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Research Article

An Investigation on the Toxic Effects of Malathion (Organophosphate Insecticide) on the *Daphnia magna* Straus, 1820 (*Crustacea, Cladocera*)

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Abstract: The toxic effects of technical grade (95%) malathion and commercial (25%) malathion were investigated on young (≤ 24 h old) *Daphnia magna* in static acute toxicity tests (24 and 48 hours). The culture of *D. magna* and toxicity tests were performed at a temperature of 24±1°C with a photoperiod of 16h light and 8h dark. To obtain a healthy population, the fecundity of each female in their life cycle were also investigated. Immobilization of daphnids were considered for determination of the 24h / 48h effective concentration (50%). The EC50 values were found by probit method 24h /48h, for technical malathion as 0.8 and 0.028 ppm., for commercial malathion as 0.11 and 0.003 ppm respectively.

Key Words: *Crustacea, Cladocera, Daphnia magna*, malathion, toxicity

Malathion (Organik Fosforlu İnsektisit)'un *Daphnia magna* Straus, 1820 (*Crustacea, Cladocera*) Üzerindeki Toksik Etkilerinin Araştırılması

Özet: Bu çalışmada *Daphnia magna* yavrularında (≤ 24 saatlik) teknik düzeydeki (%95) malathion ve ticari (%25) malathionun toksik etkileri statik, akut toksisite denemeleriyle (24 ve 48 saatlik) araştırılmıştır. *D. magna* kültürü ve toksisite denemeleri, 24±1°C sıcaklık ve 16 saat ışık / 8 saat karanlık fotoperiyodunda düzenlenmiştir. Sağlıklı bir populasyon elde etmek için, her bir dişi ferdin hayatı boyunca meydana getirdiği yavruların sayısı da araştırılmıştır. 24 / 48 saat %50 etki konsantrasyonunun (EC50) saptanması için dafnidlerin hareket durumu dikkate alınmıştır. EC50 değerleri, istatistiksel olarak probit metodu ile 24 / 48 saat sırasıyla, teknik malathion için 0.8 ve 0.028 ppm., ticari malathion için 0.11 ve 0.003 ppm bulunmuştur.

Anahtar Sözcükler: *Crustacea, Cladocera, Daphnia magna*, malathion, toksisite

Introduction

Water quality is important in our lives because it is essential to all life. If the quality of water is changed by the presence of toxicants, it is potentially harmful for all living things.

Water pollution comes from several sources, including agricultural activities, storage tank leakage, industrial waste, sewer and septic leakage, leaching from land fills, mining and many other sources. The 1990 EPA (Environmental Protection Agency) report on national water quality suggest that pollution of rivers and ponds comes mostly from agriculture (more than 50%) (1).

One of the agents that used to kill pests pollution in agriculture is pesticides. Malathion is an organophosphate pesticide and is widely used for mosquito control and also for fruit and tobacco pests. This compound may enter to the surface water and cause pollution (2, 3, 4).

An important group of freshwater invertebrates accupy a key position as invertebrate consumers in the pelagic as well as in the benthic food chains of aquatic ecosystems. Among them waterfleas of the genus *Daphnia* which belongs to the *Crustacea* classes occupy an important position in aquatic food webs, because they play a key role in determining water clarity by grazing an algae and they are an important part of the diet of fish (5).They have relatively short life cycle. The female *Daphnia* can alternate production of parthenogenetic (also called asexuel, summer) eggs and amphigonic (also called sexuel, epphipial, winter) eggs, under certain environmental conditions (6). Parthenogenetic eggs were produced and deposited regularly in the brood chamber which is the space between the upper side of the body and dorsal part of the carapace. These eggs were developed immediately and carried by the females until fully developed, looking like a miniature adults. They were swum away from the brood chamber shortly before the mother moults and they call neonates. Under certain environmental conditions such as less food, short photopeirod, over crowding and any other unsuitable conditions, male daphnids were began to produced by the female. The male daphnids are smaller than female. They have a long immobile 1st antenna. As soon as the male daphnids began to observed in the culture, female produced amphigonic eggs and sexual reproduction took place in the culture. After being fertilized, amphigonic eggs were extruded into the brood chamber. These eggs were modified to form the ephippium. Ephippium was released to the medium by maternal moulting.(6,7,8). *D. magna* and *D. pulex* are most frequently used in toxicity tests because a good laboratory culture is established by using them, they are sensitive to broad range of aquatic contaminants and are present throughout a wide range of habitat (6,9,10). *Daphnia* tests are currently the only type of freshwater invertebrate bioassay that are formarly endorsed by internatinal organizations such as US EPA, the EEC and the OECD, and that are required by virtually every country for regulatory testing (10,11). A number of investigations have been performed on *Daphnia* species for the toxic effects of various pesticides (12,13,14,15,16).

