

## MOLECULAR IDENTIFICATION OF THREE STORED PRODUCT INSECT SPECIES ON DRIED FRUITS AND THEIR CONTROL WITH THE USE OF SOME AQUEOUS PLANT EXTRACTS

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### ABSTRACT:

Mortality of three insects; red flour beetle (*Tribolium castaneum* Herbst), khapra beetle (*Trogoderma granarium* Everts) and sawtoothed grain beetle (*Oryzaephilus surinamensis* (L.)) were evaluated via some aqueous plant extracts. The identification of three examined insects was carried out via designing specific primers for each aforementioned species with the use of PCR technique. The toxicity of aqueous plant extracts; eucalyptus (*Eucalyptus camaldulensis* Dehnh), mint (*Mentha canadensis* L.) and myrtle (*Myrtus communis* L.) at three dosages (12500, 25000 and 50000) ppm for 1, 2, 3 and 7 days were tested for their insecticidal activity against examined adults on dried fruits. Percentage mortality of insect was increased with an increase of doses and exposure times, whereas, not influenced significantly by the fruit types. *T. castaneum* was the most susceptible species, followed by *T. granarium* and *O. surinamensis*. Likewise, LC<sub>50</sub> values were (3887.25, 10816.34 & 22763.48) on red flour, khapra & sawtoothed grain beetles respectively via using eucalyptus aqueous extract after 7 days on black raisin. While, higher doses required in order to kill fifty percentage of the population via myrtle aqueous extract and thus the LC<sub>50</sub> values increased to (14726.86, 18421.43 & 39016.78) on the three aforementioned species respectively.

**KEYWORDS:** Concentration, LC<sub>50</sub>, Dried Fruits, DNA Extraction, Mortality, Polymerase Chain Reaction.

### 1. INTRODUCTION

Dried fruits are among the healthiest dietary that primarily have been valued for long term stability and their sweetness that consist of refined sugar, rich source of minerals, fibers and proteins (Gyurova and Enikovam 2014). Dried fruits are also consider as an excellent resource of polyphenols and phenolic acids, that comprise the largest group of plant bioactive compounds in the diet and appear to be responsible for the health benefits (Carughi *et al.*, 2016). Dried figs include natural sugar substance, carbohydrates as a major content (73.50%), fat content around (0.56%), protein (4.67%), fibre (3.68%) and encompass essential minerals (e.g. copper, manganese, magnesium, potassium and calcium) (Gyurova and Enikovam 2014; Soni *et al.*, 2014). Whereas, raisins not only encompass important ingredients but also are consumed more than other types of dried fruits. In addition, cereal grains consider vital food source for human nutrition and have played an important role in building human civilization for thousands of years. Globally, wheat and rice crops play a significant role in economic stability throughout the world and approximately 50% of consumed calories by the whole populations count on the aforementioned crops (Poehlman and Sleper, 1995; Chowdhry *et al.*, 1998; Varshney *et al.*, 2006; Gnanamanickam 2009; Zuzana *et al.*, 2009). Thus, the demand for enhanced food production is as topical as ever due to human population growth (Zeigler and Mohanty, 2010; Ronald, 2011). High proportions of produce each year around 10-15% of the total grain production might be lost during storage processes due to insects and rodent pests in America (Sharma and Choudhary, 2008; Hill, 1990), and the annual losses can be even higher about 30% in Africa and

Asia (Hill, 1990). Insect infestation would result in the loss of nutritive value of dried fruits, reduced seed germination, and also causes changes in chemical components such as increase in moisture, free fatty acid levels and decrease in protein contents of grains (Rees, 2004 ; Das *et al.*, 2013).

The most widespread and destructive insects of stored products (i.e. cereals and dried fruits) are red flour beetle *Tribolium castaneum* Herbst, Khapra beetle *Trogoderma granarium* Everts and saw toothed grain beetle *oryzaephilus surinamensis* (L.) (Weston and Rattlingourd, 2000; Campbel *et al.*, 2004; Rees, 2004; Mishra *et al.*, 2012). Since it is difficult to identify indistinctive species on the basis of external morphological characteristics alone, especially both red flour beetle and the confused flour beetle are sympatric and morphologically similar stored-product pests, and the latter is named because of confusion over its identity as it is so similar to the former (Walter, 1990). Hence, molecular identification of species is an appropriate alternative to conventional taxonomical method (Nowaczyk *et al.*, 2009; Castalanelli *et al.*, 2011; Sorvari *et al.*, 2012; Ming *et al.*, 2014)

Furthermore, great deal of attention has been given for the adoption of improved post-harvest technology. Various methods and strategies were implemented in order to control stored product insects such the traditional chemical insecticides. Fumigants are mostly used against stored-grain insect pests because of their broad activity spectrum as well as their penetrating power on the treated products. Although using fumigants (e.g. methyl bromide and phosphine) in large scale for a long period of time might cause the appearance of resistant strains, environmental pollution, toxicity to non-target organisms, and pesticide residues on treated stored products making pesticide application a poor long term solution that raise global concern about food safety (Zettler & Cuperus 1990;

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Stephan, 2003; Benhalima *et al.*, 2004; Bughio and Wilkins, 2004).

