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Effect of profenofos pesticide on protein levels in gill and muscle of freshwater fish *Labeo rohita* (Hamilton, 1882)

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Abstract
The present study indicates a brief account of the toxic effects of pesticide profenofos on fish. Pesticides are extremely hazardous for the health of fish. Profenofos toxicity on amino acid and protein content in gill and muscle of fish *Labeo rohita* have been studied. The fish exposed to profenofos of low, medium and high concentrations of the period of 15 and 45 days. The outcomes showed decline in the protein content and expansion in the amino acid substance in gill and muscle of fish *Labeo rohita*. The significant reduction in protein content and elevated levels of amino acids is apparently indicative of the toxic effect of pesticide profenofos on biochemical parameter and organism's response to the toxic stress.

Keywords: *Labeo rohita*, pesticide, profenofos, toxicity, protein, amino acid

Introduction
Pesticides are every now and again applied to agricultural to upgrade quality and amount of food. The unrestricted, substantial utilization of manufactured synthetic pesticides brings about harmful impacts, smell of water, taste, deadly impact on different non-target creatures in aquatic environment and immediate or round about impact to clients (Kalavathy *et al.*, 2001; Sathyamoorthi *et al.*, 2019; Kumaresan *et al.*, 2019; Al-Ghanim *et al.*, 2020) [2, 3, 4, 1]. Al-Ghanim et al., (2020) [1] revealed different eco-accommodating strategies, for example, biopesticides, incorporated vermin the executives framework, utilization of neem based biopesticides and other normal pesticides are to a great extent accessible for bug the board framework, in any case, ranchers depend on the compound pesticides in a large portion of the cases on account of their great and quick impact, simple accessibility. The bug sprays structure significant contaminations of water bodies which add to the ecological issues. Precipitation, overflow and air affidavit establish the significant courses of insect poisons to amphibian biological systems. Most insect poisons at last arrive at the waterways, lakes and lakes and have been viewed as harmful to non-target living beings that occupy the water bodies near the horticultural fields (Arjmandi *et al.*, 2010; Tasneem *et al.*, 2021) [5, 6]. Notwithstanding, the escalated utilization of these synthetic substances in farming and public tasks has changed the biological equilibrium of numerous non-objective organic entities like fishes. The low solvency of manufactured pesticides incredibly adds to their high fixation in different fish lastly bioaccumulation in human body on utilization of these bio pesticide debased fish. Notwithstanding their low accessibility, pesticides show high harmfulness to creatures and people their quality in food raises different security issues (Caulibaly and Smith, 1994) [7]. All pesticides are harmful to animals and people through straightforwardly or by implication by openness or dietary admission. These organophosphate compounds were likewise often engaged with word related and unintentional harming. It was accounted that in 2013, the cumulative insecticide usage was about 3120 tonnes against 2889 tonnes in the previous year (2012). Among the synthetic pesticides, organophosphate ranked fist than other pesticide consumption (Mahmoud *et al.*, 2020) [8]. Among oceanic fauna, fish are exceptionally touchy to wide assortment of poisons particularly pesticides, which cause injurious impacts through gathering (Herger *et al.*, 1995) [10]. Pesticides collected in tissues prompts numerous physiological and biochemical changes in this manner impacting the exercises of a few chemicals and metabolites lastly causes the whole metabolic cycle upset (Chandra and Sajda, 2015). Profenofos (PFF) is a broadspectrum organophosphate habitually utilized in India for household purposes (Chatterjee *et al.*, 2021). PFF normally utilized in horticulture for controlling creepy crawlies in cotton, paddy. It is incredibly harmful to sea-going living beings through hindrance of acetylcholinesterase catalyst bringing about neurotoxicity and oxidative pressure thus, prompting demise of the organic entities (Bacchetta *et al.*, 2014; Pamanji *et al.*, 2016; Rahman *et al.*, 2020). Acute toxicity investigations of PFF have been accounted for before on normal carp, *Cyprinus carpio* (L.) (Ismail *et al.*, 2009), spotted snakehead, *Channa punctatus* (Bloch) (Pandey *et al.*, 2011) and zebrafish, *Danio rerio* (Hamilton) (Pamanji *et al.*, 2016). The aim of the present study is to understand the effect of Profenofos on protein content and amino acid content in gill and muscle of freshwater fish *Labeo rohita*.

Materials and Methods
Procurement and Rearing of Experimental Animal
Healthy *Labeo rohita* having mean weight 13-16 gm and length 10 – 14 cm were collected from PSP fish farm, at Puthur and acclimatized to laboratory conditions (29 ± 1°C). The fish were fed daily on oil-less groundnut cake. The unused food was renewed after 2 hours and water was changed daily. Prior to experimentation the fish were acclimatized to experimental tanks for at least one week.

