

## EFFECT OF IMIDACLOPRID 17.8% SL ON BEHAVIOURAL PARAMETERS OF THE FRESH WATER FISH *Catla catla*

Sumaiya Ismayil<sup>\*1</sup>, Aneykutty Joseph<sup>\*2</sup>

<sup>\*1,2</sup>Department of Marine Biology, Microbiology and Biochemistry School of Marine Sciences

Cochin University of Science and Technology, Cochin – 682016, Kerala, India.

### ABSTRACT

Use of pesticide in integrated farming in paddy field is recognized as a potential threat in aquatic organisms. The effect of sublethal toxicity of a imidacloprid 17.8% SL pesticide on behavioural parameters of a freshwater fish *Catla catla* were studied after 24 hrs, 48 hrs, 72 hrs and 96 hr of exposure. The median lethal concentration (LC<sub>50</sub>) of imidacloprid 17.8% SL for 96 hr was 4.11 ppm. The study suggests that exposure to imidacloprid 17.8% SL at low concentration results in significant behavioural alterations. Observations made after imidacloprid 17.8% SL exposure indicate unusual behaviour such as cough, yawn, fin flickering, threat, nudge and nip were also increased due to pesticide poisoning. In the present study, the pesticide imidacloprid 17.8% SL caused alterations on behavioural parameters of *C. catla* and these alterations can be used as biomarkers in pesticide contaminated aquatic environment.

### I. INTRODUCTION

Since the science of toxicology began thousands of years ago, behavioural end points have been used to study the effects of chemicals and drugs on humans and other animals. However, in aquatic toxicology the nexus of behavioural sciences with the study of toxicants has only become prominent within the last 5 decades. Recently, there was a poor understanding of how alterations in behavior may be related to ecologically relevant issues such as predation avoidance, prey capture, growth, stress resistance, reproduction and longevity. Further, the ability to achieve repeatable, quantifiable data from a large number of animals or exposure has been challenging.

Recent improvements in computer and video automation have made possible significant progress in the ease, utility, and affordability of obtaining, interpreting, and applying behavioural endpoints in a variety of applications from water quality monitoring to use in toxicity identification evaluation (Gruber *et al.*, 1997; Baldwin *et al.*, 1994). Consequently, behavioural endpoints in aquatic toxicology are shifting from being met with scepticism by investigators to being received with greater enthusiasm. One of the first comprehensive reviews on aquatic behavioural toxicology was published by Rand (1985). Over the past 20 years, the field of behavioural toxicology has grown, in part, because of increased interest in the number of species used, endpoints measured, and methods to collect and interpret data.

Numerous reviews have traced these advancements in the field of Behavioural toxicology (Gray, 1990; Little and Finger, 1990; Doving, 1991). However, the recognition of behavioural toxicology as an important tool in aquatic toxicology is most clearly seen in the acceptance of behavioural endpoints in federal regulations.

Behaviour is the product of interaction of an organism with its external environment and represents the integration and underlying physiological process and mechanisms. Behavioural plasticity is the ability of a single genotype to produce more than one alternative, potentially adaptive behaviour in response to environmental conditions. An organisms ability to alter its behavioural response to contaminant induced changes, whether internal or environmental, may significantly affect its chances for survival or reproduction.

Behavioural toxicology is a tool for hazard assessment in water pollution (Hara *et al.*, 1976). Behavioural changes in animals are indicative of internal disturbances of body functions such as inhibition of enzyme functions (Cearley, 1971), impairment in neural transmission (Veena *et al.*, 1997), and disturbances in metabolic pathways (Das and Banergee, 1980). The development of response criteria in animals varies from detailed

physiological measurements to whole animal response, especially preference or avoidance behavior (Hartwell *et al.*, 1989).

Contaminant affects a variety of behavior of many that influence growth, reproduction and survival. Behavior, however, is not routinely used in hazard assessment or in establishment of water quality criteria. Lack of test standardization and field verification of behavioural responses are major challenges that limited the acceptance of behavior as regulatory end points (Little and Finger, 1990).

