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ORIGINAL ARTICLE

The efficacy of some synthetic monoterpenes and Yucca extract for controlling *Tribolium castaneum* (Herbst) in wheat grain

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Abstract

The study's objective was to assess the efficacy and to identify the toxic mechanisms of action of some plant-derived monoterpenes and yucca extract as alternatives to chemical insecticides against the red flour beetle, *Tribolium castaneum*. Carvone, 1,8-cineole, cuminaldehyde, and linalool, as well as *Yucca schidgera* extract, were the control agents whose efficacy against the red flour beetle was tested in the laboratory and compared to malathion. The criteria for evaluating efficacy were the effects of the tested compounds on adult mortality and red flour beetle progeny. Furthermore, the effects of the control agents on some enzymes (Acetylcholinesterase, ά-amylase, and alkaline phosphatase) in *T. castaneum* were investigated. Moreover, the effect of the tested control measures on weight loss of treated wheat grain was studied. The tested substances showed a high ability to control *T. castaneum* with regard to adult mortality and offspring production, especially when used as fumigants. For adult mortality, malathion showed the highest potential against *T. castaneum* as a fumigant, followed by carvone, yucca extract, cuminaldehyde, linalool, and 1,8-cineole with LC_{50} values of 0.05, 331.5, 365.1, 372.2, 460, and 467.5 mg · 1000 cm^{-2} after 72 hours, respectively. The highest reduction in progeny was for malathion followed by carvone, linalool, cineole, yucca extract, and cuminaldehyde with reduction percentages of 100, 90, 89.3,79.4 and 65.8%, respectively. The tested compounds significantly affected acetylcholinesterase activity, alpha-amylase activity, and alkaline phosphatase in the red flour beetle. There was no significant reduction in the weight of wheat grains treated with the tested materials compared to the untreated healthy grains. Finally, the tested compounds as fumigants, especially 1,8-cineole, can be considered as effective alternatives to control the red flour beetle.

Keywords: enzymes, insecticides, progeny, red flour beetle, stored products, toxicity

Introduction

Egypt is one of the largest importers of wheat in the world. Therefore, it is very obvious that wheat grains are a product of absolute importance to Egypt and a priority for the Egyptian government (Matouk *et al.* 2017). The red flour beetle, *T. castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a widespread pest in mills, processing plants, food stores, and retail stores (Kłyś *et al.* 2017; Perkin and Oppert 2019). This type of insect can be controlled with contact insecticides applied to surfaces (Arthur 2009), aerosols applied as space treatments (Sutton *et al.* 2011), and fumigation of entire storage godowns (Campbell *et al.* 2010).

To control stored grain insects, insecticides have always been an effective and widely used solution.

However, there is an urgent need to find effective alternatives to control these types of pests due to problems caused by chemical pesticides, such as the presence of residues from these pesticides, their toxicity, and the cost of their use, especially for small producers (Chaudhari *et al.* 2021; Derbalah *et al.* 2021). Among the most important alternatives to traditional chemical insecticides are compounds derived from aromatic plants, such as monoterpenes, which can help overcome insect resistance to the action of chemical insecticides (Abdelgaleil *et al.* 2016; Ezhil-Vendan *et al.* 2017; Chaudhari *et al.* 2021; Derbalah *et al.* 2021). Due to their multiple advantages, such as being environmentally friendly, economical, easily biodegradable, and less harmful to mammals, pesticides of plant origin have attracted the attention of specialists in pest control, especially stored grain insects, to evaluate their effectiveness against these insect pests (Derbalah *et al.* 2021). Moreover, plants produce a unique assortment of secondary metabolites which have been used for many years as competitive weapons against insects (Thirumurugan *et al.* 2018). Saponins are among those secondary plant compounds. The trunk of yucca (*Yucca schidigera*) is one of the best-known sources of industrial steroidal saponins (Qu *et al.* 2018). *Yucca schidigera* Roezl (Agavaceae family) is a plant rich in these kinds of constituents. As one of the major industrial sources of steroid saponins, *Y. schidigera* is native to the deserts of southwestern United States and northern Baja California, Mexico (Miyakoshi *et al.* 2000). The commercial extracts of *Y. schidigera* are approved by the FDA as GRAS (Generally Recognized as Safe) and are widely used as animal and human food additives (Sastre *et al.* 2017). Saponins derived from different plant origins have been used to reduce grain damage and weight loss caused by insects (Singh and Kaur 2018).

