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TOXIC EFFECTS OF FOUR PLANT OILS ON *TROGODERMA GRANARIUM* EVERTS AND PHEROMONE RESPONSE

By
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Abstract

This study evaluated the insecticidal bioassays of four known plant oils against the 4th larval instar of the khapra beetle, *Trogoderma granarium* (Everts). Mortality% increased with increased concentrations of oils and exposure time LC_{25} & LC_{50} showed the efficacy of cinnamon oils, followed by black seed, lemon, and then camphor oils. They induced biochemical changes on *T. granarium* adults with LC_{50} compared to control, which were tracked by analysis of carbohydrate-hydrolysis enzymes to measure phosphatase enzymes changes post-treatment. Acid phosphatase enzyme was significantly decreased when treated with black seed, lemon, and camphor oils, but highly increased with cinnamon oil compared to control. Alkaline phosphatase was decreased significantly post-treated with black seed and lemon oils, but increased significantly after camphor and cinnamon oils treatment compared to control. Trehalase enzyme was significantly decreased, but invertase and amylase decreased significantly except in cinnamon oil, which increased significantly compared to control. All oils showed significantly less activity in acetylcholinesterase, glutathione S-transferase, and cytochrome P-450 monooxygenase compared to control. When the 4th larval instar was treated with LC_{25} of cinnamon oil (0.91%), male perception of sex pheromone and female pheromone production were impacted. Pheromone production and response were much higher in untreated beetles than in treated ones.

Key words: Khapra beetle, Cinnamon, Black seed, Lemon, Camphor, Pheromones, toxicity.

Introduction

One of the major worldwide problems is the demand for food to feed the growing population **(**FAO, 2017). Primary food supplies for humans globally are cereal crops. Thus, initiatives should be taken to guarantee the sustainability of these plant food supplies for humans. The greatest overall losses of stored products are caused by coleopteran insects (Wakil *et al*, 2021). Wheat suffered substantially more loss from khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) than other cereals and various other storage and food items, including grains and dried fruits (Elmadawy and Omar, 2022). Because the beetle can survive for extended periods of time without food that contains moisture, existing infestations can be challenging to eradicate. These beetles are relatively resistant to many surface pesticides and fumigants because they frequently enter small cracks and crevices and stay there for extended periods of time. Thus, it's crucial to stop the spread of the khapra beetle to unaffected areas (Athanassiou *et al*, 2019).

Consequently, this beetle is difficult to manage with traditional chemical pesticides that work well against other pests of stored goods. Furthermore, chemical control methods are coming under more and more constraints due to environmental issues, high costs, hazards to beneficial species, and rising insect resistance to them (Tudi *et al*, 2021). Kumar *et al*. (2021) found that the bioactive components of plant oils, especially monoterpenes, are effective in managing stored grain pests with minimal negative effects. Demirak and Canpolat (2022) also reported that such components break down quickly into non-toxic metabolites, making them effective pest agents. Additionally, Khani and Asghari (2012) and Chaubey (2019) discovered that many plant oils and their constituents have insect-repelling, anti-feedant, ovicidal, oviposition-

inhibiting, and developmental-inhibiting properties, making them promising sources of selective insecticides with little to no harm to the environment or non-target species.

Black seed (*Nigella sativa*) of Ranunculaceae family is extensively grown in Western Asia, Mediterranean countries, and Europe. Its medicinal benefits were known to the Ancient Egyptians, Greeks, and Romans (Padhye *et al*, 2008). Monoterpenes, such as p-cymene, α-thujene, γ-terpinene, carvacrol, α-pinene and β-pinene proved to be the primary oil constituents of black seed in earlier investigations (Ahmad *et al*, 2021). Another extensively cultivated plant with documented biological effects includes the lemon, *Citrus limon* (Family: Rautacae), which has insecticidal, anticancer, antioxidant, and antibacterial properties (Maqbool *et al*, 2023). *Eucalyptus camaldulensis* (Family: Myrtaceae) has antibacterial, antiviral, antifungal, anti-inflammatory, and insect-repellent properties (Salehi *et al*, 2019). Cinnamon is known for its use as a spice, with medicinal and agricultural values as an antibacterial and pharmacological agent. Also, cinnamon includes diterpenes, catechins, proanthocyanidins, lignans, mucins, tanning agents, phenolic carboxylic acids, and plant oils with insecticidal and repellent activities (Kowalska *et al*, 2021).

