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IMPACT OF SIMAZINE AND CHELATE PROPERTIES OF *SOLANAM XANTHOPIUM* IS THE FRESHWATER FISH *CIRRHINUS MRIGALA* HEMATOLOGICAL STUDIES FOR THE PERIOD OF 120 HOURS

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ABSTRACT

The pollution of environment due to use of pesticides has become an increasing problem over the last century with the development of industry, agriculture and increase in population. The organ phosphorous compounds are widely used because of their rapid biodegradability and non-persistent nature. Changes occurring in the haematological characteristic, if fishes provide a sensitive measure to assess the fish health. Further, the fish blood is a valuable diagnostic tool for the investigation of disease and physiological or metabolic alterations. Haematolgical techniques including measurements the level of WBC in blood of C. mrigala exposed to simazine, were increased, there were significant differencing mean value when compared to control fish There are no noticeable changes in the control fish, white blood corpuscles, red blood corpuscles and monitoring stress response. Administration of Solanam xanthopium results in the maximum normalization of the toxic effect of simazine thus, highlighting the protective role of Solanam xanthopium.

KEY WORDS

Cirrhinus mrigala, Hematology, Solanam xanthopium, Simazine

INTRODUCTION

Environmental pollution by various toxicants are become one of the most important problems in the world (Vijayaram *et al*., 1991). The heavy metal and pesticide contamination of aquatic system has attracted and attention of researchers to all over the world and has increased in the last decades due to extensive use of them in agricultural, chemical and industrial processes that are becoming threats to living organisms (Dutta and Dalal, 2008). Environmental contamination by pesticides has been documented in both biotic and abiotic components. The random use of different pesticides often causes lot of damage on non-target organism. Organophosphate pesticides constitute a large proportion of the total synthetic chemicals employed for the control of pests in the field of agriculture, veterinary practices and public health. The pollution of environment due to use of pesticides has become an increasing problem over the last century with the development of industry, agriculture and increase in

population. The organophosphorous compounds are widely used because of their rapid biodegradability and non-persistent nature.

Recently studies have proved that extremely low quantities of pesticides which enter the aquatic environment can affect productivity of organisms to kill eggs and larvae. The contaminations affect all groups of organisms in aquatic ecosystem like invertebrate (Meenambal and Pugazhendy 2012) non-target aquatic biota like fishes (Wadhwa et al., 1988). Simazine can be found in trace amount or at higher concentrations in soil and air. In mammals, simazine can accumulate in body fat, skin, liver, kidney, adrenal glands, overies, lung, blood and heart (Zutshi, 2003). The main target for simazine is the central nervous system. Symptoms of simazine toxicity in laboratory animals include pawing, burrowing, salivation, tremors, writhing, and seizures. In humans, high dose of simazine result simazine exerts its neurotoxic effect through voltage-dependent sodium channels and integral protein (Verma *et al*., 1998). The mechanism by which simazine affects male reproduction is unclear. Several studies have shown that simazine damages the brain, liver, and erythrocytes by causing oxidative stress (Zak, 1980).

Indiscriminate and extensive use of insecticides to protect crops possesses a serious threat to humans and the surrounding environment. Almost all pesticides are volatile in nature when applied to crops. These pesticides can be circulated into different ecosystems by different agents after entering into the environment have a deterious effect on fish and subsequently to man (Tilak *et al.,* 2005). The toxicological literature reveals that exposure of chemical can produce unexpected effects (Verma *et al*., 1998). Many researchers were baffled by these unexpected effects following exposure of individual to low levels of pesticides and other chemical toxins (Tabassum 2003).

