

RESEARCH ARTICLE

GENOTOXICITY OF SOME PLANT ESSENTIAL OILS IN COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (LEPIDOPTERA: NOCTUIDAE): THE POTENTIAL ROLE OF DETOXIFICATION ENZYMES

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ABSTRACT

Increasing incentives for discovering effective and eco-friendly bio-insecticides seem to be a very important challenge nowadays. Plant essential oils play a promising role in integrated pest-management programs. In the present study, the insecticidal activities of six essential oils from clove (*Syzygium aromaticum*), crane's-bills (*Geranium* sp.), citronella (*Cymbopogon* sp.), dill (*Anethum graveolens*), cinnamon (*Cinnamomum camphora*) and basil (*Ocimum basilicum*) were investigated against the cotton leafworm, *Spodoptera littoralis* "Boisduval" (Lepidoptera: Noctuidae). The toxicity test revealed that all the tested essential oils have noticeable insecticidal activity. The basil oil was the most efficient against the 3rd instar larvae, with the lethal concentration (LC)₅₀=0.42%; while the dill oil showed higher toxicity than the other oils against the 5th instar larvae (LC₅₀=0.49%) after 48 hours of treatment. The impact of the tested essential oils on the acetylcholinesterase (AChE), carboxylesterase (CE), and glutathione S-transferase (GST) activities was also assessed. The basil oil induced the activity of AChE in both the 3rd and the 5th instar larvae. All tested oils elevated GST activity in the 5th instar larvae, except citronella oil. The dill oil decreased CE activity in both instars. Genotoxicity analysis using comet assay revealed that all assay parameters differed significantly, compared to the control. The maximum percentage of damaged DNA was detected in larvae treated with basil followed by dill, cinnamon, clove, citronella and crane's-bills, respectively. It was concluded that these essential oils could play a promising role in controlling *S. littoralis* and providing a biological alternative to synthetic insecticides in the control programs.

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INTRODUCTION

The cotton leafworm, *Spodoptera littoralis*, is a major pest that causes severe damage to a large number of economic crops in Egypt.

It attacks numerous economically-important crops such as cotton, rice, sugarcane, and wheat throughout the year decreasing crop yields up to 100% in greenhouses and

fields^[1]. In the past, control of lepidopterous insects depended intensely on the synthetic chemical insecticides, which pose risk on human health, causing environmental contamination in addition to increasing the elaboration of resistance to the most common insecticides that have been used effortfully for long-time^[1]. The search for safe and effective new strategies is becoming crucial with emphasis on botanical insecticides^[2]. Plant extracts, especially the essential oil fraction, have been advocated as possible control agents against various insect pests for being selective, biodegradable, and non-toxic products with lower impact to non-target organisms and environmentally safe^[3]. They represent secondary metabolites, including terpenoids, alkaloids or flavonoids^[4]. They were an efficient control method when integrated into control programs^[5]. Most of the essential oils establish intense larval toxicity, developmental delay, repellency, and feeding disorders to insects^[6]. As insects could not generally develop resistance to essential oils^[7], the later was evaluated intensively against plant chewing and sucking insect pests, stored product pests, health pests, and honey bee parasitic mites^[8].

Insects respond to essential oils' toxic action either through modulating detoxification enzyme activity or increasing target site insensitivity, as in nerve conduction enzyme "acetylcholinesterase (AChE)"^[9]. Thus, the estimation of enzyme activity may elucidate its insecticidal mechanism^[10]. The most common metabolic detoxification mechanisms involved AChE, glutathione S-transferase (GST), and carboxylesterase (CE)^[11]. Effect of essential oils on detoxification enzyme activities was evaluated in *Helicoverpa armigera*, *Trichoplusia ni*, and *Paederus fuscipes*^[7,9,11]. The activities of AChE, CE, and GST showed a possible link to oil treatment even at low-toxicity level^[7,9,11].

Considering the genotoxic effect of plant essential oils, the clove oil showed an effect on gametogenesis in *Spodoptera frugiperda* ovarioles, thus affecting its reproductive

capacity^[12]. DNA damage also was estimated in *Culex quinquefasciatus* using essential oil of *Psoralea corylifolia* with larvicidal and adulticidal effect^[13]. Moreover, DNA damage and mutation were detected in *H. armigera* and *Rhyzopertha dominica* treated with various plant oils^[14].

