Effect of Alcoholic Extracts of *Mentha Piperita* Roots on *Culex Pipiens* Mosquitoes

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Abstract

The study aimed to know effects of alcohol extract of *Mentha piperita* plant roots on larva and hatching eggs of *Culex pipiens* mosquitoes after exposure to different concentrations (100, 300, 500, 700, 900) mg/l from extracts. The results appeared that percentage of larva mortalities was (37, 55, 63, 72, 91) %, (10, 24, 33, 42, 76) %, (16, 28, 41, 66, 67) % and (12, 18, 20, 23, 47) % for first, second, third and fourth phases and LC_{50} was (280), (680), (600) and (more than 900) mg/l respectively, and percentage of hatching eggs (81, 78, 76, 73, 60) % and LC_{50} (more than 900) mg/l. Also, results didn't show a significant differentiation between the percentage of larva mortalities stages but showed it among the percentage of hatching eggs in the level of probability (0.05). the concentration showed positive correlation coefficients with percentage of mortalities and negative correlation coefficient with the percentage of hatching eggs.

Introduction

Peppermint or *Mentha piperita L.* it's a herbal flowering plant belong to Lamiaceae family that widely distribution [1] which was used as food, preparing some type of tea and sweetness, flavor, medical treatment and pharmacy or as the fragrance. Also, its essential oils used as carminative, antiseptic, anti-inflammatory, myalgia, flatulence, headaches, antimicrobial, neuralgia and for the rest of the liver [2]. Most of the study referred to an essential oil that finds in it as active materials [3], and some study showed that plants as antimicrobials [4], other pointed to the plant as low antifungal [5], while a study of [6] referred to peppermint as insecticides.

This current study aimed to know the effect of alcoholic extracts of *M. piperita* plant roots on larva and hatching eggs of *Culex pipiens* mosquitoes, which most of the studies referred to this plant as insecticides [6]. Also find the effects of alcoholic extracts of root by using it and find LC_{50} on larva phases after 24 hours and on hatching eggs after 48 hours from exposing to different concentrations of the alcohol of root extract [7].

Materials and Methods

Preparation of Extracts

The methods of [8] and the way of [9] has been followed in preparing of alcoholic extracts of *M. piperita* plant roots by taken the fresh roots and washed well first with tap water then with distil-water, the roots left to dry in room temperature then crushed by electrical mill and sieved to remove solid parts. Return the powder again for grinding and sieved by using a piece of toll to gain smooth powder. The weight of 50 gm from smooth powder has mixed well with ethanolic alcohol 70 % by mixer for 10 minutes until appeared clear liquid that filtered with Millipore paper 0.45 um then with centrifuge (3000 r / min for 15 minutes). The leachate liquid has been left to dry in room temperature for many days, after that 1 gm of drying powder has mixed with distil-water to final volume 10 ml to has stock solution 0.1 % for preparing concentrations (100, 300, 500, 700, 900) per mg/l from it.

CALCULATION OF LC_{50}

To study the effects and find the toxicity of alcohol extract of mint roots on the life cycle of *C. pipiens* mosquitoes, the LC_{50} should be calculated after exposure to different concentrations (100, 300, 500, 700, 900) mg/l. individuals from 30 larva of *C. pipiens* has been located to containers 250 ml that have different concentrations with three replicates per concentration. The fourth phases of *C. pipiens* larva has been exposed to different concentrations of extracts for 24 hours, and hatching eggs for 48 hours, to study the acute effect on mosquitos' larva and eggs respectively. The LC_{50} (lethal concentration for median (half)) has been calculated by using the straight-line equation [Y= bx + a (a= intercept, b= slope)] [7] after corrected the data with Abbott equation [10].

Experimental Design and Statistical Analysis

Entirely random design was used. Data were analyzed statistically by using less significant differentiation (L.S.D) at 0.05 after subjection to the test of variance [11].

