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MOSQUITO REPELLENCE BY SMOKE OF FRESH SHOOT SYSTEMS AND DRIED POWDER OF CASSIA OBSTUSIFOLIA, STRIGA HERMONTHICA AND HYPTIS SUAVEOLENS IN COMPARISON WITH THREE STANDARD SYNTHETIC MOSQUITO REPELLENTS IN THE GHANAIAN MARKET

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Abstract

The widespread use of smoke from smouldering fresh shoot systems of plants to repel mosquitoes in most African communities has frequently resulted in fire outbreaks and respiratory disorders. The current work investigated alternative ways of using plant materials devoid of burning to protect humans against mosquito borne illnesses. Mosquito repellence of samples of Striga hermonthica, Cassia obtusifolia, and Hyptis suaveolens were studied in a linear olfactometer in comparison with Sasso and Heaven black mosquito coils, and Medisoft repellent lotion. Clean air was used as control. Percentage/mean repellence by S. hermonthica were 18.7% (2.8±0.5), 50% (7.5±0.2) and 65% (9.7±0.4) using 2, 4 and 6 units of smoke respectively. That by H. suaveolens was 28%, 71% and 83% using 2, 4 and 6 units of smoke respectively. The difference in repellence by the smoke of S. hermonthica and H. suaveolens were not significantly different (P>0.05) from those of the synthetic standard coils. Dried powdered Striga hermonthica and Hyptis suaveolens leaves also repelled a mean of 6.50±0.63 and 5.42±0.70 mosquitoes respectively and the difference between them was not significant. Comparatively, powdered leaves of both plants exhibited good repellence (P>0.05) as the standard synthetic repellents. The powdered samples could be used in place of the smoke of either plants or the standard mosquito coils to manage mosquito bites through repellence.

Key words: Mosquitoes, repellents, powdered plants, compared, standard coils

Introduction

Mosquitoes are a huge burden on society as nuisance pests (Mike, 2014) and as vectors of several diseases (Effiom et. al., 2012). They transmit diseases such as malaria, filariasis, dengue fever and Japanese encephalitis, causing millions of deaths every year (WHO, 2015). Malaria is one of the six killer diseases (Akumu et. al., 2013), and is common among children under five and pregnant women (WHO, 2015). The incidence of mosquito borne diseases is prevalent in the poorer parts of the world particularly Africa and about 90% of malaria related deaths worldwide come from sub-Sahara Africa (WHO, 2015). Protection against mosquitoes is therefore important especially in impoverished communities.

Substances that can be used for protection against mosquitoes may be natural or synthetic and grouped as repellents, feeding deterrents, toxins and growth regulators (Adeniyi et. al., 2010). There are lots of setbacks in the use of synthetic insecticides (Ogbonnia et. al., 1990; Rutledge & Day, 2002). Some synthetic mosquito-repellents cause allergy, skin and eye irritations, unpleasant smell, and respiratory disorders while others emit CFCs that deplete the ozone layer (Liu et. al., 2003; Peterson & Coats, 2001). N, N–diethyl–Meta–toluamide (DEET), a very common chemical in most insecticides (Paluch et. al., 2010) has been classified as a non-human carcinogen (Panagiotakopulu et. al., 1995). But some studies have reported cases of encephalopathy, urticaria syndrome, decreased heart rate, hypotension and anaphylaxis due to DEET (Peterson & Coats, 2001). Similarly, studies by Gunasekara (2005) found that exposure to bioallothrin that is used in some repellents causes brain and receptor damage. Also, mosquitoes continually develop resistance to most repellents (Joy et. al., 2002) thus causing the use of increasing volumes of the insecticides and the use of a mosquito net is not also sufficiently effective (Toe-Pare et. al., 2009). Smoke from burning coils is commonly used to supplement the different methods of mosquito control. Even at places where there has been mass spraying of mosquitoes, most people still use insecticide mosquito bed nets and burning coils to reduce the bites of mosquitoes (Karunamoorthi et. al., 2009). However, a study by Liu et. al. (2003) on the smoke of mosquito coil found changes within the respiratory system, epithelium and alveoli of study rats. These challenges all contribute to economic cost of combatting mosquito related challenges.

