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ORIGINAL ARTICLE

Fumigant residual impacts of *Melaleuca alternifolia* (Maid. & Betche) Cheel. (Myrtales: Myrtaceae), terpinen-4-ol, and γ-terpinene on *Sitophilus oryzae* L. (Coleoptera: Curculionidae) on germination of wheat seeds

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Abstract

The fumigation toxicity of *Melaleuca alternifolia* (Maid. & Betche) Cheel. (Myrtales: Myrtaceae) essential oil and its major fractions was studied under laboratory conditions against adults of *Sitophilus oryzae* L. (Coleoptera: Curculionidae) to protect wheat grains (*Triticum aestivum* L.) (Poales: Poaceae) from this global pest that destroys the host plant during storage. By analyzing *M. alternifolia* essential oil (EO) using GC/MS terpinen-4-ol and γ-terpinene were detected as major components. In the fumigation toxicity, *M. alternifolia* EO showed the highest toxicity (LC_{50} = 0.31 μl · l^{-1} air), followed by terpinen-4-ol (LC_{50} = 23.65 μl · l^{-1} air) and γ-terpinene was the least toxic (LC_{50} = 43.55 μl · l^{-1} air). When tested for their insecticidal activities against *S. oryzae* in stored wheat, no progeny emerged after 3 months of treatment with *M. alternifolia* EO at 10 mg · g^{-1} or with terpinen-4-ol and γ-terpinene for 2 months. However, none of these compounds could protect wheat grain from damage throughout the entire study period (4 months). Interestingly, all tested compounds at the highest application rate did not show any phytotoxic effects after 4 months of storage.

Keywords: fumigant residues, *Melaleuca alternifolia*, *Sitophilus oryzae* L., *Triticum aestivum* L.

Introduction

Stored grains are subject to loss during storage due to several causes. The most important are insects that can lead to significant financial losses of up to 5–10% of the total product each year during storage only. This translates to a loss of 1.2–2 billion tonnes per year in developing countries (Hodges 2011; Savary *et al.* 2012; Yaseen *et al.* 2019). The enormous damage during storage can either be direct (loss in the mass of products) or indirect (reduction in terms of quality and nutritive value), in addition to reduced grain germination capacity (Affognon *et al.* 2015). One of the most widespread and destructive pest of stored grain, e.g., wheat, maize, and rice, is the rice weevil *Sitophilus oryzae* Linnaeus (Coleoptera: Curculionidae). This insect causes damage through direct feeding on grain, leading to its severe deterioration and reduction in germination capacity (Ismail and Sleem 2021a). In most systems, the protection of stored grain from pests such as the rice weevil depends mainly on the use of fumigants and contact insecticides. However, problems with residues and resistance, pose a major challenge (Jagadeesan *et al.* 2018).

Currently, there is research focusing on other alternatives for management of insect pests to protect stored grains. One of these alternative approaches is the use of plant natural products such as plant derived

essential oils (EOs) which possess insecticidal and repellent properties and are a potential option for insect control in stored grains (Campolo *et al*. 2018; Singh *et al*. 2021). A perusal of the literature revealed that *Melaleuca alternifolia* EO, also known as tea tree or melaleuca oil, is widely available and has been investigated as an alternative antimicrobial, anti-inflammatory and anti-cancer agent (Yadav *et al*. 2016). There is relatively limited data available indicating that *M. alternifolia* EO is toxic to some insect species while no study has been reported concerning the activity of its main constituents. However many studies have found that the biological activities of EOs depend on their chemical composition (Jankowska *et al*. 2018). Therefore, the aim of this study was to evaluate the fumigant toxicity and efficacy of *M. alternifolia* EO and their major constituents in protecting stored wheat grains (*T. aestivum*) from *S. oryzae* infestation.

Materials and Methods

Insect population

Infested wheat grains by rice weevils *Sitophilus oryzae* was obtained from a local vendor and it was maintained in a 2 l capacity glass jar under under controlled temperature 27 ± 2°C, relative humidity 65 ± 5% (RH), and in complete darkness. From the stock culture, a separate sub culture was prepared of *S. oryzae* adults and reared on sterilized wheat grains. Two-week old, mixed-sex *S. oryzae* adults were removed from the culture using a sieve and used for fumigation bioassays.