Alkaline phosphatases are the basic phosphate absorption and secretory processes (Han *et al*, 2021). A lysosomal marker enzyme is an acid phosphatase. Moreover, these two enzymes might function as hydrolases in the final processes of digestion, gonad development, and metamorphic moults (Ghoneim *et al*, 2014). Their activity levels are low while the larvae are moulting and progressively go up after that (Miao, 2002). Insect species depend on the digestive enzymes (invertase, trehalase, and amylase) to utilize carbohydrates taken up. Kandil *et al.* (2020) used those enzymes as parameters to study the effect of some plant oils on the pink bollworm, *Pectinophora gossypiella*. Insects can diminish the sensitivity of a pesticide's target site, such as nerve conduction enzyme acetylcholinesterase (AChE), to deal with bioactive secondary metabolites (Bezerra da Silva *et al*, 2016). The detoxifying enzyme glutathione S-transferase (GST) is one that insects may use (Li *et al*, 2013) and the cytochrome p450 monooxygenase (Norris *et al*, 2018). Pheromones are substances that act as chemical messengers between members of the same species, eliciting physiological or behavioural reactions (Karlson and Luscher, 1959; Nordlund, 1981). Sex pheromones generated by specific glands of both sexes and differ from aggregation pheromones, which only attract the second sex to facilitate copulation at mating (Jacobson, 1972). Low quantities of sexual attractants are frequently effective (Burkholder, 1970). Concentration of sex pheromones, exposure time, and physiological state, such as hunger, may influence the perception and response to these chemical signals (Levinson and Bar Man, 1970).

This study aimed to evaluate the oils of cinnamon, black seed, lemon, camphor and sex pheromones in controlling *T. granarium*.

Materials and Methods

Insect culture*:* The Khapra beetle, *Trogoderma granarium*, was maintained in an incubator at the Plant Protection Research Institute, Agricultural Research Center, Dokki, for several generations in a pesticide-free environment. Medium used was sterile wheat grains with 12% moisture content. Emerged adults 120 to 150 individuals were put into liter glass muslin fabric jars with 250g of grains each at 28±2°C, 70±5% RH, & 12:12 light/dark photoperiod.

Oils: Black seed (*Nigella sativa*, Order Ranunculales, Family Ranunculaceae), lemon (*Citrus limon. Order* Sapindales, Family Rutaceae), camphor (*Eucalyptus camaldulensis*, Order Myrtales Family Myrtaceae), and cinnamon (*Cinnamomum verum*, Order Laurales, Family Lauraceae) were purchased as crude oils from the Natural Oils Extraction Unit, National Research Centre, Cairo.

Bioassay*:* Serial concentrations for all plant oils (EOs) were prepared by diluting the

crude oil in acetone to obtain different concentrations (2, 4, 8, & 16%). A sample of 10g of wheat grains was placed in a glass jar (4x5cm) and exposed to 1mL of each concentration of tested plant oils, which were then manually shaken for one minute to ensure that the test substance was evenly distributed throughout and coated all of the wheat grains and separated for an hour to let acetone evaporate. For control, 1mL of acetone was used as described. Twenty-five 2-weeks-old *T. granarium* were introduced into each prepared jar contained treated and an untreated wheat. Experiment was replicated for four times. Jars with a secure muslin cover were kept in an incubator. Mortality was measured in all tubes at 2, 4, 6, 8, &10 days after exposure. Experiments were conducted at 28±2°C & 70±5% R.H.

