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Bioaccumulation of carbamate and pyrethroid insecticides in fishes of the river Gomti at Jaunpur during breeding season

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Abstract: *The aim of the present investigation was to monitor the levels of bioaccumulation of carbamates (Carbofuran, Carbaryl and Aldicarb) and pyrethroid insecticides (Cypermethrin and Deltamethrin) in liver, brain and ovary of captured catfish Bagarius bagarius from unpolluted ponds of Gujartal, Jaunpur (reference site) and polluted river Gomti, Jaunpur during pre-monsoon or breeding phase. The carbamates were measured by high performance liquid chromatography (HPLC) and the pyrethroids by gas liquid chromatography (GLC). Results indicated that catfish B. bagarius captured from river Gomti have higher bioaccumulation of insecticides when compared to those from the reference site reflecting the degree of pesticide pollution present in those water bodies. The bioaccumulation of Carbofuran, Carbaryl and Aldicarb were significantly higher in the brain, liver and ovary in polluted fish as compared to the conspecific species captured from reference site. Likewise, the bioaccumulation of Cypermethrin and Deltamethrin was also higher in the studied tissues when compared with fish of same species captured from the reference site. Results indicated that the bioaccumulation of these insecticides cause metabolic disorder as well as affect the reproductive physiology of this species.*

Key Words: Carbamates, Pyrethroids, Tissue, Bioaccumulation, Fish.

Introduction

In recent times, the practice of extensive use of carbamate and pyrethroid insecticides to control the insects for the protection of crops is leading to disturbances in the reproductive physiology of non-target organism like fish. Carbamates like Carbaryl, Carbofuran and Aldicarb, and pyrethroids like Cypermethrin and Deltamethrin are abundantly in use in agricultural fields for the control of insect pests.

There are numbers of references available on bioaccumulation of pollutants causing reproductive inhibition (Singh and Singh, 2008 a; Singh *et al*., 2008a). Studies on sperm motility have also been done in wild population of fish species captured from polluted water (Singh and Singh, 2008 b; Singh *et al*., 2008b). Various pollutants causing endocrine disruption (Ankley *et al*., 2009; Olujimi *et al*., 2010) in fish have been reported which act as biomarkers (Bervoets *et al*., 2009; Joseph and Raj, 2011). There are reports that pollutants decrease the growth and health of fish as well as reproduction (Barrett *et al*., 2010; Hanson *et al*., 2011; Fianko *et al*., 2011). Ovarian maturity was affected by Carbofuran exposure in fish (Ram and Singh, 1988; Chatterjee *et al*., 1997; Ram *et al*., 2001; Chandra *et al*., 2004). The Carbaryl has been

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also demonstrated to change biochemical parameters (Jyoti and Narayan, 1999; Singh and Srivastava, 1998). Other reports have also demonstrated that carbamate at sublethal doses effect pheromonal mediated endocrine functions in mature *Salmo salor* (Waring and Moore, 1997).

Pyrethrins/pyrethroids are a family of compounds which either are directly derived from a species of Chrysanthemum flowers (pyrethrins) or manufactured to resemble these chemicals (pyrethroids). In general, these compounds are considered relatively safe to humans because of their low-toxicity compared with other classes of pesticides. The pyrethroids are considered to be less toxic than the pyrithrins, and are being increasingly used for agricultural purposes and crop protection to control many pests, including pests of cotton, fruit and vegetable crops. However the pyrethroids are highly toxic to aquatic organism and fish as well as to bees.

Pyrethroids are synthetic analogues of pyrethrins, the active substances in the flowers of *Chrysanthemum cinerariafolium.* Pyrethroids can be classified into two large groups. Type I pyrethroids do not contain a cyano-group in their molecules and include Allethrin, Tetramethrin, Permethrin, and Phenothrin. Type II pyrethroids contain a cyano group at the á-carbon position and include newer compounds, such as Deltamethrin, Cyphenothrin, Cypermethrin, and Fenvalerate. The two types of pyrethroids cause somewhat different symptoms of mammalian poisoning. Poisoning with type I pyrethroids is characterized by hyperexcitation, ataxia, convulsions, and eventual paralysis; poisoning with type II pyrethroids, by hypersensitivity, choreoathetosis, tremors, and paralysis. Despite differences in the symptoms, both types of pyrethroids have the same major target site; the sodium channel of nerve membrane, i.e., the channel directly responsible for generating action potentials.