Hence, plant extracts which known as biocide or green pesticides can be a substitute source of chemical pesticide due to their safe, eco- friendly, inexpensive, easy to use with more compatible properties (Risha *et al.*, 1990; Susha and Kamavar, 1993). Secondary compounds from plants include alkaloids, terpenoids, phenolic, flavonids, sesquiterpenes, limonoides and other chemicals might affect insects in various manner. They have potential to disrupt major metabolic pathways and cause fast death, act as attractants, deterrents and antifeedants (Alford *et al.*, 1987; Sarmamy *et al.*, 2011). Different types of plant extracts have been used as biocides either to eliminate or minimize the yield loss due to pests (Alford *et al.*, 1987; Tayoub *et al.*, 2012; Rahman *et al.*, 2016).

Due to the lack of sufficient studies on the use aqueous plant extracts in the fight against stored product insects especially on dried fruits this study has undertaken in order that aqueous botanical extracts can be used in broader ranges and the technique would found their way into more practical use as safe natural insecticides. Hence, the present study aimed to investigate the toxic impact of aqueous plant extracts from eucalyptus *Eucalyptus camaldulensis* Dehnh, mint *Mentha canadensis* L. and myrtle *Myrtus communis* L. on the mortality of *Tribolium confusum*, *Trogoderma granarium* and *oryzaephilus surinamensis* adult stage as well as designing specific primers for the aforementioned insects in order to be available for further studies.

## 2. MATERIALS AND METHODS

### 2.1 Insect cultures

The experiment was conducted in both biotechnology and high education laboratories of Agriculture College at Salahaddin university-Erbil. Mass cultures of red flour beetle *Tribolium castaneum* Herbst (Tenebrinidae: Coleoptera) were maintained under standard food medium of wheat flour supplemented with 5% dry yeast powder (Singh and Prakash 2015). Khapra beetle *Trogoderma granarium* Everts (Dermestidae: Coleoptera) was reared on a diet consists of whole wheat and broken wheat (Khair, 2015), whereas saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Silvanidae: Coleoptera) were maintained on rice culture (Yousif, 2012).

The diets with insects were placed in large plastic (600ml) capacity, covered with fine mesh cloth which simultaneously allow ventilation and prevent insects from escaping. All cultures were maintained in incubator at (30 ± 2°C) temperature and (60±2) relative humidity.

### 2.2 Experiment 1: Insect identification through molecular based method (PCR)

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Table (1): Primers used to confirm the identification of insect species

<i>Tribolium castaneum</i> Herbst							
Name	(Ps)bp	Length	Sequence F	length	Sequence R	TM(°C)	References
<b>Tcas-1</b>	120	21	5'-CTTCCAGGAT TTGGCATAATC-3'	21	5'-CAAAACCTAA GAGCCCAATTG-3'	57	This study
<b>AKK-1</b>	120	20	5'-GGGTGTGTAC CGCTCAGTTA-3'	20	5'-GGCGAATTGG GAATATTGAA-3'	57	Li et al, 2008
<b>TconfA</b>	150	20	5'GTGTATCTACA CACACACAC-3'	20	5'GAACCGCAA TATTGAGTTG-3'	55	Li et al, 2008
<b>TconCOI</b>	100	21	5'CTTCCTGGATTC GGAATAATC -3'	21	5'CGAACCCCTAG TAATCCAATTG-3'	57	Li et al, 2008
<i>Trogoderma granarium</i> Everts							
<b>T gra-1</b>	169	20	5'-TAGACACACG GGCCTACTTC -3'	21	5'CCTGTTAGTCCT CCTAGGGTG -3'	61	This study
<b>T gra-2</b>	193	20	5'-ATCCACCTTTA TCCGCCAAC -3'	19	5'AGGGCGGTGA TTGCTACTG -3'	59	This study
<b>T inc-3</b>	154	20	5'-TGGTTGGGGTG ATTGGAAAA-3'	23	5'-CGCAAACCTC CTTTCGATTAGA- 3'	58	This study
<b>T var-4</b>	235	20	5' TTTGACTGTGC GAAGGTAGC-3'	20	5'-ATTTCCCAATC ACCCCAACC-3'	58	This study
<i>Oryzaephilus surinamensis</i> (L.)							
<b>O sur-1</b>	223	21	5'ACAGAACTAGG AACAGCAGGT -3'	20	5'-AGAGAAGGGA GATTGAGGGA -3'	59	This study
<b>O sur-2</b>	268	21	5'ACAGAACTAGG AACAGCAGGT -3'	20	5'CTGTTCATCCTG TTCCTGCC -3'	59	This study

**2.2.1 Design and preparation of primers:** Species specific primer pairs were designed by Primer 3 (Rozen & Skaletsky 2000) (Table 1). To prepare a stock of 100 pmol/ $\mu$ l from the ordered primers, Sigma molecular biology grade water was added with the volume that was reported in the accompanying information. After vortexing and spinning, tubes were placed on ice for 30 min and to produce a 10x primer stock; 1 $\mu$ l of the stock was mixed with 9  $\mu$ l SDW for the both Forward & Reverse primers into separate tubes on ice. After that they were kept in a freezer at -20°C.