Results and Discussion

Figure (1) showed the percentage of mortalities of *C. pipiens* larva after 24 hours from exposing to different concentration of alcoholic extracts of *M. piperita* roots. The results appeared that percentage of mortalities was (1, 37, 55, 63, 72, 91) %, (0, 10, 24, 33, 42, 76) %, (0, 16, 28, 41, 66, 67) % and (0, 12, 18, 20, 23, 47) % for first, second, third and fourth phase of *C. pipiens* after exposure to concentration (control, 100, 300, 500, 700, 900) mg/l. These results may be returned to the presence of materials in alcohol root extracts that could be affected on mosquito's larva, which the study of [3], [12], [13] pointed to find compounds such as pulegone, terpenes, menthol, tannins, Flavonoids and menthone act as toxic compounds for some organisms, while in study of [14] which appeared that exposition to high concentration of plant extracts could be lead to defect nervous system with interrupted in feeding. Or it could be returned to the damage that happened to guts of larva specifically microvilli as a reason of feeding on alcohol root extract compounds [15]. Also, the study of [28] pointed to death some kind of insects because of some plant extracts have the ability for dissolving cuticle that formed the outer skeleton of insects specially uncomplete phases. The results didn't appear a significant differentiation among percentage of mortalities of *C. pipiens* larva phases but appeared variation in values of LC_{50} .

Results also pointed to find survival larva in different concentrations of alcohol root extracts which could be because of the individuals' differentiation among larva of *C. pipiens* mosquitoes [17], or there are some individuals of larva has ability to tolerant different concentration of wide range of different chemicals that could be toxic or block its growth [18]. Some study showed the capability of *C. pipiens* larva mosquitoes to adapt with aquatic environment conditions [19] and ability to become inactive for some time until return the appropriate conditions. Also, another study referred to hide or hanging larva on air bubbles that can be find in water as running away from harmful effects of chemicals [20], while many studies pointed to an individual differentiation among larva genetically is a main reason to response and adaptation to the changes that found in aquatic environment [9], [21].

The results showed in figure (2), (3), (4), (5) that the first phase was more effective by alcohol extracts than other phases and its mortalities increased with the increasing of concentrations, and, has LC_{50} (280) mg/l which is less than other phases, this could be returned to the vast feeding larva that increasing input the toxic material to the larva [9], or it returned to incomplete cuticle growth in exoskeleton which destroyed easy by high activity of compounds that extracted by alcohol [22]. Also, the fourth phase of larva (figure 5) was less effected by alcohol extracts with increasing concentration and has LC_{50} (more than 900) mg/l or proximately (1010) mg/l , that could be returned to stop larva from feeding to prepare to enter the stage of pupa [23]. Also, there is many changes that happened inside fourth phase of larva making it more resistant to the environment [8].

This study as shown in figure (6) pointed to the percentage of hatching eggs was (94, 81, 78, 76, 73, 60) % for concentration (control, 100, 300, 500, 700, 900) mg/l respectively with LC_{50} (more than 900) mg/l or proximately (1340) mg/l , means the alcohol extract has ability to effect on hatching eggs of *C. pipiens* mosquitoes after exposed to high concentration of extracts, that could be returned to some materials may be penetrated and blocked out the pores of respiration [24], or make the embryo suffered from dehydration [20], or as study of [25] could be reason for delay hatching until removed the effectors. In another hand the results appeared there were a significant differentiation (LSD=3.73) between percentage of mortalities of fourth phase which was (0, 12, 18, 20, 23, 47) % and un-hatching eggs of *C. pipiens* mosquitoes which was (6, 19, 22, 24, 27, 40) % for alcohol root plant extract concentrations (control, 100, 300, 500, 700, 900) mg/l, and the LC_{50} for hatching eggs was higher than phases of mosquitoes larva, which means the eggs more resistant and tolerant to the alcohol root extracts of *M. piperita* than even fourth face of larva as a reason of the structure of the egg shield [20].

The observed results showed that there are changing in colors of larva at different concentrations with higher movement that showed in 100-500 mg/l, also, showed disturbing motion to up and down the container than control, which could be returned to have extracts some kind of compounds that affected on nerve system [26] of larva, or the larva can't breathe [20], or some study referred to it as way to removed toxic materials from its body [27], but in another hand the larva was stable in higher concentrations with high activity than control, which could be returned to

react some compounds with effective nervous compounds in high concentration and prevent or inhibit it from over affecting on behavior of larva by inhibiting some kinds of enzymes [28].