Gas chromatography-mass spectrometry (GC-MS) analysis: Chemical composition of *Cinnamomum verum* plant oil was identified using Shimadzu GC-MS-QP 2015 plus (kyoto, Japan) with a direct capillary column HP (50m x0.25mm x0.25µm film thickness) detected by comparing peak areas with the WILEY 09, NIST14 and Tutor libraries data (Beckley *et al*, 2014).

Biochemical analysis: LC_{50} values calculated two days post-treatment were used. Ten grams of both untreated and treated adults were collected 48 hours post-treatment and kept in freezer (-20ºC) until analysis. Samples were homogenized in the distilled water (5ml/sample) using a Teflon homogenizer and centrifuged at 5000rpm for 20 min at 5ºC, and supernatant was used.

Phosphatases activity: Acid phosphatase (AP) activity was assessed using a BioAd-Wic kit (Tietz, 1986). A spectrophotometer was used to measure enzyme at wavelength 405 nm. Alkaline phosphatase (ALP) activity was evaluated using a kit from Quimica Clinical Aplicada and measured at a wavelength of 550nm with a spectrophotometer. Carbohydrate enzyme activity: Hydrolysis activities of trehalose (1.5%), starch (0.5%), & sucrose (2%) with trehalase, amylase, and invertase enzymes, respectively, were measured at optimum conditions of temperature and pH **(**Ishaaya and Swirski, 1976)**.**

Acetylcholinesterase (AChE) activity: The activity was detected according to method of Simpson *et al*. (1964) by using the substrate, acetylcholine bromide (AChBr).

Glutathione-S-Transferase (GST) activity: GST activity was determined with some modification (Habig *et al,* 1974). Larvae were homogenized in 0.1 M phosphate buffer (pH 6.5) and centrifuged at 12,000×g at 4°C for 15min. The solution reaction with 10µL enzyme stock solution, 25µL 30mM CDNB, & 25µL 50mM GSH was measured at 340nm at 25 °C for 3 minutes using a Jenway-7205 UV/Vis spectrophotometer.

Cytochrome p450 Monooxygenase activity: To determine the cytochrome p450 monooxygenase activity, the pnitroanisole odemethylation was assayed (Hansen and Hodgson, 1971).

Pheromone assay: This assessed the insecticidal efficacy of cinnamon oil on the production of female sex pheromones and male perception in adult *T. granarium* beetles. The 4^{th} larval instars were exposed to a concentration of cinnamon plant oil corresponded to 25% of (LC_{25}), and the effects were evaluated 2 days post-treatment, along with an untreated control group. Males and females were separated in pupal stages.

Four experiments were conducted on at least 50 individuals: (i) response of females to male extract versus (vs.) untreated; (ii) response of males to female extract vs. untreated; (iii) response of males to male extract vs. untreated; and (iv) response of females to female extract vs. untreated. Male and female samples were put in different olfactometers glass (15x1.5cm) vial with a rubber plug and an adjustable glass rod (Burkholder, 1970). A small piece of masking tape containing the hexane extract to be tested for pheromone production was placed at the wide inner end of a vial. The beetle that was tested for response was positioned at the bottom of the vial. Two insects were placed 3 cm apart in

each vial, and ten vials were used in each of the five replicates. All males and females were tested when 3-4 days old.

To produce pheromone extracts for male bioassays, a specified amount of hexane was added to screw-top culture tubes containing females of the appropriate age and number. They were entirely macerated after being vibrated in their tubes on a mixer. To achieve appropriate number of female equivalents (FE) per 10µl aliquot of hexane, serial extracts dilutions (0.1, 0.3, 0.5, 0.7, & 0.9 FE) were prepared using hexane. Extracts were kept in dark at -30^{o}C. Males were put into separate olfactometer vials and a female-free was kept in an incubator overnight before being tested. An extract of 20µl (0.3 insect equivalent) of either male or female or hexane (untreated) covers a piece of filter paper was placed at the vial top, and one male or female was introduced into its base and observed for behavior at 60-second intervals for 10 minutes. Only quiet and resting males were used in pheromone assays. A response could fall into one of four categories: category one was recorded when male became active and walked on vial's floor within one second of introducing stimulus; category two was recorded when male became active within the first 5 to 10 seconds of stimulus; category three was assigned when it started to walk around the bottom of the vial within six to sixty seconds of the stimulus; and category four was recorded when the male remained quiescent on the vial bottom for a period of time, which was recorded as no response. To differentiate male response threshold existed over photophase, at 2-hour intervals, five replicates of 10 (3-4-day-old) were tested against a single hexane extract of 0.3 FE versus virgin females.

