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Impact of deltamethrin toxicity on the changes in behavioural aspects of a freshwater fish, *Cirrhinus mrigala*

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Abstract
The aquatic environment is subjected to heavy and diverse pollutants load, particularly in the highly industrialized and urbanized regions throughout the globe. There is much concern over possible effects of these Deltamethrin is a synthetic pyrethroid insecticide widely used throughout the world to control different crop pests, flies, and mosquitoes etc., which effects directly or indirectly to this aquatic life. This commercial grade deltamethrin (Decis, 30%EC) was obtained from Bayer Crop Science, India Ltd., Gujarat, India. Short term definitive test by static renewal bioassay method was conducted to determine the acute toxicity (LC_{50}) of deltamethrin and freshwater fish fingerlings were exposed to different concentrations of deltamethrin ranging from 3 ul/L to 9 ul/L. LC_{50} 96 h (8 ul/L) of deltamethrin and one tenth of the 96 h LC_{50} (0.8 ul/L) was taken as the sub-lethal concentration to this behavioural toxicity study, and the fish, *Cirrhinus mrigala* weighing 5 ± 2 g were collected from the local fish farm Dharwad, Karnataka and were stored in large aquaria and providing good laboratory environmental conditions throughout the experimental period and these fishes were fed daily with commercial available fish pallets which had around 40% protein content. They were acclimatized to laboratory conditions for fifteen days. The aquarium was cleaned periodically to avoid infection to fish and 1% potassium permanganate solution was sprayed to eradicate any bacterial or fungal infection. The temperature of water in the aquaria was 29 ± 2 °C and the same was maintained throughout the course of investigation. And duration of the study was 1, 2, 3 and 4 days for lethal concentration and 1, 5, 10 and 15 days for sub-lethal concentration were chosen to observe the short-term and long-term effects of deltamethrin on the fish, *Cirrhinus mrigala.* The present study shows that behaviour condition of the fishes varied and symptoms were identified like irregular, erratic and darting swimming movements, hyper excitability, capsizing/overturning, attaching to the surface, restlessness, difficulty in breathing, loss of equilibrium and gathering around the ventilation filter up to 15 days of time period. The treatment of toxic effects of Deltamethrin on the desired fish species that is *Cirrhinus mrigala* was found suitable for the exposure based on the available literature survey. This present investigation shows that impact of Deltamethrin on the changes in behavioural aspects of a freshwater fish, *Cirrhinus mrigala* for this study. The results of the study evidenced that Deltamethrin is toxic and thus, it has led to the altered fish physiology. However the exact mechanism through which this is achieved needs to be studied further.

Keywords: Deltamethrin (Decis, 30% EC), LC_{50} 96 h, behaviour, lethal, Sub-lethal & *Cirrhinus mrigala*

1. Introduction
The history of life on earth has been the history of environment moulding the various forms according to the changing conditions. The struggle for existence between the various life forms or between the variants of particular life form gives rise to a natural biological community, which is in equilibrium with the surroundings. More recently, a rapid pace of industrialization, coupled with uncontrolled exploitation of nature, has caused continuous dumping of industrial by-products, hazardous chemicals and nuclear wastes, resulting in the pollution of the river basins, lakes and sea. In his quest for wealth and comforts, man has ignored nature's law and thus disturbed a number of natural cycles. Environment is regarded as "The sum total of all conditions and influences that affect the development and life of organisms". Each living organism from the lowest to the highest has its own environment and this is affected by changes in natural cycles. Water is essential for our survival and its pollution is a major global problem. Our rivers, lakes and sea have limits for absorbing pollutants and with an increasing world population; there is a consequent increase in the discharge of sewage, industrial and harbour wastes and dumping of garbage. (Shivaraj *et al.* 2015) [35] Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are