Conclutions

1- The percentage of mortalities of *C. pipiens* larva phases mosquitoes increased with increasing concentration of alcohol extract of *M. piperita* plant roots
2- The percentage of hatching eggs of *C. pipiens* mosquitoes decreased with increasing concentration of alcohol extract of *M. piperita* plant roots
3- The first and third phase of larva more effective by alcohol extract than other phases
4- The fourth phase of larva was more resistant to extracts than another phases of mosquitoes
5- The eggs of *C. pipiens* mosquitoes more tolerant to alcohol root extract than larva phases

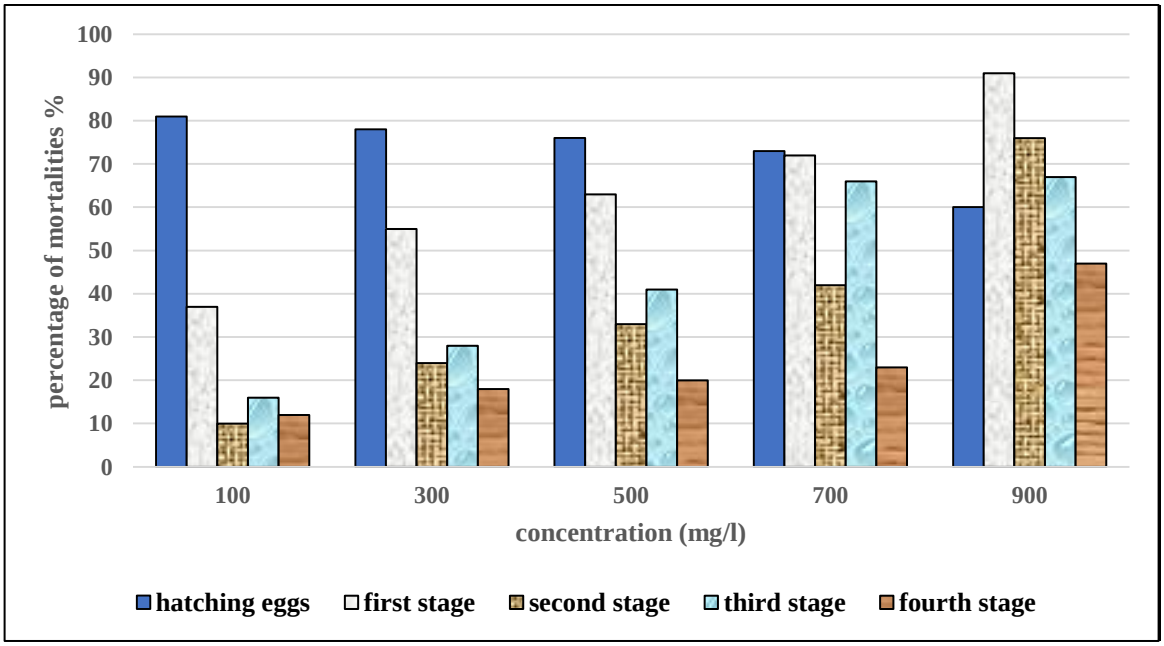

Figure (1) percentage of mortalities of *C. pipiens* larva and percentage of hatching eggs after 24 and 48 hours respectively from exposing to different concentration of *M. piperita* extracts (LSD= not found)

