J. Plant Prot. and Path., Mansoura Univ., Vol.9 (4): 253 – 259 , 2018

Potential Insecticidal Activity of some Medicinal Plants Essential Oils against Red Flour Beetle ,*Tribolium castaneum* (Herbst) (Coleoptera:Tenebrionidae).

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ABSTRACT

Tribolium castaneum is a major pest of wheat grain flour. Studies were carried out to investigate the insecticidal activity of essential oils (EOs) extracted from locally grown seven medicinal plant such as ginger *(Zingiber officinale)*, garlic (*Allium sativum*), thyme (*Thymus vulgaris*), clove (*Syzygium aromaticum*), dill (*Anethum graveolens*), eucalyptus (*Eucalyptus globulus*) , and lemon (*Citrus limon*) against *Tribolium castaneum*. In addition, Gene expression analysis for three insecticide resistance genes (Cytochrome P450 similar gene, CYP4Q4 and CYP4Q7) that mediated the detoxification mechanism, The EOs showed effective mortality of adult and larval stages of tested insect at concentration levels (6.25, 12.5 and 25) and exposure periods under laboratory conditions as compared with untreated experiments. Overall results, among essential oils *A. graveolens* and S. *aromaticum* showed highest mortality rate, followed with *T. vulgaris* and *A. sativum* while the minimum mortality rate was in *E .globules* against *T. castaneum* adult and larval stage, highest LC_{50} values was recorded in *Anethum graveolens* (LC_{50} at 1.5 hrs),it is note worthy that larvae more sensitive to EOs than adult in addition, Eos showed maximum expression level in *T. castaneum* larval stage was in Cytochrome P450 similar gene 3.38 Fold changes in mRNA, followed with CYP4Q4 and CYP4Q7 were 2.21 fold and 1.41 fold respectively as compared with reference gene (house keeping gene, β-Actin). Hence, these EOs of investigated plants may be recommended as botanical insecticide to control of stored grain insect, *T. castaneum*.

Keywords: medicinal plant, Essential oils, *T. castaneum*, mortality, Real-time-PCR, resistance genes and gene expression

INTRODUCTION

The insects attack and deteriorate the quality and the quantity of stored commodity (Ukeh, *et al* 2012 and Nadeem, *et al* 2012) resulting in significant decrease in volume, nutritional value, substantial weight loss and reasonable germination damage (Nadeem, *et al* 2012 and Phillips and Throne 2010). As indicated by the FAO about 10 to 25% of the world's harvested food is devastated every year by insects and rodent pests (Ukeh, *et al* 2012) Red flour insect, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) is secondary pest and a standout amongst dangerous stored products pests, feeding on different grain products (Mishra, *et al* 2012 and Islam and Talukdar 2005). The application of various synthetic insecticides and fumigants to stored grain products over the years led to number of problems, including the development of insecticide resistance in stored grain insect pest (Lorini and Galley, 1999 and Sousa *et al*., 2009).

Therefore, an urgent need to minimize use of synthetic pesticides and to avoid pollution of the environment and develop a natural antifeedant, deterrent, and repellent substances as environment friendly alternatives to the highly toxic chemicals for pest control during recent times . Botanicals are a promising source rich with a variety of secondary compounds including alkaloids, terpenoids, phenolics, and flavonoids that has potential as pest control agents affect insects in several ways.

Now many natural products are known to have a range of useful biological properties against insect pests (Renuga and Sahayaraj, 2009). Botanical insecticides are locally available, biodegradable, and inexpensive ways for grain pest control (Aslan, *et al*, 2005) and they are easily produced by farmers and small-scale industries (Cetin and Yanikoglu, 2006). Moreover, Plant drived materials have broad spectrum activity, do not cause resistance in insects, safe to natural enemies and without a negative impact on the environment (Negahban, *et al*, 2007).

Botanical materials as essential oils of plant origin are rich in mixtures of fungus and partial germination of grains (Mishra, *et al* 2012), Natural plant extracts as essential oils can be used as an alternative to chemical pesticides that have undesirable side effects against various stored grain insects especially *Tribolium castaneum* (Ayvaz, *et al*, 2009).

MATERIALS AND METHODS

Rearing of test insects

Rearing of heterogeneous adults of *T .castaneum* collected from different infested stored grains products like flour wheat and barley in grain stores located in the vicinity of Mansoura district, it is identity was confirmed from Department of Zoology, Entomology, faculty of Science, Mansura University, Egypt.

