Original Article

Larvicidal Activity of Essential Oil of Syzygium aromaticum (Clove) in Comparison with Its Major Constituent, Eugenol, against Anopheles stephensi

Mahmoud Osanloo 1,2, Mohammad Mehdi Sedaghat 3, Fariba Esmaeili 1, *Amir Amani 1,4

1Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran
2Students’ Scientific Research Center, Tehran University of Medical Sciences, Tehran, Iran
3Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
4Natural Products and Medicinal Plants Research Center, North Khorasan University of Medical Sciences, Bojnourd, Iran

(Received 22 May 2017; accepted 31 June 2018)

Abstract

Background: In this study, larvicidal activity of clove essential oil (EO), as a green and relatively potent larvicide, was compared with its main constituent, Eugenol, against Anopheles stephensi.

Methods: High-performance liquid chromatography (HPLC) was used to determine the amount of eugenol, major constituent of clove EO. In addition, larvicidal activity of clove EO and eugenol was evaluated against An. stephensi.

Results: The amount of eugenol in clove EO was determined as 67% using HPLC analysis. LC50 and LC90 of clove EO (57.49 and 93.14ppm, respectively) were significantly lower than those of eugenol (86.96 and 128.18 ppm, respectively).

Conclusion: EO showed more effective than its major component. Considering the lower cost of the essential oil and lower risk in occurrence of resistance in larvae, use of clove EO is preferred as larvicide in comparison with eugenol, against An. stephensi.

Keywords: Larvicidal activity, HPLC, Syzygium aromaticum, Eugenol, Essential oil, Anopheles stephensi

Introduction

More than 17% of all infectious diseases around the world are vector-borne diseases, such as dengue fever, yellow fever, and malaria (1). The number of death for such diseases is more than 1 million annually e.g. malaria caused 429000 death just in 2015 (1, 2). In order to control malaria, WHO recommends control of larva which now used in 55 countries (2). Unfortunately, due to frequent use of synthetic larvicides (such as Temephos), not only environmental pollution have appeared, but also many cases of resistance has occurred in mosquitoes around the world (3-6). Essential oils (EOs) have been suggested as alternative sources for insect control as repellents, insecticides or larvicides and they offer advantageous such as biodegradability and negligible effects on non-target specious and environment (7, 8).

Recently, comparisons of larvicidal activity of EOs with their major components have been reported (9, 10). However, there is no conclusion so fare, EOs are better or their major constituent(s) in terms of larvicidal activity.

Syzygium aromaticum (Clove) belongs to the Myrtaceae family which considers as an important medicinal plant with wide range of biological activities such as anti-bacterial or anti-oxidant activities (11, 12). Clove EO has also shown larvicidal activity against field collected larva of Ae. Aegypti with LC50 of 92.56 and 62.3ppm in two different reports (13, 14).
Eugenol, as the major constituent of clove EO, has also indicated larvicidal activity against laboratory reared and field collected of Ae. aegypti (LC50: 33 and 93.3ppm, respectively) (14, 15). Its larvicidal activity against other population of mosquito has been documented, with LC50 of 25.4, 28.14 and 30.8ppm against An. subpictus, Ae. albopictus and Cx. tritaeniorhynchus (16).

Anopheles stephensi is an important malaria vector with wide distribution in the Arabian Peninsula, the Indian subcontinent, Afghanistan and Iran (17-19). In this research, for the first time, larvicidal activity of clove EO and eugenol against An. stephensi was evaluated and compared.

Materials and Methods

Materials

High-performance liquid chromatography (HPLC) grade methanol, pure eugenol (99%) and Ethanol were supplied by Merck (Germany). Clove EO was purchased from Green Plants of Life Co (Iran).

