Physiological and toxicological effects of some plant oils on *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract:

Six plant oils of Mustard (*Brassica campestris* L.), Basil (*Ocimum basilicum* L.), Chamomile (*Matricaria recutita* L.), Anise (*Pimpinella anisum* L.), Orange (*Citrus sinensis* L.), and Rosemary (*Rosmarinus officinalis* L.) were evaluated in the laboratory for their toxicities against adults of the rust red flour beetle, *Tribolium castaneum* (Herbst) at the LC_{50} after 7 days post-treatment at 30±1° C. Toxicities of plant oils could be arranged in descending order as; Orange oil, Chamomile oil, Rosemary oil, Basil oil, Mustard oil and Anise oil was the least effective. The results of the effect of LC_{50} of Orange oil on blood cells of the larvae showed mild effect which appeared on cell membrane and the presence of empty cells. The LC_{50} of Orange oil on the total proteins, lipids and carbohydrates of the treated adults of *T. castaneum* showed significant decrease, and non-significant effect on enzymes activies **(GOT), (GPT)** and **Acetylcholinesterase**. A Significant increase in activity of **Alpha esterases, Beta esterases, Trehalase** and **Amylase** was recorded, while a significant decrease was recorded in the activity of **Invertase** enzyme.

Introduction:

The rust red flour beetle, *Tribolium castaneum* (Herbst), is a cosmopolitan and destructive beetle in the family Tenebrionidae which

mainly attacks stored grain products such as flour, cereals meal, beans, seeds, and even dried museum specimens **(Weston and Rattlingourd, 2000).** It, also, causes serious damage to dried fruits, pulses and prepared cereal foods, such as cornflake, pasta, biscuit, beans, nuts, etc. It may be considered as the most common insect pest attacking stored wheat although its pest status is considered to be secondary, requiring prior infestation by an internal feeder. It can readily infest wheat or other grains damaged during the harvesting operation. Both larvae and adults feed on grain dust and broken grain, but not the undamaged whole grains and spend its entire life-cycle outside the grain kernels **(Karunakaran *et al.*, 2004).**

During the last decades, using chemical pesticides for the control of agricultural pests has been a conventional practice. Regarding the fact that many of common pesticides can adversely affect the environment, non-target organisms, and human health; looking for safer devices of pest management have become crucial. As a consequence, these problems led researchers to look for safer natural compounds such as essential oils. Botanical derivatives, especially plant essential oils which are obtained through steam distillation of herbs and aromatic plants have been used traditionally as medicine, flavor in dishes and drinks, perfume, and as insecticides in many countries **(Pushpanathan *et al.*, 2006).** These compounds tend to have low mammalian toxicity, little environmental mal-effects and wide public acceptance **(Isman, 2000).**

Materials and Methods:

A laboratory strain of the rust red flour beetle *Tribolium castaneum* (Herbst.) was obtained from the Plant Protection Research Institute at Doki,

Giza, Egypt and reared on wheat flour in a climate chamber at 30±1°C and 65±5% R.H. for about one year in the laboratory of stored product pests of the Plant Protection Dept., Fac. of Agriculture, Benha University.

Rearing of insect culture:

The insect was reared in 1kg. glass jars, each jar contains about 250g. of sterilized and conditioned wheat flour and covered with muslin cloth fixed with rubber bands. Insect cultures were kept in the rearing room of the laboratory 28± lºC and 65±5% R.H. at number of 300 adults (1-2 weeks old) were placed in each jar and left into for egg laying. Three days later, all adults were separated from the food and the jars were kept again in the rearing room. This procedure was repeated several times in order to obtain mass rearing of the adults needed to carry out the experiments.

Adults and the developmental stages:

Eggs (0-48 hrs.old), larvae (4^{th} instar), pupae stage and adults (7-14 days-old) were used for the experiments.

Preparation of the test-insects for various treatments:

Batches of 30 adults of ***T. castaneum*** were used in all experiments of plant oils. Three replicates were used treatments.

