Eco. Env. & Cons. 26 (4) : 2020; pp. (1540-1543)
Copyright@ EM International
ISSN 0971–765X

The larvicidal activity of Ethanolic extracts of *Citrus maxima* peels, seeds against mosquito larvae in the laboratory

[1]Sawsan Fadhel Fawaz, [1]Sawsan Darweesh Jari, [2]Sienaa Muslim Al-Zurfi, [3]Rana Hassan Shatti and [3]Hasan Mousa Jaafar

[1]Department of Horticulture and Landscape Gardening, Faculty of Agriculture, University of Kerbala, Iraq
[2]Departments of Plant Protection, Faculty of Agriculture, University of Kerbala, Iraq
[3]Faculty of Agriculture, University of Kerbala, Iraq

(Received 10 April, 2020; accepted 20 May, 2020)

ABSTRACT

This research aimed to find out larvicidal activity of ethanolic extracts of the *Citrus maxima* peels; seeds were assessed in order to larvicidal activity against the mosquito larvae under the laboratory condition. Mosquito larvae were treated with different concentrations of the ethanolic extracts (10 mg/mL to 40 mg/mL. The larvae stages were more susceptible to the ethanolic extracts. Whereas, the extracts of *C. maxima* peel gave the mortality rate of 93.3 % twelve hours after the treatment at 40 mg/mL while the mortality rates were 40.0%, 60%, and 73.3 % at the lower concentration of 10 and 30 mg/mL respectively, 12h after treatment. This botanical larvicide could be used in place of the synthetic because of it is cheap, environmental-friendly, easily available biodegradable and safe.

Key words: *Mosquito, Ethanolic extracts, Citrus maxima*

Introduction

There are many diseases which spread by feeding mosquitoes to the blood, for example dengue hemorrhagic fever, Japanese encephalitis, dengue fever, malaria, and filariasis. These diseases growing in prevalence, mostly in the subtropical in addition tropical regions, and mosquitoes serve. For example, vector for several subtropical and tropical diseases cause negative effects to the human (Gutierrez, 2014). Vector simply transmit only pathogens and parasites; however, they cause allergic reaction which comprises systemic sensitivity and local skin for controlling many mosquitoes diseases, that have economic impacts and worldwide health. The based interventions on the synthetic pesticide are still essential in cases the epidemic outbreak with significant rises of mosquito adults (Suttanont *et al.,* 2009). Nevertheless, the use of indiscriminate is conventional pesticides and promoting multiple issues, such as the general development of pesticide resistance, toxic threats to the mammals, discarded effects to the environmental pollution, and on non-target organisms (Akram, 2010). Synthetic insecticides with higher quantities are common applied every year, this increases the risks to humans, many organisms, and more other environmental damage (Yang *et al.,* 2009).

In general, the plant oils are natural alternatives to insecticides (Gbolade *et al.,* 2000). In addition, the

plants normally have been found to contain several chemicals that are beneficial for controlling many insects (Robert, 2001).

Plant-derived natural products have been appealing as substitutes for using the synthetic insecticides in the integrated management programs (IMP), for reducing the health risk to the human and decrease the harmful residues to the environment. Additionally, pest resistance to the mosquitocidal plant agents have not been recorded as a several bioproducts origin. Particularly, important oils which have received substantial renewed attention is biologically active mixtures against different species of insects (Shaalan *et al.,* 2006). The resistance of mosquitoes to the synthetic insecticides, with high cost of compounds and their harmful effects on the environment has been a main challenge in vector control. Botanical pesticides can be used as alternatives to these synthetic insecticides because they are environmentally friendly, cheap, readily available, sage and biodegradable (Dehghani *et al*., 2012).

This study aimed to investigate the efficacy of the larvicidal extract of ethanol in citrus fruit on mosquito larvae. In order to control many vector diseases, especially in cases of the sensitivity of the vector to traditional industrial vehicles decreases.

Materials and Methods

Collecting the plants samples

The sample of *Citrus maxima* was obtained from the commercial market. Sample washed under tap water, and then rinsed by distilled water. Then, remove the thick cortex of the fruit by the knife. At room temperature, the *Citrus maxima* samples were air-dried for 48 h. Pulverized using an electric blender.

Extraction of *C. maxima* oil

The plant samples have been put in the container 250g. Then, samples were soaked in the methanol, at ratio (1:1) of 1 g of samples to 1 mL methanol. Samples then lifted 48h in order to stand and then filtered. Then it is concentrated in the rotary evaporator.

Phytochemical Analysis

Alcoholic plant extracts have been sent to the laboratory of the Chemistry department in Karbala University in order to phytochemical analysis and rotary evaporation. The plant sample was exposed to the rotational evaporation at the concentration of the sample until it was almost semi-solid (Harborne, 1998). Assessment of the main phytochemicals such as flavonoids, alkaloids, tannins, steroids, saponins, cyanogenic glycoside and anthraquinone was conducted. The concentrations of 100mg, 50mg, and 20mg, of each compound which were combined with the 100 mL ethanol in order to obtain the concentrations to have being applied.