Cypermethrin is one of the most important insecticides in wide scale use. It has wide uses in cotton, cereals, vegetables and fruit, for food storage, in public health and in animal husbandry. Cypermethrin, an analogue of pyrethrins is classified by the world Health Organization (WHO) as moderately hazardous (class II). It interacts with the sodium channels in nerve cells through which sodium enters the cell in order to transmit nerve signals. These channels can remain open for upto seconds compared to the normal period of a few milliseconds, after a signal has been transmitted. Cypermethrin also interferes with other receptors in the nervous system. The effect is that of long lasting trains of repetitive impulses in sense organ.

The pyrethroid pesticide, Cypermethrin is used extensively to control pests. (Bradbury and Coats, 1989) observed that pyrethroid insecticides are extremely toxic to fish during aqueous exposure. Pyrethroids are readily metabolized and have relatively short half life. Liver is an established organ in fishes (Braunbeck and Volke, 1993) and plays an important role in uptake, accumulation biotransformation and excretion of xenobiotics (Couch, 1975; Pentreath, 1976; Gluth *et al*., 1985; Kohler, 1990). Functional changes are known to be reflected in structural changes of the organ. While most of the studies are restricted to the mortality and growth rate of fishes, only a few studies have focused on their effect on different organ system. Much literature is not available on toxicity of pyrethroid Deltamethrin on the fry (Ural and Saglam, 2005) and biochemical changes in liver and gills of *Clarius batrachus* (Begum, 2005).

The present study aims to make a comparative assessment of bioaccumulation of carbamates (Carbofuran, Carbaryl and Aldicarb) and pyrethroid insecticides (Cypermethrin and Deltamethrin) in tissues of brain, liver and ovary in the female catfish *Bagarius bagarius*

collected from unpolluted ponds of Gujartal, Jaunpur (reference site) and polluted river Gomti, during the pre-monsoon (breeding phase).

Materials and Methods

The research reported herein was conducted under ethical guidelines for the treatment of animals in behavioral research and teaching (Anonymous, 1998). Female catfish *Bagarius bagarius* were caught from different sampling sites during breeding phase (water temperature 30 ± 2ºC and photoperiod 13.0L: 11.0D).

The ponds of Gujartal are situated about 30 km away from the Gomti River (city of Jaunpur) and have an area of 21 hectares. They include several ponds, such as nursery, rearing and water areas, with no apparent mixing of any type of pollutants. This site is considered the reference site (Fig. 1).

The River Gomti, is a major tributary of River Ganga which originates from the Philibhit district of Uttar Pradesh in northern India. It drains a catchment area of about 25000 km^2 and traverses a distance of about 730 km through the districts of Philibhit, Shajanpur, Sitapur, Lucknow, Barabanki, Sultanpur, Jaunpur, and Ghazipur in Uttar Pradesh, before merging with the River Ganga in the Ghazipur district, which is about 30 km northeast of Varanasi. The three major urban settlements on the banks of the river include, Lucknow, Sultanpur and Jaunpur. Untreated waste water and effluents are discharged into the river from more than 40 waste water drains at Lucknow, Jagdishpur, Sultanpur, and Jaunpur. Nayak *et al*., (1995) and Singh *et al*., (2005) reported that its river water is contaminated due to industrial effluents.

Fishes were captured by drag net with the help of local fishermen from each sampling site. Fish was kept in the ice-box and brought to laboratory for collection of brain, liver and ovary. Tissues

