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Studies on the Effect of Neemgold on Fingerlings of Common Carp, *Cyprinus Carpio*

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ABSTRACT
Neem (*Azadirachta indica*) is a medicinal plant of containing diverse chemical active substances of several biological properties. In the present study, the toxic effect of Neemgold to the fingerlings of Common carp *Cyprinus carpio* was evaluated. The aim of the present work was to study the toxic effects of Neemgold on the total protein, total carbohydrates, amount of cholesterol and sterols. The LC_{50} of Neemgold was estimated by Static renewal method. The Neemgold was 0.002ml/l, 0.0018ml/l, 0.0016ml/l, and 0.0014ml/l at 24h, 48h, 72h, and 96h respectively. The fish exhibited erratic swimming, loss of equilibrium, copious mucus secretion and hitting to the walls of test tubs prior to mortality while exposing long duration. During the present investigation we observed significant alterations in the total protein, total carbohydrates and amount of cholesterol and sterol contents in the tissue of *Cyprinus carpio* exposed to Neemgold. These results indicate that *Azadirachtin* exposure to the fish caused toxic effects.

Key words: Common carp, Neemgold, *Cyprinus carpio*, Toxicity, Fingerlings.

INTRODUCTION:

Azadirachta indica is one of the most promising medicinal plants, having a wide spectrum of biological activity, well known for its insecticidal properties (ICAR, 1993). Toxicology remains the science, which promises real opportunities to unravel some of the fascinating problems of biology by identifying chemicals and physical tools with which to probe living processes. This eco-friendly native tree of India is perhaps most researched tree in the world. Pesticides are the chemical substances used for plant protection. A pesticide is used to destroy, suppress or alter the life cycle of any pest (Bhushan *et al*., 2013). Pesticides act selectively against certain organisms without adversely affecting other group of organisms. Absolute selectivity is however difficult to achieve and also most pesticides are toxic risk to non-target organisms (Bolognesi, 2003). Farmers and agricultural laborers are direct exposed to the pesticides and are more likely to get affected by the acute toxicity of pesticides. The residues left in the crops, soil and water after use get into the human food chain. In India, the use of pesticides has now reached over 1020 tons per annum (Hicks *et al*., 1998).

Fish have been used as indicator for contamination of aquatic environment. During environmental catastrophe, fish are unable to escape from the site affected, thus bio accumulate toxic substance (Andrade *et al*., 2004). Early detection of the sub lethal effects of a chemical may be the basic element is deciding about biodegradation and revitalization methods at polluted site (Tiwari *et al*., 2003). The main route of metabolism includes the ester hydrolysis, oxidation and conjugation (Manab kumar dutta *et al*., 2015). However, the metabolism of this compound in fish is oxidative. The plant Neem tree (*Azadirachta indica*) of the family Meliaceae is native to India and was adapted to grow in Brazil (Immich *et al*., 2009). The plant contains oil with insecticidal properties. The most important source of oil is fruits which affect the insects in many ways and leaves can also be used for pest control (Schmutterer, 1988). To evaluate the biological effects of such pesticides, bioassay test were conducted to know the strength of the toxicity. Similarly we have undertaken biochemical estimations such as total protein, total carbohydrates, amount of sterol and cholesterol. Since fish tends responded to toxicants in a similar way to higher vertebrates, they can be used to screen for potential toxicants to humans. The main application of fish using a test model is to determine the distribution and effects of chemical contaminants in the aquatic environment which is useful to assess the toxic effects.

MATERIAL AND METHODS

Experimental animal

Cyprinus carpio L. is one of the important Indian major carp (Fig. 1), which together with other major carps contribute to more than 81.53% of the Inland aquaculture production in India (FAO, 2003) is being selected as test animal for the present study. It occurs abundantly in the fresh water tanks, rivers and ponds. It is largely employed for pond culture throughout the country. It is a bottom dweller and omnivorous and they feed mainly on insects, crustaceans, crawfish and benthic worms.

