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Insecticidal, Antifeedant and Repellent Effects of Five Essential Oils against Fall Armyworm, *Spodoptera frugiperda (J. E.Smith)*

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ABSTRACT

The current study aimed to assess the insecticidal effects, antifeedant and repellent activities of five essential oils; peppermint (*Mentha piperita*), cinnamon (*Cinnamomum verum*), neem (*Azadirachta indica*), camphor (*Cinnamomum camphora*) and lavender (*Lavandula angustifolia*) against the 4th instar larvae of the fall armyworm, *Spodoptera frugiperda*, under laboratory conditions, five concentrations of each oil, 10%, 5%, 2.5%, 1.25% and 0.625% (v/v) were used for these experiments. The toxicity results indicated that among all treatments, neem oil demonstrated the highest effectiveness, followed by cinnamon, peppermint, lavender and ultimately camphor oil with LC_{50} values recorded at 0.362, 0.729, 1.736, 5.704 and 11.578%, respectively. In case of antifeedant activities for the five oils it can be concluded that peppermint oil comes in first place as an antifeedant agent with feeding deterrence index (FDI) reached to 71.76% after 24h post-treatment followed by neem oil which recorded FDI reached to 58.97% followed by camphor, cinnamon oils and finally lavender oil as the FDI values lasted 40.21%, 24.67 and 10.34%, respectively. The repellency percentages of tested oils were increased based on exposure period and concentration, as neem oil had demonstrated the highest repellency percentage of 100% after 3h post-treatment at concentration 10% and after 24h of treatment at 5% concentration, while the cinnamon oil recorded 100% repellency after 24h at 10% concentration.

INTRODUCTION

Fall Armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a destructive insect responsible for significant reduction in maize production. FAW targets other plants in addition to maize as it is a polyphagous insect. It has become a significant lepidopteran pest which is characterized by its extensive distribution over the Americas, Asia, and Africa, as well as its biotic potential. Since some of the primary hosts are food crops from the Gramineae family, including corn, rice, wheat, sugar cane and sorghum, it is imperative to keep an eye on their presence and population expansion. (Goergen *et al*., 2016; Early *et al*., 2018; Sombra *et al* 2020 and Saleh *et al*., 2023).

Fall armyworms have typically been controlled with synthetic pesticides with different modes of action, including neonicotinoids and organophosphates, as well as genetically engineered (transgenic) plants (Botha *et al*., 2019). However, the extensive application of these synthetic insecticides has resulted in the development of resistance to the

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recommended ones, as well as a decrease in the populations of beneficial organisms and natural enemies. Due to these drawbacks, there was an urgent need to develop environmentally friendly methods for managing FAW by utilizing essential oils from aromatic plants alongside sustainable agricultural cultivation methods (Van den Berg and Du Plessis, 2022; Gutiérrez-Moreno *et al*., 2019 and Sisay *et al*., 2019; Alves *et al*., 2018; Ayil-Gutiérrez *et al*., 2018 and González-Castillo *et al*., 2018).

Essential oils (EOs) are botanical extracts that have evolved into plant defense mechanisms against insects and phytophagous mites. Due to their repellent, antifeedant, attractant, fumigant, and contact properties against a variety of insect pests, EOs have the potential to be novel pesticides (Isman, 2015; Campolo *et al*., 2018; Ma *et al*., 2020 and Jayaram *et al*., 2022).

The current investigation aimed to evaluate the insecticidal and sublethal effects of five essential oils peppermint, cinnamon, neem, camphor and lavender against the 4th instar larvae of the fall armyworm *S. frugiperda* under laboratory conditions.

