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Infection of Aspergillus fumigatus to freshwater crab Barytelphusa cunicularis

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Abstract:

Present study deals with isolation of fungal pathogen from lesion part of Carapace and limb of freshwater crab *Barytelphusa cunicularis* maintained in aquarium. The fungal Pathogen was isolated by using Sabouraud dextrose agar medium and incubated at 30^0C for 5 days and morphologically it was identified by staining with lacto phenol cotton blue. The major lesion was found at limb region rather than carapace region of the Crab. Identified fungal pathogen was belonging to *Aspergillus* species. Current study focus on fungal infections arises from water born Pathogen.

Keywords: *Barytelphusa cunicularis*, Pathogen, SDA, water.

Introduction:

Crabs occupy 3rd rank by virtue of its delicacy, demand and price after Shrimps and Lobsters. *Barytelphusa cunicularis* is a freshwater crab found in Godavari river of Nanded (Maharashtra). Both immature and mature males of fresh water crabs have a slender and triangular shaped abdominal flap on ventral side of the body. While the immature females have a broad and triangular shaped abdominal flap and the mature females with semi-circular shaped flap. (Kulasekarapandian *et.al,*).The Shell diseases on crustacean cuticles are due to black necrotic disease, box burnt disease, black mat syndrome, rust disease, brown spot, black spot, burn spot, tail rot disease, etc. these diseases commonly occurs in fresh water and marine crustacean.

The shell disease syndrome is progressively degradation of the cuticle and appearance of various colour spot on exoskeleton surface. (Sharmila *et. al*. (2014). Exoskeleton erosion is largely attributed to the chitinolytic activities of microorganism. Non-chitin containing layer of the cuticle erosion due to lipolytic microbial activities. (Claire *et. al*. (2002); ISSN: 0002-5109). The First time of defense of crab against parasite is of physical nature via their hard cuticle. When this parasite barrier passed then innate humeral and cellular immune reaction induced in both tissue and haemocoel result elimination of parasite.

The qualities of water control the shell disease of crustacean cuticle.The fresh water is being polluted by disposal of sewage, industrial waste and human activities. Most water bodies become contaminated due to incorporation of untreated solid and liquid waste. In India, village towns are situated near the dam. In farm various pesticide and herbicide used after rain fall these mix in water body. The water parameter also favor the infection such as temperature, salinity pH dissolved oxygen and nutrient.

The aquatic animals are highly sensitive to temperature change. Increase the temperature can lead to reduced oxygen tension in the water higher microbial growth and immune suppression resulting in higher disease. When the lower pH of water increase the solubility of nutrients like phosphate and nitrates. Many bacteria, fungi, protozoa's and microscopic algae use crustacean larvae as substrate and produce fouling problem (prevent proper folding) in exoskeleton. Protozoans interfere with gas exchange by blocking respiratory surface of egg and larvae. Protozoans do not invade the underlying tissue so they affect larvae in its movement and feeding. They found heavily on appendages and causes difficulty in swimming. Filamentous bacteria colonize a significant portion of the gill and other bacteria involve in failure in molting.

The shell disease syndromes in brachyuran crabs are characterized by damaged external manifestations of colored lesions in the exoskeleton of the crabs. Bacteria, Fungi, Virus and several other pathogens contribute the higher percentage of shell disease in crabs. Crabs with shell diseases are prone to internal damage causing variations in haemocytes count and histopathological alterations in internal tissue and organ. The infection in crabs may lead to economical loss (Joseph and Ravichandran, 2012).

Bacterial diseases of crabs are common when compared to virus and protozoan diseases. The majority of studies focus upon bacterial disease of marine crabs that produce Bacteremia's or diseases that affect the exoskeleton.

Complex populations of chitinolytic bacteria are generally associated with shell disease because they possess the enzyme chitinase i.e. capable of degradation of carapace chitin. The degradations results in typical erosion and pigmentation of lesions in the exoskeleton of crabs which are often viewed as box burnt, black spots of exoskeleton of crabs (Wang, 2011).

