Original Article

Biocontrol efficacy of some essential oils as larvicides and inhibitors of the emergence of adult *Musca domestica*

Doaa l Mohamed[1], Ahmed B Darwish[2], Heba M Aboelela[1], Mohamed M Baz[3], Ahlam F Moharam[1]

Departments of Medical Parasitology[1], Zoology[2], Entomology[3], Faculties of Medicine[1], and Science[2,3], Univerities of Benha[1,3], and Suez[2], Egypt

ABSTRACT

Background: Houseflies are global pests that promote the spread of a few harmful illnesses in humans. Compared to synthetic chemicals, phytochemical compounds are effective and safe alternative insecticides.
Objective: To evaluate the potential efficacy of *Rosmarinus officinalis*, *Piper nigrum*, *Cinnamomum verum*, *Cyperus rotundus*, *Melaleuca alternifolia*, and *Aloe vera* essential oils (EOs) as larvicides, i.e., inhibitors of *M. domestica* emergence.
Material and Methods: Housefly larvae were obtained from the insectary of the Medical and Molecular Entomology Section, Faculty of Science, Benha University. Bioassays were performed at 27±2°C and 70–80% humidity to determine the effects of variable concentrations of six EOs on larvae using ingestion and contact-treated filter paper. To determine the phytochemical composition, EOs were subjected to GC-MS analysis.
Results: All investigated EOs exhibited high to moderate toxicity against house fly larvae. Third-instar larvae suffered 100% mortality at 10% concentration of EOs in experiments using ingestion method in treated rearing medium, while 91.11-100% mortality was recorded at 10% concentration of EOs using contact-treated filter paper. The best results were obtained using *R. officinalis* and *C. verum* EOS for killing housefly larvae, delaying larval and pupal development, and increasing the inhibition rate (100%). Besides, *M. alternifolia* EO was less effective (73.3%, and 91.11 mortality) in ingestion method, and contact bioassays, respectively. The GC-MS analysis revealed that *R. officinalis* and *C. verum* EOs had more monoterpenes (α-Pinene and eucalyptol), and flavonoid compounds (cinnamonaldehyde), respectively.
Conclusion: It was concluded that *R. officinalis*, and *C. verum* EOs are effective insecticides against house fly larvae.

Keywords: adult emergence; essential oils; GC-MS; house fly; larvicides.

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Corresponding Author: Mohmed M. Baz; **Tel.:** +20 1063070572**; Email:** Mohamed.albaz@fsc.bu.edu.eg

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INTRODUCTION

Musca domestica, also known as the house fly, is a significant nuisance in the medical and veterinary fields[1]. This fly is highly prevalent and can serve as a mechanical carrier for numerous diseases[2]. It has the ability to transmit nearly 100 diseases to both humans and animals, including bacteria such as *E. coli, Shigella* spp., *Salmonella*, viruses, in its vomit or excrement, as well as protozoan and helminthic infective stages. It serves as mechanical transporters for the transmission of diseases, due to its feeding habits on human food, animal dung, sweets, garbage, and wet or decomposing material from pet waste due to its heightened sense of smell[2]. Reducing the population of these insects is a challenge.

Various categories of insecticides, including pyrethroid and organophosphate insecticides (OPs), have been widely employed for the management of *M. domestica*[3,4]. Pyrethroids exert their effects by altering the function of voltage-gated sodium channels in the central nervous system of target animals. On the other hand, OPs interfere with the activity of acetylcholinesterase, leading to disruptions in nervous system function[5]. Because these pesticides were extensively used for eradication, the house flies developed resistance[4]. They also polluted the environment, put people's health at risk, and hurt animals that weren't meant to be killed[6]. Botanicals have gained significant interest due to their ability to provide cost-effective, readily available, and environmentally safe alternatives to traditional pesticides[7]. When incorporated in pest control programs, these alternative techniques can effectively delay resistance to traditional insecticides[8]. The insecticidal activities of EOs against house flies have

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been recorded in several studies[9,10]. Some EOs contain chemicals, like limonene, myrcene, terpineol, linalool, and pulegone, which are monoterpenoids that can kill house flies[11]. So, EOs may be used instead of chemical insecticides to get rid of *M. domestica* and other insects that are harmful to humans[12].

Rosemary, scientifically known as *R. officinalis* L., is a perennial shrub widely spread in all countries, especially in the Mediterranean region. It belongs to the Lamiaceae family. Wild rosemary is found in abundance in the Rif and Middle Atlas regions of North Africa. It has been used for medicinal purposes since ancient times and is known for its antiseptic, anti-rheumatic, anti-inflammatory, anti-diabetic, anti-ulcerative, anti-bacterial, anti-fungal, anti-insect, anti-depressant, anti-inflammatory, and antioxidant properties and anticancer[13-15]. A study by Legault and Pichet[16] investigated how ß-caryophyllene improved the anti-cancer effects of α-humulene, isocaryophyllene, and paclitaxel. *R. officinalis*, *P. nigrum*, and *C. verum* oils were used along with other oils that are highly effective in combating medically important insects[17]. In our study, we investigated the potential efficacy of six EOs derived from plants commonly used in Egypt. Besides, we identified the bioactive phytochemical compounds with lethal effects against *M. domestica* larvae.

MATERIAL AND METHODS

This descriptive analytical study was conducted at the Insect Breeding Laboratory, Division of Entomology and Environment, Department of Entomology, Faculty of Science, Benha University during the period from June to November 2023.