Chemicals

The *M. alternifolia* EO used in the assessments was provided by the Egyptian Natural Co., Egypt. Terpinen-4-ol (95%) and γ-terpinene (99%) were purchased from Sigma–Aldrich Chemical Ltd. (St Louis, MO, USA).

GC/MS analysis of the *Melaleuca alternifolia* EO

Melaleuca alternifolia EO composition 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). The essential oil was diluted in solvent before being injected into the gas chromatography--mass spectrometry (GC–MS). The carrier gas was helium (flow rate of 1 ml · min^{-1}). The solvent delay was 3 min, and the 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 50°C, the column oven was initially held, then by 5/min the temperature was increased to 250°C and held for 2 min. By 30°C · min^{-1}, 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 electron ionization (EI) mass spectra were collected at the m/z range of 50–650 in full-scan mode. The temperature of the ion source was set at 200°C. Chemical constituents were identified based on their retention time (*RT*). With the mass spectra and those of Wiley 09 and NIST 14 mass spectral database the percentage of components was calculated by the GC peak area.

Fumigant toxicity

Each of the fumigation mortality assays was replicated four times for each concentration of *M. alternifolia* EO, terpinen-4-ol, and γ-terpinene. Each replication consisted of 10 mixed-sex insects (n = 40; Σn = 1,240) that were put on the bottom of the glass jars with the caps screwed on tightly. Filter papers (Whatman No. 1, cut into 4 × 5 cm strips) were treated with different concentrations and then the treated filter paper was attached to the screwed on caps of glass jars which were then sealed with air–tight lids. In the control jars, only acetone was applied on the filter papers. In all cases, the exposure times were 24, 48 and 72 h. Treated insects were incubated at 30 ± 2°C. After this time, the number of dead adults was counted. Adults were considered dead if their appendages did not move or shake when prodded with the light touch of a fine-haired brush or/and they were unable to move or walk during a 2-min observation period. Mortality in the control was not observed in any experiment. Lethal values of all tested compounds were statistically computed with a SPSS program.

Fumigant residues

The *M. alternifolia* EO, terpinen-4-ol, and γ-terpinene were tested at application rates of 0.5, 1, 5, and 10 mg · g^{-1} on wheat grain stock solutions prepared in acetone. Fifty grams of untreated, clean, sterilized, and infestation-free wheat grains were placed in glass jars (300 ml). Wheat grains were treated with tested compounds and divided into four groups. The wheat in each glass jar was treated with 1 ml of the stock solution of the test compounds. The jars were shaken manually for 3 min to distribute the compounds throughout the grain. Grains which were treated with acetone served as a control. The jars were left for 30 min to allow the solvent to evaporate completely. Twenty *S. oryzae* adults were then introduced into each jar. Each treatment and control was replicated three times. The jars were covered with cheese cloth fastened with

rubber bands to prevent the insects from escaping and to ensure proper ventilation. All jars were retained under the same conditions as above. In the first group adult mortality was examined after 1 month of treatment. The dead insects were counted and removed. The other groups of treatment were opened after 2, 3, and 4 months. The grain was sieved and the powder was discarded. The weight of remaining grains in treatments and control was recorded. The weight loss percentage was calculated from the following formula:

$$A = \left(B - \frac{C}{B} \right) \times 100,$$

where:
A – % weight loss; B – weight of uninfested grain [g]; C – weight of infested grain of control and treatment [g].

Then the efficiency of the *M. alternifolia* EO, terpinen-4-ol and γ-terpinene were calculated using this equation:

$$E = \left(A - \frac{B}{A} \right) \times 100,$$

where:
E – % efficiency of the oil; A – loss of grain in control [g]; B – loss of grain in treatment [g].

Phytotoxicity

Seed germination and seedling growth was tested using 100 randomly picked seeds from each jar treated with the highest tested concentration (10 mg · g^{-1}) of *M. alternifolia* EO, terpinen-4-ol, and γ-terpinene 4 months post treatment. Twenty seeds were kept in each plate maintaining equidistance. Plates were incubated at 25 ± 1°C. Each of the treatment combinations had three replications. The seeds were placed on a moistened filter paper in glass Petri dishes which were regularly examined for germination and the number of germinated seeds and seedling growth was recorded after 7 days.