Ecotoxicological research is focusing on studying environmental risk assessments using the comet assay (alkaline single cell gel electrophoresis), which was approved by the Committee on Mutagenicity Guidelines of the UK Department of Health (COM). It is qualitatively and quantitatively used in genotoxicity studies to assess DNA damage upon exposure to insecticides^[15], and in environmental monitoring of pollutants^[16].

The present study aimed to investigate the insecticidal toxicity of six essential oils against *S. littoralis*, with special reference to their toxic effects on the DNA and the detoxifying enzyme activities of *S. littoralis* to clarify their insecticidal mechanism. Data obtained from this study can explain to what extent we can use essential oils successfully as environmentally safe control agents in integrated pest-management (IPM) programs.

MATERIAL AND METHODS

Collection and rearing of *S. littoralis*

A laboratory susceptible strain of the cotton leafworm, *S. littoralis* was obtained as egg masses from the research division of the cotton leafworm, Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), Giza, Egypt. It was reared on castor bean leaves ever since free from any insecticidal exposure for more than 10 generations under laboratory conditions ($26 \pm 2^\circ\text{C}$, $65 \pm 10\%$ relative humidity and 8:16 light: dark photoperiod).

Plant essential oils

Six essential oils from clove (*Syzygium aromaticum*), crane's-bills (*Geranium* sp.), citronella (*Cymbopogon* sp.), dill (*Anethum graveolens*), cinnamon (*Cinnamomum camphora*), and basil (*Ocimum basilicum*) were used in this investigation. They were

obtained from Delta Aromatic Co., Ltd, Giza, Egypt.

Toxicity assay of the plant essential oils

To determine the toxicity of the essential oils, the leaf-dipping technique was followed^[14]. To determine the lethal concentration (LC₅₀) and LC₉₀, five concentrations were prepared in distilled water using 0.5% Triton™ X-100 (Sigma-Aldrich Corp., St. Louis, MO, USA), as a surfactant. For each concentration, castor leaves were dipped in each oil extract for 10 seconds then air-dried. Thirty of 3rd or 5th *S. littoralis* larval instars were placed on the treated leaf surface, while leaves dipped in the sterile water served as control. Three replicates were set up for each concentration with different patches of larvae at different times. Treated leaves were removed after 24 hours and replaced with fresh ones for additional 24 hours. Mortality percentage was recorded after 48 hours and corrected according to Abbott's formula^[17]. LC₅₀ and LC₉₀ were estimated according to Finney^[18] using "LdPLine®" software (<http://www.ehabsoft.com/ldpline>).

Assay of the detoxification enzymes

One gram of third and fifth instar larvae of *S. littoralis* treated with LC₅₀ of the essential oils under investigation was homogenized in 5.0 mL distilled water using chilled glass Teflon tissue homogenizer (ST-2 Mechanic-Preczyina, Poland). Homogenates were centrifuged at 8000 rpm for 15 minutes at 5°C; supernatants were kept at -20°C until use. Total protein content was determined according to the method of Bradford^[19]; the absorbance was measured at 595 nm using UV/visible spectrophotometer (Spectronic 1201, Milton Roy, Houston, TX, USA).

The AChE and CE activities were measured in the larval homogenates according to Simpson *et al.*^[20] and Han *et al.*^[21] using acetylcholine bromide and α -naphthyl acetate as substrates, respectively. The GST activity was determined in the larval homogenates according to the method of Habig *et al.*^[22] using 1-chloro-2,4-dinitrobenzene, the increment in the

absorbance was measured at 340 nm. All used chemicals for assaying the detoxification enzymes were purchased from Sigma-Aldrich Corp.

Comet assay

Single strand breaks in the DNA of *S. littoralis* larvae treated with LC₅₀ of selected plant essential oils were detected using the comet assay according to Singh *et al.*^[23] and Tice *et al.*^[24]. A negative control was constructed using only water and Triton™ X-100 (0.5%). For preparing single-cell suspensions, larval midgut tissue was minced using a fine scissor then incubated with collagenase. Ethylenediaminetetraacetic acid was added to stop endonuclease activity; then dimethyl sulfoxide was added to prevent DNA damage by oxidation. Cell viability was examined using trypan blue^[25]. Living cells that repelled the blue dye were counted using a light microscope. A generalized scheme for comet assay includes preparation of microscope slides with cells layered in the low melting agarose, cell lysis for liberating DNA, alkaline treatment (pH=13) to free single-stranded DNA, electrophoresis, neutralization, DNA staining using a fluorochrome stain, and visualization of comet for scoring using fluorescent microscope^[24]. The commercially available software for comet assay (Comet Assay IV, <https://www.instem.com/solutions/genetic-toxicology/comet-assay.php>) was used to calculate the amount of DNA migrated in the tail (percentage of DNA in the tail), the length of the migrated DNA (tail length), and the tail moment. The DNA damage was scored in 50-100 randomly selected nuclei for each sample (three replicates per treatment). All used chemicals for comet assay were purchased from Sigma-Aldrich Corp.