Study design: The effectiveness of six essential oils (*R. officinalis*, *P. nigrum*, *C. verum*, *C. rotundus*, *M. alternifolia*, and *A. vera*) as alternative insecticides was evaluated using two methods: ingestion in treated rearing medium, and contact-treated filter paper. The relative efficiencies (RE) of the oils were calculated to determine the best of the oils used. Utilizing GC-MS analysis, the phytochemical constituents in the investigated EOs were identified to clarify the bioactive compounds.

Essential oils: Essential oils used in all bioassays in this study were rosemary (*R. officinalis*), black pepper (*P. nigrum*), cinnamon (*C. verum*), coco grass (*C. rotundus*), tea tree (*M. alternifolia*), and aloes (*A. vera*). These oils were purchased from the Nefertiti Company for natural essential oils and herbs (Table 1).

Rearing of *M. domestica* colony: Adult houseflies were collected from Banha Vegetable Market, Qalyubiya, Egypt, placed in wooden cages measuring 30 x 30 x 30 cm3 with wire tops, and kept at room temperature (30–32°C). Diet consisted of a mixture of 10% syrup and 10% milk absorbed on cotton wool, in addition to 300 grams of mackerel in a plastic tray measuring 18 x 25 x 9 cm3. Mackerel was placed on a mixture of dry bread and ragweed, creating an ideal environment for houseflies to feed and lay their eggs.

Larvicidal activity of EOs: Bioassays were performed to determine the effects of EOs on larvae using two different exposure methods, as previously described[18]. In the first method based on ingestion and contact, five treated groups of fifteen early third-instar larvae were placed in small paper cups (5 cm in diameter and 7 cm high) that held 15 g of rearing medium. The cups were then treated with 0.5, 1.0, 2.5, 5.0, and 10% EOs mixed with 0.1 ml of Tween 20. For each treated group, untreated control groups were provided with 0.1 ml of Tween 20. The treated and untreated cups were covered with a cotton cloth tied with a rubber band to prevent larvae from escaping. Dead larvae were counted after 24 h and then 5 g of sawdust was added to each cup for pupation. The cups were kept in the laboratory until the adult insect emerges, and the timespans for larvae to pupate and pupae to become adults were recorded. The experiment was repeated three times. The second method involved contact testing. Late third larval instars were placed in cups containing 5 cm-diameter filter paper discs treated with the same concentrations of EOs as the first method. Petri dishes with treated and untreated larvae were sealed with Parafilm® to prevent larval escape and placed at room temperature. Dead larvae were counted after 24 h, and then 10 g of sawdust was added to each Petri dish for pupation. The dishes were kept in the laboratory until the adult insect emerged, and the number of timespans for larvae to pupate and pupae to become adults were recorded. The experiment was repeated three times.

Pupicidal activity: Pupicidal bioassays were conducted using previously outlined methodology[19]. For each pupicidal bioassay treatment, similar concentrations of each EO were sprayed on filter paper using a micropipette. and air dried for ten minutes to allow

Table 1. List of plant species and plant parts tested against house fly larvae and selected microbial species.

No.	Common name	Botanical name	Family	Part used
1	Rosemary	*Rosmarinus officinalis*	Lamiaceae	Leavs
2	Black pepper	*Piper nigrum*	Piperaceae	
3	Cinnamon	*Cinnamomum verum*	Lauraceae	
4	Coco Grass	*Cyperus rotundus*	Cyperaceae	
5	Tea tree	*Melaleuca alternifolia*	Myrtaceae	
6	Aloes	*Aloe vera*	Xanthorrhoeaceae	

the solvent to evaporate before adding the pupae. Fifteen, two-days old pupae were placed in a 90-mm-diameter Petri plate. The untreated control petri dishes contained distilled water. For each treatment, five triplicates were carried out. The inhibition rate percentage was used to calculate the proportion of pupicidal activity. The percentage inhibition rate (PIR) was calculated as follows: Cn - Tn / Cn × 100, where Cn is the number of freshly emerged insects in the control and Tn is the number of insects in the treated Petri plates. For five days, the emergence of each treated pupa was monitored for adulthood.

Phytochemical analysis of EOs: Thermo Scientific Trace GC Ultra/ISQ Single Quadrupole MS and TG-5MS fused silica capillary columns were used for biochemical analyses of EOs. The task was accomplished via an electronic ionizer operating at an ionization energy of 70 electron volts (eV). Helium gas was employed as the carrier gas, with a flow rate of 1 ml/min. To quantify all the identified components, a relative peak area was used. The substances were identified by comparing their retention periods and mass spectra to data from the NIST and Willy libraries on the GC/MS equipment. Single-ion chromatographic reconstructions were used to assess peak homogeneity. We used co-chromatographic analysis of reference chemicals as much as possible to confirm the retention times of GC[20].

Statistical analysis: The data were analyzed by the software, SPSS V23 (IBM, USA), for the Probit analyses to calculate the lethal concentration (LC) values and the one-way analysis of variance (ANOVA) (Post Hoc/ Turkey's HSD test). Pupicidal effectivity was calculated in terms of PIR. The significant levels were set at $P<0.05$. The relative efficacies (RE) were calculated according to the following formula: RE for LC = LC50 (LC90 or LC99) for reference oil/LC50 (LC90 or LC99) for EO.

Ethical consideration: The study was conducted according to the guidelines of the Declaration of Benha University and approved by the Ethics Committee of the Faculty of Science, Benha University (Code: BUFS-REC-2024-225Ent).