Essential Oils:

Six plant oils **(Table 1)** belonging to different families; Mustard (Brassicaceae), Basil and Rosemary (Lamiaceae), Chamomile (Asteraceae), Anise (Apiaceae) and Orange (Rutaceae) were used during these investigations. The essential oils (Basil oil, Anise oil, Orange oil and

Rosemary oil) were bought from El Captain Company ″CAP PHARM″) while Mustard oil was bought from Arab Company for Medicines and Medical Plants-Mepaco and Chamomile oil was bought from Greatco Company extracts Aromatic essential oils-Flavors and smell additives-extracts. The concentrations of plant oils (v/w %) used in this investigation were as follows:

1- ***Brassica campestris* L.:** 8, 7, 6, 4, 3, 2, 1.5, 1 and 0.75.
2- ***Ocimum basilicum* L.:** 6, 4, 3, 2 and 1.5.
3- ***Matricaria chamomillia* L.:** 6, 4, 3, 2, 1.5 and 1.
4- ***Pimpinella anisum* L.:** 6, 4, 3, 2 and 1.5.
5- ***Citrus sinensis* L.:** 4, 3, 2, 1.5, 1 and 0.75.
6- ***Rosmarinus officinalis* L.:** 6, 4, 3, 2 and 1.5.

Table (1): Plant oils used in the present investigation:

Scientific name		Family	Common name		Used part
			English	Arabic	
1	*Brassica campestris* L.	Brassicaceae	Mustard	الخردل	Seeds
2	*Ocimum basilicum* L.	Lamiaceae	Basil	الريحان	Leaves
3	*Matricaria chamomillia* L.	Asteraceae	Chamomile	البابونج	Flower
4	*Pimpinella anisum* L.	Apiaceae	Anise	الينسون	Fruits
5	*Citrus sinensis* L.	Rutaceae	Orange	البرتقال	Fruits
6	*Rosmarinus officinalis* L.	Lamiaceae	Rosemary	حصالبان	Leaves

Bioassay tests:

Insect samples were exposed to various treatments for different periods. After the exposure period has finished, mortality assessment was performed. Mortality percentages were corrected according to **Abbott's formula (1925)** as follows:

$$\text{Corrected mortality \%} = \frac{\text{\% mortality in treatment - \%mortality in control}}{\text{100 - \% mortality in control}} \times 100$$

Mortalities among treated *T. castaneum* were determined after 1, 2, 3, 5, 10, 14 and 21 days after the exposure periods.

Differential hemocyte counts:

Blood films were made from *T. castaneum* larvae after treatment which was treated with LC_{50} of Orange oil as described by **Souka (1977) and Amin (1998)** by applying one end of the slide to a drop of haemolymph. The slide was placed on a leveled surface, holding it with the thumb and index fingers .The narrow edge of a second slide was placed to touch the drop and held there till the blood has spread across it. The slide was then drawn slowly over the whole length of the first slide (45°). After the blood did spread, it should be dried by being waving rapidly in the air to prevent undue shrinkage of the cells.

Haemolymph films were stained by Geimsa as described by **Lillie and Fullmer (1976).** The dried films were fixed by methyl or ethyl alchol for 5-7 min. and then dried in air. The film was well covered by the Giemsa solution for 15 min., followed by washing in distilled water and air dried. Those were examined under the oil immersion lens of the microscope.

Physiological studies:

Biochemical studies:

A known weight of *T. castaneum* adults (0.5g) which was treated by the LC_{50} of Mustard, Orange oil and the same weight of untreated ones were kept in deep freezer. Enzyme analysis was carried out in the Plant Protection

