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Toxicity, repellency and latent effects of some medicinal oils against *Tribolium confusum* and *T. castaneum* (Coleoptera: Tenebrionidae)

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Abstract

The present investigation was conducted to determine the toxicity, repellency and latent effects of seven medicinal plant oils against the confused flour beetle, *Tribolium confusum* and the red flour beetle, *T. castaneum* (Coleoptera: Tenebrionidae). The tested oils were camphor (*Eucalyptus globulus*), guava (*Psidium guajava*), ginger (*Zingiber officinale*), turmeric (*Curcuma longa*), nigella (*Nigella sativa*), star anise (*Illicium verum*) and thyme (*Thymus vulgaris*). Results revealed that the most effective oils against *T. confusum* were turmeric, ginger, star anise, thyme and camphor which induced mortality percentages of 90.0, 70.3, 37.5, 36.4 and 35.0, respectively with the highest concentration (5ml oil/100g flour). Turmeric oil showed highly significant reduction in number of alive beetles after treatment at different concentrations and exposure times. In repellency tests, turmeric oil produced complete repellency (100%) in both multiple- and two-choice in food-preference bioassays. Moreover, it produced repellency class of V (96.67% repellency) in area-preference bioassay. Similarly, turmeric oil showed highly significant protection against the confused flour beetles' infestation up to six months (with mortality percentages ranging from 99.3% after 15 days to 96% after 180 days after oil application). But regarding *T. castaneum*, the most effective oils were ginger, turmeric, thyme, nigella and guava at mortality percentages of 75.8, 63.5, 34.7, 32.8 and 26.6, respectively with the highest concentration. Ginger oil was the most effective in reducing beetles' numbers in toxicity tests. Also, it showed highly significant repellent effects in both multiple- and two-choice tests and ranked Class V (94.17% repellency) in area-preference tests. The latent effect tests showed that ginger was the most effective oil in protecting flour from the red flour beetles' infestation. The mortality percentage was 77.7% after 15 days till 63.7% mortality after 180 days of oil application.

Keywords: Medicinal oils, *Tribolium confusum*, *T. castaneum*, toxicity, repellency, latent effect

1. Introduction

The confused flour beetle, *Tribolium confusum* and the red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae) are among the most widespread and destructive stored product pests throughout the world. They cause significant damages and weight loss of stored products, particularly in tropical and warm temperate regions [1]. Both confused and red flour beetles, known as "bran bugs", primarily attack stored grain products, such as flour and cereals [2]. Both adults and larvae feed on grains dust and broken kernels, but not the undamaged whole grain kernels. These beetles often hitchhike into the home in infested flour and can multiply into large populations [3].

Confused flour beetle and red flour beetle are the most abundant and injurious insect pests of flour mills. They infest cereals, corn, cornmeal, crackers, flour, millet, oats, rice, wheat and wheat bran, dried fruits, legume seeds, beans, milk chocolate, cottonseed, peas, pasta, powdered milk, sunflower seeds, spices, dried pet food, dried flowers, herbarium and museum specimens, also, they feed on or damage the house or furniture [3]. These beetles have chewing mouthparts but do not bite or sting, but they may elicit an allergic response [4].

The red flour beetle is reddish-brown in color and its antennae end in a three-segmented club [5]. Whereas the confused flour beetle is the same color, but its antennae end is gradually club-like, the "club" consisting of four segments. The confused flour beetle apparently received this name due to confusion over about its identity as it is so like the red flour beetle at first glance [2]. They are known as secondary pests that feed on broken kernels, seed embryos, and grain dusts [6].

Chemicals and fumigants Such as methyl bromide or phosphine play a vital role in controlling this problem, but they have been known to cause serious toxicological and environmental problems. Such chemicals were banned in many countries because of its ozone-depleting properties [7]. Moreover, development of resistance is a serious threat to the future use of these materials, and consequently, there is an urgent need to develop affordable, safe and sound pest control agents and techniques [8].

Researchers are now focused on finding alternative insecticides which will be effective, environmentally friendly, biodegradable, safer and cheaper than existing ones. Botanical materials have played an important role in traditional methods of stored product protection since time of immemorial, by mixing the stored grains with the botanical pesticides. Prakash and Rao [9] outlined 866 plant species which produced pesticide components and specified 256 biologically active ingredients. Nevertheless, the use of botanical pesticides is limited and not to a large scale. On the other hand, the use of plant-derived materials was reported by many authors. El-Sabaay [10] studied the effects of some vegetable oils against *Sitophilus granaries* and *Rhyzopertha dominica*. Tripathi *et al.* [11] evaluated the effect of *Curcuma longa* against three stored products insects. Demissie *et al.* [12] investigated the efficiency of some cooking oils against *Sitophilus zeamais*.

Botanicals have multitude of properties including: pesticidal activity, repellency, antifeedant, insect growth regulation, antifungal and antibacterial properties [13]. Moreover, they have no residues and safe for non-target organisms and human. Besides, there was no insect-resistant development and have no negative effect on seed germination [14].

Plant oils, as an important natural resource of insecticides, are generally considered broad spectrum and safe for the environment because the array of compounds they contain quickly biodegrade in the soil [8]. Their lipophilic nature facilitates their interference with basic metabolic, biochemical, physiological and behavioral functions of insects [15].

The goal of this work was to detect some medicinal botanicals as alternative to conventional insecticides in storage. Therefore, in the present study, the toxicity, repellency and latent effects of some medicinal plant oils were determined against the adult stages of two stored-products pests, *T. confusum* and *T. castaneum* (Coleoptera: Tenebrionidae).

2. Materials and Methods

2.1 Insect culture

T. confusum and *T. castaneum* adults used in the experiment were collected from infested flour (in May 2015), identified, separated and reared on wheat flour plus 5% brewer's yeast. The insects were kept at 27±1 °C and 65±5% relative humidity (RH) in continuous darkness. All experiments were carried out under the same laboratory conditions in insect laboratory at the Department of Biological and Geological Sciences, Faculty of Education, Ain Shams University, Egypt. Adults used in all experiments were 7–14 days old of mixed sex. Sterilized flour was stored at –24 °C for at least 2 days. Before the experiments, flour was kept for a week in incubators set at 27±1 °C and 55±5% RH to achieve the moisture content related to environmental RH.

Fig 1: The red flour beetle, *T. castaneum*, has 3-segmented club antennae (a); whereas the confused flour beetle, *T. confusum*, has no apparent club on the antennae (gradual club antennae) (b).

