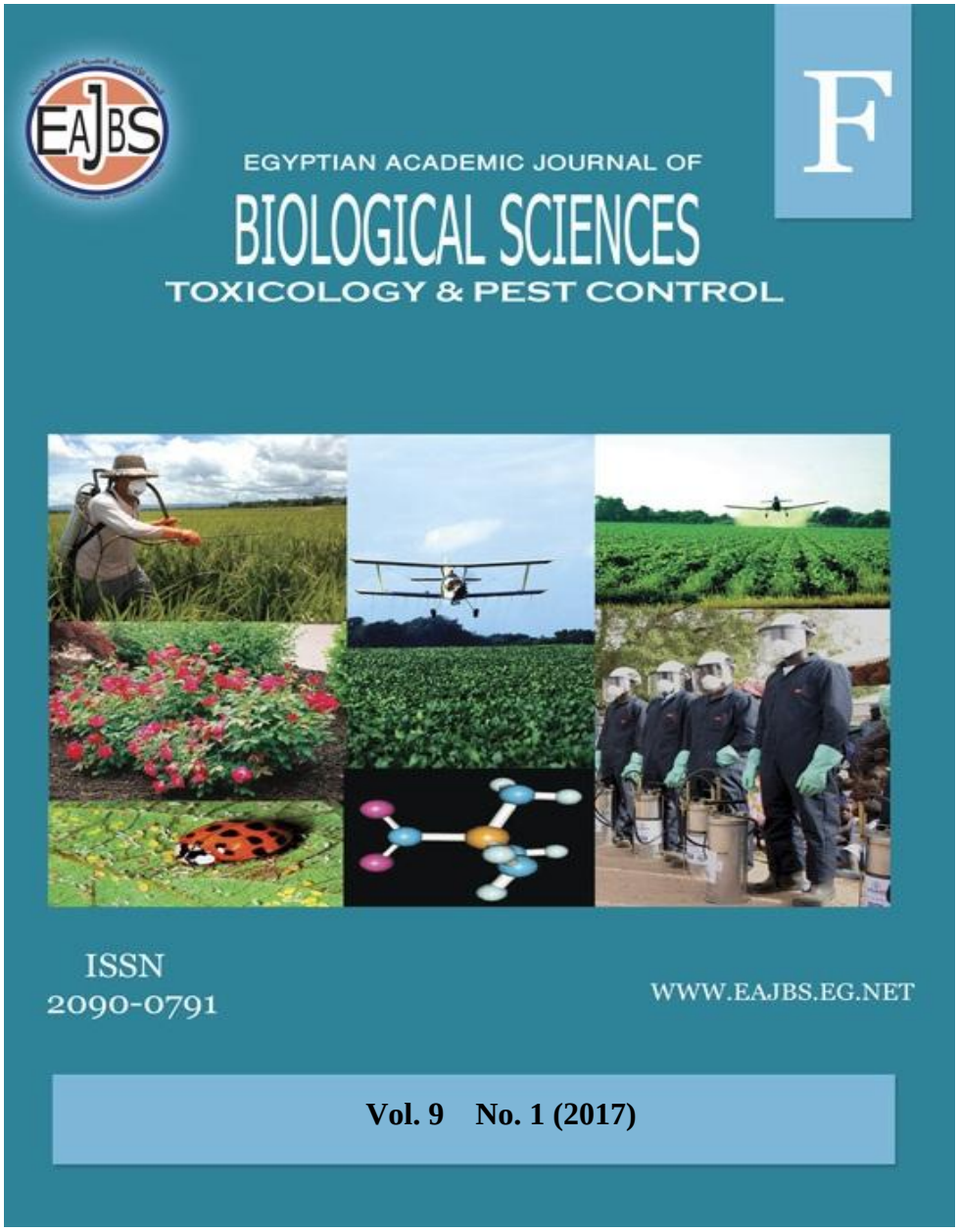

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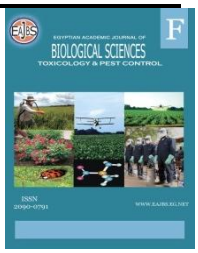

Evaluation of Two Fixed Oils *Lepidium sativum* and *Trigonella foenum-graecum* and the Volatile Oil *Cupressus macrocarpa* as Repellent Agent on Two- Spotted Spider Mite, *Tetranychus urticae* Koch Infesting Mulberry Trees and Its Impact on Silk Worm, *Bombyx mori* l.

