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Effects of Malathion and Cypermethrin on the Behaviour and Brain Protein Content in Koi Carp-(*Cyprinus Carpio*)

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Abstract: **Man has caused the deterioration of the quality of water in all our fresh water bodies by discharging undesired substances and toxicants into them. Due to the impact of pollution our fresh water resources are getting changed to mere chemical drains, as a result, the quality of fish is also getting reduced. The cosmopolitan use of pesticides for agricultural practices and public health operations for the past few decades is more hazardous than any other pollutants.Healthy adult fishes were collected from Aquarium point, Thrissur. The fishes were kept for acclimatization for a period of one week. They were fed with artificial feed once in a day. After completion of the acclimation period, fishes that showed the normal activities were only selected for the experimental purposes. Unnecessary handling of fishes was avoided. As an organophosphate pesticide Malathion and synthetic pyrethroids, cypermethrin had been selected as test toxicants for present study. The desired concentration of Malathion (0.1, 0.2, 0.3, 0.4, 0.5 ppm) was prepared by dissolving in 1litre of water.0.5 is LC 50 value. The fishes were divided into groups of four each and were exposed to the different concentrations of Malathion and cypermethrin. The distinct changes involving sequence of events occur in sub-lethal concentrations.Further, the individuals in control and test the brain were dissected out. For protein estimation by Lowry method. The statistical significance was compared between control and experimental groups by using student's t-test**

Keywords: **Pesticides, Malathion, Cypermethrin, Cyprinus carpio, Protein, brain.**

1. INTRODUCTION

The term pesticide is a composite term that includes all chemicals that are used to kill or control pests. A fundamental contributor to Green Revolution has been the development and application of pesticides for the control of a wide variety of insectivorous and herbaceous pests that would otherwise diminishes the quantity and quality of food produce. The use of pesticides coincides with the "chemical age'' which has transformed society since the 1950s (Casida, 2005). Unfortunately, with the benefits of chemistry have disrupted the predator-prey relationship.Water is one of the most essential basic natural resources to the living kingdom. In India, there has been tremendous pace in the development and sophistication of industrial and agricultural practice since independence. There is overwhelming evidence that agricultural use of pesticides has a major impact on water quality and leads to serious environmental consequences.Number of pesticide application increased year after year. The major problem with these broad spectrum modern insecticides concern their effect on both target and non-target beneficial species. Studies show that the most commonly used pesticides are the ones most likely to cause water pollution. The aquatic life is affected directly or indirectly in a number of ways, when pesticide pollution takes place. Aquatic organisms are particularly sensitive to environment contamination and pollutants may significantly damage certain physiological and biochemical processes when they enter the organs of fishes (Nemcsok et .al., 1987). Fish population however are subjected to sudden and large scale mortalities. (Jones et. al., 1987)

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Fish is a chief food source of the country. It is an excellent nutrient supplier. Fish provides high quality proteins and a wide variety of vitamins and minerals like vitamin A and D, phosphorous, magnesium, selenium, etc. It is easily digestible and complements the dietary proteins provided by cereals and legumes. They provide the essential amino acids that are present in vegetables in low quantities. Various pesticides have various effects on the fishes as observed by researches.Malathion is most commonly used organophosphate insecticides. It is widely used in the eradication programme and mosquito control programme Cypermethrin is a pyrethroid insecticide which was first synthesized in 1974. This kills insects that eat or come into contact with it. Cypermethrin works by quickly affecting the insect's central nervous system. It is also very toxic to fishes. Effect of cypermethrin on human health and the environment depends on how much cypermethrin has present and the length and frequency of exposure. It is also very highly toxic to water insects.The aquatic ecosystem is the greater part of natural environment which is facing the threat of shrinking genetic base and biodiversity due to indiscriminate use of pesticides (Rahman et.al, 2002). Synthetic pyrethroids are long-lived chemicals that interfere the functioning of nervous system. They are highly toxic to fishes and also to insects. They keep the pest population under control. Malathion is a pesticide that is widely used in agriculture, public recreation areas, public health pest control programmes such as mosquito eradication. These pesticides are found very much hazardous to the aquatic flora and fauna and hence ultimately to human beings as they depend on aquatic foods like fishes .Organophosphorus (OP) compounds are cholinesterase-inhibiting chemicals used as pesticide. Exposures to OPs cause a significant number of poisonings and deaths each year. The main objective of our studies were to study the behavioural changes immediately after the exposure to the pesticides from that of the control. To compare the toxicity of the two pesticides, by estimating the brain protein