Behaviour of aquatic organisms can also be used to identify the modes of action of chemicals. Diamond *et al.* (1990) demonstrated that the amplitude and frequency of ventilation, number and type of gill purges and frequency of erratic movement in *Lepomis macrochirus* could be used to differentiate between different groups of chemicals. The amplitude and frequency of rhythmic ventilatory movement, gill purge and erratic locomotor behaviour of captive fishes are sensitive indicators of environmental stress.

In the present study, an attempt has been made to evaluate the sub lethal effects ( $1/5^{\text{th}}$ ,  $1/10^{\text{th}}$ , and  $1/15^{\text{th}}$  of  $LC_{50}$ ) of imidacloprid 17.8% SL, on the behaviour of fish, *C. catla*

## II. MATERIALS AND METHODS

The test organisms are collected and acclimatized in the laboratory for 15 days. The temperature in the tank during the experiment was maintained at 26-27<sup>0</sup>c, pH at 7-7.5, Dissolved oxygen 6-6.8 mg/L and salinity at 0 ppt. The saturation of oxygen was maintained by giving aeration in the tank. During acclimatization period the fishes were fed with commercial fish feed. The fishes of size range 5-6 cm in length were selected for the experiment irrespective of the sex. Feeding of fishes was done one hour before changing the water and waste food and faeces removed in the tank during changing the water. Five fishes were transferred to each experimental tank. Glass tanks were used for the behavioural study. Each tank contained 5 L of water. Based on the  $LC_{50}$  values the sub lethal concentrations of imidacloprid 17.8% SL was added to each experimental tank. One tank was kept as control. Replicates were run for each concentration. The medium renewed every 24 hours for reducing the concentration of ammonia within the experimental tank (APHA, 2005). During the exposure, the altered behaviour of fish was monitored at regular intervals.

### Data Analysis

The percentage of mortalities was used for statistical analysis. The probit mortality was calculated using SPSS software 16.0. Probit values were plotted on probit paper, and the concentrations of pesticide that killed 50% of the test organism ( $LC_{50}$ ) during a 96-hour exposure with a 95% confidence limit [9]. Data from the different experiments were subjected to statistical analysis for ANOVA, using SPSS 22.0 software to determine the significance of the results.

## III. RESULT

Observations made on fish exposed to sub lethal concentrations of imidacloprid 17.8% SL indicate remarkable changes in behaviour. With increasing duration of exposure to the toxic material, the symptoms observed including coughing, yawning, partial and S jerk activity and burst swimming, following this the fish assumes a tilting position whilst performs sudden jerks. Finally, the behaviour changed to elements including aggressiveness such as nudge and nip which were increased following exposure to the toxic material. Altered rate of respiration in exposed fishes as indicated by increased frequency of cough and yawn.

Specimens of *C. catla* exposed to sublethal concentrations of imidacloprid 17.8% SL showed changes in behaviour. Uncomfortable behaviour like cough (Fig 1), yawn (Fig 2), fin flickering (Fig 3), partial jerking (Fig 4), S- jerking (Fig 5), burst swimming (fig 6) and darting movements were increased when exposed to imidacloprid 17.8% SL. The fishes were hyper excited and restless. The frequency of other kinds of unusual behaviour such as nudge (Fig 7), threat (Fig 8), and nip (Fig 9) were also increased due to pesticide poisoning. The different kinds of unusual behaviour shown by fishes were monitored and presented in Table 1. The data indicates that imidacloprid 17.8% SL exposure affected respiratory behaviour which is

manifested by elevated frequencies of cough and yawn. Discomfort movements and aggressive behaviour were also increased due to imidacloprid 17.8% SL exposure. The effect of imidacloprid 17.8% SL on fish seem to be dose dependent and time dependent as higher frequencies were recorded at higher dose and in early period of exposure. One-way ANOVA results shows that variations in the frequencies of occurrence of these behaviours in imidacloprid 17.8% SL treated fishes were found to be significant. When the time of exposure was prolonged the frequency of different behaviours were reduced but it remained higher than the control in all periods of exposure.

