PLANT ESSENTIAL OILS AND THEIR EFFECTIVENESS AGAINST *Musca domestica* LINNAEUS, 1758 AND ITS PARASITOIDS

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ABSTRACT

Pests and vector insects cause a great deal of economic and health disruption. Insect resistance and bioaccumulation are major disadvantages of the chemical control methods used against this pest. We investigated the larvicide and inhibition effect of *Citrus sinensis* (L.) Osbeck and *Mentha pulegium* Linnaeus essential oils against larvae and pupae of the housefly, *Musca domestica* Linnaeus, 1758. Their effects on a major parasitoid of houseflies; *Muscidifurax raptor* Girault & Sanders, 1910 were also investigated. Both essential oils were found to have significant insecticidal activity against larvae and pupae of houseflies. The LC_{50} of *C. sinensis* and *M. pulegium* essential oils were for larvae 3.93 and 0.71 ml/cm2, and for pupa 0.41 and 0.23 ml/cm2, respectively. Surprisingly, the parasitoids were less affected by plant-applied essential oils as compared to houseflies. After 24 and 48 hours of exposure, the LC_{50} for *C. sinensis* and *M. pulegium* essential oils was found to be 71.2 and 52.6 ml/cm2 and 45.2 and 37.6 ml/cm2, respectively. Overall, this experiment indicated that low concentrations of essential oils were found to have a significant effect on houseflies, but not parasitoids. Thus, it can be used in combination in Integrated Pest Management (IPM) programs to provide effective results.

Keywords: Bioinsecticide, vector insect, IPM, larvicide, parasitoid, housefly

ABSTRAK

Perosak dan vektor serangga menyebabkan kesan besar kepada ekonomi dan gangguan kesihatan. Kerintangan serangga dan bioakumulasi merupakan kelemahan utama dalam kawalan secara kimia ke atas serangga perosak. Kami mengkaji larvasid dan kesan perencatan minyak pati *Citrus sinensis* (L.) Osbeck dan *Mentha pulegium* Linnaeus ke atas pupa Lalat Rumah, *Musca domestica* Linnaeus, 1758. Kesan penggunakan minyak pati ke atas parasitoid utama Lalat Rumah, *Muscidifurax raptor* Girault & Sanders, 1910 juga turut dikaji. Kedua-dua minyak pati tersebut memunyai kesan signifikan ke atas aktiviti insektisid larva dan pupa Lalat Rumah. Bacaan LC_{50} *C. sinensis* dan *M. pulegium* adalah pada larva 3.93 dan 0.71 ml/cm2, serta untuk pupa 0.41 dan 0.23 ml/cm2 masing-masing. Menariknya, parasitoid adalah kurang terkesan dengan penggunaaan minyak pati dari tamanam berbanding Lalat Rumah. Selepas pendedahan 24 dan 48 jam, nilai LC_{50} menggunakan minyak pati *C. sinensis* dan *M. pulegium* yang didapati ialah 71.2 dan 52.6 ml/cm2 serta 5.2 dan 37.6 ml/cm2, masing-masing. Secara keseluruhannya, eksperimen ini menunjukkan bahawa kepekatan minyak pati rendah memberikan kesan signifikan kepada Lalat Rumah, tetapi tidak kepada parasitoid. Oleh itu, ia boleh dijadikan gabungan dalam program Pengurusan Perosak Bersepadu (PPB) untuk memberikan kesan yang efektif.

Katakunci: Bioinsektisid, serangga vektor, PPB, larvisid, parasitoid, lalat rumah

INTRODUCTION

Housefly; *Musca domestica* has the potential to serve as a mechanical vector for various diseases. There is evidence that they transmit nearly 100 diseases among human and animal populations, including bacterial infection, protozoan infection, helminthiasis, and viral infection (Malik et al. 2007). It was found that houseflies carried a deadly strain of *E. coli* in Japan (Sasaki et al. 2000; Shono & Scott 2003). *M domestica* is known to transmit several types of viruses, including polio, coxsackievirus, and enterovirus (Graczyk et al. 2001). The most effective method of controlling the transmission of vector-borne infections is to control their vectors (Graham et al. 2009; Mathew et al. 2009).

