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Research Article
Larvicidal Activity and Bio-efficacy of Some Products Against Larvae of the Housefly, *Musca domestica* (L) (Diptera: Muscidae)

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Abstract

Background and Objective: The housefly (*Musca domestica* L.) is a cosmopolitan dipterous fly and considered as one of the most important insects that transmits pathogens of a considerable medical and veterinary importance. The control of parasitic stages of such fly is a major potential target. This study was conducted to evaluate, *in vitro*, the efficacy of five compounds against the third larval stages of *M. domestica*; two natural products (peppermint oil and *Moringa oleifera* extract), two commercially used insecticides (Butox® and phoxim) and a commonly used disinfectant, Virkon®S. **Materials and Methods:** The third larval stages of *M. domestica* were collected and underwent the larval bioassay, Bait method (food-media technique). Both lethal concentrations (LC_{50} and LC_{90}) of tested compounds were detected 24 h post-treatment. Moreover, the potential effects on larval mortality, larval duration, pupal duration and adult emergence as well as detectable abnormalities were assessed. All data were statistically monitored. **Results:** It has been found that phoxim, peppermint oil and Butox® were more toxic with significant LC_{50} and LC_{90} values. Oppositely, both Virkon® S and *Moringa oliefera* extract 10% had no effect. Efficiently, the use of the peppermint oil induced a prolonged larval duration and a lower larval survival rate with prominent morphological abnormalities in all parasitic stages. Phoxim was more potential as larvicidal than Butox® with slight morphological abnormalities at the pupal stage. **Conclusion:** The present investigation revealed that compared to commonly used insecticides, the natural product, peppermint oil (*Mentha piperita*), is an effective, safe and cheap larvicidal against the third larval stages of houseflies and it could serve as an eco-friendly housefly control measure, implying that more studies dealing with other natural substances against dipterous flies of veterinary and medical importance are requested.

Key words: *Musca domestica*, control, peppermint oil, *Moringa oleifera*, insecticides

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The housefly, *Musca domestica* (L) is one of the most common household dipterous flies belonging to family Muscidae (suborder Cyclorrhapha)[1]. It is a universal pest found in several habitations and it has a global distribution except Antarctica[2,3]. It is of highly medical and veterinary potential that cause annoyance to humans by invading residencies areas, spoils food and may transmit pathogens of a considerable medical and veterinary importance[4]. Houseflies have been recognized as a mechanical vector for more than 100 potentially pathogenic bacteria including salmonellae, *Anthrax* sp., *Shigella* sp. and *Staphylococcus aureus*. Moreover, it transmits a variety of parasitic worms including ascarids and *Taenia* spp. as well as some parasitic protozoal cysts, most likely, *Entamoeba histolytica*, *Giardia lamblia* and *Balantidium coli*[5-7].

A successful control of houseflies should be based on integrated pest management program that includes all available cultural, chemical, biological and mechanical approaches against such pests. Control measures often depend on the use of chemical insecticides[8]. The in discriminative use of such materials has led to developing an insect resistance and bioaccumulation in the environment[9]. During the last decades, several insecticides have been used for the control of housefly, organophosphates, carbamates and pyrethroids[10].

Alternate measures using bio-insecticides, especially those of plant origin have been recently considered more eco-friendly[11]. Plants extract and/or essential oils are extracted from plant tissues and are documented to be used for the treatment of several human diseases, as well as the control of insects through their toxic, anti-feeding and oviposition deterrents[12]. New trends towards the use of botanical insecticides instead of chemical ones are encouraged because of their specificity, broad spectrum and ease to obtain and use[13]. Lots of plant extracts and essential oils have been used for the control of larval stages of *M. domestica*[14-17].

The overuse of chemical insecticides may lead to a resistance against muscidae and other dipterous flies as well as the disadvantages of toxicity and high costs. So, the current study aimed to evaluate the efficacy of the commercial peppermint oil, *Mentha piperita*, natural plant extract *Moringa oleifera* and a commonly used disinfectant, Virkon® S 1% as well as two commercially used insecticides Butox® and phoxim as larvicidal agents for the control of third larval instars of the housefly, *Musca domestica* (L).

