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Assessing Morphological and Behavioral Alterations in Fish Following Malathion Exposure

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Abstract:

Behavioral changes are key indicators in ecotoxicology, revealing the impact of toxic environments on aquatic organisms. This study investigated the effects of malathion, a common pesticide, on the behavior of Heteropneustes fossilis. Fish exposed to malathion showed significant behavioral changes within 30 minutes, including loss of equilibrium, erratic swimming, and increased speed. Morphological changes, such as skin discoloration, chemical deposition, and lesions, were also noted. Behavioral symptoms progressed with higher concentrations and longer exposure, leading to increased activity, air gulping due to hypoxia, and reduced resting periods. Severe symptoms, including lethargy, unresponsiveness, and extensive skin peeling, emerged over prolonged exposure. Acute toxicity testing revealed immediate surfacing behavior followed by lethargy and signs of asphyxiation. The study concludes that malathion exposure disrupts normal behavior, likely due to stress and biochemical changes, highlighting the pesticide's ecological impact.

Keywords: *Morphology, Behaviour, Fish, Melathion, Toxicology*

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* Introduction:

Fishes are very sensitive to the changes in their aquatic environment. For this reason, they are known as the bio used amidst various group of pesticide in intensive ag various pest and diseases owing to their high insecticidal property, low mammalian toxicity, low persistence and rapid biodegradability in the ecosystem. Pesticide exposure may also fatal to many non-target or occasionally leading to the death of the fish. organophosphate insecticides is being extensively used as dust, emulsion, and vapor variety of insect pests under different conditions. pesticides, may induce many significant changes in fish. Present study is aimed to review the toxicological effects on haematological parameter behavioral changes, neurotoxic, histopathological alterations, respiratory responses, bioaccumulation and chromosomal changes in fishes exposed to the organophosphate pesticide Malathion.

Malathion is an earliest, non-systemic, wide-spectrum organophosphate insecticide. Malathion may also be found in formulations with many other pesticides. However, Malathion is found to be highly toxic to various non-targeted aquatic organisms including fish. Contributing factor to the sensitivity of fish to malathion exposure seems to be its high rate of gill absorption due to the

lipophilicity. The main mode of its action is neurotoxicity, and its capacity to induce oxidative stress or alteration of antioxidant system and lipid peroxidation. Thus, the main aim of this study is to review the toxic effect of non-synthetic, malathion in fish. (Fauci, Anthony, S. *et.al*., 2008) stated that Chronic toxicity is the ability of a substance or mixture of substances to cause harmful effects over an extended period, usually upon repeated or continuous exposure, sometimes lasting for the entire life of the exposed organism.

In recent years, the high rate of increase in human population and rapid pace of industrialization have created problem of disposal of waste waters. The domestic wastes and untreated or partially treated industrial effluents, supplemented with pollutants like heavy metals, pesticides and many organic compounds, have greatly contributed to massive fish death of aquatic ecosystems. These toxic chemicals and metals have changed the quality of water that affects the fish and other aquatic organisms.

Material and Methods:

COLLECTION OF FISH SPECIMENS

Fishes were collected from river Gomti and water reservoirs in and around Lucknow, U.P. (India) with the help of local fisherman, brought to laboratory (N-26º51’59’’, E- 80º56’17’’) and acclimatized to laboratory conditions for 15days before the experiments. Stock solution of Malathion was prepared by dissolving weighed amount of salt in double distilled water.

LABORATORY WORK

For toxicity test six aquaria of 50-liter capacity were taken having 30 liters of dechlorinated tap water (Physico-chemical properties, pH = 7.6±0.2; Temp. = 26±20C; Alkalinity = 65±4.5 mg/L; Total Hardness =265±2.5 mg/L; D. O. = 7.0±0.2 mg/L).

Series of five concentrations of Malathion *viz*. 25, 50 and 75 mg/l (Toxic range was predetermined by exploratory tests) was prepared by adding calculated amount of stock solution.

One aquarium having diluent water without Malathion served as control. Adult 10 fishes of similar size and weight (average length 15± 1.5cm and weight 26.5± 2.0gm) were introduced to each test as well as control aquaria from stocking tank. Feeding was suspended 24 hours before start and throughout the experiment to avoid dissolved Malathion losses due to particulate adsorption. Proper aeration was maintained in test as well as control aquaria by air pumps and stone diffusers throughout the experiments.

PARAMETERS STUDIED

BEHAVIOUR

Experiment was carried out according to guidelines of APHA *et al*. (1998) and replicated thrice. The LC50 values of various intervals were calculated according to Trimmed Spearman Karber's Method (Hamilton *et al*., 1977) on PC. Behavioural characteristics were also recorded with respect to activity, movement, mucous secretion, skin colouration and opercular beats. The data obtained for opercular beats were statistically analyzed for student t-test and ANOVA using MINITAB software on PC.

