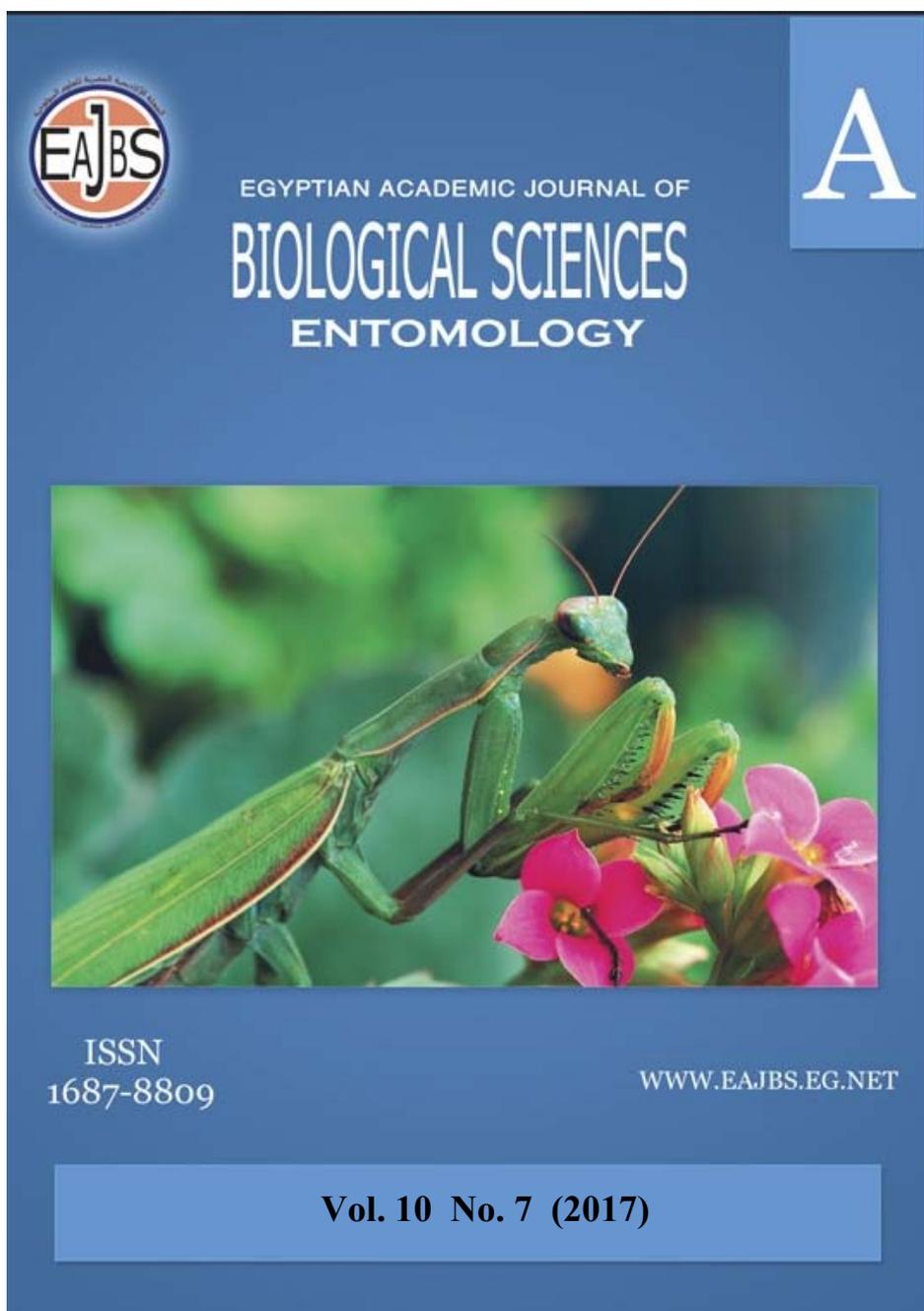


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Efficiency of Some Therapeutic Essential Oils as Antibacterial and Antioxidants on Some Biochemical Parameters of Infected Silkworm *Bombyx mori* L.

