J. Int. Environmental Application & Science, Vol. 11(1): 18-25 (2016)

The Acute Effect of Pesticides Carbaryl and Imidacloprid on *Daphnia pulex* species

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Received October 02, 2015; Accepted December 15, 2015

Abstract: The ecological risk assessment of insecticides carbaryl and imidacloprid were studied when cladoceran *Daphnia pulex* was exposed to both insecticides. The acute toxicity test of these insecticides was assessed using Median Lethal Concentration (LC_{50}) Mortality percentage and Safe concentrations. LC_{50} concentrations of carbaryl were 50.1, 33.1 and 26.4 µg/L for 24, 48 and 72 hr respectively. On the other hand, only the LC_{50} concentrations of 24 and 48 hr were reported when *D. pulex* exposed to imidacloprid which, were 1.5 and 1.09 mg/L respectively. The mortality rate of *D. pulex* was increased proportionally with the increase concentrations of insecticides. The *D. pulex* appears to be sensitive to both insecticides and this is observed from the values of safe concentrations.

Keywords: Insecticides, Carbaryl, Imidacloprid, *Daphnia pulex*, biological effect

Introduction

Water pollution caused by agricultural pesticides is a well recognized problem, as pesticides, are widely used and released into the natural environment (Hardersen & Wratten, 1998). Pesticides and other agrochemicals have been increasingly applied being much active in controlling pests, pathogens and weeds (Pereira *et al*., 2009). The majority of pesticides are designed to be used in a terrestrial environment; but nevertheless, substantial amounts of these chemical materials end up in aquatic ecosystems, in either surface or ground water (Fernandez-Alba *et al*., 2002). Non target animal population can be affected and some need more than six months for their abundance to recover after pesticide runoff that ends up in streams (Liess & Schultz, 1999).

Carbaryl is increasingly used as commonly pesticides, being within a carbamate group which they represent a successful development of organphosphorus pesticides (Downing *et al*., 2008). Organphosphorus pesticides (OPs) including carbaryl are among the most potent insecticides known and have been used throughout the world to control agricultural crop pests, for more than four decades (Smith, 1987). OPs can be taken up by organisms through the skin, the respiratory system and digestive tract (Ren *et al*., 2007). They react with acetyl cholinesterase enzymes (AChE) to inhibit the activity of the enzyme, which may induce the loss of nerve conduction ability (Christopher *et al*., 2001).

In contrast, imidacloprid insecticide is within the class of chloronicotinyl insecticides (Pestana *et al*., 2010). It is the best known stuff of these highly effective new insecticides. These nicotiniod insecticides have a selective effect on nicotinic acetyl cholinesterase receptors (Tomizawa & Casida, 1999). Water fleas are among the most preferred animals for toxicity test, especially Cladocera because of larger body sizes and high rate of reproduction (Dodson & Hanazato, 1995). They are sensitive to poor water conditions and are more commonly used for monitoring water quality (Bossuyt & Janssen, 2005). The water fleas are a zooplankton genus living in various aquatic environments all around the globe and they represent a major component of the diet of many invertebrates and vertebrates and are themselves efficient filter feeders of phytoplankton (Rousseaux *et al*., 2010). Therefore *Daphnia* spp. occupy a key position in aquatic food webs because they are often the most significant herbivore (Dodson & Hanazato, 1995).The objective of this study was to determine the acute toxicity of carbaryl and imidacloprid in their commercial available forms under constant exposure conditions.

Materials and Methods

Water quality: Filtered river water samples (freshwater) was used to inhabit water flea isolated animals and for preparation toxic solution (Lukancic *et al*., 2009). These samples were collected from

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Tigris river as natural habitat of these examined animals and was filtered by Dual water purification system with three filter housings which filters the water in the first stage and purifies it in the second. Filtered water was checked for some properties like: water temperature, hydrogen ion concentration, water conductivity and salinity.

Collection and Isolation of Crustacea

Water flea *Daphnia pulex* was obtained from biology department the College of Education Ibn-AL-Haitham, Baghdad University.

