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Keywords: hyptis suaveolens, essential oils, mosquitocidal, larvicidal.

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ABSTRACT

Essential oil was extracted from the leaves of Hyptis suaveolens by hydro-distillation. The Mosquitocidal activity, mosquito larvae (larvicidal) and mosquito repellency effect was carried-out using standard methods. Percentage yield of the essential oils was gotten as 0.05, for the larvicidal activity dose dependent mortality of the larvae was observed; there was low mortality rate at lower dosage and not significantly different from each other e.g. 6.25 and 12.50ppm had same LC_{50} and LC_{90} of 25.21 and 302.67 respectively, 25ppm had LC_{50} and LC_{90} of 9.58 and 352.46 respectively, while 50-1000ppm had LC_{50} and LC_{90} of 0.55 and 0.85 respectively. It was observed that the amount of air that enters and leaves the cage affect the rate at which mosquitoes were repelled. In a group of mosquitoes kept in a cage with all sides open (ASO) 65% of the mosquitoes were repelled within 30 minutes while for one side open (OSO) and all sides closed (ASC), 73% and 85% of the mosquitoes were repelled within the same time frame. In a second experiment, 100% of mosquitoes were repelled from the surface of rats with shaven skin where essential oil was applied. The essential oil also demonstrated dose dependent mosquitocidal activity with LC_{50} and LC_{90} values of 6 and 21ppm respectively.

Keywords: *hyptis suaveolens,* essential oils, mosquitocidal, larvicidal.

I. INTRODUCTION

A synthetic insecticide is a poisonous chemical or mixture of chemicals that is intended to prevent, repel, or kill any insect or pest. However, synthetic insecticides present hazardous impacts far beyond their intended targets. Insecticides have inherent toxicity because they are designed to kill living organisms that are considered "pests", that is any unwanted insect. Many insecticides are known to pose significant, acknowledged health risks to people—including birth defects, damage to the nervous system, disruption of hormones and endocrine systems, respiratory disorders, skin and eye irritations and various types of cancers (Agency for Toxic Substances and Diseases Registry, 1994).

Chemical repellents are important in protecting people from blood-feeding insects such as mosquito, ticks, mites, and other arthropods and may therefore also reduce transmission of arthropod borne diseases. N, N-diethyl- 3-methyl benzamide (DEET) is one of the most well-known arthropod repellents and has been in the market for almost half a century. DEET is effective against many different blood-sucking arthropods. The protection efficacy depends on the type of formulation, application pattern, species, and feeding behavior of the arthropod. DEET is generally safe for topical use if applied as recommended, although adverse effects such as serious neurologic effects have been reported. Many people consider that DEET and related compounds as a health and environmental hazard. It does not readily degrade by hydrolysis at environmental pHs and has been identified as a ubiquitous pollutant in aquatic ecosystems. Concern about the deleterious effects associated with synthetic chemicals has revived interest to explore plants as a source of natural insecticides, acaricides, and repellents for medical, veterinary and crop protection use (Abagli and Alavo, 2011).

Plants are endowed with a potential to produce a wide range of allele-chemicals that protect the plants from insect-pests. (Ramya, *et. al.*, 2008). Mechanisms were developed to repel phytophagous pests, i.e. protective hairy or waxy surfaces, hardened cuticles or tissues or the

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cellular production of repellent and toxic chemicals. In most cases these protective devices are sufficient to repel generalists among the phytophagous insects (Kiprop, *et. al.*, 2005; Din, *et.al.*, 2011).

In developing countries, Lamiaceae have traditionally been used for their insecticidal and repellent properties against several insects' species. Most of them belong to the *Hyptis* genus that includes more than 400 species that grow in the tropical regions of the world, mainly in Africa and America and are highly aromatic plants (Shenoy, *et. al.*, 2009; Benelli, *et. al.*, 2012).

