International Journal of Entomology Research
ISSN: 2455-4758; Impact Factor: RJIF 5.24
Received: 15-12-2020; Accepted: 16-01-2021; Published: 24-02-2021
www.entomologyjournals.com
Volume 6; Issue 1; 2021; Page No. 133-137

Knockdown, repellent and adulticidal activity of essential oils derived from selected aromatic plants against the filarial vector mosquito, *Culex quinquefasciatus* Say (Diptera: Culicidae)

Athira A, E Pushpalatha*
Biopesticides and Toxicology Laboratory, Department of Zoology, University of Calicut, Malappuram, Kerala, India

Abstract
Control and management of mosquito population has become all the more difficult due to the development of resistance in mosquitoes to several chemical insecticides which are continuously used in vector control programmes. Plant derived compounds are often considered as effective alternatives against mosquitoes in order to reduce the environmental impact caused by the extensive use of synthetic insecticides. The Knockdown effect and repellent activity of essential oils derived from three plants, *Melaleuca leucadendra* (L.) L., *Callistemon citrinus* Curtis and *Aegle marmelos* (L.) Corrêa) were investigated in the present study. Observations showed that essential oils from all the tested plants exhibited considerable knockdown effect on *Cx. quinquefasciatus.* In addition to this, all plant derived essential oils showed repellent activity which was significant in all concentrations. It is observed that essential oils derived from *M. leucadendra* was found to be possessing very good repellent activity and provided complete protection for six hours.

Keywords: *Culex quinquefasciatus*, essential oils, repellent activity, adulticidal activity, knockdown, *Melaleuca leucadendra*, *Callistemon citrinus*, *Aegle marmelos*

Introduction

The increased prevalence of serious diseases like malaria, Japanese encephalitis, filariasis, dengue fever, yellow fever and chikungunya transmitted by mosquitoes cause high rate of mortality and morbidity among people [1, 2]. Synthetic chemical pesticides have been used for ages for controlling mosquitoes by either killing the adults or killing mosquito larvae in their breeding sites [3]. Continuous application of synthetic pesticides, lead to the development of insecticide resistance to chemical pesticides such as DDT, Malathion, deltamethrin which is a major setback faced by the management of vector mosquitoes [4, 5]. In addition to this, application of synthetic pesticides has created several issues to the non-target organisms causing water pollution [6]. Considering the negative impact of pesticide applications, efforts have been made to develop natural products as better alternatives to synthetic insecticides. Among them, plants and plant derived substances have been widely used to repel or kill mosquitoes [7]. Essential oils, which are the major byproducts of the plant derived substances, emerged as a successful remedy for the control of mosquito mainly due to their insecticidal properties [8, 9]. Due to the presence of biologically active components in the essential oils they can effectively act as mosquito repellents [10, 11, 12] and essential oils have gained considerable attention as they contain potentially active compounds which could be effectively used against mosquito vectors [13].

Many active components isolated from plants exhibit toxic activity against mosquitoes [14, 15, 16] and other insect pests. In addition to the repellent activity, they can also be used as ovicidal agents, oviposition deterrents, Growth and reproduction inhibitors [17, 18]. In many cases, the activity of these compounds is found higher or persistent than the synthetic chemicals [19, 20]. Present study investigated the impact of essential oil extracted from selected three weed plants against *Culex quinquefasciatus* Say and attempts have been made to assess the knockdown time and repellent Activity of the essential oils.

Materials and methods

Collection of plants

Leaves of the selected aromatic plants, *Melaleuca leucadendra* (L.) L., *Callistemon citrinus* Curtis and *Aegle marmelos* (L.) Corrêa were collected from in and around of the campus of University of Calicut, Malappuram, Kerala. The taxonomic identities were confirmed at the Department of Botany, University of Calicut.

Test organism

Culex quinquefasciatus taxonomically comes under the member of *Culex pipiens* species complex. It is commonly known as 'Southern House Mosquito', which is the major vector of Bancroftian filariasis, avian malaria and arboviruses including Western Equine Encephalitis virus, St. Louis encephalitis virus, West Nile virus and Zika virus. It is considered as the primary vector *Wuchereria bancrofti*, a nematode that causes lymphatic filariasis.

