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Dual actions of certain essential oils in combating mosquito *Culex pipiens* (Diptera: Culicidae)

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Abstract: Mosquitoes are one of the most annoying and potentially dangerous insects on the planet as they are infamous for transmitting diseases such as malaria, dengue fever, Zika virus, West Nile virus, and many others. In order to protect public health and stop the spread of diseases like dengue, zika, and malaria, mosquito control is essential. The current study attempts to evaluate the toxicity and repellant qualities of lavender camphor, and garlic, essential oils against *Culex pipiens*. After 72 hours, the essential oil demonstrated good larvicidal action in a lab setting against third-instar larvae (LC50 = 341.12, 430.79, and 624.15 ppm, respectively). At all dosages, lavender oil offers a comprehensive repellant that endures longer. In contrast, there were no appreciable variations in the repellents' duration of protection against *C. pipiens*. These findings provide insight into potential use of essential oils of garlic, camphor, and lavender for the management of mosquitoes.

Key words: *Culex pipiens*; Larvicidal; Toxicity; Botanicals; Repellent activity.

1. Introduction

Insect bites and the diseases mosquitoes transmit can be avoided by the use of repellents, clothes, netting, and other personal protection gear. In particular, when applied correctly, insect repellents are both safe and effective. By keeping mosquitoes away, repellent lowers the likelihood of getting bitten. Using repellant provides people with an additional layer of protection against diseases spread by mosquitoes. It lessens the spread of illnesses carried by mosquitoes throughout the community (**Gul *et al*., 2013**). The most well-researched insect repellent now on the market for the general public is N, N-diethyl-m-toluamide, or DEET, which appears to be the most effective. Instead of killing insects, DEET is meant to be applied topically to human skin in order to repel them. DEET is currently available in formulations with doses ranging from 5% to 100% in aerosols, pump sprays, lotions, creams, liquids, sticks, roll-ons, and impregnated towelettes. Nonetheless, there are worries regarding DEET's potentially harmful effects, particularly when youngsters use it. According to **Clem *et al*. (1993)**, children who use insect repellents with high levels of DEET have had bradycardia, hypotension, slurred speech, and convulsions. Chemical-based repellents for mosquitoes have an amazing safety record, but they are toxic to the skin and nervous system, causing rashes, swelling, eye irritation, and more serious issues, such as anaphylactic shock, low blood pressure, and unusual brain swelling in children (**Shasany *et al*., 2000; Phal *et al*., 2012**). In order to get over these issues, alternate vector control strategies must be looked for. The quest for more ecologically friendly vector control techniques has been sparked by the ineffectiveness of chemical pesticides un managing the insects and the public's growing concerns about safe food and a healthy environment (**Amerasan *et al*., 2012**). Since ancient times, essential oils have been the active ingredient in the majority of significant herbal treatments. Plant-based insecticides have been widely applied to agricultural pests and, to a much lesser degree, to insects that pose a significant risk to public health and should be carefully and thoroughly screened. Plant extracts are generally more biodegradable, less dangerous, and a rich storehouse of compounds with a variety of biological activities, making them an attractive option for pest control (**Nath *et al*., 2006**).

In Egypt, several essential herbs and their by-oils act as natural insect repellents that express a high degree of repellency and toxicity against different insects (Hashem *et al*., 2018; Hashem *et al*., 2020; El-Kady *et al*., 2021; Hashem and Ramadan, 2021; Awadalla *et al*., 2023). The present investigation aims to assess the repellent and toxicity properties of the essential oils of lavender, camphor and garlic against *Culex pipiens* as medically important mosquitoes to improve its acceptability and preclude any adverse effect that could emanate from the use of synthetic bases.

