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A comparative study on the effects of two organophosphorus pesticides on the excretory function of the catfish *Heteropneustes fossilis* (Bloch)

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Abstract
In the present study, the cat fish *Heteropneustes fossilis* (Bloch) was treated with the sub-lethal doses of the organophosphorus pesticide dichlorvos and malathion for 10 days, 20 days and 30 days to observe comparative changes in excretory function among the fishes. LC_{50} value of Dichlorvos and Malathion were calculated by probit analysis (Finney, 1964) and sub-lethal concentration of 2.5 ppm and 5 ppm Dichlorvos and 0.1 ppm and 0.2 ppm Malathion were prepared by using standard technique (APHA, 1985). In this study, acclimatized fishes were divided into a control group (Group 1) which is being freed from the treatment of the pesticides and Experimental group 2 and 3 were treated with sub lethal doses of the pesticides dichlorvos and malathion respectively. A significant dose and duration dependent alteration was observed in the level of serum ammonia, serum urea, serum creatinine, and GLDH activity in the liver tissue among the experimental group of fishes exposed to dichlorvos and malathion. It was observed that the changes were significantly higher among the fishes exposed to dichlorvos compared to malathion treated fishes.

Keywords: *Heteropneustes fossilis*, Dichlorvos and Malathion, Ammonia, Urea, Creatinine, GLDH

Introduction
Pesticides are inevitable component for a country whose economy completely rests on agriculture. Due to population explosion around the globe, there is an urgent need of increase in food production to meet the demand of the people. Due to different limitations in other forms of pesticide available in the market now a days in terms of production and supply, effectiveness during short exposure period, cost-effectiveness, lack of proper studies, application techniques and awareness etc., chemical pesticides are still the reliable choice among the farmers to increase the productivity of different crops. Amongst others, organophosphorus pesticides (OPs) are the most commonly used pesticides in the world due to their rapid biodegradability. Unfortunately, OPs harm beyond their target and can contribute severe, long term population effects on terrestrial and aquatic non-target species, predominantly vertebrates. Of all pesticides, the organophosphates have the highest level of toxicity in vertebrates (Ware, 1978) [1]. However, applications of pesticides are posing great danger to aquatic environment such as fish.

Dichlorvos (76%EC) and Malathion (50% EC) are organophosphate Pesticides Registered under section 9(3) of the Insecticides Act, 1968 for use in India (CIBRC, 2016) [2]. Dichlorvos is classified by the WHO as a Class IB, 'highly hazardous'. The dermal toxicity is similar to oral toxicity, and dermal exposure is a cause for concern (WHO, 2010 [3]; Das, 2013 [4]). Malathion, one of the earliest organophosphate insecticides developed in 1950, was first registered for use in the United States in 1956 by the United States Department of Agriculture (USDA), which is now regulated by the United States Environmental Protection Agency (USEPA, 2006) [5]. The international programme on chemical safety (IPCS)/WHO (WHO, 2010) [3] hazard classification of malathion is Class III (slightly hazardous). Both the pesticides are included under anti-cholinesterase pesticides since they exert their action by inhibiting AChE activity.

A variety of studies have been carried out by various scholars on different species of fishes exposed to Malathion (Rauf, 2015 [6]; Venkataraman and Sandhya Rani, 2013 [7]; Roopavathy *et al.*, 2013 [8]; Sudha, 2012 [9]; Deka and Mahanta, 2012 [10]; Ahmad, 2012 [11]; Cristina *et al.*, 2008 [13]; Remia *et al.*, 2008 [14]; Borthakur *et al.*, 2006 [15]; Howard, 1991 [15]) and dichlorvos (Deka and Mahanta, 2015 [16], Ahmad and Gautam, 2014 [17]; Günde and Yerli, 2012 [18]; Bhat *et al.*, 2012 [19]; Velmurugan *et al.*, 2009 [20]; Jones and Davis, 1994 [21]; Verma *et al.*, 1983 [22]; Nishiuchi, 1974 [23]; Anon, 1968 [24])

Present study is aimed to carry out a comparative study on the effect of organophosphate pesticides dichlorvos and malathion on excretory function of the channel cat fish *Heteropneustes fossilis* (Bloch).