The aim of this study was to evaluate and compare the acute toxicity of malathion and malathion formulation (%25) on *Daphnia magna* in order to expand toxicity database of this pesticide in Turkey.

Materials and Methods

Preparation of *Daphnia magna* culture

Daphnia magna STRAUS 1870 were collected from a natural ponds in İzmir-Çiğli and were distinguished by the two lobe of the post abdomen and they were cultured in our laboratory. Parthenogenetic daphnids were cultured at a temperature of 24±1°C with a photoperiod of 16h light and 8h dark, in 30 x 20 x 20 cm aquariums with aged (chlorine-free) tap water. In each aquarium, the number of organisms per volume unite was approximately 1 daphnid / 2 ml of water. Daphnids were fed with 0,2 gr soy bean meal daily. In addition, they utilized protozoan and unicellular algae which were grown naturally in the aquarium. In the samples, which were taken from the daphnids aquarium, blue green algae (*Phormidium tenue*; *Lyngbya* sp; *Oscillatoria* sp.; *Microcoleus* sp.) and green algae (*Protensum* sp) were recorded. *Amoeba* sp. and *Paramecium* sp. were also observed in the culture.

In our culture condition, partenogenetic females reproduced very quickly and in the short time the aquarium filled with neonates. For obtaining a healthy growing daphnids, the density of the population must be controlled every day. The culture vessels and water were changed periodically. The mature females were transferred to the new aquarium by wide bore pipette and produced neonates. 24 h after these neonates were born, they were collected by pipette and transferred to a new aquarium. These (≤ 24 h old) neonates were used for toxicity tests.

For determining the fecundity, new mature females were transferred to the jars which containing 400 ml water in group of 40-43 individuals. The number of new borns and the number of live and dead females were recorded every second days. The mean number of neonates which were produced by each female calculated.

Preparation of the Toxicity Tests

The test materials, which were used in this study, are technical grade (95%) malathion and commercial (25%) malathion which contain active gradient of 25%. For preparing the stock solutions from the technical grade (95%) malathion, firstly, malathion was dissolved in 1 ml aceton and then according to the test concentrations it was diluted. The 24 h test concentrations, include 2.0, 3.2, 5.6, 7.0, 10.0 ppm for the initial tests, and 0.02, 0.1, 0.5, 1.0, 1.5, 2.0 ppm for definitive tests. The 48 h test concentrations were 0.1, 1.8, 3.2, 5.6, 7.0 ppm for the initial tests and 0.001, 0.005, 0.01, 0.02, 0.05 ppm for the definitive tests. For preparing the stock solutions from the commercial (25%) malathion, malathion was weighed and then according to the concentrations needed it was diluted. The 24h test concentrations were 0.1, 1.0, 3.2, 4.0, 5.6 ppm in the initial tests and 0.02, 0.1, 0.5, 1.0, 1.5, 2.0 ppm in the definitive tests.The test concentrations for 48h were 0.01, 0.05, 0.1, 0.5, 1.0 ppm in the initial tests and 0.001, 0.002, 0.004, 0.006, 0.008 ppm in the definitive tests.