The culture of the insects *Tribolium castaneum* was established and cultured on whole wheat flour media with 5% Brower's yeast in sterilized glass jars and were kept at 28±2 ºC and 70% R.H in the laboratory incubator to get the homogeneous population in limited time without exposure to insecticides.

Isolation of essential oils

In this study, seven herbal and medicinal plant species; ginger, garlic, thyme, clove, dill, eucalyptus and lemon were selected according to their ethno medicinal importance and literature survey and were collected from local markets in Alexandria Governorate in January 2015 and identified and authenticated by department of Botany, Faculty of Science - Mansoura University (Egypt). Essential oils were extracted by water distillation using a Clevenger type apparatus for 8 hrs using Acetone as solvent

Mortality rate

In order to find out the efficiency of essential oil from seven essential oils against *Tribolium castaneum*, Contact effect were evaluated on filter paper discs by treating a whatman No.1 filter paper with different concentrations (6, 25, 12.5 and 25%) of acetone based

oils paper and were allowed to dry for a reasonable time period. as described by Rehman and Schmidt (Rehman and Schmidt, 1999 and Keita, *et al* 2001). The filter papers were placed in 9 cm glass petri dishes. A volume of 5ml solution was applied to each filter paper disc in order to obtain the equal amount of applied essential oil. The 100% acetone was allowed to evaporate for 20 minutes prior to the introduction of 20 adults (15 days old) or larvae (4-6 days old). Small amount of wheat flour was provided to decrease chances of mortality due to starvation. Dishes were backed and held with rubber bands. The control petri dishes were maintained by treating the filter paper with 5mL 100% acetone only. Each experiment was replicated three times along with the control and Completely Randomized Design (Factorial) was followed. Dead insects were counted 3, 6, 12, 24, 48 and 72 hours post application. Insects which did not respond to the gentle touch of a small probe were considered dead (Su, 1976). and corrected using Abbott's formula (Abbott, 1952) and used to draw the regression line LC values according to Finney (Finney, 1952).

$$\% \text{ corrected mortality} = \frac{\% \text{ observed mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

Insect response against the plant extracts

To evaluate the molecular mechanism of botanical pesticides and their mode of action by studing the transcriptional expression levels for three cytochrome P450 detoxifying and defense genes (CYP sim, CYP 4Q4 and CYP 4Q7) and the differential display patterns in the most sensitive *T. castenum* stage (larvae) to the applied essential oils using the quantitative real- time polymerase chain reaction (qRT-PCR) analysis.

Total RNA Isolation from *Tribolium castaneum*

Total RNA was isolated from 0.1 g of *Tribolium castanum* adult and larval stages (wild and 7 plant oils treated) samples by using Tripure total RNA extraction reagent, according to manufacturer's instructions.

cDNA synthesis and Real Time PCR

Reverse transcription reactions were performed using oligo dT primer (5`- TTTTTTTTTTTTTTT-3`). Each 20 µl reaction mixture contained 2.5 µl 5X buffer, 2.5 µl MgCl2, 2.5 µl 2.5 mM dNTPs, 4 µl from oligo (dT) 10 as primer (10 pml/µl), 2.5 µl RNA and 0.2 µl (5 Unit/µl) reverse transcriptase (Promega, Germany). RT-PCR amplification was performed in a thermal cycler (Promega, Germany), programmed at 42°C for 1 hr and 72°C for 10 min, and the cDNA was then stored at -20°C for further studies (Schmittgen, *et al*, 2000). Gene manipulation according to the type of the used plant extract

Differential display –PCR was performed on the treated larvae with the different plant extracts to study the up- and down –regulated genes in response to the cytotoxicity effect of each extract.

Differential display -PCR

Amplification of cDNA fragments was performed as previously described. The reaction mixture for differential display PCR was carried out in a total volume 25 µl containing 1 µl 10 pmol of Arbitrary primers (A2 & A4) and one defense specific primer (endoglucanase 3) based on the plant genome that are mentioned in (Table 1), 2.5 µl 10 x buffer with MgCl2, 2 µl 2.5 mM dNTPs and 1 U Taq polymerase (Promega, Germany). PCR amplification was performed in a thermal cycler (Eppendorf, Germany) programmed first cycle at 94°C for 5 min, then 40 cycles as follows: 45 s at 94°C for denaturation, 1 min at 30°C for annealing and 2 min at 72°C for elongation. Finally incubate the reaction for 10 min at 72°C as final extension. DNA fragments were electrophoresed through an agarose gel. PCR products were electrophoresed through 1.5% agarose, stained with ethidium bromide; finally obtained DNA was visualized and photographed using a gel documentation system. Compare the differential display banding patterns with those of control larvae.

