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The Bioefficacy of Essential Oils against the False Stable Fly, *Muscina stabulans* (Harris) (Diptera: Muscidae)

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ABSTRACT

The distribution of false stable fly, *Muscina* stabulans (Harris) (Diptera: Muscidae) is worldwide. Control of *M. stabulans* has been seldom done; therefore, this study investigated some biological aspects post treatment of four essential oils through ingestion and contact bioassays against the 3rd larval instars of *M. stabulans*. Post treatment (PT) with 50%, the mortality% of larvae, pupae, both larvae and pupae, and adults reached 83.33, 73.33, 95.56, and 100%, respectively, for *Apium graveolens* (celery); 73.33, 75.00, 93.33, and 100.00%, respectively, for *Eruca sativa* (ruca); 80.00, 100.00, 100.00, and 100.00%, respectively, for *Lactuca sativa* (lettuce); and 63.33, 81.82, 93.33, and 90.00%, respectively, for *Raphanus sativus* (radish).The pupation and adult emergence rates PT with 50%, and LC_{50} values were 4.44, 0.00, and 21.743%; 6.67, 0.00, and 24.786%; 0.00, 0.00, and 20.909%; and 6.67, 10.00, and 31.826%, respectively. On the other hand, their toxicity indices reached 96, 84, 100, and 66%, respectively. This study indicated that *L. sativa* was the most effective oil, followed by *A. graveolens* and *E. sativa;* whereas *R. sativus* was the least effective oil. To conclude, essential oils could be used as ecofriendly fly management in organic farming and in places where conventional insecticides could not be applied.

Key words: Apium graveolens,Eruca sativa, Lactuca sativa, Raphanus sativus,oxicity indices.

Introduction

The stable fly, *Muscina stabulans* (Fallén 1817) (Diptera: Muscidae), is a cosmopolitan fly with a worldwide distribution and has medical and forensic importance in acting as a mechanical disease vector and intestinal myiasis producing fly (Shivekar et al., 2008; Patitucci et al., 2010; Wang et al., 2019). Larvae of *M. stabulans* develop on a variety of discrete and ephemeral food substrates, such as feces and carcasses. On the other hand, its larvae could prey on the immature stages of the other dipterous flies, acting as an effective control agent (Duarte et al., 2013; Zimmer et al., 2022).

Insect management mainly depends on applying of conventional insecticides which led to contaminated dairy and meat products, environmental pollution, and development of insect resistant strains. Therefore, using eco-friendly insecticides is an urgent need to reduce their health and environmental side effects. Using some available cheap plants which are usually safe to the environment and to other living organisms such as Leaves and Stems of *Lantana camara* plant against larvae of *Musca domestica* (Khater, 2012a; Fouda, et al., 2017; Ahmed et al., 2021; Iqbal et al., 2021).

The ancient Egyptians discovered the potency of essential oils (EOs) which progressed in the Middle Ages by the Arabs as they have long been used as embalmment and food preserving agents, anti-inflammatory, antimicrobial, sedative, analgesic, local anesthetic, and spasmolytic therapies (Khater, 2013, 2017, 2020).

Recently, EOs are commercially used in food, agricultural, cosmetic, perfume, and pharmaceutical industries (Khater, 2017, 2020). Plant extracts, including essential oils, meritoriously control several illnesses and parasites (Khater & Shalaby, 2008; Roni et al., 2015; Seddiek et al., 2011, 2013, 2014; Vaz et al., 2018; Khater et al., 2019, 2020; Khater, 2020; Iqbal et al., 2021; Abdel‑Meguid et al., 2022; Abosalem et al., 2022; Mohamed et al., 2022; Abd Elgawad et al., 2023; Altaf et al., 2023).

Essential oils are cost-effective, safe and biodegradable pesticides (Khater, 2012a,b, 2013) inducing deterrent, repellent, ovicidal, adulticidal, larvicidal, and insect growth regulating effects (Khater, 2003; Shalaby & Khater, 2005; Khater & Shalaby, 2008; Khater et al., 2014, 2018, 2022; Khater & Geden, 2018, 2019; Hegazy et al., 2022). The present study aimed to evaluate the lethal effects of some essential oils against the 3rd larval stage, study the pupation and adult emergence% after larval treatments, and calculate the lethal concentration values and the relative toxicities and toxicity indices.