All the experiments were carried out in 50 ml glass beakers. Ten neonates were transferred to each beaker with a pipette, and then the beakers were filled to the height of 40 ml with water. For preparing different concentrations, the necessary amount of stock solutions were added to each beaker filled with 40 ml of water and then they are closed with glass dishes. There were no feeding and no aeration during the tests. To determine the effective concentration (EC50) for each test, five concentrations and six replicants as well as control and acetone control were used. Each test container was examined daily and general condition of daphnids was observed by naked eyes. An organism was defined to be immobile if it was not able to swim within 15 sec. after gentle stirring. The number of immobilized daphnids was determined, and was controlled for mobility and behaviour under dissecting microscope.

The EC50 values for 24h and 48h were calculated using probit analysis (17). Probit method had been recommended by OECD guideline as appropriate statistical method for toxicity data analysis (11). After linearization of the dose response curve by logarithmic transformation of concentrations, 95% confidence limits and slope function were calculated to provide a consistent presentation of the toxicity data.

Results

Daphnia magna Straus culture

In our culture conditions, each female, in 8-9 weeks of her life, was produced parthenogenetically. In parthenogenetic reproduction only female neonates were produced. *Daphnia* neonates were matured after 5-6 instars during one week. The mean size of mature *Daphnia magna* female was measured as 2.27 mm lenght and 1.23 mm width (Table 1). During their parthenogenetic reproduction the mean number of neonates were recorded (Table 2). Each female *Daphnia* produced the maximum number of neonates during 5th and 6th weeks. The mean number of neonates produced 50.86 in 5th and 75.612 in 6th week (Table 2).

The Acute Toxicity Tests

At 24 h acute toxicity tests on *D. magna* (≤ 24 h old) with technical grade (95%) malathion, the mean EC50 value was found as 0.8024±0.26 ppm (Table 3). The dose response curve, together with the related equation by probit method was given in Fig. 1. In the 48h experiment with technical grade (95%) malathion, all the neonates were affected in the treatment concentrations above 0.1 ppm. The EC50 value was found as 0.0281±0.46 ppm. At 48 h, the dose response curve and the related equation by probit analysis were given in Fig. 2. For both 24h and 48 h exposures to the commercial (25%) malathion, the EC50 values were found as 0.1112±0.5 ppm and 0.0030±0.006 ppm respectively. The dose response curve and related equations by probit method were given in Fig. 3 and Fig. 4.

According to our toxicity tests with 24h and 48h exposure times on *D. magna* (≤ 24h old), it was shown that the commercial malathion (%25) was more toxic than that of the technical grade (95%) malathion.

Table 1. The measurements of the mean size (lenght and width) of mature *D. magna* for 12 days (n= The number of organisms).

Lenght x n	2.0 x 2	2.1 x 1	2.2 x 5	3.0 x 1	2.0 x 2	2.2 x 1	2.5 x 2	2.2 x 3	2.5 x 1	2.5 x 2
Width x n	1.0 x 2	1.0 x 1	1.5 x 5	1.5 x 1	1.0 x 2	1.2 x 1	1.5 x 2	1.0 x 3	1.0 x 1	1.2 x 2

Table 2. The number of youngs that produced in life span of each parthenogenetic female of *D. magna*.

Period	The Number of Mature	The Total number of Young	The Number of Young that Produced by Each Female
1st week	40	57	1.4
2nd week	39	133	3.5
3rd week	37	450	12.16
4th week	37	782	21.13
5th week	36	1831	50.86
6th week	36	2722	75.61
7th week	33	1104	33.45
8th week	31	1151	37.12
9th week	16	307	19.18

		EC10	EC50	EC90
%95 Malathion	24 h	0.0495 ± 0.90	0.8024 ± 0.26	7.0258 ± 0.1
	48 h	0.00025 ± 1.62	0.0281 ± 0.46	1.1468 ± 1.22
% 25 Malathion	24 h	0.0023 ± 1.02	0.1112 ± 0.5	2.2594 ± 1.25
	48 h	0.0010 ± 0.36	0.0030 ± 0.006	0.007 ± 0.18

Tablo 3. Results of 24h and 48h tests obtained by probit methods with malathion and malathion formulation (%25) on ***D. magna***.