Determination of eugenol contents in clove EO oil by HPLC

HPLC analysis was used to determine the amount of eugenol in clove EO. The apparatus consisted of a 30cmx 3.9mm reverse phase C18 column (Waters, Milford, USA), a pressure less injection pump (Model L-6200, Hitachi, Japan) to drive solvent and loading of samples, a UV visible detector (Model L-4000, at 280nm, Hitachi, Japan) for detection, a chromato-integrator (Model D-2500, Hitachi, Japan) for analyses. A mixture of methanol and distilled water was used as mobile phase with flow-rate of 1mL/min. Analytical procedure was started with dissolving 50µL of eugenol or 0.5mL of clove EO in 10mL of methanol, then, 20µL of this solution was injected into system at a flow-rate of 0.7mL/min. The optimum mobile phase with a methanol: water ratio of 80:20 was used for elution. By comparing peak areas of eugenol with solution of clove EO, its amount in clove EO was determined.

Evaluation of larvicidal activity

Third and fourth instar larvae of An. stephensi were used, they obtained from insectarium of Tehran University of Medical Sciences. They reared in special condition: 28±2 °C, 12:12 dark and light periods and relative humidity of 65±5%. Larvicidal bioassay was performed in line with WHO recommendation test in lab, with some modifications (20). Standard solutions were prepared by dissolving in ethanol at appropriate concentrations (i.e. eugenol 60 µL/mL and clove EO 30µL/mL). By adding 1mL from each sample (0.5%/v/v) to cups containing 199mL of no chlorine water, desired concentrations of samples were prepared. Using separated nets, 25 larvae of An. stephensi were added slowly to all containers. Dead and moribund larvae (unable to respond to stimulating agent) were counted after 24h of exposing in all cups. Larvicidal bioassays were performed in 16 repetitions at 4 different replicates at concentrations (ppm) of 12.5, 25, 50, 75, 100, 150 for clove EO and 12.5, 25, 50, 100, 150, 200 and 300 for eugenol. In each replicate, two control groups were considered having ethanol (0.5%/v/v) with similar treatments. Lethal concentrations of each sample (i.e. LC50 and LC90), were determined using a probit regression model in SPSS ver. 19 (Chicago, IL, USA).

Comparison of larvicidal activity of clove EO with eugenol

Evaluation of overlaps between confidence intervals (CI) of two groups is an easy and common approach to compare various LC values. If no overlap is observed, the difference is considered as significant (21). LC50 and LC90 of clove EO and eugenol were calculated and compared using independent sample test by SPSS.

http://jad.tums.ac.ir
Published Online: December 25, 2018
Results

Determining content of eugenol in clove EO

Results of HPLC analysis of eugenol and clove EO samples are shown in Fig. 1. Comparing the graphs, the peak related to eugenol is observed at retention time of 6.43 and 6.41min, for eugenol and clove EO, respectively. By comparing peak areas of eugenol and clove EO, percentage of eugenol in clove EO was calculated as 67%, considered relatively high.

Evaluation of larvicidal activity of clove EO and eugenol

Results of larvicidal activities of clove EO and eugenol against An. stephensi are shown in Fig. 2. Calculated LC50 and LC90 values were 57.49 and 86.96ppm for clove EO and 93.14 and 158.2ppm for eugenol, respectively (Table 1).

Larvicidal activity in both samples (i.e. clove EO and eugenol) appeared at 25ppm and enhanced with increasing the concentration of those. Nevertheless, perfect larvicidal activities were achieved at 100 and 200ppm for clove EO and eugenol, respectively. Furthermore, no overlap in CI of LC50 and LC90 for clove EO and eugenol are observed, thus, larvicidal activity of clove EO is significantly better than eugenol (Table 1). Moreover, Probit regression line of the both clove EO and eugenol are illustrated in Fig. 3.

| Specimen  | A     | B±SE  | LC50 (ppm) CI: (LCL–UCL) | LC90 (ppm) CI: (LCL–UCL) | χ2 (df) | Sig              |
|-----------|-------|-------|--------------------------|--------------------------|---------|------------------|
| Clove EO  | -2.50 | 0.04±0.002 | 57.49 (43.28–74.24)       | 86.96 (71.19–128.18)     | 47.96 (3) | 0.15 > sig*       |
| Eugenol   | -1.83 | 0.02±0.001 | 93.14 (75.60–113.33)      | 158.2 (133.85–201.20)    | 39.13 (4) | 0.15 > sig*       |

