Bulletin of the National Research Centre

RESEARCH

Open Access

Genetic expressions and evaluation of insecticidal activity of some essential oil and methomyl lannate 90% against *Spodoptera frugiperda*

Hanaa E. Sadek[1*], Huda H. Elbehery[1], Shereen Abdel-Hameed Mohamed[2] and T. E. Abd El-wahab[1]

Abstract

Background Fall armyworm (FAW) *Spodoptera frugiperda* is regarded as a major pest of various economic crops, their caterpillars are a highly destructive and have a wide host range. The application of traditional pesticides is the main strategies used for its control, that resulting to number of negative impacts of pesticides on the environment and development of pesticide resistance.

Methods This study's goal was to assess the insecticide potency of three essential oils [rosemary (*Rosmarinus officinalis* L.), lemongrass (*Cymbopogon citratus*) and Cinnamon (*Cinnamomum zeylanicum*)] and methomyl lannate 90% commercial insecticide to control *S. frugiperda* (fall armyworm) and their effects on expression of caspase-8 and inhibitor of apoptosis protein genes and expression of acetylcholinesterase (AChE) gene in fall armyworm (FAW).

Results The insecticidal activity against second larval instar of fall armyworm was evaluated with five concentrations (2.5%, 2%, 1.0%, 0.5% and 0.25%) for essential oil and four concentrations 0.4%, 0.2%, 0.15% and 0.05% for methomyl lannate 90%. The findings indicated that raising both essential oil concentrations and methomyl lannate 90% resulted in increased larval mortality at high concentration. The expression levels of Ache gene treated by low dose (0.3 µ/L) of methomyl lannate were increased (by 313%) significantly compared with the control but without significant differences.

Conclusions The tested essential oils showed insecticidal activity against the second larval instar of *S. frugiperda* death as a result of treatment with the tested oils having an effect on the genes that the pest uses to express critical processes (genes of apoptosis).

Keywords *Spodoptera frugiperda*, (*Rosmarinus officinalis* L.), Lemongrass (*Cymbopogon citratus*) and Cinnamon (*Cinnamomum zeylanicum*), Methomyl lannate 90%, Gene expression

Background

One of the very destructive pests posing a serious danger to global food security is the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (CABI 2018; FAO 2018), where its high reproduction rate, extensive migration and great dispersal ability all contributed to its economic importance. More so, their caterpillars are a highly destructive and have a wide host range; cotton, wheat, maize, sugarcane, rice, sorghum, beetroot,

*Correspondence:
Hanaa E. Sadek
hanyean2005@yahoo.com
[1] Pests and Plant Protection Department, National Research Centre, Giza, Egypt
[2] Microbial Genetics Department, National Research Centre, Giza, Egypt

soybean, cabbage, alfalfa, onion, grazing grasses, potato and tomato are among the crops the pest has been recorded on Montezano et al. (2018) and Chormule et al. (2019). Subsequently, many crops that support the lives of many people worldwide were severely damaged by this pest. The use of conventional pesticides is the method most frequently used to control pests in agriculture. However, serious negative impacts are associated with the application of conventional pesticides in controlling agricultural pests such as pesticide residues which harm the environment and pose a threat to public health due to food accumulation and ground water. Furthermore, generations that are resistant to these techniques have emerged as a result of their inconvenient use (Guedes et al. 2016; Badalamenti et al. 2021; Ahmed et al 2023).

Global demand for technologies that meet performance, selectivity, and safety standards is consequently rising, which raises awareness of different control strategies involving plant essential oils (González-Castillo et al. 2018; Ayil-Gutiérrez et al. 2018; Alves et al. 2018). Since they may reduce crop production costs, environmental harm and non-target effects, and the reliance on chemical pesticides, interest in using green pest control methods such as plant extracts and essential oils [EOs] has recently increased (Isman et al. 2020; Elbehery et al. 2022; Ibrahim et al. 2022). EO_S are mixtures of secondary metabolites, which have evolved to serve as plant defenses against insects and phytophagous mites (Isman 2015). Many researchers evaluated pesticidal plants for the control of *Spodoptera frugiperda* and supported several reporting promising results for using pesticidal plants to control fall armyworm (Isman et al. 2014). The insecticidal effective of various essential oils on *S. frugiperda* has been investigated by many authors. Likewise, previous studies have been confirmed the toxicity of rosemary pepper (*Lippia origanoides* Kunth) and lemongrass (*Cymbopogon citratus*) oils and their constituents against *S. frugiperda* (Barbosa et al. 2018; Sombra et al. 2020). It is vital to look for volatile substances that have insecticidal, deterrent, or inhibitory properties as this may improve the agriculture products, promote innovation, and expand the instruments for integrated pest management programs. So, this research sought to assess the effectiveness of essential oils [rosemary (*Rosmarinus officinalis* L.), Cinnamon (*Cinnamomum zeylanicum*) and lemongrass (*Cymbopogon citratus*)] and the commercial insecticide [methomyl lannate 90%] against larva of *S. frugiperda* in addition their effects on expression of caspase-8 and inhibitor of apoptosis protein (IAP) genes and acetylcholinesterase (AchE).

Apoptosis is pivotal for preserving tissue homeostasis; furthermore, it is responsible for destroying inglorious or harmful cells (Nirmala et al. 2015; Waldron et al. 2015). The endogenous caspase inhibitors known as inhibitor of apoptosis proteins (IAPs), which were initially discovered in the genomes of lepidopteran baculoviruses, prevent apoptosis (Ito et al. 2014). Apoptosis is negatively regulated by the protein inhibitor of apoptosis (IAP). Recent findings suggest that IAP genes could be prime candidates for RNA interference (RNAi)-mediated pest management. In the synaptic cleft, acetylcholine is hydrolyzed into choline and acetate by the enzyme acetylcholinesterase (AchE), preventing its buildup at the nerve terminal (Pang 2014; Jankowska et al. 2017; Chaudhari et al. 2021; Ruttanaphan et al. 2022; Sousa et al. 2020). As this neurotransmitter builds up, the nervous system becomes overactive, hyperactive, paralyzed, and eventually kills the insect (Abubakar et al. 2021; Chaudhari et al. 2021). Due to their complexity, essential oils [EOs] generally have effects that are distinct from those of their fundamental, pure components, making it difficult to determine their method of action (Devrjna et al. 2022). Additionally, EO components have the ability to bind non-specifically, and allostery modifies other sites on the enzyme (noncompetitive inhibitors; Devrjna et al. 2022; Lucia et al. 2021) or acts as competitive inhibitors of acetylcholine (ACh) binding to the active sites of the enzyme.