**Table-1:** Behavioural responses in *C. catla* exposed to different concentrations of imidacloprid 17.8% SL (mean frequency/5 fishes/2 hour)

Parameters	Control	0.27ppm (1/15 <sup>th</sup> of LC <sub>50</sub> )	0.41ppm (1/10 <sup>th</sup> of LC <sub>50</sub> )	0.82 ppm (1/5 <sup>th</sup> of LC <sub>50</sub> )
Cough	30±1.41	39.5±0.70*	44.5±0.70*	48.5±0.70*
Yawn	15±1.41	21±1.41*	28.5±0.70*	34.5±0.70*
Fin-flickering	161.5±2.12	183±1.41*	203±1.41*	230±2.82*
Partial jerk	43±1.41	51.5±0.70*	57±1.41*	66±2.82*
S-jerk	29.5±0.70	36±1.41*	45.5±2.12*	53.5±0.70*
Burst swimming	24.5±0.70	34±2.82*	45.5±0.70*	55±1.41*
Nudge	45.50±70	54±2.82*	63±1.41*	70.5±2.12*
Threat	74.5±2.12	84.5±2.12*	90±1.41*	97.5±2.12*
Nip	147.5±2.12	166±2.82*	183.5±2.12*	197.5±2.12*

Value ± SD \*Significance level at p<0.001

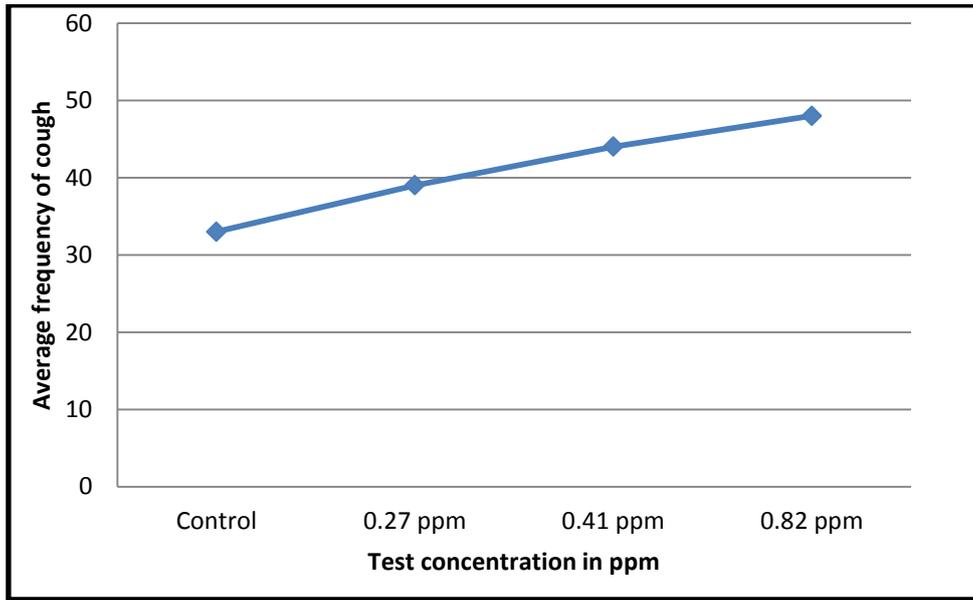


Fig-1: Average frequency of cough during exposure to imidacloprid 17.8% SL

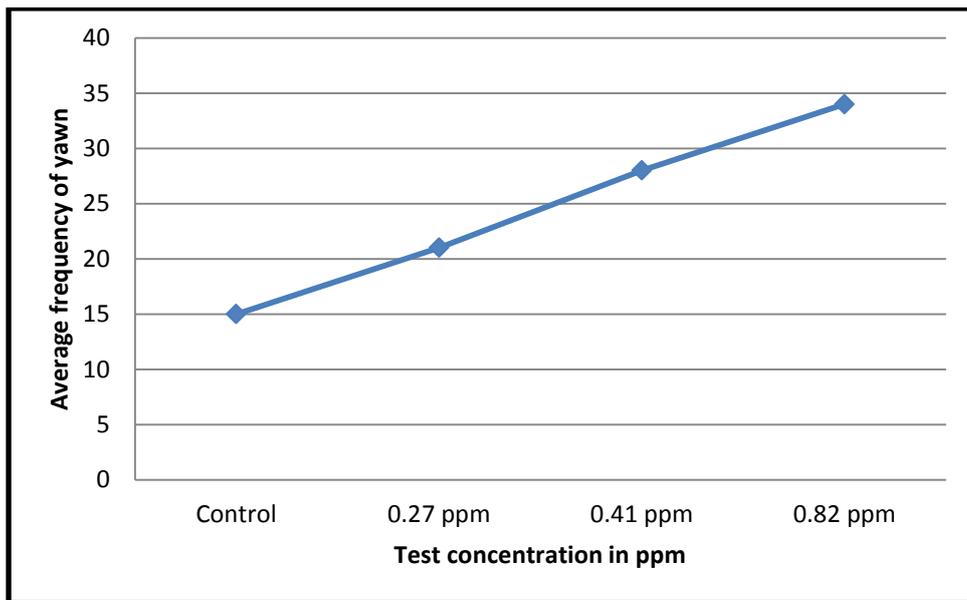


Fig-2: Average frequency of yawn during exposure to imidacloprid 17.8% SL

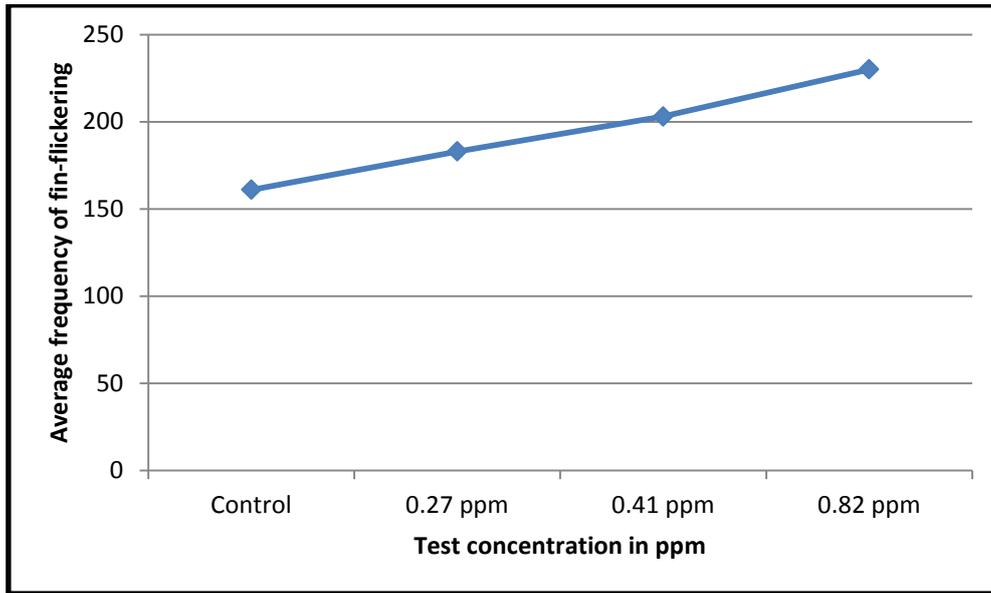


Fig-3: Average frequency of fin-flickering during exposure to imidacloprid 17.8% SL

