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Heavy mortalities in larvae of *Penaeus monodon* in hatcheries of Kakinada and Visakhapatnam coasts due to *Fusarium* and *Vibriosis*

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Abstract

Infection with luminescence bacteria in conjunction with fungal organism *Fusarium* sp. is prevalent in larvae of mysis and post larvae of *P. monodon* in hatcheries. Histopathological studies revealed the presence of *Fusarium* sp. in hepatopancreas of infected larvae. Histolytic secretions of macroconidiophores of *Fusarium* sp. destroyed carapace which developed into dome like structure to accommodate the outer extension of *Fusarium* sp. Though *Vibriosis* sp. could be treated with antibiotics, *Fusarium* caused heavy mortalities.

The outbreak of luminescence bacteria caused heavy mortalities in larvae especially in early stages of mysis and post larvae incurring losses to the hatcheries along the coasts of Kakinada and Visakhapatnam in 1999 in Andhra Pradesh. Treatment with common antibiotics like chloramphinicol, erythromycin etc., suppressed the infection but control was not absolute. Thus problems of general morbidity distress and impairment of growth of larvae remain unsolved. In view of this a study of the disease and histopathology of infected larvae were made. Several investigators reported mortalities of different species of penaeids challenged with *Fusarium* (Johnson 1974., Solangi *et al.*, 1976, Lightner 1976, Hose *et al.*, 1984, Egusa *et al.*, 1988, Karunasagar *et al.*, 1994, Anand *et al.*, 1996).

In this investigation a study is made on the histopathology of *Fusarium* sp. in the hepatopancreas in cultured Penaeid larvae of mysis stage and post larvae which displayed infection with luminescence bacteria.

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Material and methods

The mysis stage and post larvae of *P. monodon* showing luminous bacteria in larval rearing tanks were selected for the study. Many of these organisms had black spots on exoskeleton appendages and in some of them on the tail also. Abdominal flexure is common. They were processed and whole mounts were prepared. Section 6μ-8μ thickness were stained with Haidinhan's Azocaramine, aniline blue and Haidenhan's haematoxylin and Eosin. The material fixed was processed and embedded in paraffin wax.

Results

Histopathological studies of hepatopancreas in infected mysis stages and post-larvae revealed the presence of a large hyaline *Fusarium* sp. occupying 3/4 size of it (Fig. B). Macro-conidia are hyaline, with 3 to 4 septate with a distinct foot cell. Hyphae are transparent bearing phialide and macroconidia. Microconidia are enveloped in transparent slimy masses(Fig. C) denoting characteristic features of Family. Tuberculariaceae.

Bacterial infection was noted on the surface of exoskeleton (carapace), appendages abdomen and caudal region. Small to large melanized black thickenings occur on the carapace near abdomen, on eye stalks rostrum etc. The carapace near posterior thoracic region became thin, opaque, punctured with slits and developed into a dome (Fig. A) like structure accommodating extended body of *Fusarium*. Carapace became slimy with apparent histolytic activity. Some

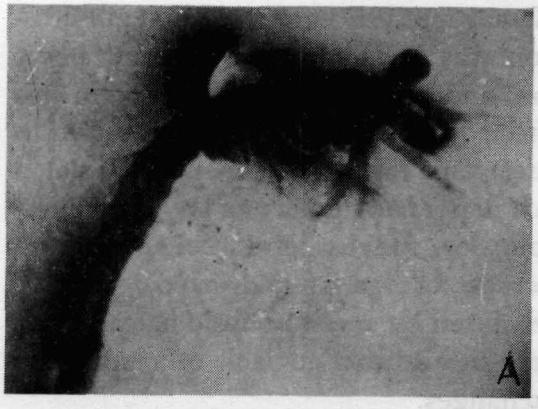

Fig. A *Mysis larvae of Penaeus monodon infected with* Fusarium *sp. showing abdominal flexure and enlargement of exoskeleton into a dome like structure.*

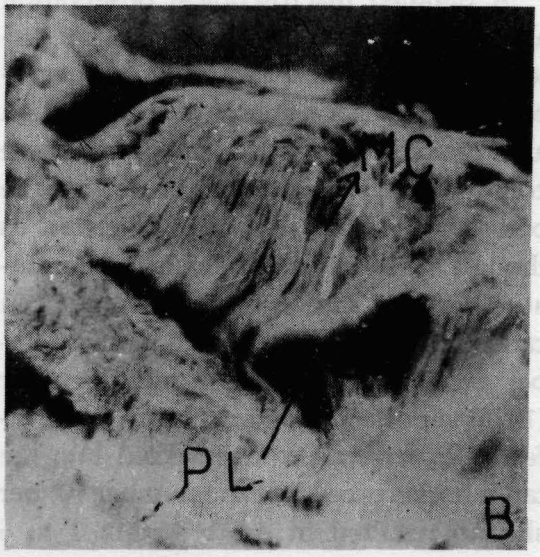

Fig. *B Fusarium sp. in the hepatopancreas of infected mysis larvae of Penaeus monodon showing Phialide (PL) and Macroconidia (MC)*

