

Development of economical mosquito repellent using marigold plant

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Abstract: Plants have been found to be rich some of food and medicine. Man has been search for new phytochemicals secondary metabolites in plants. After harvesting the flowers the remaining plant body is discarded. *Tagetes erecta* is known to possess insecticidal activity. To test the insecticidal property different parts (leaf, stem and flower) were used. The powdered material was used to make incense sticks and burnt to assess the mosquito repellent activity. The leaf powder (25%) has shown promising results when compared with a commercial product. It was found to be without side effects. The cost of production is quite less (75%) compared to commercial product.

Keywords: repellent, incense stick, insecticidal

INTRODUCTION

With over many species of mosquitoes supposed to be responsible for spreading diseases such as yellow fever, dengue hemorrhagic fever, epidemic polyarthritis, encephalitis and malaria.[1,4]According to the world Health Organization(WHO)such diseases causes more than 3 million deaths annually.[2] There are many treatments for Malaria and other mosquito transmitted diseases but it is always better to prevent the disease. Hence, the term mosquito and other repellent came in existence. Topical or other application of mosquito repellent repels the mosquitoes to bit. Mosquito repellent can be prepares synthetically or naturally. Natural mosquitoes coil: However, most synthetic chemical repellents, especially DEET, can be readily absorbed through the skin, causing many accidental poisonings, especially of children. They also can poison wildlife.DDT-Dichlorodiphenyltrichloroethane shows very harmful to environment and DEET-*N,N*-Diethyl-*meta*-toluamide is suspected to be a carcinogen, teratogen and/or mutagen. Therefore, another, preferably non-toxic, means of repelling insects is desired. Many natural Substances are known to repel insects and mosquitoes.[3].

Mosquitoes are the major vectors for the transmission of various tropical and subtropical diseases which cause devastating effects to human[5]. The most common dreadful diseases associated with mosquitoes are malaria, yellow fever, filariasis, schistosomiasis, japanese encephalitis (JE)[6] and the worst, dengue hemorrhagic fever, caused by *Aedes aegypti*[7]. Filariasis is carried by the mosquito, *Culex quinquefasciatus* which is a pantropical pest and urban vector of *Wuchereria bancrofti*[8]. Interest has been focused to control of *Aedes aegypti* and *Culex quinquefasciatus*, lies in the fact that they act as a vector of dengue and filarial fever, respectively, which is a serious public health problem in countries like India. Therefore, the studies in search of novel entities from plants to prevent proliferation of mosquito borne diseases and to protect environment from the application of chemical pesticide, the mosquito control is essential.

There are many plants which shows anti repellent activity, this is required for protection against disease. This is plant based so they are ecofriendly and does not having any side effects most of the time. Many plant-based mosquito repellents currently on the market contain essential oils from one or more of the following plants: citronella, cedar, eucalyptus, geranium, lemon-grass, peppermint, neem and soybean, marigold [9]. Because of this they are the potent source of anti repellent activity. With this they gives protection against mosquitoes and with without any kind of side effects. Synthetic mosquito coil can be dangerous because of its side effects while natural coil are effective and usefull.

Plant selection:

Taxonomical classification [10]

Table. 1 Taxonomical classification

Kingdom	Plantae
Order	Asterales
Family	Asteraceae
Genus	Tagetes
Species	<i>Tagetes erecta</i>



Fig 1. Morphological appearance of the *Tagetes erecta*

The researchers intend to use marigold plant (*Tagetes erecta*) parts as suitable components of the mosquito coil/ incense stick to be produced. It does not contain harmful chemical which are present in some commercial products it repels mosquitoes without destroying the environment. It contains a particular smell that many insects find **unappetizing**. The smell is caused by a chemical known as " **α -terthienyl**". Which lends a natural insecticidal property in marigold. Other toxic compounds available in all the ingredients are alkaloid, papain, terpenes and cyanogenic glycosides that are objectionable to human health. It also contains pyrethrin a natural compound effective as mosquito killer. Marigold is said to deter some common insect pests, as well as nematodes. Marigold are hence often in companion planting for tomato, chili, potato. Due to antibacterial thiophenes exuded by the roots, marigold should not be planted near any legume crop. Thiophenes repels aphid, white flies, maggots, and many other pests. Simultaneous steam distillation extractions (SSDE) volatiles isolated from the flower of the erecta species is believed to have higher insecticidal activity. Flower contains pyrethrin an ingredient found in many insect repellents.

