RESEARCH ARTICLE OPEN ACCESS

The Freshwater Fish Cyprinus carpio's (Linnaeus) Behavioural Reactions After Sublethal Exposure to Monocrotophos, an Organophosphorus Pesticide

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ABSTRACT
Monocrotophos, an organophosphate insecticide, was applied to common carp at varying amounts. The median fatal concentration was determined to be 0.37 ml/L after tests were conducted using the conventional procedures outlined in the APHA manual. For subacute investigations, one-third (0.052 ppm), one-fifth (0.074 ppm), and one-seventh (0.123 ppm) of the 96-hour LC50 were chosen as sublethal values. For thirty, forty-five, and sixty days, the fish were subjected to sub-lethal doses. During the experimental periods, morphological anomalies, hormonal abnormalities, and behavioural reactions were examined. Stressed fish showed altered opercular movements, darting, erratic, and irregular swimming motions, increased air gulps, atypical fin movements, hyper and hypo excitability, loss of stability, and sinking to the bottom. Although the carp were under stress, both sublethal amounts had negligible mortality. When acetylcholinesterase (AChE) activity is inhibited, excess acetylcholine (ACh) builds up in cholinergic synapses, causing hyperstimulation and stopping neural transmission (paralysis), which ultimately causes alterations in the fish's behaviour and morphology. This could be a result of monocrotophos's biotransformation of bioaccumulated monocrotophos in the tissues, which inhibits AChE activity in the brain and muscles.

Keywords: Monocrotophos, common carp, acute toxicity (96 h LC50), behavioral anomalies,

I. INTRODUCTION

Synthetic chemicals have accumulated in our dietary food products as a result of the widespread spraying of agricultural land; in India, this is believed to be 20% of food goods, or above the tolerance level, compared to only 2% worldwide. Since aquatic environments are the final destination for a wide range of suspected contaminants from both domestic and industrial sources, established examples of toxicities have been found there.

Fish, which are extremely important to humans, are among the aquatic animals that the chemicals have dangerous effects on. These substances first build up in planktons, which fish eat, and eventually end up in the skin, respiratory system, liver, kidneys, and blood of fish. In addition to a number of morphometric changes and behavioural complications, their final effects include a shift in growth rate and a sharp decline in fecundity and reproductive capacity. As a result, certain fish species have vanished from these contaminated aquatic environments (Tandon et al., 1980). Fish have not evolved to withstand such foreign substances (Ryder, 1988).

In the past, several researchers have attempted to evaluate the toxicity of monocrotophos on several fish parameters. The impact of monocrotophos on the behaviour of Anabas testudineus (Santhakumar and Balaji, 2000); leucocyte count of Channa punctatus (Seth and Saxena, 2003); erthropoietic activity of Channa punctatus (Agrahari et al., 2007); haematological indices of Clarias gariepinus (Yaji and Auta, 2007); oxidase stress and locomotor behaviour response of Gambusia affinis (Kumar, 2008); swimming velocity and average number of turns in Danio rerio (Daniela Baganz, 2004); and inhibition of Na+ K.

Because fish are extremely sensitive to even small changes in their habitat, pesticide toxicity in fish habitat has alarmed environmentalists, zoologists, health professionals, and, to some extent, the general public. This could result in fish gradually going extinct, causing ecological imbalance and the loss of future food sources.

II. MATERIAL AND METHODS

Because Cyprinus carpio communis is readily available and can withstand harsh environmental conditions, it was chosen as the experimental model for this study. The sexually mature female Cyprinus carpio communis L. was transported from the Government Fish Seed Farm at

Bagrhiani Phagon Majra village on Sirhind road, District Fatehgarh Sahib (Punjab), with a total length of 15 cm±2 cm and a weight of 171 gm±7.9 gm.

The fish were kept in a glass aquarium measuring 4'9" 1' 5" 1'0.5" for 15 days to acclimatise to laboratory conditions in dechlorinated tap water. The fish were fed floating food, and the aquarium had filters and aerators. Pellets with 32% crude protein, 4% crude fat, 5% crude fibre, 10% crude ash, 91% moisture, and 31% nitrogen-free extract. The fish's abiotic components were acclimated to laboratory conditions for 15 days in dechlorinated tap water in a glass aquarium that measured 4'9" × 1'5" × 1'0.5". The aquarium was equipped with filters and aerators, and the fish were fed floating type food. Pellets containing crude protein (32%), crude fat (4%), crude fibre (5%), crude ash (10%), moisture (91%), and nitrogen-free extract (31%). The abiotic factors of aquarium water like pH, dissolved oxygen, temperature, conductivity, alkalinity and light and dark adaptations were maintained during experiment.