Data analysis

Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's method of multiple comparison, using a statistical program for Minitab 16.0 (Minitab Ltd., Coventry, UK). Significance level was tested at $P \leq 0.05$.

RESULTS

Toxicity of plant essential oils against *S. littoralis* larvae

The obtained data showed variable toxic effects of the tested essential oils on the 3rd and 5th instar larvae of *S. littoralis* (Tables 1 and 2). Increasing essential oil concentration elevated significantly ($r=0.9$, $P\leq 0.05$) the cumulative mortality percentage of exposed *S. littoralis* larvae for 48 hours. The basil and dill oils were found to be

the most toxic oils to both larval instars followed by cinnamon, clove, and citronella; LC₅₀ values were 0.42, 0.70, 0.89, 2.29, and 2.66% for the 3rd instar larvae, and 0.62, 0.49, 0.88, 2.35, and 1.39% for the 5th instar larvae, respectively. The order of oil toxicity was fairly constant between both instars. The crane's-bills oil was the least toxic oil for both larval instars (LC₅₀ equals 4.56% and 4.52% for the 3rd and 5th instar larvae, respectively).

Table 1: Toxicity of the tested plant essential oils against the 3rd instar larvae of the cotton leafworm, *Spodoptera littoralis*.

Plant oil	LC Values (%)	95% Fiducial Limits	Slope±SE	R Values
Basil	LC ₅₀ (0.42)	(0.13-0.63)	1.38±0.15	0.94
	LC ₉₀ (43.56)	(2.79-24.06)		
Cinnamon	LC ₅₀ (0.89)	(0.44-1.69)	1.80±0.15	0.95
	LC ₉₀ (4.58)	(4.20-28.59)		
Citronella	LC ₅₀ (2.66)	(1.70-3.70)	2.32±0.22	0.96
	LC ₉₀ (9.46)	(7.87-26.75)		
Clove	LC ₅₀ (2.29)	(2.04-2.56)	2.55±0.26	0.98
	LC ₉₀ (7.32)	(6.02-9.69)		
Crane's-bills	LC ₅₀ (4.56)	(3.66-6.66)	1.33±0.25	0.99
	LC ₉₀ (41.95)	(19.89-64.01)		
Dill	LC ₅₀ (0.70)	(0.36-0.93)	2.19±0.20	0.87
	LC ₉₀ (2.68)	(2.03-5.35)		

LC: lethal concentration, SE: standard error.

Table 2: Toxicity of the tested plant essential oils against the 5th instar larvae of the cotton leafworm, *Spodoptera littoralis*.

Plant oil	LC Values (%)	95% Fiducial Limits	Slope±SE	R Values
Basil	LC ₅₀ (0.62)	(0.43-0.82)	0.94±0.10	0.99
	LC ₉₀ (14.21)	(7.10-51.07)		
Cinnamon	LC ₅₀ (0.88)	(0.70-1.09)	1.28±0.10	0.96
	LC ₉₀ (8.90)	(5.70-17.56)		
Citronella	LC ₅₀ (1.39)	(0.94-1.79)	1.30±0.20	0.94
	LC ₉₀ (13.44)	(8.90-28.06)		
Clove	LC ₅₀ (2.35)	(2.09-2.61)	2.63±0.27	0.96
	LC ₉₀ (7.23)	(5.98-9.47)		
Crane's-bills	LC ₅₀ (4.52)	(3.50-5.25)	1.13±0.24	0.97
	LC ₉₀ (61.71)	(24.06-95.27)		
Dill	LC ₅₀ (0.49)	(0.09-0.85)	0.61±0.17	0.98
	LC ₉₀ (60.58)	(15.75-63.19)		

LC: lethal concentration, SE: standard error.

