Studies on the sensitivity and biochemical response of fish, Oreochromis Mossambicus to Neem

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ABSTRACT

Aquatic inhabitants have been affected by a number of anthropogenic agents which pollute the aquatic environment. Fish, one of the aquatic inhabitants, is considered as bio indicator of aquatic pollution. The utilization and over usage of synthetic organic chemicals in the form of pesticides contaminate the aquatic environment. In recent past, plant derived products are being widely used as an alternative to pesticides for the pest management and such chemicals also affect the fish in the water bodies. In the present investigation, the plant derived compound neem is used to find out its sensitivity towards the fish to understand the threshold level to minimize the contamination of plant derived commercial compounds in order to avoid the consequences of such contamination. The widely used commercial neem product is used to determine the LC$_{50}$ level to the fish, Oreochromis mossambicus and it was 0.4ml/l. The regression equation was also derived as Y= -12.323+6.626X. While determining the LC$_{50}$ value, swimming impairment and increase of opercular activity have been observed. Besides, to understand the physiological status of O. mossambicus in the various concentrations of neem, protein was analyzed at the end of 96 hrs in the tissues such as gills, liver, muscle and brain since the protein is considered as a biochemical marker. In the various concentrations of neem at 96 hrs, protein level was significantly (P<0.05) reduced in all the tissues. The drastic level of protein decrease indicates the sensitivity of the fish towards the neem.

Key word: Fish Oreochromis mossambicus, neem, LC$_{50}$ determination, protein analysis, tissues of fish.

1. Introduction

Water bodies have been contaminated by a number of pollutants including pesticides which are excessively used in the recent past against a wide variety of insect pests in the agricultural field for high yield. Sometimes, pesticides are also applied to the fish ponds to control the fish diseases, parasites, vectors, etc. By these practices, the pesticides reach the hydrosphere through run off and concentrated in certain parts of the aquatic ecosystem or remain in solution for extended periods or adsorbed to the particulate matter and thereby deposited in the sediments as well as accumulated in the body of the aquatic animals. However their increasing use in recent years has created a range of ecological problems such as bio-magnification, resurgence and the development of insecticide resistance in pest species, etc. Synthetic insecticides are expensive for farmers and may pave way for potential risks owing to the lack of adequate technical knowledge related to their safe use. Most of the pesticides act on the respiratory and cholinergic nervous system and pose a great threat to the
inhabitants of water bodies like invertebrates and vertebrates particularly fishes since they yield income to the people and also rich sources of protein to the human kind. Fish is a very important dietary animal protein source in human nutrition. Production of aquatic species through freshwater fisheries and aquaculture for protein supply is being encouraged in developed or developing countries but in under-developed countries, it is declining due to various factors. Environmental pollution by toxicants has become one of the most important problems in the world (Chandran et al., 2005). The usage of pesticides in the Indian scenario is unavoidable in the insect pest control strategy, however, the harmful effects of the toxicants to the various organisms have to be minimized by using the minimum level of pesticides and also as an alternative, a number of plant derived products are being widely used now a days and such products are now gaining importance to utilize against pests and vectors since they have no residual and resistance problems in the target organisms. The wide usage of natural products that include plant extracts, powders, ashes, cow dung and oils elicits varying level of effectiveness (Naumann and Isman, 1995).

In recent decades, there has been a growing concern for the protection of aquatic ecosystems against the adverse effects of contaminants, as a consequence of anthropogenic activity (Oropesa et al., 2009). Though they are chiefly available and as an alternative for the pest control, the bio pesticides contaminate the river, lake, etc. and thereby aquatic inhabitants are affected. The bio pesticides derived from botanicals have also been found to be toxic to fishes (Okon, 2002). The flora of our Indian continent have rich source of potential compounds. They have broad spectrum of biological activities and medicinal properties which have been extensively reviewed in recent past (Biswa et al., 2002). Azadirachtin, one of the major components of neem, has pesticidal property and a number of works have been available in relation to the impact of bio pesticides against insect pests as target organisms. However, the work related to the influence of bio pesticides on fish, a non target organism, is very scanty. Biomarkers for water pollution are early diagnostic tools for the measurement of biological effect and environmental quality assessment (Cajaraville et al., 2000). Among the variety of biomarkers adopted in ecotoxicological investigations, there is a notable interest in the parameters related to biochemical alterations of protein. The biochemical responses appear particularly suitable for measuring stressful levels of pollutants and have long been used as indicators of stress in fish (Hattingh, 1976). The effects of neem on non-target organisms have been studied in terrestrial ecosystems, however little attention has been focused on the effects of neem in aquatic environments. Hence, the present work has been carried out to understand the influence of neem on the biochemical constituent of fish Oreochromis mossambicus.

