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J. Nat. Prod. Plant Resour., 2012, 2 (2):322-327
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

A cream formulation of an effective mosquito repellent: a topical product from lemongrass oil (*Cymbopogon citratus*) Stapf

Adeniran O. I.* **and Fabiyi E.**

Department of Chemistry, University of Abuja, Nigeria

ABSTRACT

Pure essential oil and a topical formulation from lemongrass oil (Cymbopogon citratus) Stapf. have been investigated for repellent activity against Acarus sacchari and mosquitoes respectively. To two sugar cubes were applied two drops of the oil each. The first cube 'depleted treated sample' was used after 48 h of application while the second cube (freshly treated sample) was used immediately after application. Varying doses of the topical formulation on the other hand were applied to the exposed forearm and leg of each volunteer in a mosquito-infested location. The time of the first three mosquito bites or lands were recorded. The depleted and freshly treated sugar samples exhibited 81% and 95% repellency respectively against Acarus sacchari while the topical formulation from lemongrass oil exhibited an average of [(194) min (≈3 h) for 0.5 g dose] and [(309) min (≈5 h) for 1.5 g dose] protection time against biting from mosquitoes, (about 50-80%) protection time in comparison to the activity of the best known chemical insect repellent, N, N-diethyl-m-toluamide (DEET). It can be concluded that lemongrass oil is a promising natural repellent due to its safety advantage over chemical repellents.

Keywords: Formulation, repellency, Cymbopogon citratus, Acarus sacchari.

INTRODUCTION

Insect transmitted diseases remain a major source of illness and death worldwide. Mosquitoes alone transmit disease to more than 700 million persons annually [1]. Research shows that malaria kills about 3 million persons each year, including one child every 30 seconds [1,2,3]. Although insect borne diseases currently represent a greater health problem in tropical and subtropical climate, no part of the world is immune to their risks. The worldwide threat of arthropod-transmitted diseases, with their associated morbidity and mortality, underscores the need for effective insect repellents.

Protection from arthropod bites is best achieved by avoiding infestation and using insect repellents. Insect repellents are an alternative to the use of insecticides. They may be applied to the skin to protect an individual from the bites of mosquitoes, mites, ticks and lice or, less commonly, may be used to exclude insects from an area, such as in packaging to prevent infestation of stored products. In many circumstances applying insect repellent to the skin may be the only feasible way to protect against insect bites. Given that a single bite from an infected arthropod can result in transmission of disease and the fact that people use synthetic chemical-based mosquito repellents to protect themselves from mosquito bite but in exchange get more serious problems of skin irritation, erythema, desquamation and bullae formation [4], it is important to know which repellent product can be relied on to provide predictable and

prolonged protection from insect bites. Commercially available insect repellents can be divided into two categories namely: synthetic chemicals and plant-derived essential oils. The best known chemical insect repellent is N, N-diethyl-m-toluamide, now called N, N-diethyl-3-meythyl benzamide (DEET). Many consumers, reluctant to apply DEET to their skin, deliberately seek out other repellent products.

The use of insect repellent compounds dates back to antiquity. Before the Second World War, there were only four principal repellents: oil of citronella, dimethyl phthalate, Indalone® and Rutgers 612. Other military repellent formulae for use on clothing were developed during the war, but they all failed to provide desired protection of military personnel deployed around the world. As a result, by 1956 the United States government had screened over 20,000 potential mosquito repellent compounds. In 1953, the insect repellent properties of *N, N*-diethyl-*m*-toluamide (DEET) were discovered and the first DEET product was introduced in 1956. DEET is still the most widely used mosquito repellent. It has generally been regarded as safe, but toxic effects have been recorded, including encephalopathy in children, urticaria syndrome, anaphylaxis, hypotension and decreased heart rate [5]. Several other compounds have been evaluated for repellent activity, but none have had the commercial success of DEET.

