

## TOXIC IMPACT OF THIAMETHOXAM ON THE GROWTH PERFORMANCE AND LIVER PROTEIN CONCENTRATION OF A FRESHWATER FISH *OREOCHROMIS NILOTICUS* (TREWAVAS)

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### ABSTRACT

Thiamethoxam pesticides are frequently used for pest control in agricultural fields and may reach the surrounding freshwater bodies through irrigation or rain. As a result, many non-target organisms like fish of the freshwater ecosystem are adversely affected. *Oreochromis niloticus* (Trewavas) is a common freshwater fish, which is a staple diet consumed by the people of West Bengal. The objective of the present study was to describe the effect of Thiamethoxam on the growth and liver total protein of this exotic fish. The study revealed that various sublethal doses of Thiamethoxam had significant impact on growth and liver total protein of this fish.

**Key Words:** *Oreochromis niloticus*, Thiamethoxam, Growth, Protein

### INTRODUCTION

A pesticide is any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any pest. In fish, exposure to chemical pollutants can induce either increases or decreases in haematological parameter levels. Pesticides and related chemicals destroy the delicate balance between species that characterizes the functioning ecosystem. Pesticides produce many physiological and biochemical changes in freshwater organisms. Indiscriminate and extensive uses of pesticides to protect crops possess a serious threat to humans and the surrounding environment. (Tilak *et al.* 2007). Thiamethoxam insecticide causes impairment of physiological parameters and histological features in albino rats. Therefore, these effects may influence the use of this insecticide against pests attacking vegetables in the fruit stage (Shalaby *et al.* 2010). Acda (2007) applied Thiamethoxam on three species of economically important subterranean termites in the Philippines, and feeding bioassays showed that this insecticide was repellent to *M. gilvus* and *M. losbanosensis*. Torres *et al.* (2003) reported that Thiamethoxam was very much effective at low doses for controlling white fly and aphid in cotton. Aliouane *et al.* (2008) conducted a laboratory bioassay and evaluated the effect of sublethal doses of Thiamethoxam on honey bee behaviour. Benzidane *et al.* (2010) reported a decreasing trend of locomotory activity in *Periplaneta americana* when Thiamethoxam was applied on this insect. McCornack and Ragsdale (2006) suggested Thiamethoxam as an efficient insecticide to suppress aphid population of Minnesota soybean. Thiamethoxam showed the highest rate of efficiency against whitefly (Al-Kherb 2011).

Pesticides can be circulated into different ecosystems by different agents (Weber 1977) after entering the environment like air, water, different food chains, soil and other agents (Farmer *et al.* 1972). The pesticides which are liberated into the aquatic environment have a detritus effect on fish and subsequently on man (Metelev *et al.* 1983).

Investigations have shown that changes in carbohydrate and nitrogenous metabolism in fish, induced by the stress, occurred by pesticide-induced hypoxia. These changes include depletion of proteins, glycogen and pyruvate stores from fish tissues such as liver and muscle (Laul *et al.* 1974). Black (1958) reported an elevation in lactic acid level in liver, muscle and blood and suggests that an uncontrolled entry of lactic acid into the tissues interferes with internal mechanisms, which maintain the acid-base balance. Lactic

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acid may also reduce the affinity of hemoglobin for both oxygen and carbon dioxide, diminishing the oxygen-carrying capacity of blood.

In the present study, an attempt was made to examine the sublethal toxic effect of Thiamethoxam insecticide on protein metabolism in the liver, of freshwater fish *Oreochromis niloticus*. The insecticide Thiamethoxam and fish were selected for study because the former is used often in field and the latter is an important fresh water fish and consumed by people of West Bengal as their staple diet.

## **MATERIALS AND METHODS**

**Pesticide Used:** A Thiamethoxam pesticide which is commonly used in West Bengal, India, contains Thiamethoxam 25% WG as main composition. It is a broad spectrum systemic insecticide having quick stomach and contact activity and is recommended for its use to control Brown Plant Hopper (BPH), White Backed Plant Hopper (WBPH), Green Leaf Hopper (GLH), Stem Borer, Gall Midge, Leaf Folder and Thrips in Rice as well as Aphids, Jassids, Thrips and Whiteflies in Cotton.

