IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Repellent properties of "Tulsi", *Ocimum sanctum L.* against mosquitoes.

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ABSTRACT

Repellent properties of Tulsi (***Ocimum sanctum***), leaves crude extract as well as isolated eugenol are tested in lab and field both. These phytochemicals also acted as good larvicide against Anopheles and culex larvae and obtained good results. Adult anopheles and culex tested for repellent action by crude as well as isolated eugenol showed encouraging results. Mosquito nets impregnated by eugenol also showed repelling properties. No allergic effect or any other action by phytochemicals of tulsi leaves is seen on human volunteers.

Key Words: Mosquitoes, repellent, ***Ocimum sanctum***, crude extract, eugenol, phytochemicals.

Introduction

Mosquitoes (Diptera – Culicidae) are hematophagus, nuisance and diseases transmitting insects found abundantly in India and other South Asian counties. Many deaths occur every year in India due to mosquitoes transmitted diseases like malaria, dengue, filarial, encephalitis, chicken gunea, infiltration and have been to find their way into residences and also breed nearly or in houses having open water storage tanks etc. there are many methods used for mosquitoes control. Some target larval stages while others are used to kill or repell adults. A chemical commonly used for the repellent and killing mosquitoes is

considered slightly toxic to larger animals including man. The most effective solution for malaria control efforts in the world war were plane mosquito nets and insecticide laced mosquitoes nets. Other popular methods are used nowadays for house hold mosquitoes control are small electric mats plugged into a socket, mosquito repellent vapours, repellent cream and mosquito coils all having "allethrin" and other insecticides, which are very harmful for adults as well as children. Today, there are many mosquito repellents are being used such as good knight (pyrithrine) and mortein (Allethrin) etc. which are injurious to human being in one way or the other. Hence, alternative effective and ecofriendly methods for their control, are being explored using phytochemicals.

Plants are one of the most important source of medicines and chemical molecules for the treatment of various human disorders. Some phytochemicals also act as insect repellent. "Tulsi" or "Holy basil" (***Ocimum sanctum L.***) (Labiatae) is a herbaceous sacred plant of the Hindus and is worshiped in both homes and temples. The plants is found throughout India from Himalayas to Andaman and Nicobar Islands. It played important role in the traditional Ayurvedic and Unani systems of holistic health and herbal medicine all over India. Tulsi leaves extract is considered to increase immunity power in man, hence, various brand names for "Tulsi" drops have been launched in India such as "Jolly Tulsi drop", "Dabar Tulsi drop", "Sri Tulsi drop" Dr. Morepen Tulsi drops and Ayurvedic life Tulsi Ark etc. drop. Although, Indian Materia Medica and other researches describe the use of the "Tulsi plant" in the treatment of number of ailments including bronchitis, rheumatism, pyrexia, inflammation, lower cholesterol, prevents gastric ulcers, lower fevers, improve digestion, rich supply of antioxidents, regulate blood pressure and blood sugar etc. (Satyavati, 1987 ; Nadkarni, 1976 ; Bhargava, 1981 ; Uma Devi, 1999 ; Shokeen, 2005; Singh et. al 2007) , yet its insects repellent and larvicidal properties rarely, have been investigated. Although, Dutta & Dhiman, 2020 observed attraction of mosquitoes on different colours. In view of this present studies are taken which will be very useful for devicing Tulsi based mosquito control devicings.

Materials and Methods

Following materials and methods were used for present investigations:-

A. **Cultivation of *Ocimum sanctum* (Tulsi):-** Although there are five varieties of Tulsi but for present studies two main varieties were selected for cultivation in the garden, first is the green type Sri Tulsi which is most common and second type of Krishna Tulsi which bears purple leaves. Both varieties were grown on the well irrigated and well manured (organic compost) land in the kitchen garden. The size of land was 15x10 feet. Such two land pieces were used and cultivation of Tulsi was done during August to November at Roorkee at 15°C – 30°C.

B. **Plants parts used:-** Fresh leaves of the Tulsi (***Os***) were used.

C. **Plucking the leaves:-** The Tulsi leaves were plucked by hand and washed thoroughly in water.

D. **Collection of adult mosquitoes and their larvae:-** Mosquitoes breed in stagnant water having some organic substances, such as pool, ditches, even in choked drainage water, in lakes and opened overhead water tanks etc. Adults mosquitoes were collected by specially designed "aspirator" and brought into lab and kept in fine wire gauge cages for further studies. Larvae were collected using insect collecting net from water. These were brought alive in the lab and kept for experiment purpose in troughs having water.