Materials and Methods
Specimen
Healthy and sexually mature specimen of *Heteropneustes fossilis* of equal size group (12 ± 3 cm) and average weight (12 to 15 gm) are procured from the local market and the fishes were kept for 15 days in glass aquarium containing 80 litres of fresh water in the laboratory at about water temperature 25 ± 3º c for acclimatization. Fishes are starved for 24 hours prior to the experiment and are not fed during the period of experiment (Dalela *et al.*, 1979) [25].

Pesticide
The organophosphorus pesticide dichlorvos (76% E.C) and malathion (50%vEC) were procured from the local market for present investigation purpose.

LC_{50} Calculation
A pilot experiment was done to find out the LC_{50} value of dichlorvos and malathion by probit analysis (Finney, 1964)[26] and LC_{50} for 96 hours is found to be 19 ppm for dichlorvos and 0.98 ppm for malathion. Sub-lethal concentrations of 2.5 ppm and 5 ppm for dichlorvos and 0.1 ppm and 0.2 ppm for malathion were prepared by using standard technique (APHA, 1985) [27].

Design of the experiment: In this experiment, the specimens were kept mainly in three groups namely Control Group (Group I), Experimental Group 2 and Experimental group 3 as follows.

Table 1

Investigation group	Sub group	Sub-lethal dose
Control Group	Nil	Free from the treatment of dichlorvos and malathion
Experimental Group 2	Group 2.1	Treated with sub-lethal concentration of 2.5 ppm dichlorvos
	Group 2.2	Treated with sub-lethal concentration of 5 ppm dichlorvos
Experimental Group 3	Group 3.1	Treated with sub-lethal concentration of 0.1 ppm malathion
	Group 3.2	Treated with sub-lethal concentration of 0.2 ppm malathion

Collection of sample: Blood were collected from the fishes of both the groups and serum was separated by centrifugation technique. Tissue homogenate was prepared from the collected liver tissue and was centrifuged based on the protocol of the individual experiments.

Methods of Estimation of biochemical parameters: Ammonia in serum was estimated by the method of Anken and Schiphorst (1974) [28] at the wavelength of 340 nm in spectrophotometer. Urea in Serum was estimated through Modified Berthelot Method by Fawcett and Scott (1960) [29]. GLDH activity in liver tissue was determined spectrophotometrically by adoption of the method of Doherty (1970) [30]. Arginase activity in liver tissue was estimated by the method of March *et al.* (1965) [31] and Creatinine was assayed spectrophotometrically using modified Jaffe Method (Slot, 1965) [32].

Duration of treatment: The investigation parameters were studied in control and experimental groups on 10^{th}, 20^{th} and 30^{th} day of experimental period.

Results
Serum ammonia: Changes in the mean ± SD values of serum ammonia in the *Heteropneustes fossilis* are shown in the fig-1, significance of variation and the percentage deviations of experimental groups are presented in table 1 and the fig-2 respectively.

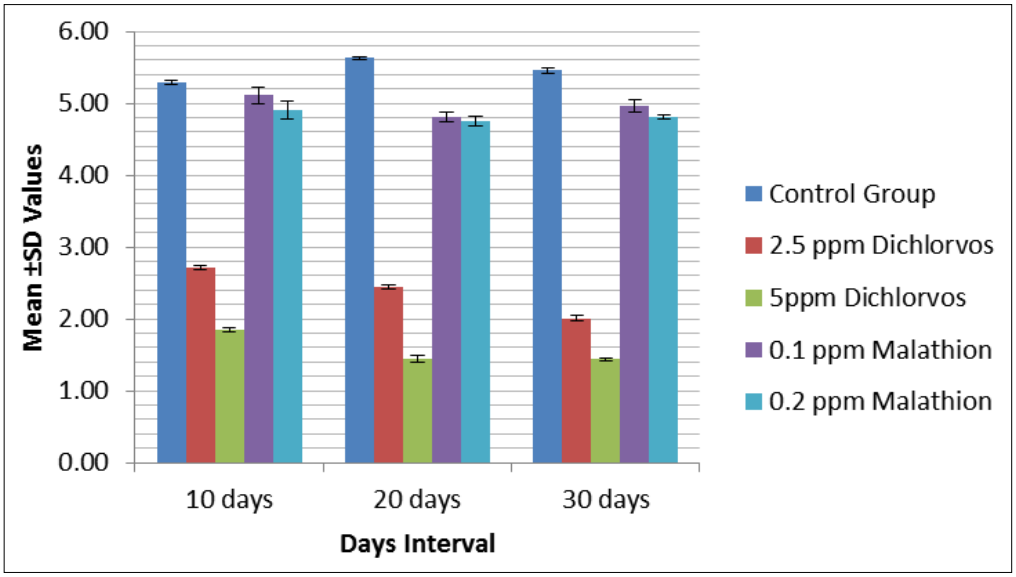

Fig 1: Presenting the mean ± SD values of serum ammonia (mg/dl) in control and experimental groups of *Heteropneustes fossilis* (Bloch) treated with sub-lethal dose of Dichlorvos and Malathion.

