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Oviposition Deterrent Activity of Some Volatile Oils against the Filaria Mosquito Vector *Culex pipiens*

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ABSTRACT

The oviposition deterrent activity of four essential oils derived from chamomile (*Matricaria recutita*), sesame (*Sesamum indicum*), jojoba (*Simmondsia chinensis*) and ginger (*Zingiber officinalis*), were evaluated against the gravid females of the filaria vector *Culex pipiens* under laboratory conditions. They were assayed at three doses (0.5, 5 and 50 ppm) where plastic cups containing 1 mL of desired oil concentration and 99 mL of distilled water were used as oviposition substrate. Results indicated that all these oils exhibited potent deterrent activity against gravid female mosquitoes with various degrees of repellency (ranging from 48.73-100%) depending on both plant species and applied dose from each plant. Likewise, Oviposition Activity Indexes (OAI) of such oils were all negative values ranged from -0.17 to -1.28 Although, present results suggesting that these oils are potential source of valuable mosquitoes oviposition deterrents, further detailed research is required for isolating and identifying their active compounds as well as other factors influencing their capacity.

Key words: Oviposition, deterrent activity, volatile oils, mosquitoes, *Culex pipiens*

INTRODUCTION

Mosquitoes are serious vector borne diseases that transmit fatal diseases such as dengue, Malaria, yellow fever, encephalitis and Filariasis to more than 700 million people annually (Taubes, 1997; Cetin *et al*., 2011). Lymphatic filariasis, worldwide disease transmitted by *Cx. pipiens,* infects 120 million people in 73 countries (Ottesen *et al*., 1997). Controlling *Cx. pipiens* populations representing one of the strategies for combating such disease but it becomes increasingly difficult because of relaying for long time on synthetic insecticides that finally lead to mosquito resistance to these insecticides (Brogdon and McAllister, 1998; Perera *et al*., 2008). Furthermore, Environmental and health influences of synthetic insecticides have stimulated the search for new more efficient and safer insecticides derived from plants (Nogueira and Palmerio, 2001).

Plants are important source of natural insecticides such as pyrethroids (pyrethrin and allethrin) and rotenoids. In the recent decades, several studies have shown that many phytochemicals have a toxic effect against insects and their immature stages by interfering in their growth, development and/or reproduction, or by producing attractive or repellent scents (ICMR, 2003). Sukumar *et al*. (1991) and Shaalan *et al*. (2005) reported many other studies carried out to

evaluate the mosquitocidal activity of phytochemicals. Identification of such novel effective secondary botanical compounds can be used as an alternative to synthetic insecticides and/or in the integrated vector control programs (Rajkumar and Jebanesan, 2008; Elango *et al*., 2009).

