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Effects of pH and the Endocrine Disrupting Chemical Cypermethrin on Organosomatic Index of Olive Barb (*Puntius sarana sarana*) Hamilton, 1822

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Abstract: Many pesticides have an effect on animals' endocrine systems. Pesticides like cypermethrin are very much resistant to biological degradation and consequently stored in the food chain. Endocrine disrupting chemicals (EDCs) can induce physiological and reproductive problems by attaching to hormone receptors and mediating their function. Abiotic variables can impact fish health in a variety of ways. Abiotic variables such as temperature, pH, water column length, turbidity, salinity, and alkalinity, are found to have a significant impact on fish reproduction. Of all these abiotic variables, pH is thought to be one of the most significant ones affecting aquatic life. In this study we documented the adverse impact of cypermethrin and extreme acidic and basic pH separately and in combination with pesticide on gonado-somatic index (GSI), Hepato-somatic index (HSI) of *Puntius sarana*. The gonado-somatic index (GSI) is an important parameter that is represented by the ratio of the percentage of gonad weight and fish weight. GSI is the change in gonad weight to the percentage mass of the fish. Hepato-somatic indices are a helpful biomarker for detecting harmful compounds and are a target for metabolism in the fish body. GSI%, HSI% decreased with the exposure of cypermethrin 25% EC. The result showed that pH also affects the GSI% and HSI% in experimental fish. Both the extreme end of pH decreases the GSI and HSI values. Whereas pH 8.5 was found to be optimal as at this pH fishes were retaining their normal GSI and HSI values. When toxicant and pH were used in combination, pH 8.5 + toxicant group fish were found to retain their normal GSI % and HSI % as control.

Keywords: Cypermethrin, EDC, Gonado-somatic index, Hepato-somatic index (HSI), pH, Pesticides

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Introduction

Many pesticides have an effect on animals' endocrine systems. Pesticides like cypermethrin are very much resistant to biological degradation and consequently stored in the food chain. Endocrine disrupting chemicals (EDCs) can induce physiological and reproductive problems by attaching to hormone receptors and mediating their function. Pesticides found their way into water bodies after being applied, causing harm to aquatic animals particularly to fish. Because of hydrophobic nature of most pesticides they can readily be mixed into the soil and then wash off into water bodies such as rivers, lakes, and ponds, resulting in aquatic contamination. When they accumulate in aquatic organisms, they may infiltrate the food chain (Prasad *et al*., 2015).

The gonado-somatic index (GSI) is an important parameter that is represented by the ratio of the percentage of gonad weight and fish weight (Duarte et al., 2007). GSI is the change in gonad weight to the percentage mass of the fish (Ghanbahadur *et al*., 2015). Liver is an organ responsible for metabolism. Hepato-somatic indices are a helpful biomarker for detecting harmful compounds and are a target for metabolism in the fish body (Goede and Barton, 1990; Viarengo *et al*., 2000; Khan, 2010; Ribeiro *et al*., 2013). The hepato-somatic index (HSI) is a ratio of the percentage of liver weight and fish mass, that is extensively applied in fisheries as a biomarker of liver energy storage and an important parameter for determining the impact of toxicants on fish (Al-Ghais, 2013, Jan and Ahmed, 2016). Vitellogenesis (i.e., the process of vitellogenin synthesis) is the link between GSI and HSI in fish and other animals (Othman *et al*., 2015; Hismayasari *et al*., 2016). According to Narra *et al*. (2015), contact of the fish with sub-lethal dosages of pesticides cause the alteration of the growth performance, survival rate, hepato-somatic index, and immunity of the fish.

Abiotic variables can impact fish health in a variety of ways. Abiotic variables that affect fish reproduction, such as temperature, pH, water column length, turbidity, salinity, and alkalinity, are found to have a significant impact (Ernst *et al*., 2005; Tiffany *et al*., 2009). Of all these abiotic variables, pH is thought to be one of the most significant ones affecting aquatic life. Fish physiology is significantly impacted by variations in water pH. Fish die at pH 4, which is an acidic state (Ikuta *et al*., 1992). At very low pH values (less than pH 3), mucus coagulation on surface of gill and consequent oxygen deficiency may be the predominant reason of death (Robinson *et al*., 1976). In this study we documented the adverse impact of Cypermethrin and extreme acidic and basic pH separately and in combination on GSI, HSI of the fish *Puntius sarana*.