Effect of the plant essential oils on the activity of the detoxification enzymes of *S. littoralis* larvae

The AChE activity increased significantly ($P \leq 0.05$) in 3rd instar larvae of *S. littoralis* upon exposure to LC₅₀ of crane's-bills, basil, dill, citronella, and clove oils in comparison with the control larvae (Table 3). The maximum fold change in enzyme activity (1.79 fold) was recorded after crane's-bills oil treatment. In contrast, the activity of AChE decreased significantly ($P \leq 0.05$) with exposure to cinnamon oil. On the other hand, a significant increase (1.37 fold) in AChE activity was recorded in the 5th instar larvae upon exposure to basil oil, while a significant decrease in the enzyme activity was observed after clove, citronella, crane's-bills, cinnamon, and dill oils treatment, as compared with the control larvae (Table 4).

A significant elevation ($P \leq 0.05$) of the activity of GST was recorded in the 3rd instar

larvae of *S. littoralis* treated with LC₅₀ of both clove and cinnamon oils in comparison with the control larvae (Table 3). However, treatment with dill, crane's-bills, basil, and citronella oils decreased significantly ($P \leq 0.05$) the activity of GST, as compared with the control larvae. The 5th instar larvae treated with all essential oils showed a significant elevation ($P \leq 0.05$) in the GST activity, except citronella that did not significantly affect ($P > 0.05$) the GST activity (Table 4).

In the 3rd instar larvae, crane's-bills oil elevated significantly CE activity ($P \leq 0.05$) followed by basil, citronella, and cinnamon oils, as compared with the control larvae (Table 3). The dill and clove oils decreased significantly the enzyme activity ($P \leq 0.05$). On the other hand, the 5th instar larvae showed a significant decrease in enzyme activity upon exposure to the basil, citronella, dill, and crane's-bills oils, while clove oil did not affect CE activity (Table 4).

Table 3: Effect of the tested plant essential oils on the acetylcholinesterase (AChE), glutathione S-transferase (GST), and carboxylesterase (CE) activities of the 3rd instar larvae of the cotton leafworm, *Spodoptera littoralis*.

	AChE activity ($\mu\text{g AChBr}/\text{min}/\text{mL}$) Mean \pm SD (Fold change)	GST activity ($\mu\text{L}/\text{min}/\text{mL}$) Mean \pm SD (Fold change)	CE activity ($\mu\text{L}/\text{min}/\text{mL}$) Mean \pm SD (Fold change)
Control	153.33 \pm 9.87 ^d	74.33 \pm 3.79 ^b	71.80 \pm 3.83 ^b
Basil oil	252.33 \pm 6.81 ^{ab} (1.64)	56.67 \pm 2.08 ^c (0.76)	81.47 \pm 3.97 ^c (1.13)
Cinnamon oil	135.00 \pm 9.54 ^d (0.88)	85.67 \pm 4.04 ^a (1.15)	76.27 \pm 3.36 ^b (1.06)
Citronella oil	221.00 \pm 6.08 ^{bc} (1.44)	55.67 \pm 2.08 ^c (0.75)	77.03 \pm 1.50 ^c (1.07)
Clove oil	191.67 \pm 9.71 ^c (1.25)	82.67 \pm 4.93 ^a (1.11)	55.87 \pm 2.36 ^d (0.78)
Crane's-bills oil	275.00 \pm 21.80 ^a (1.79)	60.33 \pm 3.21 ^c (0.81)	95.77 \pm 3.91 ^a (1.33)
Dill oil	215.33 \pm 5.51 ^c (1.40)	71.67 \pm 3.06 ^b (0.96)	44.17 \pm 4.25 ^c (0.62)

Fold change was calculated by dividing the mean of enzyme activity in each oil group with its respective value in the control group. SD: standard deviation. Columns designated by different letters are significantly different ($P \leq 0.05$).

Table 4: Effect of the tested plant essential oils on the acetylcholinesterase (AChE), glutathione S-transferase (GST), and carboxylesterase (CE) activities of the 5th instar larvae of the cotton leafworm, *Spodoptera littoralis*.

