

BIOEFFICACY OF ESSENTIAL OILS OF THYMUS VULGARIS AND EUGENIA CARYOPHYLLUS AGAINST HOUSEFLY, MUSCA DOMESTICA L.

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ABSTRACT

The housefly, *Musca domestica* L., is a cosmopolitan insect, associated with vectoring of various etiological agents. In order to search for effective control method, bioefficacy of essential oils of Thyme (*Thymus vulgaris*) and Clove leaf (*Eugenia caryophyllus*) was studied against housefly. The LC (50) 3.18 μ g/cm² value of Clove leaf oil was found highly effective as compared to LC (50) value 4.39 μ g/cm² of Thyme essential oil for inducing mortality of *M. domestica* larvae. The adulticidal activity of Thyme essential oil LC (50) 32.71 mg/dm³ was toxic than Clove leaf essential oil [LC (50) 53.10 mg/dm³]. In Attractant / repellent Bioassay, Thyme essential oil revealed 90.21% repellency as compared to 80.68% value of Clove leaf essential oil against adults of House fly. In fumigation bioassay, Thyme showed high Pupicidal activity than Clove leaf oil and in contact toxicity bioassay using topical application both the oils showed 100% pupicidal mortality. The data reveals that Clove and Thyme essential oils have excellent potential for controlling *M. domestica* population as eco-friendly approach in IPM.

INTRODUCTION

The house fly, *Musca domestica* L. is a cosmopolitan insect pest of both farm and house. This species is always found in association with humans or activities of humans. These are nuisance and can also transmit disease-causing organisms. More than 100 pathogens are associated with the house flies such as bacteria, protozoa, viruses and metazoan parasites (Barin *et al.*, 2010). The housefly is categorized as an important contributing factor in the dissemination of various infectious food-borne diseases, disease in humans and animals, such as cholera, typhoid, shigellosis, bacillary dysentery, tuberculosis and infantile diarrhea in human populations (Iwasa *et al.* 1999; Olsen *et al.*, 2001). Management and control of Housefly relied upon the use of chemical insecticides such as organochlorines, organophosphates and pyrethroids etc. There are many disadvantages associated with such synthetic pesticides like it leads to development of resistance among insects (Srinivasan *et al.*, 2008), ecological imbalances and harm to non-target organisms too. In recent years, the application of several medicinal plant products like plant extract and essential oil has drawn much attention as effective alternatives to the synthetic pesticides and chemical fertilizers. Essential oil interfere with basic metabolic, physiological, and behavioral functions of insects and some are known to affect growth, development, reproduction, or survival of insects and vectors (Hummelbrunner and Isman, 2001; Koschier and Sedy, 2001; Tripathi *et al.*, 2003). The mode of action of essential oil compounds is not fully understood, but they elicit characteristic neurotoxic symptoms including agitation,

hyperactivity, paralysis, and knockdown (Koul *et al.*, 2008).

In one of the study of Essential oil on housefly, the insecticidal activity of 34 essential oils, extracted from plants, was screened against *Musca domestica* where the *Pogostemon cablin* essential oil proved to be the most efficient at a lethal dose of 3 μ g/fly. After topical application (Roman, 2009) Oil of *Mellisa officinalis* and *Nepeta cataria* also shows promising adulticidal activity against *M. domestica* i.e. LD₅₀ 24 and 23 μ g/insect respectively. These oils can also bare further investigation for use in commercial preparation (Roman, 2008). In another study Peel oils of lemon, grapefruit and navel orange were tested for insecticidal activities against *M. domestica* and it has been found that the Grapefruit peel oil was more toxic to adults of *M. domestica* while lemon oil was more toxic to Housefly larvae (Shalaby, 1998). Thyme oil was having repellent action on Varroa mite (Natalia *et al.*, 2009). Larvicidal efficiency was determined for thymol was the lowest doses LD₅₀ 32.9 and 14.2mg/L for the third and fourth instars of *Culex quinquefasciatus*. (Roman, 2009). Bay, clove leaf has been studied and these oils shows potent insecticidal activity against all stages of *Trialeurodes vaporariorum*, these essential oils might be good candidates for naturally occurring *T. vaporariorum* control agents (Won *et al.*, 2003). It has been reported that the essential oils of lavender, can be used successfully as repellents for neonate larvae of the codling moth (Tingle and Mitchell, 1984). Some oils, such as thyme herb oil, lavender flower oil, lavender leaf oil showed very similar effects against the larvae of *T. pityocampa* (Mehmet *et al.*, 2003). Shin-Ho Kang *et al.* (2009), demonstrated clove leaf oil showed good repellency at a concentration of 0.005mg/

cm² against *Culex pipiens*. In this way, number of essential oils shows insecticidal properties against different insect pest so that there is an increase interest in developing plant origin insecticides.

