The Synergistic Effect of Two Kinds of Insecticide in Water Flea *Daphnia pulex*

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Abstract

This study aimed to investigate synergistic effect of two kind of insecticide colti 5 and cypermethrin as a mixture pesticide in a volume ratio (1:1) on *D. pulex* individuals by using different concentrations (0.002, 0.0002, 0.00002) mg/l from it. Which the results appeared that percentage of mortality for *D. pulex* individuals was (80, 66, 30) % with LC_{50} (0.00015) mg/l after 24 hours from exposure to different concentration respectively. While the percentage of mortality for *D. pulex* individuals was (95, 80, 60) % with also LC_{50} (0.00015) mg/l after 72 hours from exposure to different concentration respectively. The results observation showed that the activity, size and egg sacs were different among the concentration that used compared to control. The results showed a significant difference in level of probability (0.05), and a positive correlation coefficient between percentage of mortalities and concentrations.

Keywords: synergistic effect, LC_{50}, pesticides, colti 5, cypermethrin

Introduction

Many chemicals that found in aquatic environment can be interaction with produce secondary products that could be more toxic [1], or less toxic to the environment [2] even when it found in low concentration, also, it could be change in physical and chemical properties of water [3], or determined the kind of living form that could live in water bodies [4], for that many scientists relied on bioindicators that could be affected by very few concentrations of these pollutants [5] and has ability to show their affected by some ways. Therefore, many studies pointed to use some kind of crustaceans, which is *D. pulex* one of them as bioindicator that has a higher sensitivity to pollutants [6] and can appear obvious behavior changing in low concentration of pollutants [7].

Lambda-cyhalothrin, which is an organic insecticide synthetic pyrethroid with low solubility in water [8]. that used to kill insects which caused disease to the human such as cockroaches and mosquitoes after feeding on it [9], and has higher ability to accumulation in organisms with potential to be harmful in the environment depending on its chemical properties [10]. Cypermethrin is one from group of synthetic pyrethroid and a main pollutant that found in agriculture and domestic using water, that could be inter the aquatic environment and caused harmful to its life form such as fishes and crustaceans [11], [12]. This compound has higher stability in environment with high

activity against insects [13], it belongs to pyrethroid group which some studies referred to it as safer on environment than organophosphate pesticides [14], While many studies referred to it as very toxic in low concentration [15], [16] and tend to find in sediment and suspended matter in water [17].

This study aimed to know the synergistic effect of Lambda-cyhalothrin insecticide and cypermethrin insecticide on *D. pulex* individuals after exposed to different concentration of mixture pesticide, and know their effectiveness by find the half lethal concentration that killed 50 % from individuals.

Materials and Methods

Preparation of Mixture Pesticide

The same way of [12] and [18] with slight modulation have been used to prepared mixture pesticide that has (1:1) volume ratio from each pesticide, Lambda-cyhalothrin insecticide prepared in concentration 0.1 %. Also, cypermethrin pesticide has been prepared in concentration 0.1 % after that the stock solution from mixture pesticides has been prepared in 0.001 % concentration. Then the different concentration of mixture pesticide has been prepared from stock solution in mg/l.

Calculation of Lc_{50}

To study the synergistic effects and find the toxicity of mixture pesticide of colti 5 pesticide and cypermethrin pesticide on young individual of *D. pulex*, the LC_{50} should be found after exposed to different concentrations (0.002, 0.0002, 0.00002) mg/l. individuals from 30 young fleas of *D. pulex* has been located to containers 250 ml that have different concentrations with three replicates per concentration. The average of survival young fleas of *D. pulex* (age less 24 hours) has been taken after 24 and 72 hours from exposure to the mixture, to study the acute effect on water flea individuals. The LC_{50} (lethal concentration for median (half)) has been calculated by using the straight-line equation [Y= bx + a (a= intercept, b= slope)] [19] after corrected the data with Abbott equation [20].

Layout of Experiment with Statistical Analysis

Full random design (CRD) has been involved. Data were analyzed statistically by using less significant differences (LSD) at 0.05 after subjection to the analysis of variance [21].

