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EFFECT OF TWO ORGANOPHOSPHORUS PESTICIDES ON THE REPRODUCTIVE BIONOMICS OF FRESHWATER FAIRY SHRIMP *STREPTOCEPHALUS DICHOTOMUS* (BAIRD, 1860) (CRUSTACEA: ANOSTRACA)

Arun Kumar MS* and A Jawahar Ali
Unit of Aquaculture and Aquatic Toxicology, P.G & Research Department of Zoology, The New College, Chennai-6000 14, India.

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Abstract: This study evaluates the effects of the two organophosphorus pesticides (malathion) and (glyphosate), on the reproductive bionomics of the freshwater fairy shrimp *Streptocephalus dichotomus*. The sublethal effects at $1/5^{th}$ of 96 h LC_{50} of malathion, 2.0 ppm, and glyphosate, 0.0011 ppm on cyst production of the pre-adults were also studied. The 24 and 48 h LC_{50} values of malathion for nauplius were found to be 14 and 12 ppm; for glyphosate they were 0.026 and 0.014 ppm, respectively. The 24, 48, 72 and 96hr LC_{50} values of malathion for juveniles were 9.6, 8.0, 7.1 and 6.4 ppm; for pre-adults were 26.0, 16.8, 12.1and 9.1 ppm, respectively. Similarly the 24, 48, 72 and 96 hr LC_{50} values of glyphosate for juveniles were 0.097, 0.052, 0.032 and 0.017 ppm; for pre-adults were 0.013, 0.010, 0.007 and 0.005ppm, respectively. Animals treated with malathion (2.0 ppm) produced 41.2±12.7 cysts/female/day whereas the glyphosate exposure at 0.0011ppm resulted 95.5±12.6 cysts/female/day when compared to control (118.7±10.21cysts/female/day). An overall significant effect of pesticides on the cyst production between control and pesticide treated fairy shrimps was revealed ($P<0.001$). Of the two organophosphorus pesticides tested, glyphosate was found to be significantly more (1000 times) toxic to fairy shrimp nauplius, juveniles and preadults than malathion. However, at the sub lethal level, malathion showed higher reproductive toxicity than glyphosate.

Key Words: Fairy shrimp, malathion, glyphosate, toxicity test.

INTRODUCTION

The widespread application of synthetic pesticides over the past 50 years has led to their extensive occurrence in air, soil and water worldwide [1, 2, 3]. Such an occurrence of pesticides has the potential to exert adverse effects in humans and wildlife [4].

In the past few decades the use of organophosphorus pesticides has largely replaced organochlorine compounds for the agricultural applications. Responses to OP pesticides by aquatic animals are broad ranged depending on the compound, exposure time, water quality and the species [5]. OP pesticides are known to inhibit acetylcholinesterase (AChE E.C 3.1.1.7), which plays an important role in neurotransmission at cholinergic synapses by rapidly hydrolyzing the neurotransmitter, acetylcholine to choline and acetate [6, 7, 8]. Some OPs are highly soluble in water and can therefore easily contaminate aquatic ecosystems, thereby increasing the exposure risk of aquatic flora and fauna [9] including crustaceans and other aquatic organisms and may contribute to long term effects in the environment.

In recent years, a variety of fairy shrimp culture methods have been well developed [10, 11, 12, 13]. However information on the toxicity of OPs on fairy shrimp are limited [14, 15, 16]. Therefore an attempt has been made to evaluate the toxicity of malathion and glyphosate on the reproductive bionomics of freshwater fairy shrimp, *Streptocephalus dichotomus.*

MATERIAL AND METHODS

Chemicals

Commercial grade pesticides of malathion, (milathion 50% EC, manufactured by Insecticides India Private Limited New Delhi) and glyphosate (roundup 41% SL, manufactured by Monsanto India Private Limited New Delhi) were procured from a Agrochemical Store at Vellore, Tamil Nadu, India. Analytical grade $NaHCO_3$, $CaSO_4.2H_2O$, $MgSO_4$ and KCl were purchased from Sd-fine Chemicals Mumbai for preparation of synthetic fresh water.