The objective of the present study, was to assess the larvicidal, adulticidal, attractant/repellent, pupicidal bioassay of two plant essential oils, Thyme (*Thymus vulgaris*) and clover leaf (*Eugenia caryophyllus*) on Housefly, *M. domestica*.

MATERIALS AND METHODS

Rearing of Housefly

The mother culture of *M. domestica* was procured from National chemical Laboratory, Entomology section, Pune. This culture was maintained in laboratory conditions at temperature 28 ± 1 to 30 ± 1°C and 60-70% R.H. Adults of *M. domestica* were reared in plastic jars of 20 cm height and 10 cm width, covered with muslin cloth. A cotton swab soaked in Amul milk was offered to adults as food. The cotton swab in Petri dish also served as substrate for oviposition. The eggs were allowed to develop to hatch the larvae, in this medium up to pupal stage. Freshly emerged adults from pupae were transferred to separate containers to know the exact age of adults which is required for various bioassays.

Essential oil

The essential oil of Clove leaf (*Eugenia coryophyllus*) and Thyme (*Thymus vulgaris*) were procured from Samed Agro Services Pvt. Ltd., Maharashtra, India. These oils obtained from Aurvedic Medical shop, Rasta Peth, Pune and it used for the bioassay and it is stored at room temperature.

Bioassays

Larvicidal assay

Larvicidal assay was carried out by using residual film method as described by Busvine (1971). In this assay, for the stock solution preparation, different doses were selected, doses ranges from 10 mg- 300 mg for both the oils. Each dose was dissolved in 5ml of acetone. This solution was used as stock solution. From stock solution, 1mL of dose was used for treatment. The actual EO present in 1mL mixture was calculated using following formula (Busvine, 1971).

Dose/cm² = value present in 1 mL / area of petridish

Area of Petridish was 63.58 cm². (Actual dose was used in experiment was mentioned in Table 1). After drying, ten third instar larvae were released in each petri dish. Cotton swab soaked in milk was used as food. Acetone was used as solvent for control. For control same procedure was applied. Mortality of the housefly larvae was recorded after 24h post-exposure.

Adulticidal assay

The adulticidal assay on *M. domestica* was performed by Palacios et al. (2009) method. The jar fitted with cap having 7cm length of cotton yarn was suspended. Different doses of pure EO (essential oil) were applied to the yarn. Doses ranges from 10mg/dm³, 20mg/dm³, 40mg/dm³, 60mg/dm³, 80mg/dm³, 100mg/dm³. Each dose was applied after being dissolved in 10 µL of acetone then applied to cotton yarn. In the control jar the cotton yarn was treated with only 10 µL of acetone. Ten

4-5 days old male and female adults were released in jar. The jars were then sealed tightly and kept in a room at 26 ± 1°C for 30 min. Each test was replicated in 5 times. Mortality in each group was assessed after 30 min of exposure.

Attractant / repellent Bioassay

This assay was carried out using double choice method. twenty freshly emerged adults of mixed sexes were released in a cage (size 20×12×8 cm.) containing 2 conical flasks. For stock solution 100µl of EO was dissolved in 1mL of acetone. Acetone was used as a solvent. In treated flask, 0.1 % essential oil (i.e. 50µL from stock solution) dissolved in 5 ml of milk, while in another flask only 50µL of acetone dissolved in 5 ml of milk were used as control. The conical flask was fitted with a funnel (10cm in diameter). This assay was replicated 5 times. Number of flies attracted towards conical flask treated with essential oil as well as control conical flask were counted after 24h for the calculation of % repellency. The results expressed in terms of percentage attraction/repulsion (% R) were calculated by using the formula (Campbell 1983).

$$\% R = [100(C - T) / C]$$

Where, C = the number of flies trapped in the control flask and T = the number of flies trapped in the treated flask.

