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Environmental Study of Effect of Some Plants Extracts on Culex pipiens mosquitoes

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دراسة بيئية عن تاثير بعض المستخلصات البيئية على بعوض *Culex pipiens*

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ABSTRACT

Background

In the nature there are many plants that play important roles to control mosquitoes, but some of these plants can increased their effect after mixed with another plant. For that, this study aimed to know the effect of mixture of two types of different plants on *Culex pipiens* mosquitoes in the larva stages and the process of hatching the eggs.

Materials and methods

In this research the effect of parsley water extract (*Petroselinum crispum*) and mosses water extract (*Funaria sp.*) have been taken as mixture in ratio (1:1) to know their effect on *Culex pipiens* mosquitoes after using different concentrations (4.5, 3.5, 2.5, 1.5, 0.5) mg/l from it.

Results

The results show that percentage of mortalities for *C. pipiens* larva mosquitoes were (89, 72, 55, 42, 39)%, (89, 62, 56, 36, 38)%, (75, 67, 54, 46, 26)% and (77, 65, 63, 56, 40)% with LC_{50} (1.8, 2, 2.3, 1.3) mg/l for first, second, third and fourth stage respectively. While the percentage of hatching eggs that exposed to mixture extracts were (31, 43, 65, 71, 83)% with LC_{50} (3.2) mg/l. Results haven't shown a significant differentiation among percentage of larva mortalities stages, but they have shown among percentage of hatching eggs in level of probability (0.05), also concentrations have a positive correlation coefficient with percentage of larva mortalities stages and negative with percentage of hatching eggs.

Conclusion

The water extract mixture from water extract of parsley and mosses can be killed larva stages in low concentration and the mortality increases with increased concentration of extract, and can prevented eggs hatching.

Keywords: Larva, LC_{50}, mosses, parsley, synergistic extract.

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INTRODUCTION

In the nature there are many types of plants that have materials or compounds have ability to become food or toxic or repellent to another organisms. These materials could be nitrogenic compounds specially alkaloids or terpenoid compounds or phenolic compounds or enzymatic inhibitors or regulators growth [1] that exist as secondary metabolite compounds. Some plants producing repellent materials that it is toxic in the same time for protecting from herbivores organisms specially insects [2], with smell aromatic features that interfered with insect nervous system [3].

Parsley or *Petroselinum crispum* is aromatic plant belongs to Umbelliferae family [4], their leaves content many compounds such as apigenin as antioxidant, volatile oil and vitamins A, C and K [5], which in some studies referred to parsley plant as antifungal and antibacterial plant [6]. Parsley has widely distribution around the world. The moss or *Funaria sp.* is free living gametophyte belongs to Funariaceae family that has two types of life cycle [7], their all green parts have terpenoids, glycosides phenols and fatty acids [8]. Also have ability to work as antifungal plant diseases [9]. These mosses can be found globally in moistures, shady and firing soils. while *C. pipiens* mosquitoes can be found everywhere around the world and caused many troubles and disease to many humans and animals [10]. It belongs to culicidae family that has feeding on blood to lay eggs [11].

Around the world there were many cultures that used mixed of different plants to treat different diseases or injured, where these plants could have materials or compounds that could be toxic or nontoxic or effective or noneffective when it used separately [12], and some of these compounds sometimes became useful or toxic or deadly when it is mixed or reacted with other compounds which lead to promote of its effectiveness [13]. Some researchers study these plants such as study that mention in [14] where used extract of *Rosmarinus officinalis* plant with five antimicrobial agents such as amoxicillin to find final extract has ability to kill *staphylococcus aureus* bacteria more than used them separately. Other studies such as study that mention in [15] found the whole plant extract of *Artemisia annuab* where more effective than one compound of extracts.

This study aimed to know the effect of leaves parsley extract mixed with whole green parts of *Funaria sp.* moss extract in ratio (1:1) on hatching eggs and four stages of *C. pipiens* mosquitoes and found the half lethal concentration that killed 50% from larva or prevent hatching eggs.

