



Larvicidal efficacy of some natural products against *Culex pipiens* larvae

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Abstract:

Mosquitoes control, especially through the use of natural products, seems to be an urgent priority. In the current study, efficacy of some of these natural products such as plants extracts, organic acids, and plant oils, in monitoring to check their larvicidal effect, were evaluated by their application on 4th stage larvae of *Culex pipiens*. For plant methanol extracts; ginger, followed by garlic, showed the highest effect while onion showed the lowest efficacy. The lowest effect was found for peppermint in the oils used the highest for clove oil. Whereas, organic acids have a great effect on 4th stage larvae; citric acid has a higher effect at the concentration of 1.2 ml/100ml than acetic acid. Used materials are related to concentration and time. This research shows promising results in the use of natural products as larvicidal agents for mosquitoes, and further studies are required to examine others.

Keywords: *Culex pipiens*; Mosquitoes; Larvicidal; Ginger; Onion; Citric acid; Acetic acid; Clove oil

INTRODUCTION

According to the World Health Organization (WHO), mosquitoes are a serious threat to humans (**Ghosh et al., 2012**). Mosquitoes are responsible for significant health problems and economic losses because they are vectors of many pathogens including parasites like filarial worms, malaria protozoa, and viruses such as Dengue fever, West Nile fever (**Southgate, 1979; Hashem et al., 2017; Raveendran, 2017**).

Control of mosquitoes can be accomplished by several means. Biological control, by raising, in water channels, natural predators such as birds and fish. These predators are fed in

mosquitoes at the larval and pupal stages. Mechanical control, such as double-doors systems in front of buildings and traps for mosquitoes. Lastly, chemical control. The later approach is the most common method of controlling all stages of mosquitoes worldwide. Many chemicals have been deployed to reduce mosquitoes population (**WHO, 1982**). In mosquitoes control, chemical control is superior to the first two techniques. Nevertheless, the use of chemicals has many harmful acts such as, development of mosquitoes resistant strains, environmental toxic residues, and health hazardous (**Xue et al., 2006; Macoris et al., 2014**).

Therefore, studies have been done to use

natural products to combat mosquitoes. Plant extracts and their oils have been a strong target for this battle from ancient times until now (Ghosh et al., 2012; Chellappandian et al., 2018; Tisgratog et al., 2018). These natural products are important for reducing environmental pollutions and moreover, they are safer for all living organisms than chemicals. It is recommended to use natural products that do not harm animals, human beings and even the environment is recommended (Jacobson, 1975). Their safety comes from their natural degradation (Sun et al., 2006). Thus, Khater and Shalaby, 2008; Ali and El-Rabaa, 2010 studied using of natural products for mosquitoes control.

The aim of this study is to test some natural products against 4th stage larvae of *Culex pipiens* as larvicides.

MATERIALS AND METHODS

1. Study design

The study was performed at the latitude of 30° 35' 50.082 N and longitude 30° 59' 15.4752 E in Menofia governorate, Egypt.

2. Larvae collection

Larvae of mosquitoes were obtained by applying dipper containers (500 ml) in stagnant side channels. According to Thomas et al., 2017, the collected larvae were transported to the laboratory to conduct the experiment. *Culex pipiens* larva were identified (Gad, 1963; Harbach, 1985). Larvae in the fourth stage were selected and collected to assess the larvicidal activity of used materials in the experiment (WHO, 2005).

3. Tested materials

3.1. Plant extracts

The powdered plants (ginger, garlic and onion) were purchased from a local

market. Every plant's methanol extract was prepared according to Hossain et al., 2012. The working solution for each plant extract was prepared by weighting the powder and dissolving it. Until the required concentration of 25, 50 and 100 mg/ml has been achieved.

3.2. Natural oils extracts

In the experiment, commercial natural oils used were purchased from the market. Olive, clove, peppermint oils, all were manufactured by HEMANI, UAE. Tested oils were used at 20%, 40% and 80% (Khater et al., 2009) but herein acetone was used as an emulsifier.

3.3. Organic Acids extracts

Acids which used were acetic acid and citric acid. Acetic acid 96% manufactured by Algomhoryia, Egypt. Citric acid manufactured by Al-Nasr, Egypt. Each one was used at (0.4, 0.8 and 1.2 ml/100 ml accommodated water). Their concentrations were being graduated from low to high.

4. Experiment:

4.1. Experimental design

In addition to the negative control group, twenty 4th stage *C. pipiens* larvae were used for each trial of each concentration of each tested material. The negative control group consist of 20 larvae kept in accommodated water without any other material being included. On separate days, the experiment was conducted three times and replicated in five replicates.