Research Institute at Doki, Giza, Egypt. Insects were homogenized for biochemical analysis in a chilled glass Teflon tissue homogenizer (ST–2Mechanic- Preczyina, Poland). After homogenation, supernatants were kept in a deep freezer at -20ºC. till use for biochemical assays. Double beam ultraviolet/ visible spectrophotometer (spectronic 1201, Milton Roy Co., USA) was used to measure absorbance of colored substances or metabolic compounds. The insects were prepared as described by **Amin (1998)**. Those were homogenized in distilled water (50 mg /1 ml). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 2 ºC in a refrigerated centrifuge. The deposits were discarded and the supernatants, which are referred as enzyme extract, could be stored at least one week whithout appreciable loss of activity when stored at 5^0C. Bovine albumin standard was purchased from Stanbio laboratory (Texas, USA). Commasie brilliant blue G-250 was brought from sigma (sigma chemical co.). P- nitroanisole (purity 97%) was obtained from Ubichem Ltd. (Ham pshire), while nicotinamide ademine dinucleotide phosphate (reduced form , NADPH) was from BDH chemicals Ltd.(Poole, England). The rest of chemicals were of high quality and purchased from commercial local companies. Determining the effect of different treatments on the activity of some insect enzymes (Acetylcholinesterase determination (AChE), Non-specific esterases, Amylase, Invertase, Trehalase, GPT, GOT, Total proteins, Total lipids and Total carbohydrates.

Statistical analysis of data:

All experiments contained 3-4 replicates (insects homogenates), and the results of biochemical determinations were pooled from triplicate

determinations. The results were analyzed by one – way analysis of variance (ANOVA) using costat statistical software (cohort software, Berkeley). When the ANOVA statistics were significant (P <0.01), means were compared by the Duncan's multiple range test.

Statistical analysis:

The obtained mortality data were subjected to Probit analysis **(Finney, 1971)**, using a computer program of (**Noack and Reichmuth, 1978)**.

The Statistical analysis was carried out using ANOVA with two factors under significance level of 0.05 for the whole results using SPSS (ver.19) and data were treated as complete randomization design according to **Steel *et al.* (1997).** Multiple comparisons were carried out applying LSD values.

Results and Discussion:

Toxicity of some plant oils against *T. castaneum* adults at 30 ±1°C and 65±5% R. H.:

The lethal concentration of Mustard, Basil and Chamomile plant oils to adult stage of *T. castaneum* are shown in **Table (2).** The results showed that after 7 days post treatments for adult stage, the LC_{50} values were 3.05, 2.98 and 2.05% (v/w), respectively. The corresponding values at 14 days were significantly lower and decreased to 1.61, 2.75 and 1.59 % (v/w) for Mustard, Basil and Chamomile essential oils, respectively.

The LC_{90} values for adult stage treatment were 20.00, 5.39 and 4.78 % (v/w) at 7 days and declined to 5.43, 4.49 and 3.62% (v/w) at 14 days post treatment by Mustard, Basil and Chamomile essential oils, respectively.

The LC_{95} values of adult stage were 34.08, 6.37 and 6.09 % (v/w) at 7 days and declined to 7.67, 5.16 and 4.56% (v/w) at 14 days post treatment Mustard, Basil and Chamomile essential oils, respectively.

Table (2): Lethal concentrations of some plant oils against adult stage of *T. castaneum* at 30±1°C; 65±5% R.H. and various exposure periods:

Exposure period (Days).	Lethal concentrations (%v/w) and their 95% confidence limits			Slope ± SD	R
	LC_{50} (ml/g)	LC_{90}(ml/g)	LC_{95}(ml/g)		
Mustard (*Brassica campestris*) oil					
7 days	3.05 (2.10-4.43)	20.00 (13.78-29.04)	34.08 (23.47-49.47)	1.57±0.59	0.803
10 days	2.12 (1.47-3.05)	9.40 (6.52-13.55)	14.34 (9.95-20.68)	1.98±0.57	0.896
14 days	1.61 (1.16-2.26)	5.43 (3.89-7.59)	7.67 (5.49-10.71)	2.43±0.50	0.938
Basil (*Ocimum basilicum*) oil					
7 days	2.98 (1.63-5.42)	5.39 (2.97-9.81)	6.37 (3.50-11.61)	4.97±0.66	0.790
10 days	2.88 (2.41-3.43)	4.88 (4.09-5.83)	5.68 (4.76-6.77)	5.57±0.18	0.824
14 days	2.75 (2.33-3.25)	4.49 (3.81-5.30)	5.16 (4.38-6.10)	6.02±0.16	0.813
Chamomile (*Matricaria recutita*) oil					
7 days	2.05 (1.58-2.67)	4.78 (3.69-6.21)	6.09 (4.69-7.90)	3.48±0.32	0.872

