Annals of Parasitology 2018, 64(4), 351–360
doi: 10.17420/ap6404.171

Original papers

The insecticidal and repellent activity of ginger (*Zingiber officinale*) and eucalyptus (*Eucalyptus globulus*) essential oils against *Culex theileri* Theobald, 1903 (Diptera: Culicidae)

Samin Madreseh-Ghahfarokhi[1], Yaser Pirali[2], Azam Dehghani-Samani[3], Amir Dehghani-Samani[4]

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Azadi Square, Mashhad, Iran
[2]Department of Pathobiology, Faculty of Veterinary Medicine, Shahrekord University, Rahbar Boulevard, Shahrekord, Iran
[3]Faculty of Veterinary Medicine, Shahrekord University, Rahbar Boulevard, Shahrekord, Iran
[4]Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahrekord University, Rahbar Boulevard, Shahrekord, Iran

Corresponding Author: Amir Dehghani-Samani; e-mail: amirds2008@gmail.com

ABSTRACT. Insecticidal and repellent activity of essential oils of *Zingiber officinale* and *Eucalyptus globulus* against *Culex theileri* Theobald, 1903 as a wide-distributed species of mosquitoes in different parts of world with an important role in transmission of infectious organisms and agent were studied. Essential oils were extracted from fresh parts of plants and different concentrations of 250 µl/ml, 500 µl/ml, 750 µl/ml and 1 (undiluted extract) were prepared for each of essential oils. Insecticidal and repellent activity of essential oils against adult form of *Culex theileri* Theobald mosquitoes, collected from small pools located near the Zayande-Rood River in the Saman city, Iran, were examined via direct exposure method and Y-tube olfactometer bioassay, respectively. Results of current study, as the first study on insecticidal and repellent activity of mentioned essential oils against *Culex theileri* Theobald, show considerable values of insecticidal and repellent activity against mosquitoes, concentration of one (undiluted extract) had the highest insecticidal and repellent activity against *Culex theileri* Theobald for both of essential oils and essential oil of *Eucalyptus globulus* (66% insecticidal and 74% repellent activity) was more potent than *Zingiber officinale* (45% insecticidal and 61% repellent activity). This study shows that these essential oils can be considered as good replaces for chemical pesticides but more experiments are need for this purpose. Study on insecticidal activity of these essential oils in the field condition can be considered as a subject for next experiments.

Key words: *Culex theileri*, *Eucalyptus globulus*, *Zingiber officinale*, insecticidal, repellent

Introduction

Mosquitoes from *Culex* genus as an important vector for many important organisms, known as ecto-parasites, have a broad geographic distribution in the world [1,2]. Natural infection of *Culex* genus by West Nile virus [3,4], Sindbis arboviruses [5–7], *Dirofilaria immitis* [8–10] and Rift Valley fever virus [11,12] were reported. Same as other member of *Culex* genus, *Culex theileri* Theobald, 1903 (Diptera: Culicidae), as a widespread mosquitoes is naturally infected by West Nile virus, Rift Valley fever virus, *Dirofilaria immitis* and Sindbis arboviruses too [8–10,13].

In order to control of this potentiality in transmission of important organisms, use of pesticides is increasing every day. It has been estimated that about 2.5 million tons of pesticides are used on crops each year and the worldwide damage caused by pesticides reaches $100 billion annually [14]. The reasons for this are: (a) the high toxicity and non-biodegradable properties of pesticides and (b) the residues in soil, water resources and crops that affect public health [14].

Thus, searching for replace of chemical pesticides is necessary in order to firstly solve the problem of long term toxicity to mammals and secondly to find environmental friendly pesticides and develop techniques that can be used to reduce pesticide use while maintaining crop yields [14].

Biological control of insects and arthropods by some bacteria is a replaced way to control their population [15]. For example, the insecticidal bacterium *Bacillus thuringiensis* is a Gram-positive bacterium that produces proteinaceous inclusions during sporulation [15,16]. These inclusions can be distinguished as distinctively shaped crystals by phase-contrast microscopy. The inclusions are composed of proteins known as crystal proteins, Cry proteins, or δ-endotoxins, which are highly toxic to a wide variety of important agricultural and health-related insect pests as well as other invertebrates [15]. Because of their high specificity and their safety for the environment, crystal proteins are a valuable alternative to chem ical pesticides for control of insect pests in agriculture and forestry and in the home. It has been proposed that the rational use of *Bacillus thuringiensis* toxins will provide a variety of alternatives for insect control and for overcoming the problem of insect resistance to pesticides [15]. Adding to biological control of arthropods by Bacteria, it is seen that natural products are also excellent alternatives for chemical insecticides.

Different kinds of plants have been exploited for their medicinal use from ancient time [17], they are known as great source of medications and are used to prevention or treatment of several infections, diseases and ailments. The use of plant medicines is widely accepted in all over the world [18]. Also it is identified that several plants contain compounds that they use in preventing attack from insects. These chemicals fall into several categories, including repellents, feeding deterrents, toxins, and growth regulators [19].

Ginger (rhizome of *Zingiber officinale* Roscoe) is one of the most widely used herbal medications in oriental medicine against pain, inflammation, stomach problems, nausea, vomiting, epilepsy, sore throat, cough, common cold, bruises, wounds, liver complaints, rheumatism, muscular pains, atherosclerosis, migraine headaches, high cholesterol, ulcers, and etc. [20]. Ginger essential oil can produce from fresh rhizomes and it has many efficient effects like antibacterial, antiviral, antifungal and other properties [21,22].