Tests for median lethal concentration were conducted using the standard procedures outlined in the APHA manual (1998). The fish were exposed to a wide range of monocrotophos concentrations in order to conduct the short-term toxicity studies, also known as range finding tests.According to the guidelines provided by APHA (1998), the sublethal concentrations of 0.052 ppm, 0.074 ppm, and 0.123 ppm were created in dechlorinated water.Sixteen litres of dechlorinated water were prepared in four tanks. For 30, 45, and 60 days, six fish of roughly the same length (15±2) and weight (171±7.9) were placed in each of these aquariums. As a control, one tank was maintained.

To determine the stress caused by monocrotophos pollution, morphological and behavioural data were undertaken. The fish were routinely checked for signs of restlessness, jerking, surface movement, opercular movement, balance, and food consumption during the toxicity test. On the 30th, 45th, and 60th days of the experiment, the morphological and behavioural assessment for any variation in length, breadth, and weight was carried out.The data were all presented as mean± S.D. Students' "t" test was used to statistically analyse the comparison of the treatment and control data in order to determine the validity of the observed effect. According to SPSS software version 10, values with $p < 0.05$ were deemed statistically significant.

III. RESULTS AND DISCUSSION

Fish are ideal sentinels and test organisms to assay various stress factors and toxic exposures as fish may bioaccumlate various contaminants and play a significant role in food web biomagnifications (Ramesh Halappa, 2009). There are different scientific tools to discern and evaluate effects of environmental stress and general activity. These endpoints exposures are important because they integrate endogenous and exogenous factors that can link biochemical and physiological processes, and can provide insights into individual and community level effects of environmental contamination. The monocrotophos induced toxicity in fish adobe was studied to access the stress inducing behavioral alterations in the fish. Clinical manifestations of organophosphate poisoning depend upon the site of accumulation. The impacts of organophosphate exposure can be categorized as follows:

Muscarinic Effects	Nicotinic Effects	Central Nervous System Effects
• Hypersalivation • Sweating • Nasal Discharge • Brachycardia • Dypnoea • Coughing • Vomiting • Diarrhoea • Abdominal Pain • Polyuria	• Muscular Fasciculation • Tetany , stiff legged gait • Torricelli’s in cattle and Sheep	• Nervousness • Convulsions • Ataxia • Coma

WATER ANALYSIS

The water analysis was conducted regularly of normal as well as treated groups daily to ascertain uniform abode of normal and treated group. During the experiment, physico-chemical characteristics of the water in which the fish abode was analyzed using procedure given by APHA (1998) as water quality plays an important role in the biology of the test species. The observations are given in table 1.

TABLE 1: Water analysis after adding monocrotophos

Parameters	Control	0.052 ppm of monocrotophos	0.074 ppm of monocrotophos	0.123 ppm of monocrotophos
Temperature	27.1^0 C	26.24^0 C	26.34^0 C	26.25^0 C
pH	7.72	7.69	7.75	7.75
Dissolved Oxygen	4.21	4.28	4.23	4.20
Conductivity	314	313	308	319

The temperature of water having monocrotophos decreased slightly as compared to control during the same time. Within the toxicant concentration, this parameter did not show any definite trend. Similarly, the pH remained same as compared to the control (Table 1). The pH in two higher concentrations i.e. at 0.074ppm and 0.123ppm was higher as compared to the pH of water containing the toxicant at the level of 0.052ppm. The level of dissolved oxygen has been found to be higher in all the concentration as compared to control, but within concentrations a decreasing trend has been observed. The level of conductivity of water in the two lower concentrations is observed to be on the lower side and in the high concentration it was on the higher side as compared to the control group.

MEDIAN LETHAL CONCENTRATION FIFTY (LC_{50})

The acute toxicity of monocrotophos after 96 hours to *Cyprinus carpio communis* L. was assessed according to APHA (1998).After 96 hours of monocrotophos treatment, the percentage mortality was calculated and the values were transformed into probit scale (Finney, 1980). LC_{50} value was calculated by both graphical and arithematic methods. The probit analysis was performed by method suggested by Finney (1980) and SPPS for windows (version 10). Thus, the LC_{50} of technical grade of monocrotophos having cyclohexanone as solvent to *Cyprinus carpio* showed LC_{50} as 0.37 ml/L. The correlation coefficient 'r' came out to be 0.95 which was significant at 0.05 levels.