Hyptis suaveolens (L.Poit) is one of the aromatic and odoriferous important traditional plants belonging to the family lamiaceae, an ethno-botanically important medicinal plant. The plant has been considered as an obnoxious weed. It is a brushy erect plant with fragrant hairy cordate. (Gavani and Paarakh, 2008; Shenoy, *et. al.*, 2009; Moreira, *et. al.*, 2010; Singh, *et. al.*, 2011; Malar *et. al.*, 2012; Agarwal, *et. al.*, 2013; Noudogbessi, *et. al.*, 2013; Islam, *et. al.*, 2013; Umedum, *et. al.*, 2014; Islam, *et. al.*, 2014). *Hyptis suaveolens* is a medium aromatic shrub found in the tropics and subtropics, and distributed as the aggressive annual weedy species in the northern part of Thailand. The plant is native to tropical America but now distributed throughout the whole world from tropical to subtropical regions and, therefore, the plant is sometimes regarded as pan-tropical, it is a soft ruderal weed that normally grows along the roadsides and the wet margins of ponds. (Gavani and Paarakh, 2008; Shenoy, *et. al.*, 2009; Moreira, *et. al.*, 2010; Singh, *et. al.*, 2011; Malar, *et. al.*, 2012; Agarwal, *et. al.*, 2013; Noudogbessi, *et. al.*, 2013; Islam, *et. al.*, 2013; Umedum, *et. al.*, 2014; Islam, *et. al.*, 2014).

II. MATERIALS AND METHODS

2.1 Plant Material

Fresh *Hyptis suaveolens* leaves were used in the experiment for the extraction of essential oil via hydro-distillation.

2.2 Other Materials

Anopheles' mosquito larvae were identified and gotten from the drainage in Samaru, Zaria along Sokoto Road, Nigeria. Some of the larvae were allowed to pupate and transform to the adult mosquito.

2.3 Chemicals and Reagents

All chemicals and reagents were of analytical grade.

2.4 Equipments

Round bottom flask, water condenser, Erlenmeyer flasks, separating funnel, glass bottles with tight cover, paper tape, Cardboard papers, Aluminium cage with wire gauge, Cage made of Mosquito net with wooden support, plastic containers and petri dishes.

2.5 Methods

Plant Material Collection and Identification

The plants were collected from Samaru area of Zaria, Kaduna-Nigeria. They were then taken to the herbarium, department of Biological Science for identification. Voucher specimen number 2020 was deposited. More of the plant leaves were then plucked and taken to the laboratory for further use.

2.6 Extraction of Essential Oil from Hyptis suaveolens leaves

Hyptis suaveolens (1kg) leaves were washed and placed in round bottom flasks of the hydro-distillation set up with 1liter distilled water. The round bottom flask was then heated on heating mantle for 2 hrs. The essential oil evaporated due to the heat, it was cooled and collected as distillate (mixture of essential oil and water). This was transferred to a glass separating funnel and the essential oil separated from the water based on density. Percentage yield of the essential oil extracted was then calculated using this formula;

$$\%\ \text{yield} = \frac{mass\ of\ product}{Mass\ of\ sarting\ material} \times 100$$

2.7 Mosquito Breeding

Some of the collected mosquito larvae were taken to the laboratory washed three to four times and introduced into a clean transparent glass container; the container was placed in a mosquito cage that was prepared for the experiment. Cracker biscuit powder was sprinkled into the glass container which serves as food for the larvae. The 3rd and 4th instar larvae were collected and used according to WHO 2005 guidelines for the larvicidal bioassay. For mosquito repellency determination, the larvae were left to pupate, transform into adult stage and remain in that same cage for at least 24 hours before introducing them into another cage for repellency experiment to be carried out. They were left for 24 hours prior to the commencement of the experiment so as to be strong to be sure that whatever behavior that will be observed will not be due to their fragility.

2.8 Determination of Mosquitocidal Capability of the Essential oil

In this experiment 25 adult mosquitoes each were introduced into a separate mosquito cages and tagged 1-6. Group 1 had no essential oil and therefore served as control, group 2 had 1 ml essential oil, group 3 had 2 mls essential oil, group 4 had 3 mls essential oil, group 5 had 4 mls essential oil while group 6 had 5 mls essential oil dropped into a container in the cages according to the method described by Bulugahapitiya, *et. al.,* 2007. The mosquitocidal effects of the essential oil were observed and records taken at the end of 90 minutes in all the groups.

2.9 Determination of Mosquito Repellency Effect of Essential oil

This was carried out by modified method of Bulugahapitiya, *et. al.,* 2007. Large cages were prepared by introducing 1 ml of the essential oil into each cage containing 25 adult mosquitoes. The behavior of the mosquitoes was then observed and recorded at 30 minutes, 40 minutes, 60 minute and 90 minutes respectively. The control group was set up with pure acetone and no essential oil. The test groups were three; group one was carried out under 100% aeration, group two was done under 20% aeration and group three under 0% aeration.