The investigation of the effects of various materials used in insect pest control on the activity of some enzymes, such as acetylcholinesterase (AChE), and amylase as digestive enzymes, as well as alkaline phosphatase, which is considered a detoxifying enzyme, may play a significant role in explaining the toxic effects of these control agents (monoterpenoids and plant extracts) (Chaudhari *et al.* 2021; Derbalah *et al.* 2021). As a result, research on the biochemical effects of the tested control agents on the physiology of insects can help to identify toxic effect sites.

From this standpoint, this study aimed to evaluate the efficacy of carvone, 1, 8-cineole, cuminaldehyde, linalool, and yucca extract as possible alternatives to malathion in protecting wheat grains against *T. castaneum* based on their impact on mortality rates and the offspring of adult insects. The effect of the application method (thin-film, contact, and fumigation) of tested control agents on their efficacy against *T. castaneum* was also investigated. The effect of the tested control agents on some enzymes in the treated *T. castaneum* was also studied to try to identify the mechanism of their toxic action. Finally, the effect of the used control agents on the quantity of the treated grain with respect to the reduction in grain weight was evaluated.

Materials and Methods

Chemicals

Carvone, 1, 8-cineole, cuminaldehyde, and linalool with a purity of 99% were purchased from Sigma Aldrich, USA. Malathion with a trade name of Melason 57% EC was purchased from Kafr Elzayat Pesticides, and Chemicals Company, Egypt. *Yucca schidigera* plant extract with a trade name of Mex Yucca liquid (13.7% saponins) was obtained from Baja Yucca Company, Mexico City, Mexico with water solubility of 99.8%. All needed chemicals (5,5-dithio-bis-(2-nitrobenzoic acid and butyrylcholinesterase)) for biochemical assessments were purchased from Quimica Clinica Aplicada Company, Spain.

Rearing of insects

Whole grain flour sterilized at 60°C for 60–90 minutes served as the culture medium for the selected insect. Then, 200 grams of flour were added to each jar, and 30 unsexed beetles were transferred to each jar. The beetles were left to lay eggs and to feed in a culture environment for 3 days. Then, they were removed and added to another batch of sterile jars filled with 200 grams of flour to continue the culture. Flour containing eggs was used as a culture medium to generate adult beetles of the same age (Saleem 1990). The insect culture was kept in sterile containers in an incubator at 30 ± 2°C and 60 ±5% RH, and homogeneous insects from the culture (14 days old) were selected for further experiments.

Thin-film residue assay (without grain) of tested control agents against *Tribolium castaneum*

The efficacy of monoterpene compounds and yucca extract against the adults of *T. castaneum* as a thin-film residue (direct contact toxicity) was evaluated as described by Broussalis *et al.* (1999). Malathion was used as a standard to compare the efficacy of the used compounds and yucca extract. In acetone, series giving final concentrations of 6.4, 12.8, 19.2, 25.6 mg · 64 cm^{-2} (Petri dish area) which equal 100, 200, 300, and 400 mg · 1000 cm^{-2} for monoterpenes and yucca extract

as well as 0.001, 0.025, 0050, 0.075 mg · 64 cm^{-2} which equal 0.02, 0.04, 0.08, and 0.12 mg · 1000 cm^{-2} for malathion were prepared. One ml of each concentration was placed on the bottom of a glass Petri dish (9 cm in diameter). Before transferring the insects, the solvent was left to evaporate for 2 minutes. Twenty adults were placed in each Petri dish. Control plates were treated with acetone only. All treatments were repeated four times. Mortality ratios were recorded after 24 and 72 hours of treatment, and LC_{50} values were estimated as described by Finney (1971).

Grain treatment assay (mixing with the feeding grain)

