J. Plant Prot. and Path., Mansoura Univ., Vol.5 (9): 855-864, 2014

STUDY OF PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN MULBERRY SILKWORM FEEDING ON MULBERRY LEAVES SUPPLEMENTED WITH CAMPHOR HONEY AND OIL.

Eman M. Hassan; M.S. Ebrahem and Rehab H. Taha
Sericulture Research Department (SRD), Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC), Egypt.

ABSTRACT

This study was conducted to evaluate the physiological and biochemical changes, [glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), total soluble protein (TSP), lipid peroxidation (MDA) and protein carbonyl content (PCC)] in haemolymph of mulberry silkworm *Bombyx mori* larvae were reared on mulberry leaves treated with camphor honey and oil as a nutritional additives in the 4th and 5th instars with five concentrations. Results showed that at 4% & 5% concentrations of honey and 1% & 2% concentrations of oil exhibited the best results compared with control; larvae reared on leaves enriched with these concentrations of honey and oil showed a significant increase in GPT, GOT and TSP means and a significant decrease in free radicals (MDA & PCC) means compared with control.

INTRODUCTION

Silkworm eats only mulberry leaves to make its cocoon, producing the silk. Mulberry leaves are rich in proteins and amino acids; therefore, an increase in proteins level of mulberry leaves may lead to improvements in silk productivity. The production of high quality and quantity of natural silk depends mainly on larval feeding, suitable environmental conditions and protection from diseases (Machii and Katagiri, 1991; Parra,1991; Saha *et al*., 1995 and Dechu *et al*., 1997). The nutritional value of the mulberry leaves can be improved by enriching them with extra nutrients like glucose, glycine, egg albumin, molasses, etc., was found to increase the larval growth and improve cocoon characteristics (Sengupta *et al*., 1992).

Honey has been used as a medicine since ancient times in many cultures. The use of honey as therapeutic substance has been rediscovered by the medical profession in more recent times and its gaining acceptance as an antibacterial agent for treatment of some diseases. As a dressing on wounds, honey provides a moist healing environment, rapidly clears infection, deodorizes and reduces inflammation, edema and exudation (Efem, 1988 and Molan, 2001). Chemically, it is composed primarily of sugars and many other potentially biologically active components, such as antioxidants, which display antimutagenic activity. Actually honey varies according to their plant origin and the conditions of their production ((Wang *et al*., 2002 and Bogdanov, 1997). Camphor honey has a dark color and characteristic flavor. Previous studies reported the norisoprenoids, monoterpenes, and other volatile constituents of honey. Another study reported the flavor and free amino acid composition of camphor honeys and found diketones,

hydroxyketones, 3-hexanal, sulfur compounds, and alkanes that were characteristic of the camphor samples, as well as a high content of proline. In addition, the presence of a small amount of dehydrovomifoliol, a characteristic compound of heather honey, was also reported in camphor honey (<1 mg/kg of honey) (Ha¨usler and Montag, 1991; Bouseta *et al*., 1996 and D'Arcy *et al*., 1997).

Camphor oil and its major component 1,8-cineole have a variety of antimicrobial, immune stimulatory, anti-inflammatory, antioxidant and even analgesic and spasmolytic effects. Antimicrobial effects involve a range of bacteria, viruses and fungi. Although other plant oils may be more microbiologically active, the safety of moderate doses of CO and its broad-spectrum antimicrobial action make it an attractive alternative to pharmaceuticals (Singh *et al.*, 2009 and Angela *et al*., 2010). The successful antioxidant is the one that can prevent formation of free radicals, covert oxidants to less toxic species (Krinsky, 1992). Grune and Davies (1997) have described mechanism of antioxidant defense against free radicals; they stated that the degradation of oxidized proteins is an essential part of antioxidant defenses against free radical attack, selective degradation of oxidatively damaged proteins allow proteolytic systems to function directly in the removal of useless cellular debris and therefore prevent the accumulation of potentially toxic fragments or large aggregates of cross-linked proteins.