Laboratory maintenance of *Culex quinquefasciatus* Say.

The different larval stages of *Culex quinquefasciatus* were collected from the open drains in and around the campus of the University of Calicut, Kerala, India were brought to the laboratory and maintained at 29±2°C and 75-85% relative humidity, fewer than 14:10h light and dark cycles. The larvae were kept in plastic trays containing tap water and fed with a diet of fine powder of dog biscuits and Brewer's yeast in the ratio of 3: 1 respectively. The pupae were kept inside the standard emergence cages. The mosquitoes were identified after emergence and species confirmed before rearing. The adults were fed with 10% sucrose solution and additional blood meal was provided (using an immobilized quail) to adult females for the development of egg. A bowl containing water was kept in the emergence cages to facilitate oviposition. The eggs laid were removed from the cage and after hatching, the larvae were reared in the laboratory at room temperature.

Extraction of essential oil

Essential oils of selected three aromatic plants were extracted by steam distillation method using Clevenger apparatus. Fresh leaves of the plants were collected, washed and used for essential oil extraction using distilled water for 4-6 h. The essential oils extracted were dried over anhydrous-sodium sulfate and used for the conduct of bioassays.

Adulticidal bioassay

The bioassay was performed by following WHO protocol [21]. Different concentrations of the essential oils were prepared using ethyl alcohol and applied on Whatman No. 1 filter paper for testing the adulticidal activity. Control paper was treated with ethanol and distilled water under similar conditions. Twenty female mosquitoes (2–5 days old glucose fed, blood starved) were collected from the insect-rearing cages and gently transferred into a plastic holding tube. The mosquitoes were allowed to acclimatize in the tube for 1 h and then exposed to test paper (filter paper) for 1 h. At the end of exposure period, the mosquitoes were transferred back to the holding tube and kept 24 h for recovery period. Mortality of mosquitoes was determined at the end of 24 hr. The number of mosquitoes knocked down in the exposure tube was recorded at 5 min interval period till the last mosquito was knocked down. Knock down time (KDT) values of KDT50 and KDT90 were determined using probit analysis.

Adult repellency test

The repellent activity was conducted as per the method of WHO [22] with slight modification. Repellency bioassays were carried out in the laboratory at 29±2°C and 75–85% relative humidity. Three to four days old blood-starved 100 adult females of *Culex quinquefasciatus* mosquitoes were randomly selected and placed in an experimental cage (30 x 30 x 30 cm) and left to acclimatize for 1h. The arm of human volunteer was cleaned with ethanol. After air drying the arm of the test person, only 25cm^2 dorsal side of the skin on each arm was exposed and the remaining area being covered with gloves. The selected essential oils at different concentrations (50, 100, 150, 200 and 250 ppm) was applied and set. The control and treated arms were introduced simultaneously into the cage. The first bite by *Culex quinquefasciatus* was noted from 5 minutes for every 1h up to 6 h. Subsequently, the test arm was introduced into the cage for the same period of time and the numbers of mosquitoes that landed and attempted to feed were recorded. The experiment was conducted for three times. It was observed that there was no skin irritation by the extracts of the selected essential oils. The percentage protection was calculated by using the following formula;

$$\text{Percentage Protection} = \frac{\text{No. of bites received by control - No. of bites received by treated}}{\text{No. of bites received by control}} \text{ X100}$$

Results

Among selected plant extracts *M. leucadendra* exhibited higher knockdown effect at minimum concentration followed by, *C. citrinus*, and *A. marmelos*. It was noted that the knockdown effects of all plant extract were dose dependent and found to be increasing with increase in the concentration (Fig.1). *M. leucadendra* consistently exhibited higher knockdown effect in all concentrations. Among all the plants tested, *M. leucadendra* and *C. citrinus* exhibited gradual increase in the knockdown activity while *A. marmelos* is found to be comparatively less active (Fig. 1).