OBJECTIVES:

- Collection of plant material
- Preparation of mosquito coil/incense stick
- Check repellency test against mosquito
- Antimicrobial activity
- Oil extraction/ Preparation of cream
- Phytochemical analysis of leaf and flower extracts

MATERIAL & METHODOLOGY:

[1] PREPARATION OF MOSQUITO COIL:

A) PREPARATION OF MOSQUITO PASTE:

Marigold leaves were collected after harvesting the flowers. They were cut into smaller pieces and sundried for about 2 days. The dried parts were pounded and powdered using domestic grinder. Starch and charcoal were mixed together with water. The mixture was heated and the powder was poured into this mixture. The entire mixture was made thick and was cooled. The used flowers were collected from the temple. The flowers were sundried for 2 days and the used for powdering.

Collection of Plant Material

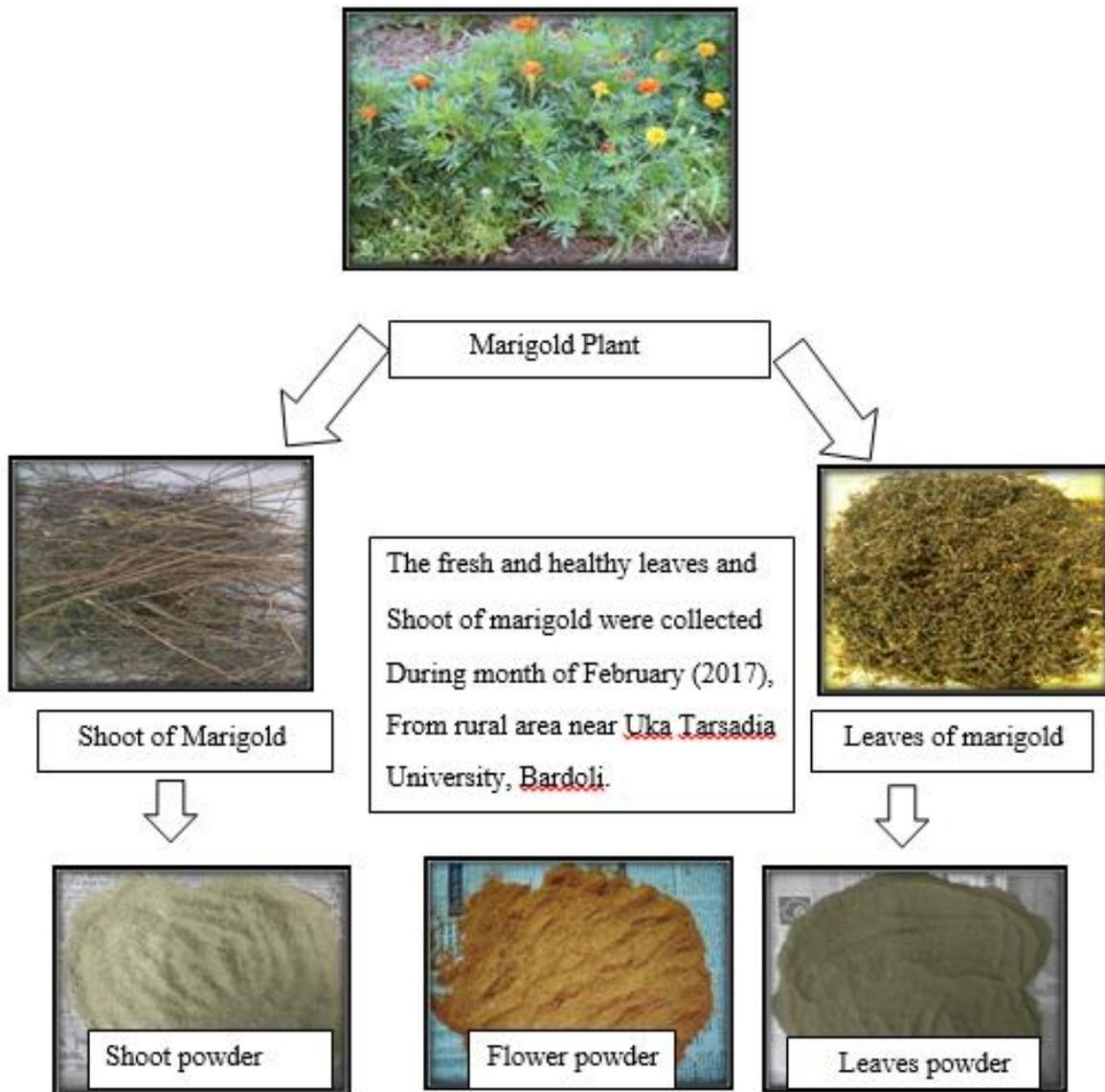


Fig 2. Collection of plant material

B) MOULDING OF MOSQUITO PASTE INTO COIL:

6*6 inches of thin sheet of metal was shaped like the usual mosquito coil. The mould was formed with a length of 66 cm and width of 7cm and height of 4cm. The thin sheet of metal was bent into a shape similar to ordinary mosquito coil. Then the mosquito paste was slowly poured into the thin sheet of metal and the paste was sundried for about 36 hours. The coil was removed from moulder.

C) REPELLENCY TEST:

Repellency test performed in a glass box of cuboidal shape with an opening so that the mosquitoes can escape through. This box mimied the room of a house, the six mosquitoes were transferred into the box. The coil was burn inside the box. The time taken by the mosquitoes try to escape or to get number or to be killed was noted.

[2] PREPARATION OF MOSQUITO INCENSES STICK:

The incense sticks were prepared using the powder at Mahavir Agarbatti Works from Vyara.



Fig 3. Preparation of incense stick of shoot, flower, leaves and mosquito coil

Cost of sticks:

Table 2. Cost of incense stick

Weight of leaf powder	330 gm	Weight of stem powder	430 gm	Weight of flower powder	50 gm
Total number of incense stick	1800	Total number of incense stick	2300	Total number of incense stick	210
Total cost of incense stick	200	Total cost of incense stick	210	Total cost of incense stick	25
Per ten stick cost	Rs 1	Per ten stick cost	Rs 1	Per Nine stick cost	Rs 1

Ingredient of sticks

Table 3. Ingredient of stick

Ingredient	Stick prepare in shoot power of marigold	Stick prepare in leaf power of marigold	Stick prepare in flower power of marigold
Plant material	25%	25%	40%
Charcoal and binding agent	75%	75%	60%
Total amount	100%	100%	100%

MOSQUITO LARVAE COLLECTION:

The mosquito larvae were collected from ditches around Uka Tarsadia University Campus, Bardoli. The mosquito larvae were maintained at room temperature.