Quantitative Real-Time –PCR (QRT-PCR)

qRT-PCR analysis was performed for three insecticidal resistant genes(Cytochrome P450 similar gene, CYP4Q4 and CYP4Q7) in *Tribolium castaneum* larvae treated with different essential

Table 1. Nucleotide sequences of primers used in differential display and quantitative real-time PCR analysis in *T. castaneum* larval stage treated with essential oils

Technique	Target Gene	Sequences (5'–3')	Annealing °C
qRT-PCR	18sRNA		
	18sRNA		
	CYP sim-F		
	CYP sim-R	AAACGGCTACCACATCCAA	
	CYP 4Q4-F	TGTTCAAAGTAAACGTGCCG	
	CYP 4Q4-R	CATCCGCAAACACAACAAAC	
	CYP 4Q7-F	AGGACTGCGAGCTGGTTTTA	
	CYP 4Q7-R	CCATTGCTGTCTCTGCGATA	
Differential display	RAPD (A2)	GAA ACGGGTGGTGATCGC	30°C
	RAPD (A4)	GGA CTGGAGTGTGATCGC	
	Endoglucanase3-F	CGGTGGTACTCCTCCTGGACCC	

Statistical analysis

The results are presented as means ± standard deviation (±SD). Data were analyzed using the statistical package for social sciences (SPSS) 16. Data were evaluated by analysis of variance (ANOVA). Statistical differences were considered significant at the $p<0.05$ level.

RESULTS

Mortality bioassay against adult

In general mortality in adult stage increased with increase of concentration and time exposure the concentration tended to increase the mortality effect in the adult stage as the concentration of used essential oils

increased and time exposure. Obtained results showed that, the highest mortality effect (100.0±0.0) was recorded in *A. graveolens* and *S. aromaticum,* followed with (95.0±8.66) in *T. vulgaris* , while the minimum mortality effect on adult insect (56.97±7.6) was in *C. limon.*

Mortality bioassay against larvae

Results reported in Table number 3 showed the mortality effect of various concentrations (0, 6.25, 12.5 and 25%) of seven natural oils on *T. castenum* larvae. As compared with malathion and untreated experiments.

Overall all the concentration tended to increase the mortality effect in the larval stage as the concentration of used essential oils increased. Obtained results showed that, the highest mortality effects were recorded as (100.0±00) mortality after 72 hrs in *C. limon* and Malathion, Worthy of the remembrance that *A. graveolens* and *S. aromaticum* arrived to (100.0±00) mortality after12 hrs.

LC_{50} percent of essential oils against *T. castenum*

By calculation the LC50 values for different essential oils against *Tribolium castenum* stages (adult and larvae) expressed by mortality bioassay are recorded tables (2, 3). According to the LC%50 in Table (4) showed that the maximium toxicity effect was recorded in *Anethum graveolens* (LC50 values at 1.5 hrs), followed with *Allium sativum*, *Thymus vulgaris*, *Syzygium aromaticum* and *Zingiber officinale* the LC50 were at 2 hrs.

Table 2. Effect of different concentrations (0, 6.25, 12.5 and 25%) of seven essential oils on mortality of *Tribolium castaneum* adult at 3, 6, 12, 24, 48 and 72 hours after treatment.