responsible for adverse effects and death in the aquatic systems. (Sunita Kanikaram *et al.* 2015) [38] Acute toxicity of a pesticide refers to the chemical's ability to cause injury to an animal from a single exposure, generally of short duration. Behaviour is the organism level manifestation of the motivational, bio-chemical, physiological and environmentally influenced state of the organism. In the laboratory, fish behaviour can be a sensitive marker of toxicant-induced stress. (Atchison *et al.* 1987) [2], (Little *et al.* 1985) [19], (Westlake 1984) [40], (David 1995) [8]. Toxic substance entering aquatic ecosystem can have a wide range of adverse effects on animal communities, not all of which can be learned from standard toxicity tests (Henry and Atchison 1986) [16]. The principal biological variables examined in the standard tests according to some authors are changes in survival, growth and reproduction rate. Studies have documented alteration in respiration, locomotion, social organization, and reproduction tendency and predator avoidance. Behaviour is an organismic level of all the above mentioned parameters including bio-chemical and physiological state of the animal under the influence of the environment. Further, behavioural study should have objectives that should (1) be easily observed in the laboratory or field (2) be sensitive to the chemicals of interest (3) be previously well described (4) be ecologically relevant to species survival and (5) integrate several sensory and or mechanical modalities. In addition, the method should be routinely available and simple to employ.so in this way the study has been carried out and the outcome of the some of the important symptoms which has been found at the time of exposure period behaviour toxicity of this and The present study shows that behaviour condition of the fishes were varies and symptoms dictated like irregular, erratic and darting swimming movements, hyper excitability, capsizing, attaching to the surface, restlessness, difficulty in breathing, loss of equilibrium and gathering around the ventilation filter up to 15 days of time period. This present investigation shows that impact of deltamethrin on the changes in behavioural aspects of a freshwater fish, *Cirrhinus mrigala* for this study the treatment of toxic effects of deltamethrin on the desired fish species that is *Cirrhinus mrigala* was found suitable for the exposure based on the available literature survey.

2. Materials and Methods

2.1 Animal selected

Cirrhinus mrigala (Hamilton) is an important edible fish with great commercial value, occurring abundantly in the freshwater tanks, rivers, reservoirs and ponds in and around Dharwad, Karnataka state. It is largely employed for pond culture throughout the country. It plays a dominant role in composite fish culture. Besides its wide availability and commercial importance, this fish is known for its adaptability to laboratory conditions and suitability to toxicity studies (Bansal *et al* 1979) [3], Kapur 1980) [18]. Hence, this fish was selected as the experimental animal for the investigation.

2.2 Procurement and maintenance of fish

Fish, *Cirrhinus mrigala* weighing 5 ± 2 g were collected form the local fish farm Dharwad, Karnataka and were stored in large aquaria. The water was aerated twice a day so as to provide oxygen. The fish were fed daily with commercial fish pallets procured from market which had around 40% protein content. They were acclimatized to laboratory conditions for fifteen days. The aquarium was cleaned periodically to avoid infection to fish and 1% potassium permanganate solution was sprayed to eradicate any bacterial or fungal infection. The temperature of water in the aquaria was 29 ± 2 °C and the same was maintained throughout the course of investigation.

2.3 Procurement of Deltamethrin

The commercial grade deltamethrin (Decis, 30%EC) was obtained from Bayer Crop Science, India Ltd., Gujarat, India. Daily requirements were taken from this stock using variable micro-pipette.

2.4 Toxicity evaluation

The percent mortality of fish in different concentrations of deltamethrin was determined at 96 h exposure. For this the experimental fish were divided into batches of ten each, and were exposed to different concentrations of deltamethrin ranging from 3 ul/1 to 9 ul/1. This range was obtained on trial and error basis. Toxicity evaluation was carried out in static water (Doudoroff *et al* 1951) [9] and mortality rate was observed and recorded in all the concentration of deltamethrin after 96 h. A batch of fish maintained alongside in freshwater medium without deltamethrin served as control the experiment was repeated thrice for accuracy. The mean values were derived following the method of (Finney Probit Kill Theory 1971) [12].

2.5 Fixation of lethal and sub-lethal concentrations

Taking into consideration of the fact that the effect of a pesticide on fish becomes consistent with in 96 h of exposure LC_{50} 96 h (8 ul/1) of deltamethrin was taken as lethal concentration to study the behavioural, physiological and biochemical responses of the fish, *Cirrhinus mrigala.* However, knowledge on the concentration of toxicant that kills 50% of the test animals in a fixed period of time could become insufficient to assess various responses of the animal to toxicant (Nobbs and Pearu 1976) [22] Further, studies on acute toxicity have significant limitations such as the occurrence of adaptation of test animal to the imposed toxicity (Stockner and Anita 1976) [37]. Hence, (Perkin 1979) [23] also viewed the need for sublethal studies because distinct changes involving a sequence of events in the responses of test animal could occur in sublethal concentration. So, one tenth of the 96 h LC_{50} (0.8 ul/1) was taken as the sublethal concentration of deltamethrin for further studies.