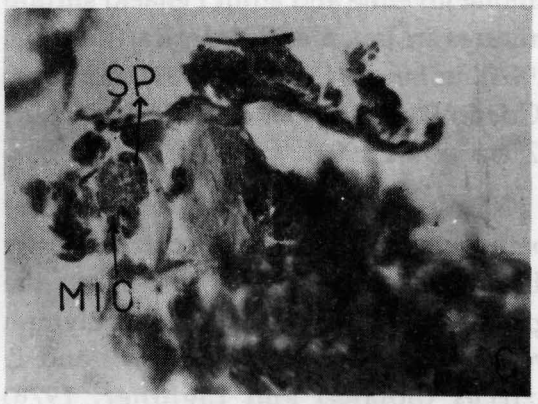

Fig. C Fusarium *sp. with microconidia (MIC) enveloped in transparent slimy masses Sporodochia (SP) in hepatopancreas of infected mysis larvae of* Penaeus monodon.

macroconidia could be observed escaping out through the slits. In histopathological studies the ventral nerve cord was pulled upwards into the clipped enclave of dorsal carapace.

A detailed macroscopical observation allows us to conclude that excessive histolytic activity and secretion of macroconidia are responsible in the dissolution and dismantling of the carapace. These conidia were found to spread outside the body though the slits of exoskeleton as though in search of new hosts to perpetuate their race. However, these condia can cause infection in hosts when their number exceeds a particular range. Lightner (1976) reported systematic growth in infected *P. setiferus* when injected into the tail muscles exceeding the number 10^4 per shrimp resulted in death within 1 to 6 days.

There are several reports of imperfect fungi *Fusarium* sp. causing high loses among juvenile and adult Penaeid shrimp cultures in Japan and America (Johnson. 1974), in Isreal (Colorini, 1989), in France (Criado Fornelio *et al.* 1988) and in China (Zhan *et al.* 1993).

Many investigators opined that pathogenecity of shrimps occurred mostly due to infections of *Fusarium* sp. in the gills resulting in clogging of haemolymph vessels of *P. japonicus, P. setiferus* and *P. aztecus* (Solangi and Lightner, 1976) haemocytic infiltration and haemocytic encapsulization and melanization in *Penaeus chinensis* (Zhan *et al.*, 1993) and nectrosis of haemolymph vessels of *Penaeus japonicus* (Souheil, 1995).

Considering the related factors of infection of larva there is enough evidence to conclude that *Fusarium* sp. from sea water enters through slits or wounds or punctures of exoskeleton caused by epizootic *Vibrio* sp. In the hepatopancreas there is fabulous growth. As stated earlier Lightner *et al.* (1979) opined that the fungus is an opportunistic pathogen and in highly susceptible species the slightest wound or abrasion may facilitate entry. However, to date nobody reported the presence of *Fusarium* sp. in hepatopancreas of diseased larvae of *P. monodon* though several investigators documented dark melanized hemocyte nodules surrounding bacteria in heart, gills, hepatopancrease gonads and muscles (Bian and Egusa, 1981, Lightner, 1981) in addition to ectodermal damage. Several workers reported on the cloudiness of hepatopancreas or midgut gland (Takahashi, *et al.* 1984) cloudiness of muscles on the 6th abdominal segments and brown spots in the gills and lymphoid organ (Takahashe *et al.* 1985, Anderson *et al.* 1988) in organisms with *Vibriosis*. In *Penaeus japonicus* infected with a low dose of spores of *Fusarium solani*, Mamoyana (1987) observed that gill invasion was lower, but the thoracic nerve cord and ventral thoracic aorta were also affected by the fungus. In *Penaeus japonicus* infected by low dose Souheil (1995) stated that under conditions of a low dose infection, slow fungus development could affect several organs and delayed death may be caused by several physiological disorders in addition to lack of respiratory exchanges.

In the present study, *Fusarium* sp. comfortably sequestered in hepatopancreas of diseased mysis stages and post larvae is

not easy to detect. *Vibriosis* although treated with antibiotics and controlled *Fusarium* sp. caused heavy mortalities of mysis stages and post larvae in hatcheries. We are in agreement with the opinion of Lightner (1983) that the incidence of shrimp diseases is closely related to the local environment where these organisms cause disease as opportunistic *Vibrio* sp. apparently establish lethal infections as a result of other primary conditions that might include nutritional diseases and extreme environmental stress. Wounds of other infection diseases or *Vibrio* infections may cause chronic sub acute condition and mortality may reach to 100% at times (Lightner, 1983).

Although the association of *Vibrios* sp. is not documented with infection of *Fusarium* sp. several investigators found the infection of luminescence bacteria causing mass mortalities of prawn larvae in hatcheries of Indonesia, Philippines and Thailand (Sunaryanto and Mariam, 1987; Lavillo - Pitogo *et al* 1990). Hung-Hung *et al.* (2001) observed that the presence of large number of *Vibriosis* in the hepatopancreas of cultured *Penaeus monodon* may be associated with growth retardation and finally led to mass mortalities.

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