Food effect on sex pheromone production and perception, treated virgin females (3-4 days old) were extracted, and tested against fed or unfed males. Unfed treated virgin females of same age were extracted and tested against fed or unfed treated males (3-4 days old) and vice versa. All pheromone assays were done at 30°C & 70% R.H., at 3 p.m.

Statistical analysis*:* Mortality was corrected by Abbott's formula (1925)**,** and its percent were statistically evaluated (Finney, 1971)**.** A LDP-line software program was used for toxicity regression lines **(**Bakr, 2000). LC_{25} & LC_{50} were determined. Data were analyzed by ANOVA followed by LSD post-hoc multiple comparison by SPSS software package version 23.

Ethical approval: This study was approved by Faculty of Science, Ain Shams University Research Ethics Committee Reference Code (ASU-SCI/ENTO/2023/4/1)

Results

The results were given in tables (1, 2, 3, 4, 5, 6, 7 & 8) and figures (1, 2 & 3)

Table 2: LC values 10 days post-treatment of *T. granarium* larvae to four oils.

Oils	Days	LC_{25}	LC_{50}	Mean ±SE	X^2	*P*	Toxicity index	Relative potency
Black seed	2	33.45	89.78	1.57±0.45	2.91	0.23	4.05%	1.09
	4	17.80	35.59	2.18±0.42	5.21	0.07	5.93%	1.18
	6	17.02	33.86	2.25±0.21	4.50	0.10	4.22%	1.17
	8	11.96	21.05	2.74±0.47	5.80	0.05	6.41%	1.55
	10	7.75	14.90	1.17±0.38	5.64	0.05	7.45%	1.55
Lemon	2	21.12	53.05	1.68±0.36	3.41	0.18	6.86%	1.85
	4	12.85	29.36	1.87±0.31	1.81	0.40	7.19%	1.43
	6	10.88	22.47	2.14±0.22	2.02	0.36	6.36%	1.77
	8	9.77	20.37	2.11±0.21	2.39	0.30	6.63%	1.6
	10	6.74	15.18	2.37±0.28	5.24	0.07	7.31%	1.52
Camphor	2	37.25	98.18	1.60±0.19	2.78	0.24	3.71%	1
	4	19.61	42.13	2.03±0.26	3.01	0.22	5.01%	1
	6	18.61	39.67	2.05±0.35	2.27	0.32	3.60%	1
	8	14.83	32.68	1.96±0.12	3.31	0.19	4.13%	1
	10	9.53	23.04	1.75±0.18	0.38	0.82	4.82%	1
Cinnamon	2	0.91	3.64	1.12±0.33	5.99	0.04	100%	26.97
	4	0.61	2.11	1.27±0.22	5.08	0.07	100%	19.97
	6	0.44	1.43	1.32±0.16	5.56	0.06	100%	27.74
	8	0.41	1.35	1.32±0.28	4.39	0.11	100%	24.21
	10	0.40	1.11	2.47±0.23	5.60	0.06	100%	20.76

Table 2: Chemical composition of Cinnamon oil (*Cinnamomum verum*).

No.	RT	Compound Name	Area %	Molecular formula	Molecular weight
1	8.86	Benzyl alcohol	12.91	C_7H_8O	108.14
2	9.18	Acetic acid, phenyl methyl ester	41.94	$C_{15}H_{12}O_4$	150.17
3	10.89	(E)-cinnamaldehyde	45.14	C_9H_8O	132

Table 3: Response of virgin *T. granarium* males and females to hexane extract emerged from cinnamon oil treated larvae.