**2.2.2 DNA extraction:** A sample size of 300 adults divided into six pooled samples of 50 individuals was used for the test; each two pooled samples represent one type of the studied insect. Pooled samples of 50 individuals were immersed into liquid nitrogen and then crushed into a fine powder using a pre-cooled sterilized mortar and pestle. The Genomic DNA was extracted from whole adults by following methods given in Allen *et al.* (2006) with some modification, before elution/resuspension into 25  $\mu$ l of Tris-EDTA buffer in the final step and stored in freezer at -20°C until it used.

**2.2.3 Polymerase chain reaction (PCR):** DNA was amplified using thermocycler Hybaid PCR machine (Applied Biosystems, 96-Well Fast Thermal Cycler) in 20 $\mu$ l reaction volume. The reaction composed of 10  $\mu$ l of PCR master mix (Gene Direx); 3  $\mu$ l of DNase RNase free water (Bioneer); 3  $\mu$ l of DNA template and 2  $\mu$ l of both forward and reverse primers (Macrogen, Ligo, USA) to make a total volume of 20  $\mu$ l as in the table (2), reaction in negative control composed of all components except DNA template. PCR reactions were run for 3 min at 94°C (initial denaturation), followed by 35 cycles of 1 min at 94°C (denaturation), 1 min 55-65°C (annealing) and 72°C for 1 min (extension). The last step was 72°C for 7 minutes (Final Extension) (Table (1)).

**2.2.4 Agarose gel electrophoresis:** Agarose gel prepared by adding 0.75 gm agarose powder (Agarose M, Bio Basic Canada INC.) to 50 ml of 1x TBE (Tris- Borate- EDTA) buffer, then carefully dissolved by microwave oven until all the particles were dissolved. After the solution cooled down, 5  $\mu$ l ethidium bromides was added and mixed gently. The gel poured into to the tray of which the comb inside it and sterilized tip was used to remove the bubbles around the comb tips and on the gel surface. After the gel had set, the tape around the tray and the comb was carefully removed to leave the well behind it to allow appropriate loading of PCR products inside it.

Later, gently 10  $\mu$ l volume of each sample were loaded onto a submerged gel that consisted of a 1.5%w/v concentration of agarose. Appropriate size marker, 8  $\mu$ l of 2-log (100-3000 bp) DNA ladder H3 RTU (Gene Direx) was loaded in the first well of the gel by micro pipette, then the gel was run at 60 V for approximately 1 hour and examined under UV light (UV Transilluminator Biostep-UST-20M-8K). The photo was taken by using digital camera (Canon; Japan).

## 2.3 Experiment 2: Plant extracts to control examined insects

**2.3.1 Preparation of aqueous extracts:** Fresh leaves of myrtle *Myrtus communis* L., eucalyptus *Eucalyptus camaldulensis* Dehnh and mint *Mentha Canadensis* L. were collected in ministry of high education and college of agriculture from Hawler region. Leaves of these plants were washed carefully with water, air dried in shade and ground into fine powder through using electric grinder (Germany). 50 g dry powder from each plant were mixed with 500 ml distilled water to prepare a 10 % stock solution. The solution

were kept overnight then filtered with muslin cloth and stored in refrigerator prior to use (Abdulhay, 2012). Stock solution were then serially diluted to obtain the desired concentrations of (5, 2.5 and 1.25) % that equivalent to (50000, 25000 & 12500) ppm subsequently.

**2.3.2 Bioactivity of the extracts:** A sample size of 720 individuals was used for the experiment. We examined the toxicity of the three afore mentioned plant extracts on a total of 240 adults of either species (*Tribolium castaneum*, *Trogoderma granarium* & *Oryzaephilus surinamensis*). Three replicates each consist of 10 insects mixed with either black raisin or fig (10 insects/ 20 g dried fruits) were sprayed with 50000, 25000 & 12500 ppm by using syringe inside 250 ml plastic container that later covered by a muslin cloth. All treatments were placed in the incubator at the optimum condition 30 $\pm$ 2 and 60 $\pm$ 2 RH. Control insects were sprayed with only water instead of aqueous extracts and kept under the same condition. Insect mortality was recorded for various concentrations after 24 h, 48h, 72h and 1week exposure.

## 2.4 Statistical Analysis

Mortality data were subjected to appropriate transformation prior analysis. Analysis of variance was used to check the significance of differences between mortality of various stages of test insects. The data were analyzed using multi-factor analysis of variance (ANOVA) in Statgraphics Centurion XV followed by Fischer's least significant difference (LSD) test to determine statistical differences between means of mortality at  $P \leq 0.05$ . Data were Arcsine transformed to minimize the variability-to achieve normal distribution.

