Comparative Toxicity of Acetamiprid and *Azadirachta indica* Leave Extract on Biochemical Components of Blood of *Labeo rohita*

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**Abstract.** *Labeo rohita* was exposed to different concentrations of acetamiprid (10, 11, 12, 13, 14 and 15 mg/L) and *Azadirachta indica* leaves extract (250, 500, 750, 1000 and 1050 mg/L) to determine their LC50. The LC50 of *A. indica* leaves extract and acetamiprid were 785.4 and 11.62 mg/L, respectively. Fish were exposed to 20% of LC50 of acetamiprid and *A. indica* leaves extract for 28 days to evaluate changes in the biochemical component of fish blood. A significant decrease in serum calcium, phosphate and albumin, and a significant increase in urea was recorded when fish were exposed to acetamiprid. In contrast, no significant alterations were observed in blood parameters of fish revealed to *A. indica* leaves extract. The results, therefore, suggest that *A. indica* leaves extract can be used safely for aquatic pests since it is non-toxic to non-target organisms.

**Key words:** LC50, biopesticide, calcium, phosphate, urea, albumin.

**INTRODUCTION**

Pesticides are used excessively all over the world to control plagues. In particular, the use of chemical pesticides is considered as an economic strategy for plague control; however, such synthetic substances are extremely hazardous to non-target species in the environment (Gaafar et al., 2010). After their application, pesticides make their way into the various aquatic ecosystems, affecting non-target inhabitants (Moraes et al., 2011) or causing ecological imbalances (Velisek et al., 2012). These toxicants may cause changes in a single cell or in a whole organism; they also produces stressful conditions with consequent alterations in cells via biological magnification in aquatic animals such as fish, and ultimately cause damages for humans through the food chain (Giari et al., 2008).

Acetamiprid is a chemical pesticide which is related to neonicotinoid family (Si et al., 2005) and is extensively utilized in agriculture for pest management in various countries (Jiao et al., 2011). Pesticides enter into aquatic ecosystems by agricultural run-off and may cause in physiological abnormalities, in aquatic organisms (Siddique and Wanule, 2010).

Many plant derived products are being used to control insects (Dubey et al., 2010). In particular, *Azadirachta indica* (neem tree) leaves, are used for pest management. Azadirachtin is the most important active substance in neem plant which is mostly present in seed, leaves, and in other parts of the neem tree (Senthil et al., 2008).

*Labeo rohita* is an edible fish and it is cultured in various farms of Indo-Pak sub-continent. This fish has significant economic value as it is a source of livelihood to numerous people (Akhtar et al., 2011). The use of growth promoters and pesticides has considerable affected the production of *L. rohita* in aquaculture systems and in wild fish populations all over the world. Additionally, the species has been considered as a model for physiology/stress studies (Ficke et al., 2007).

Blood parameters are considered as physiological indicators of animals undergoing stressful conditions such as the presence of toxicants, because blood acts as a pathophysiological reflector of the whole body (McDonald et al., 1989; Velisek et al., 2012). Particular ions such as calcium and phosphate are vital components for a broad range of biological activities in organisms. Calcium plays a role in cell adhesion, muscle contraction, acid-base balance and helps in...
triggering endocrine activities in vertebrates. Phosphate plays a crucial role in enzyme/protein components, membrane structure, metabolism and genetic information (Kumar et al., 2011a). Urea is the foremost osmolyte in fish and plays a major role in acid-base balance (Hazon et al., 2003). Albumin is the essential component in serum protein (Kovyrshina and Rudneva, 2012), it regulates blood osmotic pressure and has a role in the transportation of endogenous metabolites (bilirubin, fatty acids and hormones) and exogenous compounds (drugs) (Baker, 2002).

The present study was performed to evaluate the effect of A. indica leaves extract and acetamiprid on the biochemical parameters (calcium, phosphate, urea and albumin) and behaviour of the experimental fish L. rohita.

**MATERIALS AND METHODS**

**Collection and acclimatization of experimental fish**

Live specimens of fresh water fish (Labeo rohita) (weight 35±7.90 g) were collected from the ponds of University of Veterinary and Animal Sciences (UVAS) Pattoki. Fish samples were maintained in glass aquaria containing 70L of tap water; the organisms were acclimatized to laboratory environment and maintained at room temperature for 15 days. Continuous aeration was provided and fish were fed daily with a balanced diet. Average values of water quality parameters of tap water used during experimentation were temperature 18.25±1.48°C; DO 9.02±0.14 mg/L; pH 7.52 and EC (Electrical Conductivity) 342.9±0.23 μS. Fish were exposed to natural photoperiod. Dead fish and feces (if any) were removed from glass aquaria.

**Pesticides**

Acetamiprid was obtained from Abdullah Haseeb Group of Companies (pH 9.39), whereas Azadiracta indica (biopesticide) leaves were locally collected from garden and cleansed with dechlorinated tap water in order to remove dust. Washed leaves were air dried at room temperature, chopped with the help of grinder, and suspected for 48h at room temperature (Farah et al., 2006). The mixture was filtered after 48 h and the filtrate was used as biopesticide.

