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Study on Protein Contents and Recovery Responses in the Blood Plasma of *Channa punctatus* On Exposer to Imidacloprid (Bloch.)

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ABSTRACT: The present investigation was aimed to observe the impacts of Imidacloprid (IMI) on the protein levels in blood plasma of *Channa punctatus* and experiments were designed having control without insecticide and four treated at sublethal concentration 0.282 ppm for 24, 48, 72 and 96 hrs to assess the alterations in the level of protein in blood plasma for acute exposer. At the end of 24 hrs, minimum percent decrease was 6.90% and a maximum percent decrease was 28.77% at the end of 96 hrs of treatment were recorded. Decreased protein level was observed in blood plasma of *Channa punctatus* with an increase in the exposure periods of Imidacloprid (Confidor).

KEYWORDS: Total proteins, Imidacloprid, *Channa punctatus*.

I. INTRODUCTION

Man is using a number of chemicals and there are a number of chemicals in the environment now. Some of the chemicals are toxic to living system which get incorporated into food chain, disturb the biochemical processes occurring in the living body causing deformities, diseases and even death are called environmental toxicants (Patnaik, *et al.*, 2010). Fish are an excellent source of high-quality protein and other nutrients, vital for good health, including Iron, Calcium, Potassium, vitamin A, and Iodine. Especially for poor and food insecure populations fish and fish products are often the only source of animal protein (Jayaseelan, 2011). Selected fish is consumed by local population for their special taste and also nutritional qualities. The demand is also due to the fact that they can be kept alive in the market for a longer period in earthen pots. Imidacloprid (Confidor) is one of the most widely used insecticides in India and other parts of the World. Imidacloprid enters the target pest via ingestion or direct contact. Imidacloprid is a novel neonicotinoid insecticide which has become an important pest control agent on many crops. It is a systemic poison and shows medium soil adsorption. Imidacloprid is manufactured by Bayer crop science, since its launch in 1991, has gained registration in about 120 countries and is marketed for use on over 140 agricultural crops with annual sale of more than 600 million Euro (Cox, 2001). It is sold under many trade names which include Confidor, Admire, Gaucho, Merit, Advantage, Provado, and Winner. The proteins are most diverse bio molecule as these are of prime importance in biochemical reactions and cellular structures.

Modern agricultural practices result in indiscriminate use of various pesticides, which usually enter into the aquatic environment. Serum proteins have immunological properties in fishes and other animals as well as in human. Evidence suggests that pesticides pose potential health hazards to fish, birds, mammals and even to human beings. It is man's most important single source of high-quality protein, providing 16% of the animal protein consumed by the world's population, according to the Food and Agriculture Organisation (FAO, 1997) of the United Nations. It is a particularly important protein source in regions where livestock is relatively scarce. Fish supplies <10% of animal protein consumed in North America and Europe, but 17% in Africa, 26% in Asia and 22% in China (FAO, 2000). Toxic substances enter into human through food chain, as fish constitute an important part of protein in rural and urban areas (Rehman, *et al.*, 2002). Proteins occupy a unique position in the metabolism of cell. Proteins are used to build new tissues. Pesticides have a destructive action on all proteins. The reduced levels of protein can be due to reduction

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in protein synthesis and the liver cirrhosis (Reddy, *et al.*, 2015). Decrease of plasma protein could also result from the breakdown of protein into amino acids first and possibly into nitrogen and other elementary molecules. The present study has been undertaken to have a basic understanding of the acute toxicity of Imidacloprid insecticide on the experimental fish *Channa punctatus*.