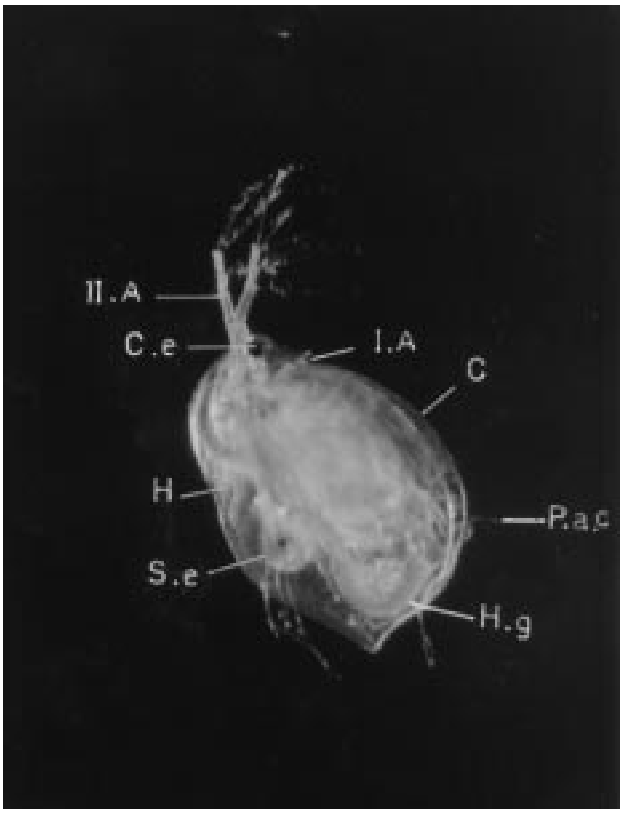

Figure 1. The dose respond curve, for 24 h with technical grade (95%) malathion on *D. magna* (≤ 24 hours) together with the linear regression produced using probit analysis.

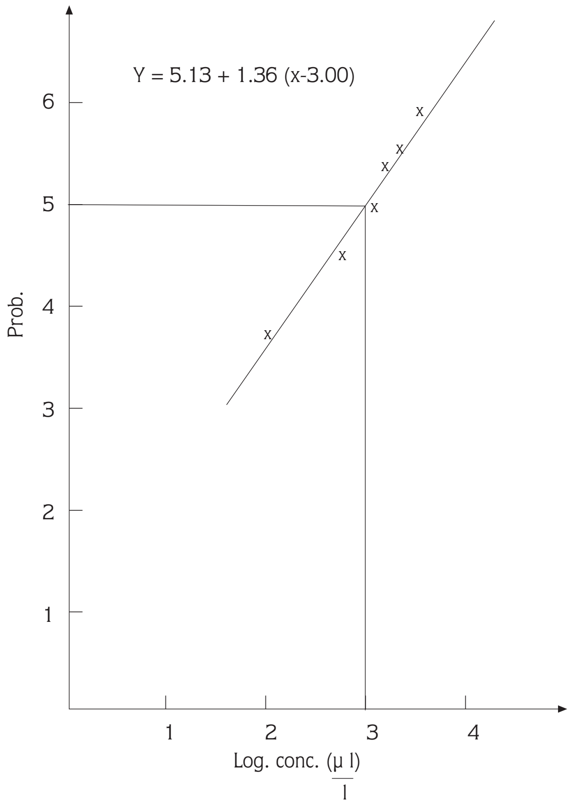

Figure 2. The dose respond curve, for 48 h with technical grade (95%) active malathion on *D. magna*. (≤ 24 hours) together with the linear regression prodused using probit analysis.