E. **Identification of mosquitoes and their larvae:-** These were identified by the incharge NMRI regional center Haridwar as well as with the help of key provided by NMRI, Delhi.

F. **Aspirator:-** A funnel of the diameter 60 mm was taken with a long tube. In this tube just behind the middle a fine wire-mesh was fitted. The base of the tube was connected with a rubber pipe having mouth holder, for sucking the air, creating the vacuum in the funnel for collection of mosquitoes or house files. During collection, mouth of the funnel was quickly placed on the sitting mosquitoes or house flies. Vacuum was created by sucking the air of the funnel by mouth holder of the tube. The mosquitoes or house flies collected

at near the wire gauge fitted in the funnel glass tube, separately collected in the plastic-jars covered at top by fine muslin cloth.

G. Wooden Box:- For the repellent effect for mosquitoes, a special type of wooden box was designed. This box was 50cm x 48cm x 53cm and divided into two parts by a central partition having a central hole. On the wall of first chamber crude extract of ***O. sanctum*** was sprayed. The second chamber was left untreated. After that, 100 mosquitoes were released in treated chamber. After releasing mosquitoes, hole of the partition wall was opened. Number of mosquitoes in both the chambers was noted at an interval of 30 minutes for two hours. The data are recorded in table-1. In second experiment in place of crude extract eugenol oil was sprayed and for the same, data were recorded in the tables.

H. Separation of eugenol:-

It was carried into following steps:

(A) Preparation of crude extract:-

1- 250 Tulsi (***Os***) fresh leaves

2- 50 mL acetone

3- A Grinder

Tulsi leaves were plucked and grinded in a grinder with acetone for 2 minutes till Tulsi leaves were crushed. The mixture was kept for 7 days. After filtering, the filtrate was placed in the dryer at 25°C to evaporate the acetone. Thus 170 mL crude extract was prepared.

(B) Steam distillation:- "Essential oils" are the volatile components associated with the aromas of many plants. In this experiment, the essential oil eugenol (the main component of leaves) was isolated from grinded leaves using the technique of steam distillation, which is often used to isolate liquid natural products from plants.

The principle of steam distillation is based on the fact that two immiscible liquids will boil at a lower temperature than the boiling points of either pure component, because the total vapour pressure of the heterogeneous mixture is simply the sum of the vapour pressures of the individual components (i. e. $P_T = P^o_A + P^o_B$, where P^o is the vapor pressure of the pure liquids). This leads to a higher vapor pressure for the mixture than would be predicted for a solution using Raoult's Law (that is $P_T = P^o_A N_A + P^o_B N_B$, where N is the mole fraction of the component in the mixture). The higher total vapor pressure leads to a lower boiling point for the mixture than for either single component. During the isolation of a liquid natural product by steam distillation, water is one of the components, and the liquid natural product being isolated (which is immiscible with water) is the other component. The product was steam distilled from the natural source grinded leaves of ***O. sanctum*** at a relatively low temperature (always less than 100 °C), thus avoiding decomposition of the product.

EXPERIMENTAL: The apparatus was assembled using a 100-mL round bottom flask as the distillation pot. The distillation pot was charged with 50 mL crude extract. The leaves were allowed to soak in the water until thoroughly wetted (about 15 min), then the mixture was distilled, the distillate being collected at the rate of about one drop every 2 – 3 minutes. After about 6 mL of distillate were collected, the distillate was extracted with 2.0-mL of CH_2Cl_2 (aka DCM), then again with (2 x 1.0 mL) of DCM. The DCM extracts were combined, dried over Na_2SO_4, and evaporated to give the product eugenol as a pale yellow oil (135 g).

$$\% \text{ Recovery} = \frac{amt\ (Eugenol\ isolated)}{amt\ (Tulsi\ leaves\ used)} = \frac{135g}{250g} \text{ x } 100 = 54\%$$

I. Eugenol ointment:

1. - Eugenol 20 mL
2. - Petroleum Vaseline 100g

The eugenol was mixed with petroleum Vaseline in the 1:5 ratios.

Eugenol + Petroleum Vaseline - → Eugenol Ointment

J. **Sprayer:-** A sprayer was filled with crude extract and eugenol oil separately. In the room and on the mosquitoes net and in the wooden box (treated part) these were sprayed whenever required. To recorded the repellent effect against the mosquitoes and house flies.