Results and Discussions

Table (1) appeared percentage of *D. pulex* mortalities after exposed 24 hours to different concentration of mixture pesticide, which the result showed that percentage of mortality was (80, 66, 30) % for concentration (0.002, 0.0002, 0.00002) mg/l, and the percentage of mortality were increased with increasing concentration in positive correlation coefficient (figure 1). These mortalities could be returned to the effective materials that found in both pesticides, where the colti 5 pesticide has some kind of materials can effect on nervous systems and caused paralysis or death to many organisms [8], while cypermethrin pesticide has a cyno-side chain that has long period of life and effectiveness on target and nontarget organisms [22]. These materials in many studies have ability to be toxic in different concentration on untargeted organism's dependent on

type of organisms [23]. The results showed that LC50 was (0.00015) mg/l, which is mean the mixture pesticide is toxicity even when it found in lower concentrations in aquatic environment, where there are many studies pointed to find many pesticides have multiple dangerous effect on aquatic life when it found together in the same water bodies as a result of combination effects [16].

Table (2) showed percentage of mortalities after exposed 72 hours to different concentration of mixture pesticide, which the results appeared that percentage of mortalities were (95, 80, 60) % for concentration (0.002, 0.0002, 0.00002) mg/l, and the percentage of mortalities were increased with increasing period of exposing to different concentration of mixture pesticide (Figure 2), also, the results showed there is a significant differentiation between two periods of mortalities but in another hand, the results in figure (2), (3) showed that LC_{50} value after 72 hours from exposure to mixture pesticide was also (0.00015) mg/l which is the same value that found after 24 hours from exposure to same concentrations of mixture pesticide. Which is the study of [12] pointed that organism with long exposure to toxic material could be decreased in his ability to resist the toxicity of materials, while study of [24] Pointed to length of exposure to chemicals that found in low concentration could be caused of accumulation these chemicals inside organism which lead sometimes to death, or could be caused damage that can't be repaired [25].

Also, the current study observation results showed that *D. pulex* individuals have small size after 72 hours compared to control after exposure to different concentrations from mixture pesticide, which Some studies mentioned to these state as physiological changes that can be appeared after long time exposed to toxic materials as a kind of resistance which protect organism temporary from the death [26], while another studies discussed a small size of individuals as kind of adaptation to survive [27].

Table (1) and (2) showed there are survival individual of *D. pulex* which was (6, 10, 21) individual/30 individual and (2, 6, 12) individual/30 individual after 24 and 72 hours from exposure to different concentration of mixture pesticide (0.002, 0.0002, 0.00002) mg/l respectively. These could be returned to individual differentiation among *D. pulex* individuals because of type of genetic mutations that can be happened normally in genetic structure of zygote [28] which lead to give some individuals sort of resistant to some kind of harmful chemicals. Where the study of [29] and study of [30] referred to the differentiation in genetic expression and mutations as a result in many differentiations between individuals of same species, which lead in differentiation and varies in response to the physical and chemical changes that can happen in environment [3].

Results showed in table (2) that egg sacs have some changes after 72 hours from exposure to mixture pesticide, which in concentration 0.00002 mg/l has change in color with normal size of sac that content few and big size of eggs than control, while concentration 0.0002 mg/l also has changed in color with small sac that content few and small size of eggs, and concentration 0.002 mg/l has change in color with smaller sac that content many and small size of eggs than control. Which some studies appeared that the organisms can be showed some type of resistant by reducing some secondary or unessential biological processes [31], in this study may be appeared as reduced in sac size or number of eggs, or it works on aggregation toxic materials in some unessential organs such as reproduction organs [32], or in many studies in essential organs such as liver [33], [34] as kind of resistant. which in this study could be mentioned to change in sacs

color. Also, few studies pointed to stop some *D. pulex* individuals from feeding and inter to temporary hybernation stage until disappeared the effector [2], [35], which the observation result showed that survival *D. pulex* individuals have small size after 72 hours from exposure than control that could be returned to stop from feeding, other studies referred to tend *D. pulex* individuals to become autogenous by producing few large eggs [36] because of unsuitable condition that surrounding it, or many small eggs when the environment where more toxic to it [37]. Some study referred to change in color and size of eggs as inter *D. pulex* individuals to auto-reproduction stage to protect it kind from extinction [38].