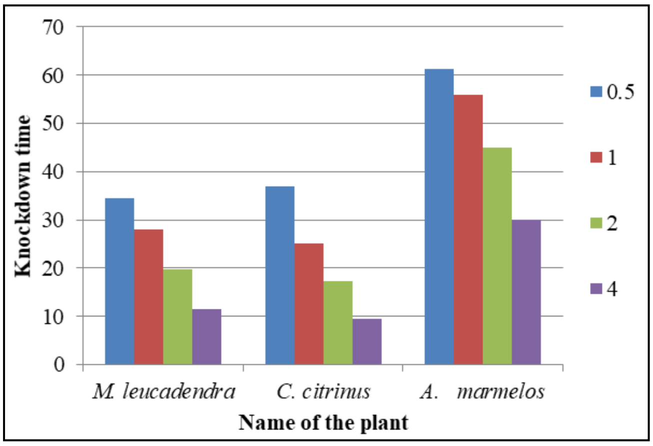

Fig 1: Knock down times required to kill 50% of the population exposed.

The mosquito repellent activity of volatile oils of the selected plant extracts were found significant in all Concentrations (Figs 2 to 6). At 10% it was found that the activity was highest for 1 hour exposure and remained stable up to 3 hours (Fig. 2). After that the activity found decreased. Among the selected plants *M. leucadendra* exhibited higher activity than the rest of the essential oils (Fig. 2). The activity of *A. marmelos* is considerably dropped between 5 and 6 hours of exposure. The trend was found similar at 25% of volatile oil (Fig. 3). It was evident that all volatile oils exhibited more than 60% protection against mosquitoes.

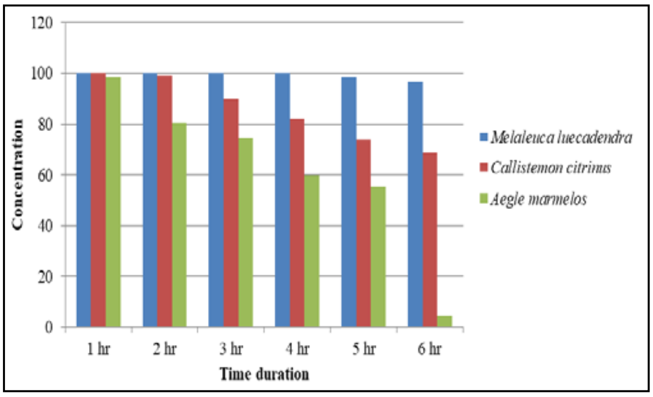

Fig 2: Adult repellency test using 10% volatile oils for 1–6 hours of application.

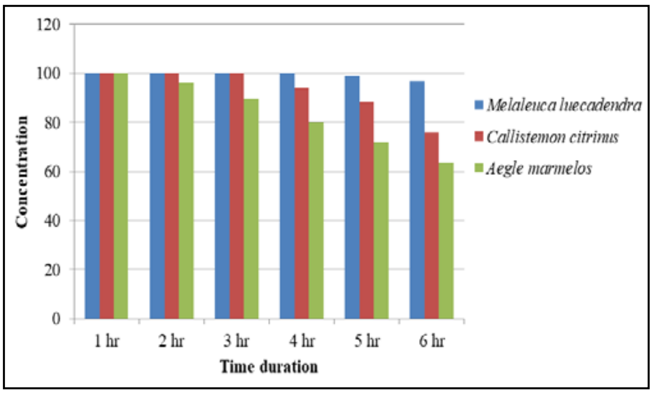

Fig 3: Adult repellency test using 25% volatile oils for 1–6 hours of application.

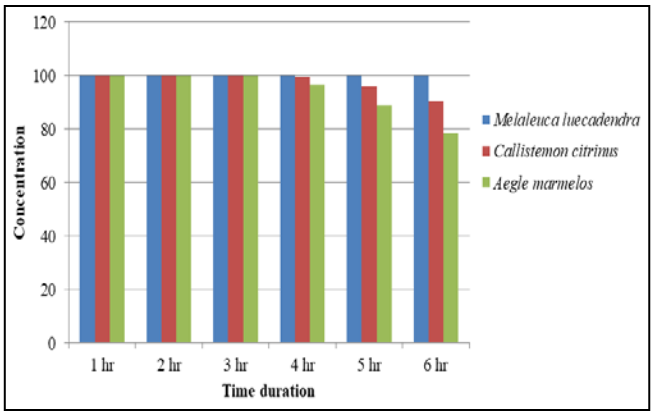

Fig 4: Adult repellency test using 50% volatile oils for 1–6 hours of application.

