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FUNGAL INFECTION IN SHRIMPS COLLECTED FROM AGNIAR ESTUARY, TAMIL NADU, INDIA

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ABSTRACT

Today shell fishes diseases are of great concern to aquaculturists as they inflict heavy losses by causing large scale mortalities resulting in huge economic losses. Among the various pathogen, fungi comes next to bacteria. As a knowledge of diseases is basic to their control, the present study was intended to find out the commonly occurring fungi in cultured shrimp farms in Tamil Nadu. Results show that 18 species of fungi could be identified in Penaeus monodon and P. indicus of which three species P. monodon, four species P. indicus. However, eventhough P. vannamei also recorded 18 fungal species only two were recorded throughout the period of culture. Nevertheless, in all the three species of Penaeus, the maximum number of fungal species (18) was uniformly recorded on the 80th day of culture.

KEY WORDS

Shrimp culture, Estuary, Fungal infection

INTRODUCTION

Wetlands although occupying only 4-6% of earth's land area are considered among the most important natural resources of the earth as they are the sources of cultural, economic and biological diversity (Sudip Mitra *et al.*, 2005). Biodegradation of organic matter in this habitat is supported by a wide variety of organisms like bacteria and fungi which are widely distributed in water and sediments of these habitats (Nambiar and Raveendran, 2006).

Fungi are important components of the ecosystem typically constituting more of biomass than bacteria and have been considered the largest biotic community after insects (Sarbhoy *et al.*, 1996). Fungi plays an indispensable role in industry, agriculture, medicine and biogeochemical cycles (Cowan, 2001; Gates *et al.*, 2005). In addition, fungi are also used in fish industry, textile, bioremediation, biofertilizers, biotechnology, *etc.* (Molina *et al.*, 1993; Keizer, 1998; Pilz and Molina, 2001; Hunt, 1999; Manoharachary *et al.*, 2005).

Today, the problems of diseases are of great concern to aquaculturists as they inflict heavy losses by causing large scale mortalities resulting in a drastic decrease in yield. Among the various pathogens, fungi come next only to bacteria. As India is poised for a large-scale development of aquaculture especially of prawns with a stress on semi intensive and intensive culture systems, it is natural to expect increasing hazards of diseases in these systems. However, diseases in fish are not understood well. What is known about fish diseases often relates to aquaria fish and more recently to farm fish (Sharma *et al.*, 2012). As a knowledge of diseases, their causative agents and etiology is basic to control, the present study was intended to find out the commonly occurring fungal pathogens that occurs in three species of prawns commonly cultured in farms using the same estuarine water.

MATERIAL AND METHODS

Sample Collection: Penaied shrimp (21 - 30 g) sample was collected during August 2017 to July 2018 by trawl net from estuarine waters of Agniar Estuary, Pudukkottai district, Tamil Nadu, India. For each collection, 8 - 20 number of fungal brown - gill infected *Penaeus* sp., were collected for further processing.

Isolation and identification of fungi: *Penaeus* sp. infected gills were washed three times in sterile physiological saline (0.85% NaCl) and inoculated on plates with Potato Dextrose Agar (PDA) medium (Himedia lab). Ampicillin and streptomycin sulphate (25 µg/ml) were added to the medium to inhibit bacterial growth. Plates were incubated for 2-4 days at 25°C and then sub-cultured on to fresh PDA plates. The single spore culture method was applied to obtain pure isolates. The isolates were maintained at 25°C on PDA slants for subsequent experiments. Fungal identification was done by lacto phenol cotton blue mount method to observe fungal morphology under light microscopy.

Histopathology method:

Routine histological techniques were used. Davidson's fixative was injected into the hepatopancreas and muscle of live fungal fouling infected shrimp in order to avoid autolysis of those organs. After injection, the whole shrimp was immersed in the same fixative for 48 h before further processing. The infected portion of brown-gill tissue were dissected and immersed in Davidson's fixative for 48 h and transferred to 70% ethanol for further processing. Sections were stained with Ehrlich's hematoxylin and Eosin stains.

RESULTS

The results of the various fungal infections in three species of prawns that were cultured are prevented in Tables 1 and 2. As evident from the table, a total of 18 species of fungi could be identified during the 120-day culture period in the three species of prawns.