2. MATERIALS AND METHODS

Koi carp are an ornamental strain of the common carp (*Cyprinus carpio*) that are kept for decorative purposes. A long-lived species, Koi carp is a highly productive fresh water fish. The fish has the ability to survive and adapted to many climates and a wide variety of environment conditions. These are omnivorous fish and will eat a wide variety of foods.Healthy juveniles were collected from Aquarium point, Thrissur. The fishes were kept for acclimatization for a period of one week. They were fed with artificial feed once in a day. Remains of feed and faecal matters were removed daily without disturbing the fishes. The range of variations in size of the fishes selected for study was also minimized by selecting those of uniform size with very marginal differences (6.01 ± 0.99 cm). Fitness of test specimens is the prime importance in the toxicity studies. After completion of the acclimation period, fishes that showed the normal activities were only selected for the experimental purposes. Unnecessary handling of fishes was avoided. As an organophosphate pesticide Malathion and synthetic pyrethroids, cypermethrin had been selected as test toxicants for present study. The desired concentration of Malathion (0.1, 0.2, 0.3, 0.4, 0.5 ppm) was prepared by dissolving in 1litre of water.0.5 is LC 50 value . The fishes were divided into groups of four each and were exposed to the different concentrations of Malathion and cypermethrin.

A batch of four healthy, disease free, laboratory acclimated fishes, irrespective of sex were released into each aquarium. The acute toxicity, lethal concentration and sub-lethal concentration were determined from the number of fishes that died during 24-hours after pesticide treatment. Thus the LC_{50} of the two pesticides was determined. Various concentrations of the Malathion and cypermethrin prepared to find out the LC_{50} were also used for observing the behavioural characteristics of the fishes. The swimming behaviour, disruption of schooling behaviour, surfacing phenomenon, opercular beats, colour changes in body, fin movements, changes on the scale, body texture, aggressiveness, loss of balance, etc. were the major parameters taken for carrying out the behavioural studies. These observations were made during the first second and the third half hours of exposure. These data were compared with the normal fishes (in control set up) without any pesticide treatment.In lethal concentration, the time is insufficient to assess various parameters taken. In acute toxicity, the studies have various limitations. The distinct changes involving sequence of events occur in sub-lethal concentrationsFurther, the individuals in control and toxicants were dissected and the brain was dissected out. For protein estimation by Lowry method (Lowery et al;1959) The statistical significance was compared between control and experimental groups by using student's t-test.

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3. OBSERVATION, RESULTS& DISCUSSION

Behavioural studies: After providing a period of one week for acclimatization, six healthy fishes were selected for conducting the behavioural studies. Here no pesticides were added, and are considered as control. A comparison of the behaviour of fishes exposed to various concentrations of Malathion and cypermethrin were also done. Behavioural characteristics are obviously sensitive indicators of toxicant's effect. It is necessary, however, to select behavioural indices of monitoring that relate to the organisms behaviour in the field in order to derive a more accurate assessment of the hazards that a contaminant pose in natural system. For studying the behaviour, the surfacing phenomenon, schooling behaviour, swimming behaviour, opecular movement, aggressiveness body texture and colour were taken. The following are the behavioural changes exhibited by the fishes in the different concentration of the pesticides.

The behavioural characteristics noted by the fishes in control are as follows.

They were very active.At the time when they were transferred to 1 litre of water, they showed an increase in opercular movements. Then later its rate reduced and then after in throughout observations, it was seen to be in a normal manner.While they were transferred to the bottle, at first they remained at the bottom without much movement.After a period of 5 minutes they started to swim actively.Sometimes during the observations, they visited at the surface, after a while they returned to the bottom. The expansion and contraction of buccal cavity was almost in a similar manner throughout the experiment. Sometimes they showed a backward movement. At first, they showed reluctance to food. But within a few minute they gradually started to feed.During the last hours of observation, i.e, on the next day, when they were fed, they quickly swallowed the food. At first, for a few minutes they were swimming independently. But later on they moved as a group i.e., showed schooling behaviourThey spent more time at the bottom of the bottle. They didn't show any type of aggressiveness. The body colour and texture was normal quite throughout the observation periods.The gill colour was same through