Identification, Classification and culturing of (*D.pulex*)

The water flea was identified by using the key (Edmondson, 1959). The cultures were maintained in filtered river water following the method described by Rousseaux *et al.* (2010). The crustacea were reared under laboratory conditions which included temperature 20±2º C. Photoperiodicity of 16 hr. light and 8 hr. for the growth and reproduction of the cladocera as recommended by Park and Yand Choi (2009). Also, the examined cladocera samples were fed by extraction of two spinach and celery leaves as described by Nebeker and Schuytema (1998).

Preparation of *D.pulex* for the toxicity test

Before exposure experiments , the gravid females of *D. pulex* were taken out and cultured individually in 120 ml glass beakers (freshwater and plant extraction) until they oviposited and healthy neonates (about 24 hr.) were systematically taken from the second and the following broods as recommended by previous study (Ren *et al.*, 2007).

Preparation of carbaryl and imidacloprid solutions for toxicity test

Two insecticide types were used in this study, the first was carbaryl (trade name Sevin) 85% wp (waterable)(manufactured by Behavar Chemical Co. ,packed in aluminum foil sac , Iranian origin). The second insecticide was imidacloprid (trade name confidor) 200SL, the concentration of this aqueous solution was 200 g/L (manufactured by Pioneers International Trading (LTD), packed in plastic bottles, Chinese origin). The insecticides were stored under refrigeration (4C°) to prevent dissociation (Wilson *et al.*, 2007).

Preparation of Carbaryl solutions for toxicity test

Astock solution of carbaryl was prepared by dissolving 0.005g (which is equal to 5000µg) in 25ml of filtered river water to obtain 200µg/ml. The purpose of using filtered river water was to avoid any decrease of the active concentration of the insecticides due to analysis (Zhu *et al*., 2009). From stock solution, four examining concentrations (25, 35, 45and 55µL) were prepared by taking 125,175,225 and 275 ml respectively and each was completed up to 1 L using again filtered river water .

Preparation of Imidacloprid solutions for toxicity test

Similar method above was used to prepare imidacloprid stock solution of again 200g/L according to previous study (Song *et al.* 1997). From this stock solution, four examined concentrations were made by transferring 5, 6, 7, and 8 ml into plastic beaker and the volume of each was completed to 1L to give 1, 1.2, 1.4 and 1.8 mg imidacloprid /L respectively.

Acute toxicity test

Acute toxicity experiments were demonstrated by exposed 4 groups of crustacea, each group consists 10 individuals with control group with the same number (Papchenkova *et al*., 2007). Acute exposure of carbaryl was conducted according to methods outlined by previous study Takahashi and Hanzato (2007). *D. pulex* exposed to 25, 35, 45, 55 carbaryl µg/L. Howver, Imidacloprid acute toxicity test was performed in accordance to standard toxicity test Kreutzweiser *et al.* (2008). The concentrations used for *D. pulex* were 1.0, 1.2, 1.4and1.8 mg/L Imidacloprid. Death of cladocera was defined according to Brennan *et al.* (2006), as the inability to swim for more than a few strokes within 15s after the gentle agitation of the test vessel and recording the presence or absence of movement.

Mortality Percentages

Mortality percentages were calculated for *D. pulex* after being exposed to carbaryl and imidacloprid insecticides for 96 hrs as applied in previous study (Nashaat, 2001).

Median Lethal Concentration (LC_{50})

Median lethal concentration was calculated for *Daphnia pulex* exposed to insecticides carbaryl during periods of 24, 48, 72and 96 hr of exposing, and calculated for the same period of exposing to imidacloprid. Probit analysis (SPSS v.13 for windows®, SPSS Inc.) was used to estimate LC_{50} values at a regular intervals for each test in acute exposure with cladocera (immobilization) (Ren *et al*., 2007).