Materials and methods

2.1. Essential Oils

Four essential oils were brought from EL CAPTAIN Company for Extracting Natural Oils, Plants, and Cosmetics "Cap Pharm", El Obor, Cairo, Egypt. Such oils were celery, *Apium graveolens* (Apiaceae); eruca, *Eruca sativa* (Brassicaceae); lettuce (*Lactuca sativa* (Asteraceae); and radish, *Raphanus sativus* (Brassicaceae).

2.2. Insects

M. stabulans were collected from Moshtohor, Toukh (30° 21′ 11.6″ N and 31° 11′ 31.5″ E), Qalyubiya Governorate, Egypt and reared at the laboratory according to a previously described procedure (Khater & Geden, 2019).

2.3. Bioassay

Early 3rd larval instars were exposed to oils via the ingestion and contact bioassay (Khater & Geden, 2019) by adding 15 larvae per replicate to a treated rearing medium in a small cup having 10 g of rearing medium treated with 1 ml of each oil concentration and Tween 20 (5% as an emulsifier); whereas the control group was treated with distilled water and Tween 20.

Five oil concentrations (2, 4, 7, 13, 25, and 50%) were applied for each oil. Each small cup was added to a larger cup having 10 g of sawdust as a dry medium for pupation and covered with a piece of cloth tied securely with a rubber band for of prevention adults escaping. Cups were maintained at 27 ± 2 °C and 80± 5% relative humidity until adult emergence (up to 16 days). The number of died larvae, pupae, and adults was recorded. The experiments were replicated three times for each concentration.

2.4. Data analysis

Via SPSS V23 (IBM, USA), the One-Way Analysis of Variance which subsequently followed by the Tukey test as well as Probit analyses were utilized for data analyses. The lethal concentrations (LC) values after larval treatments, squared (R^2), Chi-square (X^2) values, and regression equations were calculated. The p-value was significant when <0.05. The reduction% of adult emergence was calculated (Khater et al., 2009) by managing the succeeding formula: Reduction (Pre-treatment count - post treatment count/ pre- treatment count) X 100.

Results and Discussion

Even though *M. stabulans* is a species of forensic value, it acts as a mechanical disease vector and intestinal myiasis producing fly (Patitucci et al., 2010; Shivekar et al., 2008; Wang et al., 2019). Botanical extracts, including essential oils, are effective against the developmental stages of arthropod pests of medical and veterinary importance (Khater, 2012b, 2014; Khater & Geden, 2018, 2019; Baz et al., 2021; 2022a,b; Eltaly et al., 2023; Hegazy et al., 2022; Khater et al., 2009, 2011; 2013; 2014; 2018; 2022, 2023; Abdel-Ghany et al., 2023; Nabil et al., 2023).

This investigation revealed for the first time, as far as we know, the lethal and insect growth regulating (IGR) efficacy of *A. graveolens*, *E. sativa*, *L. sativa*, and *R. sativus* oils on some biological parameters of *M. stabulans;* their toxicity indices reached 96, 84, 100, and 66%, respectively. Depending on LC_{50}, *L. sativa* was the most effective oil, followed by *A. graveolens* and *E. sativa* and their relative toxicities reached 1.52, 1.46, and 1.28 times more effective than *R. sativus* (Tables 1 and 2).

Alike finding was recorded; novaluron (chitin synthesis inhibitor) is an insect growth regulator (IGR) which adversely affected growth and reproductive potential of topically treated *M. stabulans* 3rd instar larvae and prepupae (Al-Keridis & Ghoneim, 2021). A similar study documented the disruptive effects of Pyriproxyfen as IGR on the survival, developmental stages, and morphogenesis of *M. stabulans* after topical application (Hamadah, 2018).

Correspondingly, the insecticidal effect of an ethanolic extract of *Nerium oleander* leaves was recorded against 2nd larval instars of *M. stabulans* and its LC_{50} value was 113.66 ppm; such dose suspended larval and pupal durations, inhibited oviposition, decreased longevity of the survived adults, and malformed the developmental stages (El-Shazly et al., 1996).