Pupicidal bioassays

Fumigation assay

Pupicidal bioassays were performed with twenty housefly pupae (3 days old) in a 250mL conical flask (Kumar et al., 2012). The doses of oils, ranges from 20 to 100µL/250mL of air were used in fumigation assay through impregnation in a cotton swab. For Control acetone was used. Bioassay was performed at 28 ± 2°C and RH 65 ± 5%. All observations were made till six days during which emergence of adult from pupae were noted, after which the rate of pupicidal activity of the oil was calculated as the percentage reduction in adult emergence or inhibition rate (Kumar et al., 2011a). Percentage inhibition rate (% IR or PIR) was calculated as:

$$\% \text{ Inhibition Rate} = (C_n - T_n) / C_n \times 100$$

Where C_n is the number of newly emerged insects in the untreated (control) Petri plates and T_n is the number of insects in the treated Petri plates. Each treatment was replicated 5 times.

Contact toxicity by topical application

For this topical application for pupa, ten pupae placed in glass petri plates. Then different doses of essential oil ranging from 20µL to 100µL were tested using topical application method and same ranges of doses were used for control. For control acetone is used. The pupal mortality was checked by counting the number of unemerged pupae after three days of treatment (Sinthusiri et al., 2010). Five replicates were set up per treatment at room temperature. Following formula was used to calculate percentage reduction in adult emergence or inhibition rate (% IR) for pupicidal bioassays (Kumar et al., 2011a) were calculated as:

$$\% \text{ Inhibition Rate} = (C_n - T_n) / C_n \times 100$$

Where, C_n = Number of newly emerged insects in the control and T_n = Number of insect in the treated.

Statistical analysis

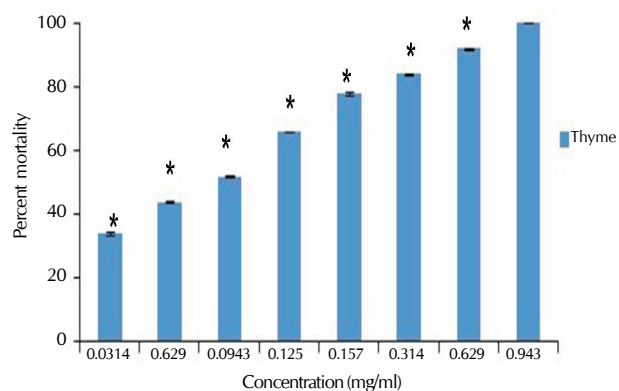


Figure 1: Larvicidal activity of Thyme essential oil on *M. domestica* larvae

Significant * $p < 0.001$ when compared to control.

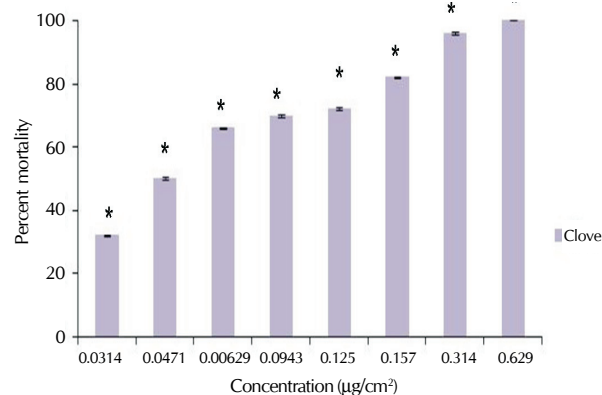


Figure 2: Larvicidal activity of Clove leaf essential oil on *M. domestica* larvae

Significant * $p < 0.001$ when compared to control.