Also, genus *Eucalyptus* that knows by over 700 species distributed throughout the world, provides variety of components extracted from its essential oil known as acaricide agent [23,24], antimicrobial and antioxidant activity of eucalyptus essential oil were also reported [25,26]. Effect of eucalyptus essential oil on respiratory bacteria and viruses, traditional use of eucalyptus in treatment of rhinosinusitis and anti-diabetic effect of eucalyptus were identified too [27–29].

In this study insecticidal and repellent activity of essential oils of *Zingiber officinale* and *Eucalyptus globulus* against *Culex theileri* Theobald as a wide-distributed species of *Culex* genus in different parts of world including Iran with an important role in transmission of mentioned organisms were studied. To the best of author's knowledge and current date, there is no study on insecticidal and repellent activity of *Zingiber officinale* and *Eucalyptus globulus* against *Culex theileri* Theobald, and this is the first survey in this way.

Materials and Methods

Extraction of essential oils. Fresh rhizomes of *Zingiber officinale* and leafs of *Eucalyptus globulus* were used to essential oil extraction. Essential oils extractions were done separately for each sample via conventional hydrodistillation method. 150 gram of each sample were crushed and added to 1000 ml distilled water in a round bottom flask. The flask was heated and the Clevenger apparatus was attached. The mixture was boiled at 100°C and then the temperature was reduced to 60°C and kept for 3 h, the recovered mixture was allowed to settle and finally essential oil was withdrawn for each sample separately [30]. In order to compare of efficiency of essential oil, percentage of essential oil yield for each plants was calculated via below equation. Essential oil yield (%) = [weight of extracted essential oil (g)/weight of sample (g)] × 100

Collection of the mosquitoes. Mature mosquitoes, which were usually flying near the surface of pools water, were collected from small pools located near the Zayande-Rood River in the Saman city, Chaharmahal Va Bakhtiari province, southwest of Iran (32°27′06″N 50°54′38″E). Insects were collected by horizontal movement of specific cylindrical nets (made of light weight metal wires as skeleton and covered by light weight cotton net with the approximately size of 1.2 m radius and 1 m length) near the surface of small pools water.

Collected mosquitoes were immediately transferred to specific glass boxes, kept in cool place away from any chemical agents and sunlight, transferred to laboratory for experiment. Note that some insects were injured during the collecting or transferring, that they were removed for experiment. Totally, about 3000–3500 health mosquitoes were collected. In order to confirmed species identification, 100 mosquitoes were chosen randomly and species identification was done under the laboratory optic loop via identification keys [31,32]. Differentially, 98% of collected mosquitoes were identified as *Culex theileri* Theobald and only about 2% were identified as *Culex pipiens*, other species of *Culex* genus. In order to deletion of biases in experiment, collected mosquitoes were immediately examined after collection. Till the experiments, mosquitoes were kept in glass boxes within dark place with 25°C temperature and about 60% humidity.

Examination groups. Different concentrations; 250 µl/ml, 500 µl/ml, 750 µl/ml and 1 (undiluted extract) were prepared for essential oil of *Zingiber officinale* and *Eucalyptus globulus* separately via combination by different amount of normal saline. In order to sure of tests validity, recommended commercial product groups with concentration of 100 mg/ml were prepared via dilution of 25 ml of Ripcord® (Cypermethrin 40%, Spiagri Company, Tehran, Iran) in 75 ml normal saline, also negative control groups were prepared from pure normal saline without any additive components.

Evaluation of insecticidal activity of essential oils. In this survey triplicate tests were done for each treatment, separately and in each replication 100 mosquitoes were examined for evaluation of insecticidal activity. In order to insecticidal activity evaluation, health mosquitoes were transferred to specific glass boxes with same condition including: temperature (25°C), humidity (about 60%), light and air circulation for each treatment. Then prepared concentrations were sprayed into boxes via small hole in wall of them. For each treatment only 15 puffs were applied. Same spray tools were prepared from same company and they were used as disposable tool and specific for each treatment. Note that all the methods were same for every groups and replications. After the 30 minutes, percentage of killed mosquitoes as insecticidal activity of each treatment were counted, then mean of insecticidal activity for each treatments were calculated by use of results of triplicate exams. After examinations, all of killed mosquitoes were carefully observed under the optic loop in order to species identification and if they were other species than *Culex theileri* Theobald, then they were deleted from results of experiments and they were not included in data and statistical analysis.

Evaluation of the repellent activity of essential oils. Repellent activity of each treatment against *Culex theileri* Theobald was studied by Y-tube olfactometer bioassay. Y-tube olfactometer consists of a glass Y-tube with a main arm (the stem) and 2 arms containing one repellent and a control in other one, where a low rate air movement is created by sucking the air in the two arms of the Y-tube with a pump connected to the stem. The essential oil sample and control are applied on a paper attached to the arms of the tube. Insects are introduced into the tube by a hole located at the center (the joint point of the three tubes). After introduction, the hole is closed with a rubber stopper and the pump is operated. After 2 minutes of exposition, the number of insects on each of the 2 tubes (treated and control) are scored to assess the percentage of repellency [33,34]. In this study, 50 mosquitoes were put into every arm for each replication (totally 100 mosquitoes in two arms) and 10 ml of each essential oil (undiluted extract) was added into treated tube and pure normal saline into control tube. Two minutes after pumping, percentages of repellent mosquitoes were reported as repellent activities for treatments, recommended commercial product and negative control groups. Note that after each application, Y-tube olfactometer was cleaned, washed and dried for next examination. Finally, pure data were analyzed statistically and insecticidal and repellent activities of each concentration of essential oils (percent values) were reported.