This study showed that *L. sativa* induced superior effect against the developmental stages of *M. stabulans*. PT of 3rd instars with *L. sativa* (50%), it effectively controlled larvae (80.00%) and completely suppressed pupal and adult development. It's LC_{50}, LC_{90}, and LC_{95} values of larval mortalities were 20.909, 50.424, and 58.791%, respectively (Tables 1 and 2 and Fig.1).

Recently, the same *L. sativa* oil used in this study was highly effective against *Cx. pipiens* 4th larvae (LC50 and LC90= 677.45 and 1344.36 ppm, respectively) (Baz et al., 2022). In contrast, it was not effective against unfed adults (7~9-day-old), the most resistant stage, of *Hyalomma dromedarii* PT with 20% using the adult immersion test (Abdel-Ghany et al., 2023).

This work revealed the efficacy of *A. graveolens* (50%) in controlling *M. stabulans* and the mortality% of larvae, pupae, larvae and pupae, and adults reached 83.33, 73.33, 95.56, and 100%, respectively. It's LC_{50}, LC_{90}, and LC_{95} values of larval mortalities were 21.743, 49.476, and 57.338%, respectively, and its toxicity index was 96.16%, it reduced the pupation (4.4%) and completely stopped the adult emergences (Tables 1 and 2 and Fig.2).

Analogously, the same *A. graveolens* oil used in this study was highly effective in our previous work against 3rd larval instars of the blowfly, *Lucilia sericata* (Diptera: Calliphoridae) (LC_{50} and LC_{90}= 4.60 and 16.94%, respectively) and negatively affected the pupating and adult emergences (Khater & Khater, 2009). *A. graveolens* oil has larvicidal effect against early 4th instars of *Ae. aegypti* and its LC_{50} and LC_{90} values were 16.10 and 29.08 ppm, respectively, PT for 24 h. it also has adulticidal effect and induced a remarkable repellent effect (100% protection time 165 min) (Kumar et al., 2014).

In addition, the methanolic extract of *A. graveolens* seeds comprises bioactive compounds of mosquitocidal activity against 4th instar of *Aedes aegypti* including sedanolide, senkyunolide-N, and

Table (1) Effect of four oils after treatment of 3rd larvae instars of *Muscina stabulan*s

Materials		larval MO%± SD	Pupal MO% ± SD	Larval and pupal MO% ± SD	Adult MO% ± SD
Control	**Conc.**	**3.33**±1.41d	**6.90**±0.71b	**10.00**±0.71f	**16.67**±1.41e
Apium graveolens	***2***	**10.00**±1.00cd	**18.52**±4.04ab	**26.67**±4.04df	**33.33**±2.00de
	4	**20.00**±1.53cd	**29.17**±4.16ab	**43.33**±4.16df	**43.33**±2.52cd
	7	**33.33**±2.31c	**35.00**±3.00ab	**56.67**±3.00bcd	**60.00**±1.53bc
	13	**50.00**±2.52b	**40.00**±3.79ab	**70.00**±3.79abc	**76.67**±3.61ab
	25	**63.33**±2.65b	**42.42**±1.73a	**78.89**±1.73ab	**93.33**±4.16a
	50	**83.33**±2.58a	**73.33**±2.16a	**95.56**±2.16a	**100.00**±0.00a
Control		**3.33**±1.41c	**6.90**±0.71c	**10.00**±0.71c	**16.67**±1.41c
Eruca sativa	**2**	**0.00**±0.58c	**27.78**±4.00bc	**27.78**±4.00bc	**33.33**±2.08bc
	4	**20.00**±4.73bc	**37.50**±4.36ab	**50.00**±4.36bc	**66.67**±6.11b
	7	**40.00**±5.13 abc	**55.56**±4.04ab	**73.33**±4.04ab	**93.33**±4.04a
	13	**53.33**±2.65abc	**64.29**±1.73ab	**83.33**±1.73a	**100.00**±2.31a
	25	**60.00**±3.06ab	**66.67**±1.15ab	**86.67**±1.15a	**100.00**±0.00a
	50	**73.33**±2.89a	**75.00**±2.65ab	**93.33**±2.65a	**100.00**±0.00a
Control		**3.33**±1.41d	**6.90**±0.71c	**10.00**±0.71c	**16.67**±1.41b
Lactuca sativa	**2**	**3.33**±0.58d	**49.43**±8.50bc	**51.11**±8.50bc	**100.00**±13.86a
	4	**23.33**±4.04cd	**100.00**±7.51a	**100.00**±7.51a	**100.00**±0.00a
	7	**43.33**±4.16bc	**100.00**±0.00a	**100.00**±0.00a	**100.00**±0.00a
	13	**56.67**±3.79ab	**100.00**±0.00a	**100.00**±0.00a	**100.00**±0.00a
	25	**66.67**±3.61ab	**100.00**±0.00a	**100.00**±0.00a	**100.00**±0.00a
	50	**80.00**±2.65a	**100.00**±0.00a	**100.00**±0.00a	**100.00**±0.00a
Control		**3.33**±1.41d	**6.90**±0.71c	**10.00**±0.71c	**16.67**±1.41b
Raphanus sativus	**2**	**13.33**±2.52d	**23.08**±4.36b	**33.33**±4.36de	**43.33**±5.20cd
	4	**20.00**±2.65bc	**36.11**±2.89b	**48.89**±2.89cd	**60.00**±5.03bcd
	7	**30.00**±3.61 abc	**47.62**±2.65ab	**63.33**±2.65bc	**63.33**±2.08abc
	13	**33.33**±2.08abc	**50.00**±1.53a	**66.67**±1.53abc	**73.33**±5.00abc
	25	**53.33**±5.57ab	**78.57**±1.73a	**90.00**±5.29ab	**83.33**±1.73ab
	50	**63.33**±2.71a	**81.82**±0.82a	**93.33**±0.82a	**90.00**±3.11a