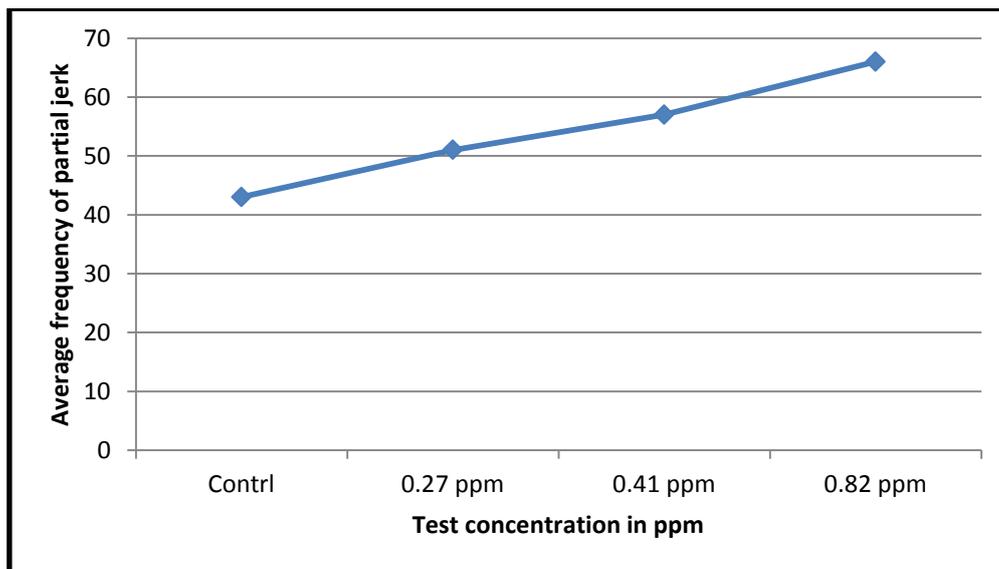


Fig-4: Average frequency of partial-jerk during exposure to imidacloprid 17.8% SL