Conclusions

1- The percentage of mortality was increased with increasing concentration of mixture pesticide which referred to the activation of two pesticides together.
2- The size of water flea individuals can be affected by the long time of exposure to mixture pesticide.
3- There are changes in color and size of sac of eggs and number of eggs after exposure to pesticides dependent on observation results which could be indicated to the effect of pesticides on the reproductive organs.
4- The color and size and number of eggs and sac eggs could be dependent on time of exposure.
5- The LC_{50} value didn't change after 24 and 72 hours from exposure to different concentrations of pesticide which means the dose could be effected on mortality of individuals and the time may be has less effect on mortality.

Table (1) percentage of *D. pulex* mortalities after 24 hours from exposed to different concentration of mixture extracts of pesticides

Concentration (mg/l)	Average of survive (Individual/30 individual)	Percentage of mortalities* %
0.002	**6**	**80**
0.0002	**10**	**66**
0.00002	**21**	**30**
Control	**30**	**0**
LSD*= 0.889		

Table (2) percentage of *D. pulex* mortalities after 72 hours from exposed to different concentration of mixture extracts of pesticides

Concentration (mg/l)	Average of survive (Indiv. /30 indiv.)	Size of individuals compare to control	Percentage of mortalities* %	Egg sacs
0.002	2	small	95	- ++ -
0.0002	6	small	80	- - -
0.00002	12	small	60	+ - ++
Control	30	normal	0	+ + +

LSD*= 1.134; r*= 0.87
Size of egg sacs compare to control: small (-), big (++)
Number of eggs compare to control: few (-), high (++)
Size of eggs compare to control: small (-), big (++)

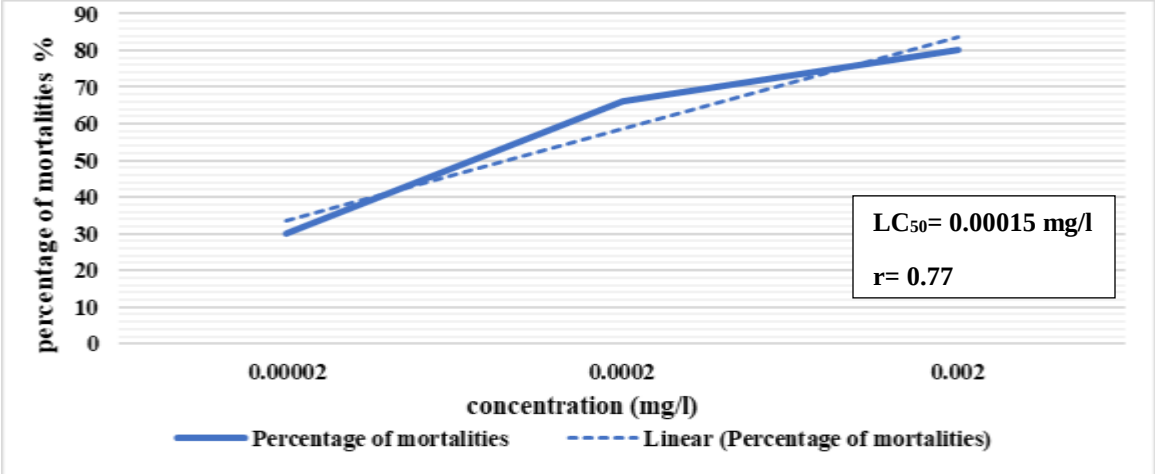

Figure (1) LC_{50} value of *D. pulex* after exposure 24 hours to several concentration of mixture pesticide