Mortality data was adjusted by Abbott's formula (Abbott, 1925) to estimate lethal concentration fifty (LC<sub>50</sub>) values and associated statistics were estimated by subjecting mortality data to the maximum likelihood program of probit analysis using SPSS software version 20. The impact of plant extracts were compared in terms of mortality and LC<sub>50</sub> values with time as the explanatory variable to evaluate mortality.

## 3. RESULTS

### 3.1 Experiment 1: Insect identification through molecular based method (PCR)

Two primer pairs were utilized for each species of *Tribolium* (*castaneum* and *confusum*) to ensure that at least one of them would have the potential to amplify the targeted gene. Agarose gel electrophoresis results indicated that the amplified species was *T. castaneum*. Consequently, the species specific primer pairs (Tcas-1) that have been designed to this study gave a clear band at 57°C with 120 base pair (bp). However, neither *T. castaneum* primer (Akk-1) nor *T. confusum* primers (TconfA and TconCOI) were capable of amplifying the DNA template (Figure 1A). Further, specific marker alongside negative control was set on the gel in order to determine the product size as well as the presence of target species. The process was repeated twice to test reliability of the new primer. Further, Species specific primers were also designed in this experiment to confirm the identity of *Trogoderma* species since specific primer can distinguish a certain species and due to the fact that all the three species of *Trogoderma* (*granarium*, *variable* and *inclusum*) morphologically similar to each other (Rees, 2004). The two specific primers for *T. granarium* (Tgr1, 169bp) and (Tgr2, 193bp). Whereas, the other two specific primers were for *T. inclusum* (Tinc, 154bp) and *T. variable* named (Tvar, 235 bp). DNA-based method revealed the species as *T. granarium* due to

the fact that both designed specific primers for this species showed clear bands on the gel (Figure 1B) Additionally, the results confirmed that *Oryzaephilus* species found in the rice was *surinamensis* when the insect template screened with species specific primer named as (Osur-1, 223 bp). Though the other primer (Osur-2, 268 bp) designed for this study was not successful in amplifying the DNA template (Figure 1C). The DNA ladder was loaded in the first line so it can be compared with the product size of the designed primer.

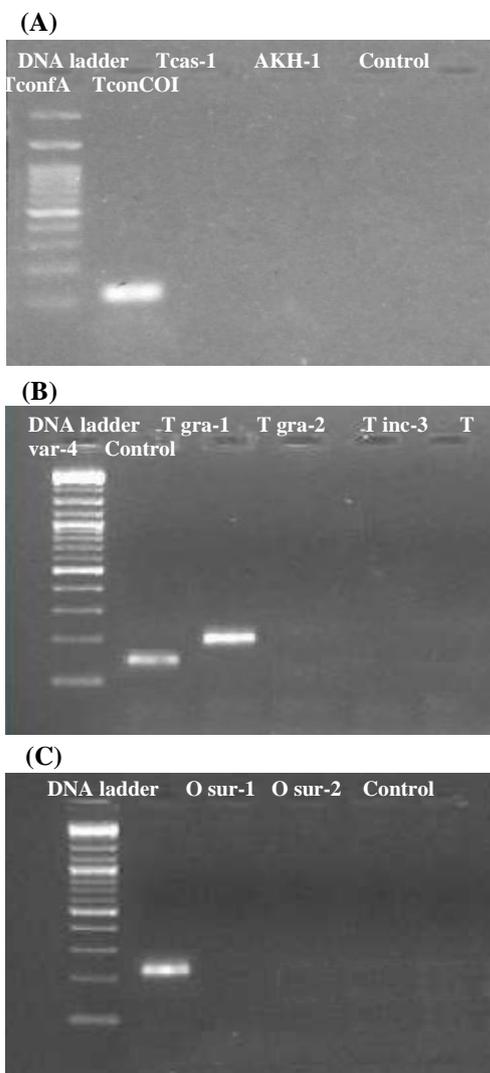


Figure 1. Gel plate of PCR product for (A) *T. castaneum* (B) *T. granarium* (C) *O. surinamensis* via using species specific primers (primer labels correspond to those in Table (1))

### 3.2 Experiment 2: Plant extracts to control examined insects

**3.2.1 Mortality of *Tribolium castaneum*:** The laboratory experiment revealed that aqueous plant extracts significantly affected the mortality of *Tribolium castaneum* ( $F_{(2,286)} = 52.65, P < 0.001$ ). Thus, the three plant extracts varied in their toxicity impact and highest mortality 44.89% achieved via using eucalyptus extract (Figure 2A). Moreover, mortality significantly influenced by exposure time ( $F_{(3,286)} = 196.93, P < 0.001$ ). As a result, fewer individuals survived when subjected for longer period of time to the extract. Highest mortality was obtained 55.12% after 7 days whereas lowest mortality was recorded after 1 day treatment 30.74% (Figure 2B).