A= intercept; B±SE= slope and standard error of the line; CI= confidence interval (0.05), UCL= Upper Confidence Limit, LCL= Lower Confidence Limit, χ2 (df)= Chi 2 and degree of freedom. *Since the significance level is less than 0.15, a heterogeneity factor is used in the calculation of confidence limits.

Fig. 1. HPLC profile of solution of eugenol and clove EO, related peak for eugenol appeared at retention time of 6.43 and 6.41min, respectively
Discussion

Determining content of eugenol in clove EO

In this study, amount of eugenol in clove EO was found as 67%, which is comparable with other reports. Reviewing other reports, content of eugenol has been reported in values more or less similar to this value: e.g. 58.29% (22), 59.29% (23), 76.8% (24), 86.61% (25) and 88.58% (26).

Evaluation of larvicidal activity of clove EO and eugenol

Obtained LC50 of clove EO and eugenol against An. stephensi were 57 and 93ppm, respectively. LC90 values were 86 and 158ppm, respectively. There are many reports about larvicidal activities of other EOs against An. stephensi. For example, larvicidal activities (LC50) of some essential oils such as Artemisia dracunculus (11.36ppm), Anethum graveolens (38.80ppm) and Kelussia odoratissima (4.77ppm) were evaluated (27-29).

Determined LC50 of clove EO in this research is lower than many reports against An. stephensi. For instance, Lawsonia inermis (69.40ppm) (30), Cionura erecta (77.30ppm) (31) and Cupressus arizonica (79.30ppm) (32), Zhumeria majdae (61.34ppm) (33). However, calculated LC50 in some other reports is lower than our reported LC50, for instance, Bunium persicum (27.72ppm) (34), Tanacetum persicum and Achillea kellarensis (48.64 and 35.42ppm respectively) (35), Satureja bachtiarica (24.27ppm) (36) and Citrus aurantium (31.20ppm) (37).

Larvicidal activity of either of clove EO and eugenol, against other species of mosquito, has already been reported. Ae. aegypti is shown to be more susceptible than Cx. quinquefasciatus when using clove EO (i.e. LC50: 92.56 vs. 124.42ppm) (13). Larvicidal activity of synthetic derivatives of eugenol against Ae. aegypti has been shown (LC50~ 62.3ppm or higher) (14).

Recently, many reports have been released...
about comparison of larvicidal activity of EOs with their major components. However, there is no conclusion so far whether EOs outperforms or their major constituent in terms of larvicidal efficacy.

LC$_{50}$ in Allium tuberosum EO against Ae. albopictus was found to be 17.9ppm, lower than that of its two major components, allyl methyl trisulfide and dimethyl trisulfide, with LC$_{50}$ of 27.5 and 36.4ppm, respectively (38). LC$_{50}$ of Ruta chalepensis EO has been evaluated against Anopheles quadriraculatus (14.9 ppm) and Aedes aegypti (22.2ppm). While, 2-undecanone, its major component, showed similar LC$_{50}$ to that of total EO against An. quadriraculatus (14.2ppm), significantly lower values against Ae. aegypti (14.37ppm) were obtained (21).

LC$_{50}$ of EO of Allium macrostemon (72.86 ppm) was better than a major constituent, methyl propyl disulfide (86.16ppm), while its efficacy was lower than the other major component, dimethyl trisulfide (36.36ppm), against Ae. albopictus (40).

Synergistic effects of constituents of some EOs are nowadays well-known when they are used as anti-fungal or anti-bacterial agents (39, 40). Our findings in this research and our previous study also show that An. stephensi is more susceptible to the clove EO or EO of Kelussia odoratissima (with LC$_{50}$ of 57.49 and 4.77ppm, respectively), compared with their major constituents, eugenol (93.14ppm) and Z- ligustilide (8.73ppm), respectively (28). A type of synergism may have occurred in larvicidal activity of the EOs too.

