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Toxicity of Malathion to the Fish *Channa punctatus*, *Heteropneustes fossilis* and *Anabas testudineus*

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Abstract: Fishes behaved very much sensibly to the changes in their aquatic ecosystem. They are known as the bio-indicator to monitor the water pollution and physical structure of the water bodies. Owing to their high insecticidal property, low mammalian toxicity, low persistence and rapid biodegradability in the ecosystem, organophosphate pesticides are extensively applied amongst various group of pesticide. Malathion is one of the thoroughly used pesticides that instigate many significant changes of fish behavior and mortality. The present study was conducted to evaluate the effects of Malathion on some behavioral changes and mortality of three indigenous fish species *Channa punctatus* (Taki), *Heteropneustes fossilis* (Shing) and *Anabas testudineus* (Koi) with three replications each species along with one control group. The effects of Malathion were observed for different exposure period (24-96 hours) with the parameters of mortality, toxicity and physical behaviors. The LC_{50} was 5.67 mg/l, 5.19 mg/l and 5.22 mg/l for *C. punctatus*, *H. fossilis* and *A. testudineus* respectively in 96 hours. The mortality was high in *C. punctatus* at 72 hours for 25.0 mg/l, in *H. fossilis* at 48 hours for 15.0 mg/l and in *A. testudineus* at 72 hours for 15 mg/l dose. The remarkable behavioral changes were recorded all three species in 24 hours of observation. All treated fishes started lying on the bottom in lower doses and produce mucus comparatively medium to high (5.0-30.0 mg/l) doses. Opening of mouth and gills were observed from 1.0 mg/l dose to the high doses in all experimented fishes. 15% of fish showed gulping before death behavior in all three experimented species for 2.5 mg/l dose. The results of present study revealed that Malathion significantly affected fish mortality and can be applied as bio-indicator for assessing pesticide toxicity to fish.

Key words: Freshwater Fish · LC_{50}, Physical Behavior · Mortality · Insecticides

INTRODUCTION

Human population is being increasing rapidly in the recent green revolution era. Simultaneously forests have been used for adopting construction and agricultural purposes that deteriorating environmental balance [1]. The emerging and increasing pollutants problem aggravating these situations much faster [2-4]. The major pollutants are domestic wastes, untreated or semitreated industries effluents and different chemicals such as pesticides used in agro-farms or safety measures [5-9]. These pollutants especially harmful chemicals are dumped or released into water bodies [10] where they affects the aquatic fauna in two ways. These substances change the water quality that is the habitat of many aquatic organisms [11]. Higher concentration than permissible limits of the chemicals claim heavy mortalities of all fauna residing in the aquatic systems. While in lower concentration of the chemicals lead to bioaccumulation and ultimately enter into food chain to human beings that cause long-term effects on human health [12-14].

Pesticides find in the aquatic ecosystem are mainly agricultural origin. Although pesticides has some effective results for its immediate action of pest control,

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the residual release contaminate the adjacent water bodies to the agro-farm. Such pesticides have been pointing as the catalyst of gradual deterioration of aquatic ecosystem [15, 16]. High degrees of pesticides concentration reduce the survival, growth and reproduction of fish [17] and cause some morphological effects as well as behavioral changes of fish [18]. The residue of pesticides also susceptible to soil microorganisms in some extent as it transported into surface and ground water from the agricultural runoff. Ultimately, the surface and ground water are highly contaminated by these agricultural dumped pesticides [19, 20]. The long-term effect of pesticides as the residue concentration (Higher or lower) found in animals in the enclosed ponds, free flowing creeks, larger water bodies like lake and estuaries [21]. It is also found in every parts of the ecosystem- soil, water, crops, fishes, even mother's milk and snow samples of the Antarctic [22].

Bangladesh gradually loses its indigenous fish species. Small indigenous fish such as *Glossogobius giuris*, *A. testudineus*, *H. fossilis*, *Clarias batrachus*, *C. punctatus* etc. are normally breeds in farm lands and small water bodies. As they are polluted the normal physiological functions, feeding and breeding behavior are interrupted by pesticides effects and in some cases, fishes become sterile [23]. Moreover, most of the pesticides are found highly toxic to other aquatic invertebrates (Phytoplankton, zooplankton, aquatic organisms) and vertebrates [24]. The adverse effect of pesticides are also observed in many aquatic invertebrates, especially the crustaceans, cladoceras, copepods and insects larvae that are the important food source of many fish species as they are more susceptible of pesticides than fishes [25]. Comprehensive application of pesticides in recent times in agro-farm and public health operation causes a high degree of pollution in aquatic ecosystem. Moreover, insect control techniques adopted with insecticides are often non-selective and most cases lead to heavy mortality of fishes and game birds [26]. Through the food web these pesticides may accumulated in human bodies and causing various diseases like heart attack, paralysis, kidney failure, brain damage, cancer and so on [27].