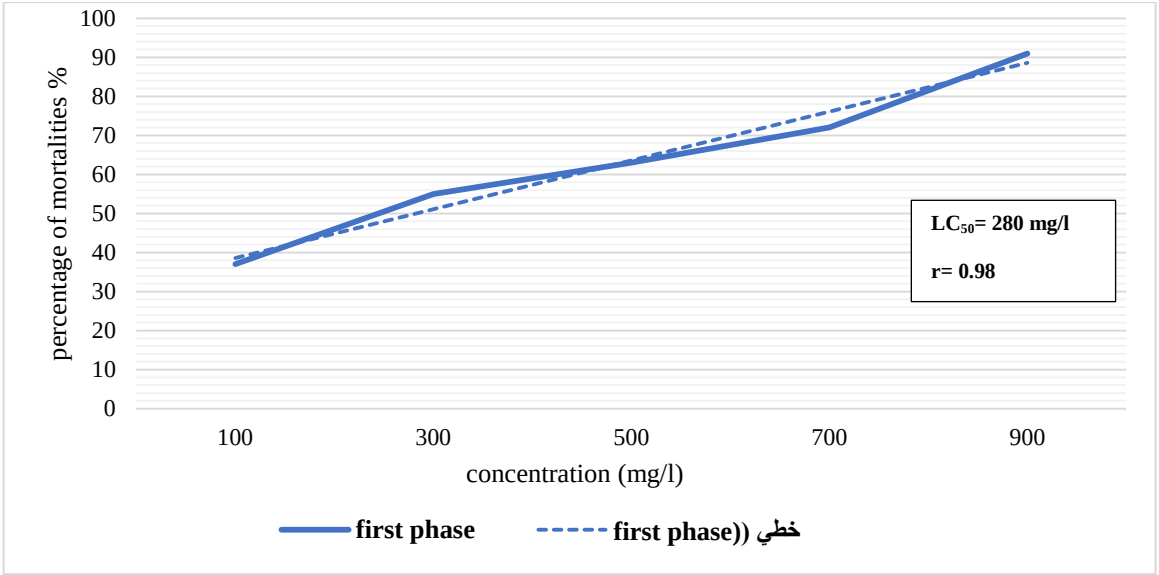

Figure (2) LC_{50} value for first phase of *C. pipiens* mosquitoes after 24 hours from exposing to different concentration on *M. piperita* alcohol root extracts.

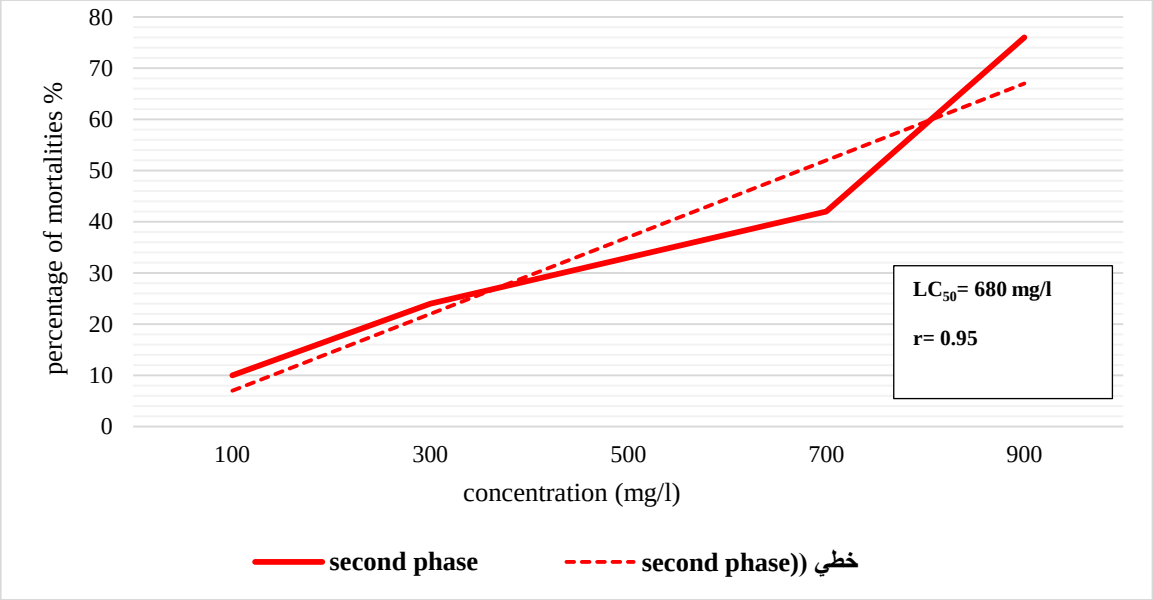

Figure (3) LC_{50} value for second phase of *C. pipiens* mosquitoes after 24 hours from exposing to different concentration on *M. piperita* alcohol root extracts.