EOs are mixtures of many constituents such as flavonoids, alkaloids, and monoterpenes (41, 42). Modes of action of mentioned constituents are different e.g. main site action of flavonoids is acetylcholinesterase (43), while alkaloids and monoterpenes target Na-K-ATPase or Na$^+$ and K$^+$ channels (44-46). This could be the main reason for occurring synergism in larvicidal activity in our study. Having mentioned that resistance against larvicides is most-ly observed when a single active agent is used compared with those having multi components (47-49).

**Conclusion**

Use of clove EO as a green larvicide against An. stephensi is preferred compared with its major constituent (Eugenol). Considering the fact that the EO is a lot cheaper than eugenol and is composed of several components, thus, has lesser chance of occurring resistance, the whole EO may be suggested as a proper larvicide.

**Acknowledgements**

This research has been supported by Students’ Scientific Research Center, Tehran University of Medical Sciences, grant No. 95-01-61-31420, and also had ethical approval by its ethical committee center, IR.TUMS.REC.1395.2480.

The authors declare that there is no conflict of interests.

**References**

1. World Health Organization (2017) Vectorborne diseases fact sheet. Available at: http://www.who.int/mediacentre/factsheets/fs387/en/
2. World Health Organization (2016) World Malaria Report. Available at: http://apps.who.int/iris/bitstream/10665/252038/1/9789241511711-eng.pdf?ua=1
3. Soltani A, Vatandoost H, Oshaghi MA, Ravasan NM, Enayati AA, Asgarian F (2015) Resistance Mechanisms of Anopheles stephensi (Diptera: Culicidae) to Temephos. J Arthropod Borne Dis. 9 (1): 71–83.
4. Vatandoost H, Hanafi-Bojd A (2005) Current resistant status of Anopheles stephensi
listen to different larvicides in Hormozgan Province, southeastern Iran, 2004. Pak J Biol Sci. 8: 1568–1570.

5. Vatandoost H, Mashayekhi M, Abaie M, Aflatoonian M, Hanafi-Bojd A, Sharifi I (2005) Monitoring of insecticides resistance in main malaria vectors in a malarious area of Kahnooj District, Kerman Province, southeastern Iran. J Vector Borne Dis. 42(3): 100–108.

6. Melo-Santos MA, Varjal-Melo JJ, Araújo AP, Gomes TC, Paiva MH, Regis LN, Furtado AF, Magalhaes T, Macoris ML, Andrighetti MT, Ayres CF (2010) Resistance to the organophosphate temephos: mechanisms, evolution and reversion in an Aedes aegypti laboratory strain from Brazil. Acta Trop. 113(2): 180–189.

7. Liu XC, Liu Q, Chen XB, Zhou L, Liu ZL (2015) Larvicidal activity of the essential oil from Tetradium glabri folium fruits and its constituents against Aedes albopictus. Pest Manag Sci. 71(11): 1582–1586.

8. Vatandoost H, Sanei Dehkordi A, Sadeghi SM, Davari B, Karimian F, Abai MR, Sedaghat MM (2012) Identification of chemical constituents and larvicidal activity of Kelussia odoratissima Mozafarian essential oil against two mosquito vectors Anopheles stephensi and Culex pipiens (Diptera: Culicidae). Exp Parasitol. 132(4): 470–474.

9. Govindarajan M, Benelli G (2016) Alpha-Humulene and beta-elemene from Syzygium zeylanicum (Myrtaceae) essential oil: highly effective and eco-friendly larvicides against Anopheles subpictus, Aedes albopictus, and Culex tritaeniorhynchus (Diptera: Culicidae). Parasitol Res. 115(7): 2771–2778.