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Materials and Methods

- **Preparation of Extract**

Used method of Al-Manhel and Niamah (2015) and modified method of Odey *et.al*., (2012) to wash and prepare parsley plant leaves, *P. crispum*. The leaves left dried in room temperature and crushed well by electrical mill and sieved to gain smooth powder that grinded again and sieved to get fine powder. The same way has been used to prepare green parts of *Funaria sp.* moss. The mixture extract prepared by taken 5 gm (2.5gm parsley powder + 2.5gm moss powder) of fine powder and mixed well with 250 ml distil water for 15 minutes in electric mixer to has net mixture extract, after that the extract was filtered in millipore paper 0.45µm. The percentage has been used to prepare stock solution 2% (20000 mg/l) that later used to prepare different concentrations (4.5, 3.5, 2.5, 1.5, 0.5) in mg/l after using dilution law.

- **Calculation of LC_{50}**

To study the synergistic effects and find the toxicity of mixture extract of parsley leaves and green parts of moss on life cycle of *C. pipiens* mosquitoes, the LC_{50} should be found after exposed to different concentrations (4.5, 3.5, 2.5, 1.5, 0.5) mg/l. individuals from 30 larva of *C. pipiens* has been located to containers 250ml that have different concentrations with three replicates per concentration. The young larva of *C. pipiens* (ageless 24 hours) has been taken after 24 hours from exposing to extracts, and after 48 hours for hatching eggs from exposing, to study the acute effect on mosquitos' larva and hatching eggs respectively. The LC_{50} (lethal concentration for median (half)) has been calculated after using the straight-line equation [Y= bx + a (a= intercept, b= slope)] [18] after corrected the data with Abbott equation [19].

Statistical Analysis

A completed randomized design (CRD) were used. Data were analyzed statistically by using the analysis of variance (ANOVA-test) then less significant differentiation (LSD) at 0.05 [20].

Results and Discussions

Figure (1) appeared the percentage of mortalities of *C. pipiens* larva mosquitoes after 24 hours from exposing to different concentrations of parsley and moss extract. Where the results pointed that percentage of mortalities were increased by increasing concentration, and showed a positive correlation coefficient, which the percentage of mortalities were (89, 72, 55, 42, 39)%, (89, 62, 56, 36, 38)%, (75, 67, 54, 46, 26)%, and (77, 65, 63, 56, 40)% for first, second, third and fourth stage after exposed to concentration (4.5, 3.5, 2.5, 1.5,

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0.5) mg/l respectively where according to some study [21] there are some types of chemical compounds that used in pest control can be more effective after mixed with another chemicals. Which current study deals with two types of extracts have many kinds of chemical compounds. Also, some study referred to parsley plant as container of inert active groups that can react and produce active materials when used with some another chemical [5]. Another study pointed to *Funaria sp.* moss as a type of bryophytes has a biological active compound sometimes work as cytotoxicity specially to cancer cells that derived from secondary metabolism which make it so strong active compound at high concentration [22].