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ABSTRACT

Laboratory bioassays of the plant fixed oils, *Lepidium sativum*, *Trigonella foenum-graecum* and volatile oil, *Cupressu smacrocarpa* as repellent against adult females of two spotted spider mite, *Tetranychus urticae* Koch which infesting mulberry trees, the predominant food source for silkworm, *Bombyx mori*. The results indicated the oils have repellent effect that may be useful in the future to control of the phytophagous mites.

Moreover, the effect of these oils was evaluated on some biological, technological and biochemical aspects of *Bombyx mori* L. All investigated oils increased larval body weight, fresh cocoon weight, cocoon shell weight, silk filaments weight and decreased the mortality percentage as compared to control. Furthermore, *C. macrocarpa* oil at all concentrations increased cocooning percentage. In the same trend, effect of used oil on total soluble protein, aspartate transaminase and alanine transaminase enzyme levels of the haemolymph of silkworm larvae were studied. Whereas, total soluble protein content increased significantly by concentration of 1% *L. sativum* and *C. macrocarpa*, respectively. Moreover, the elevations of aspartate and alanine transaminase enzymes were noticed at the highest concentrations of the tested oils. It could be recommended that, it's safe to use the tested oils for repellency of *T. urticae* which infesting mulberry trees without any dangerous effect on mulberry silkworm, *Bombyx mori* that feed on these trees. Also, these oils increased the productive characters of mulberry silkworm *B. mori* and can be used as nutritional additives to increase the silk yield.

INTRODUCTION

Sericulture development generally relates to the mulberry cultivation as mulberry leaves, *Morus alba* L. is a widespread and important fruit tree. It is also used for silkworm feed, and a source of woods in many parts of the world including Egypt. Silkworm, in particular, feed on mulberry leaves collected daily from mulberry trees found around and between treated field crops. Such polluted mulberry trees could kill or at least harm the breeds of *Bombyx mori* (Bohidar and Choubey, 2005).

The two-spotted spider mite, *Tetranychus urticae* Koch is a globally important pest on mulberry leaves and other associated field crops.

The quality and yield of mulberry plants are seriously affected by these pests, which are small, fast breeding, strongly and adaptive, and can easily develop resistance. (Wilson 1991). Some acaricides can kill *T. urticae* immediately, whereas some of them take time before inducing mortality. However, others could affect by inhibiting movement or reducing oviposition rates (Steiner *et al*., 2011). Some plant oils are also effective as a pesticide to different plant pests (Lee *et al*., 2004). Application of pesticides in agriculture to control various insects and mites not only have a positive effect for controlling pests, but also resulted in harmful effects on the natural enemies and economical insects, (Van *et al*., 2009; Kumral *et al*., 2010). Botanicals (plant oils and extracts) nowadays are said to be safe and important alternatives to pesticides, being commonly used in IPM because of their nominal effect on non-target pests and other environmentally friendly properties (Al-Dosary, 2007).

However, studies on effects of botanicals on economical insects are less. Various extracts of medicinal plants have been tested by supplementation in the silkworm *Bombyx mori* and were seen to influence the body weight, silk gland weight and the silk thread length in *Bombyx mori* (Murugan *et al*., 1998, Lotfy Lamiaa *et al.*, 2009, Hassan Eman and Saad 2012 and Zannoon *et al.* 2013). So, the aim of the present study was to evaluate the efficacy of the botanical oils from *L. sativum, T. foenum-graecum* and *C. macrocarpa* plants on *T. urticae* and the safety degree of these oils on some biological and productive characters of the mulberry silkworm, *B. mori*.