Three major types of pesticides (Chlorinated hydrocarbons, organophosphorus and carbamates) are used in present time for pest control. Among them, organophosphate (Malathion) has become on one of the widely applied insecticide. Their low solubility and rapid degradable characters makes it cherished exposure of aquatic organisms and thus it is essential to monitor it's impact on aquatic ecosystem [28]. Reports have been revealed that Malathion, even in low concentration harms fish in several ways *i.e.* alteration of growth parameters, haematological properties, swimming ability and the depletion of some biochemical parameters (Glycogen, cholesterol and total protein) [29]. Often in every case, the residue of pesticides affect some vital organs *viz.*, kidney, liver, gills, stomach, brain, muscles and reproductive organs of fishes. Especially, the functions of gill are significantly affected by the pollutants [30]. In aquatic toxicology, fishes act a significant role in toxicity testing and hazard evaluation (Indicator of pollution in water-quality management) so as rat and guinea pig used for mammalian toxicology [31, 32]. Since insecticidal toxicity is harmful in physiological activities of the aquatic organisms like fish, it is essential to investigate the effects on behavior and mortality. Therefore, to understand the toxic effect on mortality and behavior on Malathion (Hilthion 57EC) of three indigenous freshwater fish (*C. punctatus*, *H. fossilis* and *A. testudineus*) in the wetlands of Dhamrai area, Bangladesh the present study were undertaken.

MATERIALS AND METHODS

Experimental Species: Three indigenous fish species- *C. punctatus* (12-16 cm length and 24-30 gm body weight), *H. fossilis* (13-18 cm length and 25-32gm body weight) and *A. testudineus* (4.3-7.1cm length and 10.6-28.3 gm body weight) were used in the experiment. Healthy fishes were collected from the local wetlands (Beel) in Dhamrai area (Savar district), Bangladesh. Specimens (Fishes) were transported in plastic container and reared in 5 clay pots (Chari) of 18×5×10 inches in size with the capacity of 20 liters of water each. Fishes were washed with 0.5% $KMnO_4$ solution for five minutes to remove the external infections and acclimatized in a large acclimation tank with a capacity of 100 liters of tap water for a week before setting the experiments. The pH 6.5±0.02, dissolved oxygen 7.3±0.22 mg/l and temperature 26±0.17°C were maintained in the Chari with the photoperiod of 12D:12L. Fishes were fed earth worm in the adaptation period. Water of the Chari was renewed daily to produce constant effect of Malathion.

Malathion (Hilthion 57 EC) Dose Preparation: The compound was collected from the local authorized dealer of Savar. The 200 ml stock solution was prepared according to EC% active ingredient (mg/L) with the formula of (200×57)/1000 = A (A= amount of 57EC

Malathion), 200-A=DW (DW= distil water). Finally 200 ml stock solution of Malathion = (A+DW) ml. Applied dose for 20L tap water were prepare with the formula of $S_1V_1=S_2V_2$ (S_1 = 200gm/l, S_2= 1mg/l, V_1 = dose, V_2 = 20L). Desired concentrations of pesticide were poured carefully into 20 L tap water in the test aquaria (Chari) by micropipette and stirred gently with a glass rod to ensure complete mixing.

LC_{50} Determination: Acute toxicity study was conducted following the standard method of Finney [33]. The lethal concentrations (LC_{50}) were selected based on the results from a series of experimental trials (Insecticide doses 0.5, 1.0, 2.0, 2.5, 5.0, 10.0, 15.0, 20.0, 25.0 and 30.0mg/l). Each concentration was tested in two times with three replications upto four days period. Twenty fishes in each replication were maintained in each test solution along with one control group. After preliminary observation, the LC_{50} value was determined with the exposed dose of 2.5, 5.0, 10.0, 15.0, 20.0 and 25.0 mg/l. The mortality (%), behavioral changes of fishes (%) were assessed during the interval of 24 hours period following the standard methods of APHA [34].

Data Collection and Recorded: The dead fishes were removed immediately from the Chari and scored each period (24 hours) of interval. Fish considered dead as there was no visible movement and after touching the caudal peduncle has no reaction. Both control and insecticide treated fishes were observed sincerely as some fishes behave abnormally in different doses.