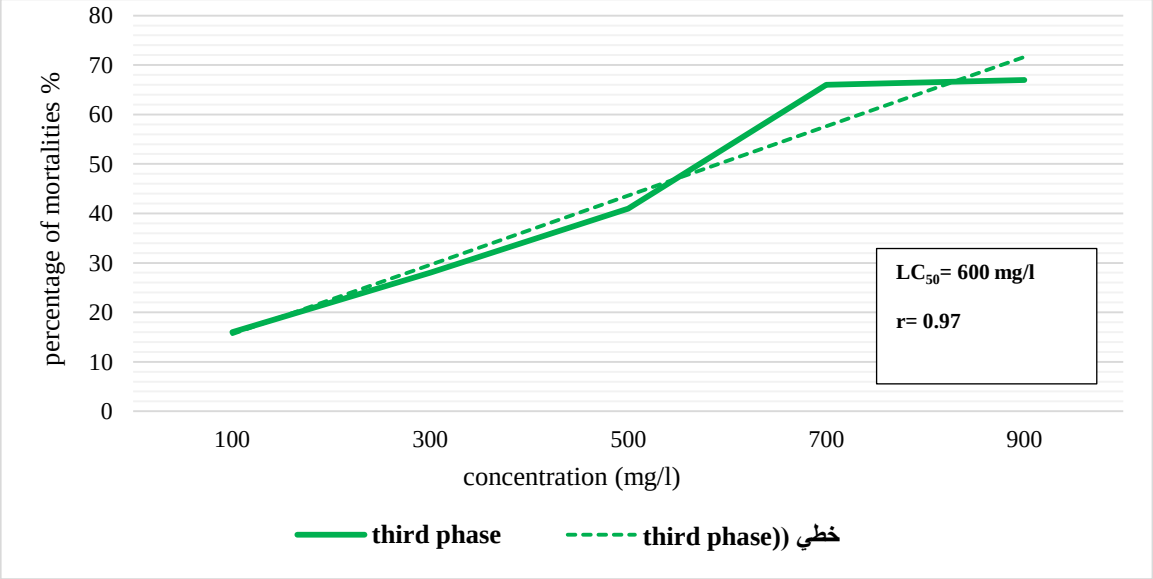

Figure (4) LC_{50} value for third phase of *C. pipiens* mosquitoes after 24 hours from exposing to different concentration on *M. piperita* alcohol root extracts.

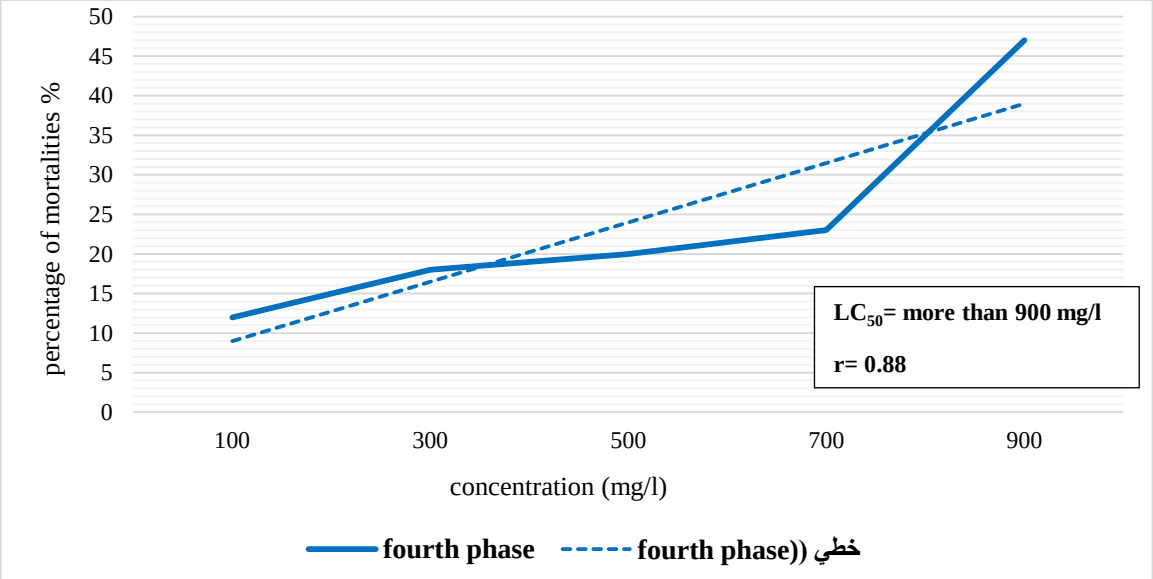

Figure (5) LC_{50} value for fourth phase of *C. pipiens* mosquitoes after 24 hours from exposing to different concentration on *M. piperita* alcohol root extracts.