This is also recommended for the control of Hoppers in Mango, Aphids in Wheat. Aphids, Jassids and Whiteflies in Okra, Psyllids in Citrus, Whiteflies in Tomato. Whiteflies and Jassids in Brinjal (Foliar application), Tea mosquito Bug in Tea, Aphids in Potato (Foliar application and soil Drench application).

The compounds used for agricultural purposes are available mainly as emulsifiable concentrates or wettable powder formulations for reconstitution as liquid sprays, but also as granules for soil applications. A limited number are also available as fogging formulations, smokes, impregnated resin strips for use indoors, and as animal or human pharmaceutical preparation.

**Experimental Design:** The live freshwater fish *Oreochromis niloticus* were collected from the market of Salt Lake, Calcutta, West Bengal. The fish were stored in glass aquaria containing tap water for seven days for acclimatization under laboratory conditions. Water was changed after every 24h. Commercial fish food was supplied to fish during acclimatization as well as treatment period. Dead fish (if any) were removed from the aquaria as soon as possible to avoid water fouling. Adult fish of nearly similar weight ( $51.20 \pm 1.26$  g) and length ( $14.52 \pm 0.16$  cm) were selected for experiments. Separate aquarium was set up for each concentration and each aquarium contained 10 fish in 20 L tap water. Water temperature was kept at 28°C during whole experimental period. Qualities of experimental water (pH= 7; dissolved oxygen= 11.91 mg.L<sup>-1</sup>; free carbon dioxide= 62.62 mg.L<sup>-1</sup>; alkalinity= 460 mg.L<sup>-1</sup>) were measured. Control groups were kept in tap water without any treatment. Based on the result of 96h LC<sub>50</sub>, *Oreochromis niloticus* were exposed to 12.5, 25, 50, 100, 150 mg.L<sup>-1</sup> concentration of Thiamethoxam for 7 days and 14 days and each concentration was replicated three times (Auta, 2001). Fish of both treated as well as control groups were killed by a severe blow on the head and liver were isolated for total protein estimation.

**Growth Study:** Growth was monitored in respect to body weight, length and breadth (Yaji and Auta, 2007). The weights of fish were measured with the help of pan balance. 10 fish were taken for each set of experiment and an average was done. Length and breadth were measured with the help of Vernier scale of the same fish which were considered for measuring the weight. Measurements were done for the control and all the Thiamethoxam dosed fish.

**Total Protein:** For total protein estimation, liver was collected from control and treated fish and homogenates were prepared with 0.1M phosphate buffer (pH 7.4) (Saito *et al.*, 1983). Estimation was made according to the method of Lowry *et al.* (1951). Bovine serum albumin was taken as standard.

**Statistical Analysis:** Correlation coefficients and its significance level and analysis of variance were used to test for difference between different levels of treatments (Zar, 2009).

## **RESULTS AND DISCUSSION**

Water pollution is recognized globally as a potential threat to both human and other animal populations which interact with the aquatic environments. At sublethal concentration of metasystox, the fish survived

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even after prolonged periods of exposure (Natarajan, 1984). The process of bioaccumulation starts when pesticides applied to agricultural land and runoff into rivers, streams and eventually the ocean and the pollutant is ingested into the body of fish.

**Table 1: Growth of *O. niloticus* exposed to sub-lethal Concentration of Thiamethoxam.**

Parameter	Control	EP	Doses of Thiamethoxam (mg.L <sup>-1</sup> )				
			12.5	25	50	100	150
No. of fish	10		10	10	10	10	10
Weight (g.)	51.20 ± 1.26	7 days	42.80 ± 1.09	36.50 ± 2.19	35.40 ± 1.28	34.60 ± 1.21	25.70 ± 2.27
		14 days	34.20 ± 0.89	33.70 ± 2.07	31.70 ± 0.83	31.10 ± 1.66	25.60 ± 0.95
		7 days	12.63 ± 0.25	12.36 ± 0.28	12.30 ± 0.21	12.12 ± 0.20	10.76 ± 0.25
		14 days	12.09 ± 0.09	11.78 ± 0.18	11.51 ± 0.16	11.46 ± 0.14	10.68 ± 0.16
Length (cm.)	14.52 ± 0.16	7 days	4.17 ± 0.08	3.69 ± 0.17	3.48 ± 0.15	3.46 ± 0.14	3.40 ± 0.13
		14 days	3.75 ± 0.07	3.53 ± 0.12	3.40 ± 0.11	3.38 ± 0.10	3.31 ± 0.11
		7 days	4.17 ± 0.08	3.69 ± 0.17	3.48 ± 0.15	3.46 ± 0.14	3.40 ± 0.13
		14 days	3.75 ± 0.07	3.53 ± 0.12	3.40 ± 0.11	3.38 ± 0.10	3.31 ± 0.11

