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Chemical composition and bioactivities of *Melaleuca alternufolia* essential oil and its main constituents against *Spodoptera littoralis* (Boisaduval, 1833)

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Abstract

Background: *Spodoptera littoralis* is mostly controlled by the use of synthetic insecticides. Nonetheless, the use of these insecticides causes a slew of issues. On this pest, the antifeedant activity of *Melaleuca alternifolia* essential oil (EO) and its two principal components was investigated.

Results: The gas chromatography–mass spectrometry (GC/MS) analysis revealed that the *M. alternifolia* EO was composed of eleven compounds. Terpinen-4-ol (40.1%) and γ-terpinene (21.9%) were chosen as the major constituents. In terms of antifeedant efficacy, treatment with *M. alternifolia* EO and these components reduced leaf consumption and the efficiency of food conversion in larvae in a concentration-dependent manner. When compared to untreated larvae, weight, growth, and pupation percentage were all significantly lower.

Conclusions: The findings show that *M. alternifolia* EO and its components, terpinen-4-ol and γ-terpinene can be effectively combined for cotton leafworm management.

Highlights

- The chemical composition of *M. alternifolia* EO was identified by GC–MS.
- The EO of *M. alternifolia* and its components, terpinen-4-ol and γ-terpinene, had potent bioactivity against *S. littoralis*.
- *M. alternifolia* EO and their components, terpinen-4-ol and γ-terpinene have great potential as a biopesticide in integrated pest management programs.

Keywords: Antifeedant activity, Food consumption, *Melaleuca alternifolia* essential oil, Terpinen-4-ol, γ-Terpinene

Background

The cotton leafworm is a destructive pest that causes significant damage and economic losses in Egypt and other nations. Larval instars are the insect's most damaging stage, capable of entirely destroying or severely reducing crop yields in over 100 economically significant species, including cereal crops, vegetables, and ornamental plants (Ismail et al. 2020). For decades, chemical synthetic insecticides have been used to control this pest, with injudicious use contributing to an increase in the likelihood of resistance evolution (Whalon et al. 2006; Ismail

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2019), as well as risks to human health, untargeted organisms, the environment, and residue issues (Sharma et al. 2019). As a result, successful pest control necessitates the development of new compounds with unique modes of action that are also low-risk. EOs have recently gained popularity as an alternative pest management method, due to its quick biodegradability, economic application, minimal toxicity to mammals, and environmental safety (Gerwick and Sparks 2014; Nollet and Rathore 2017). Furthermore, EOs are made up of a variety of compounds whose mode of action inhibits pest resistance from evolving (Nollet and Rathore 2017). On the other hand, EOs have mostly been studied as natural fumigants for the control of stored-product insects and are rarely used in lepidoptera prevention and control. The nutritional indicators and digestion were the important characteristics examined in this study to evaluate the *M. alternifolia* EO and its two main components, terpinen-4-ol and γ-terpinene, for cotton leafworm larvae.

Methods

Insect rearing

The second-instar larvae of *Spodoptera littoralis* used in bioassays were obtained from a laboratory susceptible culture in the Department of Insect Population Toxicology, Central Agricultural Pesticides Laboratory, Agriculture Research Center, Giza, Egypt. For several years, the culture was maintained at 25 ± 1 °C, 65 ± 5% relative humidity and a photoperiod of 16:8 (L:D) in the rearing chamber without being exposed to any pesticides. Larvae were fed soft, fresh castor bean leaves (*Ricinus communis* L.) in sterilized glass jars with muslin coverings. The growing larvae were transferred daily to other clean, sterile glass jars to avoid infection from feces and provided with fresh leaves for feeding. All of the experiments in this study were carried out under the same controlled conditions mentioned above.

Melaleuca alternifolia EO analyzed by gas chromatography–mass spectrometry

The composition of *Melaleuca alternifolia* essential oil was measured with a Trace GC Ultra-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m × 0.25 mm × 0.25 µm film thickness). *M. alternifolia* EO was diluted in diethyl ether before being injected to the GC/MS. The used carrier gas was helium (flow rate of 1 mL/min). The solvent delay was 3 min, and diluted sample (1 µL) was injected automatically in splitless mode with Autosampler AS1300 coupled with GC. The column oven temperature program and the separation conditions were as follows: At the temperature of 50 °C, the column oven was initially held, then by 5 °C/min, the temperature was increased to 250 °C and held for 2 min. By 30 °C/min, the final temperature was increased to 300 °C and held for 2 min. The temperatures of the injector and MS transfer line were kept at 270 and 260 °C, respectively. At 70 eV ionization voltages, the EI mass spectra were collected at the m/z range of 50–650 in full-scan mode. The temperature of ion source was set at 200 °C. Chemical constituents were identified based on their retention time (RT), with the mass spectra with those of Wiley 09 and NIST 14 mass spectral database the percentage of components was calculated by the GC peak area.