2.2 Tested plant oils

The tested medicinal plant oils selected for the present study are listed in Table 1. It includes 7 plant oils belonging to 5 different families. Medicinal oils were purchased from local market of natural herbs. Plant species selected for the present investigation are: camphor, guava, ginger, turmeric, nigella, star anise and thyme. The principal criteria in selection of the tested medicinal oils was their toxicity to stored-product insects, besides, their preferable properties as food flavorings, spices, folk medicine and as ingredients for pharmaceutical preparations.

Table 1: General information of tested plant oils.

English name	Family name	Scientific name	Arabic name
Camphor	Myrtaceae	*Eucalyptus globulus*	Kafour
Guava		*Psidium guajava*	Guava
Ginger	Zingiberaceae	*Zingiber officinale*	Zanjabeel
Turmeric		*Curcuma longa*	kurkum
Nigella	Ranunculaceae	*Nigella sativa*	Habbet El-baraka
Star anise	Schisandraceae	*Illicium verum*	Yansoon negamy
Thyme	Lamiaceae	*Thymus vulgaris*	Zaatar

2.3 Insecticidal Bioassay

The effectiveness of tested medicinal oils was evaluated against *T. confusum* and *T. castaneum* adults. In line of traditional practices carried out by small-scale farmers, susceptibility tests were conducted using food contamination method. Bioassays were performed to estimate baseline levels of susceptibility of insects to each of the tested plant oils. Concentrations of 0.5, 1.0, 1.5, 2.0, 5.0 (ml oil/100g flour) of different tested oils were prepared with acetone as a solvent. Then, prepared concentrations were mixed thoroughly with sterilized wheat flour. Treated flour was left in the air for 30 min. to ensure the complete evaporation of the solvent. Controls were prepared without any treatment. Three replicates were performed for each treatment using 200-ml plastic cups. After that, 20 adults of each species were added into each replicate. The cups were covered with muslin cloth for sufficient ventilation, then placed in an incubator set at 27±1 °C, 55±5% RH and continuous darkness. The mortality was counted after 5, 10, 15 and 20 days after exposure. Insects were considered dead when no leg or antenna movements were observed after prodding with a fine brush.

2.4 Repellency Bioassay

The repellency effect of the tested oils was evaluated against adults of *T. confusum* and *T. castaneum*, at laboratory conditions. For evaluation of potential repellency of the tested medicinal oils, two kinds of repellency bioassays were applied.

2.4.1 Food-Preference Bioassay

a) Multiple-Choice Test

Multiple- choice test was adopted from the method described by Abdel-Rahman *et al.* [16]. About 20 g- quantities of wheat flour was mixed thoroughly with 1 ml of tested oil dissolved in 1 ml acetone (Concentration of 5 ml oil/ 100 g flour). Twenty-gram flour without any additive was served as a control. Eight-quantities of the treatments (7 quantities treated with the 7 tested oils and one as a control) were arranged inside a circular plate. Flour sets were left for 30 min. in the lab temperature to ensure acetone evaporating. After that, 100 beetles of *T. confusum* were introduced into the center of the circular plate, confined by a brass ring. After 15 min. confinement, insects were released by raising the brass ring without interrupting their activity. The plate was covered tightly by stretching cling film and make some holes in it by fine needle. The plate was incubated for 48h. Each quantity of flour was then gently obtained, and the insects were counted. Insects stayed out of the flour sets were not considered. The experiment was repeated three times. All procedures were repeated for *T. castaneum* beetles [16].

b) Double-Choice Test

The same general procedure that was used for the multiple-choice experiment was used with double-choice bioassay [16]. The flour treated sets were arranged parallel to each other in a Petri dish (14-cm diameter) with tight-fitting lids. Two kinds of treatments were always compared in each Petri dish. For each Petri dish, one quantity of flour treated with tested oil, and the other quantity, on the opposite side, was untreated served as a control. Twenty adult insects were placed in the center of the Petri dish between the two sets of flour (treated and control). Insects were allowed to make a choice between treated and untreated flour for 24h in the dark in the incubator. Then, insects settled in each half were counted. Those insects which stayed out the two halves were not considered. Experiment was repeated three times for the two-insect species.

2.4.2 Area-Preference Bioassay

The Area-Preference test was adopted from the method described by Laudani *et al.* [17]. Testing substrates were prepared from 9-cm diameter filter papers (Whatman No. 1) cut into 2 halves. A quantity of 0.5 ml of the tested oil dissolved in 1 ml acetone was uniformly applied to a half of filter paper. The treated half discs were air-dried to evaporate the solvent completely. Full discs were then remade by attaching one treated have with another untreated half with cellotape. Precautions were taken to keep sufficient distance between the two halves to prevent seepage of the test samples from one half to the other one. Each filter paper was then placed in a 9-cm Petri dish. Ten beetles were released at the center of each filter paper disc and the dish was covered and incubated. For each plant oil, three replicates were used. Counting of beetles present in each half were made after 1h and at hourly intervals up to five hours and then after 24h after application.

The data were calculated to express Percentage repulsion (PR) by the formula of Talukder and Howse [18]:

$$PR\% = [(N - C)/ C] * 100$$

(where, N = number of insects present in the control half; C = total number of the insects).

The main repellency from the three replicates was averaged for 1 to 5 h and 24 h examinations. The overall average of these averages was calculated for each tested oil. Percent repellency values were assigned repellency classes according to the scale of Juliana and Su [19]: Repellency classes, (repellency rate = class): (<0.1 = 0), (0.1-20 = I), (20.1-40 = II), (40.1-60 = III), (60.1-80 = IV), and (80.1-100 = V).

2.5 Latent Effects

The investigations reported here were conducted to determine the effect of storage periods on the efficacy of the tested plant oils. The present investigation was adopted from Ahmed [20]. Large quantities of wheat flour, each of 1400g, treated with high concentration of each oil (5ml oil/ 100g flour) were prepared and kept in glass jars covered with muslin cloth. Treatment of flour with the tested oil was done by mixing for 10 min. each oil was dissolved in 5ml acetone as a solvent before treatment. Treated flour was left spread in air to allow complete evaporation of the solvent. The treated flour was divided into seven lots, each consisted of three replicates and stored for different periods under laboratory conditions. Untreated flour served as control. Control and treated flour was stored for different seven storage periods (15, 30, 60, 90, 120, 150 and 180 days), after which, 20 beetles were introduced into each replicate of the treated flour. The infested flour was incubated for two weeks. The total number of alive beetles after 2 weeks was recorded. The percentage mortality of beetles was calculated.