Thus, it is important that people use alternative and safer means to prevent effects of mosquitoes. Good repellents should have high efficacy against the vector and be user and ecologically friendly (Palanisami et. al., 2014). Natural products are usually safer to use, and need to be incorporated into mosquito repellence for human protection (Rutledge & Day, 2002). Researchers have therefore turned their focus on natural products as insecticides in battling mosquitoes and other biting diptera (Sai et. al., 2013). It has been reported that plants with insecticidal properties are widespread and can serve as new bioweapons to fight the deadly vector without complications (Akumu et. al., 2013). Plant derived mosquito repellents which are usually the commonest of natural insecticides, include volatile or essential oils (Lawal et. al., 2013; Yang & Ma, 2005). They possess phytochemicals that are biologically active or produce odours that repel insects (Palanisami et. al., 2014). Some alkaloid repellents are nicotine (Flattum & Sharkland, 1971), and physostigmine (Peterson & Coats, 2001). Terpenes also have mosquito repellence properties (Dambolena et. al., 2016; Casida & Quistad, 2006). They include (C)-Citronellol and (K)-Citronellol (Duke, 2006), pyrethrins (Casida & Quistad, 2006) limonene and mint (*Mentha* spp.) (Duke, 2006).

Studies by Palanisami et. al. (2014) and Tawatsin et. al. (2001) illustrated mosquito repellent ability of some plants. Mosquito feeding is affected by plant products such as smoke, traditionally used as repellents (Smith, 2008) and fatty acids such as capric, oleic and palmitic acids (Dhandapani & Murugan, 2011). Smoke is still the most widely used means utilized throughout the rural tropics to repel mosquitoes (Debbourn et. al., 2007; Seyoum et. al., 2002). Thus, smoke extract of *Lantana camara* leaves dissolved in methanol reportedly yielded positive results in repelling mosquitoes (Akumu et. al., 2013). Some of these plants traditionally used to drive away mosquitoes possess no health hazards (Kumuda & Yassen, 2002). However, identification of efficient non-smoke, non-synthetic repellents will be valuable in reducing the risk of fire outbreaks through the burning of repellents and also reduce cost and health risks of using synthetic repellents.

Plants such as *Hyptis suaveolens* have previously been cited to have many phytochemicals (Sharma et. al., 2013; Mbatchou & Glover, 2010; Dawet et. al., 2016), and several identified compounds depending on the influences of geographic factors and plant collection (Okonogi et. al., 2005; Noudogbessi et. al., 2009). It is an aromatic herbaceous annual shrub commonly distributed in the tropics (Noudogbessi et. al., 2009; Tennyson, 2011). Aerial parts of *Hyptis suaveolens* have been used to repel mosquitoes in Africa and South America (Abagale et. al., 2017; Sears, 1996; Seyoum et. al., 2002). Recent studies also show that extracts of the plant have larvicidal and mosquito repelling effects, and can contribute to reduce malaria incidence (Dawet et. al., 2016). *Cassia obstusifolia* is widely distributed and reported to contain many metabolites (Kewatkar, 2012; Saidou et. al., 2015). Leaves of *C. obtusifolia* are reported to have larvicidal and ovipositional deterrence against *Anopheles* mosquito (Ghosh et. al., 2011). *Striga hermonthica* is a hemi parasitic flowering plant (Hammad et. al., 2011; Jamil et. al., 2006), reported to contain many metabolites (Elshiekh and Mona, 2015; Hammad *et al*., 2011; Baba et. al., 2011). Essential oils of *Striga hermonthica* have also been reported to have mosquito repellence (Baba et. al., 2011). Therefore, in the present study mosquito repellence by dry powdered leaves of *Hyptis suaveolens*, *Cassia obstusifolia* and *Striga hermonthica*, as well as smoke from fresh shoot systems of the plants were determined and compared with repellence by three standard synthetic repellents.

