

ADULTICIDAL ACTIVITY OF ESSENTIAL OILS OF HARIDRA AND TULSI AGAINST
AEDES AEGYPTI AND ANOPHELES MOSQUITOESAkshu Sharma^{1*}, Manisha Dikshit² and Ashwani Kumar³¹Assistant Proff. in the Dept. of Agad Tantra Evum Vidhi Vaidyak SKSS, AMC Ludhiana Punjab.²Associate Proff. in the Dept. of Agad Tantra Evum Vidhi Vaidyak Rishikul Govt. College, Haridwar.³Assistant Proff.in the Dept. of Rog Nidan Shri Dhanvantri Ayurvedic College and Hospital, Chandigarh.

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ABSTRACT

Plants of *Haridra* and *Tulsi* were collected from Palampur, H.P, were collected from Rishikul Haridwar. The Plants identification was done in the department of Dravyaguna, Rishikul Campus Haridwar. Essential oils of *Curcuma longa* (Haldi) rhizome paste and *Ocimum sanctum* (Tulsi) leaves were obtained by hydro-steam distillation by Clevenger extraction methods for 4-7 hour daily for several days. A total of 250 gm of the plant materials of each plant was extracted in which 0.2gm of *Curcuma longa* and 0.1gm of *Ocimum sanctum* were obtained, which yielded 1 gm of essential oil of *Curcuma longa* and 0.5 gm of essential oil of *Ocimum sanctum* after 5 times of extraction. Three replicates were carried out along with two controls. It was observed that at end of 24 hour recovery period percent mean corrected mortality of *Ae. aegypti* was 50% (range: 45-60%) . Results of adulticidal activity essential oil of *Curcuma longa* (Haldi) against *Anopheles* mosquitoes was observed that at end of 24 hour recovery period percent mean of *Ae. eegypti* was 70% (range: 65.0-75.0%) . Adulticidal activity of essential oil of *Ocimum sanctum* against female *Aedes aegypti* was 63.3% in the range of 60-70% .Result of adulticidal activity of essential oil of *Ocimum sanctum* against adult female *Anopheles* was 71.7%.

KEYWORDS:- *Haridra*, *Tulsi*, Adulticidal Activity, *Aedes aegypti*, *Anopheles*.

INTRODUCTION

मशकाः सामुद्र, परिमण्डलो, हस्तिमशकः, कृष्णः पार्वतीय इति पञ्च;

तैर्दष्टस्य तीव्रा कण्डुर्दशशोफश्च

पार्वतीयस्तु कीटैः प्राणहरैस्तुल्यलक्षणः॥ (सु. क. 8/36)

In *Ayurveda*, the classification of mosquitoes is mentioned in *kalp sthana* of *Susruta Samhita*. There are 5 types of mosquitoes mentioned in *Susruta Samhita* in 8th chapter of *kalp sthana*. In this chapter, mosquitoes are named *mashaka*. The name of 5 types of *mashaka* are given below-*Saamudra Parimandala Hastimashaka Krishna Parvatiya* ^[1]

When bitten by these mosquitoes cause severe itching and swelling at the place of the bite.

कण्डुमान्मशकैरीषचाच्छोथस्यान्मन्दवेदन

असाध्यकीटसदृश्यमसाध्यमशकक्षतम॥ (च. चि. 23/157)

Mosquitoes bite cause itching, slight edema, and mild pain. The bite of the mosquito is like an incurable type causing signs and symptoms like those of an incurable variety of insect bites. ^[2]

कण्डुमान्मशकैरीषच्छोथस्युर्मन्दवेदन |

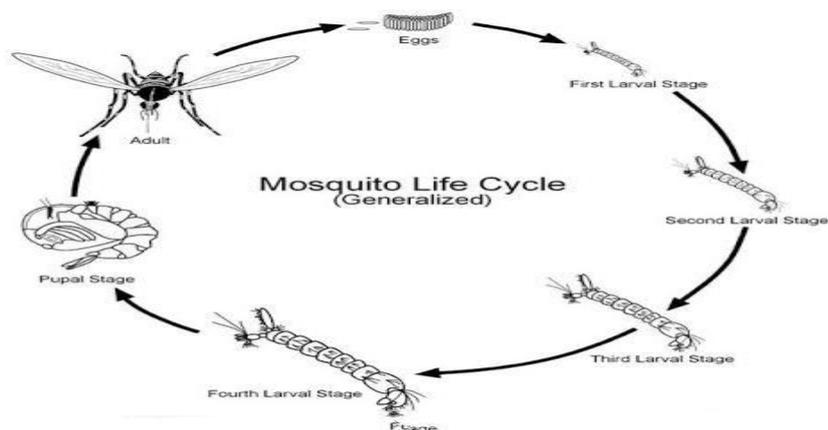
असाध्यकीटसदृश्यमसाध्यमशकक्षतम॥ (अ. स. उ 43/17)

