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Effect of Commercial Insecticide Fipronil on the Brain of *Channa striata* (BLOCH)

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Abstract
Fipronil, one of the major insecticides currently in use in agricultural fields, is playing havoc to non–target organisms especially, fishes. Though literature on the effect of pesticides on fishes is aplenty, studies on the alterations in brain tissues are scanty, if not nil. The present study evaluated the effect of the commercial formulation of the insecticide fipronil on the brain tissues of *Channa striata*. The 96 hr LC_{50} was found to be 0.16mg/l and the histo-pathological changes like vacuolation in the molecular layer, degeneration of granular and molecular layer of cerebellum, infiltration of granular cells, and hyalinization of molecular layer were observed in the brain of *Channa striata* exposed to sublethal concentrations of the insecticide for a period of 4 weeks.

Keywords
Fipronil, *Channa striata*, Brain, Histopathology

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INTRODUCTION
Fresh water ecosystem is facing a multidimensional array of threats in recent years with the concurrent loss of biodiversity [1]. It starts from household garbage through agricultural waste and ends up in industrial waste. The boom of food production is boon for humans but unfortunately it is bane for other species. The progress in agriculture sector saw the emergence of new classes of pesticides and its accumulation in soil and water is causing many health hazards to the flora and fauna of the surrounding area. The use of pesticides and the resulting ecological imbalance results in a shift in the physiological and biochemical nature of aquatic flora and fauna. Consequently, these organisms, especially the fishes help in a long way in monitoring the health status of an ecosystem. They are considered as bio-indicators of aquatic system.

Fipronil is a broad-spectrum insecticide that belongs to the phenylpyrazole chemical family. Fipronil disrupts the central nervous system of insects by blocking GABA-gated chloride channels and Glutamate-gated chloride (GluCl) channels [2]. Fipronil is gaining more attention as even its minute concentration is effective against a wide range of pests. Fipronil is toxic to crustaceans, zooplanktons and fishes. The developmental stages of fishes and many invertebrates are seriously affected with the exposure to fipronil [3].

MATERIALS AND METHODS
The insecticide selected for the study is fipronil ($C_{12}H_4C_{12}F_6N_4OS$). The International Union of Pure and Applied Chemistry (IUPAC) name for fipronil is (±)-5-amino-1-(2,6-dichloro-α, α, α-trifluoro-p-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile [3].

Commercial grade fipronil formulation Jump™ (Fipronil 80% WG) were purchased from the local agrochemical shop. Fipronil degrades rapidly in water when exposed to UV light to form fipronil-desulfinyl. Fipronil is stable to hydrolysis at pH 5 and pH 7. However, it degrades in alkaline conditions in direct proportion to increasing pH values. Fipronil and fipronil-desulfinyl are less volatile than water and can concentrate under field conditions. [3,4] Fipronil-amide is the primary residue formed from hydrolysis [4,5,6]. Fipronil-desulfinyl photo-degrades in aerated and static water with recorded half-lives of 120 (± 18) hours and 149 (± 39) hours, respectively. [6] Fipronil degradation products accumulate in riverbed sediment while the parent compound does not.[7]

The snake head murrel *Channa striata* was selected as the test specimen, as it is very common in local fresh waters and easily available. A total of 120 fishes with an average body weight of 31 - 40 gm and with an average length of 16-18 cm were employed in the present study. Healthy live fishes were collected from Kuttanadu area directly from fishermen. Before introducing the fishes in the aquarium, they were treated with 0.1% $KMnO_4$ solution to avoid dermal infection. Fishes were kept in glass aquaria, filled with clean de-chlorinated water and acclimatized for two weeks. 40 fishes were selected and divided into two groups. The first group was kept as control and the second was exposed to the pesticide. Exposure concentration was decided on the basis of 96hrs LC_{50} value of fipronil. 3 exposure durations viz., 8, 20 and 28 days were fixed. Water was renewed on every alternate day to maintain the concentration of fipronil for 28 days. As the level of toxicity is reported to vary with the interference of various extrinsic and intrinsic factors like temperature, salinity, pH, hardness of water, exposure period, density of animals, size, *etc*., precautions were taken throughout this investigation to control all these factors as far as possible.