Fig 1: **Experimental animal *Cyprinus carpio***

Procurement and maintenance

Fingerlings with 8±2cms length and 25±2grams of weight of *C. carpio* were collected from the State Fisheries Department, Bhadra Reservoir Project, Shimoga, Karnataka and were reared in a large tubs and acclimatized in tap water having dissolved oxygen 6.02±0.8 mg/L, P^H 7.4±0.2, Total hardness of 28±2 mg/L and room temperature 29±1^{o}C for 15 days prior to bringing them to laboratory. Prior to stocking in the large plastics tubs, fishes were washed with Potassium permanganate (1ppm). Fishes were fed with powdered rice bran and groundnut oil cake (3:1) and also a commercially available food then feeding was stopped 24 hours prior to expose to the test medium for acute toxicity tests. The water of the plastic tubs was changed daily once to avoid any fungal and bacterial contamination and 1% Potassium permanganate solution was sprayed to eradicate any bacterial or fungal infection. The temperature of water in the tubs was 27±1^{o}C and the same was the maintained throughout the course of investigation.

Physico-chemical characterization of water

The physico-chemical characteristics of fish habitat water were analyzed by following the methods mentioned in APHA (APHA, AWWA and WEF 2005) viz., temperature, pH, dissolved oxygen, Carbon dioxide, Total hardness as $CaCO_3$/L, Alkalinity and Chlorine. All the water quality parameters were monitored throughout the experimental period except for minimal variation which is tolerated by the fish in the wild.

Nature of Neemgold

The insecticidal ingredient found in the Neem tree is azadirachtin, a naturally accruing substance that belongs to an organic molecule called tetranortriterpenoids (Fig. 2). It is structurally similar to insect hormones called "ecdysones" which control the process of metamorphosis. It is seems to be an "ecdysone blocker". It blocks the insect's production and release of these vital hormones. It is used to control white flies, aphids, trips, fungus gnats, caterpillars, beetles, gypsy moths and others on food.

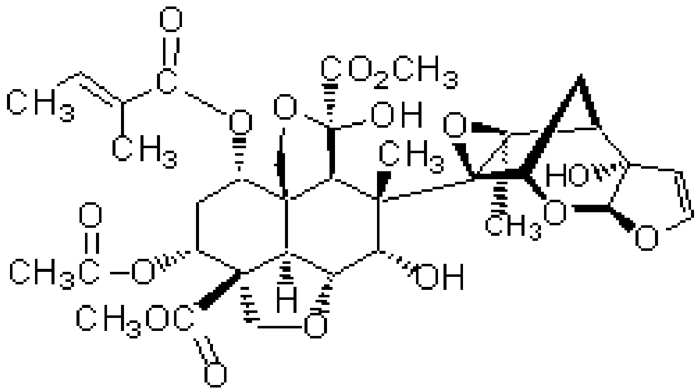

Fig. 2: The chemical structure of *Azadirachtin*

Toxicity test

The LC_{50} values for Neemgold determined by treating the fishes by Static renewal bioassay method (Benoit *et al*., 1993). In aquatic organisms the dermal route of administration is the natural way of exposure to pollutants and since the route of administration in evaluation of toxicity is usually the route through which the organism is naturally exposed to toxicants. The test animals were kept in large plastic tubs, capacity of containing 20 liter of water. The plastic tubs, which contained the test solutions of different concentrations of insecticides. The fingerlings size (8±2cms length and 25±2grams of weight) of common carp fishes were introduced into each plastic tubs and chronic test was conducted. In each tubs, 10 fishes of the same species (*Cyprinus carpio*) were introduced containing known concentration of test solution for the toxicity test, during the test period, they were not fed to avoid variation in feeding habits. The mortality was noted down for every 24hours intervals till 96hours duration. The dead animals were removed immediately and the test solution was renewed for every 24hours till 96hours along this, controls were also maintained without test chemicals. The LC_{50} were calculated by plotting percent mortality v/s toxicants concentration in a double logarithmic grid. Straight lines were fitted and concentration of toxicants at which there was 50% mortality was noted to represent LC_{50} for a given duration of exposure.

Analysis of Biochemical Parameters

The 100mg of muscle tissue was extracted by dissecting the treated and exposed fishes separately and estimation of total protein, total carbohydrates and amount of cholesterol and sterols by Lowry's method (Lowry *et al*., 1951), Hedge and Hofreiter Method (Hedge and Hofreiter, 1962), Zak's method (Zak, 1957), Liebermann-Burchard method (Liebermann, 1885) respectively.

Statistical Analysis
The statistical analysis was done for Biochemical parameters by using SPSS software. Mean ± Standard Deviation (SD) was calculated.