Essential oils are among natural compounds derived from a variety of plants and exhibited mosquitocidal activity. Although most of the mosquitocidal activity of essential oils was against larvae and adults of different mosquito species (Sukumar *et al*., 1991; Shaalan *et al*., 2005), their influence as oviposition deterrents against mosquitoes has not been studied much. A few studies on oviposition deterrent potential of these essential oils produced from different plants are available. Strong anti-oviposition activity was induced by crude ethanol extract of the Australian bottlebrush *Callistemon lanceolatus* against gravid females of *Culex. quinquefasciatus* that were given dual choice of crude ethanol extract-treated water and non-treated control water (Mohsen *et al*., 1990). Elhag (1999) indicated that neem seed kernels, *Azadirachta indica*, *Rhazya stricta*, *Heliotropium bacciferum*, *Syzygium aromaticum* and orange peels extracts significantly deterrent oviposition by gravid filaria vector females, *Cx. pipiens*, at 0.05 and 0.1% (= 50 and 100 ppm) concentrations of water and methanol extracts, respectively. Out of ten essential oils extracted from medicinal plants, essential oils of *Cinnamomum zeylanicum* was found to be highly effective in preventing egg laying by three mosquito species with an order of deterrence, *Anopheles stephensi* > *Aedes aegypti* >*Cx. quinquefasciatus* (Prajapati *et al*., 2005). Rajkumar and Jebanesan (2005) found that 0.01, 0.025, 0.05, 0.075 and 0.1% leaf extract of *Solanum trilobatum* reduced egg laying by gravid malaria vector females, *Anopheles stephensi*, from 18-99% compared to ethanol-treated controls under laboratory conditions. Essential oils extracted from 18 Thai plant species belonging to 11 families exhibited oviposition deterrent activity against *Ae. aegypti* with various degrees of repellency ranging from 16.6 to 94.7% (Tawatsin *et al*., 2006). Rajkumar and Jebanesan (2008) reported that methanol leaf extract of *Chenopodium ambrosioides* reduced egg laying by gravid filaria vector females, *Cx. quinquefasciatus*, from 27.7-90.2% at concentrations of 100, 200, 300 and 400 mg L^{-1} under laboratory conditions. Rosemary oil exhibited oviposition deterrent activity against the yellow fever vector *Ae. aegypti* (Waliwitiya *et al*., 2009). Elango *et al*. (2009) recorded effective oviposition repellency (88.26-96.93%) of the leaf acetone, ethyl acetate and methanol extracts of *Aegle marmelos*, *Andrographis lineata* and *Cocculus hirsutus* against *Anopheles subpictus* at relatively high dose (500 ppm) whilst the lowest repellency (47.14-71.09%) was recorded at 31.25 ppm. Similarly, significant difference in the number of eggs deposited by gravid malaria vector females, *Anophels gambiae* s.s., found in control cups when compared with the number of eggs found in water treated with *Ocimum kilimandscharicum* or *Ocimum suave* leaf extract (Kweka *et al*., 2010). The essential oils of peppermint (*Mentha piperita*), basil (*Ocimum basilicum*), rosemary (*Rosmarinus officinalis*), citronella (*Cymbopogon nardus*) and celery seed (*Apium graveolens*) exhibited oviposition deterrence activity against the dengue vector *Ae. aegypti* (Warikoo *et al*., 2011).

Hence, the present study was conducted to explore the oviposition deterrent potential of four essential oils, chamomile (*M. recutita*), sesame (*S. indicum*), jojoba (*S. chinensis*) and ginger (*Z. officinalis*), against the gravid females of the filaria vector *Cx. pipiens*. Although, these oils are known for their medicinal properties, they did not exhibit mosquitocidal activities except for ginger oil.

MATERIALS AND METHODS

Mosquitoes: Blood feed females of *Culex pipiens* mosquitoes were collected from the field by means of manual aspirator. Collected females were introduced directly into mosquito rearing cages (18-25 female/cage) shortly before the bioassays.

Botanical oils: Commercially available plant oils, obtained from El-Kaptain Company, were used. Such oils were chamomile (*M. recutita*), jojoba (*S. chinensis*), ginger (*Z. officinalis*) and sesame (*S. indicum*).

Bioassays: For bioassays, 1 mL from the desired concentration (0.5, 5 and 50 ppm) was introduced in plastic cup, 100 mL capacity, containing 99 mL distilled water while control received 1 mL ethanol 95% and 99 mL distilled water. Each concentration replicated 4 times plus control and all cups were randomly distributed inside mosquitoes cages containing 18-25 female mosquitoes/cage. Cages, containing female mosquitoes and chemicals, were left for 3 days and number of deposited egg rafts in both test and control cups was recorded daily until all females stopped depositing egg rafts. Number of female mosquitoes that died without depositing eggs were also recorded but not included in calculations.

The percentage of effective repellency (ER %) for each essential oil and for each dose was calculated by Xue *et al.* (2001) as follow:

$$\text{Effective repellency (\%)} = \frac{\text{NC-NT}}{\text{NC}} \times 100$$

where, ER is percent effective repellency; NC is the number of mosquito egg rafts in control cups and NT is the number of mosquito egg rafts in treated cups.

The Oviposition Activity Index (OAI) is calculated based on the formula of Kramer and Mulla (1979) as follow:

$$\text{OAI} = \frac{\text{NT-NC}}{\text{NT+NC}}$$

where, NT is no. of egg rafts in the treatment and NC is no. of egg rafts in the control. Index values lie within the range from +1 to -1. Positive values indicate that more ovipositions were observed in the treatment than in the control (indicating the oil is attractant) and conversely more ovipositions in the control than in the treatment would result in a negative OAI (indicating the oil is repellent).