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ABSTRACT

Silkworm was infected with *Bacillus thuringiensis* then treated with three concentrations (0.5, 0.75 and 1%) of three specific oils (citrus specific Orange, chamomile and Lavander) to determine their ability as antioxidants and anti-bacterial for recovering the damage occurred in silkworm hemolymph through evaluating some biochemical changes in Lipid peroxidation (MDA), Protein Carbonyl Contents (PCC) as a biomarker of damage happened in Lipids and protein, also Alanin Transaminases (ALT), Aspartate transaminases (AST) and antioxidant capacity. The results showed that (0.5 and 0.75%) of citrus oil was the most effective oil, followed by (1% and 0.75%) chamomile oil which recorded the least damage for PCC and MDA, respectively and highly antioxidant capacity however (0.5%, 0.75%) citrus oil recorded the least value in (ALT) and (AST) of the two concentrations compared with other oils and control.

INTRODUCTION

Bacterial and viral diseases are still the danger that threaten breeding in Egypt and the problems with drug resistant micro-organisms is the emergence of new diseases with no proper medication, the side effects of modern drugs have stimulated renewed interest in plants as source of new medicines (Patwardhan, *et al.* 2004). Silk is known for Queen of Fiber which is proteinecious in nature (Gnanaraj, *et al.* 2011). The mulberry silkworm, is affected by viral, fungal and protozoan pathogens among which bacterial pathogens which cause cocoon loss about 75 % of ton Das Gupta (1950). One of the main causes of mortality worldwide is the Infectious disease which represents a critical problem to health Murray and Lopez (1997). There is an equilibrium between natural antioxidant defense mechanism and reactive oxygen species (ROS), which are produced in the body as by-products by various exogenous and endogenous redox processes Uttara, *et al.* (2009). When the equilibrium is disturbed, the ROS can induce oxidative damage to various biomolecules, including carbohydrates, lipids, proteins, DNA and RNA in the human body, which is associated with cellular damage, tissue injury and genetic mutation Trachootham, *et al.* (2008). Oxidant damage is associated to photo ageing radiation toxicity, cataract formation and muscular degeneration Kolawole *et al.* (2014). Once free radicals are initiated, they can propagate by involving in chain reactions with other less reactive

types, the resulting chain reaction compounds generally survive longer in the body and thus increase the potential for cellular damage. To protect molecules against toxic free radicals and other ROS, cells have developed antioxidant defense system that include the enzymes super oxide dismutase (SOD); catalase (CAT); glutathione reductase and glutathione peroxidase, which destroy toxic peroxides and small molecules including glutathione Cheng, *et. al.*(2014). In recent years evoke interest in use of phyto-chemicals as antimicrobial agent has resulted in thorough investigation of medicinal plants Patwardhan, *et. al.* (2004) also, it is important to search for new antimicrobials, or new natural alternatives having antimicrobial activity such as plant extracts and essential oils Shah (2005). There is epidemiological evidence correlating higher intake of dietary antioxidant components with the ability to lower the rate of human mortality Bajpai *et al*(2011). Essential oils are complex mixtures of compounds, synthesized in plant tissues as secondary metabolites. Essential oils (EOs) are known for their antibacterial, antifungal, antiviral, insecticidal and antioxidant properties; due to their lipophilic character also it can interact with microbial membranes and achieve significant antimicrobial effect. Antioxidant properties of EOs have also been reported. Antioxidant compounds pose the ability to delay or inhibit the oxidation of lipids and other molecules by inhibiting the initiation or propagation of oxidation chain reactions Velioglu *et. al.*(1998).

MATERIALS AND METHODS

The present study was carried out during spring season of 2016 in Sericultural Research Department of Plant Protection Research Institute, Agricultural Research Center in Giza

Materials: Mulberry silkworm, *Bombyx mori* eggs (Egyptian hybride Giza). chamomil, Lavander and Orange oils (Cap. Pharm. Company) were used with 3 concentrations prepared according to Harvey and John (1898).

Method:

Silkworm Rearing Technique:

Silkworm rearing was carried out under laboratory conditions $28\pm 1^{\circ}\text{C}$ and $75\pm 5\%\text{R.H}$, According to Karishnaswami(1978). The egg card was incubated in an incubator at 25°C and $75\%\text{R.H}$ till hatching, newly hatched larvae were transferred to rearing trays, using cleaning nets for cleaning the rearing bed. At the beginning of 4th larval instar, larvae were divided into three groups, each group refer to type of oil treatment and divided into three sub group for concentrations (1%, 0.75% and 0.5%), each concentration with 3 replicates , each replicate contain 50 larvae. Mulberry leaves were dipped in different concentrations of different treatments every day for 5 minutes then left to dry and offered to infected larvae 3 times during 4th and 5th larval instars till cocooning. The control group was fed with mulberry leaves dipped in distilled water.