10. Liu XC, Liu Q, Chen XB, Liu QZ, Liu ZL (2015) Larvicidal activity of the essential oil of Youngia japonica aerial parts and its constituents against Aedes albopictus. Z Naturforsch C. 70(1–2): 1–6.

11. Thonggoom O, Punrattanasin N, Srisawang N, Promawan N, Thonggoom R (2016) In vitro controlled release of clove essential oil in self-assembly of amphiphilic polyethylene glycol-block-polycaprolactone. J Microencapsul. 33(3): 239–248.

12. Sebaaly C, Charcosset C, Stainmesse S, Fessi H, Greige-Gerges H (2016) Clove essential oil-in-cyclodextrin-in-liposomes in the aqueous and lyophilized states: From laboratory to large scale using a membrane contactor. Carbohydr Polym. 138: 75–85.

13. Fayemiwo KA, Adeleke MA, Okoro OP, Awojide SH, Awoniyi IO (2014) Larvicidal efficacies and chemical composition of essential oils of Pinus sylvestris and Syzygium aromaticum against mosquitoes. Asian Pac J Trop Biomed. 4(1): 30–34.

14. Barbosa JD, Silva VB, Alves PB, Gumina G, Santos RL, Sousa DP, Cavalcanti SC (2012) Structure-activity relationships of eugenol derivatives against Aedes aegypti (Diptera: Culicidae) larvae. Pest Manag Sci. 68(11): 1478–1483.

15. Cheng SS, Liu JY, Tsai KH, Chen WJ, Chang ST (2004) Chemical Composition and Mosquito Larvicidal Activity of Essential Oils from Leaves of Different Cinnamomum osmophloeum Provenances. J Agric Food Chem. 52(14): 4395–4400.

16. Govindarajan M, Rajeswary M, Hoti SL, Bhattacharyya A, Benelli G (2016) Eugenol, α-pinene and β-caryophyllene from Plantantherus barbatus essential oil as eco-friendly larvicides against malaria, dengue and Japanese encephalitis mosquito vectors. Parasitol Res. 115(2): 807–815.

17. World Health Organization W (2015) World Malaria report. Available at: https://www.who.int/malaria/publications/world-malaria-report-2015/report/en/.

18. Vatandoost H, Oshaghi M, Abaie M, Shahi M, Yaaghoobi F, Baghaii M, Hanafi-
Bojd AA, Zamani G, Townsend H (2006) Bionomics of *Anopheles stephensi* Liston in the malarious area of Hormozgan Province, southern Iran, 2002. Acta Trop. 97(2): 196–203.

19. Hanafi-Bojd AA, Vatandoost H, Oshaghi MA, Haghdoot A, Shahi M, Sedaghat MA, Abedi F, Yeryan M, Pakari A (2012) Entomological and epidemiological attributes for malaria transmission and implementation of vector control in southern Iran. Acta Trop. 121(2): 85–92.

20. World Health Organization (2005) Guidelines for laboratory and field testing of mosquito larvicides. Available at: https://apps.who.int/iris/handle/10665/69101.

21. Ali A, Demirci B, Kiyan HT, Bernier UR, Tsikolia M, Wedge DE, Khan IA, Başer KH, Tabanca N (2013) Biting deterrence, repellency, and larvicidal activity of *Ruta chalepensis* (Sapindales: Rutaceae) essential oil and its major individual constituents against mosquitoes. J Med Entomol. 50(6): 1267–1274.

22. Marya CM, Satija G, Nagpal R, Kapoor R, Ahmad A (2012) In vitro inhibitory effect of clove essential oil and its two active principles on tooth decalcification by apple juice. Int J Dent. 2012: 759618

23. Jalali N, Ariai P, Fattahi E (2016) Effect of alginate/carboxyl methyl cellulose composite coating incorporated with clove essential oil on the quality of silver carp fillet and *Escherichia coli* O157:H7 inhibition during refrigerated storage. J Food Sci Technol. 53(1): 757–765.