10 days	1.87 (1.27-2.77)	4.91 (3.32-7.25)	6.45 (4.36-9.53)	3.06±0.51	0.905
14 days	1.59 (1.19-2.14)	3.62 (2.69-4.86)	4.56 (3.40-6.13)	3.60±0.36	0.959

R = Correlation coefficient of regression line

SD= Standard deviation of the mortality regression line

Toxicity of some plant oils against *T. castaneum* adults at 30 ±1° C and 65±5% R.H.:

The lethal concentration of Anise, Orange and Rosemary plant oils to adult stage of *T. castaneum* are shown in **Table (3).** The results showed that after 7 days post treatments for adult stage the LC_{50} values were 3.12, 1.88 and 2.53% (v/w), respectively. The corresponding values at 14 days were significantly lower and decreased to 2.89, 1.52 and 1.93% (v/w) for Anise, Orange and Rosemary essential oil treatments, respectively.

The LC_{90} values of adult stage were 5.36, 7.11 and 5.96 % (v/w) at 7 days and declined to 4.81, 5.37 and 3.54% (v/w) at 14 days post treatment by Anise, Orange and Rosemary essential oils, respectively.

The LC_{95} values of adult stage were 6.24, 10.37 and 7.60 % (v/w) at 7 days and declined to 5.56, 7.68 and 4.21% (v/w) at 14 days post treatment by the three preceding essential oils, respectively.

The obtained results showed increase of mortality for different plant oils with increasing the time of exposure and increasing concentration. This result is in harmony with the results of other investigators (**Sabbour and**

Abd-El-Aziz (2010), Saeidi and Moharramipour (2013), Zandi-Sohani *et al.* (2013) and **Hossain *et al.* (2014).**

Plant oils showed relative grain protecting activity and could be included in integrated pest management (IPM) programs and their effect in reduction the progeny was the most striking. This result is in harmony with the results of **Kim *et al.* (2003), Ogendo *et al.* (2008), Nenaah and Ibrahim (2011), Nasr and Ahmed (2014), Popović *et al.* (2014), Bilal *et al.* (2015), Abdelgaleil *et al.* (2016), Darwish (2016), Ibrahim (2016)** and **Heidari *et al.* (2017).**

Table (3): Lethal concentrations of some plant oils against *T. castaneum* adults at 30±1°C; 65±5% R.H. and various exposure periods:

Exposure period (Days).	Lethal concentrations (%v/w) and their 95% confidence limits			Slope ± SD	R
	LC_{50}(ml/g)	LC_{90}(ml/g)	LC_{95}(ml/g)		
Anise (*Pimpinella anisum*) oil					
7 days	3.12 (2.62-3.72)	5.36 (4.49-6.38)	6.24 (5.24-7.44)	5.46±0.17	0.887
10 days	3.03 (2.21-4.16)	5.49 (4.00-7.54)	6.50 (4.73-8.92)	4.97±0.36	0.885
14 days	2.89 (2.49-3.37)	4.81 (4.13-5.61)	5.56 (4.77-6.48)	5.80±0.14	0.838
Orange (*Citrus sinensis*) oil					
7 days	1.88 (1.39-2.53)	7.11 (5.27-9.58)	10.37 (7.69-13.98)	2.22±0.38	0.611
10 days	1.78 (0.93-3.38)	7.28 (3.82-13.85)	10.85 (5.70-20.65)	2.09±0.86	0.770
14 days	1.52 (0.93-2.47)	5.37 (3.29-8.74)	7.68 (4.71-12.51)	2.33±0.65	0.852
Rosemary (*Rosmarinus officinalis*) oil					
7 days	2.53 (1.96-3.26)	5.96 (4.63-7.68)	7.60 (5.90-9.79)	3.45±0.29	0.966
10 days	2.28 (1.74-3.00)	5.72 (4.35-7.52)	7.42 (5.64-9.76)	3.22±0.31	0.971
14 days	1.93 (1.57-2.37)	3.54 (2.88-4.35)	4.21 (3.42-5.17)	4.86±0.20	0.974