The bite of a mosquito causes itching, mild pain and slight edema, and is an incurable type. ^[3]

Mosquito identification method^[4]

All mosquito species go through four distinct stages during their life cycle: Egg – Eggs are the first stage that is exposed to water. Larva – Larvae live in water and molts several times.

Pupa – Pupa is the stage just before emerging as an adult. Adult – The Adult stage is a stage where they fly after a short time after emerging and after their body parts have hardened. ^[5]



Mosquito Species and Their morphology^[6]

1. **Aedes mosquitoes:** *Aedes* mosquitoes are usually container breeders especially man-made containers with clean water. They complete their life cycle i.e. egg to adult stage in 6-8 days. The Egg is black and resembles a rugby ball. The larva stage, it rests at 45 degrees from the surface of the water. Adult mosquitoes have black and white marking and they prefer darker colors like black or red. They can fly short distances up to 50-100m. The bites from an *Aedes* mosquito peak after and before sunset with the change of light intensity.
2. **Anopheles mosquito:** The *Anopheles* mosquito is responsible for transmitting malaria. They prefer unpolluted and clean water. They complete their metamorphosis i.e. egg to adult stage in 6-10 days. Their egg is about 1mm long which floats on its sides. Female mosquitoes with one blood meal can lay about 50-150 eggs at a time. At the larvae stage, the larva rests parallel to the surface of the water. Whereas, adult mosquitoes mark pale and dark marks on its wing. They rest at 45-degree angle to the surface. They, usually bite at night depending upon the species, and rest indoors and outdoors.
3. **Culex mosquito:** *Culex* mosquitoes are the main vector for Japanese B – Encephalitis. The complete their life cycle i.e. egg to adult stage in 6-10 days. They breed mainly in drains and polluted stagnant water. Their egg is long and cylindrical, brown. They raft in a cemented of about 300 eggs at a time and whereas raft is usually 3-4mm long and 2-3mm wide. At the larva stage, larva rest 45 degrees from the surface of the water. Adult mosquito's thorax, legs, and veins on the wings are covered with brown colored scales, which are dull in color. The tip of the abdomen is always seen as blunt. They prefer darker colours and can fly long distances. They usually bite at night and they rest indoors before and after the meal.

Presently, the risk of contracting arthropod-borne diseases has increased due to the climate change and intensifying globalization.^[7] Malaria, a life-threatening disease transmitted by mosquitoes, is continuing to be a

major public health problem causing death and illness in children and adults around the world, especially in tropical countries. About 3.3 billion people—half of the world's population—are at risk of malaria. Every year, this leads to about 250 million malaria cases and nearly one million deaths.^[8] Agad Tantra is one of the eight branches of *Astanga Ayurveda*. It deals with Virudh aahar [food interaction], Sarp dansh [snake bite], Alark vish [dog bite] & *Keet vish* [insect bite], etc.^[9] In *Sushruta Samhita*, *Aacharya Sushruta* has mentioned *Keet visha*, in which, and he described five types of *Mashak* in kalpstan chapter-3.^[10] In *Bhavprakash Nighantu*, *Tulsi* is mentioned as anti-helminthic and *Haridra* as anti-inflammatory.^[11]

MATERIAL AND METHOD

Samples preparation

Rhizomes (Roots) parts of the *Haridra*, leaves of *Tulsi* were taken for the extraction of the essential oils. The plant materials were washed with distilled water to remove dust particles and shade dried. Essential oils of the plants were obtained by the hydro-distillation method. In Clevenger apparatus, the raw material of the shade dried was subjected to water distillation in a Clevenger apparatus for 7 h. The oil layer is separated from the aqueous phase using n-hexane with the help of a separating funnel. The anhydrous sodium sulfate is added in hexane-oil solution to remove water content absorbed by hexane. The oil is obtained by removal of n-hexane at low temperature and the samples were kept in a refrigerator at 4°C.

