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Effect of Methyl Parathion on Survival and Development of Tadpoles of Indian Cricket frog *Fejervarya limnocharis*

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ABSTRACT

Amphibian populations are declining due to various causes including pesticide contamination in natural habitat. We evaluated the effect of Methyl Parathion (MPT) an organophosphate pesticide on survival and development of common paddy field frog *Fejervarya limnocharis* in a laboratory condition. Effect of 0 µg MPT/L, 500 µg MPT/L, 1000 µg MPT/L, 1500 µg MPT/L, 2000 µg MPT/L and 3000 µg MPT/L was studied using static toxicity test for a duration of 28 days. MPT reduced the survival of tadpole. The mortality was increased with the increased concentration of pesticide. The development decreased with increased MPT concentrations. At higher concentrations, MPT induced slow development and tadpoles failed to metamorphose. It is assumed that slow development could affect the early larval life and amphibian population in the agroecosystem.

Keywords: *Cricket frog, Growth, Methyl parathion, Survival, Tadpoles*

INTRODUCTION

Amphibian population decline has been reported worldwide since the late 1960s. Several factors including pesticide contamination are considered to contribute the decline of amphibian populations [1-4]. Many amphibian species are known to dwell in agroecosystem [5-6]. Agriculture activities and management practices have induced several consequences in amphibian habitat and their habitats suffer from nitrogen pollution, agrochemical contamination and heavy metals [7-10]. Most important agrochemicals inducing the problem on amphibians are pesticides and synthetic fertilizers. Pesticides can affect many amphibians due to acute lethal and sublethal toxicities. The effects of pesticides at sublethal levels include behavioral changes, endocrine disruptions, decreased growth and development, increased developmental abnormality, susceptibility to diseases [11-15]. Many studies have showed that the pesticides might be a major cause of global amphibian population decline [16-19]. Interest in considering the effects of pesticides on amphibians emerged from the fact that the pesticides are also an important factor for global amphibian declines [8] this could have a negative impact on natural ecosystems [20].

In the Western Ghats, many amphibians are living and breeding in shallow water bodies within the vicinity of rice paddy fields. Their breeding period coincides with the application of agrochemicals, including pesticides and fertilizers [21-23]. Studies conducted by Gurushankara *et al.* [24] and Hegde and Krishnamurthy [10] have recorded high incidence of abnormal frogs in agrochemical contaminated habitats in the Western Ghats. Compared to native habitats, Patel *et al.* [25] recorded a maximum morphological abnormalities (>10.8%) in *Fejervarya limnocharis* (Indian Cricket Frog) living in highly contaminated agroecosystem of Rice paddy fields. This frogs spend most of their time in shallow aquatic habitats of rice paddy fields and concomitant water bodies and breeds during the monsoon season [22, 26] and use shallow waters of Rice paddy fields for breeding and development of tadpoles. However, the time of reproduction and larval development coincide with agrochemical applications in rice paddy field. Among the agrochemicals, organophosphate

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pesticides (OPs), a class of acetylcholinesterase inhibitors, are intensively used in agriculture to reduce insect pest population. Among the OPs, Malathion and Methylparathion (MPT) are intensively used as a potent insecticide in rice cultivation. These two insecticides together constitute 65% of total pesticides usage [21]. In general, the biological actions of OPs are due primarily to the inhibition of acetylcholinesterase (AChE), which causes a toxic and potentially lethal buildup of the neurotransmitter acetylcholine (ACh). MPT was reported to affect the reproductive system in fish, amphibians, birds, and mammals[27]. However, the effect of MPT is found to vary among different species [28], bioaccumulate and produce the negative impact on the reproductive system of frogs [29]. Since MPT is widely used along with other OPs in rice paddy fields, it is of interest to determine the effect of MPT on survival and development of common rice paddy field frog *F. limnocharis*.