R = Correlation coefficient of regression line

SD= Standard deviation of the mortality regression line

Toxicity index of various plant oils against adults of *T. castaneum* at 30 ±1° C and 65±5% R.H.:

The toxicity effect of various plant oils against adults of *T. castaneum* at the LC_{50} after 7 days post-treatment at 30±1° C could be arranged in descending order as follows: Orange oil, Chamomile oil, Rosemary oil, Basil oil, Mustard oil and Anise oil which was the least effective **(Table, 4).**

Table (4): Toxicity index of various plant oils for the adult of *T. castaneum* after 7 days post treatment at 30 ±1° C and 65±5% R.H.:

Essential oils	LC_{50} (ml/g) after 7 days	Toxicity index at LC_{50}(ml/g)	Slope ± SD	R
Orange oil	1.88	100	2.22±0.38	0.611
Chamomile oil	2.05	91.71	3.48±0.32	0.872
Rosemary oil	2.53	74.31	3.45±0.29	0.966
Basil oil	2.98	63.09	4.97±0.66	0.790
Mustard oil	3.05	61.64	1.57±0.59	0.803
Anise oil	3.12	60.26	5.46±0.17	0.887

Differential hemocyte counts:

The control group **(Fig. 1)** showed different shapes of blood cells in larvae of *T. castaneum* that appeared in form of prohaemocytes, granular cells, spherule cells and cystocytes.

The results of the effect of LC_{50} of Orange oil on *T. castaneum* larval haemocyte cells are presented in **Fig. (2).** The results showed mildeffect that appeared on cells, membrane and empty cells, also, appeared.

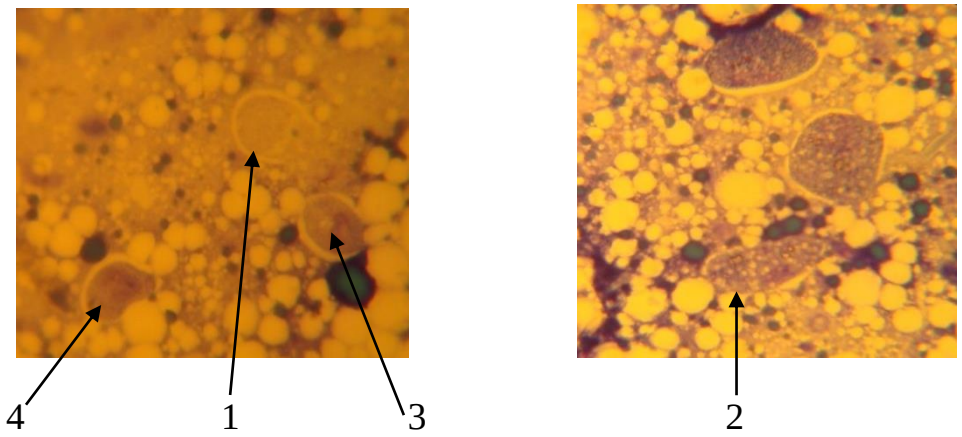

Figure (1):1- prohaemocytes, **2-** granular cells, **3-** spherule cells and **4-** cystocytes.

