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Toxicity and Biochemical Alterations of Some Essential Oils on the Rice Weevil, *Sitophilus oryzea* (l.) (Coleoptera: Curculionidae)

Draz, K. A. A.; A. A. E. Darwish and M. A. Abo-Bakr
Department of plant protection, Faculty of Agricultur, Damanhour University, Egypt

ABSTRACT

The present study aimed to investigate the effects of sublethal concentrations of nine essential oils extracted from celery (*Apium graveolens*), camphor (*Cinnamomum camphora*), garlic (*Allium sativum*), mint (*Mentha piperitaa*), basil (*Ocimum basilicum*), thyme (*Thymus vulgaris*), chamomile (*Chamaemelum nobile*), sesame (*Sesamum indicume*), jasmine (*Jasminum officinale*) and malathion insecticideon the physiological and biochemical parameters of rice weevil, *Sitophilus oryzea* L. (Coleoptera, Curculiondiae). Total protein and some enzymatic activities i.e; aspartate transaminase (AST), alanine transaminase (ALT) and acetylcholinesterase (AChE) were quantified. The Lethal Median Concentration (LC_{50}) values were estimated to be 1070, 990, 1210, 3350, 2330, 1300, 2500, 4110, 4900, 2.31 mg/kg for celery, camphor, garlic, mint, basil, thyme, chamomile, sesame, jasmine and malathion, respectively after three days of treatment. Protein content was significantly increased in case of malathion insecticide, while it was significantly decreased in case of jasmine, thyme, camphor, chamomile, mint, sesame and basil oil compared with control. Significant increase in activity of ALT observed in thyme, garlic, celery, basil, jasmine, mint, sesame, camphor and malathion insecticide, while it significantly decreased in case of chamomile oil compared with control. AST activity significantly increased in jasmine, garlic, camphor, chamomile, mint, sesame and malathion insecticide, but significantly decreased in thyme and celery oil, compared with control. Celery, chamomile, mint and basil oil significantly induced increase activity of acetylcholinesterase (AChE), while thyme, camphor and malathion insecticide caused significant decrease in enzyme activity.

Keywords: Essential oils, Rice weevil, Toxicity, Biochemical parameters.

INTRODUCTION

The rice weevil, *Sitophilus oryzae* (L.) is considered one of the main insects of cereal grains and their products (Baloch, 1992 and Rees, 1996), where causes loss in weight and quality reduction (Longstaff, 1981; Grenier *et al*., 1997 and Park *et al*., 2003). About 12% of the annual loss in wheat in Egypt is caused by the rice weevil. These losses are estimated as equivalent to half a million tons (Ministry of agriculture and land reclamation report, Egypt, 2007). Control of rice weevils conventionally depends on synthetic insecticide such as malathion to prevent grain infestation (Murray, 1979). However, weevil populations build up resistance to insecticides (Murray, 1979; Longstaff, 1988). Many alternative control techniques depend on use of botanicals (plant products) to control *S. oryzae* (Dayal *et al*., 2003). The essential oils extracted from large number of plant species have been found to contain toxic and/or repellent effects against different insects (Regnault-Roger, 1997 and Yildrim *et al*., 2001). Some of essential oils have contact and fumigant potential actions against pests (Isman, 2000). This concept revealed to broad spectrum against stored grain pests (Sahaf *et al*., 2008 and Kim *et al*., 2010). In addition other potential effect may induced i.e; antifeedant activity (Huang *et al*., 1999), repellents as documented previously by (Nerio *et al*., 2010 and Carroll *et al*., 2011) and development and growth inhibitories (Tomova *et al*., 2005 and Waliwitiya *et al*., 2008).