Materials and Methods

Fresh shoot systems of *Striga hermonthica, Hyptis Suaveolens*, and *Cassia obtusifolia*; Sasso mosquito coil (standard 1) and Heaven Black insecticide coil (standard 2), Medisoft repellent lotion (standard 3) and unsorted species of adult mosquitoes.
Solvents and reagents used in the work were purchased from BDH agents in Ghana.

Equipment used: Water bath, rotary evaporator, olfactometer, insect cages and aspirator.

Collection and Authentication of plants

Striga hermonthica were collected from among growing millet in Gongnia, a suburb of Navrongo in the Kassena-Nankana East Municipal of Ghana. Samples of both *Hyptis suaveolens* and *Cassia obtusifolia* were collected from the University for Development Studies, Navrongo campus in Ghana. Samples of the three collected plants under study were authenticated at the Department of Applied Biology of the Faculty of Applied Sciences in the University for Development Studies. The synthetic samples were purchased from the Navrongo central market.

Sample Processing

Collected leaves of each plant were dried for seven days under laboratory conditions. Each dried sample was crushed using a porcelain mortar and pestle, and the powder was put into labelled plastic zip locker. They were then stored in a freezer until required for use.

Rearing of mosquitoes

Unsorted mosquito larvae were collected from ditches, ponds and around septic tanks in Navrongo. They were transferred into a tray of distilled water and covered with a net. Powdered biscuits were used as food for the larvae to develop (Thomas, 2014).
Newly emerging adult mosquitoes were transferred into the cage using the aspirator. 10 % sucrose soaked in cotton and placed in the cage was used to feed the mosquitoes (Dube et. al., 2011).

Generation of Smoke

An amount of 15.0 g of the fresh shoot system of each plant or synthetic standard were put in a tray containing blazing charcoal to generate smoke. A glass funnel was inverted on it to direct the smoke to fill a 50 mL syringe, as one unit of smoke.

Olfactometry

A linear olfactometer,previously used in mosquito repellence studies by Abagale et. al. (2017), a modification from Paixão et. al. (2015) was used for the olfactometry. Preliminary tests were conducted using smoke and the powdered sample of each plant and repeated using the other two plants. Comparative tests were also conducted. Fifteen (15) sucrose-fed adult mosquitoes were drawn from the rearing cage into the test chamber of the olfactometer for each separate test.
In preliminary tests using smoke; 100, 200 and 300 mL each of smoke generated from each sample were used in separate tests. For each test, the smoke was introduced into one of the response chambers, while the second remained with clean air as control. In other preliminary tests using powdered samples, 1.0, 2.0 and 3.0 g each of powdered plant leaves/synthetic standard were weighed separately into one of the test chambers of the olfactometer. The second chamber was left with clean air as control for each test. For the comparative tests, the samples to be compared were placed separately, one each into each response chamber.
The transparent tops of the chambers were covered with opaque lids for 15 minutes after which the lids were taken off and the number of mosquitoes in each

chamber was counted and recorded. Each test was replicated ten (10) times and the mean number of mosquitoes repelled calculated. To avoid bias the response chambers were washed, cleaned, and aired for 10 minutes after five (5) repetitions of each test, and then the stimuli alternated in the chambers before the other five (5) repetitions to complete the test.

The repellence percentage was calculated using the formula:

$$\% \text{ repellence} = \left(\frac{control - treatment}{total\ number\ of\ mosquitoes} \times 100\right)$$

(WHO, 2009; Dube et. al., 2011).