	AChE activity ($\mu\text{g AChBr/min/mL}$) Mean \pm SD (Fold change)	GST activity ($\mu\text{L/min/mL}$) Mean \pm SD (Fold change)	CE activity ($\mu\text{L/min/mL}$) Mean \pm SD (Fold change)
Control	191.00 \pm 11.53 ^d	60.67 \pm 4.04 ^b	73.50 \pm 3.22 ^a
Basil oil	262.0 \pm 17.09 ^a (1.37)	78.00 \pm 2.00 ^{ac} (1.29)	65.67 \pm 2.58 ^b (0.89)
Cinnamon oil	170.00 \pm 10.30 ^{bc} (0.89)	72.40 \pm 2.40 ^c (1.19)	60.33 \pm 3.51 ^b (0.82)
Citronella oil	142.00 \pm 16.52 ^e (0.74)	60.00 \pm 4.36 ^{ab} (0.99)	59.43 \pm 2.40 ^b (0.81)
Clove oil	182.33 \pm 6.81 ^c (0.95)	76.00 \pm 1.50 ^c (1.25)	73.07 \pm 2.68 ^a (0.99)
Crane's-bills oil	138.33 \pm 7.64 ^e (0.72)	86.00 \pm 5.29 ^e (1.42)	38.13 \pm 1.80 ^d (0.52)
Dill oil	122.00 \pm 12.12 ^e (0.64)	99.67 \pm 3.51 ^d (1.64)	45.87 \pm 2.87 ^c (0.62)

Fold change was calculated by dividing the mean of enzyme activity in each oil group with its respective value in the control group. SD: standard deviation. Columns designated by different letters are significantly different ($P \leq 0.05$).

Effect of plant essential oils on the DNA of *S. littoralis* larvae

The effect of plant essential oils on *S. littoralis* DNA damage, as a result of comet assay, was shown in Figure “1”. The control cells showed the normal appearance of nuclei with minimal damage. Essential oils posed a slightly significant effect ($P \leq 0.05$) on DNA strand break expressed as a tail. In addition, essential oils showed variable effects on *S. littoralis* DNA in cells. The damage was either affecting the DNA by forming shorter tail (as in treatments with basil and dill oils) or is expressed in an increased tail length (as in case of the rest of the oils). In terms of DNA (%), the maximum amount of DNA was recorded in cells treated with basil oil, while treatment with crane's-bills oil showed minimal effect. Considering the tail length, it was obvious that clove, citronella and crane's-bills oils enhanced the broken DNA strand migration

forming a longer tail than that formed in case of treatment with basil, dill, and cinnamon oils, which might release larger DNA fragments that have lower migration ability, thus forming shorter tail but with more DNA.

DISCUSSION

Plant essential oils became one of the most substantial insect control agents worldwide^[26]. The flower and vegetable essential oils were tested for their anti-feeding, repellent, and oviposition-deterrent effects expressing a toxic effect on insects based on the Reregistration Eligibility Decision (RED) document issued by the Environmental Protection Agency (EPA)^[8]. In the present study, the toxic effect of some essential oils from clove, crane's-bills, citronella, dill, cinnamon, and basil proved its possible implementation as a bio-control method in IPM programs to control *S. littoralis*.