Bioassay :

Spores suspension of *B. thuringiensis* var. *kurstaki* were prepared in sterile 0.05% Tween 80 water solution. The mortality effects of the entomopathogenic bacteria of silkworm were performed by testing one concentration of spore suspension from *B. thuringiensis* (1×10^7 spores / ml).

Silkworm was transferred into the plastic containers (12 cm diameter and 23 cm height). Mulberry leaves were dipped in that concentration of *B. thuringiensis*

and distilled water as control. The experiment was replicated 3 times. Each replicate was provided with ten 5th instar larvae of *silkworm*, then were covered with muslin cloth for aeration. The containers were maintained in an incubator at 25 ± 2 , 60-70% RH.

Biochemical Analysis:

Haemolymph was collected from treated larvae at 6th day of 5th larval instar by removal of thoracic leg in eppendorf tubes 1.5 ml with small amount of phenyl thiourea crystal (PTU) (Mahmoud, 1988) as an anti-coagulant substance. The tubes were kept at -20°C, the blood samples were centrifuged at 10000rpm for 10 minutes at 5°C. The supernatant was assayed to determine

Aspartate Transaminase (AST) and Alanin Transaminase (ALT) activities:

Aspartate transaminase (AST) and Alanin transaminase (ALT) were determined colorimetrically according to the method of Reitman and Frankle (1957). AST transfer amino group from L-aspartate to α -keto acid (α -ketoglutaric acid), producing a new amino acid (L-glutamate) and a new keto acid (oxaloacetic acid). AST transfer the amino group from D,L alanine to α -keto acid (α -ketoglutaric acid), resulting in a new amino acid (L-glutamate) and a new Keto acid (pyruvic acid). Pyruvate or oxaloacetate reacts with 2,4-dinitrophenylhydrazine, forming pyruvate or oxaloacetate hydrazone which in alkaline medium form a brown color, which can be measured spectrophotometrically. The reaction mixture optical density was measured using spectrophotometer at 520 nm. The enzyme activity is expressed as U/gm body weight.

Lipid peroxidation (MDA):

Lipid peroxidation level was determined by using a diagnostic kit No. MD2529 which is based on the spectrophotometric method of Ohkawa *et al.* (1979) in which the malondialdehyde (MDA) released served as an index of lipid peroxidation. MDA was determined by measuring the thiobarbituric acid reactive species. The absorbance of the pink color was measured at 534nm.

Protein Carbonyl Content (PCC) :

Protein carbonyl was determined according to Levine *et al.* (1990).

Total Antioxidant Capacity:

The determination of the antioxidant capacity was measured using a diagnostic kit No. TA2513. Antioxidants in the sample react with a known quantity of exogenous hydrogen peroxide. The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual hydrogen peroxide is determined calorimetrically by an enzymatic reaction which involves the conversion of 3,5-dichloro-2-benzensulphonate to a colored product read at 505nm.

Statistical analysis:

The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigmasat, Statistical Soft-Ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test, and $p < 0.05$ was used to indicate significance between different groups (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Data recorded in Table (1) showed a significant variance between treatments in biochemical changes in hemolymph of infected silkworm as follow

Protein Carbonyl contents (PCC):

As shown in Table(1) A significant variance between treatments, the least damage were recorded for larvae fed on 0.5 and 0.75% Orange oil followed by chamomile oil with 1% (0.002, 0.004, 0.004 Nol mL⁻³), respectively, compared with other treatments (0.005, 0.008, 0.008, 0.0097, 0.13 and 0.018 Nol mL⁻³) for Chamomile 0.75%, Orange 1%, Control, chamomile 0.5%, Lavender 0.5% and Lavender 0.75%, respectively The highest damage recorded for Lavender with 1% that recorded (0.024 Nol mL⁻³).

These results go in line with Sarker *et al.* (1995) who found that the total protein content of the silk gland increased by feeding larvae on mulberry leaves supplemented with different nutrients and Muruga *et al.*(1998), proved that, the extracts of *T. terrestris*, *B. diffusa* and *B. niruri* plants revealed more protein fractions and a higher level of proteins in silk glands of silkworm.

