

**EVALUATING THE EFFECTIVENESS OF LOCALLY SOURCED HERBAL
INGREDIENTS AS MOSQUITO REPELLENTS*****Shivi Bhasin, Ayesha Sheikh, Prachi and Anushka Sahu**

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ABSTRACT

Mosquitoes persistent and disease-carrying insects, have always been a big nuisance to the society. To humans, they have always been agents of transmitting deadly disease like Malaria, Dengue and Zika virus which makes them a topic of global health concern. So, controlling their spread becomes need of hour and use of mosquito repellents has always been a common answer to this spread. Mosquito repellent is a substance applied to skin, clothing, or other surfaces which discourages insects particularly mosquitoes from landing or climbing on that surface. Many chemicals have been used for the purpose of mosquito repellency or killing, however they are extremely harmful for human beings. So, recently, commercial repellent products containing plant-based ingredients have gained popularity among consumers, these are commonly perceived as "safe" in comparison to long-established synthetic repellents. Based on the knowledge on traditional repellent plants obtained through ethnobotanical studies, development of new natural products is an urgent and continuous requirement. This paper intends to provide a herbal based mosquito spray developed from plant based ingredients containing Neem, Tulsi, Cinnamon, Camphor, Lantana, Citronella, Vinegar, Castor Oil, Rose Water, Essential oil and Lemongrass etc. in appropriate concentration and with high repellency frequency and good consumer safety value.

KEYWORDS: Mosquito Repellent Spray, Mosquito, Plant based ingredient, Repellent Frequency.**INTRODUCTION**

Mosquitoes are tiny but relentless vectors of disease, which have plagued humanity for centuries. They belong to Culicidae family of order Diptera. Their basic characteristics are similar to that of flies, but mosquitoes are a common cause of diseases occurring in the present day particularly in temperate climatic condition.^[1,2] Mainly in Asian countries, the mosquitoes not only create nuisance by their bite but also by their buzzing sound. "The world most dangerous animal is mosquito" and according to BBC World Service program malaria now infects approximately 110 million people annually causing 2-3 million deaths.

The World Health Organization blames global warming for increasing range of mosquitoes that are responsible for malaria, yellow fever and dengue fever, causing millions at risk. It is estimated by WHO that nearly 15,000 deaths per year at all ages occur only in Indian Peninsula due to these diseases.^[3] With the increasing number of mosquitoes in today's world a sharp rise in the number of mosquito borne diseases has been observed, as a result of which controlling mosquitoes has become a necessity in today's world. Major causes of increase in frequency of mosquito is attributed to deforestation, industrialized farming, stagnant water and

prevalence of unhygienic conditions. The attraction of mosquito towards human is due to the production of lactic acid and CO₂, which is present in our sweat.^[4] In order to minimize the chances of mosquito bites different measures like wearing long pants, applying mosquito repellents, disposing standing water and maintain hygienic conditions are undertaken. However, these measures are not enough to control mosquito spread as a result of which special products like herbal mosquito repellents are prepared to eradicate mosquito in respective place. The concept of controlling mosquito is based on human scent and in order to hide the smell of human scent, the mosquito repellent based on chemicals were designed with remarkable safety profile but these chemical based repellents have proved to be toxic against skin and nervous system, causing rashes, swelling and sometimes eye irritation. Hence, Bio based herbal mosquito repellent are preferred over chemical repellent as they are based on safe and biologically based or mainly plant based ingredients. Mainly, mosquito repellent spray are prepared by using variety of herbal ingredients like neem, lemon grass oil, tulsi, basil, camphor, rose water, vinegar etc. which have proved to be successful in increasing the efficiency of mosquito repellent.^[5,6,7] The current paper aims to develop a biological based herbal mosquito repellent spray made

from plant based products which should be good for environment, should have added benefits and should not harm individuals.