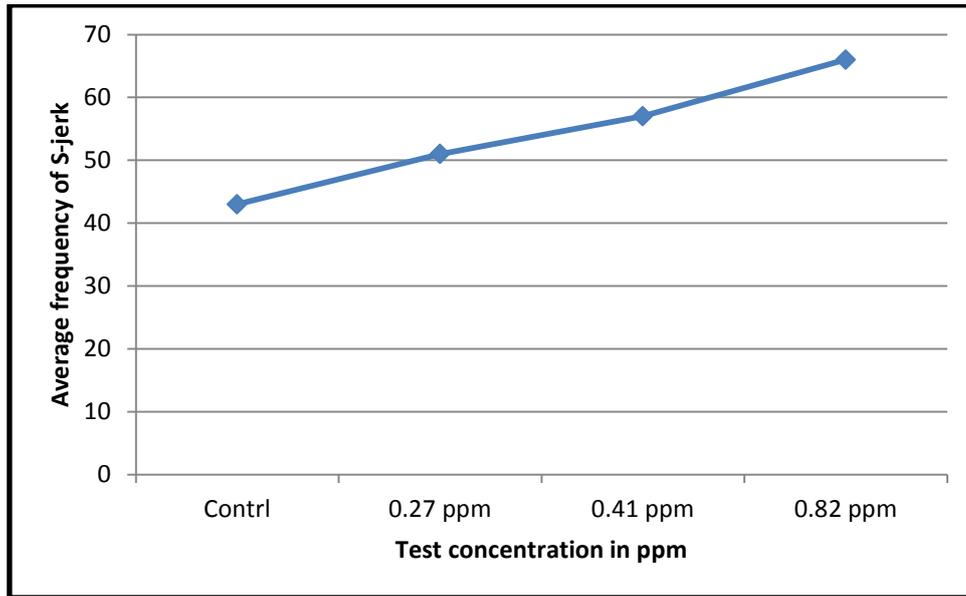


Fig-5: Average frequency of S-jerk during exposure to imidacloprid 17.8% SL

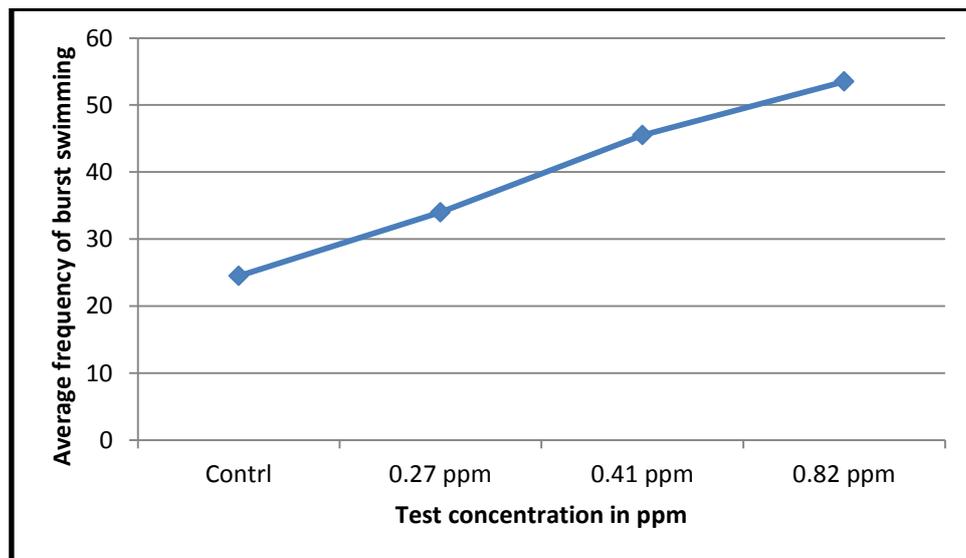


Fig-6: Average frequency of burst swimming during exposure to imidacloprid 17.8% SL