Rather than performing acute toxicity tests, responses to sublethal concentration of this toxicant should be studied to assess the effects of a toxic material on insects. Sublethal concentrations of toxic compounds may cause direct sublethal effect including development, growth, reproduction, morphological and genetic changes (Takada *et al*., 2001 and Willrich and Boethel, 2001) and indirectly through biochemical and physiological effects (Croft, 1990; Sak *et al*., 2006 and Saleem *et al*., 2013). The transaminase enzymes; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are critical in carbohydrate and protein metabolism and altered under stress conditions (Etebari *et al*., 2007). Also, acetylcholinesterase (AChE) is an important enzyme during the excitation phase of nerve conduction in the insect body; thus, target pests die when AChE enzyme in vivo is inhibited (Ramsey *et al*., 2010). This study was conducted to investigate the physiological and biochemical responses of *S. oryzae* adults to sublethal concentrations of nine essential oils and malathion in order to evaluate their efficiency in preserving wheat grains against these insect pests.

MATERIALS AND METHODS

Rice weevil, *Sitophilus oryzea* L. (Coleoptera, Curculiondiae) was the aim of this study. The stock culture was continuously reared free of any insecticidal contamination for several years at Department of Plant Protection, Faculty of Agriculture, Damanhour University, Egypt. The culture was maintained at 28 ºC ±2 and 60±5% relative humidity (RH). From the stock culture, 400-500 adults were added to a glass jar (500 ml) containing 500 g of wheat grains (Giza 172) as a culture media according to Halstead, (1963). The Jar was covered with muslin cloth fixed by rubber bands to prevent the insects from escaping and for air passage (Ahmed, 1996). Wheat grains were stored at adeep freezing for 2 weeks before use to avoid any possible infection. Adults aged from 7 to 14 days were used for experimental procedure. The tested essential oils were produced by El-Captan Company, Cairo, Egypt. The used oils were namely celery, *Apium graveolens* L.,

camphor, *Cinnamomum camphora* (L.), garlic *Allium sativum* L., mint, *Mentha piperitaa* L., basil, *Ocimum basilicum* L., thyme, *Thymus vulgaris* (L.), chamomile, *Chamaemelum nobile* (L.), sesame, *Sesamum indicume* L., Jasmine, *Jasminum officinale* L. Also, Malathion®5% dust (produced by Kafr El-Zayat Chemical and Pesticides Company, Egypt), was used apositive control.

To determine the values of LC_{50}, for the desirable oils, series of concentrations of each tested compound were prepared. Six concentrations: 500, 1000, 2000, 3000, 4000 and 6000 mg/kg grains wheat were used. Malathion was used at concentrations of 1, 2, 5, 10, 15 and 20 mg/kg. These concentrations were uniformly applied separately to 30 g of wheat grains in glass jars (5 cm diameter × 7.9 cm height). Then, thirty weevils aging 1 to 2 weeks were placed in each jar. Three replicates were used for each concentration and all jars were kept at 28±2 ºC and 65±5 % RH. Another three replicates containing untreated grains were used as control. Percentage mortality was estimated 1, 3, 7, and 14 days post-treatment. The LC_{50} values were calculated using Log dose Probit (LdP) Line (Finney, 1971).

Biochemical quantifications:

The sub-lethal concentration equivalent to LC_{50} values were used to determine biochemical responses. Weevils were exposed separately to the sub-lethal concentrations of tested compounds along with their respective controls in triplicate for three days. Live insects from each treatment were randomly selected, then weighed and used for Biochemical quantifications.

Sample Preparation:

Adult weevils, three days old after treatment were collected in Eppendorf tubes and kept at - 20 ˚C until used. The insects were weighed and the whole body was homogenized in 10 times volumes (w/v) of phosphate buffer pH 7.5 using glass homogenizer under cooling. The homogenates were centrifuged at 10000 rpm for 20 min at 4 °C. The supernatant was used for assessment.