Collection of Mosquito Larvae

Mosquito larvae were obtaining from still water bodies (Fig. 1), then placed into 500 mL of visible container containing 249 mL of dechlorinated water, with glucose feeding, and let to acclimatize. Each test concentration of one mL was putted in every single container, and then identified appropriately. The concentrations ranging (10, 50, and 100 mg/mL), with a control treatment which gave up to 249 mL of the dechlorinated water, and one mL of ethanol. Then, the concentrations were examined under the room temperature at 30˚C ± 2 °C. The mortality levels of larvae were observed 12h after the treatment, and the percentage mortality estimated as a result: total number of larvae and the number of dead larvae _ 100 / tested.

Identification of Mosquito Larvae

The mosquito larvae could be found from several aquatic insects as larvae a grouping of two characters. The thorax is wider, and they don't have a leg than the head or abdomen. Head, thorax and abdomen are the main body part of the mosquito larvae. Body regions act as basis finding for the larvae. The larvae stages were determined by using the compound microscope. In order to look at the specimen, water with larva was putting on the slide under the microscope. The study was focusing on the 3rd instar larvae of dengue-carrying mosquito. It can be distinguished the *Aedes aegypti* larvae from other larvae stages since it has a 3-branch hair tufts on each the air tube side and a single hair. While the hair tufts have 2 or more than 2 branches, the whole branches rise of the same socket. Additional, species have extra hairs, hair tufts and branches on the side of siphon or air tube (Hayes *et al.,* 1985; Bruce, 2005).

Results and Discussion

The results showed that Ethanolic extract of *C. maxima* was indicated larvicidal activity on the lar-

vae stages examined at different doses which ranged from 10- 40 mg/mL for cortex. Furthermore, the larvae dead were influenced by rising plant extracts concentrations. Table 1, showed that the per cent mortalities of the plant extract increased with increasing of the concentrations. So, these extract effects have great effects at high concentrations on mosquito larvae. No change was noticed in the control. The study showed that *C. maxima* have highest percent mortalities on mosquitoes with 93.3%, 73.3% and 60.0% mortality at 40 mg/mL, 30 mg/mL, 20 mg/mL respectively. The study agreed with the researchers who tested different plant extracts against mosquito larvae, the observations of (Akram *et al.*, 2010), who noticed that the lemon extracts and the rough lemon extracts were efficient and effective as larvicides at the lowest values of LC50 of 119.993 and 137.258 ppm respectively, 24h after the treatment. The higher concentration of plant extracts, the greater effects of larvicides. Also, the research of Mya *et al.*, 2015 was observed that the *Citrus hystrix* leaves ethanol extract concentrations at 2.4%, 2.1%, 1.8%, 1.5% and 1.2% caused the mortality of Aedes larvae in 24 h (99.5%, 85.5%, 62.5%, 26.5% and 2%) respectively. Nevertheless, 1.2% concentration proved negligible larvicidal effect. Also, indicated that the higher concentration of *C.hystrix* leave ethanol extract could be applied in order to eradication of *Aedes aegypti*.

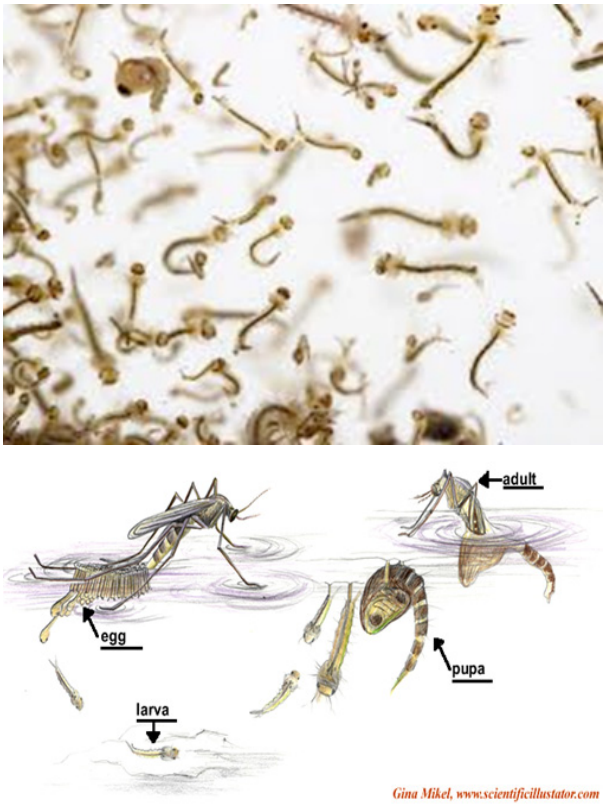

Fig. 1. Mosquito Life cycle.