Extraction of essential oils from tulsi leaves

50 gm of *Tulsi* leaves were taken in 300 ml of water and were extracted for 7-8 hours in the Clevenger apparatus. Then, the oil layer is separated from the aqueous phase using n-hexane with the help of a separating funnel. The extraction procedure was repeated several times. The samples were pooled, dried at low temperature, and kept in a refrigerator at 4°C.

Extraction of essential oils from rhizomes of haridra

50 gm of Rhizomes of *Haridra* was taken in 300 ml of water in one liter flask in Clevenger apparatus and

extracted for 7-8 hours. The essential oil was collected in a separating funnel and extracted with n-hexane. The essential oil was dried at room temperature. The process was repeated several times; samples were pooled and kept in the refrigerator at 4°C.

Field collection of mosquito Larvae and Their rearing in laboratory

Mosquito larvae of *Aedes* and *Anopheles* were collected from breeding sites in different areas in Haridwar.

- BHEL Sector 4,5
- Roadways Bus Service Station, Haridwar
- Old Industrial Area of district Haridwar
- Near Nigam Office, Haridwar
- Village Bhogpur Tall of Chilla range, District Pauni Garhwal

Laboratory rearing of the Field -collected larvae up to adult mosquito

Mosquito larvae of *Anopheles* and *Aedes* were collected from the field and brought to the laboratory and kept in the insectary at 27± 2°C with 60 to 70% relative humidity. The larvae were fed a mixture of dog biscuit and yeast powder in a 3:2 ratio as nutrients. The larvae were reared up to adult mosquitoes. The adult mosquitoes were reared in the humidified cage and fed with 10% glucose soaked into cotton. Female adult mosquitoes were separated from males and the adult female mosquitoes were used for testing for adulticidal activity.

Preparation of filter paper for adulticidal activity

0.1ml essential oil diluted in 2.5ml ethanol and applied on Whatman filter paper of 180 cm² (size:12x15cm²) and dried. Control papers were treated with ethanol under similar conditions.

Bioassays test for determination of adulticidal activity

The bioassay test for adulticidal activity was conducted using a standard protocol (WHO, 2006). Twenty female mosquitoes 2-5 days old glucose fed were collected and gently transferred into the plastic holding tubes. The mosquitoes were held in the holding tube for one hour and then exposed to test paper for one hour in the exposure tube and the knockdown of the mosquitoes

were counted at 15 minutes intervals at the end of one hour. At the end of one hour, the mosquitoes were transferred back to the holding tubes hour for a recovery period of 24 hours and a 10 % glucose solution was provided to the mosquitoes. Mortality of mosquitoes was recorded at the end of 24 hour recovery period. Three replicates were carried out along with parallel control. Percent mortality was recorded by using Abbot's formula.

$$\% \text{ mortality} = \frac{\% \text{ Test mortality} - \% \text{ Control mortality}}{100 - \% \text{ Control mortality}} \times 100$$

Data Analysis and Calculation of synergistic factor (Mix formulation)

As a methodology described earlier. In, the experiment 1st essential oil of 0. 55µL/cm² Tulsi oil and 0.27 µL / cm² Haridra were diluted in 2.5ml ethanol and applied on 180 cm² Whatman filter paper. In, the 2nd experiment 0. 55µL/cm² Haridra and 0.27 µL/cm² Tulsi were taken with same procedure used. In, the 3rd experiment the Tulsi and Haridra oil was taken at the same ratio of 1:1.

OBSERVATIONS AND RESULTS

Adulticidal activity essential oil of *curcuma Longa* and *Ocimum sanctum* against *Anopheles* and *Aedes aegypti*

The Adulticidal activity of the field-collected adult *Aedes aegypti* and *Anopheles* were carried out on Whatman impregnated paper impregnated with *Curcuma longa* essential oil. 0.1ml essential oil *Curcuma longa* diluted in 2.5ml ethanol and applied on Whatman filter paper of 180 cm² (size 12x15 cm²; concentration: 0. 55µL/cm²). Twenty adult female *Aedes aegypti* were exposed to the filter paper in the WHO tube for one hour and the number of knockdown mosquitoes was recorded at 5 min, 15min, 30, min,45 min ,and 60 min.time interval. Thereafter the mosquitoes were transferred to the recovery tube for 24 hours and the mortality of mosquitoes was recorded during 24 hour recovery period. Three replicates were carried out along with two controls. It was observed that at end of 24 hour recovery period percent mean corrected mortality of *Ae. aegypti* was 50% (range: 45-60%) .

