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Responses of *Microhyla ornata* to the Toxicity of Phoskill

Sadaf Ishaque Inamdar, Rafeek Ahmed Maniyar*

P.G. Department of Zoology, Poona College, Camp Pune-1, India

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*Corresponding Author

Tel : +91-9967836995
Fax : +91-2026453707

Email: rafeekahmedkcd@gmail.com

Summary

The lethality of phoskill on *Microhyla ornata* was determined by Static renewal bioassay method (Finney, 1971).The tadpoles of *M. ornata* were exposed to lethal concentrations of 24hrs, 48hrs, 72hrs and 96hrs for 1hr and 2hrs to record the oxygen consumption and behavioral responses respectively. The results obtained were dose dependent with slight variations. At the concentration of 24h LC_{50} the behavioral activities were found decreased within 15 minutes duration, during the 2hrs exposure period followed by increase in their resting period. Oxygen consumption also was recorded lowest compared to exposures in other concentrations.

Key Words: Phoskill, Oxygen consumption, Behavioral responses, *Microhyla ornata*, Lethal concentration

Introduction

Hazards of environmental contamination through the indiscriminate use of variety of pesticides have attracted global attention. In recent years emphasis has been placed on the use of organophosphate compounds to over come the problems caused by these toxicants to aquatic organisms (Lal et al., 1986). Phoskill, a commercial form of monocrotophos is an organophosphorus insecticide manufactured by United Phosphorus Ltd.Gujrat, is employed against agricultural pests in India by farmers, its use in developed countries has been banned long back due to its toxic and acaricidal effects. Since the insecticide is non-persistent its repeated use has become dangerous to non target organisms like fishes and amphibians (Kishore et al., 1983).The pesticide enters into aquatic environment by run off effect through rainwater. It is highly toxic orally as well as by inhalation or absorption through the skin. Its toxic action is achieved by inhibiting acetylcholinesterase; an enzyme essential for normal nerve impulse transmission. Contamination of water by pesticides either directly or indirectly leads to killing of amphibians too, ultimately affecting the diversity of amphibians.

In the present work an attempt has been made to investigate the effect of phoskill on the oxygen consumption and behaviors of *Microhyla ornata* tadpoles by exposing them to lethal doses of phoskill.

Materials and Methods

Tadpoles of *Microhyla ornata* were collected from a rain filled puddle near Pirangut Ghat (Bhoogaon) Pune, in the early monsoon months. The tadpoles were brought in plastic cans to the laboratory. The tadpoles were acclimatized in the laboratory for a week's time and then tadpoles of 30-35 stage were used for the experiments (Padhye and Ghate). 10 tubs were taken each with 1 liter of water and in each tub 10 tadpoles were released. Phoskill (toxicant) was added in each tub with different concentrations. Phoskill of 1150µl/lit, 880µl/lit, 850µl/lit, and 700µl/lit was used and LC50 found out for 24hrs.48hrs, 72hrs and 96hrs, for which Static renewal bioassay method (Finney, 1971) was followed. It was also determined by Probit analysis and Dragtedt Beheren's method (APHA, 2005).

The behavioral responses and oxygen consumption were recorded by exposing the larvae to lethal concentrations of the toxicant. Oxygen consumption was determined by Winkler's idometric method. Behavioral responses were noted by exposing the larvae for 2hrs to above mentioned concentrations.

Results and Discussion

The results obtained during the present study showed great variations in oxygen consumption and behavioral responses of *Microhyla ornata* tadpoles. The rate of whole animal oxygen consumption of control and phoskill treated tadpoles are presented in (Table -1, Figure-1).

Table-1: Effect of Phoskill on Oxygen consumption of *Microhyla ornata* tadpoles

Oxygen Consumption	24hLC_{50} (1150µl/lit)	48hLC_{50} (880µl/lit)	72hLC_{50} (850µl/lit)	96hLC_{50} (700µl/lit)
Control	14.58	14.09	13.61	13.12
SD	3.765832	1.085244	2.750648	3.671606
±SE	±1.537394	±0.443049	±1.122947	±1.498926
Exposed	6.33	8.23	10.69	12.15
SD	2.022494	2.584465	2.171969	3.537037
±SE	± 0.825679	± 1.055103	±0.8867026	±1.443989
% Change	- 56.58436	- 41.589779	- 21.454812	-7.39329

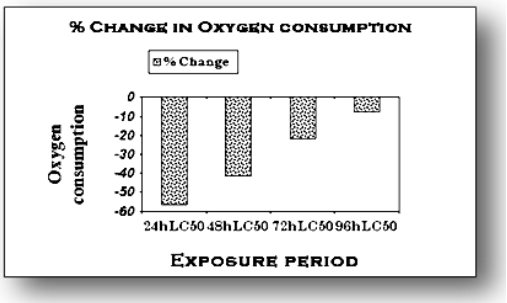

Figure-1 Graphical representation of Oxygen Consumption

According to Prashanth et al. (2003), the observed decrease in oxygen consumption in their experimental fish is due to the respiratory distress as a consequence of impairment of oxidative metabolism, similar situation is found in *M. ornata*. The toxicant from the environment enters the body of tadpoles by means of their skin and opercular movements. As the concentration of toxicant increases the respiratory stress increases which ultimately leads to decrease in oxygen consumption activity by the whole animal.