In the case of houseflies, chemical insecticides are commonly used (Hinkle & Hogsette 2021). Even though the widely used chemical method is effective, it has some major disadvantages that make it practically impossible to control insects (Göldel et al. 2020). The broad and improper use of chemical insecticides increases the probability of pest resistance (Ziaee et al. 2020). The use of synthetic pesticides has led to an increase in resistance among houseflies, long-term side effects among nontarget species, and environmental pollution1. (Acevedo et al. 2009; Nivsarkar et al. 2001; Kristensen & Jespersen 2003). Environmental problems caused by synthetic insecticides include the development of resistant insect strains, ecological imbalances, and harm to mammals (Maheswaran et al. 2008). Therefore, a continuous need to develop biologically active plant materials for larvicides exists, which will help minimize the accumulation of harmful residues in the environment, thereby reducing the hazards to humans and other organisms (Rahuman et al. 2009). Considering that plant extracts have the fewest side effects on natural enemies, there has been increasing interest in evaluating their insecticidal efficacy (Giunti et al. 2022; Nascimento et al. 2022). Also, several essential oils have been evaluated against *M. domestica* (Giunti et al. 2022; Singh & Singh 1991). Plant extracts and essential oils have been reported to be effective against houseflies (Rahuman et al. 2008). It was attempted to assess whether essential oils of *C. sinensis* and *M. pulegium* were larvicidal and inhibited pupal development against *M. domestica* in the present research.

MATERIALS AND METHODS

Plant Essential Oil

Citrus sinensis and *M. pulegium* essential oils were obtained from Shamim Daru Company (IRAN, Tehran) In order to be more stable, the essential oils of these two plants were selected based on fat. The profile analysis of the effective compounds in these essential oils was also provided by Shamim Daru Company.

Bioassay of Plant Essential Oil on Houseflies Larvae
In the bioassay test, each of the prepared plant essential oils was mixed with half a milliliter of acetone and after spraying on a filter paper, it was placed in a Petri dish. Then the diet containing 5 grams of wheat bran diet, 10 ml of water was added to the Petri dish. The treated filter paper was air-dried for 5 min before placing the larvae. Then 20 larvae (second instar) were placed on a filter paper in a Petri dish. An acetone spray was applied to the filter paper in the control sample. In the last stage, the mortality of the larvae was evaluated (Ahmadi et al. 2022).

Bioassay of Plant Essential Oil on Houseflies Pupae
For each bioassay experiment, 20 pupae were placed on a filter paper in the petri dishes. The determined concentrations of both essential oils were prepared in microliters per square centimeter and sprayed on the pupae. The treated filter paper was air-dried for 5 minutes before introducing the pupa. Control Petri were sprayed with acetone only. The percentage of reduction in the rate of emergence or inhibition of adults was done based on the method of Ahmadi et al. (2022).

Bioassay of Plant Essential Oil on Parasitoids
Filter papers were immersed in the determined concentrations of plant essential oil for 3 seconds and then exposed to the open air to dry completely. Filter papers were transferred into test tubes. 10 parasitoids were transferred to each of the test tubes. A solution of ten percent water and sugar was placed inside each of the test tubes for feeding purposes, and the lids of the test tubes were covered with a cloth net. Mortality was recorded after 24 and 48 hours.

In order to determine the exact amount of spraying of essential oils, the spray tower in the laboratory of Isfahan University of Technology was used. The experiments were done in three replicate and carried out under conditions of temperature of 25±2 and humidity of 62±2.

Statistical Analysis
Calculation of LC_{50} values and data analysis were done using POLO-PC and SAS 9.1 software. The comparison charts were made by the original 2016 64 Bit software.