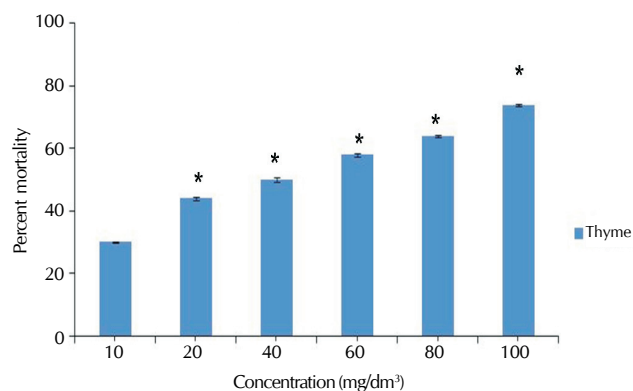


Figure 3: Adulticidal activity of Thyme essential oil on *M. domestica*

Significant * $p < 0.001$ when compared to control.

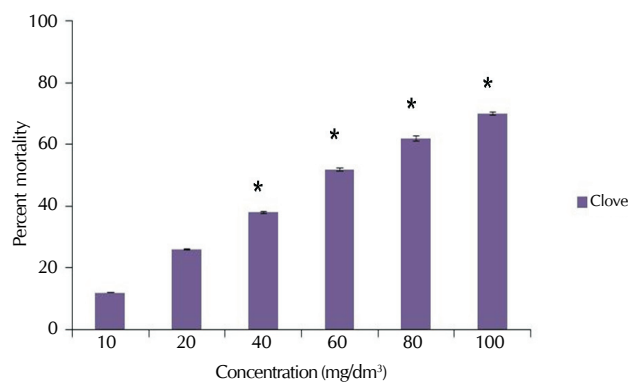


Figure 4: Adulticidal activity of Clove leaf essential oil on *M. domestica*

Significant * $p < 0.001$ when compared to control.

Data obtained from different bioassays were subjected to statistical analysis. The mean mortality data of the five replicates per dose were used to calculate the LC_{50} and LC_{90} using probit analysis. It helps to analyze the dose–mortality response. (Finney, 1952).

RESULTS

In larvicidal assay, *M. domestica* larvae treated with the essential oil of thyme and clove and the result reveals the effectiveness of the essential oils and it also confirms their potential for control of larval population of Housefly. The different doses were used to obtain mortality. Thyme and clove, leaf essential oils were tested against third instar larvae of *M. domestica*. The result of larvicidal activity of essential oil was shown in Table no. 2 with probit analysis. Clover leaf essential oil and Thyme Essential oil LC_{50} were $3.18\mu\text{g}/\text{cm}^2$ and $4.39\mu\text{g}/\text{cm}^2$ respectively. In the present residual film bioassay of Clove leaf oil was found to be highly effective as compared to Thyme essential oil in inducing mortality of larvae of *Musca domestica*. (Table 2). In figure, it was found that with increase in dose % mortality also increased. (It shows Significant * $p < 0.001$ when compared to control (Fig. 1 and 2). In adulticidal assay Thyme have exhibited significant adulticidal activity against *M. domestica* i.e. LC_{50} was 32.71

mg/dm^3 where as clove leaf essential oil shows LC_{50} was $53.10\text{ mg}/\text{dm}^3$. The result of adulticidal activity of essential oil is expressed in Table 3 with probit analysis. In this assay Thyme essential oil shows high adulticidal activity than Clove leaf essential oil (Table 3)., Further it was found that as dose concentration increases % mortality also increases. It shows Significant * $p < 0.001$ when compared to control (Fig. 3 and 4). In Attractant / repellent bioassay we used test concentration for both the oils were 0.1 %. When we used 0.5 % or 1 % test concentration we found mortality. In this assay Thyme essential oil showed 90.21% repellency as higher than clove leaf essential oil with 80.68 % of repellency at the concentration of oils at 0.1 %. (Table 4) In Pupicidal activity two different types of assay were used fumigation and contact toxicity by topical application against housefly pupae. For both the assay different range of doses of essential oil were used. In fumigation assay the LD_{50} and LD_{90} values are evaluated using probit analysis. Both the essential oils exhibit pupicidal activity. Among this Thyme have exhibited high pupicidal activity against *M. domestica* i.e. LD_{50} was $28.09\mu\text{L}/250\text{mL}$ of air, where as Clove leaf essential oil which shows LD_{50} of $41.36\mu\text{L}/250\text{mL}$ of air (Table 5 and 6). In contact toxicity bioassay using topical application both the oils i.e. thyme and clove leaf EO showed 100 % mortality in different doses ranging from $20\mu\text{L}$ to $100\mu\text{L}$.