Lipid peroxidation is the most studied biologically relevant free radical reaction. It is initiated by the attack on a fatty acid that has a sufficient reactivity to abstract a hydrogen atom from a methylene carbon in the side chain. The hydrogen atom has a single electron and its removal leaves behind an unpaired electron on the carbon atom to which it was originally attached. The resulting carbon centered lipid radical can have several fates, but the most likely one in aerobic cells is to undergo molecular rearrangement, followed by reaction with O_2 to give peroxy radical which can combine with each other if they meet or can attack membrane protein (Halliwell and Chirico, 1993). Protein carbonyl content (PCC) is the most widely used marker of oxidative modification of protein. There are several methodologies for the quantitation of PCC; in all of them 2,4-dinitrophenyl hydrazine is allowed to react with the protein carbonyls to form the corresponding hydrazone, which can be analyzed optically by radioactive counting or immunohistochemically (Chevion *et al*., 2000)

The present work was based on evaluation of the effects of camphor honey and oil as nutritional additives on some physiological and biochemical aspects such as; Glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT) enzyme activities and Total soluble protein (TSP) during the 4^{th} and 5^{th} larval instars. In the biochemical aspects, it was based on evaluation of the oxidative stress or biological damage occured in lipids and proteins of larval haemolymph because of free radical production during the 4^{th} and 5^{th} larval instars of the silkworm. Lipid peroxidation (MDA) and protein carbonyl content (PCC) are the biomarkers for this determination.

MATERIALS AND METHODS

The present study was carried out during the spring autumn of 2013 in the laboratory of Sericulture Research Department of Plant Protection Research Institute, Sharkia Branch, Agriculture Research Center. It aimed to investigate the effects of camphor honey and oil as nutritional additives on some biochemical and physiological aspects during the 4th and 5th larval instars of the silkworm.

I- Materials:

1-Mulberry silkworm, *B. mori* eggs (Egyptian hybrid Giza).

2-Camphor honey prepared by dissolving 50 ml of honey in 1000 ml of distilled water to prepare a concentration of 5%; 40 ml of honey to prepare a concentration of 4%; and so the remaining concentrations to 1%.

3- Camphor oil from *Eucalyptus* sp. leaves. Prepared by (Harvey and John 1898).

II. Methods:

Silkworm rearing technique:

Rearing of silkworm was carried out under laboratory conditions (28 ± 2°c and 70 ± 5% R.H.) without any adjustment or changes in temperature or relative humidity according to the technique of Krishnaswami (1978). The larval bed was cleaned daily. Cleaning net was used for removing the remained dried food and feces. Chicken egg cartons plates were used as montages for cocoon spinning (Zannoon and Shadia 1994).

Larvae under investigation were divided into two groups. Every group was divided into five subgroups to feed on mulberry leaves supplemented with camphor honey or camphor oil concentrations. One group of the two main groups was fed on the first day of the 4th instar after molting and the other group on the first day of the 5th instar, using 3 replicates (100 larvae) for each concentration. Mulberry leaves were dipped in the five concentrations of treated camphor oil and honey for 5 minutes and left to dry then offered to larvae. The control group of leaves was treated only with distilled water.

physiological and Biochemical measurements:

Preparation of samples for biochemical assay:-

Samples were made by removing one of the thoracic legs of the 5th instar larvae and bending the body to expose the sternum at the position of the removed leg. This ensured proper drainage of the haemolymph, and avoided any risk of internal organs to be destructed. The haemolymph of each treatment was collected in eppendorf tubes 1.5 ml with small crystal of phenyl thiourea (PTU) to prevent melanization of sample, (Mahmoud, 1988). The tubes were kept at -20°C. The blood samples were centrifuged at 10000 r.p.m for 10 minutes at 5°c. The supernatant was immediately assayed to determine glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) activities according to the method of Reitman and Frankel (1957), total soluble protein (TSP) as described by Gornall *et al.* (1949), Lipid peroxidation (MDA) according to the method of Sharma and Wadhwa (1983) and protein carbonyl content (PCC) as described by Levine *et al.* (1990).

Statistical analysis:-

The obtained results were subjected to statistical analysis of variance and the data were presented as means according to Snedecor and Cochran (1982) methods using software COSTAT program.