MATERIALS AND METHODS

Collection and rearing of flies: Adults *M. domestica* were collected from animals farm at Beni-Suef province (coordinates: 29°04'N 31°05'E), Egypt by the use of a sweeping net and transported into plastic jars to the Department of Parasitology, Faculty of Veterinary Medicine, Beni-Suef University for identification and rearing. They were maintained in plastic jars 20 cm height and 10 cm width, covered with muslin cloth at 26-28°C, relative humidity 55-60% and provided cotton swab soaked with granulated sugar and water for egg laying. Eggs were transferred to Petri dishes for hatching. Hatched larvae were transferred to jars containing larval media (yeast, dry milk powder, wheat bran and water according to the method described by Pavela[18] and checked daily until the pupal stage. Pupae moved into plastic jars containing wood dust for the emergence of adults. The obtained larvae were used in the larvicidal bioassays[19].

Natural and chemical products used in bioassay

Natural products: The commercial peppermint oil (*Mentha piperita*) was obtained from El-Gomhoria Company for Medical Pharmaceutics, Cairo, Egypt. Serial aqueous solutions were prepared to obtain the following concentrations: 0.5, 1, 3 and 5%, then stored in plastic bottles at 4°C. *Moringa olifiera* 10% ethanolic extract was prepared as 0.5 g of the extract dissolved in 5 mL distilled water[20].

Chemical products: Virkon®S (DuPont, Wilmington, USA), a common detergent was used. The active ingredients were potassium peroxy mono-sulfate 21.41%, sodium chloride 1.50% and other ingredients 77.09%. It was purchased from local commercial company and used at 1% concentration in distilled water (recommended dose).

Furthermore, two commercially acaricides were used, Butox® 5% (deltamethrin 5%), the commonly used acaricide. Phoxim 500 mg mL^{-1} (Cebacil EC 50%) were obtained from Pharma Swede Company for veterinary products and used at 1% concentration in distilled water (recommended dose).

Larvicidal bioassay: The assay was done using the Bait method (food-media technique), the standard method for evaluation[21], where the third instar larvae of *Musca domestica* were put in 250 mL glass beakers, each provided with 10 larvae and reared on food contaminated with the drug by mixing 2 g organic matters with 2 mL water containing 1 mL of the prepared concentration of each compound. Three

replicates were done. Experimental conditions of temperature 28±2°C and relative humidity 65±5% were provided. The larval mortality was evaluated 24 post-treatment using a small paint brush. No movement and the absence of the development of brownish appearance indicate dead larvae[19,22]. Survived larvae were further daily examined to estimate the effect on the larval duration, percentage of pupation and the successfully emerged adults post treatment[23,24]. Untreated flies, reared on media only supplied with water were used as a control. Morphological abnormalities of the developmental stages were recorded and photographed.

Statistical analysis: The obtained data were subjected to statistical analysis. The mean mortality data of the three replicates per dose were used to calculate the LC_{50} and LC_{90} using probit analysis[25]. Data were statistically analyzed using Statistical Package for Social Science (SPSS for Windows (IBM), version 22, Chicago, USA) to determine the variable difference between treatments. One-way analysis of variance (ANOVA) to determine the differences between means. Results were expressed as means±standard deviation and the statistical significance was determined at $p<0.05$.

RESULTS

The efficacy of peppermint oil, *Mentha piperita*, *Moringa oleifera* extract 10%, the commonly used detergent Virkon® S as well as two commercial insecticides Butox® (deltamethrin 5%) and phoxim 500 mg mL^{-1} against the third instar larvae of the housefly, *Musca domestica* was *in vitro* evaluated through the food media bioassay. It has been revealed that phoxim was found to be the most toxic for the larvae (LC_{50} = 0.45 and LC_{90} = 1.01) followed by the peppermint oil and Butox® (LC_{50} = 0.61 and 0.88, LC_{90} = 1.31 and 1.8, respectively). Surprisingly, both *Moringa oleifera* extract 10% and Virkon® S 1% had no effect and the slope ranged between 3.7 and 4.03 (Table 1).

Concerning larval and pupal durations, the use of the peppermint oil revealed a significant prolongation of both (7.33±0.57 and 7.33±0.57 days, respectively). For larvae treated with *Moringa oleifera* extract 10%, both periods were 4.33±0.57 and 5.00±0.67 days, respectively. Meanwhile, in Virkon® S-treated larvae, they were 3.66±0.57 and 4.82±0.53 days, respectively. It was observed that phoxim was more larvicidal than Butox®. In the former, larval and pupal durations were 5.66±1.15 and 6.33±0.57 days, respectively, while in the later, they were 4.46±0.51 and 5.00±0.67 days, respectively (Table 2).

Concerning the percentage of pupation, pupal duration and adult emergence, a significant reduction was observed for larvae treated with the peppermint (42.50±9.57, 7.33±0.57 and 30.00±8.16, respectively). Lower values were recorded for larvae treated with phoxim (62.50±9.57, 6.33±0.57 and 57.50±9.57, respectively) and Butox® (81.66±2.88, 5.00±0.67 and 77.33±2.52, respectively). Values in *Moringa oleifera* extract 10% and Virkon® S 1% groups were non-significant (Table 2).