Items	Untreated	Treated	Control	F- value	P-value
Male responded to female behavior	88.0±0.37^{a}	54.0±0.68^{a}	14.0±0.24^{a}	62.36	***
Female responded to male behavior	68.0±0.37^{b}	34.0±0.51^{bd}	8.0±0.37^{a}	50.30	***
Male response behavior to male	56.0±0.51^{bd}	18.0±0.37^{c}	8.0±0.37^{a}	35.63	***
Female response to female	52.0±0.37^{cd}	28.0±0.37^{cd}	10.0±0.32^{a}	35.05	***
F- value	15.37	10.35	00.73		

Same letters not significant same column (P>0.05), *** mean in same row significant (P<0.05).

Table 4: Response of virgin *T. granarium* males to females' hexane extract emerged from cinnamon oil larvae treated.

Experiments	Mean ±SE
Untreated male response behavior to treated female	62.20±0.37^{a}
Treated male response behavior to untreated female	58.0±0.37^{a}
untreated male response behavior to untreated female (control)	90.0±0.45^{b}
F- value	19.00
P- value	0.00

Table 5: Male response to pheromone concentrations of virgin females emerged from cinnamon oil larvae treated.

Pheromone concentrations	Treated	Untreated	Control	F- value	P- value
0.1	8.0±0.37^{a}	46.0±0.51^{a}	6.0±0.24^{a}	33.13	***
0.3	18.0±0.37^{a}	54.0±0.51^{a}	10.0±0.45^{a}	27.47	***
0.5	56.0±0.51^{b}	92.0±0.37^{b}	8.0±0.37^{a}	98.67	***
0.7	72.0±0.37^{c}	96.0±0.24^{b}	2.0±0.20^{a}	298.17	***
0.9	72.0±0.73^{c}	98.0±0.20^{b}	6.0±0.24^{a}	105.44	***
F- value	37.67	41.40	0.88		
P- value	0.00	0.00	0.49		

Table 6: Male response to virgin females extracted at photophase and scotophase of 2-hour intervals. Both emerged from cinnamon oil treated larvae.

Daytime of female extracts	Treated	Untreated	Control	F- value	P- value
08:00 a.m.	8.0±0.20^{af}	56.0±0.51^{ae}	8.0±0.37^{a}	52.36	***
10:00 a.m.	24.0±0.51^{bg}	64.0±0.45^{ae}	8.0±0.20^{a}	42.56	***
12:00 p.m.	44.0±0.51^{ch}	76.0±0.68^{bf}	4.0±0.24^{a}	50.05	***
14:00 p.m.	58.0±0.58^{c}	86.0±0.51^{bg}	10.0±0.45^{a}	55.40	***
16:00 p.m.	58.0±0.49^{c}	90.0±0.32^{cg}	12.0±0.20^{a}	151.13	***
18:00 p.m.	40.0±0.45^{dh}	64.0±0.24^{def}	8.0±0.37^{a}	59.20	***
20:00 p.m.	20.0±0.71^{efg}	58.0±0.58^{de}	6.0±0.24^{a}	24.13	***
22:00 p.m.	6.0±0.40^{ef}	52.0±0.37^{de}	6.0±0.32^{a}	58.78	***
F- value	17.25	09.12	00.67		
P- value	0.00	0.00	0.69		

Table 7: Males response at photophase of 2-hour intervals to a single sex pheromone of virgin females emerged from cinnamon oil treated larvae.