Statistical Analysis

The statistical mean and standard error of the mean were applied to the results. Significant differences in repellence were analysed using T test at a confidence level of 95% using Microsoft excel 2013 version

Results

In the present study smoke from the fresh aerial parts of *Hyptis suaveolens* yielded the highest repellence. Repellence by the smoke were in the order *Hyptis suaveolens* > *Striga hermonthica* > *Cassia obtusifolia* with 83, 65 and 38 % respective repellences at the maximum units of smoke used. There were significant differences (P<0.05) between the repellence by the smoke from each plant and clean air, with increasing repellence progressively with increasing volume of smoke.

Table 1: Mean numbers of adult mosquitoes repelled by varying volumes of smoke from dried powdered plants

Units (volume) of smoke	Mean of repellence ± SEM		
	H. suaveolens	***S. hermonthica***	***C. obstusifolia***
2	4.2 ±0.2	2.8±0.5	1.9±0.1
4	10.7±0.4	7.5±0.2	3.8±0.01
6	12.4± 0.3	9.7 ±0.4	5.7±0.1

Results from the tests using smoke indicated that mosquito repulsion by smoke from the natural materials increased with increasing volume of smoke (Figure 1). Comparison of the smoke of the plants in terms of percentage repellence indicates that *Hyptis suaveolens* had the highest repellence of mosquitoes, followed by *Striga hermonthica* before *Cassia obstusifolia*.

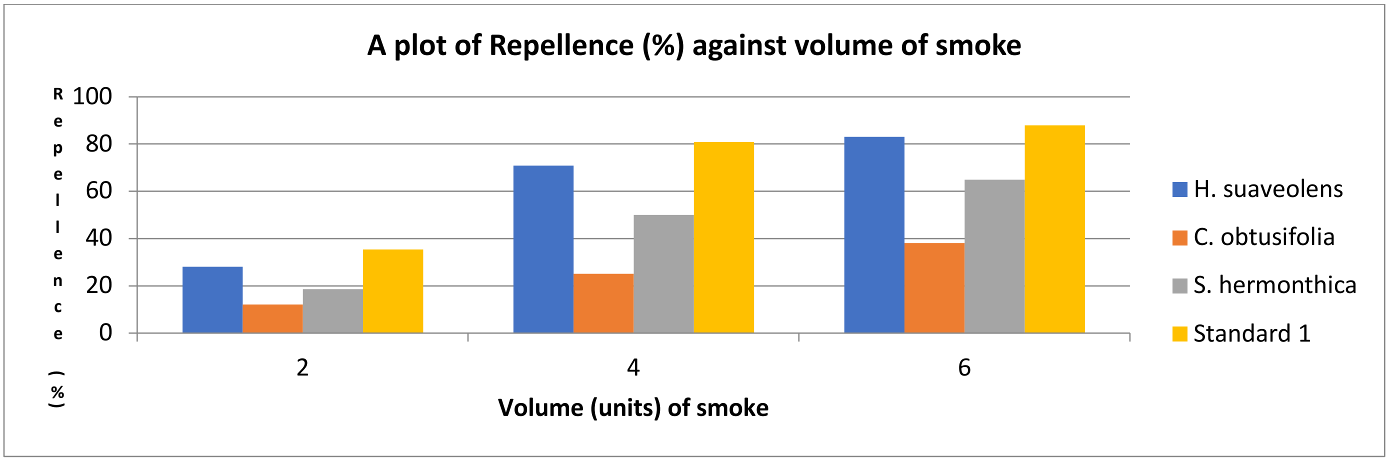

Figure 1 Graph of repellence (%) against volume of smoke

In application of the powdered plant samples in the current work, powdered *Striga hermonthica* and *Cassia obtusifolia* repelled significantly higher number of mosquitoes (Table 2): 6.50 ± 0.63 and 8.33 ± 0.82 (Mean±SE) mosquitoes compared powdered leaves of *Hyptis suaveolens* respectively. *Hyptis suaveolens* also repelled more mosquitoes (8.00 ± 0.79) compared to *Cassia obtusifolia*.