RESULTS

Bioassay of Plant Essential Oil on Housefly Larvae
The larvicidal effect of the plant essential oils studied in this experiment was determined by dose- and time-dependent toxicity. Mortality of larvae with determined concentrations was significant (F=2.79, $P<0.05$). The LC_{50} for *C. sinensis* essential oil was recorded between 93.3 and 0.71 and LC_{90} between 24.7 and 86.1 (Table 1). The LC_{50} for *M. pulegium* essential oil was 0.41 to 0.23 and LC_{90} was recorded between 0.86 and 0.36 (Table 2). LT_{50} for *C. sinensis* essential oil was recorded between 5.8 and 2.3 and for *M. pulegium* essential oil between 4.8 and 1.3. The results of the mortality probit chart show that with the increase in the concentration of both essential oils, the mortality rate increased on different days (Figure 1 & 2).

Table 1. Bioassay of the lethal effect of *C. sinensis* essential oil on house flies larvae mortality

Days	**LC95 (µL/cm²; 95% CI)**	**LC50 (µL/cm²; 95% CI)**	**χ^2 (df)**
First day	7/24 (4/14-12/18)	3/93 (2/30-6/15)	11/29 (13)
Second day	5/54 (3/52-8/79)	2/55 (1/73-7/39)	12/25 (13)
Third day	3/24 (2/41-5/49)	1/28 (0/95-1/88)	9/28 (13)
Fourth day	1/86 (1/50-2/56)	0/71 (0/51-0/93)	4/68 (13)

Table 2. Bioassay of the lethal effect of *M. pulegium* essential oil on house flies larvae mortality

Days	**LC95 (µL/cm²; 95% CI)**	**LC50 (µL/cm²; 95% CI)**	**χ^2 (df)**
First day	0/86 (0/75-1/06)	0/41 (0/35-0/45)	9/75 (13)
Second day	0/56 (0/45-0/65)	0/28 (0/23-0/31)	5/61 (13)
Third day	0/38 (0/33-0/55)	0/25 (0/22-0/27)	1/48 (13)
Fourth day	0/36 (0/32-0/39)	0/23 (0/18-0/24)	1/41 (13)