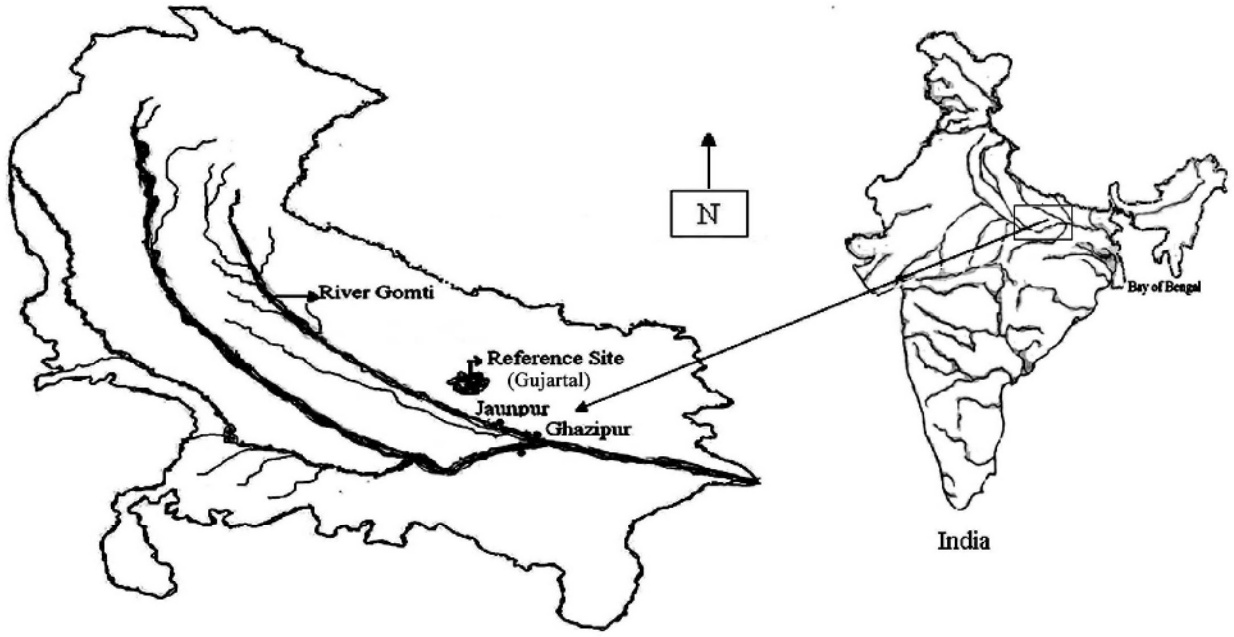

Fig. 1. Map showing sampling site of non polluted ponds Gujaratal, Jaunpur (reference site and polluted river Gomti of eastern Uttar Pradesh. 1. Gujaratal Jaunpur reference site (Lat. 25.46 N: Long. 82.44E), captured catfishes *Bagarius bagarius* (Ham.) (GSI-5.50 ±0.59). 2. River Gomti, Jaunpur: Lat. 25.46 N: Long. 82.44 E, captured conspecific female catfish (*Bagarius bagarius*) (GSI – 3.31 ± 0.26).

were kept at -20 ºC till subsequent analysis. All the samples were extracted within two days for different insecticides and cleanup for the analysis of carbamates (Carbofuran, Carbaryl and Aldicarb) and pyrethroid insecticides (Cypermethrin and Deltamethrin) and sent to AES Laboratories (P) Ltd, Phase II, NOIDA for GLC and HPLC facilities. The gonadosomatic index was calculated as gonad weight × 100/ body weight. The histology of ovary was also done for the oocyte stages (Singh *et al*., 1993).

N-Methylcarbamate Insecticide Residues Gas Chromatography was followed for the extraction and clean up procedure of carbamate pesticides.

Extraction and clean up procedure for pyrethroid insecticides were followed as per method described by Liu and Pleil (2002). Briefly, tissue (1g) or blood (1ml) was taken and 3 ml deionized water and 2g of sodium chloride added to saturate the samples. In the above mixture 6 ml of *n*-Hexane was added and vortexed for one minute. The above mixture was centrifuged at 1600 g for 30 minutes. Upper organic phase was then separated from the aqueous phase. Aqueous phase was extracted again following above procedure with 6 ml of solvent. The above organic phase was collected again and combined with above 6 ml solvent and total 12 ml organic phase was collected. The collection was passed in sodium sulphate bed and the remaining solution was evaporated to make 2 ml for GLC.

Values were expressed as ng/g of tissues (ppb) for pesticides (Mean ± SEM, n = 5). Data was analysed by student's *t*-test (Bruning and Kintz, 1977) and analysis of variance (ANOVA two-way/one-way) was done for pesticide residues analysis by Microsoft Excel tool pack data analysis (ANOVA two factor with replication).