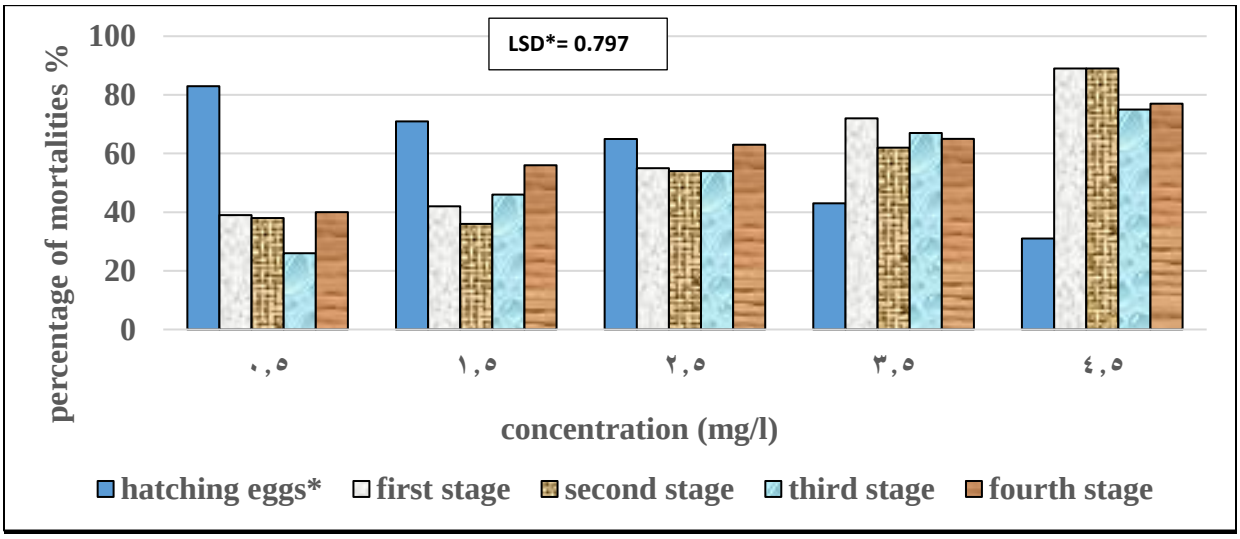

Figure (1) mortalities percentage of *C. pipiens* larva and percentage of hatching eggs after 24-48 hours exposure to different concentrations of extract.

In each larva stages there was no a significant differentiation among percentage of mortalities of each concentration, which means there is no varieties among stages of larva to response to the synergistic effects of extract, which could be referred to there is no relation among effective of extract compounds and larva activity in feeding, movement or respiratory, where the studies that mention in [23 and 24] discussed this situation as a reason of capability of compounds to exchange physical and chemical environment of the organism, which means in this case the higher or lower activity of larva have not effect on penetration of extract compounds to the larva body, and these changing in environment increased with increasing concentration that lead to have exchange on larva behavior, but this didn't deny that extract mixture has effective toxic materials that caused death to larva. Results (Figure 2, 3, 4 and 5) showed the LC_{50} were (1.8, 2, 2.3, 1.3) mg/l for first, second, third and fourth of larva stages, which could be referred that the effect of synergistic extracts could be effective and lethal even in low concentrations if it found in environment for any organisms. Some study such as studies that mention in [23 and 25] referred to less LC_{50} indicated to high effectivity of materials to kill organisms, also, it referred to find high correlation coefficients between extract concentrations and mortality percentage which

means high effective of compounds in parsley and *Funaria sp*. that can be caused mortality of mosquitoes' larva with increasing concentration.

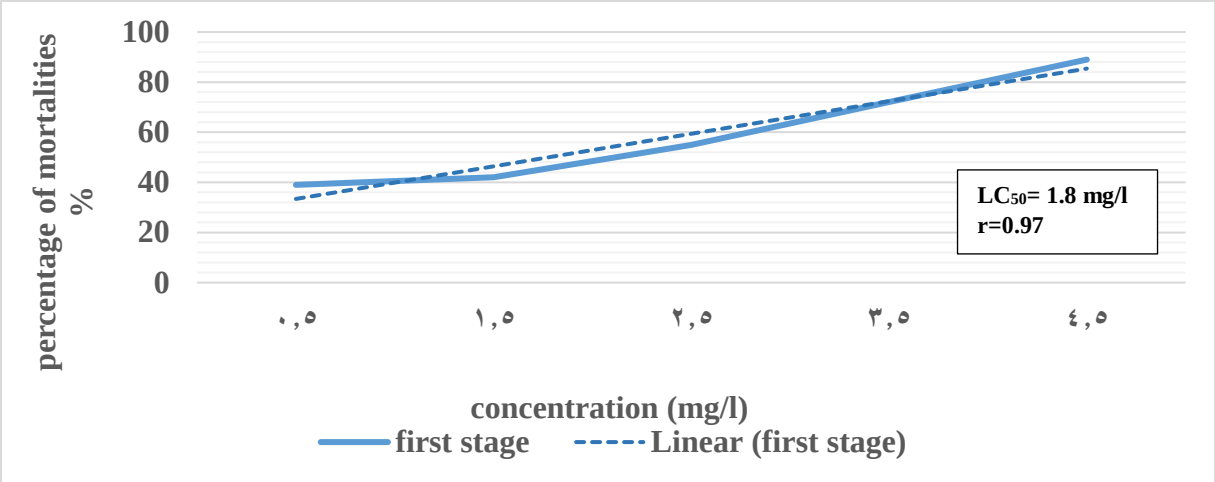

Figure (2) LC_{50} value of first stage of *C. pipiens* and linear mortalities after 24 hours from exposing to different concentration of extracts