RESULTS AND DISCUSSION

Results of this work revealed a significant increase in physiological and biochemical aspects in both 4th and 5th instars of silkworm than control, detailed results were as follows:

1- Glutamic pyruvic transaminase (**GPT):**

As shown in Table (1) data revealed that larvae fed mulberry leaves supplemented with camphor honey and oil with different concentrations in the 4th instar showed a significant increase in protein transaminase enzyme GPT in all concentrations of honey and oil than control especially 4% and 5% concentrations of honey (3.90 & 3.95 mg Pyruvate/ml) respectively than control 0.90 mg Pyruvate/ml and 1% and 2% concentrations of oil (5.77 & 4.55 mg Pyruvate/ml) respectively.

Table (1) : Effect of camphor honey and camphor oil on some physiological and biochemical aspects of the 4th instar of silkworm *B. Mori*.

Treatment	Conc.	GPT ± SE	GOT ± SE	TSP ± SE	MDA± SE	PCC ± SE
Eucalyptus honey	1%	1.49± 0.09 f	3.68± 0.14 c	11.77± 0.11 de	28.89± 0.92 ab	0.023± 0.0016 d
	2%	1.51± 0.04 f	3.68± 0.19 c	11.77± 0.91 de	27.78± 0.59 b	0.027± 0.001 d
	3%	1.51± 0.05 f	8.95± 0.13 a	15.10± 0.87 cd	24.44± 0.79 bc	0.027± 0.0007 d
	4%	3.90± 0.08 c	8.95± 0.09 a	18.43± 1.07 bc	10.00± 0.50 e	0.012± 0.0007 e
	5%	3.95± 0.06 c	8.95± 0.16 a	21.77± 0.78 ab	5.56± 0.42 e	0.011± 0.0007 e
Mean		2.47	6.84	15.77	19.33	0.020
Eucalyptus oil	1%	5.77± 0.11 a	8.95± 0.06 a	25.10± 1.13 a	10.00± 0.33 e	0.012± 0.0003 e
	2%	4.55± 0.15 b	4.58± 0.25 b	21.77± 0.11 ab	18.89± 1.15 d	0.014± 0.001 e
	3%	3.34± 0.19 d	3.68± 0.16 c	15.10± 0.38 cd	20.00± 1.55 cd	0.042± 0.001 c
	4%	3.39± 0.16 cd	3.68± 0.15 c	18.43± 0.40 bc	20.00± 1.77 cd	0.027± 0.0013 d
	5%	2.73± 0.11 e	3.68± 0.12 c	18.43± 0.29 bc	22.22± 1.07 cd	0.055± 0.001 b
Mean		3.96	4.91	19.77	18.22	0.030
Control		0.90± 0.08 g	3.68± 0.16 c	8.43 ± 0.70 e	33.33± 1.04 a	0.064± 0.001 a
LSD 0.05 (conc.)		0.561 ***	0.775 ***	3.601 ***	5.181 ***	0.005 ***

In the 5th instar as in Table (2) the 4% and 5% concentrations of honey exhibited significant increase in GPT means (4.55 & 4.58 mg Pyruvate/ml) respectively. Also the 1% and 2% concentrations of oil recorded signigicantly higher means (4.55 & 4.53 mg Pyruvate/ml) than control 0.61 mg Pyruvate/ml.

2- Glutamic oxaloacetic transaminase (**GOT):**

In the 4th instar, data exhibited a significant increase (Table, 1) in GOT means of larvae fed on mulberry leaves supplemented with camphor honey and oil especially the concentrations of honey 4% and 5% (8.95 mg pyruvate/ml) than control (3.68 mg Pyruvate/ml) and the 1% and 2% concentrations of oil (8.95 & 4.58 mg Pyruvate/ml) respectively.

Table (2): Effect of camphor honey and camphor oil on some physiological and biochemical aspects of the 5th instar of silkworm *B. Mori*.