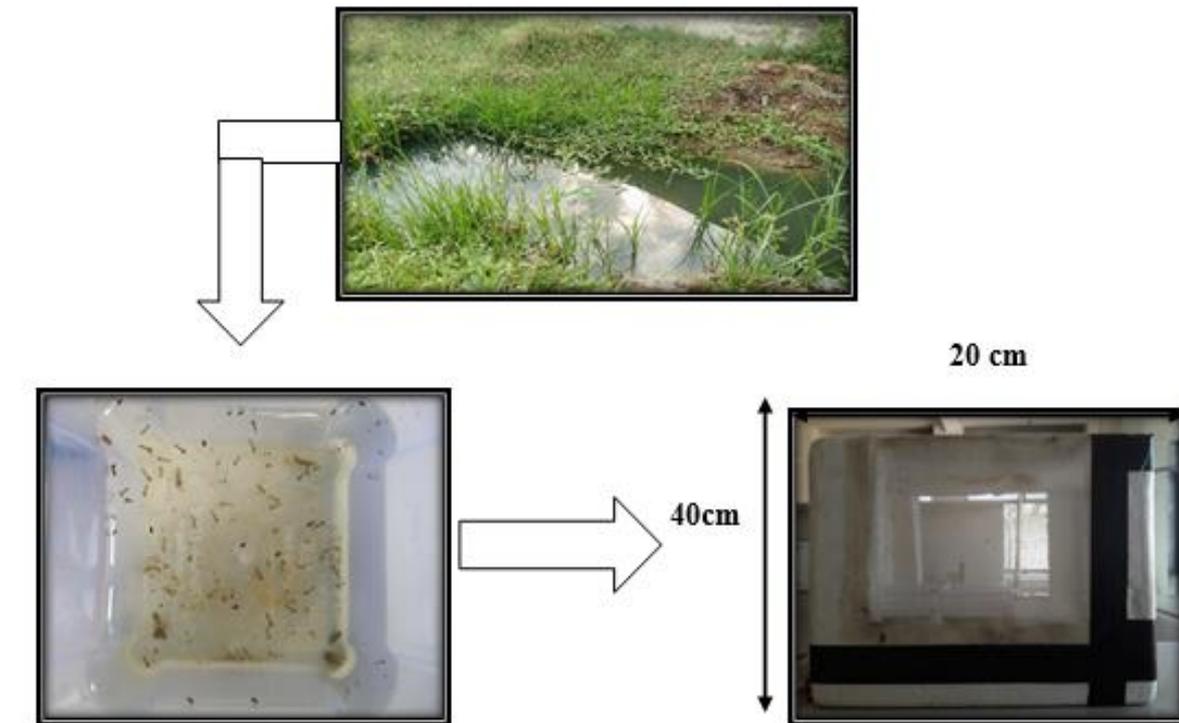


Fig 4. Collection of mosquito larvae

[3] Smoke toxicity test:

Smoke toxicity experiment was conducted in a chamber. Ten adult mosquitoes were released to the chamber and the mosquitoes were exposed to the smoke of burning incense for 45 min. and the mortality data were recorded after every 15min. The smoke toxicity was compared with the commercially available citronella incense stick as tested above. [11]

[4] Evaluation of mosquito repellent activity

Test was done by simply selecting the mosquito prone areas in the evening and night period. The public remarks were noted down after allowing them. For investigating mosquito repellent activity the prepared incense sticks were checked for causal effect such as irritation, coughing, tears were observed and recorded. Ash produced by the stick was weighed and recorded. [11]

[6] Larvicidal activity:**[A] Preparation of plant extracts:**

The prepared powder of leaf was soaked in each of water, petroleum ether, chloroform, and ethyl acetate solvents (plant material to solvent ratio was 1:10, w/v) and extracted for 24h at room temperature with shaking at 150 rpm. The extract after filtered were dried at 40°C. The dried extracts were resuspended in 1 ml of acetone.

