

REPELLENT PROPERTIES OF PHYTOCHEMICALS OF *OCIMUM SANCTUM* AGAINST HOUSEFLY, *MUSCA DOMESTICA* L.

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ABSTRACT: Tulsi (*O. sanctum*) leaves crude extracts and isolated eugenol oil are tested as repellent for houseflies. Both shown promising results as repellent, though, eugenol exhibited very good repellent property. Results suggested the use of this phytochemical as an repellent for houseflies ecofriendly.

Key Words: Repellent property, *Ocimum sanctum*, *Musca domestica*, Eugenol oil.

I. INTRODUCTION

Plants are one of the most important sources of medicines and chemical molecules for the treatment of various human disorders (Singh et. al., 2007). Some phytochemicals also act as insect repellent and fungicides. New chemical molecules are being discovered as insect repellent, antibacterial and antifungal (Arora et. al., 2007). Aromatic plants and fragrance flowers are some of nature's most beautiful creations for global benefits, medicinal plants, especially the aromatic species are the key in solving numerous inter related global issues. Medicinal plants are nontoxic, affordable locally available and help in environment preservation and restoration.

"Tulsi" (*Ocimum sanctum* L.) (Labiata) is commonly called "holy basil", and is a herbaceous plant found throughout India. Hindus worship it in both homes and temples. Though, different parts of the plant are used in the treatment of number of ailments including bronchitis rheumatism and pyrexia (Satya vati., 1987 and Madkarni, 1976). Yet its chemical molecules repellent property against insect is not very well discovered. Housefly, *Musca domestica* are a major pest species of livestock units and landfill sites. These are among the most common annoying insects around the houses that are often seen in great number during summer. These flying pests often lay eggs in warm and moist areas such as garbage bins, animal manures, rotten vegetables and fruits or other areas contaminated by any of these house flies have made them incapable of biting. But it does not mean they are harmless. These are susceptible of carrying disease causing bacteria, viruses, eggs of nematodes and cysts of many protozoans which causes diarrhoea, cholera, dysentery as *Salmonella*. *Musca domestica* is also a major pest of dairy cattle, because of its potential to spread diseases and reduce feeding efficiency and milk production in livestock. Synthetic insecticides cannot be used in organic dairy operations and hence, there is a need for alternate strategies to control these flies.

In present paper, phytochemicals of Tulsi, such as eugenol oil, are investigated showing repellent properties against houseflies.

II. MATERIAL AND METHODS

Following material and method was used during the course of study:-

- A. **Cultivation of *Ocimum sanctum* (Tulsi):**- Tulsi is commonly cultivated in the fields as well as gardens. There are two main varieties, first is the green type, Sri Tulsi which is most common and second type is Krishna Tulsi which bears purple leaves; Both the varieties were grown on well irrigated land in the kitchen garden of the first author of the paper. The land size was 15x10 feet. The plants were grown in the months of August to November at Saharanpur at 15°C to 30°C.
- B. **Plants parts used:**- Fresh leaves of Tulsi were used.
- C. **Plucking of leaves:**- The Tulsi leaves were plucked by hand and then washed thoroughly in water.
- D. **Collection of houseflies:**- *Musca domestica* the Indian houseflies, were collected from houses and other sites near bin etc. by specially designed "aspirator" and brought into lab and kept in the fine wire gauge cages for further studies.
- E. **Aspirator:**- A funnel of diameter 60mm was taken with a long rubber tube. In the tube just behind the end of funnel a fine wire mesh was fitted. The end of rubber tube fitted with a mouth holder, for sucking the air creating the vacuum in the funnel for collection of houseflies. During collection the wide mouth of the funnel was quickly placed on the sitting house flies. The vacuum was created by sucking the air of the funnel by mouth holding the mouth holder of the tube. The houseflies collected at the base of the funnel near wire mesh, separately collected in the plastic jars covered at top by fine muslin cloth.
- F. **Separation of Eugenol:**- It was carried into following steps:-
 - a. **Preparation of crude extract:**-
 - i. 250 fresh leaves of Tulsi plants,
 - ii. 50 ml acetone,
 - iii. 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 seven days. After filtering, the filtrate was placed in the dryer at 25°C to evaporated 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 isolated 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. $P_T = P^\circ_A + P^\circ_B$, where P° 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 $P_T = P^\circ_A N_A + P^\circ_B N_B$, where N is the mole fraction of the component in the mixture). The higher total vapour 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.

EXPERIMENT:-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 (135g).

$$\% \text{ Recovery} = \frac{\text{amount of Eugenol isolated}}{\text{amount of Tulsi leaves used}} = \frac{135\text{g}}{250\text{g}} \times 100 = 54\%$$

- G. Sprayer:- A sprayer was filled with crude extract and eugenol oil separately. In the room and in the wooden box (treated part), these were sprayed whenever required to record the repellent effect against the house flies.
- H. pH of the extract:- It was calculated by BDH pH papers as well as by pH meter.

III. RESULT

The experiment was carried out in the month of June 2019 to 2020, for seeing the repellency of the house flies, a wooden box was made. It had two parts, part 'A' and part 'B'. Both parts were separated by a wooden partition having a hole in the center, the crude extract of Tulsi leaves was sprayed in part 'B'. 100 houseflies were released in treated part and a watch was kept and the data were recorded in the table 1. In the first hour, maximum 45-53 houseflies moved in the untreated part 'A', in second hour 30-36 houseflies migrated and in the third hour rest houseflies moved in the untreated part 'A' of the wooden box. The experiment was repeated on dated 6, 8, 9, 10, 11, 13, 15, 16, 17 and 18-06-2019. The result of the experiment showed 97-100% repellency against houseflies with crude extract of Tulsi leaves (Table-1). In the year 2020 this experiment was repeated and again the repellency was obtained 97-100% (Table-2).

