

Evaluating Oviposition Deterrent, Larvicidal and Repellent Effects of Different Plant Extracts and Their Combinations against *Aedes aegypti*

Shahid Majeed (✉ shahid.majeed@uaf.edu.pk)

University of Agriculture Faisalabad Faculty of Agriculture <https://orcid.org/0000-0002-8402-6704>

Amna Ishaq

University of Agriculture Faisalabad

Rameen Javaid

University of Agriculture Faisalabad

Rao Muhammad Sajjad Sharif

University of Agriculture Faisalabad

Sana Iftikhar

University of Agriculture Faisalabad

Rabia Akbar

University of Agriculture Faisalabad

Abdur Rahman

University of Agriculture Faisalabad

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Abstract

Mosquitoes are significantly important in public health as they transmit many vector-borne diseases like dengue fever (DF), malaria, and filariasis to millions of people worldwide. Although there is no effective vaccination for the prevention of dengue, avoiding mosquito bites is one method to control mosquito populations. Due to the extensive uses of synthetic repellent, mosquitoes established resistance against them. The plant-based extracts are used as an alternative to targeted insecticides. Therefore, current study aimed to examine the oviposition-deterrent, larvicidal and repellent efficacy of plant-based extracts Clove (*Syzygium aromaticum*), Cinnamon (*Cinnamomum verum*), Lemon (*Citrus limon*), Orange (*Citrus sinensis*), Onion (*Allium cepa*), Garlic (*Allium sativum*), Lemon grass (*Cymbopogon citratus*), and Aloe vera (*Aloe barbadensis* Miller) against *Aedes aegypti* under laboratory conditions. The maximum repellency was observed by clove, and lemon peel plant extract compared to all other botanical plants. However, lemon, clove, garlic, onion, and garlic with lemongrass are promising for oviposition control. While, clove, lemon peel, orange and lemon peels, and lemongrass combination for larvicidal control. In terms of repellents, the most suspected repellent plants showed by the combinations of (Olive oil, lemon peel, citrus peel, lemongrass, Aloe vera), (Olive oil, lemon peel, citrus peel, lemongrass), (Olive oil, lemon peels, citrus peels, Aloe vera), such as 19.85, 19.85, 19.84 repellency value along with 100% protection time up to 1 h. The establishment of plant-based products against *Aedes aegypti*, complemented with insecticidal activity, could lead to an innovative approach to managing mosquito populations and disease vectors.

INTRODUCTION

Mosquitoes belonging to the family Culicidae are the center of entomological research due to their significance as carriers of many harmful parasitic and viral diseases that impact both humans and animals (Becker et al., 2010). Different mosquito-borne diseases, such as dengue fever (DF), hemorrhagic fever (DHF), malaria, and filariasis. Among these diseases, dengue are significant public health disease in tropical regions, especially in Asia and Africa. These diseases are transmitted to the human population through mosquito bites. World Health Organization (WHO) has recognized dengue as a critical global public health issue due to increasing concerns about viruses and mosquitoes, recurrent epidemics, and the emergence of dengue fever in new regions. Over 100 countries have reported endemic cases of dengue fever, with an estimated annual incidence of approximately 390 million new cases. These numbers have been steadily increasing, as reported by the WHO (Jayawickreme et al., 2021). *Aedes aegypti* is the major vector of dengue, for which there is no effective vaccine available for the control of these diseases. One key method to control or minimize the incidence of dengue is to avoid mosquito bites. Generally, the use of synthetic repellents, such as Pyrethrins/Pyrethroids, Organophosphates, etc. may be used to control adult mosquitoes (Richards et al., 2020). The frequent and careless utilization of synthetic repellents has led to the development of resistance in mosquito populations that have been exposed to them. As a result, these chemicals have gradually lost their ability to effectively repel mosquitoes at the recommended doses. Mosquitoes showed resistance against many synthetic

pesticides such as pyrethroids, carbamates and others is another alarming issue (Garcia et al., 2018). Besides, the extensive application of synthetic repellents resulted in various health problems, including neurological disorders, allergic reactions skin irritation, etc., These issues are coupled with potential environmental risks, DEET, which is considered the gold standard repellent, has been linked to various adverse effects such as seizures, bradycardia, vomiting, anaphylaxis, encephalopathy, and nausea. These effects are particularly notable following dermal application, especially among pregnant women and children. Some management practices such as covering standing water, removal of standing water, installing mosquito nets, awareness sessions and covering window sites to eliminate mosquito breeding sites. The vector's life cycle may be disrupted by preventing egg-laying or oviposition activity, which would therefore slow and inhibit the spread of pathogens and population growth. As a result, as part of integrated vector management programmes, potential mosquito breeding locations could be treated with oviposition deterrent substances (Ahbirami et al., 2014).