Statistical analysis

Percentage mortality of *S. oryzae* adults data was used for probit analysis to estimate the LC_{50} values (Finney 1971). All experiments in the present investigation were based on three replicates and the data were expressed as a mean of replicates ± standard error (SE). One way analysis of variance (ANOVA) with Tukey's multiple range was performed to determine the significant difference between treatments. Both probit analysis and ANOVA were performed using the SPSS (16.0 version) software program.

Results

Melaleuca alternifolia EO composition

Analysis of the chemical composition of *M alternifolia* EO (Table 1). Eleven components representing 92.6% of the *M. alternifolia* were identified by GC/MS. The main constituents of the examined *M. alternifolia* EO were terpinen-4-ol and γ-terpinene. Chemical analysis of the *M. alternifolia* EO showed that oxygenated monoterpenes are the major groups of compounds.

Fumigant toxicity

The results show that *M. alternifolia* EO, terpinen-4-ol, and γ-terpinene exhibited fumigant toxicity against the adults of *S. oryzae* (Tab. 2). Log-probit regression analysis of concentration-mortality data showed that, LC_{50} values were 12.86, 52.3, and 73.4 µl · l^{-1} air concentrations, for *M. alternifolia* EO, terpinen-4-ol, and γ-terpinene after 24 h of treatment, respectively. After 48 h of treatment the LC_{50} values were 4.02, 39.6, and 56.5 µl · l^{-1} air concentrations, respectively. After 72 h of treatment, the LC_{50} values ranged between 0.31 and 43.5 µl · l^{-1} air concentrations. The highest fumigant toxicity was observed in the case of *M. alternifolia* EO.

Fumigant residues

The results in Tables 3 and 4 clearly confirmed that the tested compounds were effective in protecting stored wheat grains. Adult mortality percentages are concentration-dependent. *Melaleuca alternifolia* EO at a concentration of 10 mg · kg^{-1} achieved full protection for 3 months and caused complete mortality (100%), i.e., no *S. oryzae* progeny emerged. At 5 mg · g^{-1} there was full protection for only 1 month, where a few adults were recorded, 22.67, 35.69 and 70.42 adults after 2, 3 and 4 months, respectively, compared with control. At the

Table 1. Chemical composition of the of *Melaleuca alternifolia* EO

Component	Area [%]	*RT
α-Pinene	5.86	2.4
α-Terpinene	10.4	13.0
Limonene	1.2	1.0
p-Cymene	1.20	2.6
1,8-Cineole	1.83	5.1
γ-Terpinene	21.9	28.0
Terpinolene	3.24	3.1
Terpinen-4-ol	40.1	48.0
α-Terpineol	6.91	2.4
o-Cymene	5.0	9.0

RT – retention time [min]; EO – essential oils

Table 2. LC_{50} values of *Melaleuca alternifolia* essential oils, terpinen-4-ol, and γ-terpinene against *Sitophilus oryzae* adults at three different exposure periods

Treatment	Exposure period [h]	Slope ± SE	LC_{50} (95% CL) [μl · l^{-1} air]	χ^2 ($df = 4$)	*p*-value
M. alternifolia	24	1.37 ± 0.16	12.86 (8.43–17.50)	0.02	0.48
	48	1.14 ± 1.10	4.02 (2.95–5.25)	3.47	0.34
	72	0.83 ± 0.14	0.31 (0.07–0.66)	0.16	0.75
Terpinen-4-ol	24	2.9 ± 0.30	52.3 (46.1–61.1)	3.07	0.41
	48	1.69 ± 0.42	39.6 (22.3–62.7)	3.26	0.34
	72	3.2 ± 0.28	23.6 (19.5–28.4)	3.24	0.37
γ-Terpinene	24	1.46 ± 0.27	73.4 (59.0–98.5)	0.44	0.69
	48	2.5 ± 0.45	56.5 (43.8–69.5)	1.43	0.96
	72	1.62 ± 0.36	43.5 (30.4–58.5)	2.65	0.86

lowest concentration (0.5 mg · kg^{-1}), *M. alternifolia* EO caused the highest mortality (57.21%) after 1 month of exposure. The wheat grains treated with terpinen-4-ol and γ-terpinene at a concentration of 10 mg · kg^{-1} gave full protection from adult infestation for 2 months. After that, adult infestation started to appear in 5.34 and 10.44 adults after 3 and 4 storage months, respectively, for terpinen-4-ol and 13.85 and 16.81, respectively, for γ-terpinene. Compared with untreated stored wheat grain, the infection rate after 1 month was 159.60% while the infection rates after 2, 3 and 4 months were 315.71, 555.68 and 712.95, respectively (Table 3). A high of weight loss during storge periods was 50.60, 85.27, 94.64, and 96.75% from the first till the 4th month in the control. In contrast, all tested compounds significantly reduced the grain weight loss ($p > 0.05$). The results clearly indicated that *M. alternifolia* EO was the most effective followed by terpinen-4-ol while γ-terpinene was the least effective in protecting stored wheat grains (Tab. 4).