Result and Discussion:

Behavior is a crucial indicator in ecotoxicology, revealing the impacts of toxic environments on aquatic organisms. Fish exposed to malathion, a common pesticide, exhibited significant behavioral changes. These changes, observable within 30 minutes of exposure, included loss of equilibrium, erratic swimming, and increased speed compared to the control group. During this study, we documented the specific site of malathion compounds action on *H. fossilis*. Among morphological changes, discoloration of skin, chemical deposition on skin and aquarium, lesions were recorded and effects were concentration dependent

Immediate Behavioral Responses

Upon exposure to malathion, fish demonstrated a loss of equilibrium and exhibited irregular, erratic, and darting swimming movements. Additionally, their opercular (gill cover) movement increased from 46.14±3.99/min to 54.84±2.23/min under hypoxic conditions.

Resting Period and Activity Levels

The normal resting period between swimming actions in the control group was 252.02±2.88 seconds. However, this period decreased with higher concentrations of malathion, indicating heightened activity levels and reduced rest in exposed fish.

Air Gulping and Hypoxia

Fish exposed to malathion showed a significant increase in air gulping due to hypoxia. The control group exhibited an average of 1.15±0.36 air gulps per 15 minutes. When exposed to 25 mg/L of malathion, the fish demonstrated an average of 2.52±0.45 air gulps per 15 minutes. When it is exposed with concentration of 50 mg/L, the fish showed an average of 3.61±0.50 air gulps per 15 minutes. The highest concentration, 75 mg/L, resulted in an average of 5.76±0.52 air gulps per 15 minutes.

Progression of Behavioral Symptoms

Fish exposed to malathion exhibited progressively severe symptoms based on concentration and exposure duration. At a concentration of 25 mg/L, behavioral changes and clinical symptoms

appeared after 5 hours. When the concentration was increased to 50 mg/L, symptoms appeared after just 2 hours.

Over 15 to 30 days, fish exhibited increased surfacing and air gulping, restlessness, erratic swimming, and loose schooling. They also showed lethargy, reduced activity, and unresponsiveness, along with increased mucus secretion and opercular beat rate. Other symptoms included hemorrhage, color fading, skin peeling, and rashes. After 60 days, these symptoms worsened, and additional observations included fast swimming, jerky movements, and loss of balance. There was extensive skin peeling, increased hemorrhages, and skin rashes, particularly in the opercular and caudal regions.

Based on concentration, at 25 mg/L Malathion, fish were calm, remained near the bottom, and exhibited minimal symptoms. At 50 mg/L and 75 mg/L Malathion, fish were more active, swam near the surface, and exhibited vertical hanging behaviors.

Table 1: Effect of Malathion doses on different behavioral parameters of Heteropneustes *fossilis*

Duration of exposure	**Loss of balance**		**Surfacing**		**Circling**		**Mucous secretion**		**Hyperactivity**		**Schooling**		**Tail wagging**	
	C	**E**	**C**	**E**	**C**	**E**	**C**	**E**	**C**	**E**	**C**	**E**	**C**	**E**
15 days	-	++	+	+++	-	+	-	++	-	+++	+++	+	+	++
30 days	-	++	+	++	-	++	-	+++	-	+++	+++	+	+	++
60 days	-	+++	+	++	-	++	-	+++	-	+++	+++	+	+	+++

Table-2: Effects of Malathion exposure on general behavioral pattern of fresh water fish *Heteropneustes fossilis*

PARAMETERS	**CONTROL**	**25 mg/L**	**50mg/L**	**75 mg/L**
Resting period (second)	251.02±2.88	224.93±2.48	198.15±4.04	184.84±2.15
Opercular movement (1min)	43.51±3.03	46.14±3.99	51.9±2.17	54.84±2.23
Air gulp (15 mins)	1.14±0.36	2.50±0.45	3.59±0.50	5.73±0.52
S-jerk (15 mins)	20.53±1.37	23.05±1.O9	31.56±1.77	35.07±2.04

C-Control, E-Exposed (Nil); + (Less); ++ (Moderate); +++ (Prominent)

During acute toxicity testing, fish displayed immediate surfacing movements for the first 1-20 minutes, followed by gradual lethargy and settling at the bottom. Signs of asphyxiation were evident, including occasional jumps to gulp air. Fish also showed sensitivity to external stimuli,

causing rapid, jerky movements.

The behavioral changes are the manifestation of motivational, biochemical, physiological and environmentally influenced state of the organism. Warner *et al*. (1966) commented that "The behavioral activity of an organism represents the final integrated results of a diversified biochemical and physiological processes". Thus, a single behavioral parameter is generally more comprehensive than a physiological or biochemical parameter. Fishes exposed to sublethal concentrations of pesticides showed increase in swimming activity as compared to control. This resulted in decrease in resting period. This increase in swimming activity may be due to disruption of schooling behavior which occurs because of the stress of the toxicant (Venkata *et al*., 2008). Similar alteration was also observed by Yaji *et al.* (2011) in *Orechromis niloticus* treated with cypermethrin.

The fishes of the control were calm and preferred to confine themselves to the bottom of the aquarium whereas pesticide treated fishes were found active and mostly swimming near the upper surface of water and also found hanging vertically most of the time in water. These findings can be correlated with the findings of Narwaria and Saksena (2012).

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