Treatment	Conc.	GPT ± SE	GOT ± SE	TSP ± SE	MDA ± SE	PCC ± SE
***Eucalyptus* honey**	**1%**	2.17± 0.08 bc	3.68± 0.09 c	21.77± 0.44 de	24.44± 0.29 bc	0.073± 0.0013 ab
	2%	2.72± 0.10 b	8.95± 0.19 a	15.10± 0.50 f	25.00± 0.62 b	0.059± 0.005 cd
	3%	4.54± 0.15 a	3.68± 0.05 c	25.10± 1.17 cd	22.22± 1.08 bcd	0.041± 0.0007 e
	4%	4.55± 0.14 a	4.55± 0.19 b	28.43± 1.11 bc	21.11± 0.48 cde	0.014 ± 0.0007 f
	5%	4.58± 0.14 a	8.95± 0.02 a	38.43± 1.73 a	17.78± 0.77 ef g	0.011± 0.0003 f
Mean		3.71	5.96	25.77	22.11	0.040
***Eucalyptus* oil**	**1%**	4.55± 0.14 a	8.95± 0.06 a	38.43± 0.69 a	15.56± 0.37 g	0.012± 0.0007 f
	2%	4.53± 0.16 a	8.95± 0.14 a	31.77± 0.82 b	17.00± 0.67 f g	0.036± 0.001 e
	3%	3.95± 0.14 a	3.68± 0.18 c	25.10± 0.45 cd	20.00± 0.48 de f	0.055 ± 0.0007 d
	4%	3.95± 0.09 a	8.95± 0.17 a	21.77± 0.81 de	24.00± 0.79 bc	0.067± 0.0013 bc
	5%	1.51± 0.18 c	8.95± 0.09 a	18.43± 1.22 ef	22.22± 0.46 bcd	0.073± 0.0007 ab
Mean		3.70	7.90	27.10	19.76	0.049
Control		0.61± 0.12 d	3.68± 0.16 c	15.10± 0.76 f	30.00± 0.90 a	0.077 ± 0.001 a
LSD 0.05 (conc.)		0.681 ***	0.679 ***	4.869 ***	3.403 ***	0.009 ***

As shown in Table (2), nearly parallel results were noticed; the 5th instar larvae reffered a significant increase in GOT means than control in most concentrations. The 4% and 5% concentrations of honey (4.55, 8.95 mg Pyruvate/ml) respectively than control (3.68 mg Pyruvate/ml) and the 1% and 2% concentrations of oil (8.95 mg Pyruvate/ml).

Serafino *et al*. (2008) suggested that, camphor oil might be useful as a cell-mediated immuno-regulatory agent in immune-suppressive pathologies, infectious diseases.

3-Total soluble proteins (TSP):

Obtained data in Table (1) cleared that larvae of the 4th larval instar fed on mulberry leaves treated with the 4% and 5% concentrations of camphor honey recorded the highest TSP values (18.43 & 21.77 mg/ml of serum) compared with control (8.43 mg/ml of serum). The 1% and 2% concentrations of camphor oil revealed higher TSP means (25.10 & 21.77 mg/ml of serum) respectively than other concentrations and control.

Larvae of the 5th larval instar fed on camphor honey and oil exhibited significant increase in TSP values than control (15.10 mg/ml of serum) especially the concentrations of honey 4% and 5% (28.43 & 38.43 mg/ml of serum) respectively and the 1% and 2% concentrations of camphor oil (38.43 & 31.77 mg/ml of serum) respectively.

These results explained with Das *et al.* (2004) revealed that detection of the highest level of soluble proteins after fourth molt and simultaneous gradual increase in the free amino acids in the silk gland up to the end of larval stage reflected the possibility of active function of the protein synthesis mechanism in the silk gland. Nagata and Kobayashi (1990), cleared that, the nutritional richness in the diet influenced the accumulation of stored proteins in the haemolymph of the silkworm larvae i.e. the quantity of stored proteins in the silkworm larvae fed on low protein were less than the standard diet ones, but the larvae fed on optimal level of protein showed a higher levels of storage protein.

4- Lipid peroxidation (MDA):

The levels of lipid peroxidation of larval haemolymph in the 4th and 5th larval instars of larvae reared on enriched mulberry leaves with either camphor honey or oil with different concentrations compared with control were shown in Tables (1, 2). In the 4th instar, the MDA content in larvae reared on mulberry leaves supplemented with different concentrations of camphor honey were significantly decreased as shown in Table (1) especially the 4% and 5% concentrations (10 & 5.56 nmol./ ml of serum) respectively compared with control (33.33 nmol./ ml of serum). In case of camphor oil as shown in Table (1), the concentrations 1% and 2% were significantly decreased MDA content (10 & 18.89 nmol./ ml of serum) respectively compared with control.