2.6 Data Analysis

Control and checks mortalities were zero and no corrections were necessary. Results were expressed as mean ± standard deviation (SD). The statistical significance of differences between means was determined by student "t- Test" for paired observations (treated and control). The level of significance was stated to be highly significant ($P<0.01$), significant ($P<0.05$) or insignificant ($P>0.05$). In all cases, the percentages of change were calculated using the following equation:

Percentage of change = [(test-control)/ control]*100

3. Results

3.1 Toxicity of tested oils against *T. confusum* and *T. castaneum*

The susceptibility of *T. confusum* and *T. castaneum* to seven medicinal plant oils (camphor, guava, ginger, turmeric, nigella, star anise and thyme) were tested. Results of the toxicity tests are tabulated in Table 2, 3 and 4 and illustrated graphically in Fig. 2. In all toxicity tests, there was no mortality recorded for all control replicates, thus there is no need for the correction of the recorded mortalities of the treated ones.

For *T. confusum*, statistical analysis of obtained data revealed that at low concentrations, camphor, ginger and turmeric oils showed highly significant ($p<0.01$) effects on beetles' mortality, but, guava and star anise oils showed insignificant ($p>0.05$) effects. Whereas, at higher concentrations, camphor, ginger, turmeric and thyme oils showed highly significant ($p<0.01$) effects while guava, nigella and star anise oils showed significant ($p<0.05$) effects on the mortality of treated beetles.

For *T. castaneum* beetles and with low oil concentrations, guava, ginger, turmeric, nigella and thyme oils showed significant (p<0.05) effects on beetle' mortalities, whereas, camphor and star anise showed insignificant (p>0.05) effects. But, with higher concentrations, camphor, guava, ginger and turmeric oils showed highly significant effects and the effects was significant (p<0.05) with star anise and thyme.

In general, results indicated that the most effective oils against *T. confusum* were turmeric, ginger, star anise, thyme and camphor which induced mortality percentages of 90.0, 70.3, 37.5, 36.4 and 35.0, respectively with the highest concentration (5ml oil/100g flour). For *T. castaneum*, with the same highest oil concentration used, the most effective oils were ginger, turmeric, thyme, nigella and guava at mortality percentages of 75.8, 63.5, 34.7, 32.8 and 26.6, respectively. The rest of tested oils had lower effects. Results also revealed that the toxicity was dose-dependent; as the oil concentration increased, the mortality percentages also increased (Table 4). Similarly, the toxicities of the tested oils were increased as the exposure period increased. In conclusion, turmeric was the most effective against *T. confusum*, whereas, ginger was the most effective to *T. castaneum*.

Table 2: Susceptibility of *T. confusum* to the tested medicinal oils after 5-, 10, 15- and 20-days after treatments with concentrations of 0.5, 1.0, 1.5, 2.0 and 5.0 ml oil/ 100g flour, under laboratory conditions.

Treatment	**Conc.**	**Mortality%(1)**					***t*-test**	
		5d	**10d**	**15d**	**20d**	**Mean ± SD**	***p*-value**	**Significance level(2)**
Control	0	0	0	0	0	0		
Camphor	0.5	10	15.6	18.9	22.2	16.7 ± 5.2	0.0077	**
	1	14.4	18.9	24.4	30	21.9 ± 6.8	0.0074	**
	1.5	18.9	24.4	31.1	37.8	28.1± 8.2	0.0064	**
	2	23.3	28.9	33.3	43.3	32.2 ±8.5	0.0047	**
	5	25.6	32.2	37.8	44.4	35.0 ±8.0	0.0032	**
	Mean	18.4	24.0	29.1	35.5	26.8 ± 7.3	0.0052	**
Guava	0.5	0	0	2.2	6.7	2.2 ± 3.2	0.2536	ns
	1	3.3	4.4	5.6	10.0	5.8 ± 2.9	0.0286	*
	1.5	5.6	6.7	11.1	15.6	9.8 ± 4.6	0.0236	*
	2	5.6	11.1	15.6	22.2	13.6 ± 7.0	0.0304	*
	5	10	13.3	21.1	26.7	17.8 ± 7.6	0.0182	*
	Mean	4.9	7.1	11.1	16.2	9.8 ± 4.9	0.0290	*
Ginger	0.5	21.1	26.7	33.3	40	30.3 ± 8.2	0.0051	**
	1	23.3	31.1	40	44.4	34.7 ± 9.4	0.0051	**
	1.5	39	45.5	60.9	63.3	52.2 ± 11.8	0.0031	**
	2	48.3	53.3	67.8	75.6	61.3 ± 12.6	0.0023	**
	5	58.9	65.6	73.3	83.3	70.3 ± 10.5	0.0009	**
	Mean	38.1	44.4	55.1	61.3	49.7 ± 10.4	0.0024	**
Turmeric	0.5	26.7	33.3	43.3	53.3	39.2 ± 11.6	0.0067	**
	1	31.1	41.1	48.9	66.7	47.0 ± 15.0	0.0083	**
	1.5	41.1	54.4	63.3	73.3	58.0 ± 13.7	0.0034	**
	2	54.4	63.3	72.2	78.9	67.2 ± 10.7	0.0011	**
	5	72.2	87.8	100	100	90.0 ± 13.2	0.0009	**
	Mean	45.1	56.0	65.5	74.4	60.3 ± 12.6	0.0024	**
Nigella	0.5	3.3	6.7	11.1	14.4	8.9 ± 4.9	0.0357	*
	1	7.8	12.2	16.7	20	14.2 ±5.3	0.0129	*
	1.5	6.7	12.2	15.6	21.1	13.9 ± 6.0	0.0193	*
	2	11.1	16.7	21.1	26.7	18.9 ± 6.6	0.0106	*
	5	13.3	21.1	25.6	31.3	22.8 ± 7.6	0.0092	**
	Mean	8.4	13.8	18.0	22.7	15.7 ± 6.1	0.0140	*
Star anise	0.5	1.11	15.6	20	26.7	15.9 ± 10.8	0.0612	ns
	1	14.4	16.7	23.3	31.1	21.4 ± 7.5	0.0107	*
	1.5	15.6	21.1	27.8	35.6	25.0 ± 8.6	0.0102	*
	2	21.1	26.7	33.3	40	30.3 ± 8.2	0.0051	**
	5	25.6	32.2	40	52.2	37.5 ± 11.4	0.0072	**
	Mean	15.6	22.5	28.9	37.1	26.0 ± 9.2	0.0109	*
Thyme	0.5	8.9	13.3	16.7	22.2	15.3 ± 5.6	0.0122	*
	1	11.1	17.8	22.2	30	20.3 ± 7.9	0.0145	*
	1.5	14.4	21.1	30	35.6	25.3 ± 9.4	0.0126	*
	2	21.1	28.9	34.3	43.3	31.9 ± 9.3	0.0064	**
	5	26.7	32.2	38.9	47.8	36.4 ± 9.1	0.0041	**
	Mean	16.4	22.7	28.4	35.8	25.8 ± 8.2	0.0082	**

(1)Average mortality of three replicates for each treatment, 20 beetles per replicate.