Table-1: Fungal Infection on *Penaeus* species collected from Agniar Estuary

S. No.	Species	*Penaeus monodon*						*Penaeus indicus*						*Penaeus vannamei*					
		20	40	60	80	100	120	20	40	60	80	100	120	20	40	60	80	100	120
1.	*Mucor racemosus*	+	-	-	+	+	-	-	+	+	+	+	-	-	-	+	+	+	+
2.	*Rhizopus nigricans*	+	+	++	++	+	+	+	+	+	++	+	+	-	-	-	-	-	-
3.	*Rhizopus oryzae*	-	+	++	++	+	+	+	+	+	++	+	-	+	+	+	+	-	-
4.	*Rhizopus stolonifer*	-	+	-	++	+	+	-	+	+	++	+	-	+	+	+	++	-	-
5.	*Aspergillus flavus*	+	-	-	++	+	+	+	+	+	++	+	+	-	-	+	++	+	+
6.	*Aspergillus fumigatus*	-	+	-	++	+	+	-	-	+	++	+	-	+	+	+	++	+	+
7.	*Aspergillus japonicus*	-	-	-	+	+	+	+	+	+	++	-	-	+	-	++	+	+	+
8.	*Aspergillus niger*	+	+	+	+	+	+	-	+	+	++	+	+	+	+	+	++	+	+
9.	*Aspergillus terreus*	+	+	+	++	+	-	+	+	+	++	+	-	+	+	++	+	-	-
10.	*Aspergillus versicolor*	-	+	++	++	+	-	-	+	+	++	+	-	-	+	++	+	+	-
11.	*Fusarium quaeductum*	-	-	+	++	+	-	-	-	+	++	+	-	+	-	-	+	+	-
12.	*Fusarium oxysporum*	-	-	+	++	+	-	+	+	+	++	-	-	-	-	-	+	+	+
13.	*Fusarium solani*	+	+	+	++	+	-	-	-	++	++	+	+	+	-	+	+	-	-
14.	*Penicillium chrysogenum*	+	+	-	-	+	+	+	+	+	++	+	+	+	+	+	++	-	-
15.	*Penicillium griseofulvum*	+	+	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-
16.	*Penicillium oxalicum*	-	+	++	++	+	-	-	-	+	-	+	-	+	+	+	++	+	-
17.	*Trichoderma harzianum*	-	+	+	++	++	-	-	-	-	++	-	-	+	+	+	++	+	-
18.	*Trichoderma viride*	-	-	+	++	++	-	+	+	+	+	+	+	-	-	+	+	-	-

'-'represents no growth, '+' represents average growth, '++' represents over growth

As far as *Penaeus monodon* was concerned, 18 species of fungi were identified belonging to six genera (*Mucor, Rhizopus, Aspergillus, Fusarium, Penicillium* and *Trichoderma*. Of these, three species were found to occur throughout the period of investigation (*Rhizopus nigricans, Aspergillus niger* and *Fusarium solani*). *Rhizopus nigricans* recorded their overgrowth between the 60th and 80th days, while *Fusarium solani* recorded an overgrowth in the 80th day and *A. niger* was uniformly recorded throughout the period of study.

Among the genus *Rhizopus*, *R. nigricans* was noticed throughout and recorded an overgrowth on the 60th and 80th days; *R. oryzae* which was absent only on the 10th day also recorded an overgrowth on the 60th day and 80th days and *R. stolonifer* which was recorded from the 80th-120th day recorded an overgrowth on the 80th day. Thus, it appears that they preferred the 80th day to record their highest counts.

Within the genus *Aspergillus*, only *A. niger* was recorded throughout the period of culture. A perusal of the Table-1 reveals four species of *Aspergillus* (*A. flavus, A. fumigatus, A. terreus and A. vesicolor*) recorded overgrowths on the 80th day of culture. However, *A. japonicus* which was recorded from the 80th day, showed a uniform growth till the 120th day.

Among the genus *Fusarium,* none of the species were recorded throughout. However, all the three species that were observed recorded an overgrowth on the 80th day of culture.

Within the genus *Penicillium* also, none of the species were recorded throughout the culture period. While *P. chrysogenum* and *P. griseofulvum* were not recorded between the 60th and 80th days of culture, *P. oxalicum* was not observed during the 10th and 120th day of culture. However, it recorded an overgrowth during the 60th and 80th day of culture.

Among the two species that were recorded in the genus *Trichoderma*, none of them were recorded throughout. However, both the species recorded an overgrowth on the 80th and 100th days of culture.

Thus, an overall comparison reveals that on the 20th day of culture, eight species of fungi were recorded in *P. monodon.* This increased to 12 species on the 40th day and almost remained stable till the 60th day (11 species). However, the number increased to 16 and 18 species on the 80th and 100th day only to decrease again on the 120th day (9 species).