Methods

Chemicals and materials

The three tested essential oils of rosemary (*Rosmarinus officinalis* L.), lemongrass (*Cymbopogon citratus*) and cinnamon (*Cinnamomum zeylanicum*) were obtained from oil extraction unit, National Research Centre, Cairo, Egypt, whereas methomyl lannate 90% commercial insecticide was bought from Al Gomhoria Pharm. Ind., Cairo, Egypt.

Rearing of *Spodoptera frugiperda*

Spodoptera frugiperda were collected from Assuit Governorate, Upper Egypt, through regular field visitations of various farmers' summer harvests. The infested maize plant was collected and transported to pests and Plant Protection Department, National Research Centre to investigate. The collected larvae maintained in glass jar and provided with fresh castor leaves for nutrition until the pupation. The pupae were incubated till adult emergence. The adults were transferred to glass jar (1000 ml) containing 10% of honey solution as food supply, provided with paper towels for oviposition. The deposited eggs were collected daily and placed in rearing jar. After 4 days, the newly hatched larvae were provided daily with castor leaves as food. Fall Armyworm (FAW) were reared under laboratory condition for several generations before bioassay. The second larval instars of *S. frugiperda* were obtained from the colony for the experiments. The

insects rearing and experiments were carried out under laboratory conditions (26 ± 2 °C, 66 ± 5% RH).

Bioassays

The Dipping technique was applied to assess the insecticidal activity of the essential oils (EOs) of rosemary *Rosmarinus officinalis* L.), lemongrass (*Cymbopogon citratus*) and Cinnamon (*Cinnamomum zeylanicum*) at concentrations 2.5, 2, 1.0, 0.5 and 0.25 v/v % of EOs in water/Tween 80 mixture) prepared according to Sharaby (1988).The range of tested concentrations was selected based on previous studies (Sombra et al 2020; Elbehery and Ibrahim 2022). The fresh castor leaf was immersed for 20 s in each series of the tested concentrations of essential oils (EO_S) of each prepared oil separately then left to air dry. Distilled water with few drops of neutral detergent (tween 80) is considered as the negative control. Three replicates per treatment were executed and using five larva of second larval instar of fall armyworm (FAW) per replicate. The previous steps were repeated with using the commercial pesticide (methomyl lannate 90%) with concentrations 0.4%, 0.2%, 0.15% and 0.05%. Data of mortality was submitted to Probit analysis after 72 h of application in order to evaluate the median lethal concentration response. (LC50) of each treatment. The estimated LC50 values of each treatment were used to determine its effects on expression of *caspase-8* gene and inhibitor of apoptosis protein (IAP) genes and expression of *AChE* gene in armyworm (FAW).

Analyzing the expression of apoptotic genes and *Acetylcholinesterase* gene in *Spodoptera frugiperda*

Total RNA isolation

The usual TRIzol® Reagent extraction procedure was used to obtain total RNA from all of the insects' tissues (Invitrogen, Germany). Briefly, 50–100 mg of tissue samples were homogenized in 1 ml of TRIzol® Reagent. The homogenized material was then incubated at room temperature for 15 min. For each 1 ml of TRIzol® Reagent, 0.2 ml of chloroform were added. The samples were then vortexed violently for 15 s and incubated at room temperature for 3 min. The samples were centrifuged for 15 min at 4 °C for no more than 12,000 × g. Following centrifugation, the mixture was divided into a colorless upper aqueous phase and a lower red, phenol–chloroform phase. Only RNA continued to exist in the aqueous phase. As a result, the upper aqueous phase was carefully moved into a new tube without disturbing the interphase. By mixing isopropyl alcohol with the aqueous phase, the RNA was precipitated. For every 1 ml of the TRIzol® Reagent that was used for the first homogenization, isopropyl alcohol (0.5 ml) was added. The samples were then centrifuged at no more than 12,000 × g for 10 min at 4 °C after being incubated for 10 min at a temperature between 15 and 30 °C. Before centrifugation, the RNA was precipitated, which was frequently undetectable, and it created a gel-like pellet on the tube's side and bottom. The supernatant was removed completely. The RNA pellet was washed once with 1 ml of 75% ethanol. The samples were combined by vertexing and centrifuged for 5 min at a max of 7500 × g. The supernatant was removed and RNA pellet was air-dried for 10 min. RNA was dissolved by many times pipetting solution via a pipette tip in water that had been treated with diethylpyrocarbonate (DEPC). To digest DNA residues, total RNA was treated with 1 U of RQ1 RNAse-free DNAse from Invitrogen (Germany), then resuspended in DEPC-treated water. The 260/280 nm ratio (between 1.8 and 2.1) was used to determine the purity of total RNA. Ethidium bromide-stain analysis of the 28S and 18S bands by formaldehyde-containing agarose gel electrophoresis further ensured integrity. Aliquots of reverse transcription (RT) were used immediately. Otherwise, they were stored at -80 °C.

RT reaction for reversed transcription

Using the RevertAidTM First Strand cDNA Synthesis Kit (MBI Fermentas, Germany), the whole Poly (A) + RNA isolated from insect tissues was reverse-transcribed into cDNA in a total volume of 20 l. The master mix (MM), a reaction mixture, received 5 g of total RNA. The MM contains 10 mM of each dNTP, 50 M of oligo-dT primer, 40 M of $MgCl_2$, and 5x RT buffer (50 M KCl, 10 M Tris-HCl, pH 8.3), 20 U of ribonuclease inhibitor (50 U of M- MuLV reverse transcriptase and a 50 kDa recombinant enzyme that suppresses RNase activity were also included. After combining all samples, centrifuge them at 1000 g for 30 s in a thermocycler (Biometra GmbH, Göttingen, Germany). 10 min at 25 °C, 1 h at 42 °C, and lastly 5 min at 99 °C were used to complete the RT reaction. Prior to using the RT preparations for real-time polymerase chain reaction-based DNA amplification (RT-PCR), they were flash-cooled in an ice chamber.