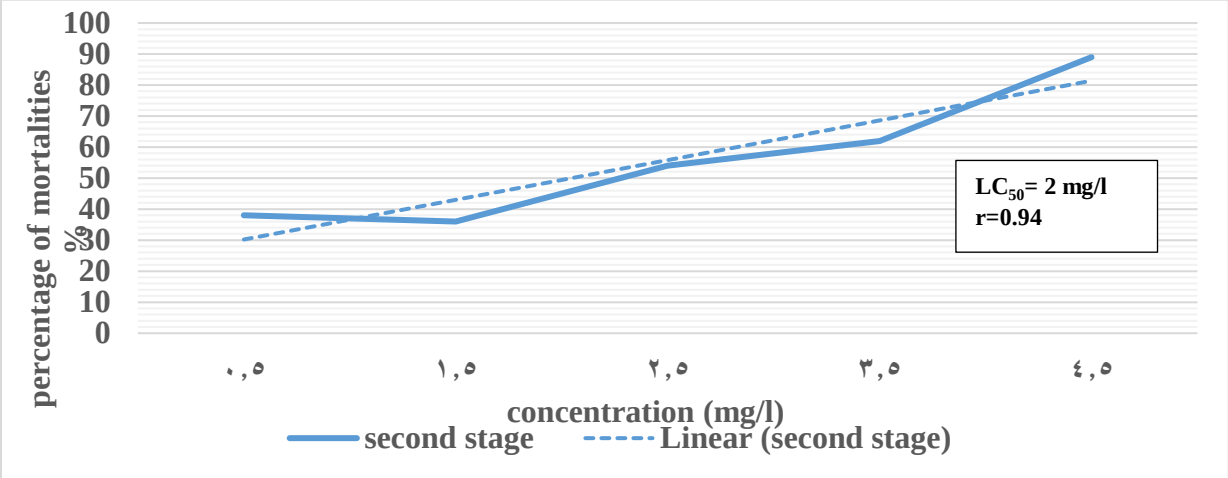

Figure (3) LC_{50} value of second stage of *C. pipiens* and linear mortalities after 24 hours from exposing to different concentration of extracts

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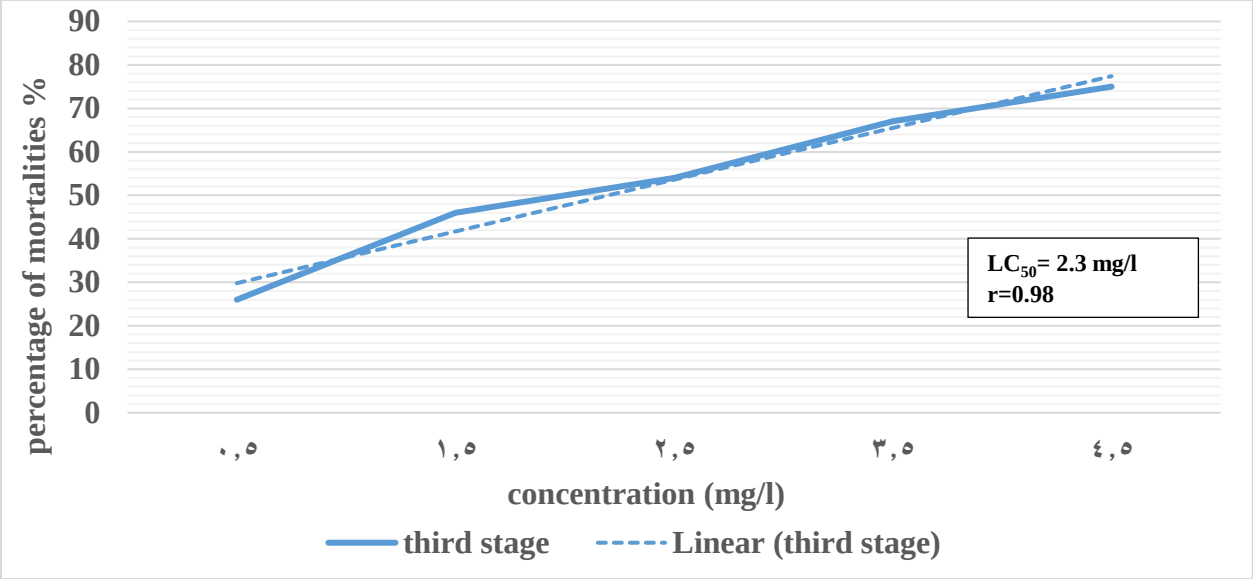

Figure (4) LC_{50} value of third stage of *C. pipiens* and linear mortalities after 24 hours from exposing to different concentration of extracts