Cymbopogon citratus (lemongrass) belonging to the family Poaceae is a genus of about 55 species of grasses, native to warm temperate and tropical regions of the Old World and Oceania. Lemongrass is native to the Philippines where it is locally known as *tanglad*. It is a tall perennial grass. Common names include lemon grass, lemongrass, barbed wire grass, silky heads, citronella grass or fever grass amongst many others. Lemongrass is commonly used in teas, soups, and curries. It is also suitable for poultry, fish, beef, and seafood. It is often used as a tea in African countries such as Togo and the Democratic Republic of Congo and Latin American countries such as Mexico.

Lemongrass oil is the essential oil obtained from the aerial part of *Cymbopogon citratus*. The plant has been widely recognized for its enthnobotanical and medicinal usefulness [6]. The insecticidal [7], antimicrobial [8], and the therapeutic properties [9] of its oil and extracts have been reported. Trado-medicinal preparations of the oil have been used both internally ftor alleviating colds and fever symptoms [10] and externally to treat skin eruptions, wound and bruises [11]. Plant essential oils in general have been recognized as an important natural source of pesticides – insecticides [12,13], larvicides [14], and repellents [15,16,].

Personal protection by the use of repellents is of considerable importance within the integrated disease vector control endeavor. In this light, the present study attempts to ascertain the degree of repellency of lemongrass oil against sugar ants (*Acarus sacchari*) with a view to extrapolating to other arthropods and to evaluate the protection affordable against mosquitoes by a topical formulation of lemongrass oil prepared in petroleum jelly base with a view to providing an herbal repellent with long lasting protection, safe for human life, human and domestic animal skin with no side effect and no feedback of environmental ill effect, as an alternative to synthetic chemical repellents.

MATERIALS AND METHODS

Collection of plant materials

Leaves of *Cymbopogon citratus* were collected in Adankolo area of Lokoja in Kogi State, Nigeria. The leaves were screened and unwanted leaves were removed before using them. The remaining leaves were used fresh as they were obtained from the garden.

Extraction of lemongrass essential oil

The extraction of lemongrass oil was carried out using a mixture of 900 g of the fresh leaves of *Cymbopogon citratus* and distilled water which was subjected to steam distillation. The steam and the volatile oil generated in the process were collected after passing through a condenser, in a separate flask. The mixture of oil and water obtained from the steam distillation was separated with a separating funnel. The recovered oil after the separation was dried over anhydrous sodium sulphate and kept in the refrigerator at 4^{o}C before use.

Preparation of repellent product

Pure petroleum jelly (22.73 g) was accurately weighed into a 50 ml wide-mouth glass bottle and melted in water bath at temperature of about 50^{o}C. At this stage, 1.73 g of the lemongrass oil was transferred into the molten

petroleum jelly. The mixture, in a screwed, covered bottle, was swirled properly to ensure that the oil is uniformly mixed with the petroleum jelly. The product (7.6% w/w cream) is then taken for repellency test.

Methods of conducting repellent test

The test insects used during investigation of the action and effectiveness of the pure lemongrass oil were the common sugar ants "*Acarus sacchari*". The insects (totaling 65) were cautiously captured in a netted box to prevent them from escaping and starved for about 24 h under laboratory condition [(28±2)^{o}C and (78±2)% relative humidity]. Two untreated sugar cubes were carefully introduced into the box containing the captured insects. All the insects were allowed to feed on the sugar for about 48 h.

At this point, the remaining sugar cube (about 20%) was removed from the box, and the insects were starved for another 48 hours. After this starvation period within the confinement, a 'depleted treated' sample (that has been allowed to stand for about 48 h after treatment) was placed in the box and the number of insects hovering round it at 15 minutes intervals was recorded. After about 180 minutes, a sugar cube freshly treated with two drops of the lemongrass oil was used to replace the 'depleted treated' cube in the box and the same procedure followed (Table 1).