Real-Time Polymerase Chain Reaction (RT-PCR)

The number of copies of the insects' cDNA were counted using the StepOneTM Real-Time PCR System from Applied Biosystems (Thermo Fisher Scientific, Waltham, MA, USA). The following ingredients were used to set up the PCR reactions: 6.5 ml of distilled water, 5 ml of cDNA template, 12.5 ml of 1 SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd.), 0.5 ml of 0.2 M sense primer, and 0.2 M antisense primer. The reaction programmed has three steps assigned to it. The first phase took place for three minutes at 95.0 °C. The second phase was made up of 40 cycles, with each cycle having three steps: (a) 15 s at 95 °C, (b)

Table 1 Apoptosis-related gene expression levels can be determined utilizing real-time quantitative PCR studies using certain primer sequences

Gene	Primer sequence (5′ to 3′)	GenBank (accession no)
β-actin	F: AGTCATTCAGTCATGCCCCA R: AACATACTGCCTCCGAACCA	MN044625.1
Caspase-8	F: AGACAATCCCAGCGACTTGA R:CCAGTTGGGGTTGTGCTTAG	XM_050703228.1
IAP	F: TGTGCGCAAATTGAACGGTA R: CGGCGAGTTCTTCAGGTTTT	AF186378.1

IAP Apoptosis protein gene

Table 2 Sequence of primers used in real-time quantitative PCR reactions to determine the expression level of *Ache* gene

Gene	Primer sequence (5′ to 3′)	GenBank (accession no)
β-actin	F: AGTCATTCAGTCATGCCCCA R: AACATACTGCCTCCGAACCA	MN044625.1
AChE	F:TGGCTTTGCAATGGGTGAAA R:CGGACAATGTACAGCTTCGG	KU985167.1

30 s at 55 °C, and (c) 30 s at 72.0 °C. The third stage was made up of 71 cycles that began at 60.0 °C and grew by roughly 0.5 °C every 10 s until reaching 95.0 °C. A melting curve analysis was carried out at 95.0 °C at the conclusion of each qRT-PCR to evaluate the effectiveness of the primers utilized. There was a distilled water control in each experiment. The sequences of particular primers for the genes utilized are reported in Tables 1 and 2 according to Macours et al. (2003).

A melting curve analysis was performed at 95.0 °C following each qPCR to assess the quality of the utilized primers. The 2−ΔΔCt method was used to determine the relative quantification of the target to the reference.

$$\Delta C_{\mathrm{Ttest}} = C_{\mathrm{T}}\left(\text{target, test}\right) - C_{\mathrm{T}}(\text{reference, test}),$$

$$\Delta \mathrm{CT}_{(\text{calibrator})} = \mathrm{C_T})\ \text{target, calibrator} - \mathrm{C_T}(\text{reference, calibrator}),$$

$$\Delta\Delta \mathrm{CT} = \Delta \mathrm{C_{T(Test)}} - \Delta \mathrm{C_{T\ (calibrator)}}$$

Statistical analysis

LC50 values determined by Finney (1971) Probit analysis method. Duncan's test ($P < 0.05$) was used to examine the collected data using one-way analysis of variance (ANOVA). Means ± SEM were used to express all data from biochemical and molecular genetics investigations. The Statistical Package for Social Sciences (SPSS 0.26 for Windows) was used to analyze the data.

Results

Bioassay

The results show that increasing the concentrations resulted in increased the larval mortality at high concentration. The highest concentration (2.5%) of Cinnamon and Rosemary oils caused the same percentage mortality (66.67%), while the Cinnamon oil caused 26.67% mortality when the larvae fed on castor leaf treated with the lowest concentration (0.25%) (Table 3, Fig 1). Likewise, these were supported by the findings that the Caspase-8 gene was overexpressed following treatment of *C. citratus*, but at lower levels compared to *R. officinalis* L. and *C. zeylanicum* and the control. The highest concentration (0.4%) caused 60% mortality after 3 days treatment (Table 4, Fig 2). This result proposes that components of essential oils [EOs] contribute to the activity against *S. frugiperda*. LC50 is calculated after three days from treatment and determined with 1.68 % for lemongrass and

Table 3 Insecticidal activity (%) of different tested oils against second larval instar of *S. frugiperda* post 72 h of treatment

Oil concentrations	Cinnamon				Lemon grass				Rosemary			
	Mean of larval mortality%											
	Mean ± SE											
2.5%	66.67	±	6.67	a	100.00	±	0.00	a	66.67	±	24.04	a
2%	46.67	±	17.64	ab	40.00	±	20.00	b	26.67	±	6.67	b
1%	40.00	±	11.55	ab	20.00	±	11.55	bc	20.00	±	0.00	b
0.5%	33.33	±	6.67	b	13.33	±	6.67	bc	13.33	±	6.67	b
0.25%	26.67	±	6.67	bc	6.67	±	6.67	bc	6.67	±	6.67	b
control	0.00	±	0.00	c	0.00	±	0.00	c	0.00	±	0.00	b
F	5.11**				13.157**				4.75**			

Means in a column followed with various letters are significantly different at 5% level of probability

** = Highly significant

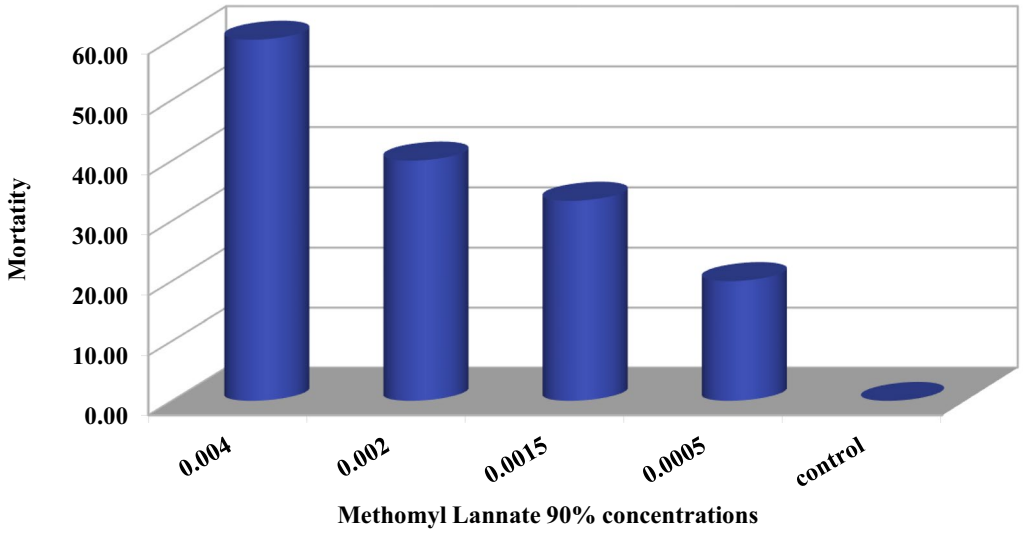

Fig. 1 Percentage mortality of second larval instar of *S. frugiperda* post 72 h of treatment with methomyl lannate 90%