Pesticides use is known to cause serious environmental problems, especially in the dry season, because during the period, the dilution capacity of the water systems is low thus increasing the risk of high concentration of toxic chemicals. In addition, the dry season is often the critical period of many animals especially fish and birds. Fish stocks suffer from natural mortality and high fishing pressure at the end of the dry season-contamination of water by pesticides either directly or indirectly can lead to sift kills, reduced fish productivity of devoted concentration of undesirable chemical in edible dish tissue which can be affect the health of humans eating these fishes (Rodrigues *et al.,* 2001). Pesticides are well recognized as an economic approach to control pests, at the same time such chemicals are highly toxic to other species in the environment (Tamizhazhagan, 2015)

Worldwide herbicide usage has increased dramatically during the past two decades, coinciding with changes in farming pesticides and increasingly intensive agriculture (Vasantharaja and Pugazhendy, 2012). As a consequence, residuals number of herbicides and their metabolites have been found in drinking water and food (Venkatesan and Pugazhendy., 2012). Chemical pesticides have contributed greatly to the increase of yields in agriculture by controlling pest and disease and also towards clacking the insect-borne disease (malaria, dengue, encephalitis, filariasis, etc.) in human health sector (Rahman and Siddiqui, 2006). In tropical countries, crop loss is even more served because the prevailing high temperature and humidity are high conductive to rapid multiplication of pest (Vettrivel and Pugazhendy., 2013). Thus, the application of wide variety of pesticides on crop plants is necessary in the tropics to combat pests and vector borne diseases. However, the sporadic use has been leading to significant consequences not only public health but also to food quality resulting in an impact load on the environment and hence the development of pests resistance (Ajayi and Ajimoko.,2005). Atrazine was introduced in the 1950s and since that time has become commonplace in agricultural and forestry application. For instance, atrazine is used to control annual grasses and broad-leaved weeds in selected vegetables and cereal crops, vines, fruits, orchards, citrus groves, sugarcane, grassland and forestry. It is the most widely used herbicide in the world, with between 70,000 and 90,000 tons applied per year (Chopra *et al.,* 2001).

It is well known that pesticides in aquatic environments have a toxic effect on organisms, especially on fish. Haematological techniques are the most common method to determine the sub-lethal effects of the pollutants (Tamizhazhagan and Pugazhendy, 2016). Thus, blood parameters such as RBC (Red Blood Cell), HB (Haemoglobin), HCT (Haematocrit), MCV (Mean Cellular Volume), MCH (Mean Cellular Haemoglobin Concentration), and PLT (Thrombocytes) are the most common criteria used in the toxicity studies on fish. As an indicator of pollution, blood parameters are used in order to diagnose and describe the general health condition of some fish. Besides, this type of index reflects certain ecological changes in the environment (Meenambal and Pugazhendy 2012).

Pesticides being used in agricultural tracts are realised into the environment and come into human contact directly or indirectly. Increasing incidence of cancer, chronic kidney disease, suppression of the immune system, sterility among males and female's endocrine disorders, neurological and behavioural disorders, especially among children's have been attributed to chronic pesticides poisoning (Tamizhazhagan et al 2016). In biological systems, the balance between both endogenous and exogenous pro-oxidant factors versus antioxidant defense can be used to assess oxidative damage induced by different classes of chemical pollutants (Tamizhazhagan and Pugazhendy.,2017) changes of antioxidant enzymes activity may disappear a change in the ROS within the cells. Therefore, these enzymes can be used as biomarkers for oxidative stress (Prabakaran and Pugazhendy 2014). Aquatic organisms are usually more sensitive than terrestrial and may be better experimental subject to evaluate sublethal effect of oxidative stress (Abraham, 2004).

MATERIALS AND METHODS

Collection and maintenance of the experimental Animal

The fresh water fish *Cirrhinus mrigala* were collected from the fish farm located in Pinnaloor at Navarathna form, Cuddalore district. The fish were brought to the laboratory and transferred to the rectangular cement tanks (100 × 175) of 500 liters capacity containing chlorine free aerated well water, fishes of the same size and weight were used irrespective of their sex for the experiments.

Toxicity Studies

Acute toxicity tests conducted to measure the impact of toxicity studies, the renewal technique of acute static test was adopted in which fish were periodically exposed to the concentrations of the same composition, usually once in every 24 hours by transferring the animals from one test chamber to another (Committee on methods for toxicity test with aquatic organisms, 1975). The LC_{50} was a statistical estimation of the concentration of toxic material in water that killed 50 percent of the test animals under experimental conditions with specific time intervals (Spragu, 1971). This value was ideally suited for toxicity studies as it gave more acceptable and reproducible concentration required to affect 50 percent of the organic than any other value (Pickering and Henderson, 1996).