(2) (Significance level: ns, insignificant (P>0.05); *, significant (P<0.05); **, highly significant (P<0.01).

Table 3: Susceptibility of *T. castaneum* to the tested medicinal oils after 5-, 10, 15- and 20-days after treatments with concentrations of 0.5, 1.0, 1.5, 2.0 and 5.0 ml oil/ 100g flour, under laboratory conditions

Treatment	Conc.	Mortality% (1)					*t*-test	
		5d	10d	15d	20d	Mean ± SD	*P*-value	Significance level (2)
Control		0	0	0	0	0		
Camphor	0.5	0	0	2.2	4.4	1.7 ± 2.1	0.2152	ns
	1	0	2.2	3.3	7.8	3.3 ± 3.3	0.1360	ns
	1.5	2.2	6.7	8.9	12.2	7.5 ± 4.2	0.0374	*
	2	5.6	10	11.1	16.7	10.9 ± 4.6	0.0177	*
	5	11.1	15.6	17.8	21.1	16.4 ± 4.2	0.0044	**
	Mean	3.8	6.9	8.7	12.4	7.9 ± 3.6	0.0	**
Guava	0.5	5.5	10	14.4	18.9	12.2 ± 5.8	0.0241	*
	1	8.9	12.2	16.7	20.0	14.5 ± 4.9	0.0097	**
	1.5	12.2	18.9	22.2	25.6	19.7 ± 5.7	0.0062	**
	2	14.4	21.1	23.3	28.9	21.9 ± 6.0	0.0053	**
	5	18.9	23.3	30	34.3	26.6 ± 6.9	0.0044	**
	Mean	12.0	17.1	21.3	25.5	19.0 ± 5.8	0.0	**
Ginger	0.5	16.7	21.1	26.7	40	26.1 ± 10.1	0.0141	*
	1	32.2	40	46.9	61.1	45.1 ± 12.3	0.0052	**
	1.5	42.2	51	56.7	65.6	53.9 ± 9.8	0.0016	**
	2	55.6	60	66.7	78.9	65.3 ± 10.2	0.0010	**
	5	65.6	70	76.6	90.9	75.8 ± 11.1	0.0008	**
	Mean	42.5	48.4	54.7	67.3	53.2 ± 10.6	0.0	**
Turmeric	0.5	13.3	21.1	26.7	34.4	23.9 ± 8.9	0.0127	*
	1	25.6	31.1	37.8	44.4	34.7 ± 8.2	0.0034	**
	1.5	28.9	34.4	44.4	54.4	40.5 ± 11.3	0.0055	**
	2	33.3	41.1	47.8	56.7	44.7 ± 9.9	0.0029	**
	5	50.3	59.1	67.8	76.7	63.5 ± 11.4	0.0015	**
	Mean	30.3	37.4	44.9	53.3	41.5 ± 9.9	0.0036	**
Nigella	0.5	7.8	12.2	14.4	21.1	13.9 ± 5.5	0.0153	*
	1	11.1	16.7	18.9	27.8	18.6 ± 6.9	0.0127	*
	1.5	13.3	21.1	27.8	32.2	23.6 ± 8.2	0.0106	*
	2	17.8	24.4	31.1	36.7	27.5 ± 8.2	0.0067	**
	5	22.2	26.7	36.7	45.6	32.8 ± 10.5	0.0082	**
	Mean	14.4	20.2	25.8	32.7	23.3 ± 7.8	0.0094	**
Star anise	0.5	1.1	4.4	6.7	10	5.6 ± 3.8	0.0596	ns
	1	4.4	7.8	10	15.6	9.5 ± 4.7	0.0277	*
	1.5	5.6	10	14.4	17.8	12.0 ± 5.3	0.0204	*
	2	7.8	11.1	17.8	23.3	15.0 ± 6.9	0.0227	*
	5	11.1	15.6	21.1	26.7	18.6 ± 6.8	0.0118	*
	Mean	6.0	9.8	14.0	18.7	12.1 ± 5.5	0.0213	*
Thyme	0.5	10	13.3	18.9	23.3	16.4 ± 5.9	0.0115	*
	1	12.2	16.7	21.1	25.6	18.9 ± 5.8	0.0072	**
	1.5	16.7	20	23.3	40	25.0 ± 10.4	0.0169	*
	2	17.8	24.4	30	43.3	28.9 ± 10.8	0.0129	*
	5	22.2	28.9	37.8	50	34.7 ± 12.0	0.0103	*
	Mean	15.8	20.7	26.2	36.4	24.8 ± 8.9	0.0113	*

(1) Average mortality of three replicates for each treatment, 20 beetles per replicate.
(2) (Significance level: ns, insignificant ($P>0.05$); *, significant ($P<0.05$); **, highly significant ($P<0.01$).

Table 4: Toxicities of the tested medicinal oils against *T. confusum* and *T. castaneum* at different concentrations and exposure time.