Lipid Peroxidation (MDA):

The results indicated that, the level of Lipid peroxidation produced in hemolymph of treated infected larvae decreased in all different treatments compared with control also, in different concentrations with a significant variance, however larvae fed on mulberry leaves supplemented with (0.5% and 0.75%) Orange oil showed the least damage (118.3 and 120.7 nol mol⁻¹ of serum), respectively which supported by finding of Niesink *et al.* (1995) as they reported that free radicals can induce a wide range of effects such as membrane damage, inactivation of enzymes and cell. Also, with Abdoljala Marjani *et al.* (2012) who reported that MDA decreased by using higher doses of permanent oil, Andallu and Varadacharyulu (2003) observed that mulberry leaves significantly decreased Lipid peroxidation and increased the activity of almost all the antioxidant enzymes in all rates. Also, confirmed by Djamel (2015) who concluded that citrus EOs could be used as a way of inhibiting the growth of common causes of food poisoning and used as a natural preservatives to reduce Lipid Peroxidation. Reddy and Venkatappa (2016) who approved that, larvae Infected with *Staphylococcus aureus* in fifth larval instar causes significant increase in MDA concentration, acid phosphatase and phenoloxidase activity, Also Dubovskiy *et al.*(2008) who confirmed that, infection with *Bacillus thuringiensis* cause increasing the activities of some SOD, GST and MDA.

Total antioxidant capacity:

The essential oils exhibited a significant variance between treatments in its ability to donate a hydrogen atom to recover the damage occurred due to bacterial infection where, hemolymph of larvae fed on mulberry leaves supplemented with 0.5% and 0.75% orange oil recorded the highest value without a significant variance between them followed by 1% of chamomile (25.17, 24.57 and 23.87 Mm/L), respectively. The least value recorded for control (15.10 Mm/L). Values of other concentrations arranged (23.83, 23.83, 23.50, 21.53, 16.90 and 16.67 Mm/L) for 1%, orange, (0.75% and 0.5%, chamomile), (0.5%, 0.75% and 1% Lavender), respectively. The previous data confirmed by Suh *et al.*(2010) who suggested that, the development of new bioactive products with potential applications to reduce oxidative stress in living organisms involving ROS as well as play a vital role in insect organisms against oxidative damage of undesirable conditions. Also, Neuza *et*

al. (2016) who approved that the orange seed oils can be used as specialty oils in diet, as they contain considerable amounts of bioactive compounds and antioxidants, Malacrida *et al.* (2012) who found that, the highest antioxidant activity resulted from the oil obtained from Pera-rio orange seeds that contain higher DPPH scavenging activity. A possible role for orange peel oil as a dietary antioxidant in decreasing lipid peroxidation in blood plasma could be associated with high concentrations of terpenic aldehydes. Lado *et al.*, 2004; Chikhi *et al.*, 2012 who reported that the major constituent of orange peel oil is limonene and its antioxidant activities have been reported Roberto *et al.*, 2009.

Table(1): Effect of feeding infected larvae with mulberry leaves supplemented with different concentrations of essential oils in biochemical changes of heamolymph.

Treatments	Parameters	PCC NoI mL ⁻³	MDA NoI mL ⁻³	AC Mm/L
Orange Oil	1%	0.008 ^e	155.3 ^d	23.83 ^c
	0.75%	0.004 ^h	120.7 ^{fg}	24.57 ^{ab}
	0.5%	0.002 ⁱ	118.3 ^g	25.17 ^a
Chamomile Oil	1%	0.004 ^g	131.6 ^{ef}	23.87 ^{bc}
	0.75%	0.005 ^f	138.3 ^e	23.83 ^c
	0.5%	0.0097 ^d	162.7 ^d	23.50 ^c
Lavender Oil	1%	0.024 ^a	256 ^a	16.67 ^e
	0.75%	0.018 ^b	210.3 ^b	16.90 ^e
	0.5%	0.013 ^c	186.7 ^c	21.53 ^d
Control		0.008 ^e	263.7 ^a	15.10 ^f
P		<.0001		<.0001
LSD 0.05		0.0001		0.733

Alanin Transaminase (ALT):

Data exhibited that, the larvae fed on mulberry leaves supplemented with orange oil recorded the least values between 3 concentrations with a significant variance, they recorded (97.33, 115.66, 177.33 U/L) for 0.5, 0.75 and 1%, respectively. While larvae fed on mulberry leaves supplemented with Lavender oil showed the highest value with significant variance between them (212.33, 232.33, 258 U/L), respectively compared to other treatment. No significant variance was observed between larvae fed on mulberry leaves supplemented with chamomile oil (184.66, 175.33, 171.33 U/L) for the previous concentrations.