Statistical analysis. The analyzed data were expressed as the mean±standard error of the mean (SEM) using Sigma stat (version 3.1) software. Groups were compared using one-way ANOVA for repeated measurements. A value of ($P \leq 0.05$) was considered significant.

Results

Insecticidal activity of essential oils

In this study, percentage of essential oil yield for *Zingiber officinale* and *Eucalyptus globulus* were 0.213% and 0.235%, respectively. Different concentrations of essential oil of *Zingiber officinale* had great insecticidal activity against *Culex theileri* Theobald versus negative control groups. There

Table 1. Insecticidal activity or percentage of killed mosquitoes (mean±SEM)% of different concentration for each essential oil versus negative and positive control groups

Groups	*Zingiber officinalis*	*Eucalyptus globulus*
250 µl/ml	19.85±2.33*[a], (P=0.024)	27.41±2.75[a], (P=0.035)
500 µl/ml	24.39±1.15[b], (P=0.027)	39.52±3.06[b], (P=0.028)
750 µl/ml	35.62±3.72[c], (P=0.035)	46.39±2.78[c], (P=0.027)
1 (undiluted extract)	44.98±2.36[d], (P=0.026)	66.23±4.02[d], (P=0.025)
Negative Control	0[e], (P=0.022)	0[e], (P=0.039)
Positive Control	100[f], (P=0.038)	100[f], (P=0.033)

*Presence of different superscript lowercase letters (a-f) shows the significant differences (P≤0.05) between efficacies of different concentrations (rows) of each essential oil (column).

were significant differences (P≤0.05) in insecticidal activity of different concentrations and it is efficacy was more sensible at the higher concentrations and highest insecticidal activity was seen at the concentration of 1 (undiluted extract). Highest amounts of increases in it's insecticidal activity were observed between concentration of 500 µl/ml and 750 µl/ml, and between concentration of 750 µl/ml and 1 (undiluted extract). Whole of the mosquitoes in recommended commercial product groups were killed due to exposure by chemical insecticide agent and whole of the mosquitoes in negative control groups were survived after the experiment (Table 1).

Considerable values of insecticidal activity of essential oil of *Eucalyptus globulus* against *Culex theileri* Theobald were also observed. Same as the ginger, there were significant differences (P≤0.05) in insecticidal activity of different concentrations of eucalyptus essential oil and it is efficacy was more sensible at the higher concentrations and the highest insecticidal activity was seen at the concentration of 1 (undiluted extract) too. But highest amounts of increase in it's efficacy were observed between concentration of 250 µl/ml and 500 µl/ml, and between concentration of 750 µl/ml and 1 (undiluted extract). Results of negative and recommended commercial product groups were just like the results of ginger treatments (Table 1).

Statistical comparison between results of both groups shows that in the same concentrations, insecticidal activity of eucalyptus essential oil was significantly (P≤0.05) higher than ginger, this was common for all of treatments, and in the whole of concentrations eucalyptus was more potent than ginger. This difference was more obvious for concentration 1 (undiluted extract) of essential oils, and for this concentration, insecticidal activity of eucalyptus against *Culex theileri* Theobald was about 1.5 fold more than ginger (Fig. 1).

Table 2. Repellent activity or percentage of repelled mosquitoes (mean±SEM)% of different concentration for each essential oil versus negative and positive control groups

Groups	*Zingiber officinalis*	*Eucalyptus globulus*
250 µl/ml	37.26±3.58*[a], (P=0.031)	49.67±3.19[a], (P=0.025)
500 µl/ml	46.83±2.94[b], (P=0.023)	58.75±4.21[b], (P=0.038)
750 µl/ml	50.14±1.88[c], (P=0.042)	67.03±3.56[c], (P=0.029)
1 (undiluted extract)	61.07±3.53[d], (P=0.015)	74.16±5.27[d], (P=0.036)
Negative Control	5.18±1.69[e], (P=0.038)	4.46±0.72[e], (P=0.021)

*Presence of different superscript lowercase letters (a-f) shows the significant differences (P≤0.05) between efficacies of different concentrations (rows) of each essential oil (column).