Essential oil	Concentration (%)	Mean mortality± S.E Time (hrs) 3hr	6hr	12hr	24hr	48hr	72hr
C. limon	Cont.	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	6.25%	0.00±0.00	0.00±0.00	3.33±2.887	11.67±7.64	15.0±5.0	21.67±7.6
	12.5%-	0.00±0.00	1.67±2.89	5.0±5.0	13.33±2.134	18.33±5.77	31.67±2.9
	25%	8.33±2.887	21.67±2.9	23.33±2.887	33.33±2.89	41.667±2.89	56.67±7.6
E .globulus	Cont.	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.67±2.89
	6.25%	0.33±0.577	15.0±10.0	23.33±12.58	30.0±10.0	35.0±15.0	50.0±10.0
	12.5%-	0.00±0.00	26.67±2.9	31.667±2.89	41.67±2.89	51.667±2.89	65.0±5.0
	25%	66.67±18.9	75.0±22.9	78.33±20.82	81.667±15.	83.33±12.58	90.0±10.0
A. sativum	Cont.	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	6.25%	0.0±0.0	1.67±2.89	5.0±0.0	13.3±2.887	16.667±2.89	26.67±2.9
	12.5%-	0.0±0.0	3.3±2.887	13.33±7.638	23.33±2.887	30.0±0.0	50.0±5.0
	25%	56.667±16	66.67±16.	75.0±17.32	78.3±15.28	80.0±18.028	86.7±12.5
A. graveolens	Cont.	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	6.25%	0.00±0.00	0.00±0.00	0.00±0.00	6.67±2.887	11.667±2.89	21.67±2.9
	12.5%	11.667±7.6	23.33±10.	35.0±13.229	53.33±10.4	60.0±13.229	73.33±10.
	25%	90.0±5.0	90.0±5.0	93.33±2.887	95.0±5.0	98.33±2.887	100.0±0.0
Z. officinale	Cont.	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	6.25%	0.00±0.00	10.0±5.0	11.67±2.887	20.0±5.00	23.33±2.887	31.67±2.89
	12.5%-	5.0±5.00	13.3±2.89	18.33±2.887	23.33±2.89	31.667±2.9	45.00±5.0
	25%	51.67±2.9	63.3±5.77	68.33±2.887	68.33±2.89	73.33±2.887	80.0±5.00
T. vulgaris	Cont.	0.00±0.00	0.00±0.00	0.00±2.886	0.00±0.00	0.00±0.00	0.00±0.0
	6.25%	.00±.00	.00±.00	3.33±2.8868	6.667±2.89	6.667±2.887	15.0±5.00
	12.5%-	1.667±2.9	6.67±2.89	16.6667±2.89	21.67±2.89	30.05.0±2.9	38.33±5.8
	25%	28.33±23.	45.0±13.2	56.667±17.56	66.67±30.5	73.33±25.17	95.0±8.66
S. aromaticum	Cont.	.00±.00	.00±.00	.00±.00	.00±.00	.00±.00	.00±.00
	6.25%	.00±.00	.00± .00	8.33±2.8868	13.3±2.887	20.00±5.0	26.67±2.9
	12.5%-	3.33±2.887	8.33±2.89	18.33±2.887	33.33±2.89	43.33±2.887	50.0±5.0
	25%	80.00±.00	83.3±2.89	90.0±5.0	96.67±2.89	96.667±2.89	100.0 .00
Malathion	Cont.	.00±.00	.00±.00	.00±.00	.00±.00	.00±.00	.00±.00
	6.25%	10.0±5.00	15.0±5.00	40.0±5.0	65.0±5.00	86.667±12.58	98.3±2.89
	12.5%-	8.33±2.887	10.0±.00	16.667±2.887	65.0±13.23	91.667±14.43	98.3±2.89
	25%	20.0±8.66	28.3±15.3	83.3±2.887	98.33±2.89	100.0 .00	100.0 .00

[a]**Values are means ± SD, and least-significant difference test (LSD) at significance level $P \leq 0.05$**

Differential display

According to the differential display results Figure1 (A, B & C) the expression patters of amplified cDNA fragments using three different raped and specific resistant primers in *T. castenum* larvae after two days of treatement with different essential oils showed that there are up and down expressed bands depending on the insecticidal effect of applied essential oils. The expression pattern for A2 primer (fig.1A) in the *T. castaneum* treated lravae showed up regulation bands appeared; *Allium sativum* bands at 550 bp, *Citrus limon* bands were at 600, 700 and 800bp, *Thymus vulgaris* at 200 bp, *Anethum graveolens* at 800bp while down regulation bands; *Allium sativum* at 320, 350 and 600bp, *Eucalyptus globulus* at 550bp and *Syzygium aromaticum* at 300bp.

And the expression pattern for A4 primer (fig.1B) in the *T. castaneum* treated larvae with showed up regulation bands appeared, *Citrus limon* bands were at 180 and 500bp, *Anethum graveolens* at 180bp while down regulation bands; *Citrus limon* band at 650bp, *Eucalyptus globulus* and *Syzygium aromaticum* at 700bp

Table 3. Effect of different plant extracts concentrations (0, 6.25, 12.5 and 25%) on mortality of *Tribolium castaneum* larvae at3, 6, 12, 24, 48 and 72 hours after treatment