Efficiency measures of nutritional indices

To evaluate the effect of *M. alternifolia* EO (purchased from local market, Egyptian Natural Co.), terpinen-4-ol, and γ-terpinene were purchased from Sigma–Aldrich Chemical Ltd. (St. Louis, MO, USA) on the nutritional physiology in larvae of *S. littoralis*. Fresh castor bean leaves were used to prepare the leaf discs (8 cm diameter). A monopan balance was used to measure all of the weights, which were accurate to 0.1 mg (Sartorius GMBH, Type: A 120 S). For 20 s, leaf discs were dipped in five concentrations of each *M. alternifolia* EO, terpinen-4-ol and γ-terpinene (250, 500, 1000, 2000, and 4000 mg/L). Control leaves were submerged in diluted water containing Triton X-100. Newly molted second-instar larvae (< 24 h) were chosen and weighed after being denied nourishment for 4 h. This experiment was conducted in 4 replications, each with 10 larvae/concentration. Larvae were allowed to feed on treated leaves for 72 h, which were changed every 24 h and replaced with fresh treated leaves, after which they were allowed to feed on untreated leaves. Each replicate's feces and unconsumed treated leaves were weighed every 24 h during the 72-h feeding period. Farrar et al.'s formula (1989) was used to calculate nutritional indicators as follows:

Approximate digestibility:

$$\mathrm{AD}\,(\%) = (C-F)/C \times 100,$$

Efficiency of conversion of ingested food:

$$\mathrm{ECI}\,(\%) = G/C \times 100,$$

Efficiency of conversion of digested food:

$$\mathrm{ECD}\,(\%) = G/(C-F) \times 100,$$

Consumption rate:

$$\mathrm{CR}\,(\mathrm{mg/mg/day}) = C/TA,$$

Relative growth rate:

$$\mathrm{RGR}\,(\mathrm{mg/mg/day}) = G/TA.$$

where *A* weight of larvae during the feeding period (mg), *C* weight of food consumed (mg), *F* weight of feces produced (mg), *G* weight gain of larvae (mg), *T* the duration of feeding period (day).

The larvae that survived were then transferred to sterilized glass jars, fed fresh untreated leaves, and monitored daily until pupation. When larvae were probed with fine brush and did not move, they were assumed to be dead. The larval growth index was calculated as follows (Itoyama et al. 1999):

Larval growth rate:

$$LG = P/T$$

where *P* pupation (%), *T* the duration of the larval period (day).

Antifeedant activity

M. alternifolia EO, terpinen-4-ol, and γ-terpinene were investigated for antifeedant activity against second instar larvae *S. littoralis* (> 24 h) at concentrations of 250, 500, 1000, 2000, and 4000 mg/L. The feed deterrent index was calculated using the formula by Pavela et al. (2008):

Feeding deterrence:

$$\mathrm{FD}(\%) = C-T/C+T \times 100.$$

where *C* food consumed in control, *T* food consumed in treatment.

Statistical analysis

The data were calculated as mean ± SE and analyzed statistically. One-way analysis of variance (ANOVA) was used to evaluate statistically significant differences between individual means using SAS software. Mean values were analyzed with Tukey's test at the 0.05 level of probability or less.

Results

The chemical components of the *M. alternifolia* EO

GC/MS revealed 11 aromatic components, accounting for 92.6 percent of the total oil (Table 1). The main components of *M. alternifolia* EO were terpinen-4-ol (40.1%), γ-terpinene (21.9%), and other minor components. The oxygenated monoterpenes were the most common chemical group in the oil's chemical composition.

Nutritional indices

All nutritional indicators of different concentrations of *M. alternifolia* EO, terpinen-4-ol, and γ-terpinene on the feeding efficiency of *S. littoralis* larvae were significantly reduced when compared to the control (Table 2). The weight gain was the lowest in larvae treated with *M. alternifolia* EO. All treatments led to a significant decrease in weight gain ($P \leq 0.05$) than the control group due to lower consumption rate (CR) and relative growth rate (RGR). In treated larvae, there was a significant decrease in digestibility (AD), with significant differences from untreated larvae. The efficiency of converting ingested food (ECI) and conversion of digested food (ECD) values decreased with increasing concentration. *M. alternifolia* EO was the most effective at a concentration of 4000 mg/L, showing the lowest values of ECI (4.29%) and ECD (4.51%).