II. MATERIAL AND METHODS

Healthy fresh water fish, *Channa punctatus* (av. length 08.40 ± 1.14 to 10.50 ± 1.56 cm and av. weight 12.14 ± 0.45 to 15.54 ± 0.48 gm) of both sexes were purchased from local market of Amalner, district, Jalgaon and used as the test animal, carefully brought to the laboratory avoiding any injury to the fish during transport. The fish were disinfected with 0.01% solution of $KmNO_4$ for two minutes and then transferred to a 45 L ($60 \times 30 \times 45$ cm) capacity glass aquarium to acclimatized for 15 days in laboratory conditions before use. The tank water was aerated continuously and food was provided daily in the morning in the form of chopped goat liver, meat, chicken as well as pieces of earthworm. The dead animals were removed as soon as possible from the test container to prevent water fouling. After acclimatization, fish were kept in different concentrations of Imidacloprid in to 5 L capacity plastic tubs for toxicity evaluation. The commercial grade Imidacloprid (1-[6-chloro-3-pyridylmethyl]-N-nitroimidazolidin-2-ylideneamine), chemical formula is $C_9H_{10}ClN_5O_2$ and the Chemical Abstracts Service (CAS) registry number 138261-41-3 (CCME, 2007) was chosen for the experiment and was obtained from the local market, Amalner. The toxicity of the Imidacloprid on the fresh water fish, *Channa punctatus* was assessed and determined LC_{10} , LC_{50} and LC_{90} for 24, 48, 72 and 96 hrs by probit analysis as per Organisation for Economic Co-operation and Development (OECD) guidelines for testing of chemicals (OECD 203 "Fish, acute toxicity test", 2010). After completion of experimental and control groups, five fish were taken without anesthesia after an interval of 24, 48, 72 and 96 hrs for biochemical study and five fish were kept for recovery study. For this study blood samples were taken by heart puncturing as well as posterior caudal vein using Ethylene-diaminetetraacetate (EDTA) as anticoagulant (Schmitt, *et al.*, 1999). The blood was immediately centrifuged at 1500 rpm for 15 min. Plasma was then removed and stored at $4^\circ C$ prior to immediate determination for various blood parameters. Total Proteins (TP) estimated by the Biuret method (Reinhold, 1953) by using kits which are produced by Autospan, Span Diagnostics, Ltd, Surat, Gujarat State-India. Total Protein contents was determined by using Biochemical analyser (Quantimate-RM 2012, Tulip Diagnostics, (P) Ltd. India, Mfg. by B.S.I., Italy) as per biochemical test kits manual. Experimental work was repeated at least five times. The obtained results were analyzed by statistically using two-way analysis of variance (ANOVA) by using Microsoft Excel, 2007.