MATERIALS AND METHODS

Insect Rearing:

Spodoptera frugiperda was obtained from Syngenta Company, Kaha Research Station, Egypt. The rearing method was described by Eldesouky *et al.,* (2024). Egg masses were incubated at 25±5 °C and 65±5% relative humidity in glass jars covered with muslin cloth. Once the eggs turned into a black coloration, indicating ready to hatch, fresh castor leaves (*Ricinus communis* L.) were supplied daily as a food source for the neonate larvae. 2nd instar larvae were then transferred to plastic containers (30 cm in diameter × 20 cm in height) to undergo their first molt and given fresh caster leaves every day. Upon reaching the last instar, the larvae were moved to uncontaminated containers, given moistened sawdust that was 4 cm thick, and allowed to pupate. Pupae were collected every day and placed on glass jars until the adult moths emerged, and the glass jar was changed every day. Moths were fed on 10% honey solution hung the glass jar and changed daily.

Tested Essential Oils:

Peppermint, cinnamon, neem, camphor, and lavender were acquired from the Oil Extraction Unit located at the National Research Center in Egypt.

Bioassay Tests:

Toxicity Test:

The experiment was carried out on the 4th instar larvae of *S. frugiperda* to estimate the effectiveness of the tested oils by employing the leaf dipping technique. Five concentrations of each oil 10, 5, 2.5, 1.25, and 0.625% (v/v) were prepared in distilled water with 10 ml/L of Tween-80 added as an emulsifier agent. The leaves of the castor bean were immersed in the above-mentioned concentrations for duration of 20 seconds for each oil and left till dried on filter papers for 30 s. The treated leaves were placed in glass jars (500 g) and coated with gauze. Leaves treated with water containing tween 80 were used as a control. In each jar there were 10 larvae. Each treatment was replicated three times.After 48 hours of feeding on treated leaves, until the end of the experiment, the larvae were fed on fresh, untreated leaves. Mortality percentages were recorded after 1, 2, 3, 4, 5, and 6 days, and adjusted using the Abbott formula (1925). The Ld-p line program was used to determine the LC_{25}, LC_{50}, and LC_{90} values in accordance with Finney (1971).

Antifeedant Activity Test:

The feeding inhibition effect of the five concentrations of each oil 10%, 5%, 2.5%, 1.25%, and 0625% on the 4th instar larvae of *S. frugiperda* was investigated. Leaves of the castor bean were dipped in each concentration and then left for air dry. The control leaves were treated with water containing only tween 80. Ten larvae (n = 30) were maintained in each of the three replicates. After 24 hours post-treatment, the rate of leaf consumption at which the treated and control leaves were recorded. The feeding deterrence index (FDI) was determined using the following formula (Rahman *et al.,* 2022):

$$FDI = [(C - T) / (C + T)] \times 100$$

Where C and T represent the weights of control and treated leaves that *S. frugiperda* consumed, respectively.

Repellency Activity Test:

The repellent effectiveness of five investigated EOs was assessed against the 4th instar larvae of *S. frugiperda*. For this purpose, a small petri dish cover (1 cm height and 6 cm diameter) containing ten 4th instar larvae of *S. frugiperda* and treated leaves (treated with the tested EOs with five concentrations as mentioned above) was inserted into a large one (5cm height and 15 cm diameter) contained water Afterward, the petri dish was wrapped. For the control, leaves were soaked in water containing Tween 80, and three replicates were conducted for each treatment. The number of larvae that left the petri dish and fell into the water in the large petri dish was counted 1, 2, 3 and 24 hours after treatment and the percentage of insect repellency was determined using the following formula (McDonald *et al.,* 1970 and Hasyim *et al.,* 2014):

$$PR= ((NC-NT)/ (NC+NT))*100$$

Where PR= Percent repellency
NC=Number of insects in control
NT=Number of insects in treatment

For each oil the value of repellency was estimated and allocated to repellency classes according to the scale of Jilani and Su (1983) and Hasyim *et al*., (2014); repellency classes from 0 to V: class 0 (PR ≤ 0.1%), class I (PR = 0.1 - 20%), class II (PR = 20.1 - 40%), class III (40.1 - 60%), class IV (60.1 - 80%) and class V (80.1 - 100%).

Statistical Analysis:

To evaluate the sublethal concentrations LC_{25}, LC_{50}, and LC_{90} of tested oils against *S. frugiperda* the Probit analysis was used (Finney, 1971).

