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STUDIES ON MOSQUITOCIDAL ACTIVITY OF GERANIUM OIL (*PELARGONIUM GRAVEOLENS*) AGAINST *CULEX QUINQUEFASCIATUS* SAY (DIPTERA: CULICIDAE)

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Abstract.

Mosquitoes are the most important single group of insects in terms of public health. Mosquitoes-borne disease is endemic in more than 100 countries, causing mortality of nearly two million people every year. Who has been declared mosquitoes are serious human disease causing insects which transmit many dreadful diseases. *Culex quinquefasciatus* more commonly called the southern house mosquito is the principal vector of lymphatic filariasis caused by *Wuchereria bancrofti* and a potential vector of Dirofilariaimmitis. Larviciding is a successful way of reducing mosquito densities in their breeding places before they emerge into adults. The wide use of conventional chemical insecticides, such as Malathion and DDT, against adult mosquitoes has shown promising results in combating the spread of mosquitoes. Phytochemical are widely used as biocontrol agent against vector mosquitoes. Present study was undertaken to evaluate the mosquitocidal activity of Geranium oil against *Culex quinquefasciatus*. This study was conducted in the laboratory to evaluate the Geranium oil with different concentration (1% and 2%). The mosquitocidal activity was recorded of 24 hours, under laboratory condition 98% larval mortality was observed in 1st instar of *Culex quinquefasciatus*, after the treatment of Geranium oil at 2% concentration; where as in 3rd and 4th instar larval mortality were 94% and 90% at 2% treatment respectively. The pupal mortality was 81% at 2% oil treatment. Adult mortality was 64% after the treatment; the adult emergence was drastically reduced after the treatment of Geranium oil. The larval duration was greatly extended up to 4 days after the treatment of Geranium oil (2%) than other concentration. Pupal duration also extended after the treatment of Geranium oil than control. Fecundity and egg hatchability also reduced after the treatment of Geranium oil 37%. Ovipositional deterrency was observed after the treatment of plant extract at 2%. Adult repellency was 67% after the treatment of Geranium oil and biting deterrency also increased after the treatment of oil (1% < 2% < 4%). Larval pupal intermediate were very high after the treatment of Geranium oil.

Key words

Culex quinquefasciatus, geranium oil, larvicide, pupicide, adulticide, pupal duration, adult duration.

1. Introduction

Mosquitoes are the most important group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, lymphatic filariasis, dengue , Japanese encephalitis etc., resulting in millions of death every year (Das and Mukherjee, 2006). The order Dipteral presents an array of insects which more than any other group poses the greatest challenge to human and veterinary health as vectors of diseases *culex* is a genus of mosquito and is important in the females of several species are blood eating pests, about 3500 species of mosquitoes have been described worldwide. The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine and organophosphate compounds. But this has not been very successful due to human, technical, operational ecological and economic factors. Chemical insecticides have continued to be commonly used for controlling mosquitoes in many parts of the world. Initially their use was focused on the control of mosquitoes, either by killing or repelling them. However, the appearance of mosquito resistance to conventional insecticides together with public concern about the safety and availability of the insecticides have prompted the necessity to search for alternative insecticides that would be environmentally acceptable and less costly. Therefore, in recent years the use of environment friendly and easily biodegradable natural insecticides of plant origin has received renewed importance for diseases control. Botanicals with mosquitocidal properties such as general toxicant, repellents, growth and reproductive inhibitors and oviposition- deterrents have been projected as potent alternative natural insecticides in future mosquito control programmers (Sukumar *et al*., 1991). Essential oils are defined as, any volatile oil(s) that have strong aromatic components which provides distinctive odour, flavour or scent to a plant. Oils are volatile substances found in a various plants. Botanical based oils were the first preservatives used by man, their natural state within plant tissues and as oils obtained by water distillation. The uses of commercial, essential oils are used in four primary ways: Pharmaceuticals enhancers in many food products, odorantsin fragrances and insecticides. Essential oil play an important role in controlling several mosquito species. In general, essential oils from plants have been considered important natural resource to act as insectides. In recent years, attempts are being made to identify plants, inclouding herbs and weeds, for their insecticidal property with a view to find out suitable alternatives to replace hazardous synthetic pesticides utilized in large scale in India.