III. RESULT AND DISCUSSION

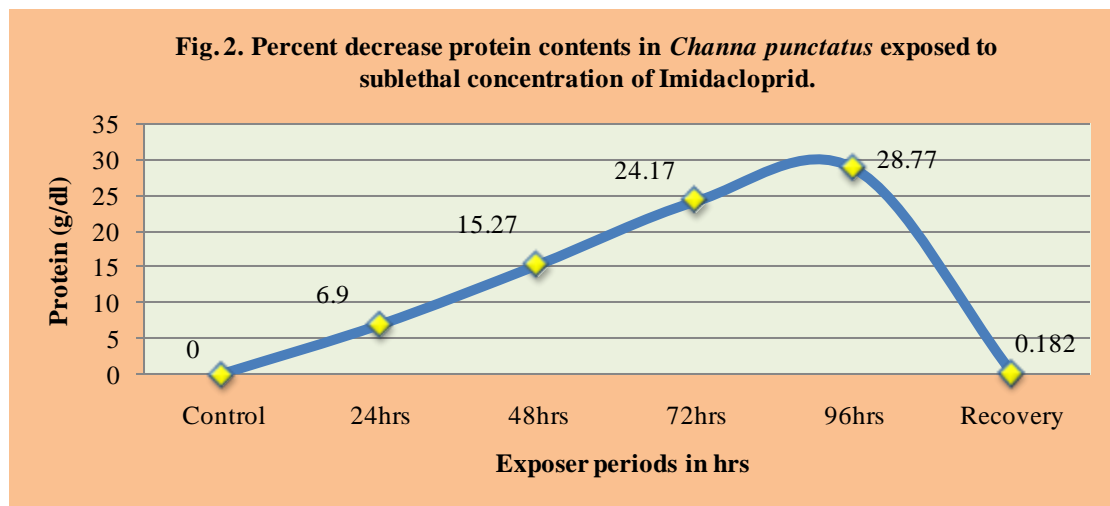
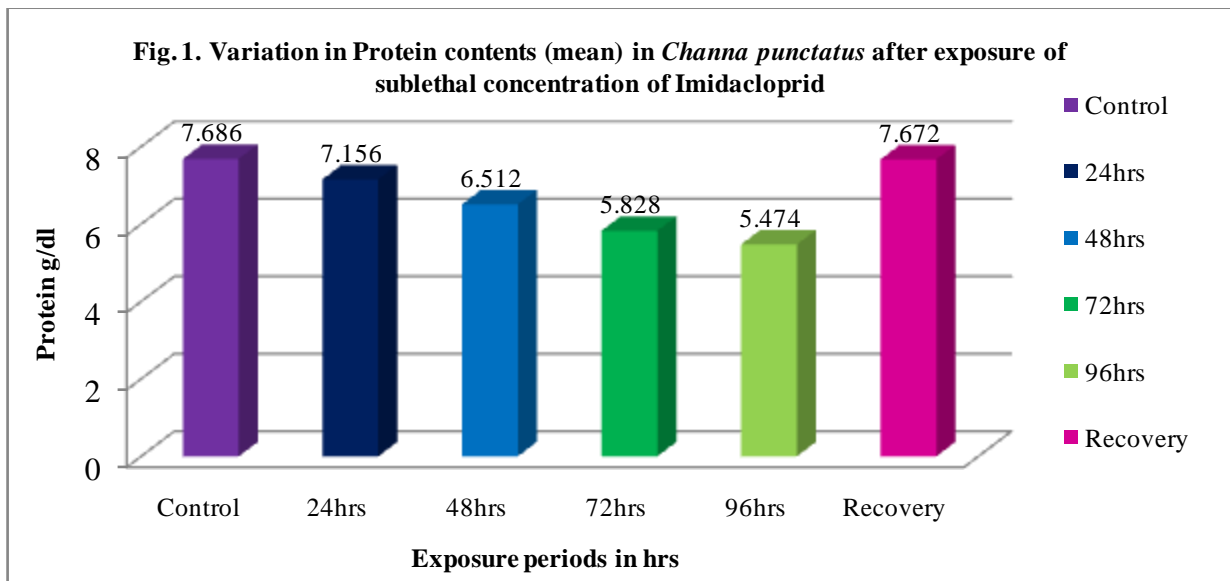
Kandeepan (2014) showed normal values of blood plasma protein such as 2.2 ± 0.4 mg/dl, 3.1 ± 0.18 mg/dl, 4.1 ± 0.23 mg/dl and 3.9 ± 0.3 mg/dl in *Channa striatus*, *Cyprinus carpio*, *Catla catla* and *Labeo rohita* respectively. Acharya and Mohanty (2014) noticed serum protein (g/dl) 4.22 ± 0.27 as well as 4.46 ± 0.32 in normal male and female respectively of walking Cat Fish, *Clarias batrachus* (Linnaeus, 1758). Kumar and Dahiya (2013) noticed serum protein level in control fish 8.40 ± 0.58 ($\mu g/g$) and reduced serum protein level 8.00 ± 2.54 ($\mu g/g$) (4.76%), 7.33 ± 4.34 ($\mu g/g$) (11.90%) and 6.34 ± 0.72 ($\mu g/g$) (24.06%) at concentrations 0.01, 0.02, 0.04 ppm of Zinc respectively after 45 days were observed in *Cirrhinus mrigala*. During the recovery period plasma showed normal protein level. Many pesticides have been reported to produce a plasma protein changes in fish at sublethal levels (Partapnand Bonga, 1990; Desai, *et al.*, 2002; Velisek, *et al.*, 2009; Abdul, *et al.*, 2010; Kumar and Dahiya, 2013 and Reddy, *et al.*, 2015). Total Protein level in plasma of *C. punctatus* exposed to sublethal concentration of Imidacloprid toxicity was found to be decreased moderately in experimental fish than the control. The difference between control and the experimental fish after 24 hrs, 48 hrs, 72 hrs and 96 hrs were statistically significant at $p < 0.05$. The variations and percent decrease in total proteins content is graphically represented in Fig. 1 as well as 2 respectively. Mean value of total protein content were in control, after 24hrs, 48 hrs, 72 hrs as well as 96 hrs and after recovery 7.686 ± 0.328 g/dl, 7.156 ± 0.237 g/dl, 6.512 ± 0.182 g/dl, 5.828 ± 0.223 g/dl, 5.474 ± 0.180 g/dl and 7.672 ± 0.189 g/dl respectively. Total protein decreased as compared to the control value where it was $6.90\% < 15.27\% < 24.17\% < 28.77\%$ and 0.182% after 24 hrs, 48 hrs, 72 hrs as well as 96 hrs of exposure and after 10 days recovery (Table 1). The decrease was significant ($P < 0.05$) in exposed fish. The