Table 4 Insecticidal activity (%) of different methomyl lannate 90% insecticide against second larval instar of *S. frugiperda* post 72 h of treatment

Oil concentrations	Methomyl lannate 90%			
	Mean of larval mortality%			
	Mean ± SE			
0.40%	60.00	±	0.00	a
0.20%	40.00	±	0.00	b
0.15%	33.33	±	6.67	b
0.05%	20.00	±	0.00	c
control	0.00	±	0.00	d
F	56.50**			

Means in a column followed with various letters are significantly different at 5% level of probability

** = Highly significant

cinnamon, while 2.59% and 0.3% for both rosemary and methomyl lannate 90%. The obtained results confirmed that lemongrass (*C. citratus*) and cinnamon (*C. zeylanicum*) presented the highest insecticidal activity against the evaluated larvae followed by *R. officinalis* L. which had largest LC50.

Acetylcholinesterase (Ace) gene expression changes in insect tissues after exposure to various biological pesticide dosage (methomyl lannate 90%)

The *Acetylcholinesterase* (Ache) gene expression alterations were examined and shown in Fig. 3, which is a key enzyme in the nervous system of insects that ends nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetyl- choline. When compared to the control but without significant changes, the expression levels of the Ache gene were elevated (by 313%) in the treated by the concentration (0.3%) of methomyl lannate 90% pesticide. Two *acetylcholinesterase* genes, ace1 and ace2, were characterized from the transcriptome and genome of *S. frugiperda.* A spatial-temporal expression analysis revealed that Sfruace1 and Sfruace2 both exhibited elevated expression in the heads among all larval tissues and in third instar larvae among all developmental stages. Importantly, in all tissues and developmental stages analyzed, Sfruace1 expression is markedly higher than Sfruace2 expression. Spinosad treatment of the leaf worm *Spodoptera littoralis* led to the maximum level of change percentage of ACHE activity after 24 h and the lowest level after 72 h.

Apoptotic gene expression analysis using quantitative real-time PCR (RT-qPCR)

Figure 4 presents a summary of the findings from the RT-qPCR gene expression investigation. According to three different treatments, the genes encoding apoptotic proteins were identified (Fig. 4). *Cymbopogon citratus* (1.68%) had higher expression levels of the caspase-8 gene than *Rosmarinus officinalis L.* (2.59%) and *Cinnamomum zeylanicum* (1.67%) when compared to the control. However, the levels of expression alterations IAP gene (Fig. 5) highest levels of expression in control insects' tissues while *R. officinalis* L. 2.59% thin *C. zeylanicum* 1.67% and *C. citratus* 1.68% were lower than compare with control was highest. The expression levels of tested gene *Caspase-8* were overexpressed in *C. citratus* 1.68% but in lower levels than with control while the expression levels of tested gene IAP gene were overexpressed in

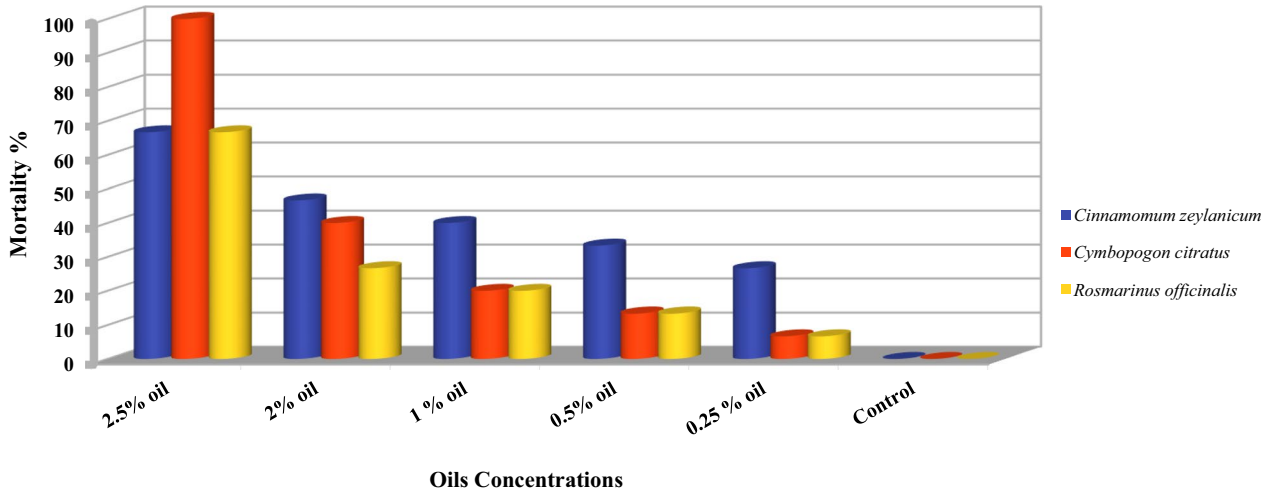

Fig. 2 Percentage mortality of second larval instar of *S. frugiperda* post 72 h of treatment with tested oils