Statistical analysis: SPSS version 16 was used. One-way Analysis of Variance (ANOVA) was used for the multiple concentration tests and for percent mortality to determine significant treatment differences. Results with $p<0.05$ were considered to be statistically significant.

RESULTS

All the oils exhibited oviposition deterrent activity against gravid females of *Cx. pipiens* mosquitoes but with different levels of activity depending on both oils and applied dose (Table 1-3). In general, gravid female mosquitoes deposited more egg rafts in cups devoid of oils "control" compared to treated cups and such difference in the percentage of deposited egg rafts between test and control is not significantly different ($p>0.05$) except for the jojoba oil ($df = 3$, $F = 5.87$, $p<0.05$) in particular the 50 ppm dose (Table 1). Females of *Cx. pipiens* deposited more egg rafts in lower doses, 0.5 and 5 ppm, than the higher dose, 50 ppm, (Table 1, 2) indicating that the latter one is the most active for all plant species derived oils. Unlike chamomile and sesame oils, larger dose 50 ppm of both ginger and jojoba completely deterrent gravid females to deposit egg rafts

Table 1: Oviposition deterrent activity of volatile oils against gravid females *Culex pipiens*

	Mean percentage of egg rafts±standard error			
Oils	Control	0.5 ppm	5 ppm	50 ppm
Matricaria recutita (chamomile)	45.0±15.35[a]	30±12.32[a]	21.0±5.1[a]	4±3.7[a]
Sesamum indicum (sesame)	43.0±9.0[a]	14±2.10[a]	31.0±10[a]	12±2.88[a]
Simmondsia chinensis (jojoba)	53.5±15.37[ab]	17±6.60[a]	29.5±6.8[a]	0±0.0[ab]
Zingiber officinalis (ginger)	47.0±13.19[a]	24±8.32[a]	29.0±13.66[a]	0.0[a]

[a]Rows are not significantly different at $p>0.05$ by one way ANOVA with Tukey's multiple range test, [b]Rows are significantly different at $p<0.05$ by one way ANOVA with Tukey's multiple range test

Table 2: Effective repellency of volatile oils against gravid females *Culex pipiens*

	Mean percentage of effective repellency (ER)±standard error		
Oils	0.5 ppm	5 ppm	50 ppm
Matricaria recutita (chamomile)	69.59±3.7	81.61±6.4	93.33±6.6
Sesamum indicum (sesame)	60.36±4.38	48.73±13.09	74.31±20.1
Simmondsia chinensis (jojoba)	69.44±16.07	58.33±12.26	100.00±0.0
Zingiber officinalis (ginger)	66.66±18.25	72.00±11.57	100.00±0.0

Table 3: Oviposition activity index (OAI) of the volatile oils against gravid females *Culex pipiens*

	Mean oviposition activity index (OAI)±standard error		
Oils	0.5 ppm	5 ppm	50 ppm
Matricaria recutita (chamomile)	-1.28±1.29	-0.18±0.4	-0.89±0.11
Sesamum indicum (sesame)	-0.5±0.04	-0.17±0.23	-0.64±0.28
Simmondsia chinensis (jojoba)	-0.41±0.27	-0.21±0.27	-1±0.0
Zingiber officinalis (ginger)	-0.41±0.28	-0.44±0.27	-1±0.0

(Table 2) and they were more potent than chamomile and sesame oils. The oviposition activity indices for all doses of all oils (Table 3) were negative and ranged from -0.17 to -1.28 which means that these oils have oviposition deterrent activity and the more the OAI value, the greater the oviposition deterrent activity. Accordingly, the present results categorized these oils into 2 groups: the first one showed strong oviposition deterrent activity including oils that showed OAI>-0.5 up to = -1 such as the 50 ppm dose of the 4 oils whilst the second group showed moderate oviposition deterrent activity including oils that showed OAI = -0.5 such as the other doses, 0.5 and 5 ppm, of the 4 oils except for the 0.5 ppm of chamomile oil.