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decreased plasma protein was directly proportional to the exposure periods. Kumar, *et al.* (1997) observed reduced protein content in blood serum profiles in air breathing catfish, *Clarias batrachus* after sublethal effect of Endosulfan and Cypermethrin. Kumar, *et al.* (2011) observed serum protein in control fish such as 3.58 ± 0.595 mg/dl, 3.57 ± 0.639 mg/dl, 3.586 ± 0.64 mg/dl as well as 3.958 ± 0.71 mg/dl and reduced serum protein were 3.25 ± 0.58 mg/dl (-9.22), 3.01 ± 0.539 mg/dl (-15.69), 2.66 ± 0.48 mg/dl (-25.82) and 2.232 ± 0.399 mg/dl (-43.61) at the end of 8, 16, 24 and 32 days respectively were observed in *Cirrhinus mrigala* after sublethal concentrations of Chromium exposure. Serum total protein (mg/dl) for control 2.85 ± 0.90 , 3.83 ± 0.58 as well as 1.95 ± 0.07 and for treated 0.85 ± 0.08 , 2.85 ± 0.68 as well as 1.65 ± 0.21 at 2, 4 and 8 days sublethal (120 mg/l) Imidacloprid treated fish *Labeo rohita* observed by Qadir *et al.* (2014). Similar reduction in protein has been reported in *Channa punctatus* exposed to Cypermethrin (Das, *et al.*, 1999). There were also decreased protein levels in *Catla catla* exposed to Fenevalerate (Susan, *et al.*, 1999). All these changes are similar to following investigations. Several authors showed similar results reduction in RBCs, Hb and increase WBCs in different species of fish exposed to pesticides.



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Table 1. Variation in Protein content (mean) in *Channa punctatus* exposed to sublethal concentration of Imidacloprid.

Exposure period (hrs)	Protein (g/dl)	Decrease in %
Control	7.686±0.328	-
24	7.156±0.237	6.90*
48	6.512±0.182	15.27*
72	5.828±0.223	24.17*
96	5.474±0.180	28.77*
Recovery	7.672±0.189	0.182

Data are represented as mean±SD.

Data were analyzed through two-way ANOVA.

*Significant (P < 0.05), when treated groups were compared with control.

IV. CONCLUSION

In conclusion, alterations of total proteins in blood plasma can change the body metabolism of the fresh water fish *Channa punctatus* on acute exposure to sublethal Imidacloprid (Confidor). Fish with low protein values (due to insecticide or any toxicants) is not fit for human consumption. The present investigation showed a need for continuous pollution assessment study of aquatic organisms in fresh water body.

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