Materials and Methods

Experimental Fish:

The healthy freshwater *Puntius sarana* (100 ± 5 g; 17-19 cm) (Fig. 1) were obtained from a local fish reservoir of Sasan, near Taki Road, Barasat, West Bengal, India. To eliminate any dermal adhesion, fish were subjected to potassium permanganate solution (0.5 per cent w/v) for 1 min. The fish were acclimatised for two weeks at room temperature (32 ± 1°C) in a rectangle glass aquarium (500 L) containing de-chlorinated aerated tap water. During this time, fish were given 3% of their body weight with commercial meal pellets on a regular basis. To avoid polluting the water, as quickly as possible, dead fish were removed. The fish were fasted for 24 h before being tested for toxicity, and they were starved during the experiment. Quality parameters of water were measured prior to the experiment with water quality test kit Aquaponics (Table 1).

Toxicant and Experimental design:

Cyper 25 was used in present study (Fig. 2). Before exposure of fishes to pesticides, the water quality was examined in accordance with the APHA guidelines. For pH treatment, 10 fishes each were assigned to five different pH medium (7, 5, 6, 8.5 and 9.5) where pH 7 group considered as control. For toxicity test 10 fishes each were assigned to sublethal (1/10 of LC_{50}) dose of Cyperethrin where fishes exposed to nontoxic water was considered as control. LC_{50} dose of Cyper 25 for *Puntius sarana* was considered as 3.65 μg/l. For sub lethal test the fishes were assigned to six groups of ten specimens for each group (Table 2). pH regulation of water was done by using sodium hydroxide and hydrochloric acid.

Fig. 1: *Puntius sarana* (Experiential fish).

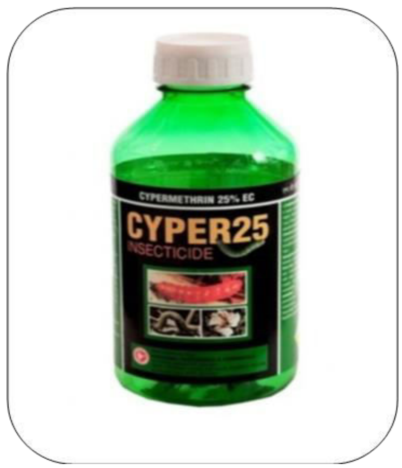


Fig. 2: Toxicant used in present investigation (Cyper 25).

Table 1: Water quality parameters

Parameters	Range
Temperature	29-31^{o}C
pH	7.3-7.6
Dissolved O_2	6.1- 6.7 mg/l (average)
Nitrate	0.18 mg/l (average)
Free CO_2	4.5 mg/l
Alkalinity	118-132 mg/l (as $CaCO_3$)
Total Hardness	108-116 mg/l (as $CaCO_3$)

Table 2: Sub lethal experimental groups

	Group	Parameters
10 fish/ group Exposure period: 15 days	1 (Control)	pH 7 with no Cypermethrin
	2	pH7 + Sublethal concentration Cypermethrin
	3	pH5 + Sublethal concentration Cypermethrin
	4	pH6 + Sublethal concentration Cypermethrin
	5	pH8.5 + Sublethal concentration Cypermethrin
	6	pH9.5 + Sublethal concentration Cypermethrin

Table 3: GSI% and HSI% of *Puntius sarana* subjected to sublethal concentration of Cypermethrin 25% EC

		Male	Female
GSI%	Control	2.14±0.05	4.32±0.12
	Toxicant (sublethal dose)	1.62±0.06*	3.16±0.04*
HIS%	Control	5.46±0.04	5.12±0.06
	Toxicant (sublethal dose)	3.84±0.05*	4.04±0.05*

Data represent Mean±SE; * indicates $p<0.05$

For sublethal tests one tenth concentration of 96 h LC_{50} value was considered. Every 24 h, the aquaria's water was replaced and the varied concentrations of cypermethrin, as well as the pH, were restored as described above. Because the pyrethroids utilised were solubilized in acetone, in the control tank, an equivalent volume of acetone was maintained.