Means followed by the same letter in the same column were not significantly different by ANOVA (P > 0:05).
Conc. Concentrations %; MO%: mortality %; SD: standard deviation

Table (2) Lethal concentration values of oils after treatment the larvae of *Muscina stabula*s

Essential oil	LC50 Upper Lower	LC_{90} Upper Lower	LC_{95} Upper Lower	LC_{99} Upper Lower	Relative toxicity	Toxicity index	X^2 df sig	Equation R2
Apium graveolens	21.743	49.476	57.338	72.086	1.46	96.16	11.664	0.800
	13.447	35.112	40.455	50.271			5	Y=0.06x +-2
	36.959	95.748	113.214	146.182			.040[a]	
Eruca sativa	24.786	57.435	66.691	84.053	1.28	84.36	24.420	0.638
	10.223	35.182	40.584	50.387			5	Y=0.06x+-2
	100.498	350.518	423.068	559.491			.000[a]	
Lactuca sativa	20.909	50.424	58.791	74.487	1.52	100.00	23.192	0.657
	7.610	31.627	36.698	45.876			5	Y=0.06x+-2
	60.939	211.208	255.545	339.047			.000[a]	
Raphanus sativus	31.826	72.702	84.290	106.026	1.00	65.70	8.286	0.720
	21.291	50.054	57.545	71.427			5	Y=0.05+-2
	59.866	158.797	187.506	241.527			.141[a]	

Relative toxicity = LC_{50} of the least toxic compound / LC_{50} of the tested compound.
Toxicity index = LC_{50} of the most toxic compound ×100 / LC_{50} of the tested compound.
The most toxic oil has given 100 units on the toxicity index scale
LC: lethal values; **X2**: Chi: squared; df: degree of freedom; Sig. significance; R^2: R-squared is a goodness-of-fit measure in case of linear regression models