MATERIALS AND METHODS

The active ingredient in a mosquito repellent is primarily responsible for its usefulness. The criteria for selection of plant materials to be used in mosquito repellent suggests that the selected material should discourage insect attack on the treated area for many hours and on different types of surfaces. The material should also be able to work under a variety of different environmental conditions, should be non-toxic and should not cause irritation when applied to human or animal skin. Apart from this, the material used in mosquito spray should also be cosmetically acceptable and should have a pleasant odor, taste, and feel. The material should be relatively of low cost and should be effective against other common types of insects, such as flies and particularly against mosquitoes. In our product we have used the variety of ingredients like lemon, Neem, Tulsi, Cinnamon, Camphor, Lantana, Citronella, Vinegar, Castor Oil, Rose Water, Essential Oil and Lemongrass etc. We have used containers like spray bottle for storage, mortar and pestle for grinding and crushing to make slurry and a appropriate label showing it's usage and ingredients, the preparation process of the spray includes following-

- (a) **Extraction of the Material:** Leaves of different plant material were collected, dried and weighed according to their respective concentration for preparing different formulations. In the current investigation leaves of Neem, Tulsi, Cinnamon, Camphor, Lantana, Citronella and Lemongrass etc. were taken, dried, weighed and grinded. Five formulations of the spray were prepared where plant ingredients in the different proportion were taken (Table 1).
- (b) **Preparation of the Spray:** The grinded part of the above mentioned ingredients Neem, Tulsi, Cinnamon, Camphor, Lantana, Citronella, Vinegar and Lemongrass etc. were taken mixed and were added to a liquid mixture of Castor Oil, Rose Water and Essential Oil etc. After filtering the above material different concentrations of the above mentioned ingredients were taken and different formulations of spray were prepared using these ingredients. The overall active ingredients in the spray were pure and rose water was applied to the mixture for fragrance, until the final amount was 100ml. After this mechanical stirrer was used to blend all the ingredients & eventually, the mixture was poured into plastic containers, after which the spray was set (Fig. 1).



Fig. 1: Plant Material for Preparation of Mosquito Spray.

- (c) **Mosquito Repellency Test:** The test for Mosquito repellency test was performed by simply selecting the mosquito prone areas in the evening and night hours near the bushes in the garden, laboratory and public places to check it's effectively. Landing and probing behavior of mosquitoes signifies the end point and the effectively of repellent. The efficacy of the repellent of this aromatic solution against mosquitoes was done by counting number of mosquito bites per unit time, and percentage repellencies were calculated and statistically confirmed by counting.^[8,9,10] Different formulations taking different concentrations of plant extracts were prepared from these material and were tested for efficiency (Table 1).
- (d) **Statistical Analysis:** The mosquito repellency was calculated for different concentrations and solutions of tested plant products through usage of following formula-

$$\% \text{ Repellency} = \left\{ \left(\text{No. Control} - \text{No. Test Product} \right) / \text{No. Control} \right\} * 100 \text{ (Palsson \& Jaenson 1999)}$$

Table 1: Different Formulations of Mosquito Spray Prepared with Different Ingredients.

Serial No.	Contents	Quantity				
		F ₁	F ₂	F ₃	F ₄	F ₅
1.	Neem	100 gm	200 gm	300 gm	250 gm	500 gm
2.	Tulsi	100 gm	50 gm	150 gm	200 gm	250 gm
3.	Cinnamon	20 gm	30 gm	30 gm	25 gm	50 gm
4.	Castor Oil	10 ml	5 ml	10 ml	10 ml	10 ml

5.	Camphor	3 piece	5 piece	3 piece	3 piece	5 piece
6.	Citronella	60 gm	80 gm	100 gm	150 gm	200 gm
7.	Vinegar	15 ml	10 ml	10 ml	15 ml	20 ml
8.	Lemongrass	20 gm	30 gm	30 gm	40 gm	50 gm
9.	Rose Water	20 ml	25 ml	30 ml	20 ml	60 ml
10.	Lantana Oil	5 ml	7 ml	15 ml	5 ml	20 ml

RESULTS

The Method implied for study was based on collection of mosquitoes, a dark room was selected where the number of mosquitoes was counted, before applying spray. After, applying the spray the number of mosquitoes were counted in the same room continuously for 120 minutes with respective time duration. Then, the % of Repellency

was calculated in different time duration for different formulations of the spray (Table 2). It was reported that formulation with maximum amount of Ingredients showed maximum effectiveness (Fig.1). The repellency frequency for different formulations was calculated and the results obtained are as follows-

Table 2: Repellency Frequency of different Formulations of Spray.

