

Individual and Combined Acute Lethal Toxicity of Monocrotophos and 2,4-D on the Juveniles of *Eetroplus suratensis* (Bloch) (Pisces: Cichlidae)

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The 48 h individual and combined lethal toxicity of monocrotophos (insecticide) and 2,4-D (herbicide) on the juveniles of *Eetroplus suratensis* were determined. The 48 h LC₅₀ of monocrotophos was 5.85 mg.l⁻¹ (5.02 to 6.69) and that of 2,4-D was 267.4 mg.l⁻¹ (228.93 to 305.86). The median additive index and its range for the combined toxicity were worked out. The calculated value was -0.185 (-0.541 to 0.083). A simple or strictly additive toxicity is indicated for the mixture of monocrotophos and 2,4-D. This shows an enhanced potential of the individual toxicant for pollution in the natural bodies when they are applied sequentially and even simultaneously.

Key words: Monocrotophos; 2,4-D; combined lethal toxicity; *Eetroplus suratensis*

In the natural aquatic systems, fishes are exposed simultaneously to more than one biocide or contaminant because some chemicals are applied continuously and are highly persistent or others are applied as combinations to increase efficacy or reduce costs (Marking, 1977). The problems of toxicity of mixtures of pesticides on fish have been recognized and notable among the studies are those of Krieger & Lee (1973), Macek (1975), Marking & Dawson (1975), Marking & Mauck (1975), Statham (1975), Statham & Lech (1976) and Fabacher *et al.* (1976).

No attempts seem to have been made so far to study the combined toxicity of insecticides like monocrotophos, phosphamidon, hinosan, malathion and the herbicide 2,4-D which are very commonly used sequentially or even simultaneously in the paddy fields of Kuttanad, an ecological hotspot in Kerala. Although herbicides in general are moderately toxic to fish, the possibility of enhanced toxicity (synergism) with an insecticide has not been taken into consideration. An attempt is made in the present study to understand the individual and combined toxicity of monocrotophos

and 2,4-D on the juveniles of the indigenous cichlid species, *Eetroplus suratensis*, a highly favoured food fish of the region. The present study is based on concentration addition model or simple similar action model of Broderius (1991).

Materials and Methods

The weedicide 2,4 dichlorophenoxy acetic acid (80%) is a selective phenoxy herbicide mainly used to control the aquatic and semi-aquatic weeds in the paddy fields. The insecticide 'Nuvacron' is a water soluble organophosphate concentrate containing 360 g monocrotophos (0,0-dimethyl-0-(2)-methyl-carbonyl-1 methyl vinyl phosphate) as active ingredient in a kilogram of product. It is a broad spectrum, systemic and contact insecticide-cum-acaricide, mainly used against the insect pests of paddy.

Early juveniles of *E. suratensis* of uniform size (total length 2±02 cm and weight 139±15 mg) were collected from the pollution free low saline ponds (<5 ppt) of the College of Fisheries, Panangad. The test animals were acclimatized to freshwater by

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gradual dilution with well water without any mortality. They were fed *ad libitum* twice daily on *Artemia* larvae. The 48 h acute lethal toxicity tests (48 h LC_{50}) were carried out by the static bioassay method (Sprague 1973) with 12 h replenishment of test concentrations (pH, 6.9-7.45; dissolved oxygen, 5-7.5 mg.l⁻¹ and temperature 27±1°C). The experiments were conducted in triplicate using 5 l glass troughs with 10 fish each and 4 litres of test solution. The average animal load factor was 348 mg.l⁻¹. The juveniles were taken at random from the stock and starved for 24 h prior to the experiment.

After exploratory tests, seven concentrations of monocrotophos from 4 mg.l⁻¹ (no mortality) to 9 mg.l⁻¹ (100% mortality) were selected for the 48 h LC_{50} test. Similarly, seven concentrations of 2,4-D from 150 mg.l⁻¹ (no mortality) to 400 (100% mortality) were selected for the final test.

