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Acute Toxicity of Monocrotophos on Histological Alterations in the Anomuran Crab, *Emerita asiatica* (H. Milne Edwards, 1837)

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Abstract **- This study characterized the acute toxicity of Monocrotophos on histological alterations in the different organs like gills, Hepatopancreas and ovary of Anomuran Crab, Emerita asiatica. Though Emerita asiatica is not a commercially viable crab, but it plays a vital role in the coastal environment to maintain a stable marine ecosystem. Several steps and precautions measures should be taken to consume these members of the marine food chain to have aunwavering ecosystem and also to protect this species from extinction. Thus, it can be concluded that the use of Monocrotophos which has been legally banned in India is justified. It has been proved by several workers and has been conformed in present investigation that use of this organophosphate causes serious damage to the vital organ of sand crabs gill, hepatopancreas and ovary. The utilization of Monocrotophos should be minimized in the agricultural field area near to the coastal ecosystem.**
Keywords**: *Emerita asiatica*, Food Chain, Monocrotophos, Marine Ecosystem, Toxicity**

I. INTRODUCTION

Aquaculture is the practise of raising aquatic organisms in water, including fish, shellfish, and plants (freshwater or marine). Food fish, game fish, bait fish, ornamental fish, crustaceans, mollusks, algae, sea vegetables, research animals, and fish eggs are all produced by aquaculture. Aquaculture also includes growing plant species utilised in a variety of food, pharmaceutical, nutritional, and biotechnology goods, as well as producing ornamental fish for the aquarium trade. A type of aquaculture known as "stock restoration" or "enhancement" involves releasing fish and shellfish from hatcheries into the wild in order to repopulate wild populations or coastal environments like oyster reefs. The sector of food production with the highest rate of growth globally is aquaculture, which grew 9.5% between 1990 and 2000 and 6.2% yearly between 2000 and 2012 [1].

Aquaculture is used to breed, grow, and harvest animals and plants in many kinds of aquatic habitats [2]. Rebuilding populations of threatened and endangered animals uses it. Aquaculture is a technique for producing food and other commercial goods, reestablishing habits, and replenishing wild stocks; it entails the rearing of plants and animals with the goal of producing food for humans, enhancing commercially valuable stocks for recreational fishing, recovering endangered species, and producing bait and ornamental species. Sustainable aquaculture is becoming more popular all over the world. In comparison to livestock, fish and aquatic creatures in general are a considerably healthier source of protein. The production of marine capture fisheries, which climbed from 81.2 million tonnes to 84.4 million tonnes in 2018, was mostly responsible for the rise.

About 74% of the entire production of the catch fisheries was produced by the top 20 producing nations [3]. Aquaculture has roots in ancient China and may be at least 4,000 years old. Asia currently produces the majority of the world's aquaculture. The globalization of trade and the advantageous economics of larger-scale intensive farming have been the primary drivers of the fast development in the production of carnivorous species like salmon, shrimp, and catfish [4]. Public aquaculture plays a minimal or nonexistent role in many countries, with the majority of aquaculture produce used directly as human food. India is the second-largest producer of cultured fish.

India's culture system is based on a combination of 3-6 species. Freshwater aquaculture now makes up more than 95% of all aquaculture production in India, where it has increased six and a half times over the past 20 years. To help feed the globe, aquaculture is necessary. According to the UN, there will be 9.7 billion people on the earth by 2050, and this increase will place tremendous strain on food supply in general and fish production in particular. Due to harmful fishing methods and overfishing, 33% of wild fish stocks have already reached their biological limit.

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Approximately 5% of the world's total fish production and 7% of the total aquaculture production are being produced in India. The potential for growth in India is enormous, and the nation is on the verge of rapid advancement in fisheries and aquaculture. About 3.32% of India's exports are seafood, and the sector ranks fourth in terms of net foreign exchange contributions. Indian aquaculture is mainly through the freshwater Indian major carps. The coastal aquaculture is mainly by the shrimps to the extent of around 2, 50,000 tones [8]. Indian coastal aquaculture, though traditional in some parts of India in the low-lying brackish water areas of Kerala, Karnataka, West Bengal, Odisha and Goa, the farming has emerged as an important activity in improved manner in recent years. Various technological advancements in the feed and seed production and on other inputs have prompted the farmers to take up the aquaculture in improved manner and new areas have been brought into coastal farming and phenomenal growth was witnessed till the mid of 1990s. 1.3 Fresh water aquaculture. About 2.36 million ha of ponds and tanks are used in India's freshwater aquaculture industry, which produces almost 55% of the nation's fish [9].

Emerita asiatica, also referred to as sand crabs, sand bugs, sand fleas, or mole crabs, is a tiny genus of decapod crustaceans. These tiny creatures utilize their antennae for filter feeding and burrow in the sand in the swash zone. The sole sand crab that lives on the sandy beaches along the Madras coast is Emerita asiatica. Typically, specimens are discovered on the beach's wave-washed area, hidden under loose sand. This anomuran crab is continuously reproducing, according to [10].

But nothing is understood about its sexual biology. Emerita asiatica is a skilled burrower, able to bury itself entirely in under 1.5 seconds. (Kenneth) Unlike mud shrimp, Emerita digs tail-first into the sand, using its periopods to scrape the sand away from beneath its body. As support for the digging limbs during this activity, the carapace is forced into the sand. Emerita must bury herself in the proper position before the wave has passed in order to be safe from predators. The digging requires the sand to be fluidized by wave movement. The Sand Crab moves up and down the beach based on the tide and lives beneath the sand's surface.

The crab rises to the surface and spreads its antennae to feed as each wave comes and goes [11]. Because of this, it is vulnerable to raptors like the sander ling. These birds actively monitor the area of the beach where the oncoming waves wash over it, using their beak to probe the softer sand. As each wave breaks, the sand crab dives deeper beneath the sand, increasing the likelihood that it will be out of the birds' reach. The bird scrambles along the edge of the waves to increase its chance of catching sand crabs. Willets, godwits, surf scoters, black-bellied plovers, and curlews are some of the other birds that consume sand crabs. The intermediate stages of numerous parasitic worms are hosts to the crabs.

These are given to predators when they eat the crabs, and if enough of the worms are consumed, the predator has been known to be killed [12]. The Californian coast is home to the barred surfperch (*Amphistichus argenteus*), which eats a lot of sand crabs. The crabs are taken from the shore by commercial bait fisheries and used as bait by surf fishermen. The newly mounted soft-shelled sand crabs are preserved as bait, and the hard-shelled crabs are released back into the water.