Pesticide

Organophosphorus insecticide profenofos (commercial name is Selecron) purchased from local agro chemist shop was used for the present study.

Experimental Design

A total of 40 fishes (10 fishes per aquarium) were separated as four groups. The following experimental groups were conducted in the freshwater fish *Labeo rohita* for the period of 45 days. Sub lethal doses preferred on previous references.

Group 1 *Labeo rohita*, without any pesticide exposure (control)

Group 2 *Labeo rohita*, on exposure to 0.50 ppb Profenofos for a period of 15 and 45 days

Group 3 *Labeo rohita*, on exposure to 1.00 ppb Profenofos for a period of 15 and 45 days

Group 4 *Labeo rohita*, on exposure to 1.50 ppb Profenofos for a period of 15 and 45 days

At the end of experiment, the control and experimental fishes were sacrificed. The gill and muscle were removed from both control and treated fishes for biochemical estimation.

Estimation of Protein and Amino acid

Protein contents in the tissues were estimated by the method of Lowry *et al*. (1951) [17]. Total free amino acids content of the tissue were estimated by the method of Moore and Stein (1954).

Statistical Analysis

The values are expressed as mean ± SD. Data were statistically analyzed by Analysis of Variance (ANOVA) along with Duncan's Multiple Range Test (DMRT) (Duncan, 1957) [19] which was applied to find out significant difference between various treatment means and control means for the observed parameters.

Results

The present results revealed that profenofos induced alterations are time dependent, tissue specific and they point to altered protein metabolism has shown significant elevation of amino acid and decrease the levels of protein in gill and muscle of *Labeo rohita* exposed to low, medium and high sublethal concentrations of profenofos for the period of 15 and 45 days. The reduction in protein content is directly proportional to the exposure period of the toxicant. The obtained biochemical estimation values of gill and muscle were subjected to statistical analysis and showed significant values at $P<0.05$. The increase in the amino acid content in the gill and muscle of the *Labeo rohita* exposed to profenofos for 15 and 45 days in low, medium and high concentrations were estimated. Protein and amino acid content in the gill and muscle are shown in Table 1, 2, 3 and 4. The content of amino acid in gill of treated fish shows a gradually increase in amino acid level than that of amino acid content in the muscle. On the other hand, the elevation of amino acid was observed at all exposure periods of gill and muscle.

Table 1: Changes of protein levels in gill of *Labeo rohita* exposed to Profenofos.

Treatments	15 days (Protein level (mg/g)	45 days (Protein level (mg/g)
Control	110.30 ± 8.31^{c}	113.40 ± 8.73^{c}
Low	91.70 ± 7.04^{b}	85.18 ± 6.45^{b}
Medium	81.62 ± 6.53^{ab}	74.60 ± 5.71^{a}
High	78.85 ± 5.40^{a}	69.36 ± 5.20^{a}

All the values mean ± SD of six observations.
Values which are not sharing common superscript differ significantly at 5% ($p<0.05$)
Duncan multiple range test (DMRT)

Table 2: Changes of protein levels in muscle of *Labeo rohita* exposed to Profenofos.

Treatments	15 days (Protein level (mg/g)	45 days (Protein level (mg/g)
Control	70.30 ± 5.20^{a}	68.42 ± 5.30^{c}
Low	65.32 ± 5.04^{a}	59.26 ± 4.43^{b}
Medium	63.74 ± 4.92^{a}	55.32 ± 4.30^{ab}
High	60.48 ± 4.76^{a}	50.20 ± 3.90^{a}

All the values mean ± SD of six observations.
Values which are not sharing common superscript differ significantly at 5% ($p<0.05$)
Duncan multiple range test (DMRT)

Table 3: Changes of Amino acid levels in gill of *Labeo rohita* exposed to Profenofos.

Treatments	15 days (Amino acid level mg/g)	45 days (Amino acid level mg/g)
Control	3.11 ± 0.23^{a}	3.10 ± 0.25^{a}
Low	4.27 ± 0.32^{d}	6.36 ± 0.50^{b}
Medium	4.80 ± 0.37^{c}	8.95 ± 0.72^{c}
High	6.30 ± 0.40^{d}	13.09 ± 0.79^{d}

All the values mean ± SD of six observations.
Values which are not sharing common superscript differ significantly at 5% ($p<0.05$)
Duncan multiple range test (DMRT)

Table 4: Changes of Amino acid levels in muscle of *Labeo rohita* exposed to Profenofos