All the above methods were repeated three times.

2.10 Determination of Mosquito Repellency Activity of Essential oil of Hyptis suaveolens leaves on Rats

Mosquitoes (25 adults) were introduced into each three separate cages grouped I, II and III. Restrained rats (3) each were also introduced into the cages. The rats had their back hairs scrapped off and essential oil (0.5 ml) applied on the bare skin of group one (test rats). The other two groups served as control. Group two (II) had no essential oil applied to the scrapped skins but glycerine. Group three (III) had no essential oil or glycerine applied to their bare skins. The number of mosquito bites were counted at the end of 90 minutes by observing red spot on the scrapped area due to the effect of bite on the rats' bare skin.

2.11 Test for Larvicidal Effect of the Essential oil (WHO, 2005)

Essential oil concentrations of 6.25 ppm, 12.50 ppm, 25 ppm, 50 ppm, 100ppm, and 1000ppm were prepared from 1% stock solution. For each of the above concentrations using 25 larvae in their 3rd and 4th instar, numbers of dead larvae were counted after 6 hours, 12 hours and 24 hours. A control group was set up for the same timing without the essential oil.

III. RESULTS

3.1 Extracted Essential Oil from Hyptis Suaveolens Leaves

The quantity of essential oil obtained from three (3) independent extractions using 1 kg in each case of leaves of *Hyptis suaveolens* is presented in Table 1. An average quantity of 0.4-0.5 mls was extracted and the average yield was 0.1%. The oil is a clear liquid with physical appearance of pale yellow.

Table 1: Extracted Essential oil from *Hyptis suaveolens* leaves

Weight of leaves used	Quantity of oil obtained
One (1) kilogram	0.5 mls
One (1) kilogram	0.6 mls
One (1) kilogram	0.4 mls
Average quantity extracted	0.5 mls
Percentage yield of essential oil extracted	0.05

3.2 Mosquitocidal capability of Essential oil from Hyptis suaveolens leaves

Essential oil from *Hyptis suaveolens* leaves showed mosquitocidal activity against adult mosquitoes. At the end of 90-minute observation of mosquitocidal effect, all the mosquitoes in different groups except those in control group were not active. In the first cage, that is, the group with 1 ml essential oil 16 % were knockdown (dead) while the remaining 84% were seen resting on the wall, all far from the petri dish where the essential oil was placed. In the group with 2mls essential oil, 72% were inactive far away from the essential oil while 28% were knockdown (dead). As the concentration increases the percentage of dead mosquitoes increases and the inactive ones in all cases were seen far away from the essential oil. At 3mls 36% were knockdown (dead) and 64% weakened while at 4mls, 40% were knockdown (dead) and 60% weakened. At 5mls, 44% were knockdown (dead) while 56% were weakened (not active).

Table 2: Mosquitocidal potential of the *Hyptis suaveolens essential* oil

Amount of essential oil (mls).	Time (mins)	Percentage dead (%)	Percentage inactive (%)
0	90	0	0
1	90	16	84
2	90	28	72
3	90	36	64
4	90	40	60
5	90	44	56

N = 25. LC_{50} = 6.20 and LC_{90} = 21.28ppm

3.3 Repellency property of Hyptis suaveolens Essential oil

The mosquito repellency property of *Hyptis suaveolens* essential oil under 100% Aeration is presented in table 3. Essential oil from *Hyptis suaveolens* leaves had strong repellency effect against mosquitoes. In this study 1 ml of the essential oil repelled 65.32% of 25 adult mosquitoes within a period of 30 minutes. At 40 minutes 73.32% of the mosquitoes were repelled, at 1-hour post application, 77.32% of the mosquitoes were completely repelled from the source of the essential oil not only were they repelled but were weakened as they were observed to be resting on the wall of the net far away from the source of the essential oil.