Table 1: Larvicidal activity of clove leaf and thyme essential oil on larvae of *M. domestica*

Sr. No.	Essential Oil	LC ₅₀ (µg/cm ³)	95% Confidential limit LCL	(LC ₅₀) UCL	Regression Equation	LC ₉₀ (µg/cm ³)	Chi square
1	Clove leaf	3.18	2.72	3.62	Y = 2.03x + 3.97	13.60	8.24*(7)
2	Thyme	4.39	3.66	5.12	Y = 1.64x + 3.94	26.42	9.94*(7)

Means + SEM, df degree of freedom, * Significant at p < 0.05.

Table 2: Adulticidal activity by clove leaf and thyme essential oil on *M. domestica* Adult

Sr.No.	Essential oil	LC ₅₀ (mg/dm ³)	95% Confidential limit LCL	(LC ₅₀) UCL	Regression Equation	LC ₉₀ (mg/dm ³)	Chi square
1	Clove leaf	53.10	45.74	62.59	Y = 1.65x + 2.15	317.23	1.21* (5)
2	Thyme	32.71	25.11	41.13	Y = 1.03x + 3.43	568.17	2.33* (5)

Means + SEM, df degree of freedom, * Significant at p < 0.05.

Table 3: Attractant / repellent bioassay of thyme, clove leaf essential oil

Name of Essential oil	Mean No. of flies attracted in Control	Mean No. of flies attracted in treated	% Repellency
Clove leaf	15.8 ± 0.66	2.6 ± 0.67	80.68
Thyme	17.2 ± 0.8	1.6 ± 0.4	90.21

Means + SEM

Table 4: Oviposition deterrence activity.

Essential oil	Mean no. of eggs in Control	Mean no. of eggs in Treated	% oviposition deterrence
Thyme	75.4 ± 1.56	47 ± 0.31	37.57
Clove leaf	74.4 ± 1.28	59 ± 0.44	20.56

Means + SEM

DISCUSSION

In larvicidal assay, it was found that clove leaf oil was highly effective as compared to Thyme essential oil. It was also found that as the doses of both essential oils were increased the % mortality was also increased. The mechanism of action of essential oils to insects is not clear but is reported to be diverse. In fact, it is difficult to make exact comparisons with other studies due to large variation in oil composition, target insect, mode/scale of experimentation, different exposure times and concentrations employed (Kumar *et al.*, 2011 a). So it reflected by different affectivity of same oil in various assays. Clove oil toxicity to different types of insects varies considerably. Other studies indicate that clove oil and eugenol can be effective at controlling mites, termites and mosquitoes at lower application rates. Eugenol kills mosquito larvae (specifically *Ochlerotatus caspius*) with LC₅₀ values of 7.53 mg/L for 24 hours and 5.57 mg/L for 48 hours (Knio KM, Usta J *et al.*, 2008). In *Aedes aegypti*, the LC₅₀ was 33 mg/L (unspecified exposure duration). Eugenol induced 100 percent mortality in mosquitoes *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* at a dose of 7 L/ha in 30-35 minutes (Bhatnagar M, Kapur K *et al.*, 1993). Another study found that clove bud oil killed 100% of termites (*C. formosanus*) in 2 days at 50 µg/cm² (2 kg/ha) (Zhu BC, 2001). Roman (2009), observed larvicidal efficiency for thymol was the lowest doses LD₅₀ 32.9 and 14.2 mg/L for the third and fourth instars of *Culex quinquefasciatus*.

Where as, in adulticidal assay it was found that EOs from medicinal plants such as *Eugenia coryophyllus* and *Thymus vulgaris* were good options for use as housefly fumigants, provoking death of the adult insect within a short period of

time. Similar finding on mortality of housefly (*M. domestica*) were also obtained by Khan and Ahmed (2000) using crude extract of *Azadirachta indica*. Sukontason *et al.*, (2004) reported toxicity of *Eucalyptol* extract against *M. domestica*. Singh and Singh (1991) screened 31 essential oils from different botanical sources against *M. domestica* and reported mortality of the species. Thymol is very perspective for the development of botanical pesticides suitable for fighting against mosquitoes (Pavela, 2009) as well as against houseflies (Pavela, 2008). The effective insecticide was the EO derived from *C. sinensis*, followed by *C. aurantium* and *E. cinerea*, with LC₅₀ values of 3.9, 4.8, and 5.5 mg/dm³ of air respectively. EOs derived from other citrus species was also very effective against *M. domestica*, with LC₅₀ values ranging from 6.5 to 7 mg/dm³. However, some EOs could kill some individual of *M. domestica* adults within 30 minutes (Palacios S. *et al.*, 2009).

