

Pesticide induced changes in the proximate composition of a freshwater fish for estimating maximum allowable toxicant concentration of the pesticide under tropical conditions

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ABSTRACT

The proximate composition is used to assess the nature and extent of pesticide induced pathogenesis in *Etroplus maculatus*, which is a true denizen in the paddy fields of Kuttanad. *E. maculatus* was subjected to long term exposure to sublethal concentrations of monocrotophos, which is a widely used organophosphate pesticide in the paddy fields of Kuttanad. The results showed that, the Maximum Allowable Toxicant Concentration (MATC) end points of carbohydrate and ash gave sensitive end points whereas that of protein and fat was less sensitive. For monitoring early changes, biochemical biomarkers can be used as sensitive end points. The application factors (AF), derived from the laboratory studies can be directly assigned to the field conditions of Kuttanad, as the experimental fishes are the true denizens of Kuttanad water bodies. If we know the application factor of one pesticide, the MATC for other related pesticides could be easily calculated provided its 48 h LC₅₀ is known. This knowledge of fundamental toxicological and pathological processes is not only important for the regulations of chemicals that are potential aquatic pollutants, but also for the researchers who are involved in similar field studies.

Keywords: Maximum Allowable Toxicant Concentration (MATC), Monocrotophos, Pesticide induced changes, Proximate composition, Sublethal toxicity

Introduction

Seasonal utilization of paddy fields for fish culture is quite common in Kerala and West Bengal. In recent years, with the advent of high yielding varieties of paddy, the use of pesticides has become very popular. According to the data compiled by Kuttanad water balance study project, 485 t of pesticides were applied in Kuttanad on an annual basis of which, 370 t were used for the *puncha* crop alone (KWBS, 1990). Dimecron, Monocrotophos, Henosan, Thymet, Fernoxan and Nuvacron are the major components of the pesticides being used in Kuttanad.

Pesticides in the aquatic environment affect various physiological and biochemical aspects of fishes (Webb and Brett, 1973; Palanichamy *et al.*, 1986, 1987; Baskaran *et al.*, 1987; Seshagiri Rao *et al.*, 1987; Vasanthi and Ramasamy, 1987; Vasanthi *et al.*, 1990). Many studies have reported decline in body proximate composition of fishes exposed to different pesticides (Sivaprasanna Rao and Ramana Rao, 1979; Rath and Misra, 1980; Palanichamy *et al.*, 1986, 1987; Vasanthi and Ramaswamy, 1987; Seshagiri Rao *et al.*, 1987; Babu *et al.*, 1988; Malla Reddy and Bashamohideen, 1988; Begum and Vijayaraghavan, 1995). Hence water pollution can lead to different changes, ranging

from biochemical alternations in single cell to changes in whole populations. In general, the end points used in toxicity studies are mortality, survival and growth with acute toxicity tests. These parameters are quite appropriate, but for long-term sublethal concentrations, these relevant parameters are difficult to ascertain. Hence in the present study, proximate composition of freshwater fish was analysed to estimate the maximum allowable toxicant concentration of the pesticide. The proximate composition was used to assess the nature and extent of pesticide induced pathogenesis in *Etroplus maculatus*, which is a true denizen in the paddy fields of Kuttanad. *E. maculatus* was subjected to long term exposure to sublethal concentrations of monocrotophos, which is a widely used organophosphate pesticide in the paddy fields of Kuttanad.

Materials and methods

The experiments on the lethal and sublethal toxicity of monocrotophos in the juveniles of *Etroplus maculatus* were conducted for 48 h and 30 days, respectively during the period of investigation. Juveniles of *E. maculatus* collected from pollution free ponds (47.5 ± 9.0 mm in total length and 330 ± 80 mg weight) were used for monocrotophos exposure. During these periods, they were

fed fresh clam meat *ad libitum* once a day. Based on the LC₅₀ values (Mercy *et al.*, 2000), five nominal concentrations of the pesticides were selected for sublethal toxicity (30 days) studies. Maximum and minimum sublethal concentrations were chosen based on Konar (1969) and Sprague (1973).