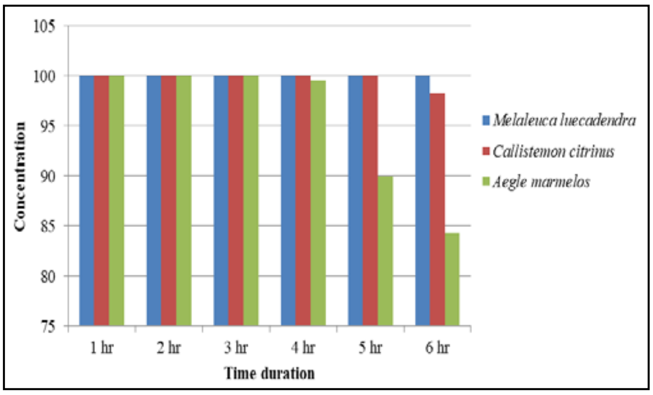

Fig 5: Adult repellency test using 70% volatile oils for 1–6 hours of application.

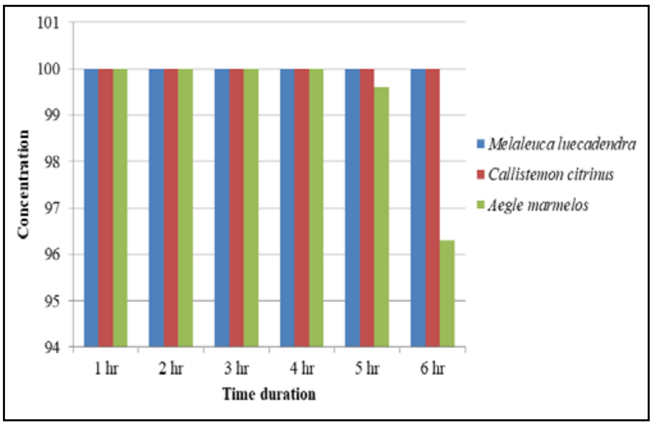

Fig 6: Adult repellency test using 100% volatile oils for 1–6 hours of application.

The observations revealed that the activity of *A. marmelos* was significantly increased at 25% (Fig. 3). At 50%, all the plant extracts exhibited the maximum activity from 1 hour to 3 hours (Fig. 4) and except for *M. leucadendra*, the activities of all plant extracts were found to be decreasing. The maximum protection was provided by all extracts was nearly up to 4 hours of exposure except marginal decrease in the activity of *A. marmelos*. The activity of *A. marmelos* exhibited significant reduction. Almost all plant extracts exhibited maximum repellent activity from 1 hour to 5 hours exposure with the exception of a slight decrease of activity exhibited by essential oils from *A. marmelos*.

In the case of 70 and 100% concentrations all plants showed maximum (100%) repellent activity up to three hours (Figs 5 & 6). Later the activity of *A. marmelos* was observed to be reducing in 70% concentration in four hours (Fig. 5), but in 100% all plants exhibited maximum activity up to 4 hours (Fig. 6). At 5th hour onwards, the activity of *A. marmelos* significantly reduced at 70% concentration (Fig. 5). In addition to the reduced activity of *A. marmelos*, *C. citrinus* also showed reduced activity at 70% concentration.

Discussion

Due to the extensive use of many chemical pesticides against mosquito vectors and its consequent ill effects has instigated a renewed interest in search of botanicals and most of the studies have suggested that plant derived compounds and phytochemicals could be used as a better alternative for mosquito control [23]. Until now, many plant extracts and essential oils derived from plants exhibited commendable larvicidal and repellent activity against several mosquito species [24, 25, 26, 27]. In the present study different volatile oils extracted from selected plants exhibited distinct knockdown and larvicidal activity against *Cx. quinquefasciatus*. Among the plants, *M. leucadendra* showed highest repellent activity followed by *C. citrinus* and *A. marmelos*. The increased repellent activity of *M. leucadendra*, *C. citrinus* and *A. marmelos* may be due to their aromatic properties [28].

