Effect of Monocrotophos, an Organophosphorus Pesticide on the Growth of Tadpoles of *Limnonectis Limnocharis*, a Rice Field Frog

Sanjay Kumar Swain, Malaya Ranjan Mahananda*

Department of Environmental Sciences, Sambalpur University, Odisha -768019, India

*Corresponding Author: malaya_env@rediffmail.com

Abstract

The effect of monocrotophos an organophosphorus insecticide on the growth of tadpoles of a dominant rice field anuran species (*Limnonectis limnocharis*) was studied. Prior to growth experiment a 96h toxicity test was conducted in different concentrations of monocrotophos to calculate LC₅₀ value by probit analysis. The 96h of LC₅₀ value for the tadpoles was found to be 0.59 mg l⁻¹. Based on the result of toxicity test and recommended field agricultural dose (0.60 mg l⁻¹) three sublethal concentration of monocrotophos were taken to study the growth of three days old tadpoles up to metamorphic climax stage and it was compared with that of the controls. Analysis of data revealed that after 35 days the length and body weight of tadpoles decreased by 7.8, 13 and 21.4% and by 6.03, 6.74 and 8.8% in 0.25, 0.30 and 0.35 mg l⁻¹ respectively from the control condition. The decrease in length and body weight of tadpoles also caused increase in duration of metamorphosis. The metamorphic climax was reached after 38, 41 and 44 days in 0.25, 0.30 and 0.35 mg l⁻¹ monocrotophos respectively, instead of 35 days in control condition. The above results indicates that application of pesticides even below the recommended dose cause ecological problems and non target beneficial species are victimized. This finding supports that organic farming and controlled use of pesticides for conservation of frogs in the paddy field.

Keywords

*Limnonectis Limnocharis*, Tadpole, Growth, Metamorphosis, Monocrotophos

1. Introduction

Paddy (Rice) is the most important cereal crop of the world [1]. In India paddy is cultivated in about 22% of the gross cropped area. It covers about 47% of the gross cropped area in Odissa state [2]. The major insect pests and related diseases are culminated in paddy during the rainy to early winter period i.e. from July to November. A diversity of pesticides and their residues have been recorded in a wide variety of aquatic habitats [3] exposing the inhabitants to these harmful substances Organophosphorous insecticides are less persistence and toxic than organochlorine and carbamate type insecticides. But they also create several problems like others, which lead to the imbalance in the ecosystem [4]. The two most important problems are: (i) some of the pest organisms particularly the insects have developed resistance to the chemicals. (ii) Non-target beneficial organisms like frog, bird and earthworm etc.[5-7] are also affected by the pesticides.

Amphibians are integral component of many ecosystem and they may constitute the highest fraction of vertebrate biomass in some ecosystems [8] and occupy a critical position in the ecological pyramid, as they are major predators of insect and other pest [9-10]. Because of their beneficial role, especially from agricultural point of view they are often refer to as “farmer’s friend”. A decline in amphibian population can have an adverse effect in agriculture and other areas. It is generally believed that increasing use of pesticides and fertilizers are one f the major causes responsible for depletion of the population of anurans all over the world [7, 11]. There have been numerous reports suggesting that the populations of anuran species in a wide array of geographical regions and habitats have apparently declined or have experienced range reductions by adverse effects of pesticides [12-14].

The most of the paddy fields of Orissa, the organophosphorous insecticides particularly monocrotophos, malathion, phorate and fenitothion are commonly used against the insecticide pests. The amphibian species generally seen in paddy fields of Orissa are as follows: *Limnonectis limnocharis*, *Haplobatrachus tigrinus* and *Euphlyctis cyanophlyctis* [11]. Among these *Limnonectis limnocharis* is the dominant species followed by *Euphlyctis cyanophlyctis* and *Haplobatrachus tigrinus*. Basing on the above fact the present work was undertaken to study the effect of a widely used organophosphorus insecticide (monocrotophos) on the tadpoles of a dominant anuran species (*Limnonectis limnocharis*) found in the paddy fields of Orissa.
2. Materials and Methods

Amicron 36% SL, a trade name of monocrotophos, manufactured by All India Medica Corporation, Noroda, Ahmedabad, India was selected as the test chemical in the present investigation. It is a water soluble concentrate containing 36% w/w, 0-0-dimethyl-0(2 methyl carbonyl-1 methyl-vinyl) phosphate. It is a contact insecticide and acaricide, effective against sucking, chewing and manning type of insect pests (thrips, aphides, mites, scales, borers, caterpillars, beetles and hoppers) of crops like paddy, maize, sugarcane, cotton, gram etc.