Serum ammonia was observed to be in decreasing trend among the fishes of both the experimental group compared to control group of fishes on 10^{th}, 20^{th} and 30^{th} day of exposure to dichlorvos and malathion. However, group 2 fishes exposed to sub-lethal dichlorvos shown significantly ($p< 0.01$) lower serum ammonia level compared to group 3 fishes treated with sub-lethal malathion for the entire study duration.

Table 1: Presenting the significance of difference in the mean values of serum ammonia (mg/dl) between the control group of fishes and experimental groups of fishes treated with sub-lethal dose of dichlorvos and malathion as well as between two different experimental groups of fish *Heteropneustes*

fossilis (Bloch) at different days' interval. Here, the significance test is based on the student's t test for difference of mean given by $t = \frac{X_1 - X_2}{\sqrt{(SEM_1)^2 + (SEM_2)^2}}$, tabulated value of t at 0.01 is 2.42.

Table 2

t between Groups of		Days Interval		
		10 days	20 days	30 days
Group I and Group 2.1	t	292.36	398.88	281.56
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group I and Group 2.2	t	385.67	340.36	396.89
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group 2.1 and Group 2.2	t	104.60	76.74	51.01
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group I and Group 3.1	t	6.51	55.22	24.20
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group I and Group 3.2	t	13.15	56.75	56.74
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group 3.1 and Group 3.2	t	5.47	3.34	7.55
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group 2.1 and Group 3.1	t	92.67	151.36	141.32
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group 2.2 and Group 3.2	t	107.53	174.87	326.15
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00

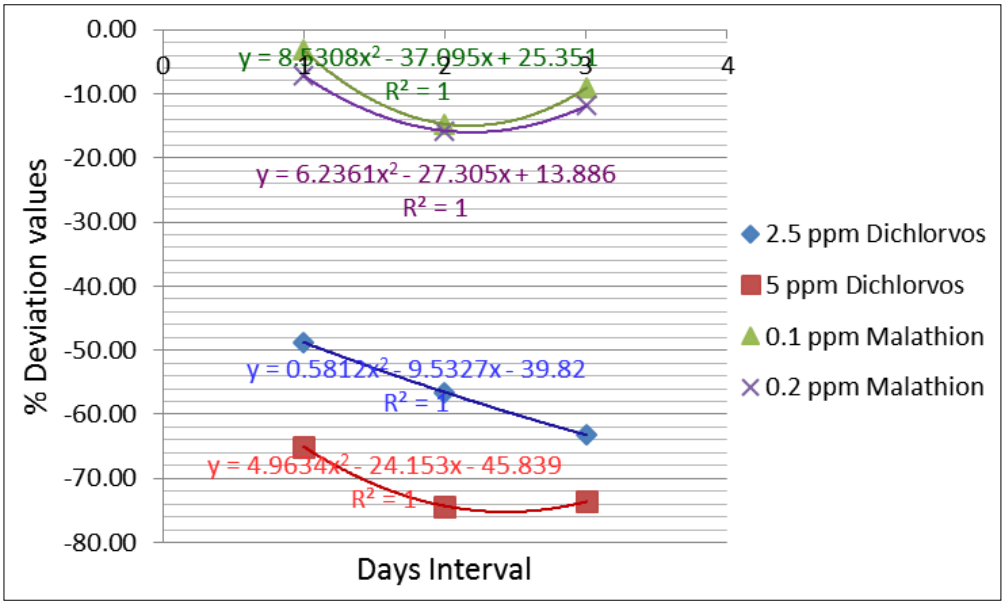

Fig 2: Presenting the % deviation of serum ammonia (mg/dl) in experimental groups of *Heteropneustes fossilis* (Bloch) treated with sub-lethal doses of dichlorvos and malathion from control group.