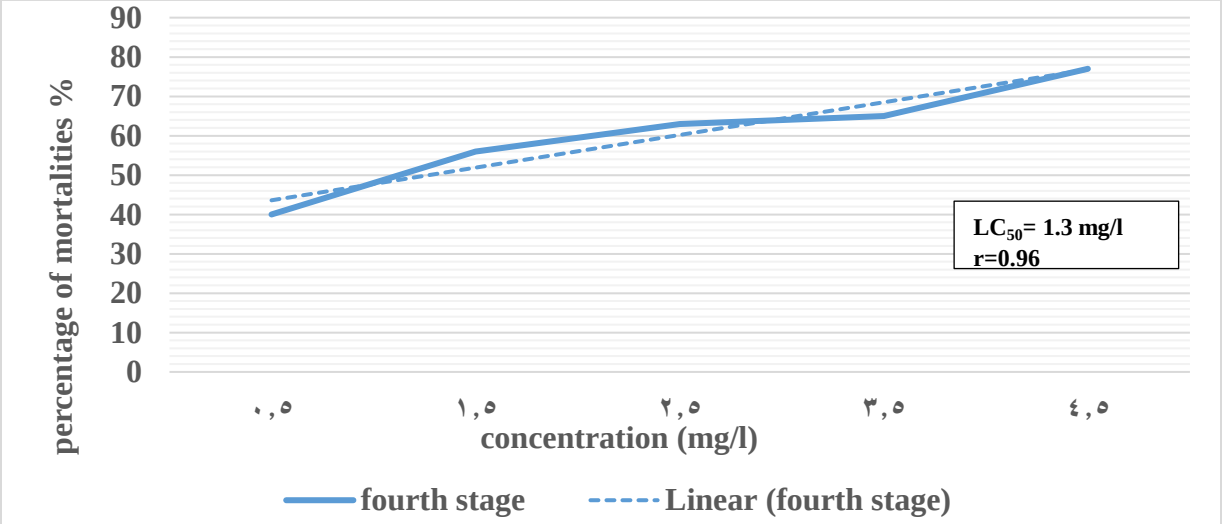

Figure (5) LC_{50} value of fourth stage of *C. pipiens* and linear mortalities after 24 hours from exposing to different concentration of extracts.

Figures (1 and 6) appeared the hatching eggs that treated with extracts, which the results showed the percentage of hatching eggs compared with control were (31, 43, 65, 71, 83)% for concentrations (4.5, 3.5, 2.5, 1.5, 0.5) mg/l respectively with LC_{50} (3.2) mg/l. Also, the results showed a significant differentiation among percentage of hatching eggs, which could be referred to find synergistic effect of extracts on eggs. According to the study that mention in [26] the mosquitoes' eggs can be affected by some chemicals because of their structure that caused penetrate some kind of materials. In the other hands the results pointed to find a negative correlation coefficient (r= -0.98), where the percentage of

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hatching eggs decreased with increasing concentration that may be indicated on effects of extracts components on eggs which un-hatching eggs increased with increasing concentration, the study of [27] mention to these state as caused of collecting chemical compounds on the shield of eggs that could be destroyed some weak sites that find on outer layer that covered eggs which is lead to loss air that kept between two layers [28], or changing in surface tension that didn't make egg rafts floating [29] and these state increasing with concentration of effective materials.