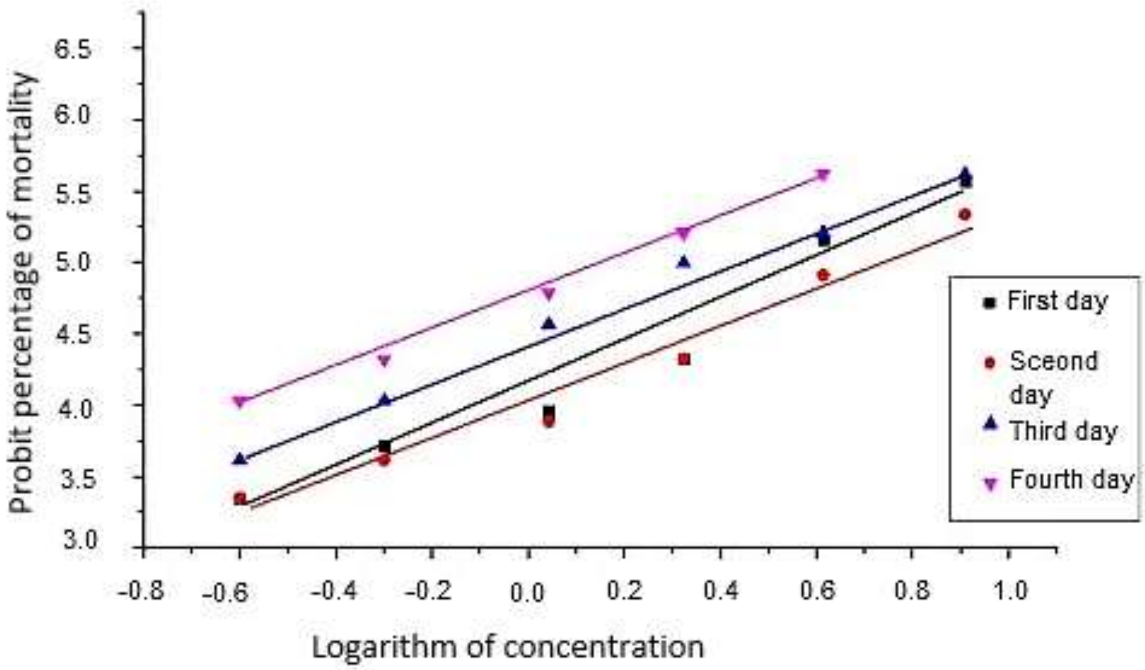

Figure 1. Probit plots of mortality-logarithm of *C. sinensis* essential oil concentration on housefly larvae

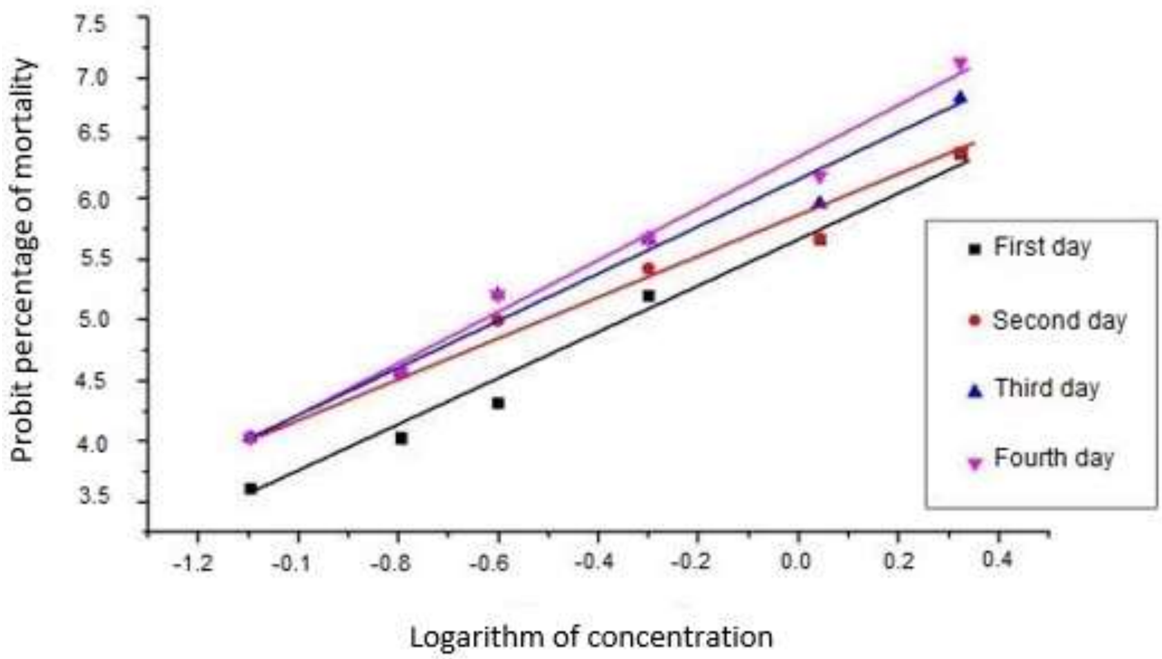

Figure 2. Probit plots of mortality-logarithm of *M. pulegium* essential oil concentration on housefly larvae

Bioassay of Plant Essential Oil on House Fly Pupae

The bioassay tests showed inhibition of housefly pupae from 72 to 27% for orange essential oil (Table 3) and 52 to 100% for oregano essential oil (Table 4). The regression diagram shows that there is a positive correlation between the concentration of both plant essential oils and the percentage inhibition of pupae (Figures 3 & 4).