RESULTS

Larvicidal activity: All tested EOs had significantly higher mortality rates than the controls. The percentage of dead larvae in EO-treated rearing medium at the high concentration (10%) was 100% compared to 2.2% in control groups. At a concentration of 5%, *R. officinalis* and *C. verum* oils resulted in a 100% mortality rate (Table 2). The LC50 values for 50%, which is the median lethal concentration of EOs, were 0.77, 1.17, 0.87, 1.42, 2.28, and 1.79%, respectively (Table 3).

Using the treated filters ingestion technique at a concentration of 10%, *R. officinalis*, *P. nigrum*, and *C. verum*, caused 100% larval mortality on contact, while the *C. rotundus*, *M. alternifolia* and *A. vera* caused 95.56, 91.11, and 95.56% mortality compared to 4.4% in controls (Table 4). Our results revealed that LC50 of *R. officinalis* and *C. verum* were 1.02%, and 1.51%, respectively, i.e., more toxic to house fly larvae than oils from *M. alternifolia* (3.48%). According to LC50, RE of the five oils were 2.96, 1.95, 2.62, 1.61, and 1.27 times, respectively, i.e., higher than that of *M. alternifolia* (1.00) using treated ingestion medium technique (Table 3).

Table 2. Mortality of house fly larvae after exposure to different concentrations of EOS using treated rearing medium.

Parameter		Conc. (%)	Mortality% (Mean ± SE)					
			R. officinalis	*P. nigrum*	*C. verum*	*C. rotundus*	*M. alternifolia*	*A. vera*
Mortality (%)		0.0	2.20±0.00^{eA}	2.20±0.00^{eA}	2.20±0.00^{eA}	2.20±0.00^{eA}	2.20±0.00^{eA}	2.20±0.00^{eA}
		0.5	33.33±3.85^{dA}	22.22±2.22^{dB}	28.89±2.22^{dA}	17.78±2.22^{eBC}	11.11±2.22^{eD}	15.55±2.22^{eCD}
		1.0	62.22±5.88^{cA}	48.89±2.22^{cB}	57.78±8.01^{cA}	33.33±3.85^{dC}	22.22±2.22^{dD}	26.67±0.00^{dD}
		2.5	88.89±4.44^{bA}	73.33±3.85^{bB}	84.45±2.22^{bA}	66.67±3.85^{cC}	48.89±5.88^{cE}	55.56±5.88^{cD}
		5.0	100.0±0.00^{aA}	95.55±2.22^{aA}	100.0±0.00^{aA}	95.55±2.22^{bA}	73.33±3.85^{bC}	86.67±3.85^{bB}
		10	100.0±0.00^{aA}	100.0±0.00^{aA}	100.0±0.00^{aA}	100.0±0.00^{aA}	100.0±0.00^{aA}	100.0±0.00^{aA}
Development (day)	Larvae	0.0	4.67±0.33^{dA}	4.67±0.33^{dA}	4.67±0.33^{dA}	4.67±0.33^{dA}	4.67±0.33^{eA}	4.67±0.33^{eA}
		0.5	7.33±0.33^{cA}	6.33±0.33^{cB}	7.00±0.58^{cA}	6.00±0.58^{eB}	5.33±0.33^{dC}	5.33±0.33^{dC}
		1.0	10.00±0.58^{bA}	8.33±0.33^{bC}	9.33±0.88^{bB}	8.67±0.33^{bC}	7.67±0.33^{cD}	7.67±0.33^{cD}
		2.5	12.00±0.00^{aA}	10.00±0.58^{aC}	11.00±0.58^{aB}	10.33±0.33^{aC}	9.33±0.33^{bD}	9.33±0.33^{bD}
		5.0	0.00±0.00^{eC}	10.33±0.33^{aA}	0.00±0.00^{eC}	10.33±0.33^{aA}	9.67±0.33^{aB}	10.33±0.33^{aA}
		10	0.00±0.00^{eA}	0.00±0.00^{eA}	0.00±0.00^{eA}	0.00±0.00^{eA}	0.00±0.00^{fA}	0.00±0.00^{fA}
	Pupa	0.0	3.33±0.33^{dA}	3.33±0.33^{dA}	3.33±0.33^{dA}	3.33±0.33^{dA}	3.33±0.33^{eA}	3.33±0.33^{dA}
		0.5	4.67±0.33^{cA}	4.33±0.33^{cAB}	4.67±0.33^{cA}	4.67±0.33^{cA}	4.00±0.58^{dB}	4.67±0.33^{cA}
		1.0	7.67±0.33^{bA}	6.00±0.00^{bB}	5.67±0.33^{bB}	6.00±0.58^{bB}	4.67±0.33^{cC}	6.00±0.58^{bB}
		2.5	10.00±0.00^{aA}	8.00±0.00^{aB}	7.67±0.33^{aBC}	8.00±0.58^{aB}	7.33±0.33^{bC}	8.00±0.58^{aB}
		5.0	0.00±0.00^{eC}	8.33±0.33^{aA}	0.00±0.00^{eC}	8.33±0.33^{aA}	7.67±0.33^{aB}	8.00±0.00^{aAB}
		10	0.00±0.00^{eA}	0.00±0.00^{eA}	0.00±0.00^{eA}	0.00±0.00^{eA}	0.00±0.00^{fA}	0.00±0.00^{eA}

a, b, c: There is no significant difference between any two means for each parameter within the same column having the same superscript letter; **A, B, C:** There is no significant difference between any two means within the same row having the same superscript letter.

Table 3. Lethal concentrations of six essential oils against housefly larvae after treatment with ingestion technique.