The Effects on Behaviour and Morphological Structure

Morphological effects of malathion for all concentrations on *D. magna* (≤ 24h old) were observed as the immobility of the antenna and legs, deformation of carapace (Fig.5). Affected daphnids were swam slowly to the bottom and to the surface for a few times and then they gathered to the bottom and showed trembling behaviour.

Discussion

A number of conditions such as temperature, photoperiod, food level, aquarium size and crowding are fundamental for experimental success (7,8,18,19,20). In our culture conditions *D. magna* neonates were molted every day or every second day. They became mature in a week time after 5th-6th moulting. According to Peters and Bernardi (1987), daphnids had matured after 4th-7th instars at 15-25 °C. Green (1961) showed that

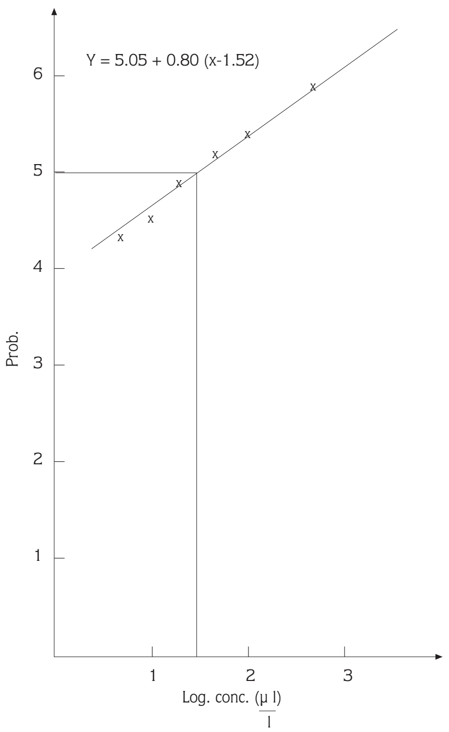

Figure 3. The dose respond curve, for 24 h with commercial (25%) malathion on ***D. magna***. (≤ 24 hours), together with the linear regression produced using probit analysis.

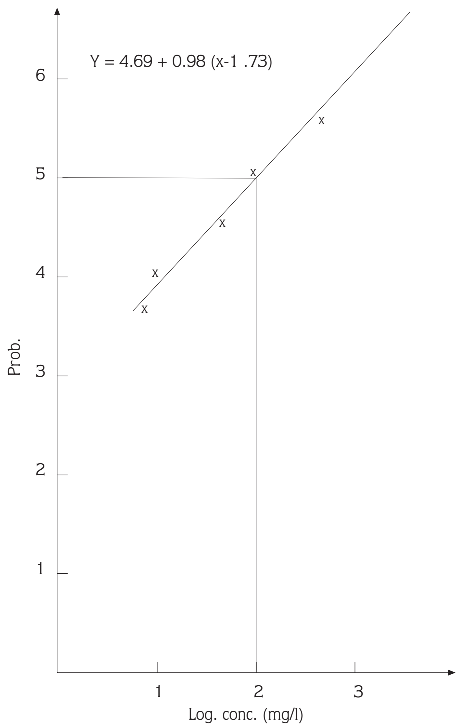

Figure 4. The dose respond curve, for 48 h with commercial (25%) malathion on ***D. magna***. (≤ 24 hours) together with the linear regression produced using probit analysis.

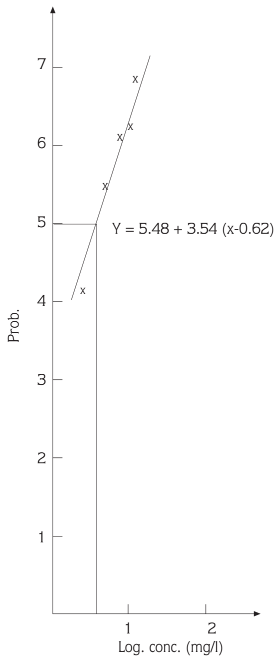

Figure 5. The dose respond curve, for 48 h with commercial (25%) malathion on ***D. magna***. (≤ 24 hours), together with the linear regression produced using probit analysis.

