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Repellant activity of plant essential oils formulation against three disease causing mosquito vectors

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A plant oil formulation (POF) consisting of calamus, cinnamon, citronella, clove, eucalyptus, lemon, menthe, orange oils and natural Camphor 10% was evaluated for its repellant potential against *Aedes aegypti* (L.), *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say at 250 and 15.25 ppm. At 250 ppm concentrations the POF provided 100% protection from bites of *Anopheles stephensi* up to a mean time of 6 hours and 30 minutes while for *C. quinquefasciatus* and *A. aegypti*, it was 5 h 30 min. At 15.62 ppm it also exhibited more than 74% mean protection against *C. quinquefasciatus* and *A. aegypti* while it was 62.88% effective against *A. stephensi*. Ethanol which was used for suspension was kept as negative control and DEET (25 mg) served as positive control. The study concludes that this formulation could be used to repel these insects.

Key words: Biting protection, DEET, *Aedes aegypti*, *Anopheles stephensi*, *Culex quinquefasciatus*

Introduction

Mosquitoes act as vectors for the transmission of dangerous diseases like malaria, lymphatic filariasis and viral diseases which lead to morbidity, mortality, social and economical loss (Becker *et al*., 2003). Personal protective measures, including repellants are widely used to prevent the transmission of arthropod-borne diseases by minimizing the contact between humans and vectors. In contrast to vaccine and chemoprophylaxis as means of personal protection, repellants are considered as convenient, inexpensive and affordable with advantages in protection against a wide range of vectors (WHO, 1995). Different volatile organic molecules have the potential to inhibit the scent

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orientation of insects in confined environmental conditions and therefore can be efficiently used as repellants against mosquito populations (Oyedele *et al*., 2002; Kline *et al*., 2003; Achee *et al*., 2009; Drapeau *et al*., 2009). Repellancy is one of the commonly used methods to prevent mosquitoes and other blood-sucking pests from biting. Repellants are applied directly on the skin or to clothing and other fabrics such as bed nets and anti-mosquito screens. Repellants evaporate much more quickly than most insecticides. The prolonged use of commercially developed mosquito coils, mats and vapourizers which contains synthetic pyrethroids like pyrethrin, allethrin and paraallethrin and chemical such as DEET may cause harmful effects (Bell *et al*., 2002); also, the mosquitoes develop resistance to these chemicals (Magurayni *et al*., 2009). In the commercial mosquito vapourizers, the synthetic and chemical insecticides are dissolved in deodourized kerosene, which also causes side effects to the user. In search of alternative and safe methods to protect from mosquito bites, products from essential oils are emerging as good mosquito repellants and they are effective, safe and also low cost (Mittal *et al*., 2003; Senthil Nathan *et al.*, 2004).

Researchers all over the world have reported the repellant activity of essential oils derived from plants against the adults of various mosquito species. Volatile oils from *Acorus calamus* showed repellant activity against *A. Aegypti* (Tariq and Quadri, 2001); oil from *Cinnamomum camphora, C. zeylanicum*, and *Citrus limon* (Amer and Mehlhorn, 2006), *C. zeylanicum* (Prajapati *et al*., 2005) form chemical repellants against *A. aegypti, An. stephensi* and *C. quinquefasciatus.* Oil from *Citronella spp*. has shown repellant activity against *A. aegypti* (Nazli *et al.*, 2008).