Results and Discussion

Analysis of variance (TW-ANOVA) revealed that the concentration of carbamates and pyrethroids varied in the tissue of captured

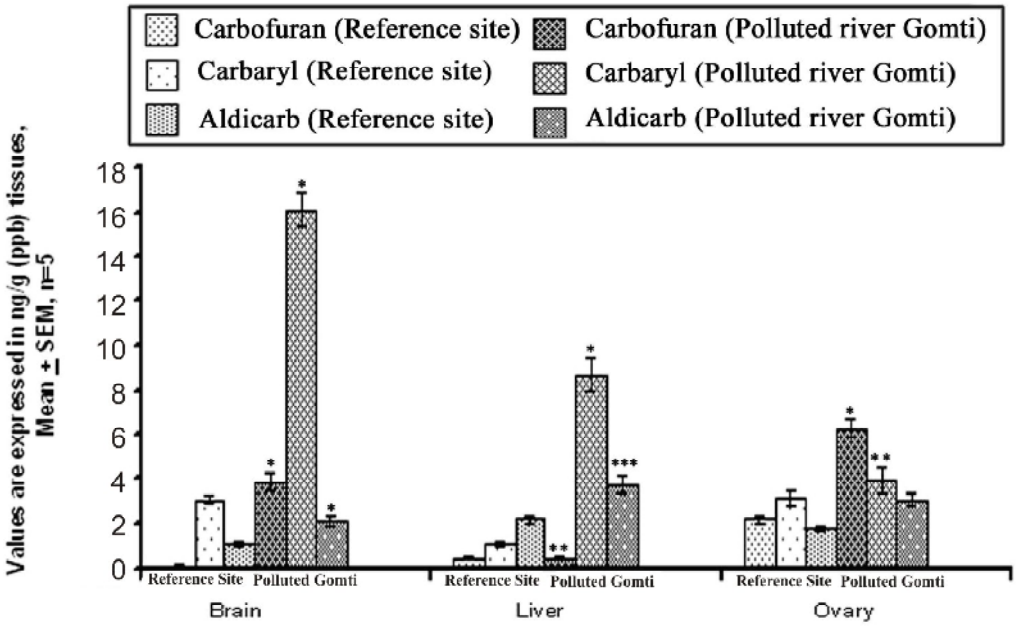

Fig. 2. Comparison and bioaccumulation level of carbamates (Carbofuran, Carbaryl and Aldicarb) in tissues of female catfish (*Bagarius bagarius*) between reference site and polluted river Gomti during breeding season. Results were compared from reference site fish to same species of polluted site fish by Students *t*- test. The level of significance (P) - * $P < 0.001$; ** $P < 0.05$; *** $P < 0.005$. ANOVA (TW): tissues $F = 39.93$ $P < 0.001$; carbamates $F = 10.38$ $P < 0.05$; tissue × carbamates $F = 65.32$ $P < 0.05$.