Hematological Studies

The blood was mixed well with the EDTA solution by using a needle and this sample was used for determining the Red Blood Corpuscle Count (RBC), Total Leucocyte Count (TLC) and Haemoglobin content (Hb). For RBC count, a method devised by Yokayama and later modified by Christensen *et al.,* was followed. For counting the total number of WBC, the pipette with white lead was used. The number of cells present in the four large corner squares marked by capital letter *'L'* was counted and multiplied by 10^3which give the total number of WBC per cubic millimeter of blood. Haemoglobin determination is the quickest means for detecting anaemia. However, many factors are known to influence the haemoglobin level. The Sahli Hellige method was followed for haemoglobin determination. Sahlis pipette was filled slightly above the 20mm mark. The pipette was wiped with a filter paper or cotton to remove excess blood and the volume was adjusted to exactly 20 $mm^3$by blotting the tip. The blood was expelled into a calibrated (transmission) test tube

containing 2 ml of 0.1 N HCl. The pipette was rinsed several times in the acid solution. The sample was allowed to stand for 15 min. In the control fish, the erythrocytes were oval in shape with elongated nucleus fish, exposed to sublethal concentration of Monocrotophos showed abnormal size. Reduction in the volume of the cytoplasm of cells and swelling of nuclei were observed in fish exposed to concentration.

RESULTS

In the present investigation simazine treated fish *C. mrigala* (group-2) showed the decreases in RBC (Red blood cells) content when compared to control (group-1). The percentage changes are -12.98, -25.76, -33.95, -40.72 and -45.80 for 24, 48, 72, 96 and 120 hours respectively. Whereas in the simazine and *Solanam xanthopium* treated fish (group-3) record significant recovery when compared with control the percent regains are -4.25, -7.36, -9.87, -13.06 and -15.75 for 24 to 120 hours respectively. In the group 3 compared with group 2 recorded regained RBC content, the percent changes are +10.04, +24.78, +36.45, +46.67 and +55.44 for 24, 48, 72, 96 and 120 hours respectively. While in fish of *C. mrigala* exposed to *Solanam xanthopium* alone, when compared with control, a slight variation is noticed. The percentage changes in *Solanam xanthopium* (group-4) are +0.56, +0.80, +1.28, +1.85 and +2.26 for 24, 48, 72, 96 and 120 hours respectively. The recorded RBC content in blood cell counts for four groups are statistically significant at 1% and 5% levels (Table 1).

The level of WBC in blood of *C. mrigala* exposed to simazine, were increased, there were significant differencing mean value when compared to control fish (group-1). There are no noticeable changes in the control fish. The increased percent changes are +35.59, +52.70, +71.67, +80.66 and +83.53 for 24, 48, 72, 96 and 120 hours respectively. Whereas in the simazine along with *Solanam xanthopium* treated fish (group-3) record significant recovery from the effect of simazine compared with control. The percentage recoveries are +9.08, +12.96, +15.79, +18.46 and +19.95 for 24, 48, 72, 96 and 120 hours respectively. The simazine exposed fish (group-3) has slightly decreased when compared with (group-2) the percentage changes are -19.55, -26.03, -32.55, -34.43 and -34.64 120 hrs.

While in the fish exposed to *Solanam xanthopium* alone (group-4), no changes are noticed, and it is equal to control. The percentage changes in *Solanam xanthopium* alone (group-4) are +0.32, +0.56, +0.71, +1.03 and +1.35 for 24 to120 hours respectively. The WBC content in blood cells count for groups 2, 3 and 4 are statistically significant at 1% and 5% levels (Table 1). The result showed a significant reduction of Hb activity in blood *C. mrigala.*The group-2 simazine sublethal concentration exposed fish, the Hb content was decreased when compared with control values (group-1). The percent changes are -14.68, -20.88, -25.67, -32.04 and -39.87 for 24 to 120 hours respectively. The investigation of Hb activity in simazine along with *Solanam xanthopium* was gradually recovered when compared to group-1. The percent recoveries are -5.44, -8.72, -9.38, -11.12 and -13.31 for 24, 48, 72, 96 and 120 hours respectively.