RESULTS AND DISCUSSION

Toxicity of Tested Oils Against the Fourth Instar Larvae of *Spodoptera frugiperda:*

Data from treatment of the 4th instar larvae of FAW with the five tested oils; peppermint, cinnamon, neem, camphor, and lavender on antifeedant, and repellent activities under laboratory conditions, were tabulated in Figure 1 and indicated the following effects on the tested instar.

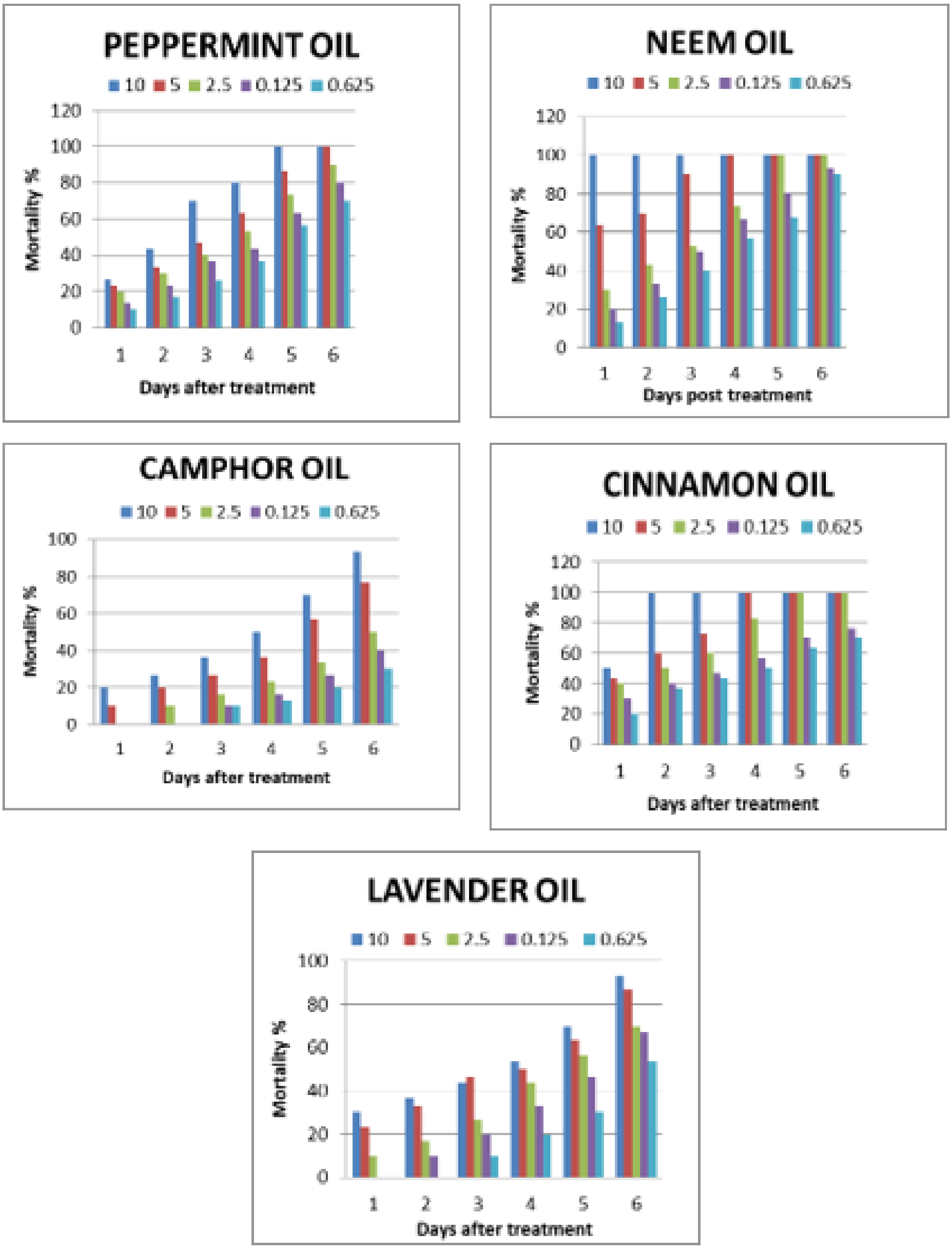

Fig. 1: Mortality percentages of the 4th larval instar of *Spodoptera frugiperda* treated with five tested oils.