Table 1 MALATHION

0.1ppm	0.2ppm	0.3ppm	0.4ppm	0.5ppm
Active	Activ	Was active at beginning then reduced	Less active	Less active
No restlessness was shown	Sometimes showed restlessness	Showed some type of restlessness towards the end of study	On exposure showed restlessness	Showed high range of restlessness
Opercular movements increased at first. Later came to normal	Variation in opercular movements was noted	Increased opercular movements	Increased opercular rate	Opercular movements was very high
Preferred to stay at bottom	Mostly stayed at bottom. Stayed at the surface for few more minutes than control	Both up and down movements were shown	Stayed more time at the surface. Also stayed at the bottom	Just after a few minutes of exposure preferred to stay at surface.
Schooling behaviour was shown	Schooling behaviour was exhibited	Schooling behaviour was shown for a few seconds	Preferred to stay independently. Aggressive was shown	Moved independently. Aggressiveness increased
No change in body colour	No change in body colour	Slight paleness in body colour	Became more pale in body colouration	Body colour changed to reddish
Swallowed the feed quickly	Showed reluctance to feed. Later took it	Took the feed suddenly	At first took the feed then spitted	Refused the feed
Normal mucus was secreted	No change in scales	Mucus secretion on body and scales started to shed	Shedding of scales in the beginning itself	Shedding of scales and mucus secretion increased

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Table 2 CYPERMETHRIN

0.1ppm	0.2ppm	0.3ppm	0.4ppm	0.5ppm
Active throughout the observation	Active	Active	Was active during early stages of exposure	Soon became inactive after exposure
No restlessness was shown	No restless movements	Sometimes dashed on the walls of the bottle	Some sort of restlessness to the end of observation	Was restless and dashed on the walls. Soon lost balance
At first stayed at the surface. Later preferred to stay at bottom	Often visited the surface	Spent more time on the surface	Preferred to stay on the surface	From the time of exposure spent on the surface
Operular movements was not so high	Widening of buccal cavity was more while staying at the surface	Sometimes showed increased opercular movements	Towards the end, increased opercular movements and widening of the buccal cavity while at surface	Opercular movement was high. Widening of buccal cavity was more
No schooling behaviour at first. Later was exhibited sometimes	Mostly preferred to stay independently	Schooling behaviour was exhibited	Rarely exhibited schooling behaviour	Schooling behaviour was not at all exhibited
No changes in the body colour	Slight paleness appeared	Body became more pale	Paleness in body colour was shown	Paleness appeared rapidly
Shedding of scales was seen	Shed scales	Shedding of scales was noted	More scales were shed	Majority of scales were shed
Swallowed the feed only after sometimes	Took the feed quickly and then spited out	After a long time swallowed the feed	Took the feed quickly	Showed reluctance to feed
All survived even after 24 hours	All survived	All survived	After 12 hours the number came to half. Obtained the LC_{50} value	All fishes died within 5 hours

It may be suggested that Organo-phosphorous insecticides are inhibitors of choline esterases. The acetyl choline hydrolysis to choline and acetate by acetyl choline esterases. As esterase gets inhibited this process does not occur. This leads to the production of a co-active substance buy CNS.this reduces oxygen and finally leads to death.

After 48 hours of observation, 90 days of toxicity studies were done for protein estimation in the brain . Then the results were compared with that of the control. Total protein in brain tissues were presented in tables 3 & 4 The protein content in brain, shows the decreasing trends. In the brain the decrease in protein levels were 4.5%, 15.5%, 21.5% at 21st day, 60th day and 90th day respectively ($P \leq 0.01$).while treated with Malathion .While treated with cypermethrin decreasing trend was as follows: on 21st day 7.2% on 60th day 11.6% and 90th day 24.8% ($P \leq 0.01$).

Table 3 Total protein concentration in brain (gm/gm) in control and experimental groups while treated with malathion

Period	Control	Treated	Value of 't' Statistics
0 day	0.4217 ± 0.0030	0.4101 ± 0.0045	6.91
After 21 days	0.4206 ± 0.0020	0.4041 ± 0.0048	10.86**
After 60 days	0.4110 ± 0.0102	0.3720 ± 0.0025	10.54**
After 90 days	0.4109 ± 0.0045	0.3516 ± 0.0036	30.84**

Table 4 Total protein concentration in brain(gm/gm) in control and experimental groups while treated with cypermethrin

Period	Control	Treated	Value of 't' Statistics
0 day	0.5906 ± 0.0113	0.5618 ± 0.015	6.776
After 21 days	0.6009 ± 0.0056	0.5123 ± 0.0072	29.14**
After 60 days	0.6003 ± 0.0119	0.4710 ± 0.0052	28.74**
After 90 days	0.6000 ± 0.0051	0.4211 ± 0.0088	53.21**