Table 1: Adulticidal activity of essential oil of *Curcuma longa* (Haldi) against *Aedes* mosquitoes.

Concentration	Replicate No.	No. of mosquitoes exposed	Nos. of mosquitoes knock down in minutes					Mortality in 24 hour recovery period	Percent (%)		
			5	15	30	45	60		Mortality in 24 hour	Corrected mortality in 24 hour	Mean corrected mortality (Range)*
0. 55µL/cm ²	R1	20	2	4	5	7	12	12	60	60	50 (45-60)
	R2	20	3	4	5	6	9	9	45	45	
	R3	20	3	4	5	6	9	9	45	45	
Control	C-1	20	0	0	0	0	0	0	0	0	0
	C-2	20	0	0	0	0	0	0	0	0	

0.1ml essential oil diluted in 2.5ml ethanol and applied on 180 cm² on Whatman filter paper * Figure in parenthesis indicates the range

The Adulticidal activity of essential oil of *Curcuma longa* against adult female *Anopheles* was carried out as method reported earlier. Twenty adult female *Anopheles* mosquitoes were exposed at Whatman filter paper of 180 cm² (size 12x15 cm²; concentration: 0. 55µL/cm²) in WHO tube for one hour and number of knock down

mosquitoes were recorded. Thereafter the adult *Anopheles* mosquitoes were transferred to the WHO recovery tube and mortality of the *Anopheles* mosquitoes were recorded at end of 24 hour recovery period. Results of adulticidal activity essential oil of *Curcuma longa* (Haldi) against *Anopheles* mosquitoes is shown in table-7. It was observed that at end of 24 hour recovery period percent mean corrected mortality of *Ae. egypti* was 70% (range: 65.0-75.0%) .

Table 2: Adulticidal activity essential oil of *Curcuma longa* (Haldi) against *Anopheles* mosquitoes.

Concentration	Replicate No.	No. of mosquitoes exposed	Nos. of mosquitoes knock down in minutes					Mortality in 24 hour recovery period	Percent (%)		
			5	15	30	45	60		Mortality in 24 hour	Corrected mortality in 24 hour	Mean corrected mortality (Range)*
0. 55µL/cm ²	R1	20	2	5	6	10	15	15	75	75	70 (65-75)
	R2	20	2	5	6	10	14	14	70	70	
	R3	20	2	5	6	10	13	13	65	65	
Control	C-1	20	0	0	0	0	0	0	0	0	0
	C-2	20	0	0	0	0	0	0	0	0	

0.1ml essential oil diluted in 2.5ml ethanol and applied on 180 cm² on Whatman filter paper * Figure in parenthesis indicates the range

Adulticidal activity of essential oil of *Ocimum sanctum* against female *Aedes aegypti* was carried out at a

concentration of 0. 55µL/cm² as the method reported earlier. It was observed that the mean corrected mortality of adult *Aedes aegypti* was 63.3% in the range of 60-70%.

Table 2: Adulticidal activity essential oil of *Ocimum sanctum* (Tulsi) against *Aedes* mosquitoes.

Concentration	Replicate	No. of mosquitoes exposed	Nos of mosquitoes knock down in minutes					Mortality in 24 hour recovery period	% mortality in 24 hour	% corrected mortality in 24 hour	Mean % corrected mortality (Range)*
			5	15	30	45	60				
0. 55µL/cm ²	R1	20	5	8	9	9	14	14	70	70	63.3 (60-70)
	R2	20	5	7	9	10	12	12	60	60	
	R3	20	5	7	9	10	12	12	60	60	
Control	C-1	20	0	0	0	0	0	0	0	0	0
	C-2	20	0	0	0	0	0	0	0	0	

0.1ml essential oil diluted in 2.5ml ethanol and applied on 180 cm² on Whatman filter paper * Figure in parenthesis indicates range

Result of adulticidal activity of essential oil of *Ocimum sanctum* against adult female *Anopheles* is shown in

table -3. It was observed that percent corrected mortality was in range 70-80 %. Percent corrected mean mortality of *Anopheles* was 71.7%. No mortality of *Anopheles* was recorded in control group.

Table 3: Adulticidal activity essential oil of *Ocimum sanctum* (Tulsi) against *Anopheles* mosquitoes.