2. Materials and methods

Fish, Oreochromis mossambicus, ranging from 10±5 gms, were collected from the local hatchery and brought to laboratory in a aerated polythene bag for acclimatization. The fish were maintained in a cement tank and considered as stock culture. They were fed with standard fish feed and ground nut cake paste. After 15-20 days, the acclimatized fish were selected and used to find out the dose response relationship for the commercial neem product. The biocide, plant derived product, neem was purchased from the local market. The commercial neem product was considered as stock solution. For experimental purpose, uniform sizes of plastic troughs were used. From the stock of biocide, different concentrations such as 0.3 ml/l, 0.4 ml/l, 0.41 ml/l, 0.43 ml/l, 0.5 ml/l, were taken and mixed with the water taken in the selected troughs. The troughs were marked 1 to 5 and then, selected fish were exposed to each concentration for 96 hrs. One day prior to experiment the
fish were kept under starvation (Jones 1972). Control was maintained simultaneously. During the experimental period aquatic medium and test solution of various concentrations were renewed daily till 96 hrs. The level of medium was maintained constantly throughout the study period (i.e., 1 gm/l) and at the same time no feed was provided. Behavioural observations and mortality rate were recorded till the completion of 96hrs. Using the mortality rate, Probit analysis was carried out (Finney, 1971).

Fish were exposed to various concentrations (0.25ml/l, 0.3ml/l, 0.4ml/l, 0.43ml/l, and 0.45ml/l) of neem to study the biochemical status of fish during the stress condition for 96hrs. Fish exposed to various concentrations were collected separately and sacrificed for the removal of tissues such as brain, gills, muscles and liver which were kept in the ice box till the protein assay was made. The separated tissues from the fish of various concentrations were subjected to protein analysis by the method of Lowry et al., (1951). The results obtained were represented in the graph and to understand the significance of the results, t-test was performed. The significant level was observed at P<0.05 level.

3. Results

The lethal concentration of neem at 50% mortality level and regression equation were derived by using Probit analysis and they were 0.4ml/l (Fig.1) and \( Y = -12.323 + 6.626X \) respectively. The LC\(_{50}\) calculation was also confirmed with Trimed Spearman-Karber method (Software available freely). The 95% upper and lower limits were 0.39ml/l and 0.42ml/l respectively.

![Figure 1: Log concentration Vs Probit Kill](image)

Protein was analyzed in the tissues such as brain, liver, gills, and muscles of *O. mossambicus* exposed to various concentrations of neem (Fig.2). The analysis of protein in brain showed the reduction level in all the treatments and maximum of 97.91% was observed in 0.45ml/l treatment at 96hrs and the lowest level of 79.36% was observed in 0.25ml/l treatment. The trend of decreasing level was noticed in all the treatments. Similarly in the liver, the protein level was also decreased but the percent of protein level was lesser than the level observed in the brain in 0.45 ml/l treatment. However in liver, the maximum reduction of 96.08% was
observed in 0.25ml/l at 96hrs treatment whereas in all other treatments the level of protein decrease was above 80% except in 0.43ml/l and 0.45ml/l treatment where the reduction level was 61% and 50% respectively. Among the brain and liver, the brain showed maximum decrease of protein and it is evident that the brain is sensitive to the concentrations of neem used for the present investigation and at the same time the liver is probably less susceptible to the neem than the brain regions. The gill showed maximum of 77.09% decrease in the 0.3ml/l of treatment at 96 hrs and in the other treatments particularly 0.4ml/l showed decrease of 87.86%. However, in 0.25ml/l and 0.43ml/l treatments, the decrease was very less when compared to the rest of the treatments. Similar to gills, the muscle also showed decreasing level of protein in all the treatments. In 0.43ml/l treatment, the analysis revealed very low level of protein decrease i.e., 4.87% and in 0.25ml/l treatment also showed decrease of protein by 56%. In other treatment particularly in 0.45ml/l of concentration the protein showed 86% of decrease. All the changes in the treatments showed statistical significant.