[B] Biological assay:

Different concentrations of extracts was prepared in distilled water. All experimental exposures were made in petri-plate. Twenty-five (25) larvae were collected with a Pasteur pipette and placed in a petri-plates containing various concentration of crude extracts. Control test was performed with distilled water only. The petri-plate were covered with muslin cloth

to avoid entry of any foreign material. The observed mortality was recorded at 24 h of exposure to test solution. From this data with respect to mortality, if any, was checked and percentage mortality was recorded. [12]

$$\text{Percentage of mortality} = 1 - \frac{\text{Population in treated plot after treatment}}{\text{Population in control plot after treatment}} \times 100\%$$

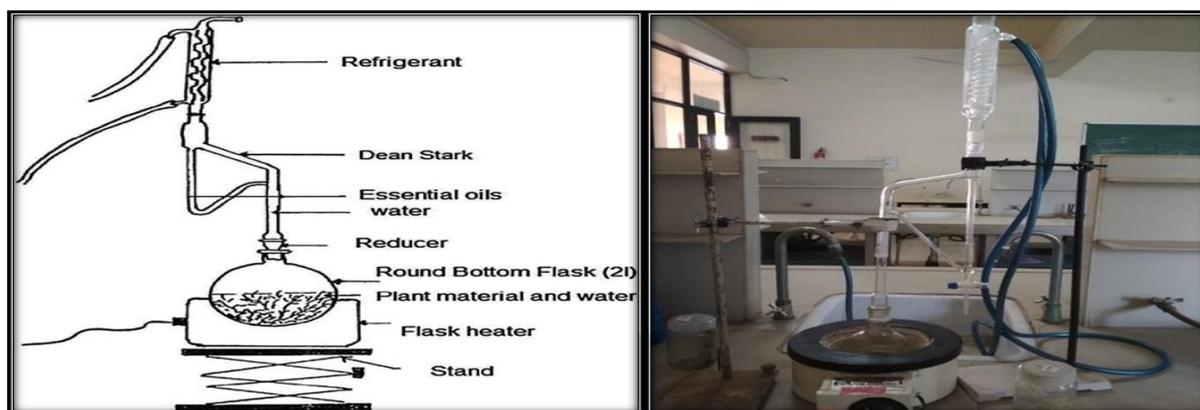
[7] Oil extraction using Clevenger apparatus:

Fig 5. Description of Clevenger apparatus

Water distillation is an ancient technique for extraction of volatile oils. It is used worldwide for its simplicity but requires high energy consumption for heating and cooling. The powered material (15g) leaf was put in round bottom flask and distilled water was added (100 ml). The mixture was allowed to boil. The water steam after sufficient boiling brought. The essential oil from the leaf material simultaneous and gets collected in the condenser. The essential oil with water was collected after cooling.

[C] Preparation of mosquito repellent cream:

Material:

Rice, Lemon, Vitamin E, Corn flour

Procedure:

2 tbsp. white rice was washed it properly and 2-3 cups of water was added. It was cooked on a heater it becomes thick and mashy. Then it was strained using a strainer. After straining a creamy and slightly thick paste was obtained $\frac{1}{2}$ lemon was squeezed into it $\frac{1}{2}$ tsp corn flour and 2 vitamin E capsules were added. Then 1tsp marigold oil was added and was air tight container a in refrigerator for up to 7-10 days. [13]

RESULTS:**Effect of repellence on mosquito smoke toxicity test:**

Table. 4 Results Effect of repellence on mosquito smoke toxicity test

Shoot powder	Control (sleep well)	Test 1	Test 2	Leaves powder	Control (sleep well)	Test 1	Test 2
No of Mosquitos	25	25	30	No of Mosquitos	25	25	30
No. of dead mosquitoes	25	25	30	No. of dead mosquitoes	25	25	30
Time (min.)	5	25	27	Time (min.)	5	21	22
Temperature °C	31	31	31	Temperature °C	33	33	33
Burning time (min)	30	46	45	Burning time (min)	31	45	46

Investigating mosquito repellent activity

Table 5: Investigation mosquito repellent activity

Table. 6 and 7 : Results of Larvicidal activity using plant extract and the effect reported from people