Sublethal exposure was done in a static system where water and pesticide medium were renewed every 24 h to obtain the desired pesticide concentration. A control, free of pesticide, was also maintained in each experiment. All the treatments were made in triplicates. Ten healthy specimens of *E. maculatus*, chosen at random from the acclimated stock were reared in 32 l of water in seasoned cement cisterns. The ratio of the animal wet weight to water volume ranged from 0.4899 - 2.7875 gm l⁻¹. The tanks were covered with plastic mesh nets to prevent escape of the fishes by jumping. All the experiments were conducted at an ambient temperature of 28 ± 2 °C. Dissolved oxygen, pH and temperature were measured immediately before and after the pesticide inoculation using standard procedures. While running the bioassay, the animals were closely observed for their general behaviour, health and number.

After 30 days sublethal toxicity experiment, five fishes from each exposure were selected for proximate analysis. Crude protein (Kjeldahl nitrogen x 6.25) and ash contents (residue left after heating samples at 550 °C in a muffle furnace for 6 h) were determined by AOAC (1984) methods.

Table 1. The LC₅₀ of monocrotophos on *E. maculatus* (Mercy *et al.*, 2000) and sublethal concentrations

Species	Pesticide	48 h LC ₅₀ (ppm)	Sublethal concentrations (mg l ⁻¹)					
<i>E. maculatus</i>	Monocrotophos	3.36	0.0	0.1	0.3	0.6	1.0	1.5

Lipids were extracted using soxhlet extraction method (AOAC, 1984). Carbohydrate concentration was indirectly determined using Knauer's procedure (Knauer *et al.*, 1994) as given below.

Percentage of carbohydrate = 100 - (% of protein + % of lipid + % of ash).

Specimens of *E. maculatus* were also collected from the paddy fields and polders of Kuttanad, during the month of February – March (the period when the pesticide application is maximum) and were subjected to proximate analysis.

Statistical analysis

The treatment means of various indices were subjected to one-way analysis of variance on IBM-PC microcomputer based on CRD programmes.

Based on the significance of values in ANOVA test, no observable effect concentration (NOEC - is the concentration

at which no significant change in the proximate composition of the body) and least observable effect concentration (LOEC - in which there are significant changes in the proximate composition from that of the control) were determined.

Based on these NOEC and LOEC values, maximum allowable toxicant concentration (MATC- the concentration in which the animal can tolerate for survival and reproduction without much effect on its metabolic activities) level is calculated using the formula

$$\text{MATC} = (\text{NOEC} \times \text{LOEC})^{1/2}$$

From this MATC value, application factor was calculated using the formula (Mount and Stephan, 1967)

$$\text{AF} = \frac{\text{MATC}}{48 \text{ h LC}_{50}}$$

The application factor (AF), worked out for one species can be used for other related species also, provided their 48 h LC₅₀ is known. From this, the MATC can be conveniently found out. This will be an important tool for the farmers in deciding the concentrations of pesticides before its application in the paddy fields.

Results

The LC₅₀ of monocrotophos on *E. maculatus* (Mercy *et al.*, 2000) and sublethal concentrations chosen for the experiments are given in Table 1.

Crude protein, crude fat and carbohydrate contents of the whole body of *E. maculatus* decreased when fish were exposed to various sublethal concentrations of monocrotophos, whereas the ash levels increased with increasing concentrations. Proximate composition of *E. maculatus* at the end of the 30 days exposure to various sublethal concentrations of monocrotophos is given in Table 2. Carcass proximate composition of fish collected from the paddy fields of Kuttanad showed variation from that under sublethal studies (Table 3).

Physico-chemical parameters

Weekly mean temperature, pH and DO values ranged from 28.0 to 28.4 °C, 6.8 to 7.5 and 6.2 to 7.1 mg l⁻¹ respectively.

Proximate analysis

Comparison of treatment means of *Etroplus maculatus* to various sublethal concentrations of monocrotophos, were done, based on critical difference (Table 4).

Table 2. Proximate composition (Mean* ± SD) of *E. maculatus* at the end of 30 days exposure to various sublethal concentrations of monocrotophos.