The overall behaviour of an animal is complex interaction of a number of specific behaviours. In fishes, the most common sub-lethal effects are behavioural changes. The behaviour of fishes in pesticide polluted water varies according to the test concentration (Sahai, 1990). The behavioural changes taking place in the fish to the toxicant is a result of an inbuilt mechanism to overcome the physiological stress. The hyper excitability of the fish invariably in different concentrations of pesticides may be due to the hindrance in the nervous system Aswathi et al .,1984.Aquatic organisms are particularly sensitive to environmental contamination and pollutants may significantly damage certain physiological and biochemical processes when they enter the organs of fishes (Nemcook et. al, 1987).

When fishes were exposed to different concentrations of pesticides they showed different behavioural changes which extends from mild to chronic (Sahai 1990).At the same time the behaviour of fishes in the control was also noted and was behaving in a normal manner. The fishes were more resistant to Malathion than cypermethrin. They showed more restlessness when they were exposed to different concentrations of cypermethrin. When the fish were exposed to the lethal concentration of cypermethrin, they migrated immediately to the bottom of the tank. The migration of the fish to the bottom of the tank following the addition of cypermethrin clearly indicates the avoidance behaviour of the fish, which was reported by, Murthy in trout. The migration of the fish to the bottom of the tank following the addition of cypermethrin clearly indicates the avoidance behaviour of the fish. The surfacing phenomenon of fish observed under exposure might be due to hypoxic condition of the fish as reported by Ruparelia et.al .,1995. The increased surfacing during the initial periods of exposure to cypermethrin concentrations suggests an elevated rate of metabolism. Changes in ventilation rate and surfacing frequencies are the general symptoms noticed in the fish after exposure to pesticide and these activities help the fish to avoid contact with poison and fight against stress. Chronic exposure of finfish to aroclor was found to induce surfacing phenomenon of fish as pointed out by Drummond.

The tendency to jump out of the water and frequent visit to the surface for gulping the atmospheric air was more in cypermethrin treated fishes (Sambasiva Rao,1999). Thus shows the reduction of oxygen in the water due to the chemical changes. The decrease in opercular movement and corresponding increase in frequency of surfacing of fish clearly indicates that fish adaptively shifts towards aerial respiration (by obtaining atmospheric oxygen) and the fish tries to avoid contact with the pesticide through gill and operculum wide chamber.Changes in the swimming behaviour and morphology were also noted. The schooling behaviour was observed to be disrupted in the first day itself and the fish occupied twice the area than that of the control group Schooling behaviour was disturbed while applying pesticides (Ware,2000; Davi,2003). They were spread out and appeared to be swimming independent of one another. In higher concentrations of pesticides fishes exhibited notable changes in the scales, body colouration, also (Murty, 1986). As the concentration of pesticides increased the mucous secretion around the body increased. This is to reduce the toxic effect of the pesticides. That means it is a defensive mechanism. The secretions of mucous may help in protecting the vital organs of the fish like gills and skin against heavy metals and pesticide toxicity (M P.Bandyopadhyay & A.Ajith kumar, 2002)

Engulfment of the air along with quick opercular movements could be regarded as an indication of enhanced rate of respiration and oxygen deficiency. The surfacing phenomenon of the exposed fishes might be due to hypoxic condition. More rapid repeated opening and closing of the mouth and opercular coverings was shown by the fishes exposed to cypermethrin (Gangolli ,1999). A misbalanced state in fishes was noted in fishes exposed to cypermethrin. The overall behavioural changes exhibited by the fishes exposed to cypermethrin were more while that of the fishes exposed to Malathion. The behavioural changes exhibited by the pesticide treated fishes may be due to the disorder of the central nervous system(Farmer et al,1995).