Serum urea

Alterations in the mean ± SD values of Serum urea in control and experimental groups of the cat fish *Heteropneustes fossilis* are depicted in the fig 3 and the significance of variation and percentage deviations of experimental groups 2 and 3 are presented in the table 2 and fig 4 respectively.

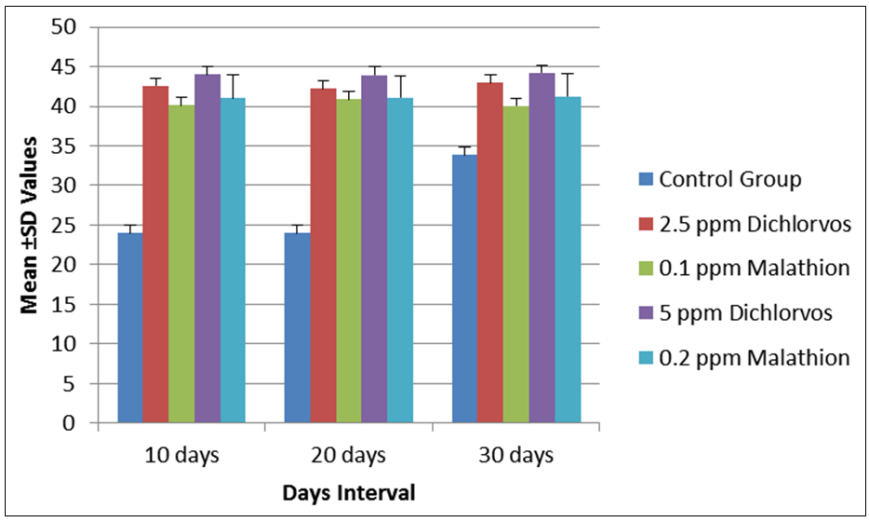

Fig 3: Presenting the mean ± SD values of Serum urea (mg/dl) in control and experimental group of *Heteropneustes fossilis* (Bloch) treated with sub-lethal dose of Dichlorvos and Malathion.

An increase in the level of serum urea was recorded among the fishes administered with sub-lethal dichlorvos and malathion throughout the experimental period of 30 days in comparison to the control group of fishes. It was observed that level of urea was comparatively higher in the Group 2 fishes exposed to sub-lethal dichlorvos than group 3 fishes treated with sub lethal malathion during 10^{th} to 30^{th} day of the study period.

Table 2: Presenting the significance of difference in the mean values of serum urea (mg/dl) between the control group of fishes and experimental groups of fishes treated with sub-lethal dose of dichlorvos and malathion as well as between two different experimental groups of fish *Heteropneustes fossilis* (Bloch) at different days' interval. Here, the significance test is based on the student's t test for difference of mean given by

$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{(SEM_1)^2 + (SEM_2)^2}}$, tabulated value of t at 0.01 is 2.42.

Table 3

t between Groups of		Days Interval of treatment		
		10 days	20 days	30 days
Group I and Group 2.1	t	29.01	28.07	12.58
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group I and Group 2.2	t	27.43	27.66	12.38
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group 2.1 and Group 2.2	t	1.60	1.87	1.43
	p	>0.01	>0.01	>0.01
	df	38.00	38.00	38.00
Group I and Group 3.1	t	22.09	24.48	8.51
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group I and Group 3.2	t	24.23	25.74	9.30
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group 3.1 and Group 3.2	t	0.94	0.25	1.47
	p	>0.01	>0.01	>0.01
	df	38.00	38.00	38.00
Group 2.1 and Group 3.1	t	2.70	1.54	3.79
	p	<0.01	>0.01	<0.01
	df	38.00	38.00	38.00
Group 2.2 and Group 3.2	t	3.15	3.09	3.14
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00