Energy component	Nominal monocrotophos concentrations (ppm)					
	T ₁ (0.0)	T ₂ (0.1)	T ₃ (0.3)	T ₄ (0.6)	T ₅ (1.0)	T ₆ (1.5)
Crude fat	11.55 ± 0.17a	11.51 ± 0.17a	11.45 ± 0.11a,b	11.30 ± 0.09a,b*	10.98 ± 0.29b**	9.75 ± 0.58c
Crude protein	68.88 ± 0.16a	68.79 ± 0.18a	68.62 ± 0.21a	68.55 ± 0.44a	68.41 ± 0.27a*	67.52 ± 0.57b**
Ash	11.28 ± 0.63a	11.55 ± 0.42a	11.86 ± 0.52a	12.24 ± 0.79a	14.31 ± 0.67a*	17.70 ± 0.61b**
Carbohydrate	8.29 ± 0.25a	8.15 ± 0.21a	8.07 ± 0.19a	7.91 ± 0.23a*	6.30 ± 0.17b**	5.03 ± 0.41c

Values with the same superscripts are not significantly different

* Average of 3 values (expressed on dry wt. basis)

* NOEC

** LOEC

Table 3. Proximate composition (% on weight basis) of *E. maculatus* collected from the paddy fields of Kuttanand.

Fish species	Crude Fat	Crude Protein	Ash	Carbohydrate
<i>E. maculatus</i>	8.82 ± 0.12	64.58 ± 0.19	25.83 ± 1.51	0.77 ± 0.25

Table 4. Comparison of treatment means of *E. maculatus* to various sublethal concentrations

Component	Critical difference	Mean					
		T ₁ 0.0 ppm	T ₂ 0.1 ppm	T ₃ 0.3 ppm	T ₄ 0.6 ppm	T ₅ 1.0 ppm	T ₆ 1.5 ppm
<i>Crude fat</i>	0.5104	11.55	11.51	11.45	11.30*	10.98*	9.75*
<i>Crude protein</i>	0.6023	66.88	68.79	68.62	68.55	68.41	67.52*
<i>Ash</i>	1.0993	11.28	11.55	11.86	12.24	14.31*	17.70*
<i>Carbohydrate</i>	0.4520	08.29	08.15	08.07	07.91	06.30	05.03

* Significantly different

It may be inferred that, there is no significant change in the composition of fat, ash and carbohydrate upto 0.6 ppm concentration whereas it is not significant upto 1ppm concentration exposure in the case of protein. On this basis, the NOEC, LOEC and MATC are selected (Table 5).

of monocrotophos in the present investigation indicated the utilization of all these energy components when fish is under stress. It does not mean that all the substances are simultaneously utilized. When the principal and immediate energy source gets depleted, other sources exhibit a proportional depletion as the metabolism of these energy

Table 5. Maximum allowable toxicant concentration (MATC) values (safe level concentration) calculated based on the carcass proximate analysis of *E. maculatus* exposed to monocrotophos and their corresponding application factor (AF)

Energy component	Pesticide	Fish species	NOEC (ppm)	LOEC (ppm)	MATC (ppm)	48 h LC ₅₀ (ppm)	AF = $\frac{\text{MATC}}{48 \text{ h LC}_{50}}$
Fat	Monocrotophos	<i>E. maculatus</i>	0.6	1.0	0.7746	3.36	0.2305
Protein	“	“	1.0	1.5	1.2247	“	0.3645
Ash	“	“	0.6	1.0	0.7746	“	0.2305
Carbohydrate	“	“	0.6	1.0	0.7746	“	0.2305

Discussion

Fat, protein and carbohydrate, which constitute the major components of the body, play an important role in energy metabolism. This is affected by environmental factors like water pollution (Palanichamy *et al.*, 1986).

Decrease in fat, protein and carbohydrate content of *E. maculatus* exposed to different sublethal concentrations

components are linked through a common metabolic pathway *i.e.*, the tricarboxylic acid (TCA) cycle, (Arunachalam *et al.*, 1990).