Table 2: Comparative mean number of adult mosquitoes repelled by dried powdered leaves

Test	Stimuli	Mean ± SEM	P
Dried powdered *leaves* compared	*Striga hermonthica*	6.50 ± 0.63	NS*
	Hyptis suaveolens	5.42 ± 0.70	
Dried powdered leaves compared	*Striga hermonthica*	8.33 ± 0.82	0.0009
	Cassia obtusifolia	3.91 ± 0.48	
Dried powdered leaves compared	*Hyptis suaveolens*	8.00 ± 0.79	0.0314
	Cassia obtusifolia	5.42 ± 0.54	

*NS, not significant

Table 3 Comparative mean numbers of adult mosquitoes repelled by varying amounts of standard synthetic materials

Volume (units) of smoke (mL) or mass of sample (g)	Mean of repellence ± SEM		
	Standard 1	Standard 2	Standard 3
2/ 1	5.3 ± 0.2	2.0 ± 0.3	5.7 ± 0.5
4/ 2	12.2 ± 0.3	7.5 ± 0.6	8.1 ± 0.2
6/ 3	13.3 ± 0.2	12.3 ± 0.1	15.3 ± 0.5

Smoke from the plant materials was therefore statistically as good as that of the synthetic coil. However, in comparison with smoke from *Cassia obtusifolia*, that from the synthetic material was a better repellent with a significant difference (P>0.05) between them (Table 4).

Table 4 Comparative responses of adult mosquitoes to natural and synthetics samples

Test	Stimuli	Mean± SEM	P
Smoke	*Cassia obtusifolia* leaves	5.0 ± 0.30	0.0049 (Significant)
	Standard 1	10 ± 0.10	
Smoke	*Hyptis Suaveolens* leaves	6.8 ± 0.30	NS
	Standard 1	8.2 ± 0.10	
Smoke	*Striga hermonthica* leaves	6.7 ± 0.40	NS
	Standard 1	8.3 ± 0.10	
Dried powdered	*Striga hermonthica* leaves	4.5 ± 0.47	NS
	Standard 2	5.2 ± 0.58	
Dried Powdered	*Hyptis suaveolens* leaves	3.9 ± 0.57	NS
	Standard 2	5.3 ± 0.63	
Dried Powdered	*Hyptis suaveolens leaves*	3.9 ± 0.57	NS
	Standard 3	9.1 ± 0.54	

Discussion

Insects perceive repellents by smell and this causes them to move away from the odour source (Lutz et. al., 2014). Smoke generated from burning of plant material for mosquito repellence can disguise human kairomones, disrupt convectional currents affecting host location and/or cause the mosquitoes to desiccate (Noor et. al., 2008; Smith, 2008). Six (6) units of the *Hyptis suaveolens* smoke yielded 83 % repellence with a significant difference (P<0.05) between the repellence by 2 and 6 units of smoke from the plant. Smoke from *Striga hermonthica* also had a fairly high repellence; 2 units of smoke had 18.7 %, 4 units had 50 % and 6 units yielded 65 % repellence and each repellence was significantly different (P<0.05) from the others.

Earlier studies by others researches had already indicated that repellency was concentration dependent. A study on dried leaf extract of *Hyptis suaveolens* reported a high repellency of 90 % at high concentration with a significant difference (P<0.05) between low and high concentration of the leaf extract (Joseph, 2011). Also, Ayange-kaa et. al. (2015) observed that low concentration (50 mg/ml) had low repellence (5.0 %) and higher concentration (500 mg/mL) had higher repellence (53.1%).

A comparative test of repellence by the smoke of the three standard mosquito repellents and that from the plants is presented in table 4. The difference in repellence between the smoke of *Hyptis suaveolens* as well as *Striga hermonthica* and that of standard 1 were not statically significant (P>0.05). The coil contains esbiothrin, an insect repelling compound derived from pyrethoid, with a fast knock down effect on arthropods as the active ingredient (WHO, 2002) and the plant material reportedly contains various secondary metabolites (Abagale et. al., 2017; Koua, 2011). Some classes of secondary metabolites in the plants have been described to have mosquito repellency (Sai et. al., 2013; Debbourn et. al. 2007; Sears, 1996; Smith & Secoy, 1983) and are expected to have contributed to mosquito repellence by the plants. Alkaloids are insecticidal even at low concentration (Debbourn et. al., 2007). They release insecticidal smoke that repels mosquitoes through direct toxicity (Sears, 1996). Other compounds such as methone, limonene, beta pinene, alpha pinene and linaliol have also been isolated for mosquito repellence (Libro et. al., 2016).