Daytime of male extracts	Treated	Untreated	Control	F- value	P- value
08:00 a.m.	4.0±0.24^{af}	50.0±0.45^{agij}	4.0±0.24^{a}	66.13	***
10:00 a.m.	16.0±0.40^{aeg}	58.0±0.49^{af}	14.0±0.24^{a}	40.26	***
12:00 p.m.	44.0±0.51^{b}	66.0±0.24^{bf}	14.0±0.51^{a}	35.24	***
14:00 p.m.	50.0±0.45^{b}	74.0±0.51^{b}	8.0±0.37^{a}	55.80	***
16:00 p.m.	54.0±0.51^{b}	92.0±0.37^{c}	18.0±0.20^{b}	93.36	***
18:00 p.m.	46.0±0.51^{b}	54.0±0.51^{dfgh}	10.0±0.32^{a}	26.58	***
20:00 p.m.	28.0±0.37^{ce}	44.0±0.51^{ehi}	8.0±0.20^{a}	22.18	***
22:00 p.m.	10.0±0.45^{dfg}	40.0±0.45^{ej}	12.0±0.58^{a}	11.41	***
F- value	19.93	14.47	1.50		
P- value	0.00	0.00	0.21		

Table 8: Effect of food on sex pheromone and perception by *T. granarium* emerged from cinnamon oil treated larvae.

Females extracts	Male response	Untreated	Treated	control	F- value	P-value
Fed female	Fed male	90.0±0.45^{a}	58.0±0.37^{a}	4.0±0.24^{a}	141.70	***
Non-fed female	Fed male	70.0±0.32^{b}	24.0±0.51^{b}	8.0±0.37^{a}	62.16	***
Fed female	Non-fed male	66.0±0.24^{b}	18.0±0.37^{b}	10.0±0.32^{a}	91.73	***
Non-fed female	Non-fed male	38.0±0.37^{c}	6.0±0.24^{c}	6.0±0.24^{a}	39.39	***
F- value		36.69	33.13	00.74		
P- value		0.00	0.00	0.54		

Discussion

In the present study, the death rate of *T. granarium* 4th stage larvae was proportional to concentrations (2, 4, 8, &16%) and time exposure (2, 4, 6, 8, & 10 days) of plant oils tested, showing that cinnamon oil was the most effective one than others in terms of mortality rates. Toxicity indexes were 7.45, 7.31, & 4.82 % for black seed, lemon, and camphor, respectively, compared to cinnamon (100%), with a LC_{90} value of 50.33%. This agreed with Qari and Abdelfattah (2017), who reported that oils' alteration differed among compounds with variations in mortality rates and might be applied in controlling *Rhyzopertha dominica* in stored goods. Abdel-Fattah and Boraei (2017) found that the oils fumigant toxicity on insects increased with increasing content and exposure duration. Chaudhari *et al.* (2021) reported that oil may be applied as a safe pesticide for some insects in stored items as well as a fumigant and repellant. They added that cinnamon, apart from having anti-inflammatories properties, useful in organic farming as a viable alternative to the pesticides used for plant protection. Kowalska *et al.* (2021) reported that natural products consisted of the European Green Deal objectives for the restriction of chemical pesticides.

In the present study, GC-MS analysis of *C. verum* showed that its oil is composed of 3 components: cinnamaldehyde (45.14%), acetic acid, phenyl methyl ester, (41.95%), and benzyl alcohol, or α-Cresol (12.91%) which account for 100% of the total composition. Also, the present study compared to the negative control, the acid phosphatase enzyme significantly decreased when treated with black seed, lemon, and camphor oils, but highly increased with cinnamon oil. Alkaline phosphatase decreased significantly in samples treated with black seed and lemon oils but, increased significantly with camphor and cinnamon oils. Activity ratios of acid to alkaline phosphatase ranged from 1.08-0.71 to 1.09-0.60, respectively. This agreed with Qari *et al*. (2017), they reported that acid and alkaline phosphatases were significantly increased with chlorpyrifos, moderately increased with *Zingiber officinale* and *Origanum majorana* but reduced with *Citrus aurantium*.