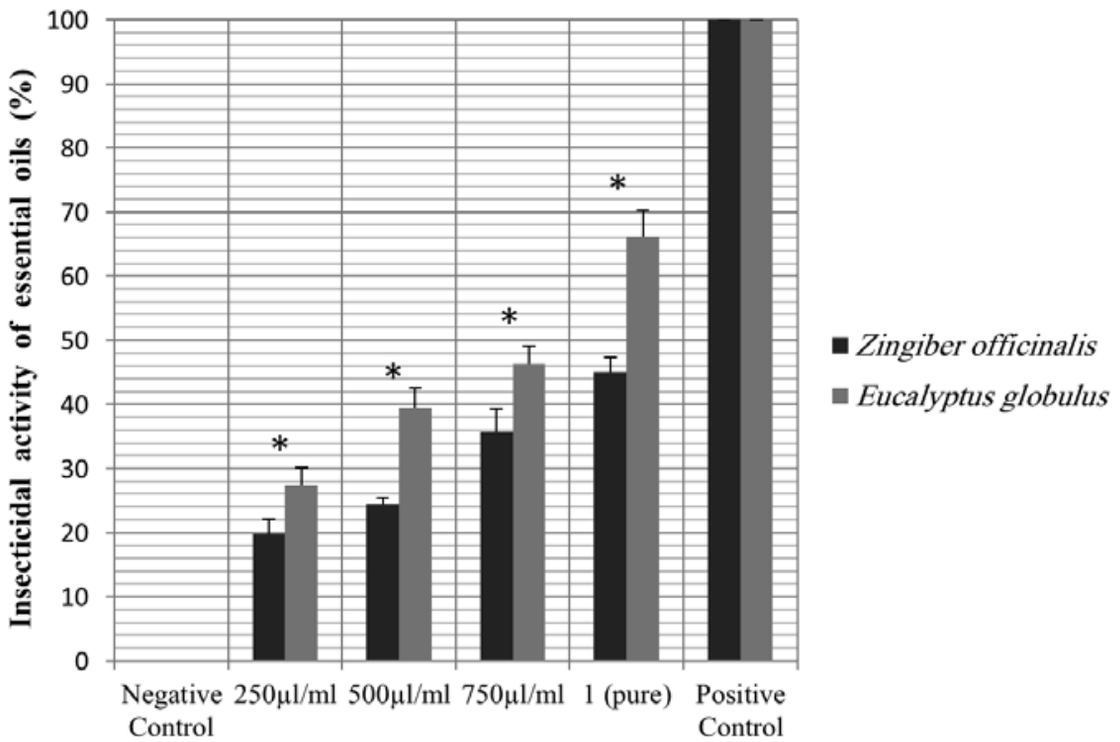

Fig. 1. Comparison of insecticidal activity (percentage of killed mosquitoes) for different essential oils in different concentrations. Presence of * in every treatment groups shows the significant differences (P≤0.05) between efficacies of different essential oils (column) in each treatment group (different concentrations and control groups).

Repellent activity of essential oils

Repellent activity of ginger essential oil against *Culex theileri* was different for each concentration, its efficacy was more at the higher concentrations; and highest repellent activity was seen at the concentration of 1 (undiluted extract), also increase in repellent activity between concentration of 750 µl/ml and 1 (undiluted extract) was more sensible than others (Table 2). Same results were observed for eucalyptus essential oil and highest repellent activity was seen at the concentration of 1 (undiluted extract), but increase in repellent activity between concentration of 500 µl/ml and 750 µl/ml was more sensible than others (Table 2).

Comparison between results of both groups shows that in the same concentrations, repellent activity of eucalyptus essential oil was significantly (P≤0.05) higher than ginger, this was common for all of treatments and in the whole of concentrations, eucalyptus was more potent than ginger. This difference was more sensible for concentration 750µl/ml of essential oils, in this concentration, repellent activity of eucalyptus against *Culex theileri* Theobald was about 1.33 fold more than ginger (Fig. 2).

Discussion

Natural products, such as essential oils which are produced by the secondary metabolisms of herbs and are used in human consumption as functional food, food additives, medicines, nutritional supplements and the manufacture of cosmetics due to their properties [35] are good candidates for replace of chemical products. In this study for the first time insecticidal and repellent activity of essential oils of *Zingiber officinale* and *Eucalyptus globulus* against *Culex theileri* Theobald as a wide-distributed species of mosquitoes in different parts of world with an important role in transmission of zoonosis were studied.

To the best of authors knowledge and current date, there are no data about insecticidal and/or repellent activity of any essential oil against *Culex theileri* Theobald, but some studies were done about insecticidal activity of some essential oils against other members of *Culex* genus, for example: larvicidal and repellent properties of some essential oils against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi were studied and essential oils of *Cymbopogan citrates*, *Cinnamo-*