Entomopathogens *Bacillus* genus (*Bacillus thuringiensis israelensis*) and predatory fishes (*Gambusia affinis*) are biological strategies to control the vector of mosquito and larval populations respectively. Since they can shorten the lifespan of mosquitoes or decrease the success of female mosquitoes in sucking blood from human (Favia et al., 2007). Larvicidal and adulticidal methods, along with eliminating breeding sites, are keys to eliminate the dengue population in the absence of a vaccine. Insect repellents offer indoor and outdoor protection from biting insects (Tawatsin et al., 2006).

Various plant-based insect repellents have been extensively utilized to overcome the mosquitoes, owing to their ability to emit highly volatile substances that effectively repel or deter these biting insects from approaching their hosts. The primary purpose of these compounds (alkaloids, phenols, terpenoids, steroids, and phenolics) within plants is to serve as a defense mechanism against mosquitoes. Furthermore, these compounds exhibit repellent or deterrent properties against mosquitoes and other biting Diptera. Certain chemicals released by these plants have the ability to bind to Odor binding proteins (OBPs) present in the insects' antennae, signaling the insects to move away from the compounds (Venkatachalam and Jebanesan, 2001). Plants essential oils act as another alternate method to control mosquito as they have high concentration of chemicals that are harmless and effective in mosquito control. Insecticides made from plant extracts have long been regarded as a valuable natural resource (Govindarajan and Karuppannan, 2011). Comparable evidence for these chemicals' ability to repel mosquitoes suggests that they should be the subject of future study. Considering the significance evidence of plant essential oils in prior research, the present study aims to examine the ovicidal, larvicidal and repellent activities of oil/water-based extracts of 8 different plants against the *Aedes aegypti*.

METHODS

Plant species

Eight plant species were selected for this study because most of them are known or used traditionally as mosquito repellents. They were Clove (*Syzygium aromaticum*), Cinnamon (*Cinnamomum verum*), Lemon

(*Citrus limon*), Orange (*Citrus sinensis*), Onion (*Allium cepa*), Garlic (*Allium sativum*), Lemon grass (*Cymbopogon citratus*), Aloe vera (*Aloe barbadensis* Miller)

Mosquitoes Rearing

Aedes aegypti larvae were collected from fish pond area of the University of Agriculture, Faisalabad, and were shifted into plastic trays (22 x 6 x 6 cubic cm) at $27 \pm 2^{\circ}\text{C}$ temperature, $65\% \pm 5\%$ RH, and a photoperiod of 12L: 12D in Insect Chemical Ecology Laboratory, Department of Entomology, University of Agriculture, Faisalabad. The fish food (tetramine[®]) was used as mosquito larval diet. The Lab susceptible mosquito population were used for experiments.

Ovicidal Bioassay

The experiment cups, partially filled with distilled water and filter papers, were placed in cages. In each cup, 5 ml of each plant extracts were added such as (50g lemon peels), (50g lemon peels + 10g lemongrass), (50g garlic), (50g garlic + 10g lemongrass), (50g onion), (50g onion + 10g lemongrass), (50g clove), (50g clove + 10g lemongrass), (50g cinnamon), (50g cinnamon + 10g lemongrass), (50g blended Aloe vera) and (50g lemongrass), while in the separate cup, only distilled water was added as a control. 40 female mosquitos were released into cages, and the number of eggs laid on filter papers in each cup was counted.

Larvicidal Bioassay

Different plant extracts comprise (50g lemon peels), (50g lemon peels + 10g lemongrass), (50g garlic), (50g garlic + 10g lemongrass), (50g onion), (50g onion + 10g lemongrass), (50g clove), (50g clove + 10g lemongrass), (50g cinnamon), (50g cinnamon + 10g lemongrass), (50g lemon peels + 50g orange peels), (50g garlic + 50g onion) and (50g clove + 50g cinnamon) were used to observe the mortality of larval. The transparent plastic trays (500ml capacity) containing 500ml distilled water and 10 larvae were used for each experiment. Each treatment has three replications including control treatment against each treatment. The data was collected after 24h.