Table 3: Repellency property of *Hyptis suaveolens* essential oil at 100% aeration

Amount of essential oil (mls)	Time (Mins)	Active Mean/SD	Percentage Active (%)	Inactive Mean/SD	Percentage Inactive(%)	Mortality (%)
1	30	8.67 ± 1.25^{b}	34.68	16.33 ± 1.25^{a}	65.32	0
1	40	6.67 ± 1.25^{ab}	26.68	18.33 ± 1.25^{ab}	73.32	0
1	60	5.67 ± 1.25^{a}	22.68	19.33 ± 1.25^{b}	77.32	0
1	90	7.33 ± 0.94^{ab}	29.32	17.66 ± 0.94^{ab}	70.64	0

Group one (1), N=25.LC_{50} = 0.061 and LC_{90} = 183.078ppm. Means with different letters are statistically different ($p \leq 0.05$)

Table 4 shows the result of group two (2) conducted under 20% aeration. At 30 minutes after application, 73.32% of 25 adult mosquitoes were repelled from the source of the oil. The increase in percentage repellency within the same time frame may be due to level of aeration that is less in this second group which builds up the concentration of the essential oil considering its volatility.

Table 4: Repellency property of *Hyptis suaveolens* Essential oil 20% aeration

Amount of essential oil (mls)	Time (Mins)	Active Mean/SD	Percentage Active (%)	Inactive Mean/SD	Percentage Inactive (%)	Mortality Mean/SD	Percentage Mortality (%)
1	30	6.67 ± 1.57^{b}	26.68	18.33 ± 2.05^{a}	73.32	0	0
1	40	3.00 ± 2.16^{a}	12.00	22.00 ± 2.16^{b}	88.00	0	0
1	60	0.33 ± 0.47^{a}	1.32	23.33 ± 0.47^{b}	93.32	1.33 ± 0.47	5.32
1	90	0.00 ± 0.00^{a}	0.00	22.67 ± 0.94^{b}	90.68	2.33 ± 1.33	9.32

Group two (2), N =25. LC_{50} = 0.572 and LC_{90} = 2. 005ppm. Means with different letters are statistically different ($p \leq 0.05$).

A third group recorded in Table 5 was done under 0% aeration; the repellency effect was higher in this group. At 30-minute post application 85.32% were repelled from the essential oil, at 40 minutes 97.32% were repelled at 60 minutes there was 100% (88% repelled and 12% dead) repellency and at the end of 90 minutes 100% repellency (84% repelled and 16% dead) was recorded also. In all groups the repelled mosquitoes were weak, unable to fly and resting on the wall of the cage far away from the essential oil.

Table 5: Repellency effect of *Hyptis suaveolens* Essential oil 0% aeration

Amount of essential oil(mls)	Time (Mins)	Active M/SD	Percentage Active (%)	Inactive M/SD	Percentage Inactive (%)	Mortality Mean/SD	Percentage Mortality (%)
1	30	3.67 ± 0.47^{b}	14.68	21.33 ± 0.47^{ab}	85.32	0.00	0.00
1	40	0.67 ± 0.47^{a}	2.68	24.33 ± 0.47^{c}	97.32	0.00	0.00
1	60	0.00 ± 0.00^{a}	0.00	22.00 ± 0.00^{b}	88.00	3.00 ± 0.00	12.00
1	90	0.00 ± 0.00^{a}	0.00	21.00 ± 0.00^{a}	84.00	4.00 ± 0.00	16.00

Group three (3), N =25.LC_{50} = 0.511 and LC_{90} = 1.178ppm. Means with different letters are statistically different ($p \leq 0.05$)

3.4 Control groups

Control was set up for each of the three group without essential oil but conditions remain unchanged (aeration of 100%, 20% and 0%) and in all cases the mosquitoes were actively flying through out, the results are shown in tables 6, 7 and 8.

Table 6: Repellency property of *Hyptis suaveolens* Essential oil at 100% aeration (control)

Amount of essential oil (mls).	Time (Mins.)	Percentage Repellency (%)	Percentage Mortality (%)
0	30	0	0
0	40	0	0
0	60	0	0
0	90	0	0

N= 25

At the end of 90 minutes there was 100% repellency in the first group, this has been proven by zero number of bites on shaven dorsal part of rats. In the second group with 0.5mls glycerine on the bare skin there was 46.67% of bites at the end of 90 minutes. In the third group with nothing applied on the bare skin of the rats, 45.33% bites were recorded at the end of 90 minutes. Table 9 shows the summary of this experiment.