Test organisms

S.dichotomus were originally collected from the temporary ponds of Putlur, Thiruvallur District and maintained in the laboratory for cyst (resting eggs) production. Toxicity tests were performed on three different life stages namely nauplii (0.4±0.07mm), juveniles (6.3±0.57mm), and pre-adults (12.6±1.15mm). Cysts were hatched into nauplii by following the procedures of [12]. All fairy shrimps used in toxicity tests were hatched in moderately hard synthetic freshwater (EPA Medium) which was prepared by dissolving 96 mg of $NaHCO_3$, 60 mg of $CaSO_4.2H_2O$, 60

***Corresponding Author:**
Arun Kumar MS,
Unit of Aquaculture and Aquatic Toxicology,
P.G & Research Department of Zoology,
The New College, Chennai 6000 14. India.

mg of $MgSO_4$ and 4 mg of KCl per litre of distilled water [17]. Cysts were placed in a 100 mL conical flask with 50 mL of synthetic freshwater and hatched with fluorescence light illumination at 27°C [18]. The free-swimming nauplii (24 h old) were randomly selected for toxicity tests. Juveniles and sexually matured pre-adults of *S. dichotomus* were reared in clean glass tanks containing ten litres of dechlorinated tap water with adequate aeration. The fairy shrimps were fed with a mixture of *Spirulina* powder and dried yeast dissolved in dechlorinated tap water [19].

Static acute toxicity test

Toxicity tests were conducted in polystyrene 24 well plates (2.0mL capacity); stock solutions of malathion and glyphosate were prepared in synthetic freshwater and its dilutions were made as per the requirements. Based on the range finding tests, five desired concentrations of each pesticide, 10, 20, 40, 60 and 80 ppm for malathion and 0.01, 0.02, 0.03, 0.04 and 0.05 ppm for glyphosate were selected, ten nauplii per well were allowed in 2.0ml of test solution; experiments were carried out in triplicates. The nauplii were not fed during the exposure period. Animals were recorded as being dead if no discernible movements were observed during 10 seconds of observation period under a dissection microscope.

Ninety six hour static bioassays were conducted with fairy shrimp juveniles and pre-adults in 250 mL plastic disposable cups. The following malathion and glyphosate concentrations were tested: 5, 10, 15, 20 and 25 ppm, 10, 20, 40, 60 and 80 ppm; 0.02, 0.04, 0.06, 0.08 and 0.1 ppm, 0.005, 0.01, 0.015, 0.02 and 0.025 ppm on juveniles and pre-adults, respectively. Test animals without toxicant served as control. Three replicates for each concentration (with 10 test organisms each in 100 mL test solutions) were tested. During exposure no food was offered to test organisms.

The mortality of the nauplii, juveniles and pre-adults were checked after specific periods of exposure (24, 48, 72, and 96 hrs). At the end of each test, the number of dead animals were counted and discarded. The LC_{50} values were calculated using probit analysis method [20].

Chronic toxicity tests

Chronic toxicity tests were carried out over a period of 30 days. Test animals (1:1 sex ratio) were introduced into test vessels of 250 mL disposable cups containing 100 mL test solution. Continuous aeration was provided throughout the experiment and the medium was renewed 100% on daily basis with appropriate level of each pesticide. The animals were fed with a mixture of 10 mg *Spirulina* powder and yeast granules dissolved in 1 litre dechlorinated water [21]. Based on 96 hr LC_{50} (malathion, 9.1 ppm; glyphosate 0.0055ppm), the fairy shrimp pre-adults were exposed to a sub-lethal concentration of (1/5th of 96 hrs LC_{50}) malathion (2.0ppm) and glyphosate (0.0011 ppm). A control without any pesticide was also concurrently maintained. Every 24 hrs the cysts were collected using 120µ sieve and carefully counted under dissection microscope and fecundity rate of per female per day was recorded.

Statistical Analysis

Data on % mortality were used to calculate the 24, 48, 72 and 96 hr LC_{50} by probit analysis [20]. Data obtained from chronic tests were subjected to repeated measures analysis of variance to detect the significant difference ($P < 0.001$) on cyst production between treated and control groups using SPSS 13.0 [22].

RESULTS AND DISCUSSION

Acute toxicity of malathion and glyphosate to the fairy shrimp nauplii, juveniles and pre-adults

As shown in Table 1, the 24 hr and 48 hr LC_{50} values (with 95% confidence limits) of malathion and glyphosate to fairy shrimp nauplii were found to be 14.1 and 12.3 ppm and 0.026 and 0.014 ppm, respectively. After 24, 48, 72 and 96 hrs exposures, the LC_{50} values (with 95% confidence limits) of malathion to the juveniles were found to be 9.6, 8.0, 7.1 and 6.4 ppm, respectively, and the LC_{50} values to pre-adults were found to be 26.0, 16.8, 12.1 and 9.1 ppm, respectively. The 24, 48, 72 and 96 hr LC_{50} values of glyphosate to the juveniles were 0.097, 0.052, 0.032 and 0.017 ppm, respectively, and to pre-adults were 0.013, 0.010, 0.007 and 0.005 ppm, respectively. According to Lahr *et al.,* [14] the 48hr LC_{50} value of malathion was found to be 67.7 ppm in *S. sudanicus* nauplii. Whereas the 24hr LC_{50} value of malathion in *S. proboscidius* nauplii was reported to be 81.5 ppm [23] and 6.4 ppm [15]. Likewise, Ripley *et al.,*[16] observed that 24hr LC_{50} value of malathion for *Branchinecta sandiegonensis* was 24.5 ppm. These values were higher than the 48hr LC_{50} value estimated for *S. dichotomus* in the present study.