Statistical Analysis: Recorded data were analyzed with different statistical software. χ^2 values were determined through probit analysis by using the computer package Poloplus, version 0.03. The doses were converted to logarithms and the significance level was set up at ($p<0.05$). LC_{50} and LC_{90} values were calculated by the same software. The cumulative mortality (%) and graphical presentation were done by using the Microsoft office Excel 2007 software.

RESULTS

Mortality: Ten different concentration doses (0.5, 1.0, 2.0, 2.5, 5.0, 10.0, 15.0, 20.0 25.0 and 30.0 mg/l) of Malathion were exposed to three different indigenous fishes (*C. punctatus, H. fossilis* and *A. testudineus*) for a period of 96 hours with four observations (24H, 48H, 72H and 96H). The results of different observation revealed different mortality rate ranged from 0 to 100 % in various doses of concentration, suggesting that mortality rate fully depends on insecticide dose and time. The cumulative mortality of three fishes presented in Figure 1. Result shows that mortality rate of *C. punctatus* had gone higher (40% in 24H to 98% in 96H at the dose of 30 mg/l) with the risen of exposed duration and doses of Malathion. Lower doses upto 2.0 mg/l had no remarkable effect on mortality. Mortality rate was high form 5.0 mg/l to 30.0 mg/l doses as the exposure time gone up and it fluctuated in the moderate doses from (5.0 mg/l to 20.0 mg/l) from 15 to 35, 25 to 50, 35 to 70 and 43 to 87 in application time of 24H, 48H, 72H and 96H respectively (Fig. 1A). *H. fossilis* was more sensitive in Malathion than other two species of fish as 65% fishes were died in 30.0 mg/l dose within 24H. This percentage scored 100% in 96H. Alike *C. punctatus* lower doses upto 2.5 mg/l claimed lower mortality rate. This rate became higher from 5.0 to 30.0 mg/l doses where maximum number of *H. fossilis* dead in 10.0 mg/l dose at 96H (Fig. 1B). *A. testudineus* on the other hand, no effect was found in 0.5 mg/l dose and only 8% of fish was died in 1.0 mg/l dose at 24H period after that no fish was died upto 96H period. Almost similar number of fish in this species was dead between 2.5 and 5.0 mg/l dose within the four days period. Fifty percent mortality of *A. testudineus* was recorded at the higher dose (30.0 mg/l) at 24H which reached upto 97% at 96H period. Maximum number dead of fish in this species was scored in 10.0 mg/l dose at 96H time (Fig. 1C).

Sub-Lethal Concentration: The Sub-Lethal concentration of three treated fish species were tested with six different doses (2.5, 5.0, 10.0, 15.0, 20.0 and 25.0 mg/l) at four different (24H, 48H, 72H and 96H) exposure time. The LC_{50}, value, LC_{90}, value, 95% confidence limits, slope and chi-square values of Malathion for three different treated fish species were shown in Table 1. The LC_{50} values of Malathion in three different fish species at 24H and 48H shows that short exposure time demand bigger amount of insecticides as the toxicity values of 48H (12.50 mg/l for *C. punctatus*, 10.78 mg/l for *H. fossilis* and 10.73 mg/l for *A. testudineus*) were almost triple time higher than those of 24H (32.85, 26.14 and 22.05 mg/l for *C. punctatus*, *H. fossilis* and *A. testudineus* respectively) (Fig. 2). The LC_{50} values of Malathion for all three species of treated fish were gradually decreased along with time. The LC_{90} values also followed the same ratio of toxicity of Malathion at 24H and 48H (Table 1). In case of 72H and 96H, the LC_{50} values were almost same in the treated three species of fishes. The results were 7.92, 7.05 and 7.20 mg/l for *C. punctatus*, *H. fossilis* and *A. testudineus*