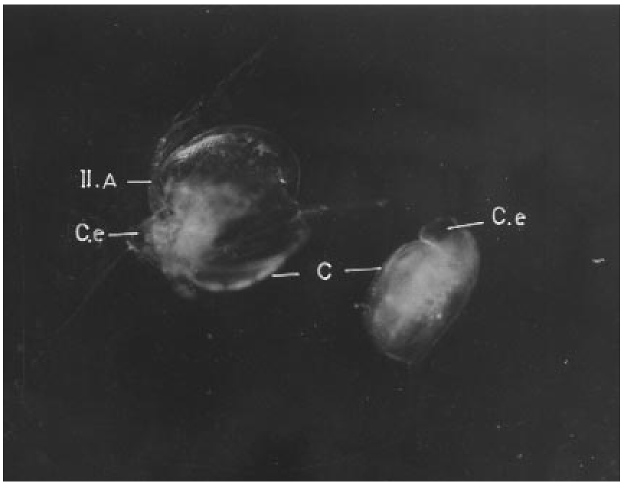

Figure 6. ***Daphnia magna***, Left: Effected with malathion, Right: Control II.A: II. Antenna, C.e: Compound eye, C: Carapace.

maturation time had been 4-7 days. Our results were in accordance their results.

In our toxicological experiments on *D. magna* neonates (≤ 24h old) for 24 h and 48 h exposure tests, the values of EC50 for technical grade malathion were found as 0.802 and 0.028 ppm respectively. For the commercial malathion the EC50 for 24h and 48h exposure tests were 0.111 and 0.003 ppm, respectively. The commercial malathion was more toxic than that of the technical grade malathion. The similar results were also observed on Rana ridibunda larvae (21). It was stated that additive material are more toxic in commercial formulation (21). Lilius et al., (1994) reported the EC50 values on *D. magna* for 24h tests as 1.07 mM (=0.353 ppm). However, we found it as 0.802 ppm. The difference between these two EC50 might have been due to the differences in the condition of experiments and genotype of populations. Because, their test condition were at 12h light and 12h dark photoperiod and M4 culture medium (12). Indeed, it has been shown that maternal nutrition and composition of the culture medium, effect the sensitivity of the young and this may contribute to inter-laboratory variability of the toxicity test results (10,22). In addition, different *D. magna* genotypes exposed to certain toxicants could response differently (23). Our toxicity tests have been performed using native species. It is proposed that the organisms which use in toxicity tests should be native or local species (24).

The toxicity effect of malathion in the 24h were reported as 3.18 µg/l (=0.00318 ppm) for *Ceriodaphnia dubia* (14). LC50 of malathion for *Culex pipiens* larvae was 0.0027-0.0043 ppm (25). It was 0.5 mg/l for *Mugil capito* (26). Lilius et al (1994) indicated that *D. magna* was more sensitive than fish hepatocyte cells in treatment with malathion. *Daphnia* species had shown high sensitivity against different pestisides according to 24 h and 48 h toxicity tests. The EC50 values for paraquate tests were 9.91–2.57 mg/l and for glyphosate 94.87-66.18 mg/l on *Daphnia spinulata*, respectively (15). The EC50 values of carbaryl on D. obtusa were reported as 0.015-0.115 mg/l (13).

It was shown that malathion (organophosphate pesticide) has toxic to *D. magna* by acetyl cholinesterase inhibition (12,27,28). According to our tests, morphological effects of malathion for all concentrations on *D. magna* were observed as the immobility of the antenna and legs, deformation of carapace. Dodson and Hanazato (1995) has also shown that carbaryl has some effects on swimming behavior and morphologic structure of daphnids.

Conclusion

In this study the acute toxicity of Malathion, which is an organophosphorous pesticide was determined on *D. magna*. The values of EC50 (24h-48h) were found for technical grade malathion as 0.8 and 0.028 ppm., for commercial malathion as 0.11 and 0.003 ppm, respectively. According to these results the commercial Malathion is more toxic than technical grade malathion.

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