Assay of total protein levels:

The total protein level was determined colorimetrically according to the method of Lowry *et al*. (1951). Ten microliters of supernatants were completed to 0.2 ml by sodium phosphate buffer pH 8, then thoroughly mixed with 1 ml of reagent C (50 ml of 2% Na_2CO_3 in 0.1N NaOH containing 0.5 g sodium or potassium tartrate plus 1ml of 0.1 g $CuSO_4$ in a liter of distilled water). The mixture was kept for 10 min at room temperature. Hunderd µL of folin reagent (Freshly prepared by diluting folin reagent 1:1 v/v H_2o) was added and immediately mixed. The samples were kept for further 30 min at room temperature. The developed blue color was measured at 750 nm against blank. Protein concentration was expressed as mg/ml wet tissue. Bovine serum albumin (BSA) was used as a standard.

Assay of (ALT) and (AST) activities:

ALT and AST activities were measured according to the method of Reitman and Frankel (1957). Hunderd µL of enzyme source was mixed with 0.50 ml of ALT substrate. Similarly, for determination of AST the same volume of enzyme source was mixed with 0.50 ml of AST substrate. Then, 0.50 ml of 2, 4-dinitrophenyl hydrazine solution was added and the mixtures were left stand for 20 min at room temperature. Then, 5.0 ml of 0.4 N NaOH was added and mixed well and allowed to stand at room temperature for 5 minutes. The optical density was read at 505 nm after setting the blank. The enzyme activity was expressed as U/ml.

Assay of acetylcholinesterase (AChE) activity:

Enzyme activity was determined according to the method of Ellman *et al*. (1961). In test tube, 2.9 ml of 0.1M sodium phosphate buffer (pH 8.0), 0.1 ml of 0.1mM 5,5 dithiobis (2-nitro benzoic acid) (DTNB) reagent and 20 µL of supernatant were mixed. To the above mixture, 20 µL of 0.075M acetylthiocholine iodide (ATChI) were added. The optical density of the developed yellow color was measured at 412 nm after 10 min against blank. The specific activity was calculated as µmole of substrate hydrolyzed per mg protein per min.

Data were subjected to one-way analysis of variance (ANOVA) using the SPSS version 12 software. Means were separated using SNK method (Steel and Torrie, 1980) and the results were considered statistically significant at 0.05 levels.

RESULTS AND DISCUSSION

Acute toxicity:

Data shown in Table (1) illustrate LC_{50} values after 3 days of treatment. The highest effective oil was camphor oil accounting for 990 ppm followed by celery, garlic, thyme, basil, chamomile, sesame, respectively, while the lowest one was jasmine oil (4900 ppm). Also, malathion was more effective against *S. oryze* (2.31 ppm)compared with desirable essential oils.

Table 1. LC_{50} values of tested essential oils and malathion after 3 days of treatment against rice weevil, *S. oryzae*.

Tested compounds	LC_{50} (mg/kg)
Jasmine	4900
Thyme	1300
Garlic	1210
Camphor	990
Celery	1070
Chamomile	2500
Mint	3350
Sesame	4110
Basil	2330
Malathion	2.31

These results showed that camphor oil was the most effective oil, while jasmine was the least effective in agreement with the results of Hamed *et al*., 2012. Also, Arabi *et al*., 2008 reported that mortality of *Sitophilus oryzae* was increased with the increase of the concentrations of oils that contain camphor and 1,8-cineole and increased the time of exposure. Richards, (1978) reported that essential oils of plant origin are

highly lipophilic and therefore have the ability to penetrate the cuticle of insects. By this method the plant material apart from its odor, may have also acted as a contact poison.

Total Protein levels:

The total protein levels significantly changed after oils treatments (Table 2). Significantly decrease in the total protein was found in camphor (-38.39%), followed by chamomile (-36.39%), sesame (-31.52%), mint (-29.23%), thyme (-28.08%),basil (-16.05%) and jasmine (-15.47%), respectively. However, garlic (-1.72%) and celery (-1.15%) did not cause significant change in total protein levels compared with control. Nathan *et al*., 2008 reported that, the reduction in protein content is a common phenomenon in insects after treatment with toxic compounds. It is likely that, the insect degrades proteins to resultant compensation for the lower energy caused by stress (Nath *et al*., 1997). Data in Table (2) illustrate the highest significant increase in protein levels associated with malathion treatment, while camphor induced significant decrease in protein levels. However, the lowest oil caused significant decreased in protein levels was jasmine. Abo El-Makarem *et al*., 2015 found examined essential oils imposed alteration in protein levels in homogenate of granary weevil, *Sitophilus granarius* (L.) in order as follows: basil > anise > clove, respectively. Our results are supported by a number of reports, where toxicity of essential oil lead to reduced protein content of insects such (Smirle *et al*.,1996; Caballero *et al*., 2008 and War *et al*., 2011). The reduction of body protein levels in weevils treated with sublethal doses of basil is indicative of reduced protein synthesis and low assimilation of food and low amino acid uptake for protein synthesis during insecticide stress (Ribeiro *et al*., 2001).

On the other hand, the total protein levels were significantly increased in malathion treatment (23.78%), it seems that the increase in the protein levels in malathion treated weevils could be the result of an elevation in tissues metabolic activity to compensate the stress caused by the insecticides. Fluctuation in the protein level in the insects treated with insecticides was reported by several authors. Shakeet and Bakshi, (2010) found that the monocrotophos led to highly significant decrease in protein level of the surface grasshopper, *Chrotogonus trachypterus*.

Table 2. Total protein levels(mg/ml) in rice weevil, *Sitophilus oryzea* treated with some essential oils.

Tested compounds	Total protein (mg/ml)	% of D*
Control	0.349^{b} ± 0.001	-
Jasmine	0.295^{c} ± 0.002	-15.47
Thyme	0.251^{d} ± 0.013	-28.08
Garlic	0.343^{b} ± 0.006	-1.72
Camphor	0.215^{e} ± 0.011	-38.39
Celery	0.345^{b} ± 0.011	-1.15
Chamomile	0.222^{e} ± 0.012	-36.39
Mint	0.247^{d} ± 0.004	-29.23
Sesame	0.239^{d} ± 0.013	-31.52
Basil	0.293^{c} ± 0.002	-16.05
Malathion	0.432^{a} ± 0.008	23.78

Values are mean ± standard error; n=3; means within each column followed by the same letter were not significantly different; at 0.05 levels.

***% of D: percent of increase or decrease of control = (Control-Treatment)/Control * 100.**

ALT and AST activities:

Activities of ALT and AST are tabulated in Table (3). Significant increase in activity of ALT observed as follows: camphor (139.94 %), followed by sesame (137.83%), celery (133.28%), garlic (117.28%), mint (116.48%), malathion (114.44%), jasmine (89.39%), thyme (81.98%) and basil (65.43%), respectively. While there were significantly decrease in activity of ALT recorded for chamomile (-21.35%) compared with control. In addition, AST activity significantly increased by jasmine (73.89%), followed by sesame (68.81%), mint (58.87%), chamomile (26.30%), garlic (23.07%), camphor (17.19%) and malathion (13.56%) respectively. while, AST activity significantly decreased by celery (-13.77%) and thyme (-11.36%). Basil oil did not cause significant change in activity of AST (7.26%) compared with control.

Table 3. Activities of ALT and AST in rice weevil, *Sitophilus oryzea* followed by some essential oils treatment.

Tested compounds	ALT (U/ml)	% of D*	AST (U/ml)	% of D*
Control	5.400^{e} ±0.327		11.747^{f} ± 0.484	-
Jasmine	10.227^{c} ±0.678	89.39	20.427^{a} ± 0.774	73.89
Thyme	9.827^{cd} ±0.591	81.98	10.413^{g} ± 0.686	-11.36
Garlic	11.733^{b} ±0.249	117.28	14.457^{cd} ± 0.323	23.07
Camphor	12.957^{a} ±0.530	139.94	13.767^{de} ± 0.668	17.19
Celery	12.597^{ab} ±0.705	133.28	10.130^{g} ± 0.408	-13.77
Chamomile	4.247^{f} ±0.519	-21.35	14.837^{c} ± 0.533	26.30
Mint	11.690^{b} ±0.754	116.48	18.663^{b} ± 0.590	58.87
Sesame	12.843^{a} ±0.409	137.83	19.830^{a} ± 0.709	68.81
Basil	8.933^{d} ±0.472	65.43	12.600^{f}± 0.618	7.26
Malathion	11.580^{b} ±0.787	114.44	13.340^{e} ± 0.553	13.56

Values are mean ± standard error; n=3; means within each column followed by the same letter were not significantly different; at 0.05 levels.