The domoic acid-producing diatoms (Pseudo-nitzschia spp.) that occasionally generate poisonous blooms off the coast of California have been identified as an indicator species for the sand crabs[13]. Barrell-shaped body Emerita Asiatica. Due to its robust exoskeleton, which enables it to roll in waves and tidal currents by keeping its limbs close to the body, its feathery antennae are able to sift plankton and other debris out of the swash. (Kenneth) The majority of the time, men are smaller than females, and in some species, like the *Emerita rathbunae*, the males are physically linked to the females' legs.

Depending on the species, female carapace lengths range from 8 to 37 millimeters (0.31 to 1.46), while male carapace lengths range from 2.5 millimeters (0.0098) in *E. rathbunae* and *E. talpoida* to similar to those of females in *E. austroafricana* [14]. A very rich source of protein is sand crab. It is among the best dietary sources of protein that can be found. It includes selenium, riboflavin (vitamin B2), copper, phosphorus, and long-chain omega-3 fatty acids as shown in Fig. 1.

Dorsal view

Ventral view

Fig. 1 Photograph showing the Dorsal and ventral view of sand crab, *Emerita asiatica*

The only species of the genus Emerita that may be found on the sand beaches of the Indian peninsula is the sand crab Emerita asiatica. The initial research on Emerita species concentrated on distribution patterns and how they related to biological and physical factors. Males in this species frequently exhibit neoteny, and it has also been observed that males are typically smaller than females.

The Pacific Sand Crab is a tiny crustacean that can reach lengths of up to 35 mm (1.4 in) and widths of 25 mm (1.0 in). The orange egg mass carried under the telson, which is approximately twice as large as the male and frequently used to identify the female (Sivakumar et al., a and b, 2014). The adult lacks claws and spines is sand-colored and is well-camouflaged. It contains three pleopod pairs and five pairs of legs. Periodically, sand crabs moult, leaving their exoskeletons to wash up on the shore. The sand crab has an extended dome shape that is intended for quick burrowing, and it is well adapted to live in the sand, which presents as an unstable substrate. The eyes are elevated above the sand's surface on lengthy stalks, and the antennules are likewise lengthened [15].

These come together to form a tube that directs water via the gills downward. The retractable antennae are substantially longer. They also extend above the sand's surface when there is water overhead to collect food scraps. To aid in digging, as well as for use in gathering food and transferring it into the mouth, the legs and uropods have hairy edges [16]. A great source of superior protein is seafood. Global importance can be found in fish, shellfish, and other aquatic creatures that can be used as food and feed. Like meat and poultry, they are a good source of high-quality proteins. The majority of man's nourishment comes from the land where he lives, which takes up a quarter of the planet's surface.

However, marine fisheries only provide 14% of the animal protein consumed by people [17]. The fish industry is currently our nation's eighth-largest source of foreign exchange revenue. Additionally, this industry provides both direct and indirect employment for millions of individuals [18]. The majority of Indians continue to eat food that is produced at home using whole, broken, or powdered grains such rice, wheat, maize, various millets, and pulses. In addition to the variations in eating habits across the country, a large amount of food is wasted, primarily due to inadequate skill in dish preparation [19]. According to [20], recipes for fish, prawns, lobster, oyster, mussel, dried anchovies, and mole have been prepared.

One of the widely used organophosphorus insecticides in agricultural and animal husbandry is Monocrotophos [21]. Due to its extreme toxicity toward both beneficial and non-target insects like fish, birds, honeybees, and bees, Monocrotophos has been banned from usage in industrialized nations. (http://www.panuk.org/Pestnews/actives/monocrot.htm) The non-specific organophosphate insecticide Monocrotophos (MCP) is frequently used to protect crops including rice, maize, sugarcane, cotton, soybeans, groundnuts, vegetables, etc. from pests [22]. In addition to killing pests efficiently, this insecticide has adverse effects on a number of ecological groups that are not intended targets, including healthy humans [23], aquatic animals, birds, and mammals.

[(E)-3-hydroxy-Nmethylcrotonamide (8 Cl); dimethyl phosphate ester with (E)-dimethyl-1-methyl-3-(methyl amino)-3-oxo-1-propenyl phosphate (9Cl)] Monocrotophos Dimethyl phosphate ester with (E)-dimethyl-1-methyl-3-(methyl amino)-3-oxo-1-propenyl phosphate (9Cl); Trade names include, among others; Wide-ranging antibiotics include Nuvacron and Azodrin. Pests include rootworms, cockroaches, leaf folders, and leaf hoppers. Insecticides, acaricides, and organophosphate insecticides are also available [24]. Asia is the continent with the largest population in the world, according to statistics. The top three regions for MCP usage are Africa (43%), South America, and India. 26%, 15%, and 9%, respectively, come from China (15%) and Southeast Asia (9%). On a worldwide level, 90% of the time on a global scale Andhra Pradesh is a state in India. MCP is primarily consumed in

the states of Uttar Pradesh and Punjab. [25]. Monocrotophos has been shown to have hyperglycemic and stress genic effects, with hyperglycemia and hyper lactatemia caused by Monocrotophos being reduced by pre-treatment with atropine [26]. From the foregoing accounts there is no study on the toxicity effect of pesticides on this species for a decade. Even though the sand crabs not an economic important one, but it plays a significant role in the marine ecosystem. It also otherwise called as marine Bioindicators. Hence the present investigation has been taken for toxicity effect of Monocrotophos on this crab.

II. MATERIALS AND METHODS

A. Collection and Maintenance of Sand Crab (Emerita asiatica)

The animals were collected from Besant Nagar beach, 15 kmaway from Chennai, Tamil Nadu, India (Fig. 2). The sand crabs were collected from the intertidal surf zone during the early morning. The animals in the collection were both male and female by handpicking method. More than fifty sand crabs were collected in a polythene bag containing wet sand and transported to the laboratory. The intermediate sizes were discovered in the swash zone. The specimens were acclimatized to the laboratory condition in a plastic tray with adequate aeration and water for five days. The water and sand were added on daily basic for their survival days (Fig. 3).

B. Identification of Male and Female Sand Crabs

The endogenous males of the Indian species Emerita asiatica exhibit an overall simplification of the appendages that is consistent with their tiny size. The vas deferens, on the other hand, is bloated with spermatophore components and the testis is well formed. The endogenous males also have two noticeable sperm sacs or genital papilla. Pleopods and the females' genital apertures on the coxae of the third pair of legs were used to identify them. The sand crab females lay several kinds of colourful eggs at various times. These eggs were regarded as egg-bearing females or females since they were linked to the pleopods [27].