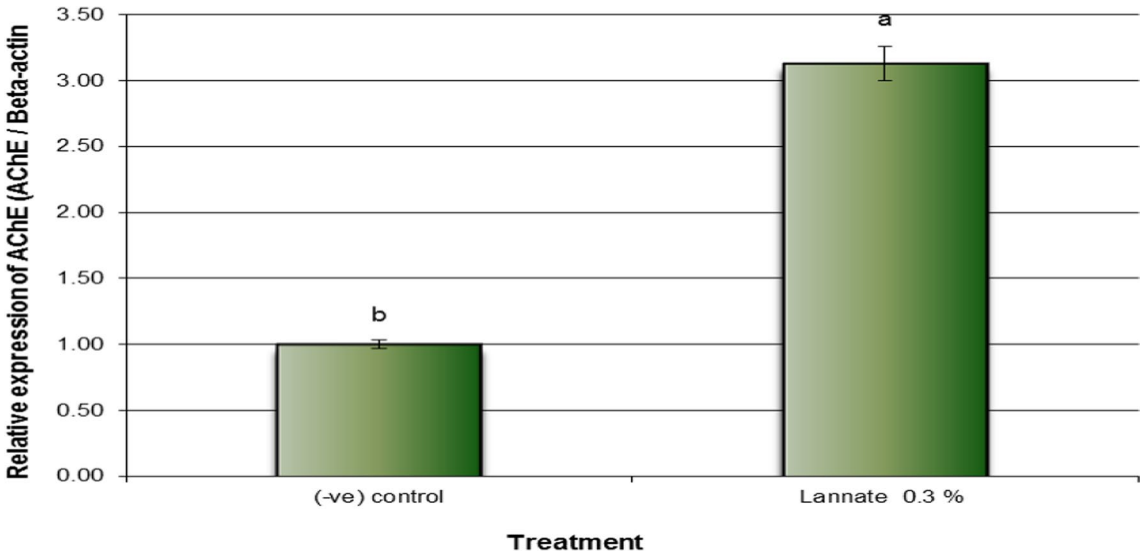

Fig. 3 Altered expression of the *AchE* gene in *S. frugiperda* larvae tissues after treatment with 0.3% methomyl lannate 90%. **a**, **b**: Mean values within tissues were significantly different (a: P0.01, b: P0.05) when superscript letters with opposite meanings were used. Data are shown as mean SEM

control compare with treatments Rosemary 2.59%, *C. citratus* 1.68% and *C. zeylanicum* 1.67% were lowest expression levels.

The larvae were exposed to a bacterium that affected the genes that express essential functions (genes of apoptosis), causing anomalies and PTM's death.

Discussion

Bioassay

Information regarding the biocidal ability of extracts and essential oils was supported by the results obtained utilizing rosemary, lemongrass, and cinnamon essential oils. The statistical analysis confirm that all EOs showed insecticidal efficacy on the second larval instars of pest, demonstrating the high mortality rates and quick action of the lemongrass (Cymbopogon citratus) essential oil, which resulted in 100% mortality after 3 days after treatment. Similarly, *Zengiber officinale* and *C. citratus* emerged as the most promising essential oils for the management of *S. frugiperda* larvae among the evaluated essential oils, according to Knaak et al. (2013). The Lemongrass (*C. citratus*) essential oil was toxic to *S. frugiperda*, corroborating results in other studies in which the pesticidal activity of essential oil for FAW from other

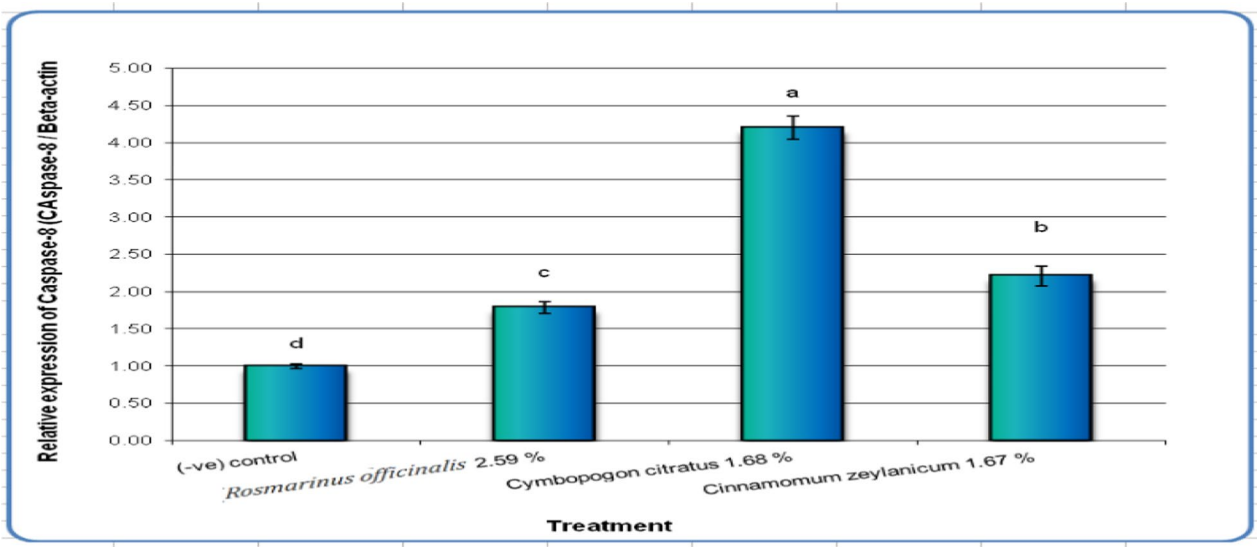

Fig. 4 Expression changes *Caspase-8* and *IAP gene* in *S. frugiperda* larvae tissues that had been exposed to various essential oils. The data is shown as mean SEM. **a**, **b**, **c**, **d** Mean values within tissues with different superscript letters demonstrated a difference that is statistically significant (a: P0.01, b: P0.05)