Results of larvicidal activity preform by using Clevenger oil

Sr.No.	Areas	Reported given by people	Remarks	Extract	Concentration ($\mu\text{g/ml}$)	No. of larvae	Time duration	Percent age of mortality
						Exposed Dead		
1	Hostel	Mosquitoes escaped ,Less irritation, coughing, tears. No headache	Mosquito repelled	Distilled water	50 $\mu\text{g/ml}$	25 0	24h	0%
					100 $\mu\text{g/ml}$	25 0	24h	0%
				Petroleum ether	50 $\mu\text{g/ml}$	25 1	24h	4%
					100 $\mu\text{g/ml}$	25 2	24h	8%
2	Tarsadi village	Mosquitoes escaped ,No irritation, coughing, tears	Mosquito repelled	Chloroform	50 $\mu\text{g/ml}$	25 1	24h	4%
					100 $\mu\text{g/ml}$	25 4	24h	16%
				Ethyl acetate	50 $\mu\text{g/ml}$	25 0	24h	0%
					100 $\mu\text{g/ml}$	25 0	24h	0%

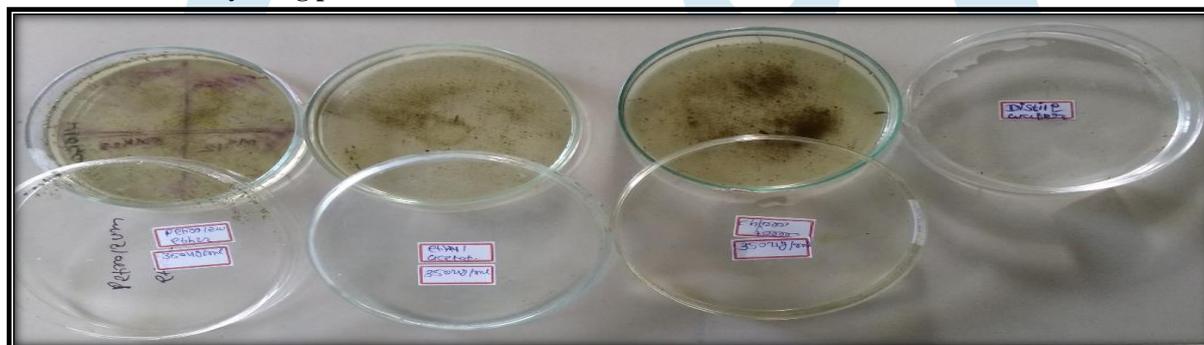
Results of Larvicidal activity using plant extract:

Fig. 6: Results of larvicidal activity

DISCUSSION:

Commercial mosquito repellents contain various synthetic chemicals which have been shown to be toxic to the uses. Of recently plant based products are being explored for human welfare in addition to food. Marigold (African) is a plant grown mainly forests. Flower which are used in temples for worshipping. After worshipping the plant are discarded. The entire marigold plant has been considered to be having insecticidal properties. With this view, the discarded plant material of marigold has been utilized with the present study to prepare incense sticks and coils. Which was tested for mosquito repellency which might to a larger extent save as form mosquito mediated diseases. The flower which are discarded by the temples after worshipping were also utilized.

The incense sticks were prepared using the power from both leaves and stem part. As a control commercially available incense sticks (sleep well brand) were used. The control and the experimental products were subjected to smoke toxicity test against mosquitos.

The control was able to kill the mosquito (25 no.) in just five minutes and look 31 minutes for complete burning. The sticks prepared with leaf and stem powder look 46 and 45 minutes respectively for complete burning and killed 25 mosquito with in 21 and 25 minutes respectively. The control when burnt in the room caused headache in some individual and irritation in the eyes of some individuals where as the experimental product did not cause any such symptoms. The unit cost of commercially available stick is Rs 1.50 where as the cost of production per stick using stem, leaf and flower powder was around 10 paisa per stick. We consider the selling cost as 4 times the production cost. The cost per experimental stick would be just 40 poise. The test could not be conducted for the extract of flower powder due to non- availability of mosquitoes.