While in the group 3 compared with group 1, the recover effect of *Solanam xanthopium* has great significant with group-3 there are +7.73, +15.33, +22.00, +30.79 and +44.20 for 24 to 120 hours respectively. While in the fish exposed to *Solanam xanthopium alone* (group-4), slight variations are noticed compared with control (group-1). The percentage changes in *Solanam xanthopium* alone exposed group-4 are +0.24, +0.51, +0.74, +0.89 and +1.25 for 24, 48, 72, 96 and 120 hours respectively. The Hb content in blood cell counts for groups 2, 3 and 4 are statistically significant at 1% and 5% levels (Table 1).

In the present investigation, (group-2) showed the level of PCV value was decreased when compared to group-1. The percent changes for group-2 are -10.60, -19.25, -26.55, -30.75 and -33.33 for 24, 48, 72, 96 and 120 hours respectively.

While in the fish exposed to group-3, the PCV content decreases when compared to group-1. The percent changes are -4.96, -7.80, -9.89, -11.52 and -13.14 for 24 to 120 hours respectively. The level of recover effect of *Solanam xanthopium* in group-3 shows maximum

activity when compare to group-2 +6.31, +14.18, +22.68, +27.77 and +30.28 for 24, 48, 72, 96 and 120 hours respectively, (Table 1)

During the present investigation were observed significant alteration in the MCV content in the blood of *C. mrigala* exposed to simazine at different concentrations of simazine exposed fish (group-2) which showed a slight increase in the MCV value when compared to control fish. There are no noticeable changes in the control fish. The percent changes are +4.89, +8.50, +11.20, +17.27 and +23.89 for 24, 48, 72, 96 and 120 hours respectively. The simazine along with *Solanam xanthopium* treated fish (group-3) recorded significant recoveries from the effect of simazine (group-2). The percent recoveries are +0.98, +1.58, +2.14, +2.59 and +3.15 for 24, 48, 72, 96 and 120 hours respectively. In the supplementary feed exposed group regained the percent changes are -3.72, -6.39, -8.54, -12.52 and -16.54 for 24 to 120 hours respectively. In the fish exposed to *Solanam xanthopium* alone (group-4), slightly decrease no changes occur and it is equal to normal. The percentage changes in *Solanam xanthopium* (group-4) are +1.05, -0.53, -0.94, -1.17 and -1.57 for 24 to 120 hours respectively. The MCV values in blood cell countsfor groups 2, 3 and 4 are statistically significant at 1% and 5% levels (Table 2).

The simazine exposed fish (group-2) shows a slight increase in the MCH content when compared to control fish (group-1). There are no noticeable changes in the control fish. The percent changes are +5.76, +8.11, +10.32, +14.11 and +14.97 for 24, 48, 72, 96 and 120 hours respectively. The simazine along with *Solanam xanthopium* treated fish (group-3) recorded significant recoveries from the effect of simazine group-2 (Table 2)

The activity of MCHC in blood of fish *C. mrigala* exposed to simazine content decreases when exposed to group-2 compared with control fish group-1. The decreased percent changes for group-2 are -2.78, -3.76, -4.91, -6.23 and -9.91 for 24, 48, 72, 96 and 120 hours respectively. In group 3, the increased percentage recoveries are -0.61, -1.30, -1.88, -2.00 and -2.45 for 24, 48, 72, 96 and 120 hours. The *Solanam xanthopium* exposed to (group-3) fish has slightly increased when compared with group-2. In group-4, there are no markable changes. The slight variations are recorded like +.020, +0.37, +0.54, +0.67 and +0.81 for 120 hours respectively. The recorded MCHC content of all 4 groups was statistically significant at 1% and 5% levels (Table 2).