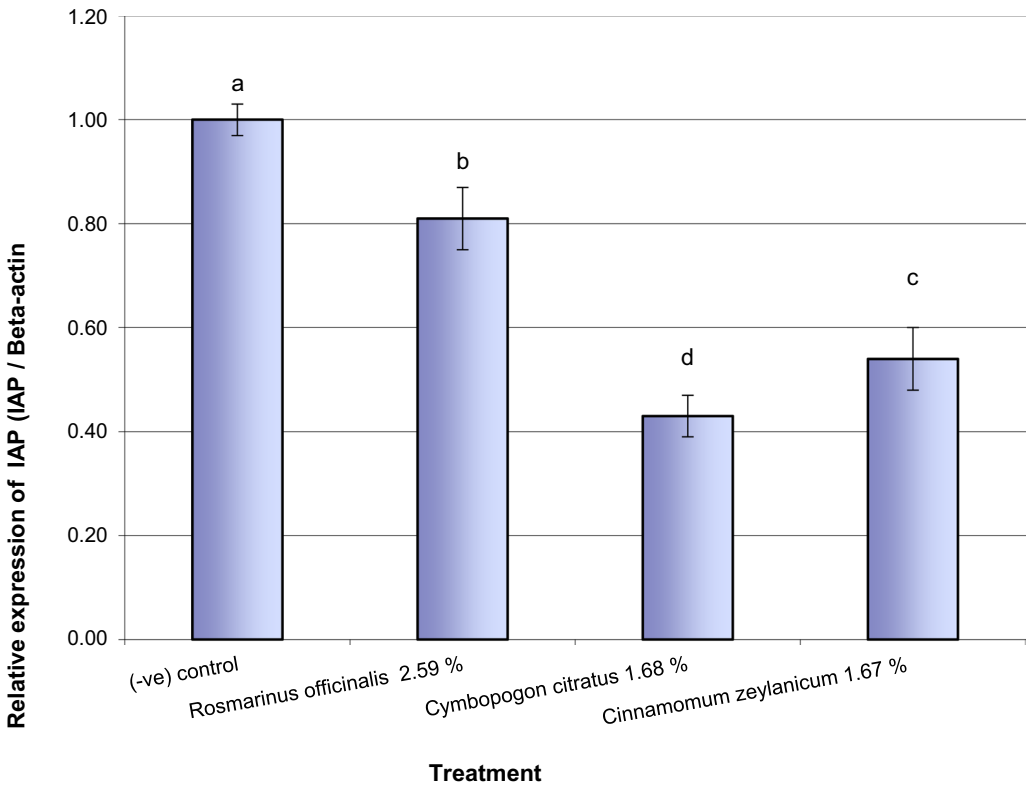

Fig. 5 *IAP gene* expression changes in *S. frugiperda* larvae tissues that have been exposed to various essential oils. The data are shown as mean SEM. **a**, **b**, **c**, and **d** Mean values within tissues with distinct superscript letters demonstrated a statistically significant difference (a: P0.01, b: P0.05)

plant species of the genus *Cymbopogon* was verified, such as *Cymbopogon flexuosus* (Oliveira et al. 2018) *Cymbopogon winterianus* (da Silva et al. 2015, Silva et al. 2016, Silva et al. 2017, Silva et al. 2018).

Monteiro et al. 2020 mentioned that the rapid action of some oils against insects is indicative of neurotoxicity, thus suggesting that the action of *Ocimum gratissimum* essential oil may be associated with a neurotoxic response. In the same vein, mortality increased as concentration of pesticide methomyl lannate 90% increased. Knaak et al. (2013) confirmed the findings of this investigation by demonstrating that the *C. citratus* oil produced the lowest LC50 (0.19 L/cm^2) when compared to the other treatments. According to Sombra et al. 2020, Rosemary pepper (Lippia origanoides) essential oil displayed increased larvicidal activity after application and reduced the mortality of *S. frugiperda* larvae from 60.41 to 0.1% after 6 h of treatment. methomyl lannate 90% Insecticide appeared toxicity to larva of *S. frugiperda* and had 0.3% LC50.

Acetylcholinesterase (Ace) gene expression changes in insect tissues after exposure to various biological pesticide dosage (methomyl lannate 90%)

This result is in line with (Boaventura et al. 2020, 2021), who reported that target site insensitivity is one of the fall armyworm's insecticide resistance mechanisms. This occurs when a small number of highly conserved point mutations in genes that encode for receptor target sites, like acetylcholinesterase (AChE), which develops resistance to organophosphates and carbamates, occur, in a study by Gao et al. (2023).

According to research by Megahed et al. (2013), Spinosad is a natural pesticide that is recognized as an alternative biocide; however, it is yet unknown why sublethal Spinosad exposure affects development.

Apoptotic gene expression analysis using quantitative real-time PCR (RT-qPCR)

These findings support (Mohamed et al. 2023) B (Bt::Bs1), C (Bt::Bl), and D (Bt::Bs2) which are three fusants. They were evaluated for the expression of genes associated with apoptosis in PTM and contrasted with A (Bt) and control. The findings demonstrated that tissues from the (PTM) after exposure to several pesticides that are biological in nature, including *Caspase-16, Dronc,* and *Dredd* genes, had considerably higher levels of gene expression in apoptosis-related tissues. The highest expression of apoptosis-related genes was seen in larvae tissues treated with the bio-insecticides Dronc, Dredd, Caspase-16, and Fusants D (Bt::Bs2).

Hussein et al. (2014) used real-time PCR to assess the expression levels of caspase-8 and telomerase reverse transcriptase. Tissue samples were used to generate the amplification plots of fluorescence intensity versus PCR cycle, the melting curves for TERT and caspase-8 mRNA, and caspase-8 expression was significantly lower in all groups than in the control group ($P=0.001$) in every instance. In terms of caspase-8 expression, Group III considerably outperformed, while Groups IV and V did not (p2 = 0.992, 1.000 and 1.000, respectively), Group II did (p2 = 0.031). Although group V's caspase-8 expression was significantly lower (p3 = 0.038), it did not differ from groups IV and III's significantly (p3 = 0.098). Groups V and IV did not differ from one another significantly (p4 = 0.996). According to Alam et al. (2016), the ethanolic extract of U. lactuca also appeared to be more effective than the aqueous one at lowering the expression of the apoptotic genes Bax and Caspase-3. According to their theory, U. lactuca's well-known total phenolics, flavonoids, and sulfated polysaccharides are the main catalysts behind this powerful antioxidant defense system. To counteract the deadly effects of irradiation, the commonly available green alga U. lactuca appears to be a promising antioxidant and anti-apoptotic agent. Feline panleukopenia disease was discovered in cat tissues by Awad et al. (2018) using qRT-PCR gene expression analysis, DNA fragmentation testing, and an apoptosis assay.

Conclusions

The assessment of insecticidal activity of EOs of rosemary (Rosmarinus officinalis L.), lemon grass (*Cymbopogon citratus*) and cinnamon (*Cinnamomum zeylanicum*) and methomyl lannate 90% commercial insecticide is crucial for implementing appropriate management strategies for *S. frugiperd*a (fall armyworm). All tested essential oils showed insecticidal activity on the second larval instars of pest, highlighting the fast action and high rate of pest mortality occurred by the lemon grass (*Cymbopogon citratus*) essential oil which caused 100% mortality after 3 days after treatment. The highest concentration (2.5%) of cinnamon and rosemary oils caused the same percentage mortality (66.67%). The expression levels of the tested gene caspase-8 were overexpressed in *C. citratus* by 1.68%, however at a lower level than with the control, whereas the expression levels of the tested gene IAP were overexpressed in the control. When compared to the control, the concentration (0.3%) of methomyl lannate 90% pesticide significantly (P0.05) and (P0.01) elevated the expression levels of the Ache gene (by 313%). As a result, an alternative to the traditional care of *S. frugiperda* is to use the tested essential oils.

Abbreviations
FAW Fall armyworm
AChE *Acetylcholinesterase*

Acknowledgements
Many thanks to National Research Centre for experimental facilities during this work.

