Longitudinal evaluation of repellent activity of *Ocimum gratissimum* (Labiatae) volatile oil against *Simulium damnosum*

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To determine the repellent activity of Ocimum gratissimum *volatile oil against* Simulium damnosum *(blackflies), a 12 month (January-December 2003) field study was conducted in three onchocerciasis endemic communities (Idomido, Obio camp, and Ikot Adaha) in Ini Local Government Area of Akwa Ibom State, Nigeria. The result revealed that topical application of 20% (v/v) concentration of the oil with liquid paraffin as a base, reduced the biting rate of* S. damnosum *by 90.2, 81.6, and 79.7%, in Idomido, Obiocamp, and Ikot Adaha respectively. The oil gave protection against the bite of* S. damnosum *for at least 3 h. A total of 710 adults* S. damnosum *were caught by individuals treated with Ocimum oil, as against 4296 caught by the control group. When the flies caught by the treated individuals were dissected none of them was infected with microfilariae of* Onchocerca volvulus*. Human-vector contact and onchocerciasis transmission could be reduced by the topical application of the volatile oil during the peak biting periods of the vector.*

Key words: volatile oil - *Ocimum gratissimum* - blackfly *Simulium damnosum* - repellent activity

Insect-transmitted diseases remains a major source of morbidity and mortality globally. In Nigeria, *Onchocerca volvulus* the causative agent of onchocerciasis (river blindness), is transmitted primarily by the blackflies (*Simulium damnosum*) complex (WHO 1995, Aisen et al. 2004, Opara et al. 2005). Onchocerciasis is both a public health hazard and socio-economic problem of considerable magnitude in Nigeria (Opara et al. 2005). They cause itching and disfiguring skin disease, serious eye lesions, and blindness (WHO 1995). The habit of *S. damnosum,* crawling on the skin of the individual constitute an intolerable nuisance, their painful bite lead to blood loss and serve as portal for viruses, bacteria, protozoa, and nematodes which the flies may carry on their bodies.

The strategy for control of onchocerciasis is based on destruction of the vector's larvae, prevention of vector migration, and contact with human host, killing the adult filarial and microfilarial worms in the human host. Several attempts have been made to use DDT, larvicide, and chlorinated hydrocarbon insectide in the control of blackflies. Despite a remarkable reduction in the blackfly population the epidemiological results are not impressive (Walsh 1970, WHO 1995). The strategy being used to control onchocerciasis in Nigeria is mass drug administration (MDA) through community directed treatment with ivermectin. Ivermectin has a positive effect in reducing microfilariae load in the infected individuals, it reduces transmission of infection, prevents onchocercal blindness, and skin disease (Boaten et al. 1998, Ndyomugyenyi et al. 2004).

Control of the parasites vector though desirable, is not feasible in view of the expansive areas infested and the long flight range of the fly, which facilitates the reinvasion of treated communities by flies from untreated contagious communities (Aisen et al. 2004). The worldwide threat of arthropod-transmitted disease, with their associated morbidity and mortality underscores the need for effective insect repellent. The quest to make human less attractive to blackflies has stimulated research on blackfly behaviour and control. A reduction in human vector contact through repellent action would significantly reduce the transmission of infective larvae to human hosts.

Extracts from plant sources have been shown to possess insecticidal (Iwuala & Osisiogu 1981, Pathake & Dixit 1988, Anyanwu & Uloko 1997, Oda et al. 1997, Ayanwu & Amefule 2001) and repellent (Bernard 1999, Tawatsin et al. 2001, Aisen et al. 2004) properties. There is lack of information on the effectiveness of repellents against blackfly attacks. This study investigates the repellency of volatile oil derived from *Ocimum gratissimum* against biting female adult of *S. damnosum.* This is in a search for effective and affordable natural products to be used in the control of onchocerciasis.