Table 3. Bioassay of the lethality and inhibitory effect of *C. sinensis* essential oil on the mortality of house fly larvae and pupae

Concentrations of Essential Oils	Average Lethal Time for Larvae (LT_{50})	Percentage Inhibition Rate of Pupae
0/16	5/2	27/3
0/25	5/8	36/4
0/50	4/9	54/5
1/01	3/3	59/1
2/01	2/3	72/1

Table 4. Bioassay of the lethality and inhibitory effect of *M. pulegium* essential oil on the mortality of house fly larvae and pupae

Concentrations of Essential Oils	Average Lethal Time for Larvae (LT_{50})	Percentage Inhibition Rate of Pupae
0/16	4/8	52/1
0/25	3/1	70/4
0/50	2/9	87/1
1/01	2/6	90/1
2/01	1/3	100

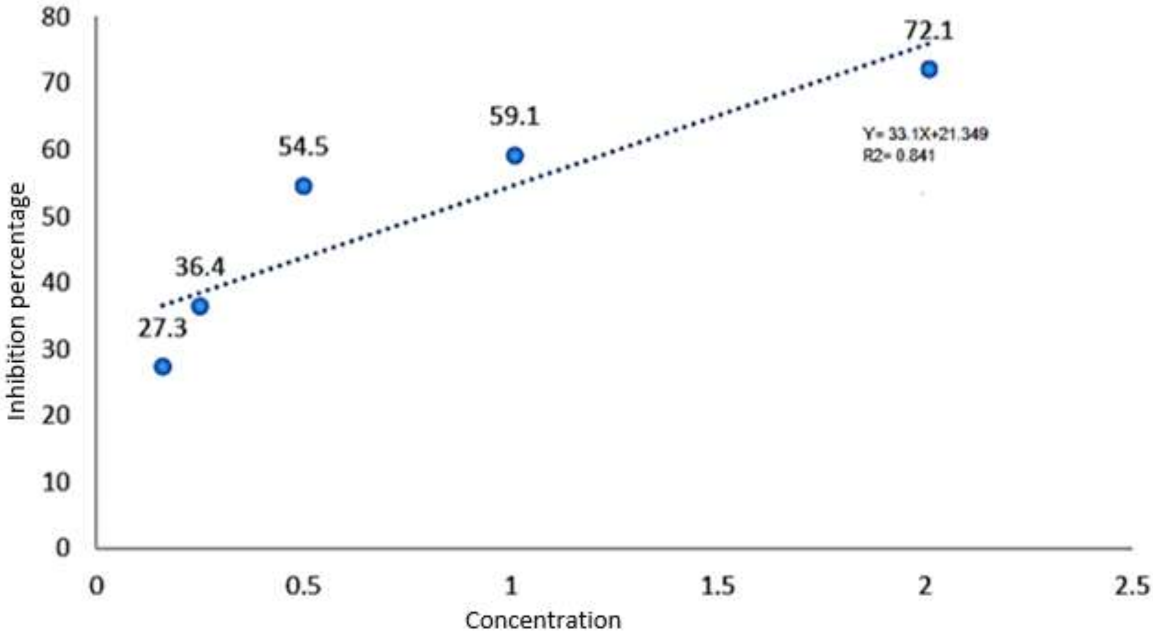

Figure 3. Regression of different concentrations of *C. sinensis* essential oil and pupal inhibition percentage in housefly