As shown in Table (2), the 5th instar larvae reared on mulberry leaves enriched with camphor honey and oil revealed a significant decrease in means of MDA content in all concentrations compared with control (30 nmol./ ml of serum), especially the 4% and 5% concentrations of honey (21.11 & 17.78 nmol./ ml of serum) respectively, and the concentrations of oil 1% and 2% (15.56 & 17 nmol./ ml of serum) respectively.

Bee-honey may be a strong factor for antioxidant activity and free radical depression. These biochemical effects of bee-honey feeding might be owing to the presence of various components which represent antioxidant and antibacterial agents such as phenolics, gluconic acid, ascorbic acid, hydroxyl methyl furaldehyde and the enzymes glucose oxidase, catalase and peroxidase as documented by Wang *et al*. (2002) who stated that diatery supplementation of these components to larvae improved their immunity to oxidative stress and enhanced larval vitality. These results are in agreement

also with Gheldof *et al.* (2002) stated that, antioxidant analysis of the different honey fractions suggested that the water – soluble fraction contained most of the antioxidant components including gluconic acid; ascorbic acid; hydroxymethylfuraldehyde and the enzymes glucose oxidase, catalase and peroxidase.

5- Protein carbonyl content (PCC):

As illustrated in Tables (1, 2), that refer to the level of protein carbonyl content in larval haemolymph of the 4th and 5th larvae reared on mulberry leaves supplemented with camphor honey and oil. Both honey and oil concentrations decrease PCC means significantly compared with control.

In the 4th instar, as shown in Table (1) the 4% and 5% concentrations of camphor honey exhibited significantly the least PCC (0.012 & 0.011 nmol./ ml of serum) respectively compared with control (0.064 nmol./ ml of serum). At the same time, all camphor oil concentrations decrease PCC in larval haemolymph compared with control especially the concentrations 1% and 2% (0.012 & 0.014 nmol./ ml of serum) respectively.

In the 5th instar, nearly parallel results were noticed as in Table (2); all camphor honey and oil concentrations decrease PCC means significantly compared with control (0.077 nmol./ ml of serum).

The above results were supported with Agarwal and sohal (1993) and Sohal *et al*. (1995) postulated that proteins in tissues of old animals are more sensitive to oxidative damage than tissues of young animals and factors or physiological conditions that lead to an increase in the life span of animals also cause a decrease in the intracellular levels of oxidized proteins (measured as protein carbonyl content), and vice vesa. The carbonyl content of proteins in widely different animal species and tissues increases almost exponentially as a function of animal age (Stadtman and Levine, 2001).

Conclusively, *Eucalyptus* oil and its major component 1,8-cineole have a variety of antimicrobial, immune stimulatory, anti-inflammatory, antioxidant and even analgesic and spasmolytic effects. Antimicrobial effects involve a range of bacteria, viruses and fungi. Although other plant oils may be more microbiologically active, the safety of moderate doses of EO and its broad-spectrum antimicrobial action make it an attractive alternative to pharmaceuticals. EO can react biologically as an antioxidant; The free radical scavenging capability of *E. tereticornis* oil from fresh or decaying leaves and separate oil constituents was studied against superoxide anion and hydroxyl radical. Both oils showed strong antioxidant ability (Singh *et al.*, 2009 and Angela *et al*., 2010).

According to the above results, it can be concluded that, mulberry leaves fortified with *Eucalyptus* honey in increase concentrations (the 4th & 5th concentration) and *Eucalyptus oil* (the 1st & 2nd concentration) were proved to be more efficient in rearing silkworm as it improved protein enzymes activities (GPT & GOT) and total soluble protein of the larvae and this improvement was positively correlated with decreased free radicals (MDA & PCC) and increased antioxidant defense in the larval haemolymph, hence, the viability of larvae increased, the silk yield in commercial silkworm rearing increased also.

REFERENCES

Agarwal, S. and sohal, R. S. (1993):"Relationship between aging and susceptibility to protein oxidative damage". Biochem. Biophys. Res. Commun. 194, pp. 1203-1206.

Angela, E; Sadlon, N. D; Davis, W. And Lamson, M.S. (2010): "Immune-modifying and antimicrobial effects of Eucalyptus oil and simple inhalation devices". Altern. Med. Rev. 2010; 15 (1) :33-47.