The recommended field agricultural dose of monocrotophos for paddy crops as provided in the leaflet specification is 1250ml formulation diluted to 750L of water sprayed to over an area of one hectare. This is equivalent to 1.67 ml l⁻¹ formulations or 0.60 mg l⁻¹ active ingredient of monocrotophos. Basing on this recommended rate, toxicity test on tadpoles (larvae) of *Limnonectis limnocharis* was conducted in different concentrations of monocrotophos. For this experiment plastic dishes of 40x30x25 cm size having 5L capacity was used. The various concentrations of monocrotophos used were 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.9 mg l⁻¹. These concentrations were prepared using appropriate volume of conditioned water. For each concentration three replicates of above sized plastic dishes having three liters of respective concentration were kept and in each replicate twenty tadpoles (3 day old) were kept. The larvae were fed every day with adequate amount of mixed food diet consisting of boiled *Amaranthus tricolor* leaves, chicken egg yolk and cooked minced goat meat in a ratio of 5:1:1 [15]. During the test period the tadpoles from each replicate were transferred to another container having freshly prepared respective concentrations of monocrotophos. The mortality was recorded daily during the time of transfer and it was continued for 4 days. After 4 days LC₅₀ value was calculated from the mortality data using Finney’s probit analysis [16].

3. Results

3.1. Toxicity Test

The sublethal concentrations (0.25, 0.30, and 0.35 mg l⁻¹) of monocrotophos were prepared using appropriate volume of conditioned water and control (0.0 mg l⁻¹) was prepared using only conditioned water as suggested by Nace and Richards [17]. Three liter of each concentration were kept in plastic dishes (40x30x25 cm) in five replicates. To each replicate five 3 day old tadpoles of approximately equal size and weight were kept. The initial length and body weight were measured. The tadpoles were given every day with adequate amount of mixed diet [15]. On every alternate day the tadpoles from each replicate were transferred to another container having freshly prepared respective concentrations of monocrotophos. During the time of transfer, the tadpole growth was measured in terms of body weight and length and emergence of fore limbs, hind limbs and deformation of body, if any was examined.

### Table 1. 96hr toxicity test of 3 days old *Limnonectis limnocharis* tadpoles at different concentrations of monocrotophos

| Conc. Of Monocrotophos(ppm) | % of Mortality | Log(+1) dose | Experimental probit | Expected probit | Working probit | LC₅₀ conc. arrived from graph |
|-----------------------------|----------------|--------------|---------------------|----------------|---------------|-----------------------------|
| 0.0                         | 0              | -            | -                   | -              | -             | -                           |
| 0.1                         | 0              | 0.00         | -                   | -              | -             | -                           |
| 0.2                         | 1.0            | 0.30         | 1.85                | 2.10           | 3.467         |
| 0.3                         | 20.0           | 0.50         | 4.15                | 3.94           | 4.195         |
| 0.4                         | 56.0           | 0.60         | 5.15                | 5.52           | 5.127         |
| 0.5                         | 88.0           | 0.70         | 6.25                | 6.00           | 6.160         |
| 0.6                         | 98.0           | 0.80         | 7.07                | 6.85           | 7.033         | 0.59ppm                     |
| 0.7                         | 99.4           | 0.85         | 7.52                | 7.40           | 7.319         |
| 0.8                         | 99.8           | 0.90         | 7.82                | 7.65           | 7.073         |
| 0.9                         | 99.9           | 0.95         | 8.04                | 8.80           | 100.0         |
| 1.0                         | 100.0          | 1.00         | -                   | -              | -             |
3.2. Growth of Tadpoles