Eugenol sprayer:- The 25 % solution of eugenol oil was placed in the bottle with a wick. With this wick the eugenol liquid was raised up side. There is a hot plate to diffuse this eugenol oil in the atmosphere for the repellent effect of the mosquitoes.

K. **pH of the extracts:-** The pH was calculated by pH paper. BDH pH papers were used.

L. **Impregnation of mosquito net with eugenol oil:-** Eugenol oil was sprayed on the mosquito nets with the help of hand sprayer and treated net was kept in shadow for drying. After 4-6 hrs it was ready for use.

Results

Repellent property of eugenol oil against mosquitoes such as *Culex* and *Anopheles*

Repellent and larvicidal properties of ***Ocimum sanctum*** (Tulsi) extract against mosquitoes larvae and adults were worked out in the laboratory as well as in field. The repellent property was observed during May to September when the mosquitoes population reaches on peak in this region. The experiments were conducted on the Culex as well as on Anopheles mosquitoes. Larvicidal and repellent properties were examined using crude extract, acetone extract, aqueous extract as well as pure eugenol oil.

For the repellency of the mosquitoes such as Culex and Anopheles, a wooden box was made as described in materials and methods. It had two parts 'A' and 'B' separated by a partition of wooden plank having a hole in the center. 100 mosquitoes were used for experimental purpose. In the part 'B' the extracts of Tulsi leaves were sprayed and part 'A' was kept untreated. The experiment was carried out in the month of May to September. The data were recorded and maintained in the tables.

Repellency against Culex mosquitoes:- The experiment was carried out in the month of May to September during the year 2019 and 2020. In the experimental cage the crude extract was sprayed in the part 'B'. 100 Culex mosquitoes were released in the treated part 'B' as said above. The observations regarding migration of mosquitoes from treated to untreated part were keenly taken and the data are recorded in the table-1, which clearly depict that after 3 hours 98% to 100% mosquitoes were repelled in the untreated part 'A' of the wooden box. In the second hour of the experiment, maximum numbers of mosquitoes were repelled. In the second experiment pure eugenol (4-allyl 2-methoxyphenol) was sprayed in the part 'B' of the wooden box. After that again 100 Culex adults were released in the treated part and observations were made for the repellent behavior of the mosquitoes and the data are recorded in Table-2. The data of afore said table depict that cent percent mosquitoes population migrated from treated 'B' part to untreated 'A' pan of the wooden box within 3 hours. The result showed the magnitude of the eugenol repellent property.

Repellency against Anopheles mosquitoes:- The repellency effect against Anopheles mosquitoes were also carried out during 2019 to 2020. In this experiment, the crude extract of Tulsi leaves was sprayed in the part 'B' of the wooden box like that the previous experiment and 100 Anopheles mosquitoes were released in it. The data of the repellency were recorded and presented in the table-3. The data showed that 98% to 100% Anopheles mosquitoes were repelled into the untreated part 'A' of the box after 3 hours. In the second experiment, pure eugenol (4-allyl 2-methoxyphenol) was sprayed in the part 'B' of the wooden box. And again 100 Anopheles adults were released in the treated part and observations were made on the repellency property of the mosquitoes and the data are recorded in table-4, which showed that in the first hr. of the experiment maximum 49-53 percent mosquitoes population migrated from treated part 'B' to untreated part 'A' of the wooden box within 3 hours. The result showed the magnitude of the eugenol repellent property like that of Culex mosquitoes.

Larvicidal activity against mosquitoes larvae:- Larvicidal activity were recorded for the larvae of (mixed population) Culex and Anopheles during 2009 with Tulsi leaves extract and pure eugenol. For this 50 larvae were kept in the petri dish and 10 ml crude extract was sprayed on

the surface of the petri dish and data were recorded. Within 4 hours, 96% to 100% larvae were killed (Table-5). This experiment also done with eugenol, which showed that cent percent mosquito larvae were killed when pure eugenol oil was sprayed on the water surface (Table-6).

Repellent property using mosquitoes nets impregnated by eugenol oil during 2009:- In another experiment, two mosquito nets were taken and on these, eugenol oil was sprayed with the help of a hand sprayer and the nets were dried under the shadow area for 4 to 6 hrs. Then, these were used during different nights. These nets were repeatedly used for many days. Observation were made giving continuous sitting for 4 to 6 hours with the help of a helper and the data regarding mosquitoes appearance on the nets were recorded in table-7 The observations showed that, generally, mosquitoes were repelled by the smell of eugenol oil sprayed on nets. Only in few instances single mosquito came near the net and at once took flight away.