2.6 Fixation of exposure periods

Since the duration of exposure is having a great influence on the toxicity of a pesticide on an organism. The effects of lethal and sublethal concentration of deltamethrin were studied at different periods of exposure in order to understand influence of time over toxicity. In the lethal concentration 1, 2, 3 and 4 days and in the sublethal concentration 1, 5, 10 and 15 days were chosen to observe the short-term and long term effects of deltamethrin on the fish, *Cirrhinus mrigala.*

2.7 Experimental design

After the determination of 96 h LC_{50} further studies in this investigation were carried out on the gill, muscle and liver at 1, 2, 3 and 4 days of exposure to lethal and 1,5,10 and 15 days of exposure to the sublethal concentration of deltamethrin. Selection of the gill, muscle, and liver in fish was to understand the difference in the effects of deltamethrin in different tissues. Prior to each experiment, fishes were exposed to their respective lethal and sublethal concentration of deltamethrin and were maintained in these concentrations up to the stipulated period of exposure. At the end of exposure the fishes were stunned to death and the target organs were dissected out from each animal using sterilized instrument. The organs were weighed on an electrical semi-microbalance and transferred into ice-jacketed micro beakers containing fish ringer solution. The fish ringer was prepared as per the composition given by (Ekenberg 1958) [11]. An equilibration time of 15 min was allotted to the organs to regain normalcy from a state of shock, if any, due to the handling and dissecting procedures.

3. Results

3.1 Normal fish

Control fishes maintained a fairly compact school, covering about one third of the bottom during the first seven days of the 20 days experiment. By seventh day, the school became less compact covering up to two-third of the tank area. Fishes were observed to scrap the bottom surface. When startled, they instantly formed a school that was maintained briefly. They were sensitive to light and moved to the bottom of the tank when light was passed into the tank. Except a less response to form a dense school towards the end of the study, no other extraordinary behaviour was observed.

3.2 Treated fish

When the fish were exposed to the lethal concentration of deltamethrin, they migrated immediately to the bottom of the tank. The schooling behaviour was observed to be disrupted on the first day itself and the fish occupied twice the area than that of the control group. They were spread out and appeared to be swimming independent of one another. This was followed by irregular, erratic and darting movements with imbalanced swimming activity. The fish exhibited peculiar behaviour of trying to leap out from the pesticide medium, which can be viewed as an escape phenomenon. The frequency of surfacing phenomenon was greater on the second day of exposure wherein the fish frequently come to the water surface. Respiratory disruption was observed in the normal ventilating cycle (cough and yawn) with a more rapid, repeated opening and closing of the mouth and opercular coverings. Partially extended fins and wide opening of the opercular coverings accompanied by hyperextension of all fins were found and the fish was in a state of excitement on the third day. The swimming behaviour was in a cork screw pattern rotating along horizontal axis and followed by 'S' jerk, partial jerk, sudden, rapid, non-directed spurt of forward movement (burst swimming). The fish progressively showed signs of tiredness and lost positive rheotaxis characterized by weakness and apathy. On the day 4 they lost their equilibrium and response, to external stimuli such as touch and light followed by drowning to the bottom. They often barrel-rolled or spiralled at intervals and engulfed the air through mouth before respiration ceased. Prior to death, the pectoral and pelvic fins of affected fish were spread forward (anteriorly), while swimming movements and respiration rate declined. The fish eventually died with their mouth and operculum wide opened. A change in colour of the gill lamellae from reddish to light brown with coagulation of mucus on gill lamellae was seen in dead fish. In sub lethal treatment, the schooling behaviour of the fish was slowly disrupted during the first day. The ventilation rate was increased but hyperactivity, excitement, hyperventilation etc, were not much influenced on exposure to the sub lethal concentration of deltamethrin at day 5 and 10. Further, the fish at 15 days of exposure exhibited balanced swimming and active feeding.