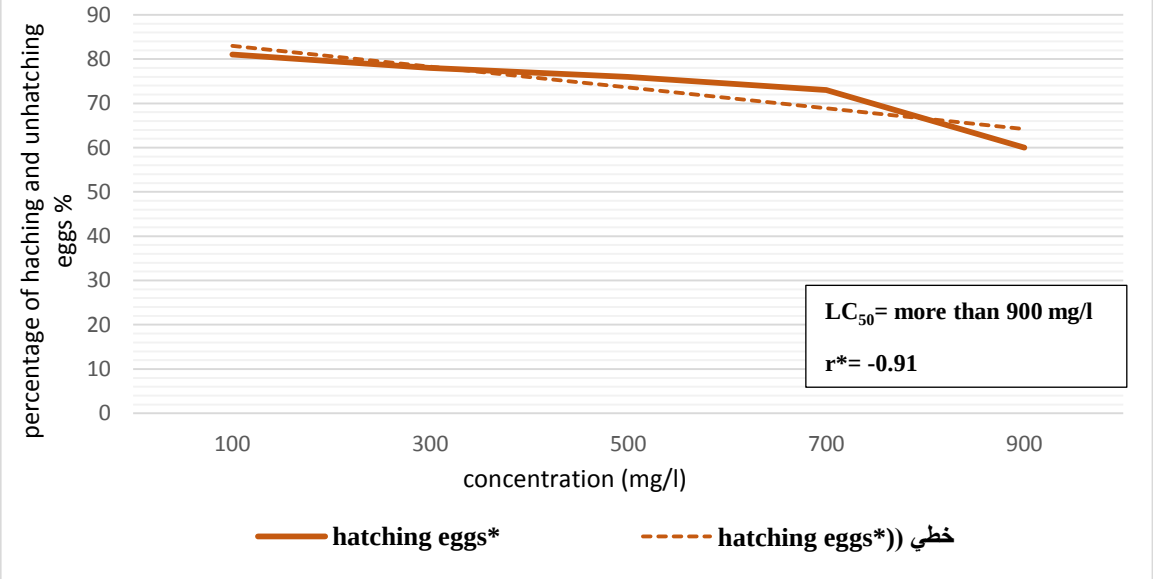

Figure (6) LC_{50} value for hatching eggs of *C. pipiens* mosquitoes after 48 hours from exposing to different concentration on *M. piperita* alcohol root extracts.

CONFLICT OF INTERESTS

There are no conflicts of interest.

References

[1] G. Iscan, N. Kirimer, M. Kurkcuoglu, K. H. C. Baser and F. Demirci, "Antimicrobial Screening of *Mentha piperita* Essential Oils". *J. Agric. Food Chem.,* 50, 3943-3946, 2002.

[2] A. Stojanova, P. Paraskevova, C. H. Anastassov, "A Comparative Investigation on the Essential Oil Composition of Two Bulgarian Cultivars of *Mentha piperita* L", *J. Essent. Oil Res.,* 12, 438-440, 2000.

[3] A. Aflatuni, K. Heikkinen, P. Tomperry, J. Jalonen, K. Laine, "Variation in the Extract Composition of Mints of Different Origin Cultivated in Finland", *J. Essent. Oil Res.,* 12, 462-466, 2000.

[4] C. Tassou, K. Koutsoumanis, G. J. E. Nychas, "Inhibition of *Salmonella enteritidis* and *Staphylococcus aureus* in Nutrient Broth by Mint Essential Oils". *Food Res. Int.,* 33, 273-280, 2000.

[5] Q. Delespaul, B. G. Billereck, C. G. Roques, G. Michel, C. M. Vinuales, J. M. Bessiere, "The Antifungal Activity of Essential Oils as Determined by Screening Methods", *J. Essent. Oil Res.,* 12, 256-266, 2000.