2. Materials and Methods

2.1 Plant oil

The Geranium oil (*Pelargonium graveolens*), are purchased from MPDA, Udhagamandalam, The Nilgiris, Tamilnadu and reserved in dark glass bottles at a low temperature (15°C) until use. In preparing test concentrations, plant oil was volumetrically diluted in water. Plant oil was dissolved with an emulsifier (0.1% Tween 80).

2.2 Mosquito culture

Mosquito larvae/eggs of *Culex quinquefasciatus* have been collected in an around Ooty. The mosquito colonies were maintained at 27 ± 2^0C, 75-85% relative humidity index a 14:10 light/dark photo period cycle (Murugan and Jeyabalan, 1999).

2.3 Larvicidal and Pupicidal assays

Larvae tested for the present study was obtained from our laboratory culture. Freshly hatched or moulted larvae were used for the bioassay tests. The required quantity of plant oil concentrations were mixed thoroughly with 200 ml of rearing water in 500ml plastic troughs. One hundred early fourth instars mosquito larvae were released into each trough. Larvae food consisted of 1g of finely ground dog biscuits per day per trough. Dried coconut midribs were place over water as the substratum for pupation. The plastic trough containing 200 ml of rearing water served as the control. Dead larvae and pupae was removed and counted at 24 h intervals. Observations on larval and pupal mortality were recorded. The experiment was replicated five times. Percentage mortality observed in the control was subtracted from that observed in the treatments (Abbot, 1925). The day from moulting of the larvae to pupation and to adulthood was noted. Fecundity was assessed by counting the number of eggs laid during the life span by control and experimental mosquitoes. The larvae and pupal duration of treated and control individuals were compared and developmental rates were determined.

2.4 Adulticidal assay

Culex quinquefasciatus fresh adults were exposing to filter paper treated with different concentration of plant oil. The paper was keep inside the beaker. Muslin cloth covering the beaker was also treated. Control insects were exposed only to distilled water with water treated paper and muslin cloth. Mortality count was taken after 24h (Sharma *et al*., 1992).

2.5 Ovicidal assay

Culex quinquefasciatus eggs were released in water. The test oil was added in desired quantities and hatching were observed for one week. The eggs were then exposed to deoxygenated water and the numbers of hatching eggs were recorded. Percentage hatching was compared with the control in which only distilled water was used (Sharma *et al*., 1992).

2.6 Biting deterrency activity

The percentage protection in relation to dose method was used (WHO, 1996). Blood starved female *Culex quinquefasciatus* (100 nos), 3 - 4 days old, was kept in a net cage (45x30x45 cm^2). The arm of the test person was cleaned with isopropanol. After air drying the arm, a 25 mc^2 area of the dorsal side of the skin was exposed, the remaining portion was covered by rubber gloves. The plant oil was dissolved in water, where distilled water served as control. Different concentration of the plant oil was applied. The control and treated arms was introduced simultaneously into the cage. The numbers of bites was count over 5 minute from 6 pm to 6 am. The experiment was conducted five times.

2.7 Statistical analysis

All data was subject to analysis of variance and the treatment mean was separated by Duncan's Multiple Range Test (Duncan, 1955). Statistical analysis was carried out using the (Statistical Package Social Science) SPSS software, version 16.0.

3. Results

The table 1 shows that the effect of geranium oil on the larval mortality of *Culex quinquefasciatus* after that treatment of geranium oil at 2 % the larval mortality of 1^{st} instar larvae was 100 %, 2^{nd} instar was 98 %, 3^{rd} instar was 94 % and the 4^{th} instar was 90%.no dead larvae were observed in the negative control (water) and geranium oil produced significant mortality. The table 2 shows that the pupal, adult mortality and adult emergence of *Culex quinquefaciatus* after the treatment of Geronium oil, At 2 % concentration the pupal mortality was 81 %, adult mortality was 64% and adult emergence was 49%, The geranium oil with higher concentrations, was found to be most effective for pupicidal activity against *Culex quinquefaciatus*. The adulticidal efficacy was observed in geranium oil. The percentage of larval mortalities and inhibition of adult emergence was singnificant with the tested plant oil. The table 3 shows that the effect of geranium oil on adult repellency and ovipositional deterrency of *Culex quinquefaciatus* after the treatment of geranium oil at 2% concentration the adult repellency was 67% ovipositional deterrence was 74%. Table 4 shows that the developmental duration of *Culex quinquefasciatus* after the treatment of geranium oil at 2 % concentration the larval duration of 1st instar larvae was 6.3 days, 2 nd instar was 8.7 days, 3 rd instar was 9.7 days and the 4 th instar was 10.5 days. The table 5 shows that the developmental duration of *Culex quinquefasciatus* after the treatment of geranium oil at 2% concentration the total pupal duration was 9.9 days and total adult duration was 19 days. The geranium oil showed more than 50 % pupal mortality. Larval mortality was recorded in geranium oil. The order of the larvicidal efficacy of plant oils after 24 hours of geranium oil. Table 6 shows that the effect of geranium oil on fecundity and egg hatchability of *Culex quinquefasciatus* after the treatment of Geranium oil at 2% concentration the fecundity was 69% and egg hatchability was 37.Table 7 shows that the effect of geranium oil against *Culex quinquefasciatus* after the treatment of geranium oil at 2% concentration the larval- pupal intermediate was 45%.

