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Original article

Sub-lethal effect of synthetic pyrethroid pesticide on metabolic enzymes and protein profile of non-target Zebra fish, *Danio rerio*

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ABSTRACT

Extensive application of pesticide in agricultural field affects the enzymatic activity of non-target animals, including fishes. In this study, the impact of sublethal concentration of fenvalerate on marker enzymes of freshwater Zebra fish was evaluated. Pesticide-induced stress can specifically affect non target fishes, through elevated level of reactive oxygen species which is responsible for biochemical, cell metabolism and physiological activities. The oxidative stress mediated by fenvalerate at sub lethal concentrations after 28 days of exposure of Zebra fish. Following 28 days of exposure of pesticide, catalase, superoxide dismutase, aspartate amino transferases, alanine amino transferase, alkaline phosphatase and acid phosphatase were assessed. Results revealed reduction of superoxide dismutase activity after 28 days of exposure in sub lethal concentration of fenvalerate in liver and gills. In liver, catalase activity was found to be less in fenvalerate exposed fish than control fish. In liver, increase of 75.75% aspartate amino transferase and 38% increase in alanine amino transferase in gills. SGPT activity was relatively higher than SGOT suggests more contribution of phyruvalate than oxaloacetate formation. Fenvalerate induced changes in acid phosphatase and alkaline phosphatase activity in the liver and gills of Zebra fish after four weeks of exposure. Fenvalerate induced expression of various stress proteins in gill, liver, followed by muscle. Some proteins lost its intensity due to fenvalerate toxicity. Result revealed that enzyme assays and SDS-PAGE analysis for protein subunits determination is relevant tool to monitor stress in freshwater ecosystem. The findings suggest that in monitoring fenvalerate toxicity programme, enzyme activities can be potent diagnostic tool for fenvalerate induced toxicity.

1. Introduction

Pesticides are frequently applied to agricultural commodities to enhance quality and quantity of food. The unrestricted, heavy use of synthetic chemical pesticides results in deleterious effects, odour of water, taste, lethal effect on various non-target organisms in aquatic environment and direct or indirect effect to users (Kalavathy et al., 2001; Sathyamoorthi et al., 2019; Kumaresan et al., 2019). Although various eco-friendly methodologies such as, biopesticides, integrated pest management system, application of neem based biopesticides and other natural pesticides are largely available for pest management system, however, farmers rely on the chemical pesticides in most of the cases because of their good and immediate effect, easy availability.

Fish accumulate various fold higher concentration of chemical pesticide residues than the surrounding water in aquatic environment. Severe contamination of aquatic environment by chemical pesticides can cause chronic and acute poisoning of fish and other organisms. The accumulated pesticides damage skeletal system, various vital organs of fish and cause biochemical alterations in fish. The pesticide hazard to aquatic organism is further increased by biomagnification of the synthetic pesticides from water by aquatic

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organisms (Murty, 1986; Sathyamoorthi et al., 2018; Ravichandran et al., 2018; Sathyamoorthi et al., 2017). The pesticide causing epilepsy, cancer, kidney and liver dysfunction, leukemia, somatic growth depression, testicular cancer and decreased fertility (Scheel et al., 1996). The low solubility of synthetic pesticides greatly contributes to their high concentration in various fish and finally bioaccumulation in human body on consumption of these biopesticide contaminated fish. Despite their low availability, pesticides exhibit high toxicity to animals and humans their presence in food raises various safety issues (Caulibaly and Smith, 1994).