2. Materials and methods

Mosquito Colony: The mosquito used in this study was *Culex pipiens* L., which was provided by the Medical Entomology institute. It was reared for several generations in the Medical Entomology Insectary, Animal house, Faculty of Science, Al-Azhar University, under controlled conditions at a temperature of 27±2°C, relative humidity 70±10% and 12-12 light-dark regimen. Adult mosquitoes were kept in wooden cages and daily provided with sponge pieces soaked in 10% sucrose solution for a period of 3-4 days post-emergence. After this period, females were allowed to take a blood meal from a pigeon host which is necessary for laying eggs **(Kasap and Demirhan, 1992).**

Experimental bioassay

Larvicidal activity of oils

For larvicidal activity, the tested oils were dissolved in 2 drops of $Tween_{80}$ as emulsifier to facilitate the dissolving of tested material in water. Different range of concentrations of each concerned extract was prepared in order to detect mortalities. All tested materials were performed in 250ml of dechlorinated tap water contained in 400ml plastic cups. Then, third 3^{rd} instar larvae (25 larvae) were put immediately into plastic cups contained different concentrations of extracts. Three replicates were usually used for each tested concentration. All plastic cups were incubated under controlled conditions of mosquito colony and subsequently mortality was recorded. Control larvae received 0.1 ml of methanol or 2 drops of Tween80 in 250ml water. Mortality was recorded daily and dead larvae and pupae removed until adult emergence. All values calculated as Mean±SD. The larvae were observed daily until pupation and adult emergence to estimate the following parameters.

Larval mortality was indicated by a failure to respond to mechanical stimulation (Williams *et al*, 1986). Larval mortality percent was estimated by using the following equation (Briggs 1960):

$$\text{Larval mortality } \% = A - B / A \times 100$$

Where: A = number of tested larvae; B = number of tested pupa.

Repellent effects

Standard cages (30×30×30 cm) were used to test the repellent activity of the selected plant extracts. Prepare different concentrations. The concentration was directly applied onto 5×6 cm of ventral surface of pigeon after feathers removal from the abdomen to evaluate the repellency against *C. pipiens* compared with control (Johnson Wax Egypt) as a negative control. After 10 minutes, the treated pigeons were placed in the cages containing 50 *C. pipiens* starved females (5-7d-old) for three hours. Control tests were carried out alongside with the treatments using solvents. Each test was repeated three times to get a mean value of repellent activity.

After treatments, the number of unfed females were counted and calculated according to Abbott (1925):

$$\text{Repellency \%} = [\% A - \% B / 100 - \% B] \times 100$$

Where: A = percent of unfed females in treatment; B = percent of unfed females in control.

Statistical analysis

The data were analyzed using the sofware SPSS V23 (IBM, USA) to perform Probit analyses to compute lethal concentration (LC) values and the one-way analysis of variance (ANOVA) (Post Hoc/Turkey's HSD test). The significance threshold was established at $P < 0.05$.

3. Results

All the essential oils exhibited larvicidal activity against the mosquito larvae. The mortality of the larvae of *C. pipiens* from 0 to 72 hours of observation in laboratory conditions was found to be dosage dependent (Figure 1), and the LC_{50} values for the oils of lavender, camphor and garlic were found to have statistically significant difference (Table 1). Also, the mortality effect was found to be exposure time dependent, with percentage mortality significant different revealed in some time intervals. The lethal concentration enough to kill 50% (LC_{50}) of the larvae exposed varied with exposure time in tested oils (Table 1). In both LC_{50} and confidence limits, the lowest values were found in lavender (341.12) following camphor (430.79) and garlic (624.15) (Table 1).

Table (1). LC_{50} (ppm) and confidence limits of three essential oils against *Culex pipiens* larvae after 0, 24, 48 and 72 hours

Essential oils	Time (h)	LC_{50} (Lower – Upper)	Slope	X^2 (*p* -value)
Lavender oil	0	1194.66 (712.77-6440.01	1.41±0.39	0.94 (0.63)
	24	674.32 (468.36-2177.65)	1.18±0.34	2.43 (0.29)
	48	409.89 (338.56-571.98)	1.61±0.34	3.52 (0.17)
	72	341.12 (272.89-511.68)	2.11±0.35	6.53 (0.04)
Camphor oil	0	3217.48 (2573.98-4059.75)	0.91±0.39	1.18 (0.55)
	24	1099.67 (879.74-1374.58)	0.89±0.34	0.97 (0.61)
	48	541.51 (416.88-996.21)	1.44±0.34	5.44 (0.07)
	72	430.79 (344.63-646.19)	1.86±0.35	10.42 (0.01)
Garlic oil	0	1531.77 (886.14-9688.15)	2.24±0.63	0.08 (0.96)
	24	1281.93 (799.46-4845.82)	1.97±0.49	0.25 (0.88)
	48	871.47 (657.86-1592.56)	2.75±0.54	0.74 (0.69)
	72	624.15 (526.32-835.45)	3.15±0.49	0.69 (0.71)