Bogdanov, S. (1997):"Nature and origin of the antibacterial substances in honey". Lebensmittal Wissenschaft and Technology. 30:7, 748-753.

Bouseta, A.; Scheirman, V. and Collins, S. (1996): "Flavor and free amino acid composition of lavender and eucalyptus honeys." *J. Food Sci*., *61*, 683-687, 694.

Chevion, M.; Berenshtein, E. and Stadtman, E. R. (2000):"Human studies related to protein oxidation: protein carbonyl content as a marker of damage". Free Radic. Res. 2000 Nov.; 33 Suppl: S99-108.

D'Arcy, B. R.; Rintoul, G. B.; Rowland, C. Y. and Blackman, A. J. (1997):"Composition of Australian honey extractives. I. Norisoprenoids, monoterpenes, and other natural volatiles from blue gum (*Eucalyptus leucoxylon*) and yellow box (*Eucalyptus melliodora*) honeys." *J. Agric. Food Chem*. *45*, 1834-1843.

Das, P.; Bhattacharya, P. R.; Unni, B. G. and Deka, P. C. (2004): Consumption and utilization of food (*Persea bombycina*) in relation to larval development and silk protein biosynthesis by semi-domesticated muga silkworm, *Antheraea assama*. *Indian J. Seric.*, 43 (1):57-65.

Dechu, P. S.; Govindon, R.; Devaiah, M. C. and Swamy, T. K. N. (1997):" Effect of antibiotics on growth and cocoon Parameters of silkworm *Bombyx mori* L. mysore". J. Agric. science, 31 (1): 41-46.

Efem, S. E. (1988):"Clinical observation on the wound healing properties of honey". Br J Surg. Jul; 75 (7): 679-81.

Gheldof, N.; Wang, X. H. And Engeseth, N.J. (2002):"Identification and quantification of antioxidant components of honeys from various floral sources". J. Agric. Food chem. Oct. 9;50 (21): 5870-7.

Gornall, A. G.; Bardawil, C. D. and David, M. M. (1949): Determination of serum protein by means of bruit reaction. *Journal of Biological Chem.,* 177: 751-766.

Grune, T. and Davies, K. J. (1997):"Breakdown of oxidized proteins as a part of secondary antioxidant defenses in mammalian cells". Biol. Factors, 6 (2): 165-72.

Ha¨usler, M. and Montag, A. (1991):"Flavor related constituents of honey. IV. Occurrence and honey-type-specific distribution of the flavor precursor (S)-dehydrovomifoliol." *Dtsch. Lebensm.* Rundsch.87, 35-36.

Halliwell, B. and Chirico, S. (1993):"Lipid peroxidation: Its mechanism, measurement and significance". Am. J. Clin. Nutr. 57:715S-25S.

Harvey, W. F. and John, U. L. (1898): King's American Dispensatory. 19 Edition, Third Revision Vol.2, The Ohio Valley, Company.

Krinsky, N. I. (1992):"Mechanism of action of biological antioxidants". Proc. Soc. Exp. Biol. Med., 200: 248-254.

Krishnaswami, S. (1978): New technology of silkworm rearing. *Central Sericulture Researches and Training, Inst*., Mysore Bull., 2:1- 10.

Levine, R. L.; Garland, D.; Oliver, C. N. and Climent, I. (1990):"Detemination of carbonyl content in oxidatively modified proteins". Methods Enzymol, 186, 464-474.

Machii, H. and Katagiri, K. (1991):"Varietal differences in nutritive values of mulberry leaves for rearing silkworms". JARQ, 25: 202-208.

Mahmoud, S. M. (1988): Activation of silk secretion by silkworm, *Philosamia ricini* and *Bombyx. mori* after applying antibiotics. Ph. D. Thesis, Faculty Agric., Cairo University, Egypt.

Molan, P. C. (2001):"Potentialof honey in the treatment of wounds and burns". Am. J. Clin. Dermatol. 2(1): 9-13.

Nagata, M. and Kobayashi, J. (1990): Effect of nutrition on storage protein concentrations in the larval haemolymph of the silkworm *Bombyx mori*. J.Seric. Science Jpn., 59:469-474.