Treatment	Mortality %	
	T. castaneum	*T. confusum*
Control	0.0	0
Camphor	8.0	26.8
Guava	19.0	9.8
Ginger	53.3	49.7
Turmeric	41.5	60.3
Nigella	23.3	15.2
Star anise	12.1	26.5
Thyme	24.8	25.8
Concentration		
0.5	14.2	18.7
1	20.6	23.6
1.5	26.0	30.3
2	30.6	36.9
5	38.4	43.9
Exposure time		
5 days	17.8	21.3
10 days	22.9	27.2
15 days	28.0	33.5
20 days	35.2	40.4

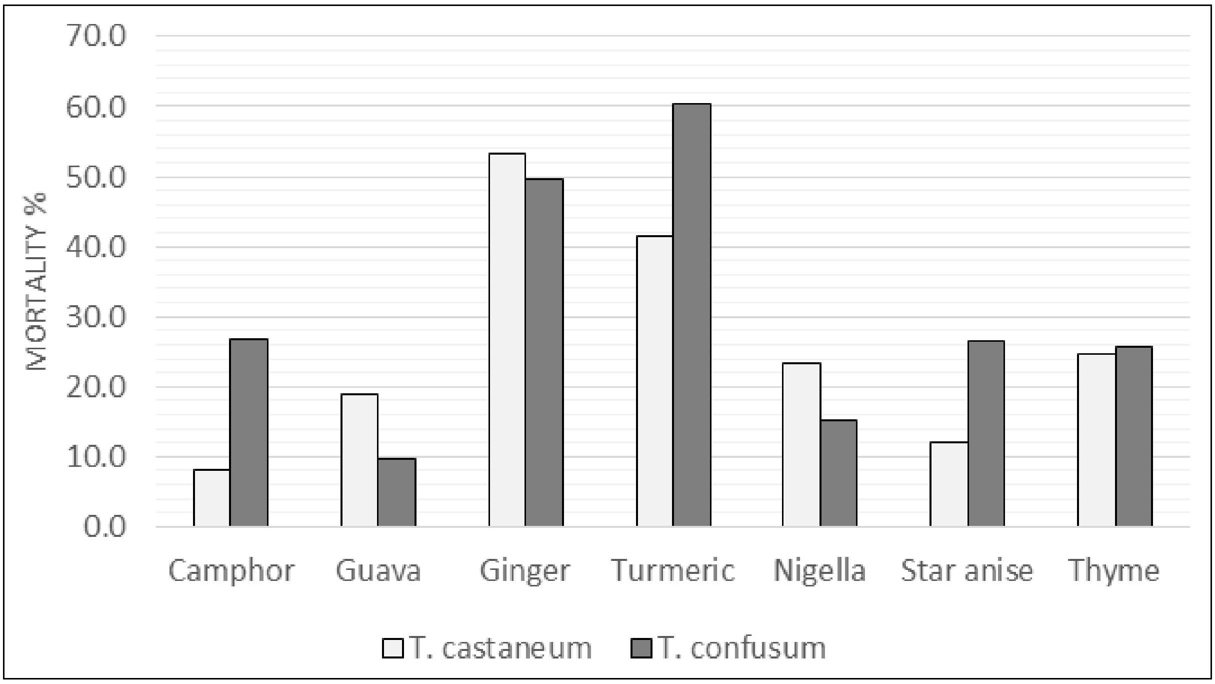

Fig 2: Toxicities of the tested medicinal oils against *T. confusum* and *T. castaneum* at laboratory conditions.

3.2 Repellency bioassay

The repellent effect of the above mentioned botanical oils against *T. confusum* and *T. castaneum* was also studied with two different repellency bioassays.

3.2.1 Food-preference bioassay

The food-preference of the two tested beetles were assayed toward the seven plant oils via two different tests.

a) Multiple-choice tests

Results of multiple-choice tests of *T. confusum* and *T. castaneum* are tabulated in Table 5.

Table 5: Repellency effect of the tested oils on food preference of *T. confusum* and *T. castaneum* as evaluated by using the Multiple-choice method, at laboratory conditions.

Tested insect	Treatment(1)	Beetles found in sections %			%Change	*t-Test*	
		Min.	Max.	Mean ± SD		*p*-value	Significant level (2)
T. confusum	Control	33.3	40	36.7 ± 2.8			
	Camphor	0	5.3	2.7 ± 2.4	-92.8	0.0007	**
	Guava	24	33.3	27.9 ± 4.2	-23.7	0.0584	n.s.
	Ginger	0	1.3	0.3 ± 0.7	-99.1	0	**
	Turmeric	0	0	0	-100	0.0001	**
	Nigella	6.7	13.3	9.3 ± 2.8	-74.6	0.0015	*
	Star anise	0	7.1	3.8 ± 3.1	-89.7	0	**
	Thyme	14.7	24	19.0 ± 3.9	-48.2	0.0031	*
T. castaneum	Control	24	33.3	29.0 ± 3.9			
	Camphor	9.1	16	11.6 ± 3.2	-60.0	0.0032	*
	Guava	17.3	22.7	19.3 ± 2.6	-33.4	0.0507	n.s.
	Ginger	0	0	0	-100	0.0007	**
	Turmeric	0	2.6	0.9 ± 1.2	-96.6	0.0010	**
	Nigella	6.7	18.7	12.7 ± 4.9	-56.3	0.0015	*
	Star anise	0	8	4.3 ± 3.7	-85.1	0.0009	**
	Thyme	10.6	26.7	17.7 ± 6.8	-39.1	0.1277	n.s.

(1) Three replicates for each treatment, 100 beetles per replicate.
(2) Significance level: ns, insignificant ($P>0.05$); *, significant ($P<0.05$); **, highly significant ($P<0.01$).

In case of *T. confusum*, the obtained data summarized that the mean percentage of beetles' number found in the control sections was 36.7%. Results showed that all the tested oils produced a reduction in the mean number of beetles found in treated sections as compared with the control. Statistical analysis revealed that the reduction in treated beetles was highly significant ($p<0.01$) with camphor, ginger and star anise, and significant ($p<0.05$) reduction in case of turmeric, nigella and thyme oils, whereas, there were insignificant ($p>0.05$) reduction with guava oil application.
For *T. castaneum*, results revealed that ginger and nigella had a highly significant ($p<0.01$) effect on reduction of beetles in treated sections as compared with the control. While, the reduction was significant ($p<0.05$) with camphor, turmeric and star anise, but there were insignificant ($p>0.05$) reduction in number with guava and thyme oils.
In conclusion, For *T. confusum*, turmeric was the most effective oil in prevention of beetles' presence in the treated sections (100% prevention), followed by ginger, camphor and star anise with 99.1, 92.8 and 89.7% prevention, respectively.
For *T. castaneum*, the best oil for prevention of beetle's presence in the treated area was ginger oil (100% prevention) followed by turmeric and star anise with 96.6 and 85.1% prevention, respectively, as compared with the controls.

b) Two-choice tests

Table 6: Repellency effect of the tested oils on food preference of *T. confusum* and *T. castaneum* as evaluated by using the Two-choice method, at laboratory conditions.