1. Effect of Geranium oil on the larval mortality of *Culex quinquefasciatus*

S. No	Treatment	Concentration (%)	1st Instar	2nd Instar	3rd Instar	4th Instar
1.	Control		00^{d}	00^{d}	00^{d}	00^{d}
2.	Geranium oil	0.5%	37^{c}	34^{c}	30^{c}	28^{c}
		1%	60^{b}	57^{b}	52b	50b
		2%	100^{a}	98^{a}	94^{a}	90^{a}

Within a column means followed by the same letters are not significantly different at 5% level by DMRT

2. Effect of Geranium oil on pupa and adult of *Culex quinquefasciatus*

S. No	Treatment	Concentration (%)	Pupal mortality (%)	Pupation (%)	Adult mortality (%)	Adult emergence (%)
1.	Control		00^{d}	100^{a}	00^{d}	96^{a}
2.	Geranium oil	0.5%	37^{c}	84^{b}	30^{c}	78^{b}
		1%	54^{b}	67^{c}	45^{b}	62^{c}
		2%	81^{a}	52^{d}	64^{a}	49^{d}

Within a column means followed by the same letters are not significantly different at 5% level by DMRT

3. Effect of Geranium oil on adult repellency and ovipositional deterrency of *Culex quinquefasciatus*

S. No	Treatment	Concentration (%)	Adult Repellency (%)	Ovipositional deterrency (%)
1.	Control		00^{d}	00^{d}
2.	Geranium oil	0.5%	38^{c}	34^{c}
		1%	48^{b}	53^{b}
		2%	67^{a}	74^{a}

Within a column means followed by the same letters are not significantly different at 5% level by DMRT

4. Developmental duration of *Culex quinquefasciatus* after the treatment of Geranium oil

S.NO	Treatment	Concentration (%)	Total larval duration (days)			
			1st Instar	2nd Instar	3rd Instar	4th Instar
1.	Control		1.6^{d}	2.9^{d}	3.1^{d}	3.6^{d}
2.	Geranium oil	0.5%	3.1^{c}	5.5^{c}	5.7^{c}	6.0^{c}
		1%	4.5^{b}	7.3^{b}	7.9^{b}	8.5^{b}
		2%	6.3^{a}	8.7^{a}	9.7^{a}	10.5^{a}

Within a column means followed by the same letters are not significantly different at 5% level by DMRT

5. Pupal and Adult duration of *Culex quinquefasciatus* after the treatment of Geranium oil

S.No	Treatment	Concentration (%)	Total pupal duration (days)	Total adult duration (days)
1.	Control		3.1[d]	71[a]
2.	Geranium oil	0.5%	5.9[c]	47[b]
		1%	7.9[b]	32[c]
		2%	9.9a	19d

Within a column means followed by the same letters are not significantly different at 5% level by DMRT

6. Effect of Geranium oil on fecundity and egg hatchability of *Culex quinquefasciatus*

S. No	Treatment	Concentration (%)	Fecundity (No of eggs)	Egg hatchability (%)
1.	Control		248[a]	97[a]
2.	Geranium oil	0.5%	115[b]	59[b]
		1%	84[c]	48[c]
		2%	69[d]	37[d]

Within a column means followed by the same letters are not significantly different at 5% level by DMRT

7. **Effect of Geranium oil against *Culex quinquefasciatus***

S. No	Treatment	Concentration (%)	Larval-Pupal Intermediate (%)
1.	Control		00[d]
2.	Geranium oil	0.5%	17[c]
		1%	31[b]
		2%	45[a]

