwined to form colonies. The colonies prominently exhibit conidiophores and conidial heads, which are crucial for reproduction. The conidial heads are typically radiating, splitting into several poorly defined

columns, though some display a distinctly columnar structure. The conidia are globose to sub-globose in shape, covering the entire surface of the conidiophore. These structural features contribute to the organism's efficient spore dispersal mechanism. Additionally, the SEM observations emphasize the intricate details of the conidia and spores, critical for fungal identification. This detailed morphology highlights the unique features of Aspergillus sp (Fig 4).

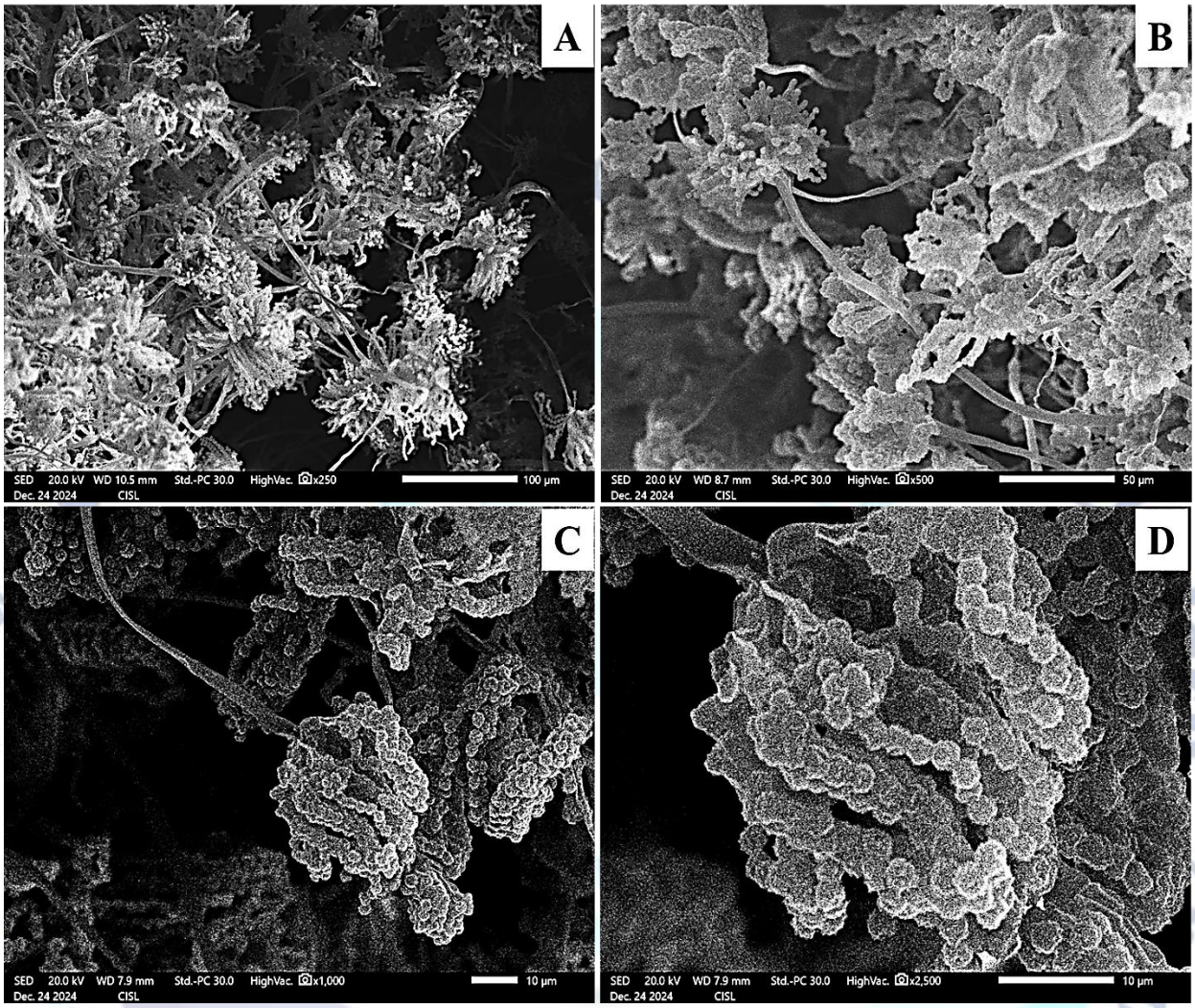

Figure 4: shows the detailed features of *Aspergillus* sp., showing numerous intertwined hyphae forming colonies. The colonies display conidiophores with radiating or columnar conidial heads and globose to sub-globose conidia covering their surface. These features enhance spore dispersal efficiency and are critical for fungal identification.

3.5 Prevalence of black gill disease

Black gill illness was shown to be common in wild black tiger shrimp (*Penaeus monodon*) from the coastal region of Mudasalodai between July 2023 and June 2024. Disease prevalence varied according to data gathered throughout the four different seasons of the monsoon, post-monsoon, summer, and pre-monsoon. The number of cases recorded during the monsoon season was 11, followed by 15 cases in the post-monsoon