BEHAVIORAL OBSERVATIONS

Basic knowledge of exposure related behavioral alterations relevant for ecotoxicological assays remain scarce and systems that have the ability to link toxicology data with swimming and avoidance behaviour are needed.

Despite historical shortcomings, movement analysis and quantification of locomotion has progressed over the past years mainly due to continued improvement of computer processors and digital imaging methods. As a result, acceptability of behavioral tests for hazard evaluation has increased as technology has improved the quantification of behavioral observation (Kondaiah and Murty, 1994). A variety of methods have been used in order to quantify the movement data, including hand scoring through direct observations (Teather *et al*. 2001; Wibe *et al*., 2001) and computer digitization into X,Y coordinate data (Suzuki *et al*., 2003).

Behavioral alterations can be measured as end points for sub lethal toxicity, which serve as a tool for environmental risk assessment and analysis of toxicological impacts. Numerous technical and biological factors have made sub lethal effects on fish behavior difficult to quantify as the changes in fish behavior was observed at threshold dose where apparent changes take place without any mortality. The dose of a pesticide determines the degree of effect it produces and immediate fish response can be studied by the parameters e.g. resting period, air gulps, opercular movements and fin movements.

The present investigations have recorded a significant shift in the behavior of *Cyprinus carpio communis* L. when exposed to three sub lethal concentrations (0.052 ppm, 0.074 ppm, 0.123 ppm) of monocrotophos. The observations were made for sixty days regularly once a day at the time of change of pesticide. The following observations were made:-

GROWTH PARAMETERS

The growth expressed in weight was recorded at the beginning of the experiment and then weight was studied at the end of 30^{th}, 45^{th} and 60^{th} days of the experiment (Fig.1).

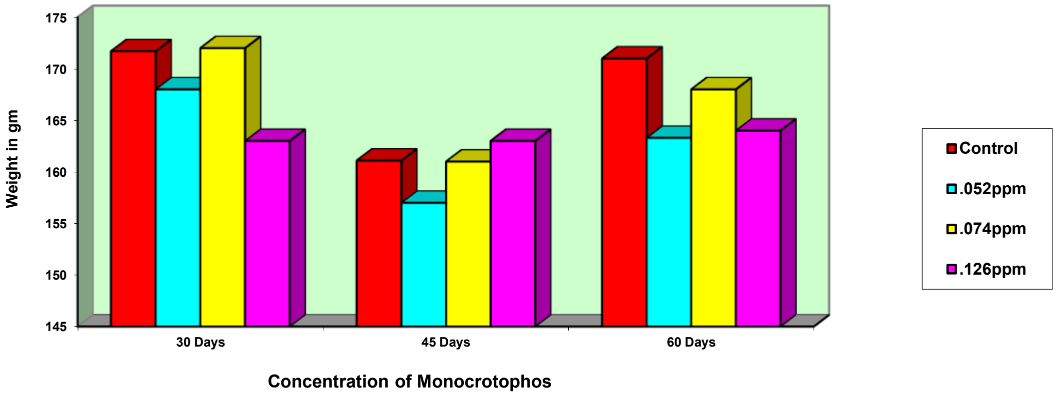

Fig.1: Weight variation in *Cyprinus carpio communis* L. with sub lethal doses of monocrotophos

On 30th day of exposure by monocrotophos the mean weight in control was significantly higher (171±7.94 g) as compared to the mean weight in fish exposed to 0.052 ppm concentration of monocrotophos (168±2 g) . The paired t-test shows significant decrease in weight and p value is 0.56. At 0.074 ppm concentration of monocrotophos, the mean weight in the treated fish was (172±3 g) which was almost equal to that of control. The paired t-test showed p value as 0.84 which is significant but at highest dose of 0.123 ppm, control mean of treated fish (163±3.61g) was significantly lower with p value as 0.62.