Treatments	15 days (Amino acid level mg/g)	45 days (Amino acid level mg/g)
Control	2.21 ± 0.20^{a}	2.27 ± 0.21^{a}
Low	2.81 ± 0.25^{b}	3.80 ± 0.30^{b}
Medium	3.20 ± 0.26^{c}	4.22 ± 0.35^{b}
High	4.40 ± 5.05^{d}	5.49 ± 0.50^{c}

All the values mean ± SD of six observations.
Values which are not sharing common superscript differ significantly at 5% ($p<0.05$)
Duncan multiple range test (DMRT)

Discussion

The steadily expanding utilization of pesticides causes synthetic contamination results potential wellbeing perils to live stock, specifically to fish, frogs, birds and warm blooded animals. Proteins are complicated substance with high sub-atomic compound weight structure the underlying system, yet in addition cog wheels and switches of the working component in the living compensation body. Proteins are valuable for the polypeptide chains of amino corrosive particles. The proteins are valuable for the vehicle and capacity. Explicit proteins transport numerous little particle and particles. A protein was intricate that directs the arrangement of neural organizations in higher living beings. The essential capacity of protein food is to supply the amino acids required for the development, fix and general upkeep of the primary and reactant apparatuses of living (Sangeeta Sharma and Agarwal, 2004) [20]. The diminished protein content may likewise be because of tissue annihilation, unsettling influence of cell division and ensuing weakness in protein manufactured apparatus (Bradbury, 1987) [21]. The current work demonstrates that pesticides caused changes in the protein digestion of fish treated tissues. The modified portability and low substance of proteins mirrors an adjustment of the pace of amalgamation and debasement of protein. Proteins are predominantly associated with the design of the cell. During stress conditions they are a wellspring of energy as fish need more energy to detoxify the poison and to conquer pressure (Naqvi *et al.*, 2017) [22].

Channa punctatus exposed to various concentrations of chloryrifos for the periods of 15 and 45 days showed decrease the protein and elevated levels of amino acid in different tissues like liver, kidney and brain (Revathi *et al.*, 2020) [23]. Depletion of protein and increased levels of free amino acids in the muscle of fish *Channa punctatus* exposed to lethal and sublethal concentrations of insecticides chlorantraniliprole (Nagarajulu *et al*. 2018). Naqvi *et al.*, (2017) [22] reported that disturbance in the physiological activity of fish *Oreochromis mossambicus* exposed to Pesticides which intern caused alteration in the protein content in tissues. Chandra and Sajda, (2015) [9] reported that protein metabolism decreased in the kidney, liver and gills of *Clarias batrachus* exposed to Pesticide Rogorin. Simillarly, protein levels were decreased and amino acid contents increased significantly in gill, liver and kidney of Cyprinus carpio exposed to sublethal concentration of pharmaceutical effluent (Muthulingam *et al.*, 2011) [24].

Rahman et al., (2020) [12] reported that decrease the level serum protein of fish *Cyprinus carpio* exposed to profenofos which compared to diet croup of *Pelargonium graveolens*. Ghazala et al., (2019) [28] observed that the toxic effects of Profenofos exhibited negative effects on protein metabolism in muscle of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*. The decreased protein level was observed in the liver and muscles tissue of Oreochromis niloticus at sublethal concentration of profenofos (Sharafeldin *et al.*, (2015) [27]. Jagadeesan and Darcus (2012) [26] reported that the decrease in total protein content in turn affects the *Catla catla* exposed to profenofos toxicity. Protein levels was decreased of vital organs such as brain, gill, kidney, liver and muscle exposed to profenofos of fresh water fish *Labeo rohita* (Nagaraju and Rathnamma, 2013) [32].

The current discoveries upheld by Francis and Muthulingam (2018) [30] revealed decline in protein and expansion in aminoacid levels in every one of the tissues of *Channa striatus* presented to sublethal concentration of lead acetic acid derivation. A pesticidal mixture utilized againat *Clarias batrachus* incited changes in protein content (Jha and Verma, 2002) [29]. Thenmozhi *et al.*, (2011) investigated that malathion diminished protein substance in *Labeo rohita*. Exhaustion of protein movement in indian significant carp *Labeo rohita* treated with intense grouping of pesticide malathion (Sivanandan *et al.*, 2021) [31].

A few different examinations additionally uncovered an abatement in protein profiles and expansion in amino acid with organophosphate compounds. This large number of examinations support the current investigation of diminishing pattern of proteins and expanding pattern of amino acid in the tissues of fish *Labeo rohita* exposed to Profenofos

Conclusion

It tends to be determined from the current discoveries that the profenofos exposed to *Labeo rohita* caused pressure condition and energy emergencies which thusly modify protein digestion.

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