MATERIALS AND METHODS

Experimental Design

More than 800 pre-feeding tadpoles of *F. limnocharis* from different egg clutches were collected from natural pristine habitats (Loc; 13° 18`-75° 25` and 13°-22` - 75° 28`; altitude range: 720-1060 m MSL), remotely located away from human activities and crop lands. Tadpoles were collected in the month of July and were transported to the laboratory and maintained in reconstituted water in a large container (100 L) until tadpoles reach Gosner stage 25 [30]. Reconstituted water was formulated by dissolving 96 mg $NaHCO_3$, 60 mg $CaSO_4.2H_2O$, 60mg $MgSO_4.7H_2O$ and 4 mg KCl with a liter of deionized water. This reconstituted water was used throughout the experiment. Tadpoles were maintained on a 14:10 hour light: dark cycle at a room temperature of 22-25 °C throughout the experiment. Tadpoles were fed boiled spinach, provided ad libitum following methods of Sabnis and Kuthe [31].

We used Methyl parathion (O, O-dimethyl O-4-nitrophenyl phosphorothioate) 50% EC (Registration No. 5-11(34) methyl parathion (EC)-2, Batch No. CC4111, Bayer AG Germany), obtained from the local market as a test chemical. We followed a factorial design to test the toxicity of MPT on tadpoles. This comprises 0 (control), Acetone control, 500, 1000, 1500, 2000 and 3000 µg MPT/L as test concentrations. We consider these concentrations as environmentally realistic as field concentration of MPT immediately after application found to vary between 1000 to 3500 µg/L in different rice paddy fields of the area. Analytical grade Acetone (purity 99%, Product No 33515, Batch No NL 27616403 v, Qualigens Fine Chemicals) was used to dissolve and make the stock solution of MPT. The LC50 of methyl parathion for tadpoles at 24, 48, 72 and 96 hours were found to be 11.06, 8.86, 7.52 and 6.50 mg/L respectively [32].

Since we have collected pre-feeding tadpoles hatched out from different egg masses, a random selection of twenty tadpoles (Gosner stage 25) were picked up to expose to each test solution. Each tadpole of all test groups is exposed to test solution in a separate inert polyethylene circular container (Diameter: 20 cm, Depth: 8 cm, Volume: 1500 mL) containing 1 liter of test solution. As a result, each group comprises 20 experimental containers with one tadpole in it. (i.e total 7 test concentrations x 20 tadpoles in 140 containers). At an interval of 7 days, until the 28th day, the length, body mass and changes in Gosner stage were recorded. The length of the tadpole was measured using an electronic calipers and body mass was recorded with the help of electronic balance (Model: Anamed: M-300, precision 0.001 g). The total length and body mass (BM) of tadpoles at the beginning of the experiment were found to be 20.0 ± 0.65 mm and 0.11 ± 0.012 g respectively. The total number of tadpoles surviving at the end of 28 days was recorded and average days up to which tadpoles survive under a selective exposure of MPT was considered as mean survival time in days. To maintain constant test concentration and to reduce the load of excretory material and decaying food in the test media, the test solution was changed once in two days with the same concentration of MPT. The dosing solutions were formulated immediately before changing the solution in the tadpole containers. Tadpole were fed with boiled spinach ad libitum (≈ 100 mg/tadpole/day).

For all the statistical analysis, the response given out by each tadpole from a group is pooled. Since the differences in data recorded for control and acetone control are insignificant ($\rho > 0.50$), we did not consider data registered for acetone treated tadpoles to any statistical calculations. We used ANOVA to check the significance of differences in survival, the increment in length, and the body mass of tadpoles between controls and treatment groups. The correlation between test concentration and response was estimated using Karl Pearson Correlation. *SPSS ver. 20* was used for all statistical analysis.

RESULTS AND DISCUSSION

Tadpole Survival

Tadpole survival was 99.84% in control followed by 60%, 35%, 25%, 20% and 10% recorded in 500 µg MPT/L, 1000 µg MPT/L, 1500 µg MPT/L, 2000 µg MPT/L and 3000 µg MPT/L respectively for a period of 28 days. For the exposure of 3000 µg MPT/L, tadpoles survived only up to 7 days. Figure 1 presents the survival time (days) of tadpole against MPT concentrations used in the experiment. Similar to tadpole survival (%), survival time in different treatment groups have decreased constantly with the increase in concentration of MPT ($r = -0.94$, $\rho = 0.006$). Survival time recorded for different treatment groups showed significant differences ($F_{5, 14} = 207.54$, $p = 0.0001$).