Exploratory tests for combined toxicity were carried out with 1:1 ratio of the individual 48 h LC_{50} values and their proportionately decreasing combinations based on the method of Marking (1977) and five combinations ranging from 2.5 mg.l⁻¹ monocrotophos and 125 mg.l⁻¹ 2,4-D (no mortality) to 4.25 mg.l⁻¹ monocrotophos and 212.5 mg.l⁻¹ 2,4-D (100% mortality) were selected for the 48 h LC_{50} study.

The 48 h LC_{50} values and their 95% fiducial limits were calculated by linear regression analysis after probit transformation of mean mortality and log₁₀ transformation of the test concentrations (Finney, 1971).

The sum of biological activity (S) was calculated based on the 'toxic unit' as defined by Sprague & Ramsey (1965) as, $S = (Am/Ai) + (Bm/Bi)$, where A and B are toxicants, i and m are toxicities (48 h LC_{50}) of the individual toxicants and mixtures respectively. The sum (S) could function as an index of additive toxicity, except that values greater than 1 are not linear with values less than 1. Hence the "additive

index" values of Marking (1977) were calculated as, Additive index = $(1/S) - 1$ for $S \leq 1$ and Additives index = $S(-1) + 1$ for $S \geq 1$.

Using this method the significance of variation from zero can be determined by substituting the 95% fiducial limits for the different LC_{50} values in the equations to establish a range of additive index values. The additive, greater than additive and less than additive toxicities are represented by zero, positive and negative values respectively. Whenever an index overlaps zero, additive toxicity is assumed (Marking, 1977).

Results and Discussion

The calculated 48 h LC_{50} value of monocrotophos to early juveniles of *E. suratensis* was 5.86 mg.l⁻¹ (5.02-6.69) and of 2,4-D, 267.4 mg.l⁻¹ (228.9-305.86) (Fig.1 a,b). The juveniles of *E. suratensis* are more tolerant to monocrotophos when compared with juveniles of *E. maculatus* whose 48 h LC_{50} value is 3.36 mg.l⁻¹ (2.95-3.82) (Mercy *et al.*, in press). Elezovic *et al.* (1994) reported the 48 h LC_{50} of 2,4-D on juveniles of common carp as 295 mg.l⁻¹ (262 - 312.5) and 96 h LC_{50} as 270 mg.l⁻¹ (259.2-286.5) at 20±1°C and compares well with the present 48 h LC_{50} value of 267 mg.l⁻¹ (228.9-305.9) at 27±1°C.

The results of the 48 h LC_{50} values and their 95% fiducial limits (individually and in combination), range of the sum of biological activity and the range of additive index values are given in Table 1. The probit

Table 1. Individual and combined 48 h LC_{50} (mg.l⁻¹) and their 95% fiducial limits in *E. suratensis* juveniles

Toxicant	48 h LC_{50} 95% fiducial limits		'S' value (range)	Additive index (range)
	Individually	In combination		
2,4-D	267.40 (228.9-305.8)	165.65 (147.35-183.95)	1.185 (0.923-1.541)	-0.185 (-0.541-0.083)
Monocrotophos	5.86 (5.02-6.69)	3.31 (2.95-3.70)		