RESULTS
The physico-chemical characteristics of water sample found as follows, temperature 26±1^oC, pH 7.4±0.2 at 29^oC, dissolved oxygen 6.02±0.8mg/L, Carbon dioxide 3.8±1.0mg/L, Total hardness 28±2mg as $CaCO_3$/L, Alkalinity 40±10mg/L, Chlorine 12.78±1.0mg/L. LC_{50} values were decreased with the increase in chemical exposal duration. It is evident from results that the percent mortality of the fingerlings of common carp. The present study showed that during the exposure of Neemgold, the 24h LC_{50} was 0.002ml/l, at 48 and 72h it was 0.0018ml/l and 0.0016ml/l respectively, which was decreased to 0.0014ml/l after 96h of exposure (Table. 1; Fig. 3). Toxicity test with fingerlings is valuable for assessing potential impacts on growth, reproduction and survival of fish in polluted environment and are important tools for good environmental monitoring. The fingerlings exhibited normal swimming behavior in the control condition while erratic and jerky swimming was observed among in the treated media. They became restless, aggregated at one corner of the tub. These changes may be related to the consequent alteration in the physiological process due to chemical exposure. At 96h exposure the pectoral and pelvic fins were expanded and they rolled vertically prior to death. In some cases the fingerlings exhibited inconsistent jumping, frequent surfacing, secreted mucus from whole body, loss of equilibrium and decrease in opercula beat. The fish exhibited peculiar behavior of trying to leap out from pesticide medium which can be viewed as an escaping phenomenon. They often spiral rolled at intervals and finally the fishes sank to bottom with their least operculum movements and died with their mouth opened.
During the present investigation we observed significant alterations in the total protein, total carbohydrates and amount of cholesterol and sterol contents in the tissue of *Cyprinus carpio* exposed to Neemgold. In the present study, the amount of protein estimated in control fish was 7.293±0.267mg/g whereas in Neemgold 4.573±0.021mg/g (Table. 2). Neemgold treated fishes showed significantly lower protein levels when compared to untreated control group (Fig. 4). The amount of carbohydrate estimated in control and treated group were 7.183±0.300mg/g and 5.06±0.07mg/g respectively (Table. 2). The highest reduction of carbohydrate was noticed at Neemgold after 96hrs of exposure (Fig. 5). The total cholesterol estimated in the control group was 0.413±0.015mg/g whereas Neemgold treated tissue was 0.167±0.015mg/g respectively (Table. 2). The exposure of Neemgold caused significant decrease in total sterol in the tissues. The amount of total sterol was 0.917±0.006mg/g, and 0.513±0.015mg/g in control and Neemgold treated fishes respectively (Table. 2). Neemgold exposure caused a significant decrease in the level of cholesterol and sterol contents in the tissues of treated group when compared to control group (Fig. 6&7).

Table 1. ***LC_{50} values of Neemgold on C. carpio from 24h to 96h time intervals (in ml)***

Pesticides	24h	46h	72h	96h
Azardirechtin	0.0020	0.0018	0.0016	0.0014

Table 2. ***Effect of Neemgold on biochemical parameters in fingerlings of C. carpio (Average±SD in mg/g)***

Parameters	Protein	Carbohydrates	Cholesterol	Sterol
Control	7.293±0.267	7.183±0.300	0.413±0.015	0.917±0.006
Neemgold	4.573±0.021*	5.06±0.07*	0.167±0.015*	0.513±0.015*