Determination of Organosomatic Indices:

HSI% and GSI% were determined in three different conditions, such as fishes exposed to sublethal dose of cypermethrin 25% EC, fishes exposed to different pH medium (7, 5, 6, 8.5, 9.5) and fishes exposed to different pH medium with sublethal concentration of cypermethrin 25% EC. In all three cases fishes exposed to neutral (pH 7) water without toxicant acted as control. After 15 days of exposure at different experimental condition as stated above the weight of the fish from control and treated fish was determined for somatic indices analysis. A wild dose of Benzocaine was used to anaesthetize the fish as directed by Larid and Oswald (1975), sedated, and dissected. After dissection, the liver and gonads were taken out and moisture was dried using a blotting paper. The weights of the liver and the gonads were measured using an electronic weighing balance (WENSER) and recorded in grams. The hepato-somatic index was computed as a percentage of the fish's total weight divided by the liver weight (Ariweriokuma et al., 2011), whereas the gonado-somatic index was calculated as the percentage of gonad mass to total mass of the fish (Abdel-Latiff *et al*., 2020).

The organosomatic indices of fish were calculated as follows:

Hepato-somatic index (HSI%) = Weight of liver/ Weight of the fish x 100

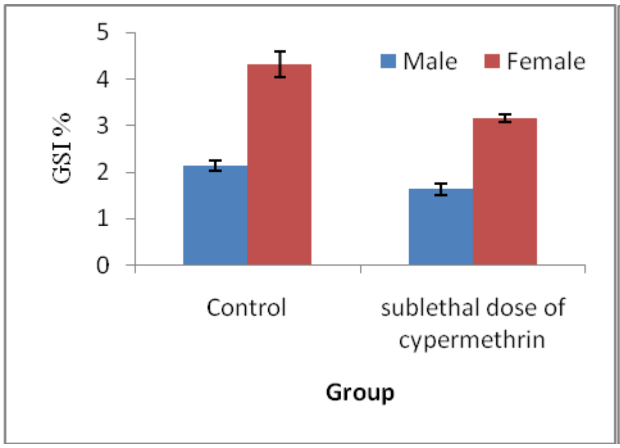
Gonado-somatic index (GSI%) = Weight of gonad /Weight of the fish x 100

Statistical analysis:

Data of all experiments are represented as mean ± standard error. Apart from descriptive statistics of the outcome variables, appropriate multivariate statistic such as One Way ANOVA, LSD, t-test was done with Minitab, version 17 Software to understand the causal relationship between parameters.

Results

GSI% was found to be increased in female than male in different experimental groups whereas no significant difference was observed for HSI% in both sexes of fish (Table 3, Fig. 3). A significant decrease ($p<0.05$) in GSI% (1.62 ± 0.06 in male and 3.16 ± 0.04 in female) and HSI% (3.84 ± 0.05 in male and 4.04 ± 0.05 in female) was found in



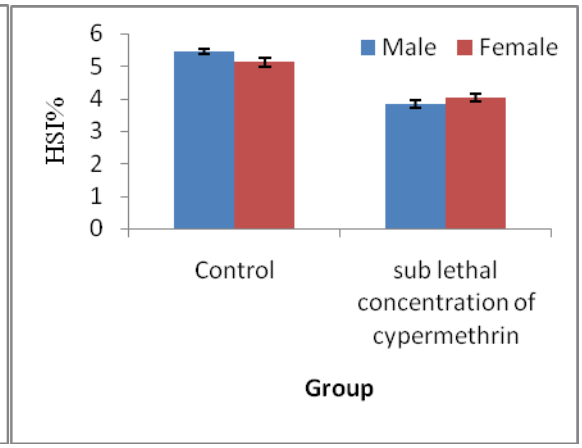


Fig. 3: GSI% (a) and HSI% (b) of *Puntius sarana* exposed to sublethal concentrstion of Cypermethrin 25% EC. Data represented as Mean ±SE.

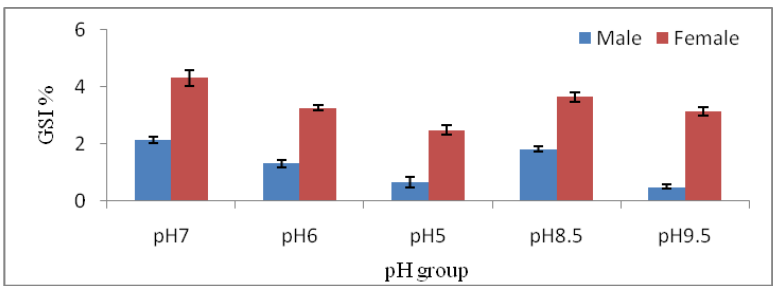


Fig. 4: GSI% of *Puntius sarana* exposed to different pH. Data represented as Mean ±SE.