On the basis of toxicity test the recommended agricultural dose, three sublethal (0.25, 0.30, and 0.35 mg l\(^{-1}\)) concentrations of monocrotophos were chosen. Effect of these three concentrations on the length and body weight of \textit{L. limnocharis}, larvae during metamorphosis were studied and compared with controlled condition. The results of the study are presented in Table 2 and 3. The three days old tadpoles initially taken for the experiment had on an average (mean ± SD, 7.28 ± 0.37 mm length and 10.3 ± 0.308 mg body weight). After 35 days the tadpoles in control reached a length of 32.3 ± 0.57mm, whereas during the same period the tadpoles exposed to 0.25, 0.30 and 0.35 ppm were respectively reached 28.78 ± 0.43, 28.1 ± 0.29 and 25.36± 0.65 mm in their length. This showed that compared to the control condition the length was decreased by 7.8%, 13% and 21.4% in 0.25, 0.30 and 0.35 mg l\(^{-1}\), respectively. Like length, the body weight of tadpoles after 35 days decreased about 6.03, 6.74 and 8.8% respectively. The two way analysis of variance (ANOVA) test was conducted to compare the difference in length and body weight of \textit{L. limnocharis} tadpoles exposed to different concentrations of monocrotophos taking 35 days of development into consideration. ANOVA test also indicated significant decrease in length and body weight causing delay in tadpole growth. Besides significant decrease in length and body weight, a delay in tadpole growth was also noticed exposed to monocrotophos. The hind limbs emerged on 15\(^{th}\), 21\(^{st}\) and 24\(^{th}\) day and the fore limb developed on 13\(^{th}\), 31\(^{st}\) and 35\(^{th}\) day respectively in 0.25, 0.30 and 0.35 mg l\(^{-1}\) concentration of monocrotophos. As a result of which metamorphic climax was reached by 38, 41 and 44 days in the three different concentrations respectively with compared to 35 days in control condition.

Table 2. Effect of Monocrotophos (Amicron 36% SL) on the development (Total length in mm) of Limnonectis limnocharis tadpoles during metamorphosis

| Days | Concentration of monocrotophos exposed |
|------|---------------------------------------|
|      | 0ppm | 0.25ppm | 0.30ppm | 0.35ppm |
| 3    | 7.28±0.37 | 7.28±0.37* | 7.28±0.37* | 7.28±0.37* |
| 6    | 21.7±0.367 | 20.22±0.277* (-6.8) | 17.7±0.717* (-18.43) | 15.82±0.717* (-27.09) |
| 9    | 24.0±0.1 | 22.34±0.415* (-6.9) | 19.58±0.676* (-18.41) | 16.26±0.618* (-32.2) |
| 12   | 25.5±0.533 | 23.24±0.391* (-8.8) | 21.14±0.336* (-17.0) | 17.78±0.443* (-30.2) |
| 15   | 28.8±0.836 | 27.82±0.311* (-3.4) | 25.30±0.57* (-12.15) | 20.96±0.456* (-27.22) |
| 18   | 30.4±0.717 | 27.84±0.502* (-8.4) | 25.76±0.487* (-15.2) | 21.6±0.651* (-28.9) |
| 21   | 30.44±0.770 | 28.02±0.570* (-7.92) | 26.14±0.415* (-14.1) | 22.9±0.463* (-24.7) |
| 24   | 30.40±0.717 | 28.46±0.676* (-6.3) | 26.14±0.336* (-14.0) | 23.18±0.571* (-23.7) |
| 27   | 30.56±0.696 | 28.60±0.651* (-6.4) | 27.1±0.339* (-11.3) | 24.14±0.336* (-21.0) |
| 30   | 30.72±0.501 | 28.90±0.418* (-5.9) | 27.50±0.367* (-10.4) | 24.5±0.43* (-20.2) |
| 33   | 31.02±0.370 | 29.10±0.651* (-6.1) | 27.42±0.454* (-11.6) | 24.9±0.254* (-19.7) |
| 35   | 32.3±0.570 (MC) | 28.78±0.438* (-7.8) | 28.1±0.294* (-13.0) | 25.36±0.650* (-21.4) |
| 38   | 30.98±0.501 (MC) | 28.02±0.526* (MC) | 28.18±0.294* (MC) | 25.82±0.715* (MC) |
| 41   | 32.3±0.570 (MC) | 28.18±0.294* (MC) | 28.18±0.294* (MC) | 26.26±0.736* (MC) |

Data are presented as Mean± SD of 5 replicates.* indicate p<0.05 in comparison to control (0 ppm). MC: metamorphic climax. The value in the parentheses indicates the percentage increase/decrease over control.
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Table 3. Effect of Monocrotophos (Amicron 36% SL) on the development (total body weight in mg) of Limnonectis limnocharis tadpoles during metamorphosis.