Tested insects	Treatment(1)	% Beetles (Mean ± SD)		t-test	
		Control	Treated	*p*-value	Significance level(2)
T. confusum	Camphor	86.7 ± 10.4	13.3 ± 10.4	0.0258	*
	Guava	35.0 ± 10.0	65.0 ± 10.0	0.1217	ns
	Ginger	95.0 ± 8.7	5.0 ± 8.7	0.0121	*
	Turmeric	100.0	0	0	**
	Nigella	55.0 ± 5.0	45.0 ± 5.0	0.2254	ns
	Star anise	85.0 ± 13.2	15.0 ± 13.2	0.0445	*
	Thyme	33.3 ± 7.6	66.7 ± 7.6	0.0634	ns
T. castaneum	Camphor	63.3 ± 10.4	36.7 ± 10.4	0.1567	*
	Guava	53.3 ± 12.6	12.6 ± 12.6	0.9614	ns
	Ginger	100.0	0	0	**
	Turmeric	91.7 ± 7.6	8.3 ± 7.6	0.0110	**
	Nigella	48.3 ± 7.6	51.7 ± 7.6	0.7418	ns
	Star anise	90.0 ± 10.0	10.0 ±	0.0202	*
	Thyme	56.7 ± 18.9	43.3 ±	0.6039	ns

(1) Three replicates for each treatment, 20 beetles per replicate.
(2) Significance level: ns, insignificant (P>0.05); *, significant (P<0.05); **, highly significant (P<0.01).

The obtained data in Table 6 revealed that turmeric was the most effective oil which produced complete repellency (100%) to *T. confusum* in the treated sections, as compared with the control ones. Ginger, camphor and star anise showed significant repellency toward *T. confusum* with repellency of 95.0, 86.7 and 85.0%, respectively. While, guava and nigella oils showed insignificant repellency toward the same beetle.
For *T. castaneum*, ginger oil produced complete repellency (100%), followed by turmeric oil with 91.7% repellency. Star anise and camphor showed significant (p<0.05) repellency, whereas guava and nigella oils recorded in significant effect (p>0.05).
In conclusion, Turmeric was the most effective repellent against *T. confusum*, while, ginger oil was the most effective for *T. castaneum* repellency.

3.2.2 Area-preference bioassay
The obtained results showed that in case of *T. confusum* beetle, the most effective repellent was turmeric oil, generating repellency class V (96.67%), followed by ginger and camphor. The least effective oil in repellent activity was thyme oil, with repellency class of I (Table 7). For *T. castaneum*, ginger oil was the most effective repellent (Class V), followed by turmeric and star anise with almost the same rank. Thyme had the second rank with Class IV, followed by camphor, guava and nigella with repellency class III.

Table 7: Repellency effect of the tested oils on food preference of *T. confusum* and *T. castaneum* as evaluated by using the Area-preference test after 6-time intervals, at laboratory conditions.

Tested insects	Treatment	Average repellency (hours after treatment) (1)						Overall average%	Repellency class (2)
		1h	2h	3h	4h	5h	24h		
T. confusum	Camphor	86.7	76.7	86.7	83.3	75	80	81.40	V
	Guava	35	40	36.7	30	31.7	28.3	33.62	II
	Ginger	91.7	90	96.7	98.3	98.3	100	95.83	V
	Turmeric	90	95	98.3	96.7	100	100	96.67	V
	Nigella	51.7	58.3	60	56.7	53.3	60	56.67	III
	Star anise	80	78.3	81.7	80	78.3	80	79.72	IV
	Thyme	33.3	16.7	13.3	16.7	15	15	18.33	I
T. castaneum	Camphor	58.3	56.7	55	50	61.7	61.7	57.23	III
	Guava	50	50	56.7	60	56.7	48.3	53.62	III
	Ginger	91.7	88.3	90	96.7	98.3	100	94.17	V
	Turmeric	91.7	85	95	93.3	96.7	95	92.78	V
	Nigella	53.3	51.7	63.3	65	53.3	51.5	56.35	III
	Star anise	75	80	86.7	86.7	88.3	90	84.45	V
	Thyme	56.7	58.3	60	61.7	71.7	70	63.07	IV

(1) Average of three replicates, 10 beetles per replicate.
(2) Repellency class: repellency rate < 0.1 = class 0, 0.1 - 20 =I, 20.1 - 40=II, 40.1 - 60=III, 60.1 - 80=IV, 80.1 - 100=V.

3.3 Latent effect
The effect of storage periods on the efficiency of tested oils against the two beetles' species was evaluated. The results are tabulated in Table 8 and illustrated graphically in Fig. 3 and 4.

Table 8: Latent effect of the tested oils against infestation with *T. confusum* and *T. castaneum* at different storage periods.

Tested insects	Storage period (1)	Mortality % ± SD						
		Camphor	**Guava**	**Ginger**	**Turmeric**	**Nigella**	**Star anise**	**Thyme**
T. confusum	15-d	34.3** ± 4.0	22.0** ± 2.6	69.5** ± 4.3	99.3** ± 1.2	26.2** ± 3.5	43.0** ± 3.0	35.0** ± 4.4
	30-d	37.7** ± 6.8	27.3** ± 5.5	68.7** ± 3.2	100.0**	19.3** ± 2.1	33.0** ± 2.0	37.** ± 6.1
	60-d	36.0** ± 3.6	20.7** ±2.5	58.7** ± 1.5	98.0** ± 2.6	18.0** ± 1.0	26.3* ± 5.5	35.0** ± 2.0
	90-d	31.0** ± 2.0	17.7** ± 2.1	55.0** ± 5.3	98.0** ± 2.0	18.3** ± 2.1	22.7** ± 3.8	34.7** ± 2.5
	120-d	28.3** ± 2.1	16.7* ± 5.7	52.3** ± 3.1	97.3** ± 2.5	13.0** ± 2.0	16.7* ± 4.7	25.3* ± 4.5
	150-d	24.7** ± 3.2	16.3* ± 5.0	51.7* ± 12.6	95.7** ± 5.1	10.3** ± 1.5	10.0* ± 2.6	26.0** ± 3.6
	180-d	19.0** ± 1.0	9.3* ± 2.1	53.3** ± 4.7	96.** ± 2.1	9.3** ± 1.5	7.3^{ns} ± 3.2	20.0** ± 3.0
T. castaneum	15-d	18.9** ± 1.1	34.0** ± 3.6	77.7** ± 2.1	68.7** ± 2.1	33.3** ± 3.1	18.0** ± 3.0	38.3** ± 1.5
	30-d	19.7** ± 2.5	24.3** ± 3.1	81.0** ± 4.0	66.7** ± 3.2	32.7** ± 1.5	17.0* ± 3.6	32.3** ± 3.1
	60-d	13.0** ± 2.0	13.0* ± 4.0	75.7** ± 5.1	71.3** ± 9.6	24.7* ± 4.7	13.0** ± 2.0	25.3** ± 3.8
	90-d	11.0* ± 2.0	11.0* ± 2.6	75.0** ± 7.2	63.7** ± 4.2	24.0* ± 4.4	8.7* ± 1.5	22.3* ± 4.5
	120-d	9.3* ± 2.5	5.3* ± 2.1	68.7** ± 4.0	67.7** ± 6.1	20.3** ± 3.1	6.0** ± 1.0	26.3** ± 3.1
	150-d	9.3* ± 2.1	1.0 ^{ns} ± 1.0	58.7** ± 3.2	65.3** ± 5.5	18.7** ± 3.1	3.7 ^{ns} ± 2.1	20.0* ± 4.4
	180-d	6.3* ± 1.5	0.0 ^{ns}	63.7** ± 6.0	57.3** ± 2.5	12.0* ± 3.6	1.0 ^{ns} ± 1.0	17.7** ± 1.2