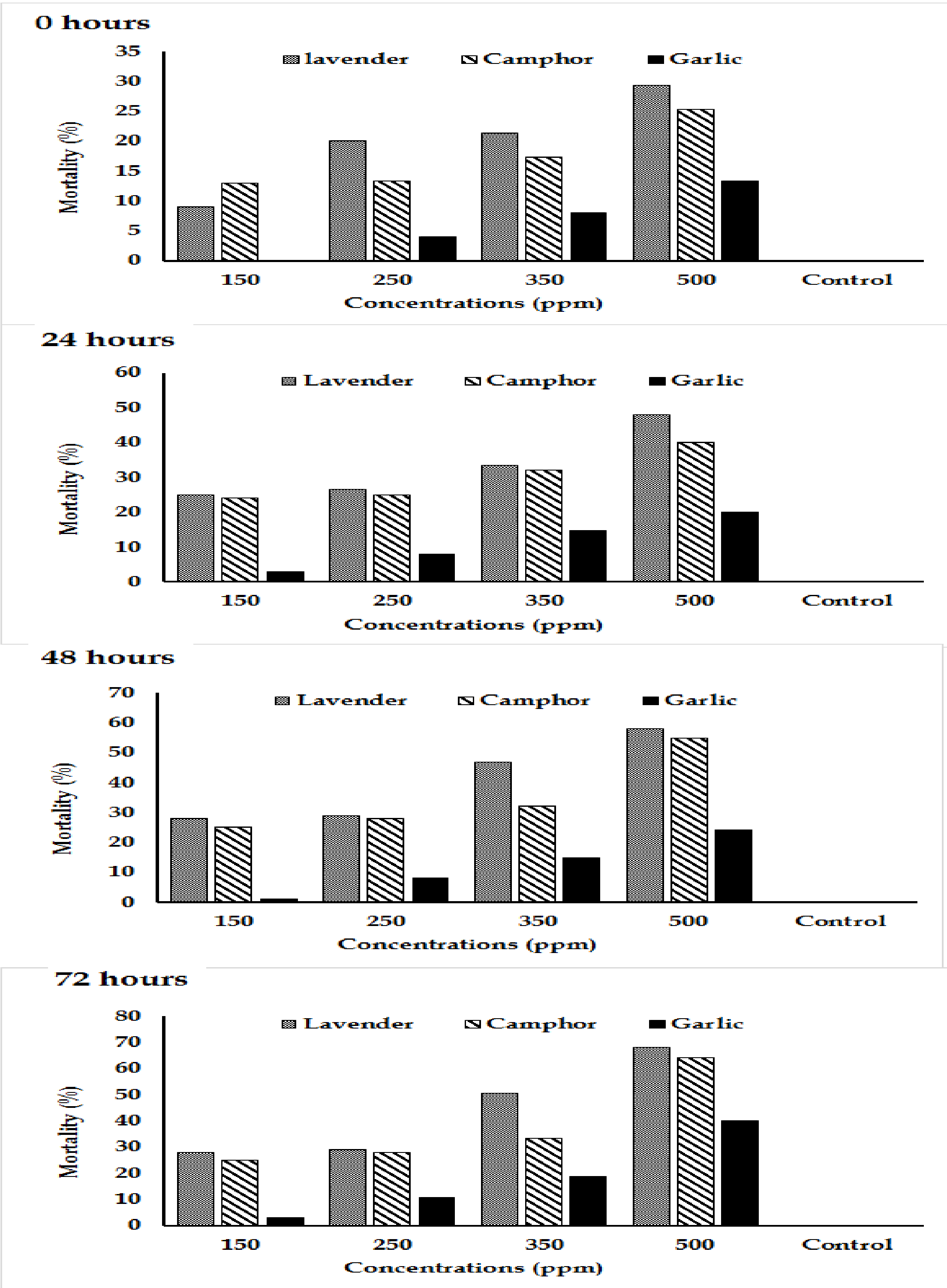

Fig. (1). Effect of dosage on mortality of lavender, camphor and garlic oils against *Culex pipiens* larvae under laboratory conditions after 0, 24, 48 and 72 hours

The results for plant oils repellency against *C. pipiens* were summarized in Figure 2. Lavender oil provides a complete repellent that