Table 4: GSI% and HSI% of *Puntiussarana*exposed to different pH. *, indicates P<0.05, each value represents the mean ± SE

		pH 7	pH 6	pH 5	pH 8.5	pH 9.5
GSI%	Male	2.14±0.05	1.32±0.06*	0.66±0.08*	1.82±0.04*	0.50±0.03*
	Female	4.32±0.12	3.26±0.04*	2.50±0.07*	3.66±0.07*	3.14±0.07*
HIS%	Male	5.46±0.04	3.82±0.04*	2.78±0.04*	4.94±0.05*	2.54±0.05*
	Female	5.12±0.06	3.62±0.04*	2.74±0.05*	4.64±0.08*	2.26±0.04*

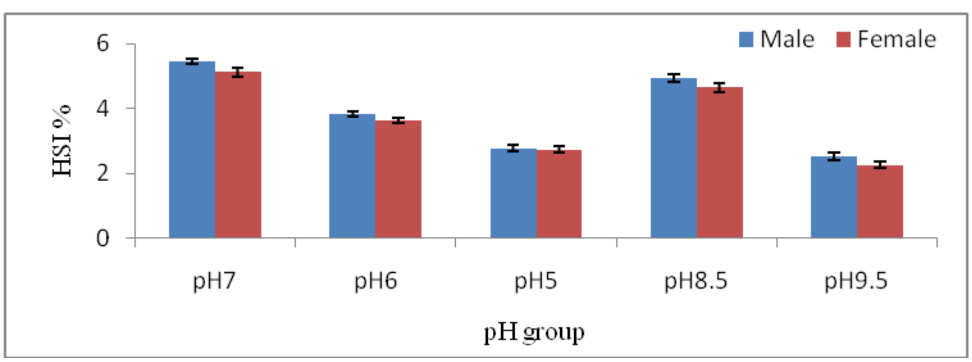


Fig. 5: HSI% of *Puntius sarana* exposed to different pH. Data represented as Mean ±SE.

Table 5: One Way ANOVA table for GSI% and HSI% of *Puntiussarana*exposed to different pH

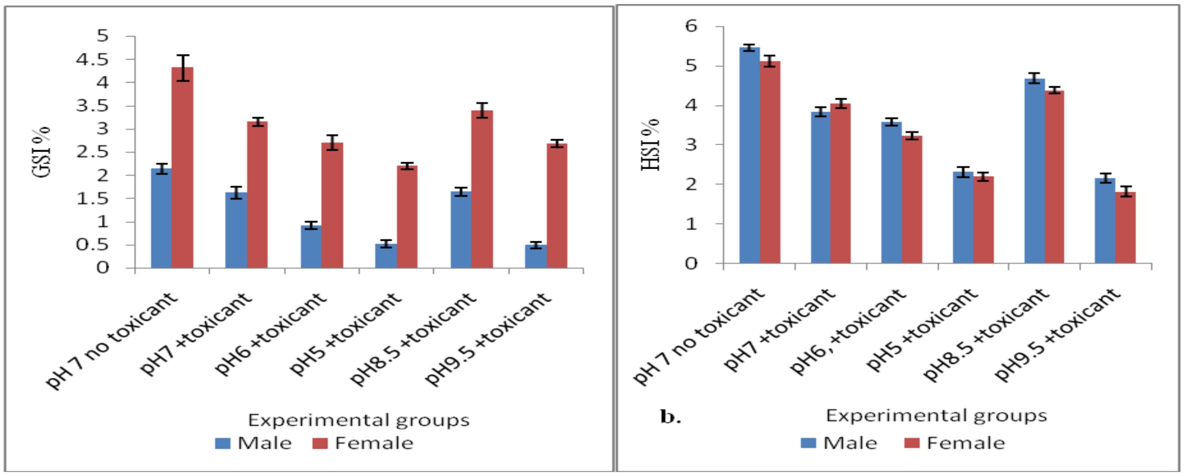
ANOVA							
			Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
GSI%	**Male**	Between Groups	10.126	4	2.532	168.773	0.000
		Within Groups	0.300	20	0.015		
		Total	10.426	24			
	Female	Between Groups	9.042	4	2.260	70.199	0.000
		Within Groups	0.644	20	0.032		
		Total	9.686	24			
HSI%	**Male**	Between Groups	33.126	4	8.282	862.667	0.000
		Within Groups	0.192	20	0.010		
		Total	33.318	24			
	Female	Between Groups	29.494	4	7.373	542.162	0.000
		Within Groups	0.272	20	0.014		
		Total	29.766	24			