Length and Body Mass of Tadpoles

In control group, the length of the tadpoles over the time have followed the typical curvature of normal development of anuran tadpoles (Figure 2); after a continuous increase and once tadpole have reached a threshold level (around 21st day; stage 42) the length did not show increase. Tadpoles developed limb buds and reduction in tail length has occurred. A similar trend was also recorded in tadpoles treated with 500 µg MPT/L. However, froglet did not emerge at the end. The length of the tadpoles recorded at the end of the experiment for control and 500 µg MPT/L did not show any significant differences ($F_{1, 5} = 2.18$, $p = 0.206$). In 1000 µg MPT/L, 1500 µg MPT/L and 2000 µg MPT/L exposure, surviving tadpole showed a continuous increase in their length and did not follow observed pattern for control and 500 µg MPT/L exposures. There is a significant differences in length recorded for control and 1000 µg MPT/L ($F_{1, 5} = 9.1$, $p = 0.015$), 1500 µg MPT/L ($F_{1, 5} = 12.41$, $p = 0.0075$), and 2000 µg MPT/L ($F_{1, 5} = 14.78$, $p = 0.005$), while in 3000 µg MPT/L, all tadpoles were dead in 7 days.

Body Mass has followed the similar trend as exhibited for length (Figure 3). In control and 500 µg MPT/L exposure, tadpoles exhibited the normal growth pattern and did not exhibit considerable difference to that of control ($F_{1, 5} = 3.27$, $p = 0.109$). In 1000 µg MPT/L, 1500 µg MPT/L and 2000 µg MPT/L exposure concentration the BM has continuously increased and differ considerably compared to control ($F_{1, 5} = 10.56$, $p = 0.011$, $F_{1, 5} = 26.28$, $p = 0.0001$, $F_{1, 5} = 4.94$, $\rho = 0.05$ respectively). While in 3000 µg MPT/L, tadpole showed a decrease in BM before all of them were dead by 7th day.

In control, we observed that the tadpoles have completed Gosner stage 46 and froglets have emerged. While in 500 µg MPT/L tadpoles have grown upto 43rd stage in the same duration. In 1000 µg MPT/L, 1500 µg MPT/L, 2000 µg MPT/L and 3000 µg MPT/L, tadpoles attained maximum growth of Gosner stage 37, 38, 33 and 27 respectively by the time all the tadpoles in control have emerged out as froglets.

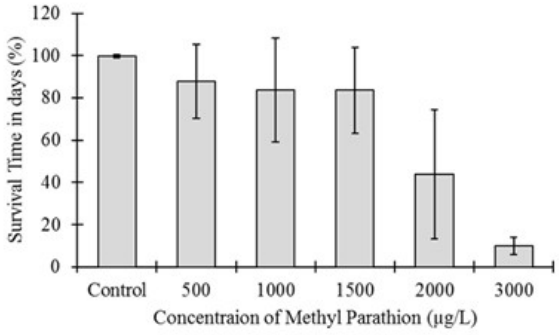

Figure 1. Mean survival time (day ± 1 SD) of tadpoles recorded for 28 days in control and those exposed to different concentrations of MPT

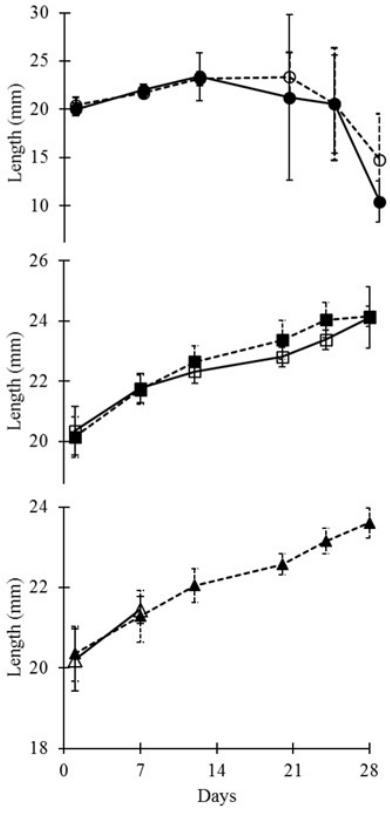

Figure 2. Length (mm ± 1 SD) of tadpoles recorded with treatment of different test concentrations of MPT for 28 days (Note: —●— = control; ---○---- = 500 µg MPT/L; ---■--- = 1000 µg MPT/L; —□— = 1500 µg MPT/L; ---▲--- = 2000 µg MPT/L and —△— = 3000 µg MPT/L)