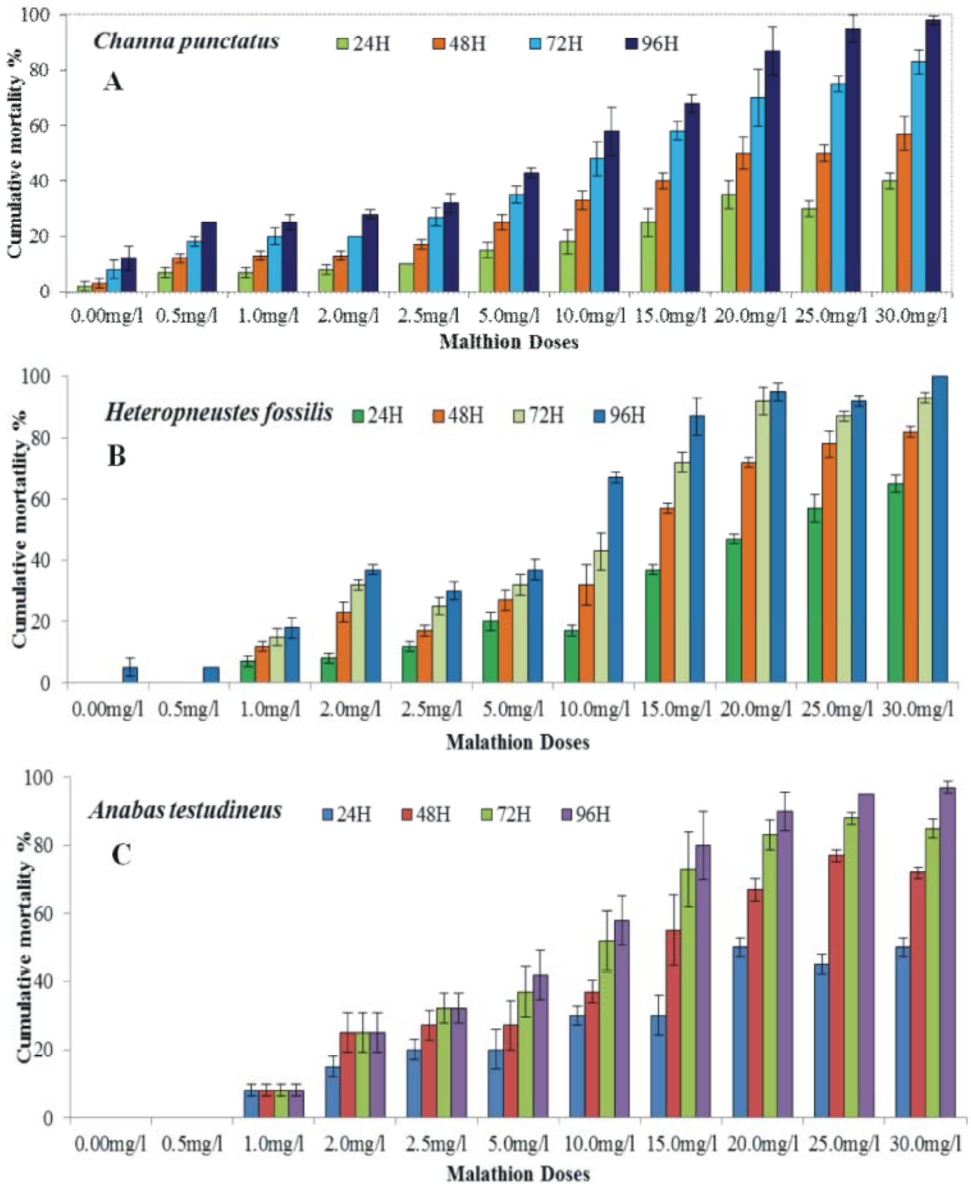

Fig. 1: Cumulative mortality (%) of three indigenous fish species under ten different treated doses of Malathion

respectively at 72H observation period and 5.67, 5.19 and 5.22 mg/l *C. punctatus*, *H. fossilis* and *A. testudineus* respectively at 96H. Similar results were found in LC_{90} values of Malathion in the mention three fish species though for *C. punctatus*, 72H observation period shows a little bit higher LC_{90} values than those of other two fish species (Table 1). It revealed a direct relationship between exposure duration and concentration of Malathion.

Behavior Study: The fishes showed typical changes in behavioral against Malathion in different treated doses observed at 24 hours with different parameters (Erratic movement, lying on the bottom, lethargic movement, downward movement, opening of mouth and gills, gulping before death and profuse mucus production). The mean (%) of behavioral changes of the three species of fish is presented in Table 2. It shows that all three species of fish experienced progressive erratic movement along with high exposed doses. Maximum 30% of *C. punctatus*, 31% of *H. fossilis* and 25% of *A. testudineus* moved erratically at 30.0 mg/l dose. The most interesting result observed in Lying on the bottom behavior where highest percentage (20% of *C. punctatus* at 1.0 mg/l, 21% of *H. fossilis* and *A. testudineus* at 0.5 and 2.0 mg/l dose respectively)

Table 1: Malathion toxicity against three species of fish in different exposure time