Repellency Bioassay of *Aedes aegypti*

Various mixtures of plant extracts such as (Olive oil 50ml + lemon peel 5g + Citrus peel 5g + Lemongrass 5g), (Olive oil 50ml + lemon peel 5g + Lemongrass 5g), (Olive oil 50ml + lemongrass 5g + Citrus peel 5g), (Olive oil 50g, lemon peel 5g, and citrus peel 5g) + (Olive oil 50g and lemon peel 5g) and (Olive oil and citrus peel), (Olive oil 50ml + Cloves 5g + Cinnamon 5g + Lemongrass 5g), (Olive oil 50ml + Cinnamon 5g + Lemongrass 5g), (Olive oil 50ml + Cloves 5g + Cinnamon 5g), (Olive oil 50ml + lemon grass 5g + cloves 5g), (Olive oil 50ml + cinnamon 5g + lemongrass 5g + cloves 5g), (Olive oil 50ml and cinnamon 5g) and (Olive oil 50ml and cloves 5g), (Olive oil 50ml + garlic 5g + onion 5g + Lemongrass 5g), (Olive oil 50ml + garlic 5g + lemongrass 5g), (Olive oil 50ml + onion 5g + garlic 5g), (Olive oil 50ml, onion 5g, garlic 5g and lemongrass 5g), (Olive oil 50ml and garlic 5g), (Olive oil 50ml and onion 5g), (Olive oil 50ml and lemongrass 5g), and (Olive oil 50ml and Aloe vera gel 50ml) were used to observe the attractant and repellent effect for 1 hour. Behavioral experiments were carried out in experimental cages having starved

20 females, in treatment and control cages separately. Botanical extracts were sprayed on upper side of hand as well as the left hand keep intact for control treatment. After the application of extracts (2 ml each), both arms were exposed to mosquitoes for observations of mosquito landing for 1 hour with one-minute intervals in each experiment. For each set of experiments, three replications were carried out.

Statistical Analysis

Collected data were subjected to statistical analysis to determine the activity of plant extracts against *Aedes aegypti* using One-way ANOVA with Tukey HSD test.

RESULTS

Oviposition deterrent

The results showed that different extracts had significant effects on oviposition of *Aedes aegypti* ($p < 0.05$). The analyzed results revealed that maximum oviposition (334.67 eggs) were counted in Lemongrass, (248 eggs) in Aloe vera, 160 eggs in cinnamon, 83.34 eggs in clove + lemon grass, 29 eggs in cinnamon + lemon grass, 22 eggs in onion + lemon grass, 19 eggs in lemon + lemon grass, 17.67 eggs in onion. While minimum oviposition 14.67 eggs were counted in clove, 12.67 eggs in garlic + lemongrass, 4.34 eggs in lemon and 2 eggs in garlic (Fig. 1).

Larval behavior

The results were analyzed which showed that different extracts had significant effects on the mortality of *Aedes aegypti* larvae ($p < 0.00$). The analyzed results revealed that maximum larval mortality 10 was observed in orange + lemon peel, lemon peel, and clove followed by 9.67 mortality observed by garlic + lemon grass, and clove and cinnamon. While minimum mortality 5.67 against onion + lemon grass followed by 7.67 against cinnamon + lemon grass. Other extracts like onion, cinnamon, clove + lemon grass, garlic, lemon peel + lemon grass, and garlic and onion showed 7.83, 8.5, 8.33, 8.33, 9, and 9 respectively. While in control treatment no mortality was found (Fig. 2).

Citrus and Lemon based extracts

The results showed repellent efficacy all citrus based extracts against *Aedes aegypti*. The analysis of variance indicated that different combinations of citrus peels extracts had significant impact ($p \leq 0.000$) against attraction activity of females. The maximum repellency (19.85) was observed by extract with combinations of (Olive oil, Lemon peels, citrus peels, lemongrass and Aloe vera), while minimum repellency rate (18.67) was observed by (Olive oil, Lemon peels, lemongrass and Aloe vera) against adult mosquitoes (Fig. 3).

Onion and Garlic based extracts

The results showed repellent efficacy all onion and garlic-based extracts against *Aedes aegypti*. The analysis of variance indicated that different combinations of onion and garlic-based extracts had

significant impact ($p \leq 0.000$) against female attraction and repulsion of females. The maximum repellency 19.61 was observed on Olive oil, Onion, Lemongrass and Aloe vera, while minimum repellency rate 19.33 was observed on olive oil against adult mosquitoes shown in Figure. 4.