Concentration	Replicate	No. of mosquitoes exposed	Nos of mosquitoes knock down in minutes					Mortality in 24 hour recovery period	% mortality in 24 hour	% corrected mortality in 24 hour	Mean % corrected mortality (Range)*
			5	15	30	45	60				
0. 55µL/cm ²	R1	20	3	4	5	6	13	13	65	65	71.7 (65-75)
	R2	20	2	5	5	6	14	14	70	70	
	R3	20	3	4	5	9	116	16	80	80	
Control	C-1	20	0	0	0	0	0	0	0	0	0
	C-2	20	0	0	0	0	0	0	0	0	

0.1ml essential oil diluted in 2.5ml ethanol and applied on 180 cm² on Whatman filter paper * Figure in parenthesis indicates range

Adulticidal activity essential oil of *curcuma Longa* and *Ocimum sanctum* against *aedes aegypti*

The Result of the adulticidal test (Mix Formulation) of the field-collected *Aedes aegypti* against impregnated paper (WHO) using *Curcuma longa essential oil and Ocimum sanctum* is given in table-4,5 and 6. Three replicates were carried out along with one control. Knock downtime of *Aedes aegypti* at five min intervals was recorded. First, knock down of *Ae. Aegypti* was recorded

at 5 min. In three replicates knockdown of *Ae. Aegypti* was started between 5 and 60 min. *Ae. Aegypti* at 24 hr. recovery period was 12 out of 20 exposed mosquitoes in 3 replicates (20 mosquitoes each).

The result for the test (Mix Formulation) collected *Aedes aegypti* against impregnated paper (WHO) using *Curcuma longa essential oil and Ocimum sanctum* is shown in the table -10. The data in the table shows, that there was 66.7% knockdown immortality was obtained in 24hrs. The percent corrected mortality range for the 3 replicas is 60-75% (shown in table 10).

Table 4: Adulticidal activity of mix formulation of essential oil of Tulsi and Haridra against *Aedes* mosquitoes.

Concentration	Replicates	No. of mosquitoes exposed	Nos of mosquitoes knock down in minutes					Mortality in 24 hour recovery period	% mortality in 24 hour	% corrected mortality in 24 hour	Mean % corrected mortality (Range)
			5	15	30	45	60				
0.55µL/cm ² Tulsi 0.27 µL / cm ² Haridra	R1	20	6	8	10	10	15	15	75	75	66.7 (60-75)
	R2	20	5	7	9	12	13	13	65	65	
	R3	20	4	7	9	10	12	12	60	60	
Control	C-1	20	0	0	0	0	0	0	0	0	0
	C-2	20	0	0	0	0	0	0	0	0	

The result for the test (Mix Formulation) collected *Aedes aegypti* against impregnated paper (WHO) using *Curcuma longa essential oil and Ocimum sanctum* is shown in the table -5. The data in table shows, that there

was 56.6% knockdown immortality was obtained in 24hrs. The percent corrected mortality range for the 3 replicas is 45-60 % (shown in table 5).

Table 5: Adulticidal activity of mix formulation of essential oil of Tulsi and Haridra against *Aedes* mosquitoes.

Concentration	Replicates	No. of mosquitoes exposed	Nos of mosquitoes knock down in minutes					Mortality in 24 hour recovery period	% mortality in 24 hour	% corrected mortality in 24 hour	Mean % corrected mortality (Range)
			5	15	30	45	60				
0.55µL/cm ² Tulsi 0.27 µL / cm ² Haridra	R1	20	3	4	7	8	14	14	70	70	56.6 (45-60)
	R2	20	2	5	6	7	11	11	55	55	
	R3	20	3	4	5	7	9	9	45	45	
Control	C-1	20	0	0	0	0	0	0	0	0	0
	C-2	20	0	0	0	0	0	0	0	0	

The result for the test (Mix Formulation) collected *Aedes aegypti* against impregnated paper (WHO) using *Curcuma longa essential oil and Ocimum sanctum* is shown in table -6. The data in table shows, that there

was 66.6% knockdown immortality was obtained in 24hrs. The percent corrected mortality range for the 3 replicas is 60-70 % (shown in table 6).

Table 6: Adulticidal activity of mix formulation of essential oil of Tulsi and Haridra against *Aedes* mosquitoes.