Table 1 Chemical composition of *Melaleuca alternifolia* EO

Component	Area%	*RT
α-Pinene	5.86	2.4
α-Terpinene	10.4	13
Limonene	1.2	1.0
p-Cymene	1.20	2.6
1,8-Cineole	1.83	5.1
γ-Terpinene	21.9	28
Terpinolene	3.24	3.1
Terpinen-4-ol	40.1	48
α-Terpineol	6.91	2.4
o-Cymene	5.0	9.0

*Retention time (min)

Antifeedant activity

The results in Table 3 show that the antifeedant activity on second instar larvae of *S. littoralis* reveal different values according to treatments and concentrations. *M. alternifolia* EO treatment at all concentrations (250, 500, 1000, 2000, and 4000 mg/L) was higher than terpinen-4-ol, and γ-terpinen treatments as compared to the untreated control. The most significant increase has been found with 4000 mg/ L of *M. alternifolia* EO.

Larval growth

Table 4 shows that the treatments with *M. alternifolia* EO, terpinen-4-ol, and γ-terpinene greatly slowed larval growth after 72 h of treatment even at the lowest concentration (250 mg/L). Larval growth index (LG) values steadily decreased with increasing concentrations tested for all treatments. The most effective was *M. alternifolia* EO, followed by terpinen-4-ol, and the least effective was γ-terpinene, with LG values of 1.08, 1.86, and 3.51%, respectively, at 4000 mg/L.

Discussion

The quality and quantity of food consumed by insects can influence their growth, development, and reproduction (Dmitriew 2011). *S. littoralis* larvae fed on treated leaves showed a lower consumption rate (CI) than larvae

Table 2 Nutritional indices of 2nd instar *Spodoptera littoralis* larvae fed for 72 h on treated castor bean leaves by *Melaleuca alternifolia* EO, terpinen-4-ol, and γ-terpinene at different concentrations

Treatment	Concentration (mg/L)	AD (%)	ECI (%)	ECD (%)	CR (mg/mg/day)	RGR (mg/mg/day)
Melaleuca alternifolia	4000	76.11 ± 0.60c	4.29 ± 0.79d	4.51 ± 0.57 cd	1.21 ± 0.06f	0.33 ± 0.48 h
	2000	76.93 ± 1.66bc	5.15 ± 0.39d	6.13 ± 0.47c	1.29 ± 0.03f	0.45 ± 0.53 fg
	1000	79.63 ± 2.72bc	7.22 ± 0.73c	8.17 ± 0.62c	1.38 ± 0.23e	0.49 ± 0.65 fg
	500	82.31 ± 0.35b	9.87 ± 0.16c	13.14 ± 0.34b	1.46 ± 0.12d	0.55 ± 0.82de
	250	83.17 ± 2.22b	10.43 ± 1.65c	15.66 ± 1.46ab	1.51 ± 0.17c	0.58 ± 0.30de
Terpinen-4-ol	4000	85.43 ± 0.35b	5.90 ± 0.51 cd	6.21 ± 0.20c	1.33 ± 0.88e	0.48 ± 0.78 fg
	2000	86.44 ± 1.21b	6.65 ± 0.68c	7.03 ± 0.26c	1.37 ± 0.33e	0.52 ± 0.26ef
	1000	88.06 ± 0.28b	8.11 ± 0.19c	8.44 ± 0.76c	1.47 ± 0.59d	0.56 ± 0.12de
	500	89.66 ± 0.61ab	12.92 ± 1.62b	13.13 ± 0.37b	1.58 ± 0.93c	0.63 ± 0.25 cd
	250	90.00 ± 0.89a	13.14 ± 0.37b	13.95 ± 0.49b	1.65 ± 0.45b	0.65 ± 0.22bc
γ-Terpinene	4000	88.35 ± 1.37b	9.95 ± 0.64c	11.82 ± 1.08b	1.49 ± 0.22d	0.55 ± 0.72de
	2000	89.26 ± 1.21b	12.42 ± 0.51b	13.23 ± 0.65b	1.56 ± 0.28c	0.58 ± 0.41de
	1000	89.76 ± 0.30ab	13.15 ± 0.68b	13.39 ± 0.11b	1.60 ± 0.31b	0.60 ± 0.47 cd
	500	92.07 ± 1.37a	14.02 ± 1.95b	14.21 ± 0.57b	1.66 ± 0.15b	0.67 ± 0.26bc
	250	92.47 ± 0.61a	15.59 ± 0.29ab	15.73 ± 1.37ab	1.71 ± 0.23a	0.70 ± 0.35ab
Control		96.51 ± 0.30a	17.66 ± 0.90a	20.06 ± 0.98a	1.75 ± 0.18a	0.77 ± 0.29a