MATERIALS AND METHODS

This work was carried out at laboratories of Sericulture, Acarology and Physiology Research Departments, Plant Protection Research Institute, Sharkiya Province, Egypt.

Three plants were used in this study, *Lepidium sativum* seeds (F:Brassicaceae), *Trigonellafoenum-graecum* seeds *(F: Fabaceae)*and *Cupressus macrocarpa* leaves(F: Cupressaceae).The fixed oils were extracted from *Lepidium sativum* and *Trigonella foenum-graecum*plants, while the volatile oil was extracted from *Cupressus macrocarpa* plant and all oils were applied by three concentrations (1, 0.5 and 0.25 %)

Extraction technique of fixed oils:

Extraction of the fixed oils was performed from crushed plant parts and the dry powdered plants were macerated in petroleum-ether (60/80) according to (Hussien and Shoukry, 1997). The petroleum-ether extracts were filtered through anhydrous sodium sulphate. A rotary evaporator apparatus was used to remove the solvent, and the oils were stored under nitrogen gas until use.

Extraction technique of volatile oils: The volatile oil was extracted by steam distillation using 500 g of dried plant in 500 ml of water for 4-6 h following the method described by (Weaver *et al*. 1994).The distillate was extract received over anhydrous sodium sulphate in special receiver which yielded 1.5- 2.0 % (V/W). The oils were stored in dark bottles in the refrigerator.

Preparation of mite culture:- The mass rearing of two spotted spider mite, *T. urticae* was cultured on the lower surface of mulberry leaves (*Morus alba* L.) placed on moistened cotton pads. The colonies were maintained at room temperature under laboratory conditions. The mulberry leaves were examined every few hours and replaced with fresh ones when over-crowding of mites and yellow leaves were observed.

The repellent effect on *Tetranychus urticae* Koch adult females: Mulberry leaves were cut into discs (5 cm in diameter) of the asymmetrical portion along midrib obtained per each disc. One half portion of the leaf disc was carefully dipped within the tested concentration; the other half remains dry. Serial successive concentrations of each extract were prepared (0.25, 0.5, 1 and 1.25). Each treatment included 3 replicate and control. Twenty adult *T. urticae* females were transferred from the culture with a fine brush into the center of each leaf disc. The mites left to move freely across the two portions of the disc, then counted every (12, 24, 48, 72 and 96) hours. The rearing units were kept at room temperature where mite survival and repellency (number of mites off the discs) recorded daily for 96 hours. All bioassays were conducted under the same environmental conditions. After 12, 24, 48, 72 and 96 hours past, the number of mites in each treatment was assessed under Stereomicroscope and counted. The repellency index (RI) was calculated according to Pascual–Villalobos and Robledo, (1998) formula:

RI= (C-T/C+T)×100

Where: C= the number of mites on control diet. T = the number of mites on treated died.

Effect of the botanical oils on silkworm, *Bombyx mori* L. Silkworm rearing techniques:

The mulberry silkworm, *Bombyxmori* (H1 x KK x G2 x V2 hybrid) eggs were obtained fromSericulture Research Department of Plant Protection Research Institute (PPRI), Agriculture Research Center (ARC), Giza, Egypt. Larvae were fed four times\ day with mulberry leaves (*Morus alba* L.) from hatching to cocooning. Rearing procedures were achieved under laboratory conditions of 25 ± 2°c and 70 ± 5 % RH according to the techniques of (Krishnaswami 1978).Half number of the resulted cocoons of each replicate was oven driedat 80 ºC for 6 hr and used to study the technological characters, while the other half of cocoons was used for the biological studies.

Treatment of plant extracted oils:

Newlymolted 5thlarval instar was divided into two groups; untreated group (control) and group treated with three plants extracted oils; *L.sativum*, *T.foenum-graecum*and*C.macrocarpa*oils with three different concentrations. Each treatment and control was included 3 replicates each of 50 larvae. The control group was fed on mulberry leaves dipped only in tap water.Mulberry leaves were dipped in oil concentrations for one minute and left to dry and offered to treated groups.