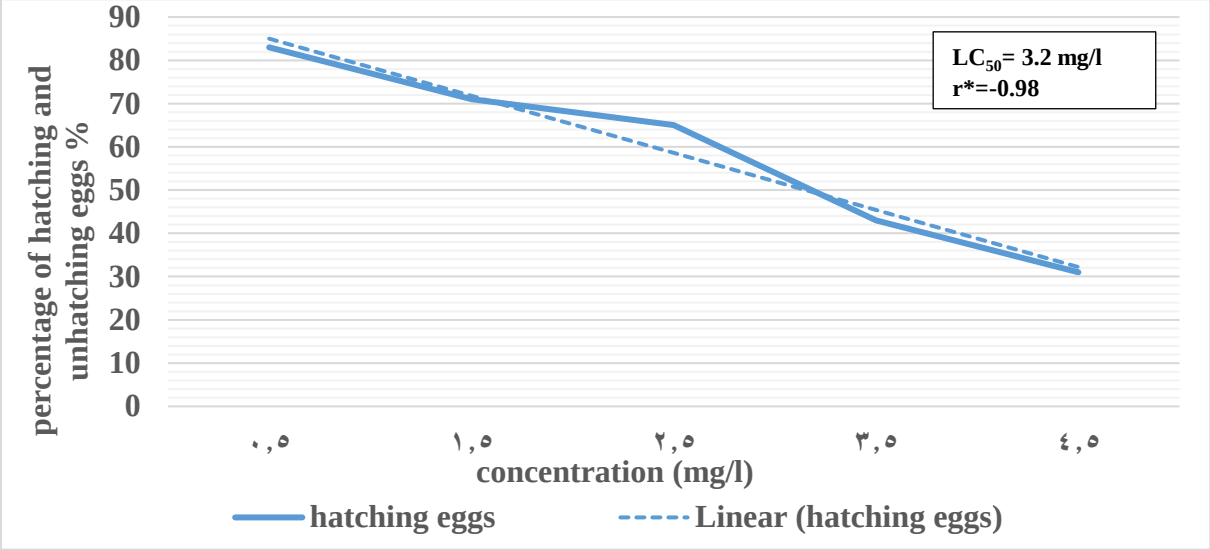

Figure (6) LC_{50} value of hatching eggs of *C. pipiens* after 48 hours from exposing to different concentration of extracts

Conclusions

1- The mixture extract has higher effects on egg and larva mosquitoes in low concentration.
2- The extract has been more affected on hatching eggs, where the hatching decreased with increasing concentration.
3- The extract had effect on eggs and larva phases in different concentration.

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Conflict of interest

There are no conflicts of interest.

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الخلاصة

مقدمة

تتواجد في الطبيعة أنواع عديدة من النباتات التي تلعب دور مهم في السيطرة على البعوض، لكن بعض هذه النباتات يكون تاثيرها اكبر عند خلطها مع نباتات أخرى لذلك هدفت هذه الدراسة الى معرفة تاثير هذه النباتات بعد خلطها مع بعضها على الاطوار اليرقية لبعوض *Culex pipiens* وعلى عملية فقس البيض.

المواد وطرق العمل

في هذا البحث تم اخذ خليط من المستخلص المائي نبات المعدنوس *Petroselinum crispum* والمستخلص المائي لجنس الحزازيات *Funaria sp.* بنسبة (1:1) لمعرفة تاثيره على دورة حياة بعوض *Culex pipiens* بعد التعرض الى تراكيز مختلفة من هذا المستخلص (4.5، 3.5، 2.5، 0.5,1.5) ملغم/ لتر.

النتائج

اظهرت النتائج ان النسبة المئوية لموت يرقات بعوض *C. pipiens* كانت (89 ، 72 ، 55 ،42 ، 39) % و (89، 62 ، 56 ، 36 ، 38) % و (75 ، 67 ، 54 ، 46 ، 26) % و (77 ، 65 ، 63 ، 56 ، 40) % وقيمة LC_{50} كانت (1.8 ، 2 ، 2.3 ، 1.3) ملغم / لتر لكل من الطور الاول والثاني والثالث والرابع على التوالي. بينما كانت النسبة المئويه لفقس البيض المعرضة للمستخلصات الخليطة (31 ، 43 ، 65 ، 71 ، 83) % وقيمة LC_{50} كانت (3.2) ملغم / لتر. كما اشارت نتائج المشاهدات الى ان بالغات البعوض غير المعرضة للمستخلصات الخليطة من قبل لها انجذاب قليل الى اماكن الغذاء المعاملة بالمستخلصات كلما زاد التركيز. اظهرت النتائج وجود فروقات معنوية بين النتائج عند مستوى احتمالية 0.05 ، ووجود معامل ارتباط ايجابي مع النسب المئوية لهلاك الاطوار اليرقية ومعامل ارتباط سلبي مع النسب المئوية لفقس البيض.

الاستنتاجات

يمكن للمستخلص المائي الناتج من خلط المستخلص المائي للمعدنوس والحزازيات ان يقتل الاطوار اليرقية بتراكيز قليلة وتزداد نسبة القتل كلما زاد التركيز وكذلك بالنسبة الى منعه لفقس البيض.

الكلمات المفتاحية: يرقات، LC_{50} ، حزازيات ، معدنوس ، مستخلص تعاضدي.