Besides, the present activity of carbohydrate-hydrolysis enzymes (Trehalase, Inver-tase, & Amylase) in adult *T. granarium* exposed to the LC_{50} of all oils calculated after 2 days post-treatment showed that trehalase enzyme was significantly decreased by all oils. But enzymes invertase and amylase decreased significantly, except for cinnamon oil, which significantly increased. This agreed agreement with Shakoori *et al.* (2018), who reported that trehalase, amylase, and invertase activities reduced the esfenvalerate sublethal dosage compared to negative control ones.

In the present study, enzymes significantly inhibited treated beetles as compared to control. This agreed with Almadiy and Nenaah (2022), who found that oils inhibited acetylcholine esterase in *T. granarium* larvae. Younes *et al*. (2011) reported that AChE activity changed response to medicinal plant oils. Also, this agreed with Abd EL-Aziz and El-Sayed (2009), who tested six essential oils against *T. confusum* last larval instar and adults, found that suppression of detoxification enzymes became less effective. However, Gupta *et al.* (2023) reported a significant decrease in GST activity in larvae of *Callosobruchus maculatus* and *C. chinensis*.

In the present study, treated males tested against treated females, the highest degree of response (54%) was obtained. The responses in the negative and positive controls were 88% & 14%, respectively. Behavior of treated males towards treated females was characterized by a series of increased excitation levels, as males became active within a second after stimulus administration and moved to the vial floor (category 1), but, virgin female extract treated with LC_{25} oil generated substantially less pheromone than negative control. When females were tested against males, the response ratio was 34%, and tho-

se of the negative and positive controls was 68%, & 8%, respectively. Reaction occurred when male became active within the first 5-10 seconds after introducing stimulus (category 2). Reaction of treated females to a male's extract treated with LC_{25} oil was markedly reduced to a lower titer of pheromone production compared to the negative control.

In the present study, male and female response behavior towards females showed (18%), but negative male responses were 56% & 8%, respectively. When evaluated against the same sex, treated females responded at 28%, but negative & positive control responses were 52% & 10%, respectively, for males and females (category 3). Furthermore, untreated males showed a response of (62.20±0.37) to treated females, compared to a response of (58.0±0.37) in untreated males towards treated female beetles. Both showed a highly significant difference compared to negative males to negative females (90.0±0.45). Responses of negative groups showed significance, but female responses towards males compared to male responses to males or also responses to males compared to female responses to their own sex and vice versa didn't show significant differences. Untreated groups showed much greater responsiveness and pheromone production than did the treated ones, without significant difference compared to the solvent.

Generally, female pheromones are sexual ones that attract and lure males for mating. Six species of *Trogoderma* exhibited glandular epithelia in the seventh abdominal sternites (Hammack *et al*, 1973). The glandular tissues are the source of producing pheromones. While in calling postures, females of *Trogoderma* species display the abdomen sternites, and perhaps this activity helped pheromones spread across the surrounding area. The other pheromone that males emit appeared to be an aggregation pheromone produced by them to gather with the population for feeding. This agreed with Abdu *et al*. (1985), who used radiation against the rust-red flour beetle; Chen *et al*. (1988), who used juvenile hormone analogue and fenoxycarb on *Ips paraconfusus* and Bakr *et al*. (2010), who used *T. castaneum*. Besides, Ruther *et al*. (2007) reported that sex pheromones produced by an untreated male attracted females in the parasitic jewel wasp, *Nasonia vitripennis*.

In the present study, *Cinnamomum verum* oil showed a higher significant impact on male responsiveness to sex pheromones than it did on female pheromone production in the treated sexes. Thus, LC_{25} oil treated male 4^{th} larval instars had a greater impact on pheromone-producing glands than treated females. In the same line, in treated sexes, the response of the male was significantly increased with increasing concentrations of female pheromone extract, as observed by El-Barky *et al*. (2012) on *Tribolium castaneum* adults produced from treated 4^{th} larval instars by pyriproxyfen.

In the present study, male response to pheromone increased gradually with increased the titers of treated and untreated virgin female extract concentrations. Treated male reaction was 8, 18, 56, 72, & 72% at 0.1, 0.3, 0.5, 0.7, & 0.9 FE, and the untreated one was 46, 54, 92, 96, & 98%, significantly different compared to control. This agreed with Nagata *et al*. (1972), who used *Adoxophyes fasciata* and found that male response was significantly increased with increased female pheromone concentration.