Within a column means followed by the same letters are not significantly different at 5% level by DMRT

4. Discussion

Mosquito as are serious threat to public health, transmitting several dangerous diseases for over two billion people in the tropics. There has been a large increase in the insecticide resistance of this vector and has become a global problem. Insecticides residues in the environment, as a result of chemical insecticide usage, have turned the researchers' attention towards natural products (Murty and Jamil, 1987). In the past years, the plant kingdom has been of great interest as a potential source of insecticidal products. Many species in the plant kingdom synthesize a variety of secondary metabolites which play a vital role in defense of plants against insects/mosquitoes. Botanicals have widespread insecticidal properties and will obviously work as a new weapon, and in future may act as suitable alternative product to fight against vector mosquitos (Ghosh, 2012). In may conclude that natural products from plants of insecticidal and medicinal values have higher efficiency in reducing mosquito menace due to their repellent toxicity. Further in-depth laboratory studies and field bioassays are needed as the present study indicated that there is scope to use local plants to control and repellent mosquito control. This study recommends that this plant could from safe and ecofriendly alternative to synthetic pesticides. These results are encouraging for developing new natural mosquitocidal products from plant oil thereby offering an alternative to synthetic products.

Geranium oil is more effective in controlling several previous studies suggested that both *C. colocynthis* and *P. tomentosa* could be used as natural larvicidal products against several vector larval stages (Mullai *et al*., 2007; Asiry, 2015). However, the lesser efficacy recorded in the current study could be attributed to the variation in the chemical composition of the effective essential oils found in these local varieties of both plant. The effectiveness of the Geranium oil against the 4th instar larvae of *Culex quinquefasciatus* could be attributed to the chemical composition of its essential oils. *Rhanterium epapposum* (Arfaj) is commonly used in folk medicine in rural of areas of Saudi Arabia as a remedy for gastrointestinal disturbances, skin infections, and most importantly as an insecticide (Younis *et al*., 2008; Phondani *et al*., 2016). In a recent study (Awad *et al*., 2016) recorded the major constituents of essential oils of R. *epapposum* leaves, which included limonene, sabinene, - pinene, - myrcene, im addition to other constituents in lesser percentages. Due to aquatic condition, they cause adverse effects on the environment and human health and hence, this finding not only to control the spreading of mosquito but also biodegradable and easily available in low cost (Nasir *et al*., 2017) plants possessing bioactive compounds are main the culprit against mortality of *Ae. Aegypti* which was suggested after experimented on roots of *Rubia cordifolia*, with LC_{50} and LC_{90} values of 3.86 and 8.28 ppm for larvae, and 3.92 and 8.05 ppm for pupae of *A. aegypti*. Further thease group isolated alizarin from roots of *R. cordifolia*, which is the main source to destroy the larvae/ pupa of mosquito. Larvicidal and pupicidal actions of methanol leaf extract of *Tephrosia purpurea* was also observed again *A. aegypti* and found that the LC_{50} values of 1st instar to 4th larval instars were 139.24, 176.24, 219,28, 256.27, and 326.29 ppm, respectively whereas LC_{50} value of pupa was 326.29 ppm. The results so obtained also having similarities to who have taken the direct leaf and stems extracts of *Parthenium hysterophorus* against *A. aegypti* and confirmed as potential natural larvicidal agent (Amir *et al*., 2017).