Essential oils from leaves of *Cymbopogon nardus* and *Cymbopogon sp.* showed repellant activity against *A. aegypti*, *A. stephensi, A. culicifacies* and *C. quinquefasciatus* (Ansari and Razdan, 1995; Tyagi *et al*., 1998; Trongtokit *et al.*, 2005); *Eucalyptus globulus* oil revealed repellant activity against *A. aegypti* (Choi *et al.*, 2002), *A. albopictus* (Yang and Ma, 2005), *C. pipiens pallens, A. stephensi* and *C. quinquefasciatus* (Amer and Mehlhorn, 2006), *Mentha piperita* oil exhibited repellant activity against *A. stephensi, A. aegypti* and *C. quinqeufasciatus* (Pathak *et al.*, 2000; Amer and Mehlhorn, 2006) while oil from *M. spicata* and *Citrus sinensis* showed repellency against *A. albopictus* (Yang and Ma, 2005) and *C. pipiens molestus* (Traboulsi et al., 2005). Rajkumar and Jebanesan (2007) showed the repellant activity of essential oils from *Centella asiatica, Ipomoea cairica, Momordica charantia, Psidium guajava* and *Tridax procumbens* against the malarial vector, *A. stephensi.*

Combination of different oils such as *Syzygium* spp., *Cymbopogon* spp., *Eucalyptus* spp., *Pelargonium* spp., *Lavandula* spp., *Mentha* spp., *Santalum* spp. and *Thymus* spp. exhibited repellant activity against *A. aegypti* (Girgenti and Suss, 2002). Recently, essential oil formulations have been reported to have repellant activity against *A. albopictus* and *C. quinquefasciatus* (Cliek *et al*., 2010), *A. gambiae* and *Anopheles funestus* (Oparaocha *et al*., 2010). Lawal *et al*. (2012) reported that different formulations from six essential oils showed repellant activity against *A. gabmbiae*. The present study was aimed at evaluating the repellant activity of an essential oil formulation comprising eight oils against *A. aegypti*, *A. stephensi* and *C. Quinquefasciatus.*

Materials and methods

Based on preliminary screening (Manimaran *et al*., 2012), the following oils were selected for this study. The plant oil formulation (POF) consisted of calamus (*Acorus calamus* Linn.) oil – 3%, cinnamon (*Cinnamomum veerum* Presl.) oil – 13%, citronella (*Cymbopogon nardus* Rendle) oil – 12%, clove (*Myrtus caryophyllus* L.) oil – 24%, eucalyptus (*Eucalyptus globulus* Labil.) oil – 6%, lemon (*Citrus limon* (L.) Burm. F.) oil – 7%, mentha (*Mentha piperita* L.) oil – 10%, orange (*Citrus sinensis* (L.) Osbeck) oil – 25% and natural Camphor 10% (10 g in 100 mL).

The repellancy of volatile oils was evaluated using the human-bait technique (Schreck and McGovern, 1989; WHO, 2009). The repellant activity was determined by the percentage of protection in relation to dose method (WHO, 1996). Three to four days old, blood starved 100 adult female *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* mosquitoes were kept in respective net cages (45 cm x 30 cm x 45 cm). The forearms of the test persons were cleaned with distilled water. After air drying, one arm of the test person (only 2.5 cm^2 dorsal side of the skin) was exposed and the remaining area being covered by rubber gloves. The POF was dissolved in ethanol to make 15.25 and 250 ppm concentrations. About 1 mL of the POF was applied to the exposed part of the forearm of volunteers. One volunteer applied with ethanol served as negative control. DEET (25 mg) was used as positive control.

The periodicity of feeding pattern differed from species to species. According to the feeding time, the repellancy tests were conducted. At each testing period the hands were introduced inside the test cage and exposed to the adult mosquitoes for 5 min duration from 6:00 h until 18:00 h for *Aedes aegypti* and *Anopheles stephensi* and from 20:00 h until 06:00 h for *Culex quinquefasciatus*. Each test was replicated six times. The number of mosquitoes

landing on both the control and treated hands was recorded at every 5 minutes exposure period with the help of a timer.

$$\text{Percentage of mosquitoes fed} = \frac{\text{No. of mosquitoes fed}}{\text{Total no. of mosquitoes released}} \text{ x } 100$$

Percentage of protection = Percentage of mosquitoes fed - 100

Results

The results of repellant activity of POF on the forearm of the volunteers in the laboratory against *Anopheles stephensi* are presented in Table 1. At 250 ppm, the POF provided 100% protection from bites of *Anopheles stephensi* up to a mean time of 6 h 30 min. The control gave protection for only about 30 min. The study clearly showed that the application of 250 ppm concentration of the POF provided 83.19 % biting protection from *Anopheles stephensi*. DEET (25 mg) recorded the biting protection for 5.30 h and it showed 80.34% biting protection.