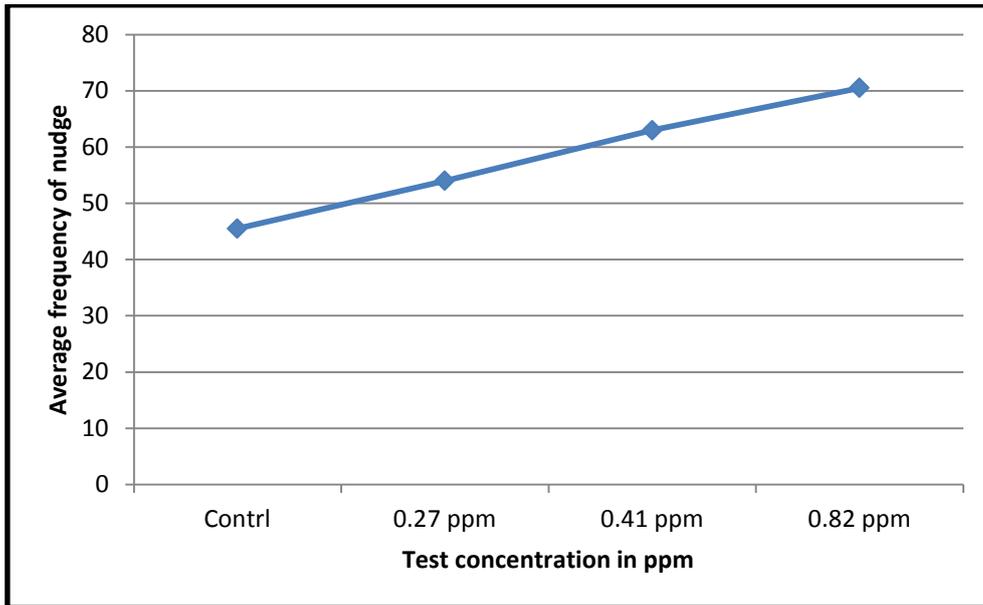


Fig-7: Average frequency of nudge during exposure to imidacloprid 17.8% SL

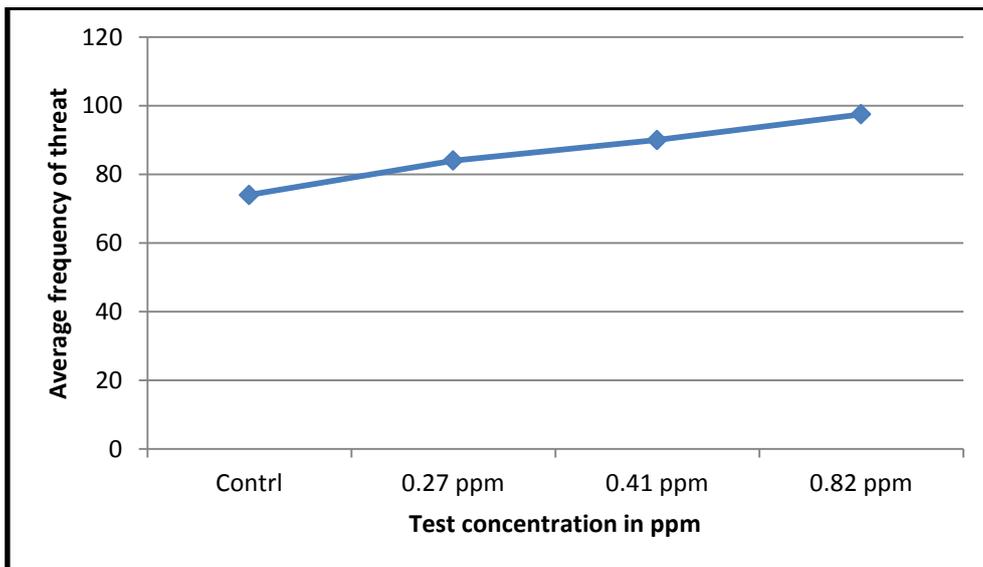


Fig-8: Average frequency of threat during exposure to imidacloprid 17.8% SL

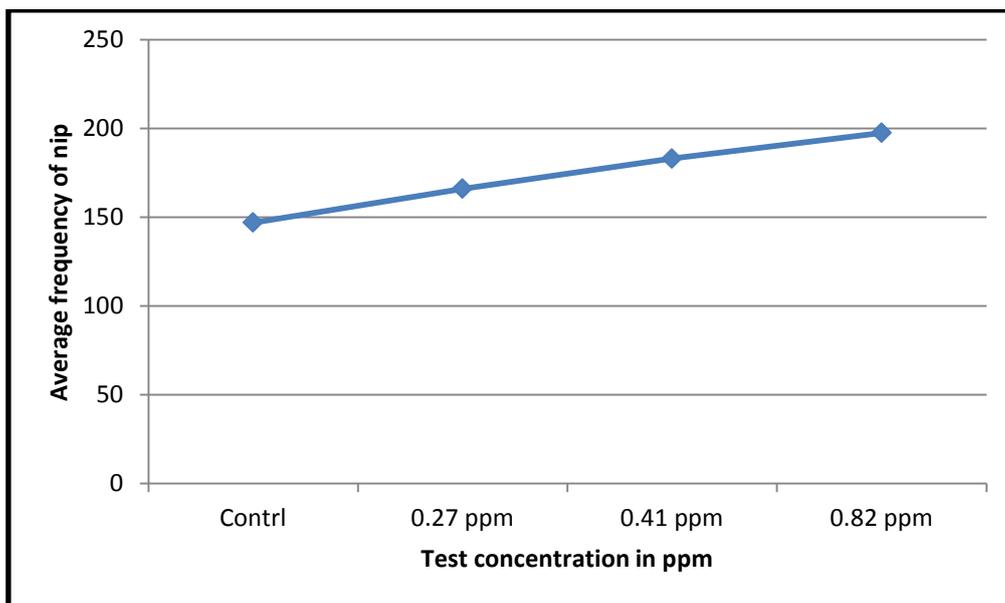


Fig-9: Average frequency of nip during exposure to imidacloprid 17.8% SL

#### IV. DISCUSSION

Pesticides are widely used in agriculture and there is a need for tools to monitor the impact of these pesticides on fish population. Production of fish in rice fields is almost as primitive as the practice of rice culture itself. Rice farming with- fish culture is a type of duo culture farming system in which rice is the sole enterprise and fishes are taken to initiate additional for extra income. Rice-cum fish culture is practiced in many rice-growing belts of the world including China, Bangladesh, Malaysia, Korea, Indonesia, Philippines, Thailand and India. Rearing of fish along with paddy is an older farming practice adopted in India. It has largely been practiced in a traditional primitive way in the coastal areas of the country. There is a growing concern worldwide over the indiscriminate use of chemicals, resulting in environmental pollution and toxicity risk to on-targeted organisms (Bayo, 2011).