Plant	LC50 (Low-Up,)	LC90 (Low-Up.)	LC95 (Low-Up.)	RF	Slope ± SE	X^2 (*P* value)
R. officinalis	0.77 (0.66-0.89)	2.35 (1.98-2.95)	3.22 (2.61-4.29)	2.96	2.654±0.243	2.285 (0.515)
P. nigrum	1.17 (1.00-1.36)	4.52 (3.61-6.10)	6.63 (5.05-9.60)	1.95	2.185±0.195	4.764 (0.189)
C. verum	0.87 (0.75-1.00)	2.66 (2.24-3.33)	3.65 (2.96-4.82)	2.62	2.646±0.229	4.368 (0.224)
C. rotundus	1.42 (0.98-1.99)	4.41 (3.33-8.15)	6.07 (4.56-12.56)	1.61	2.612±0.192	7.831 (0.049)
M. alternifolia	2.28 (1.42-3.59)	7.99 (6.08-19.49)	11.41 (8.89-32.55)	1.00	2.349±0.168	11.594 (0.008)*
A. vera	1.79 (1.15-2.68)	6.10 (4.58-13.15)	8.63 (6.55-21.35)	1.27	2.410±0.175	9.906 (0.019)*

Low-Up.: Lower and upper confidence limit; **RE:** Relative efficacies; **Slope±SE:** Regression line equation; X^2: Chi-square; ***:** Significant (P<0.05).

Table 4. Mortality of house fly larvae after exposure to different concentrations of EOS using treated filter paper technique.

Parameter		Conc. (%)	Mortality% (Mean ± SE)					
			R. officinalis	***P. nigrum***	***C. verum***	***C. rotundus***	***M. alternifolia***	***A. vera***
Mortality (%)		0.0	4.40 ± 0.00^{eA}	4.40 ± 0.00^{eA}	4.40 ± 0.00^{eA}	4.40 ± 0.00^{eA}	4.40 ± 0.00^{eA}	4.40 ± 0.00^{eA}
		0.5	26.67 ± 3.85^{dA}	15.56 ± 4.44^{eB}	24.44 ± 5.88^{eA}	11.11 ± 5.88^{eBC}	6.67 ± 3.85^{eC}	11.11 ± 5.88^{eBC}
		1.0	53.33 ± 3.85^{cA}	37.78 ± 5.88^{dB}	53.33 ± 11.55^{dA}	24.44 ± 5.88^{dC}	13.33 ± 6.67^{dD}	22.22 ± 4.45^{dC}
		2.5	80.00 ± 0.00^{bA}	66.67 ± 3.85^{cB}	75.56 ± 5.88^{cA}	57.78 ± 2.22^{cC}	33.33 ± 3.85^{cE}	51.11 ± 9.69^{cD}
		5.0	95.56 ± 4.44^{aA}	91.11 ± 4.44^{bA}	93.33 ± 3.85^{bA}	84.44 ± 5.88^{bB}	66.67 ± 3.85^{bC}	80.00 ± 0.00^{bB}
		10	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}	100.0 ± 0.00^{aA}	95.56 ± 4.44^{aAB}	91.11 ± 4.44^{aB}	95.56 ± 4.44^{aAB}
Development (day)	**Larvae**	0.0	4.33 ± 0.33^{eA}	4.33 ± 0.33^{eA}	4.33 ± 0.33^{dA}	4.33 ± 0.33^{eA}	4.33 ± 0.33^{eA}	4.33 ± 0.33^{eA}
		0.5	7.00 ± 0.58^{dA}	6.00 ± 0.58^{dB}	6.33 ± 0.33^{cB}	5.33 ± 0.33^{dC}	4.67 ± 0.33^{cD}	5.33 ± 0.33^{dC}
		1.0	9.33 ± 0.33^{cA}	8.00 ± 0.58^{cB}	9.00 ± 0.58^{bA}	8.00 ± 0.58^{cB}	7.00 ± 0.58^{bC}	7.67 ± 0.33^{cB}
		2.5	11.33 ± 0.67^{bA}	9.33 ± 0.33^{bC}	10.33 ± 0.67^{aB}	9.67 ± 0.88^{bC}	8.67 ± 0.88^{aD}	8.67 ± 0.33^{bD}
		5.0	12.00 ± 0.00^{aA}	10.00 ± 0.58^{aC}	10.67 ± 0.33^{aB}	10.00 ± 0.00^{aC}	9.00 ± 0.58^{aD}	9.67 ± 0.33^{aC}
		10	0.00 ± 0.00^{fC}	0.00 ± 0.00^{fC}	0.00 ± 0.00^{eC}	10.00 ± 0.00^{aA}	9.00 ± 0.00^{aB}	10.00 ± 0.00^{aA}
	Pupa	0.0	3.00 ± 0.58^{eA}	3.00 ± 0.58^{eA}	3.00 ± 0.58^{dA}	3.00 ± 0.58^{dA}	3.00 ± 0.58^{eA}	3.00 ± 0.58^{eA}
		0.5	4.33 ± 0.33^{dAB}	4.00 ± 0.00^{dBC}	4.33 ± 0.33^{cAB}	4.67 ± 0.33^{cA}	3.67 ± 0.33^{dC}	4.00 ± 0.58^{dBC}
		1.0	7.33 ± 0.33^{cA}	5.67 ± 0.33^{cBC}	5.33 ± 0.67^{bC}	6.00 ± 0.58^{bB}	4.33 ± 0.33^{cD}	5.33 ± 0.33^{cC}
		2.5	9.33 ± 0.33^{bA}	7.33 ± 0.33^{bBC}	7.67 ± 0.33^{aB}	7.67 ± 0.33^{aB}	7.00 ± 0.00^{bC}	7.33 ± 0.67^{bBC}
		5.0	10.0 ± 0.0^{aA}	8.33 ± 0.33^{aB}	7.67 ± 0.33^{aCD}	8.00 ± 0.00^{aBC}	7.33 ± 0.33^{abD}	7.33 ± 0.67^{bD}
		10	0.00 ± 0.00^{fB}	0.00 ± 0.00^{fB}	0.00 ± 0.00^{eB}	8.00 ± 0.00^{aA}	7.67 ± 0.33^{aA}	8.00 ± 0.00^{aA}