Table 6: Post hoc Test (LSD) Multiple Comparison table for GSI% and HSI% of*Puntiussarana* exposed to different pH

GSI%				HIS%			
Dependent Variable	**(I)**	**(J)**	**Mean Difference (I-J)**	**Dependent Variable**	**(I)**	**(J)**	**Mean Difference (I-J)**
Male	pH7	pH 6	0.82*	**Male**	pH 7	pH 6	1.64*
		pH 5	1.48*			pH 5	2.68*
		pH 8.5	0.32*			pH 8.5	0.52*
		pH 9.5	1.64*			pH 9.5	2.92*
Female	pH7	pH 6	1.06*	**Female**	pH 7	pH 6	1.50*
		pH 5	1.82*			pH 5	2.38*
		pH 8.5	0.66*			pH 8.5	0.48*
		pH 9.5	1.18*			pH 9.5	2.86*

*indicates p<0.05

both sexes of fish compared to the control when they were exposed to sublethal dose of Cypermethrin 25% EC (Table 3, Fig. 3). When fishes were subjected to different acidic pH medium, GSI% and HSI % decreased significantly ($p<0.05$) as pH decreases below 7 in both male and female fish. In basic medium, a decrease in GSI% and HSI% was recorded whereas at pH 8.5 the GSI% and HSI% was found to be retained as control in both male and female (Table 4, Figs. 4, 5). In all treatments the mean difference with the control was found to be significant at the 0.05 level. ANOVA indicated significant difference both in HSI% and GSI% ($p<0.001$) between experimental groups with the F value of male- 862.67, female- 542.16 and male- 168.77, female- 70.20, respectively (Table 5). In both male and female, the mean difference of GSI and HSI value with the control group were recorded lowest when the fishes were subjected to pH 8.5 medium (Table 6). When the fishes were exposed to sublethal dose of cypermethrin 25% EC at different pH medium the GSI% and HSI % decreased significantly ($p<0.05$) in acidic medium and in extreme basic medium (pH 9.5) compared to the control group (Table 7, Fig. 6). ANOVA showed a significant difference both in HSI% and GSI% ($p<0.001$) between groups with the F value



(a) (b)

Fig. 6: (a) GSI% and (b) HSI% of *Puntius sarana* exposed to sublethal dose of cypermethrin 25% EC in different pH. Data represented as Mean±SE.

Table 7: GSI% and HSI% of *Puntius sarana* exposed to sublethal dose of cypermethrin 25% EC in different pH

		pH 7 - toxicant	pH 7 + toxicant	pH 6 + toxicant	pH 5 + toxicant	pH 8.5 + toxicant	pH 9.5 + toxicant
GSI%	**Male**	2.14±0.05	1.62±0.06*	0.92±0.04*	0.52±0.04*	1.64±0.04*	0.50±0.03*
	Female	4.32±0.12	3.16±0.04*	2.70±0.07*	2.20±0.03*	3.40±0.07*	2.68±0.04*
HSI%	**Male**	5.46±0.04	3.84±0.05*	3.58±0.04*	2.32±0.06*	4.68±0.07*	2.16±0.*05
	Female	5.12±0.06	4.04±0.05*	3.24±0.04*	2.20±0.04*	4.38±0.04*	1.82±0.06*

* indicates P<0.05. Each value presented as Mean ± SE

Table 8: ONE WAY ANOVA TABLE for GSI% and HSI% of *Puntius sarana* exposed to sublethal dose of cypermethrin 25% EC in different pH