24. Jirovetz L, Buchbauer G, Stoilova I, Stoynanova A, Krastanov A, Schmidt E (2006) Chemical composition and antioxidant properties of clove leaf essential oil. J Agric Food Chem. 54(17): 6303–6307.

25. Tian BL, Liu QZ, Liu ZL, Li P, Wang JW (2015) Insecticidal Potential of Clove Essential Oil and Its Constituents on *Cacopsylla chinensis* (Hemiptera: Psyllidae) in Laboratory and Field. J Econ Entomol. 108(3): 957–961.

26. Chaieb K, Hajlaoui H, Zmantar T, Kahl-Nakbi AB, Rouabha M, Mahdouani K, Bakhrouf A (2007) The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzygium aromaticum* L. Myrtaceae): a short review. Phytother Res. 21(6): 501–506.

27. Osanloo M, Amani A, Sereshti H, Abai MR, Esmaeili F, Sedaghat MM (2017) Preparation and optimization nanoeulsion of Tarragon (*Artemisia dracunculus*) essential oil as effective herbal larvicide against *Anopheles stephensi*. Ind Crops Prod. 109: 214–219.

28. Osanloo M, Amani A, Sereshti H, Shayeghi M, Sedaghat MM (2017) Extraction and chemical composition essential oil of *Kelussia odoratissima* and comparison its larvicidal activity with Z-ligustilide (Major Constituent) against *Anopheles stephensi*. J Entomol Zool Stud. 5(4): 611–616.

29. Osanloo M, Sereshti H, Sedaghat MM, Amani A (2017) Nanoeulsion of Dill essential oil as a green and potent larvicide against *Anopheles stephensi*. Environ Sci Pollut Res Int. 25(7): 6466–6473.

30. Khanavi M, Vatandoost H, Khosravi Dehaghi N, Sanei Dehkordi A, Sedaghat MM, Hadjiakhoondi A, Hadjiakhoondi F (2013) Larvicidal activities of some Iranian native plants against the main malaria vector, *Anopheles stephensi*. Acta Med Iran. 51(3): 141–147.