On the 45th day of experiment, the mean control weight of fish was 161.1 +8.08 g. It was significantly higher than the treated mean weight at 0.052 ppm (157±7.5 g) and 0.123 ppm (161±6.6g) with p value as 0.0059 and 0.92 respectively. At 0.074 ppm of concentration of monocrotophos, the treated mean weight (163±4.9 g) is higher than the control mean weight but was less significant than 0.052 ppm and 0.123 ppm with p value as 0.43.

On 60th day of exposure, the control mean weight was (171± 3.6 g). The treated mean weight at 0.052ppm (163.3± 4.16 g), 0.074 ppm (168±2.6 g) and 0.123 ppm (164±4.5 g) was significantly lower than the control mean weight with p values as 0.13, 0.22 and 4.5 respectively.

On the basis of above data it can be concluded that there is significant decrease in weight at lowest (0.052 ppm) and highest (0.123 ppm) concentrations as compared to middle dose on 30th, 45th as well as 60th day of experimental period. Barton and Iwama (1991) observed that in long them chronic stress slows or stops the fish growth. Skripsky and Loosli (1994) also confirm the above study as it was shown that body weight at high doses of monocrotophos got reduced. The reduction in oxygen consumption, feeding energetic and weight were clearly marked in studies made by Palanivelu and Vijayavel (2004) in *Oreochromis mossambicus* upon exposure to urea. Hyper extension of fins, dullness in body colour and fish body became lean towards abdomen and carp under stress were observed with time and concentration in experimental periods. In general, fish poisoned with anticholinesterase insecticides show signs of muscle paralysis, especially of the fins and respiratory apparatus, hyperactivity and loss of balance. Leaning of fish indicates reduced feeding behavior and diversion of fish metabolism towards adaptability to the toxic media. Feeding preferences were affected and consumption of food in fish was impaired and reduced (Chawanrat, 2007).

OPERCULAR MOVEMENTS

When the fish was exposed to toxic water, it has been observed that the opercular movements per minute increased (Fig.2) to maximum at the lowest concentration, when the monocrotophos was acting as toxicant plus the allergen. Toor and Kaur (1974) exposed *Cyprinus carpio communis* L. to different organophosphates and found increased opercular movements per minute.

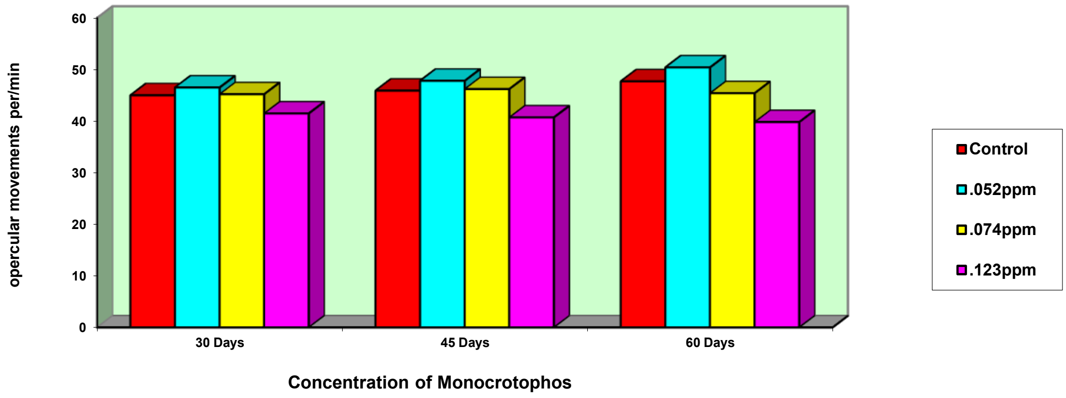

Fig.2: Variation in opercular movement per minute of *Cyprinus carpio communis* L. with sub lethal doses of monocrotophos

It has been observed that on the average, there is slight increase in opercular movements per minute. However, at 0.123 ppm of concentration (highest concentration of monocrotophos) the fish activity is reduced to minimum and showed maximum decrease in opercular movements per minute. The lowest value of opercular movements per minute is on 60th day at 0.123 ppm of monocrotophos exposure. Such decline in opercular movement at high doses was also reported in studies by Singh *et al,* (2009) on common carp upon exposure to dimethoate. Decrease in opercular movement probably helps in reducing absorption through gills.