To evaluate the efficacy of the tested substances as grain treatments against *T. castaneum* (exposure through the grain feeding) compared to malathion, a series of concentrations of 5, 10, 15, 20 mg · 20 g^{-1} grain which equal 250, 500, 750, and 1000 mg · kg^{-1} for monterpenes and yucca extract as well as 0.001, 0.002, 0.004 and 0.006 mg · 20 g^{-1} grain which equal 0.05, 0.1, 0.2, and 0.3 mg · kg^{-1} for malathion were prepared in acetone. As described by Derbalah *et al.* (2021), 20 grams of broken wheat were placed in 300 ml glass jars. Then, 1 ml from each concentration of the tested substances was mixed with broken wheat grains in the glass jars. Jars used as controls received only acetone as treatment. Each jar was manually shaken for ~5 minutes to ensure that the treatments were evenly distributed throughout the grain mass. To ensure complete evaporation of the solvent, the jars were left open for 30 minutes. Then 20 unsexed adults of *T. castaneum* were added to each jar. For each treatment and control, four replicates were created. To keep insects out and ensure proper ventilation, the jars were covered with cotton cloth and secured with rubber bands. After 24 and 72 hours of treatment, all dead insects were counted and removed, and adult mortality was calculated. All jars were kept under the same conditions after the last mortality count, and the number of adult *T. castaneum* insects was counted after 6 weeks. The following equation, as reported by El-Lakwah *et al.* (1992) was used to calculate the percentage reduction in the number of progeny:

$$\%\ \text{reduction} = \left(1 - \frac{x}{y}\right) \times 100,$$

where: x – the number of adults emerging from treatment, y – the number of adults emerging from control.

To evaluate the effect of tested treatments on the loss in grain weight, the treated grain was sieved and the powder was discarded. After that, the weight of the grains was recorded in the treatments and control. An untreated control free of insects was maintained in the weight loss experiment to check for any possible natural weight losses. No weight loss was observed (data not shown). To calculate the percentage of weight loss the following equation reported by Harris and Landlady (1978) was used:

$$\%\ \text{weight loss} = \left(W_u - \frac{W_i}{W_u}\right) \times 100,$$

where: W_u – weight of noninfested grain, W_i – weight of infested grain.

Then, the efficacy (E) of the treatments as a percent of loss in grain weight was calculated using the equation, as described by Harris and Lindblad (1978):

$$E = \left(LC - \frac{LT}{LC}\right) \times 100\%,$$

where: LC – loss of grain in control and LT – loss of grain in treatment.

Fumigation treatment assay

The examined substances were evaluated as fumigants against adults of *T. castaneum* (insects exposed to the vapor of the tested control agents) following the method of Huang *et al.* (2000) with some modifications. Several concentrations of monoterpenes and yucca extract (200, 250, 290, and 330 mg · 1000 cm^{-3}) and 20, 40, 80, and 120 mg · cm^{-3} for malathion were tested. One liter glass jars were equipped as steaming chambers and 1 ml of each concentration of the tested concentration was applied to pieces of filter paper (2 × 3 cm) and left to dry and then placed on the bottom surface of screw cap jars using double face stickers. Although malathion is not a used as fumigant other organophosphorus insecticides such as dichlorovos were used as a space or vapor treatment to control stored products. As a result, the fumigant activity of malathion as an organophosphorus insecticide was investigated in this study. To prevent insects from coming into direct contact with the tested item, vaseline was applied to the inside of the jar's neck. The caps were tightly screwed to the jars after adding 20 adults of *T. castaneum* insects to each one. The control insects were housed under identical conditions, but without the tested substances. Four replicates were prepared for each treatment. Mortality in adult insects after 24, and 72 hours of treatment was determined as well as LC_{50} values (Finney 1971).

Biochemical effects of the used control agents

The adults of *T. castaneum* were treated with the tested control agents at a concentration equivalent to the LC_{50} value after 72 hours when used as a grain treatment to assess enzyme activity. Live adults of *T. castaneum* (0.5 g) were homogenized separately

using a Teflon glass tissue homogenizer in 5 ml of 0.1 M ice-cold phosphate buffer (pH 7.0). The homogenates were centrifuged for 20 minutes at 4°C at 5000 rpm, and the supernatants were used for the enzyme activity assay.

Acetlycholinesterase (AChE) activitiy assay

Acetlycholinesterase activitiy (AChE) was determined following the method of Knedel and Bottger (1967). The enzyme activity assay was carried out by the addition of 18 ml of R_1 (buffer) to one vial of R_2 (DTNB). Then, 11 ml of R_1 was added to one vial of R_3 (BTC) and mixed gently until dissolution. To prepare the monoreagent, 2 ml of R_3 were added to one vial of reconstituted R_2 and gently mixed. Then the reagent was allowed to reach room temperature before use. After that, 1 ml of monoreagent was added using a pipette in a test tube, followed by the addition of 20 µl of the sample. The solution was transferred to the measuring cuvette of the spectrophotometer after mixing it well. The initial absorbance was recorded and, using a stopwatch, the absorbance was recorded again after 30, 60, and 90 seconds at a wavelength of 405 nm against a blank. The concentration of AChE in serum was determined according to the following equation:

$$\text{AChE (U/L)} = 22710 \times \frac{\Delta A}{30\ \text{sec}},$$

where: ΔA is the mean absorbance.