With regard to fungal infection in *P. indicus,* a total of 18 species belonging to the same six genera that were noticed in *P. monodon* were recorded. Among the 18 species, only four species (*R. nigricans, A. flavus, P. chrysogenum* and *T. viride*) were recorded throughout the period of culture.

The genus *Mucor* was represented by a single species *M. racemosus* which was not recorded during the 20th and 120th day of culture. On other days, it showed uniform growth.

The genus *Rhizopus* was represented by three species of which only one (*R. nigricaus*) was recorded throughout. It recorded an overgrowth on the 80th day of culture. The other two species (*R. oryzae* and *R. stolonifer*) when present, recorded overgrowth on the 80th day.

The genus *Aspergillus* was recorded by six species of which only one (*A. flavus*) was recorded throughout. It recorded an increased growth on the 80th day of culture. The remaining species of *Aspergillus*, eventhough were not recorded throughout the period of culture also recorded their overgrowth on the 80th day when present.

Among the three species of *Fusarium* that were recorded, none were recorded throughout the period of culture. However, here also, all the three species when present, recorded overgrowth on the 80th day of culture.

Within the genus *Penicillium*, among the three species that were recorded, only one (*P. chrysogenum*) occurred throughout and recorded an overgrowth on the 80th day. The other two species, when present, also recorded their overgrowth on the 80th day of culture.

Among the two species of the genus *Trichoderma* that were observed, *T. viride* was recorded throughout showing a uniform growth throughout the days of culture. The other species (*T. harzianum*) recorded overgrowth on the 80th day of culture.

Thus, an overall comparison reveals that there was a gradual increase in the number of fungal infections as the days progressed till about the 80th day of culture (18 species) followed by a decline till the 120th day (6 species).

The fungal infections that were recorded on *P. vannamei* are presented in Tables 1 & 2 which reveals again that a total of 18 species belonging to 6 genera that were observed in other prawns. However, among these, only two species were recorded throughout the period of culture (*A. fumigatus* and *A. niger*).

The genus *Mucor* was represented by a single species *M. racemosus* which was recorded only from the 60th till the 120th day of culture. At that time, it is recorded an average growth.

The genus *Rhizopus* was represented by two species none of which were recorded throughout the period of culture. Both the species (*R. oryzae* and *R. stolonifer*) were recorded from the 20th till the 80th day of culture; while *R. stolonifer* recorded an overgrowth on the 80th day; *R. oryzae* recorded a uniformly average growth throughout.

Among the six species of genus *Aspergillus* that were noticed, two were recorded throughout the period of culture (*A. fumigatus* and *A. niger*). Further, both the species recorded an overgrowth on the 80th day of culture; while *A. flavus* which was recorded from the 60th to the 120th day of culture also recorded an over growth on the 80th day. The other species (*A. japonicus, A. terreus* and *A. versicolor*) recorded an overgrowth on the 60th day of culture.

The genus *Fusarium* was represented by three species, none of which were recorded throughout the period of culture. Further, when present, all the three species showed an average growth. The genus *Penicillium* was also represented by two species both of which were not recorded throughout the period of culture. However, when present, both the species (*P. chrysogenum* and *P. oxalicum*) recorded an overgrowth on the 80th day. The genus *Trichoderma* was also represented by two species of which none occurred throughout the period of culture. While *T. viride* was recorded only on two occasions, *T. harzianum* was recorded between the 20th and 100th day of culture recording an overgrowth on the 80th day.

Thus, an overall comparison reveals that as the days progressed the number of fungi infecting prawns also increased culminating in a peak on the 80th day recording 16 infections followed by a decreasing trend till the 120th day (6 species).

A perusal of literature reveals that Velmurugan and Ayyaru (2014) while studying the fungal diversity in three species of cultured prawns reported the presence of 20 fungal species that belonged to six genera. This study is comparable to the present investigation in which 18 species of fungi belonging to 6 genera could be identified.

In the present study, in *Penaeus monodon*, three species were perennial (*Rhizopus nigncans, Aspergillus niger* and *Fusarium solani*) while in *P. indicus,* four species were perennial (*R. nigricans, A. flavus, P. chrysogenum* and *Trichoderma viridae*) and in *P. vannameri,* two species were perennial (*A. fumigatus* and *A. niger*). Thus, in all the three species of prawn, *Aspergillus* was found to dominate. A similar dominance of *Aspergillus* in prawn was also reported by Velmurugan and Ayyaru (2014). Karunasagar *et al.* (2004) however, suggested that eventhough about 500 fungal species have been isolated from marine and estuarine conditions, only a few were found to be pathogenic to prawns. In the present study, *Fusarium* species could be isolated in all the three species of prawns. Reports reveal that *Fusariosis* and black gill disease are caused by *Fusarium* and can affect all the development stages of prawn (Ramaiah, 2006). He also suggested that *Fusarium* sp. are usually opportunistic pathogens which however can cause mortalities. The present investigation also revealed the presence of *Penicillium* sp. Pillai (1982, 1986) in his observations on various prawn diseases, reported that deep mycosis is produced by *Penicillium* in *P. monodon*. Khoa *et al.* (2004) isolated *Fusarium* sp. in *P. monodon* grown in various farms in Vietnam which brought about blackgill disease resulting in high mortality.