Cloves and Cinnamon based extracts

The results showed repellent efficacy all clove and cinnamon based extracts against *Aedes aegypti*. The analysis of variance indicate that different combinations of clove and cinnamon based extracts had significant impact ($p < 0.000$) against attraction of females. The maximum repellency 19.85 was observed on Olive oil and Cloves, while minimum repellency rate 19.45 was observed on Olive oil, Cloves, Cinnamon and Aloe vera against adult mosquitoes shown in Fig. 5.

DISCUSSION

The studies on oviposition deterrent activity of chemical compounds and insect repellents have been carried out against mosquito vectors. In a recent study, Xue et al. (2006) also noted the effectiveness (76–100% repellency) of 21 commercial insect repellent products (at 0.1 percent concentration) against *Ae. albopictus*. These products included 12 botanicals, 6 deet-based, and 3 synthetic organics. As for the plant extracts, Sharma et al. (2022) observed that oils from *Citrus reticulata* (Kinnow) and *Citrus limon* (Lemon), and *Citrus limetta* (Musambi), against mosquito eggs. After 24 hours, the LC50 and LC90 values of the essential oils in the leaves were 23-3.45 and 81-15.24 ppm and 35-3.55 and 81 – 10 ppm, while the corresponding values for the peels were 16-3 and 77 – 18 ppm and 29 – 3 and 68-9ppm. Similarly, Tawatsin et al. (2006) investigated that each essential oil displayed varying degrees of oviposition deterrent effects against *Ae. aegypti*, from 16.6–94.7%, while DEET and IR3535 showed no repellent activity. The current results are similar to the above findings who have investigated the ovipositional deterrent efficacy of plant-based extracts against of *Aedes aegypti* mosquito. Among all previously mentioned extracts combination, lemon, clove, garlic, onion, and garlic with lemongrass, are very promising for oviposition control.

Plants-based extracts having oviposition deterrent and larvicidal effects against *Ae. aegypti* would be of interest as plant-based products for the control of mosquitoes. Abanit et al. (2022) determined lethal concentrations of galangal rhizome and lime leaf extract against larvae of *Aedes aegypti* mosquito larvae. Lime leaf extract at 650 ppm (LC50) killed 52%, and also at 1100 ppm (LC90) killed 92% larvae. Galangal root extract had similar effects. When combined, LC50 extracts killed 76%, while LC90 extracts eradicated all larvae. Similarly, Yearsi and Munawaroh (2021) demonstrated that garlic extract killed *Aedes aegypti* larvae. The LC50, or the amount needed to kill 50% of the larvae, is 0.24% in the first instar, 0.15% in the second instar, 0.16% in the third instar, and 0.24% in the fourth instar. Furthermore, Kurniasih et al. (2021), determined the larval mortality from lemongrass and orange (*Citrus Sinensis*). Lemongrass exhibited a lower LC50 value of 35.1%, while orange had a higher LC50 value of 790.9%. Correspondingly, Pandiyan et al., (2019) investigated the synergistic effect of essential oils from *Syzygium aromaticum* (clove), *Illicium verum* (star anise) and *Trachyspermum ammi* (Ajwain) against *Aedes aegypti* mosquito larvae. The hydro-distillated oils were tested against larvae of *Ae. aegypti*. The binary combinations of

EOs were prepared and concluded that these mixtures exhibited larvicidal properties with highest 60.89 mgL^{-1} LC_{50} values from cloves oil and other two oils. About 13 different formulations of plant based larvicidal combinations have been evaluated in this present study. Among these plant extracts, clove, lemon peel, orange and lemon peels, clove and cinnamon, garlic and lemongrass are very promising for larvicidal control.

Research has shown that plants-based extracts used as repellents and are also safe for human life, with no harmful effect on human and domestic animal skin with and no feedback of environmental ill effect. Deletre et al. 2013 reported the repellent properties of 20 essential oils against malaria vector. Many studies have proven that limonene extracted from citrus peel was a fragrance agent (Singh et al., 2021), limonene created fragrance and also used for mosquitoes repelled application (Hue et al., 2022). Similarly, Phasomkusolsil and Soonwera (2016), demonstrated that formulation consisted of *Cymbopogon citratus* (lemon grass), *Syzygium aromaticum* (cloves), essential oils indicated high repellency, among them strong effective repellency was observed by lemongrass by using arm in cage method. In another research, Amer and Mehlhorn (2006), investigated the repellent effects of 41 essential oils against *Anopheles stephensi*, *Ae. aegypti*, and *Culex quinquefasciatus*. In terms of the repellency, lemon peel extract (0.35 mg/cm^2) and citrus peel extract (0.38 mg/cm^2) combinations exhibited the highest efficacy provided longer protection times as compared to the single original lime extract. Utomo and Supriyatna, (2014) tested repellent efficacy of natural geranium, lemongrass, lavender, and lemon oils combined with aloe vera gel and mentioned that these oil as an alternative to different commercial mosquito repellent lotions contain harmful DEET. The best results with 15% essential oils showed over 50% effectiveness for geranium, citronella, and lavender oils up to six hours, while lemon oil lasted two hours.