Statistically the mean of control (45.0±6.18) on 30th day is higher than mean treated opercular movements per minute at 0.123ppm (41.47 ± 6.38) with significant p value as 0.123. The treated mean value at 0.052 ppm (46.5 ± 6.4) and 0.074 ppm (45.20 ± 5.94) found to be higher than control mean opercular movements per minute significant at p equal to 0.0085 and 0.0026 respectively.

On 45th day of experimental period, the control mean opercular movement per minute value is (45.9 ± 7.4). The treated mean values at 0.052 ppm (47.8 ± 7.9), 0.074 ppm (46.27 ± 7.63) and 0.123 ppm (40.71 ± 7.05) are all significant with p values as 0.045, 0.0010 and 0.0001 respectively. Decrease in opercular movements per minute at 0.123 ppm concentration of monocrotophos has been recorded.

The opercular movement per minute decreased at 0.123 ppm (39.83 ± 5.57) and 0.074 ppm (45.47 ± 7.16) with significant p values as 0.0001 and 0.0001 respectively as compared to the control mean opecular movement per minute (47.7 ± 7.47) on 60th day of monocrotophos exposure. However, at 0.052 ppm of monocrotophos concentration, the opercular movements per minute (50.46 ± 8.50) showed increase and p value as 0.006 which is not significant.

The rapid opercular movements leaping out of water and thick mucous covering over the whole body surface were also observed by Pathan *et al*.,(2009); Pandey *et al*.,(2008) and Ismail *et al*.,(2008); Ganeshwade *et al*.,(2006) support the present study by reporting increased opercular movements and coughing in common carp exposed to industrial effluents.

FIN MOVEMENTS

Dorsal fin movement per minute of the fish is related with the equilibrium and the balance maintained by the fish which is decreased as the concentration is increased due to muscular dystrophy at higher concentration (Fig.3).

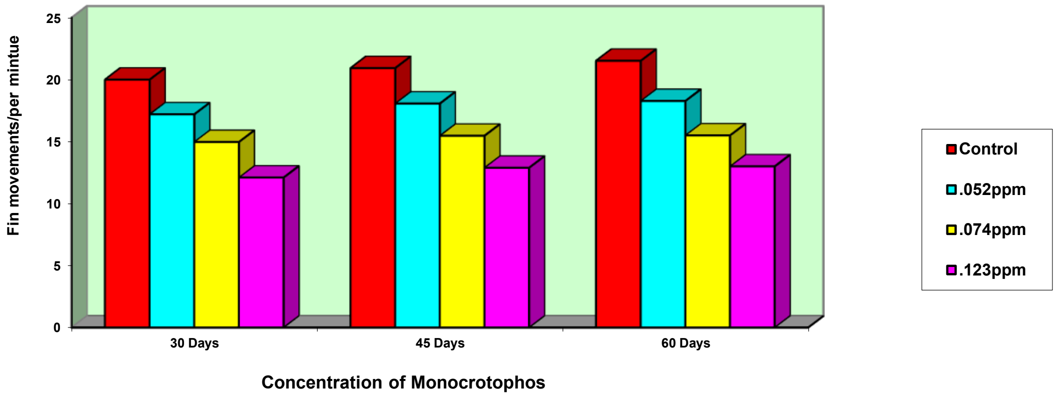

Fig.3: Variation in fin movements per minute in *Cyprinus carpio communis* L. with sub lethal doses of monocrotophos

As is evident in Fig.3, the fin movements per minute showed decrease in treated fish (17.20 ± 6.22) at 0.052 ppm, (14.97 ± 5.67) at 0.074 ppm, (12.10 ± 5.18) at 0.123 ppm with p value as 0.0001 for all concentrations as compared to control (20.0 ± 7.05) on 30th day of experiment.

On 45th day of experiment, the mean control fin movement per minute (20.93 ± 6.77) is higher than mean treated fin movements per minute which are (18.07 ± 6.20, p=0.001) at 0.052 ppm, (15.47 ± 5.61, p=0.0001) at 0.074 ppm and (12.89 ± 5.10, p=0.0001) at 0.123ppm of concentration. All these results are found to be statistically significant.

Similar observations are recorded on the 60th day of experimental period. Here, the control mean fin movement per minute is (21.52 ± 6.25) and the mean treated fin movements per minute are (18.28 ± 5.28, P=0.0001) at 0.052 ppm, (15.50 ± 5.14, p= 0.0001) at 0.074 ppm and (13.0 ± 4.62, p=0.0001) at 0.123ppm of monocrotophos treatment.