An organophosphate pesticide is Monocrotophos. With the confirmed letter, it was purchased from a nearby pesticide retailer. It has been outlawed in the United States, the European Union, India, and many other nations due to its severe toxicity to both humans and birds. Monocrotophos is a reasonably inexpensive insecticide that is primarily used in agriculture. But it's also regularly employed as a suicide instrument. It is applied on cucumbers as a pesticide. The contaminant that caused the deaths of 23 schoolchildren in Bihar, India, is thought to be Monocrotophos. In July 2013, they consumed a government-provided school lunch in the Indian region of Saran, which was cooked in oil stored in the pesticide's container. Its chemical name is $C_6H_{12}O_6$(12 hydrogen atoms, six carbon and oxygen atoms) Fig. 4.

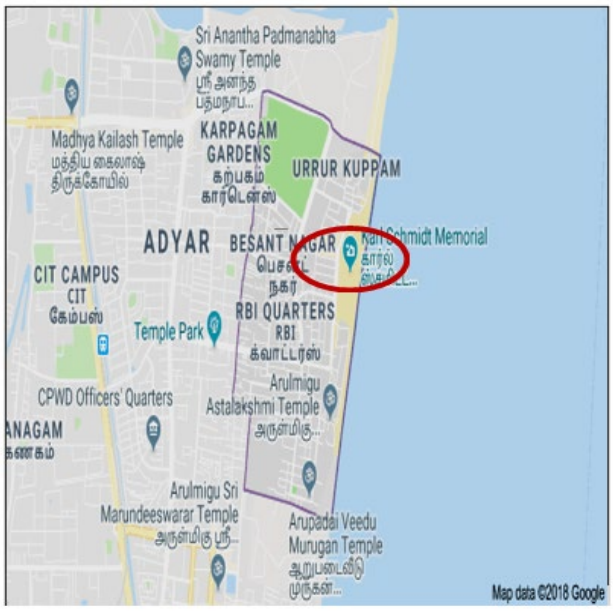


Fig. 2 Map showing the collection site – Besant Nagar beach

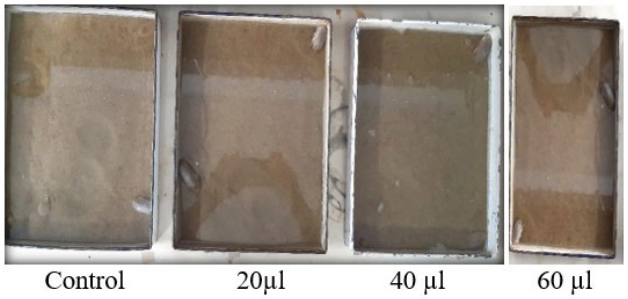


Fig. 3 Acclimatization of sand crabs,*Emerita asiatica* in different trays

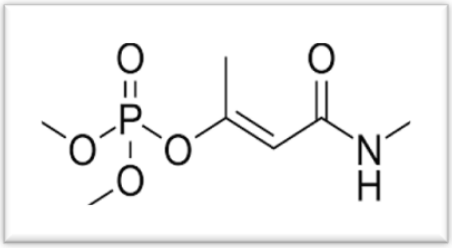


Fig. 4 Structure of Monocrotophos

C. Toxicological Assay

Emerita asiatica was used in lethal experiments with 10 sand crabs in each tray to ascertain the toxicity effect of Monocrotophos on sand crabs. Five days were spent acclimatizing the sand crabs. Each tray had an aeration system, and the hydrological conditions were the same throughout. In accordance with the Organization for Economic Co-operation and Development standard procedure, 20 l of Monocrotophos was diluted in 1 litre of distilled water for a concentration of 20 ml/L to calculate the 24-hour LD50 of these species. 40 ml of Monocrotophos were diluted to a 40 ml/Concentration in 1 litre of distilled water. Monocrotophos was diluted to a concentration of 60 ml/L in 1 litre of distilled water.

First, due to the data on the toxicity of these species of Monocrotophos, small-scale preliminary studies were carried out to determine the fatal concentration of this pesticide on this species. A micropipette was used to introduce 3 concentrations of Monocrotophos (20, 40, and 60 l) around the margins of each tray based on this information. To avoid the impacts of metabolites and sand crab waste organic matter, all of the water in the trays were swapped throughout the experiment, containing the same concentration of Monocrotophos, in accordance with the method utilized (static-renewal test condition). Mortality rates were calculated after removing the dead crabs from the tray at intervals of 0, 30, 60, 90, 120, 150, and 180 minutes. Acute toxicity experiments were conducted, the nominal Monocrotophos concentration expected to cause 100% mortality of sand crabs within 5 hours (5-h LD50), and the degree of toxicity were determined [28].

D. Histological Investigation of Sand Crab, Emerita asiatica

For histological investigation, organs such as Gills, Hepatopancreas and Ovaries were dissected out from control as well as experimental sand crabs. The histology of these organs in normal crabs was also studied.Davidson's alcohol formalin acetic acid fixativewas used to fix the organs. Later, the organs such as the gills, hepatopancreas, and ovaries were cut and stored in screw-capped bottles containing the fixative before being transferred using standard protocols.

The sand crab was anaesthetized or chilled and immediately fixed with appropriate Davidson's alcohol formalin acetic acid fixative. The Sand Crab was sacrificed and the target organs (gills, hepatopancreas, and ovaries) were dissected out. It was immediately placed into the fixative and carefully labeled, and it was recommended to take at least ten volumes of fixative for each volume of tissue samples (ratio of 1part tissue to at least 9 parts formalin). Sample was allowed to fix for at least 24-48 hours before processing.

E. Processing of Tissue and Staining

A variety of tissues were cleansed, dried, and prepared for paraffin cutting at room temperature. The tissues were graded twice in 95 percent alcohol and absolute alcohol, cleansed in a solution of absolute alcohol and chloroform (1:1 v/v), and then passed twice I. They were also dehydrated in two changes of 70% alcohol for one hour each. In contrast to xylene, chloroform does not promote tissue stiffening or brittleness. The tissues were cleaned and then submerged in a 1:1 mixture of chloroform and paraffin wax for an entire night at room temperature. The tissues were soaked with ceresin for one hour each in three changes of paraffin wax with melting temperatures ranging from 58 to 60ºC.

The transverse slices were cut at a thickness of 5 to 7 m using a manual rotatory microtome. After being deparaffinized in xylene, the sections were hydrated and stained with Harris alum hematoxylin before being counterstained with 1% alcoholic eosin (Preece, 1972). Before being mounted in DPX through xylene with a glass cover slip, staining slices were dehydrated in a graduated series of alcohol.

F. Light Microscopy and Photomicrography

A Carl Zeiss binocular compound microscope was used to examine the histological sections. Cellular measurements were conducted using a Carl Zeiss microscope equipped with a calibrated ocular micrometer scale with a 10 m resolution. Photographs were shot using a digital camera (Nikon) connected to a Carl Zeiss microscope equipped with a projection eyepiece of 10 X and objectives of 10, 20, 40, and 100 X. The enlarged prints' magnification was estimated using an ocular and a stage micrometer.