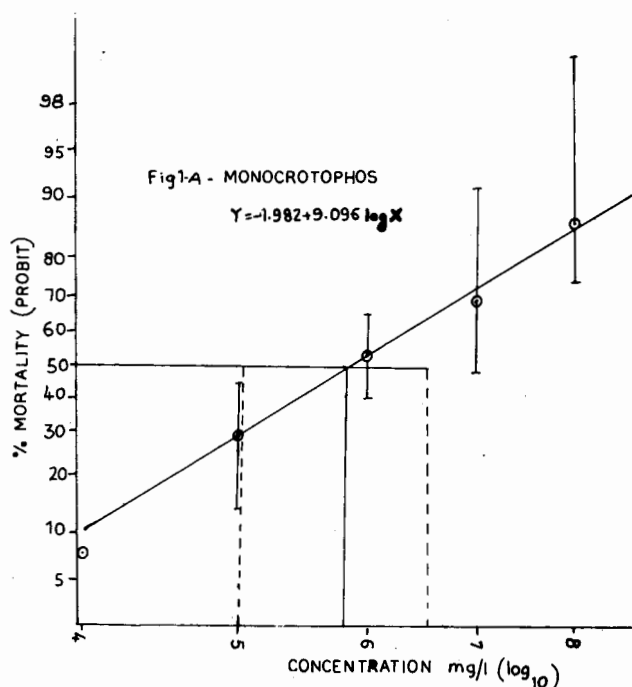


Fig. 1a. 48h LC50 values (95% fiducial limits) of monocrotophos in juveniles of *E. suratensis*.

diagrams for the individual toxicities in the mixture are given in Fig. 2a&b. The sum of

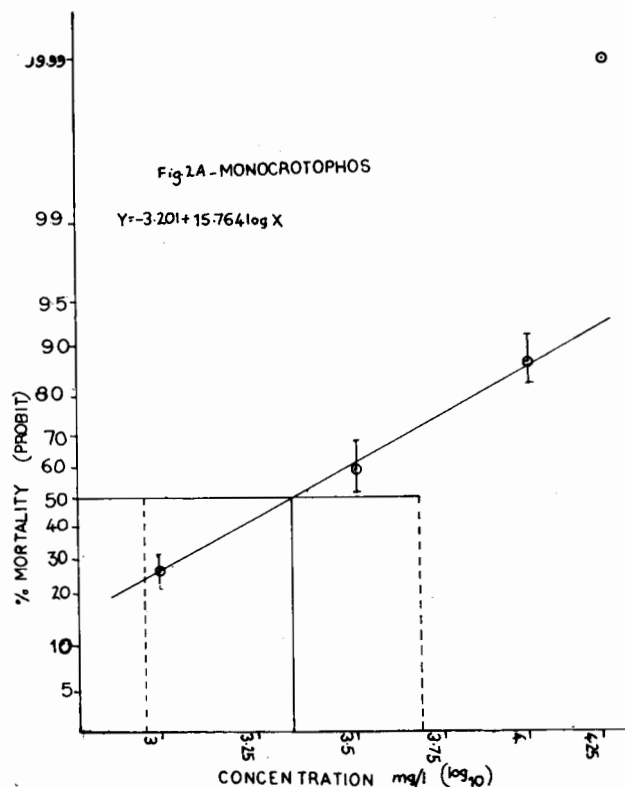


Fig. 2a. 48h LC50 values (95% fiducial limits) of monocrotophos in juveniles of *E. suratensis* in the monocrotophos - 2, 4-D combination.

biological activity and its range are represented in Fig.3.