ANOVA							
			Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
GSI %	**Male**	Between Groups	11.406	5	2.281	240.119	0.000
		Within Groups	0.228	24	0.009		
		Total	11.634	29			
	Female	Between Groups	13.626	5	2.725	111.230	0.000
		Within Groups	0.588	24	0.025		
		Total	14.214	29			
HSI %	**Male**	Between Groups	41.819	5	8.364	669.099	0.000
		Within Groups	0.300	24	0.012		
		Total	42.119	29			
	Female	Between Groups	41.319	5	8.264	688.644	0.000
		Within Groups	0.288	24	0.012		
		Total	41.607	29			

Table 9: Post hoc Test (LSD) Multiple Comparison table for GSI% and HSI% of *Puntius sarana* exposed to sublethal dose of cypermethrin 25% EC in different pH

GSI%				HSI%			
Dependent Variable	**(I)**	**(J)**	**Mean Difference (I-J)**	**Dependent Variable**	**(I)**	**(J)**	**Mean Difference (I-J)**
Male	pH7, no toxicant	pH 7, tox	0.52*	**Male**	pH7, no toxicant	pH 7, tox	1.62*
		pH 6, tox	1.22*			pH 6, tox	1.88*
		pH 5, tox	1.62*			pH 5, tox	3.14*
		pH 8.5,tox	0.50*			pH 8.5, tox	0.78*
		pH 9.5,tox	1.64*			pH 9.5, tox	3.30*
Female	Ph7, no toxicant	pH 7, tox	1.16*	**Female**	Ph7, no toxicant	pH 7, tox	1.08*
		pH 6, tox	1.62*			pH 6, tox	1.88*
		pH 5, tox	2.12*			pH 5, tox	2.92*
		pH 8.5, tox	0.92*			pH 8.5, tox	0.74*
		pH 9.5, tox	1.64*			pH 9.5, tox	3.30*

* indicates $p<0.05$

of male- 669.10, female- 668.64 and male- 240.12, female- 111.23, respectively (Table 8). LSD showed that the mean difference between the values of HSI% and GSI % in fishes exposed to pH 8.5 and control was lowest compared to the difference of control to other treatment groups (Table 9).

Discussion

Gonadosomatic index is an indirect method of studying the reproductive capabilities in spawning season in fishes. Regular seasonal cyclical changes in weight of gonads were evident especially in female fish. These characteristic changes are indication of the spawning season (Qasim, 1973). The gonads (testis and ovary) are found to be extensively sensitive to toxic insecticides. A significant decrease in GSI% and HSI% was recorded in the present study. Similar decrease in GSI value was reported in *Channa puntatus* exposed to fentrothion and carbonyl (Saxena, 1986). Reports on *C. carpio* treated with BHC (De Boeck *et al.*, 2001; David *et al.*, 2004) and on *Sarotherodon mossambicus* exposed to Malathion (Shukla *et al.*, 1984) are in good agreement with the findings of the present investigation.

The GSI and HSI level decreased in *Oreochromis mossambicus* treated with micronutrient Librel (Sadekarpawar and Parikh, 2013). This supports the present study in which the GSI% and HSI% decreased with different concentration of cypermethrin. This reduction may be occurred due to the negative impact induced by cypermethrin on the production of protein in the liver and their storage in the ovary. Pesticides are known to affect the testis in vertebrates resulting significant decrease in the GSI or per cent weight of the testis (Sehgal and Pandey, 1984). Similar results were noticed in *S. mossambicus* subjected to dimecron (Lakhani and Pandey, 1985) and the exposure with fenitrothion and carbofuran to *Channa puntatus* (Mani and Saxena, 1985). The present study showed that pH also affects the GSI% and HSI% in experimental fish. Both the extreme end of pH decreases the GSI and HSI values. Whereas pH 8.5 is found to be optimal as at this pH fishes were retaining their normal GSI and HSI values. When toxicant and pH were used in combination pH 8.5 + toxicant group were found to retain its normal GSI % and HSI % as control.

Conclusion

GSI%, and HSI% decreased with the exposure to cypermethrin 25% EC. Acidic water was found to have more adverse effect on the above parameters than basic water. It has been found that in basic water at pH 8.5, GSI%, HSI% became close to the percentage of the control for the above parameters. At pH 8.5 fishes were found to overcome the adverse effect of pesticide to significant extent.

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