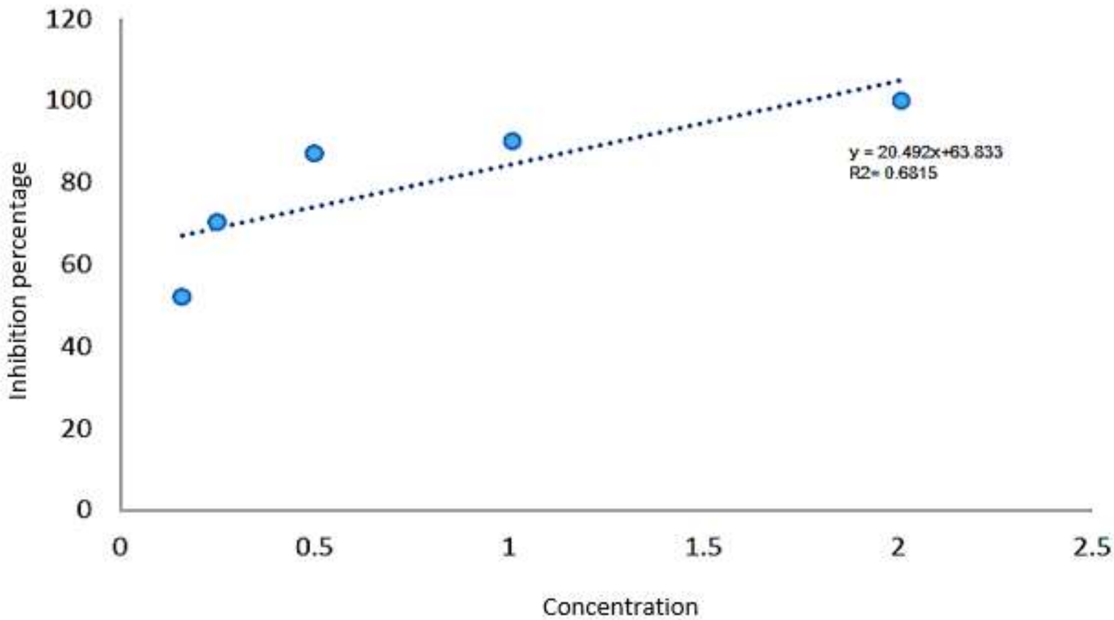

Figure 4. Regression of different concentrations of *M. pulegium* essential oil and pupal inhibition percentage in housefly

Bioassay of Plant Essential Oil on The Parasitoid *Muscidifurax raptor*

Bioassay for M. raptor parasitoid with *C. sinensis* essential oil with different tested concentrations was significant (F=3.23, $P<0.05$). The lethal concentration of LC_{50} and LC_{95} in the exposure period of 24 hours was reported as 2.71 and 2.92, respectively. While 6.52 and 7.70 were recorded during 48 hours (Table 5). Bioassay for this parasitoid with *M. pulegium* essential oil with different tested concentrations was significant (F=3.23, $P<0.05$). The lethal concentrations of LC_{50} and LC_{95} in the 24-hour exposure period were reported as 2.45 and 9.54, respectively. While during 48 hours it was recorded 6.37 and 7.47 respectively (Table 6). The results of the mortality probit chart show that with the increase in the concentration of both essential oils examined in this research, the mortality rate increased on different days (Figures 5 & 6).

Table 5. Bioassay of the lethal effect of *C. sinensis* essential oil on the mortality of the parasitoid *Muscidifurax raptor*

Days	**LC_{95} (µL/cm^2; 95% CI)**	**LC_{50} (µL/cm^2; 95% CI)**	**χ^2 (df)**
First day	92/2 (83/45-109/14)	71/2 (66/69-78/51)	6/12 (7)
Second day	70/7 (65/98-78/10)	52/6 (49/54-55/68)	6/18 (7)

Table 6. Bioassay of the lethal effect of *M. pulegium* essential oil on the mortality of the parasitoid *Muscidifurax raptor*

Days	**LC_{95} (µL/cm^2; 95% CI)**	**LC_{50} (µL/cm^2; 95% CI)**	**χ^2 (df)**
First day	54/9 (51/71-58/14)	45/2 (43/69-51/1)	7/24 (7)
Second day	47/7 (44/36-49/45)	37/6 (35/4-39/4)	7/97 (7)