| Days | Concentration of monocrotophos exposed |
|------|----------------------------------------|
|      | 0ppm | 0.25ppm | 0.30ppm | 0.35ppm |
| 3    | 10.3±0.308 | 10.3±0.308 | 10.3±0.308 | 10.3±0.308 |
| 6    | 24.11±0.796 | 21.57±0.973* (-10.53) | 19.06±0.364* (-20.94) | 16.70±0.543* (-30.7) |
| 9    | 72.85±1.063 | 69.69±0.523* (-4.33) | 67.51±0.790* (-7.33) | 65.00±0.707* (-10.7) |
| 12   | 92.216±0.676 | 89.80±0.647* (-2.61) | 87.90±0.627* (-4.68) | 85.58±0.575* (-7.19) |
| 15   | 133.89±0.797 | 130.89±0.478* (-2.24) | 129.06±0.453* (-3.6) | 127.03±0.593* (-5.12) |
| 18   | 203.97±0.805 | 199.68±0.601* (-2.1) | 196.74±0.454* (-3.5) | 193.93±1.266* (-4.9) |
| 21   | 207.54±1.096 | 201.03±0.501* (-3.13) | 199.95±1.521* (-3.6) | 197.39±0.789* (-4.8) |
| 24   | 220.14±0.773 | 204.20±0.799* (-7.24) | 215.10±0.720* (-2.2) | 212.60±0.896* (-3.4) |
| 27   | 231.10±0.858 | 218.01±0.651* (-5.66) | 225.40±0.762* (-4.46) | 222.44±0.740* (-3.7) |
| 30   | 235.55±0.717 | 228.37±0.678* (-3.04) | 229.76±0.885* (-2.4) | 225.70±1.021* (-4.1) |
| 33   | 245.11±0.825 | 232.40±0.695* (-5.18) | 236.49±0.861* (-5.5) | 231.23±0.891* (-5.6) |
| 35   | 258.17±1.052 | 242.58±0.794* (6.03) | 240.76±0.982* (-6.74) | 235.51±0.915* (-8.8) |
| 38   | 248.61±0.782* (-3.7) | 243.90±0.802** (5.52) | 238.31±0.736* (7.69) |
| 41   | 250.91±0.916* (MC) | 246.66±0.944* (MC) | 240.89±0.541* |
| 44   | | | | 242.73±0.592 (MC) |

Data are presented as Mean± SD of 5 replicates. * indicate p<0.05 in comparison to control (0 ppm). MC: metamorphic climax. The value in the parentheses indicates the percentage increase/decrease over control.

4. Discussion

The susceptibility of the tadpoles and adults to the agrochemicals of various kinds has been reported by several workers. Body et al.[18] have studied the possible DDT resistance in natural population of cricket frog, Acris crepitans. They have also observed a large number of dead fauna, including the Southern cricket frog Acris gryllus, green frog Hyla cinerea, fowlers toad, Bufo woodhousiei and leopard frog Rana pipiens in ponds or nearby treated cotton fields in Mississippi delta. Mulla et al.[19] have reported that field application of endrin, dieldrin and toxaphene was fatal to the bull frog Rana catesbeiana and the toads Bufo boreas and Seaphiopus hammondii. Besides this, the adverse effect of PP, DDT on tadpoles of Rana temporaria [20], rogor on tadpoles of Rana tigerina [21-22], metacid and ekalux and tadpoles of the skipper frog Rana cyanophlyctis [23] and endosulphan, malathion and furadon on tadpoles of Rana hexadactyla [24] have also been reported.

The present work shows that the growth, duration of metamorphosis of larvae of Limnonectis limnocharis were seriously affected even at three sublethal concentrations (0.25, 0.30 and 0.35 mg l⁻¹) of monocrotophos (Amicron 36% SC) which was much below the LC₅₀ value (0.59 mg l⁻¹) and also the recommended agricultural rate of application (0.6 mg l⁻¹). The pesticides not only affect the length of larval period but also the size at metamorphosis. This indicates that when pesticides are applied to paddy fields, many beneficial non target organisms like L. limnocharis frogs seen in these fields are most likely to be affected or victimized. This can eventually leads to adverse effects on the natural ecological balance and consequently on agriculture. From conservation and sustainability point of view, time has come to evaluate purely organic farming with organic part use of fertilizer with modern agricultural practices.

5. Conclusion

Application of pesticides even below the recommended dose cause ecological problems and non target beneficial species are victimized. Exposed to agro-chemicals (viz., synthetic fertilizers, pesticides, etc.) reduced survivability of tadpoles and low froglet emergence due to exposure to these agrochemicals could contribute both for decline of the species and possible associated ecological imbalance. This finding supports that organic farming and controlled use of pesticides for conservation of frogs in the paddy field.
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