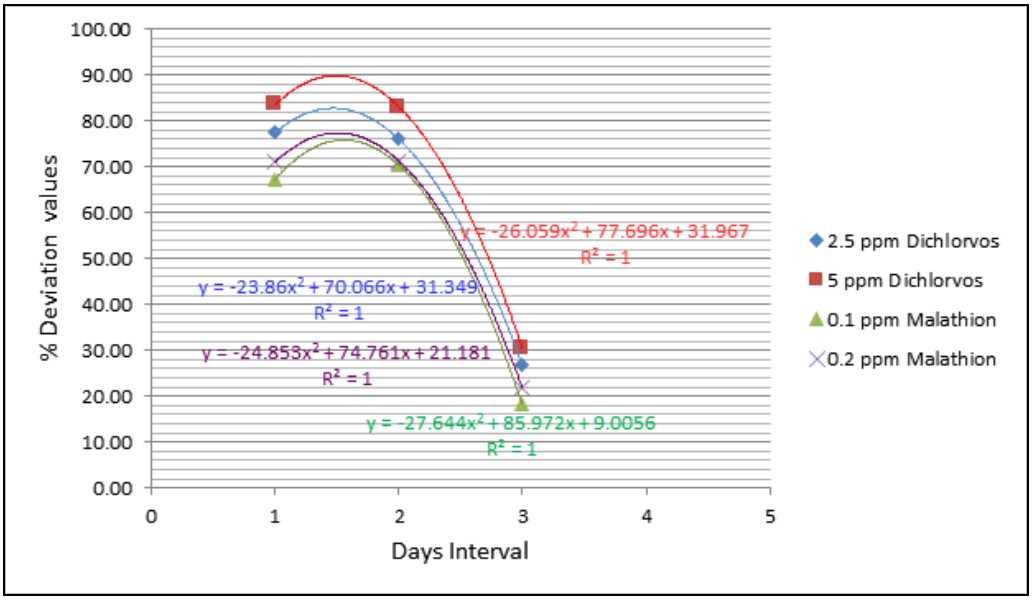

Fig 4: Presenting the % deviation values of Serum urea (mg/dl) in experimental groups of *Heteropneustes fossilis* (Bloch) treated with sub-lethal doses of dichlorvos and malathion from control group.

Glutamate Dehydrogenase (GLDH) activity

Variations in the mean ± SD values of GLDH activity in the *Heteropneustes fossilis* are demonstrated in the fig 5, significance of variation and the percentage deviations of different experimental groups are depicted in the table 3 and fig 6 respectively.

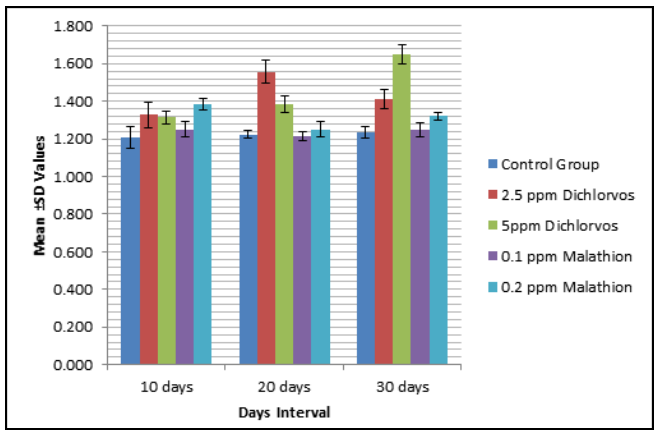

Fig 5: Presenting the mean ± SD values of GLDH (U/mg) in control and experimental group of *Heteropneustes fossilis* (Bloch) treated with sub-lethal dose of Dichlorvos and Malathion.

GLDH activity in liver tissue of the fishes showed a significant ($p< 0.01$) increase among the fishes exposed to sub-lethal dichlorvos and malathion compared to control group of fishes. A significant ($p< 0.01$) increasing trend in the mean ± SD values of GLDH activity was noticed in the fishes of experimental group 2 treated with sub-lethal concentration of 5 ppm dichlorvos from 10th day onwards up to 30th day with maximum level of GLDH activity on 30th day compared to any other investigating group.

Table 3: Presenting the significance of difference in the mean values of GLDH (U/mg) activity between the control group of fishes and experimental groups of fishes treated with sub-lethal dose of dichlorvos and malathion as well as between two different experimental groups of fish *Heteropneustes fossilis* (Bloch) at different days' interval. Here, the significance test is based on the student's t test for difference of mean given by

$t = \frac{X_1 - X_2}{\sqrt{(SEM_1)^2 + (SEM_2)^2}}$, tabulated value of t at 0.01 is 2.42.