The decrease in fin movements which results in lowering of the swimming speed has been reported by many worker like *Ballesteros et al*.,(2009) in *Jenynsia multidentata* upon exposure to endosulphan; Jakka *et al*.,(2007) in *Gambusia affinis* upon exposure to mercury; Venkateshwara Rao *et al*.,(2007) in *Atremia salina* upon exposure to profenofos; Kavita and Venkateshwara (2007) in *Gambusia affinis* upon exposure to monocrotophos.

AIR GULPS

Gulping air at the surface of the water is frequently referred to as piping. Piping is an abnormal behavior and is indicative of severe hypoxia. The hypoxic condition of fish is state of distress and may show a sign of trying to leave the water, by jumping out.

When pesticide is added in the water tank, water becomes toxic and the fish tries to come up on the surface to engulf air bubbles. Piping represents on effort by the fish to utilize oxygen from a thin surface layer of the water that is supersaturated with dissolved atmospheric oxygen.

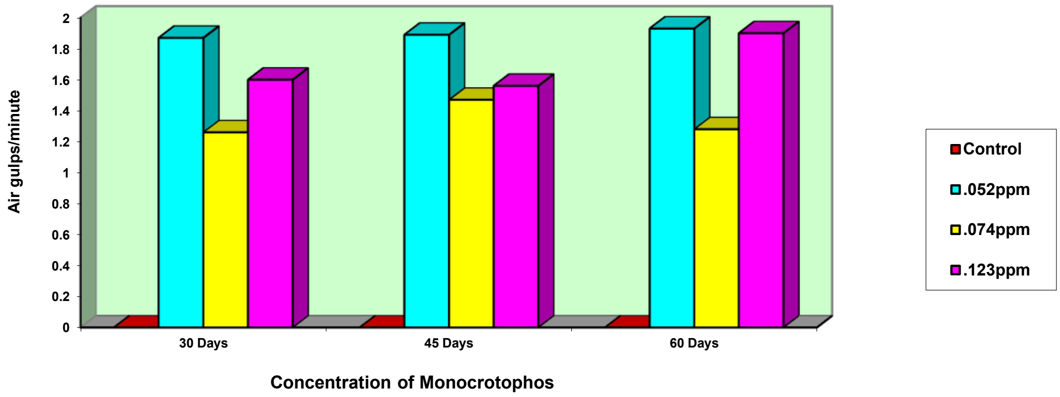

Fig.4: Variation in air gulps per minute in *Cyprinus carpio communis* L. with sub lethal doses of monocrotophos

The process of piping showed increase at concentration of monocrotophos on 0.052 ppm at 30th, 45th as well as 60th day of experimental period. A little decrease at 0.074 ppm as compared to 0.052 ppm and again a rise at 0.123 ppm of monocrotophos treatment has been noticed (Fig.4).

The increased surfacing and air gulping under the stress created by the pesticides has been reported by earlier workers like Singh *et al*.,(2009) on common carp; Pathan et al.,(2009) on *Rasbora daniconius* and Santhakumar and Balaji (2000) on *Anabas testudineas.*

The overall behaviour of the fish has showed anorexia or loss of normal feeding activity which resulted in reduction in weight. The abnormalities like tail walking i.e. swimming movement in which the fish does not swim in a normal horizontal plane, assuming an oblique position with the head directed toward the surface have been observed. Abnormal positioning by the affected fish is due to loss of control over equilibrium, and it may appear to swim backwards as an attempt to correct its posture. *Cyprinus carpio communis* L. in treated tanks are holding their fins into the body which is a sign of uncomfortable condition. It may represent the early signs of impending disease or is considered to be general sign of depression.

The drastic changes have been observed at lowest and highest concentration of pesticide (0.052 ppm and 0.123 ppm) where, behaviour is more erratic. At low concentration, the fish did not follow the dose response relationship. This relationship does not hold true in allergic relationship. Allergic reactions are special kinds of changes in the immune system and they are not really toxic response. The difference between allergic and toxic reactions is that a toxic effect is directly the result of the toxic chemical acting on cells, whereas, allergic response is the result of a chemical stimulation by the body to the release natural chemicals which are in turn directly responsible for apparent effects. Thus, in an allergic reaction, the chemical acts merely as trigger. Therefore, here also low concentration is challenging the fish immune system but at highest concentration there is long resting period and the fish is more lethargic showing less movements.