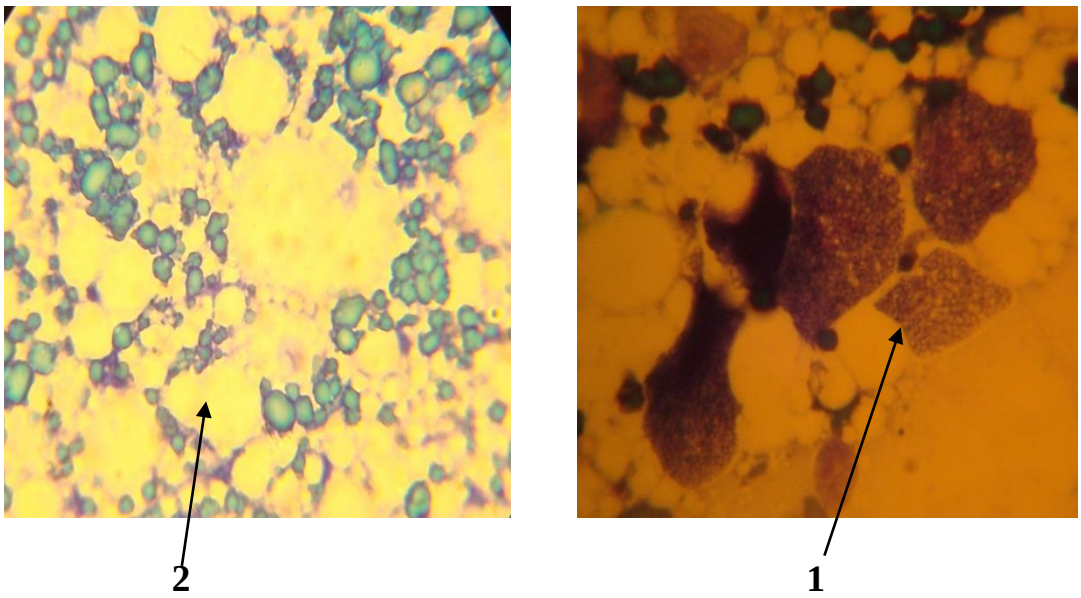

Figure (2): 1- mildeffect that appeared on cells membrane and **2-** empty cells which appeared.

Physiological studies:

Biochemical studies:

Effect of different treatments on the total proteins, lipids and carbohydrates of the treated *T. castaneum* adults:

Data on the total proteins, carbohydrates and lipids shown in **Table (5)** revealed, significant decrease in total proteins content after treatment by Orange oil which recorded 13.53 mg/g.b.w., compared to the control treatment (19.70 mg/g.b.w.) The rate of decrease was -31.32. Total carbohydrates content was significantly reduced by Orange oil treatment which recorded 9.53 mg/g.b.w., compared to the control treatment (12.43 mg/g.b.w.) this decrease was -23.33. On the other hand, the obtained results in the same table indicated that total lipids decreased significantly by orange oil treatment which gave 3.39 mg/g.b.w. and the control treatment was 5.23 mg/g.b.w; the decrease percentage was -35.18.

Table (5): Total proteins, lipids and carbohydrates in adult of *T. castaneum* after exposure to different treatments:

Treatment	Total proteins		Total carbohydrates		Total lipids	
	Activity (mean)	Percent of increase or decrease (%)	Activity (mean)	Percent of increase or decrease (%)	Activity (mean)	Percent of increase or decrease (%)
Orange oil	13.53^{b}	-31.32	9.53^{c}	-23.33	3.39^{b}	-35.18
Control	19.70^{a}	-	12.43^{a}	-	5.23^{a}	-
S.E.	0.50		0.33		0.13	

Effect of different treatments on the activies of enzymes of *T. castaneum* adults:

Treatments of *T. castaneum* adults affected the activity of different enzymes. **Table (6)** indicated, non-significant decrease in the activity of **(GOT)** after Orange oil treatments being 974.33 uM/g.b.wt., compared to 1010.67 uM/g.b.wt. in control treatment; the decrease of **(GOT)** by Orange oil was -3.6%. On the other hand, the obtained results of **(GPT)** indicated non-significant increase in the enzyme activity by Orange oil treatment as it recorded 382.33 uM/g.b.wt., compared 372 uM/g.b.wt. in control; the increase of **(GPT)** after Orange oil treatment measured 2.78% than control.

A Significant increase in **Alpha esterases** activity occurred due to Orange oil treatments, being 320 mg/g.b.w. compared to 285.67 mg/g.b.w. in case of control adults; the increasing percentage was 12.02% than control. Significant increase occurred in β-estrase enzyme activity under Orange oil treatment. Treated adults recorded (548.67 mg/g.b.w.) compared to 468 mg/g.b.w. in the control treatment; this increase was 17.23% **(Table, 6)**.