[6] V. Pavlidou, L. Karpouhtsis, G. Franzios, A. Zambetaki, Z. Scouras and P. Mavragani-Tsipidou, "Insecticidal and Genotoxic Effects of Essential Oils of Greek sage, *Salvia fruticosa*, and Mint, *Mentha pulegium*, on *Drosophila melanogaster* and Bactrocera oleae (Diptera: Tephritidae)", *J. Agric. Urban Entomol.,* Vol. 21, No. 1, 2004.

[7] S. Kitvatanachai, C. Apiwathnasorn, S. Leemingsawat, W. Wongwit and H. J. Overgaard, "Determination of lead toxicity in *Culex quinquefasciatus* mosquitoes in the laboratory", *Sou. eas. Asi. J. of Trop. Medi. Pu. Heal.,* 36 (4), 2005.

[8] A. Alouani, N. Rehimi and N. Soltani, "Larvicidal Activity of a Neem Tree Extract (Azadirachtin) Against Mosquito Larvae in the Republic of Algeria", *Jord. Jo. of Biologic. Scienc.,* 2 (1): 15 - 22, 2009.

[9] H. Matoug, B. Merabti, W. Tadjer, D. El Bah and M. L. Ouakid, "Biological Control Test of Ethanol Extracts of *Peganum Harmala* (L.) on The Mortality and Development of *Culex Pipiens* (Diptera)", *Worl. Jo. of Environme. Biosc.,* 6 (6): 15-19, 2017.

[10] W. S. Abbott, "A method of computing the effectiveness of an insecticide", *J. Econ. Entomol.,* 18(2): 265-7, 1925.

[11] J. Devore, N. Farnum and J. Doi, "Applied Statistics for Engineers and Scientists. Cengage Learning", third edition, San Luis Obispo, California Polytechnic State University, Printed in the United States of America, pp: 656, 2014.

[12] P. Gardiner, "Peppermint (*Mentha piperita*)", The Longwood Herbal Task Force, The Center for Holistic Pediatric Education and Research, PP:1-22, 2000.

[13] European Medicines Agency (EMA), "Assessment Report on *Mentha piperita* L., Folium", Evaluation of Medicines for Human Use, 7 Westferry Circus, Canary Wharf, London, E14 4HB, UK, 2008.

[14] O. Koul, S. Walia and G. S. Dhaliwal, "Essential Oils as Green Pesticides: Potential and Constraints", *Biopestic. Int.,* 4(1): 63–84, 2008.

[15] M. Kjanijou, K. Jiraungkoorskul, P. Kosai and W. Jiraungkoorskul, "Effect of *Murraya paniculata* Leaf Extract Against *Culex quinquefasciatus* Larva", *Asi. Jo. of Biolog. Scien.,* 5: 201-208, 2012.

[16] C. Wang, N. Singh and R. Cooper, "Efficacy of an Essential Oil-Based Pesticide for Controlling Bed Bug (*Cimex lectularius*) Infestations in Apartment Buildings", *Insects,* 5, 849-859, 2014.

[17] C. Berticat, G. Boquien, M. Raymond and C. Chevillion, "Insecticide resistance genes induce a mating competition cost in *Culex pipiens* mosquitoes", *Genet. Res., Camb.,* 79, pp. 41-47, 2002.

[18] F. EL-Akhal, S. Maniar, A. El Bachir, F. Chafiqa, A. Badoc and L. A. El Ouali, "Resistance of *Culex pipiens* (Diptera: Culicidae) to Organophosphate Insecticides in Centeral Morocco", *Internati. Jo. of Toxicolo. and Pharmaco. Re.,* 8 (4); 263-568, 2016.

[19] F. Cui, C. L. Qiao, B. C. Shen, M. Marquine, M. Weill and M. Raymond, "Genetic differentiation of *Culex pipiens* (Diptera: Culicidae) in China", *Bulle. of Entomo. Re.,* 97, 291-297, 2007.

[20] N. Becker, D. Petric, M. Zgomba, C. Boase, M. B. Madon, C. Dahl and A. Kaiser, "Mosquitoes and Their Control", Chapter 2: Biology of Mosquitoes, 9, DOI 10.1007/ 978-3-540-92874-4-2., ISBN: 978-3-540-92873-7, 2010.