REVIEW OF LITERATURE:

Ghaware A. U. et.al (2015) studied the impact of bacterial and fungal infection on edible crab *Paratelphusa jacauemantti* (Rathbun). The edible crab *Paratelphusa jacauemantii* important fresh water sources in Maharashtra and focus on impact of bacterial and fungal infections on edible crabs and discussed histological and hematological changes.

Joseph (2014) studied on shell disease in the freshwater crab, *Barytelphusa cunicularis*. The isolation and identification of bacterial and fungal pathogen from lesion carapace and limb of the freshwater Mananthavady, Waynad, Kerala. The fungal pathogen was isolated from sabouraud dextrose agar medium and morphologically identified using lacto phenol cotton blue stain.

Veeruraj (2008) studied the antibacterial activity of haemolymph extracts from six different species of crabs. Two positive controls amphicillin and erythromycin were also used. The crab haemolymph of crude sample tested against gram +ve and gram –ve pathogenic bacteria. This work has shown that the haemolymph of crabs would be a good source of antimicrobial agent.

Anbuchezhian *et. al* (2009) studied the influence of crab haemolymph on clinical pathogens. Marine organisms are capable of surviving and growing in habitats of extremes. In crustaceans, the defense system against microbes rests largely on cellular activities performed by hemolytic such as phagocytosis encapsulation, Antibacterial activity was detected some species showed maximum zone of inhibition in the haemolymph some show low zone of inhibition. This study shows that the haemolymph of crabs not potential antibiotics.

Joseph and Ravichandran (2012) studied the shell diseases of brachyuran crabs shell fishes like crustaceans and mollusks are often prone to shell diseases. Among crustaceans crabs pathogen and various environmental stresses crabs are infected in higher extend than any other crustacean shell disease syndrome in crabs is

characterized by dialoged external manifestations of colored lesion in exoskeletons bacteria, virus, fungi and several other pathogens influence the higher percent of shell disease in crabs, infection in crab lead to great economic loss.

Materials and Methods

Collection of Crabs

-A healthy growing live crabs (*Barytelphusa cunicularis*) was collected from Vishnupuri dam in Nanded (Maharashtra) from the local fisherman these crabs was fully mature. These crabs widely distributed in India.

Maintenance of crabs:

These collected crabs are maintained in laboratory condition by providing food, light and aerated water. Crabs are omnivorous animal. In natural condition, it feeds on whatever available in River, and pond water. While maintaining the crabs in laboratory condition, dead Prawns, Poha were provided.

Preparation of media:

The media are species specific for proper growth of fungus. 3gm.Sabouraud dextrose powder + 3 gm. agar powder dissolved in 100mL of distilled water. Agar powder was added for solidification of media. The pH was adjusted by adding Acid (HCL) and Base (NaoH) and autoclaved at 121^0 C for 20 minutes and 15 lbs pressure. Due to autoclave contaminations were removed. After autoclave these media pour in petri plates under laminar air flow, for avoiding contaminations. At low temperature these plates were solidify. These plates kept 37^0 C for 24 hours, if their will any contaminations or colonies, these plates were discarded. In plates, if any contaminations after incubation these plates were not used for inoculation of culture. The cultures were taken from the exoskeleton of crabs with the help of cotton swab. These plates were kept 3 -4 days for incubation.

Isolation of fungi:

After 4 – 5 days of incubation colonies were observed. one colony was taken from these plate on slide with the help of needle. For the identification of fungi lacto phenol cotton blue (LPCB) stain was used. These stains are specific for fungi.

- Phenol kill any live organism
- Lactic acid – It Deserves fungal structures cotton blue stained the fungal cell wall.
- The cotton blue stained the fungal cell wall (Chitin).