*significant at $P < 0.0001$

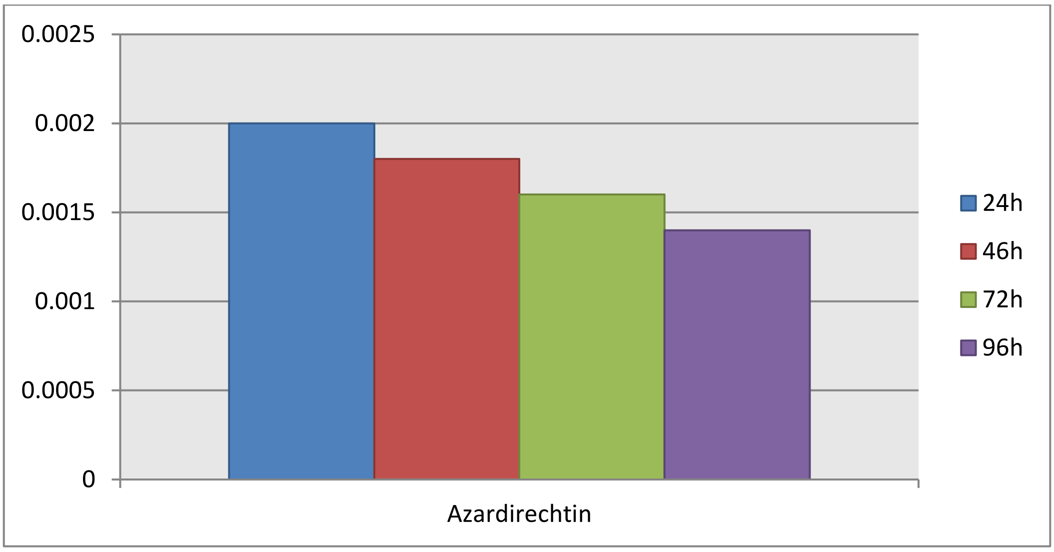

Fig 3. ***LC_{50} values of Neemgold for C. carpio from 24h to 96h time intervals***

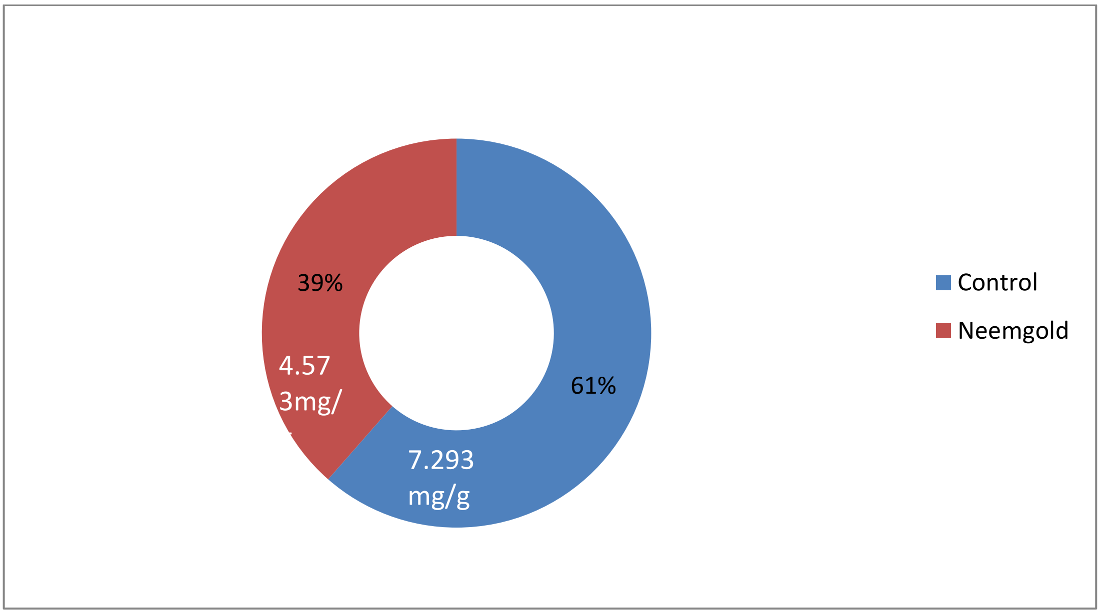

Fig 4. ***Total protein in the muscle tissue of experimental animal*** *(in mg/g)*