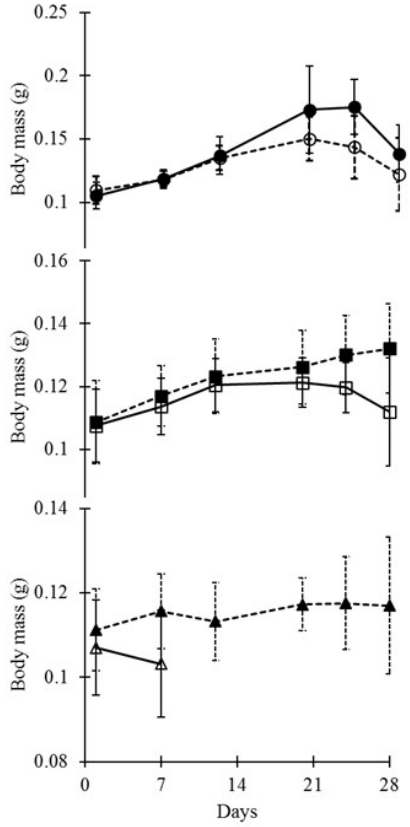

Figure 3. Body mass (g ± 1 SD) of tadpoles recorded with treatment of different test concentrations of MPT for 28 days (Note: —●— = control; ---○---- = 500 µg MPT/L; ---■--- = 1000 µg MPT/L; —□— = 1500 µg MPT/L; ---▲--- = 2000 µg MPT/L and —△— = 3000 µg MPT/L)

Methyl Parathion and its active metabolite methyl paraoxon are affecting the development of amphibians [1, 33]. However, such information of MPT is not available for *F. limnocharis*. In the present study, we observed a drastic reduction in survival over the time. MPT possess a half-life 175 days (at pH range 1-5, 20°C) and aqueous photolysis half-life range from 8 to 38 days. Since tadpoles of *F. limnocharis* complete their aquatic life around 28-36 days, this pesticide can produce a negative effect on the development of the tadpoles.

The length, body mass and increment in larval stage are considered as the indicator of growth [34-36]. In the present study, continuous monitoring of developmental rate over the time showed that MPT reduced the development of tadpoles. Further, this negative influence is correlated with the concentration of MPT. A similar observation of adverse effects on food consumption, survival of tadpoles and delay in metamorphosis were also observed in the same species by exposing to malathion and combinations with other pesticides [22, 23]. At low concentration exposure (500 µg MPT/L, 1000 µg MPT/L and 1500 µg MPT/L), the tadpole mortality was not significantly high. However, low body mass and development (as indicated by Gosner stage) are going to persist, and tadpole will have a long duration of early life. Given such possibilities of MPT contamination in the natural condition and if they are forced to a long larval life, then tadpole may face a severe threat of desiccation of water bodies and other environmental changes occurring over the season.

CONCLUSIONS

As the larval stages of frog in shallow water and pesticide applications to cropland coincides, tadpoles living in rice paddy croplands have higher chances of being exposed to pesticides. The pesticide Methyl parathion at environmentally realistic concentrations reduced the development and survivability of tadpoles of *F. limnocharis.* This has been indicated by significant differences in developmental parameters like length and body mass of tadpoles, growth increments and survival rate and time of tadpoles among the tested different concentrations of MPT. At low levels of MPT, although mortality is low, the tadpoles exhibited slow growth. It is believed that if they are forced for a long duration of larval life, then tadpole may face a severe threat of desiccation of water bodies and other environmental changes occurring over the season.

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