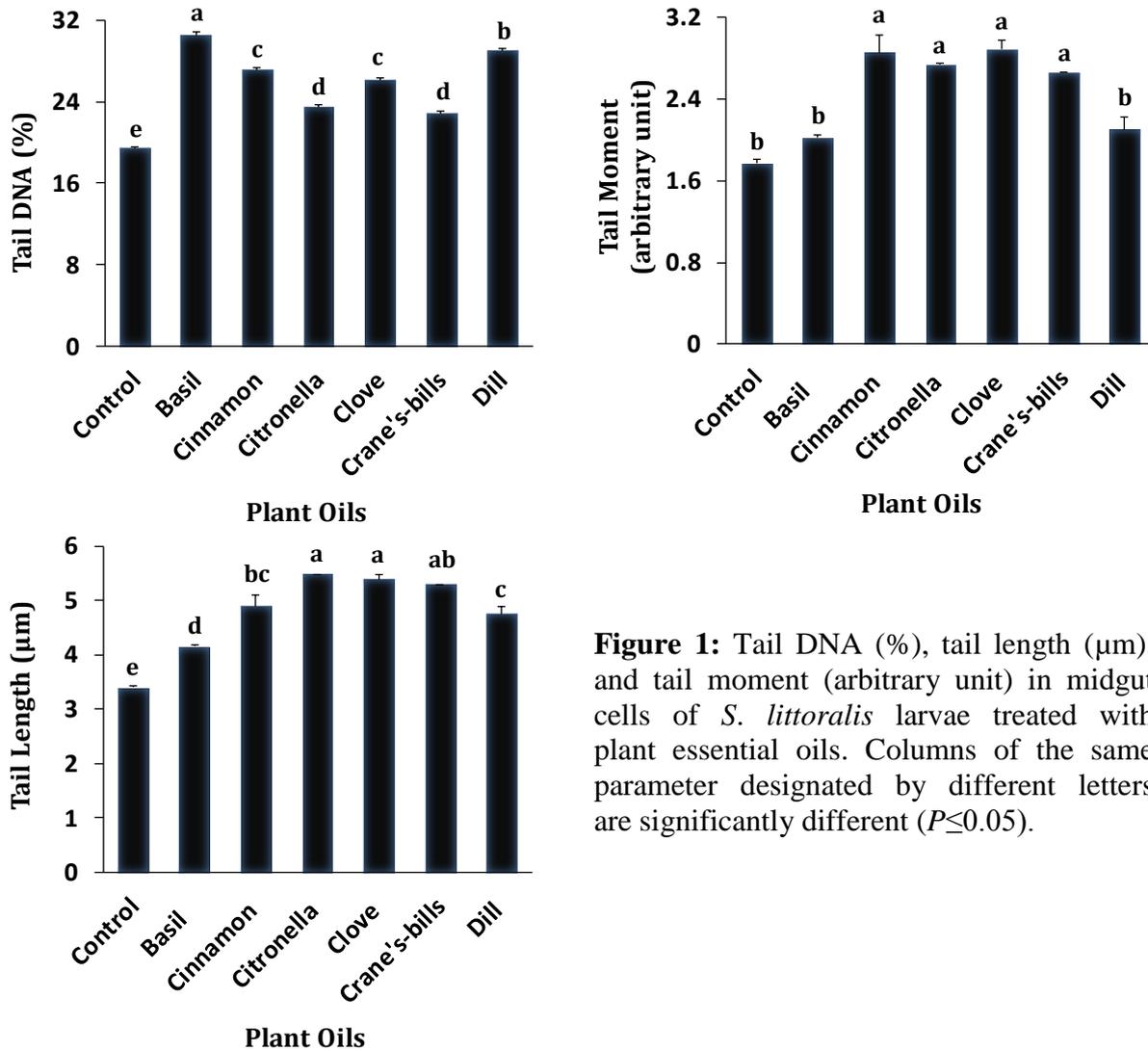


Figure 1: Tail DNA (%), tail length (µm), and tail moment (arbitrary unit) in midgut cells of *S. littoralis* larvae treated with plant essential oils. Columns of the same parameter designated by different letters are significantly different ($P \leq 0.05$).

It is valuable to highlight that the recorded 3rd and 5th instar larval mortality, upon treatment with the tested essential oils, emphasized its promising insecticidal activity within the first 48 hours after application. Such a short duration for achieving mortality (lethal time) is a beneficial character for a chemical agent against polyphagous pest larvae; hence preventing the destructive defoliating effect of larval activity. Similar results were obtained after treating the 4th instar larvae of *S. littoralis* with castor and camphor oils; a highly toxic effect was recorded^[27]. The clove oil showed a toxic effect on *Tineola bisselliella* after treatment to woolen fabrics, the effect was not persistent, thus a multiple application strategy was recommended^[28]. Lingathurai

et al.^[29] recorded a highly toxic effect of many essential oils against larvae of the oblique banded leaf roller, *Choristoneura rosaceana* and *Trichoplusia ni*. In addition, *Thymus vulgaris*, *Syzygium aromaticum*, *Cymbopogon citrates*, *Cinnamomum cassia*, and *Cymbopogon nardus* residues were recorded as toxic oils against *T. ni*^[30]. The effect of the clove and citronella oil combination increased the repellency and toxicity of these essential oils against *T. bisselliella*^[28]. Essential oils, such as basil, may express repellent, toxic, or oviposition deterrent effect on various insects (Dryophthoridae, Curculionidae, Bostrichidae, Tenebrionidae, Bruchidae, and Gelechiidae)^[31,32]. Similar results were obtained in attaining up to 100% 3rd instar

larval mortality of the fall armyworm “*S. frugiperda*” through ingestion bioassay^[33].