Author contributions
This work was carried out in collaboration between all authors. HES involved in writing—review and editing, writing methodology, formal analysis, data curation, and conceptualization. HHE involved in conceptualization, methodology, investigation, validation, formal analysis, data curation, writing—review and editing, and writing—original draft. SAHM involved in investigation, methodology, writing—review and editing, and formal analysis. TEAE involved in resources, conceptualization, investigation, methodology, supervision, writing—original draft, and writing—review and editing. All authors have read and approved the manuscript.

Funding
This work was supported by National research center.

Availability of data and materials
All data are available in the article, and the materials used in this work are of high quality and grade.

Declarations

Ethics approval and consent to participate
The manuscript does not contain studies involving human participants, human data, or human tissue.

Consent for publication
Not applicable.

Competing interests
All authors declare that they have no competing interest.

Received: 28 November 2023 Accepted: 8 January 2024
Published online: 22 January 2024

References

Abubakar MU, Abubakar D (2021) Characterization of acetylcholinesterase from various sources: a mini re. J Envi Bio and Toxi 4(1):24–30. https://doi.org/10.54987/jebat.v4i1.581

Ahmed HA, Nassrallah AA, Abdel-Raheem MA, Elbehery HH (2023) Lemon peel essential oil and its nano-formulation to control *Agrotis ipsilon* (Lepidoptera: Noctuidae). Sci Rep 13(1):17922

Alam SS, Abd El-Kader HAM, Abd El-Rahim AH, Hamed SM, Saber AA (2016) The protective role of *Ulva lactuca* against genotoxic and biochemical effects induced by γ-irradiation in rats. Int J Pharma Sci Rev Res 37:40–48

Awad RA, Khalil WK, Attallah AG (2018) Feline panleukopenia viral infection in cats: application of some molecular methods used for its diagnosis. J Genet Eng Biotechnol 16(2):491–497. https://doi.org/10.1016/j.jgeb.2018.08.001

Ayil-Gutiérrez BA, Sánchez-Teyer LF, Vazquez-Flota F, Monforte-González M, Tamayo-Ordóñez Y, Tamayo-Ordóñez MC, Rivera G (2018) Biological effects of natural products against *Spodoptera* spp. Crop Prot 114:195–207. https://doi.org/10.1016/j.cropro.2018.08.032

Badalamenti N, Ilardi V, Bruno M, Pavela R, Boukouvala MC, Kavallieratos NG, Maggi F, Canale A, Benelli G (2021) Chemical composition and broad-spectrum insecticidal activity of the flower essential oil from an ancient sicilian food plant *Ridolfia segetum*. Agriculture 11:304

Barbosa MS, Dias BB, Guerra MS, da Costa VGH (2018) Applying plant oils to control fall armyworm *(Spodoptera frugiperda*) in corn. Aust J Crop Sci 12(4):557–562. https://doi.org/10.21475/ajcs.18.12.04.pne822

Boaventura D, Bolzan A, Padova FE, Okuma DM, Omoto C, Nauen R (2020) Detection of a ryanodine receptor target-site mutation in diamide insecticide resistant fall armyworm *Spodoptera frugiperda*. Pest Manage Sci 76(1):47–54. https://doi.org/10.1002/ps.5505

Boaventura D, Buer B, Hamaekers N, Maiwald F, Nauen R (2021) Toxicological and molecular profiling of insecticide resistance in a Brazilian strain of fall armyworm resistant to Bt Cry1 proteins. Pest Manag Sci 77(8):3713–3726. https://doi.org/10.1002/ps.6061

CABI 2018. *Spodoptera frugiperda* (fall armyworm) invasive species compendium. Wallingford, UK: CAB international. https://www.cabi.org/isc/datasheet/29810

Chaudhari AK, Singh VK, Kedia A, Das S, Dubey NK (2021) Essential oils and their bioactive compounds as eco-friendly novel green pesticides for management of storage insect pests: prospects and retrospect's. Env Sci Pollu Res 28:18918–18940. https://doi.org/10.1007/s11356-021-12841-w

Chormule A, Shejawal N, Sharanabasappa CM, Asokan R, Swamy HM, Studies Z (2019) First report of the fall Armyworm, *Spodoptera frugiperda* (JE Smith) (Lepidoptera, Noctuidae) on sugarcane and other crops from Maharashtra, India. J Entomol Zool Stud 7(1):114–117

da Silva JL, de Souza PE, Alves E, Pinto JEBP, Bertolucci SKV, Freitas MLO, Resende MLV (2015) Essential oil of *Cymbopogon flexuosus, Vernonia polyanthes* and potassium phosphite in control of bean anthracnose. J Med Plants Res 9(8):243–253. https://doi.org/10.5897/JMPR2014.5718

Devrnja N, Milutinović M, Savić J (2022) When scent becomes a weapon—plant essential oils as potent bioinsecticides. Sustainability 14(11):6847. https://doi.org/10.3390/su14116847

Elbehery HH, Ibrahim SS (2022) Cinnamon essential oil loaded β-cyclodextrin/gum Arabic nanoparticles affecting life table parameters of potato tuber moth, *Phthorimaea operculella* (Zeller). Bio Agri Bio 42:102349. https://doi.org/10.2174/1573413719666221202110952

FAO (2018) Integrated management of the fall armyworm on maize a guide for farmer field schools aaain Africa. http://www.fao.org/faostat/en/

Finney DJ (1971) Probit analysis, 3rd edn. Cambridge University Press, New York

Gao J, Gong LF, Wang HH, Zhao R, Xiao X, Tian XY, Gu SH (2023) Expression and functional analysis of ace1 and ace2 reveal their differential roles in larval growth and insecticide sensitivity in *Spodoptera frugiperda*. J Pest Sci. https://doi.org/10.21203/rs.3.rs-2404478/v1

González-Castillo JA, Quezada-D'Angelo TP, Silva-Aguayo GI, Moya-Elizondo EA (2018) Effect of saponins of *Quillaja saponaria* extracts in combination with *Pseudomonas protegens* to control *Gaeumannomyces graminis* var. *tritici* in wheat. Chil J Agri Res 78:378–390. https://doi.org/10.4067/S0718-58392018000300378

Guedes RNC, Smagghe G, Stark JD, Desneux N (2016) Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. Annu Rev Entomol 61:43–62

Hussein NAEM, El-Toukhy MAEF, Kazem AH, Ali MES, Ahmad MAER, Ghazy HMR, El-Din AMG (2014) Protective and therapeutic effects of cannabis plant extract on liver cancer induced by dimethyl nitrosamine in mice. Alex J Med 50(3):241–251. https://doi.org/10.1016/j.ajme.2014.02.003