				CI (95%)					
				LC_{50}		LC_{90}			
Fish species	Obs. (hours)	LC_{50} mg/l	LC_{90} mg/l	Lower	Upper	Lower	Upper	Slope ±SE	χ^2
Channa punctatus	24	32.85	549.73	21.83	76.27	172.25	8496.2	1.04±0.22	3.33
	48	12.50	128.26	9.78	16.62	67.60	426.70	1.26±0.20	3.26
	72	7.92	59.61	4.56	11.88	29.96	408.31	1.46±0.20	6.72
	96	5.67	31.17	3.57	7.69	20.21	120.54	1.73±0.21	8.53
Heteropneustes fossilis	24	26.14	187.50	19.95	40.63	94.37	689.01	1.49±0.24	0.95
	48	10.78	65.39	8.85	13.19	43.43	126.66	1.63±0.21	3.57
	72	7.05	36.02	3.57	10.89	19.88	209.13	1.80±0.21	11.34
	96	5.19	24.01	2.63	7.63	14.95	73.99	1.92±0.22	16.01
Anabas testudineus	24	22.05	132.86	15.08	49.62	55.88	1766.9	1.64±0.23	6.51
	48	10.73	56.65	6.58	17.87	28.40	496.35	1.77±0.21	10.68
	72	7.20	38.93	2.34	13.07	18.71	1350.6	1.74±0.21	17.74
	96	5.22	25.88	1.46	8.77	14.02	263.09	1.84±0.21	16.25

d.f. = 4 (for each observation), LC = Lethal concentration, CI = Confidence interval