Table 1. Repellant Activity of the Plant Oil Formulation Against the Adult *Anopheles stephensi*

Observation Time	**Mean number of mosquitoes Landing / Feeding on volunteers**			
	Plant Oil (250 ppm)	Plant Oil (15.62 ppm)	Ethanol (Control)	DEET (25 mg)
18:00 – 19:00	0	0	0	0
19:01 – 20:00	0	0	6.83 ± 0.40	0
20:01 – 21:00	0	3.33 ± 0.51	8.33 ± 1.03	0
21:01 – 22:00	0	4.33 ± 0.51	7.66 ± 1.03	0
22:01 – 23:00	0	4.16 ± 0.40	6.33 ± 0.51	0
23:01 – 24:00	0	3.16 ± 0.40	8.33 ± 0.51	2.17±0.41
00:01 – 01:00	4.00 ± 0.63	5.00 ± 0.63	8.16 ± 0.75	2.50 ±0.55
01:01 – 02:00	3.16 ± 0.40	3.66 ± 0.81	7.33 ± 1.03	2.67 ± 0.82
02:01 – 03:00	3.16 ± 0.51	4.83 ± 0.75	4.16 ± 0.75	3.17±0.75
03:01 – 04:00	2.00 ± 0.63	3.16 ± 0.81	6.83 ±0.40	3.66±0.81
04:01 – 05:00	2.16 ± 0.40	3.16 ± 0.75	7.66 ± 1.03	3.16±0.40
05:01 – 06:00	2.33 ± 0.51	2.33 ± 0.51	4.50 ± 0.83	2.33±0.51
Total (% mosquito fed)	16.81±1.58	37.12 ± 1.03	76.12 ± 0.98	19.66±1.50
% of Protection	83.19	62.88	23.88	80.34
Mean protection time (Min)	390.00	150	30	330.00

Values represent Mean ± Standard deviation of six replicates.

*Value represent Mean ± SD of the means

The repellant activity of the POF against *Culex quinquefasciatus* is given in Table 2. At 250 ppm 100% protection from the bites of *Culex quinquefasciatus* up to a mean time of 5 h 30 min was observed. The control protected only 30 min from mosquito bite or landing. The POF at 250 ppm provided 87.19% biting protection from *Culex quinquefasciatus.* DEET (25 mg) recorded the biting protection for 5.30 h and it showed 81.53% biting protection.

Table 2. Repellant Activity of the Plant Oil Formulation Against the Adult *Culex quinquefasciatus*

Observation Time	**Mean number of mosquitoes Landing / Feeding on volunteers**			
	Plant Oil (250 ppm)	Plant Oil (15.62 ppm)		Ethanol (Control)
18:00 – 19:00	0	0	0	0
19:01 – 20:00	0	0	5.16 ± 0.75	0
20:01 – 21:00	0	0	7.16 ± 0.75	0
21:01 – 22:00	0	2.33 ± 0.51	8.16 ± 0.75	0
22:01 – 23:00	0	1.16 ± 0.40	7.33 ± 1.03	0
23:01 – 24:00	3.00 ± 0. 63	2.00 ± 0.63	8.33 ± 1.03	1.833±0.41
00:01 – 01:00	2.16 ± 0.40	3.50 ± 0.83	9.66 ± 0.75	2.00 ±0.63
01:01 – 02:00	2.33 ± 0.51	2.16 ± 0.40	7.33 ± 1.03	2.33 ± 0.52
02:01 – 03:00	1.16 ± 0.40	4.33 ± 0.51	4.50 ± 0.83	2.66±0.52
03:01 – 04:00	2.00 ± 0.63	2.83 ± 0.40	8.16 ±0.75	2.83±0.75
04:01 – 05:00	1.16 ± 0.40	2.00 ± 0.63	6.33 ± 51	3.16±0.40
05:01 – 06:00	1.00 ± 0.63	3.16 ± 0.40	5.50 ± 0.83	3.66±0.82
Total (mosquito fed %)*	12.81 ± 0.83	23.47 ± 1.03	75.62 ± 0.98	18.47±1.44
% of Protection	87.19	76.53	24.38	81.53
Mean protection time (Min)	330	180	60	330.00