a, b, c: There is no significant difference between any two means for each parameter within the same column having the same superscript letter; **A, B, C:** There is no significant difference between any two means within the same row having the same superscript letter.

Using treated filters technique RE of LC50 values were 3.41, 2.30, 3.19, 1.66, and 1.50 times, respectively, i.e., higher than that of *M. alternifolia* (1.00) (Table 5).

Pupicidal activity: Bioassay of *M. domestica* pupae exposed to varying doses of the six EOs showed differential efficiency (Table 6). Following five days, the PIR at various concentrations of six essential oils ranged from 53.33 (*M. alternifolia*) to 100% (*C. verum*). A higher concentration of all essential oils resulted in a higher PIR. At a 10% concentration, all EOs had a high PIR value, ranging from 86.67% (*R. officinalis*) to 100% (*R. officinalis* and *C. verum*). In pupicidal bioassays, the emergence of adult flies was inhibited by 91.11–100% at the highest concentration (10%) of *R. officinalis*, *P. nigrum*, *C. verum*, and *C. rotundus*. *M. alternifolia* oil performed the worst, with a PIR value between 77.78 and 86.67% at 5% and 10% concentrations.

Phytochemical analysis: Metabolomics analysis of *R. officinalis*, and *C. verum* EOs using GC-MS analysis identified various compounds such as terpenes, fatty acids, esters, flavonoid, and phenols in the oils of the two EOs. Table (7) shows that *R. officinalis* EO contained 19 compounds with abundance of Eucalyptol (20.65%), α-Pinene (17.85%) and (+)-2-Bornanone (14.65%). Table (8) shows that *C. verum* oil had 21 compounds, of which the highest concentrations were Cinnamaldehyde (56.12%), Linalool (18.02%), and (E)-Cinnamylacetate (10.22%).

Table 5. Lethal concentrations of six essential oils against housefly larvae after contact treatment using filter paper technique.

Plant	LC50 (Low-Up,)	LC90 (Low-Up.)	LC95 (Low-Up.)	RF	Slope ± SE	X^2 (*P* value)
R. officinalis	1.02 (0.87-1.17)	3.64 (3.08-4.47)	5.22 (4.27-6.72)	3.41	2.322±0.171	0.790 (0.851)
P. nigrum	1.51 (1.33-1.72)	4.82 (4.04-6.00)	6.70 (5.44-8.73)	2.30	2.547±0.186	3.743 (0.290)
C. verum	1.09 (0.94-1.26)	4.00 (3.31-5.08)	5.77 (4.59-7.76)	3.19	2.277±0.182	3.607 (0.307)
C. rotundus	2.10 (1.85-2.37)	7.02 (5.88-8.71)	9.88 (8.03-12.82)	1.66	2.443±0.165	0.305 (0.958)
M. alternifolia	3.48 (3.07-3.96)	10.93 (8.98-14.05)	15.11 (11.99-20.42)	1.00	2.579±0.195	2.303 (0.456)
A. vera	2.32 (2.04-2.64)	7.94 (6.58-10.04)	11.25 (9.01-14.92)	1.50	2.401±0.168	1.565 (0.667)

Low-Up.: Lower and upper confidence limit; **RE:** Relative efficacies; **Slope±SE:** Regression line equation; X^2: Chi-square.

Table 6. The PIR% against housefly pupae after exposure to different concentrations of essential oils for contact toxicity assay.

Plant	Concentration (µ/L)					
	Control	**0.5**	**1.0**	**2.5**	**5.0**	**10.0**
R. officinalis	0.0±0^{aF}	75.55±2.22^{aE}	86.67±3.85^{aD}	91.11±2.22^{aC}	95.55±2.22^{aB}	100±0.00^{aA}
P. nigrum	0.0±0^{aF}	68.89±2.22^{bD}	71.11±2.22^{bC}	71.11±2.22^{cC}	86.67±3.85^{cB}	95.55±2.22^{bA}
C. verum	0.0±0^{aF}	71.11±2.22^{bD}	73.33±3.85^{bD}	84.45±2.22^{bC}	91.11±2.22^{bB}	100.0±0.00^{aA}
C. rotundus	0.0±0^{aF}	62.22±2.22^{cD}	64.45±2.22^{cD}	71.11±2.22^{cC}	84.45±2.22^{dB}	91.11±2.22^{cA}
M. alternifolia	0.0±0^{aF}	53.33±3.85^{eE}	62.22±2.22^{cD}	68.89±4.44^{dC}	77.78±2.22^{eB}	86.67±3.85^{dA}
A. vera	0.0±0^{aF}	57.78±2.22^{dE}	64.45±2.22^{cD}	68.89±2.22^{dC}	80.00±3.85^{eB}	88.89±2.22^{dA}

a, b, c: There is no significant difference between any two means that have the same superscript letter within the same column. **A, B, C:** There is no significant difference between any two means that have the same superscript letter within the same row.