Essential oil	Concentration (%)	Mean mortality± S.E Time (hrs) 3hr	6hr	12hr	24hr	48hr	72hr
C. limon	Cont.	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	6.25%	7.0±7.55	18.3±17.56	26.67±17.56	51.67±25.17	70.0±26.46	95.0±5.0
	12.5%-	45.0±8.67	85.0±5.0	88.3±2.89	90.0±0.0	96.67±2.89	100.0±0.0
	25%	63.3±16.1	88.3±2.89	91.67±2.9	96.67±2.89	98.3±2.8	100.0±0.0
E .globulus	Cont.	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.0±0.0
	6.25%	8.33±٧.٦٣ 8	25.0±10.0	46.67±30.14	75.0±25.0	81.67±23.63	86.67±18.93
	12.5%-	21.67±24.66	51.67±7.64	80.0±5.0	86.67±2.89	98.3±2.8	100.0±0.0
	25%	56.67±17.55	73.3±10.41	86.67±5.77	88.3±2.89	100.0±0.0	100.0±0.0
A. sativum	Cont.	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	6.25%	6.67±7.64	10.0±5.0	18.3±12.58	33.3±17.56	73.3±25.17	83.3±15.28
	12.5%-	36.67±7.64	81.67±7.64	86.67±7.64	93.3±11.55	100.0±0.0	100.0±0.0
	25%	76.67±2.89	91.67±2.89	98.3±2.89	100.0±0.0	100.0±0.0	100.0±0.0
A.graveolens	Cont.	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	6.25%	16.67±17.56	45.0±27.84	68.3±22.55	81.67±20.21	93.3±5.8	100.0±0.0
	12.5%-	50.0±5.0	85.0±5.0	91.67±2.89	100.0±0.0	100.0±0.0	100.0±0.0
	25%	65.0±5.0	95.0±5.0	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Z.officinale	Cont.	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	6.25%	13.3±12.58	25.0±13.23	41.67±17.56	55.0±20.0	68.3±23.63	83.3±15.27
	12.5%-	50.0±5.0	60.0±7.64	71.67±2.89	85.0±5.0	98.3±2.89	100.0±0.0
	25%	63.3±7.64	76.67±7.64	96.67±2.89	100.0±0.0	100.0±0.0	100.0±0.0
T. vulgaris	Cont.	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
	6.25%	25.0±5.0	50.0±25.0	65.0±37.75	75.0±25.0	83.3±15.28	93.3±7.64
	12.5%-	40.0±5.0	76.67±2.89	91.67±2.89	100.0±0.0	100.0±0.0	100.0±0.0
	25%	73.3±2.89	90.0±5.0	98.3±2.89	100.0±0.0	100.0±0.0	100.0±0.0
S.aromaticum	Cont.	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	6.25%	20.0±22.9	38.3±40.0	50.0±45.83	56.67±43.1	71.67±27.54	78.3±25.66
	12.5%-	55.0±5.0	75.0±5.0	85.0±5.0	95.0±8.66	98.0±2.89	100.0±0.0
	25%	83.3±2.889	98.3±2.889	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Malathion	Cont.	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
	6.25%	1.67±2.89	6.67± 7.64	20.0±5.0	35.0±5.0	56.67±10.41	78.3±10.41
	12.5%-	5.0±5.0	31.67±7.64	60.0±10.0	71.67±10.41	90.0±10.0	98.3±2.89
	25%	23.3±7.64	66.67±7.64	93.3±5.0	83.3±7.64	98.3±2.89	100.0± 0.0

[a]**Values are means ± SD, and least-significant difference test (LSD) at significance level P ≤ 0.05**

Table 4. LC_{50} values for seven essential oils exhibiting >50% mortality against *T. castenum*

Essential oils	*Concentration (%)*	LC_{50} Time (hrs) Adult	Larvae
C. limon	control	NE	NE
	6.25%	NE	30
	12.50%	42	3
	25.00%	2	2
E .globulus	control	NE	NE
	6.25%	NE	15
	12.50%	24	6
	25.00%	3	3
A. sativum	control	NE	NE
	6.25%	NE	33
	12.50%	50	5
	25.00%	3	2
A. graveolens	control	NE	NE
	6.25%	NE	10
	12.50%	30	3
	25.00%	2	1.5
Z. officinale	control	NE	NE
	6.25%	NE	25
	12.50%	24	4
	25.00%	2.5	2
T. vulgaris	control	NE	NE
	6.25%	NE	12
	12.50%	NE	4
	25.00%	24	2
S. aromaticum	control	NE	NE
	6.25%	NE	37
	12.50%	45	3
	25.00%	2	2
Malathion	control	NE	NE
	6.25%	23	38
	12.50%	21	10
	25.00%	12	4.5

NE, not effective; *LC_{50} values (concentration causing 50% mortality); 4-5 concentrations were used to calculate the LC_{50} values; CI= confidence interval and n= 3 replicates of 30 insects