MATERIALS AND METHODS

Study area - The study was conducted in three communities (Idomido, Obio Camp, and Ikot Adaha) of Ini Local Government Area of Akwa Ibom State, Nigeria. Akwa Ibom State is located between latitudes 4 $N33^1$–$5N35^1$ and longitudes $7E35^1$–$8E25^1$ in the Southeastern Nigeria. The state lies within the tropical rainforest belt of Southern Nigeria. It is characterized by humid tropical climate, with annual rainfall reaching 3000 mm. The state has uniform temperature regime with annual values of 20.4 to 35.7°C. The state is characterized by the presence of numerous ecologic and zoogeographically important high gradient streams and rivers (Usip et al. 2003). Men are predominantly subsistence agriculture farmers, hunters, timber cutters, and white sand diggers. These activities increase

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man-fly contact hours. Market days are festive and resting periods. The state had been surveyed for onchocerciasis previously (Usip 2004).

Ethical clearance - The study was approved by the Ministry of Health, Akwa Ibom State, Nigeria. Informed consent was obtained from individuals and the communities involved.

Oil extraction - Volatile oil of *O. gratissimum* was extracted by steam distillation in a Clevenger-type apparatus as described previously (Craveiro et al. 1976).

Catching methods - Biting adult female *S. damnosum* were caught using human bait at Idomido, Obio Camp, and Ikot Adaha from January to December 2003. Each station was sampled four times a month. Fly catching was conducted between 7 a.m. and 6 p.m. by two fly collectors working alternately. A total of four fly collectors were recruited for each station, two fly collectors acted as control, while the other two were the test collectors.

Repellent test procedure - To determine the repellent activity of the oil extract, dilution of the stock oil was made with liquid paraffin (v/v) at 20% concentration. About 3 ml of the dilution repellent was applied topically on both legs and forearms of each of the two test individuals. The other two individuals that served as control applied ordinary paraffin oil and sat with the two test individuals for the duration of the experiments. The test and the control individuals sat approximately 5 m apart. The fly collectors sat or stood with their hands and legs exposed. Any fly perching on the exposed part was caught before it fed by inverting a small glass tube over it. The caps of the tubes were then immediately replaced. All tubes containing flies were labeled to indicate time, date, and place of capture. Each fly was caught in a different tube. The process was repeated the next day at the other stations using different fly collectors from the locality.

Dissection method - The blackflies caught by individuals treated with *O. gratissimum* oil were dissected to distinguish nulliparous and parous females (Cupp & Collins 1979). Nulliparous flies were discarded because they do not transmit *O. volvulus* (Duke 1968). Further dissection of the abdomen, thorax, and head was continued if a fly was found to be parous to check for the presence of filarial worms.

Data analysis - The repellency in the three stations was compared using the two-way analysis of variance (ANOVA). Percentage repellency was calculated by the method of Sharma and Ansari (1994) and Yap et al. (1998).

RESULTS AND DISCUSSION

The repellent properties of *O. gratissimum* volatile oil on the biting rate of adult *S. damnosum* is presented in Table I. Result indicates that topical application of the volatile oil reduces the biting rate of *S. damnosum* in a given human population. An annual biting rate of 1248 vs 192; 1572 vs 288; and 1476 vs 300 bites per person per year for control and test individuals were obtained from Idomido, Obio Camp, and Ikot Adaha respectively. The relative repellency is shown in Table II. Idomido 90.2% (range 80-93.8%); Obio Camp 81.6% (range 70.4 - 91%); and Ikot Adaha 79.7% (range 69.7-88.8%). There was a significant ($P < 0.05$) difference in the relative repellency at the three stations. The results of the protection time of *O. gratissimum* oil against the bite of *S. damnosum* is presented in Table III. There was no bite by the *S. damnosum* for at least 3 h after the topical application of the volatile oil extract. However, after 4 h of application the repellency fell to > 90% in Obio Camp and Ikot Adaha. The diurnal biting rate of parous flies from the three stations is shown in the Figure. The biting cycle showed a bimodal peak of activity. There was a small peak between 10 and 11 a.m. and a pronounced evening peak between 4 and 5 p.m. There was a significant difference ($P < 0.05$) in diurnal biting rates of flies at the three sites.