Danio rerio (Zebra fish) is a freshwater, tropical fish, small in size that is available in Asia and is considered as a good model organism in the field of developmental genetics and ecotoxicology (Ravichandran et al., 2017; Arasu et al., 2017a, Arasu et al., 2017b; Ravichandran et al., 2016; Arasu et al., 2016). The advantages of Zebra fish are its ease of maintenance, rapid development, in the laboratory condition, and also have high fecundity rate. Also the genomic information on Zebra fish was reported earlier (Zhu et al., 1985; Chaurasia et al., 2016a; Kumaresan et al., 2016; Chaurasia et al., 2016b; Kumaresan et al., 2015a). Recently, *Danio rerio*, has been used as a model organism to study diuron and diazinon induced toxicity (Velki et al., 2019). The whole genome sequence information and richness of bioinformatics data on the genetic characterization of Zebra fish makes it an ideal organism for analyzing pesticide toxicity and analysis of marker enzymes (Arockiaraj et al., 2015a,b; Palanisamy et al., 2015). Due to synthetic pesticide toxicity variations in the activity of enzymes were reported previously in various non targeted organisms, including fishes. Of various marker enzymes, catalase (CAT) and superoxide dismutase (SOD) are important to find oxidative stress caused by pesticide such as, fenvalerate. These enzymes are highly useful marker enzymes to explore the impact of toxic materials such as pesticides to aquatic organisms (Zaki et al., 2008; Chaurasia et al., 2015; Kumaresan et al., 2015b; Rao et al., 2015). Considering the earlier work of fenvalerate induced mortality on Zebra fish, the objective of this work was to evaluate how pesticide affect physiology of Zebra fish as a model organism. In this study fenvalerate induced oxidative stress was investigated at sub lethal concentrations in Zebra fish.

2. Materials and methods

2.1. Experimental animal

In the present investigation, Zebra fish was used as the experimental animal which was maintained in glass aquarium ($50 \times 25 \times 30$ cm. A total of 250 Zebra fish was maintained and acclimatized for 15 days prior to the experiment. One set of control experiment (n = 20) and three sets of experimental fish (n = 20) were maintained. About 14 h light and 10 h dark cycle was maintained. Control and experimental animal was fed with tubefex worm and pellet feed twice in a day. An aerator was attached with a mechanical air compressor to ensure continuous air supply. The experimental animal was subjected to sub lethal concentrations for 96 h at 30 ± 2 °C (1/10th LC_{50} value of 96 h). Toxicity analysis was made previously to determine lethal and sub lethal concentration of fenvalerate. All experimental animals were examined previously and all were free from any visible parasites externally (AFS-FHS, 2003). Experiment was performed for 28 days subjected to enzyme assays and protein profile analysis.

2.2. Samples and total protein content

Experimental and control group of animals were randomly selected and tissue, gill and liver were dissected out. About 0.2 g of tissues was homogenized individually with 2.0 ml sodium phosphate buffer (0.1 M, pH 7.4). It was centrifuged at 10,000*g* for 15 min. The supernatant was used for enzyme assay and estimation of total protein content. Total protein content of liver, gill and muscle was performed as described earlier (Lowry et al., 1951). Bovine Serum Albumin (100–1000 μg) was used to prepare standard curve.

2.3. Superoxide dismutase (SOD) assay

SOD assay of the gill and liver tissues of Zebra fish was performed (Bewley, 1996). The principle of this method is based on photochemical reduction of Nitroblue tetrazolium (NBT). Then the sample was read at 560 nm against reagent blank.

2.4. Catalase (CAT) assay

Catalase assay was performed in gill and liver samples. Briefly, the reagent mixture containing sodium phosphate buffer (pH 7.4, 0.1 M, 0.5 mM EDTA, 10 mM H_2O_2 and 0.2 ml sample). Reaction was performed at 37 °C and the sample was read at 240 nm (Lushchak and Bagnyukova, 2006).

2.5. Aspartate amino transferases and alanine amino transferases (SGPT and SGOT)

Blood sample was collected from experimental animal. About 10 Zebra fish was used for blood collection and collection was made between anal fin and caudal fin. The sample was centrifuged in a refrigerated centrifuge at 10,000*g* for 10 min and serum was stored at – SGOT and SGPT were assayed as suggested by Reitmann and Frankel (1957). About 0.2 ml sample from both control and experimental animal was added and reaction was initiated. Finally, the sample was read at 505 nm and enzyme units were calculated using a calibration curve.

2.6. Acid and alkaline phosphatase assay

Alkaline phosphatase (E.C. 3.1.3.1) assay was performed as described previously by Garen and Levinthal (1960) with little modifications. The enzyme assay mixture comprising bicarbonate buffer (0.1 M, pH 9.2), 0.1 M para-Nitrophenylphosphate (p-NPP) as substrate, sample (0.2 ml) and 0.1 M $MgCl_2$. Then the reaction mixture was incubated at 25 °C for 15 min and terminated using 0.1 N NaOH. One unit of ALP activity was defined as nanomoles of p-nitrophenol released/min/mg protein at specific temperature (25 °C). Acid phosphatase (E.C. 3.1.3.2) activity was carried out same like alkaline phosphatase except the application buffer in acidic range (acetate buffer, 0.2 M, pH 5) in place of bicarbonate buffer.