3. Discussion

Contamination of aquatic bodies by a variety of chemicals is an ever increasing menace. Owing to such incidence, an impact of contaminants on aquatic inhabitants has been evident. In recent years, plant derived chemicals have been used against pests which in turn caused the effects that mimic the effect of pollutants. In the present work, the neem product though it is a biodegradable and low toxicity in nature, it causes undesirable consequences in the form of behavioural and biochemical response in the different tissues of *Oreochromis mossambicus*. The LC$_{50}$ level of neem elicited behavioural responses in various ways. While determining the lethal concentration, some of the fish becomes moribund state and the movement is very much restricted. Sometimes, the fish swims laterally and some time swims circular manner either left or right side. The fish turns upside down and moves slowly. When the fish is disturbed it moved very fast and then lay flatly on the surface of the water. This may be due to the loss of muscular coordination which contributes to the less effective swimming. The low doses of LC$_{50}$ value elicited responses like increase in opercular activity, bulging of
abdomen, etc., are similar to effect of other pollutants used in the fish farming such as carbamates, organophosphates, etc. However, Winkaler et al., (2007) have reported that the neem is less toxic nature which may be due to the ingredients present in the preparation of emulsion. Tiwari and Singh (2006) also noticed similar behavioural changes in *Channa punctatus* exposed to *Nerium indicum* leaf extract.

The biochemical analysis of protein showed heterogeneous responses in the tissues such as gills, brain, liver and muscle of fish, *O.mossambicus*. The neem drastically reduces the protein level in the tissues at the end of 96 hrs. The decreasing level may be due to the fact that during stress condition, the protein level may be utilized to withstand the different concentrations of neem since protein is a major source of energy. The decrease of protein is also supported the view of Holbrook (1980) who have reported that the decreased level of amino acid incorporation and disaggregation of polysomes lead to the decrease of protein synthesis. The present work also supports the view of Durairaj and Selvarajen (1992). They have suggested that the decrease of protein in the brain, liver, muscle and gill is due to the decrease of RNA in the tissues of fish.

The protein decrease may be initiated to maintain homoeostasis or the response may reflect a breakdown of some physiological functions. Similar type of protein reduction is reported in the infected fish on the 10th, 20th and 30th day (Haney et al., 1992). The depletion of protein fraction in liver and muscle might have been due to their degradation and possible utilization for metabolic purposes. Bradbury et al. (1987) have pointed out that the decreased protein content might also be attributed to the destruction or necrosis of cells and consequent impairment in the protein synthesis machinery. According to Tiwari and Singh (2006) the decreases in protein level in the liver and muscle of fish exposed to neem might have resulted from high protein hydrolytic activity due to elevation of protease enzyme activity in both the tissues. The responses of muscle protein appear particularly suitable for measuring stressful level of pollutants and have been used as indicator of stress in fishes. Schmidt Nielson (1975) stated that the decreased tendency of total protein may also be due to the metabolic utilization of the Keto acids to gluconeogenesis. Moreover, Adham (1992) has observed that the methanolic extract of the fruit of neem, *Melia azedarach* as well as water suspension of its milled dried leaves on serum protein pattern of the freshwater catfish, *Clarias lazera* cause disturbances in the levels of circulating proteins during the administration of both neem products. These are expressed as hyperproteinemia as well as disproteinemia. The study clearly indicates the alterations of protein in different tissues at 96 hr period and thus adversely affects the behavior and physiology of fish. The investigation also reveals the sensitiveness of the toxicant to the fish even at the low level in aquatic environment.

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**4. References**