DISCUSSION

Haemotological and biochemical profiles of blood can provide important information about internal environment of the organisms (Das and Mukherjee,2001). Pesticides are known to alter the blood parameters of fish. A significant decrease in RBC, Hb and PCV content in the present investigation of simazine exposed fish were observed. Which may be due to pesticides simazine induce changes which give evidence for decrese in test fishes. The decreased erythrocyte count may be due to the disruptive action on the erythropoietic tissue which in turn affected by the cell viability.

The decreasing RBC, Ht, and Hb values indicate that RBCs are being destroyed by the leucocytosis in erythrocytic anaemia with subsequent erythroblastosis (Dikshith *et al*., 1978) on the other hand; increase in Ht level has been reported as a result of oxygen deficiency (Dutta et al.,2006). In this study, treated fishes eventually suffered a hypochromic, macrocytic anaemic condition that is attributable to the swelling of the RBCs, haemodilution and impaired Hb synthesis. A decrease in the number of RBC, haemoglobin and haematocrit values of diazinon exposed fish was reported by (Abraham ,2004) and related it to destruction of cells and decrease in size of cells due to the adverse effects of pesticide.

The present investigation shows an increased level of WBC content in simazine treated fish throughout the exposure period of 24 to 120 hours. The increase in WBC counts recorded in this research when compared with other parameters could be due to the attempt of the fish to fight against the pollutant and this augmented the production of more WBC to improve the health status of the fishes which however, agreed with the reports of Adeyemo, (2005) ;(Meenambal and Pugazhendy 2012:

Vasantharaja and Pugazhendy 2012)The increase in WBC count can be correlated with a survival and recovery of the fishes exposed to the toxicant (Ghosh et al ., 1999). That increase in WBCs count occurred as a pathological response since these WBCs play a great role during infestation by stimulating the haemopoietic tissues and the immune system by producing antibodies and chemical substances working as defense against infection. The WBC showed greatest sensitivity to changes in the environment of leucocytes was lymphocytes. Leucocytosis is evidenced in present study, by the increase in total leucocyte count during simazine intoxication. Similar increase in WBC counts was found in fish *H. fossilis* during nickel intoxication (Gill et al 1991).

Blood cell indices like mean corpuscular volume MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) seem to be changes that are more sensitive and can cause reversible changes in the homeostatic system of fish. Fluctuations in these indices correspond with values of RBC count, haemoglobin concentration and packed cell volume. In the study a significant increase in these indices was noticed in *C. mrigala* after exposure to simazine. A similar response was noted in common carp and other freshwater fish exposed to acute toxic level of pesticides (Rahman, 2016). The pesticide may change the functions of vital organs like liver and kidneys, disrupting the homeostatic condition of the body. Similar observations were reported by Ajayi *et al*. (2005). Haematological parameters Changes might have been brought about by simazine as an anemic condition due to decreased synthesis of Hb and RBC number in bone marrow cells. Significant reduction in haemoglobin in experimental animals might be destruction in haemoglobin has been reported by Vasantharaja *et al*., (2012). The investigation of MCV and MCH in blood levels increased and MCHC levels decreased in sublethal concentration in simazine in freshwater fish *C. mrigala.* The increase in MCH and MCV values with decreased in MCHC perhaps is due to toxic substances in the medium causing differences in haemopoietic activity (Kaur and Dhavan, 1996).

Changes in glucose concentration are most often associated with renal injury. Plasma concentration of glucose is regulated by complex interaction of hormones such as glucagon and cortisol (Khan et al 2002). In the present investigation shows that the activities of ALT and AST increased as the concentration of simazine increased in all the organs tested in a dose dependent pattern. Similar result was also reported by (Lakhani, and Pandey, 1985). When they exposed *Cyprinus carpio* to diazinon for 96 hrs which produced depressed activities in the enzymes (AST, ALT, ACP and ALP). The decrease in total proteins could be attributed in part to the damaging effects of pesticide on liver cells as confirmed by the increase in the activities of serum AST and ALT observed in this study. A decline in serum total protein level was reported in fish *R. quelen* (Borges *et al*., 2007) and *O. niloticus* (Oner *et al*., 2008) in response to simazine and copper exposure, respectively.