Table 7. The major chemical constituents of *R. officinalis* essential oil.

No.	RT	Compound name	Area (%)	MW	M.F.
1	2.02	Cyclobutane, 1,1-dimethyl-2-octyl	0.47	196	$C_{14}H_{28}$
2	4.63	Tricyclene	1.63	136	$C_{10}H_{16}$
3	4.70	β-Pinene	0.32	136	$C_{10}H_{16}$
4	4.87	α-Pinene	17.85	136	$C_{10}H_{16}$
5	5.25	Camphene	8.04	136	$C_{10}H_{16}$
6	5.93	β-Pinene	6.69	136	$C_{10}H_{16}$
7	6.25	7-Methyl-3-methylene-1,6-octadiene	0.42	136	$C_{10}H_{16}$
8	6.77	3-Carene	3.53	136	$C_{10}H_{16}$
9	6.94	7-Oxabicyclo[2.2.1]heptane, 1-methyl-4-(1-methylethyl)	0.32	154	$C_{10}H_{18}O$
10	7.23	O-Cymene	4.49	134	$C_{10}H_{14}$
11	7.42	Eucalyptol	20.65	154	$C_{10}H_{18}$
12	8.22	γ-Terpinene	2.96	136	$C_{10}H_{16}$
13	9.59	1,6-Octadien-3-ol, 3,7-dimethyl-	1.54	154	$C_{10}H_{18}O$
14	10.95	(+)-2-Bornanone	14.65	152	$C_{10}H_{16}O$
15	11.54	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, exo-	11.32	154	$C_{10}H_{18}O$
16	11.84	endo-Borneol	0.46	154	$C_{10}H_{18}O$
17	12.13	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	1.98	154	$C_{10}H_{18}O$
18	12.64	α-Terpineol	2.0	154	$C_{10}H_{18}O$
19	12.78	Estragole	0.68	148	$C_{10}H_{12}O$

RT: Retention time; **MW:** Molecular weight; **MF:** Molecular formula.

Table 8. The major chemical constituents of *C. verum* essential oil.

No.	RT	Compound name	Area (%)	MW	M.F.
1	4.62	Alpha-Pinene	0.5	136	$C_{10}H_{16}$
2	4.57	Camphene	0.08	136	$C_{10}H_{16}$
3	4.87	Sabinene	0.31	136	$C_{10}H_{16}$
4	5.25	Beta-Pinene	0.07	136	$C_{10}H_{16}$
5	5.93	1,4-Cineole	0.18	154	$C_{10}H_{18}O$
6	6.25	Delta-3-carene	0.37	136	$C_{10}H_{16}$
7	6.77	O-cymene	3.31	134	$C_{10}H_{14}$
8	6.94	Limonene	0.19	136	$C_{10}H_{16}$
9	7.23	p-Cymene	0.25	134	$C_{10}H_{14}$
10	7.42	1,8-cineole	1.02	154	$C_{10}H_{18}O$
11	8.22	Benzyl alcohol	0.14	108	C_7H_8O
12	9.59	Linalool	18.02	154	$C_{10}H_{18}O$
13	10.95	γ-Terpinene	0.15	136	$C_{10}H_{16}$
14	11.54	Cinnamaldehyde	56.12	132	C_9H8O
15	12.11	Eugenol	6.37	164	$C_{10}H_{12}O_2$
16	12.64	Geraniol	0.21	154	$C_{10}H_{18}O$
17	12.78	Cinnamyl acetate	1.48	176	$C_{11}H_{12}O_2$
18	13.12	Alpha-humulene	0.19	204	$C_{15}H_{24}$
19	13.57	(E)-Cinnamylacetate	10.22	176	$C_{11}H_{12}O_2$
20	14.22	Eugenyl acetate	0.35	206	$C_{12}H_{14}O_3$
21	14.56	Benzyl benzoate	0.37	212	$C_{14}H_{12}O_2$

RT: Retention time; **MW:** Molecular weight; **MF:** Molecular formula.

DISCUSSION

Plants produce many phytochemical compounds as biopesticides that are more target-specific, organic, biodegradable, and not harmful to animals, unlike other chemically manufactured products. Two studies[21,22] suggested that certain phytochemical compounds also delay or reduce the pests' ability to metabolize insecticides. Thus, considering the significance of plant-based essential oils, we examined the effectiveness of *R. officinalis*, *P. nigrum*, *C. verum*, *C. rotundus*, *M. alternifolia*, and *A. vera* EOs against developmental stages of *M. domestica*.

Several researchers[6,18,23,24] studied the insecticidal properties of plant-derived EOs to control houseflies and other harmful pests. Our current results indicated that as the length of exposure and oil concentration of all the botanical extracts examined increased, so did the number of *M. domestica* larvae that died. Additionally, all 10% EO concentrations were more effective compared to 5%, 2.5%, 1.0, and 0.5% concentrations. In accordance, other studies[25,26] showed that treatments with essential oils (EOs) significantly prolonged the development time of larvae and pupae. Abdel-Baki *et al*.[27] also found that longer periods of exposure to EOs such as *Foeniculum vulgare*, *C. verum*, *Allium sativum*, *Capsicum annum*, *Mentha piperita*, and *Urtica dioica* had a greater effect on *M. domestica*. Other EOs of *Allium sativum*, *Azadirachta indica*, *Cinnamomum cassia, Eucalyptus camaldulensis, Piper nigrum*, and *Thevetia peruviana* were proved to kill or stop flies from laying eggs, as well as stop and delay housefly larvae development[28]. Later, Yousef *et al*.[29] showed that *Mentha piperita* extended the larval and pupal stages of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). It was claimed that EOs might be able to change the insects' physical characteristics[30], such as diminishing their food consumption, stopping digestive enzymes from working[29], and maybe even prolonging the development period[30,31].