Means ± SE within the same column having the same letter are not statistically different from each other, $P \leq 0.05$ according to Tukey's test. Data are averages of 4 replicates of 10 larvae each

AD approximate digestibility, *ECI* efficiency of conversion of ingested food, *ECD* efficiency of conversion of digested food, *CR* consumption rate, *RGR* relative growth rate

Table 3 Antifeedant activity (FD) of 2nd instar *Spodoptera littoralis* larvae fed for 72 h on treated castor bean leaves by *Melaleuca alternifolia* EO, terpinen-4-ol and γ-terpinene at different concentrations

Treatment	Concentration (mg/L)				
	4000	2000	1000	500	250
Melaleuca alternifolia	67.85 ± 1.70a	55.83 ± 1.74a	49.62 ± 1.29a	40.16 ± 1.80a	34.62 ± 1.76a
Terpinen-4-ol	56.88 ± 1.82b	46.81 ± 1.73b	39.53 ± 1.72b	34.53 ± 1.87b	29.62 ± 0.99ab
γ-Terpinene	50.67 ± 1.02bc	43.45 ± 1.11bc	36.28 ± 1.44bc	31.00 ± 1.57bc	22.81 ± 0.64bc
Control	0.0 ± 0.0d	0.0 ± 0.0d	0.0 ± 0.0d	0.0 ± 0.0d	0.0 ± 0.0d

Means ± SE within the same column having the same letter are not statistically different from each other, $P \leq 0.05$ according to Tukey's test. Data are averages of 4 replicates of 10 larvae each

fed on untreated leaves, according to the feeding indices studied at varied concentrations of *M. alternifolia* EO and two of its main components. According to the findings, reduced CI is associated with slower larval growth, which is most likely owing to longer food retention in the gut in order to maximize approximate digestibility (AD) to satisfy increasing nutritional requirements (Akhtar and Isman 2004). In treated larvae, the levels of converting ingested food (ECI) and converting digested food (ECD) also reduced significantly, suggesting that plant allelochemicals are toxic to the peritrophic membrane and that damage to the midgut's cellular surfaces has occurred (Mukherjee 2002; Sun et al. 2019; Braga et al. 2020). As a result, the EO of *M. alternifolia* is high in bioactive compounds (terpinen-4-ol and γ-terpinene) that have an antifeedant impact on insects and can be employed as natural insecticides (El-Wakeil 2013; Thomsen et al. 2013; Liao et al. 2017; Dehsheikh et al. 2020; Manfron et al. 2021).

Table 4 Larval growth index (LG) of 2nd instar *Spodoptera littoralis* larvae fed for 72 h on treated castor bean leaves by *Melaleuca alternifolia* EO, terpinen-4-ol, and γ-terpinene at different concentrations

Treatment	Concentration (mg/L)	Pupation (%)	Larval growth index (LGI)
Melaleuca alternifolia	4000	30.0 ± 1.5 g	1.08
	2000	42.1 ± 4.6f	2.23
	1000	48.7 ± 2.3e	2.51
	500	55.3 ± 1.7d	3.36
	250	61.5 ± 2.2c	3.59
Terpinen-4-ol	4000	46.9 ± 3.6d	1.86
	2000	51.4 ± 3.0d	2.54
	1000	57.4 ± 1.8c	2.93
	500	68.2 ± 4.1b	4.65
	250	75.2 ± 5.5b	5.25
γ-Terpinene	4000	54.2 ± 2.2c	3.51
	2000	62.8 ± 2.4c	4.00
	1000	70.0 ± 3.4b	4.67
	500	88.4 ± 3.3b	5.78
	250	93.3 ± 6.6a	6.00
Control		95.6 ± 4.7a	6.69

Means ± SE within the same column having the same letter are not statistically different from each other, $P \leq 0.05$ according to Tukey's test. Data are averages of 4 replicates of 10 larvae each

Conclusions

Finally, the *M. alternifolia* EO and its components, terpinen-4-ol, and γ-terpinene had potent antifeedant activity on *S. littoralis* via effects on important metabolic processes. As a result, *M. alternifolia* EO and its compounds should be used as natural insecticides in IPM to combat the cotton leafworm.

Abbreviations

EO: Essential oil; AD: Approximate digestibility; ECI: Efficiency of conversion of ingested food; ECD: Efficiency of conversion of digested food; CR: Consumption rate; RGR: Relative growth rate; FD: Feeding deterrent index; LG: Larval growth index.