Data in Figure 1 demonstrated the mortality of 4th larval instar of *S. frugiperda* treated with five plant oils: neem, peppermint, cinnamon, lavender, and camphor. Results indicated that the mortality percentage increased with higher concentrations and exposure time. Neem oil showed the most efficient oil on the larvae as the mortality reached 100% after 24 hour post-treatment at 10% concentration, and the cinnamon oil came in the second place with 100% mortality percentage after 2 days of treatment at the same concentration, followed with peppermint oil that recorded mortality percentage 100% after 5 days post-treatment, and finally place came lavender and camphor oils with same values of mortality percentage 93.33% after 6 days of treatment. The present results agreed with the results which were reported by Dimetry *et al.,* (2019) who evaluated the efficacy of these essential oils, peppermint, thyme, camphor, and sage, on the larvae of *Agrotis ipsilon*. Among the tested oils results indicated that peppermint demonstrated the highest effectiveness one, while thyme oil was the least effective, with LC_{50} values of 0.45%, 0.60%, 0.73% and 0.86 % for peppermint, camphor, sage and thyme, respectively after 96 hours of treatment. Also, Altaf *et al.,* (2022) evaluated the insecticidal effect of chlorantraniliprole (synthetic pesticide) on the larvae of FAW in maize compared with different concentrations of neem leaf extract (50 ppm and 100 ppm). Results indicated that these compounds had a significant ($P < 0.001$) impact on FAW larval mortality percentages. On the 5^{th} day after treatment, the recommended dosage of the insecticide chlorantraniliprole (50 ml/100 liters of water) demonstrated 71.0% FAW mortality, which increased to 82.0% on the 7^{th} day. FAW larval mortality was 59.0% on the fifth day of application and 72.0% on the seventh day when neem was applied at a higher concentration (100 ppm). These results suggest chlorantraniliprole as a useful pesticide for managing FAW larvae in maize crops; however, neem extract at a concentration of 100 parts per million also demonstrated good efficacy against this pest. Neem leaf extract can be applied in integrated pest management programs for FAW, as botanical solutions are generally safer for both humans and the environment compared to chemical pesticides.

Table 1: Lethal concentrations values of the five tested oils against the 4^{th} larval instar of *Spodoptera frugiperda* after 4 days post-treatment under laboratory conditions.

Treatments	Lethal concentrations % (v/v) and their 95% confidence limits			Slope ± SE
	LC_{25}	LC_{50}	LC_{90}	
Cinnamon	0.276 (0.152-0.501)	0.729 (0.401-1.324)	4.617 (2.542-8.387)	1.607±0.132
Peppermint	0.338 (0.142-0.805)	1.736 (0.730-4.130)	38.836 (16.327-92.374)	0.954±0.192
Neem	0.046 (0.015-0.147)	0.362 (0.114-1.149)	17.946 (5.649-57.005)	0.756±0.256
Lavender	0.727 (0.245-2.159	5.704 (1.919-16.951	286.076 (96.264-850.154)	0.758±0.241
Camphor	2.229 (0.880-5.646)	11.578 (4.570-29.329)	264.929 (104.584-671.115)	0.946±0.206