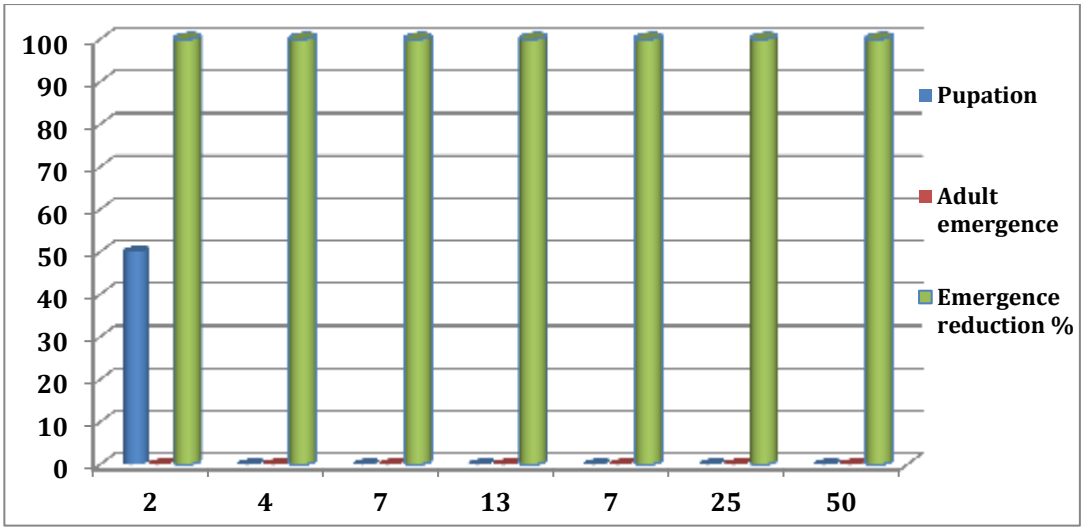

Fig. (1) Effect of *Lactuca sativa* on pupation, adult emergence, and emergence reduction (%) after treatment of 3rd larval instars of *Muscina stabulas*

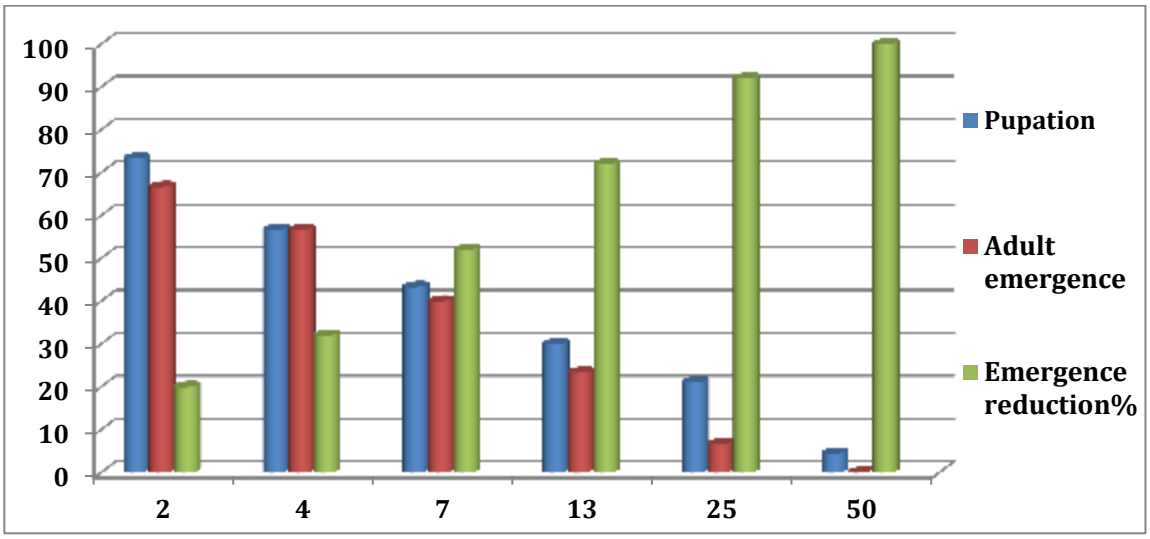

Fig. (2) Effect of *Apium graveolens* on pupation, adult emergence and emergence reduction (%) after treatment of 3rd larval instars of *Muscina stabulas*