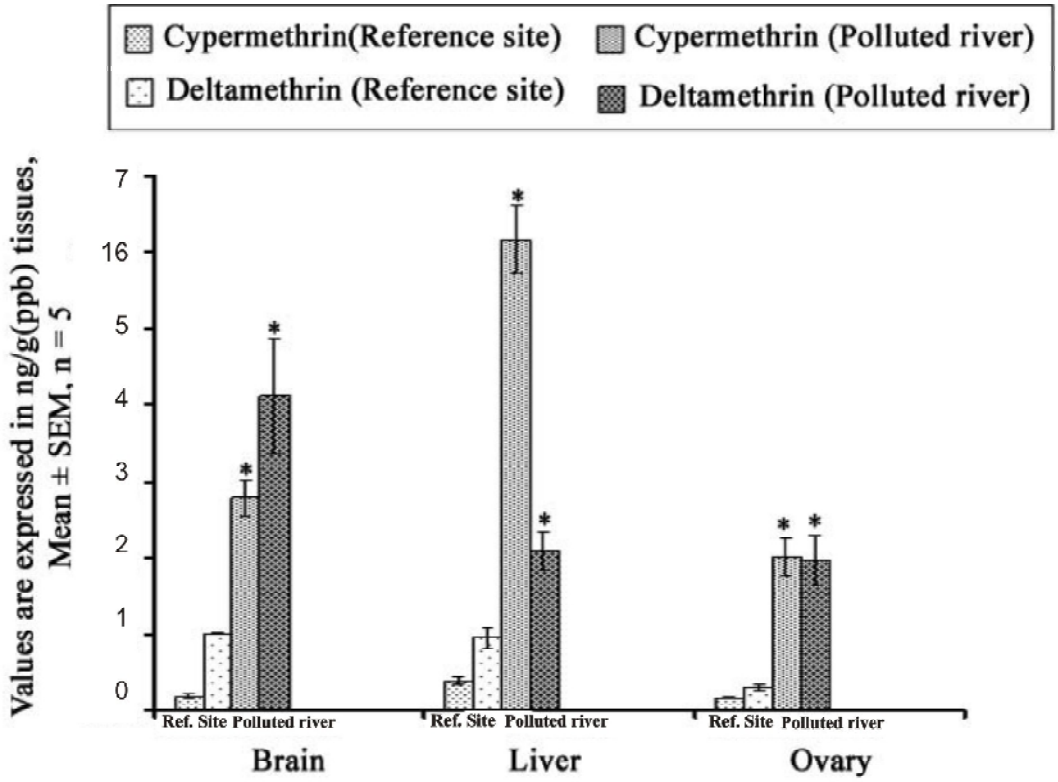

Fig. 3. Comparison and bioaccumulation level of pyrethroids (Cypermethrin and Deltamethrin) in tissues of female catfish (*Bagarius bagarius*) between reference site and polluted river Gomti during breeding season. Results were compared from reference site fish to same species of polluted site by Students *t*- test. The level of significance (P) - * $P < 0.001$. ANOVA (TW): tissues $F = 88.79 P < 0.05$; pyrethroids $F = 20.17$ $P < 0.001$; tissue × pyrethroids $F = 17.99$ $P < 0.001$.

female catfish *B. bagarius* from that of reference site and polluted river Gomti. The values are given for carbamates and pyrethroids (Figs 2, 3).

Results indicated that catfish *B. bagarius* captured from river Gomti have higher bioaccumulation of insecticides when compared to those from the reference site reflecting the degree of pesticide pollution present in those water bodies. The bioaccumulation of Carbofuran, Carbaryl and Aldicarb was significantly higher in the brain, liver and ovary in polluted fish as compared to the conspecific species captured from reference site. Likewise, the bioaccumulation of Cypermethrin and Deltamethrin was also higher in the studied tissues when compared with fish of same species captured from the reference site. Results indicated that the bioaccumulation of these insecticides cause metabolic disorder as well as affect the reproductive physiology of this species.

The present studies have indicated that carbamates and pyrethroids bioaccumulated much higher in catfish, *B. bagarius* captured from polluted river as compared to conspecific species captured from non-polluted ponds of Gujartal, Jaunpur or reference site. The histological examination of ovary of such polluted fish indicated having large number of immature oocytes as compared to fish ovary captured from reference site. This indicated that the toxicant perturbed the hormonal imbalance thereby disrupting the reproductive physiology

of this species. Such studies have also been reported by Singh and Singh (2008 a) which support our findings during breeding phase. The bioaccumulation of Carbaryl was more in the brain, liver and ovary as compared to Carbofuran and Aldicarb in the fish captured from polluted site. The liver showed much more accumulation as compared to other tissues. Reports of Singh and Singh (2008 b) have indicated that Cypermethrin causes inhibition of oocyte maturation as well as atretic follicles in the ovaries and gross condensation of spermatogenic cells in testis after Cypermethrin exposure at sublethal concentration in another catfish, *Heteropneustes fossilis*. It means insecticides like carbamates and pyrethroids also decrease the growth of fishes by hormonal imbalance.

Long term exposure of organisms to pesticides means a continuous health hazard for the population. So, human population is at high risk by consuming these toxicated fishes. This implies that one should take the necessary protection in the application of pesticides to protect the life of fish and other aquatic fauna.

Deltamethrin is highly toxic synthetic pyrethroid pesticide widely used in agriculture. It is also heavily used in the mosquito control programs, which necessitates in-depth subchronic and chronic toxicity tests to fish species and to nontarget species to be under taken. In addition, potential risk from Deltamethrin metabolites should be investigated to get a more complete picture in terms of toxicity. The low toxicity of Deltamethrin to mammals may be misleading at this point in terms of ecotoxicology and lead to extrapolation problems to aquatic species.

Organochlorines, organophosphates, carbamates and synthetic pyrethroids are the common agricultural insecticides. All insecticides vary in degree of biodegradability and toxicity to fish. Fenvalerate, one of the pyrethroids effectively used for pest control in cotton and variety of other crops, is highly toxic to fish. Bioaccumulation of xenobiotics in aquatic fishes is increasing alarmingly and this is also increasing the potential human health hazard. A variety of fish species show absorbance and accumulation of many contaminants such as pesticides and bactericides, polychlorinated biphenyls and heavy metals. Due to the lipophylic characteristics of many of the insecticides, they (specially pyrethroids) are absorbed by fish, even at low concentrations in the water. The toxicity caused to fish may not be immediately harmful, but most of the insecticides disturb haematological and metabolic process by inhibition/activation of enzymatic activities and cause tissue damage.

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