In the present study, the plant essential oils induced larval mortality during the first 24 to 48 hours indicating their potency against *S. littoralis* larvae. Topical application was also considered as another assay for evaluating the toxicity of the essential oils against *S. littoralis*^[34]. The essential oils toxicity was evaluated and promising results were obtained against various insect orders including Hymenoptera, Isoptera, Coleoptera, and Diptera^[35]. Considering order susceptibility, lepidopteran species were more susceptible to the essential oils than coleopterans^[35]. The essential oils toxicity was evaluated against *S. exigua* at 24, 48, and 72 hours causing more than 80% larval mortality during the first 24 hours with the incidence of death during the first minute post-treatment^[36].

Agrochemical metabolism involves many enzymes including cytochrome P450 monooxygenases, GST and carboxyl/cholinesterases. The activity of these enzymes explained insect's complex immune modulation through the activation of the detoxification enzymes in order to metabolize secondary plant metabolites^[37]. In the present study, the activity of some detoxifying enzymes was measured in *S. littoralis* larvae treated with LC₅₀ of the essential oils investigated herein. The elevation in GST enzyme activity was recorded in the 5th instar larvae; a more tolerant stage compared to the 3rd one, the latter expressed a decrease in enzyme activity emphasizing the possible role of GST in essential oil detoxification. In accordance to our findings, GST enzyme activity decreased in the cotton bollworm “*H. armigera*” treated with LC₅₀ of *Artemisia annua* essential oil^[38]. *S. littoralis* treatment with camphor oil also led to modulation of physiological response including the activity of lipase, α -amylase, and glucose-6-phosphate dehydrogenase enzymes^[27]. The GST activity was a good

candidate as a biomarker in monitoring plant extracts and insecticide-exposure in *Callosobruchus maculatus* and *S. littoralis*^[39].

During the observation period in the present study, apparent symptoms were ranging from hyperactivity and convulsions, followed by paralysis or even morbidity and eventually death. These observations together with a significant change in AChE enzyme activity suggested a neurotoxic mode of action. Neurotoxicity symptoms including variable uncontrolled movements were observed immediately after larval treatment accompanied by larval paralysis 5.0 minutes post-treatment with recovery during 3-6 hours, assuming enzymatic detoxification of the oil's bioactive compounds^[40,41].

The CE-isozymes are a group of α/β hydrolase enzymes hydrolyzing carboxylester-containing compounds, such as agrochemicals, through addition of water molecule. Meanwhile, they have a dual function in lipid and xenobiotic metabolism including pesticides^[42]. The enzyme either remains bound to the hydrolysis by-product or hence is aged; its catalytic activity is lost, or it is released “regenerated” to retain its catalytic activity^[43]. Since CE in insects exist in isoforms; so, the hydrolytic activity differs according to substrate used^[43]. The variation in enzyme activity in the present work after essential oil treatment to larval food may be attributed for using one substrate; difference in isozyme pattern requires using variable substrates to quantify all isozymes activity. So, insect CE-isozymes abundance and activity contribute to the toxicity of agrochemicals containing ester group. CE is considered as one of the possible mechanisms conferring resistance to essential oils in *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae) resistant strain^[44]; such situation of cross-resistance is responsible for the tolerance towards the essential oils. It is of a major concern for the researchers to investigate the role of CE in xenobiotic metabolism and insecticide detoxification.

The variation in esterase activity between CE and AChE may be because some individuals may have more CE titers than AChE, and vice versa^[45]. Different oils have different active ingredients with different stereoisomers, hence a stereospecific enzyme activity^[46]. The significant induction of detoxifying enzymes upon treatment with some of the tested oils may consider, to some extent, one of the possible mechanisms of incidence of resistance. Therefore, the pest immune modulation through affecting variable metabolic pathways against xenobiotics including secondary plant metabolites should gain more attention^[47]. Meanwhile, the development of insect resistance is expected to be slow for being biodegradable, posing less stress on the environment or pests, besides having low mammalian toxicity^[41]. Generally speaking, induction and inhibition of detoxification enzymes are possible in response to allelochemicals; however, the role of detoxification enzymes was validated, in few cases, at the biochemical level only^[47].