All biological activities are regulated by enzymes and hormones, which are also proteins. Assessment of protein and enzymes activities can be considered as a diagnostic tool to determine the physiological status of cells or tissues (Manoj, 1999). Alterations of ALT and AST activities of fish resulting from toxicant or contaminant effect in various organs of fish have been reported Gill *et al*., (1991) and Begum, (2004).

Enzyme activities are considered as sensitive biochemical indicators and widely used to assess the health of the organism in aquatic toxicology (Gul *et al*., 2004). Several soluble enzymes of blood serum have been considered as indicators of the hepatic dysfunction and damage. Among the array of enzymes used the aspirate aminotransferase (AST) and alanine aminotransferase (ALT) are widely used to detect the tissue damage caused by the toxicants (Jung *et al*., 2003). In fish, liver is the major organ for arsenic toxicity and plays a major role in uptake, accumulation, biotransformation and excretion of toxicant (Datta *et al*., 2007. AST and ALT in conjunction with LDH has been found to be involved in gluconeogenesis from amino

acids and the effects of changes in the activities of the transaminases (Meenambal and Pugazhendy 2012). Increase in the transaminases is an immune mechanism, which occurs at the initial stages of diseased condition (Chopra *et al*., 2001). Increased activity of acid phosphatase and alkaline phosphatase in blood plasma as an indicator of hepatic tissue damage and dysfunction have been shown in fish following the exposure of pesticides (Maruthanayagam and Sharmila, 2004).

The evaluation of haematological and biochemical characteristics in fish has become an important means of understanding normal and pathological processes and toxicological impacts. Blood indices are often subject to variation depending upon stress and environmental factors (Hlavova, 1993). More biochemical toxicological research has been conducted on mammals than on fish. However, not surprisingly, many biochemical similarities exist among vertebrate species (Mezin and Hale, 2000). Alkaloids generally exert pharmacological activity particularly in mammals such as humans. Even today many of our most commonly used drugs are alkaloids from natural sources and new alkaloidal drugs are still being developed for clinical use (e.g., taxol from *Taxus baccat*). Most alkaloid with biological activity in humans affects the nervous system, particularly the action of the chemical transmitters, e.g., acetylcholine, epinephrine, norepinephrine, γ-aminobutyric acid (GABA), dopamine and serotonin (Prabakaran *et al*., 2011). This enzyme is known to play a key role in the polyol pathway, by catalyzing the reduction of the glucose to sorbitol, which under normal conditions cannot diffuse out of cell membranes. Because of the intracellular accumulation of sorbitol, the chronic complications (such as neuropathy, retinopathy and cataracts) of diabetes can occur. Apigenin and luteolin were shown to possess antihyperglycaemic (Orton et al 2006) and antioxidant activity (Parvathi *et al*., 2000)

CONCLUSION

Changes occurring in the haematological characteristic, if fishes provide a sensitive measure to assess the fish health. Further, the fish blood is a valuable diagnostic tool for the investigation of disease and physiological or metabolic alterations. Haematolgical techniques including measurements of PCV, white blood corpuscles, red blood corpuscles and monitoring stress response. Administration of *Solanam xanthopium* results in the maximum normalization of the toxic effect of simazine thus, highlighting the protective role of *Solanam xanthopium*.

Table 1: Variations of RBC (×10^6/mm^3), WBC (×10^3/mm^3), Hb (g/L) and PCV (%) values in the freshwater fish *C. mrigala* exposed to simazine and *Solanam xanthopium* for 120 hours