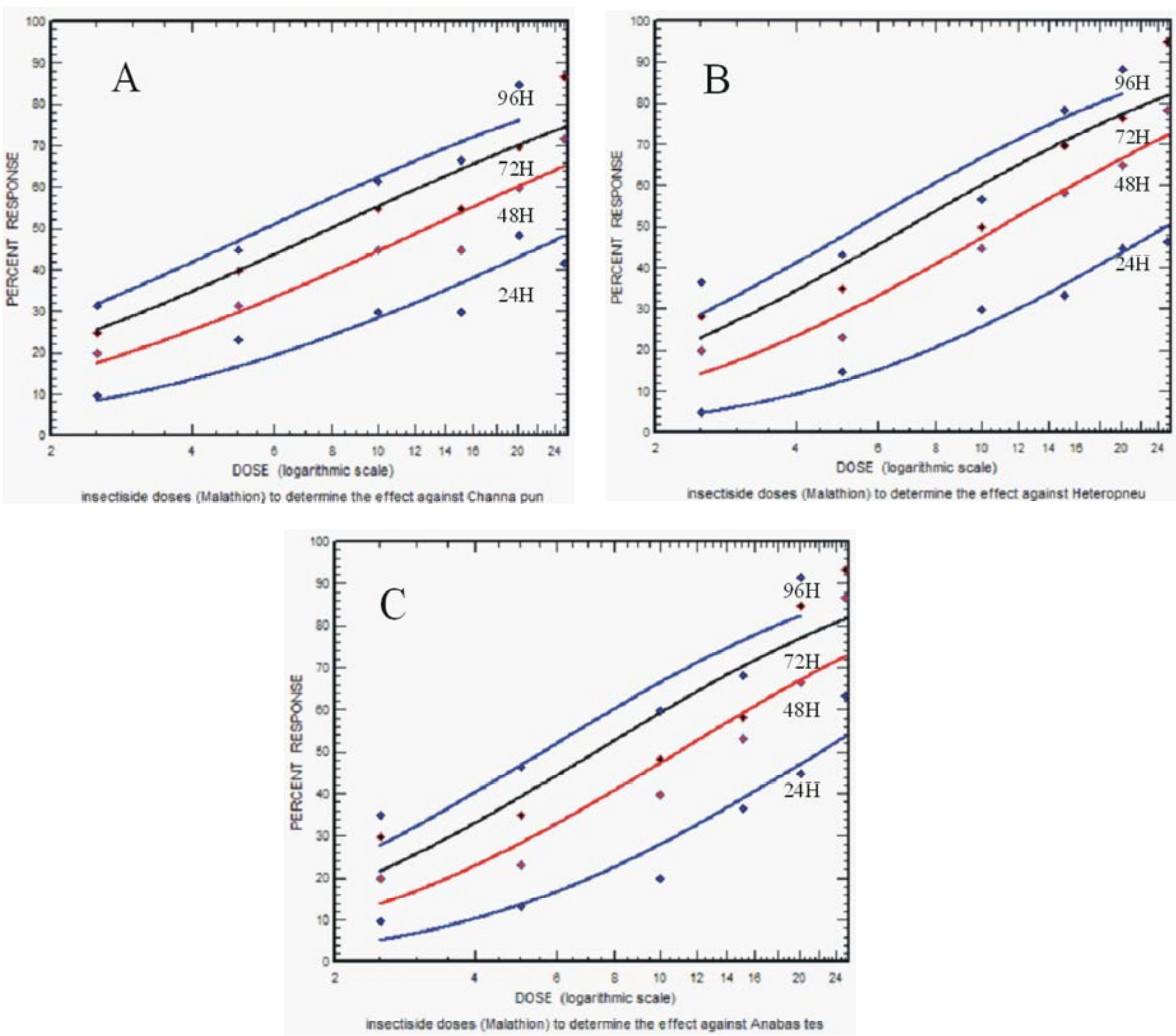

Fig. 2: LS_{50} values for Malathion at B = *Heteropneustes fossilis*, C = *Anabas testtudineus*

Table 2: Behavioral changes of three species of fishes (Mean% and Standard Error) against Malathion under different treated doses

	Channa punctatus							*Heteropneustes fossilis*							*Anabas testudineus*						
	Er.M.	Ly.B.	Le.M.	Do.M.	Op.M.	Gu.D.	Pr.M.	Er.M.	Ly.B.	Le.M.	Do.M.	Op.M.	Gu.D.	Pr.M.	Er.M.	Ly.B.	Le.M.	Do.M.	Op.M.	Gu.D.	Pr.M.
Dose	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE	M±SE
0.0	1.76	6.67	0.00	1.67	0.00	1.67	1.67	1.67	3.33	0.00	1.67	0.00	1.67	3.33	1.67	0.00	0.00	1.67	0.00	1.67	0.00
	±1.67	±3.33	±0.00	±1.67	±0.00	±1.67	±1.67	±1.67	±1.67	±0.00	±1.67	±0.00	±1.67	±1.67	±1.67	±0.00	±0.00	±1.67	±0.00	±1.67	±0.00
0.5	11.67	15.00	6.67	3.33	3.33	1.67	13.33	10.00	21.67	3.33	1.67	3.33	1.67	11.67	10.00	5.00	3.33	1.67	3.33	1.67	11.67
	±4.41	±2.89	±1.67	±1.67	±1.67	±1.67	±1.67	±2.89	±1.67	±1.67	±1.67	±1.67	±1.67	±1.67	±2.89	±0.00	±1.67	±1.67	±1.67	±1.67	±1.67
1.0	18.33	20.00	13.33	3.33	11.67	6.67	25.00	18.33	20.00	5.00	3.33	10.00	6.67	25.00	18.33	18.33	5.00	3.33	10.00	6.67	20.00
	±3.33	±2.89	±1.67	±1.67	±1.67	±1.67	±5.00	±3.33	±5.00	±2.89	±1.67	±0.00	±1.67	±2.89	±3.33	±1.67	±2.89	±1.67	±0.00	±1.67	±0.00
2.0	21.67	11.67	13.33	8.33	10.00	10.00	18.33	21.67	21.67	1.67	1.67	8.33	6.67	20.00	21.67	21.67	1.67	1.67	8.33	6.67	18.33
	±4.41	±1.6	±1.67	±1.67	±2.89	±0.00	±1.67	±1.67	±1.67	±1.67	±1.67	±1.67	±1.67	±2.89	±1.67	±1.67	±1.67	±1.67	±1.67	±3.33	±1.67
2.5	20.00	8.33	10.00	6.67	13.33	15.00	25.00	18.33	5.00	1.67	5.00	11.67	15.00	20.00	18.33	5.00	3.33	5.00	10.00	15.00	18.33
	±2.88	±1.67	±0.00	±1.67	±1.67	±0.00	±5.78	±3.33	±0.00	±1.67	±0.00	±1.67	±0.00	±2.89	±3.33	±0.00	±1.67	±0.00	±0.00	±0.00	±1.67
5.0	16.6	6.67	8.33	8.33	11.67	11.67	33.33	16.67	5.00	5.00	6.67	10.00	11.67	31.67	16.67	0.00	5.00	6.67	10.00	11.67	26.67
	±1.67	±1.67	±1.67	±1.67	±1.67	±1.67	±1.67	±1.67	±0.00	±0.00	±1.67	±0.00	±1.67	±1.67	±1.67	±0.00	±0.00	±1.67	±0.00	±1.67	±1.67
10.0	21.6	1.67	6.67	6.67	10.00	10.00	31.67	20.00	1.67	3.33	3.33	10.00	10.00	31.67	15.00	0.00	3.33	3.33	10.00	10.00	23.33
	±1.67	±1.67	±1.67	±1.67	±000	±0.00	±3.33	±0.00	±1.67	±1.67	±1.67	±0.00	±0.00	±1.67	±0.00	±0.00	±1.67	±1.67	±0.00	±0.00	±3.33
15.0	20.0	0.00	5.00	5.00	10.00	10.00	31.67	21.67	0.00	1.67	3.33	6.67	8.33	26.67	20.00	0.00	1.67	3.33	6.67	8.33	21.67
	±0.00	±0.00	±0.00	±0.00	±000	±0.00	±3.33	±1.67	±0.00	±1.67	±1.67	±3.33	±1.67	±1.67	±0.00	±0.00	±1.67	±1.67	±3.33	±1.67	±1.67
20.0	25.0	0.00	5.00	5.00	10.00	10.00	31.67	28.33	0.00	1.67	3.33	10.00	10.00	28.33	26.67	0.00	1.67	3.33	10.00	10.00	26.67
	±0.00	±0.00	±0.00	±0.00	±0.00	±0.00	±3.33	±1.67	±0.00	±1.67	±1.67	±0.00	±0.00	±1.67	±1.67	±0.00	±.167	±1.67	±0.00	±0.00	±1.67
25.0	28.33	0.00	3.33	3.33	10.00	10.00	31.67	30.00	0.00	3.33	3.33	10.00	10.00	31.67	23.33	0.00	3.33	3.33	10.00	8.33	21.67
	±4.41	±0.00	±1.67	±1.67	±0.00	±0.00	±3.33	±5.77	±0.00	±1.67	±1.67	±0.00	±0.00	±3.33	±1.67	±0.00	±1.67	±1.67	±0.00	±1.67	±3.33
30.0	30.00	0.00	6.67	5.00	10.00	10.00	33.33	31.67	0.00	1.67	5.00	10.00	10.00	28.33	25.00	0.00	1.67	5.00	8.33	10.00	26.67
	±2.88	±0.00	±1.67	±0.00	±0.00	±0.00	±4.41	±4.41	±0.00	±1.67	±0.00	±0.00	±0.00	±3.33	±0.00	±0.00	±1.67	±0.00	±1.67	±0.00	±4.41