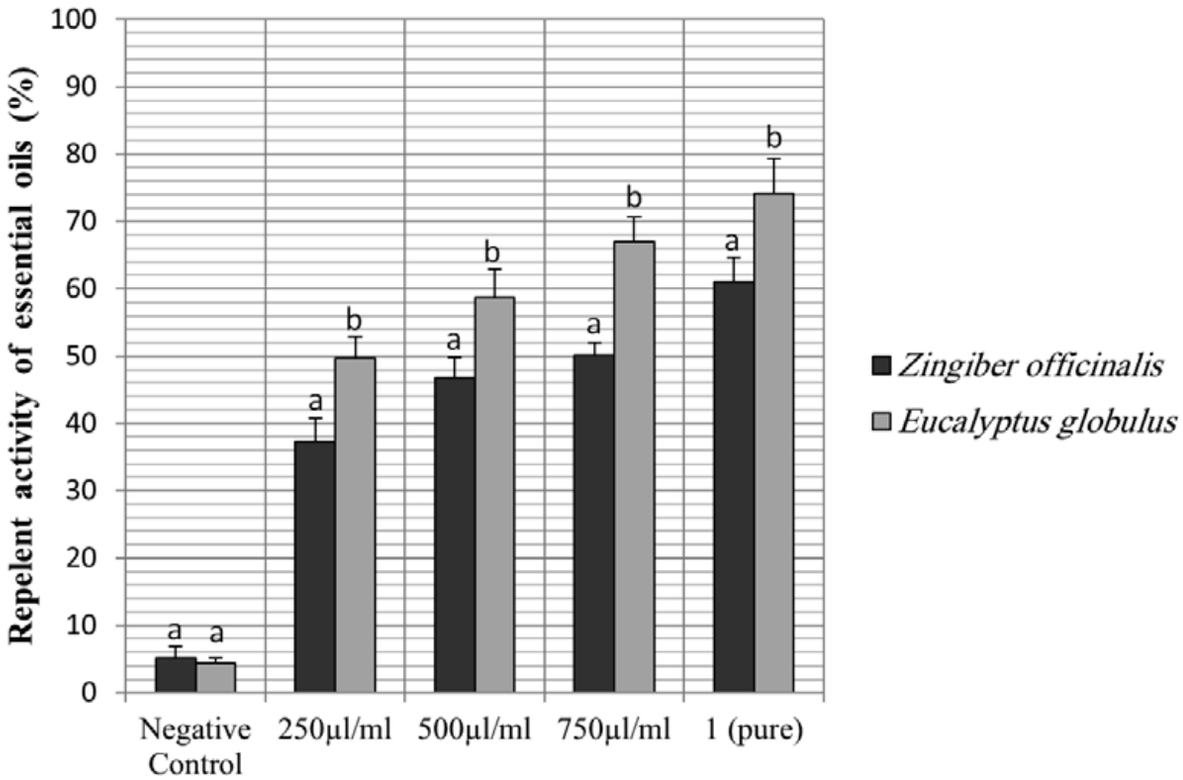

Fig. 2. Comparison of repellent activity (percentage of repelled mosquitoes) for different essential oils in different concentrations. Presence of different lowercase letters (a-b) in every treatment groups shows the significant differences (P≤0.05) between efficacies of different essential oils (columns) in each treatment (different concentrations and negative control group).

mum zeylanicum, *Rosmarinus officinalis* and *Zingiber officinale* were identified to have most effect on examined insects [36]. Results of current study agree with them and show the considerable insecticidal and repellent activities against *Culex theileri* Theobald for essential oil of *Zingiber officinale* and also for essential oil of *Eucalyptus globulus*.

In the other study, larvicidal and repellent activity of essential oil of *Zingiber officinale* against *Culex quinquefasciatus* was studied and it was reported that essential oil of ginger served as a potential larvicidal and repellent agent against *Culex quinquefasciatus* [37]. Results of current study agree with them and show the considerable values of insecticidal/repellent activities for examined essential oils against *Culex theileri* Theobald, too.

Acaricidal and repellent activities of eucalyptus essential oil were studied against the poultry red mite, *Dermanyssus gallinae* (Acari: Mesostigmata) and it is efficacies were reported about 90% and 94% for acaricidal and repellent activity respectively [23], in current study insecticidal and repellent activities of eucalyptus essential oil against *Culex theileri* Theobald were determined about 66% and 74% respectively, that maybe its lowest performances occurred due to different resistance between these insects species or due to different methods of essential oil extraction.

Pesticide and/or repellent activity of eucalyptus essential oil against *Acanthoscelides obtectus* (Say) [38], *Culex quinquefasciatus* [39], housefly, *Musca domestica* [40], *Pediculus humanus capitis* (Anoplura: Pediculidae) [41] and *Lutzomyia longipalpis* [42] were reported by different authors. Results of current study agree with them and show considerable insecticidal/repellent activity for eucalyptus essential oil.

Presence of components such as 1,8-cineole, citronellal, citronellol, citronellyl acetate, p-cymene, eucamalol, limonene, linalool, a-pinene, g-terpinene, a-terpineol, alloocimene, andaromadendrene in eucalyptus essential oil were reported [43], which maybe insecticidal and repellent activity of this essential oil against *Culex theileri* Theobald are

presented due to presence of these components, but more studies and GC-MS analysis are need in this way in order to find the responsible components.

There are little data about insecticidal and/or repellent activity of ginger essential oil against insects. Insecticidal, repellent and oviposition-deterrent activity of ginger essential oil against *Anopheles stephensi* and *Aedes aegypti* [44] and larvae of *Spodoptera littoralis* [45] were reported in different experiments and different amount of efficacies were reported. Results of current study agree with them and show insecticidal/repellent activity of essential oil of ginger against *Culex theileri* Theobald.

Presence of bioactive compounds such as: gingerols, shogaols, diarylheptanoids, phenylbutenoids, flavanoids, diterpenoids and sesquiterpenoids in ginger essential oil were reported [46], which maybe insecticidal and repellent activity of this essential oil against *Culex theileri* Theobald are observed because of these components presence, but more studies and GC-MS analysis are need in this way in order to find the responsible components.

Insecticidal and/or acaricidal effect of *Micromeria fruticosa* L., *Nepeta racemosa* L. and *Origanum vulgare* L. (Lamiaceae) essential oils against *Tetranychus urticae* Koch and *Bemisia tabaci* Genn were reported in other study [47]. Also it is identified that essential oils of *Cuminum cyminum*, *Pimenta dioica* and *Ocimum basilicum* have different values of acaricidal effects against the cattle tick *Rhipicephalus (Boophilus) microplus* [48]. Acaricidal activity of essential oils of *Lippia graveolens*, *Rosmarinus officinalis*, and *Allium sativum* against *Rhipicephalus microplus* were also identified [49]. Acaricidal properties of *Artemisia absinthium* and *Tanacetum vulgare* essential oils against *Tetranychus urticae* Koch were also studied [50]. Whole of mentioned studies are showed that plants essential oils are excellent candidates for replace of chemical pesticides. Results of current study agree with them and show the considerable values of insecticidal and repellent activities against *Culex theileri* Theobald for essential oils of *Zingiber officinale* and *Eucalyptus globulus*. Results of current study and similar studies show that essential oils can be considered as good replaces for chemical insecticidal and or repellent agents.