Ibrahim SS, Abou-Elseoud WS, Elbehery HH, Hassan ML (2022) Chitosan-cellulose nanoencapsulation systems for enhancing the insecticidal activity of citronella essential oil against the cotton leafworm *Spodoptera littoralis*. Ind Crops Prod 184:115089. https://doi.org/10.1016/j.indcrop.2022.115089

Isman MB (2015) A renaissance for botanical insecticides? Pest Manag Sci 71:1587–1590. https://doi.org/10.1002/ps.4088

Isman MB (2020) Botanical insecticides in the twenty-first century: fulfilling their promise? Ann Rev Entomol 65:233–249. https://doi.org/10.1146/annurev-ento-011019-025010

Isman MB, Grieneisen ML (2014) Botanical insecticide research: many publications, limited useful data. Trends Plant Sci 19(3):140–145. https://doi.org/10.1016/j.tplants.2013.11.005

Ito H, Bando H, Shimada T, Katsuma S (2014) The BIR and BIR-like domains of Bombyx mori nucleopolyhedroviral IAP2 protein are required for efficient viral propagation. Bioch Biophys Res Com 454(4):581–587. https://doi.org/10.1016/j.bbrc.2014.10.132

Jankowska M, Rogalska J, Wyszkowska J, Stankiewicz M (2017) Molecular targets for components of essential oils in the insect nervous system: a review. Molecules 23(1):34. https://doi.org/10.3390/molecules23010034

Knaak N, Wiest SL, Andreis TF, Fiuza LM (2013) Toxicity of essential oils to the larvae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J Biopestic 6(1):49

Lucia A, Guzmán E (2021) Emulsions containing essential oils, their components or volatile semi chemicals as promising tools for insect pest and pathogen management. Adv Colloid Interface Sci 287:102330. https://doi.org/10.1016/j.cis.2020.102330

Macours N, Hens K, Francis C, De Loof A, Huybrechts R (2003) Molecular evidence for the expression of angiotensin converting enzyme in hemocytes of *Locusta migratoria*: stimulation by bacterial lipopolysaccharide challenge. J Insect Phys 49(8):739–746. https://doi.org/10.1016/s0022-1910(03)00110-0

Megahed MMM, El-Tawil MF, El-Bamby MMM, Abouamer WL (2013) Biochemical effects of certain bioinsecticides on cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera:Noctuidae). Res J Agri Biol Sci 9(6):308–317

Mohamed SAH, Kh AEAS, Moawad SS, Attallah AG (2023) Genetic improvement of some microorganisms to increase the effect of bio-control on the potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae). Egypt J Biol Pest Cont 33(1):12. https://doi.org/10.1186/s41938-023-00648-5

Monteiro IN, dos Santos MO, de Oliveira AKM, Favero S, Garcia NZT, Fernandes YML, Matias R (2020) Chemical analysis and insecticidal activity of *Ocimum gratissimum* essential oil and its major constituent against *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae). Res Soc Devel 9(11):e4999119787–e4999119787. https://doi.org/10.33448/rsd-v9i11.9787

Montezano DG, Sosa-Gómez DR, Specht A, Roque-Specht VF, Sousa-Silva JC, Paula-Moraes SD, Peterson JA, Hunt TE (2018) Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. Afr Entomol 26(2):286–300. https://doi.org/10.4001/003.026.0286

Nirmala X, Schetelig MF, Zimowska GJ, Zhou L, Handler AM (2015) Pro-apoptotic gene regulation and its activation by gamma-irradiation in the Caribbean fruit fly, *Anastrepha suspensa*. Apoptosis 20:1–9. https://doi.org/10.1007/s10495-014-1055-3

Oliveira ERD, Alves DS, Carvalho GA, Oliveira BMRGD, Aazza S, Bertolucci SKV (2018) Toxicity of *Cymbopogon flexuosus* essential oil and citral for *Spodoptera frugiperda*. Ciência e Agrotecnologia 42:408–419. https://doi.org/10.1590/1413-70542018424013918

Pang YP (2014) Insect acetylcholinesterase as a target for effective and environmentally safe insecticides. Advances in insect physiology. Academic Press, pp 435–494

Ruttanaphan T, Bullangpoti vol, (2022) The potential use of thymol and (R)-(+)-pulegone as detoxifying enzyme inhibitors against *Spodoptera litura* (Lepidoptera: Noctuidae). Phytoparasitica 50(4):913–920. https://doi.org/10.1007/s12600-022-00989-1

Sharaby A (1988) Effect of orange, *Citrus sinensis* (L.) peel oil on reproduction in *Phthorimaea operculella* (Zell.). Int J Trop Insect Sci 9:201–203

Silva SM, Cunha JPARD, Carvalho SMD, Zandonadi CHS, Martins RC, Chang R (2017) *Ocimum basilicum* essential oil combined with deltamethrin to improve the management of *Spodoptera frugiperda*. Ciência e Agrotecnol 41:665–675. https://doi.org/10.1590/1413-70542017416016317

Silva CTS, Wanderley-Teixeira V, Cunha FM, Oliveira JV, Dutra KA, Navarro DF, Teixeira AAC (2018) Effects of citronella oil (*Cymbopogon winterianus* Jowitt ex Bor) on *Spodoptera frugiperda* (JE Smith) midgut and fat body. Biotech Histochem 93(1):36–48. https://doi.org/10.1080/10520295.2017.1379612

Sombra KE, de Aguiar CV, de Oliveira SJ, Barbosa MG, Zocolo GJ, Pastori PL (2020) Potential pesticide of three essential oils against *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae). Chil J Agri Res 80(4):617–628. https://doi.org/10.4067/S0718-58392020000400617

Sousa PA, Neto J, Bastos MM, Aguiar AA (2020) Eugenol and Pulegone as potential biorational alternatives for *Trioza erytreae* (Hemiptera: Triozidae) control: preliminary results on nymphal toxicity and applicability on Citrus limon. J Nat Pesti Res 1:100004. https://doi.org/10.1016/j.napere.2022.100004

Waldron JA, Jones CI, Towler BP, Pashler AL, Grima DP, Hebbes S, Newbury SF (2015) Xrn1/Pacman affects apoptosis and regulates expression of hid and reaper. Biol Open 4(5):649–660. https://doi.org/10.1242/bio.201410199

Publisher's Note