Er. M. = erratic movement, Ly. B. = lying on the bottom, Le. M. = lethargic movement, Do. M. = downward movement, Op. M. = opening of mouth and gills, Gu. D. = gulping before death, Pr. M. = profuse mucus production

of fishes lied on the bottom at lower doses (0.5-2.0 mg/l). Lethargic and downward movement were observed at lower to medium (1.0-10.0 mg/l) concentration doses and the percentages were not cross 10% of all treated fish species (Table 2). Similar fashioned was observed in opening of mouth and gills behavior in case of *C. punctatus* and *A. testudineus*, where *H. fossilis* showed progressively higher percentage with higher doses. More interestingly, the equal percentages (15 %) of all three species of treated fishes exposed gulping before death behavior at 2.5 mg/l concentration dose however, the percentages were almost equal (10%) at rest of the higher concentration doses (from 10.0-30.0 mg/l). The percentage of profuse mucus production fishes were progressively higher (10-30 %) up to 10.0 mg/l concentration doses in all three species of experimented fishes and these percentages were constant though the high doses of Malathion were applied in the test chari.

DISCUSSION

The cumulative mortality of the three treated species of fish was gone higher associated with the observation period. The result of the present study showed that 40% of *C. punctatus* died in 30.0 mg/l dose at 24H and at the end of 96H the mortality touched at 98% compared with control group where only 12% fish died at that period. *H. fossilis* followed the same trend of mortality and *A. testudineus* showed the similar pattern of mortality in 30.0 mg/l dose with the figure of 50% mortality that followed of 72%, 85% and 97% at 48H, 72H and 96H respectively. A similar fashion of mortality was found in the experiment of Thenmozhi *et al*. [35]. They studied on *Labeo rohita* to observe the impact of Malathion on their mortality and biochemical changes.