The DNA damage resulted in the present study after essential oil treatment is considered low to mild damage based on visual scoring of comet assay, as it did not lead to formation of long tails, the situation that is common with strong genotoxicants as some insecticides^[48] or oxidizing agents like hydrogen peroxide^[49]. The ideal phenotype of an apoptotic cell, with more than 80% DNA fragmented outside the cell, was not recorded at any of the examined cells; indicating that there might be a DNA repair process that is responsible for protecting the cell^[49]. The relation between the incidence of DNA damage and plant essential oil treatment in insects was validated in some insects^[13,50]. Treatment of adult *C. quinquefasciatus* mosquito with essential oils of *P. corylifolia* induced an increase in DNA damage with increasing comet tail length with 6.713% and 8.864% DNA damage^[13]. In addition, treatment of *R. dominica* with essential oils of *Citrus*

aurantium, *Eruca sativa*, *Zingiber officinale*, and *Origanum majorana* increased significantly the DNA damage^[50].

The information obtained in the present study, through integrating biomarker assays, might form a holistic and judicious analysis of the impact of plant-derived chemicals (as plant essential oils) on the target organism, as well as it highlighted its environmental safety. The correlation between comet, biomarkers, and toxicological response provides an assessment method of the insect response toward essential oil application, which may be considered in risk assessment concerning evaluating the decision of its future application in an IPM program.

In conclusion, the presented research highlighted the promising potential of the examined essential oils against larvae of *S. littoralis*, indicating the possibility of using these six essential oils in the development of new bio-pesticides in implementing an efficient IPM program. Further studies are recommended to evaluate the efficacy of these essential oils in the field, and to determine their safety on non-target insects.

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CONFLICT OF INTEREST

The authors declare no potential financial conflict of interest.

AUTHORS' CONTRIBUTIONS

YAF, HME, and HMH planned the study and designed all experiments. YAF and HMH carried out the experiments and performed the statistical analysis, summarized, discussed, and interpreted the results. YAF, HME, and HMH drafted the manuscript. All authors read and approved the manuscript.

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السُّمية الجينية لبعض الزيوت النباتية الأساسية في حشرة دودة ورق القطن
SPODOPTERA LITTORALIS (LEPIDOPTERA: NOCTUIDAE)
النشاط المحتمل لإنزيمات إزالة السموم

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تشكل زيادة الحافز لاكتشاف مبيدات حشرية فعالة وصديقة للبيئة تحديًا مهمًا في الوقت الحاضر. وقد وجد أن الزيوت النباتية الأساسية تلعب دورًا هامًا وواعدًا إذا ما استخدمت كعنصر في برامج مكافحة متكاملة. وفي هذه الدراسة تم تقييم الفاعلية السمية لستة طرز من الزيوت النباتية العطرية من القرنفل، والجيرانيوم، والسترونيلا، والشبث، والقرفة، والريحان تجاه يرقات دودة ورق القطن. وقد تأكد من خلال الدراسة التأثير السمي لكل الزيوت المستخدمة، حيث وجد أن زيت الريحان أكثرها سُمية تجاه الطور اليرقي الثالث، بينما أظهر زيت الشبث سُمية أعلى من كل الزيوت المستخدمة وذلك بعد مرور 48 ساعة تجاه الطور اليرقي الخامس. وقد أظهرت الدراسة التأثيرات البيوكيميائية للزيوت على نشاط بعض الإنزيمات مثل إنزيم الأستيل كولينستيريز، وإنزيم الكربوكسيل إستيريز، وإنزيم الجلوتاثيون ترانسفيريز. وقد سببت المعاملات بزيت الريحان تزايد في نشاط إنزيم الأستيل كولينستيريز في كل من الطورين اليرقي الثالث واليرقي الخامس. وأظهرت كل الزيوت قيد الدراسة تزايد ملحوظ في معدل نشاط إنزيم الجلوتاثيون ترانسفيريز في معاملات الطور اليرقي الخامس، فيما عدا زيت السترنيلا. وسببت المعاملة بزيت الشبث تناقص في نشاط إنزيم الكربوكسيل إستيريز في كل من الطورين اليرقي الثالث واليرقي الخامس. وبدراسة السُّمية الجينية للزيوت قيد الدراسة، باستخدام تقنية الكوميت، لوحظت دلائل للتأثير على المحتوى الجيني في كل المعاملات، حيث كان أعلى معدل للسُّمية الجينية (من الأعلى إلى الأقل) في اليرقات التي تم معاملةها باستخدام زيوت الريحان، والقرفة، والقرنفل، والسترونيلا، والشبث على التوالي. ويستنتج من هذه الدراسة التأثير المحتمل لبعض الزيوت الأساسية كبديل آمن وواعد للمبيدات الكيميائية في برامج مكافحة حشرة دودة ورق القطن.