2.7. Sodium dodecyl sulphate polyacrylamide gel electrophoresis

SDS-PAGE analysis was used to determine fenvalerate induced protein profile changes in various organs of Zebra fish under stress conditions at sub-lethal concentration. Gills, liver and gut sample was analyzed from control and experimental group.

2.8. Statistical analysis

One way analysis of variance (ANOVA) was applied to explore the impact of fenvalerate on metabolic enzyme changes. The p-value (<0.05) was confirmed the significant of changes in metabolism due to fenvalerate toxicity at 95% level.

3. Results and discussion

The damage of cellular components and tissues is because of oxidative stress, mainly due to the reaction oxygen species and oxygen free radicals (Valavanidis et al., 2006). Each organisms have various anti oxidative defense mechanism, mainly composed of both non enzymatic and enzymatic components (Venancio et al., 2013). Antioxidant defense mechanism comprising enzymes such as, catalase, superoxide dismutase. The SOD is the set of mettaoenzymes that significantly play as antioxidant and comprise the important primary defense mechanism against the toxic properties of superoxide radicals in organisms (Kohen and Nwani, 2010). In this study, SOD activity was 1.038 ± 0.0017 U/ml in the liver of control fish and in fenvalerate exposed fish it lowered as 1.033 ± 0.0009 U/ml. Results revealed reduction of SOD activity after 28 days of treatment in sub lethal concentration of fenvalerate. In gills, observed decreased SOD activities in experimental fenvalerate exposed fish than control fish. But the reduction of SOD activity was not statistically significant ($P > 0.05$) (Fig. 1). Catalase involves the removal of hydrogen peroxide into molecular oxygen and water (Van der Oost et al., 2003). In the present study, CAT activity was decreased significantly in the fenvalerate exposed fish than control fish ($P < 0.05$) both in liver and gill (see Figs. 2–4).

In liver, CAT activity was 3.028 ± 0.019 U/ml in the control fish, whereas decreased level (2.826 ± 0.31 U/ml) was observed in fenvalerate treated fish. Recently Stara et al. (2012) assessed chronic exposure of prometryne in *Cyprinus carpio* L. and changes in SOD activity was reported after 60 days of exposure in gill and brain. However, variation in CAT was observed at higher concentration (80 μg/l) in intestine and liver after 60 days. CAT enzyme activity in the tissues of organs may be in response of H_2O_2 produced by the catalytic activity of SOD (Rossi et al., 2011; Toni et al., 2011).

In this study increased activity of SGPT and SGOT was observed at sub lethal exposure of fenvalerate. In liver, increases of 75.75% SGPT and 38% raise in gill tissue was observed. The elevated level of either SGPT or SGOT suggests increased synthesis of aminoacids or increased transamination process from fatty acids or glucose during fenvalerate intoxication. In this study, SGPT activity was relatively higher than SGOT suggests more contribution of phyruvalate than oxaloacetate formation. In *Channa gachua* sub lethal concentration of dichlorous increased the level of SGPT and SGOT activity (Koul et al., 2007). Tilak et al. (2009) reported increased level of SGPT and SGOT when *Channa punctatus* (Bloch) exposed to alachlor. In *Labeo rohita*, SGPT and SGOT in blood and liver increased after treatment with endosulfan (Saravanan et al., 2010).

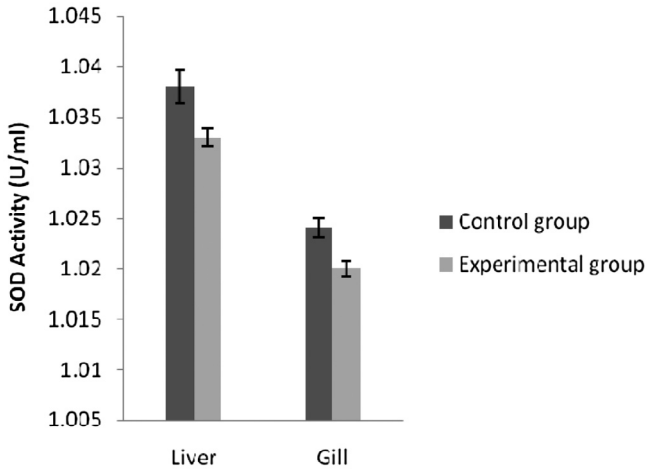

Fig. 1. Effect of pesticide on SOD activity. Error bar shows standard deviation and reporesents mean value of three experiments.

