Organophosphorus Pesticides Toxicity on Brine Shrimp, Artemia

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Abstract

Present investigation discusses the toxic effects of two organophosphorus pesticides (Malathion) and (Glyphosate) on brine shrimp, Artemia in static acute toxicity tests (24hr and 48hr). Toxicity tests were performed at a temperature of 27±1°C with a photoperiod of 12h light and 12h dark. Ten healthy nauplii were exposed to five different concentrations of Malathion (10, 20, 40, 60 and 80 ppm) and Glyphosate (0.01, 0.02, 0.03, 0.04 and 0.05 ppm) along with a control group. Finney's Probit analysis was used to determine the 24/48h LC50 values and were found to be 58.3 and 17.3 ppm, 0.028 and 0.019 ppm for Malathion and Glyphosate, respectively. Of the two pesticides tested Glyphosate was found to be significantly more toxic to Artemia nauplii than that of Malathion (P<0.05).

1. INTRODUCTION

In recent years, synthetic pesticides are extensively being used to ensure crop yields, quality, and the variety and quantities of these substances accidently discharged into aquatic ecosystems which in turn lead to various adverse consequences on the non-target organism [1]. Organophosphorus pesticides (OPs) have largely replaced organochlorine compounds in the intensive agricultural activities because of their less persistent life and easy detoxification in animal tissues [2].

The mode of action of OPs arises by inhibiting the enzyme acetylcholinesterase (ACHE, E.C. 3.1.1.7), which is responsible for conducting the nerve impulse during neurotransmission as well as plays a vital role in the development of neurons and network formation in central nervous system [3-6]. Moreover, OPs also act as an endocrine disruptor and known to produce genotoxic, immunotoxic effects and oxidative stress which is incapable of increasing the levels of reactive oxygen species (ROS) in aquatic organisms [7-13].

Presently, organophosphates are considered to be highly toxic to many aquatic organisms [14-17,13]. It should be noted that, over the last two decades, the brine shrimp Artemia has gained popularity as test organism for short term bioassays because of its ease culture, short generation time, cosmopolitan in distribution and its commercial availability as resting eggs. This cyst was suggested as an attractive alternative to standard invertebrate stock cultures, since test animals can be hatched synchronously [26-29]. Like other organisms, Artemia also tend to bioaccumulate the toxicants and subsequently transferred them to higher trophic levels in the food chain. Therefore, it is necessary to know the relationship between Artemia and its tolerance range of various pollutants, so that the cultures can be used more safely and effectively [30,31]. In the present study, we investigated the toxic effect of two highest market selling organophosphorus pesticides namely Malathion and Glyphosate on Artemia.

2. MATERIAL AND METHODS

2.1 Chemicals

Commercial grade pesticides of Malathion (Malathion 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 Agrochemical Store at Vellore, Tamil Nadu, India. Analytical grade NaCl, Na2SO4, KCl, KBr, Na2B4O7·10H2O, MgCl2·6H2O, CaCl2·2H2O, SiCl4·6H2O and NaHCO3 were purchased from Himedia India for preparation of synthetic seawater.

Stock solutions of Malathion and Glyphosate were prepared in synthetic seawater. Temperature was maintained at 28±1°C and the pH was adjusted to 8.3 throughout hatching and the glass container was supplied with strong aeration. The light (2000lux) was provided by a fluorescent lamp placed near the hatching vessels. The photoperiod was maintained at 16:8D:L. After 24hr of incubation, the free swimming nauplii were attracted towards the lights source (phototactic behavior) and randomly collected for toxicity tests.