In the present investigation, distinct morphological abnormalities were recorded in larvae treated with peppermint oil and phoxim. Regarding the effect of the peppermint oil, all larval stages of the flies exhibited clear morphological alterations. They became darker and the cuticle became thinner. Pupae showed several morphological

Table 1: Lethal concentrations LC_{50} and LC_{90} of some natural and chemical products against the third instar larvae of *Musca domestica*

Treatments	Groups	Larval survival (%)	Larval duration (days)±SD	Pupation (%)	Pupal duration (days)	Adult emergence (%)
Mentha pipertia	0.5%	21.66±2.88^{c}	7.33±0.57^{d}	42.50±9.57^{e}	7.33±0.57^{d}	30.00±8.16^{e}
Moringa oleifera extract	10%	100.00±0.00^{a}	4.33±0.57^{b}	95.00±5.77^{b}	5.00±0.67^{b}	95.00±5.77^{b}
Virkon® S	1%	100.00±0.00^{a}	3.66±0.57^{a}	95.00±5.77^{b}	4.82±0.53^{a}	95.00±5.77^{b}
Butox® 5%	1%	53.30±5.77^{b}	4.46±0.51^{b}	81.66±2.88^{c}	5.00±0.67^{b}	77.33±2.52^{c}
Phoxim 500 mg mL^{-1}	1%	16.60±5.77^{d}	5.66±1.15^{c}	62.50±9.57^{d}	6.33±0.57^{c}	57.50±9.57^{d}
Water	Control	100.00±0.00^{a}	3.33±0.57^{a}	100.00±0.00^{a}	4.43±0.50^{a}	100.00±0.00^{a}

LC: Lethal concentration, CI: Confidence interval

Table 2: Biological effects of some natural and chemical products on the third instar larvae of *Musca domestica* treated in food media technique (Bait method)

Treatments	LC_{50}	95% CI	LC_{90}	95% CI	Slope
Mentha pipertia	0.61	0.54-0.68	1.31	1.12-1.66	3.9
Moringa oleifera extract 10%	0.00	0.00	0.00	0.00	0.0
Virkon® S	0.00	0.00	0.00	0.00	0.0
Butox® 5%	0.88	0.79-0.98	1.80	1.57-2.26	4.03
Phoxim500 mg mL^{-1}	0.45	0.36-0.52	1.01	0.86-1.29	3.7

Data expressed as Mean±SD, significance at $p<0.05$ between different superscripts

Fig. 1(a-b): Percentage of the emergence of adult flies from larvae, (a) Larvae treated with the peppermint oil revealed a significant reduction in the percentage of adult emergence and (b) Control untreated larvae showing a high percentage of adults emergence

Fig. 2(a-i): Morphological alterations in the third stage larvae, pupae and adult stages of *Musca domestica* treated with peppermint oil and phoxim, (a) Larvae treated with peppermint oil were darker and with smooth cuticle, (b) Larvae treated with phoxim insecticide appeared more or less normal, (c) Control untreated larvae revealed creamy white, segmented and worm-like, (d) Morphological abnormalities of pupae treated with peppermint oil were darker, shrunken, with an irregular body shape and some of them were larval-pupal intermediate forms (arrow), (e) Pupae treated with phoxim appeared darker in color, (f) Normal control pupae appeared barrel-shaped with normal brown coloration, (g) An emerged adult fly from larvae treated with peppermint oil appeared wingless, (h) An emerged adult fly from larvae treated with peppermint oil either dead and small-sized or survived with an extensive deformed body configuration, particularly in the abdomen and (i) An emerged adult fly from control untreated larvae

deteriorations consisted of being darker in color, shrunken, deformed body shape and the appearance of larval-pupal intermediate forms. The deformed pupae did not metamorphose into adults. Emerged adults were dead/small-sized or survived wingless with a great deformed body configuration, particularly in the abdomen. In phoxim-treated larvae, minor morphological abnormalities are found larvae were more or less normal in color and appearance. Moreover, pupae were darker compared to normal ones (Fig. 1, 2).