Larvae were collected from their natural habitat (local water accumulations and ponds) and kept at room temperature in late summer in the laboratory and exposed to natural daylight. The observation took place with the naked eye, and the aid of a stereo-microscope

every 12 hours. Records were collected 3 days post application (dpa) (WHO, 2005; Thomas et al., 2017).

Mortality signs: *C. pipiens* larvae in the incubated containers that are diagnosed as dead display no active movement (up and down) and with no reaction, even with shaking or needle touching.

Mortality percent: Counting and recording all dead 4th stage larvae of *C. pipiens* in all experimental trials according to formula of (Abbott, 1925; Thomas et al., 2017).

$$\text{Mortality \%} = \frac{X-Y}{X} \times 100$$

X= the number of living larvae in the control group.

Y= the number of living larvae in the treated group.

X-Y= the number of larvae killed by the treatments used.

5. Statistical analysis

The findings obtained were statistically evaluated by using mean, standard deviation (SD), standard error of the mean (SEM), and one-way analysis of variance (ANOVA) test, followed by Dunnett's test, and a *p* value of 0.05 and 0.01 which was considered to be significant by using the SPSS software (SPSS, 2007). Significance of the differences between tested and control groups was determined for every tested product. A *p* value of ≤ 0.05 and ≤ 0.01 was considered statistically significant.

RESULTS

The most effective concentration when use ginger extract were 50 and 100 mg, with active moved larvae at 3rd dpa (7.66 ± 0.211) and (7.33 ± 0.211) with 50 and 100 mg concentration, respectively. Onion extract at 100 mg at 3rd dpa ($9.5 \pm$

0.224) showed close readings to garlic extract. The control group displaying active motion ranged from (96 ± 0) to (98 ± 0) in all of extracts (Table 1). When used at high concentrations ginger extract shows a higher larvicidal effect at 3rd dpa. The plant extracts were concentration-dependent as mosquitoes larvicides.

Where, with the use of plant oils, a reduction in the movement of active larvae is observed in olive oil, at 2nd dpa. Clove oil on 3rd dpa gave more effective readings as active larvae at 80% concentration were (2.66 ± 0.211). Peppermint oil is concentration related, showing low actively moving larvae (9.5 ± 0.224) at higher concentration (80 %) at 2nd dpa and decreased to approximately half (4.66 ± 0.211) at 3rd dpa (Table 2).

However, the use of acids shows a dramatic decrease in motile active larvae. Both citric and acetic acids, when used in their two high concentrations showing similar results (6.6 ± 0.211) & (4.8 ± 0.167) of citric acid and (6.6 ± 0.211) & (4.6 ± 0.211) of acetic acid in concentration of (0.8 & 1.2) respectively at 2nd dpa. While at the 3rd dpa citric acid results showed a more successful effect, the number of active larvae were much more lower (being 1 ± 0 or 00 ± 00) (Table 3).

DISCUSSION

In combating several pathogens transmitted by these vectors, control of insects, especially mosquitoes, is a necessity. The use of safe and environmentally friendly natural products is a trend to reduce environmental contamination and resolve the problem of mosquitoes strains that are resistant to chemicals (Zayed et al., 2006; Macoris et al., 2014). *Culex pipiens* is distributed in Egypt (Mostafa et al., 2002; Morsy et

al., 2004). The larvae of mosquitoes are good target to be destroyed because of their immobility and concentration in one location (Ali and El-Rabaa, 2010).

In the current study, ginger powder showed a greater larvicidal effect at 3rd dpa when used by high concentrations. Plant extracts (i.e. ginger, garlic and onion) as larvicidal agents were concentration-dependent. Our findings are in line with other studies; several studies have been applied on garlic, onion and ginger to detect the medicinal role on various arthropods; especially mosquitoes (Iqbal et al., 2018). The effectiveness of these plants extracts (i.e. ginger, garlic, onion) may result from their high content of sulfur and lectins that interact with protein receptors in the gut of insects thus hinder normal absorption, in addition to interfering with the larval respiration process due to deprivation of oxygen leading to death of the larvae (Upadhyay and Singh, 2012). Bilal et al., 2017 estimated the larvicidal activity of *Citrus sinensis*, mint (*Mentha spicata*) and ginger rhizome (*Zingiber officinale*) against *Aedes (Stegomyia) albopictus* in Pakistan. *Alium sativum* ethanolic extract destroys larvae of *Culex quinquefasciatus* (Kalu et al., 2010). Although the garlic aqueous extract was toxic for eggs and larvae of *Aedes aegypti* (Rim and Jee, 2006). As garlic contains lectins (Junnilla et al., 2015) used the microencapsulated garlic oil with an attractive toxic sugar baits (ATSB) resulting in an effective pesticide against *Aedes albopictus*. The effectiveness of onion may be attributed to the same process of garlic. Onion and garlic, also as acaricidal agents, have activity on different stages of *Boophilus annulatus* stages with high concentrations (Aboelhadid et al., 2013). A point should

be noted, the variation between different studies in the form of the extract (e.g. water-extract, ethanol extract, methanol extract) may result in variation in results.