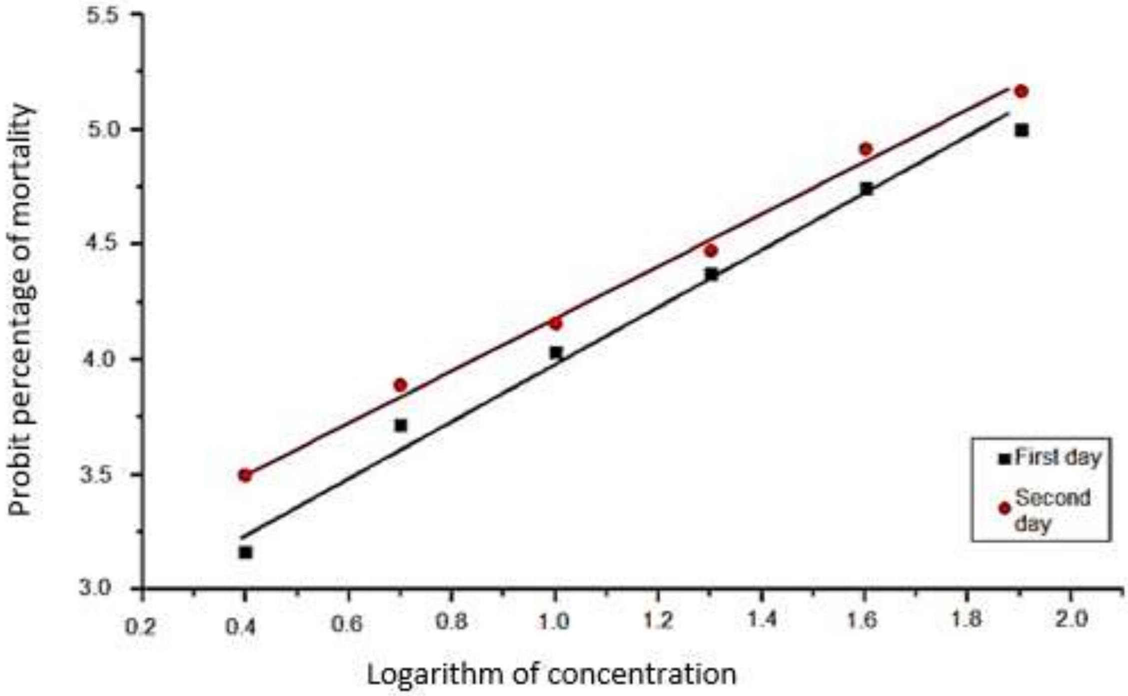

Figure 5. Probit plots of mortality-logarithm of *C. sinensis* essential oil concentration on parasitoid