In conclusion, in current study eucalyptus essential oil had the highest insecticidal (66%) and repellent (74%) activates against *Culex theileri* Theobald and for ginger essential oil insecticidal and repellent activities was 39% and 62%, respectively. This study shows considerable values of insecticidal and repellent activity against *Culex theileri* Theobald for both of essential oils, so they can be considered as potent lethal/repellent agents for biological control of mosquitos, but more studies are need for this purpose. Study on lethal/repellent activity of other plants essential oils against *Culex theileri* Theobald and study on insecticidal activity of these essential oils in the field condition can be considered as a subject for next experiments can be considered as a subject for next experiments.

Acknowledgments

Authors are grateful to Prof. Yaser Pirali (Designer and adviser during the study), Dr. Azam Dehghani Samani: Chief Technology Officer (CTO) of Tolou Tejarat Khavar Company and all of the persons that helped them to do this research.

References

[1] Becker N., Zgomba M., Petric D., Dahl C., Boase C., Lane J., Kaiser A. 2003. Mosquitoes and their control. Springer, New York, USA. doi:10.1007/978-1-4757-5897-9

[2] Demirci B., Lee Y., Lanzaro G.C., Alten B. 2012. Identification and characterization of single nucleotide polymorphisms (SNPs) in *Culex theileri* (Diptera: Culicidae). *Journal of Medical Entomology* 49: 581-588. doi:10.1603/me11139

[3] Balenghien T., Vazeille M., Grandadam M., Schaffner F., Zeller H., Reiter P., Sabatier P., Fouque F., Bicout D.J. 2008. Vector competence of some French *Culex* and *Aedes* mosquitoes for West Nile virus. *Vector-Borne and Zoonotic Diseases* 8: 589-596. doi:10.1089/vbz.2007.0266

[4] Votýpka J., Šeblová V., Rádrová J. 2008. Spread of the West Nile virus vector *Culex modestus* and the potential malaria vector *Anopheles hyrcanus* in central Europe. *Journal of Vector Ecology* 33: 269-277. doi:10.3376/1081-1710-33.2.269

[5] Brummer-Korvenkontio M., Vapalahti O., Kuusisto P., Saikku P., Manni T., Koskela P., Nygren T., Brummer-Korvenkontio H., Vaheri A. 2002. Epidemiology of Sindbis virus infections in Finland 1981-1996: possible factors explaining a peculiar disease pattern. *Epidemiology and Infection* 129: 335-345. doi:10.1017/s0950268802007409

[6] Turell M.J. 2012. Members of the *Culex pipiens* complex as vectors of viruses. *Journal of the*

American Mosquito Control Association 28: 123-126. doi:10.2987/8756-971x-28.4.123

[7] Hesson J.C., Rettich F., Merdić E., Vignjević G., Östman Ö., Schäfer M., Schaffner F., Foussadier R., Besnard G., Medlock J., Scholte E.-J., Lundström J.O. 2014. The arbovirus vector *Culex torrentium* is more prevalent than *Culex pipiens* in northern and central Europe. *Medical and Veterinary Entomology* 28: 179-186. doi:10.1111/mve.12024

[8] Santa-Ana M., Khadem M., Capela R. 2006. Natural infection of *Culex theileri* (Diptera: Culicidae) with *Dirofilaria immitis* (Nematoda: Filarioidea) on Madeira Island, Portugal. *Journal of Medical Entomology* 43: 104-106. doi:10.1093/jmedent/43.1.104

[9] Azari-Hamidian S., Yaghoobi-Ershadi M.R., Javadian E., Abai M.R., Mobedi I., Linton Y.M., Harbach R.E. 2009. Distribution and ecology of mosquitoes in a focus of dirofilariasis in northwestern Iran, with the first finding of filarial larvae in naturally infected local mosquitoes. *Medical and Veterinary Entomology* 23: 111-121. doi:10.1111/j.1365-2915.2009.00802.x

[10] Yildirim A., Inci A., Duzlu O., Biskin Z., Ica A., Sahin I. 2011. *Aedes vexans* and *Culex pipiens* as the potential vectors of *Dirofilaria immitis* in Central Turkey. *Veterinary Parasitology* 178: 143-147. doi:10.1016/j.vetpar.2010.12.023

[11] Diallo M., Lochouarn L., Ba K., Sall A.A., Mondo M., Girault L., Mathiot C. 2000. First isolation of the Rift Valley fever virus from *Culex poicilipes* (Diptera: Culicidae) in nature. *The American Journal of Tropical Medicine and Hygiene* 62: 702-704. doi:10.4269/ajtmh.2000.62.702

[12] Moutailler S., Krida G., Schaffner F., Vazeille M., Failloux A.B. 2008. Potential vectors of Rift Valley fever virus in the Mediterranean region. *Vector-borne and Zoonotic Diseases* 8: 749-754. doi:10.1089/vbz.2008.0009

[13] Şimşek F.M. 2004. Seasonal larval and adult population dynamics and breeding habitat diversity of *Culex theileri* Theobald, 1903 (Diptera: Culicidae) in the Gölbaşı district, Ankara, Turkey. *Turkish Journal of Zoology* 28: 337-344.