Safe Concentration (SC)

Safe concentration was calculated by using the following equation (Al-Obaidy 2000):

$$SC = \frac{LC50(24hr)*0.3}{[LC50(24hr)/LC50(48hr)]^{x}}$$

x=2 or 3

Results and Discussions

Testing water quality

Table (1) shows the measurements of the physical and chemical parameters that were reported for testing water. The aim of measuring these parameters is to insure the suitability of using this type of water in rearing the animals and the range of them agree with what was found by other study Dwyer *et al*. (2005).

Table 1. Physical and chemical parameters of filtered river water

Physical and chemical parameters	Range	Mean Value
Temperature ºC	18-22	20
Hydrogen ion conc. (pH)	7.9-7.97	7.93
Electrical conductivity µs/cm	980-1028	1004
Salinity (ppt)	0.6-0.63	0.61

Acute toxicity test of insecticides (Carbaryl and Imidacloprid)

Median Lethal Concentration (LC_{50}) of *D. pulex exposed* to carbaryl

The experiment of calculating Median Lethal Concentration (LC_{50}) of *D. pulex* was carried out in 24, 48 ,72 and 96hr. , then Median Lethal Concentration (LC_{50}) values were only obtained in periods 24, 48and72 hr. (Table 2) and Fig: (1, 2 and 3) .

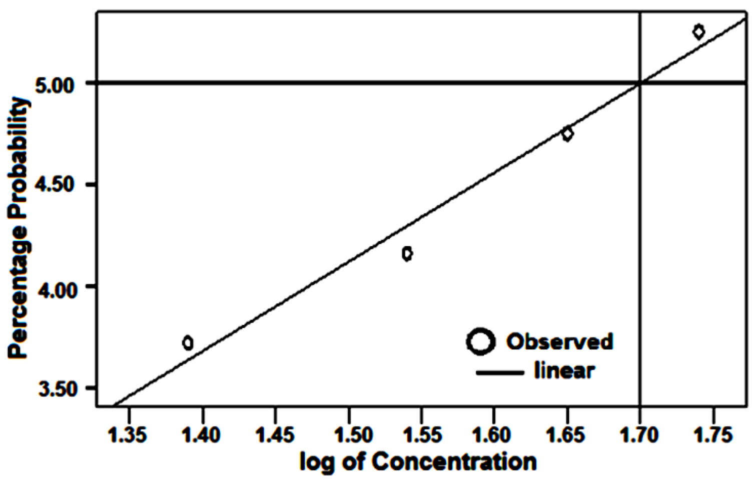

Figure 1. Toxicity curve of the insecticide carbaryl after 24 hr. of exposure

Table 2. *D. pulex* acute test: LC_{50} values (µg/L) for Carbaryl and Imidacloprid

Species	Insecticides	Median Lethal Concentration LC_{50} 24 hr.	48 hr.	72 hr.
D.pulex	Carbaryl	50.1	33.1	26.4
D.pulex	Imidacloprid	1.5	1.09	-