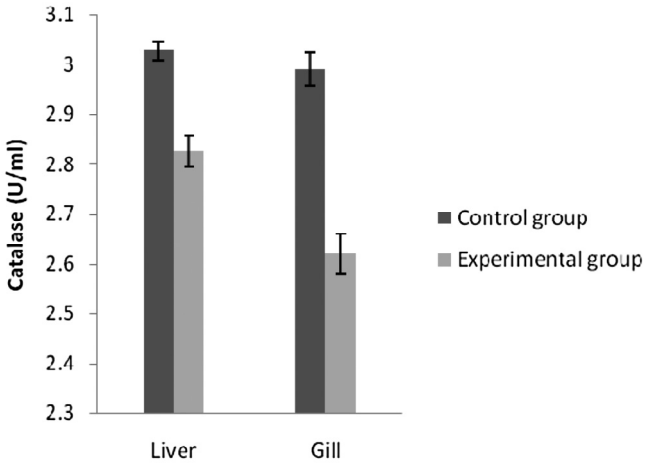

Fig. 2. Effect of pesticide on CAT activity. Error bar shows standard deviation and reporesents mean value of three experiments.

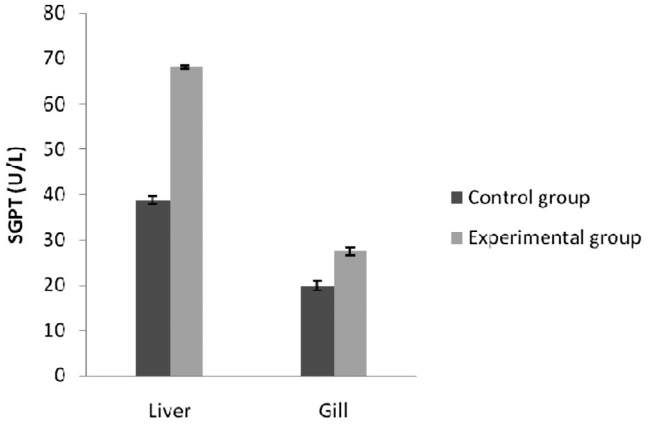

Fig. 3. Effect of pesticide on SGPT activity. Error bar shows standard deviation and reporesents mean value of three experiments.

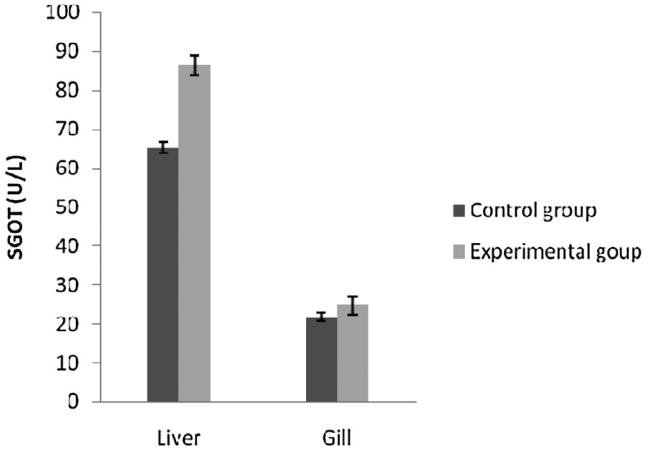

Fig. 4. Effect of pesticide on SGOT activity. Error bar shows standard deviation and reporesents mean value of three experiments.

In this study fenvlerate induced alterations in alkaline phosphatase activity in the liver of Zebra fish after four weeks of exposure at sub lethal concentrations. Similarly, in the catfish, *Heteropneustes fossilis* upon fenvalerate treat, elevated level of ALP was reported. After 28 days of exposure experimental animal

responded 17% of reduced ALP activity than control. The observed ALP activity was lower in the experimental Zebra fish in liver is statistically significant ($P < 0.05$). In control fish, liver sample showed 143 ± 5.3 U/ml and it was 132 ± 4.9 U/ml in experimental animal (Fig. 5).