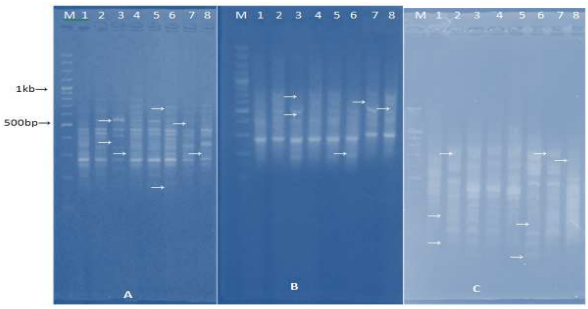

Figure 1. differential display band patterns of mRNA in *T. castenum* larvae after two days of treatment with different essential oils was amplified using A2 (a), A4 (b) and Endoglucanase F (c) oligonucleotide primers. Amplified cDNA fragments were electrophoresed on a 1.5 % agarose gel and visualized by gel decomentation system. While 10kb DNA ladder; lane1: Control. *T. castenum* larvae treated with *Zingiber officinale* (lanes2):, with *Allium sativum* (lanes3); with *Citrus limon* (lanes4), with *Thymus vulgaris* (lane5):, with *Anethum graveolens* (lane 6); with *Eucalyptus globulus* (lane 7): with *Syzygium aromaticum* (lane8)

Finally The epression pattern for endoglucanase primer (fig.1C) in the *T. castaneum* lrava treated with showed up regulation bands appeared; *Zingiber officinale* at 250, 300, 750bp, *Allium sativum*; with *Citrus limon*, with *Thymus vulgaris* at 450bp, *Anethum*

graveolens at 150, 250bp, *Eucalyptus globulus* at 750bp and *Syzygium aromaticum* at 700bp, while down regulation bands*; Zingiber officinale* at 550bp, *Anethum graveolens* at 200, 300bp and, *Eucalyptus globulus* at 250, 350bp

qRT_PCR analysis result

qRT-PCR analysis for three insecticidal resistant genes (Cytochrome P450 similar gene, CYP4Q4 and CYP4Q7) in *Tribolium castaneum* larva treated with seven essential oils; *Zingiber officinale*, *Allium sativum*, *Citrus limon*, *Thymus vulgaris, Anethum graveolens, Eucalyptus globulus* and *Syzygium aromaticum* as natural insecticidal agent and the analysis was performed at three different times after treatment (3, 6 and 12 hrs)

According to the qRT-PCR results Figure2, maximum relative expression level of Cytochrome P450 similar gene was 3.3820 ±0.2778 Fold changes in mRNA, followed with CYP4Q4 and CYP4Q7 were 2.2143 ±. 03386 and 1.4157± .03386 fold respectively as compared with reference gene (house keeping gene, β-Actin).