The acute toxicity (LC_{50} and LC_{90} values) of Malathion of the present study is presented in Table 1. Salwa and Ella [36] conducted an experiment on toxicity of Malathion and its impact on the activity of acetylcholinesterase in different tissues of grass carp, *Ctenopharyngodon idella*. They reported that the acute toxicity (LC_{50} values) were 3.73 mg/l, 2.84 mg/l, 2.44 mg/l and 2.14 mg/l at 24H, 48H, 72H and 96H respectively for that species. They also reported that fishes were showed typical changes in behavior with various concentration doses including progressive lethargicity, loss of equilibrium, difficulty in respiration, exhibited convulsions, dashing against the wall of the test aquaria and bottom settling before death. It supported the pattern of the present result. Rauf [37] reported in his investigation, the acute toxicity of Malathion as an aquatic pollutant on the behavior and hematological indices in Indian carp (*Cirrhinus mrigala*) that the LC_{50} values of Malathion at 1H, 24H, 48H, 72H and 96H were 14.55 mg/l, 12.48 mg/l, 11.56 mg/l, 10.85 mg/l and 9.32 mg/l respectively following the findings of the current study.

Toxicity of Malathion against various species of fishes had been studies by many authors. Ahmad [38] reported that the LC_{50} values of Malathion for *Clarias gariepinus* was 8.22 mg/l at 96H, according to Kabeer *et al.* [39] the result was 5.8 ppm for *Tilapia mossambica* at 48H, Naserabad *et al.* [40] noticed that the sub-lethal concentration (LC_{50} values) was 4.71 mg/l for *Carassius auratus* at 96H, Martinez and Leyhe [41] found the LC_{50} was 11.8 mg/l for *Ameiurus melas* at 96H, Pandey *et al.* [42] showed 6.65 ppm for *C. punctatus* at 96H, Sharmin *et al.* [43] reported 15.24 ppm for *Cyprinus carpio* at 96H, Begum and Mithra [44] found 10.7 ppm for *H. fossilis* at 96H of Malathion toxicity that supported the results of the present study. Sambasiva Rao [45] published report on pesticide impact on fish metabolism (*Clarius batrachus* and *C. punctatus*) where the LC_{50} value of carbaryl for *C. batrachus* was 13.24 ppm and 8.5 ppm for *C. punctatus* at 48H period which is relatively lower than the present study. The LC_{50} of Malathion for *C. punctatus*, *H. fossilis* and *A. testudineus* were 5.67 mg/l, 5.19 mg/l and 5.22 mg/l respectively at 96H compared with the study of Vittozi and De Angelis [46]. Where they summarized that the LC_{50} values of Malathion was 0.09 to 22.09 ppm at 96H for different species of fish. The differences of toxicity in various fish species was due to differences in absorbed doses of pesticides, their accumulation, biotransformation and excretion. Metabolic pathways differences among species may result in different pattern of biotransformation that leading to more or less toxic metabolites [47]. The toxic effect of pesticides also depends on length and weight, corporal surface to body weight ratio as well as breathing rate of fishes [48-50].

During the toxicity tests, various fish species have exhibited several abnormal behavioral responses, such as rendering sluggishness and erratic swimming making them more susceptible to be preyed, reduced food consumption, bottom lying, convulsions, gulping and mucus profuseness after all tendency to escape from the Chari (Test aquaria) [51]. Pesticides also disrupted the schooling behavior of fish [52] due to dangling, erratic and lethargic movement and agitated swimming [53]. Pesticides make the fishes stressful an immune-compromised that drives them more susceptible and vulnerable to diseases, secondary infections and pathogens [54, 55]. The ultimate results of those hyper excitability became paralysis and death of the fish. Marigoudar *et al.* [56] studied respiratory and behavioural responses in *Labeo rohita* against Cypermehrin, where they observed darting, erratic and irregular swimming movements, equilibrium loss, hyper excitability and sinking to bottom of that species that followed the present observation. Mollah *et al.* [57] suggested that concentration (Perfekthion) doses and exposure media including exposure time induced the behavioral changes of *A. testudineus* supporting the results of the present study. Available evidences of some studies indicate that even a little amount of some toxicants (Pesticides) cause abnormal behavioral changes of fishes through impaired perceptive acuity [58]. The observed results of the present study also somewhat similar with those of different species of fishes treated with various insecticides [59, 60].

Form the result of the present study it can be concluded that toxicity of Malathion ultimately imposed hazardous effects on the indigenous fish species (*C. punctatus, H. fossilis* and *A. testudineus*) which are non-target and economically important by showing quick and lethal response to the toxicant. These adverse effects work as a catalyst for being endangered or eradicated those fish species from the experimented area.

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