Parra, J.R.P. (1991):"Consumoe utilizaçào de alimentos por insetos". In: PANIZZI, A.R., Parra, J.R.P. Ecologia nutricional de insetose suas Implicaçòes no manejo de pragas. 359 P. Manole, Sào Paulo.

Reitman, S. M. D. and Frankel, S. (1957): A colorimetric method for the determination of serum glutamic-oxaloacetic and glutamic-pyruvic transaminase. Ann. J. Clin. Pathol., 28: 56-62.

Saha, A. K.; Rahaman, M. S.; Saha, B. N.; Rahman, M. and Das, D. K. (1995):"Effect of antibiotics on the growth and rearing Performance of silkworm *Bombyx mori* L.". J. Asiatic Soc. Bangladesh Sci., 21 (2): 289-292.

Sengupta, K.; Singh, BD. and Mustafi, JC. (1992): Role of vitamins in silkworm nutrition. Indian J. of Seri. 11(1): 11-19.

Serafino A; Sinibaldi v. P and Andreola F. (2008):" Stimulatory effect of Eucalyptus essential oil on innate cell-mediated immune response." BMC Immunol; 9:17.

Sharma, S. P. and Wadhwa, R. (1983):"Effect of butylated hydroxy tolwene on the life span of *Drosophila bipectinata*". Mechanisms of aging and development, 23: 67-74.

Singh H. P; Mittal S. And Kaur S. (2009): " characterization and antioxidant activity of essential oils from fresh and decaying leaves of *Eucalyptus tereticornis*". J. Agri. Food Chem. 2009; 57:6962-6966.

Snedecor, G. W. and Cochran, W. G. (1982): *Statistical Methods*. Iowa State University Press, Amer. Iowa, USA.

Sohal, R. S.; Agarwal, S. and Sohal, B. H. (1995):"Oxidative stress and aging in the Mongolian gerbil (*Meriones unguiculatus*)". Mech. Ageing Dev. 81, pp. 15-25.

Stadtman, E. R. and Levine, R. L. (2001):"Oxidative modification of proteins during aging". Experimental Gerontology, 36, 1495-1502.

Wang, X. H.; Andrae, L. and Engeseth, N. J. (2002):" Antimutagenic effect of various honeys and sugars against Trp-p-1." J. Agric. Food Chem. 6; 50 (23), 6923:8.

Zannoon, A. A. and Shadia M. O. (1994): Efficiency of certain natural materials as mountages for mulberry silkworm, *Bombyx mori* L. Egypt. J. Appl. Sci., 9 (8): 691-696.

دراسة التغيرات الفسيولوجية والبيوكيميائية لديدان الحرير التوتية تغذت على ورق التوت المدعم بعسل الكافور وزيت الكافور

إيمان محمود حسان ، محمود سعد ابراهيم و رحاب حسنى طه
قسم بحوث الحرير، معهد بحوث وقاية النباتات، مركز البحوث الزراعية، الدقى، مصر.

أجريت هذه الدراسة لتقييم بعض التغيرات الفسيولوجية والبيوكيميائية لديدان الحرير التوتية نتيجة لتغذيتها على أوراق التوت المعاملة بعسل وزيت الكافور فى العمرين الرابع والخامس بتركيزات مختلفة. وقد تمت دراسة نشاط الأنزيمات الناقلة للبروتين (GOT, GPT) ، والمحتوى الكلى للبروتين (TSP) ، مستوى العوامل المؤكسدة كمحتوى ثنائي ألدهيد المالون (MDA) ، ومحتوى البروتين الكربونيلى (PCC) فى دم اليرقات.

وقد لوحظ تأثر الصفات الفسيولوجية والبيوكيميائية السابقة الذكر بصورة أيجابية حيث تحسن نشاط الأنزيمات الناقلة للبروتين والمحتوى الكلى للبروتين بصورة ملحوظة كما انخفض مستوى العوامل المؤكسدة بنسبة كبيرة فى جميع المعاملات نتيجة للتغذية بعسل وزيت الكافور بتركيزاته المختلفة وخاصة التركيزين الرابع والخامس للعسل (٤%، ٥%) والتركيزين الأول والثانى لزيت الكافور (١%، ٢%) مقارنة بمجموعة الكنترول فى العمرين الرابع والخامس.