(-): no LC_{50} was calculated

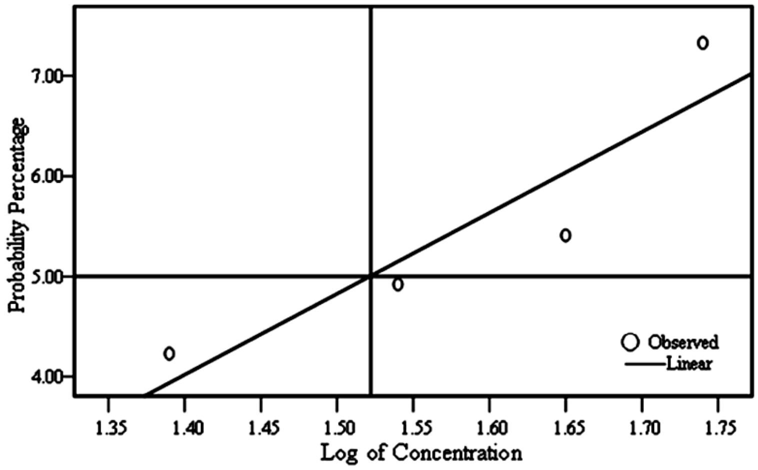

Figure 2. Toxicity curve of the insecticide carbaryl after 48 hr. of exposure

The values of carbaryl LC_{50} are higher than those reported by Takahashi and Hanazato (2007), in which the highest LC_{50} was 26.48 µg / L in 48 hr. Whereas other study (Coors *et al*. 2009) had reported that LC_{50} ranges from 6 µg/L to 12.5 µg/L. In contrast Peterson (2001) found that 96hr. LC_{50} values ranged from 11.1 µg/L to 61 µg/L when he exposed stream invertebrates to carbaryl. Concentrations (as low as 50 µg/L) are lethal to 50% of cladoceran zooplanctons (Chang, 2005).The LC_{50} values in the studies which are mentioned previously are near to LC_{50} values which were reported in this study for *D. pulex*.

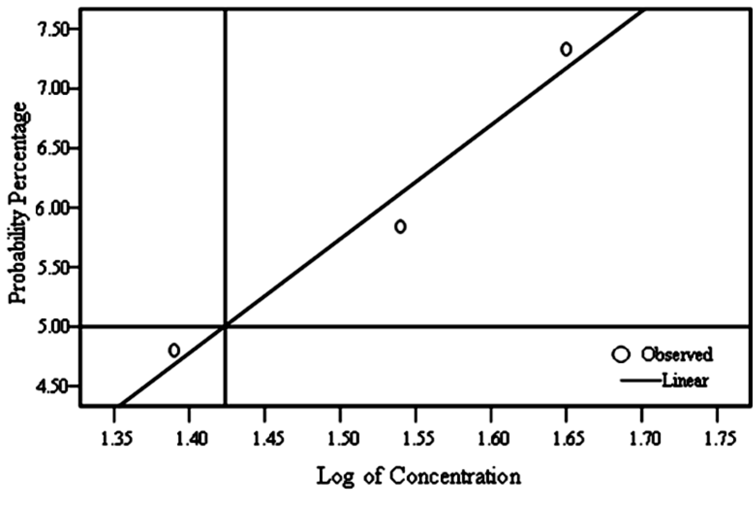

Figure 3. Toxicity curve of the insecticide carbaryl after 72 hr. of exposure.

Median Lethal Concentration (LC_{50}) of *D. pulex* exposed to imidacloprid

The Median Lethal Concentration (LC_{50}) values of *D.pulex* exposed to imidacloprid were 1.5 mg/L and 1.09 mg/L for 24 hr. and 48 hr. respectively as shown previously in table(2) and figures (4,5).

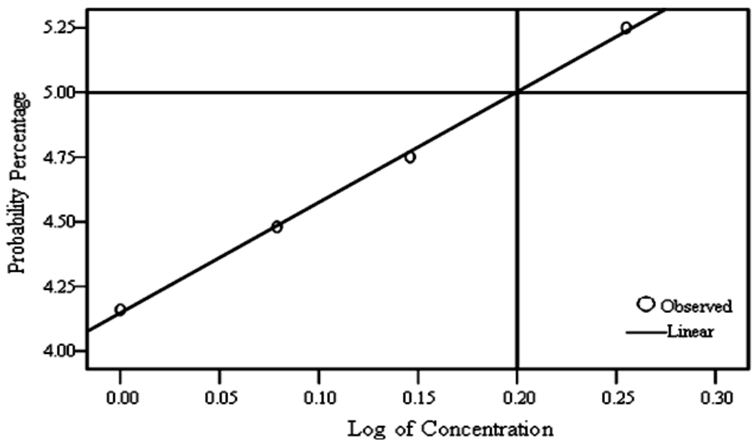

Figure 4. Toxicity curve of the insecticide imidacloprid after 24 hr. of exposure