III. RESULTS

The present investigation revealed some interesting facts. After introducing the pesticide Monocrotophos in different concentrations on the different groups of sand crabs it was reacted immediately. The crabs were started to swim restlessly and inverted its carapace towards the sand grains. The crabs secreted the foams in the anterior region to survive in the experimental trays (Fig. 5).

Fig. 5 Formation of foam from the gill region by the sand crabs to avoid suffocation due to Monocrotophos

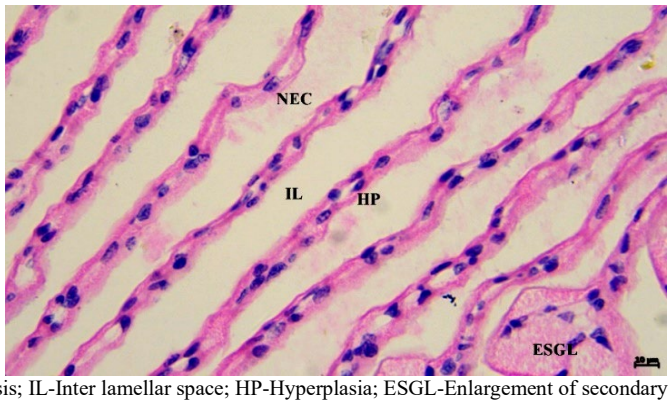
Fig. 6 Acute toxicity of Monocrotophos on carapace and Ovaries

At one stage the original color of the carapace and ovaries has been changed into blue color (Fig. 6). Among the three categories the 60 µl concentration, the crabs were started to die quickly. In the case of 20 µl concentration the crabs were survived for a period of more than four hours. Whereas in 40 µl concentration the crabs were moderately survived (Fig.3, 4). Toxicity effects severely damaged the gills, hepatopancreas and ovaries.

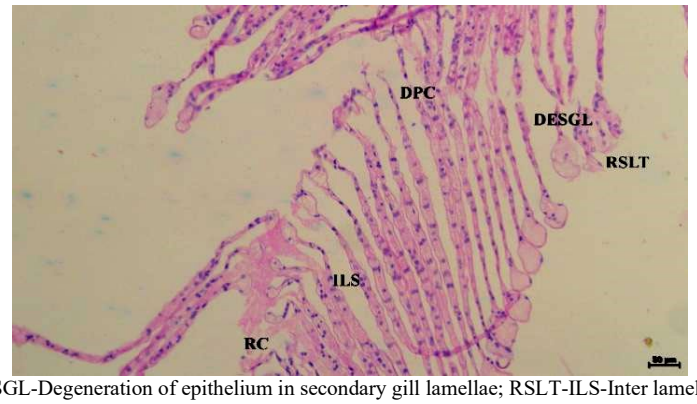
A. Histological Changes in Gill

Detail microscopic examination has revealed the structure of gill as the primary gill lamellae with the appropriate supporting axis with gill lamellae on each side of it. Normal structure of gill is seen. The surface is covered with squamous epithelium normal cells are observed separated by the mucous cells. In the experimental samples that is after the exposure of sand crab with Monocrotophos that is organopesticide has revealed an abnormal structure.

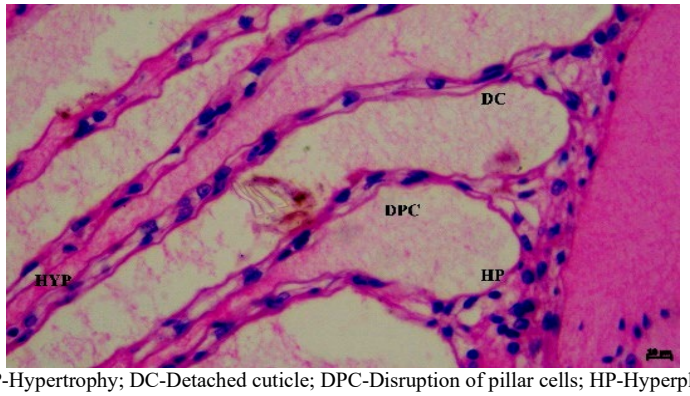
The lamellae, or large flattened plates, that make up *Emerita asiatica* gills were organized serially in pairs along a control gill stem. Primary gill lamellae, which further divide into secondary gill lamellae or filaments, form the centre axis of gill tissue. A thin cuticle covering the whole outside of the control gill is seen. There is a continuous layer of epithelial cells below the cuticle. Pillar cells may join the lamellae (Figs.7 and 8). The interlamellar gap increased and became tightly packed with granular material at a concentration of 20 l, and the gill structure was lost (Figs. 9 and 10). The rupture of the pillar cells causes the gill lamellae to collapse in exposed crab.



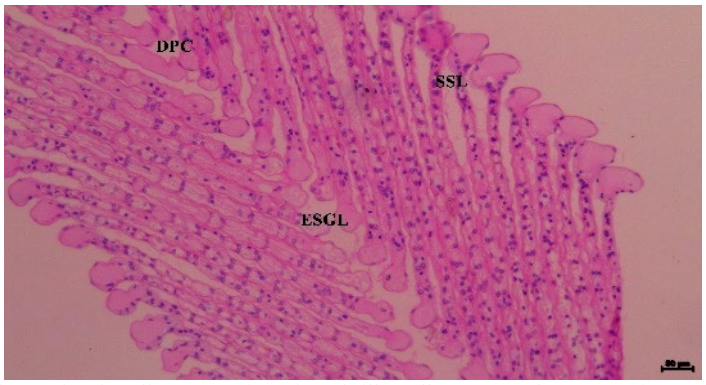
NEC-Necrosis; IL-Inter lamellar space; HP-Hyperplasia; ESGL-Enlargement of secondary gill lamellae
Fig. 7 Photograph showing the gills of control sand crab at 40X magnification



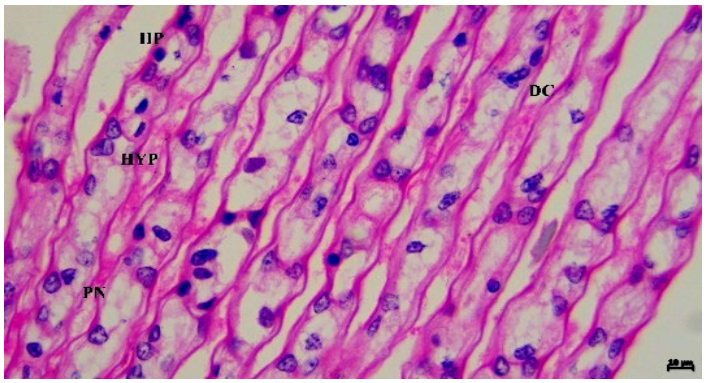
DPC-Disruption of pillar cells; DESGL-Degeneration of epithelium in secondary gill lamellae; RSLT-ILS-Inter lamellar space, RC-Rupture of capillaries
Fig. 8 Photograph showing the gills of sand crab treated with 20 µl Monocrotophos at 10X magnification