DISCUSSION

Several types of chemical insecticides have been used against housefly stages. Unfortunately, the indiscriminative uses of those have led to a universal resistance[3]. The present study clearly indicated that the peppermint oil was highly potential larvicide as an eco-friendly alternative for the control of *Musca domestica* in habitations. Similar results were obtained by Sajfrtova *et al*.[26] and Kumar *et al*.[19], who recorded 100% mortality. Furthermore, Morey and Khandagle[27] indicated that the peppermint oil had a promising larvicidal effect against *M. domestica*. The insecticidal action of essential oils is related to their active recorded natural pesticide ingredients reported for *M. piperita* as menthol, menthone and limonene[28,29]. Currently, phoxim was the most toxic to larvae followed by the peppermint oil and Butox®. Meanwhile, both Virkon® S 1% and *Moringa oliefera* extract 10% had no effect. Concomitantly, Abdel Razik[17] found out that methomyl was the most toxic larvicide followed by deltamethrin. This could be attributed to being that phoxim was not commonly used in the farm, while collected houseflies were continuously subjected to Butox® as a routine use of acaricides (with the development of resistance is highly possible). Virkon® S is primarily a disinfectant exhibiting no effect on the flies' larvae. Oppositely, Kaufman *et al*.[30] recorded a dramatic increase in permethrin and beta-cyfluthrin tolerance by both adult and larvae.

A significant prolongation of larval duration as well as a reduction in the survival rate of larvae treated with the peppermint oil followed by phoxim, Butox® and *Moringa oliefera*, while Virkon® S seemed to have no effect. Such result was in agreement with those obtained by Mansour *et al*.[31] and El-Kholy *et al*.[32], who used ethanolic plant extracts of *Piper nigrum*. Moreover, Khater and Shalaby[33] reported the same findings on *Culex pipiens* on treatment with *Boswellia serrata* and *Trigonella foenum-grecum*.

Referring to the percentage of pupation, a significant reduction in groups treated with peppermint oil followed by phoxim and Butox®. Both Virkon® S and *Moringa oliefera* had a minor effect. Meanwhile, the pupal duration significantly increased in groups treated with peppermint oil and phoxim. Concomitant findings are recorded by Bobi *et al*.[34] and Bosly[35] on using the peppermint oil.

The percentage of adults emergence was significantly reduced in groups treated with Butox®, phoxim and peppermint oil, respectively. The same results are given by Abdel Halim and Morsy[15], who used volatile oils of *C. macrocarpa* and *A. officinarum* against *Synthesiomyia nudiseta*. Moreover, Kumar *et al*.[19] found that *M. piperita* completely suppressed the adults emergence. Gamil *et al*.[36] revealed a significant reduction in adults emergence on using indoxacarb. Furthermore, El-Sherbini and Hanykamel[37] reported that housefly larvae treated with *Fortunella crassifolia* showed a significant reduction in both the pupation percentage and adult emergence. Currently, distinct malformations of larvae, pupae and adults post treated with peppermint oil and phoxim, otherwise, other compounds had minor effects. Pupae were darker, irregular body shape (some larval-pupal intermediate forms appeared). Adults were dead/small-sized and wingless. On the other hand, phoxim only induced black-coloured pupae. The abnormalities could be attributed to hormonal disturbance that inhibit the process of metamorphosis as a result of the muscle paralysis[38]. Those findings run with data published by Bosly[35]. Furthermore, Khater and Shalaby[33], Khater and Kahter[38] and Mansour *et al*.[31] recorded various morphological abnormalities in all stages of *Culex pipiens*, *Lucilia sericata* and *M. domestica* treated with essential oils. Similarly, Sexena *et al*.[39] reported developmental abnormalities in larvae of *Cnaphalocrocis medinalis* post treatment with 50% neem oil and Halawa *et al*.[40] who denoted that the insecticides, Beticol, Biosad, Elsan, Lufox and Mani, induced different morphological abnormalities against pupae of *Bactrocera zonata*.

CONCLUSION

Five compounds were used to control houseflies larvae. The peppermint oil was found to be the most effective one against all stages of the life cycle of the dipterous fly, followed by phoxim and Butox®. *Moringa oliefera* 10% extract and Virkon® S seemed to have no effect. The holistic and eco-friendly usage of the peppermint oil, as a novel approach, associated with a significant reduction in *M. domastica* populations in the livestock farms, therefore, further investigations are requested to establish the efficacy under field conditions as a better alternative to chemical insecticides.

SIGNIFICANCE STATEMENTS

This study clarified the effect of various substances, natural, chemical and a commercially used disinfectant against the larvae of the housefly, *Musca domestica*. The peppermint oil was the most effective suggesting the trend of the use of natural products against common flies in Egypt and similar countries. The low price, the high larvicidal affect as well as the absence of side effects are highly welcomed.

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