Alpha-amylase activity assay

The activity of this enzyme was estimated following the method described by Caraway (1959). The determination procedure was carried out by the addition of 0.5 ml of the buffered substrate to two test tubes. Then, 0.01 ml of the sample was added to the first tube, while the second tube served as a blank. The content of the sample tube was mixed well and incubated for 7.5 min at 37°C. After that, 0.5 ml of working reagent was added and mixed well, followed by the addition of 4 ml of distilled water. The absorbance of the solution was recorded at 660 nm against distilled water. The activity of amylase was estimated as described in the following equation:

$$\alpha\text{-Amylase activity} \left(\frac{\text{U}}{\text{L}}\right) =$$

$$= \left(A_{\text{blank}} - \frac{A_{\text{sample}}}{A_{\text{blank}}}\right) \times 1480.$$

Alkaline phosphatase (ALP) activity assay

Alkaline phosphatase activity was determined following the method described by Belfield and Goldberg (1971). The procedure was started by the addition of 0.5 ml of the buffer substrate to three clean dry test tubes. Then, 0.025 ml of the standard and the same volume from the sample were added to the first and second tubes, respectively. Then, mixtures in the first and second tubes were incubated at 37°C for exactly 20 min, while the third tube served as a blank. Then, the reaction was stopped by the addition of 0.25 ml of the enzyme inhibitor of (4-aminoantipyrine and sodium arsenate) to each tube with a vortex shaken at 400 rpm for 3 min. Finally, 0.25 ml of the color developing reagent was added and the test tubes were allowed to stand for 5 minutes in the dark before the absorbance was recorded at a wavelength of 510 nm using reagent blank (buffer substrate). The activity of alkaline phosphatase was calculated according to the following equation:

$$\alpha\text{-Alkaline phosphatase (IU/L)} = \frac{A_{\text{sample}}}{A_{\text{standart}}} \times 75.$$

Data analysis

Statistical analysis of this data was performed using SPSS version 23.0. LC_{50} values were calculated by analyzing mortality rates versus concentrations using probit analysis (Finney 1971). If the 95% confidence limits did not overlap, the LC_{50} values were considered significantly different. One-way analysis of variance was used to examine breed data, weight loss, and grain quality (ANOVA). The student Newman-Keuls test (SNK) was used to find significant differences between the means, and a difference of $p = 0.05$ was considered significant.

Results

The effectiveness of the control agents tested against *Tribolium castaneum*

The efficacy of the examined monoterpenes and plant extract was evaluated against *T. castaneum* based on adult mortality in thin-film, grain, and fumigation treatments (Tables 1–3). Table 1 shows the efficacy of compounds and plant extract applied as a thin film treatment. Malathion showed the highest potential against *T. castaneum*, followed by carvone, yucca extract, cuminaldehyde, linalool, and 1,8-cineole with LC_{50} values of 0.07, 404.9, 526.4, 572.4, 737.4, and 1186.4 mg · 1000 cm^{-2} after 24 hours, respectively. After 72 hours of treatment, malathion was still the most efficient against *T. castaneum*, followed by carvone, 1,8-cineole, yucca extract, cuminaldehyde, and linalool with LC_{50} values of 0.05, 331.5, 365.1, 372.2, 460, and 467.5 mg · 1000 cm^{-2}, respectively.

The insecticidal effect of the tested control agents used as a grain treatment against *T. castaneum* based on the adult mortality rate is shown in Table 2. The results showed that malathion had the highest activity against *T. castaneum*, followed by linalool, carvone, cuminaldhyde, 1, 8-cineole and yucca extract, with LC_{50} values of 0.08, 491, 606.6, 3191.3, 3763.3, and 10311.8 mg · kg^{-1} after 24 hours, respectively. However, after 72 hours of treatment, malathion continued to be the best against *T. castaneum*, followed by linalool carvone, 1.8 cineole, yucca extract, and cuminaldehyde with LC_{50} values of 0.06, 358.9, 474.9, 1672.3, 1777.9 and 2824.8 mg · kg^{-1}, respectively.