As mosquitoes replants and larvicidal, plant oils can be used alone or as in combination with others (Moraes et al., 2009; Soonwera and Phasomkusolsil, 2015; Prabakaran et al., 2017). Soonwera and Phasomkusolsil, 2016 found that both *Cymbopogon citratus* (lemongrass) and *Syzygium aromaticum* (clove) oils have significant larvicidal effects on the larvae of *Aedes aegypti* and *Anopheles dirus*. Sanei-Dehkordi et al., 2016 reported the natural larvicidal effect of the limonene of peel essential oil of *Citrus aurantium* against *Anopheles stephensi* in Iran. Whereas the microencapsulated oils of *Alpinia galanga*, *Citrus grandis* and *C. aurantifolia* used by Misni et al., 2017 as lotion formulations to prolong their repellent effect against *Culex quinquefasciatus* mosquitoes in Malaysia. Kumar et al., 2011 proved that the essential oil of peppermint is effective larvicidal and has a repellent action against *Aedes aegypti*. Clove oil showed a larvicidal effect on *Aedes gambiae* larvae, and its effect was concentration dependent, its chemical composition was mainly Eugenol (Thomas et al., 2017). The larvicidal effect of plant oils may be attributed to the dissemination of these substances (such as Eugenol), which resulted in some toxic derivatives that have worse action on larvae (Upadhyay and Singh, 2012; Araujo et al., 2016) and these substances and derivatives may damage the cuticle of the larvae, and/or might be due to alteration with the respiration process of the larvae by changing the surface tension at the respiratory surface of the larvae.

The used acids demonstrated greater larvicidal efficacy compared with plant extracts. The higher effect was observed by using acetic acid in our results. Using of acids as larvicidal agents for mosquitoes have been used and described before (**Adhikari et al., 2012**). Acetic acid is one of the simplest organic acids. Here, "organic" means a compound which has carbon molecules. It is a naturally occurring material present in limited quantities in all plants. Vinegar indicates concentrations of up to 8%, when the concentration is high enough to be considered acetic acid, and it is a pesticide and herbicide used to kill weeds (**Daniel and Miller, 2015**). Organic glacial acetic acid has demonstrated efficacy against *Culex* and *Anopheles* larva (**Adhikari et al., 2012**), which agrees with our results. Citric acid is another organic acid. Citrus plants derivatives may be used alone or in combination with other acids and emulsifiers as natural mosquitoes replants (**Xue et al., 2006; Bilal et al., 2012**). The benefits of using organic acids as mosquitoes larvicidals; their availability, can be used in field conditions, and safety for the environment. The mode of action of organic acids as mosquitoes larvicidal may be presumed to have a toxic effect on the larva outer cuticle. Which results in larvae loss most of their self-defenses. So, the continuous growth is altered. At this point, the life cycle ceased.

The current research can be considered as a preliminary study to assess the effectiveness of the used natural products as larvicides of the mosquitoes larvae. In order to confirm the results herein and evaluate the active principles and their lethal concentrations (e.g. LC50 and LC90), toxicological and pharmacological studies should be performed.

In conclusion as larvicidal agents; the most effective product used in the current study was citric acid followed by the clove and olive oils. Then after; acetic acid, followed by peppermint oil. The powdered plant materials (ginger, onion) showed the lowest efficacy than recorded by others (acids and oils). These products as larvicides showed concentration and time relationship. Mosquitoes have both public and veterinary importance. Using of natural and safe products to combat insects is a promising method to control such threads.

Conflict of interests/Competing interests None.

Ethical considerations

In accordance with WHO guides for larval bioassays. No animals and humans were involved.

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Table (1): Efficacy of different powdered plant extracts used against *Culex pipiens* 4th stage larvae