[14] Isman M.B., Machial C.M. 2006. Pesticides based on plant essential oils: from traditional practice to commercialization. *Advances in Phytomedicine* 3: 29-44. doi:10.1016/s1572-557x(06)03002-9

[15] Roh J.Y., Choi J.Y., Li M.S., Jin B.R., Je, Y.H. 2007. *Bacillus thuringiensis* as a specific, safe, and effective tool for insect pest control. *Journal of Microbiology and Biotechnology* 17: 547-559.

[16] Höfte H., Whiteley, H.R. 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. *Microbiological Reviews* 53: 242-255.

[17] Chopra A., Doiphode V.V. 2002. Ayurvedic medicine: core concept, therapeutic principles, and current relevance. *Medical Clinics of North America* 86: 75-89. doi:10.1016/s0025-7125(03)00073-7

[18] Ayyanar M., Ignacimuthu S. 2011. Ethnobotanical survey of medicinal plants commonly used by Kani tribals in Tirunelveli hills of Western Ghats, India. *Journal of Ethnopharmacology* 134: 851-864. doi:10.1016/j.jep.2011.01.029

[19] Maia M.F., Moore S.J. 2011. Plant-based insect repellents: a review of their efficacy, development and testing. *Malaria Journal* 10 (Suppl.): s11. doi:10.1186/1475-2875-10-s1-s11

[20] Shukla Y., Singh M. 2007. Cancer preventive properties of ginger: a brief review. *Food and Chemical Toxicology* 45: 683-690. doi:10.1016/j.fct.2006.11.002

[21] Singh G., Maurya S., Catalan C., De Lampasona M.P. 2005. Studies on essential oils, Part 42: chemical, antifungal, antioxidant and sprout suppressant studies on ginger essential oil and its oleoresin. *Flavour and Fragrance Journal* 20: 1-6. doi:10.1002/ffj.1373

[22] Koch C., Reichling J., Schneele J., Schnitzler P. 2008. Inhibitory effect of essential oils against herpes simplex virus type 2. *Phytomedicine* 15: 71-78. doi:10.1016/j.phymed.2007.09.003

[23] Dehghani-Samani A., Madreseh-Ghahfarokhi S., Dehghani-Samani A., Pirali-Kheirabadi K. 2015. Acaricidal and repellent activities of essential oil of *Eucalyptus globulus* against *Dermanyssus gallinae* (Acari: Mesostigmata). *Journal of HerbMed Pharmacology* 4: 81-84.

[24] Brooker M.I.H., Kleinig D.A. 2006. Field guide to *Eucalyptus*. 3rd ed. Bloomings Books, Melbourne.

[25] Vázquez G., Fontenla E., Santos J., Freire M.S., González-Álvarez J., Antorrena G. 2008. Antioxidant activity and phenolic content of chestnut (*Castanea sativa*) shell and eucalyptus (*Eucalyptus globulus*) bark extracts. *Industrial Crops and Products* 28: 279-285. doi:10.1016/j.indcrop.2008.03.003

[26] Gilles M., Zhao J., An M., Agboola S. 2010. Chemical composition and antimicrobial properties of essential oils of three Australian *Eucalyptus* species. *Food Chemistry* 119: 731-737. doi:10.1016/j.foodchem.2009.07.021

[27] Guo R., Cantery P.H., Ernst E. 2006. Herbal medicines for the treatment of rhinosinusitis: a systematic review. *Otolaryngology-Head and Neck Surgery* 135: 496-506. doi:10.1016/j.otohns.2006.06.1254

[28] Cermelli C., Fabio A., Fabio G., Quaglio P. 2008. Effect of eucalyptus essential oil on respiratory bacteria and viruses. *Current Microbiology* 56: 89-92. doi:10.1007/s00284-007-9045-0

[29] Mahmoudzadeh-Sagheb H., Heidari Z., Bokaeian M., Moudi B. 2010. Antidiabetic effects of *Eucalyptus globulus* on pancreatic islets: a stereological study. *Folia Morphologica* 69: 112-118.

[30] Blažević I., Mastelic J. 2009. Glucosinolate

degradation products and other bound and free volatiles in the leaves and roots of radish (*Raphanus sativus* L.). *Food Chemistry* 113: 96-102. doi:10.1016/j.foodchem.2008.07.029

[31] Snell A.E. 2005. Identification keys to larval and adult female mosquitoes (Diptera: Culicidae) of New Zealand. *New Zealand Journal of Zoology* 32: 99-110. doi:10.1080/03014223.2005.9518401

[32] Azari-Hamidian S., Harbach R.E. 2009. Keys to the adult females and fourth-instar larvae of the mosquitoes of Iran (Diptera: Culicidae). *Zootaxa* 2078: 1-33.

[33] Geier M., Boeckh J. 1999. A new Y-tube olfactometer for mosquitoes to measure the attractiveness of host odours. *Entomologia Experimentalis et Applicata* 92: 9-19. doi:10.1046/j.1570-7458.1999.00519.x

[34] Koul O., Singh G., Singh R., Singh J. 2007. Mortality and reproductive performance of *Tribolium castaneum* exposed to *anethole vapours* at high temperature. *Biopesticides International* 3: 126-137.