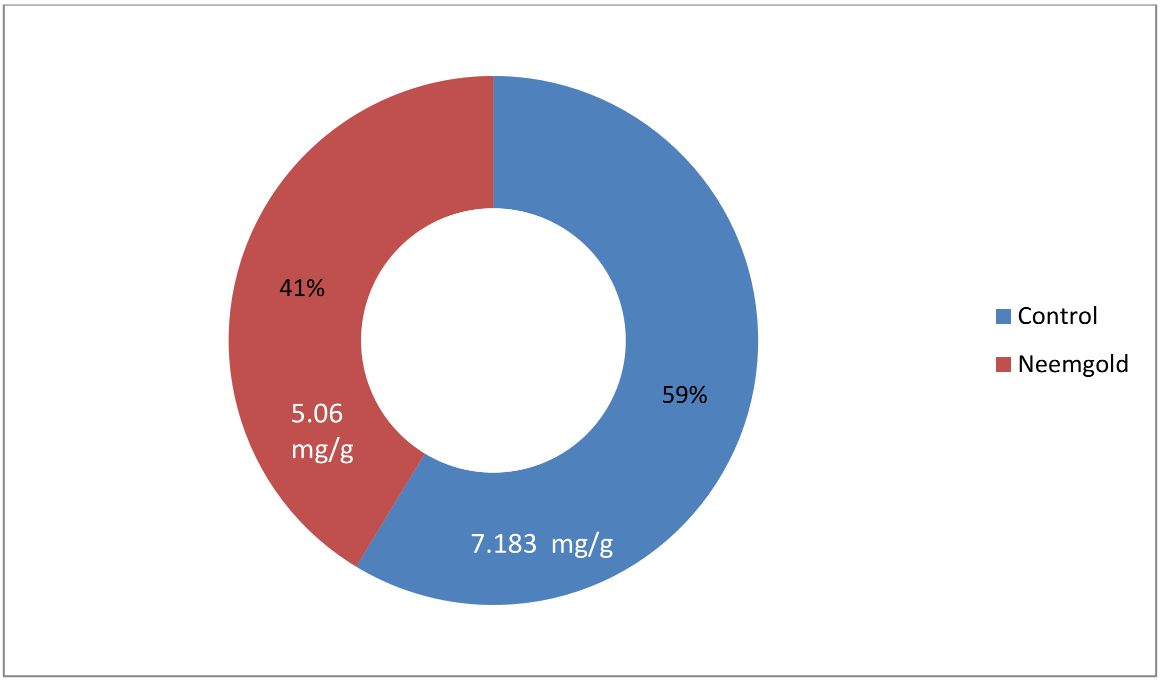

Fig 5. ***Total carbohydrates in the muscle tissue of experimental animal*** *(in mg/g)*

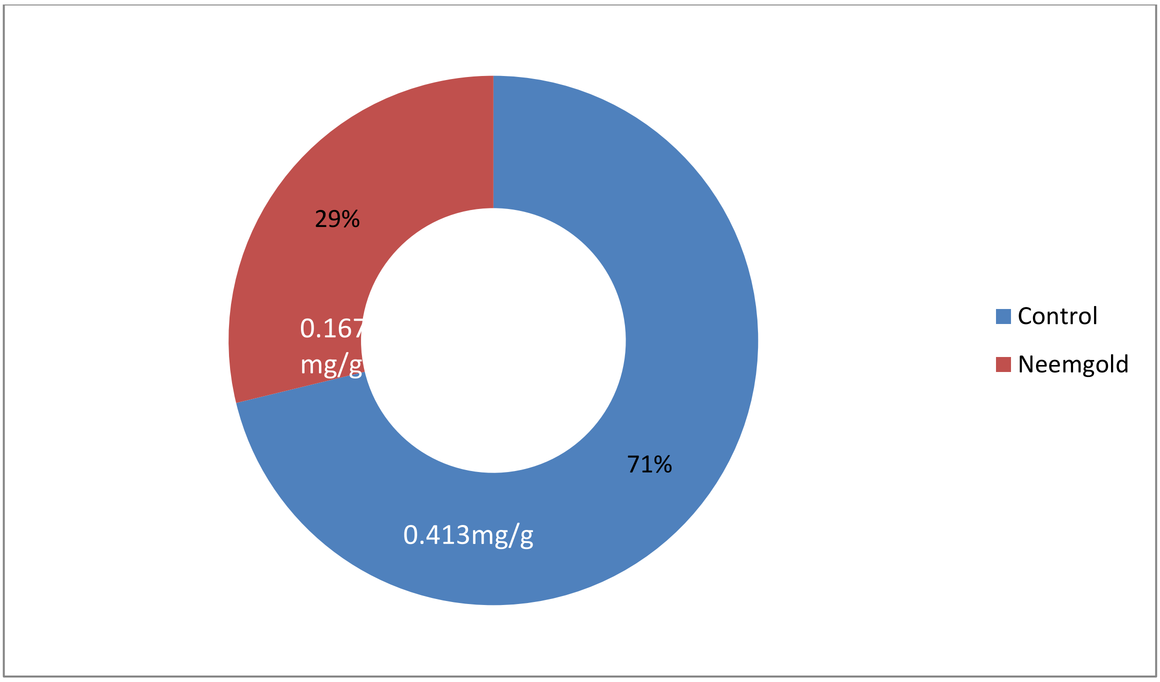

Fig 6. ***Total Cholesterol in the muscle tissue of experimental animal*** *(in mg/g)*