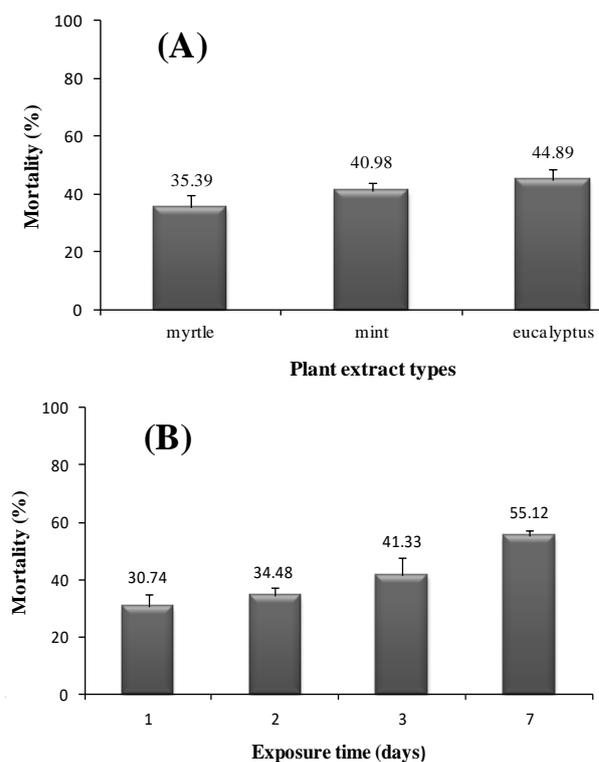


Figure 2. Percentage mortality of red flour beetle in relation to (A) plant extract types and (B) exposure times

Likewise, plant extract concentration ( $F_{(3,286)} = 1142.89, P < 0.001$ ) significantly affected adult mortality. The most efficient dose was 50000 ppm which recorded 62.21% , followed by 25000 ppm and 12500 ppm which recorded 52.22% & 44.16 mortality respectively. Further, whether the insects were subjected to extracts with either black raisin or fig fruit didn't have any influence on adult mortality either separately ( $F_{(1,286)} = 0.22, P = 0.64$ ) or in interaction with other extract doses ( $F_{(3,286)} = 2.45, P = 0.07$ ).

In response to present data, mortality rate significantly influenced by the combination between plant extract dose and experimental time ( $F_{(6,286)} = 9.63, P < 0.001$ , Figure 3), maximum mortality was observed after 7 days treatment and were 82.95, 68.54 and 57.35% for 50000, 25000 and 12500 ppm aqueous concentration respectively. While, control treatment after 7 days recorded lowest mortality 11.64% when sprayed with water instead of aqueous plant extracts.

The interaction terms between plant extract type and doses were significant ( $F_{(6,286)} = 7.05, P < 0.001$ ). Maximum adult mortality reached at 50000 ppm concentration for all the three plants. Thus, eucalyptus achieved 68.98% mortality which significantly differs from the other two extracts (mint & myrtle) (Table 2).

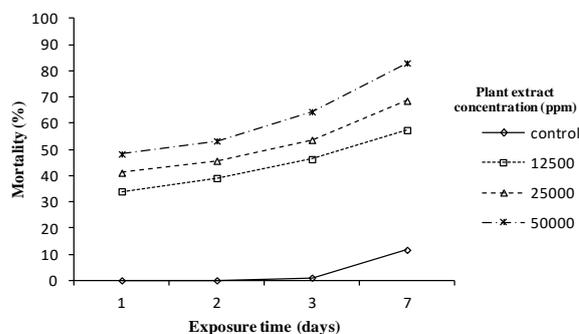


Figure 3. The impact of both extract concentrations and exposure times on percentage mortality of red flour beetle

**3.2.2 Mortality of *Trogoderma granarium*:** Mortality was significantly affected by the type of aqueous extract ( $F_{(2,287)} = 75.05, P < 0.001$ ), mint aqueous extract resulted in highest adult mortality 42.76% followed by eucalyptus 41.34% which were not significantly differ from each other & while myrtle extract produced lowest 30.89% that significantly different from the other two plant extracts. Moreover, adult survival significantly influenced by exposure time ( $F_{(3,287)} = 158.69, P < 0.001$ , Figure 4A). Hence, highest mortality 52.24% recorded after 7 days, whereas lowest was more than 27.41% after one day. Adult capability to escape from mortality was negatively in dependant to extract concentrations ( $F_{(3,287)} = 538.98, P < 0.001$ ). Thus, high dose (50000 ppm) achieved 53.79% mortality but percentage mortality reduced with dosage reduction (Figure 4B). Whereas, Percentage mortality was not significantly affected by the types of dried fruits alone ( $F_{(1,287)} = 25.4, P = 0.091$ ) or via interaction with any other main factors in the experiment.