Sidharta [24] reported a 48hr LC_{50} value of 1.00 ppm for malathion (Technical grade) to *Artemia salina.* Nelson and Roline [25] documented a 24hr LC_{50} value of 0.00318 ppm for *Ceriodaphnia dubia.* However, Jensen and Gaufin [26] found out a 96 hr LC_{50} value of 50 ppm for stonefly naiads. Post [27] documented the toxic effects of malathion on different species of fish and reported its 96hr LC_{50} values: fatheathed minnow (9.0 ppm), largemouth bass (0.285 ppm) and rainbow trout (0.17 ppm). Goodman *et al.,* [28] found that the 96hr LC_{50} for estuarine *mysid Mysidopsis* Bahia varied with the age of the test animals and ranged from 2.6 to

3.1 ppm. The 48hr LC_{50} of technical grade and commercial grade malathion to *D. magna* were 0.028 ppm and 0.003 ppm, respectively [29]. In an earlier study, Lahr [30] opined that the fairy shrimp *S. sudanicus* was most sensitive to a variety of insecticides.

Table 1: Median lethal concentrations (ppm) (with 95% confidence limits) of malathion and glyphosate to different stages of fairy shrimp, *S. dichotomus*

Pesticide	Life stage	Exposure duration 24hr	48hr	72hr	96hr
Malathion (ppm)	Nauplii	14.1 (11.85-16.87)	12.3 (10.28-14.93)	- (no data)	- (no data)
	Juveniles	9.6 (8.83-10.47)	8.0 (6.68-8.81)	7.1 (6.44-7.97)	6.4 (5.94-7.05)
	Pre-adults	26.0 (23.15-29.21)	16.8 (14.48-19.58)	12.1 (10.44-14.02)	9.1 (7.93-10.57)
Glyphosate (ppm)	Nauplii	0.026 (0.021-0.031)	0.014 (0.012-0.016)	- (no data)	- (no data)
	Juveniles	0.097 (0.066-0.142)	0.052 (0.040-0.068)	0.032 (0.026-0.038)	0.017 (0.015-0.020)
	Pre-adults	0.013 (0.013-0.014)	0.010 (0.009-0.012)	0.007 (0.006-0.008)	0.005 (0.004-0.006)

Very little information is available on acute toxicity of glyphosate on fairy shrimp. As summarized in the Table 1, the 24 and 48hr LC_{50} values for *S. dichotomus* nauplii and 96hr LC_{50} values for juveniles and pre-adults were determined as 0.026 and 0.014 ppm for nauplii and 0.017 ppm for juveniles and 0.005 ppm for pre-adults, respectively. From these results it is clear that glyphosate was more toxic to test organisms than that of malathion. A previous study conducted by Fochtman *et al.*,[31] has demonstrated that the 24hr LC_{50} value for glyphosate on *T. platyurus* was 0.35 ppm. While 24hr LC_{50} value of glyphosate on *B. sandiegonensis* was 0.0118 ppm [16]. Whereas Boonsoong and Bullangpoti [32] reported a 24hrs LC_{50} value of 0.319 ppm for glyphosate on *B. thailandensis* nauplii.

According to Alberdi *et al.*,[33] the 48hr LC_{50} value of glyphosate was found to be 42 ppm in *D. magna*. However, Raipulis *et al.*,[34] have reported almost five times higher 48hr LC_{50} for the same species (250 ppm). Likewise, Servizi *et al.*,[35] reported a 96hr LC_{50} value 25.5 ppm for glyphosate to *D. pulex*. The 96hr LC_{50} values for juveniles and pre-adults of the present study were lower than 96hr LC_{50} value of 1.05 ppm for *Oreochromis niloticus* [36]. The LC_{50} values observed in this study for different life stages of fairy shrimp exposed against malathion and glyphosate were comparatively lower, which may be due to differences in experimental conditions and species sensitivity. Further, the results also reveal that the respiratory mechanism and feeding behaviour of juveniles and pre-adults which is different when compare to that of nauplius.