There are other variables that could cause pupation failure and adult emergence failure. Among them are unsaturated fatty acids that speed up the melanization process, the hardening of the opercular suture, and not allowing the adult insect to emerge from the pupation pouch[27].

Kökdener[25] showed that *C. verum* has high PIR values (97.4-100%), while that of *M. piperita* oil was moderate with a value ranging from 63.15% to 92.10% according to concentrations of the tested EO. Giving the results obtained by Abdel-Bakı *et al*.[27], *M. domestica* pupae were completely killed by a 10% concentration of EO from *F. vulgare*. We conducted a pupicidal experiment using contact toxicity against *M. domestica* pupae, and revealed significant variations in growth and development inhibition when exposed to variable concentrations of the investigated EOs.

On the other hand, researchers reported using EOs with insecticides to create a synergistic effect. For example, treatment against *Spodoptera frugiperda* by deltamethrin and *Ocimum basilicum* worked better together than either one by itself and cut the LD50 of deltamethrin used by 80%[32]. A study conducted by Suwannayod[33] also found that mixing EOs with pyrethroid insecticides (permethrin and deltamethrin) made the mixture more poisonous against blowflies and the house fly. From this standpoint, we recommend using a combination of EOs with pyrethroid insecticides to enhance the insecticide's effectiveness. This alternative strategy will be helpful in developing a formula for effective fly control management. Finally, our results revealed that the tested EOs were toxic to housefly larvae using the ingestion method more than contact-treated filter paper. This method shows the superiority of applying essential oils to control housefly larvae through food rather than contact.

In conclusion, *R. officinalis* and *C. verum* EOs showed potential efficiency against house flies' larvae. We recommended further studies to document using EOs as larvicides or synergists to enhance the efficacy of insecticides.

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REFERENCES

1. Scott JG, Warren WC, Beukeboom LW, Bopp D, Clark AG, Giers SD, *et al*. Genome of the house fly, *Musca domestica* L.: A global vector of diseases with adaptations to a septic environment. Genome Biol 2014; 15:466.
2. Issa R. *Musca domestica* acts as transport vector hosts. BNRC 2019; 43(1):1-5.
3. Freeman JC, Ross DH, Scott JG. Insecticide resistance monitoring of house fly populations from the United States. Pestic Biochem Physiol 2019; 158:61-68.
4. Scott JG. Evolution of resistance to pyrethroid insecticides in *Musca domestica*. Pest Manag Sci 2017; 73(4):716-722.
5. Sparks TC, Nauen R. IRAC: Mode of action, classification and insecticide resistance management. Pestic Biochem Physiol 2015; 121:122-128.
6. Khater H, Geden CJ. Efficacy and repellency of some essential oils and their blends against larval and adult house flies, *Musca domestica* L. (Diptera: Muscidae). J Vector Ecol 2019; 44(2):256-263.
7. Bajda SA, De Clercq P, Van Leeuwen T. Selectivity and molecular stress responses to classical and botanical acaricides in the predatory mite *Phytoseiulus persimilis*