Responses of aquatic organisms are broad- ranged depending on the toxic compound, exposure time, water quality and the species (Fisher, 1991). Fishes are continuously exposed to comparatively low concentrations of pesticides affecting their behavioural responses. Mortality is obviously not the only endpoint to consider and there is a growing interest in the development of behavioural markers to assess the sub-lethal affects of toxicant. According to Drummond and Russom (1990) behaviour is considered a promising tool in ecotoxicology and these studies are becoming prominent in toxicity assessment in unicellular organisms (Tadehl and Hader, 2001), fish (Hansen *et al.*, 1999) and even rodents (Omo *et al.*, 1997). Locomotory behaviour is commonly affected by contaminants (Mel and Pathiratne, 2005) and the pattern of fish swimming is a highly organized species specific response. Various methods were employed to investigate the altered locomotor observations were reported in r behaviour of stressed organisms.

The behavioural changes in the exposed fish, in the present study appear to be the manifestation of imidacloprid 17.8% SL toxicity. Upon exposure to imidacloprid 17.8% SL, increase in surfacing and gulping of surface water appears to be an attempt by the fish to avoid breathing in the poisoned water and increasing unusual behaviour like cough, yawn, fin flickering etc. Similar observations were reported in *Anabas testudiensis* after exposure to pesticide monocrotophos by Santhakumar and Balaji (2000).

Moreover the hypotoxic conditions also contribute to increase unusual respiratory behavior as reported by Radhaiah and Rao (1998) in *Tilapia mossambica* after exposure to pesticide fenvalerate. Hypotoxic conditions arises primarily due to damage of gills of fenvalerate exposed *Cirrhinus mrigala* (Velmurugan *et al.*, 2007). Gill damage due to imidacloprid 17.8% SL was also observed in the present study.

Ganeshwade *et al.* (2006) have been reported increased coughing in the common carp exposed to industrial effluents. The observations in the present study agree with these reports. In fact coughing responses is shown to have direct relationship was shown by *C. catla* when exposed to various sub lethal concentrations of imidacloprid 17.8% SL. Following the initial restlessness the fishes calm down in lower concentrations after some time, but at higher concentrations fishes showed restlessness throughout the experimental period.

The observed changes in behaviour of *C. catla* may be due to a direct manifestation of the disturbances in physiological mechanisms, which initiates, maintain and terminate behaviour. According to Marler and Hamilton (1966), erratic swimming, jerking, and aggressive behaviours observed after the exposure may be due to the effect of imidacloprid 17.8% SL on central nervous system. Available evidence indicates that the imidacloprid pesticide exert an insidious effect on the central nervous system (Peakall, 1969). The change in the swimming behaviour might be due to the damage inflicted on the lateral line sensory cells of the fish by the pesticide that entered through their pores in the lateral line system. Further research is necessary to study the effect of imidacloprid 17.8% SL and their combinations on the behaviour of fish in the laboratory as well as field conditions.

## V. CONCLUSION

The result of the present study indicate that imidacloprid 17.8% SL exposure during sublethal treatment induces significant changes in the behavioural parameters of *C. catla*. The behavioural changes in *C. catla* under the three test concentrations are observed. The observed behaviors include cough, yawn, fin-flickering, partial jerk, S-jerk, burst swimming, nudge, threat and nip. The frequency of all these were significantly ( $p < 0.001$ ) higher than in the control fishes. The frequency of occurrence of observed behaviours remained in the same significant level throughout the period of observation. The findings of the present study provide a better understanding of the toxicological endpoint of imidacloprid 17.8% SL and to ascertain a safer level of these pesticide in the aquatic environment.

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