Repellent action of *Cymbopogon citratus* cream

The formulation was screened for repellency against mosquitoes under laboratory conditions by using human-bait method during night period [17] (Oyedele *et al.*, 2002)**.**

Before application of the repellent, the forearms and legs of human volunteers were washed and rinsed thoroughly with distilled water. The right forearm and leg were used for the treatment while the left forearm and leg of each volunteer were used for control. Different doses of the formulated cream were applied thinly to each of the three sets of volunteers' bare skin on the forearm and from knee to foot. The mosquito repellence was determined in triplicate experiments of three volunteers per experiment. After application, each volunteer was placed in a separate classroom in the same environment. The period of application of the cream formulation to the time of first landing or bite of mosquitoes on the treated parts as well as two subsequent bites or landings for each volunteer was noted and compared to the control bite.

RESULTS AND DISSCUSION

Table 1: Repellent activities of lemongrass oil against sugar ants (*Acarus sacchari*)

	Number of insects (ants) attracted to each sample		
Time(mins)	Untreated sample	Depleted treated sample	Freshly treated sample
15	30	0	0
30	36	1	0
45	38	3	0
60	39	5	0
75	39	6	0
90	40	8	0
105	42	7	1
120	44	9	1
135	47	9	1
150	51	10	2
165	53	12	3
180	61	12	3

Table 2: The repellent activity of topical cream formulation of *Cymbopogon citratus* essential oil against mosquito bite

Average Time Between Application and Bite In Minutes for Various Doses of Cream Per Application			
volunteers	0.5 g	1.0 g	1.5 g
Set 1	179	235	302
Set 2	201	233	311
Set 3	203	235	315

The result of the repellent activity of the pure lemongrass oil against sugar ants, *Acarus sacchari* is shown in table 1. The result obtained shows that the untreated sugar sample had 100% attraction for the insects (0% repellency). The second sample was treated and subjected to test after 48 h of treatment, with the same set of insects. This attracted twelve (12) insects as the highest number at a time after a period of 180 min (≈82% repellency). The third sample was treated and used immediately after treatment following the same procedure. After a period of 180 mins, the highest number of insects found around the sample at a time was three (3) (≈95% repellency). Within 48 h, a great number of the insects were found dead and the remaining insects were weak. Their death may be due partly to the insecticidal properties [7] of the lemongrass oil and partly to starvation.