season. The summer season showed a reduction in prevalence, with 5 cases, while the pre-monsoon season recorded 8 cases. The total prevalence across all seasons amounted to 39 cases, indicating the overall burden of black gill disease in the Mudasalodai region during the study period (Table 1 & 2).

Table 1: shows the prevalence of black gill disease in wild black tiger shrimp, *Penaeus monodon*.

Station	Monsoon	Post Monsoon	Summer	Pre Monsoon	Grand Total
Mudasalodai	11	15	5	8	39

Table 2: shows the seasonal intensity of the black gill disease in wild black tiger shrimp, *Penaeus monodon*.

Seasons	No of shrimp Examined	No of shrimp Infected	Mean Intensity	Prevalence (%)
Monsoon	1011	11	0.0109	1.09
Post Monsoon	992	15	0.0151	1.51
Summer	1015	5	0.0049	0.49
Pre Monsoon	998	8	0.0080	0.80
Grand Total	4016	39	0.0097	0.97

The monsoon season had the highest number of infected shrimp (11 out of 1,011 examined) with a mean intensity of 0.0109. The post-monsoon season showed a slightly higher infection count (15 out of 992 examined), with a mean intensity of 0.0151. Summer had the lowest infection rate, with only 5 infected shrimp out of 1,015 examined, and a mean intensity of 0.0049. The pre-monsoon season recorded 8 infected shrimp out of 998 examined, with a mean intensity of 0.0080. In total, 4,016 shrimp were examined, with 39 infections, and a mean intensity of 0.0097. This data provides insights into the seasonal variation in shrimp infection rates and the intensity of the infections (Fig. 5).