HYP-Hypertrophy; DC-Detached cuticle; DPC-Disruption of pillar cells; HP-Hyperplasia
Fig. 9 Photograph showing the gills of sand crab treated with 20 µl Monocrotophos at 40X magnification

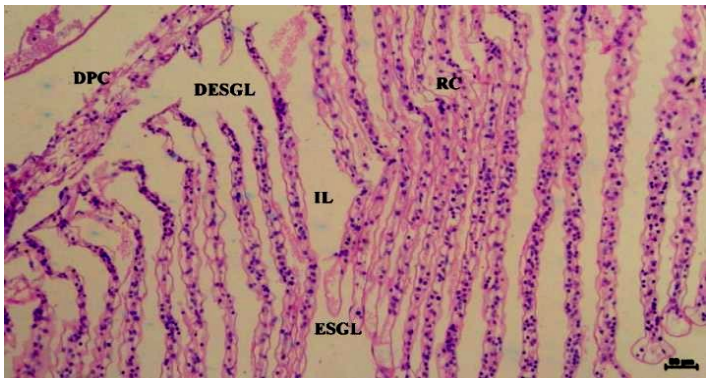


SSL-Swelling of secondary lamellae; DPC-Disruption of pillar cells; ESGL-Enlargement of secondary gill lamellae
Fig. 10 Photograph showing the gills of sand crab treated with 40µl Monocrotophos at 10X magnification

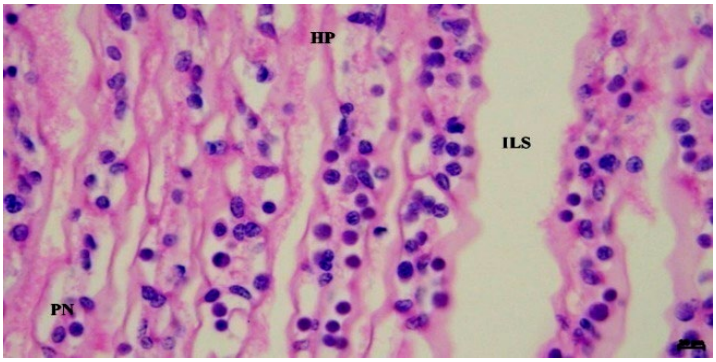


HP-hyperplasia; HYP-hypertrophy; DC-detached cuticle; PN-pyknotic nuclei

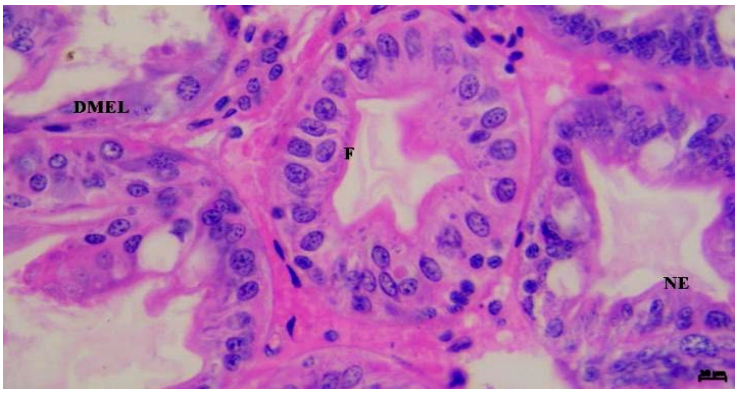
Fig. 11 Photograph showing the gills of sand crabtreated with 40µl Monocrotophos at 40Xmagnification



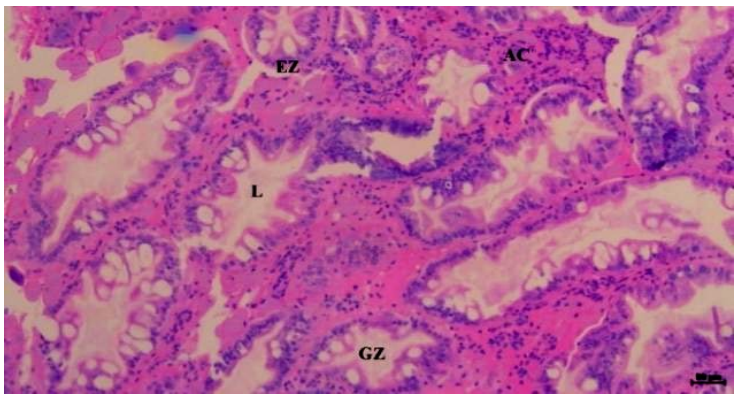
DESGL-degeneration of epithelium in secondarygill lamellae; RC-ruptures of capillaries;
ESGL-Enlargement of secondary lamellae; IL-inter lamellar space; DPC-disruption of pill of cells
Fig. 12 Photograph showing the gills of sand crab treated with 60µl Monocrotophos at 10X magnification



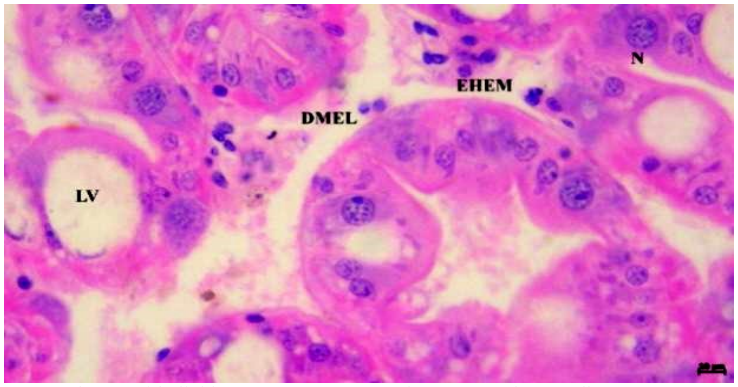
HP-Hyperplasia; PN-Pyknotic nuclei; ILS-Inter lamellar space
Fig. 13 Photograph showing the gills of sand crab treated with 60µl Monocrotophos at 40X magnification