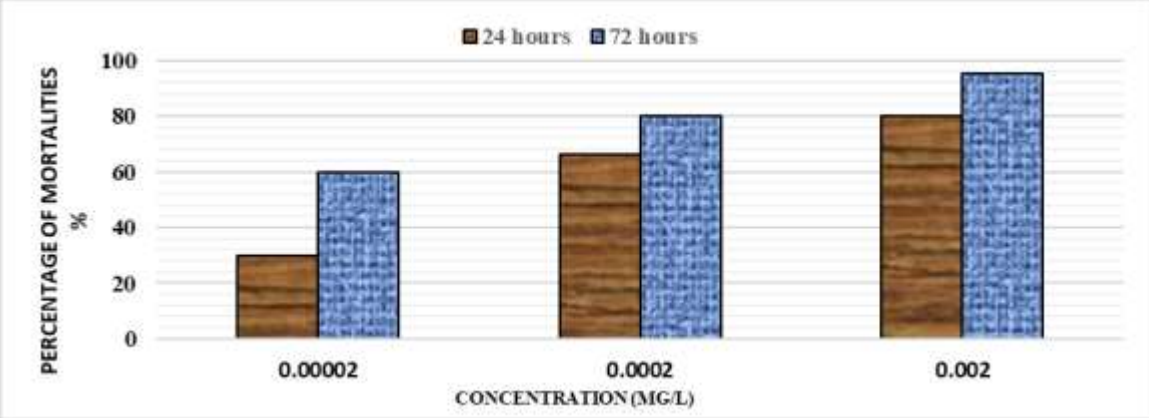

Figure (2) percentage of mortalities of *D. pulex* after 24 and 72 hours from exposure to different concentration of mixture pesticide (LSD= 1.254).

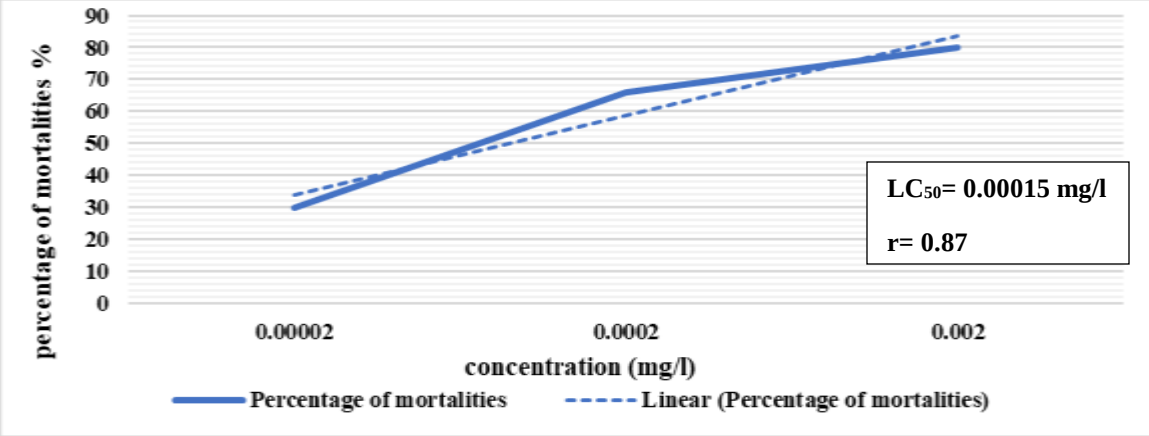

Figure (3) LC_{50} value of *D. pulex* after 72 hours from exposure to different concentration of mixture pesticide

Conflict of Interests.

There are non-conflicts of interest .

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الخلاصة

تناولت هذه الدراسة التحري عن التاثير التعاضدي للمبيد الحشري colti 5 والمبيد الحشري cypermethrin كمبيد خليط بنسبة حجم (1:1) على افراد برغوث الماء *D. pulex* بعد التعرض الى تراكيز مختلفة منه (0.002 , 0.0002 , 0.00002) ملغم/ لتر. اذ اظهرت النتائج ان النسبة المئوية لهلاك افراد *D. pulex* كانت (80 , 66 , 30) % وقيمة LC_{50} كانت (0.00015) ملغم / لتر بعد التعرض 24 ساعة الى تركيز مختلفة من المبيد الخليط على التوالي. بينما النسبة المئويه لهلاك افراد *D. pulex* كانت (95 , 80 , 60) % وقيمة LC_{50} كانت ايضا (0.00015) ملغم / لتر بعد 72 ساعة من التعرض الى تراكيز مختلفة من المبيد. كما اشارت نتائج المشاهدات الى ان فعالية وحجم و كيس البيض لافراد برغوث الماء كانت مختلفة بين التراكيز المستخدمة مقارنة بالسيطرة. كما اظهرت النتائج وجود فروقات معنوية عند مستوى احتمالية 0.05 , وكذلك وجود معامل ارتباط ايجابي بين النسبة المئوية لهلاك افراد برغوث الماء والتراكيز المستخدمة.

الكلمات الدالة: تاثير تازري, LC_{50} , مبيدات , colti 5 , cypermethrin