In the present study *Cyprinus carpio communius* L. showed alterations in various behavioural responses especially frequent surfacing, mucus secretion, change in skin color, hyperexcitation, erratic swimming , jerky movements, loss of balance, increased defecation along with the changes already explained which include decreased opercular movements per minute, weight, fin movements per minute(swimming speed) and increased air gulps per minute.

Needless to say these behavioural changes observed in exposed common carps are manifested by monocrotophos toxicity. Upon pesticide exposure increase in surfacing and gulping of surface waters appears to be an attempt by the fish to avoid breathing in toxic water. Similar observation has been reported in *Anabas testudineus* after exposure to monocrotophos (Santhakuar and Balaji, 2000). Moreover, hypoxic condition also contributes to increased surfacing (Radhaia *et al*., 1998). Hypoxic condition arises primarily due to damage of gills of pesticides exposed fish which hampers oxygen uptake (Velmurugan *et al*., 2007; Pandey *et al*., 2008). This situation further continued intensely throughout the test periods, which is in accordance with the observations made by Ural and Simsek (2006).

Certainly decreased opercular movement probably helps in reducing absorption of toxic substances through gills. This results in reduced rate of oxygen consumption as is evident in the present study. Reduction in oxygen consumption in *Cyprinus carpio communis* has also been reported after sblethal exposure of dimethoate (De Mel *et al*., 2005) and antimony chloride (Chen *et al*., 2007). Ganeshwade *et al*., (2006); Omitoyin *et al*.,(2006); and Yadav *et al*., (2007) too reported increased opercular rate and coughing at high doses of various pesticide which has been confirmed by the present studies. Erratic movements and abnormal swimming triggered by deficiency in nervous and muscular coordination may be due to the accumulation of acetylcholine in synaptic and neuromuscular junctions (Rao *et al*., 2006). Tremors, gradual loss of equilibrium and drowning are caused by adverse effects of organophosphate on central nervous system. Increased mucus secretion after dimethoate exposure is probably the adaptive response to counteract the irritating effect of pesticide on body surface and mucus membrane (Srivastava *et al*.,2007; Chawanrat *et al*.,2007).Changes in body color to pale white may be caused by impairment of pituitary functions on inhibition of acetylcholine by monocrotophos (Vasalt and Patil, 2005). This is reflected by the reduction in number and size of chromatophores and pigment content in them (Ram *et al*., 2001). Body color changes have also been observed in *Cyprinus carpio* after exposure to mercuric chloride (Masud *et al*., 2005). Defecation is increased in exposed fishes due to organophosphate toxicity involving hyper stimulation of muscarinic receptors in smooth muscles of the end organs viz., gastrointestinal tract and secretory glands (Bonita, 2004).

It is concluded that deleterious changes occurred in the fish behavior with increase of toxicant in its abode. The quick behavior response to mild stressor in aquatic abode is the indicator in the form of decreased fin movement, opercular movements, general fish activity and increase air gulping in a confined area. The above symptoms may be due to inhibition of acetylcholinesterase (AChE) activity leading to accumulation of acetylcholine (ACh) in cholinergic synapses ensuing hyperstimulation (Timchalk et al., 2002). The primary molecular mechanism of action of the OP pesticides is inhibition of AChE activity, a widely distributed serine esterase. AChE hydrolyses ACh into choline and acetic acid and is responsible for the removal of the neurotransmitter ACh from the synaptic cleft through hydrolysis (Dembele, K.*et al.,* 2000). Activity of AChE system is vital to normal behavior and muscular function and represents a prime target on which some toxicants can exert a detrimental effect. Once bound, organophosphorus compounds are considered irreversible inhibitors, as recovery usually depends on new enzyme synthesis. An excess secretion of mucus in fish forms a nonspecific response against toxicants, thereby probably reducing the toxicant contact (Deb and Das, 2013). Mucus also forms a barrier between the body and the toxic medium, to minimize its irritating effect, or to scavenge it through epidermal mucus (Rao, 2006). In natural waters the fish might have shifted from site of toxicologically changed abode to an open area. This reflex behavior of fish is quick and certain to sense out slight variation in aquatic system. Therefore, it becomes very important to quantify fish behaviour which reflect its direct effect on nervous system of exposed fish and which can be used as a biomarker to study environmental changes in the fish.

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