Concentration	Replicates	No. of mosquitoes exposed	Nos of mosquitoes knock down in minutes					Mortality in 24 hour recovery period	% mortality in 24 hour	% corrected mortality in 24 hour	Mean % corrected mortality (Range)
			5	15	30	45	60				
0.55µL/cm ² Tulsi 0.27 µL / cm ² Haridra	R1	20	2	4	6	7	15	15	70	70	66.6 (60-70)
	R2	20	2	5	6	7	11	11	60	60	
	R3	20	2	4	6	7	15	15	70	70	
Control	C-1	20	0	0	0	0	0	0	0	0	0
	C-2	20	0	0	0	0	0	0	0	0	

From the above observations Mix formulation results using *Curcuma longa essential oil and Ocimum sanctum essential oil* (Table 4, 5 and 6) do not show any significant results.

Adulticidal activity essential oil of *Curcuma Longa* and *Ocimum sanctum* against *Anopheles* and *Aedes aegypti*

The Adulticidal activity of the field-collected adult *Aedes aegypti* and *Anopheles* were carried out on Whatman impregnated paper impregnated with *Curcuma longa essential oil*. 0.1ml essential oil *Curcuma longa* diluted in 2.5ml ethanol and applied on Whatman filter paper of 180 cm² (size 12x15 cm²; concentration: 0. 55µL/cm²). Twenty adult female *Aedes aegypti* were exposed to the filter paper in the WHO tube for one hour and the number of knockdown mosquitoes was recorded at 5 min, 15min, 30, min,45 min ,and 60 min. time interval. Thereafter the mosquitoes were transferred to the recovery tube for 24 hours and the mortality of mosquitoes was recorded during 24 hour recovery period. Three replicates were carried out along with two controls. It was observed that at end of 24 hour recovery period percent mean corrected mortality of *Ae. aegypti* was 50% (range: 45-60%). The Adulticidal activity of essential oil of *Curcuma longa* against adult female

Anopheles was carried out as a method reported earlier. Twenty adult female *Anopheles* mosquitoes were exposed to Whatman filter paper of 180 cm² (size 12x15 cm²; concentration: 0. 55µL/cm²) in a WHO tube for one hour and the number of knock-down mosquitoes were recorded. Thereafter the adult *Anopheles* mosquitoes was transferred to the WHO recovery tube and mortality of the *Anopheles* mosquitoes were recorded at end of 24 hour recovery period. Results of adulticidal activity essential oil of *Curcuma longa* (Haldi) against *Anopheles* mosquitoes are shown in table-7. It was observed that at end of 24 hour recovery period percent mean corrected mortality of *Ae. aegypti* was 70% (range: 65.0-75.0%). The Adulticidal activity of essential oil of *Ocimum sanctum* against female *Aedes aegypti* was carried out at a concentration of 0. 55µL/cm² as the method reported earlier. Result. It was observed that the mean corrected mortality of adult *Aedes aegypti* was 63.3% in range of 60-70%. The Result of adulticidal activity of essential oil of *Ocimum sanctum* against adult female *Anopheles* is shown in the figure. It was observed that percent corrected mortality was in the range 70-80 %. The Percent corrected mean mortality of *Anopheles* was 71.7%. No mortality of *Anopheles* was recorded in the control group.

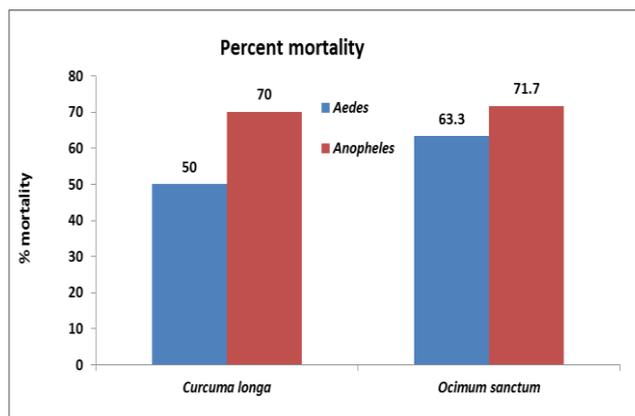


Figure 1: Adulticidal activity of essential oil of *Curcuma Longa* and *Ocimum sanctum* against *Aedes* and *Anopheles* mosquitoes.

From the study, we came to know that in the case of adulticidal activity in *Aedes aegypti*, *Tulsi* shows better results as compared to *Haridra*, whereas in the case of *Anopheles mosquito* both *Haridra* and *Tulsi oil* shows good results.

After, the adulticidal study we have done mix formulation to observe the synergistic effect, but this experiment does not show any significant results.

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