Data in Table 1 showed the LC_{25}, LC_{50}, and LC_{90} values of the various tested oils. Data showed that neem oil was the most effective one against *S. frugiperda* larvae of the 4^{th} instar, followed by cinnamon, peppermint, lavender and camphor. The values of LC_{50} were 0.362, 0.729, 1.736, 5.704, and 11.578% for neem, cinnamon, peppermint, lavender and camphor oils, respectively. Said *et al.,* (2019) investigated the effectiveness of five essential oils; lavender, camphor, mint, clove, and rosemary with four concentrations (5%, 10%, 15%, and 20%) on the 3^{rd} instar larvae of *Galleria mellonella*. The results revealed that eucalyptus, clove, and mint oils exhibited the lowest toxicity, with LC_{50} values (9.45%, 11.45%, and 13.61%, respectively). Conversely, lavender and rosemary oils showed higher toxicity, with LC_{50} values (7.11% and 6.45%, respectively). In a related study, Sharaby and El-Nojiban, (2015) demonstrated LC_{50} values for other five essential oils; garlic, mint, cumin, caraway, and parsley, highlighting differences in their insecticidal activity. The findings revealed that mint oil was the 2nd most effective oil among the tested oils. Its LC_{50} value as a contact toxicant was recorded (0.032) %. Moreover, the LC_{50} values for Its stomach toxicity against larvae and pupae were very similar, measured at 0.160% and 0.148%, respectively.

Our findings proved that the essential oils can be significant in managing pests as a botanical pesticides, and they can be good alternatives to synthetic pesticides because they are repellent, attractant, and fumigant, and have contact properties against a variety of insect pests as found by Rohimatun & Laba, (2013); Campolo *et al.,* (2018); Ma *et al.,* (2020) and Jayaram *et al.,* (2022); and Wangrawa *et al.,* (2024).

Antifeedant Activities of the Five Tested Oils against 4th larval instar of *Spodoptera frugiperda:*

The feeding deterrence index of neem, lavender, peppermint, cinnamon, and camphor on 4th larval instar of *S. frugiperda* after 24 hours post-treatment is shown in Table 2. From these results, it can be concluded that peppermint oil came in first place as an antifeedant agent with a feeding deterrence index reached 71.76% after 24 hours post-treatment at a concentration 10%. In the second place came neem oil which recorded FDI 58.97% followed by camphor, cinnamon oils and finally lavender oil as the FDI values lasted 40.21%, 24.67%, and 10.34% respectively, at 10% concentration.

Table 2: Antifeedant effect of five tested essential oils against the 4th larval instar of *Spodoptera frugiperda* after 24 h. of exposure under laboratory conditions.

Treatments	**Feeding Deterrence Index (FDI)**				
	Conc. % (v/v)				
	10	5	2.5	1.25	0.625
Neem	58.97	21.35	13.94	7.61	0.77
Cinnamon	24.67	21.77	11.39	7.28	0.29
Peppermint	71.76	47.74	28.34	18.33	13.82
Lavender	10.34	3.16	0.480	0.288	0.696
Camphor	40.21	17.13	13.57	5.31	0.383

According to Eldesouky *et al.,* (2024) who evaluated the toxicity and sublethal impacts of *C. citratus,* and *M. piperita* essential oils on the antifeedant, biological, and biochemical activities of *S. frugiperda* in controlled lab conditions. The results illustrated remarkable antifeedant activities on 3rd larval instars of *S. frugiperda* after 48 h post treatment at LC_{10} and LC_{30} concentrations. *C. citratus* EO (FDI = 35.17% and 43.06%) showed greater feeding deterrent effect than *M. piperita* EO (FDI = 30.67% and 39.01%) at LC_{10} and LC_{30} values, respectively. Essential oils can prevent feeding in addition to being toxic to various species. Jeon and Tak (2024) investigated the antifeedant and insecticidal effects of 29 widely available essential oils. According to the results of their chemical analysis, the impact of essential oil mixtures on feeding deterrence and habituation was also examined. Using no-choice tests, the FDI was assessed in binary mixtures at a 1:1 (w:w) ratio to estimate the combination effects of fennel sweet with other EOs. Among the tested mixes, the combination of marjoram and fennel sweet had the largest synergistic impact (X2=258.6), followed by cypress, cinnamon, sandal wood, ylang ylang, and basil oils (X2>40.0). Concerning the two active antifeedants/toxicants, fennel sweet oil's feeding deterrent was enhanced by clove bud oil (X2=34.8), however, lemongrass oil showed an additive interaction (X2=1.4). It's interesting to note that when combined with fennel sweet oil, neem oil, which had a noticeable feeding deterrent effect, demonstrated an antagonistic impact.