2.2 Test organisms

Artemia resting eggs (cysts) were collected from the salt pans of Veppalodai, Tuticorin, Tamil Nadu, India. Cysts were hatched into nauplii by following the standard ARC procedures (Artemia Reference Centre, State University of Ghent, Belgium). About 10g of the Artemia cysts were incubated in a cylindrical glass tube containing 100 ml of synthetic seawater (36pprt) which was prepared by dissolving 21.03 g of NaCl, 3.52 g of Na2SO4, 0.61 g of KCl, 0.080 g of KBr, 0.034 g of Na2B4O7·10H2O, 9.50 g of MgCl2·6H2O, 1.32 g of CaCl2·2H2O, 0.02 g of SrCl2·6H2O and 0.17 g of NaHCO3 per liter of MILLI-Q water. Temperature was maintained at 28±1°C and the pH was adjusted to 8.3 throughout hatching and the glass container was supplied with strong aeration. The light (2000lux) was provided by a fluorescent lamp placed near the hatching vessels. The photoperiod was maintained at 16:8D:L. After 24hr of incubation, the free swimming nauplii were attracted towards the lights source (phototactic behavior) and randomly collected for toxicity tests.

2.3 Static acute toxicity test

Static acute toxicity tests were conducted in 250 ml test vessels at a temperature of 27±1°C with a photoperiod of 12h light and 12h dark. Stock solutions of Malathion and Glyphosate were prepared in synthetic seawater and its dilutions were made as per the requirements. Based on the range finding tests, five different concentrations of each pesticide, 10, 20, 40, 60 and 80 ppm, respectively for Malathion and 0.01, 0.02, 0.03, 0.04 and 0.05 ppm, respectively for Glyphosate were selected; ten nauplii per vessel were allowed in 100 ml of test solutions. Experiments were carried out in triplicates. The nauplii were not fed during the exposure period. The mortality of the nauplius was checked after specific exposure periods namely 24 and 48 hrs. At the end of each observation period, the number of dead animals were counted under dissection microscope and discarded.

2.4 Statistical Analysis

Data on percentage mortality were used to calculate the 24 and 48hr LC50 values by Probit analysis method [32]. Analysis of variances (ANOVA) was used to compare the mortality (%) values of Malathion and Glyphosate to
Artemia nauplii after 24 and 48 hrs using SPSS ver. 13.0 IBM software [33].

3. RESULTS

The 24- and 48-hrs LC50 values for Artemia nauplii exposed to Malathion and Glyphosate were depicted in Table 1. The 24 and 48hr LC50 values of Malathion to Artemia nauplii were 58.3 and 17.3 ppm, respectively and those for glyphosate were 0.028 and 0.019 ppm, respectively.

Table 1: The LC50 values of Malathion and Glyphosate to Artemia nauplii after 24 and 48hrs exposure

<table>
<thead>
<tr>
<th>S. No</th>
<th>Pesticides</th>
<th>LC50 24hr</th>
<th>LC50 48hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Malathion</td>
<td>58.3 ppm</td>
<td>17.3 ppm</td>
</tr>
<tr>
<td>2</td>
<td>Glyphosate</td>
<td>0.028 ppm</td>
<td>0.019 ppm</td>
</tr>
</tbody>
</table>

An overall significant effect of Malathion and Glyphosate on the mortality of Artemia nauplii was revealed (P < 0.05; Tables 2 & 3). Different concentrations of Malathion and Glyphosate also had significant effect (P < 0.05).

Table 2: Comparative toxicity (mortality %) of Malathion and Glyphosate to Artemia nauplii after 24hrs exposure (ANOVA)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Source</th>
<th>Sum of Squares (mortality %)</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malathion vs Artemia nauplii</td>
<td>Between groups</td>
<td>63.067</td>
<td>4</td>
<td>15.767</td>
<td>236.50</td>
<td>.000*</td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>0.667</td>
<td>10</td>
<td>0.067</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>63.733</td>
<td>14</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Glyphosate vs Artemia nauplii</td>
<td>Between groups</td>
<td>71.067</td>
<td>4</td>
<td>17.767</td>
<td>133.250</td>
<td>.000*</td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>1.333</td>
<td>10</td>
<td>0.133</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>72.400</td>
<td>14</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Note: *Significant level is expressed at 95% confidence interval (P < 0.05)