DISCUSSION

Based on phenomenon of either selection or rejection of oviposition sites by the sensory receptors located on their antennae (Davis and Bowen, 1994), researchers investigated and employed this behavior as one of the mosquito control measures. The present study is one of these trials and revealed that essential oils of *M. recutita* (chamomile), *S. indicum* (sesame), *S. chinensis* (jojoba) and *Z. officinalis* (ginger) produced oviposition deterrent activity against gravid females *Cx. pipiens*. Such activity depended on both plant species and applied oil concentration (Table 1-3). The deterrent activity was directly proportional with applied oils concentration. Gravid females deposited fewer egg rafts in case of 50 ppm compared to 0.5 and 5 ppm and could be considered as

applicable dose. Furthermore, the ER and OAI (Table 2, 3) indicated that *Cx. pipiens* gravid females were sensitive to the odour of these oils and highly effective in preventing egg laying. The reason could be due to a variety of chemicals compounds in these oils that their degradation within the oviposition medium producing secondary metabolites acting independently or jointly to inhibit mosquitoes from laying eggs.

The present findings are compatible with other previously laboratory screened essential oils and derivatives. For instance, Warikoo *et al.* (2011) showed that essential oils of peppermint (*M. piperita*), basil (*O. basilicum*), rosemary (*R. officinalis*) and citronella (*C. nardus*) produced 100% oviposition deterrence activity against the dengue vector *Ae. aegypti* when pure oils (concentration of 100 % = 100000 ppm) were applied in the oviposition medium whilst present study showed similar activity but at a very smaller dose (50 ppm), in particular the ginger and jojoba essential oils. Similarly, *Imperata cylindrica* showed 100% anti-oviposition activity against *Cx. quinquefasciatus* at 1000 ppm (Mohsen *et al.*, 1995) that is 20 times larger than the present concentration (50 ppm). Furthermore, the present findings are 10 times smaller than those of Elango *et al.* (2009) who mentioned that effective oviposition repellency (88.26-96.93%) of the leaf acetone, ethyl acetate and methanol extracts of *A. marmelos*, *A. lineata* and *C. hirsutus* against *An. subpictus* was at relatively high dose (500 ppm). The anti-oviposition activity induced by crude ethanol extract of the Australian bottlebrush *C. lanceolatus* against gravid females of *Cx. quinquefasciatus* at 10-100 mg L^{-1} (Mohsen *et al.*, 1990) is in coincidence with the present results. Likewise, Elhag (1999) mentioned that neem seed kernels, *A. indica*, *R. stricta*, *H. bacciferum*, *S. aromaticum* and orange peels extracts significantly deterrent gravid filaria vector females, *Cx. pipiens*, at 50 and 100 ppm of water and methanol extracts respectively. In contrast to the present study, inflorescence oil of *Piper marginatum* did not stop the oviposition of *Ae. aegypti* females when assayed at 50 ppm (Autran *et al.*, 2009) indicating with much the potential of the present results.

Essential oils of ginger, *Z. officinale*, exhibited larvicidal (Govindarajan, 2011), adulticidal (Dadji *et al.*, 2011), repellent (Govindarajan, 2011) activities and oviposition deterrent activity (Tawatsin *et al.*, 2006). The present oviposition deterrent activity results of *Z. officinale* are not comparable to previous study conducted by Prajapati *et al.* (2005) that indicated that essential oil of *Z. officinale* was effective in preventing egg laying by three mosquito species at a very small dose ranged from 106.7-126.7 μg L^{-1} = 0.106-0.126 mg L^{-1}. Furthermore, Tawatsin *et al.* (2006) found that the ginger oil, *Z. officinale*, produced 90% deterrent activity against *Ae. aegypti* gravid mosquitoes at a dose (0.01% =10 ppm) 5 times lower than the dose applied in the present study, 50 ppm but produced 100% deterrent activity.

Results of the resent study suggest that these oils are effective and promising oviposition deterrents against *Cx. pipiens* mosquitoes and could be useful in the search for new natural mosquitocidal products. Consequently, more research is required to determine active compounds in such oils, their mode of action, their activity against wide range of mosquito species and their residual capacity in natural environment.

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