ALP activity in liver may cause alterations in glycogen content. ALP in liver effectively inactivates phosphorylase enzymes, thus involving glycogen synthesis (Parthasarathi and Karuppasamy, 1998). The toxic pollutants may interact with regulators and co-factors and may inhibit enzyme activity. The low activity of acid and alkaline phosphotase in pesticide treated Zebra fish indicated damage in cell organelles such as, membrane transport system and endoplasmic reticulum. The variation in acid phosphatase activity in the fenvalerate exposed Zebra fish for 28 days is described in Fig. 6. In this study, acid phosphatase level significantly decreased in fenvalerate exposed Zebra fish. In *C. punctatus*, decreased level of acid posphatase was observed after the exposure of fenvalerate. Accumulation of toxic materials in the liver beyond tolerant limit causes various enzymatic changes. The decreased trend of acid phosphatase activity in fish was mainly due to changes in the mitochondrial membrane function or due to increased glycogenolysis (Parthasarathi and Karuppasamy, 1998).

Xenobiotics are well known substances to induce mutagenesis and various oxidative stress by mediating free radicals or reactive oxygen species (Tabrez and Ahmad, 2011b). In fish, antioxidant property changes due to various stress, however, high concentration of zenobiotics, may alter SOD enzyme activity (Ballesteros et al., 2009). The alteration of antioxidant enzymes in fishes in various tissues showed the variation in oxidative stress (Oruc and Usta, 2007). In *Clarias batrachus,* carbofuran treated fish showed elevated level of SGPT and SGOT in liver tissue and muscle (Begum, 2004). Kaneko (1989) reported that differences in enzyme production mainly occur due to impaired circulation, cell death, and obstruction of normal excretory route in fish. It could be noted that variations in SGOT and SGPT activity is mainly due to damage of liver cells. These clearly illustrated that Zebra fish was under stress due to fenvalerate toxicity. The present finding concludes exposure of Zebra fish to fenvalerate caused alterations in oxidative stress.

In this study, fenvalerate induced expression of various xenobiotic stress proteins inorder to survive in the toxic environment. SDS-PAGE revealed variations in the protein profile of the gill of control fish and fenvalerate treated fish and showed variations in protein profile between control and fenvalerate exposed Zebra fish. The protein sub units with the molecular weight of 43 kDa, 69 kDa ad 92 kDa was high intensity in fenvalerate treated fish than control fish. Also high intensity protein subunits were appeared at 153 kDa, 160 kDa, 165 kDa, 172 kDa, 189 kDa, 190 kDa and 198 kDa (Fig. 7a). In the muscle of fenvalerate exposed fish, a high intense protein sub unit was observed at 205 kDa. Also, a protein subunit with molecular weight of 50 kDa and 51 kDa were observed in the muscle of fenvalerate exposed Zebra fish (Fig. 7b). The selected xenobiotics induced to synthesize number

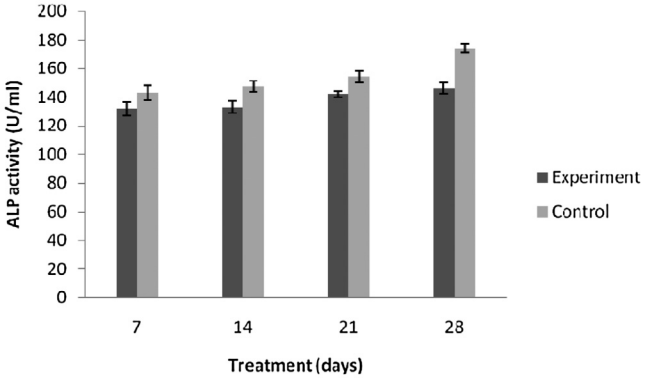

Fig. 5. ALP activity of liver sample of Zebra fish exposed to fenvalerate at sub lethal concentrations.

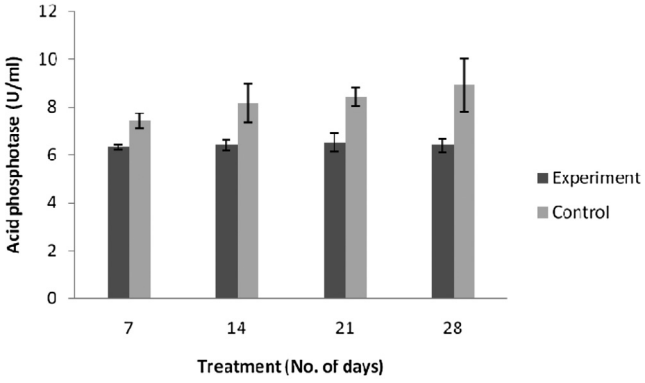

Fig. 6. Acid phosphatase activity of liver sample of Zebra fish exposed to fenvalerate at sub lethal concentrations.