***% of D: percent of increase or decrease of control = (Control-Treatment)/Control * 100.**

According to Ali *et al*., 2013 the transaminase enzymes (ALT and AST) are mitochondrial enzymes which transfer an amino group from amino acid to keto acid. They are released in the haemolymph of insects only when the cells are damaged or destroyed. Data in Table (3) showed that camphor oil caused the highest

significant increase in activity of ALT, while basil oil caused the lowest significant increase in activity of ALT, but chamomile oil caused highest significant decrease in activity of ALT. Abo El-Makarem *et al*., 2015 proved that, clove oil caused highest significant increase in activity of ALT, while basil oil induced the lowest activity. Also, the data shown in Table (3) illustrate jasmine oil caused significantly increase in activity of AST, while malathion insecticide caused significantly increase in activity of AST. Abo El-Makarem *et al*., 2015 found basil oil was the highest oil caused significantly increase in activity of AST. This result might suggests that, essential oil application enhanced transaminase enzyme activity due to toxic stress. Increased transaminase activity might have been required by weevils to metabolize amino acids to obtain energy under stress. In fact, the varying effect of plant extracts on AST and ALT activities might be due to the effect on the synthesis or functional levels of these enzymes directly or indirectly by altering the cytomorphology of the cells (Nath, 2000).

Acetylcholinesterase (AChE) activity.

Data shown in Table (4) dipicte that mint (40.44%), followed by basil (30.77%), chamomile (28.65%) and celery (18.77%) significantly increased in activity of acetylcholinesterase (AChE), while malathion (-66.83%), followed by camphor (-14.39%) and thyme (-12.28%), caused significant decrease in activity of enzyme, but jasmine (-6.63%), garlic (-0.99) and sesame (-2.39%), did not cause significant change in enzyme activity compared with control.

Table 4. Activity of AChE in homogenate of the rice weevil, *Sitophilus oryzea* treated with some essential oils.

Tested compounds	Activity(µmole. mg^{-1}. min^{-1})	% of D*
Control	$1.417^{c} \pm 0.066$	-
Jasmine	$1.323^{cd} \pm 0.106$	-6.63
Thyme	$1.243^{d} \pm 0.082$	-12.28
Garlic	$1.403^{cd} \pm 0.135$	-0.99
Camphor	$1.213^{d} \pm 0.076$	-14.39
Celery	$1.683^{b} \pm 0.077$	18.77
Chamomile	$1.823^{ab} \pm 0.118$	28.65
Mint	$1.990^{a} \pm 0.119$	40.44
Sesame	$1.383^{cd} \pm 0.117$	-2.39
Basil	$1.853^{ab} \pm 0.045$	30.77
Malathion	$0.470^{e} \pm 0.080$	-66.83

Values are mean ± standard error; n=3; means within each column followed by the same letter were not significantly different; at 0.05 levels.