[35] Zengin H., Baysal A.H. 2014. Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. *Molecules* 19: 17773-17798. doi:10.3390/molecules191117773

[36] Govindarajan M. 2011. Larvicidal and repellent properties of some essential oils against *Culex tritaeniorhynchus* Giles and *Anopheles subpictus* Grassi (Diptera: Culicidae). *Asian Pacific Journal of Tropical Medicine* 4: 106-111. doi:10.1016/s1995- 7645(11)60047-3

[37] Pushpanathan T., Jebanesan A., Govindarajan M. 2008. The essential oil of *Zingiber officinalis* Linn (Zingiberaceae) as a mosquito larvicidal and repellent agent against the filarial vector *Culex quinquefasciatus* Say (Diptera: Culicidae). *Parasitology Research* 102:1289-1291. doi:10.1007/s00436-008-0907-6

[38] Papachristos D.P., Karamanoli K.I., Stamopoulos D.C., Menkissoglu-Spiroudi U. 2004. The relationship between the chemical composition of three essential oils and their insecticidal activity against *Acanthoscelides obtectus* (Say). *Pest Management Science* 60: 514-520. doi:10.1002/ps.798

[39] Mandal S. 2011. Repellent activity of *Eucalyptus* and *Azadirachta indica* seed oil against the filarial mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae) in India. *Asian Pacific Journal of Tropical Biomedicine* 1 (Suppl.): S109-112. doi:10.1016/s2221-1691(11)60135-4

[40] Kumar P., Mishra S., Malik A., Satya S. 2012. Compositional analysis and insecticidal activity of *Eucalyptus globulus* (family: Myrtaceae) essential oil against housefly (*Musca domestica*). *Acta Tropica* 122: 212-218. doi:10.1016/j.actatropica.2012.01.015

[41] Yang Y.C., Choi H.Y., Choi W.S., Clark J.M., Ahn Y.J. 2004. Ovicidal and adulticidal activity of *Eucalyptus globulus* leaf oil terpenoids against *Pediculus humanus capitis* (Anoplura: Pediculidae). *Journal of Agricultural and Food Chemistry* 52: 2507-2511. doi:10.1021/jf0354803

[42] Maciel M.V., Morais S.M.L., Bevilaqua C.M., Silva R.A., Barros R.S., Sousa R.N., Sousa L.C., Brito E.S., Souza-Neto M.A. 2010. Chemical composition of *Eucalyptus* spp. essential oils and their insecticidal effects on *Lutzomyia longipalpis*. *Veterinary Parasitology* 167: 1-7. doi:10.1016/j.vetpar.2009.09.053

[43] Liu X., Chen Q., Wang Z., Xie L., Xu Z. 2008. Allelopathic effects of essential oil from *Eucalyptus grandis×E. urophylla* on pathogenic fungi and pest insects. *Frontiers of Forestry in China* 3: 232-236. doi:10.1007/s11461-008-0036-5

[44] Prajapati V., Tripathi A.K., Aggarwal K.K., Khanuja S.P.S. 2005. Insecticidal, repellent and oviposition-deterrent activity of selected essential oils against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus*. *Bioresource Technology* 96: 1749-1757. doi:10.1016/j.biortech.2005.01.007

[45] Pavela R. 2005. Insecticidal activity of some essential oils against larvae of *Spodoptera littoralis*. *Fitoterapia* 76: 691-696. doi:10.1016/j.fitote.2005.06.001

[46] Sivasothy Y., Chong W.K., Hamid A., Eldeen I.M., Sulaiman S.F., Awang K. 2011. Essential oils of *Zingiber officinale* var. rubrum Theilade and their antibacterial activities. *Food Chemistry* 124: 514-517. doi:10.1016/j.foodchem.2010.06.062

[47] Çalmaşur O., Aslan I., Şahin F. 2006. Insecticidal and acaricidal effect of three Lamiaceae plant essential oils against *Tetranychus urticae* Koch and *Bemisia tabaci* Genn. *Industrial Crops and Products* 23: 140-146. doi:10.1016/j.indcrop.2005.05.003

[48] Martinez-Velazquez M., Castillo-Herrera G.A., Rosario-Cruz R., Flores-Fernandez J.M., Lopez-Ramirez J., Hernandez-Gutierrez R., del Carmen Lugo-Cervantes E. 2011. Acaricidal effect and chemical composition of essential oils extracted from *Cuminum cyminum*, *Pimenta dioica* and *Ocimum basilicum* against the cattle tick *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). *Parasitology research* 108:481-487. doi:10.1007/s00436-010-2069-6

[49] Martinez-Velazquez M., Rosario-Cruz R., Castillo-Herrera G., Flores-Fernandez J.M., Alvarez A.H., Lugo-Cervantes E. 2011. Acaricidal effect of essential oils from *Lippia graveolens* (Lamiales: Verbenaceae), *Rosmarinus officinalis* (Lamiales: Lamiaceae), and *Allium sativum* (Liliales: Liliaceae) against *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae). *Journal of Medical Entomology* 48: 822-

827. doi:10.1603/ME10140
[50] Chiasson H., Bélanger A., Bostanian N., Vincent C., Poliquin A. 2001. Acaricidal properties of *Artemisia absinthium* and *Tanacetum vulgare* (Asteraceae) essential oils obtained by three methods of extraction. *Journal of Economic Entomology* 94: 167-171. doi:10.1603/0022-0493-94.1.167

Received 24 February 2018
Accepted 16 July 2018