Blood	Groups	Hours of exposure				
		24	48	72	96	120
RBC	Group-I Control	1.248 ± 0.009	1.250 ± 0.005	1.246 ± 0.004	1.240 ± 0.008	1.238 ± 0.006
	Group-IIsimazine	1.086** ± 0.006	0.928** ± 0.005	0.823** ± 0.006	0.735** ± 0.004	0.671** ± 0.007
	% COC	% -12.98	% -25.76	% -33.95	% -40.72	% -45.80
	Group-IIIsimazine+*Solanam xanthopium*	1.195** ± 0.004	1.158** ± 0.008	1.123** ± 0.007	1.078** ± 0.005	1.043 ± 0.006
	% COC	% -4.25	% -7.36	% -9.87	% -13.06	% -15.75
	% COT	% +10.04	% +24.78	% +36.45	% +46.67	% +55.44
	Group-IV *Solanam xanthopium*	1.255^{NS} ± 0.005	1.260^{NS} ± 0.006	1.262^{NS} ± 0.005	1.263^{NS} ± 0.004	1.266^{NS} ± 0.006
	% COC	% +0.56	% +0.80	% +1.28	% +1.85	% +2.26
WBC	Group-I Control	1.256 ± 0.025	1.258 ± 0.032	1.260 ± 0.027	1.262 ± 0.031	1.263 ± 0.039
	Group-IIsimazine	1.703** ± 0.028	1.921** ± 0.029	2.163** ± 0.031	2.280** ± 0.028	2.318** ± 0.031
	% COC	% +35.59	% +52.70	% +71.67	% +80.66	% +83.53
	Group-IIIsimazine+*Solanam xanthopium*	1.370* ± 0.028	1.421* ± 0.029	1.459** ± 0.031	1.495** ± 0.028	1.515** ± 0.031
	% COC	% +9.08	% +12.96	% +15.79	% +18.46	% +19.95
	% COT	% -19.55	% -26.03	% -32.55	% -34.43	% -34.64
	Group-IV *Solanam xanthopium*	1.260^{NS} ± 0.036	1.265^{NS} ± 0.025	1.269^{NS} ± 0.031	1.275^{NS} ± 0.038	1.280^{NS} ± 0.037
	% COC	% +0.32	% +0.56	% +0.71	% +1.03	% +1.35
Hb	Group-I Control	3.345 ± 0.035	3.348 ± 0.047	3.352 ± 0.058	3.355 ± 0.031	3.357 ± 0.038
	Group-IIsimazine	2.936** ± 0.038	2.649** ± 0.040	2.491** ± 0.031	2.280** ± 0.047	2.018** ± 0.033
	% COC	% -14.68	% -20.88	% -25.67	% -32.04	% -39.87
	Group-IIIsimazine+*Solanam xanthopium*	3.163* ± 0.036	3.056** ± 0.040	3.039** ± 0.058	2.982** ± 0.049	2.910 ± 0.058
	% COC	% -5.44	% -8.72	% -9.38	% -11.12	% -13.31
	% COT	% +7.73	% +15.33	% +22.00	% +30.79	% +44.20
	Group-IV *Solanam xanthopium*	3.353^{NS} ± 0.049	3.365^{NS} ± 0.051	3.377^{NS} ± 0.046	3.385^{NS} ± 0.052	3.399^{NS} ± 0.065
	% COC	% +0.24	% +0.51	% +0.74	% +0.89	% +1.25
PCV	Group-I Control	28.938 ± 0.531	28.943 ± 0.430	28.945 ± 0.574	28.947 ± 0.429	28.946 ± 0.634
	Group-IIsimazine	25.870** ± 0.446	23.370** ± 0.548	21.261** ± 0.633	20.045** ± 0.424	19.298** ± 0.588
	% COC	% -10.60	% -19.25	% -26.55	% -30.75	% -33.33
	Group-IIIsimazine+*Solanam xanthopium*	27.503^{NS} ± 0.397	26.685* ± 0.456	26.083* ± 0.584	25.611** ± 0.428	25.141** ± 0.532
	% COC	% -4.96	% -7.80	% -9.89	% -11.52	% -13.14
	% COT	% +6.31	% +14.18	% +22.68	% +27.77	% +30.28
	Group-IV *Solanam xanthopium*	28.950^{NS} ± 0.427	28.963^{NS} ± 0.630	28.977^{NS} ± 0.477	28.990^{NS} ± 0.548	28.999^{NS} ± 0.473
	% COC	% +0.04	% +0.07	% +0.11	% +0.15	% +0.18

Values are mean ± S.E-Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at ** P<0.01 levels. (+,-) denotes decreased and increased. % COC (change over control); % COT (change over treated).