Non-significant decrease in the activity of **Acetylcholinesterase** enzyme occurred under Orange oil treatment. That recorded 187 µg/ Br/g/min. compared 193.33 µg/ Br/g/min in control. The decrease in this enzyme was -3.27%. As for Invertase enzyme, these was a significant decrease in its activity after Orange oil treatment, it measured 212 µg Glu./g/min compared to 348.67 µg Glu./g/min in control; this decrease was -39.2%.

Non-significant decrease in **Trehalase** activity under Orange oil treatment, that recorded 70.97 µg Glu./g/min compared to 72.7 µg Glu./g/min in control, showing a decrease in the enzyme of -2.38%.

By measuring the **Amylase** activity, it showed significant increase after Orange oil treatment, being 60.43 µgGlu./g/min, compared to control treatment (54.7 µgGlu./g/min); this increase was 10.48%.

As a conclusion, it could be stated that the essential plant oils caused inhibition of acetylcholine esterase enzyme activity as the major site of action of enzymes *in Tribolium castaneum* as suggested by **Rajendran and Sriranjini (2008)** and **Mikhaiel (2011).**

Table (6): Enzymes activity by Orange oil in *T. castaneum* adults after treatments:

Treatment	Activity (mean)	percent of increase or decrease(%)	Activity (mean)	percent of increase or decrease(%)
	(GOT) uM/g.b.wt.		**(GPT) uM/g.b.wt.**	
Orange oil	974.33[a]	-3.6	382.33[a]	2.78
Control	1010.67[a]	-	372[a]	-
S.E.	15.4		8.81	
α-esterase (mg/g.b.w.)			**β-esterase (mg/g.b.w.)**	
Orange oil	320[a]	12.02	548.67[a]	17.23
Control	285.67[b]	-	468[b]	-
S.E.	6.02		8.7	
Acetylcholinesterase (µg/ Br/g/min.)			**Invertase (µgGlu./g/min.)**	
Orange oil	187[b]	-3.27	212[c]	-39.2
Control	193.33[b]	-	348.67[a]	-
S.E.	4.47		7.57	
Trehalase (µgGlu./g/min)			**Amylase (µgGlu./g/min)**	
Orange oil	70.97[b]	-2.38	60.43[a]	10.48
Control	72.7[b]	-	54.7[b]	-
S.E.	1.02		1.05	

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التأثير الفسيولوجى والسام لبعض الزيوت النباتية على حشرة خنفساء الدقيق الكستنائية (الصدئية) (رتبة غمدية الأجنحة وعائلة Tenebrionidae)

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أجريتٌ هذه الدراسة بمعمل أبحاث أفات المواد المخزونة - بقسم وقاية النبات - كلية الزراعة جامعة بنها، وذلك بغرض دراسة فاعلية ستة من الزيوت النباتية وهى : زيت الخردل، الريحان، البابونج، الينسون،البرتقال وزيت الحصالبان وكذلك تأثيرها الفسيولوجى على الحشرة الكاملة لخنفساء الدقيق الكستنائية عند درجة حرارة30 ±1° مئوية و 65 ± 5% رطوبة نسبية.

ولقد تم ترتيب الزيوت ترتيباً تنازلياً على حسب تأثيرها السام بناءً على التركيز النصفى المميت بعد سبعة ايام من المعاملة بالتركيزات المختلفة لكل زيت،وكانت كالتالى: زيت البرتقال، زيت البابونج، زيت روزماري، زيت الريحان، زيت الخردل وزيت اليانسون على التوالى.

وقد أظهرت النتائج أنه عند المعاملة بالتركيز النصفى المميت (LC_{50}) لزيت البرتقال تأثير على جدار خلايا الدم فى اليرقات كما ظهرت الخلايا فارغة من محتواها الخلوى كما أنه أدى أيضاً إلى حدوث إنخفاضاً معنوياً فى محتوى الحشرات الكاملة من البروتينات الكلية والدهون والكربوهيدرات وكذلك أنزيم الأنفرتيز، بينما لم يكن هناك تأثيراً على مادة الأستيل كولين أستيريز و (GPT) ,(GOT) بينما أدى إلى زيادة معنوية فى أنزيمات ألفا وبيتا أستيريز وأميليز وتريهاليز.