Serial No.	Formulations (F)	0-20 Minutes (%)	20-40 Minutes (%)	40-60 Minutes (%)	60-80 Minutes (%)	80-100 Minutes (%)	100-120 Minutes (%)
1.	F ₁	76.74	55.81	34.88	18.60	12.79	9.30
2.	F ₂	83.72	50.00	32.55	27.90	16.27	18.60
3.	F ₃	84.88	73.25	55.00	48.82	39.53	20.93
4.	F ₄	81.39	68.90	58.13	43.02	32.55	19.76
5.	F ₅	100	97.60	94.18	91.86	90.69	88.33

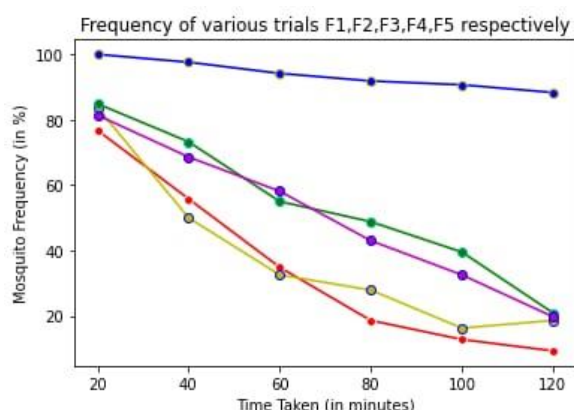


Fig. 1: Repellency Frequency of different Formulations of Spray.

DISCUSSION

Insects are very commonly known for their aggravating characteristics of biting, stinging, contaminating and spreading disease so science has always helped in finding ways to contain them.^[11] Mosquitoes are attracted toward human beings due to production of lactic acid and carbon dioxide in their sweat. Actually, the smell of sweat is perceived by the mosquitoes, their they are attracted to human blood. Human blood is majorly required by female mosquitoes to maintain their reproductive state that is why female mosquitoes have sucking and piercing mouth parts.^[8] Various anthropogenic activities like deforestation, industrialization and destruction of natural habitat has led an increase in number of mosquitoes requiring the use of special products. Production of different mosquito repellents having varied repellent frequencies have been designed, out of which natural mosquito repellent were

reported to be helpful in masking human scent.^[12] However, natural repellents are preferred on artificial relents as they do not contain any chemicals, are less harmful to the skin and do not irritate the body also. These naturally made mosquito repellent are mainly made from plant based material, are less expensive, easily available, are widely accepted and hence are extensively used particularly in developing countries. Since, these natural & plant based mosquito spray contain essential oil and other important ingredients with repellent properties as valuable natural resource they have proved to be very effective in protecting from mosquito bites. By, testing these individual compounds derived from plant extracts it is revealed that these compounds are effective against mosquitoes.^[13,14] In the current investigation five formulations have been prepared by using different concentration of Neem, Tulsi, Cinnamon, Camphor, Lantana, Citronella, Vinegar, Castor Oil, Rose Water, Essential Oil and Lemongrass etc. Results of the study indicate that due to the presence of certain specific compounds and combination of these ingredients is most beneficial for controlling mosquito bites (Table 2).

Neem (*Azadirachta Indica*) is one of the most important ingredient of mosquito spray as it contains many important photochemical like glycoproteins, terpenes, flavonoids, phenols, tannins, nimbins, saponins, catechines, azadirachtin and galic acid. Neem also contains Azadirachtin, Nimbolin, Nimbin, Nimbidol, Sodium Nimbin, Gedunin, Salanin and Quercetin. Neem leaves & neem formulations exhibiting antibacterial and antifodent activities. Studies report that emulsified formulation of neem oil contain strong larvicidal activities and is effective against various stages

of mosquito larva and different species of mosquitoes. Neem Powders obtained from neem leaves contains 40% Azadirachtin has also proved to be effective against mosquitoes.^[15,16,17]

Similarly, Tulsi (*Ocimum sanctum*) is an aromatic herb which is majorly found in tropical regions of Asia. It is widely cultivated in India however, lesser about it is known in the western regions of the world. The leading phytochemical compounds in holy basil leaf include eugenol (volatile oil), ursolic acid (triterpenoid) and rosmarinic acid (Phenylpropanoid) however, other active compounds include caryophyllene and oleanolic acid. Seeds contain fixed oils having linoleic & linolenic acid which are effective against various insecticides.^[8] Findings on the photochemical of Tulsi leaves and their use as insect repellent are new and will alarm the pharmaceutical companies to use them for human welfare and for controlling various ailments and nuisance creatures such as mosquitoes [18,19, 20].