Values represent Mean ± Standard deviation of six replicates.
*Value represent Mean ± SD of the means

At 250 ppm of POF, it provided 100% protection from bites of *Aedes aegypti* up to a mean time of 5 h 30 min. The control recorded only 30 min protection from mosquito bite or landing. The POF at 250 ppm concentration provided 87.03 % biting protection from *Aedes aegypti* (Table 3). DEET (25 mg) recorded the biting protection for 5.30 h and it showed 84.33% biting protection.

Allergic reaction study clearly indicated that the POF did not show any allergic effects such as itching, breathing difficulty, or headache to the volunteers.

Table 3. Repellant Activity of the Plant Oil Formulation Against the Adult *Aedes aegypti*

Observation Time	Number of Mosquitoes Landing / Feeding on Volunteers			
	Plant Oil (250 ppm)	Plant Oil (15.62 ppm)	Ethanol (Control)	DEET (25 mg)
06:00 – 07:00	0	0	0	0
07:01 – 08:00	0	0	7.33 ± 1.03	0
08:01 – 09:00	0	0	9.16 ± 0.75	0
09:01 – 10:00	0	2.16 ± 0.75	8.33 ±1.03	0
10:01 – 11:00	0	1.33 ± 0.51	6.83 ± 0.40	0
11:01 – 12:00	2.33 ± 0. 51	2.33 ± 0.51	5.16 ± 0.75	1.5±0.57
12:01 – 13:00	3.33 ± 0.51	5.00 ± 0.63	8.33 ±1.03	1.67±0.52
13:01 – 14:00	1.50 ± 0.63	3.33 ± 0.51	4.50 ± 0.83	2.00±0.63
14:01 – 15:00	2.16 ± 0.40	3.50 ± 0.83	6.83 ± 0.40	2.33±0.52
15:01 – 16:00	1.33± 0.51	2.16 ± 0.75	7.66 ±1.03	2.67±0.52
16:01 – 17:00	1.16 ± 0.40	2.16 ± 0.75	6.16 ± 0.75	3.0±0.63
17:01 – 18:00	1.16 ± 0.40	3.33 ± 0.51	5.15 ± 0.75	2.5±0.55
Total (Mosquito fed %)	12.97 ± 1.58	25.14 ± 1.03	75.45 ± 0.98	15.67±1.22
% of Protection	87.03	74.86	24.55	84.33
Mean protection time (Min)	330	180	30	330.00

Values represent Mean ± Standard deviation of six replicates.
*Value represent Mean ± SD of the means

Discussion

The present study clearly revealed that the formulation at 250 ppm offered a mean protection time of more than 330 minutes (i.e. 5h. 30 min) against the mosquito species tested. The result of this study coincides with the earlier results of Das *et al*. (2003) who reported that *Zanthoxylum limonella* and *Citrus aurantifolia* essential oils when mixed with mustard oil exhibited a mean protection time of more than 302 min against *Aedes* (S.) *alpobictus* at 30% concentration. Complex mixture of five essential oils recorded more than 450 minutes protection time against *A. agypti*, *C. Quinquefasciatus* and *A. stephensi* (Amer and Mehlhorn, 2006). Khandagle *et al*. (2011) reported that essential oil from rhizome of *Zingiber officinalis* recorded 300 min protection from biting of *A. aegypti* and *C. quinquefasciatus*. An oil formulation containing *Nepeta cataria* was tested against black flies, *Simulium decorum* and mosquito *Aedes intrudens* for its repellant activity. The formulation