The efficacy of the tested compounds and yucca extract applied as a fumigant treatment against *T. castaneum*, is shown in Table 3. Even though malathion is not a fumigant, it was the most effective compound against *T. castaneum*, followed by 1,8-cineole, carvone, cumenaldehyde, and yucca extract with LC_{50} values of 32.7, 138.5, 233.8, 237.9, 284.4, and 356.6 mg · 1000 cm^{-3} after 24 hours, respectively. After 72 hours of treatment, results indicated that malathion was the best treatment against *T. castaneum*, followed by 1,8-cineole, carvone, linalool, cuminaldehyde, and yucca extract with LC_{50} values of 14.1, 23.4, 205.7, 209.6, 249.8, and 312.1 mg · 1000 cm^{-3}, respectively.

Table 1. The efficacy of evaluated control agents as a thin film treatment on adult mortality of *Tribolium castaneum*

Treatments	Time [h]	LC_{50} [a] [mg · 1000 cm^{-2}] lower-upper	Slope	Chi-square	Degree of freedom [df]
Malathion	24	0.07 (0.05–0.08)	0.27	0.99	2.0
	72	0.05 (0.04–0.06)	0.34	3.16	2.0
Cineole	24	1186.4 (561.3–2226.3)	1.10	1.48	2.0
	72	365.1 (271.1–749.4)	1.33	0.57	2.0
Carvone	24	404.9 (336.5–556.2)	2.58	0.35	2.0
	72	331.5 (279.6–426.6)	2.41	4.78	2.0
Linalool	24	737.4 (471.6–1080.7)	1.56	0.63	2.0
	72	467.5 (331.2–1256.8)	1.35	2.40	2.0
Cuminaldehyde	24	572.4 (443.1–1028.6)	2.79	1.38	2.0
	72	460.0 (370.4–697.8)	2.47	0.04	2.0
Yucca schidigera extract	24	526.4 (378.3–1241.2)	1.63	0.02	2.0
	72	372.3 (290.7–609.3)	1.68	0.57	2.0

[a]the concentration causing 50% mortality in *T. castaneum*. If the 95% confidence limits did not overlap, the LC_{50} values were considered significantly different

Table 2. The efficacy of evaluated control agents as grain treatment on adult mortality of *Tribolium castaneum*

Treatments	Time [h]	LC_{50} [a] [mg · 1000 cm^{-2}] lower-upper	Slope	Chi-square	Degree of freedom [df]
Malathion	24	0.08 (0.02–0.19)	2.9	6.93	2.0
	72	0.06 (0.05–0.07)	4.3	2.29	2.0
1,8 Cineole	24	3763.3 (1641.4–11895)	1.2	0.11	2.0
	72	1672.3 (1078–7206.2)	1.4	1.11	2.0
Carvone	24	606.6 (514.8–722.9)	2.4	0.15	2.0
	72	474.9 (404.1–545.5)	2.8	3.52	2.0
Linalool	24	491.0 (414.1–569.7)	2.6	2.72	2.0
	72	358.9 (285.9–421.6)	2.6	4.0	2.0
Cuminaldehyde	24	3191.3 (1668.6–6196.4)	1.8	2.38	2.0
	72	2824.8 (1489.3–4786.1)	1.4	1.32	2.0
Yucca schidigera extract	24	10311.8 (10099.7–10528.3)	0.6	0.11	2.0
	72	1777.9 (1035.9–2828.0)	1.0	1.31	2.0

[a]the concentration causing 50% mortality in *T. castaneum* adults. If the 95% confidence limits did not overlap, the LC_{50} values were considered significantly different

Table 3. The efficacy of evaluated control agents as fumigation treatment on adult mortality of *Tribolium castaneum*

Treatments	Time [h]	LC_{50} [a] [mg · 1000 cm^{-2}] lower-upper	Slope	Chi-square	Degree of freedom [df]
Malathion	24	32.7 (28.9–36.9)	0.6	0.17	2.0
	72	14.1 (4.9–40.4)	2.7	1.02	2.0
1,8-Cineole	24	138.5 (114.5–188.9)	4.2	2.06	2.0
	72	23.4 (18.9–28.7)	1.4	8.65	2.0
Carvone	24	237.9 (188.6–264.7)	5.0	1.15	2.0
	72	205.7 (166.6–225.5)	7.6	4.61	2.0
Linalool	24	233.8 (200.8–254.2)	6.6	0.39	2.0
	72	209.6 (170.4–229.9)	7.4	0.06	2.0
Cuminaldehyde	24	284.4 (266.5–307.8)	8.7	0.37	2.0
	72	249.8 (227.2–268.4)	7.7	0.65	2.0
Yucca schidigera extract	24	356.6 (316.3–522.9)	6.1	0.34	2.0
	72	312.1 (281.8–400.4)	5.4	0.94	2.0