A summary of the result of catches and dissection of *S. damnosum* is shown in Table IV. A total of 710 *S. damnosum* flies captured by the treated individuals were

TABLE I

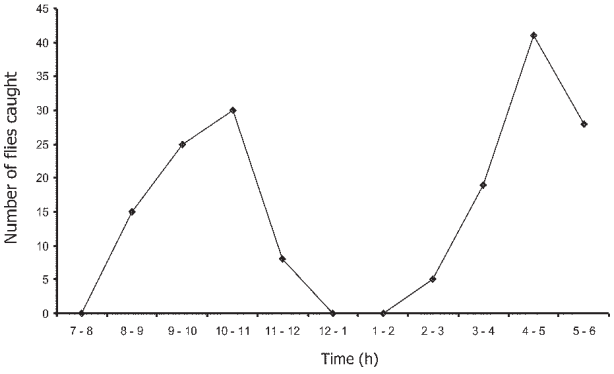
Monthly and annual biting rate of *Simulium damnosum* on treated and control individuals from Idomido, Obio Camp, and Ikot Adaha

	Idomido		Obio Camp		Ikot Adaha	
Months	Control	Treated	Control	Treated	Control	Treated
January	35	7	44	8	45	10
February	93	9	123	13	113	16
March	104	9	134	12	125	14
April	107	11	140	27	128	24
May	112	13	147	24	137	28
June	118	11	151	30	141	32
July	130	10	162	48	155	38
August	142	13	173	41	162	41
September	144	9	196	50	185	56
October	166	17	179	23	165	20
November	71	8	82	8	90	14
December	26	5	41	4	30	7
Total	1248	122	1572	288	1476	300
	$P < 0.05$		$P < 0.05$		$P < 0.05$	

TABLE II

Monthly and annual percentage repellency of volatile oil of *Ocimum gratissimum* against *Simulium damnosum*

Months	Idomido	Obio Camp	Ikot Adaha
January	80.0	81.8	77.8
February	90.3	89.4	85.8
March	91.4	91.0	88.8
April	89.7	80.7	81.3
May	88.4	83.7	79.6
June	90.7	80.1	77.3
July	92.3	70.4	75.5
August	90.8	76.3	74.7
September	93.8	87.2	69.7
October	89.8	74.5	87.8
November	88.7	87.1	84.4
December	80.7	90.2	76.7
Total	90.2	81.6	79.7



Diurnal biting pattern of black flies attracted to man (three stations combined).

dissected. None of these flies was infected (containing L_1 L_2 and L_3 larvae) with *O. volvulus*. Whereas 4296 *S. damnosum* flies were caught by the control individuals, the result of the dissection will be presented elsewhere.

No skin irritation, rashes, hot sensation were observed on the arms and legs of the treated volunteers throughout the 12 months study period.

Protection against arthropod bites is best achieved by avoiding infested habitats wearing protective clothings and applying insect repellent (Curtis 1992). The result of the present study has shown that the volatile oil derived from *O. gratissimum* to be very effective against adult *S. damnosum*. This finding is consistent with the result of other investigators (Das et al. 1985, 2003, Tawatsin et al. 2001, Aisen et al. 2004). Multiple factors play part in determining the effectiveness of any repellent. These include the species of the biting organism, the users age, sex, level of activity and biochemical attractiveness to the biting insect, ambient temperature, humidity, and wind (Maibech et al. 1996, Fradin 1998, Golenda et al. 1999). These factors acting singly or collectively might have accounted for the varied degree of repellency observed at the stations, since a repellent may not protect all users equally (Das & Anseri 2003). A single application of 20% (v/v) concentration of the oil resulted in protection from *S. damnosum* bite for 3 h. This finding agrees with the documented report of Aisen et al. (2004). However, studies carried out by Tawatsin et al. (2001) revealed that the oil of *O. americanum* provided absolute protection for 8 h against mosquitoes when 5% vanillin was added to the *Ocimum* oil. It is possible that the same protection effect could be achieved against *S. damnosum* bite if vanillin was added to *O. gratissimum* oil. The report of Chokechaijaroenporm et al. (1994) had also shown that the volatile oil obtained from *O. gratissimum* exhibited the greatest repellency against mosquitoes when compared with other ocimum species.