**Determination of LC50 values**

For determination of lethal concentration (96 h LC50) of acetamiprid and A. indica leaves extract in L. rohita (APHA, 2005), five acetamiprid concentrations 10, 11, 12, 13, 14mg/L and five A. indica leaves extract concentrations 250, 500, 750, 1000 and 1250 mg/L were used. Test was conducted in duplicates and each concentration considered 10 fish in 70 L of water; a control treatment without pesticide was performed simultaneously. Fish were deprived of food 24 h prior and during the trial. Test aquaria were examined regularly and the mortality percentage was observed at 24, 48, 72 and 96 h. Experimental data were analyzed by probit analysis method (Finey, 1971). Chi-square test exhibited that all values were appropriate at 0.05 probability levels.

**Evaluation of blood parameters**

For sub-lethal toxicity test, L. rohita were maintained in glass aquaria containing 70 L of water (10 fish per aquarium). There were one control and two experimental groups. Treatments were also performed by duplicate. Fish were fed daily *ad libitum*. In experimental groups, fish were exposed to 20% LC50 at 96 h of acetamiprid (2.2 mg/L) and A. indica leaves extract (150 mg/L) for twenty eight days. Temperature, dissolved oxygen (DO), pH and electrical conductivity (EC) were monitored weekly. Media (water) was replaced on alternate days and to renew the media, continuous aeration was provided with air pumps (Qayyum et al., 2005).

**Blood collection and analysis of blood components**

Blood of fish was collected directly from the fish’s heart with non-heparinized syringes at 7th, 14th, 21st and 28th days of exposure from both control and experimental groups. Blood samples were centrifuged at 3000 rpm for 15min using a microcentrifuge (Model: Scan speed mini, 2010, Denmark) (Monteiro et al., 2006; Moraes et al., 2011). Serum was used to analyze the biochemical parameters (calcium, phosphate, albumin and urea).
Analysis of biochemical parameters
Calcium (Analyticon Fluitest CA CPC, Germany), phosphate (Fluitest Phos, Germany), urea (Crescent diagnostics catCS612, Saudi Arabia) and albumin (Crescent diagnostics, catCS60, Saudi Arabia) were analyzed by using commercial kits following the manufacturers specifications. Blanks, standards and samples were prepared for biochemical analyses and a spectrophotometer was used to measure the absorbance (Model: spectroscan 80D UV-VIS Spectrophotometer).

Statistical analysis
SPSS software 16 was used to analyze the data. The entire data were expressed as means ±S.E. One way ANOVA (Analysis of Variance) was used to find out the significances between different multiple groups. Results of Tukey Duncan post hoc test showed that significance level was 0.05.

RESULTS

LC50 value of A. indica leaves extract (785.4 mg/L) was 67.59 fold-times higher than acetamiprid (11.62 mg/L). Results of biochemical parameters of the control and experimental fish (L. rohita) demonstrated that acetamiprid significantly (p<0.001) enhanced the serum urea level but lowered serum calcium, phosphate and albumin levels compared to the control group. A. indica leaves extract did not result in any significant changes (p>0.05) in biochemical blood parameters of L. rohita (Fig.1).

Fish exposed to acetamiprid registered significant decreases (p<0.05) in serum calcium, phosphate and albumin concentration at all sampling dates compared to the control, but the most drastic diminution occurred at 28th day of exposure to the synthetic pesticide, in which calcium levels decreased by 84%, phosphate level decreased by 90.6%, albumin level decreased by 84.3% compared to the control (Fig. 1). Fish exposed to acetamiprid registered a significant increase (p<0.05) in serum urea level and a 56% increase occurred at 28th day of exposure. No significant differences for calcium, phosphate, urea and albumin levels were detected among fish treated with A. indica and the control at any of the sampling dates.

DISCUSSION

Biopesticides are gaining momentum as they are environment friendly and can replace some of the chemical pesticides (Israel et al., 2008). Biopesticides are not much harmful to the environment (Ghimerya et al., 2009). In the present study, 96 h LC50 value of A. indica leaves extract was higher than acetamiprid. This illustrated that
biopesticides are less hazardous to non-target organisms as compared to the chemical pesticide. Similarly, Israel et al. (2008) evaluated 96 h LC50 of A. indica based pesticide azadirachtin for Poecilia reticulate and found that LC50 value has higher than that of the chemical pesticide deltamethrin.

The estimation of biochemical parameters in fish has become an imperative approach to understand normal and pathological activities of aquatic organisms as well as toxicological impacts (Sudova et al., 2009). Pesticides have potential to induce oxidative stress in fish (Monteiro et al., 2006). Siddiqui and Wanule (2010) illustrated that exposure of acetamiprid increased the leucocyte concentration of Channa punctatus, which induced further tissue damage. Bhatkar and Dhande (2000) explained that exposure of furdan (chemical pesticide) caused alterations in hematology of L. rohita.