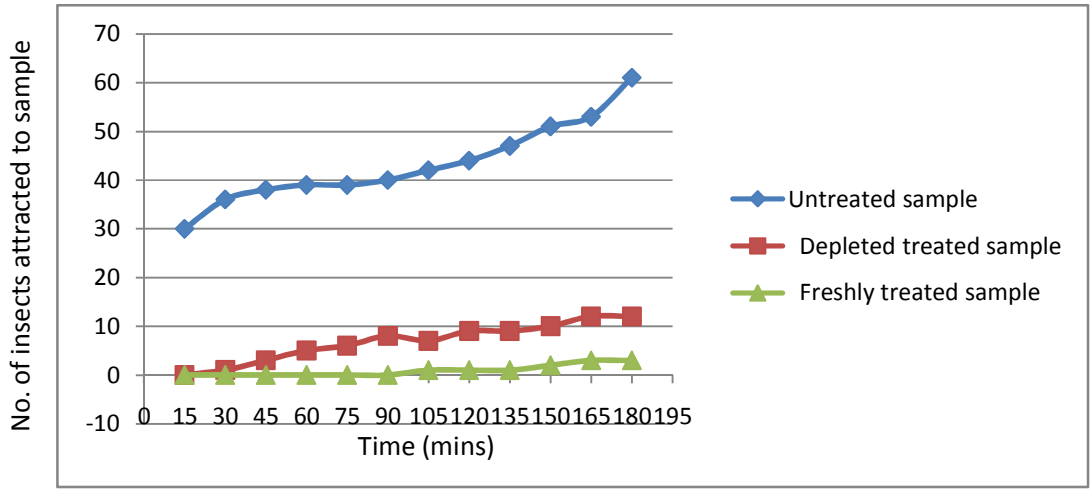

Fig 1: Chart showing number of attracted insects to sample

The result from skin repellent activity of the cream formulation of lemongrass oil against mosquitoes is summarized in table 2. The product produced is found to be 7.6% w/w and the repellent effect of the oil in the product reduces with time as might be expected. The vapour phase-mediated repellency of an essential oil fades with time obviously due to diffusion of the vapour in the local convectional air currents, resulting in its gradual reduction and elimination from the vicinity. Volatile oils diffuse faster from liquids (solutions) than from semisolid preparations [16]. The slow release of the volatile repellent ingredient of lemongrass oil from the oleaginous formulation can thus be attributed to the lipophilic affinity of the oil and its constituents for the base, as well as the viscosity of the base. The mosquito bite-deterrent effect of the cream formulation of the oil is very promising for topical use, and the preparation can be reapplied should a longer effect be required without untoward effect being produced.

As reported by Geier *et al*. [18], lactic acid is present in warm-blooded animal body odor and sweat, and is attractive to female mosquitoes. In behavioral studies lactic acid is essential to attraction of *Aedes aegypti*, but lactic acid by itself is only mildly attractive, indicating synergism with other unidentified human odor components. Further evidence for the role of lactic acid in host seeking comes from studies examining mosquito physiology following a blood meal. Host-seeking behavior in *Aedes aegypti* stops after taking a blood meal. It has been found that following a blood meal, the sensitivity of lactic acid sensitive neurons drops, and this drop is co-incident with the cessation of host-seeking behavior. Lactic acid sensitivity returns to normal after oviposition [19]. This serves to support the hypothesis that host seeking behavior may be modified by affecting the lactic acid receptor mechanisms of a mosquito. Although the mode of action of *cymbopogon* oil is not yet established it might be that it masks lactic acid present in warm-blooded animal body odour and sweat thereby affecting the sensitivity of mosquitoes to the presence of host.

Research has shown that only product containing DEET offer long lasting protection (80 to 90% protection for about 360 minutes when the concentration is about 20% or less) after a single application. Although, the repellent effects of herbal essential oils do not usually last as long as synthetic chemical which can protect from mosquito bite for up to 6 h [20] essential oil repellents are safe for human life, human and domestic animal skin with no side effect and

no feedback of environmental ill effect. With regard to the formulation, it favorably competes with DEET-based products in that it produces up to 80% protection for more than 3 h at 0.5 g and about 5 h at 1.5 g doses respectively (Table 2) perhaps due to its slow release form the base. Smith, [21] proved that the length of time a repellent remained effective depended on the rate at which it was lost by rubbing, evaporation or absorption. There is no evidence that repellents become less effective by dilution with sweat, carbon dioxide or water, which is attractive to insects. Continuous application of DEET causes in folding of the epidermis with fewer hairs and a thickened dermis with more vascularity [22]. The lemongrass extract (lemongrass essential oil) did not cause such discomfort or skin irritation to the volunteers.

CONCLUSION

From the test performed, the result obtained shows that lemongrass essential oil can equally provide substantial protection against mosquitoes and insect bite like that of the synthetic based DEET which has been in existence worldwide since 1957 and it is the only repellent that is believed to give at least 80 to 90% protection against the worldwide threat of arthropod transmitted diseases. To create even stronger natural oil insect repellence that will last up to 6 h per application it is hoped that mixing lemongrass oil with such oils as eucalyptus will meet this requirement.

Recommendations

Protection against arthropod bites is best achieved by avoiding infested habitats, wearing protective clothing's and applying insect repellents. The insect repellent that are currently available to consumers are either synthetic chemicals or are derived from plants. The protection provided by lemongrass essential oil is proportional to the logarithm of the dose per application. Research has shown that concentration above 3% could provide protection for about 3 to 4 h, which is averagely acceptable and it does not cause any skin irritation when used on different volunteers during an experiment. It is therefore recommended that people should consider the use of lemongrass oil as an insect and mosquito repellent apart from its other health benefits. This is due to the fact that lemongrass is a fast growing plant, and the extraction process is relatively cheap thereby reducing the dependence on expensive imported products.

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