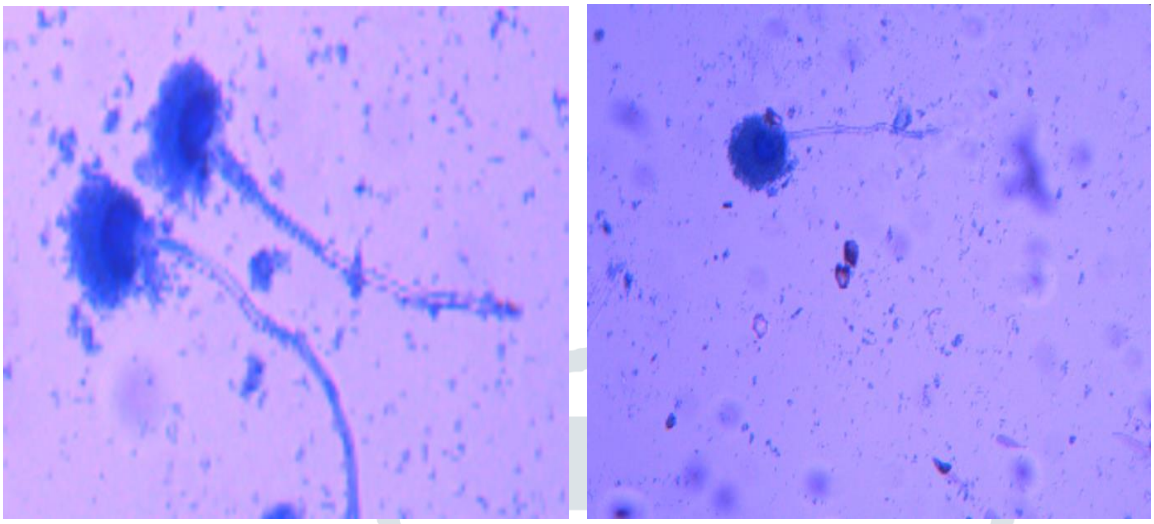

Fig1. fungi under the microscope

Result:

Identification of fungi: microscopic observations of isolated fungi shows that,

1) Globuse shaped and smooth walled conidial surface.

2) Conidiophore and hyphae.

Discussion:

(F. R. Sharmila Joseph, N. P. Latha, S. Ravichandran, A. R. Sudha Devi) The bacterial flora was isolated by pour plate method and phenotypic identification based in morphological features and biochemical test. The fungal pathogens were isolated from SDA medium and morphologically identify using LPCB stain. Identified isolates belonging to nine groups of bacteria and one fungal pathogen from infested surface and two bacterial genera from the infested limb.Current study based on identification and isolation of fungal pathogen on carapace of *Barytelphusa cunicularis* was higher load of fungal pathogen (Aspergillus fumigates) on carapace than limb.

Mohammed E. Sayed. Enany, Mohammed E Abas E Att2 and Mohamed M. E1 T antawyz were studied on bacterial pathogen in summer season form shell. diseased crab was isolated by using tryptic say agar media. Isolated bacteria from shell gills haemolymph, hepatopancreases and muscles. the isolated bacteria were identified by the biophysical and biochemical method.

The infestions of micro-organisms were higher on cuticle and lower amount on the muscles. This study based on identification and isolation of fungi by using SDA agar medium and identification of isolated fungi based on the staining.

Gaware A. U. and Jadhav (2015) also explained the variety of stressors can lead disease to their severity. Pollution poor water quality, hypoxia, temperature extremes, over expatiation have all been implicated in various outbreaks. This review focuses on impact of bacterial and fungal infection on edible crabs and histological and hematological changes in their body are discussed. The current study was based on isolation and identification of fungi pathogen from fresh water crabs of normal environmental condition i.e. captive maintences.

Conclusion:

The present research work carried on the isolation of fungal pathogens from the body carapace of freshwater crab *Barytelphusa cunicularis* maintained in the laboratory conditions recorded that there was only a single fungal pathogen associated with this crab. The present strain belonging to *Aspergillus fumigatus.*

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