In the other experiment pure eugenol was sprayed in part 'B' of the box, 100 houseflies were released in the treated part 'B'. During the year 2019-2020 as mentioned in the tables-3 and 4, in first hr. 58-67 houseflies migrated in untreated part 'A' of wooden box from the treated part. In second hr. 28-35 flies moved in untreated part 'A' while in third hr. rest flies shifted in the untreated part 'A'. Thus, as a total after 3rd hr. 99-100 % repellency was shown by eugenol oil (Table-3 and 4).

This experiment showed that the Tulsi (*Ocimum sanctum* L.) has good repellent effect against the house flies (*Musca domestica* L.)

Table – 1: Repellent property of houseflies (*Musca domestica*) with crude extract.

Sr. No.	Date of release Houseflies	No. of Houseflies released in treated part 'B'	No. of Houseflies found in untreated part 'A'				No. of Houseflies found in treated part after 3 hrs.
			1hr	2hr	3hr	total	
1.	06-06-2019	100	50	32	18	100	02
2.	08-06-2019	100	52	30	17	99	01
3.	09-06-2019	100	50	36	12	98	02
4.	10-06-2019	100	50	34	15	99	01
5.	11-06-2019	100	53	30	17	100	-
6.	13-06-2019	100	45	32	20	97	03
7.	15-06-2019	100	49	30	21	100	-
8.	16-06-2019	100	51	35	14	100	-
9.	17-06-2019	100	49	31	19	99	01
10.	18-06-2019	100	48	30	22	100	-

Table – 2: Repellent property of houseflies (*Musca domestica*) with crude extract.

Sr. No.	Date of release Houseflies	No. of Houseflies released in treated part 'B'	No. of Houseflies found in untreated part 'A'				No. of Houseflies found in treated part after 3 hrs.
			1hr	2hr	3hr	total	
1.	01-06-2020	100	51	33	15	99	01
2.	03-06-2020	100	53	32	15	100	-
3.	10-06-2020	100	50	34	16	100	-
4.	15-06-2020	100	49	34	15	99	01
5.	16-06-2020	100	50	30	17	97	03
6.	20-06-2020	100	55	31	13	99	01
7.	25-06-2020	100	49	33	18	100	-
8.	26-06-2020	100	50	34	16	100	-
9.	27-06-2020	100	52	30	16	99	02
10.	28-06-2020	100	50	33	17	100	-

Table – 3: Repellent property of houseflies (*Musca domestica*) with pure eugenol.

Sr. No.	Date of release Houseflies	No. of Houseflies released in treated part 'B'	No. of Houseflies found in untreated part 'A'				No. of Houseflies found in treated part after 3 hrs.
			1hr	2hr	3hr	total	
1.	20-06-2019	100	60	35	05	100	-
2.	22-06-2019	100	65	32	03	100	-
3.	23-06-2019	100	61	28	11	100	-
4.	24-06-2019	100	57	28	14	99	01
5.	25-06-2019	100	61	32	07	100	-
6.	27-06-2019	100	65	30	05	100	-
7.	29-06-2019	100	63	33	04	100	-
8.	30-06-2019	100	67	28	05	100	-
9.	01-07-2019	100	58	29	03	100	-
10.	02-07-2019	100	59	31	10	100	-

Table – 4: Repellent property of houseflies (*Musca domestica*) with pure eugenol.

Sr. No.	Date of release Houseflies	No. of Houseflies released in treated part 'B'	No. of Houseflies found in untreated part 'A'				No. of Houseflies found in treated part after 3 hrs.
			1hr	2hr	3hr	total	
1.	12-07-2020	100	61	35	04	100	-
2.	15-07-2020	100	62	32	06	100	-
3.	19-07-2020	100	58	29	03	100	-
4.	21-07-2020	100	63	33	04	100	01
5.	22-07-2020	100	57	28	14	100	-
6.	27-07-2020	100	65	30	05	100	-
7.	30-07-2020	100	58	30	02	91	01
8.	31-07-2020	100	67	28	05	100	-
9.	02-08-2020	100	61	32	07	100	-
10.	05-08-2020	100	59	31	10	100	-

IV. DISCUSSION

Repellency against houseflies revealed that in first hr. 58-67 houseflies migrated in untreated part of wooden box. In second hr. 28-35 flies moved in untreated part while in third hr. 3-14 flies shifted in the untreated part. Thus, as a total after 3rd hr. 99-100% repellency was shown by eugenol oil. On the other hand, insecticides as residual sprays may pose a problem for children and may lead invisible poisoning. Baiting with safer chemicals / material is a choice for abatement of house fly (Jerome *et. al.*, 2002) worked on houseflies and tell that boric acid inhibit the development of the Houseflies. In the present study the pure eugenol showed 100% repellency against houseflies as said above. Sohail *et. al.*, (2005) used non-insecticide baits for households as an important component of IPM for house fly, because houseflies has ability to resist chemicals tested for routine toxicological studies. Further investigations are needed to improve and add the material readily available for the environmental safer control of house fly.

V. ACKNOWLEDGEMENT

The authors are thankful to the head of chemistry department and Zoology Department M.S. College, Saharanpur for providing lab facilities and CDRI, Lucknow for the identification of chemical molecule (Eugenol oil), isolated from the Tulsi leaves.

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