Athias-Henriot (Acari: Phytoseiidae). Pest Manag Sci 2021; 78:881-895.
8. Wandjou JGN, Baldassarri C, Ferrati M, Maggi F, Pavela R, Tsabang N, *et al*. Essential oils from Cameroonian aromatic plants as effective insecticides against mosquitoes, houseflies, and moths. Plants (Basel). 2022; 11(18):2353.
9. Benelli G, Pavela R, Giordani C, Casettari L, Curzi G, Cappellacci L, *et al*. Acute and sub-lethal toxicity of eight essential oils of commercial interest against the filariasis mosquito *Culex quinquefasciatus* and the housefly *Musca domestica*. Ind Crop Prod 2018; 112:668-680.
10. Ahsaei SM, Rodríguez-Rojo S, Salgado M, Cocero MJ, Talebi-Jahromi K, Amoabediny G. Insecticidal activity of spray dried microencapsulated essential oils of *Rosmarinus officinalis* and *Zataria multiflora* against *Tribolium confusum*. Crop Prot 2020; 128:104996.
11. Chintalchere JM, Dar MA, Raut KD, Pandit RS. Bioefficacy of lemongrass and tea tree essential oils against house fly, *Musca domestica*. Proc Natl Acad Sci India Sect B Biol Sci 2021; 91(2):307-318.
12. Campos EV, Proença PL, Oliveira JL, Bakshi M, Abhilash P, Fraceto LF. Use of botanical insecticides for sustainable agriculture: future perspectives. Ecol Indic 2019; 105:483-495.
13. Rodríguez-Yoldi MJ. Anti-inflammatory and antioxidant properties of plant extracts. Antioxidants 2021; 10(6):921.
14. Soonwera M, Moungthipmalai T, Puwanard C, Sittichok S, Sinthusiri J, Passara H. Adulticidal synergy of two plant essential oils and their major constituents against the housefly *Musca domestica* and bioassay on non-target species. Heliyon 2024; 10(5):e26910.
15. Cossetin LF, Santi EMT, Garlet QI, Matos AFIM, De Souza TP, Loebens L, *et al*. Comparing the efficacy of nutmeg essential oil and a chemical pesticide against *Musca domestica* and *Chrysomya albiceps* for selecting a new insecticide agent against synantropic vectors. Exp Parasitol 2021; 225:108104.
16. Legault J, Pichette A. Potentiating effect of beta-caryophyllene on anticancer activity of alpha-humulene, isocaryophyllene and paclitaxel. J Pharmacol 2007; 57(12):1643-1647.
17. Vinturelle R, Mattos C, Meloni J, Nogueira J, Nunes MJ, Vaz IS, *et al*. *In vitro* evaluation of essential oils derived from *Piper nigrum* (Piperaceae) and *Citrus limonum* (Rutaceae) against the tick *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae). Biochem Res Int 2017; 5342947.
18. Xie Y, Jin H, Yang X, Gu Q, Zhang, D. Toxicity of the essential oil from *Thymus serpyllum* and thymol to larvae and pupae of the housefly *Musca domestica* L. (Diptera: Muscidae). Environ Sci Pollut Res 2020; 27, 35330-35340.
19. Kumar P, Mishra S, Malik A, Satya S. Housefly (*Musca domestica* L.) control potential of *Cymbopogon citratus* Stapf. (Poales: Poaceae) essential oil and monoterpenes (citral and 1, 8-cineole). J Parasitol Res 2013; 112:69-76.
20. Ashmawy NA, Salem MZM, El-Hefny M. Antibacterial activity of the bioactive compounds identified in three woody plants against some pathogenic bacteria. Microb Pathog 2018; 121:331-340.
21. Wang Z, Zhao Z, Cheng X, Liu S, Wei Q, Scott IM. Conifer flavonoid compounds inhibit detoxification enzymes and synergize insecticides. Pestic Biochem Phys 2016; 127:1-7.
22. Yoon J, Tak JH. Cuticular property affects the insecticidal synergy of major constituents in thyme oil against houseflies, *Musca domestica*. Sci Rep 2023 4;13(1):12654.
23. Baz MM, Eltaly RI, Debboun M, Selim A, Radwan IT, Ahmed N, *et al*. The contact/fumigant adulticidal effect of Egyptian oils against the house fly, *Musca domestica* (Diptera: Muscidae). Int J Vet Sci 2023; 12(2):192-198.
24. Zahoor MK, Zahoor MA, Mubarik MS, Rizvi H, Majeed HN, Zulhussnain M, *et al*. Insecticidal, biological and biochemical response of *Musca domestica* (Diptera: Muscidae) to some indigenous weed plant extracts. Saudi J Biol Sci 2020; 27(1):106-116.
25. Kökdener M. Larvicidal and pupicidal effects of some essential oils against *Musca domestica* Linnaeus, 1758 (Diptera: Muscidae). Int J Agr Env Food Sci 2023; 7(1):234-243.
26. Senthoorraja R, Subaharan K, Manjunath S, Pragadheesh VS, Bakthavatsalam N, Mohan MG, *et al*. Electrophysiological, behavioral and biochemical effect of *Ocimum basilicum* oil and its constituents methyl chavicol and linalool on *Musca domestica* L. Environ Sci Pollut Res Int 2021; 28(36):50565-50578.
27. Abdel-Baki AAS, Aboelhadıd SM, Sokmen A, Al-Quraıshy S, Hassan AO, Kamel AA. Larvicidal and pupicidal activities of *Foeniculum vulgare* essential oil, trans-anethole and fenchone against house fly *Musca domestica* and their inhibitory effect on acetyl cholinestrase. Entomol Res 2021; 51:568-577.
28. Khan H AA. Toxicity, repellent and oviposition deterrent effects of select essential oils against the house fly *Musca domestica*. J Asia-Pacific Entomol 2021; 24(1):15-20.
29. Yousef DA, Bayoumi AE, Dimetry NZ, Amin AH, Hoballah EM. Evaluating effect of pepper mint oil (*Mentha pipreta*) and its nano-formulations on some enzymatic activities and bionomics of cotton leaf worm *Spodoptera littoralis*. Univ J Agric Sci. 2018; 26(2):1977-1991.
30. Bezzar-Bendjazia R, Kilani-Morakchi S, Maroua F, Aribi N. Azadirachtin induced larval avoidance and antifeeding by disruption of food intake and digestive enzymes in *Drosophila melanogaster* (Diptera: Drosophilidae). Pestic Biochem Phys 2017; 143:135-140.
31. Mohamed HS, Fahmy MM, Attia MM, Rabab M, El Khateeb M, Shalaby HA, *et al*. The insecticidal activity of two medicinal plants (*Commiphora molmol*) and (*Balanites aegyptiaca*) against the Blowfly *Lucilia sericata* (Diptera: Calliphoridae). Int J Adv Res Bio Sci 2016; 3(3):144-158.
32. Silva S, Rodrigues da Cunha JPA, Malfitano de Carvalho S, Zandonadi CHS, Martins RC, Chang R. *Ocimum basilicum* essential oil combined with deltamethrin to improve the management of *Spodoptera frugiperda*. Cienc. Agrotec. 2017, 41:665–675
33. Suwannayod S, Sukontason KL, Pitasawat B, Junkum A, Limsopatham K, Jones MK, *et al*. Synergistic toxicity of plant essential oils combined with pyrethroid insecticides against blow flies and the house fly. Insects 2019; 10(6):178.