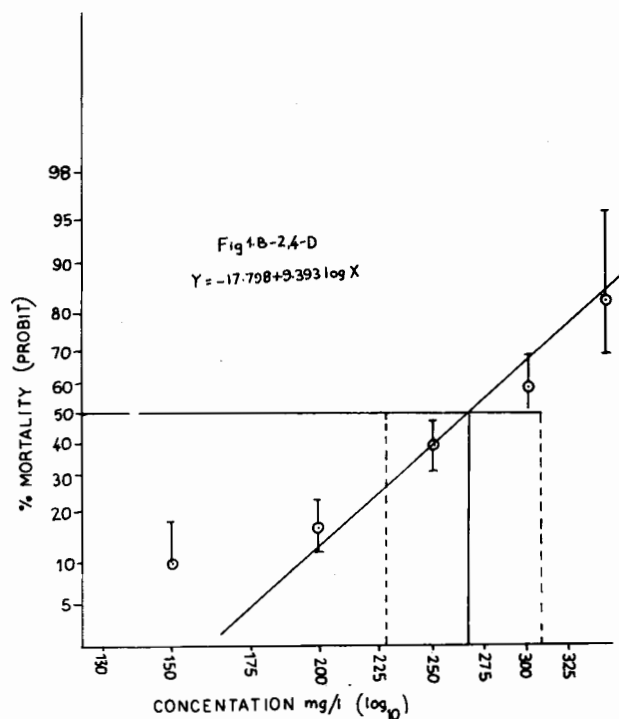


Fig. 1b. 48h LC50 values (95% fiducial limits) of 2, 4-D in juveniles of *E. suratensis*.

In the present study, the median additive index value was -0.185 denoting less than additive toxicity for the mixture. But the additive index range was -0.541 to +0.083. Since the index range overlaps zero, a simple or strictly additive toxicity is assumed (Marking, 1977) for the mixture of monocrotophos and 2,4-D on the early juveniles of *E. suratensis* at $27 \pm 1^\circ\text{C}$ (Magnification factor $\times 1$). Alabaster & Lloyd (1982) reported that while some data on the acute lethal toxicity of mixtures of pesticides and other substances to fish showed that joint action was close to additive, a relatively high proportion showed that it was several fold more than additive.

Lichenstein *et al.* (1973) found that interaction between parathion and 2,4-D was more than additive ($\times 3.2$) when tested on

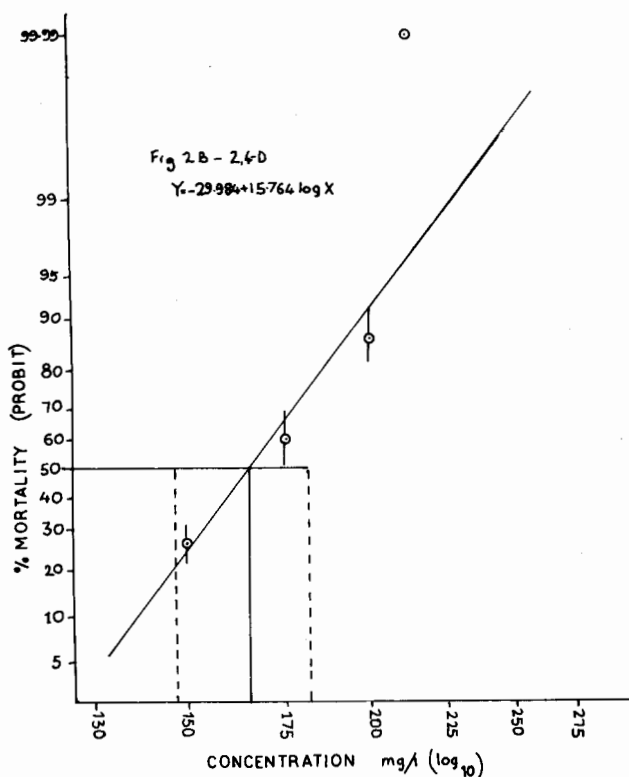


Fig. 2B. 48h LC₅₀ values (95% fiducial limits) of 2, 4-D in juveniles of *E. suratensis* in the monocrotophos-2, 4-D combination.

third instar of mosquito larvae while it was approximately additive for DDT and 2,4-D. Macek (1975) exposed blue gill (*Lepomis macrochirus*) to 29 different mixtures of pairs of pesticides. He found that the average value of the results was slightly more than additive. Marking & Mauck (1975) working on rainbow trout with seven insecticides and 20 combinations, found that in nine combinations, the response was not significantly different from additive.