Present study reports the highest repellent activity of *M. leucadendra* among all selected plant extracts and it has been reported that *Melaleuca* spp. (*M. leucadendra* and *M. quinquenervia*) were efficient against three mosquito species [29]. Based on a comparative analysis it was reported that *Cx. quinquefasciatus* is the most susceptible mosquito species than *Ae. aegypti* and *An. stephensi* [29]. In present study the protection period of *M. leucadendra* extended up to 6 hours of exposure and studies have confirmed that *Melaleuca* oils exhibit protection time up to 8 hours against mosquito species [29].

The activity of *C. citrinus* is found to be lesser than *M. leucadendra* but the activity can extend up to 6 hours of exposure. The repellent activity of *C. citrinus* can be justified by its higher larvicidal activity against mosquitoes particularly on *Cx. quinquefasciatus* [30]. In the present study, leaves of *C. citrinus* showed lesser activity than leaf extracts of *M. leucadendra*. The reduction in the repellent activity of *C. citrinus* is may be due to the fact that fruits are the most preferable source of these active metabolites for the extraction of essential oils than leaves [30] and the higher activity of fruit essential oils of *C. citrinus* is probably due to the synergistic activity of the minor components [30,31]. The effectiveness of these plant extracts could be attributed to the presence of phytochemical compounds which can act as insecticides against mosquitoes [32].

The differential repellent activity of three essential oils extracted from different plants is supported by the fact that insecticidal activity of plants extracts varies according to the plant species, target organism, geographical varieties, parts used and the extraction method used [32, 33]. Among the studied plants, *A. marmelos* was the least active plant extract against *Cx. quinquefasciatus*. Although *A. marmelos* exerted lower activity in the present study it's a well-known plant extract against larvae of *An. stephensi* and *Ae. Aegypti* [34, 35]. The repellent activity of *A. marmelos* is in line with some reports [36, 37] as essential oils from leaves of *A. marmelos* exhibit repellent activity against *Ae. Aegypti* and *Cx. quinquefasciatus*. Although laboratory experiments can only be regarded as indicative, we herein suggest the use of essential oils extracted from *M. leucadendra*, *C. citrinus* and *A. marmelos*. In light of the results from the present study it can be suggested that the extracts from *M. leucadendra* and *C. citrinus* have significant repellent activity against *Cx. quinquefasciatus* and the findings of the present study recommend plant based essential oils for the control of mosquito vectors.

Conclusion

The findings of the present study show that essential oils derived from the three aromatic plants exhibit comparable knockdown and repellent activity against the filarial vector, *Cx. quinquefasciatus*. Application of the essential oils of all the selected plants extends personal protection up to 6 h except a slight decrease in activity registered for *A. marmelos.* Formulations based on these essential oils and incorporation of further purified fractions can be employed as a better ecofriendly alternative to ward off the filarial vector and mosquitoes in general.

Acknowledgements

We thank the University Grants Commission, New Delhi for the financial support through UGC-MRP program.