Trongtokit et al. (2004) used clove oil and prepared 10 experimental repellent products in cream or gel form against *Anopheles* and *Aedes aegypti* Gel (E) having 10% clove oil, and gel (B) having 20% clove oil showed significant 4–5 hours of protection against three mosquito species. Ahmed and Aujla (2012) observed the repellency of Aloe vera, Olive oil, Onion, Garlic to examine their repellency against *Aedes aegypti* and the experiment showed the remarkable result mean number of landing mosquito in both control and experimental units was 1.8 and 3.36 respectively 30 and 120 minutes. The present study is similar to above mentioned who have demonstrated essential oil efficacy from different plant-based extracts as the best protection against mosquito bites. Among the tested 30 plant-based extracts, the combinations of (Olive oil, lemon peel, citrus peel, lemongrass, Aloe vera), (Olive oil, lemon peel, citrus peel, lemongrass), (Olive oil, lemon peels, citrus peels, Aloe vera), (Olive oil, onion, lemongrass, Aloe vera), (Olive oil, clove, lemongrass, Aloe vera), (Olive oil, lemongrass), were found to possess the highest repellency values 19.85, 19.85, 19.84, 19.83, 19.79, 19.78 respectively with 100% protection time up to 1 h.

Conclusion

Plant-based natural extracts are still used traditionally against mosquito bite throughout worldwide. Knowledge regarding new natural products, derived from plants is important for controlling *Ae. aegypti* populations. As indicated by this review, natural plants-based extracts combined oils have demonstrated repellent and lethal effects on adult and it could emerge as encouraging scope for controlling dengue vector. It's also important to mention that throughout the experimentation, no harmful outcomes like, rashes, dermatitis, skin irritation, or any allergic reactions were not seen on the skin of the volunteers who were subjected to the various extracts mentioned earlier.

Abbreviations

- *Ae. aegypti*
- *Aedes aegypti*
- LG
- Lemongrass
- EO
- Essential oil

Declarations

Ethical Approval and Consent to Human Participation

The present research used insects where no formal review for ethics needed.

Consent for publication

Not applicable

Availability of data and materials

The datasets employed and/or examined in the present study can be obtained from the corresponding author upon a reasonable request.

Competing interests

The author declares that he/she has no competing interests.

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Not applicable

Authors' contributions

All authors read and approved the final manuscript.