20% eugenol based chemical vaporizer envelops room with its fragrance, repelled mosquitoes, driving them outside the living environment.

Thus both crude extract as well as eugenol oil of Tulsi leaves are good repellent and larvicide against mosquitoes and their larvae. Moreover, Tulsi leaves extract either in crude form or pure form is ecofriendly and does not cause any pollution hazard as well as ill effect for human being. Hence, their use is suggested for mosquitoes control.

TABLE – 1

Repellent property of mosquitoes (Culex) with crude extract

Sr. No.	Date of release of mosquitoes	No. of mosquitoes released in treated part 'B'	No. of mosquitoes found in untreated part 'A'				No. of mosquitoes found in treaded part after 3 hrs.
			1hr.	2hr.	3hr.	Total	
1.	26-05-2019	100	30	52	18	98	02
2.	27-05-2019	100	31	51	18	100	-
3.	28-05-2019	100	35	56	09	100	-
4.	29-05-2019	100	32	56	12	100	-
5.	30-05-2019	100	32	55	12	99	01
6.	31-05-2019	100	36	54	10	100	-
7.	01-06-2019	100	34	55	10	99	01
8.	02-06-2019	100	32	52	16	100	-
9.	03-06-2019	100	39	53	18	100	-
10.	04-06-2019	100	35	52	23	100	-
11.	02-07-2020	100	31	52	17	100	-
12.	03-07-2020	100	30	50	18	98	02
13.	05-07-2020	100	34	55	11	100	-
14.	06-07-2020	100	33	54	13	100	-
15.	07-07-2020	100	31	55	14	99	-
16.	08-07-2020	100	35	55	09	100	01
17.	10-07-2020	100	36	54	10	100	-
18.	11-07-2020	100	33	56	11	100	-
19.	12-07-2020	100	39	53	8	100	-
20.	13-07-2020	100	33	53	24	100	-

TABLE-2

Repellent property of mosquitoes (Culex) with eugenol oil

Sr. No.	Date of release of mosquitoes	No. of mosquitoes released in treated part 'B'	No. of mosquitoes found in untreated part 'A'				No. of mosquitoes found in treaded part after 3 hrs.
			1hr.	2hr.	3hr.	Total	
1.	06-06-2019	100	50	32	18	100	-
2.	07-06-2019	100	50	30	20	100	-
3.	08-06-2019	100	56	33	17	100	-
4.	09-06-2019	100	52	29	19	100	-
5.	10-06-2019	100	51	30	19	100	-
6.	12-06-2019	100	61	28	11	100	-
7.	15-06-2019	100	60	25	15	100	-
8.	16-06-2019	100	59	31	10	100	-
9.	17-06-2019	100	59	30	12	100	-
10.	20-06-2019	100	58	20	17	100	-
11.	15-07-2020	100	51	31	18	100	-
12.	16-07-2020	100	52	28	20	100	-
13.	18-07-2020	100	55	34	11	100	-
14.	19-07-2020	100	50	30	20	100	-
15.	21-07-2020	100	61	28	11	100	-
16.	22-07-2020	100	59	31	10	100	-
17.	23-07-2020	100	51	30	19	100	-
18.	24-07-2020	100	58	20	17	100	-
19.	25-07-2020	100	55	29	16	100	-
20.	26-07-2020	100	59	30	12	100	-

TABLE-3

Repellent property of mosquitoes (Anopheles) with crude oil extract

Sr. No.	Date of release of mosquitoes	No. of mosquitoes released in treated part 'B'	No. of mosquitoes found in untreated part 'A'				No. of mosquitoes found in treaded part after 3 hrs.
			1hr.	2hr.	3hr.	Total	
1.	10-05-2019	100	29	49	22	100	-
2.	11-05-2019	100	30	48	22	100	-
3.	12-05-2019	100	34	50	16	100	-
4.	13-05-2019	100	30	51	18	99	01
5.	14-05-2019	100	32	50	18	100	-
6.	15-05-2019	100	29	53	18	100	-
7.	16-05-2019	100	31	49	20	100	-
8.	17-05-2019	100	33	52	14	99	01
9.	18-06-2019	100	30	54	16	100	-
10.	19-06-2019	100	32	51	16	99	01
11.	28-07-2020	100	33	45	22	100	-
12.	29-07-2020	100	31	47	22	100	-
13.	30-07-2020	100	34	55	11	100	-
14.	31-07-2020	100	28	53	19	100	-
15.	02-08-2020	100	29	50	21	100	-
16.	03-08-2020	100	32	53	15	100	-
17.	04-08-2020	100	30	49	20	99	01
18.	05-08-2020	100	32	52	14	98	02
19.	06-08-2020	100	33	51	16	100	-
20.	07-08-2020	100	31	52	17	100	01