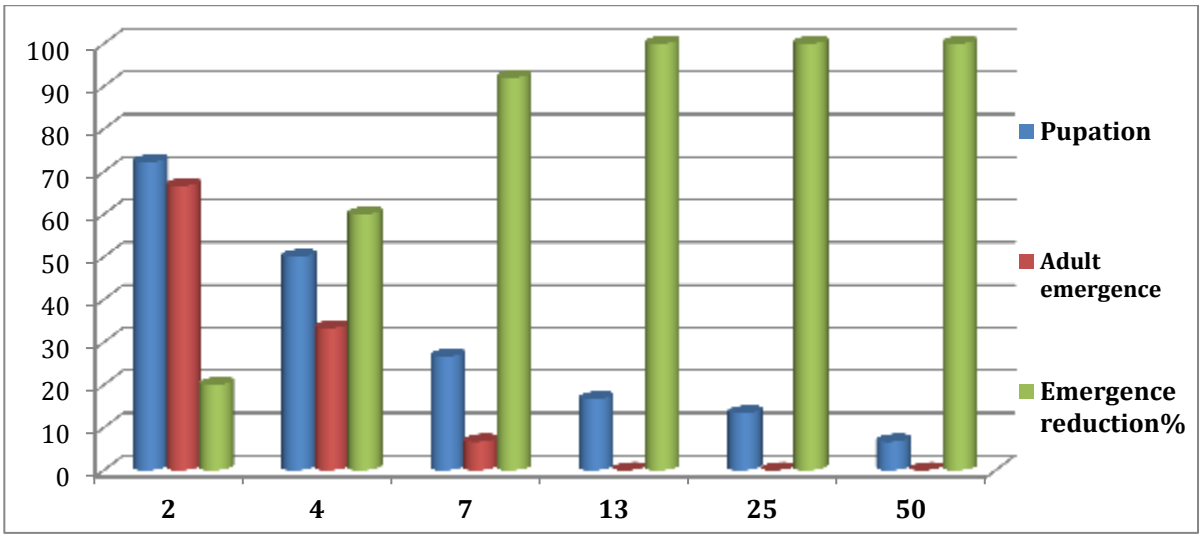

Fig. (3) Effect of *Eruca sativa* on pupation, adult emergence, and emergence reduction (%) after treatment of 3rd larval instars of *Muscina stabulas*

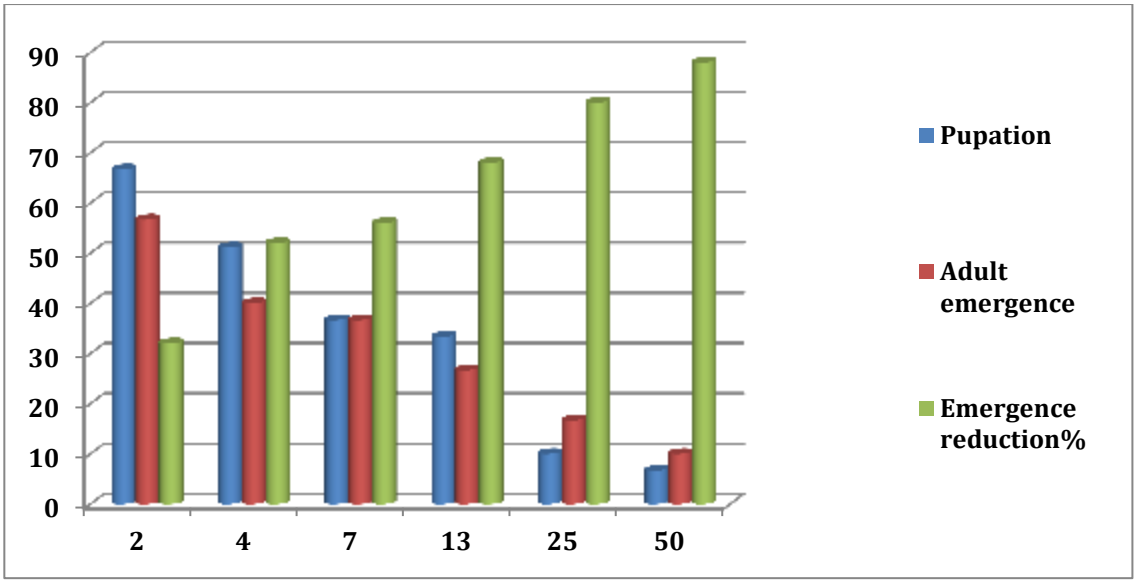

Fig. (4) Effect of *Raphanus sativus* on pupation, adult emergence, and emergence reduction (%) after treatment of 3rd larval instars of *Muscina stabulas*

senkyunolide- J (Momin & Nair, 2001). *A. graveolens* oil contains major constituents such as lactones, flavonoids, and terpenoids, which could play a role in its toxicity (Kumar et al., 2014).