Proteins are naturally occurring polymer of high molecular weight consisting predominantly of amino acids linked by peptide bonds. Proteins account for more than 50% of the organic constituent of protoplasm. They are the major component of the dry material by weight of the living organisms and they are among the most important functional components of the living cell.In our study the observations indicate the disruption of protein metabolism in tissues such as brain exposed to sub lethal concentration of malathion and cypermethrin It would be seen that pesticide toxicity stimulate proteolysis in tissues by activating protease enzymes. Protein depletion in tissues may constitute a physiological mechanism and may play a role of compensatory mechanism under pesticidal stress to provide intermediates to kreb cycle or to enhance osmolarity of the body fluids during the pesticidal stress (Yasmeen, 1986; Rajeswari, 1986).Structural

proteins contribute to mechanical structure of organs and tissues or they may constitute the bulk of a natural structure. Contractile proteins are responsible for active movements of living organisms. Proteins are also involved in biochemical defenses (Antibodies, interferons etc) Rao and Ramaneswari, 2000). The decrease in protein content of organochlorine intoxicated ispods also indicates a physiological adaptability to compensate for pesticie stress (Riberio, *et. al*., 2001).

The present observations corroborated with many previous studies. The decline in problem may be related to impaired food intake, increased energy cost of homeostasis or detoxification during stress. The detoxification process of endosulphan may lead to disruption of active site of proteins and structural variations in the aminoacids or proteins by binding up of the complex molecules. To overcome the stress condition, animal require high energy and this energy demand may have led to the utilization of these energy yielding compounds, that is, gluconeogenesis increases to meet the high energy demands. Decrease in protein content may also be due to a mechanism of lipoprotein formation which will be used to repair damaged cells and tissue organells (Sancho, *et. al*., 1998; Rambabu and Rao, 1994; Van Brummelen and Stuijifxand, 1993; Van Brummelen, *et. al*., 1996, a; Reddy, *et. al*., 1991). According to Praveen, *et. al*., (1987) it is probable that the protein is catabolized for entry into TCA cycle to cope with energy demands augmented during pesticide stress. Various proteins are involved in blood clotting system (,Riberro, *et. al.*, 2001).

In particular many organochlorine pesticides have been known to suppress the protein concentration in tissues. The same effect was reported in various animal models and fish like *Cirrhinus mrigala* (Murthy and Devi, 1982) *Channa punctatus* (Swaroop, *et. al*., 1981) exposed to endosulphan. Short-term exposure of Oziotelphusa *senex senex* to endosulphan caused a significant reduction in total proteins of gills and various tissues of fish (Naidu, 1985). Depletion of tissue proteins in animal models exposed to various toxicants have been reported by several investigators (Ramlingam and Ramlingam, 1982; Rao and Rameswari 2000). The decrease in protein content of organochlorine intoxicated isopods also indicates a physiological adaptability to compensate for pesticide stress (Riberio, *et. al*., 2001)

4. CONCLUSION

Water pollutants such as sewage, detergents, toxic heavy metals, fertilizers, pesticides, hazardous organic substances, etc., has became a serious threat to the aquatic life. Many toxicants disrupts complex fish behaviours, such as predators avoidance, reproductive and social behaviours etc. Behaviour links physiological function and the behavioural indicators of toxicity appears ideal for assessing the effects of the pollutants on fish population. The most commonly observed links with behavioural disruption include cholinesterases (ChE) inhibition, altered brain neurotransmitter levels, sensory deprivation, impaired gonadal or thyroid hormone levels. Organophoshorous insecticides are cholinesterase inhibitors they affect the physiology which can be noted from the behaviour.

Misbalanced states in fishes were noted in fishes exposed to cypermethrin. Soon the fishes came to a lateral position and were not able to swim as those fishes in control. But the fishes treated in Malathion showed jerky and erratic movements after sometimes of the exposure. Irregular, erratic and darting movements followed this with imbalanced swimming activity. The fish exhibited peculiar behaviour of trying to leap out from the pesticide medium, as an escaping phenomenon. The frequency of surfacing phenomenon was greater on the second half of the exposure wherein the fish frequently come to the water surface. The behavioural changes exhibited by the pesticide treated fishes may be due to the disorder of the central nervous system. It is clear from the behavioural changes that had been noted in them resulting in their death. Death may be due to the internal metabolic problems which would have been caused by the toxicant in the pesticides. As in that of the behavioural studies and the estimation of brain protein reveals that cypermethrin is more toxic to fishes than Malathion. Even though there is not much difference in the brain protein content of the fishes treated in two pesticides the effect of these pesticides was understood from the behavioural changes shown by them. Certain essential behaviours that are essential for the fitness and survival in natural ecosystem get eliminated as a result of toxicant exposure. The disruptions of sensory, hormonal, neurological and metabolic system have a much great implications for many fish behaviours. A multidisciplinary study added important insight into the mechanisms of behavioural alterations. However, little toxicological research has sought to integrate the behavioural effects of toxicants with physiological processes.

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