exhibited over 4 hrs protection from bites (Spero *et al*., 2008). Oyedele *et al*. (2002) reported that the products (ointment and cream) from lemon grass oil exhibited 2-3 hrs protection from biting of *A. aegypti* at a concentration of 15%v/w. In the present investigation, the concentration of 250 ppm of the POF exhibited 83.19% protection against *Anopheles stephensi*. The concentration of 250 ppm of the POF exhibited 87.19 % protection against *Culex quinquefasciatus*. The POF at 250 ppm concentration exhibited 87.03% protection against *Aedes aegypti* bite. Amer and Mehlhorn (2006) reported that a mixture of essential oils provided biting protection against *A. aegypti* , *C. Quinquefasciatus* and *A. stephensi.* The essential oil formulation comprising of *E. globulus, O. basilicum, C. citratus , C. sinensis, A. indica* (Neem) and *H. suaveolens* exhibited 72.22% protection (2 h) from biting of *A. gambiae* at 10% concentration (Lawal *et al*., 2012). *E. globulus* and *Ocimum basilicum* mixed in soybean oil exhibited 99% biting protection from *A. aegypti* and *A. dirus* with protection time of 210 minutes (Sritabutra *et al*., 2011). The repellant activity in the present study may be due to the presence of E. *globulus, C. citrates* and *C. sinensis* which were used in the formulation along with other essential oils. Similarly Trongtokit *et al*. (2005) reported that the essential oils from *C. nardus, Pogostemon cablin, Syzygium aromaticum* and *Zanthoxylum limonella* effectively provided 100% repellancy for 2 hrs from biting of *A. aegypti*. Combination of different oils such as *Syzygium* spp., *Cymbopogon* spp., *Eucalyptus* spp., *Pelargonium* spp., *Lavandula* spp., *Mentha* spp., *Santalum* spp., and *Thymus* spp. exhibited repellant activity against *Ae. Aegypti* (Girgenti and Suss, 2002). The repellant activity of plant products may be due to the active principles present in the plants which block mosquito sensory organs with heavy, bulky molecules (Ned Roze, 2012).

In the present investigation the essential oil formulation showed repellant activity against *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti*. In this study the repellant activity depends on the concentration of POF (250 ppm and 15.62 ppm). The present findings corroborate with the findings of Lawal *et al*. (2012) who reported that the percentage protection was concentration dependent manner. Higher concentration showed higher protection than the lower concentration. Similarly, Venkatachalam and Jebanesan (2001) reported that *Feronia elephantum* exhibited the total percentage protection of 45.8 % at 1.0 mg/cm^2 and 59.0 % at 2.5 mg/cm^2. Essential oil formulation comprising of *Ocimum gratissimum*, with olive oil at 30% concentration showed more than 96% repellency while 20% concentration recorded less than 75% repellency against *A. gambiae* and *Anopheles funestus* (Oparaocha *et al.,* 2010).

The POF contains some chemical compounds like eugenol in clove oil, 1,8-cineole in *Eucalyptus globules*, citronellal in lemon grass, menthol and pulegone in menthe oil, cinnamaldehyde, eugenol and cinnamyl acetate in cinnamon oil, several terpenes and geraniol in lemon oil. These maybe responsible for repellant activity of five essential oils containing monoterpenes such as eucalyptol, geraniol, limonene, linalool, menthone, linalyl acetate and menthyl acetate which has been shown effective against blood sucking bug, *Rhodnius prolixus* which is a vector of Chagas disease in many Latin American nations (Sfara *et al.*, 2009). Choi *et al.* (2002) reported that the presence of α-terpinene, carvacrol and thymol in *Lavandula officinalis* and *R. officinalis* were responsible for the repellant activity against *C. pipiens pallens* adults.

Conclusion

The plant oil formulation exhibited repellant activity against the tested vector mosquitoes more than 300 minutes. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and has no adverse environment effects.

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