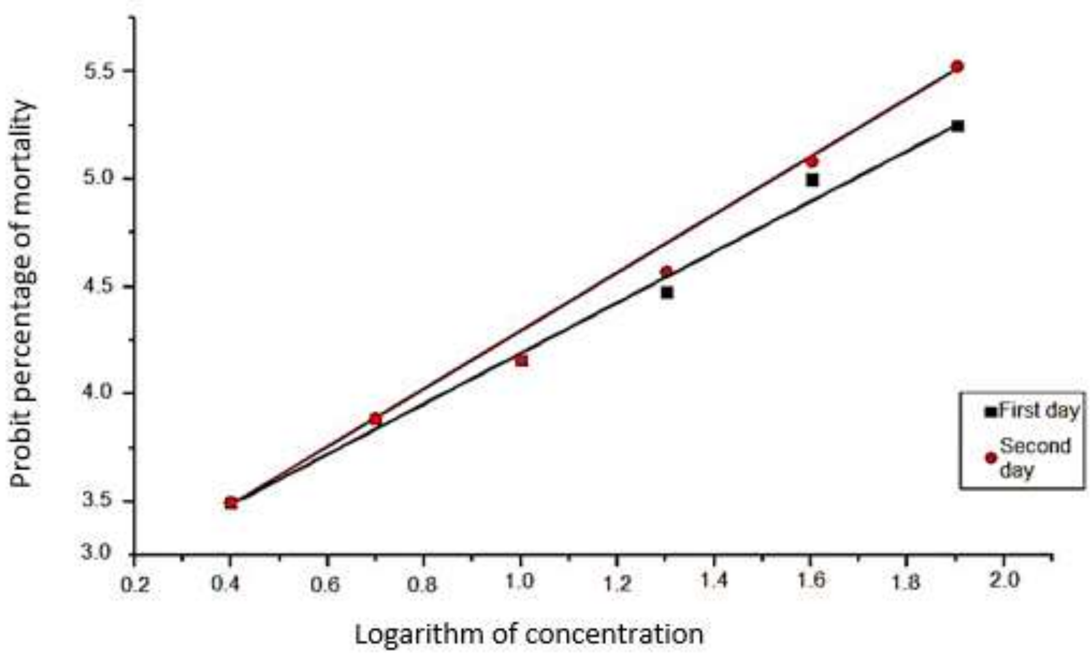

Figure 6. Probit plots of mortality-logarithm of *M. pulegium* essential oil concentration on parasitoid

DISCUSSION

In recent years, bioinsecticides, particularly those derived from plants, have increasingly been evaluated in controlling the population of insects that pose a threat to human health (Siriwattanarungsee et al. 2008). The natural products of plants and their derivatives offer alternative insecticides because they contain a rich source of bioactive chemicals that are helpful in the control of insects (Khalaf et al. 2009). The toxic effects of plant extracts and dipterans have been the subject of numerous studies (Muhammed et al. 2022; Nisar et al. 2021). The result of the present work illustrated the significant insecticidal activity of plants essential oils against larvae and pupae of houseflies. The LC_{50} of *C. sinensis* and *M. pulegium* essential oils were determined to be for larvae 3.93 and 0.71 ml/cm2, and for pupa 0.41 and 0.23 ml/cm2 respectively. The parasitoids were less affected by plant-applied essential oils compared to houseflies. After 24 and 48 hours, the LC_{50} for *C. sinensis* and *M. pulegium* essential oils was found to be 71.2 and 52.6 ml/cm2 and 45.2 and 37.6 ml/cm2, respectively. In this experiment, low concentrations of essential oils were found to have a significant impact on houseflies, but were not harmful to parasitoids. With evaluating contact toxicity and fumigation bioassays, the insecticidal activity of essential oil *M. pulegium* and *C. sinensis* against houseflies larvae and pupae have been shown. The significant activity of sinensis essential oil against larvae and pupae of houseflies, which leads to its use as an environmentally friendly method of controlling houseflies, have been determined (Bora et. 2020; Hu et al. 2017; Kumar et. 2012; Salem et al. 2018; Ziaee et al. 2020). Furthermore, essential oils have shown to be highly selective particularly when used as fumigants, which does not affect parasitoid behavior. Their residues usually appear harmless after one week and do not cause sublethal effects against parasitoids (González et al. 2013).

CONCLUSION

The essential oils have been found to have insecticidal and repellent properties, which can be used to deter houseflies from entering a space, as well as killing houseflies that may already be present. Additionally, these essential oils are ecofriendly, meaning they are not harmful to the environment, making them an ideal candidate for a housefly management program. This study found that these essential oils could be useful for an ecofriendly housefly management program and can be evaluated for their residual value and economic viability. Studies on the active principles of these plants essential oils may offer new methods for developing a housefly control product.

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Conflict of Interest
The authors declare that they have no conflict of interest.

Ethics Declarations
No ethical issue required for this research

Data Availability Statement
This manuscript has no associated data

Authors' Contributions
MK and AJZ conceived this research and designed experiments; AJZ, EM and MR participated in the design and interpretation of the data; MK performed experiments and analysis; MK wrote the paper and AJZ participated in the revisions of it. All authors read and approved the final version of this manuscript.

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