In the present study, all the three species of prawn recorded maximum growth of fungi at around the 80th day of culture. A similar observation was also reported by Velmurugan and Ayyaru (2014). This might probably be attributed to the differences in the water exchange that is usually done in various farms. Nevertheless, the incidence of various fungi in all the three species of prawns analysed reveals the need to incorporate mycological examination to be an integral part of monitoring the health of prawns to prevent economic losses as well as prevention of diseases to handlers and human consumers.

REFERENCES

Cowan, A. (2001). *Fungi - Life Support for Ecosystem.* Essential ARB, 4: 1-5.

Gates, G. M., Ratkowsky, D. A. and Grove, S. J. (2005). A Comparison of macrofungi in young Silvicultural regeneration and mature forest at the warra LTER site in southern forests of Tasmania. *Tasforests*. 16: 127-152.

Hunt, G. A. (1999). 'Assessing Macrofungi of special concern for Conservation in Forested Ecosystem'. In: *Proceedings of Biology and Management of species and habitats at risk.* University College of the Cariboo, Kamloops, p. 779.

Karunasagar, I., Karunasagar, I. and Umesha, R. K. (2004). Microbial diseases in shrimp aquaculture. In: *Marine Microbiology: Facets and Opportunities.* National Institute of Oceanography, Goa, India (N. Ramaiah, ed.). pp. 165-186.

Keizer, G. J. (1998). *The Complete Encyclopedia of Mushrooms*. Rebo Publishers, Netherland. p. 268.

Khoa, L. V., Hatai, K., Aoki, T. (2004). *Fusarium incarnatum* isolated from black tiger shrimp, *Penaeus monodon* Fabricius with black gill disease cultured in Vietnam. *J. Fish Dis.*, 27: 507-515.

Manoharachary, C. S., Singh, K. R., Adholeya, A., Suryanarayanan, T. S., Rawat, S. and Johri, B. N. (2005). Fungal Biodiversity, distribution, conservation and prospecting of fungi from India. *Curr. Sci.*, 89: 59-70.

Molina, R., O'Dell, T., Luoma, D., Amaranthus M. Castellano and Russell, K. (1993). Biology, Ecology and Social aspects of Wild Edible Mushrooms in the Forests of the Pacific North-west: A preface of Managing Commercial Harvest. US Dept of Agriculture Forest Service, Pacific Northwest Research Station, United States, p. 45.

Nambiar, G. R. and Raveendran, K. (2006). A checklist of marine fungi from Kerala State. *Am. Euras. J. Bot.*, 1: 73-77.

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Pillai, C. T. (1986). *Handbook on diagnosis and control of fungal diseases in finfish and shelfish culture.* Sudarsana Publications, Ernakulam. p. 62.

Pillai, C. T. (1982). Studies on the finfish and shell fish diseases. Ph.D. Thesis. University of Cochin, Kerala, India.

Pilz, D. and Molina, R. (2001). Commercial harvests of edible mushrooms from the forests of the Pacific North-West United States: Issues, Management and Monitoring for sustainability. *Forest Ecology and Management,* pp. 1-14.

Ramaiah, N. (2006). A review on fungal diseases of algae, marine fishes, shrimps and corals. *Indian J. Mar. Sci.,* 35: 380-387.

Sarbhoy, A. K., Agarwal, D. K. and Varshney, J. L. (1996). *Fungi of India 1982-1992*. CBS Publishers and Distributors, New Delhi.

Sharma, M., Shrivastav, A. B., Sahni, Y. P. and Pandey, G. (2012). Overviews of the treatment and control of common fish diseases. *Inter. Res. J. Pharma.* 3: 123-127.

Sudip Mitra, Wassmann, R. and Gukk, P. (2005). An approval of global wetland area and its organism carbon stock. *Curr. Sci.,* 88: 25-33.

Velmurugan, K. and Ayyaru, G. (2014). Culturable fungal diversity of brown-gill disease in three *Penaeus* species. *Int. J. Res. Marine Sciences.* 3: 1-4.

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