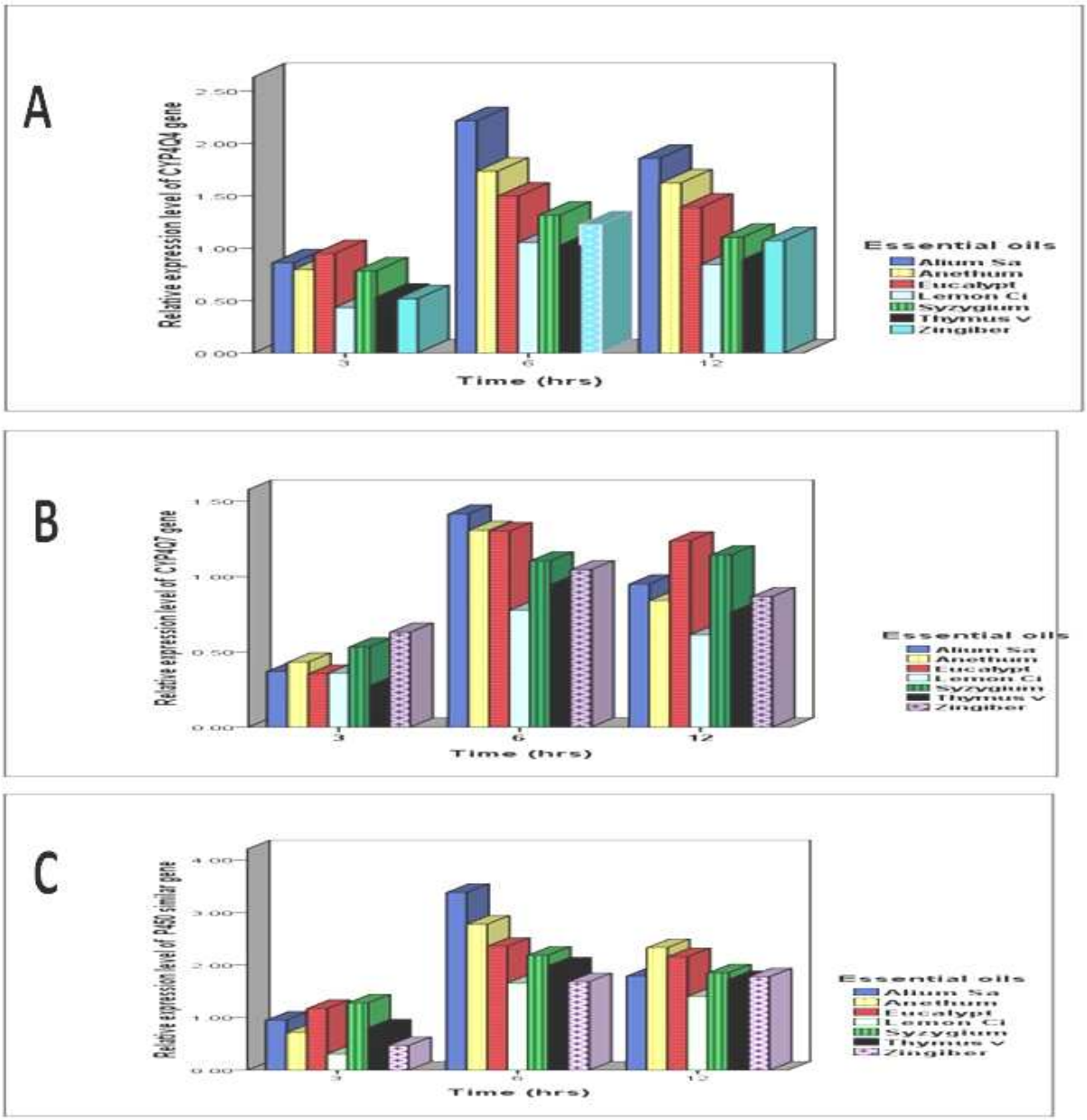

Figure 2. Relative expression of CYP4Q4 gene (a), CYP4Q7 gene (b) and P 450 similar gene (c) in *T. castenum* larva at three times (3, 6 and 12hrs) after treatment with different essential oils; *Zingiber officinalem, Alium sativum, Citrus limon, Thymus vulgaries, Anethum graveolens*, *Eucalyptus globulus* and *Syzygium aromatic*

DISCUSSION

experiments proved a definite impact of plant oils towards inducing mortality and repellency against the adult and larval stages of stored grain insect pest, *Tribolium castaneum*; the highest mortality rate in adult stage (100.0±0.0) was observed in *A. graveolens* and *S. aromaticum,* followed with (95.0±8.66) in *T. vulgaris*, while the minimum mortality effect on adult insect (56.97±7.6) was in *C. limon* .Mean while the

highest mortality rate in larval stage (100.0±00) was observed after 72 hrs in all used oils, Worthy of the remembrance that *A. graveolens* and *S. aromaticum* arrived to (100.0±00) mortality after12 hrs. Another experiments (Mehmet Karakas 2016) was focused on the toxicity effects of dill and purple basil leaf extracts on wheat granary weevil *S. granarius* and proved that Average mortality percentage of wheat granary weevil at 24, 48 and 72 hours after treatment (HAT) indicated that dill leaf extract (22.08%) possessed more toxic effect comparing purple basil leaf extract (10.65%). Mortality percentages were directly proportional to the time after treatment. While the repellency rates of insects were influenced by the concentrations of extracts shown in. Repellency rate did not increase proportionally with the doses. The highest mean repellency effect was found with 15% purple basil extract (52.24%).

According to qRT-PCR analysis results showed increase in expression level of three insecticide resistance genes (CYP4Q4, CYP4Q7 and Cytochrome P450 similar genes) in the *T. castaneum* larvae subjected to the studied at different periods. Eos showed maximum expression level in *T. castaneum* larval stage was in Cytochrome P450 similar gene 3.38 Fold changes in mRNA, followed with CYP4Q4 and CYP4Q7 were 2.21 fold and 1.41 fold respectively as compared with reference gene (house keeping gene, β-Actin).