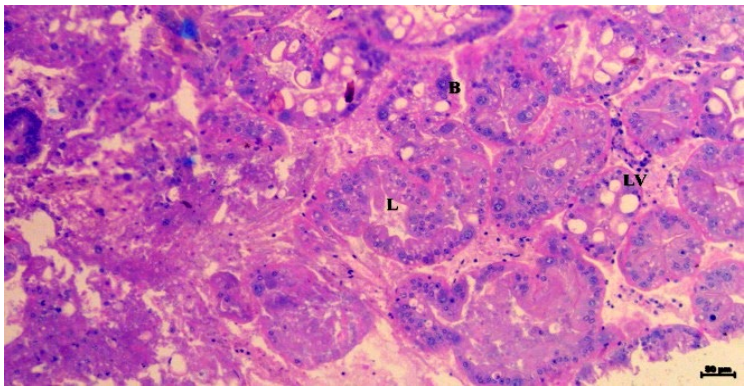
DMEL-Damaged myoepithelial layer; NE-Necrosis; F-Fibrillar cells
Fig. 14 Photograph showing the hepatopancreas of control sand crab at 40X magnification



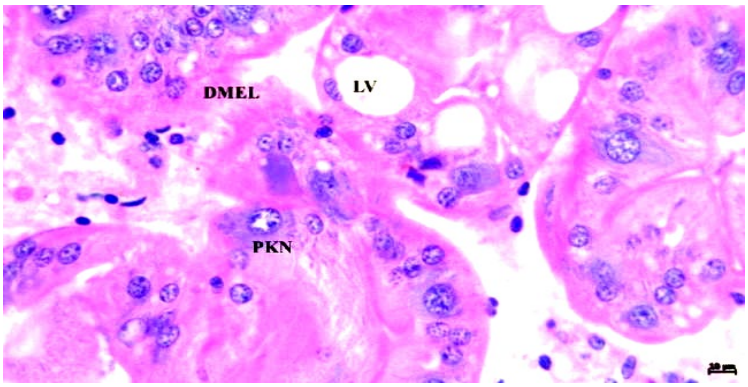
EZ-embryonic zone; AC-absorptive cells; L-lumen; GZ-germinal zone
Fig. 15 Photograph showing the hepatopancreas of sand crab treated with 20µl Monocrotophosat 10X magnification



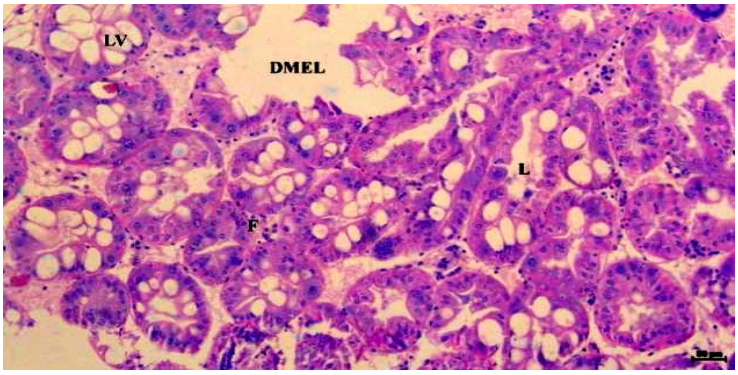
DMEL-Damaged myoepithelial layer; LV-Large vacuole; EHEM-Elongatedhaematocytes; N-Nucleus
Fig. 16 Photograph showing the hepatopancreas ofsand crab treated with 20µl Monocrotophos at 40Xmagnification



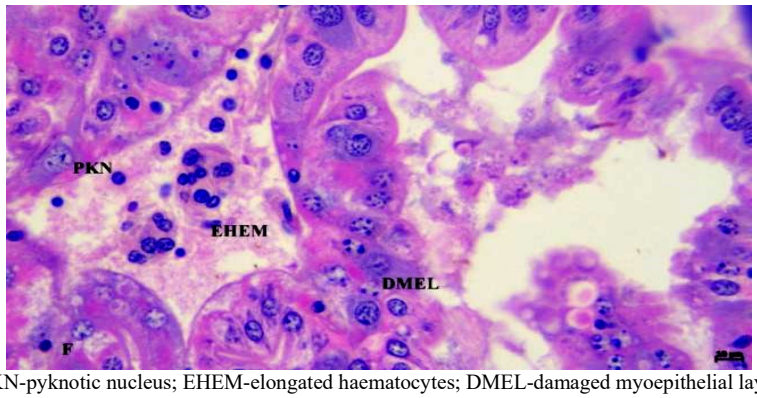
B-βcells; L-Lumen; LV-Largevacuole
Fig. 17 Photograph showing the hepatopancreas of sand crab treated with 40µl Monocrotophos at 10X magnification



DMEL- damaged myoepithelial layer; LV- large vacuole; PKN-Phyknoticnucleus
Fig. 18 Photograph showing the hepatopancreas of sand crab treated with 40µl Monocrotophos at 40X magnification



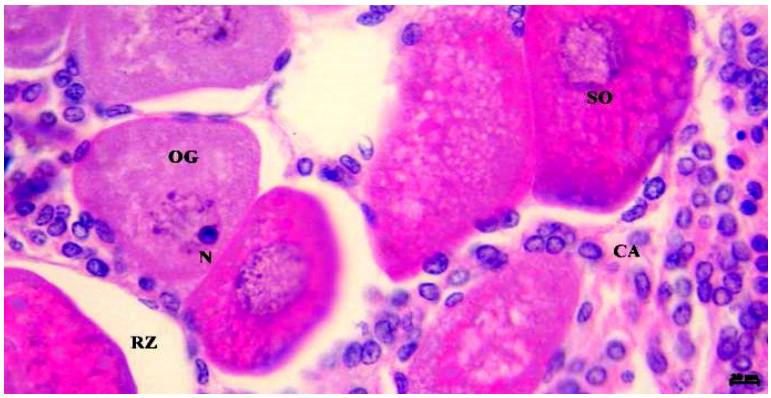
DMEL- Damaged myoepithelial layer; F- Fibrillar cells; L- Lumen; LV-Largevacuole
Fig. 19 Photograph showing the hepatopancreas of sand crab treated with 60µl Monocrotophos at 10X magnification



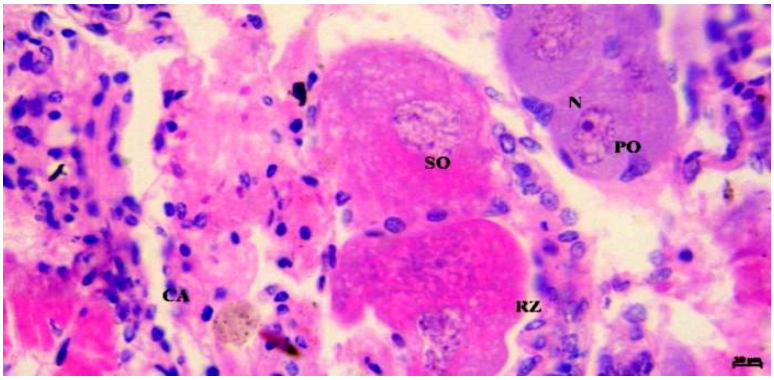
PKN-pyknotic nucleus; EHEM-elongated haematocytes; DMEL-damaged myoepithelial layer
Fig. 20 Photograph showing the hepatopancreas of sand crab treated with 60µl Monocrotophos at 40X magnification