Table 3: Comparative toxicity (mortality %) of Malathion and Glyphosate to Artemia nauplii after 48hrs exposure (ANOVA)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Source</th>
<th>Sum of Squares (mortality %)</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malathion vs Artemia nauplii</td>
<td>Between groups</td>
<td>71.600</td>
<td>4</td>
<td>17.900</td>
<td>134.250</td>
<td>.000*</td>
</tr>
<tr>
<td></td>
<td>Within groups</td>
<td>1.333</td>
<td>10</td>
<td>0.133</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>72.933</td>
<td>14</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Results showed differences in sensitivity of Artemia against two pesticides namely Malathion and Glyphosate (Table 1). The 24 and 48hr LC50 values of Malathion on Artemia nauplii were 58.3 and 17.3 ppm, respectively. In an earlier study, a group researcher reported 24hr LC50 value of 81.5 ppm for Malathion to the freshwater anostracan, Streptocephalus proboscidius [41]. A group of scientists have found 24hr LC50 value of 67.7 ppm for Malathion to S. sudanicus nauplii [42]. However, some of them reported 24hr LC50 value of 6.4 ppm for Malathion to S. proboscidius nauplii [43]. Likewise, a researcher observed a 24hr LC50 value of 24.5 ppm for Malathion to Branchinecta sandiegogenus [44]. Table 4 summarizes the toxicity of Malathion and Glyphosate used in earlier studies as well as in the present study to different brachiono-pods.

Table 4: Toxicity of (LC50) of Malathion and Glyphosate to branchiopods

Note: *Significant level is expressed at 95% confidence interval (P < 0.05)
According to a study, the 48hr LC50 value of Malathion (Technical grade) to Artemia salina was 1.00 ppm [45]. At the same time, two researchers observed 48hrs LC50 value of was 1.23 ppm to S. dichotomus, which is lower than the value observed in the present study [2]. However, in other study, they documented a 24hr LC50 value of 0.00318 ppm for Ceriodaphnia dubia [46]. The 48hrs LC50 values of technical and commercial grades of Malathion to D. magna were 0.028 ppm and 0.003 ppm, respectively [47].

As shown in Table 1, the 24 and 48hr LC50 values of Glyphosate to Artemia nauplii were 0.028 and 0.019 ppm, respectively. From these results it is obvious that Glyphosate was significantly more toxic to Artemia nauplii than Malathion (P < 0.05). A group of researchers has demonstrated that the 24hr LC50 value for Glyphosate on anastrean, Thamnocephalus platyurus was 0.35 ppm [48]. However, some of them observed 24hr LC50 value of 0.0118 ppm for Glyphosate to B. Sandiegogenonis [44]. Whereas reported 24hr LC50 value of 0.319 ppm for Glyphosate to B. Thailandensis nauplii. Fairy shrimp, S. dichotomus exposed to Glyphosate showed an LC50 of 0.14 ppm, which is lower than the present study of 0.019 ppm for brine shrimp, Artemia in 48 hours [2]. A scientist observed that Artemia franciscana exposed to Glyphosate and Zinc in combination produced an increase in lethality compared to shrimp exposed only to Glyphosate and Zinc separately [39].

According to some study, the 48hr LC50 value of Glyphosate was 42 ppm to D. magna [49]. A group of scientists has reported almost five times for the same species higher at 48hr LC50 (250 ppm) [50]. A researcher also reported that fish and aquatic invertebrates were more sensitive to Glyphosate than terrestrial organisms [51]. The LC50 values observed in the present investigation for Artemia 24hr old nauplii (instar I) exposed against Malathion and Glyphosate were comparatively lower, which might be due to differences in experimental conditions and sensitivity of the species. Toxicity of a xenobiotic is governed by many factors, such as water temperature, purity of the toxin, life stage of organism, size of the individual etc. From the results, it was clear that Artemia sp. is more sensitive to the exposure of the organophosphates Glyphosate than that of Malathion. The residues of organophosphate can be easily accumulated in the tissues of Artemia either by direct contact or by ingestion and the secondary effects might be observable in predators of Artemia in aquatic environments [31]. In conclusion, the branchiopod microcrustacean Artemia proved to be an excellent ecotoxicological bioindicator for marine and saline environments polluted with organophosphate residues and may be considered as an important bio monitoring tool for future toxicological analysis.

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REFERENCES