Phytotoxicity

The effect of fumigant residues of tested compounds on seed wheat germination and seedling growth was studied (Tab. 5). Seed germination percentages were 90, 91.46 and 94.11% for *M. alternifolia* EO, terpinen-4-ol, and γ-terpinene, respectively, compared with 98.95% in the control. Also, there was no visual abnormality in the morphology of the plants and they appeared as healthy as those in the control.

Discussion

The incidence of *S. oryzae* L. resistance to conventional insecticides and persistent infestation problems of stored grain has lead to the search for more effective treatments (Ismail and Sleem 2021b). EOs possess insecticidal and repellent properties and are a potential option for insect control in stored grains (Campolo *et al.* 2018). Therefore, the aim of this study was to evaluate the fumigant toxicity and efficacy of *M. alternifolia* EO and their major constituents in protecting stored wheat grains (*T. aestivum* L.) from *S. oryzae* infestation.

Chemical composition analysis of *M. alternifolia* EO showed that terpinen-4-ol and γ-terpinene were the main components. This result was in line with Ibáñez and Blázquez (2019) who found that the main component in *M. alternifolia* EO was terpinen-4-ol (28.37%) followed by γ-terpinene (15.60%).

Fumigation activity of EO *M. alternifolia* and their major constituents were examined against the adults of *S. oryzae*. *M. alternifolia* EO showed highly effective fumigant toxicity, followed by terpinen-4-ol while γ-terpinene was the least effective. These results were supported by Fang *et al.* (2016), Liao *et al.* (2017), Jankowska *et al.* (2018) and Yang *et al.* (2020). They reported that the EOs' activity may primarily be due to the synergy between their components (Jankowska *et al.* 2018). Terpinen-4-ol was the most active ingredient of *M. alternifolia* EO on *Sarcoptes scabiei* (Fang *et al.* 2016), on *Helicoverpa armigera* (Liao *et al.* 2017), and on maize weevils, *Sitophilus zeamais* (Motschulsky) (Yang *et al.* 2020).

All tested compounds neither showed any adverse effect on germination of wheat seeds nor exhibited any deleterious effect on seedling growth of wheat. Also, there were no visual abnormalities in the morphology of the plants of all the treatments. The plants appeared as healthy as those of the control. These results indicate the nonphytotoxic nature of these compounds and show their future use as botanical insecticides. Thus, the farmer can store wheat treated with these compounds for extended storage periods.

Table 3. Reduction percentage of *Sitophilus oryzae* on wheat grains treated with *Melaleuca alternifolia* essential oils, terpinen-4-ol, and γ-terpinene at different concentrations