[a]the concentration causing 50% mortality in *T. castaneum* adults. If the 95 confidence limits did not overlap, the LC_{50} values were considered significantly different

The efficacy of the examined control agents against adults of *T. castaneum* was improved by increasing exposure time under different treatment methods. The mortality rate of each of the tested control agents also increased by increasing their concentrations under different treatment methods. Fumigation was the best treatment for controlling the red flour beetle in wheat, compared to grain and thin-film treatments.

The effect on progeny and weight loss of wheat grain

The efficacy of the used compounds and yucca extract on the offspring of *T. castaneum* adults is shown in Table 4. The results showed a significant decrease in the offspring of *T. castaneum* adults treated with the examined compounds and yucca extract relative to the untreated insects (Table 4). The results additionally indicated that the reduction in the progeny of *T. castaneum* is directly proportional to the concentration of the tested control agents. Malathion was the most effective treatment of the tested compounds in reducing the progeny of *T. castaneum*, followed by carvone, linalool, yucca extract, 1.8 cineole, and cuminaldehyde, respectively. Table 4, also shows that the tested compounds and yucca extract significantly reduced the weight loss of the treated grain compared to the untreated grain. The lowest grain weight loss was for wheat grain treated with malathion, followed by yucca extract, carvone, 1, 8-cineole, linalool, and cuminaldehyde, respectively.

The biochemical effects of the control agents that were tested

Table 5 compares the activities of acetylcholinesterase, alpha-amylase, and alkaline phosphatase enzymes in mature *T. castaneum* treated with the examined monoterpenes and yucca extract to those of an untreated control. Adult insects given yucca extract and 1,8 cineole had lower acetylcholinesterase activity than untreated controls, whereas adults given carvone, linalool, and cuminaldehyde had higher activity. For *T. castaneum* adults, treated with all the examined monoterpenes and yucca extract, amalyase activity was lower than in untreated controls. *T. castaneum* adults treated with yucca extract, caminaldehyde, linalool, carvone, and 1,8 cineole displayed lower alkaline phosphatase activity than untreated controls.

Discussion

The results in this study showed that *Yucca schidgera* extract was effective for the control of red flour beetle in relation to mortality of adults and offspring inhibition. The insecticidal activity of *Y. schidgera* extract against *T. castaneum* may be attributed to the presence of saponins known to have a toxic effect against this insect (Bakr and Gad 2021).

Monoterpenes as environmentally friendly control agents, having basic components of plant essential oils, can be used in some important strategies for controlling stored product insects (Abdelgaleil *et al.*

Table 4. The effect of the used control agents on the progeny of *Tribolium castaneum* and weight loss in wheat grain

Treatments	Conc. [mg · kg^{-1}]	Mean No. of emerged adults ± SE	% Reduction in progeny	Mean weight ± SE	% Weight loss
Malathion	0.05	1.30hij ± 0.33	94.65	19.98a ± 0.01	0.08
	0.1	0.33ij ± 0.02	98.6	20.00a ± 0.0	0.0
	0.2	0.00j	100	20.00a ± 0.0	0.0
	0.3	0.00j	100	20.00a ± 0.0	0.0
1,8 Cineole	250	13.3bcde ± 2.40	45.2	18.41cdef ± 0.12	1.83
	500	9c–h ± 1.15	62.9	18.93bcde ± 0.02	1.20
	750	7.6c–j ± 1.45	68.7	19.02bde ± 0.01	1.07
	1000	5fghij ± 1.15	79.4	19.88a ± 0.01	0.25
Carvone	250	12.3bcdef ± 0.88	49.3	18.54cdef ± 0.10	1.68
	500	8c–i ± 1.15	67.1	19.00bcd ± 0.02	1.20
	750	5.6e–j ± 0.66	76.9	19.54ab ± 0.07	0.76
	1000	2.3ghij ± 0.88	90	19.92a ± 0.01	0.31
Linalool	250	15.3bc ± 1.45	37	18.13ef ± 0.03	2.03
	500	10cdefg ± 2.30	58.8	18.95bcde ± 0.02	1.18
	750	5.6e–j ± 0.66	76.9	19.72ab ± 0.05	0.51
	1000	2.6ghij ± 1.45	89.3	19.90a ± 0.04	0.33
Cuminaldehyde	250	20ab ± 0.57	17.6	18.01f ± 0.23	2.26
	500	14.3bcd ± 1.45	41.1	18.30def ± 0.18	1.96
	750	10.6cdef ± 1.20	56.3	19.00bcd ± 0.23	1.23
	1000	8.3c–h ± 0.88	65.8	19.53ab ± 0.23	0.90
Yucca schidigera extract	250	11cdef ± 1.52	54.7	19.18abc ± 0.19	1.05
	500	9.6cdefg ± 1.20	60.5	19.71ab ± 0.13	0.49
	750	6.6d–j ± 0.66	72.8	19.76ab ± 0.11	0.40
	1000	5fghij ± 0.57	79.4	19.91a ± 0.17	0.35
Control	0.00	24. a ± 2.0	0.00	17.84f ± 0.35	10.8