Aspartate Transaminase(AST):

Obtained data in Table (2) cleared that, there was a significant variance between treatments. Larvae fed on mulberry leaves supplemented with 0.5, 0.75 and 1% Lavender oil showed the highest value (413.66, 392.66, 428.66, U/L) respectively, while the control recorded (434.66 U/L). The least value recorded for larvae fed on orange oil with 0.5 and 0.75% concentrations(272, 278.66 U/L). From the previous results bacterial infection increase the level of transaminases enzymes and dealing with therapeutic essential oil recovered the damage the more effective treatments were 0.5 and 0.75% orange oil, respectively. These results in agreement with Kaneko *et al.*(1997) who reported that, the activities of ALT and AST have been shown to be increased following injury. Inagaki *et al.* (2012) concluded that, ALT activity in the silkworm heamolymph was increased by the injection of various

cytotoxic drugs. Reddy and Venkatappa (2016) cleared that, Protein concentration in haemolymph markedly increased in infected larvae following appearance of disease symptoms. *Staphylococcus aureus* has ability to altering antioxidant levels such as superoxide. Khalil *et al.*, 2015 approved that some enzymes ALT, AST were increased in infection with CCL4 and decreased by treatment with peppermint oil.

Table(2): Effect of feeding infected larvae with mulberry leaves supplemented with different concentrations of essential oils on ALT and AST.

parameters		ALT	AST
Treatments		U/L	U/L
Orange Oil	1%	177.33 ^d	387.66 ^c
	0.75%	115.66 ^e	278.66 ^e
	0.5%	97.33 ^f	272 ^e
Chamomile Oil	1%	171.33 ^d	334 ^d
	0.75%	175.33 ^d	386 ^c
	0.5%	184.66 ^d	392 ^c
Lavender Oil	1%	258 ^a	428.66 ^{ab}
	0.75%	232.33 ^b	413.66 ^b
	0.5%	212.33 ^c	392.66 ^c
Control		268 ^a	434.66 ^a
P		<.0001	<.0001
LSD 0.05		13.7	16.8

Conclusion: It could be suggested that, bacterial infection produce damage in silkworm haemolymph as, MDA, PCC and increase ALT,AST. Orange oil with the least concentration repair the damage occurred inside silkworm and reduce ALT, AST due to it possess antioxidant potential which could be protective against Oxidative damage.

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ARABIC SUMMARY

كفاءة بعض الزيوت العلاجية كمضاد للبكتيريا و مضاد للأكسدة علي بعض التغيرات البيوكيميائية في دودة الحرير التوتية المريضة *Bombyx mori* L.

مروى نبيل مصطفي و إيمان محمود حسان.

قسم بحوث الحرير، معهد وقاية النباتات، مركز البحوث الزراعية

تم إصابة دودة الحرير التوتية ب بكتريا *Bacillus thuringiensis* و معاملتها ب ثلاث تركيزات (0.5, 0.75, 1%) لثلاث أنواع من الزيوت (citrus specific, Orange, Chamomile and Lavander) لتقييم قدرة الزيوت كمضادات للأكسدة و مضاد للبكتيريا علي التغلب علي الضرر الناتج عن الإصابة عن طريق تقدير بعض التغيرات الكيميائية مثل Lipid Peroxidation (MDA), Protein Carbonil Content (PCC), و أيضا Antioxidant Capacity (ALT), (AST) . و قد أظهرت النتائج أن التركيزين (0.5, 0.75 %) من زيت الموالح كانا أكثر تأثيرا تبعه التركيزين (1, 0.75%) من زيت الكاموميل حيث سجلت هذه التركيزات أقل ضرر في (MDA), (PCC) و أعلى قيمة في قدرتها كمضادة للأكسدة بينما التركيزين (0.5, 0.75%) من زيت الموالح أظهرت أقل قيمة في (ALT), (AST) مقارنة ب الزيوت الأخرى و الكنترول.