The potential volatile oils extracted from Citronella grass and hairy basil have proved to be tropical relents against both day & Night biting mosquitoes. The Citronella oil contains several components which are repellent in nature & give protection for 3-4 hours. The characteristic smell of oil comes from four major components like Citronellal, Eugenol, Geraniol and limonene.^[8] Citronella oil contains several monoterpenes (aldehyde, ketone, alcohol) & compounds like citronellylacetate, geranylacetate, lemonol, linalool & neral.^[14] Studies report the use of Citronella & Vanillin oil increases the mosquito repellent frequency of mosquitoes; however the repellent frequency reported was less than that of chemical based spray.^[8,21,22]

Lantana (*Lantana Camara*) oil and Crude Extract are used as natural fumigants against many insects and mosquitoes. The leading phytochemical compounds present in Lantana include mainly triterpenoids, Oleanonic acid, Lantadene A, Lantadene B, Lantalenic acid, Icterogenin and 4,5 -dihydroxy-3,7-dimethoxyflavone, D-glucopyranoside, Camaroside and these compounds are responsible or providing Lantana with maximum repellent activities. The qualitative analysis of Lanatana shows the presence of alkaloids, glycosides, carbohydrates, flavonoids and tannins etc. which make lantana a good mosquito repellent.^[23,24,25,26]

The leaves of Lemongrass (*Cymbopogen citrates*. L) have lemon like odor because of the presence of an essential oil which has citral as its main constituent similar to that of lemon peel. The major constituents of lemongrass leaf are terpineol, dipentene, limonene, α -terpineol, citronellol, methyl heptenone, dipentene, geraniol, limonene, nerol, farnesol and triterpenoids. Studies have shown that the presence of these ingredients in lemongrass have increased its repellent frequency.^[8,27] Reports also suggest that Lemongrass (*Cymbopogon*

spp.) produce the most used natural repellents in the world.^[28]

Cinnamon contains eugenol, an aromatic compound which is commonly used as an insect repellent. The cinnamon spray is a natural repellent which kills mosquito larva and eggs. It includes cinnamaldehyde, cinnamic acid, cinnamate, cinnamyl acetate, trans-cinnamaldehyde and eugenol. Several studies have reported that cinnamon essential oils have larvicidal, ovicidal, adulticidal & repellent activities against mosquitoes.^[29,30]

The chemical constituents of Camphor contain D-Camphor, Linanool, Cineole, Limonene, ∞ -Pinene, β -Mycene, Camphene, Saffrole and ∞ -terpineol. However, the exact chemical composition varies depending on the part, origin & environment in which Camphor is grown. Camphor is believed to disrupt the mosquito's ability to detect host odors by stimulating their olfactory receptors with a strong unpleasant scent. Several studies have reported the effect of Camphor against insects & mosquitoes when used in appropriate concentration hence camphor was used along with other ingredients for preparation of mosquito spray.^[31,32,33]

Vinegar is one of the best repellent against mosquitoes and other insects as it contains acetic acid, water, organic acid, esters, alcohol, vitamins, mineral salts, amino acid and compound which make it a good repellent. Studies reported that the use of vinegar in insecticides has proved that vinegar harms mulberry plants which has promoted the use of vinegar as insecticide with pest resistance and has proved to be helpful in inducing mulberry plant growth.^[34,35]

Castor oil, is a natural and non-toxic compound which has powerful pest-repellent properties making it an effective and environmentally responsible choice for controlling insect and garden pest. Castor oil contains many components including triglycerides molecule along with omega fatty acid, flavonoids, vitamin E, phenolic acid, amino acid, terpenoids and phytosterols. It also contain unsaturated hydroxylated 12-hydroxy, 9-octadecenoic acid which combining work as mosquito repellent. Different studies report the use of castor seed cake in making mosquito repellent spray where castor oil has increased the repellent frequency of the spray.^[36,37] The addition of rose water in mosquito spray has also proved to be beneficial for its preservation as it works as a flavoring agent and imparts durability to the spray.^[38]

Table 3: Major Ingredients of Mosquito Spray.