EP= Exposure Period; ± SE

**Table 2: Rate of changes in Growth (%) of dosed *O. niloticus*.**

Concentration (mg.L <sup>-1</sup> )	EP	Rate of Change (%)		
		Weight	Length	Breadth
12.5	7days	16.40	13.00	11.50
	14days	33.20	16.70	20.40
25	7days	28.70	14.90	21.70
	14days	34.20	18.90	25.10
50	7days	30.90	15.30	26.10
	14days	38.10	20.70	27.80
100	7days	32.40	16.50	26.50
	14days	39.30	21.10	28.20
150	7days	49.80	25.90	27.80

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14days	50.00	26.40	29.70
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**EP= Exposure Period**

**Table 3: Relation between body weight, length and breadth of *O. niloticus*.**

Variables	r-value	r <sup>2</sup>	Regression Equation	t-value	F-value
Weight-Length (Before Treatment)	0.786	0.6182	Y=7.385+0.130X	7.15*	507.74
Weight-Length (After Treatment)	0.808	0.6530	Y=8.061+0.113X	7.26*	246.00
Weight-Breadth (Before Treatment)	0.564	0.3186	Y=2.620+0.038X	3.81*	890.01
Weight-Breadth (After Treatment)	0.761	0.5797	Y=2.045+0.046X	6.21*	480.19

**\* Significant (p < 0.05)**

**Table 4: Level of total protein in Liver of fresh water fish *O. niloticus* after exposure to different concentrations of Thiamethoxam.**

Parameter	Tissue	Control	EP	Doses of Thiamethoxam (mg.L <sup>-1</sup> )				
				12.5	25	50	100	150
Protein (mg.g <sup>-1</sup> )	Liver	40.19 ± 0.92	7days	22.10 ± 0.78 r= 0.91 t= 3.82*	24.91 ± 0.67 r= 0.94 t= 4.70*	30.89 ± 0.66 r= 0.94 t= 4.70*	24.28 ± 0.48 r= 0.95 t= 5.20*	26.31 ± 0.60 r= 0.90 t= 3.58*
			14days	21.18 ± 0.59 r= 0.92 t= 4.11*	22.25 ± 0.76 r= 0.92 t= 4.11*	28.49 ± 0.52 r= 0.92 t= 4.11*	13.44 ± 0.45 r= 0.94 t= 4.70*	22.88 ± 0.69 r= 0.96 t= 5.88*

**EP= Exposure Period; ± SE**

**\* Significant (p < 0.05)**

The studies reveal that weight of the fish was gradually decreased with the increase of doses of Thiamethoxam (Table 1). When length and breadth were considered, it was observed that these two parameters showed a gradual decrease in measurement in comparison to the control fish.

Average weight, length and breadth of control fish were 51.20 ± 1.26 g; 14.52 ± 0.16 cm, and 4.71 ± 0.09 cm respectively. The rate of weight loss in different doses were 16.4% at 12.5 mgL<sup>-1</sup> for 7d Exposure Period (EP), 28.7% at 25mg.L<sup>-1</sup> for 7d EP, 30.9% at 50mgL<sup>-1</sup> for 7d EP, 32.4% at 100mgL<sup>-1</sup> for 7d EP,

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49.8% at  $150\text{mgL}^{-1}$  for 7d EP, similar trends were observed in an exposure for 14 days also (Table 2). Length and breadth also showed similar pattern of decreasing rate with increase in pesticide doses.