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Figures

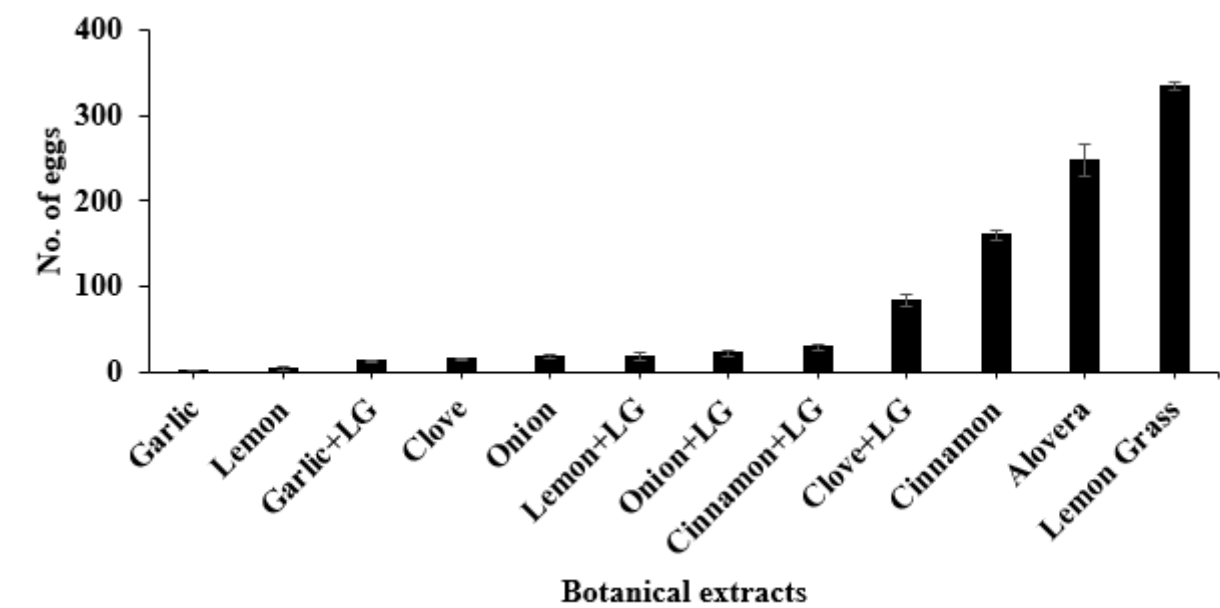


Figure 1

Oviposition Deterrent Bioassay

The figure represents the average number of eggs laid by female *Aedes aegypti*. The bars represented the standard errors and mean values of number of eggs. The X-axis represents botanical extracts, while the Y-axis represents the total number of eggs. LG abbreviated as lemongrass.

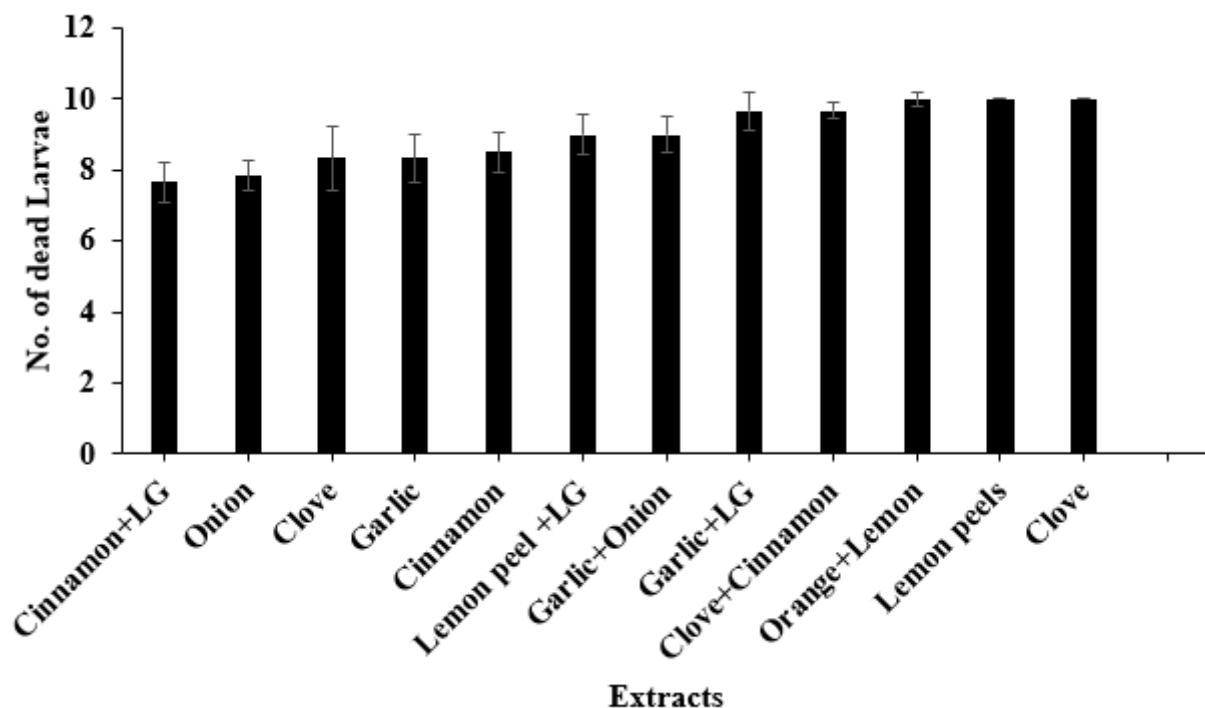


Figure 2

Larvicidal Bioassay

The figure illustrates average number of dead larvae of *Ae. aegypti*. Bars showed the mean values of dead numbers and capped lines above the bars shows standard errors value of dead larvae. The X-axis represents extracts Y-axis represents number of dead mosquitoes.