TABLE-4

Repellent property of mosquitoes (Anopheles) with eugenol oil

Sr. No.	Date of release of mosquitoes	No. of mosquitoes released in treated part 'B'	No. of mosquitoes found in untreated part 'A'				No. of mosquitoes found in treaded part after 3 hrs.
			1hr.	2hr.	3hr.	total	
1.	20-06-2019	100	49	25	26	100	-
2.	21-06-2019	100	49	30	21	100	-
3.	23-06-2019	100	51	35	14	100	-
4.	24-06-2019	100	50	36	14	100	-
5.	25-06-2019	100	52	30	18	100	-
6.	02-07-2019	100	51	31	18	100	-
7.	03-07-2019	100	49	29	22	100	-
8.	04-07-2019	100	53	30	17	100	-
9.	08-07-2019	100	52	29	19	100	-
10.	09-07-2019	100	51	32	17	100	-
11.	10-08-2020	100	50	24	26	100	-
12.	11-08-2020	100	52	28	20	100	-
13.	12-08-2020	100	51	31	18	100	-
14.	13-08-2020	100	50	34	16	100	-
15.	15-08-2020	100	49	33	18	100	-
16.	16-08-2020	100	50	31	19	100	-
17.	17-08-2020	100	51	29	20	100	-
18.	18-08-2020	100	51	31	18	100	-
19.	19-08-2020	100	53	30	17	100	-
20.	22-08-2020	100	50	34	16	100	-

TABLE-5

Larvicidal activity against mosquitoes larvae with crude extract.

Sr. No.	Date of release of mosquitoe larvae	No. of larvae released	No. of larvae found dead				Mortality%
			2hr.	3hr.	4hr.	total	
1.	21-07-2020	50	11	26	12	49	98
2.	22-07-2020	50	09	28	13	50	100
3.	23-07-2020	50	12	24	12	48	96
4.	24-07-2020	50	12	24	14	50	100
5.	25-07-2020	50	12	27	10	48	96
6.	26-07-2020	50	10	28	12	50	100
7.	27-07-2020	50	11	24	15	50	100
8.	28-07-2020	50	12	25	13	50	100
9.	29-07-2020	50	13	27	09	49	98
10.	30-07-2020	50	11	26	12	49	98

TABLE-6

Larvicidal activity against mosquitoes larvae with eugenol oil.

Sr. No.	Date of release of mosquitoe larvae	No. of larvae released	No. of larvae found dead				Mortality%
			2hr.	3hr.	4hr.	total	
1.	11-08-2020	50	25	15	10	50	100
2.	12-08-2020	50	24	16	09	50	100
3.	13-08-2020	50	25	20	04	49	98
4.	14-08-2020	50	26	19	05	50	100
5.	15-08-2020	50	23	15	12	50	100
6.	16-08-2020	50	27	14	09	50	100
7.	17-08-2020	50	27	13	10	50	100
8.	18-08-2020	50	26	14	10	50	100
9.	19-08-2020	50	25	15	05	50	100
10.	20-08-2020	50	26	15	09	50	100

Table-7

Repellent property using mosquitoes nets impregnated by eugenol oil during 11-07-2009

Sr. No.	No. of Hours after which observation were made	No. of Mosquitoes observed on net.
1	1	Nil
2	2	Nil
3	3	Nil
4	4	Came and went away
5	5	Nil
6	6	Nil
7	7	Came and went away
8	8	Nil
9	9	Nil
10	10	Nil