Table 9: Summary of repellency effect of *Hyptis suaveolens* Essential oil

Amount of essential oil (mls).	Time (Mins)	Percentage Repellency (%)	Percentage Mortality (%)
0	30	0	0
0	40	0	0
0	60	0	0
0	90	0	0

3.5 Larvicidal Property of Essential oil from Hyptis suaveolens

Hyptis suaveolens essential oil has shown very strong larvicidal properties against mosquito larvae. There is low mortality rate at lower dosage and not significantly different from each other. At a concentration of 6.25ppm the mortality rate is 4% over a period of 6 hours, same concentration at 12 and 24 hours gave mortality rate of 11%. The Lc50 and Lc90 at this concentration were 25.206 and 302.669 respectively and a regression equation of Y= 818.6x-161.36. At a concentration of 12.50ppm a mortality rate 4% was recorded at 6 hours, at 12 and 24 hours 13% mortality were recorded, same Lc50, Lc90 and regression equation with 6.25ppm. At concentration of 25.00ppm 20% mortality rate was recorded for 6 hours and 30% for both 12 and 24 hours, the Lc50, Lc90 and regression equation are 9.575, 352.458 and Y=0.7264x+0.2465 respectively. There are no significant differences at 50ppm, 100ppm and 1000ppm, the mortality rate was 100% for the period of 6 hours, 12 hours and 24 hours with Lc50, Lc90 and a regression equation of 0.549, 0.853 and Y=0.7264x+0.2465 respectively. There was no record of death in the control even after 24 hours. The summary of the larvicidal properties are recorded in Table 10.

Table 10: Larvicidal property of essential oil from *Hyptis suaveolens* leaves

Concentrations (ppm).	Time (hours)	Percentage Mortality (%)	Lc50	Lc90	Regression Equation
6.25	6	4	25.206	302.669	Y=818.6x-161.36
	12	11			
	24	11			
12.50	6	4	25.206	302.669	Y=818.6x-161.36
	12	13			
	24	13			
25	6	20	9.575	352.458	Y=1695.8x-360.07
	12	30			
	24	30			
50	6	100	0.549	0.853	Y=0.7264x+0.2465
	12	100			
	24	100			
100	6	100	0.549	0.853	Y=0.7264x+0.2465
	12	100			
	24	100			
1000	6	100	0.549	0.853	Y=0.7264x+0.2465
	12	100			
	24	100			
Control	6	0			
	12	0			
	24	0			

Larvicidal overall $Lc_{50} = 3.579$

$Lc_{90} = 13.828$

Overall Regression equation=$y = 0.2868e^{0.1402x}$

Overall $R^2 = 0.9959$

IV. DISCUSSION

The clear liquid oil with physical appearance of pale yellow is same with all previous work. Essential oil yield of about 0.4-0.6ml/kg (equivalent to 0.1%) got in this studies is same with the work of Raizada (2006) who got a percentage yield of 0.1 from the same plant, never the less it is low compared with the work of the following researchers; Iwalokun *et. al.*, (2012) a percentage yield of 0.31 and Okonogi, *et. al.*, (2005) percentage yield of 0.21; The difference in percentage yield could be due to the moisture level of the leaves and the chemotypic profile of the *Hyptis suaveolens* strains analysed (Iwalokun, *et. al.*, 2012). Latitude, altitude, soil composition, climate and genetic composition are factors that have been implicated for chemotype variations in *Hyptis suaveolens* and other species of *Hyptis* as well as other aromatic herbs belonging to the Lamiaceae family (Iwalokun, *et. al.*, 2012). Various bioactive compounds were recovered from the essential oil samples of this chemotypes with variations in yield, composition and pharmacological effects (Iwalokun, *et. al.,* 2012). Mandal, *et. al.*, (2007) got the following percentage yield from the same plant using several solvents which are Steam distillation (yield: 0.24%), petroleum ether extract (yield: 1.6%) and ethanol extract (yield: 2.64%); Bachheti, *et. al.*, (2013) got a percentage yield of 17.44 from seed oil and not leave oil; Shenoy, *et. al.*, (2009) reported percentage yield of 4.78% for Petroleum ether, 8.52% for Solvent ether, 3.30% for Chloroform, 5.48% for Alcohol and 15.22% for Chloroform water; Gavani and Paarakh, (2008) got a percentage yield of 4.86% methanol extract. The difference in these studies and the present work could be due to the different solvents used and in one case different part of the plant, apart from the above mentioned factors.