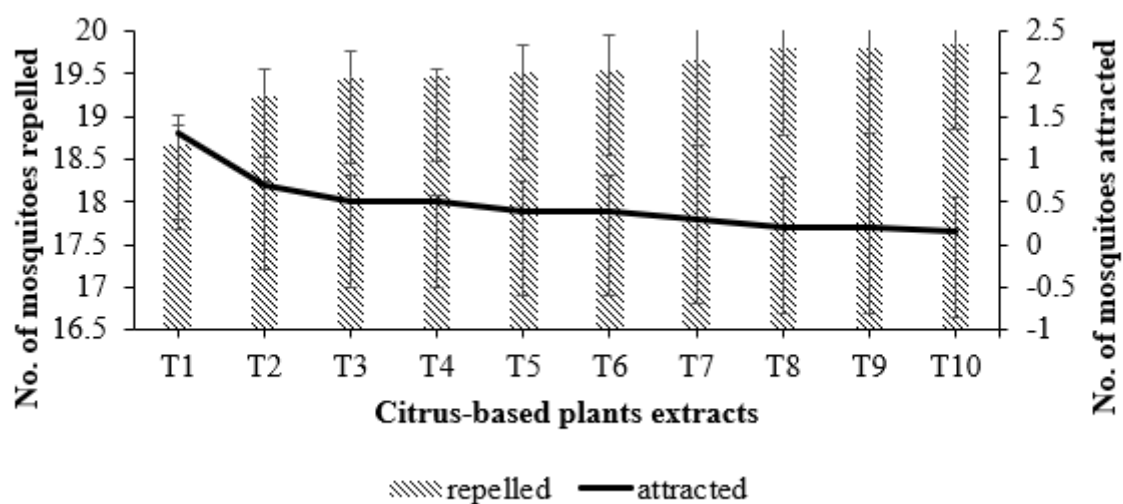


Figure 3

Citrus and lemon peel-based Extracts

showed T1= Olive oil, lemon peels, lemongrass, Aloe vera, T2= Olive oil, citrus peel, lemongrass, Aloe vera, T3= Olive oil, citrus peel, T4= Olive oil, T5= Olive oil, Aloe vera, T6= Olive oil, lemon peel, T7= Olive oil, lemongrass, T8= Olive oil, lemon peels, citrus peels, Aloe vera, T9= Olive oil, lemon peel, citrus peel, lemongrass, T10= Olive oil, lemon peel, citrus peel, lemongrass, Aloe vera. Bars showed the mean values of repelled numbers of mosquito and black line showed attracted number of mosquito and capped lines above the bars and line shows standard errors value of repelled and attracted mosquito. The X-axis represents the total number of citrus based extracts (T1...T10), Y-axis represents the total number of repelled mosquitoes and secondary axis represents the total number of attracted mosquitoes.