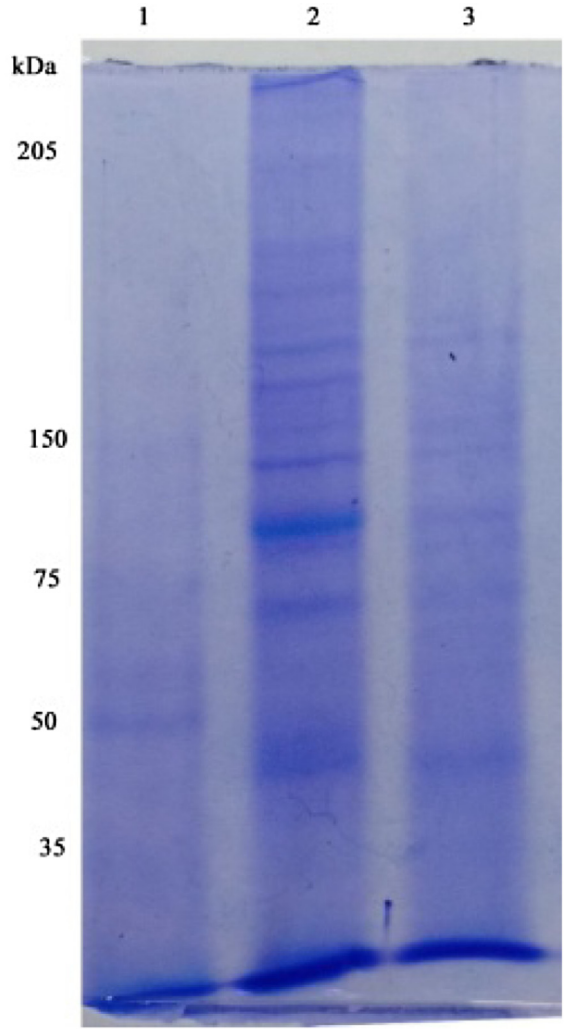

Fig. 7a. SDS-PAGE analysis of fenvalerate induced protein profile changes in the gills of Zebra fish under stress conditions at sub-lethal concentration (1 – protein molecular weight marker; Lane 2 – fenvalerate exposed gills; Lane 3 – control fish).

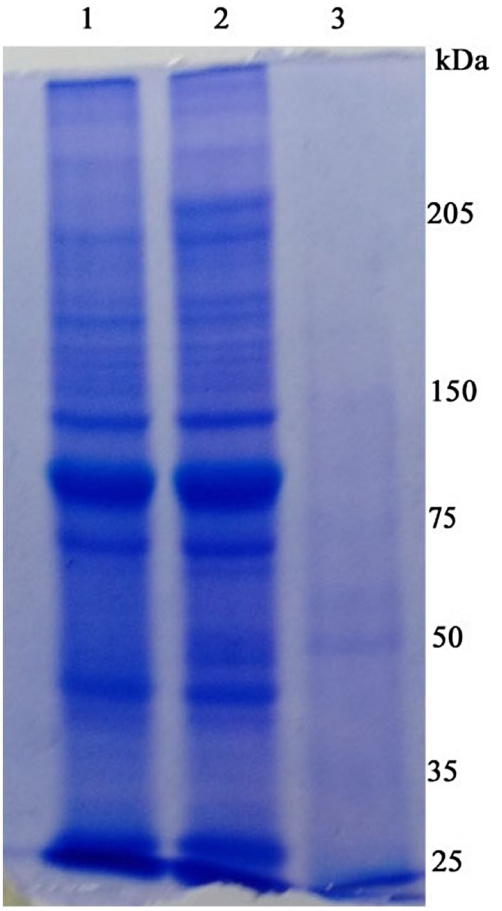

Fig. 7b. SDS-PAGE analysis of fenvalerate induced protein profile changes in the muscle of Zebra fish under stress conditions at sub-lethal concentration (1 – muscle from the control fish, 2 – muscle from the fenvalerate exposed fish; 3 – protein molecular weight marker).