Present study also revealed a significant ( $p < 0.05$ ) relationship between weight-length and weight-breadth in both pesticides treated and untreated fish (Table 3). The body weight and length of the fish were positively related according to the regression equation  $Y = 7.385 + 0.130X$  ( $r = 0.786$ ,  $p < 0.05$ ) before and  $Y = 8.061 + 0.113X$  ( $r = 0.808$ ,  $p < 0.05$ ) after the treatment of the Thiamethoxam. Whereas, body weight and breadth were positively correlated before ( $Y = 2.620 + 0.038X$ ;  $r = 0.564$ ,  $r = 0.564$ ,  $p < 0.05$ ) and after ( $Y = 2.045 + 0.046X$ ;  $r = 0.761$ ,  $p < 0.05$ ) the treatment of Thiamethoxam.

After the completion of 7 and 14 days of exposure of different doses of Thiamethoxam, liver total protein was studied. The data of the biochemical analysis is given in Table 4. Fish expose to Thiamethoxam exhibited a significant ( $p < 0.05$ ) dose dependent increase in liver protein. Levels of protein were  $22.10 \pm 0.78 \text{ mg.g}^{-1}$  (Dose =  $12.5 \text{ mg.L}^{-1}$ ; EP = 7d),  $21.18 \pm 0.59 \text{ mg.g}^{-1}$  (Dose =  $12.5 \text{ mg.L}^{-1}$ ; EP = 14d),  $24.91 \pm 0.67 \text{ mg.g}^{-1}$  (Dose =  $25 \text{ mg.L}^{-1}$ ; EP = 7d),  $22.25 \pm 0.76 \text{ mg.g}^{-1}$  (Dose =  $25 \text{ mg.L}^{-1}$ ; EP = 14d),  $30.89 \pm 0.66 \text{ mg.g}^{-1}$  (Dose =  $50 \text{ mg.L}^{-1}$ ; EP = 7d),  $28.49 \pm 0.52 \text{ mg.g}^{-1}$  (Dose =  $50 \text{ mg.L}^{-1}$ ; EP = 14d). A significant decrease in the protein level was observed with the increase of doses of pesticide. Levels of protein were  $24.28 \pm 0.48 \text{ mg.g}^{-1}$  (Dose =  $100 \text{ mg.L}^{-1}$ ; EP = 7d),  $13.44 \pm 0.45 \text{ mg.g}^{-1}$  (Dose =  $100 \text{ mg.L}^{-1}$ ; EP = 14d). But further increase in the dose showed slightly increase in the protein level (Table 4).

Prolonged exposure of *Oreochromis niloticus* to Thiamethoxam recorded a significant reduction ( $p < 0.05$ ) in weight gain as well as length and breadth of the fish. Such reduction in growth rate in *O. niloticus* was probably due to suppressive effect of toxicant or increased activity in an attempt to avoid the polluted water by the fish. Similar observations were made by Yaji and Auta (2007) in case of *Clarias gariepinus*. The study shows that exposure of *Clarias gariepinus* to low concentration of Monocrotophos affects the physiology of this fish which over time affected the food acceptability and loss in weight.

Proteins are mainly involved in the architecture of the cell. During chronic period of stress they are also a source of energy (Umminger, 1977). The study revealed that there was a significant ( $p < 0.05$ ) increase in the total protein between  $12.5 \text{ mg.L}^{-1}$ ,  $25 \text{ mg.L}^{-1}$  and  $50 \text{ mg.L}^{-1}$  doses of Thiamethoxam which was followed by a significant decreased ( $p < 0.05$ ) in the protein level in liver at  $100 \text{ mg.L}^{-1}$  of pesticide. Again the protein level was increased at  $150 \text{ mg.L}^{-1}$  dose. This primary increase in the protein level was probably due to check the effect of toxicant and try to recover from the stress of pesticide at lower doses. When the dose was increased, there was decrease in liver protein level. That may be due to depletion of protein fraction in liver, may have been due to their degradation and possible utilization of degraded products for metabolic purpose. This was in conformity with the effects of organophosphate on *Channa punctatus* (Tripathi et al., 2003). Singh et al., (1996) have also reported decline in protein constituent in different fish tissue exposed to sublethal concentration of insecticides.

The study also revealed that weight, length and breadth showed a decreasing trend in all doses of pesticide though there was a variation in protein level at different dosed fish. That was probably to cope up with the increasing doses so that the fish could overcome the toxic effect of Thiamethoxam to make a balance between growth and metabolism. This was in conformity with the effects of thiodon pesticides on *Clarias gariepinus* (Aguigwo, 2002).

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