Extract (mg extract/ml)	1st dpa	2nd dpa	3rd dpa
Ginger 25	50.3 ± 0.211*	14.66 ± 0.211	8.5 ± 0.224*
Ginger 50	40.33 ± 0.211*	11.66 ± 0.211	7.66 ± 0.211*
Ginger 100	34.66 ± 0.211*	9.33 ± 0.33**	7.33 ± 0.211*
Control	99 ± 0	98.5 ± 0.2	96 ± 0
Garlic 25	49.8 ± 0.167	19.6 ± 0.211*	13.8 ± 0.4**
Garlic 50	39.6 ± 0.211*	17 ± 0	12.8 ± 0.167
Garlic 100	34.8 ± 0.167	14.6 ± 0.211*	8.6 ± 0.211*
Control	99.6 ± 0.21	99 ± 0	97.6 ± 0.2
Onion 25	49.5 ± 0.22**	29.6 ± 0.211*	14.3 ± 0.211*
Onion 50	40 ± 0	24.8 ± 0.167	11.6 ± 0.211*
Onion 100	29.8 ± 0.167*	24.6 ± 0.211*	9.5 ± 0.224*
Control	99.8 ± 0.1	98 ± 0	98 ± 0

Data represent the active moving larvae (as the mean ± SEM of the different trials)

*P≤0.05, significant to control group; **P≤0.01, significant compared with control negative group (ANOVA followed by Dunnett's test).

dpa= day post application

Table (2): Efficacy of different oils of plants used against *Culex pipiens* 4th stage larvae

Oils %	1st dpa	2nd dpa	3rd dpa
Olive oil 20%	96.6 ± 0.211	7.5 ± 0.224*	5.5 ± 0.34*
Olive oil 40%	95 ± 0.25*	4.5 ± 0.224*	3.5 ± 0.224*
Olive oil 80%	89.6 ± 0.422**	3.5 ± 0.224*	2.8 ± 0.167
Control	99.5 ± 0.2	99 ± 0.0	98.5 ± 0.22
Clove oil 20%	95.5 ± 0.22*	11.83 ± 0.167	5 ± 0.0
Clove oil 40%	95 ± 0.258*	8.83 ± 0.167	4 ± 0.25*
Clove oil 80%	91 ± 0.365**	6.6 ± 0.22*	2.66 ± 0.211
Control	98 ± 0.0	97 ± 0.0	97.5 ± 0.22
Peppermint 20%	94.6 ± 0.211*	14.16 ± 0.4**	6.5 ± 0.224*
Peppermint 40%	93.3 ± 0.211*	11.6 ± 0.211*	6.16 ± 0.167
Peppermint 80%	90.5 ± 0.34**	9.5 ± 0.224*	4.66 ± 0.211*
Control	98 ± 0	97.8 ± 0.1	97 ± 0

Table (3): Efficacy of different organic acids used against *Culex pipiens* 4th stage larvae

Acids (ml acid/ 100 ml water)	1st dpa	2nd dpa	3rd dpa
Citric acid (0.4)	44±0.167*	9.8± 0.167	2.8±0.167
Citric acid (0.8)	39.8±0.167*	6.66± 0.211*	1 ± 0.0
Citric acid (1.2)	29.5±0.34**	4.8 ± 0.167	0 ± 0.0
Control	98±0	98±0.1	96±0.21
Acetic acid (0.4)	85.3± 0.21*	7.3± 0.211*	6.1± 0.167*
Acetic acid (0.8)	79.8± 0.167	6.6± 0.211*	5.6± 0.211*
Acetic acid (1.2)	69.5± 0.224*	4.6± 0.211*	4.16± 0.33**
Control	99±0.0	98.5± 0.2	98 ± 0.0

الملخص العربي

فاعلية بعض المنتجات الطبيعية ضد يرقات البعوضة المنزلية الشائعة

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مكافحة البعوض، خاصة بإستخدام المنتجات الطبيعية، أصبحت ذات أهمية كبيرة. في الدراسة الحالية تم تقييم فعالية بعض هذه المنتجات الطبيعية مثل المستخلصات النباتية والأحماض العضوية والزيوت النباتية من خلال تطبيقها على يرقات المرحلة الرابعة من البعوضة المنزلية الشائعة للكشف عن تأثيرها على تلك اليرقات. بالنسبة للمستخلصات النباتية أظهر الزنجبيل أعلى تأثير يليه الثوم وأظهر البصل أقل فعالية. أما الأحماض العضوية. اما بالنسبة للزيوت النباتية فكان أقل تأثير في الزيوت المستخدمة للنعناع وأعلى تأثير كان لزيت القرنفل. أظهرت الأحماض العضوية فاعلية كبيرة على يرقات المرحلة الرابعة و كان لحمض الستريك (الليمون) تأثير أعلى بتركيز 1.2 مل/ 100 مل من حمض الأسيتيك (الخل). المواد المستخدمة كانت تأثيرها مرتبط مع التركيز المستخدم و زمن التعرض لها. تُظهر هذه الدراسة نتائج واعدة لاستخدام المنتجات الطبيعية كعوامل مبيدة ليرقات البعوض، وهناك حاجة إلى مزيد من الدراسات للتحقيق في المنتجات الطبيعية المستخدمة و مثيلاتها.