Histopathological examination

Histological sections of the brain of the control and exposed fishes were taken by adopting the procedure described by Humason. The whole brain was dissected out from the control and fipronil treated fishes. Fishes from both control and exposed groups were analysed simultaneously. Brain was removed and rinsed with physiological saline solution (0.9% NaCl) to remove blood mucus and debris adhering to the tissues. Fixative used was bouin's fluid. Sections were cut at 5µ thickness, stained with haematoxylin and counter stained with eosin. Sections were mounted in DPX and coloured micro photographs were taken using light microscope Olympus CH20*i* attached with camera NikonD800 with microscope attachment.

RESULTS AND DISCUSSION

Channa striata in control group showed clear neural cells with distinct nuclei [Fig.1]. There was no significant variation between the controls. The histoarchitecture remained more or less intact in control fishes. No sign of vacuolation or degeneration noticed in control fishes.

Brain histo-architecture of 8-day exposed fishes showed remarkable variations in neural tissues with the formation vacuoles. On 20th day dark stained degenerative neural cells of granular and molecular layer were noticed. [Fig.2]. 28-day exposed fishes showed more degenerative changes and increased necrotic condition of neural cells with infiltration of granular layer and hyalinization of molecular layer [Fig.3].

In toxicological studies, histopathological studies remain as the primary tool for the evaluation of pathological changes in tissue. The study of sublethal toxicity of different toxicant in fishes attain a considerable attention as it explains the toxicity level in each organ. In the present study the histopathological responses of the fish exposed to the sublethal concentration of the pesticide fipronil showed severe damages in the brain tissue. The damages caused to the different exposure durations varied from the formation vacuoles, dark stained degenerative neural cells of granular and molecular layer and degenerative changes and increased necrotic condition of neural cells with infiltration of granular layer and hyalinization of molecular layer in 8, 20- and 28-day exposure durations respectively. These changes in the histo-architecture of the brain of the fipronil exposed fish *C. striata* suggest a progression of changes with exposure duration. Mild vacuolar changes in the brain of Indian major carp exposed to hexachlorocyclohexane was reported earlier (Basanta kumar Das and Subhas Chandra Mukherjee, 2000) [8]. Mononuclear infiltration, neuronal degeneration and severe spongiosis in the brain of *C. gariepinus* exposed to Cypermethin were reported by Ayoola and Ajani (2008).[9]

It is common knowledge that any impairment in the tissue of a region in the brain may lead to the curtailment of that function which was managed by the region in the fish brain. Brain and spinal cord being the important organs in controlling the movements of the fish, the signs of stress, loss of equilibrium and erratic swimming which increased progressively with exposure duration in the present

study could only be attributed to the degenerative changes noted in the histo-architecture of the brain of the fipronil exposed fishes. Fipronil is toxic to many aquatic organisms especially in the developmental stages [10]. The environmental fate of fipronil shows that, residue of fipronil in the soil remained for three years though its concentration among samples varied greatly [11]. Ardeshir *et. al*., [12] reported some alterations in the distribution of purkinje cells in brain tissues of Caspian white fish exposed to fipronil.

Bradburry *et.a*l. (1987)[13] observed tremors and convulsions in fenvalerate exposed rainbow trout. Histological changes like vascular dilation in endosulfan exposed *A. testudinius* (Sajitha Bhaskar, 1994)[14], scatterly arranged cells, severe necrosis and loss of differentiation in the brain cells of malathion exposed *O. punctatus* (Pugazhvendran *et al*., 2008)[15] are all corroborating the present findings in the brain of fipronil exoposed *C. striata*.

CONCLUSION

Sub-lethal concentration of the pesticide fipronil caused severe changes in the histo-architecture of the fish C. striata and the changes in the brain progressed with the duration of exposure. The histopathological changes were manifested in the fish through the signs of respiratory stress, loss of equilibrium and erratic swimming. All these behavioural changes and associated changes in the histo-architecture of the pesticide exposed fish call for the restrictive use of the pesticide in the environment.