Nauplius requires small quantity of water for their respiration and also for metabolic activity because it possesses yolk protein for their nourishment but in the case of juveniles and pre-adults they require large quantity of water for their respiration and metabolic activity. The continuous intake of pesticide through water which affects the chloride cells present in the throcapods and it also leads to discomfort of respiratory pigment to carry dissolved oxygen in the water and prolonged exposure leads to anorexia condition.

Sub lethal effects of malathion and glyphosate on cyst production in the fairy shrimp pre-adults

The 1/5th sub lethal effect of malathion (2.0 ppm) and glyphosate (0.0011 ppm) on fairy shrimp pre-adults was also apparent from data on reproductive performance (Fig.1). A significant effect of the toxicity of malathion and glyphosate on the fecundity of test animals was revealed ($P < 0.001$). In the case of control, the number of cysts produced/female/day ranged between 95.33±7.02 and 135.66±11.6; in the case of test animals exposed to malathion, it varied between 24.33±2.57 and 83.33±5.05; the fecundity of test animals exposed to glyphosate had a minimum of 63.66±6.02 and a maximum of 115.33±8.32.

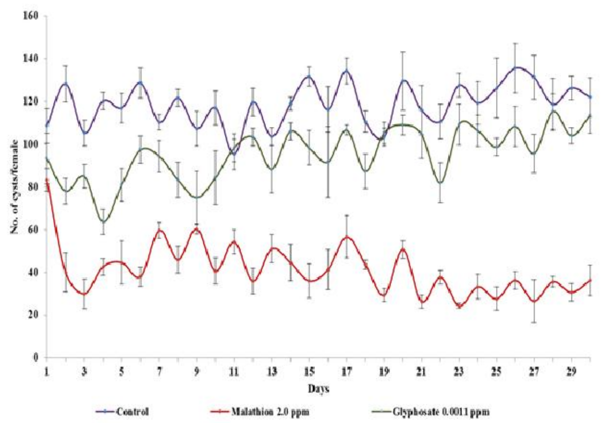

Figure 1: Cyst Production (No. cysts/ female/day) of *S. dichotomus* at sublethal concentration of malathion and glyphosate. Each point represents the mean of three replicates of 3 animals

Test animals exposed to sub lethal concentration of malathion yielded a mean cyst number of 41.2±12.7 cysts/female/day, while those treated with glyphosate produced 95.5±12.6 cysts/female/day, when compared to control

(118.7±10.2 cysts/female/day). Cunningham [37] investigated the effects of the insecticide Dimilin (TH 6040) on different life stages of brine shrimp under static renewal test conditions. In one experiment, brine shrimp reproduction was evaluated by exposing brine shrimp pairs and monitoring the number of nauplii produced. Wong *et al.*,[38] observed the reproductive performances of *M. macrocopa* at a sublethal concentration of 0.01µg/L of malathion and reported that the offspring production was reduced to about 40%. Day and Kaushik [39] recorded the reproductive performances of *D. galeata menodotae* when exposed to 0.01 and 0.05 µg/l of fenvalerate. They observed a significant reduction in the number of broods, mean brood size, and no. of young ones produced per female.

A reduction in reproduction was also reported by Ferrando *et al.*,[40] in *D. magna* when animals were exposed to the organophosphorus pesticide, fenitrothion at a concentration of 0.011µg/l and higher. Similarly Villarroel *et al.*,[41] observed the reduction in no. of offspring's in *D. magna* when exposed to tetradifon. Hernandez *et al.*,[42] recorded a decreased offspring production in *D. pulex* and *Diphnanosoma birgei* when exposed to higher concentrations of malathion. In aquatic systems malathion causes many adverse effects on animals such as affecting migration and behavior; inappropriate reproduction timings, decreased growth, abortion, decreased enzyme activities and egg production etc. [43].

To our knowledge, this is the first study focusing on effects of organophosphorus pesticides in freshwater fairy shrimp *S. dichotomus*. Freshwater fairy shrimp is widely used as live food in aquaculture (44, 12, 46, 47, 48, 49, 51, 52]. Thus, effect of organophosphate pesticides on this important anostracan also needs to be taken as a matter of serious concern. Hence, the fairy shrimp *S. dichotomus* could be taken as bioindicators of pesticide contamination and thereby a tool for bio-monitoring in vernal pools. In conclusion, organophosphorus pesticide contamination is risk to aqautic and terrestrial wildlife and this fact should be taken into consideration when this pesticides are used in agriculture and non-agricultural landscapes. Biological methods could be employed for controlling insects and weeds instead of using synthetic pesticides in order to protect the natural environment.

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