In comparison, the differences in repellence between *Hyptis suaveolens* and *Striga hermonthica* were not significant (NS) (Table 2). *H. suaveolens* leaf material was found to be the second efficient and that of *C. obtusifolia* leaves had the least repellence, hence the difference in repellence potential was not appreciable. Previous studies indicated that 10 % of the essential oils of *Hyptis suaveolens* yielded 92 % repellence (Abagli & Alavo, 2011), and according to Baba et. al. (2011) *S. hermonthica* oil has good repellent potential on *Anopheles gambiae*. The lotion (standard 3) was also highly repellent to the mosquitoes (Table 3). The mean number of mosquitoes repelled was in the range of 5.7±0.5 - 15.3±0.5. Oils and lotions are usually applied on the body to keep mosquitoes away.

Various *Casia* species have been published to contain alkaloids, tannins and other secondary metabolites relevant to mosquito repellence but *Cassia obstusifolia* was generally found to have low repellency (12-38 %). The leaves of *Cassia tora* have been reported to have mosquito repellence, showing a protection time of 1 hour (Tehri & Singh, 2015). *Cassia occidentalis* has also been reported to have toxicity to adult *An. stephensi* mosquitoes (Rajarajan & Rajasekaran, 2014) and studies have also shown that leaves of *Cassia obtusifolia* have strong larvicidal ability at 25 mg/l (Ghosh et. al., 2011). However, it was found in the current work that dried powdered leaves of *Cassia obtusifolia* and the smoke produced from fresh leaves of the plant were both weakly repellent to mosquitoes.

From the comparison (Table 4), mosquito repellence of the standard products was not significantly different from those of the powdered samples of *Hyptis suaveolens* and *Striga hermonthica* and also the smoke generated from them. Processing the powder is more economical and its use is less risky hence recommended in place of the standard coil or smoke from the fresh plants. Display of dried powdered repellent plant material would be a safer approach to repel mosquitoes and prevent malaria yet avoid numerous requirements (WHO, 2008) for a material to be valuable as a mosquito repellent for application on human or animal skin.

Conclusion and Recommendation

There was no significant difference (P>0.05) between the repellence by smoke of the standard repellents and that of *Hyptis suaveolens* and *Striga hermonthica*. Also, there was no significant difference (P>0.05) between repellence by smoke from *H. suaveolens* and that of *Striga hermonthica*. Thus, smoke of *S. hermonthica* and *H. suaveolens* has proved to be as potent repellents as the standard products in repelling mosquitoes.

Repellence by the powdered plant materials were in the order *Striga hermonthica* > *Hyptis suaveolens* > *Cassia obtusifolia*. The difference in repulsion between both powdered plant material and *Cassia obtusifolia* were significant.

Both the powdered dry leaves of the plants and smoke generated from them equally had statistically good repellence as the synthetic standard repellents. Thus, the plants could be used as powder and not smouldered without significant difference in repellent effect. This will reduce the possibility of fire outbreaks through the burning of repellents, and preservation of the plant products is easy and cheaper. It will also reduce the economic burden of purchasing synthetic repellents as well as the health risk associated with their use. Hence dried and powdered *Striga hermonthica* and *Striga* *hermonthica* could be provided in rooms to repel mosquitoes during sleep.

Recommendation for further study

i. Toxicity studies of the plants according to the way of use are required.

ii. Synergistic studies between *Hyptis suaveolens* and *Striga hermonthica* is required to verify any better potencies.

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