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Author contributions

SMI subject selection, study design, carried out the experiments, paper writing, collecting and interpretation of the data. NAS and TFW helped in statistical and chemical analysis. NSh was involved in methodology supervision. All authors read and approved the final manuscript.

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Declarations

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Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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References

Akhtar Y, Isman MB (2004) Comparative growth inhibitory and antifeedant effects of plant extracts and pure allelochemicals on four phytophagous insect species. J Appl Entomol 128:32–38

Braga VAA, Cruz GDS, Guedes GA, Silva CTDS, Santos AA (2020) Effect of essential oils of *Mentha spicata* L. and *Melaleuca alternifolia* Cheel on the midgut of *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae). Acta Histochem 122:151529. https://doi.org/10.1016/j.acthis.2020.151529

Dehsheikh AB, Sourestani MM, Dehsheikh PB, Mottaghipisheh J, Vitalini S, Iriti M (2020) Monoterpenes: Essential oil components with valuable features. Mini Rev Med Chem 20:958–974. https://doi.org/10.2174/1389557520666200122144703

Dmitriew CM (2011) The evolution of growth trajectories: what limits growth rate? Biol Rev 86:97–116. https://doi.org/10.1111/j.1469-185X.2010.00136.x

El-Wakeil NE (2013) Botanical pesticides and their mode of action. Gesunde Pflanz 65:125–149

Farrar RR, Barbour JD, Kenedy GG (1989) Quantifing food consumption and growth in insects. Ann Entomol Soc Am 82:593–598

Gerwick BC, Sparks TC (2014) Natural products for pest control: an analysis of their role, value and future. Pest Manag Sci 70:1169–1185. https://doi.org/10.1002/ps.3744

Ismail SM (2019) Field evaluation of recommended compounds to control some pests attacking cotton and their side effects on associated predators. J Biol Chem Res 36:1–12

Ismail S, Abdel-Galil F, Hafez S, AbuEl-Ghiet U (2020) Influence of some insecticides on the incidence of common Lepidopterous insect-pests in field cotton. Egypt Acad J Biol Sci 12:23–30. https://doi.org/10.21608/eajbsf.2020.73377

Itoyama K, Kawahira Y, Murata M, Tojo S (1999) Fluctuations of some characteristics in the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae) reared under different diets. Appl Entomol Zool 34:315–321

Liao M, Xiao JJ, Zhou LJ et al (2017) Chemical composition, insecticidal and biochemical effects of *Melaleuca alternifolia* essential oil on the *Helicoverpa armigera*. J Appl Entomol 141:721–728. https://doi.org/10.1111/jen.12397

Manfron J, Raman V, Khan IA, Farago PV (2021) Essential oils of *Baccharis*: chemical composition and biological activities. In: Fernandes GW, Oki Y, Barbosa M (eds) Baccharis. Springer, Cham. https://doi.org/10.1007/978-3-030-83511-8_10

Mukherjee S (2002) Influence of plant allelochemicals on growth rate, nutritional physiology and mid-gut esterase activity in fifth instar larvae of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Invert Reprod Dev 43:125–132. https://doi.org/10.1080/07924259.2003.9652531

Nollet LML, Rathore HS (2017) Essential oils for pest control. Green pesticides handbook, pp. 572

Pavela R, Vrchotova N, Sera B (2008) Growth inhibitory effect of extracts from Reynoutria sp. plants against *Spodoptera littoralis* larva. Agrociencia 42:573–584

Sharma A, Kumar V, Shahzad B et al (2019) Worldwide pesticide usage and its impacts on ecosystem. SN Appl Sci 1:1446. https://doi.org/10.1007/s42452-019-1485-1

Sun Z, Shi Q, Li Q, Wang R et al (2019) Identification of a cytochrome P450 *CYP6AB60 g*ene associated with tolerance to multi-plant allelochemicals from a polyphagous caterpillar tobacco cutworm (*Spodoptera litura)*. Pestic Biochem Physiol 154:60–66. https://doi.org/10.1016/j.pestbp.2018.12.006

Thomsen NA, Hammer KA, Riley TV, Van Belkum A, Carson CF (2013) Effect of habituation to tea tree (*Melaleuca alternifolia*) oil on the subsequent susceptibility of staphylococcus spp. to antimicrobials, triclosan, tea tree oil, terpinen-4-ol and carvacrol. Int J Antimicrob Agents 41:343–351

Whalon ME, Mota-Sanchez D, Hollingworth RM (2006) Analysis of global pesticide resistance in arthropods. CAB eBooks, p 10. https://doi.org/10.1079/9781845933531.0005

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