Table 4

t between Groups of		Days Interval of treatment		
		10 days	20 days	30 days
Group I and Group 2.1	t	6.03	23.37	13.29
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group I and Group 2.2	t	6.71	14.13	31.79
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group 2.1 and Group 2.2	t	0.81	10.06	14.93
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group I and Group 3.1	t	2.66	1.30	1.11
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group I and Group 3.2	t	11.84	2.92	10.22
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group 3.1 and Group 3.2	t	11.96	3.74	7.23
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group 2.1 and Group 3.1	t	4.46	23.60	11.38
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group 2.2 and Group 3.2	t	6.64	9.83	26.73
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00

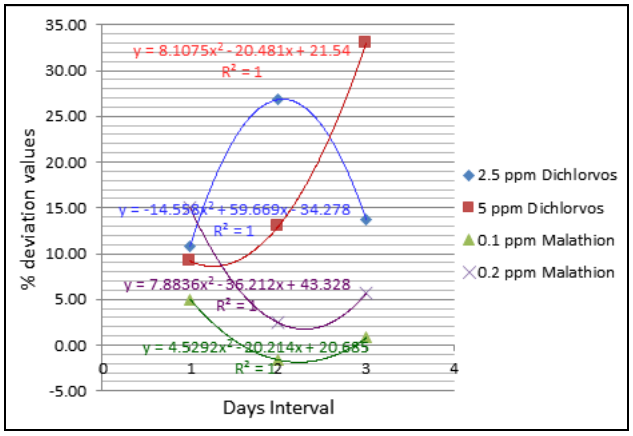

Fig 6: Presenting the % deviation values of GLDH (U/mg) in experimental groups of *Heteropneustes fossilis* (Bloch) treated with sub-lethal doses of dichlorvos and malathion from control group.

Creatinine

A shift in the mean ± SD values of creatinine in the *Heteropneustes fossilis* are shown in the fig 7, significance of variation and the percentage deviations between different experimental groups are revealed in the table 4 and fig 8 respectively.

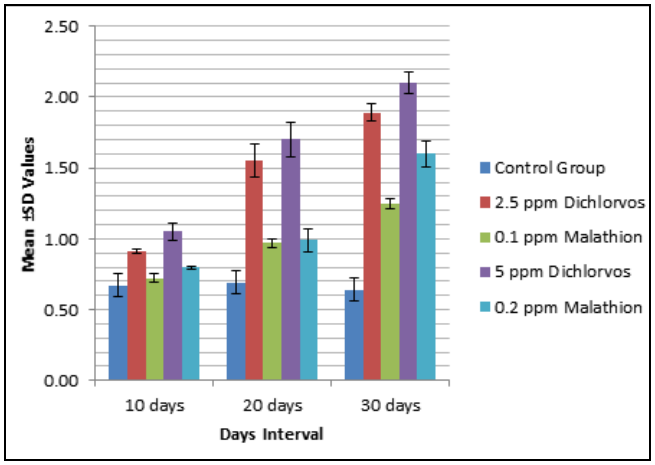

Fig 7: Presenting the mean ± SD values of serum creatinine (mg/dl) in control and experimental groups of *Heteropneustes fossilis* (Bloch) treated with sub-lethal dose of Dichlorvos and Malathion.

An increasing trend in the mean ± SD values serum creatinine was observed in the group of fishes administered with sub-lethal dose of dichlorvos and malathion from 10th day onwards and reached maximum value on 30th day compared to control group of fishes. A significant ($p< 0.01$) decrease in the level of serum creatinine was registered among the fishes of experimental group 3 compared to experimental group 2 from 10th to 20th day of experimental period.

Table 4: Presenting the significance of difference in the mean values of serum creatinine (mg/dl) between the control group of fishes and experimental groups of fishes treated with sub-lethal dose of dichlorvos and malathion as well as between two different experimental groups of fish *Heteropneustes fossilis* (Bloch) at different days' interval.

Here, the significance test is based on the student's t test for difference of mean given by

$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{(SEM_1)^2 + (SEM_2)^2}}$, tabulated value of t at 0.01 is 2.42.