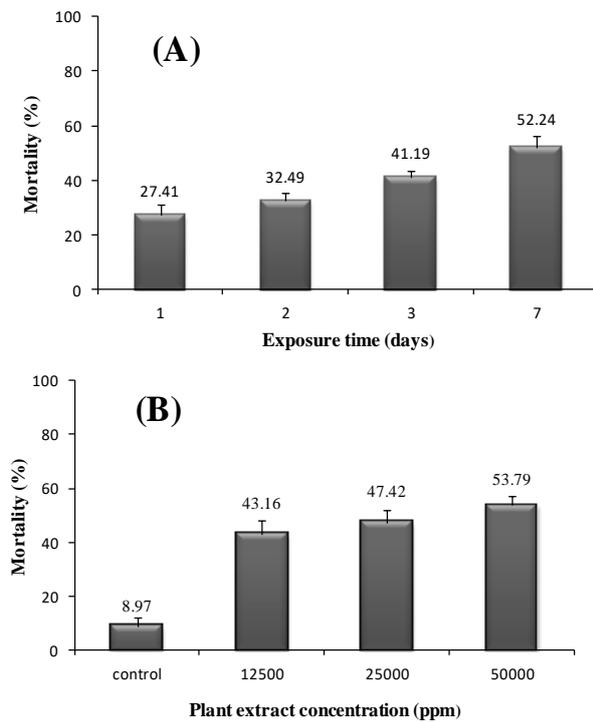


Figure 4. Percentage mortality of khapra beetle in response to (A) exposure times and (B) plant extract concentrations

Interactions term between not only plant extract concentration and exposure times were significant ( $F_{(9,287)} = 9.8, p < 0.001$ , Figure 5), but also survivorship influenced by both plant extract types and doses ( $F_{(6,287)} = 18.16, p < 0.001$ , Table 2).

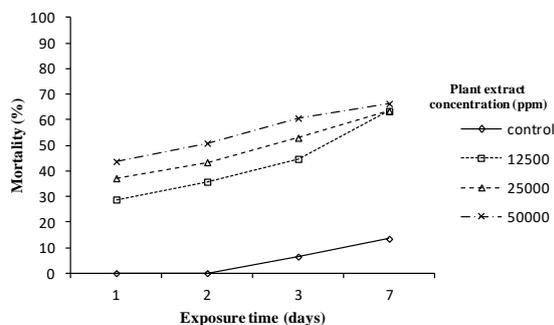


Figure 5. The impact of both extract concentrations and exposure times on percentage mortality of khapra beetle

**3.2.3 Mortality of *Oryzaephilus surinamensis*:** The laboratory test via utilizing different plant extracts significantly affected percentage mortality of sawtoothed grain beetle ( $F_{(2,287)} = 6.39, P < 0.001$ ). Adult treatment with eucalyptus aqueous extract achieved highest mortality (30.29%) which didn't differ significantly from mint extract (27.78%) (Figure 6A). In the same way, percentage mortality significantly influenced by plant concentrations ( $F_{(3,287)} = 323.86, P < 0.001$ ). Thus, highest percentage of mortality was 43.34 recorded by using 50000ppm (Figure 6B).

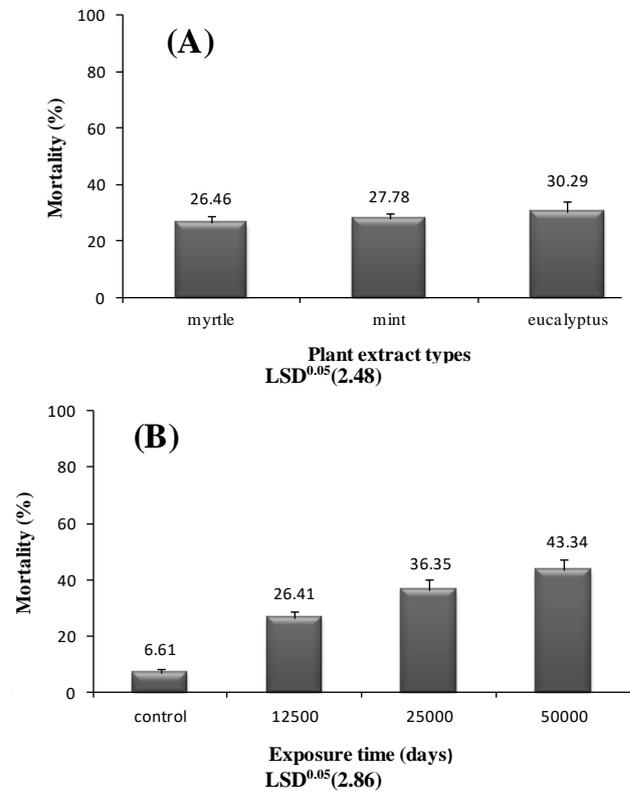


Figure 6. Percentage mortality of sawtoothed grain beetle in accordance to (A) plant extract types and (B) exposure times

In addition, the results obtained from the experiment indicated that not only the exposure duration alone had significant influence on survival ( $F_{(3,287)} = 97.62, P < 0.001$ ) but also via interaction with aqueous plant extract types ( $F_{(6,287)} = 6.39, P < 0.002$ ) had significant impact on adult mortality. Therefore, (44.64, 40.52 and 36.19) % mortality were observed after 7 days on eucalyptus, mint and myrtle respectively (Figure 7).