lasts longer at all concentrations. However, there were not significant differences in the protection time among the repellents against *C. pipiens* (P> 0.05).

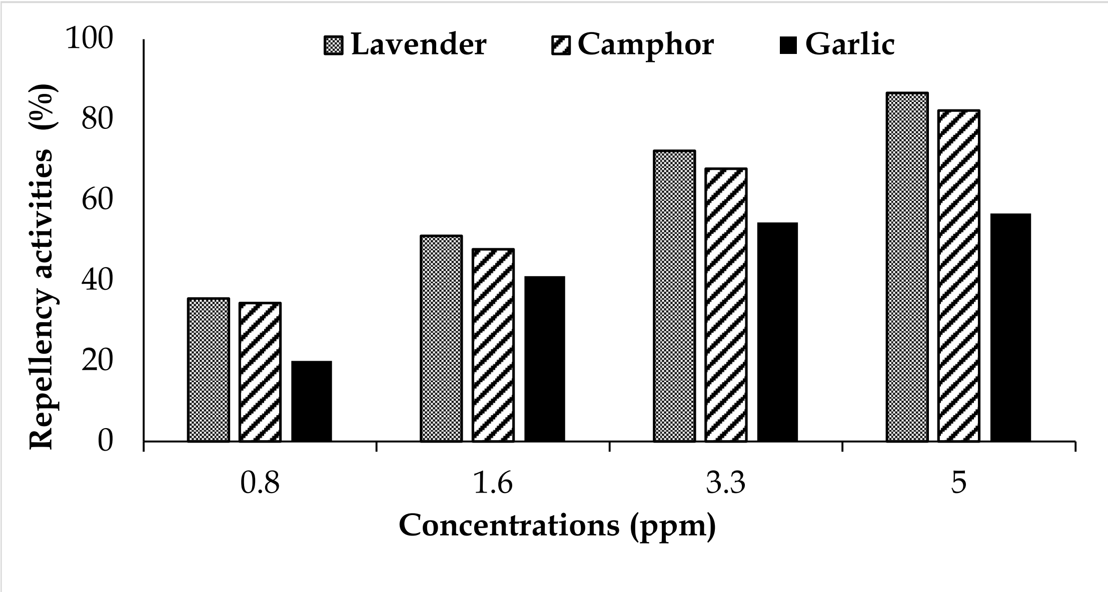

Fig. (2). Mosquito repellent activities of lavender, camphor and garlic oils against *Culex pipiens* larvae. Each oil was triplicately tested