DISCUSSION

Only few attempts have been made so far, to observe repellent properties of some phytochemicals against mosquitoes and house flies. Experimental data for present studies for repellency properties of ***Ocimum sanctum*** revealed that after three hrs. 98-100 percent ***Culex*** mosquitoes were repelled by crude extract spray in treated part. The same experiment was repeated with eugenol oil which indicates the 100 percent repellent property for ***Culex*** and ***Anopheles*** both. With crude extract of Tulsi leaves, 96 to 100% larvicidal activities were recorded for the larvae of (mixed population) ***Culex*** and ***Anopheles*** both. Mosquitoes net impregnated with eugenol oil used during nights showed almost cent present repellency. Only in few instances single mosquitoes came near the net and at once took flight away. Thus, both crude extract as well as eugenol oil of Tulsi leaves are found good repellent and larvicide against mosquitoes and their larvae. Morever, Tulsi leaves extract either in crude form or pure form is ecofriendly and does not cause any pollution hazard as well as ill effect for human being. 20% eugenol based chemical vaporizer envelops room with its fragrance, repels mosquitoes, driving them outside the living environment. Present studies are supported by the work on phytochemicals use against mosquitoes as repellent. Bhatnagar et. al, (1993) find out the insecticidal properties of essential oils and major constituents of aromatic plants, ***Ocimum basilicum*** Linnaeus flies. Thus, there is slight variation in repellent and larvicidal properties of different phytochemicals used against mosquitoes, house flies and other insects. In the present study the new phytochemical was introduced as β-sitosterol-D-glycoside. It is supported by Mizanur Rahman (2009).

Further, the hemolytic assay proved that the protein present in the extract of Tulsi leaves was not allergic to human as there was no hemolysis in the RBCs.

Thus, present findings on the Tulsi leaves phytochemicals and their use as insect repellent are new and will arm the pharmaceutical companies to use them for human welfare for controlling various ailments and nuisance creatures such as mosquitoes. These studies also open the door for further investigations.

CONCLUSION

Mosquitoes are sanguivorous disease transmitting insects, act as vector for malaria germs (***Plasmodium***) dengue virus, Encephalitis and Chicken guinea viruses and elephantiasis nematode (***Wuchereria bancrofti***). Plant phytochemicals have been tested time to time as repellent and larvicidals. Holy basil (Tulsi) ***Ocimum sanctum*** leaves extract (crude as well as isolated eugenol) is tested during present studies. Both crude extract as well as eugenol oil acted good repellent for adult mosquitoes species (Culex and Anopheles). Encouraging results are obtained from leaf extracted eugenol oil. Eugenol oil impregnated mosquitoes nets also tried and found repelling action for mosquitoes and safe for human beings. Experiments conducted resulted promising repellent action of crude extract eugenol. Larvicidal properties of both the crude extract and eugenol tested in lab, has given very good results. Hence, use of Tulsi leaves phytochemicals is suggested as repellent and larvicidal. ***O. sanctum*** Linnaeus evaluated against ***Anopheles stephensi*** Liston, ***Aedes aegypti*** Linnaeus and ***Culex quinquefasciatus*** (Say) mosquito species under laboratory conditions. The bioassay tests revealed that the essential oil of ***O. basilicum*** and its major constituent, methyl chavicol are more effective as compared to ***O. sanctum***. Dosages of 0.003 ml/43.0 cm^2 of essential oil and 0.001 ml/43.0 cm^2 of methyl chavicol extracted from ***O. basilicum*** induced 100 per cent mortality in all the three mosquito species in a period ranging from 10 to 25 minutes. Mandavgance et. al (2005) prepared the cow dung based herbal mosquito repellent. In present study the eugenol ointment (EO) and evaporator were made for mosquitoes and found good repellent. Further, investigations are needed to improve and add the materials readily available for the environmental safer control mosquitoes. Anees (2008) used the acetone, chloroform, ethyl acetate, hexane, and methanol leaf and flower extracts of ***Ocimum sanctum*** against fourth instar larvae of ***Aedes aegypti*** and ***Culex quinquefasciatus***. The highest larval mortality was found in leaf extract of ***O. sanctum*** against the larvae of ***A. aegypti*** and ***C. quinquefasciatus.*** Knio et al (2008) find out Larvicidal activity of essential oils extracted from commonly used herbs in Lebanon against the seaside mosquito, ***Ochlerotatus caspius***. Kamaraj *et. al,* (2008) also supported with different plant extract observed screening for antifeedant and larvicidal activity of plant extracts against ***Helicoverpa***

armigera (Hibner), ***Sylepta derogata*** (F.) and ***Anopheles stephensi*** (Liston). Jerome *et. al* (2010) worked on house flies and said that boric acid inhibit the development of the House.

Acknowledgement

The authors are thankful to the Principal and heads Chemistry and Zoology departments for laboratory facilities and to the incharge NMRI regional centre Haridwar for the identification of mosquitoes species and their larvae, authors are also thankful to the director NMRI Delhi for providing identification Key material.

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