Initial number of adults was 20 individuals · jar^{-1}; initial weight of wheat grains was 20 g; different letters indicate significant differences by Fisher's LSD at $p \leq 0.05$

Table 5. The effect of evaluated control agents on acetylcholinesterase (AChE), amylase, and alkaline phosphatase activity in treated *Tribolium castaneum*

Treatments	AChE activity [U · L^{-1}]	Amylase activity [U · L^{-1}]	Alkaline phosphatase activity [U · L^{-1}]
1,8 Cineole	10141.2d ± 193	346.3ab ±11.27	122.6b ±0.85
Carvone	11506.2ab ± 157.6	362.6a ± 2.26	124.6ab ± 1.42
Linalool	11854.6a ± 138.1	293.9ab ± 39	115.5c ± 0.57
Cuminaldehyde	12157.6a ± 161.9	257.8b ± 21.6	125.6ab ± 2.27
Yucca schidigera extract	10756.9cd ± 141.4	342.9ab ± 7.44	120.3bc ± 1.12
Control	11120.4bc ± 192.0	373.8a ± 6.84	130.5a ± 1.99

Different letters indicate significant differences by Fisher's LSD at $p \leq 0.05$

2016; Ezhil-Vendan *et al.* 2017; Chaudhari *et al.* 2021; Derbalah *et al.* 2021). The monoterpene compounds used in this study showed high efficacy against *T. castaneum*, both in terms of adult and offspring mortality rates. This is supported by what was shown by Saad *et al.* (2019) who reported that monoterpenes such as mentthone and terpeniene showed high toxicity against the adults of red flour beetle. Also, Derbalah *et al.* (2021) reported that monoterpenes such as linalool, carvone, cuminaldhyde and cineole were effective

against *Sitophilus oryzae*. Moreover, Devi *et al.* (2020) observed that essential oils (containing monoterpenes such as geraniol, citral, citronellal or geranyl acetate) extracted from *Cymbopogon* species can be used as an effective management tool against *T. castaneum*. Jemâa *et al.* (2012) indicated that *Laurus nobilis* essential oil had good repellency against *T. castaneum,* and *Lasioderma serricorne*. In addition, Karabörklü *et al.* (2010) reported that *L. nobilis* oil had a strong toxicity against *T. castaneum*. Similarly, Jemâa *et al.* (2012) saw that Moroccan *L. nobilis* oil showed higher efficacy against *Rhyzopertha dominica* and *T. castaneum* than Tunisian and Algerian *L. nobilis* oils. According to some previous studies, the toxic effects of essential oils isolated from the plant against stored pests is due to the presence of monoterpenes as essential components in these oils (Abdelgaleil *et al.* 2016) and secondary metabolites (Yildirim *et al.* 2012a, 2012b). Furthermore, in previous studies on some essential oils, the main components, namely, monoterpenes, have shown contact toxicity against *T. castaneum*, which is consistent with the findings of this study, which showed that the monoterpenes studied have contact toxicity (Kanda *et al.* 2017; Saad *et al.* 2019).