In Attractant / repellent Bioassay, Thyme showed higher repellent activity at 0.1% concentration as compared to Clove leaf EO. In relative studies the significant repellent effect of thyme oil, has been known from the work of other authors as well. Park *et al.* (2005) determined the repellent efficiency of five monoterpenes (C, p-cymene, L, alpha-terpinene and T) derived from the essential oil of *T. vulgaris* against the mosquito *Culex pipiens pallens*. It is proved that *T. vulgaris* as efficient repellent against different mosquito species. The essential oils obtained from *Ocimum gratissimum* L., *T. serpyllum* L showed 100 % repellent activity at 2 % concentration (Singh and Singh, 1990). In another study The essential oil of *M. piperita* exhibited 96.8 % repellency, while that of *Z. officinalis*, 84.9 %, followed by *C. verum*, 77.9 %, and the essential oil of was still good, though significantly lower, i.e., 63 %, at the concentration of 1 % (Morey *et al.*, 2012). Present study also shows close agreement with the other authors.

In Pupicidal bioassays, due to scanty information on their studies, for both types of assays, contact toxicity and fumigation and other practical difficulties (different insects, methodology), it was difficult to make a comparisons between them. In the present study, significant mortality and inhibition of pupae was observed in both bioassays. In fumigation toxicity thyme essential oil showed high Pupicidal activity whereas contact toxicity assay both oils showed 100% mortality. In other studies, in fumigation experiments, clove oil produced 100% mortality in Japanese termites at 0.5 µL/L of air. (Park and Shin, 2005). In one of the study Kumar *et al.* (2012) which showed contact toxicity assay for pupae of houseflies, *E. globulus* essential oil showed the percentage inhibition rate (PIR) of

Table 5: Fumigation assay for clove leaf essential oil.

Sr. No.	Dose (μ L)	% of IR	LD ₅₀	95%Confidential limit (LD ₅₀) LCL	UCL	Regression Equation	LD ₉₀	Chi square
1	20	35	41.36	33.60	48.81	Y = 2.43x + 1.58	256.69	5.44*(5)
2	40	45						
3	60	55						
4	80	65						
5	100	80						

Table 6: Fumigation assay for thyme essential oil

Sr. No.	Dose (μ L)	% of IR	LD ₅₀	95%Confidential limit (LD ₅₀) LCL	UCL	Regression Equation	LD 90	Chi square
1	20	45	28.09	20.28	34.56	Y = 2.78x + 1.52	193.75	3.81* (5)
2	40	55						
3	60	65						
4	80	75						
5	100	85						

36.0–93.0 % at different concentrations. Thus thyme oil was found to be effective with 80 % suppression in emergence of adult houseflies at its maximum concentration. In fumigation assay for housefly pupae, PIR values between 67.9 and 100% were obtained at different concentrations of *E. globulus* essential oil (Kumar *et al.*, 2012). Similarly in Pupicidal bioassays, crude oils of *M. piperita* and *E. globulus* suppressed the emergence of adult flies by 100 % (Kumar *et al.*, 2011). Our observations also supported by another studies. Dimiaty *et al.* (2003) found shrinkage of the pupae and folding of the wings of adults as a result of treatment of third instar larvae of *M. domestica* with *P. nigra* volatile oil. In one of the study, treatment of third instar of *C. albiceps* with *T. vulgaris* and *Z. officinale* produce larval pupal intermediate and adults with crumpled wings. In present study it was found that thyme essential oil showed high Pupicidal activity than clover leaf essential oil. Where as in contact toxicity bioassay using topical application both the oils showed 100 % mortality in different doses ranging from 20 μ L to 100 μ L same doses as fumigation assay.

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