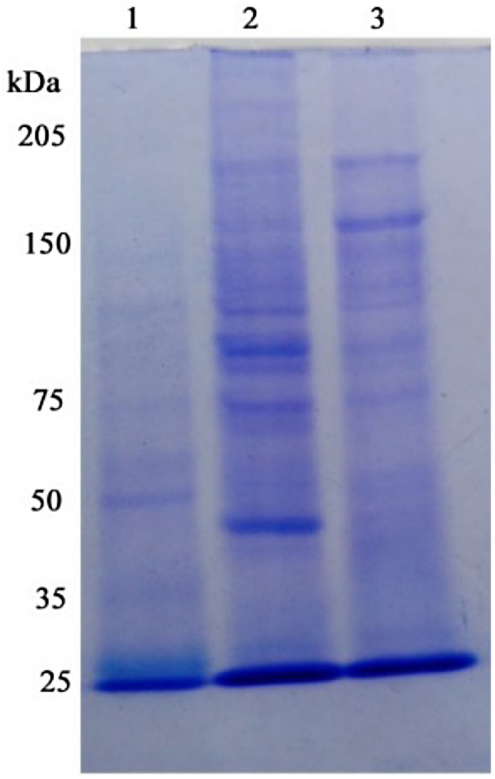

Fig. 7c. SDS-PAGE analysis of fenvalerate induced protein profile changes in the liver of Zebra fish under stress conditions at sub-lethal concentration (1 – protein molecular weight marker, 2 – liver from fenvalerate exposed fish, 3 – liver from control fish).

of stress proteins in gills than muscle. This clearly reveals, gill is most susceptible to fenvalerate toxicity in the environment. Liver is one of the most affect organs due to fenvalerate toxicity and the variations of protein sub unit prove that Zebra fish is under stress conditions. Fenvalerate induced changes in liver and shows high intensity protein subunits at 44 kDa, 75 kDa, 103 kDa and 121 kDa. In other hand, less dense protein bands (150 kDa and 168 kDa) were observed in the liver stipulated protein degradation in this vital organ. A subunit with molecular weight 205 kDa was observed only in the fenvalerate treated fish which was not detected in the control fish (Fig. 7c). The variation in the subunit of gill, muscle and liver protein may due to expression of some stress genes. The mechanism of action of pesticides and and fenvalerate on protein synthesis was hypothesized. The used pesticides may significantly inhibit the expression of gene or various genes or may activate other set of genes to produce mRNAs which may subsequently be translated into stress induced proteins to survive in the pesticide stress environs (Ksenia et al., 2008; Daniel et al., 2004). Moon et al. (2016) reported the molecular and biochemical differences in Zebra fish larvae due to endosulfan toxicity. Morphological changes, including shortened tail and curved spines were reported due to endosulfan toxicity. Many biomarkers were reported to measure environmental toxicity in ecosystem. Previous applications of biomarkers have mainly involved primarily laboratory (Wolkers et al., 1996) or pointsource contaminated field sites rather than highly complex contaminated ecosystem (Adams et al., 1992a). In these days, various environmental problems are not much related to point-source contamination, but not highly relevant to point-source contamination, but to highly complex systems that may be contaminated by various non-point sources inputs. SDS-PAGE determination of proteins in control and fenvalerate exposed Zebra fish in various organs are useful to determine point-source contamination and complex contaminations. Recently, Muhammad et al. (2018) used SDS-PAGE analysis as an important biomarker for various toxicological studies in fishes.

4. Conclusions

Exposure of pesticides on non-targeted fish population continuously causes various biochemical and metabolic changes. Also, consumption of this fish pose health hazard to the human population. The present finding revealed fenvalerate induced impairment in metabolism in Zebra fish. An increase in the activities of SGPT, SGOT indicating injury in the liver, inflammatory disease or hepatic damage. The variations in the enzyme activities of SOD, CAT, ALP and Acid Phosphatase indicated mitochondrial disruption and tissue damage as a result of fenvalerate induced stress. Also, sub lethal level of fenvalerate exposure at longer time induced changes in protein metabolism. The findings suggest that in monitoring fenvalerate toxicity programme, enzyme activities can be potent diagnostic tool for fenvalerate induced toxicity.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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