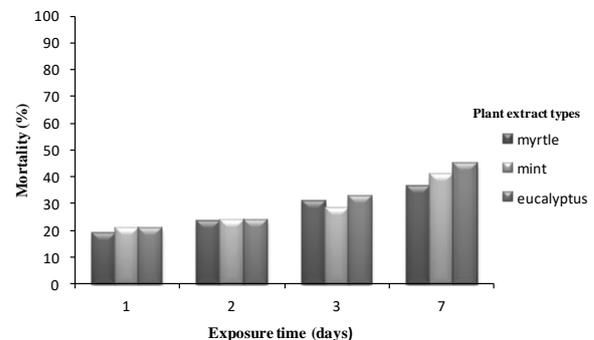


Figure 7. Percentage mortality of saw toothed grain beetle in relation to plant extract types and exposure times

The interaction between doses and extract types were significant ( $F_{(3,287)} = 323.86, P < 0.001$ ). Highest mortality (46.16%) was recorded on eucalyptus at 50000 ppm (Table 2). Moreover, when the three stored product insects treated with various plant extracts and doses the adult of red flour beetle was the most susceptible

species followed by khapra beetle then saw-toothed grain beetle (Table 2).

Table (2): Comparison of the insecticidal activity between plant extract types and doses on percentage mortality of *T. castaneum*, *T. granarium* and *O. surinamensis*

Plant extract	Concentration (ppm)	Red flour beetle	Khapra beetle	Sawtoothed grain beetle	Average
Eucalyptus	0	4.98	8.85	9.49	38.84
	12500	47.74	41.89	27.29	
	25000	57.89	53.06	38.22	
	50000	68.98	61.55	46.16	
Mint	0	2.64	9.49	8.82	37.61
	12500	48.98	46.86	25.94	
	25000	53.07	52.92	33.80	
	50000	64.44	61.72	42.57	
Myrtle	0	1.87	8.57	1.53	30.91
	12500	35.76	36.17	26.00	
	25000	45.69	38.10	37.03	
	50000	58.20	40.71	41.29	
<b>Average</b>		40.85	38.33	27.44	
<b>LSD</b>		2.53	4.62	2.66	

**3.2.4 Comparison between examined insect species on the basis of LC<sub>50</sub> values:** The toxicity of different plant extracts were evaluated via LC<sub>50</sub> values on red flour beetle, khapra beetle and saw-toothed grain beetle, Table . The LC<sub>50</sub> values revealed that saw-toothed grain beetle adults were more tolerant botanical insecticides than the other two species within various plant extracts and exposure times. Thus, higher doses must utilize in order to achieve 50% mortality, followed by khapra and finally red flour beetle. The LC<sub>50</sub> values of saw toothed grain beetle, khapra and red flour beetle ranged between 68713.87, 49607.16 and 44348.78 to 22763.48, 276.55 and 87.97 for the three species respectively (Table 3).

Table 3. The LC<sub>50</sub> values in (ppm/days) using various aqueous plants on the adults of three stored product insects

Plant extract	Dried fruit	Time (days)	Redflour beetle	Khapra beetle	Saw-toothed grain beetle
Eucalyptus	Black raisin	1	32936.93	40369.71	54562.63
		2	26881.96	33117.25	46623.76
		3	15918.70	24301.11	35703.78
		7	3887.25	10816.34	22763.48
	Fig	1	36412.21	43770.43	68713.87
		2	28552.75	33730.87	65655.19
		3	16454.51	25510.70	46233.39
		7	87.97	2532.38	42912.62
Mint	Black raisin	1	29167.18	41148.83	64633.34
		2	24872.52	36602.86	61888.20
		3	21261.02	27278.95	59063.88
		7	11053.97	3144.76	45048.41
	Fig	1	34882.33	36129.12	63205.28
		2	29030.82	317116.5	57941.60
		3	24068.72	116971.5	48305.43
		7	11972.13	995.02	34330.48

Myrtle	Black raisin	1	44348.78	49607.16	67814.80
		2	39600.78	41544.77	54076.79
		3	28730.72	37220.31	41008.472
		7	14726.86	18421.43	39016.78
	Fig	1	37338.90	37798.22	56359.29
		2	36628.66	27675.42	51807.99
		3	30972.84	17836.72	42023.97
		7	19199.63	276.55	28551.57

#### 4. DISCUSSION

The molecular data from this study reveal that specific primers can be used to confirm the identity of other similar morphologically indistinct species. Hence, species-specific PCR technique was efficient to separate *Tribolium castaneum* from *T. confusum*. Similar to our finding, species-specific PCR technique was used for the separation of six species within *Tribolium* which were *T. castaneum*, *T. confusum*, *T. destructor*, *T. madens*, *T. freemani* and *T. brevicornis* of stored products (Zhang *et al.*, 2016). Moreover, PCR-RFLP has been developed to distinguish *Tribolium* flour beetles based on a partial 28S rRNA gene sequence (Zhang *et al.*, 2014). In addition, a study revealed that of out of 45 primer pairs designed only 19 pairs produced successful amplification when five strains of *T. castaneum* were screened to develop molecular genetic markers (Pai *et al.*, 2003).