When the tadpoles were exposed to different lethal concentration of phoskill as stated above, great observationss were made with respect to their behavioral activities as shown in (Table -2).

Table-2: Behavioral changes of tadpoles

Sr.No	Responses	24hLC$_{50}$ (1150µl/lit)					48hLC$_{50}$ (880µl/lit)					72 hLC$_{50}$ (850µl/lit)					96hLC$_{50}$ (700µl/lit)				
	Time of Exposure	1-15 Min	15-30 Min	30-60 Min	60-90 Min	90-120 Min	1-15 Min	15-30 Min	30-60 Min	60-90 Min	90-120 Min	1-15 Min	15-30 Min	30-60 Min	60-90 Min	90-120 Min	1-15 Min	15-30 Min	30-60 Min	60-90 Min	90-120 Min
A	Locomotory Changes																				
1	Restlessness	✓	✓	✓	-	-	✓	✓	-	-	-	-	✓	-	-	-	✓	-	-	-	-
2	Erratic movement	✓	-	-	O	O	-	-	-	O	-	-	-	-	O	O	-	-	-	-	-
3	Fast movement	✓	-	-	-	-	✓	-	-	-	-	✓	-	-	-	-	✓	-	-	-	-
4	Position change	✓	-	-	O	O	✓	-	-	-	O	✓	✓	-	-	O	✓	✓	O	-	-
5	Direction changes	✓	F	F	O	-	-	✓	-	O	O	✓	-	-	O	O	✓	-	-	-	O
6	Sudden swimming	✓	✓	F	F	F	-	-	-	✓	✓	✓	-	-	O	O	✓	-	-	-	O
7	Surfacing	✓	F	F	F	F	✓	F	F	F	F	-	✓	✓	✓	✓	-	-	✓	✓	✓
8	Sustaintial swimming	-	-	✓	F	F	-	-	-	O	O	-	-	-	✓	✓	-	-	-	-	✓
9	Hyperactivities	✓	✓	-	-	-	✓	-	-	-	-	-	-	O	-	O	-	-	-	-	-
B	Neurological Changes																				
1	Jerk movements	-	-	-	O	O	-	-	O	-	O	-	-	-	-	O	-	-	-	-	O
2	Frightened	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	Balance loss	F	F	F	F	F	✓	✓	-	F	F	-	✓	✓	✓	✓	-	-	-	✓	✓
C	Morbidity																				
1	Reduced swimming	-	-	✓	✓	✓	-	✓	✓	✓	✓	-	-	✓	✓	✓	-	-	-	-	-
2	Slow movements	-	✓	✓	✓	✓	-	-	✓	✓	✓	✓	✓	-	-	-	✓	✓	-	-	✓
3	Stationary	-	-	✓	-	✓	-	-	-	O	O	-	-	✓	✓	✓	-	-	✓	✓	✓

[O =occasional, ✓ =observed, F =frequent, - = not seen]

The behavioral responses were noted under three heads as locomotory, neurological and morbidity for 2hrs time duration. Under the locomotory responses at 24hLC$_{50}$ in first 1hr restlessness was evident which is also noticed in first 30 min of 48hLC$_{50}$, 72hLC$_{50}$ and first 15 min of 96hLC$_{50}$. Erratic movements, direction change, and position change were observed in first 15-30 min in all concentrations. These responses exhibited by tadpoles might be due to the irritation, inflammation and injuries to skin by toxicant. Surfacing was observed frequently after each 15 min in all concentrations. Surfacing phenomenon shown by the tadpoles might be to gulp maximum possible air to ease the tension (Sangli and Kanabur, 2002). Neurological changes were noted in the form of jerks and loss of balance during the exposure period which might be due to the influence of toxicant on sensory motor interaction of nervous system, the toxicant may be acting as a neurotoxin leading to disorganization of nervous coordination (Ram and Gopal, 1991). Frightened movements were not observed in any of the above concentrations. Morbidity responses were observed in the form of reduced swimming, slow movement and stationary in 24hLC$_{50}$, 48hLC$_{50}$ and 72hLC$_{50}$ in first hr of exposure period and in 2nd hr of exposure

the same responses were observed at 96hrsLC_{50}. Morbidity responses exhibited by the tadpoles in the form of the fatigue ness and loss of equilibrium are due to the damage caused to the region in brain associated with the maintenance of equilibrium (Rao and Rao, 1987; Prashanth, 2002; Shivakumar and David, 2004).

Conclusion

From the above results it may be concluded that the effect of phoskill is highly toxic on the experimental animals, the tadpoles of *M. ornate*. The larvae and freshly hatched frog lets are totally dependent on aquatic environment from where they can reach land environment easily after metamorphosis for which they cannot inhabit big water bodies like pond and lakes, instead depend on the water puddles that are temporarily formed and will last only till mosoon. The water collected in such puddles is the run off water carrying large amount of such pollutants from their surrounding agricultural fields which may be considered as an obstacle in flourishing of the experimental animal leading to mass killing of tadpoles as one of the reasons affecting the diversity of species .

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