Significant differences in the carcass proximate composition of *E. maculatus* exposed to monocrotophos may be the direct result of reduced food consumption and food conversion efficiency as was observed in pin fish

(*Lagodon rhomboides*) exposed to sublethal concentrations of bleached kraft mill effluent (Stonir and Livingston, 1978) in rohu juveniles exposed to sublethal concentration of phenol (Nair and Sherief, 1998) and also in rohu juveniles exposed to sublethal concentrations of monocrotophos (Ramani, 2001), *Etroplus maculatus* exposed to phosphamidon, and *Anabas testudineus* exposed to monocrotophos and phosphamidon (Sulekha, 2002; Sulekha and Mercy, 2009).

In the present study, significant decrease ($p < 0.05$) in protein content was observed at 1.5 ppm (highest concentration). Protein is the source of energy during chronic conditions of stress (Umminger, 1970) and a number of studies have reported a decline in protein composition in different organs of fishes treated with pesticides (Premadas and Anderson, 1963; Sivaprasanna Rao and Ramana Rao, 1979; Bakthavalsalam, 1980; Ram and Sathyanasan, 1984; Ganguly *et al.*, 1997; Ramani, 2001). Depletion in protein level in the body might be due to diversion of energy when the animal was under toxic condition as reported by Manoharan and Subbiah (1982).

The significant ($p < 0.05$) decrease in fat and carbohydrate content was observed in 1.0 and 1.5 ppm (higher concentrations). Grant and Mehrle (1970) reported a similar decline in fat content of fish during exposure to pesticides. Hanes *et al.* (1968) and Pasley *et al.* (1968) opined that the elevated mitochondrial activity following pesticide exposure resulted in high fat utilization in fishes. Present observation also supports their view. Carbohydrate is the principal and immediate source of energy. This decrease of carbohydrate indicated its immediate utilization under stress.

Various studies indicated that the different body organs also exhibited a reduction in fat, protein and carbohydrate composition of fishes during exposure to different pesticides. Arunachalam *et al.* (1990) observed that protein, carbohydrate, and lipid content of muscle, liver, gill and intestine of *M. vittatus* decreased when fish were exposed to malathion, thiodon, and carbaryl. Sheela and Muniandy (1992) found that the protein, carbohydrate and lipid content of gill, muscle, liver and intestine of *Channa striatus* decreased with increasing sublethal concentrations of phosphamidon. Sheela *et al.* (1992) reported similar results in the same organs in *Channa punctatus* treated with fenvalerate. The effects of ekalux on protein and carbohydrate values of muscle, liver and brain of *E. maculatus* were found to decrease while the concentration and the period of exposure kept increasing (Nelson and Kumar, 1996).

Carcass proximate composition of *E. maculatus* collected from Kuttanad showed that the fat and protein content are less than 1.5 ppm monocrotophos treated *E. maculatus* under the laboratory conditions. But the carbohydrate content of *E. maculatus* collected from Kuttanad was less than that is present in the highest sublethal concentrations (1.5 ppm). In the case of ash content, Kuttanad fishes showed higher level than the fishes of the highest sublethal concentrations (1.5 ppm and 1.0 ppm monocrotophos).

For *E. maculatus*, MATC end points of carbohydrate and ash gave sensitive end points whereas that of protein and fat were less sensitive. To monitor early changes, biochemical biomarkers can be used as sensitive end points. The application factors (AF), derived from the laboratory studies can be directly assigned to the field conditions of Kuttanad, as the experimented fishes are the true denizens of Kuttanad water bodies.

The NOEC, LOEC and MATC values so obtained in respect of *E. maculatus* used for experiments could be useful in assessing the water quality status and the pollution levels of Kuttanad ecosystem as the fish is typical inhabitant of Kuttanad water bodies and is recruited from the parent population inhabiting the same ecosystem itself. Due to their non-migratory nature, they are prone to all source pollutants added to the aquatic environment of Kuttanad. The changes noticed in histology of their gills, liver and kidney would manifest the intensity of pollution in the medium of their inhabitation (Sulekha, 2002; Sulekha and Mercy, 2009)

The application factor derived from the laboratory studies can be directly applied to the field situations, as the fishes are the true inhabitants of Kuttanad. Knowing the application factor of one pesticide, the MATC for other related pesticides could be easily calculated provided its 48 h LC_{50} is known. This knowledge of fundamental toxicological and pathological processes is not only important for the regulations of chemicals that are potential aquatic pollutants, but also for the researchers who are involved in similar field studies.

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