31. Mozaffari E, Abai MR, Khanavi M, Vatandoost H, Sedaghat MM, Moridnia A, Saber-Navaei M, Sanei-Dehkordi A, Rafi F (2014) Chemical Composition, Larvicidal Activity of...
cidal and Repellency Properties of Cion-
nura erecta (L.) Griseb. Against Malaria Vector, Anopheles stephensi Liston (Diptera: Culicidae). J Arthropod Borne Dis. 8(2): 147–155
32. Sedaghat MM, Dehkordi AS, Khanavi M, Abai MR, Mohtarami F, Vatandoost H (2011) Chemical composition and larvicidal activity of essential oil of Cupressus arizonica E.L. Greene against malaria vector Anopheles stephensi Liston (Diptera: Culicidae). Pharmacognosy Res. 3(2): 135–139.
33. Sanei-Dehkordi A, Soleimani-Ahmadi M, Akbarzadeh K, Salim Abadi Y, Paksa A, Gorouhi MA, MohamMadi-Azni S (2016) Chemical composition and mosquito larvicidal properties of essential oil from leaves of an Iranian indigenous plant Zhumeria majdae. J Essent Oil Bear Pl. 19(6): 1454–1461.
34. Sanei-Dehkordi A, Vatandoost H, Abaei MR, Davari B, Sedaghat MM (2016) Chemical composition and larvicidal activity of Bunium persicum essential oil against two important mosquitoes vectors. J Essent Oil Bear Pl. 19(2): 349–357.
35. Soleimani-Ahmadi M, Sanei-Dehkordi A, Turkhi H, Madani A, Abadi YS, Paksa A, Gorouhi MA, Rashid G (2017) Phytochemical Properties and Insecticidal Potential of Volatile Oils from Tanacetum persicum and Achillea kfellalensis Against Two Medically Important Mosquitoes. J Essent Oil Bear Pl. 20(5): 1254–1265.
36. Soleimani-Ahmadi M, Abtahi SM, Madani A, Paksa A, Abadi YS, Gorouhi MA, Sanei-Dehkordi A (2017) Phytochemical profile and mosquito larvicidal activity of the essential oil from aerial parts of Satureja bachitarica Bunge against malaria and lymphatic filariasis vectors. J Essent Oil Bear Pl. 20(2): 328–336.
37. Sanei-Dehkordi A, Sedaghat MM, Vatandoost H, Abai MR (2016) Chemical compositions of the peel essential oil of Citrus aurantium and its natural larvicidal activity against the malaria vector Anopheles stephensi (Diptera: Culicidae) in comparison with Citrus paradisi. J Arthropod Borne Dis. 10(4): 577–585.
38. Liu XC, Zhou L, Liu Q, Liu ZL (2015) Laboratory Evaluation of Larvicidal Activity of the Essential oil of Allium tuberosum Roots and its Selected Major Constituent Compounds Against Aedes albopictus (Diptera: Culicidae). J Med Entomol. 52(3): 437–441.
39. Samber N, Khan A, Varma A, Manzoor N (2015) Synergistic anti-candidal activity and mode of action of Mentha piperita essential oil and its major components. Pharm Biol. 53(10): 1496–504.
40. Wongsupan K, Phanthong P, Bunyaphatsara N, Srisukh V, Chomnawang MT (2014) Synergistic interaction and mode of action of Citrus hystrix essential oil against bacteria causing periodontal diseases. Pharm Biol. 52(3): 273–280.
41. Alaççek A, Bozkurt M, Çabuk M (2004) The effect of a mixture of herbal essential oils, an organic acid or a probiotic on broiler performance. S Afr J Anim Sci. 34(4): 217–222.
42. Helander IM, Alakomi H-L, Latva-Kala K, Mattila-Sandholm T, Pol I, Smid EJ, Gorris L, Wright A (1998) Characterization of the action of selected essential oil components on Gram-negative bacteria. J Agric Food Chem. 46 (9): 3590–3595.
43. Perumalsamy H, Jang MJ, Kim JR, Kadarkarai M, Ahn YJ (2015) Larvicidal activity and possible mode of action of four flavonoids and two fatty acids identified in Millettia pinnata seed toward three mosquito species. Parasit Vectors. 8(1): 237.
44. Isman MB (2006) Botanical insecticides, deterrents, and repellents in modern...
agriculture and an increasingly regulated world. Annu Rev Entomol. 51: 45–66.

45. Lucia A, Zerba E, Masuh H (2013) Knockdown and larvicidal activity of six monoterpenes against Aedes aegypti (Diptera: Culicidae) and their structure-activity relationships. Parasitol Res. 112 (12): 4267–4272.

46. Rajashekar Y, Shivanandappa T (2017) Mode of Action of the Natural Insecticide, Decaleside Involves Sodium Pump Inhibition. PloS One. 12(1): e0170836.

47. Araujo AF, Ribeiro-Paes JT, Deus JT, Cavalcanti SC, Nunes Rde S, Alves PB, Macoris ML (2016) Larvicidal activity of Syzygium aromaticum (L.) Merr and Citrus sinensis (L.) Osbeck essential oils and their antagonistic effects with temephos in resistant populations of Aedes aegypti. Mem Inst Oswaldo Cruz. 111(7): 443–449.

48. Intirach J, Junkum A, Tuetun B, Choochote W, Chaithong U, Jitpakdi A, Riyong D, Champakaew D, Pitasawat B (2012) Chemical constituents and combined larvicidal effects of selected essential oils against Anopheles cracens (Diptera: Culicidae). Psyche J Entomol. 2012: ID 591616.

49. Okumu FO, Knols BG, Fillinger U (2007) Larvicidal effects of a neem (Azadirachta indica) oil formulation on the malaria vector Anopheles gambiae. Malar J. 6 (1): 63.