Treatment	Application rate [mg · kg^{-1}]	Storage period [month]							
		1		2		3		4	
		progeny production	% reduction	progeny production	% reduction	progeny production	% reduction	progeny production	% reduction
M. alternifolia	0.5	68.29 ± 6.47 cA	57.21 ± 8.12 bB	147.19 ± 10.39 fB	53.38 ± 5.08 cB	282.00 ± 20.32 gC	49.25 ± 3.35 bB	390.78 ± 17.59 hD	45.19 ± 5.53 cA
	1.0	58.67 ± 5.52 bA	63.24 ± 5.48 cB	125.00 ± 4.87 eB	60.41 ± 2.15 dB	238.11 ± 6.35 fC	57.15 ± 3.29 cA	322.50 ± 6.04 gD	54.77 ± 4.18 dA
	5.0	0.0 ± 0.0 aA	100.0 ± 0.0 dB	22.67 ± 3.74 bB	95.82 ± 5.60 gA	35.69 ± 3.74 aC	93.58 ± 4.18 fA	70.42 ± 4.22 bD	90.12 ± 6.25 hA
	10.0	0.0 ± 0.0 aA	100.0 ± 0.0 dB	0.0 ± 0.0 aA	100.0 ± 0.0 hB	0.0 ± 0.0 aA	100.0 ± 0.0 gB	13.54 ± 1.74 aB	98.11 ± 23.8 hA
Terpinen-4-ol	0.5	85.99 ± 5.40 eA	46.12 ± 6.23 aB	180.14 ± 4.02 iB	42.94 ± 5.11 bB	340.45 ± 24.5 iC	38.73 ± 4.16 aA	490.15 ± 24.95 kD	31.25 ± 3.69 bA
	1.0	75.32 ± 3.10 dA	52.81 ± 2.34 bB	157.85 ± 6.3 gB	50.16 ± 3.74 cB	290.00 ± 13.3 hC	47.81 ± 3.00 bA	426.89 ± 17.87 iD	40.12 ± 7.21 cA
	5.0	0.0 ± 0.0 aA	100.0 ± 0.0 dA	55.09 ± 4.17 cB	82.55 ± 5.34 fC	107.74 ± 9.23 dC	80.61 ± 4.45 eC	159.85 ± 17.5 eD	77.58 ± 6.47 fB
	10.0	0.0 ± 0.0 aA	100.0 ± 0.0 dC	0.0 ± 0.0 aA	100.0 ± 0.0 hC	49.14 ± 4.00 bB	91.16 ± 7.21fB	84.84 ± 5.52 cC	88.10 ± 3.12 gA
γ-Terpinene	0.5	94.42 ± 11.32 fA	40.84 ± 2.11 aC	207.19 ± 10.11 jB	34.37 ± 2.20 aB	390.14 ± 13.34 jC	29.79 ± 3.20 aAB	564.05 ± 43.89 lD	20.89 ± 3.60 aA
	1.0	79.88 ± 4.22 eA	49.95 ± 6.47 bB	173.42 ± 24.54 hB	45.07 ± 10.85 bB	337.57 ± 3.56 iC	39.25 ± 3.00 aA	465.50 ± 5.52 jD	34.71 ± 3.83 bA
	5.0	0.0 ± 0.0 aA	100.0 ± 0.0 dC	65.30 ± 5.48 dB	79.32 ± 8.72 eB	128.72 ± 11.02 eC	76.84 ± 6.15 dB	219.00 ± 22.5 fD	69.28 ± 3.15 eA
	10.0	0.0 ± 0.0 aA	100.0 ± 0.0 dB	0.0 ± 0.0 aA	100.0 ± 0.0 hB	66.98 ± 5.08 cB	87.95 ± 3.12 eA	109.63 ± 5.31 dC	84.63 ± 5.34 gA
Control	0.0	159.60 ± 13.63 gA		315.71 ± 17.5 kB		555.68 ± 23.44 kC		712.95 ± 27.6 mD	

Values are means ±SE; within each column, means with the same lowercase letter are not significantly different ($p > 0.05$); within each row, means with the same uppercase letter are not significantly different ($p > 0.05$)

Table 4. Efficacy of *Melaleuca alternifolia* essential oils, terpinen-4-ol, and γ-terpinene in protecting wheat grains against *Sitophilus oryzae* adults under storage conditions