In the present study, acetamiprid decreased serum calcium content which caused a subsequent hypocalcemia in L. rohita, while A. indica leaves extract did not cause significant changes. Earlier studies have also demonstrated the decrease in calcium value of fish exposed to cypermethrin (Mishra et al., 2005) and deltamethrin (Srivastav et al., 2010). Velisek et al. (2012) also illustrated decline in calcium level in common carp which is exposed to simazine and decrease in calcium cause hypocalcemia in fish. Hypocalcemia has been noticed in other species exposed to high concentrations of pesticides such as Clarias batrachus, Heteropneustes fossilis and Oreochromis mossambicus (Singh and Srivastava, 1998). Calcium plays a vital role in blood coagulation and regulates the permeability of the cell membrane to inorganic ions and water. It also plays a role in the development of action potential in nerves and muscles and also maintained the membrane potential (Kumar et al., 2011b). Pesticides such as organophosphates, carbamates and neonicotinoids inhibit the enzyme acetylcholinesterase that causes skeletal muscle paralysis and death from asphyxiation in the organisms exposed to these insecticides (Singh et al., 1996). Thus, the decrease in calcium in L. rohita exposed to the chemical pesticide could be evidence of disturbance in the physiological function of fish.

In the present study, acetamiprid significantly decreased the serum phosphate in L. rohita, causing hypophosphatemia. This represents the toxic effects of acetamiprid to L. rohita. In contrast, there was non-significant decline in phosphate value of L. rohita exposed to A. indica leaves. The inorganic phosphate is the main source of energy exchange and it plays a crucial role as a chief cytoplasmic buffer. Both hypo and hyperphosphatemia have been observed in fish treated with several chemical pesticides (Singh and Srivastava, 1998) signifying that pesticides are lipophilic in nature and they access easily through the membrane of mitochondria and restrain the enzymes occupied in electron transport chain which affects the phosphorylating capability of mitochondria.

The chemical pesticide caused hypophosphatemia and hypocacalcaemia in catfish, affecting the homeostasis of calcium and phosphate. Calcium and phosphate are the importations for the vitellogenin production, which is a lipophosphoprotein mainly documented in females; thus chemical pesticides severely affect the fish’s reproductive physiology (Srivastav et al., 1997).

The significant urea increase in fish treated with acetamiprid, suggests that the pesticide may have caused physiological damage in kidney tissue. Patnaik (2010) reported a considerable increase in urea level of Clarias batrachus exposed to sevin. Gaafar (2010) illustrated that increase in urea level affects the kidney tissue with the chronic treatment of ediphenphos in Nile tilapia. Organs such as intestine, gill and kidney are primarily responsible to regulate the water and ionic movement between external and internal environment of fish and these organs are greatly vulnerable to poisonous effects of pesticides (Hazon et al., 2003).

The significant albumin decrease in fish treated with acetamiprid, suggests that the pesticide may have cause renal disease and liver insufficiency. Prusty (2011) also observed the decrease in albumin level of L. rohita which is treated with chemical pesticide fenvalerate and noticed that under stress situation, numerous animals mobilize protein as an energy source during the oxidation process of amino acids. Patnaik (2010) also noticed significant decreases in albumin levels and protein levels, probably because of tissue repair,
increased energy cost of homeostasis, impaired food intake and detoxification mechanisms during stress. The other reasons for the decrease of protein and albumin level are the liver cirrhosis or kidney nephritis and inhibition of protein synthesis. Velisek et al. (2012) described significant decrease in albumin after the exposure of simazine in common carp. The decline in albumin content signifies that chemical pesticide could have caused malnutrition, renal disease, liver inefficiency and may induce harmful impacts on immune system. The pesticide caused stress in fish which degrades the protein. Non-significant decrease in fish treated with A. indica leaves extract, proves that biopesticide is safe for non-target organisms.

In the present study, analysis of biochemical parameters such as calcium, phosphate, urea and albumin revealed significant difference in fish treated with acetamiprid as compared to control and fish treated with A. indica leaves extract had non-significant changes alterations in its blood parameters and behavior. This suggests that biopesticides are less hazardous and safer to environment as compared to toxic chemical pesticides (Ansari and Ahmad, 2010).

In the present study, fish L. rohita exposed to acetamiprid exhibited extreme behavioral changes. Fish displayed stress throughout the experiment and attempted to escape the aquaria. Irregular, rapid movement and loss of balance of fish was observed throughout the experiment. Tiwari et al. (2012) noticed similar behavioral alterations in L. rohita treated with cypermethrin. Velisek, (2008) also observed similar behavioral alterations such as movement loss and increased rate of respiration in rainbow trout and common carp exposed to metribuzin. Fish treated with A. indica leaves extract were exempted from such stressful situation. Fish moved in groups in aquaria and sometimes they reached to water surface to consume air.

Comparison of toxicity potential of chemical and biopesticide shows that A. indica leaves extract can be utilized as eco-friendly plant based pesticide to restrain pests on crop fields as an alternative of detrimental and harmful chemical pesticides. Acetamiprid in contrast, should be mentioned in list of toxic pesticide as it brings changes in biochemical parameters such as calcium, phosphate, albumin and urea of aquatic ecosystem inhabitants such as fish.

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