4. Discussion

The larval source management is mostly done with frequent application of organophosphate (Berlin Rubin *et al*., 2020) and insect growth regulators **(Gonzalez and Harburguer 2020; Gunathilaka 2020)**. So, taking up plant- and fungal-based natural products in improved synthesis in small-scale trials can pave the way to manage insecticide resistance and reduce vector abundance. The results of this investigation have demonstrated that the oils under test have an effect on the mortality rates of mosquitoes. Various nontoxic, biodegradable essential oils (EOs) are present in plant extracts as active ingredients that can be used to effectively treat illnesses and get rid of harmful pests. Even though they work well as insecticides, barely 5% of pesticides used globally are biopesticides. Biopesticides are predicted to surpass chemical pesticides in the near future at an average annual growth rate of 9–20% because of several characteristics that make them easier to use, like their lack of environmental toxicity. The modification of the plant-based biolarvicides is of paramount importance to extend their longevity in the environment by making it in slow-release technology, to maintain the effectiveness of the botanical larvicides, repeated application is needed frequently **(Yang *et al*., 2002)**. In recent years, non-particle technologies have enhanced natural products to be more stable and effective **(Hashem *et al*., 2018; Hashem *et al*., 2020)**. The known insecticidal compounds found in the volatile oils of some Ocimum species are methylcinnamate, methylchavicol **(Bowers and Nishida, 1980)** and eugenol **(Sharma and Saxena, 1986)**. Other chemicals which have been reported to have insect-repellent activity are thymol, carvacrol **(Mansour *et al*., 1986)**, camphor **(Masui and Kochi, 1974)**, caryophyllene oxide **(Hubert and Wiemer, 1985)**, cineole, limonene, and myrcene **(Nutting *et al.*, 1974)**.

In this study, the tested repellent has also been found to be effective. The test samples' repellant activities differed significantly from one another. Consequently, the repellency activity of the studied oils against C. pipiens was examined. According to **Soonwera and Phasomkusolsil (2015)**, the plant Cymbopogon citratus, also known as lemongrass, is widely used as an insect repellent throughout the world, and the formulations examined cover the range of concentrations that are commercially accessible. On the other hand, the compound was more of a repellent than a feed deterrent if the protection period was lengthy but the proportion of repellency was low **(Amer and Mehlhorn, 2006)**. A recipe that maintains the aromatic ingredients on the skin for a long time is required for commercial repellent solutions that use essential oils. A group of researchers assessed the repelling qualities of an essential oil blend in three foundation materials. In a trial conducted by **Barnard and Xue (2004)**, Bite Blocker, a commercial formulation comprising glycerin, lecithin, vanillin, coconut oil, geranium, and

2% soybean oil, demonstrated comparable repellency to DEET, offering 7.2 hours of mean protection against dengue vectors and nuisance biting mosquitoes. The 30% (v/v) Ocimum gratissimum in olive oil base was found to have the highest average percentage repellencies at the World Bank Estate, Ihitte, and Umuekunne centers, with 97.2%, 95.7%, and 96.3%, respectively, according to **Oparaocha *et al*. (2010)**. In mustard and coconut oil bases, **Das *et al*. (2003)** examined the repellent qualities of Zanthoxylum limonella, Citrus aurantifolia, and petroleum ether extract of Z. limonella (fruits) as repellant against Aedes albopictus. Coconut oil is an essential element in both cooking and cosmetics. More so than any other dietary oil, it has therapeutic effects. According to **Agarwal and Lakshmi (2013)**, coconut oil is used in traditional medicine to cure a wide range of illnesses, such as skin infections, gonorrhea, bronchial asthma, allergic reactions, and malnourishment. Meanwhile, soybean oil is a rich source of vitamin E. Vitamin E is essential to protect the body fat from oxidation and to scavenge the free radicals and therefore helps to prevent their potential effect upon chronic diseases such as coronary heart diseases and cancer **(Lu and Liu, 2002)**.

Since pesticide resistance has made a vaccination against dengue ineffective, one is now lacking. Despite this, wearing insect repellent can help prevent direct contact with mosquitoes and provide protection from bites. There are numerous repellents available in the market that are sold commercially. The number and species of organisms trying to bite, the user's natural attraction to bloodsucking arthropods, the frequency, dosage, and consistency of application, and the potential host's overall activity level are just a few of the variables that affect how effective any repellent is **(Schreck, 1995)**. While the plants mentioned in this article have been shown to have mosquito-repelling properties and toxicity levels, they require reapplication to be effective over several hours. For the period the test was conducted and even several hours after the experiment, no skin irritation or dermatitis was observed on the thuman subject's treated skin after applyingthe repellent treatments. An insect repellent of plant origin ought to be well-defined and harmless to humans and other non-target organisms.

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