B. Histological Changes in Ovaries

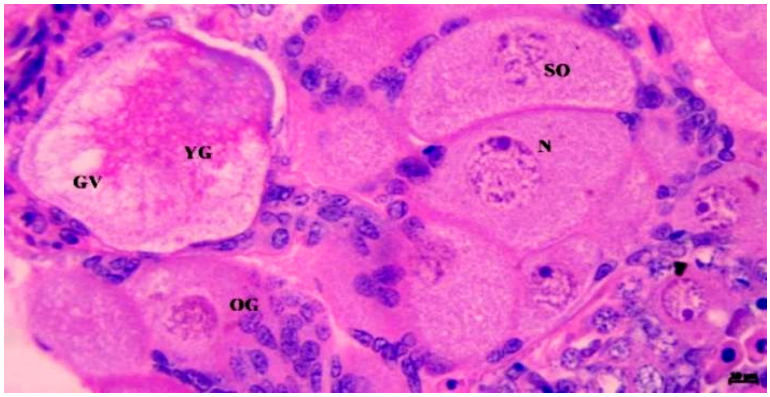
After 4 hours of exposure to a greater concentration of 60 l, the modifications include enlarged gill lamellae, extensive hemocytic infiltration, and hemocoel packed with coarse, amorphous to fibrous debris (Figs. 11, 12 and 13). The histology findings in crabs treated with Monocrotophos in three different concentrations show that the volume and quality of yolk in vitellogenic-sized follicles gradually decline, with the yolk becoming more watery (Fig. 21). Only a small quantity of yolk is present in follicles in the ovaries. Cortical alveoli (yolk vesicles) in the oocytes are frequently broken up or dispersed (Fig. 22). The vitelline membrane (chorion) of oocytes is frequently smooth and continuous, in contrast to oocyte atresia. However, diminished yolk formation frequently comes with at least a minimal level of oocyte atresia. Somatic cells are visible surrounding primary oocytes in the staining as well (Fig. 23). Stage 2 primary oocytes and previtellogenic oocytes exhibit the most pronounced staining. Stage 2 primary oocytes are primarily found in the middle of the ovary, whereas stage 1 primary oocytes and oogonia are found on the ovary's perimeter. A uniform layer of follicular cells surrounds the zona radiata, which is thick on vitellogenic primary oocytes. Primary oocytes that are previtellogenic lack a thick zona radiata. We find an atretic oocyte. As a result of peritoneal cell invasion, a granulomatous inflammatory response is noted. The zona radiata appears to be thinner, and there are some invaginations that are visible, which point to the beginning of the atresia process. A disorganised follicular layer is another sign of atresia (Fig. 24).



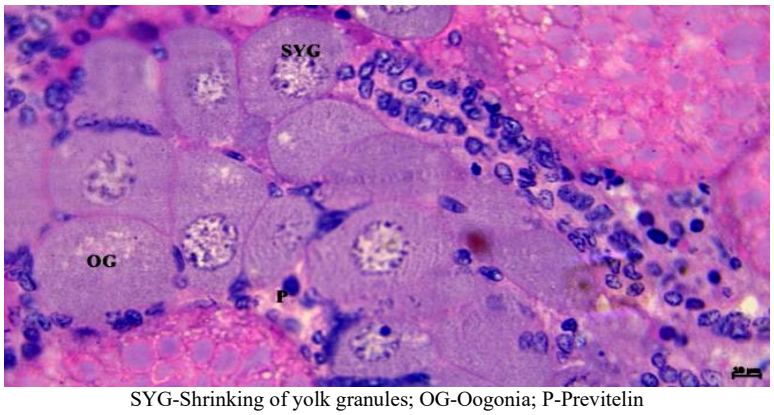
OG-Oogonia; SO-Secondary Oocyte; RZ-Radiata zone; CA-Cartical Alveoli
Fig. 21 Photograph showing the ovary of controlsand crab at 40X magnification



N-Nucleus; SO-Secondary Oocyte; PO-Primary Oocyte; RZ - Radiata Zone
Fig. 22 Photograph showing the ovary of sand crab treated with 20µl Monocrotophos at 40X magnification



GV-Germinal vesicle; YG-Yolk Granules; OG-Oogonia; SO-Secondary Oocyte; N-Nucleus
Fig. 23 Photograph showing the ovary of sand crab treated with 40µl Monocrotophos at 40X magnification



SYG-Shrinking of yolk granules; OG-Oogonia; P-Previtelin
Fig. 24 Photograph showing the ovary of sand crab treated with 60µl Monocrotophos at 40X magnification