4. Discussion

The migration of the fish to the bottom of the tank following the addition of deltamethrin, clearly indicates the avoidance behaviour of the fish as observed in trout which was reported by (Murthy 1987) [21]. (Sprague and Drury 1969) [36] have observed the avoidance nature by rainbow trout and Atlantic salmon on exposure to four pollutants viz., Alkyl benzene, Sulfonate (AVS), Phenolchlorine and Kraft pulp effluent reported by (Belitginer and Freeman 1983) [4], (Hartwell *et al.* 1989) [15], (David 1995) [8], (Sadanand 2003) [27], (Prashant *et al.* 2002) [24] in various species of fish. Gold fish has been observed to avoid fenitrothion at low concentration of 10 mg/1 (Scheter 1975) [32]. It has been also reported by (Folmar 1976) [13] that Rainbow trout can detect and avoid copper sulphate, dalapon, 2, 4-D (DMA), xylene and acrolein. The lethal concentration of herbicide glyphosate has been avoided by Rainbow trout (Hildebrand *et al.* 1982) [17]. When abate was applied to river Oti in Ghana to control similum larvae, fish found at that site were observed to show avoidance reaction (Adban and Samman 1980) [1], (Prashant 2002) [24], (Shivakumar 2004) [34]. Disruption of schooling behaviour of the fish, due to the lethal and sub lethal stress of the toxicant, results in increased swimming activity, and entails increased expenditure of energy (Murthy 1987) [21]. A change in the normal physiological and bio-chemical aspects in the treated fish in the present study could be attributed to the disruption of the schooling behaviour of the fish, which in turn leads to hyper activities as suggested by (Murthy 1987) [21]. (Weis and Weis 1974) [39] Have reported that carbaryl has a marked effect on the schooling behaviour of the Atlantic silverside. Loss of such behaviour following pesticide exposures has been observed by many workers (Drummond *et al.* 1986) [10], (Shivakumar 2004) [34], (Shivakumar and David 2005) [33].

The erratic swimming of the treated fish indicates loss of equilibrium. It is likely that the region in the brain which is associated with the maintenance of equilibrium should have been affected (Mehrle and Mayer 1975) [20], (Drummond *et al.* 1986) [10]. Loss of equilibrium and erratic swimming are reported in blue gills exposed to dursban (Mehrle and Mayer 1975) [20]. Excited and erratic movements were observed by (Rao *et al.* 2003) [26]. (Radhaiah and Jayantharao 1988) [25], (Henry and Atchinson 1986) [16]. Increase in fin "flickers" observed in the treated fish is not uncommon (Drummond *et al.* 1986) [10]. These behavioural changes were seen in the present investigation also.

The surfacing phenomenon of fish observed under deltamethrin exposure might either be due to hypoxic condition of the fish as reported by (Radhaiah and Jayantha Rao 1988) [25], (Sambasiva Rao and Chandrasekara Rao 1984) [30]. This fact is clearly evidenced in the present study. Chronic exposure of fin fish to alchlor was found to induce surfacing phenomenon of fish as pointed out by (Hansen *et al.* 1972) [14]. (Drummond *et al.* 1986) [10], have recorded similar observation in fathead minnow treated with different chemical groups.

The increased ventilation rate by rapid, repeated opening and closing of the mouth and opercular coverings accompanied by partially extended fins (coughing) was observed in the present study. This could be due to clearance of the accumulated mucus debris in the gill region for proper breathing as suggested by (Carlson and Drummond 1978) [7], cough and

yawns seem to be a more extreme effort to do the same, Similar situation was observed by (Carlson *et al.* 1982) [6] in the bluegill, *Lepomis mahrochirus.* (Schaumburg *et al.* 1967) [31] have noticed a direct relationship between the frequency of coughing and the time of exposure in rainbow trout. Coughing frequency in coho salmon was increased, with increasing concentration of fenitrothion (Bull and Me Inemey 1974) [5].
The hyperexcitability of the fish invariable the lethal and sub lethal exposure of deltamethrin may probably be hindrance in the functioning of the enzyme AChE in relation to nervous system as suggested by many authors and It leads to accumulation of acetylcholine which is likely to cause prolonged excitatory post synaptic potential. This may first lead to stimulation and later cause a block in the cholinergic system. (Sadanand and David *et al.* 2005) [28], has observed hyperactivity, in juveniles of *Mugil cephalus* exposed to lindane, to affect central nervous system (Mehrle and Mayer 1975) [20] reported the state of neuromotor system in the exposed fish. According to (Sambasiva Rao and Chandrasekara Rao 1987) [29], (David 1995) [8] behavioural patterns are also influenced by bio-chemical changes at the tissue level. The significant alterations observed in the bio-chemical constituents of gill, liver and muscle in the present investigation corroborate with the above view that bio-chemical change at the tissue level of the dosed fish contribute to the abnormal behaviour of the fish.

5. Conclusion
The results of the study evidenced that the analysis of data from the present investigation evidenced that Deltamethrin is highly toxic and had profound impact on behaviour and respiration in the Freshwater fish, *Cirrhinus mrigala* in both lethal and sub lethal concentrations. Thus, it has led to the altered fish physiology and due to some of the variation in the oxygen consumption in Deltamethrin exposed fish is probably due to impaired oxidative metabolism and Deltamethrin induced stress. However the exact mechanism through which this is achieved needs to be studied further.

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