This study revealed that *E. sativa* (50%) was lethal to larvae and pupae, and adults of *M. stabulans* (73.33, 75.00, 93.33, and 100.00%, respectively) and its LC_{50}, LC_{90}, and LC_{95} values reached 24.786, 57.435, and 66.691%, respectively, and its toxicity index was 84.36%, it reduced the pupation and completely suppressed adult emergences (Tables 1 and 2 and Fig.3).

A related study showed the lethal effect of *E. sativa* against 4th larval instars of *Culex pipiens* (Diptera: Culicidae) (LC50= 86.06, ppm) and reported a remarkable reduction of the pupation rate (6.67%), and inhibited adult emergences PT of larvae with 1000 ppm (Khater & Shalaby, 2008).

Also, *E. sativa* induced a complete (100%) aphicidal effect against the rose aphid, *Macrosiphum rosae* and the black bean aphid, *Aphis fabae*, PT with 1% (Alghamdi, 2018). A comparable study reported that petroleum ether and acetone seed extracts of arugula seeds (*Eruca vesicaria,* Brassicaceae) were effective contact and stomach poison against the adult granary weevil, *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrochidae) and the lesser grain borer, *Sitophilus granarius* Linnaeus (Coleoptera: Curculionidae) and their LC_{50} values reached 0.722, 0.620, 0.622, and 0.475 ml/ kg, respectively, 5 days PT and inhibited their first generations (Mohanny et al., 2020).

This research paper brought to light that the developmental stages of *M. stabulans* were susceptible to *R. sativus* (50%) because larval, pupal, larval and pupal, and adult mortalities reached 63.33, 81.82, 93.33, and 90.00%, respectively. On the other hand, its LC_{50}, LC_{90}, and LC_{95} values reached 24.786, 57.435, and 66.691%, respectively. It was the least effective oil as its toxicity index reached 65.70%, but it also lowered the pupation (6.67%) and adversely affected adult emergences (88%) at its sublethal concentrations (Tables 2 and 3 and Fig.4).

A similar study showed that *R. sativus* oil has contact/fumigant adulticidal effect against the house fly, *Musca domestica* (Diptera: Muscidae) (LC_{50} and LC_{90}= 0.25 and 1.38%, respectively) and its median lethal time PT with 2% was 16.44 min (Baz et al., 2023). Moreover, it was effective against 3rd larval instars of *L. sericata* (LC_{50} and LC_{90}= 6.93 and 46.86%, respectively) and adversely affected both pupating and adult emergences (Khater & Khater, 2009). Alike finding indicated that *R. sativus* (2%) induced complete (100%) aphicidal effect against *M. rosae* and *A. fabae* (Alghamdi, 2018).

Chemical constituents of *R. sativus* Linn. roots were effectively toxic against adults and 2nd instar nymphs of *A. gossypii* in vitro and its methylene chloride fraction was highly potent against both adults and nymphs (LC_{50} = 386.63 and 309.43 ppm, respectively), followed by ethyl acetate fraction (LC50 = 394.9 and 334.37 ppm, respectively). Whereas, the sub-lethal concentrations of such root extracts inhibited transaminases and alkaline phosphatase of *A. gossypii* (Ibrahim et al., 2020).

Conclusion

Filth flies such as *M. stabulans* have medical and veterinary importance and this study revealed for the first time, according to our knowledge, the efficacy of four essential oils against it. *L. sativa* was the most toxic oil, followed by *A. graveolens* and *E. sativa*, whereas *R. sativus* was the least effective oil. Such oils drastically affected treated larvae and reduced the

pupation and adult emergence rates. Essentials oils could be used as eco-friendly fly management tool in organic farming and in places where conventional insecticides could not be applied. Future studies could be directed toward improving the oil formulations for prolonged persistence in the environment and their application in the field after studying their ecotoxological side views.

Ethical approval

The protocol of this study had been reviewed and approved by the by the Ethical Committee of Zagazig University: Institutional animal care and Use committee, ZU-IACUC; the approval number is ZU-IACUC/2/F/197/2023

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