(1) Three replicates were used for each treatment, 20 beetles per replicate.
(2) Significance level: ns, insignificant (*P*>0.05); *, significant (*P*<0.05); **, highly significant (*P*<0.01) as compared with the control.

Mortality percentages were 0% for all control replicates at different storage periods.

Turmeric oil produced a highly significant effect ($p<0.01$) in the mortality percentages of treated *T. confusum* beetles after all the experimental storage periods (15, 30, 60, 90, 120, 150 and 180 days after application) with mortality percentages ranging from 99.3% after 15 days to 96% after 180 days after oil application. Whereas, the least effective oil was guava with 22% mortality after 15 days and terminated with 9.3 mortality after 180 days after oil treatment.

In case of *T. castaneum* beetles, ginger was the most effective oil in protecting flour from infestation. The mortality percentage was 77.7% after 15 days till 63.7% mortality after 180 days of oil application. Followed by turmeric oil with almost the same effects in protection. But the least effective oils in flour protection from *T. castaneum* infestation were camphor, guava and star anise oils.

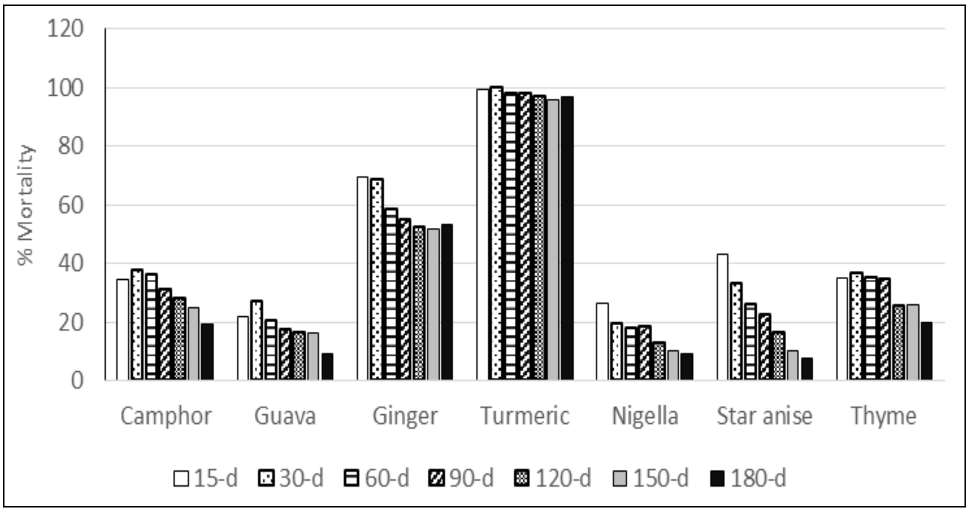

Fig 3: Effect of storage periods of treated flour on the efficiency of the tested oils against infestation with *T. confusum*, at laboratory conditions.

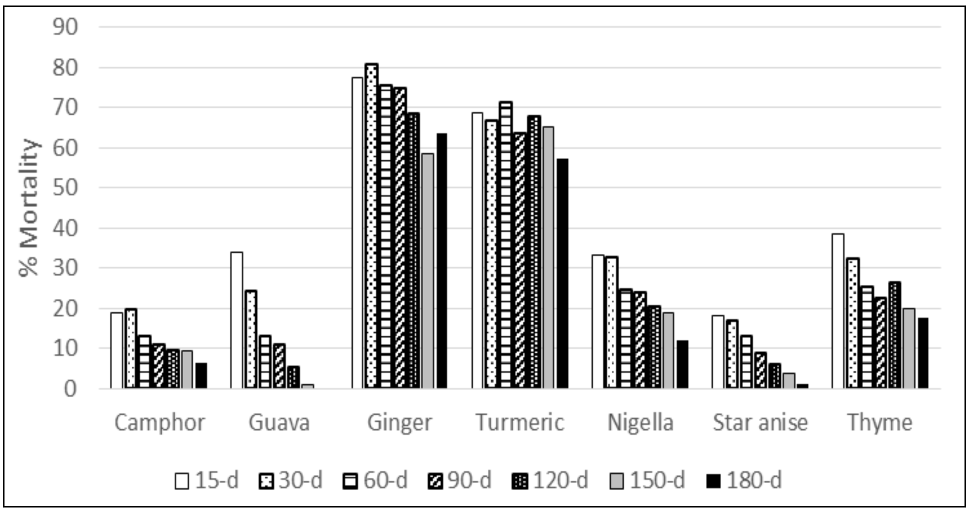

Fig 4: Effect of storage periods of treated flour on the efficiency of the tested oils against infestation with *T. castaneum*, at laboratory conditions.

4. Discussion

Protection of stored grains and grain products from insect infestations is the major problem that concern all nations in the world. *T. confusum* and *T. castaneum* are among economical important pests attacking a lot number of stored products. So, it is important to search for good protective materials for the stored products against such beetles' infestation. In the present study, seven medicinal plant oils were chosen for their promising effects reviewing from the literature. The tested plant oils were reported to have medicinal value and are not toxic to vertebrates [21].