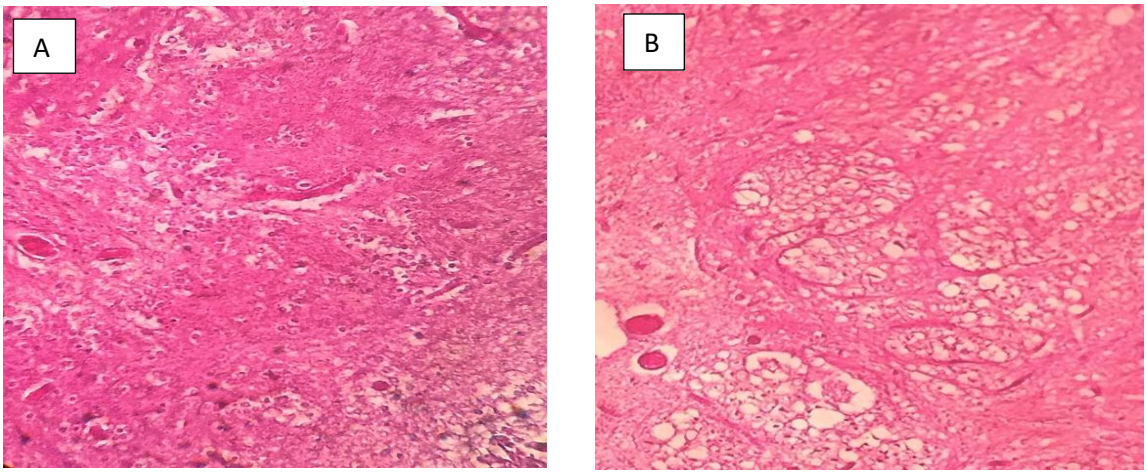

FIG.1 Brain histopathology of *Channa striata,*. A(100X) & B(400X)(control),Normal histology of brain

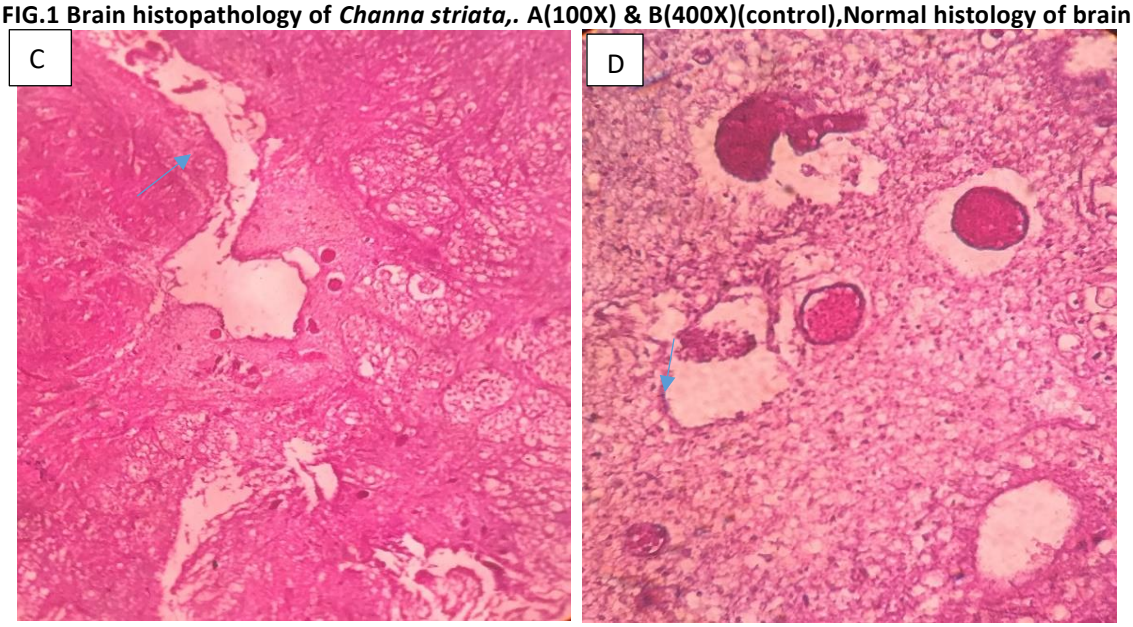

FIG.2.On 8th day *C.striata* exposed to fipronil , C & D showing vacuolation in the molecular layer(H&E), (400X)