Serial No.	Ingredients	Botanical Name	Family	Uses
1.	Neem	<i>Azadirachta indica</i>	Mahogany	It acts on insects by repelling them, by inhibiting their feeding, and by disrupting their growth,
3.	Tulsi	<i>Ocimum Tenuiflorum</i>	Lamiaceae family	Anti-cancer properties, bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases.
4.	Rose water	<i>Rosa indica</i>	Rosaceae family	Prevent wounds such as burns and cuts becoming infected
5.	Cinnamon	<i>Cinnamomum verum</i>	Lauraceae family.	Acts as antibacterial, anti-viral and anti-fungal.
6.	Castor Oil	<i>Ricinus communis</i>	Ricinus	Used as a natural skin moisturizer and in denture treatment.
7.	Citronella	<i>Cymbopogon nardus</i>	Cardiopteridaceae	Helps in preventing mosquito bites for a short period of time.
8.	Lemongrass	<i>Cymbopogon Citrates</i>	Gramineae Family	Used in production of mosquito spray
9.	Camphor	Cinnamomum camphora	Laurel family	toxic to insects and is thus sometimes used as a repellent.
10.	Vinegar	-	-	Cooking, baking, cleaning and weed control and may aid weight loss and lower blood sugar and cholesterol.
11.	Essential Oil	(Lantana oil)	Poaceae	For skin care & aromatherapy to help relieve stress, anxiety, and depression.

Over the years, researchers have demonstrated that effectiveness of repellents can be improved by synergizing the repellent with a base or fixative materials such as vanillin, salicylic acid, mustard and coconut oils.^[39,40,41] However, the effectiveness of the repellents depends on multiple factors including the type of repellents, formulation of ingredients, mode of application, environmental factors, the attractiveness of individual people to insects, loss due to removal by perspiration and abrasion, insect sensitivity to repellents, and the biting density of insects.^[42,43,44] Five different formulations of mosquito spray were prepared using similar ingredients in different concentrations (Table 1). The results indicate that mosquito spray Formulation 5 (F₅) showed maximum repellency frequency where as mosquito spray Formulation 1 (F₁) showed minimum frequency. This is attributed to the fact that F₅ has maximum content of ingredients which have high repellent frequency (Neem, Tulsi, Cinnamon, Camphor, Lantana, Citronella, Vinegar, Castor Oil, Rose Water, Essential Oil and Lemongrass etc.). On the contrary mosquito spray F₁ contains these ingredients in low concentration so, its repellent frequency and effectiveness is minimum. Several studies show that the mosquito spray containing combination of Neem, Tulsi, Cinnamon, Camphor, Lantana, Citronella, Vinegar, Castor Oil, Rose Water, Essential Oil and Lemongrass etc. in different concentration have proved to be effective.^[45,46,47,48]

CONCLUSION

Diseases like Malaria, Dengue and Filariasis which spread primarily through mosquito bite are a major

concern and are major public issue in the entire world. The world's scientific community has been successful to control these disease by controlling individual mosquito bites by the use of various mosquito repellents. However, from health point of view we need to avoid commercial and synthetic mosquito repellents available in the market as these repellents contain synthetic and harmful compounds such as organophosphorus, carbamate, N,N-diethyl-3-methylbenzamide (DEET) and Dichloro Diphenyl Trichloromethane (DDT), which are carcinogenic, non-eco friendly, and harmful for human world thus creating a critical condition for human health. The use of mosquito repellents naturally obtained from medicinal plants is an efficient and healthy process. The use of naturally and mediated plants is beneficial for humans and is friendly to our environment as it checks the spreading of mosquitoes thus restricting mosquito borne diseases. Using natural sources as a repellent is a brilliant and less costly idea as natural and herbal repellent has more benefits as compared to chemical based repellent. Thus, it was envisaged that if individually any plant based ingredient can be effective then the synergistic effect of these ingredients will surely be more pronounced. Thus, In the current investigation five formulations have been prepared by using different concentration of Neem, Tulsi, Cinnamon, Camphor, Lantana, Citronella, Vinegar, Castor Oil, Rose Water, Essential Oil and Lemongrass etc. Results indicate that combination of these ingredients is most beneficial for controlling mosquito bites. Mosquito spray with different formulations have been used out of which formulation having maximum concentration of these ingredient was reported with maximum repellent frequency. Thus it can

be concluded that the blended extract solution has the potential to be used as a repellent against mosquitoes without posing any harm to human health.

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