With these observation, we can say that marigold plant displays mosquito repellent activity and it can be utilized to produce. Eco-friendly and cost effective mosquito repellent products in the form of incense sticks. A very important point is that the experimental products have not shown any side effect on the users..

The larvicidal activity was also checked using the extracts prepared in petroleum ether, chloroform and ethyl acetate at the rate of 50 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$. The chloroform extract has shown 16% percent mortality of larvae against the concentration 100 $\mu\text{g/ml}$.

Larvicidal activity was also checked using the extract of leaves in distilled water and methanol using Clevenger apparatus. The oil obtained was diluted 10 times with distilled water and tested against mosquito larvae. The methanol extract showed better larvicidal activity in terms of time taken.

REFERENCES:

- [1]. Narsinh LT, Sandhya PM, Reena AP, Madhavi MI. Mosquito Larvicidal potential of some extracts obtained from the marine organisms –Prawn and Sea cucumber. *Indian Journal of Marine Sciences*, 33(3): 303-306., 2004
- [2]. Hindustan AA, Kishor KR, Chitta SK, Krishna PU, Ravindra BV, Chandra SK, Anil KG, Vamsi MG. Formulation and Evaluation of Home Made Poly Herbal Liquids Mosquito Repellent. *JITPS*, 1(2):98-105., 2019
- [3]. Braverman Y, Chizov-Ginzburg A, Mullens, BA. Mosquito repellent attracts *Culicoides imicola* (Diptera: Ceratopogonidae). *Journal of Medical Entomology*, 36(1): 113– 115., 1999
- [4]. Mohini M, Satya NN, Dhananjay KT. Evaluation of antimosquito properties of essential oils. *Journal of Scientific and Industrial Research*, 64: 129-133., 2005
- [5]. Service MW. Management of vector. In: Youdewei A, Service N, editors. *Pest and Vector Management in the Tropics*. England: Longman Ltd., pp. 7–20., 1983
- [6]. Das MK, Ansari MA. Evaluation of repellent action of *Cymbopogon martinii martinii* Stapf var *sofia* oil against *Anopheles sundanicus* in tribal villages of Car Nicobar Island, Andaman and Nicobar Islands, India. *J Vector Borne Dis*. 40:100–4., 2003 [[PubMed](#)]
- [7]. Udonsi JK. The status of human filariasis in relation to clinical signs in endemic areas of the Niger Delta. *Ann Trop Med Parasitol*. 80:425–32., 1986 [[PubMed](#)]
- [8]. Samuel T, Jayakumar M, William SJ. *Culex* mosquito: An overview. In: William SJ, editor. *Defeating the Public Enemy, the Mosquito: A Real Challenge*. Chennai: Loyola Publications., pp. 95–116., 2007
- [9]. Donald R, Bernard, Ruidexue., Laboratory Evaluation of Mosquito Repellents against *Aedes albopictus*, *Culex nigripapua* and *Ochlerotatus triseriatus* (Diptera: Culicidae) *Journal of Medical Entomology* 41 (4): 727., 2004
- [10]. Gopi, G., Elumalai, A., & Jayasri, P. A concise review on *Tagetes erecta* *International Journal of Phytopharmacy Research* 3(1): 16-19., 2012
- [11]. Anjali Rawani, Anupam Ghosh, Subrata Laskar, Goutam Chandra Aliphatic Amide from Seeds of *Carica papaya* as Mosquito Larvicide, Pupicide, Adulticide, Repellent and Smoke Toxicant. *Journal of Mosquito Research*, 2012, Vol.2, No.2, 8-18, 2012
- [12]. Malik, B. R., Malik, M. K., & Balakrishnan, N. Evaluation of larvicidal activity of the different extracts against important species of mosquito: *Anopheles stephensi* *Journal of Parasitology and Vector Biology* 6(1): 11-15., 2014
- [13]. Bhide, M. S. S., More, M. B. H., & Prabhakar, M. S. *World Journal of Pharmaceutical Research*, 2014