References

1. Jang YS, Kim MK, Ahn YS, Lee HS. Larvicidal activity of Brazilian plant against *Aedesaegypti* and *Culexpipiens* (Diptera: Culicidae). Agric. Chem. Biotechnol. 2002; 4:131-134.
2. Benelli G, Lo Iacono A, Canale A, Mehlhorn H. Mosquito vectors and the spread of cancer: An overlooked connection? Parasitol. Res. 2016; 115(6):2131-2137.
3. Joshep CC, Ndoils MM, Malima RC, Nkuniya NHM. Larvicidal and mosquitocidal extracts, a coumrin, isoflavonoids and petrocarpans from Neorautanenia mitis. Trans. R. Soc. Trop. Med. Hyg. 2004; 98:451-455.
4. Tabashnik BE. Evolution of resistance to Bacillus thuringiensis. Annu. Rev. Entomol. 1994; 39:47-79.
5. Yang P, Yajun MA, Shuiqing Z. Adulticidal activity of five essential oils against Culex pipiens quinquefasciatus. J. Pestic. Sci. 2005; 30:84-89.
6. Dua VK, Pandey AC, Dash APM. Adulticidal activity of essential oil of Lantana camara leaves against mosquitoes. Ind. J. Med. Res. 2010; 131:434-439.
7. Soonwera M. Efficacy of essential oils from Citrus plants against mosquito vectors *Aedesaegypti* (Linn.) and *Culex quinquefasciatus* (Say). Int. J. Agri. Tech. 2015; 11(3):669-681.
8. Sukumar K, Perich MJ, Boobar LR, Botanical derivatives in mosquito control: a review. J. Am. Mosq. Contr. Assoc. 1991; 7:210-237.
9. Cheng SS, Chang HT, Chang ST, Tsai KH, Chen WJ. Bioactivity of selected plant essential oils against yellow fever mosquitoes *Aedesaegypti* larvae. Biores. Technol. 2003; 89:99-102.
10. Babu R, Murugan K. Interactive effect of neem seed kernel and neem gum extracts on the control of *Culex quinquefasciatus* Say. Neem Newsletter. 1998; 15:9-11.
11. Venkatachalam MR, Jebanesan A. Repellent activity of *Ferronia elephantum* Corr. (Rutaceae) leaf extracts against *Aedesaegypti*. Biores. Tech. 2001a; 76:287-288.
12. Venkatachalam MR, Jebanesan A. Larvicidal activity of *Hydrocotyle javanica* Thunb. (Apiaceae) extract against *Culex quinquefasciatus*. *J. Exp. Zool*. 2001b; 4:99-101.
13. Kim SI, Shin OK, Song C, Cho KY, Ahn YJ. Insecticidal activities of aromatic plant extracts against four agricultural insects. Agri. Chem. Biotech. 2001; 44:23-26.
14. Cheng S, Liu J, Tsai K, Chen W, Chang S. Chemical composition and mosquito larvicidal activity of essential oils from leaves of different *Cinnamomum osmophloeum* provenances. J. Agric. Food Chem. 2004; 52:4395-4400.
15. Rahuman AA, Venkatesan P, Gopalakrishnan G. Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrulluscolocynthis* (Linn.) Schrad. *Parasitol. Res*. 2008; 103:1383-1390.
16. Mathew N, Anitha MG, Bala TSL, Sivakumar SM, Narmadha R, Kalyanasundaram M *et al*. Larvicidal activity of *Saracaindica*, *Nyctanthes arbortristis* and *Clitoriaternatea* extracts against three mosquito vector species. *Parasitol. Res.* 2009; 104:1017-1025.
17. Rajkumar S, Jebanesan A. Repellency of volatile oils from *Moschosma polystachyum* and *Solanum xanthocarpum* against filarial vector *Culex quinquefasciatus* Say. *Tropical. Biomed*. 2005; 22:139-142.
18. Pushpanathan T, Jebanesan A, Govindarajan M. Larvicidal, ovicidal and repellent activities of *Cymbopogancitrates* Stapf (Graminae) essential oil against the filarial mosquito *Culex quinquefasciatus* (Say) (Diptera: Culicidae). *Tropical. Biomed*. 2006; 23:208-212.
19. Moore SJ, Lenglet A, Hill N. Field evaluation of three plant-based insect repellents against malaria vectors in VacaDiez province, the Bolivian Amazon. *J. Am. Mosq. Contr. Assoc.* 2002; 18:107-110.
20. Omolo MO, Okinyo D, Ndiege IO, Lwande W, Hassanali A. Repellency of essential oils of some Kenyan plants against *Anopheles gambiae*. *Phytochemistry*. 2004; 65:2797-2802.
21. WHO Report of the WHO informal consultation on the evaluation on the testing of insecticides. CDT/WHO PES/IC/. 1996; 96(1):69.
22. WHO Guidelines for efficacy testing of mosquito repellents for human skin. WHO/HTM/NTD/ WHOPES/, 2009, 4. Control of neglected tropical diseases, World Health Organization, Geneva.
23. Pierre DY, Okechukwu EC, Nchiwan NE. Larvicidal and phytochemical properties of *Callistemon rigidus* R. Br. (Myrtaceae) leaf solvent extracts against three vector mosquitoes. *J.Vect. Bor. Dis*. 2014; 51(3):216-