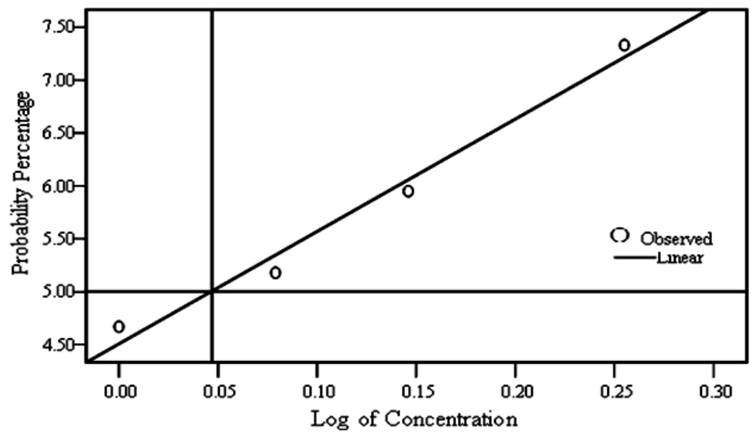

Figure 5. Toxicity curve of the insecticide imidacloprid after 48 hr. of exposure

The results showed high susceptibility of *D. pulex* toward imidacloprid as judged by LC_{50} and were less than the standard toxicity test which fluctuate between 10 – 85 mg/L, 17.4 mg/L to *D. magna* at 20º C (EC_{50} or LC_{50} 48 hr). (Song *et al*., 1997).Whereas the value of LC_{50} 48 hr. in this study was 1.09 mg/L for *D. pulex*. Therefore imidacloprid has toxicity value higher than those reported by the standard toxicity test and this was observed from the lower values of LC_{50} which were obtained in this study during 48 hr.

Several aquatic invertebrates appear to be sensitive to imidacloprid. Tomlin (1997) reported a 48 hr. EC_{50} (immobility) of 85 mg /L for *D.magna*. While another study obtained a value varied from 65 to 133 mg/L for *Daphnia* sp (Sanchez-Bayo & Goka, 2006). The LC_{50} values of the present study were near to the LC_{50} values obtained by Jemec *et al*. (2007) who determined LC_{50} in 48 hr. (as LOLC: lowest observed lethal concentration) for *D. magna* 10 mg/L. Also, LC_{50} in 48 hr for two non-target crustacean species (*Acellus aquaticus* & *Gammarus fossarum)* were: 8.5 mg/L, and 1 mg/L, respectively (Lukancic *et al*., 2009). In general, it seems likely that the different life stages, end points used, an increased temperature, length of exposure periods and the species of organisms are responsible for toxicity exhibition (Dwyer *et al.*, 2005).

Mortality percentages of *D.pulex* exposed to insecticide Carbaryl insecticide

Figure 6 demonstrate the mortality percentage (10%) of *D. pulex* exposed to carbaryl at concentration of 25µg/L and (60%) at concentration of 55µg/L after24 hr. of exposure

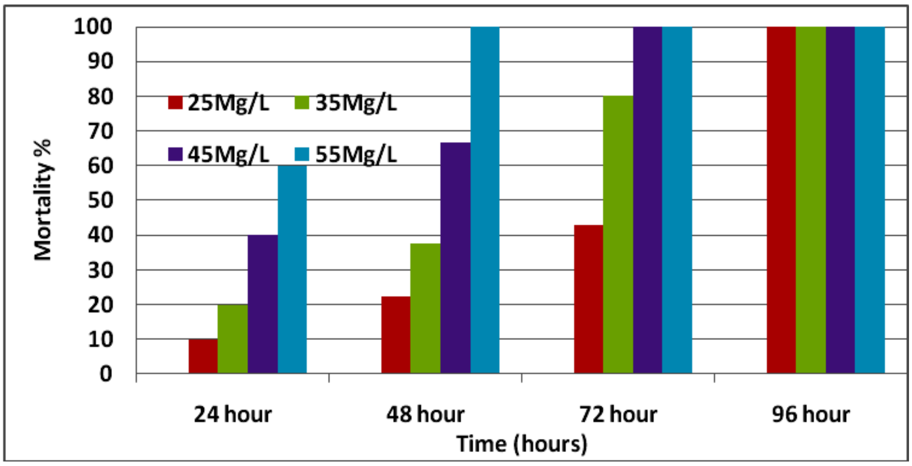

Figure 6. Mortality percentages of *D. pulex* exposed to insecticide carbaryl insecticide

Mortality percentage of *D.pulex* exposed to insecticide Imidacloprid insecticide

Figure 7 demonstrates the mortality percentage of *D. pulex* exposed to an acute concentration of the insecticide imidacloprid insecticide during exposure period of 96hr. *D. pulex* had 20% mortality in 1mg/L during 24hr while reached a value of 80% after 72 hr. at the same concentration. After 24hr.of exposure to 1.8mg/L imidacloprid, the mortality reached 60%.