Repellency Activities of the Five Tested Oils against 4th Larval Instar of *Spodoptera frugiperda:*

The percent repellency of 4th instar larvae of *Spodoptera frugiperda* at concentrations, 0.625%, 1.25%, 2.5%, 5% and 10% of neem, peppermint, cinnamon, lavender, and camphor essential oils, after 1, 2, 3 and 24h of treatment are presented in Figure 2. The obtained findings indicated that the repellency percentage increased with increasing exposure time and concentration. The highest rate of repellency 100% was recorded at 10% concentration after 3 hours post-treatment for neem oil, and it recorded 100% after 24 hours at concentration 5% for the same oil belonged to class V, while the cinnamon oil recorded 100% repellency percentage after 24 hours at concentration 10% fitted class V. For the other oils they achieved lower repellency percentage than neem and cinnamon oils, thus reached 66.67% for peppermint oil lined class IV, 42.86% for lavender oil reached class III, and 25% for camphor oil belonged to class II, respectively at 10% concentration after 24 hours of exposure time.

Akter *et al.* (2024) determined the effect of black cumin, castor, neem and sesame on FAW, According to findings, the repellency rate among the treatments, 10% concentration of castor oil exhibited the highest repellency rate at 93.33% among the various treatments, whereas the 5% concentration of sesame oil demonstrated the lowest rate at 56.67%. Comparably, among the evaluated oil treatments, dosages, and interval durations, 10% castor oil lined in class V showed the highest statistical repellency (88.33%), while 5% sesame oil concentration fitted in class II showed the lowest (40.00%). Similarly, when oils, dosages, and interval times were examined for their repellent properties, 10% of castor oil showed the maximum repellency (80.00%), which is classified as class IV, while the lowest repellency observed, at 43.33%, was recorded with 5% sesame oil, which fell into class III. Additionally, Braga *et al.,* (2020) found that the metabolites in *A. indica* affected the repellent activity at various phases of *S. frugiperda*, which is consistent with the current study's findings. These results are also consistent with those of Shu *et al.,* (2021), who found that azadirachtin, a plant derived from neem, inhibited growth and caused mortality in *S. frugiperda* larvae.

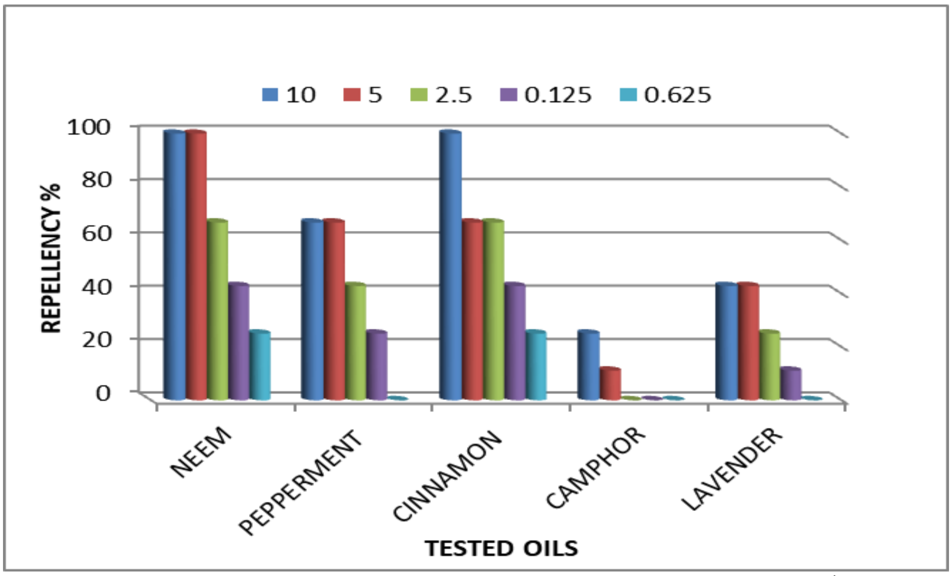

Fig.2: Repellent effect of five tested oils against *Spodoptera frugiperda* 4th larval instar after 24 h post-treatment at five concentrations under laboratory conditions.