Mosquitocidal capability against adult mosquitoes could be due to the presence of terpenes in the essential oil as reported by Bala *et. al.*, (2020). Terpenes are generally known to have insecticidal capability. Earlier work done by Olotuah, (2013) showed that essential oil from *Hyptis suaveolens* leaves has insecticidal activities against several insects. Adda, *et. al.*, (2011) also showed that essential oil from *Hyptis suaveolens* leaves has larvicidal activity against some insects, all these could be as a result of terpenes in the plant as reported by Bala *et. al.*, (2020)

The strong repellent activity against mosquitoes as seen in this work is still connected to the presence of terpenes. This agrees with the work of Benelli, *et. al.,* (2012) where the essential oil repels some insects such as *Sitophilus granarius* which kept distance from source of the essential oil that was applied on white man filter paper. As expected the repellency effect is concentration dependant, this goes in line with the work of Abagli and Alavo (2011) which showed that essential oil from *Hyptis suaveolens* has strong repellency property towards mosquitoes. The higher the concentration the higher the repellency property. In their work 97% of mosquitoes were repelled within a period of 15 minutes which demonstrated same effectiveness as DEET. The overall lethal concentration at 50% (Lc_{50}) is 3.336ppm and Lc_{90} is 0.878ppm. A significant ($\leq$ 0.05) repellent activity with differences in repellency rates is a function of both concentration and observation time, this is in perfect agreement with the work of Conti *et. al.*, (2011), these authors showed that differences in repellency rates, were as a function of both concentration and observation time. Singh *et. al.,* (2011) also showed that *Hyptis suaveolens* has strong ability to repel mosquitoes, these authors proved that plant placement, smoldering (dry and fresh leaves, which gives 90% repellency), spraying (essential volatile oil) and sticks formation of *Hyptis suaveolens* leaves repels mosquitoes strongly due to the presence of intense pungency of the leaves. In their study they were able to prove that *Hyptis suaveolens* is an effective plant to repel mosquito as well as various insects.

Strong larvicidal properties against mosquito larvae due to the presence of terpenes are concentration dependant. Lower concentrations gave lower mortality rates and not significantly ($\leq$ 0.05) different from each other, at higher concentrations mortality rates was higher and there were no significant ($\leq$ 0.05) differences between higher concentrations. These are further confirmed by giving same Lc_{50} and Lc_{90} for lower concentration (25.206 and 302.669 respectively for both 6.25ppm and 12.50ppm) and same Lc_{50} and Lc_{90} for higher concentrations (0.549 and 0.853 respectively for 50-1000ppm). The work of Conti *et. al.,* (2011) also showed that there is no significant difference between lower concentrations, at dosages ranging from 250 to 350 ppm, mortality rates were lower and not significantly different from each other. Arivoli and Samuel, (2011) also showed that essential oil of *Hyptis suaveolens* has larvicidal activities against mosquito larvae, which is concentration dependant as was seen in this research work.

V. CONCLUSION

Percentage yield of 0.05. This is averagely sufficient using hydro-distillation. Essential oil from *Hyptis suaveolens* leaves has shown mosquitocidal activity. It has strong repellency effect against adult mosquitoes. It also has very strong larvicidal properties.

Since terpenes have been revealed from previous studies to be present in the essential oil from the leaves of this plant (*Hyptis suaveolens*) and the oil has successfully killed and repelled adult mosquitoes and at the same time eliminated mosquito larvae, it then suggests that the activities of the essential oil is as a result of the terpenes contained in the oil.

Considering the insecticidal properties of essential oil from *Hyptis suaveolens* leaves, we therefore recommend that the essential oil be used in subsequent work to make soap, perfume and or mosquito repellent popularly known as mosquito coil (right concentration of the essential oil mixed with saw dust or any other dust that can be used). Further studies could be done by extracting the components of the essential oil and test each one of them to know the particular compound(s) that are responsible for the mosquitocidal, larvicidal and repellency properties. This information could help in determining the possibility of using the oil to make insecticides or use it as a component of fumigant to eliminate all unwanted target insects.

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