RT-PCR is a very sensitive, accurate, and reliably quantitative method for gene expression analysis, detection of plant viruses, and elucidation of viral propagation profiles inside infected tissues (Peters, *et al*, 2004, Varga and James, 2005and Hafez, *et al*, 2013). The _-actin gene has long been used as a universal internal control for gene expression studies (Nicot, *et al*, 2005), Thus, it is essential that prior to the experiment, all reference genes are validated to confirm the stability of their expression under certain experimental conditions, and to prevent inaccurate data interpretation and subsequent incorrect conclusions (Bustin, 2002 and Gutierrez, *et al*, 2008)

CONCLUSION

In general the present investigation of studied Eos; *Zingiber officinale*, *Allium sativum*, *Citrus limon*, *Thymus vulgaris*, *Anethum graveolens*, *Eucalyptus globulus* and *Syzygium aromaticum* found high mortality and repellent rates against *T. castaneum* adult and larval stages. Based on insecticidal efficacy of EOs may be recommended as nonphytotoxic herbal insecticides against *Tribolium castaneum.*

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تقييم كفاءة مستخلصات بعض النباتات الطبية المحلية كمبيدات حشرية في مكافحة خنفساء الدقيق الصدئية

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تعتبر خنفساء الدقيق الصدئية (*Tribolium castaneum*) ويرقاتها من بين أخطر آفات الحبوب المخزونة التى تصيب القمح كأحد أهم السلع الإستراتيجية فى مصر وكذلك منتجاته بالأضافة إلى انواع اخرى من الحبوب المخزونة كالفول السوداني والبن والتوابل والفواكه المجففة مخلفة خسائر اقتصادية كبيرة لايمكن تعويضها .حيث تتغذى عليها و بالاخص على أجنة تلك الحبوب، وتكسبها رائحة مميزة غير مرغوب فيها، فضلا عن تلويثها لها بمخلفاتها وجلود الإنسلاخ والأفراد الميته منها. الهدف من الدراسة:- حيث هدفت تلك الدراسة إلى تحرى تأثير أستخدام تركيزات مختلفة من الزيوت العطرية (EOs) المستخلصة من سبعة نباتات طبية مزروعة محليًا و هى الزنجبيل (*Z. officinale*) والثوم (*A. sativum*) والزعتر (*T. vulgaris*) والقرنفل (*S. aromaticum*) والشبت (*A. graveolens*) والكافور (*E .globulus*) والليمون (*C. limon*) كمبيدات حشرية طبيعية من أصل نباتى لمكافحة خنفساء الدقيق الصدئية و يرقاتها طريقة البحث :- و بتربية الحشرة الكاملة على دقيق القمح بالمعمل حسب الطرق الموصى بها و تعريض الطور الكامل و كذلك اليرقات لتركيزات مختلفة من الزيوت العطرية (6.25, 12.5 and 25%) محل الدراسة ثم حساب نسبة القتل فى عدد الحشرات و اليرقات المعرضة عند أوقات مختلفة (3, 6, 12, 24, 48 and 72hrs) من بداية التعرض النتائج:- و قد أظهرت النتائج وجود فروق معنوية بين تركيزات الزيوت المستخدمة و كذلك أظهرت النتائج بشكل عام أن أعلى تأثير للقتل فى أطوار الحشرة و اليرقه كان من أستخدام المستخلص الزيتى للشبت و القرنفل و يليهم فى التاثير كانت زيوت الزعتر و الثوم و بينما أظهر زيت الكافور أقلهم فى نسبة القتل لطور الحشرة و كذلك طور اليرقة الا أنه لوحظ أن معدل حساسية اليرقات و تأثيرها و موت أفرادها كان أعلى و أسرع مقارنة بطور الحشرة الكاملة و بإجراء التحليل البيوجزيئى لقياس نسب التعبير الجينى لثلاثة جينات(P450، CYP4Q4 و CYP4Q7) من جينات مقامة اليرقات لتاثير سمية المبيدات بأستخدام تقنية الريال تايم (qRT-PCR) الكمية لقياس مستوى التعبير الجينى لتلك الجينات لتركيزات الزيوت. و قد أظهرت نتائج القياس ان أقصى مستوى للتعبير الجينى فى طور اليرقات لكونها أكثر حساسية من الحشرة ذاتها كان في جين (P450) بتضاعف مستوى التعبير الجينى إلى ٣.٣٨ ضعف ، تلى ذلك تضاعف مستوى التعبير لجينى (CYP4Q4 and CYP4Q7) إلى (1.41 and 2.21) على التوالى و ذلك مقارنة مع الجين المرجعي (house keeping gene, β-Actin). ومن ثم ، فقد أوصت الدراسة إن هذه الزيوت العطرية المختبرة يمكن أستخدامها بفعالية عالية كمبيد حشري نباتي لمكافحة حشرة الحبوب المخزنة (*Tribolium castaneum*) و يرقاتها.