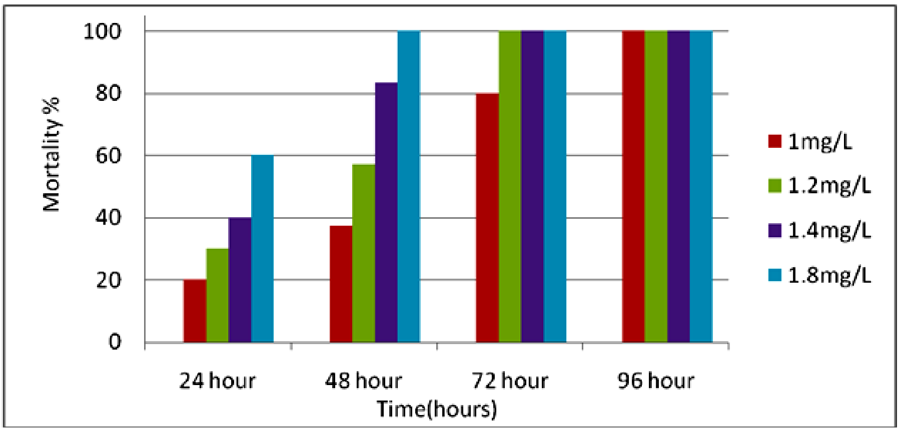

Figure 7. Mortality percentage of *D. pulex* exposed to insecticide imidacloprid

The result showed that the mortality percentages had increase with increased concentration of insecticides for species of cladocera. This agrees with the study of Takahashi and Hanazato (2007) in which they found that if the test period prolonged 72 hr (3days), for example, the sensitivity of animals should increase greatly. Therefore, in the present study, the acute exposure prolonged to 96hr (4days) and this proved insecticides' toxicity.

In previous studies in acute exposure where no food was added they found that carbaryl had the ability to pass the animals membranes (Overmyer & Nobelt ,2003).In other study, they found that imidocloprid had an effect on what is known as (ETS/R) ratios.(ETS: Electron transport system and R: Respiration value of crustacean) (Lukancic *et al.*, 2009). Therefore it had noxious effect on these vital processes through high short term concentrations.

The movement behavior which was observed in *D. pulex* included at first slowly, then the animals became stiff and settle on the bottom of the beakers. The same thing was observed by Ren *et al*. (2007) when they exposed *D. magna* to different OPs and they suggested that the changes in the movement behavior of cladocera could be considered as an early warning system for aquatic environmental quality. In addition the biological response of organisms to toxicant can vary among different labs (Lin, 2009).

Safe concentrations of insecticides Carbaryl and Imidacloprid to *D. pulex*

Table 3 shows the values of safe concentrations of the used insecticides for examined samples.

Table 3. The values of safe concentrations of Carbaryl and Imidacloprid to *D. pulex* .

Species	Type of Insecticides	Safe Con. When x=2	Safe Con. When x=3
D.pulex	Carbaryl	6.6	4.4
D.pulex	Imidacloprid	0.24	0.13

The *D.pulex* is found to be less tolerant to both insecticides which will support the use of such species to distinguish between the toxic effect and the hyper contamination (Schuytema *et al*., 1996). A study had examined the safe concentrations of Al-Daura refinery waste to two types of cladocera (*D. magna* and *S. exspinonus)* and reported higher for *S. exspinosus* than that of *D. magna* (Al-Obaidy, 2000).

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