The net results obtained from different toxicity and repellency tests of the medicinal oils against *T. confusum* beetles concluded that the turmeric oil showed highly significant reduction in number of alive beetles after treatment at different concentrations and exposure times. Complete mortality was achieved with turmeric oil at a concentration of 5ml oil/100g flour after 15 and 20 days after oil application. In repellency tests, turmeric oil exhibited highly significant repellent effect in both multiple- and two-choice in food-preference bioassays. Moreover, it produced repellency class of V in area-preference bioassay. Similarly, turmeric oil showed highly significant protection against beetles' infestation after different storage periods (from 15 days till 180 days after treatment). Ginger oil came in the second rank in the high efficiency. Whereas, the least effective oils were guava and thyme against *T. confusum* beetles, as compared with the controls.

For *T. castaneum,* ginger oil was the most effective oil in reducing beetles' numbers in toxicity tests at different concentrations and exposure periods. In repellency tests, ginger oil also showed highly significant repellent effects in both multiple- and two-choice tests and ranked Class V in area-preference tests. Ginger oil came in the second rank in toxicity but have almost the same repellency effects of ginger oil against *T. castaneum* beetles. Besides, ginger oil highly protected flour against *T. castaneum* infestation. On the other hand, the least effective oils were guava and thyme oils.

The present investigation showed pronounced increase in mortality for most tested oils with increasing both oil concentrations and time of exposure. That results are in accordance with those of Abdel-Rahman *et al.* [16] and Alagarmalai *et al.* [22].

Results of toxicity tests are in harmony with those of Al Qahtani *et al.* [23] who reported that ginger was the most potent plant against *O. surinamensis*. Also, Al-Jabr [24] who reported that complete mortality of *Oryzaephilus surinamensis* was achieved by *C. camphora, M. chamomill* and, *M. viridis*, while, *P. amygdalus* and *C winterianus* gave complete mortality of *T. castaneum* after two weeks of application. Also, Khani and Heydarian [1] studied the fumigant and repellent properties of sesquiterpene-rich essential oils from *Teucrium polium capitatum* against *T. castaneum* and *C. maculatus* adults. Authors found that in all cases, considerable differences in mortality of insect to essential oil vapor were observed in different concentrations and exposure times. They concluded that sesquiterpene-rich essential oils could be used as a potential control agent for stored-product insects.

Results of repellency bioassays showed that most of the tested medicinal oils exhibited promising repellents against two *Tribolium* beetles. According to the role stated by Egwunyenga *et al.* [25], at least repellency of class III (40.1-60% repellency) is the standard for promising repellents, in area-preference tests. Repellency results are in accordance with the findings of Abdel-Rahman [16] who found that turmeric produced a repellency of class III against *C. cautella* larvae. Besides, Tooba *et al.* [26] stated that *Eucalyptus* sp. showed significant repellency effects against *T. castaneum*. Britto *et al.* [27] found that *Zingiber officinale* and *Ocimum sanctum* possess antifeedant properties on the adult of *T. castaneum*.

Present findings also indicate that *Tribolium* beetles were present in some treatments in two-choice conditions, whereas when given a choice among numerous treatments avoid presence in treated flour in multiple-choice tests, as happened with nigella oil application. However, a close relation between toxicity and repellency is not always the case. Although guava and thyme oils had significant repellent effect (repellency class III and IV, respectively) in area-preference tests against beetles, it had low toxic effects against *T. castaneum* and had insignificant repellent effect in food-preference tests. These findings are in harmony with those of Talukder and Howse [18] who found that *A. polystachya* seed extract was highly toxic to *C. chinensis* but was week repellent for this insect. Also, Wang *et al.* [28] found that, among six essential oils tested, *C. funebris* oil had highly repellency effects on *L. bostrychophila* adults, but its fumigant toxicity was significantly lower than those of other five plant oils; thus, authors suggested that active compounds that acted as repellents and fumigants might be chemically different.

The toxic effect of the tested plant oils on adult beetles may come from inhibiting insect feeding [29], or through destroying the insect mid gut [30] reducing the food ingestion by insects, causing reduction in growth and finally death. In addition, plant oil vapors can act indirectly by masking the stimulating action of the desired stored product [31]. These observations are in accordance with Regnault-Roger [32] who stated that the activities of natural plant materials are manifold, and they induce fumigant and contact toxicity as well as antifeedant or repellent effects.

Grain damage level up to 100% have been found in some grain samples from farm stores after 6-8 months of storage [33]. The use of plant oils for storage protection is sustainable; it can be continuously propagated year after year, biodegradable, and do not have any negative impact on the environment. The latent effect tests showed that turmeric oil have highly protective effects against *T. confusum* infestation in storage, whereas, ginger oil followed by turmeric oil had significant protective effects against *T. castaneum* infestation.

The present results indicated that the protection of flour against *Tribolium* beetles infestation up to 6 months turmeric or ginger oils was mainly due to insect repellency from the treated flour and hence the beetles died by starvation. The observation of the present study showed that the beetles trying to escape away from turmeric- or ginger-treated flour and the repellency tests marked the turmeric and ginger oils as of class V of repellency.

The present results are in harmony with that of Gautam *et al.* [34] who reported that clove gave the minimum adult emergence of *C. analis* after a 5-month storage period. However, El-Lakwah *et al.* [35] found that clove buds extract resulted in complete inhibition of S. oryzae, *R. dominica* and *T. castaneum* population for 12 months. On the other hand, Sighamony *et al.* [36] who found that the clove oil provided protection to wheat against *S. oryzae* and *R. dominica* up to only 60 and 30 days of exposure, respectively, and the potency decreased gradually with increasing storage time.

On the other hand, loss in some oils toxicity when mixed with flour and stored up to six months may be attributed to the evaporation and gradual degradation of the oil components during storage. The persistence of oils in stored flour could be enhanced by developing suitable formulations combining with natural plant materials.

5. Conclusion

From the present results it can be concluded that turmeric and ginger oils were the most promising medicinal oils against *T. confusum* and *T. castaneum* at laboratory conditions. Their contact toxicity and repellency effects make these oils good candidates as stored-grain protectants against such beetles' infestation. Besides, these botanical oils are available in Egypt and potentially could offer a cheap and easy natural control method. The use of formulation based on these plants oils could reduce the use of chemical insecticides with human food supplies. However, additional experiments are required to identify the chemical compounds responsible for toxic effects, also to clarify botanical oils properties and their potential toxicity against different insect species in various environments.

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