The results of the various bioassay methods used in this study revealed that the insecticidal activity of the monoterpenes tested against *T. castanum* was highly dependent on the method used. The toxicity of monoterpenes as fumigants in this study was higher than contact and thin-film toxicities. These differences may be a result of the differences in their chemical and physical properties (Adel *et al.* 2015). Also, the higher fumigant toxicity of the tested compounds than other bioassay methods may be due to the high vapor pressure of the tested compounds which helped to increase their effectiveness as a fumigation treatment against *T. castaneum* compared to other bioassay methods. Moreover, the low efficacy of the examined compounds as a grain treatment assay can be due to the fact that mixing these compounds with grains may reduce their toxic effects due to the interaction or antagonism between them and semiochemicals from the stored grains (Adel *et al.* 2015).

In this study, yucca extract and 1,8-cineole decreased AChE activity in treated adults of *T. castaneum* compared to the untreated control. Consequently, the toxic effects of these chemicals on *T. castaneum* may be caused by a suppression of AChE activity similar to that of malathion (Chaudhari *et al.* 2021). There is agreement between the results obtained from this study and those of Ishaaya *et al.* (2007), who explained that the toxic action of essential oils and biocides on insects is due to the effect on the nervous system, as treatment with these essential oils causes behavioral symptoms similar to those of organophosphorus insecticides or AChE inhibitors (Chaudhari *et al.* 2021).

In this study, a decrease in the activity of the α-amylase in *T. castaneum* treated with monoterpenes and yucca extract was recorded. Therefore, the insecticidal effect of these monoterpenes against *T. castaneum* is most likely due to the inhibition of amylase's action as a digesting enzyme. Inactivation of this type of enzyme leads to intestinal obstruction, resulting in nutrient misuse, growth retardation, and death due to starvation (Mehrabadi *et al.* 2011). The reduction of α-amylase activity by natural products primarily containing monoterpenes can be attributed to the fact that these compounds inhibit the action of insect gut enzymes such as α-amylases and proteinases (Tatun *et al.* 2014) or their cytotoxic effect on epithelial cells of the midgut that synthesis α -amylase (Jbilou *et al.* 2008).

The activity of the ALP enzyme in *T. castaneum* adults treated with the tested monoterpenes and yucca extract was decreased compared to the untreated control. Therefore, the toxic effects of cuminaldehyde, carvone, 1, 8-cineole, linalool, cuminaldhyde, and yucca extract against *T. castaneum* may be due to the inhibition of phosphatase activity (Ramachandran *et al.* 2021). Insect detoxification enzyme is one of the defenses against foreign compounds which helps maintain the normal insect physiological functions (Li and Liu 2007). Thus, inhibiting the activity of this type of enzyme, such as ALP, leads to an increase in the sensitivity of these insect pests to these compounds (Abd-Elaziz and El-Sayed 2009).

In summary, the potential toxic effects or insecticidal activities of the tested control agents against *T. castaneum* may be via inhibition of AChE, alpha-amylase, and ALP enzymes, individually or together. On the other hand, the increase of AChE activity in adults treated with carvone, linalool, and cuminaldhyde compared to the untreated control may lead to a decrease in the efficiency of cholinergic neurotransmission, which ultimately leads to insect death (Ferreira *et al.* 2012).

Providing good quality grains of wheat is of great importance in Egypt and worldwide. In Egypt, the quality of wheat grain is greatly affected by the length of the wheat storage period. The decrease in grain weight and quality as a result of insect attack is one of the main problems of grain storage (Rayhan *et al.* 2014). In this study, the examined compounds and yucca extract significantly reduced the loss of grain weight. The results of this study are in line with Torres *et al.* (2014) who revealed that natural oils, which are mainly made up of monoterpenes, reduce grain weight loss induced by *S. zeamais* (L.). Moreover, Brari and Kumar (2020) found that two pure monoterpenes, citronellol and geraniol, reduced the weight loss of treated rice grain.

In spite of large-scale production of natural products, such as terpenes, they present a significant scientific and technological challenge. However, one

promising approach to tackle this problem is chemical synthesis inside nano-capsules. Recent application of different delivery strategies viz. nanoencapsulation, active packaging, and polymer-based coating effectively addressed these challenges and improved the bioefficacy and controlled release of essential oils (Maurya *et al.* 2021).

Conclusions

The tested control agents were highly effective against *T. castaneum*, for both adult mortality and progeny production. The toxin's mechanism of action of the tested substances against *T. castaneum* was due to the occurrence of some biochemical changes in the activity of the selected enzymes. The high efficacy of the tested monoterpenes, especially 1,8-cineole, against *T. castaneum* as fumigants, suggests that they could be used as effective alternatives to insecticides in the fight against *T. castaneum*.

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