***% of D: percent of increase or decrease of control = (Control-Treatment)/Control * 100.**

Acetylcholinesterase is a key enzyme that terminates nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine in the nervous system (Lopez and Pascual-Villalobos, 2010). It has been widely accepted that inhibition of AChE in cholinergic synapses of the nervous system is the primary mechanism of acute toxicity of insecticides. Data shown in Table (4) depicte that mint oil was the highest oil caused significantly increase in activity of AChE, while celery was the lowest oil caused significantly increase in activity. Malathion insecticide was the highest compound caused significantly decrease in activity of AChE in agreement with that obtained by (O'Brien, 1967 and Mosleh *et al*., 2011). They found that, organophosphorus insecticides showed higher inhibiting effect than plant essential oils because they are considered specific inhibitors of cholinesterase. Thyme oil was the lowest oil caused significantly decrease in activity of AChE. Abo El-Makarem *et al*., 2015 found that, anise oil was the highest oil caused significantly decrease in activity of AChE, basil was of lowest one. Camphor and thyme oils might act by interfering with the passage of impulses in the insect nervous system. The ability of AChE to hydrolyze acetylcholine, the buildup of concentration of the acetylcholine in the synapse and excessive neuro excitation are the results of prolonged binding of acetylcholine to its post synaptic receptor (Lopez and Pascual-Villalobos, 2010). Previous works indicated that monoterpenoids in most plant essential oils cause insect mortality by inhibiting acetylcholinesterase enzyme (Gracza, 1985; Grundy and Still, 1985 and Lopez and Pascual- Villalobos, 2010).

CONCLUSION

These findings indicate that, the desirable essential oils showed toxicity against rice weevil, *Sitophilus oryzea* L. lower than these induced by malathion. Moreover, other biochemical alterations were induced by these treatments. So, more research must be done before recommendation to use these products as alterative agents in stored grain pests control.

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السمية والتغيرات البيوكيميائية لبعض الزيوت الطيارة علي حشرة سوسة الأرز *Sitophilus oryzea* (Coleoptera: Curculionidae)

خليل عبد الله دراز، عدنان عبد الفتاح السيد درويش و محمد عبد الناصر ابو بكر

قسم وقاية النبات، كلية الزراعة، جامعة دمنهور

تهدف هذه الدراسة الي اختبار تاثيرالتركيزات تحت المميتة لتسعة زيوت مستخلصة من نباتات الكافور، الكرفس، الثوم، الزعتر، الريحان، البابونج، النعناع، السمسم، الياسمين وكذلك مبيد الملاثيون علي التاثيرات الفسيولوجية والبيوكيميائية لسوسة الارز. قيم التركيزات المميتة لنصف الحشرات للمركبات المختبرة بعد ثلاثة ايام من التعرض هي 1070، 990، 1210، 3350، 2330، 1300، 2500، 4110، 4900 و 2.31 مجم/ كجم لزيت الكرفس، الكافور، الثوم، النعناع، الريحان، الزعتر، البابونج، السمسم، الياسمين ومبيد الملاثيون علي الترتيب. تم قياس المحتوي البروتيني بالاضافة الي قياس نشاط انزيمات Alanine transaminase، Aspartate transaminase وAcetylcholineesterase. اوضحت النتائج ان المحتوي البروتيني لجسم الحشرة زاد معنويا بعد المعاملة بمبيد الملاثيون بينما قل معنويا بعد المعاملة بزيت الياسمين، الزعتر، الكافور، البابونج، النعناع، السمسم والريحان مقارنة بالكنترول. وقد لوحظ زيادة نشاط انزيم (ALT) معنويا بعد المعاملة بزيت الزعتر، الثوم، الكرفس، الريحان، الياسمين، النعناع، السمسم، الكافور ومبيد الملاثيون بينما قل نشاط الانزيم معنويا بعد المعاملة بزيت البابونج مقارنة بالكنترول. بعد المعاملة بزيت الياسمين، الثوم، الكافور، البابونج، النعناع، السمسم ومبيد الملاثيون لوحظ زيادة نشاط انزيم (AST) معنويا لكن قل نشاط الانزيم معنويا بعد المعاملة بزيت الزعترو الكرفس مقارنة بالكنترول. ايضا اوضحت النتائج زيادة نشاط انزيم (AChE) معنويا بعد المعاملة بزيت الكرفس، البابونج، النعناع والريحان بينما قل نشاط الانزيم معنويا بعد المعاملة بزيت الزعتر، الكافور ومبيد الملاثيون.لذا هذه الزيوت تحتاج لمزيد من البحث قبل التوصية باستخدمها في مكافحة افات الحبوب المخزونة.