Solon & Nair (1970) working with fathead minnow (*Pimephales promelas*) found that joint action of linear alkyl benzene sulphonates (LAS) and organophosphate pesticides was more than additive for parathion, ronnel and trithion, additive for guthion and less than additive for dicapton. Marking & Dawson (1975) measured the 96 h LC₅₀ of malathion and delnav to *L. macrochirus* and found that joint action was markedly more than additive (x 8.2). Similarly, Fabacher *et al.* (1976) showed that

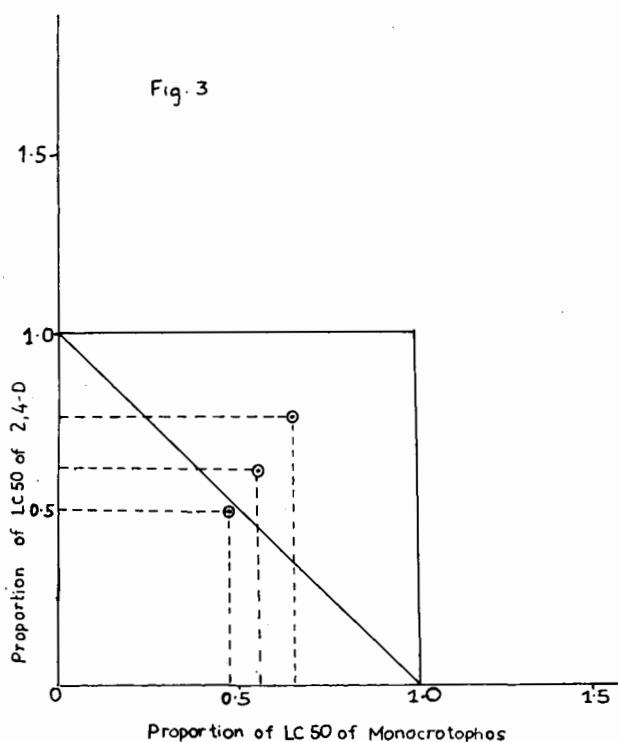


Fig. 3. The sum of biological activity and its range for the monocrotophos-2, 4-D combination.

mortality of mosquito fish, *Gambusia affinis*, in a mixture of methyl parathion and a defoliant, tributyl phosphorotrithioate, was several fold more than additive. Mirex clearly increased DDT toxicity to larvae of the salt marsh fish *Adinia xenica* (Koenig, 1977). Combination of quinalphos and phenthoate showed synergistic toxicity to tilapia at 96 h LC₅₀ (Durairaj & Selvarajan, 1995).

Ferguson & Bringham (1966) working with mosquito fish (*G. affinis*) exposed to different combinations of endrin, DDT, toxaphene and methyl parathion found less than additive toxic effect. Similar results were obtained by Ludke *et al.* (1972) working with several species of freshwater fish and mixtures of parathion and aldrin. Krieger & Lee (1973) showed that simultaneous treatment with diquat and an insecticide (DDT, aldrin or parathion) did not affect the toxicity of the insecticide.

Many theories have been put forth regarding the interactions among toxicants of

dissimilar chemical nature that may either aggravate or alleviate the toxicity of individual pollutant. The interactions may arise from alterations in the absorption, distribution, biotransformation or excretion of one toxicant by the other (Broderius, 1991). Inhibition of detoxification seems to be the most popular among the projected theories especially in the case of additive toxicity. Hence, interactive joint toxicity is not directly predictable from the toxicity of the separate components (Broderius, 1991). Thus, the knowledge of the combined effect can be determined only by experimentation.

Individually, monocrotophos is 'toxic' while 2,4-D is 'moderately toxic' to juveniles of *E. suratensis* based on the classification of Sprague (1973). But the strictly additive nature of their combined toxicity and the sequential or even simultaneous use in the ecosystem increases their potential for pollution. Hence, more systematic experimentations with biocide pairs and mixtures, both at the acute (lethal) and chronic (sublethal) response levels, are required to know the intricacies of joint toxicity at work in the Kuttanad water bodies.

The third author is grateful to ICAR for financial assistance. The authors wish to thank the Dean, College of Fisheries for providing facilities and the publication forms part of her M.F.Sc. research work in the Kerala Agricultural University.

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