In the present study, treated females extracted early at 8a.m. stimulated 8% of males, with a low pheromone titer. Two hours later, at 10a.m., more pheromone was produced with considerable increase up to 24% of males responded to treated female. The maximum pheromone titer generated by treated females at that time was caused peak of treated male response (58%) between 14 p.m. & 16 p.m. At 18 p.m., male response to treated females decreased to 40%. At night (scotophase) continued to decline to 20% at 20.00p.m. Although the maximal pheromone production in untreated virgin female extract was 90% at both photophase and sc-

otophase, yet it was substantially greater in treated virgin female extract at both phases at 16.00p.m. In fact, treated females consistently produced far more pheromones than negative and positive control females.

In the present study, from early morning to midafternoon, there was an increase in male response, which dropped in late afternoon with lowest level at (22.00p.m.). By 16p.m. in the evening, highest response (54%) was reached. For both photophase and scootphase, maximum pheromone response in untreated virgin males was 92%, despite the fact that their reactivity to pheromone was much higher than that of treated ones. Responses of treated male at each time showed a significant effect compared to negative and control ones (solvent only). This agreed with Hammack and Burkholder (1976), they reported that *Trogoderma glabrum* activity was photo-periodically regulated and circadian in nature. Raina and Kempe (1990) found that the behavior of cabbage looper, *Trichoplusia ni* have an endogenous rhythm. This agreed with Obeng-Ofori and Coaker (1990), they found that all pheromones caused a periodicity of response, with a peak between 10.00 a.m. & 18.00p.m., in *T. castaneum*, *T. confusum*, *R. dominica*, and *Prostephanus trunkates.* During photophase, female pheromone content coincided with male response in *T. granarium*, as well as in *R. dominica* (Bashir *et al*, 2003). This would serve the practical purpose of harmonizing female pheromone release and mate-receptiveness with the time when male attraction would be at its maximum. So, in managing insects by pheromones impacts behavioral must be considered in certain daily activity.

In the present study, highest sex pheromone titer by treated females and highest level of treated male response were (58%). But, reverse occurred when food was not allowed to the treated ones (6%). Sex pheromone extracts from non-fed females excited either allowed or not allowed food to males, but male response showed a higher significance (24%) for food than without food (6%).

Intermediate levels of pheromone production and male response occurred when one sex was introduced to food or not. Responsiveness and pheromone production were significantly higher in untreated groups than in treated ones. This agreed with El-Barky *et al*. (2012), who reported similar findings in adult *T. castaneum* that had emerged from pyriproxyfen treated 4th larval instars. But, it disagreed with Mccluskey *et al*. (1969), who found that starved and untreated male *Periplaneta americana* responded to sex pheromone till death, and Ali (2010), who found that starvation didn't affect either production or responsiveness to pheromones in both sexes of *Attagenus fasciatus*.

Conclusion

The outcome data proved that cinnamon oil was the most promising one than either black seed lemon or camphor. This fact was indicated by carbohydrate-hydrolysis, phosphatase, acetylcholinesterase, glutathione S-transferase, and cytochrome P-450 monooxygenase enzymes activity monitored treatment changes. Sex pheromone production and male perception were influenced in treated adults compared to untreated ones.

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Explanation of figures

Fig. 1: Phosphatases activity in *Trogoderma granarium* adults treated with LC_{50} of black seed, lemon, camphor, and cinnamon oils.
Fig. 2: Carbohydrates enzymes activity of *T. granarium* adults treated with LC_{50} of black seed, lemon, camphor, and cinnamon oils.
Fig. 3: Acetylcholinesterase, Glutathione S-Transferase, and Cytochrome p450 Monooxygenase activities on adults treated with LC_{50} of black seed, lemon, camphor, and cinnamon oils.