In a study about seed extracts from remaining citrus varieties against Aedes. albopictus was the notice that seed extracts were less effective at low doses; nevertheless, the higher doses of seed extracts were biologically effective against *Aedes albopictus*.

The conclusion and recommendation

The conclusion

1. The larvicidal activity of the Citrus- maxima extract showed that the larvae are demonstrated by a high levels of mortality comparison with the control treatment.
2. Citrus- maxima extract is eco-friendly. This group can be applied for controlling mosquito larvae.
3. The plant derivatives are possible sources of some bio active agents for control many species of mosquito in the future.

The recommendation

We are recommending using this plant extract against another organism, because of its larvicidal activity against mosquito and it was economic, en-

Table 1. The per cent mortality of ethanol cortex extract of *Citrus maxima* on the larvae 12h after treatment.

Concentrations (mg/mL)	Total no. of the larvae treated	No. of the larvae dead	Death (%)
W3Control	30	0	0.0
10	30	12	40.0
20	30	18	60.0
30	30	22	73.3
40	30	28	93.3
L.S. D	8.3	7.3	10.6

vironment-friendly, easily available biodegradable and safe.

References

Akram, W., Khan, H.A., Hafeez, F., Bilal, H., Kim, Y.K. and Lee, J.J. 2010. Potential of citrus seed extracts against dengue fever mosquito, Aedes albopictus (Skuse)(Culicidae: Diptera). *Pak J Bot.* 42(4): 3343-3348.

Bruce, A. 2005. Field identification of adult and larval mosquitoes.

Dehghani, M., Ahmadi, K. and Zohdi, H. 2012. Evaluation of some plant extracts and conventional insecticides against *Trialeurodesva porariorum* (westwood) (homoptera: Aleyrodidae) in greenhouse condition. *Munis Entomol & Zool.* 7 : 828–836.

Gbolade, A.A., Dyedele, A.D., Sosan, M.B., Adewayin, F.B. and Soyela, O.I. 2000. Mosquito repellent activities of essential oils from two Nigerian Ocimum species. *J. Trop. Med. Plants.* 1 : 146-148.

Gutierrez, P.M., Antepuesto, A.N., Eugenio, B.A.L. and Santos, M.F.L. 2014. Larvicidal activity of selected plant extracts against the dengue vector Aedes aegypti mosquito. *Int Res J Biol Sci*. 3(4) : 23-32.

Harborne, 1998. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, London: Kluwer Academic.

Harborne, J.B. 1984. *Phytochemical methods. A guide to modern techniques of plant analysis*. Chapman and Hall, London, UK

Hayes, R.O., Maxwell, E.L., Mitchell, C.J. and Woodzick, T.L. 1985. Detection, identification, and classification of mosquito larval habitats using remote sensing scanners in earth-orbiting satellites. *Bulletin of the World Health Organization*. 63(2) : 361.

Mya, M.M., Aye, Y.Y., Oo, A.W. and Saxena, R.K. 2015. Effect of Citrus hystrix DC leaves ethanol extract on larvae of *Aedes aegypti. Journal of Biological Engineering Research and Review.* 2(2) : 1-6.

Shaalan, E.A.S., Canyon, D.V., Bowden, B., Younes, M.W.F., Abdet, W.H. and Mansoin, A.H. 2006. Efficacy of botanical extracts from *Callitris glaucophylla* against Aedes aegypti and *Cules annulirostris* mosquitoes. *Trop. Biomed.* 2 : 180-185.

Suttanont, N., Choochote, W., Tuetun, B., Junkum, A., Jitpakdi, A., Chaithong, V. and Riyong, D. and Pitasawat, B. 2009. Chemical composition and larvicidal activity of edible plant derived essential oils against the pyrethroid–susceptible and resistant strains of *Aedes Egypti* (Diptera: Culicidae) dep. Of para. chia ma. Univers.

Sutthanont, N., Choochote, W., Tuetun, B., Junkum, A., Jitpakdi, A., Chaithong, U., Riyong, D. and Pitasawat, B. 2010. Chemical composition and larvicidal activity of edible plant derived essential oils against the pyrethroid susceptible andresistant strains of *Aedes aegypti* (Diptera: Culicidae). *Journal of Vector Ecology.* 35(1) : 106-115.

Yang, T., L. Liang, F. Guiming, S. Zhong, G. Ding, R. Xu, G. Zhu, N. Shi, F. Fan and Liu, Q. 2009. Epidemiology and vector efficiency during a dengue fever outbreak in Cixi, Zhejiang province, China. *J. Vect. Ecol.* 34 (1): 148-154.