223.
24. Govindarajan M, Rajeswary M, Amsath A. Larvicidal properties of *Caesalpinia pulcherrima* (Family: Fabaceae) against *Culex tritaeniorhynchus*, *Aedesalbopictus* and *Anopheles subpictus* (Diptera: Culicidae). *Int. J. Pur. Appl. Zool*. 2013; 1(1):15-23.
25. Mokua GN, Innocent E, Mbwambo HZ, Lwande W, Bwire JO, Hassanali A *et al*. Larvicidal and brine shrimp activities of *Vitexschiliebenii* extracts and isolated phyto ecdysteroids on *Anopheles gambiae* Giles S.S larvae. *J. Appl. Pharm. Sci*. 2013; 3(5):91-95.
26. Mousumi K, Anjali R, Goutam C. Evaluation of mosquito larvicidal activities of seed coat extract of *Cassia sophera* L. *J. Mosq. Res*. 2013; 3(11):76-81.
27. Danga YSP, Nukenine EN, Esimone CO, Younoussa L. Larvicidal and pupicidal toxicities of *Plectranthusglandulosus* and *Callistemon rigidus* leaf essential oils against three mosquito species. *J. Mosq. Res*. 2014; 4(2):5-14.
28. Zhu BCR, Henderson G, Chen F, Fei H, Laine RA. Evaluation of vetiver oil and seven insect-active essential oilsagainst the Formosan subterranean termite. *J. Chem. Ecol*. 2001; 27:1617-1625.
29. Amer A, Mehlhorn H. Larvicidal effects of various essential oils against *Aedes*, *Anopheles*, and *Culex* larvae (Diptera, Culicidae). *Parasitol. Res.* 2006; 99:466-472.
30. An NTG, Huong LT, Satyal P, Tai TA, Dai DN, Hung NH *et al*. Mosquito Larvicidal Activity, Antimicrobial Activity, and Chemical Compositions of Essential Oils from Four Species of Myrtaceae from Central Vietnam. *Plants*. 2020; 9(4):544.
31. Scalerandi E, Flores GA, Palacio M, Defagó MT, Carpinella MC, Valladares G. Understanding synergistic toxicity of terpenes as insecticides: Contribution of metabolic detoxification in Musca domestica. Front. Plant Sci. 2018; 9:1579.
32. Palanikumar M, Pravin Y, Navaneethan M, Mahendren S, Mohanraj RS, Dhanakkodi B. *Callistemon citrinus* (Myrtaceae) methanolic leaf extract: a potent mosquitocidal agent for controlling dengue vector mosquito *Aedesaegypti* (Diptera: Culicidae). J. Entom. Zool. Stud. 2017; 5(3):1051-1059.
33. Shaalan E, Canyon DV, Faried MW, Abdel-Wahab H, Mansoura A. A review of botanical phytochemicals with mosquitocidal potential. *Env. Int.* 2005; 31:1149-1166.
34. Patil SV, Patil CD, Salunkhe RB, Salunke BK. Larvicidal activities of six plants extracts against two mosquito species, *Aedesaegypti* and *Anopheles stephensi*. *Trop. Biomed*. 2010; 27:360-365.
35. Kaliyamoorthy DASS, Mariappan P. Larvicidal Activity of *Aegle marmelos*, *Coleus aromaticus* and *Vitexnegundo* leaf extract against filarial vector *Culex quinquefasciatus*. *Turk. Tarımve. Doga. Bilimleri*. 2014; 6:858-862.
36. Sarma R, Mahanta S, Khanikor B. Insecticidal Activities of the Essential Oil of *Aegle marmelos* (Linnaeus, 1800) against *Aedesaegypti* (Linnaeus, 1762) and *Culex quinquefasciatus* (Say, 1823).*Uni. J. Agri. Res*. 2017; 5:304-311.
37. Athira A, Pushpalatha E. Essential oils of a few aromatic plants and their potential as knockdown, repellent and adulticidal agent to the filarial vector, Culex quinquefasciatus Say (Diptera: Culicidae). International Journal of Mosquito Research. 2021; 8(1):35-40.