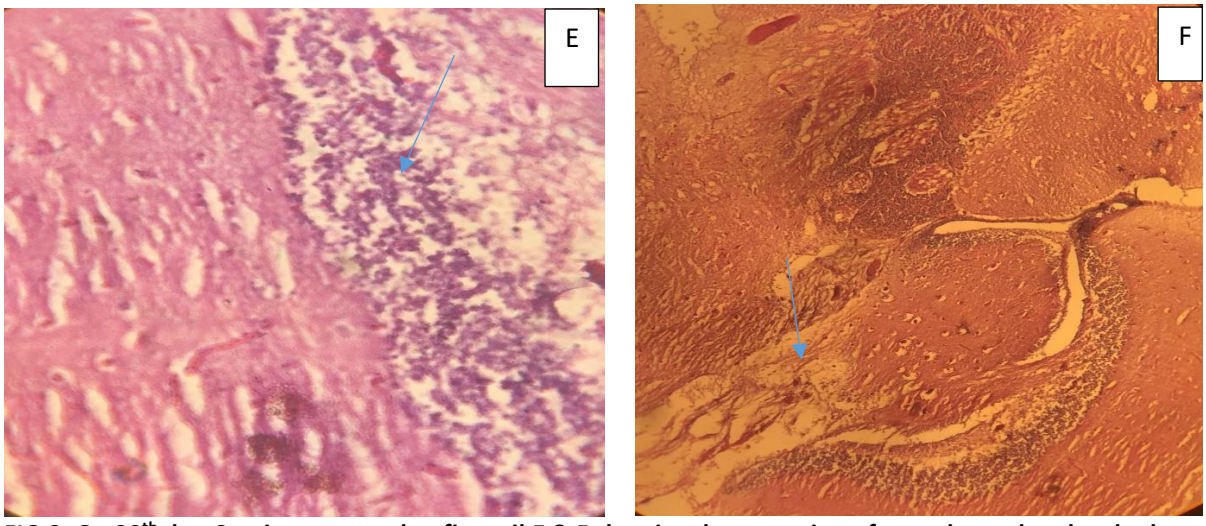

FIG.3 .On 20th day *C.striata* exposed to fipronil,E & F showing degeneration of granular and molecular layer of cerebellum(H&E)400X

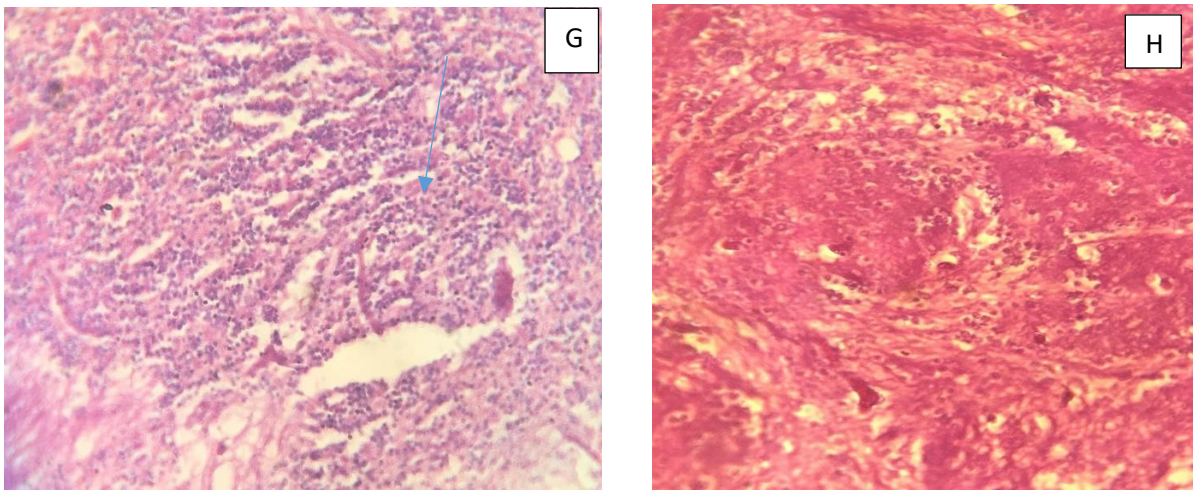

FIG.4.On 28th day *C.striata* exposed to fipronil, G&H Showing infiltration of granular cells, hyalinization of molecular layer(H & E)400X

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