Treatment	Application rate [mg · kg^{-1}]	Storage period [month]							
		1		2		3		4	
		weight loss [%]	efficiency [%]	weight loss [%]	efficiency [%]	weight loss [%]	efficiency [%]	weight loss [%]	efficiency [%]
M. alternifolia	0.5	43.60 ± 6.47 dA	48.87 ± 6.73 aA	45.56 ± 10.39 cA	47.74 ± 5.08 aA	52.41 ± 3.35 dB	44.62 ± 2.54 bA	55.51 ± 4.02 dB	43.21 ± 5.53 bA
	1.0	17.08 ± 2.22 bA	66.25 ± 1.26 bB	30.10 ± 2.37 bB	64.70 ± 4.67 bB	35.25 ± 3.22 cB	62.28 ± 4.41 cB	43.95 ± 13.1 cC	55.03 ± 4.52 cA
	5.0	0.0 ± 0.0 aA	100.0 ± 0.0 eB	3.99 ± 3.69 aA	95.32 ± 1.12 dA	4.58 ± 1.99 aA	95.16 ± 7.42 eA	6.89 ± 2.89 aA	92.95 ± 2.02 fA
	10.0	0.0 ± 0.0 aA	100.0 ± 0.0 eB	0.0 ± 0.0 aA	100.0 ± 0.0 eB	0.0 ± 0.0 aA	100.0 ± 0.0 fB	3.70 ± 1.56 aA	96.21 ± 5.77 fA
Terpinen-4-ol	0.5	39.55 ± 5.40 cdA	70.75 ± 4.42 cB	36.67 ± 2.41 bA	66.58 ± 14.3 bB	63.30 ± 14.5 eB	33.11 ± 3.10 aA	68.11 ± 6.67 eB	30.32 ± 2.66 aA
	1.0	52.27 ± 4.87 eA	86.62 ± 5.44 dB	50.59 ± 6.34 dA	83.26 ± 2.10 cB	49.17 ± 17.5 dA	48.05 ± 6.89 bA	52.35 ± 42.32 dA	46.45 ± 9.94 bA
	5.0	0.0 ± 0.0 aA	100.0 ± 0.0 eB	87.90 ± 6.84 eC	85.96 ± 6.68 cA	15.63 ± 5.53 bB	83.48 ± 4.53 dA	20.97 ± 3.62 bB	78.55 ± 6.47 dA
	10.0	0.0 ± 0.0 aA	100.0 ± 0.0 eB	0.0 ± 0.0 aA	100.0 ± 0.0 eB	5.34 ± 3.07 aA	94.36 ± 3.96 eA	10.44 ± 2.46 aA	89.32 ± 6.67 eA
γ-Terpinene	0.5	36.21 ± 4.18 cA	67.98 ± 3.74 bB	34.61 ± 4.47 bA	63.52 ± 14.3 bB	66.23 ± 13.34 eB	30.00 ± 2.20 aA	69.64 ± 2.83 eB	28.76 ± 3.33 aA
	1.0	49.49 ± 8.74 deAB	83.30 ± 4.12 dB	45.13 ± 7.54 cA	80.71 ± 18.2 cB	53.71 ± 15.3 dB	43.25 ± 10.4 bA	57.48 ± 3.34 dB	41.19 ± 3.17 bA
	5.0	0.0 ± 0.0 aA	100.0 ± 0.0 eC	85.06 ± 2.35 eC	83.84 ± 15.5 cB	19.44 ± 5.16 bB	79.46 ± 9.49 dA	22.54 ± 1.98 bB	76.94 ± 4.36 dA
	10.0	0.0 ± 0.0 aA	100.0 ± 0.0 eB	0.0 ± 0.0 aA	100.0 ± 0.0 eB	13.85 ± 2.01 bB	85.37 ± 4.75 dA	16.81 ± 3.13 bB	82.80 ± 2.14 eA
Control		50.60 ± 2.08 eA		85.27 ± 2.35 eB		94.64 ± 4.29 fC		96.75 ± 7.21 fC	

Values are means ±SE; within each column, means with the same lowercase letter are not significantly different ($p > 0.05$); within each row, means with the same uppercase letter are not significantly different ($p > 0.05$)

Table 5. Phytotoxicity of *Melaleuca alternifolia* essential oils, terpinen-4-ol, and γ-terpinene on seed germination and seedling growth of wheat

Treatment	Germination [%]	Radical [cm]	Plumule [cm]
M. alternifolia	90.00 ± 0.28 c	3.12 ± 0.11 b	2.02 ± 0.20 c
Terpinen-4-ol	91.46 ± 0.17 c	3.28 ± 0.14 b	2.35 ± 0.05 c
γ-Terpinene	94.11 ± 0.11 b	3.50 ± 0.17 a	2.49 ± 0.02 b
Control	98.95 ± 0.40 a	4.33 ± 0.13 a	3.16 ± 0.08 a

Values are means ±SE; within each column, means with the same lowercase letter are not significantly different ($p > 0.05$)

Conclusions

This work did not only show efficacy of *M. alternifolia* EO, terpinen-4-ol and γ-terpinene in protecting wheat grains from *S. oryzae* infestation, but also there were no adverse effects on seed germination or seedling growth. Therefore, these results indicate great potential for the development of *M. alternifolia* components as part of an integrated pest management strategy and this requires further studies.

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