Table 2: Variations of MCV (fL), MCH (pg) and MCHC (%) content in the freshwater fish *C. mrigala* exposed tosimazine and *Solanam xanthopium* for 120 hours

Blood	Groups	Hours of exposure 24	48	72	96	120
MCV	Group-I Control	231.878 ± 1.808	232.096 ± 1.663	232.301 ± 1.272	232.543 ± 1.358	232.712 ± 1.869
	Group-IIsimazine	243.209** ± 1.971	251.828** ± 2.061	258.330** ± 1.988	272.717** ± 2.393	287.603** ± 1.389
	% COC	% +4.89	% +8.50	% +11.20	% +17.27	% +23.89
	Group-IIIsimazine+*Solanam xanthopium*	234.148^{NS} ± 1.610	235.743^{NS} ± 1.630	237.264* ± 1.236	238.575* ± 1.471	240.041 ± 1.055
	% COC	% +0.98	% +1.58	% +2.14	% +2.59	% +3.15
	% COT	% -3.72	% -6.39	% -8.54	% -12.52	% -16.54
	Group-IV *Solanam xanthopium*	234.326^{NS} ± 2.877	230.870^{NS} ± 1.855	230.110^{NS} ± 1.973	229.830^{NS} ± 2.067	229.066^{NS} ± 1.826
	% COC	% +1.05	% -0.53	% -0.94	% -1.17	% -1.57
MCH	Group-I Control	26.502 ± 0.494	26.680 ± 0.568	26.802 ± 0.406	26.966 ± 0.394	27.030 ± 0.483
	Group-IIsimazine	28.030* ± 0.315	28.845* ± 0.478	29.567** ± 0.411	30.770** ± 0.378	31.076** ± 0.514
	% COC	% +5.76	% +8.11	% +10.32	% +14.11	% +14.97
	Group-IIIsimazine+*Solanam xanthopium*	27.068^{NS} ± 0.495	27.790^{NS} ± 0.339	28.061^{NS} ± 0.427	28.660* ± 0.430	28.908 ± 0.365
	% COC	% +2.13	% +4.16	% +4.70	% +6.16	% +6.90
	% COT	% -3.43	% -3.66	% -5.09	% -6.86	% -6.95
	Group-IV *Solanam xanthopium*	26.580^{NS} ± 0.443	26.803^{NS} ± 0.503	26.959^{NS} ± 0.428	27.161^{NS} ± 0.569	27.218^{NS} ± 0.505
	% COC	% +0.29	% +0.46	% +0.58	% +0.61	% +0.69
MCHC	Group-I Control	11.562 ± 0.066	11.570 ± 0.075	11.585 ± 0.067	11.592 ± 0.073	11.599 ± 0.059
	Group-IIsimazine	11.240* ± 0.054	11.135** ± 0.049	11.016** ± 0.066	10.870** ± 0.050	10.450** ± 0.070
	% COC	% -2.78	% -3.76	% -4.91	% -6.23	% -9.91
	Group-IIIsimazine+*Solanam xanthopium*	11.486^{NS} ± 0.046	11.420^{NS} ± 0.059	11.367* ± 0.048	11.360* ± 0.053	11.315 ± 0.050
	% COC	% -0.61	% -1.30	% -1.88	% -2.00	% -2.45
	% COT	% +2.19	% +2.56	% +8.79	% +8.79	% +8.83
	Group-IV *Solanam xanthopium*	11.585^{NS} ± 0.067	11.613^{NS} ± 0.071	11.648^{NS} ± 0.052	11.670^{NS} ± 0.072	11.693^{NS} ± 0.061
	% COC	% +0.20	% +0.37	% +0.54	% +0.67	% +0.81

Values are mean ± S.E-Mean of six individual observations; and student t-test. Significant at *P<0.05; Significant at ** P<0.01 levels. (+,-) denotes decreased and increased. % COC (change over control); % COT (change over treated).

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