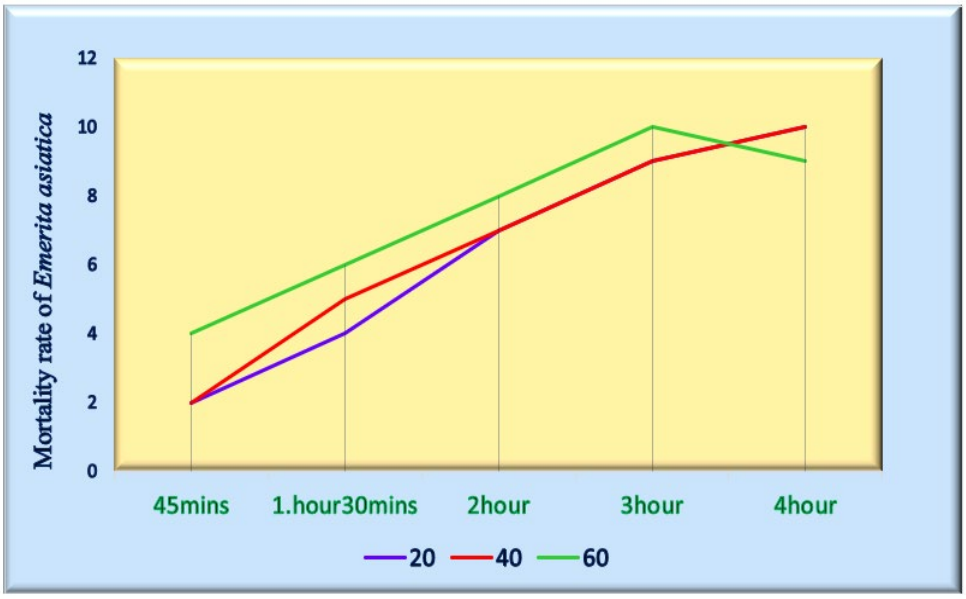


Fig. 25 Graph showing the toxicity effect of Monocrotophos on *Emerita asiatica*

IV. DISCUSSION

Hypoxia, respiratory failure, and issues with the ionic and acid-base balance are all possible outcomes of the histological changes in the gill [29]. The observed histological effects of hyperplasia, necrosis, and lamellar aneurysms in the exposed crab in response to sub lethal concentrations of Monocrotophos are compatible with changes in the gill surfaces and increased mucus production. Under the stress of Monocrotophos, a pesticide, changes in the architecture of the gill would change its ability to diffuse oxygen, leading to hypoxic conditions, making respiration challenging for E. asiatica in a marine environment. Our findings imply that impairment to gas exchange mechanisms as a result of the observed gill diseases underlies Monocrotophos' deadly effect.

In the current investigation, after 4 hours of exposure to a greater dose, epithelial lifting, edema, necrosis, fusion of nearby secondary lamellae, and haemorrhage at primary lamellae were seen in the crabs' gills. Ultimately, all of the crabs died (Fig. 25). The irritants' primary harmful effects are epithelial necrosis and gill epithelium rupture. Due to stress brought on by environmental changes and pathological substances that encourage the development of mucus cells, the animal's defense mechanisms include increased mucus secretion. In that it reduces the amount of exposed gill surface area, lifting of the epithelium, lamellar fusion, and club-shaped lamellae may be protective[30].

In addition to being a digestive organ with the capacity for absorption, digesting, storage, and secretion, the hepatopancreas is a key location for biotransformation and detoxification in crustaceans. In the current study, the hepatopancreas displayed changes in the F and B cells in low concentrations of Monocrotophos, and cells were found clumped and intercellular spaces invisible in medium concentrations. Cells were also found in the high concentrations of Monocrotophos exposed E. asiatica to show general degeneration, loss of tubule structures, vacuolation, star-shaped lumen, and necrosis of cells. Due to morphological alterations in the tubular epithelial cells, where certain cells shrank in height from a typical columnar height to a low cuboidal form, the star shape of the lumen was largely lost.

The growth of B-cells in the dosed crabs is one of the most obvious alterations in the current study, showing a high rate of hepatopancreatic excretion. When xenobiotics enter the hepatopancreatic tubules, they may accumulate and be eliminated differently because many F-cells are transformed into B-cells. In various metabolic processes in crustaceans, the hepatopancreas is crucial. The structural changes caused by Monocrotophos included a decrease in the tubular epithelium's cellular height, a reduction in secretary and lipid vacuoles, hemocyte infiltration, atrophy, pyknotic nuclei, cytolysis, and the melanized encapsulation of necrotic tissues [31].

According to [29], *Macrobrachiumlamerrei* subjected to low (0.0065 ppm) and high (0.0215 ppm) copper concentrations underwent alterations such as elongation of hepatopancreatic cells and shrunken cells. The hepatopancreas and gills of Penaeus indicus exposed to Zn at a low concentration of 100 ppb underwent destructive and degenerative alterations [27]. Since the hepatopancreas is the primary organ for storage, metabolism, and detoxification, the observed histological alterations in this organ may be caused by pesticide accumulation. It is possible that exposure to Monocrotophos in crabs impacted the tissue integrity as evidenced by the rupture of basal laminae found in the hepatopancreatic tubules.

Hemocytes are the most crucial type of cellular defense in crustaceans, so abnormal infiltration of hemocytes in the interstitial sinuses found in the hepatopancreas of test

animals suggests that the mechanism of cellular/host defense was in operation to neutralize the tissue damage caused by Monocrotophos [29]. The development of necrotic hepatopancreatic tubules in test crabs shows that the hepatopancreas of E. asiatica subjected to the maximum sub-lethal dosage of Monocrotophos experienced cell deformation, disintegration, and death. As a result, Monocrotophos toxicity disrupted the hepatopancreas' normal integrity and led to tissue destruction in E. asiatica. When exposed to sub lethal concentrations of Monocrotophos, the muscles of E. asiatica showed significant alterations in the tissue.

Muscle degeneration, necroses of muscle fibres with haemorrhages, and RBC-like pigmented cells are among the pathological findings [23]. The structural changes observed in the muscle tissue, such as atrophy, necrosis, wavy appearance and granular material between the muscle fibres, fragmentation, loss of muscle structure, and the appearance of basophilic deposits of the muscle fibres, were caused by crab exposure to sublethal concentrations. Pollutants had an immediate effect on the muscle epidermis during pesticide exposure. Pigmented cells are an important component of the chronic inflammatory response.

The current study supported a similar report by [22] in the muscle tissues of *Artemia urmiana* in response to carbamates pesticide, which resulted in degeneration, Zenkers necrosis of muscle fibre with haemorrhages, and RBC like cells. The exposure of *Labeorohita* to hexachlorocyclohexane caused muscle bundle separation and intracellular edoema in the muscle tissues [14]. When Oreochromis mossambicus muscle tissues were exposed to dimethoate, similar observations were made [17]. [18]. documented histopathological changes in the muscle tissues of Heteropneustes fossils exposed to polluted river water. As a result, we can conclude that the use of Monocrotophos should be limited in the agricultural field area of the coastal ecosystem.

In conclusion, the present investigation is the first, latest and the only report on the histological toxicity effect of Monocrotophos on histological alterations in the anomuran crab, *Emerita asiatica.* Though *Emerita asiatica* is not a commercially viable crab, but it plays a vital role in the environment to maintain a stable marine ecosystem. Several steps and precautions measures should be taken to conserve these members of the marine food chain to have a stable ecosystem and also to protect this species from extinction.

V. CONCLUSION

In the conclusion, this study on Sand crab (*Emerita asiatica*) is the recent study that is characterizing the toxicity effect of Monocrotophos on the different organs like gills, Hepatopancreas and ovary by histological investigations. Though Emerita asiatica is not a commercially viable crab, but it plays a vital role in the environment to maintain a stable marine ecosystem. Several steps and precautions measures should be taken to conserve these members of the marine food chain to have a stable ecosystem and also to protect this species from extinction. Thus, it can be concluded that the use of Monocrotophos which has been legally banned in India is justified. It has been proved by several workers and has been conformed in present investigation that use of this organophosphate causes serious damage to the vital organ of sand crabs gill, hepatopancreas and ovary. The utilization of Monocrotophos should be minimized in the agricultural field area of coastal ecosystem.

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