Blackflies (*simulium* species) often bite continuously from dawn to dusk, but seldom at the same sustained level throughout the day (Crosskey 1990). The result of this study shows that biting activity manifested itself in a bimodal pattern with an early morning peak (10-11 a.m.) and late afternoon peak (4-5 p.m.) that is more pronounced. This finding is consistent with previous reports (Potter & Collins 1988, Kutin et al. 2004, Opara et al. 2005). The apparent diurnal variation in numbers of adult flies may have epidemiologic implications since their biting corresponds to periods of peak human out door activities. It

TABLE IV

Summary of annual result of dissection in the three stations

Variables	Idomido	Obio Camp	Ikot Adaha
Persons day worked	48	48	48
Total flies caught	122	288	300
Average daily catch/person	2.54	6.00	6.25
No. of dissected flies	122	288	300
% of dissected flies	100	100	100
No. of parous flies	110	265	264
% of parous flies	90.2	92.0	88.0
Total no. of infected flies	0	0	0

TABLE III

Relative repellency of volatile oil of *Ocimum gratissimum* against *Simulium damnosum*

Stations	Control [a]	Treated [a]	% repellency after application			
			3 h	4 h	5 h	6 h
Idomido	104 ± 40.7	10 ± 3.02	100	100	91.3	50.3
Obio camp	131 ± 48.4	24 ± 15.2	100	96.4	85.7	64.5
Ikot Adaha	123 ± 45.3	25 ± 13.9	100	98.5	82.9	60.4

a: number of *S. damnosum* bites (Mean ± SD)

was observed in the study area that farming, fishing, and hunting are done in the early morning and late afternoon which corresponds to the peak biting period of the vector. With the volatile oil providing 3-4 h of protections at 20% (v/v) concentration, topical application of these oils at the peak biting periods (morning and evening) could effectively provide protection for 4-6 h of the average 8 working hours of a Nigerian farmer (Aisen et al. 2004). The repellent action would further reduce the vector's access to the already reduced load of microfilariae achieved through ivermectin treatment thereby reducing disease transmission. The transmission of onchocerciasis varies with location and season and may be influenced by the longevity of the fly and its ability to support the development of *O. volvulus* (Henry & Meredith 1990). The fly to human ratio and the availability of microfilariae reserve in the human population may also affect infectivity rates. Remarkably, none of the dissected flies caught by treated individuals haboured any microfilariae. It is possible that infected flies avoided volatile oil of *O. gratissimum*. Active ingredient of most repellent act as neurotoxins or respiratory toxins to insect (Anyanwu & Amefule 2001). These ingredients decrease the fecundity and fertility of the insects and the development of parasites in the insects (Haiba 1996, Holetz et al. 2003). Since no adverse skin reaction was observed throughout the period of this investigation, it might imply that volatile oil of *O. gratissimum* is non-toxic, non-sensitising, and non-irritant. We did not carry out any dermatological test to confirm these observations.

In conclusion, this longitudinal study has clearly demonstrated the potential of volatile oil derived from *O. gratissimum* for use as topical repellent against biting adult *S. damnosum*. These results are very promising in formulating a potent and affordable natural product in the control of onchocerciasis transmission, prevention of intolerable nuisance created by the fly crawling on the skin and their painful bite. This can be integrated into the ongoing mass distribution of ivermectin in onchocerciasis endemic communities.

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