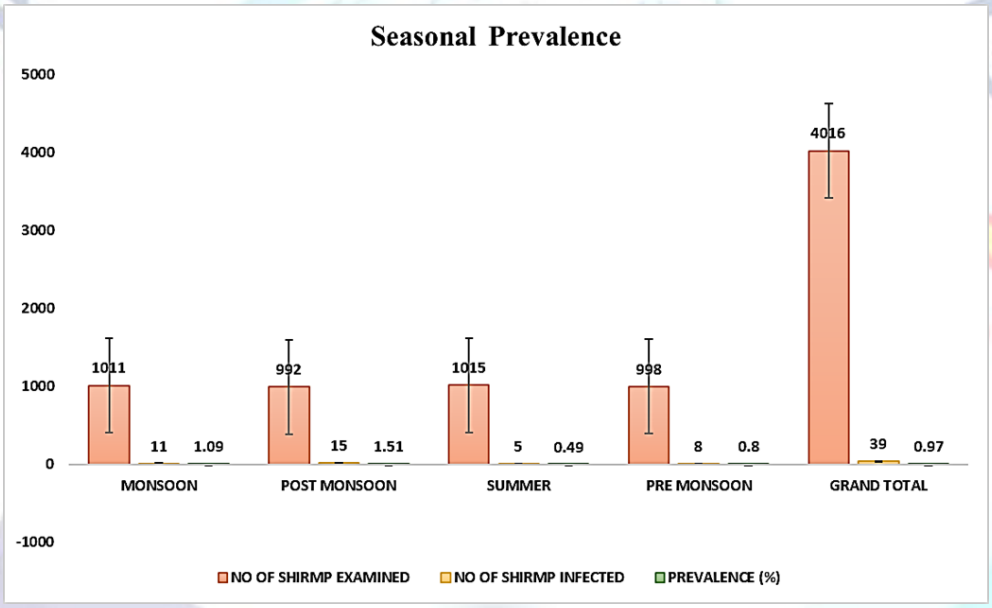

Figure 5: shows the intensity of the black gill infestation over the period of 2023 to 2024

4. Discussion

The findings from this study on black gill disease in wild-caught *Penaeus monodon* highlight its severe impact on the gills, which serve as vital respiratory organs. Fungal pathogens, particularly those from the *Aspergillus* genus, were identified as the primary causative agents. This observation aligns with earlier studies that noted fungal infections as a significant issue in shrimp aquaculture, affecting respiratory efficiency and survival rates (Pramanik & Mohanty, 2015; Karthikeyan et al., 2015). Histopathological observations from this study revealed encapsulated fungal hyphae, haemocytic infiltration, and coagulation necrosis, consistent with findings in *Litopenaeus vannamei* infected by *Aspergillus awamori*. Fungal invasion disrupted gill function, leading to chronic mortality. SEM analysis corroborated these results by illustrating the morphological features of *Aspergillus* sp., including its conidiophores and spore-producing structures, critical for fungal identification and pathogenicity (Karthikeyan et al., 2015). Fungi can be secondary infection also in other diseased shrimps like loose shell syndrome (Gopalakrishnan and Parida, 2005)

Environmental factors significantly influence the progression and severity of black gill disease. Pramanik and Mohanty (2015) demonstrated that higher stocking densities in aquaculture systems correlate with increased disease prevalence. Elevated densities exacerbate stress

in Shrimp populations are more stressed by elevated densities, which makes them more vulnerable to fungal infections. These patterns are in line with research from aquaculture systems in Odisha, India, where fungus outbreaks were more likely due to inadequate water quality control.

Previous reports identified multiple environmental and biological factors contributing to black gill disease, including chemical pollutants and inadequate aquaculture practices. For instance, Lavilla et al. (2000) suggested that contaminants like oil and heavy metals might exacerbate gill damage. However, the current study emphasizes fungal pathogens, particularly *Aspergillus* sp., as the primary causative agents in wild populations. This insight underscores the necessity of monitoring wild-caught shrimp used for broodstock to prevent disease transmission to cultured systems (Velmurugan and Gopalakrishnan, 2014).

This study suggests that adopting better management practices—such as maintaining optimal water quality, reducing organic loads, and ensuring adequate space in aquaculture systems—could mitigate black gill disease risks. Additionally, the development of disease-resistant shrimp strains and stringent biosecurity protocols in hatcheries could offer long-term solutions (Pramanik & Mohanty, 2015; Karthikeyan et al., 2015; Dewangan et al., 2015). The study concludes that Aspergillus sp. and black gill illness in wild *Penaeus monodon* are clearly related, and that environmental stressors play a major part in the development of the disease. These results lay the groundwork for methods to manage and stop outbreaks in populations of wild and cultivated shrimp.

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Author's contribution

Murari Jha: Writing – original draft. Ayyaru Gopalakrishnan: Supervision, Investigation, Financial acquisition. Vasanthan Koothan: Methodology, Data curation, Manuscript editing. Keerthika Ganesan: Formal data analysis. Vijayanand Packiaraj: Supervision, Investigation. Naresh Kumar Dewangan: Formal analysis.

Ethics approval

The study did not require ethical clearance because it does not involve human or animal, so not applicable

Conflict of interest statement

Authors declare that they do not have any conflict of interest.

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