Table 5

t between Groups of		Days Interval of treatment		
		10 days	20 days	30 days
Group I and Group 2.1	t	-14.22	-26.80	-53.81
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group I and Group 2.2	t	-17.95	-30.23	-57.34
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group 2.1 and Group 2.2	t	-10.04	-3.87	-9.59
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group I and Group 3.1	t	-3.02	-14.15	-29.54
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group I and Group 3.2	t	-7.78	-11.93	-34.39
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group 3.1 and Group 3.2	t	-10.46	-1.21	-15.87
	p	<0.01	>0.01	<0.01
	df	38.00	38.00	38.00
Group 2.1 and Group 3.1	t	24.14	21.64	40.25
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00
Group 2.2 and Group 3.2	t	18.58	21.79	18.71
	p	<0.01	<0.01	<0.01
	df	38.00	38.00	38.00

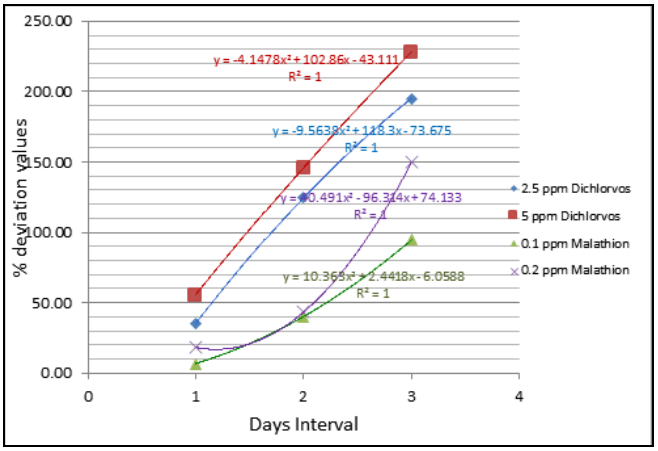

Fig 8: Presenting the % deviation of serum creatinine (mg/dl) in experimental groups of *Heteropneustes fossilis* (Bloch) treated with sub-lethal doses of dichlorvos and malathion from control group.

Discussion

The present investigation indicates that administration of dichlorvos and malathion to the cat fish *Heteropneustes fossilis* caused significant alterations in excretory functions like serum ammonia, serum urea, serum creatinine and GLDH activity. The serum ammonia was significantly ($p< 0.01$) decreased in experimental fishes exposed to dichlorvos than the malathion treated fishes. The level of serum urea, serum creatinine and activity of GLDH were also found to be higher in the experimental group of fishes treated with

dichlorvos in comparison to the malathion administered group. These results suggest that among dichlorvos and malathion treated fishes the impact of dichlorvos is greater than the malathion on the experimental group of fishes. Although comparative study between the effect of malathion and dichlorvos is still scanty, yet deleterious effect of these pesticides to fish observed in the present study are in agreement with scattered reports of investigations by previous researches which also indicated that the exposure to dichlorvos, malathion and other pesticides led to induce severe physiological and biochemical disturbances in experimental animals. Demael *et al.* (1990) [33] recorded significant effect of dichlorvos on kidney of *Cyprinus carpio* with altered kidney function. Cristina *et al.* (2008) [12] observed histological alterations in kidney with changes in the glomeruli size of *Carassius auratus gibelio* exposed to malathion. Many researchers reported significant increase in serum urea and serum creatinine in fishes exposed to different doses of pesticides (Ahmad and Gautam, 2014 [17]; Lakshmanan *et al.*, 2013 [34]; Koul *et al.*, 2007 [35]). Zaki *et al*. (2009) [36] also found an increase in serum urea and serum creatinine level in the fish *Clarias gariepinus* exposed to 4.5 mg/l malathion for 98 hours. Jabbar *et al.* (1990) [37] also observed enhanced GLDH level on the rat liver after administering the pesticide malathion for short term (24 hrs) and as well as long term (4 weeks) duration. Thoker *et al. (*2016) [38] also reported elevated blood urea and creatinine due to kidney damage caused by administering malathion to the fish *Channa punctatus* (Bloch.). The results of present investigation are in conformity with the results observed by earlier workers and support the view of dichlorvos toxicity in fresh water fishes. The present study also indicates moderately toxic nature of malathion by causing some amount of disturbances in the biochemical parameters used to assess the excretory function. It also indicates greater intensity of effect of dichlorvos compared to malathion in *Heteropneustes fossilis* (Bloch), a freshwater fish.

Conclusion

The present investigation suggests that exposure to the organophosphate pesticide dichlorvos and malathion to *Heteropneustes fossilis* brought significant alterations in excretory functions like serum ammonia, serum urea, serum creatinine and GLDH activity. Alterations in these biochemical parameters were observed to be more in dichlorvos treated fishes than the malathion treated fishes.

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