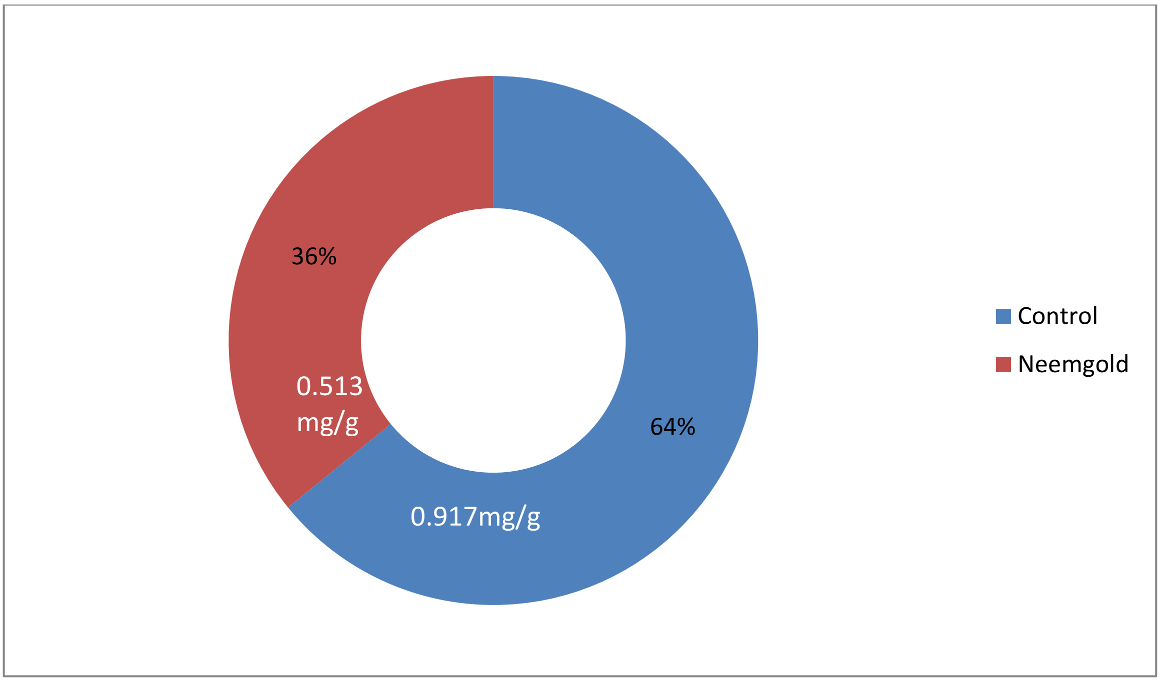

Fig 7. *Total sterols in the muscle tissue of experimental animal* *(in mg/g)*

DISCUSSION

Pesticides produce numerous physiological and biochemical changes in aquatic organism and particularly in fishes by influences the activities of several antioxidant enzymes (Regoli and Principato, 1995). Pesticides also affect the basic genetic materials (DNA and RNA), total protein, total free amino acids, and carbohydrates (Ansari and Kumar, 1986). This provides the impetus to investigate the toxic effects of sub-lethal exposure to Neemgold on different biochemical parameters in the tissues of fingerlings of common carp, *Cyprinus carpio*. LC_{50} values were decreased with the increase on exposure time. Toxicity test with fingerlings is valuable for assessing potential impacts on growth, reproduction and survival of fish in polluted environment and are important tools for good environmental monitoring. The fingerlings exhibited normal swimming behavior in the control while erratic and jerky swimming was observed among fingerlings in the treated condition. They became restless, aggregated at one corner of the tub. These changes may be related to the consequent alteration in the physiological process. At 96h exposure the pectoral and pelvic fins were expanded and they rolled vertically prior to death. During present investigation we observed that significant alterations in the total protein, total carbohydrates, and amount of cholesterol and sterol contents in the tissue of *Cyprinus carpio* exposed to Neemgold.

The depletion of the protein fraction in tissues might be due to their degradation and the possible utilization of degraded products for metabolic purposes. The amount of carbohydrate estimated in control, Neemgold treated. Liver is an important organ of detoxification and biotransformation process and due to these reasons the hepatic cells are damaged severely. Hence it effects on secretion of bile juice, it affects on carbohydrate metabolism. The exposure of Neemgold caused significant decrease in total cholesterol and total sterol in the tissue.

During the present study we concluded that the fingerlings of common carp are very sensitive to Neemgold because the reduction in all the macromolecules was significantly reduced in aquatic environment and significantly affect its population. Therefore, these pesticides should be used with great caution and in a sustainable way so that it may not be hazardous to aquatic environment and human beings. Moreover, extensive investigations should be done for their safe use. Consequently, it is important to identify the impact of these products have on certain biochemical parameters. Water pollution biomarkers are early diagnostic tools for measuring biological effects and assessing environmental quality.

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