[21] E. V. Shaikevich, E. B. Vinogradova, A. Bouattour and A. P. G. Almeida, "Genetic diversity of *Culex pipiens* mosquitoes in distinct populations from Europe: contribution of *Cx. quinquefasciatus* in Mediterranean populations", *Parasi. Vectr.,* 9: 47, 2016.

[22] N. Djeghader, H. Boudjelida A. Bouaziz and N. Soltani, "Biological effects of a benzoylphenylurea derivative (Novaluron) on larvae of *Culex pipiens* (Diptera: Culicidae)", *Advanc. in Applie. Scien. Re.,* 4 (4):449-456, 2013.

[23] N. Noori, B. G. Lockaby and L. Kalin, "Larval development of *Culex quinquefasciatus* in water with low to moderate pollution levels", *Jo. of Vecto. Ecolo.,* 40 (2): 208-220, 2015.

[24] T. Gajmer, R. Singh, R. K. Saini, S. B. Kalidhar, "Effect of methanolic extracts of neem (*Azadirachta indica* A. Juss) and bakain (*Melia azedarach* L.) seeds on oviposition and egg hatching of *Earias vittella* (Fab.) (Lepidoptera: Noctuidae)". *J. Appl. Entomol.,* 126, 238–243, 2002.

[25] D. Dris, F. Tine-Djebbar, H. Bouabida, and N. Soltani, "Chemical composition and activity of an *Ocimum basilicum* essential oil on *Culex pipiens* larvae: Toxicological, biometrical and biochemical aspects", *Sout. Afr. Jo. of Bota.,* 113: 362–369, 2017.

[26] B. Al-Anzi, V. Sapin, C. Waters, K. Zinn, R. J. Wyman, and S. Benzer, "Obesity-blocking neurons in *Drosophila*", *Neuron,* 63: 329-341, 2009.

[27] A. Rivero, A. Magaud, A. Nicot and J. Ve´ Zilier, "Energetic Cost of Insecticide Resistance in *Culex pipiens* Mosquitoes", *J. Med. Entomol.,* 48 (3): 694-700, 2011.

[28] Z. Wang, H. Perumalsamy, X. Wang, and Y. J. Ahn, "Toxicity and possible mechanisms of action of honokiol from *Magnolia denudata* seeds against four mosquito species", *Sci. Rep.,* 9: 411, 2019.

الخلاصة

تناولت هذه الدراسة معرفة تاثير المستخلص الكحولي لجذور نبات النعناع على يرقات وفقس بيض بعوض *C. pipiens* بعد التعرض الى تراكيز مختلفة من المستخلص (١٠٠, ٣٠٠, ٥٠٠, ٧٠٠, ٩٠٠) ملغم /لتر. اذ اظهرت النتائج ان النسبة المئوية لموت اليرقات كانت (٣٧ , ٥٥ , ٦٣ , ٧٢ , ٩١) % و (١٠ , ٢٤ , ٣٣ , ٤٢ , ٧٦) % و (١٦ , ٢٨ , ٤١ , ٦٦ , ٦٧) % و (١٢ , ١٨ , ٢٠ , ٢٣ , ٤٧) % لكل من الطور الاول والثاني والثالث والرابع وان قيمة LC_{50} كانت (٢٨٠) و (٦٨٠) و (٦٠٠) و (اكثر من ٩٠٠) ملغم/ لتر على التوالي, وان النسبة المئوية لفقس البيض (٨١ , ٧٨, ٧٦, ٧٣, ٦٠) % وقيمة LC_{50} كانت (اكثر من ٩٠٠) ملغم/ لتر. كذلك لم تظهر النتائج وجود فروق معنوية بين النسبة المئويه لهلاك الاطوار اليرقية لكنها اظهرت فروق معنوية بين النسبة المئوية لفقس البيض عند مستوى احتمالية ٠.٠٥ , كما انها اظهرت وجود معامل ارتباط ايجابي بين التركيز مع النسبة المئوية لهلاك اليرقات ومعامل ارتباط سلبي مع النسبة المئوية لفقس البيض.

الكلمات المفتاحية: فقس البيض, نعناع , LC_{50} , يرقة.