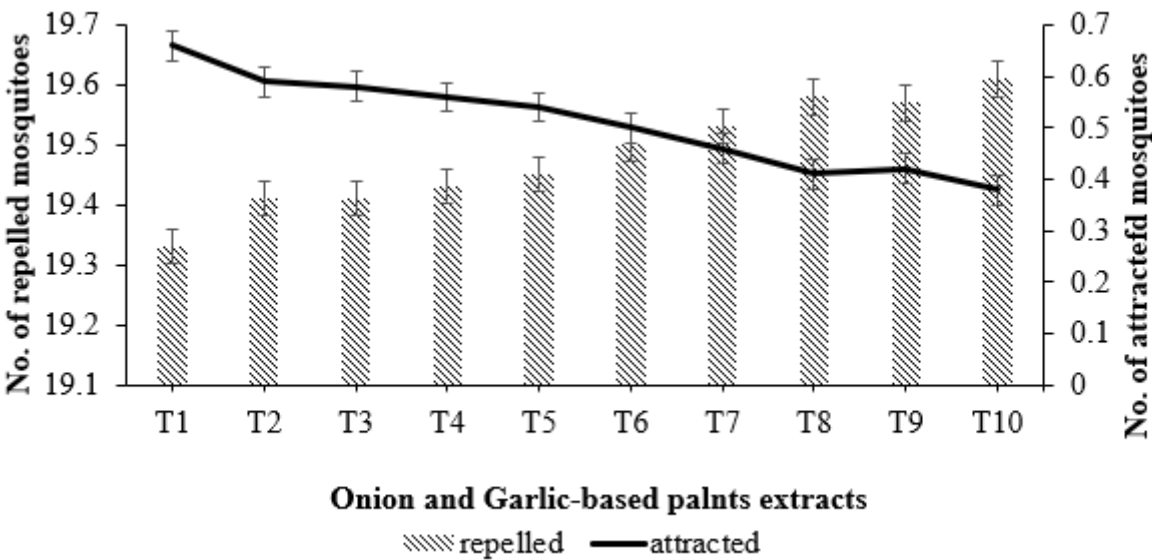


Figure 4

Onion and Garlic-based Extracts

Showed T1= Olive oil, T2= Olive oil, onion, garlic, lemongrass, Aloe vera, T3= Olive oil, garlic, lemongrass, Aloe vera, T4= Olive oil, garlic, lemongrass, T5= Olive oil, Aloe vera, T6= Olive oil, garlic, T7= Olive oil, onion, garlic, lemongrass, T8= Olive oil, lemongrass, T9= Olive oil, onion, T10= Olive oil, onion, lemongrass, Aloe vera. Bars showed the mean values of repelled numbers, black line shows number of attracted mosquito and capped lines above the bars and line shows standard errors value of repelled and attracted mosquito. The X-axis represents the total number of onion and garlic-based extracts (T1...T10), Y-axis represents the number of repelled mosquitoes and secondary axis represents the number of attracted mosquitoes.

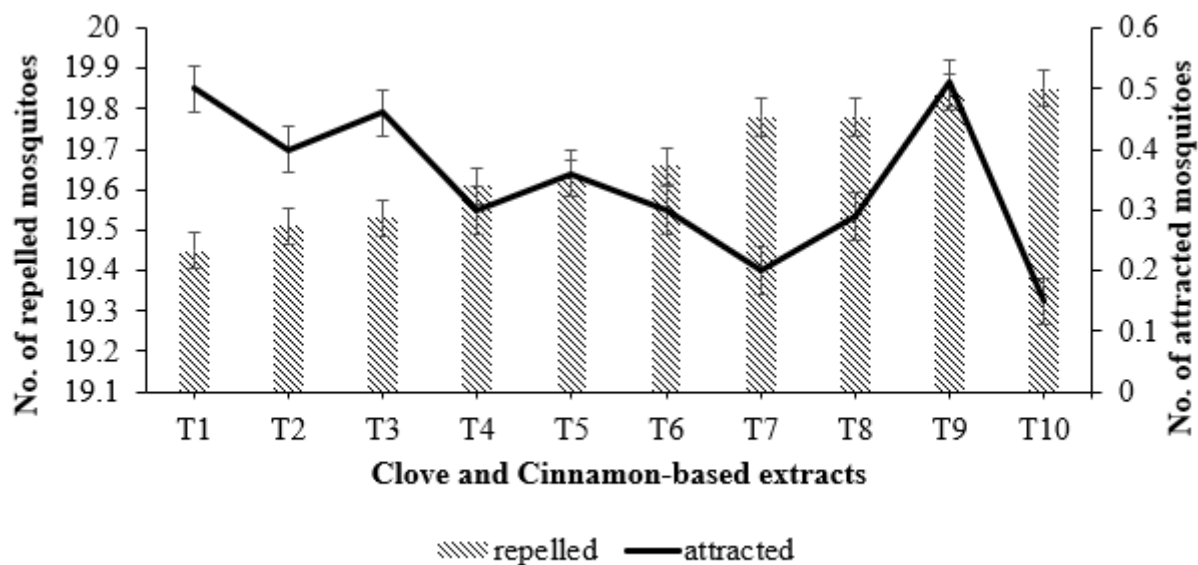


Figure 5

Clove and Cinnamon-based Extracts

Showned T1= Olive oil, cloves, cinnamon, Aloe vera, T2= Olive oil, Aloe vera, T3= Olive oil, cinnamon, lemongrass, Aloe vera, T4= Olive oil, cinnamon, clove, lemongrass, Aloe vera, T5= Olive oil, cinnamon, T6= Olive oil, lemongrass, T7= Olive oil, cinnamon, clove, lemongrass, T8= Olive oil, clove, lemongrass, Aloe vera, T9= Olive oil, T10= Olive oil, clove. Bars showed the mean values of repelled numbers, black line shows number of attracted mosquito and capped lines above the bars and line shows standard errors value of repelled and attracted mosquito. The X-axis represents the total number of clove and cinnamon based extracts (T1...T10), Y-axis represents the number of repelled mosquitoes and secondary axis represents the number of attracted mosquitoes.