Essential oils such as Geranium oil have been found promising in killing mosquito larvae (James *et al*., 1992; Shaalan *et al*., 2005). The results of the present study were comparable with the 24 hours LC $_{50}$ values of recent studies. Samuel *et al*. (2011) screened six plant oils (lemon grass, palmarosa, geranium, tulsi, rosemary and menthal) against larvae of *Aedes aegypti* and found that palmarosa exhibited the highest activity and lc50 value was 13.96 ppm. Youssif *et al*. (2011) tested the cinnamon; white camphor and wintergreen oil for activity against the larvae of *Culex quinquefaciatus* and LC_{50} values were 58.41, 42.98 and 81.32mg/l respectively. Pugazhvendan and Elumali (2013) indicated camphor, clove and eucalyptus oil to exhibit 70,100 and 100% activity against the larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefaciatus* respectively.The larvicidal effect of the Eos was evaluated according to WHO guidelines (World Health Organization 2005). Due to field sampling constraints at VK7 and based on previous study where Garanium oil EO appears to be the most active (Wangrawa *et al*., 2015), Granium oil EO only was used against the VK7 field collected dilutions in distilled water from a stock Granium oil to produce a mortality ranged from 10 to 100 % In the present study, Granium oil showed good activity against *Culex quinquefasciatus* and caused high mortality. Geranium oil produced enhanced mortality with increasing concentrations of extracts and further, it has been noticed that ethanol extracts possessed strong activity than others. Globally, the people are always searching the eco-friendly alternative options to control the mosquitoes. For that, exploring of floral biodiversity is preferred, as they contain vest repository secondary metabolites. The tested plant, *C. philippinum* has larvicidal and pupicidal activity against *An. stephensi* which may be due to the presence of active biological compounds including terpenoidos, flavonoid, alkaloids and phenoilcs etc. (Wang *et al*., 2018). The above mentioned compounds may jointly or independently contribute to impact on larvicidal and pupicidal activity against *An. Stephensi* and a similar report was given by Subarani *et al*. (2013) in which aqueous and solvent leaf extracts of *Catharanthus roseus* is able to impact on *An. stephensi* (Subarani *et al*., 2013).

All the essential oils from Geranium tested had larvicidal and pupicidal activity against *Cx. quinquefasciatus*. All the essential oils induced 100% mortality against *Cx. quinquefasciatus* larvae at 60 minutes and had pupicidal activity against both *Cx. quenquefasciatus* pupae by causing 100% mortality at 48hours. The essential oil from Geranium proved to be the most effective against *Culex quenquefasciatus* (Jatan *et al.*, 2003) also found Z. *cassumunar* oil to be the effective against mosquito larvae with a LC_{50} value less than 200 ug/ml. Further more, the essential oils from *B. rotunda*, *C. zedoaria*, *E. littoralis*, *Z. ottensii* and *Z. zerumb* also exhibited high larvicidal activity against *Ae. Aegypht* larvae (Isa *et al.*, 2012) also found *B. rotunda* exhibited insecticidal properties.

Yang *et al.* (2003) studied the adulcidal activity of five essential oils against *Culex pipines*. They found that the Rutaceae oil obtained from *Citrus sinesis* was the most effective adulcidal treatment. Other plant species that are reported to possess adulticidal activity includes; *Curcuma aromatic* against *Ae. Aegypti* (Choochate *et al*., 2005). The adulticidal activity of ethanol extract of *Apium graveolens* seeds against *Aedes aegypti* has been reported (Choochote *et al*., 2004). Dua *et al*. (2010) reported that adulticidal activity of essential oil of leaves of *lantana camara* against *Ae. aegypit*, *Cx. quinquefasciatus*, *An. Culicifacies*, *An. Fluvialitis* and *An. Stephensi*, LD_{50} values were 0.06, 0.05, 0.05, 0.05 and 0.06 mg/cm ^{2} while LD_{90} value were 0.10, 0.10, 0.09, 0.09 and 0.10 mg/cm^2 respectively. Whereas KDT_{50} values were 20, 18,15,12,14 min and KDT_{90} values were 35,28,25,18 and 23 min against *Ae.aegypti*, *Cx quinquefasciatus*, *An. culicifacies*, *An. Fluviatilis* and *An. stephensi*, respectively on 0.208 mg/cm ^{2} impregnated papers. The target of many insecticides is the nervous is available on mode of particular, acetylcholine esterase. However little information is available on mode of adulcidal activity of essential oil. Ware (1994) has reported that the only way volatile insecticidal can enter the pest's body is through the respiratory system. The oil demonstrated the efficacy in eggs, larval and pupal stages, but no effect was observed in case of the adults as an adulticidal agent. Larvicides or ovicides or pupicides kill the respective developmental stages in their breeding habit before they can mature into adults, hence they can minimize the application of other adulticiding chemicals (Requel *et al*., 2007). The eggs of *C. quinquefaciatus* were more susceptible then other developmental stages as the encapsulation of the eggs actually increased exposure to stresses by holding embryos in stressful condition that larvae or other stages could easily avoid through passive dispersal or vertical migration (Fox *et al*., 2001).