Likewise, the laboratory experiment was also able to develop specific primers for both *Trogoderma granarium* and *Oryzaephilus surinamensis* species. Three primers were sufficient to distinguish three species of *Trogoderma*. Hence, DNA polymorphic of *T. grosuirium*, *T. variable* & *T. glabrum* was evaluated on the basis of molecular weight (Yulin *et al.*,

1999). Also, the sawtoothed grain beetle *O. surinamensis* along with the merchants grain beetle *O. mercator* were separated via using universal primers and species identity were confirmed as *O. surinamensis* after sequencing (Sorvari et al., 2012).

The aqueous plant extracts in this study showed clear insecticidal effects on the three examined stored product insects. The results of the present study are in agreement with some earlier studies Ciepielewska et al. (2005), who found that aqueous plant extracts prepared by pouring 10 g of macerate plant with 100 ml water were capable of protecting stored commodities against *T. castaneum*. In addition, Ahmad et al. (2013) prepared (1:10) plant extracts via mixing 50 g powder of ginger *Zingiber officinale* with 500 ml acetone as solvent. The results revealed that highest concentration of ginger was more toxic and reduced khapra population significantly inside wheat grains. Also, 83.3 and 73.3 % mortality were achieved on sawtoothed grain beetle via using 0.125 dose after 2 and 1 week respectively within mint oil (Al-Jabar 2006).

The aqueous botanical extracts might affected the insects either via contact method through the cuticle or might disrupted the normal respiratory activities of these insects or could influenced the insect via feeding on treated dried fruits with the toxic extracts. Thus, the abovementioned methods alone or together resulted in the death of the test insects. Whereas, the respond of the three stored product insects vary towards the toxic impact of the aqueous extracts probably in accordance to chemical composition of the plants as well as insect susceptibility. The powerful impact of *Eucalyptus camaldulensis* might refer to the presence of various phytochemicals in the aqueous extract (e.g. tannins, saponins, glycosides, steroids and anthraquinones) (Sani et al., 2014). Therefore, the chemical ingredients of the plant have the potential to function as antimicrobial, insecticidal and insect repellent (Muell and Olugbade, 1996; Batish et al., 2008). In similar study, the toxicity of *Eucalyptus camaldulensis* leaves were examined against three stored product beetles (*Tribolium castaneum*, *Sitophilus zeamais* and *Callosobruchus maculatus*). The botanical extracts exhibited more insecticidal toxicity against *T. castaneum* than to *C. maculatus* and *S. zeamais* (Channoo et al., 2002). On the other hand, adult resistant might refer to the hard exoskeleton and less moisture content and however, *O. surinamensis* are small in size but consider as strong insect that has built up resistant to various insecticides and thus are often more difficult to kill and apply control methods on it in comparison with other stored product beetles (Heather & Wilson, 1983; Walbank & Collins 2003). Similar to our finding, the extracts of various plants; *Azadirachta indica*, *Zanthoxylum zanthoxyloides*, *Anacardium occidentale* and *Moringa oleifera* evoked high mortality effect on *Sitophilus oryzae*, *Ryzopertha dominica* and *Oryzaephilus mercator* but *O. mercator* was more tolerant to botanical extracts than *S. oryzae* and *R. dominica* (Kileke and Ogungbite, 2014). However, the finding of the current study is inconsistent with Al-Jabr (2006) who found that *O. surinamensis* is more susceptible to various oil plants than *T. castaneum*. As a result, when the toxicity of essential oil from various plants were tested complete mortality of saw toothed grain beetle was achieved at concentration 0.5% while required higher dose 1% to achieve complete mortality for red flour beetle.

Similar to our finding Mohamad and Abdelgaleil (2008) examined the insecticidal activity of eight plant extracts on *T. castaneum* and the result indicated that *Mentha microphylla* (LC<sub>50</sub> = 0.01mg/cm<sup>2</sup>) exhibited highest insecticidal activity, followed by *Eucalyptus camaldulensis* (LC<sub>50</sub> = 0.15 mg/cm<sup>2</sup>). In a similar study conducted by Mamun et al. (2009) aqueous plant extracts of (bazna, ghora-

neem, hijal, karanje, mahogoni and neem) utilized against red flour beetle with four different concentrations (5.0, 7.5, 10.0 and 12.5%) and various exposure times. The relative toxicity increased with an increase of both doses and exposure times. Thus, the result indicated that neem extract was the most toxic and the LC<sub>50</sub> values were (51.9, 6.11 and 4.05) after 1, 2 & 3 days respectively.

## 5. CONCLUSIONS

This study revealed that aqueous plant extracts possess toxic principles with significant insecticidal effects and could be a potential dried fruits protectant against various species of stored product. Further, among the tested plants, eucalyptus exhibited highest toxicity followed by mint and myrtle. Aqueous plant extracts can be utilized instead of alcoholic extract for insect treatment because not only it is feasible and cheap but also would not leave any unfavourable chemical residues on treated products. In addition, molecular based method consider as a vital complementary to the traditional method in order to achieve more precise identification

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