CONCLUSION

Results of this study concluded that the EOs of neem, peppermint, lavender, camphor and cinnamon had significant toxic effects against the 4th instar larvae of *S. frugiperda*.

Furthermore, their antifeedant and repellent activities highlight their potential to be used to develop bioinsecticides, repellents, and antifeedant formulations based on the plant botanical oils. Therefore, we can consider these essential oils used in this study to be a natural alternative control method for *S. frugiperda.*

Declarations

Ethical Approval: This study has been approved by the Research Ethics Committee of Faculty of Agriculture, Benha University, Egypt (Approval No. REC – FOABU. 14/00037).

Competing Interests: The authors declare that they have no competing interests.

Authors' Contributions: MSKh did the conceptualization. MSKh contributed to the formal analysis. MSKh and HShR wrote the original draft. MSKh and HShR did the writing–review and approved the final manuscript. All authors read and approved the final manuscript.

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Availability of Data and Materials: All datasets analyzed and described during the present study are available.

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ARABIC SUMMARY

التأثيرات السامة، المانعة للتغذية و الطاردة لخمسة زيوت عطرية ضد دودة الحشد الخريفية
تحت الظروف المعملية*Spodoptera fragiperda (J. E.Smith)*

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هدفت هذه الدراسة إلى تقييم التأثيرات السامة، المانعة للتغذية، والطاردة لخمسة زيوت عطرية هي: زيت النعناع (*Mentha piperita*) ، زيت القرفة (*Cinnamomum verum*) ، زيت النيم(*Azadirachta indica*) ، زيت الكافور(*Cinnamomum camphora*) ، وزيت اللافندر(*Lavandula angustifolia*) ضد يرقات العمر الرابع لدودة الحشد *الخريفية Spodoptera frugiperda* تحت ظروف المعمل. تم استخدام خمس تركيزات مختلفة من كل زيت (10%، 5%، 2.5%، 1.25%، و0.625% (v/v).

أظهرت النتائج أن زيت النيم كان الأعلى فعالية كمبيد حشري، حيث سجل أقل قيمة للتركيز (LC_{50}) حيث بلغت 0.362%، تلاه زيت القرفة (0.729%)، ثم النعناع (1.736%)، فاللافندر (5.704%)، وأخيرًا زيت الكافور (11.578%).

وفيما يتعلق بتأثير الزيوت كمانعات للتغذية، أظهر زيت النعناع أعلى تأثير كمانع للتغذية، حيث بلغ نسبة (FDI) 71.76% بعد 24 ساعة من المعاملة، تلاه زيت النيم (58.97%)، ثم زيت الكافور (40.21%)، فزيت القرفة (24.67%)، وأخيرًا زيت اللافندر (10.34%).

أما التأثير الطارد، فقد تبين أن فعالية الزيوت تزداد بزيادة التركيز وطول فترة التعرض. حيث سُجلت أعلى نسبة طرد (100%) عند استخدام تركيز 10% بعد 3 ساعات من المعالجة، وكذلك عند تركيز 5% لزيت النيم بعد 24 ساعة. كما سجل زيت القرفة نسبة طرد بلغت 100% بعد 24 ساعة عند تركيز 10%.

تشير هذه النتائج إلى فعالية الزيوت العطرية المدروسة، خاصة زيت النيم والنعناع، كبدائل واعدة للمبيدات الكيميائية في إدارة الآفات الزراعية بطريقة آمنة وصديقة للبيئة.