Most adults were emerged incompletely or left their tarsi attached in the pupal exuvia (Salesh *et al*., 1981; Mahyoub *et al.*, 1949-1956). In other words, the results thus may confirm the unsuitability of larvae mortality records as a criterion for evaluating the efficacy of such compounds as they more Juvenilizing effects than toxic mode of action (Saleh *et al*., 1989). Therefore, the biological effects of the present IGRs and plant extracts were expressed as the percentage of larvae that do not develop into successfully emerging adults or the inhibition of adult emergence (WHO, 2005).The different in susceptibility to the two different essential oil as ovicides are due to the different rate of intake, penetration through the egg-membrane, detoxicationetc (Valarmathy *et al*., 2011). Mosquitoes vary in their response to essential oil and it has already been established that the sensitivity of different development stages of the same species of mosquito could be quite different for the same compound. Again essential oil can be inhaled, ingested or absorbed by the skin of insects. Therefore, the different toxicity levels in different developmental stages were due to those physiological as well as morphological variations (Roger *et al*., 1997).

Culex quniquefasciatus was found to be more susceptible towards this phytochemical insecticides property when compared to *Ae. albohpicus.* Since dengue virus involves transovarial transmission (Lee and Rohani, 2005) preventing egg lying and egg hatching might be one of the strategy in controlling spreading this disease. Mosquito control in the larval is worthwhile to minimize the emergence of the adult population and thereby reduce the risk of spreading vector-borne diseases. The mosquitoes breeding in small habitats such as small pounds, marches, ditches, pools, drains, water containers and any other utensils holding water can easily be managed with locally available resources, in this study, essential oil of Garanium were tried in the laboratory against immature and adult stages of *Culex quinquefasciatus* mosquitoes to collect baseline data for the development of eco-products. The mortality rate of 2nd and 4th instar larvae and pupae varied significantly based on a concentration of the plant extract tested and period of exposure. In the present study, analysis reveals that mortality of Geranium oil was effective against *Culex quniquefasciatus* during the larval and pupal stage. In general, the mortality rate from 1st to 4th instar were cumulatively decreasing which indicate that the extracts created a more toxic environment during the very beginning stages of mosquitoes (that have treated). Exploring new pest management strategy by using insect growth disruptors represents an environmentally friendly option. These novel insecticides act on selective biochemical sites in insects such as chitin synthesis inhibitors, or juvenile hormone analogues and ecdysone agonists, which effect the hormonal regulation of different processes (Berghiche *et al*., 2008; Suman *et al*., 2013). Some insects exposed to such compounds may die due to abnormal regulation of hormone mediated cell or organ development (Benelli *et al*., 2014).

Our result showed that oils of Granium have significant repellent activity against *Cx. quinquefasciatus* mosquito. However, the repellent effect was decreased as time increased which was in the line with an earlier report by (Govere *et al*., 2000) who reported *citronella* (*Cymbopogon excavates*) oil gave 100% repellency for 2 hour. When it was evaluated in the laboratory against *A. arabiensis* and its repellency decreased to 59.3% after 4hour. Another study conducted by Ansari *et al*. (2000) showed that the peppermint (*Mentha piperita*) oil gave 94.1% protection for 6 hours, while mylol oil gave 95% protection for 7.2 hours (Govere *et al*., 2001) also showed that the alcohol plant extract of Cymbopogon excavates and *Pelargonium reniforme* provided 66.7% and 63.3%protection against *A. arabiensis* for 3 hours, respectively. Some drops of a neem (*Azadirachta indica*) oil vaporized from a mat at the door repel mosquitoes, keeping them off for approximately five to seven hours (Luthi *et al*., 2008).

The repellent activites of geranium oil was comparable to previously screened plant using the pine (*Pinus longifolia*) oil (Ansari *et al*., 2005) who was reported to have storng repellent action against mosquitoes as it provided 100% protection against *An.culicifacies* and 97% protection against *Cx. quinquefasciatus* for nine hours, respectively. The five most effective oils were those of litsea (*Litsea cubeba*), Cajeput (*Melaleuca leucadendron),* Niaouli (*Melaleuca nervia),* Voilet (*Viola odorata*) and Catnip (*Nepeta cataria*), which induced a protection time of 8 hours at the maximum and a 100% repellency against Aedes, *Anopheles* and *Culex* mosquitoes (Amer *et al*., 2006). Nagpal *et al*. (2001) reported that *An. Culicifacies* and *Cx. quinquefaciatus* mosquitoes were unable to bite the protected person within 4 hours after his her application of neem products, which were safe, and better than any other repellents without adverse reactions. It is very common in the literature to see reports on essential oils with mosquito repellent activity without a corresponding bioassay-directed fractionation of the oils to specifically attribute the mosquito repellent activity of individual compounds. Often the oil activity is reported only and we are left to speculate on which individual constituents are responsible for the activity of the oil as a whole. Plant-arthropod interactions can be mediated by various compounds that are promising candidates for disease vector control. Especially, plant essential oils have been proposed for mosquito control and repelling (Boschitz and Grunewald, 1994 and Amer and Mehlhorn, 2006). In addition, a recent study suggested the use of chitosan-based elicitors for the induction of insect-repelling activity (Wongchai *et al*., 2013). Plant- arthropod interactions can be quantified by the exposition of humans and animals or by olfactometry, Amer and Mehlhorn (2006) used human exposition to test 40 plant essential oils against mosquitoes, thereby counting the landing and the biting mosquitoes. Another approach used two-field olfactometry and the preference index to determine repellent activity of autotroph and heteroph *chenopodium rubrum* plant cell cultures (Wongchai *et al.,* 2013). Finally, olfactometry can also use Y tube and multi-arm airflow olfactometers besides electro-antennography (Stanczyk *et al.,* 2013). These different methods can help to find new repellents replacing DEET, since the well-established mosquito repellent DEET should not be used on humans when pregnant or breastfeeding or for children under 3 years of age (Koren *et al.,* 2003). Geranium oil has proved their utility for various medicinal uses since time immemorial. The bioefficacy of plant Eos against mosquitoes as growth inhibitors, larvicides, adulticides, and repellent or oviposition deterrents have been reported by many researchers (Sukumar *et al.,* 1991; Tyagi *et al.,* 2016). Phukerd *et al.* (2013) found larvicidal and pupicidal activities of EOs from *Culex quinquefasciatus.* The EOs examined here showed an array of different bioactivities against *Ae. aegypti* that can be considered useful for mosquito control. The EOs of *Lippia* (Verbenaceae) exhibited pupicidal, adulticidal and oviposition- deterrent activity. These effects may be explained by the chemical composition of the EOs of this genus, which has been previously reported (Terblanche and Kornelius, 1996; Folashade and Omoreige, 2012). The most common components of *Lippia* Eos are thymol, carvacrol, geranial, linalool, p-cymenence, carvone, neral, limonence, b-caryophyllene, caryophyllene oxide, myrcene and c-terpinene (Vera *et al.,* 2014). Specifically, of the 2 *Lippia* species evaluated in our study, the EOs with the greatest pupicidal, adulticial and repellency activity were obtained from *L. origanoides* while *L. alba* exhibited a greater capacity to deter ovipostion in female *C. quinquefasciatus.* Insecticidal properties of Geranium oil against adult mosquitoes have been reported by many workers (Panella *et al.,* 2005). Geranium oil is reported to possess insecticidal activity against stored grain pest, vegetables crops pest, mosquito larvae and antifungal, repellent and other biological activities.

5. Conclusion

Using plant derivatives (botanicals) is one of the alternatives to persistent, non-target, very toxic synthetics. Essential oils are a group of botanicals. In addition to the general advantages of botanicals, redundancy, presence of numerous analogues of one compound, which is known to increase efficacy by synergism and slows the onset of insecticide resistance is a characteristic of essential oils. The major constituents are terpenoids in those that have been used as larvicides or repellents against mosquitoes. Sources are from several plant species across more than 10 families dominated by the Labiateae (Lamiaceae) and Asteraceae. They have been found effective as larvicides. Repellency tests have shown these oils as providing protection for several hours, and in some cases comparable to the protection from synthetic DEET. As repellents, essential oils reduce human-mosquito contact, ensuring protection, one of the two strategic approaches to malaria control. Essential oils are usually safe to humans and the environment. Insecticides of plant origin are expected to be target selective and biodegradable leading to fewer harmful effects on human and other animals and are environmentally safe as compared to synthetic compounds. The Geranium oil are dose dependent and the mortality of the larvae increased as the doses of the sample were increased from 0.25% to 2%. When estimating lethal concentration of the larval forms, it is noted that as the larval developmental stage increases, the mortality also increases.

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