Biological aspects:

Larval mortality percentage, grown larval weight (g.) and cocooning percentage (%) were calculated. Weight of fresh cocoon (g.), weight of cocoon shell (g.) and silk content ratio (%) were recorded. Silk content ratio (%) was calculated according to Tanaka (1964)formula:

$$\textbf{Silk content ratio (\%)} = \frac{\text{Weight of cocoon shell x 100}}{\text{Weight of fresh cocoon}}$$

Technological characters:

The cocoons spun by treated and untreated larvae were collected, oven-dried and reeled using the individual reeling machine. The length (m.), weight (g.) and size (dn.) of reeled silk filaments were measured and tabulated in Table (4).The size of reeled silk filaments by denier (dn.) was calculated according to the following equation (Tanaka, 1964).

$$\textbf{Size of silk filament (dn.)} = \frac{\textbf{Weight of filament (g)} \times 9000}{\textbf{Length of filament (m)}}$$

Statistical analysis: All data were analyzed using analysis of variance (ANOVA) and Least Significant Difference Test (LSD) was employed to compare the treatment means (P = 0.05)

using the SAS program 2010 (SAS Institute Inc., Cary, NC).

Biochemical studies: For haemolymph preparation three random samples of the 8th day of the 5th instar larvae were selected from each replicate and the haemolymph was collected by cutting one of the prologs in micro-tubes and 1 mg phenylthiourea added immediately to prevent melanization. Samples were centrifuged at 14.000 rpm for 10 min. The supernatant was removed and kept in -20ºC for analysis. Data were subjected to statistical analyses using the software package Cost at Statistical Software (2005) a product of Cohort Software, Monterey, California, USA.

Determination of total soluble protein: Protein was measured in the supernatant as total soluble protein calorimetrically according to the method described by (Gornall *et al.* 1949).

AST and ALT enzyme activities: colorimetric determination of the activities of Asprtat transaminase (AST) and Alanine transaminase (ALT) were measured in the supernatant according to method of (Reitman and Frankle 1957)

RESULTS AND DISCUSSION

Effect of plant oils on *T. urticae* Koch adult females:

Repellency might be used to protect the host from mite infestations by repelling mites from the leaves. Data given in Table (1) showed that *L. sativum* oil recorded the highest repellency percentage averaged (76.68%) followed by *T. foenum-graecum* averaged (77.4%) and volatile oil , *C. macrocarpa* averaged (74.64%) for repellent activity against adult females of two spotted spider mite, *T. urticae* by repellency bioassay after 96 h. Mean total repellency of *L. sativum* showed the highest repellency percentage averaged (57.66%) followed by *T. foenum-graecum* averaged (55.67%) and volatile oil , *C. macrocarpa* averaged (54.98%).

Table 1: Repellency rate of Three Plant Oils Against Two-spotted Spider Mite, *Tetranychus urticae* Adults on Mulberry leaf discs:

Treatments	Concentration	Time after treatment										
		12hr		24hr		48hr		72hr		96hr		% Mean total repellency
		No. L.	% (Mean ± sd) Repelled	No. L.	% (Mean ± sd) Repelled	No. L.	% (Mean ± sd) Repelled	No. L.	% (Mean ± sd) Repelled	No. L.	% (Mean ± sd) Repelled	
Lepidium sativum	**0.25**	36	24.3±2.8c	29	33.4±2.3c	24	41.9±7.4c	17	55.2±15.6b	10	70.2±11.4a	45d
	0.50	26	39.0± 5.0b	22	45.4±7.1bc	22	45.0±3.8bc	15	59.3±11.7ab	10	70.4±11.0a	51.82cd
	0.75	25	40.7±7.4b	19	50.9±9.7ab	20	49.0±9.9bc	11	67.9±8.4ab	7	77.9±4.5a	57.28bc
	1.0	17	55.3±2.6a	18	52.8±6.2ab	16	57.1±9.9ab	8	75.5±3.9a	6	81.1±8.4a	64.36ab
	1.25	15	59.6±6.5a	14	61.4±6.6a	12	66.0±6.4a	7	78.4±10.1a	5	83.8±4.9a	69.84a
Mean	**-**	**23.8**	**43.78**	**20.4**	**48.78**	**18.8**	**51.8**	**11.6**	**67.26**	**7.6**	**76.68**	**57.66**
Untreated	**-**	**59**	**-**	**58**	**-**	**58**	**-**	**57**	**-**	**56**	**-**	**-**
L.S.D.	**-**	**-**	**9.54**	**-**	**12.35**	**-**	**14.23**	**-**	**19.35**	**-**	**15.58**	**11.28**
Cupressus macrocarpa	**0.25**	41	18.5±7.8d	33	27.5±4.4c	26	38.4±6.8bc	18	53.6±17.7b	11	68.1±14.8a	41.22c
	0.50	27	37.3±3.7c	23	43.6±7.4b	23	43.3±2.7bc	15	59.3±11.7ab	11	68.2±14.5a	50.34bc
	0.75	24	42.3±5.4bc	19	50.9±9.7b	20	49.0±9.9bc	12	65.5±6.6ab	8	75.2±4.2a	56.58ab
	1.0	21	47.6±5.6ab	19	50.7±4.0ab	17	54.8±7.6ab	10	70.1±5.6ab	7	77.9±4.5a	60.22ab
	1.25	17	55.3±2.6a	16	57.0±7.2a	14	61.2±3.6a	8	75.4±4.9a	5	83.8±4.9a	67.875a
Mean	**-**	**26**	**40.2**	**22**	**45.94**	**20**	**49.34**	**12.6**	**64.78**	**8.4**	**74.64**	**54.98**
Untreated	**-**	**59**	**-**	**58**	**-**	**58**	**-**	**57**	**-**	**56**	**-**	**-**
L.S.D.	**-**	**-**	**9.66**	**-**	**12.49**	**-**	**12.51**	**-**	**19.06**	**-**	**18.03**	**10.22**
Trigonellafoenumgraecum	**0.25**	44	15.3±10d	34	26.3±6.6c	28	35.4±9.9b	19	51.0±13.7b	12	65.4±11.6b	38.68c
	0.50	26	39.0±5.0c	24	41.9±8.8b	23	43.2±4.3b	16	57.2±12.5b	9	73.1±12.8ab	50.88b
	0.75	27	37.3±3.7bc	22	45.2±6.0b	22	45.0±3.8b	12	65.5±6.6ab	7	77.9±4.5ab	54.14b
	1.0	19	51.7±8.0ab	19	51.0±9.1ab	15	59.1±6.7a	7	78.3±3.8a	4	86.8±5.1a	65.38a
	1.25	15	59.6±6.5a	14	61.4±6.6a	12	66.0±6.4a	8	75.4±4.9a	5	83.8±4.9a	69.16a
Mean	**-**	**26.2**	**40.58**	**22.6**	**45.16**	**20**	**49.74**	**12.4**	**65.48**	**7.4**	**77.4**	**55.67**
Untreated	**-**	**59**	**-**	**58**	**-**	**58**	**-**	**57**	**-**	**56**	**-**	**-**
L.S.D.	**-**	**-**	**12.72**	**-**	**13.70**	**-**	**11.98**	**-**	**16.80**	**-**	**15.63**	**9.0**

These results were in agreement with that obtained by Zhang *et al*. (2013) who concluded that percentage of repellency showed more fluctuations with the processing time and El-Khayat *et al.*, (2014) indicated that the hexane extract caused high repellency rate percentage averaged 78.75% over 96 hours after application in 2500 ppm concentration followed by acetone, ethanol and water extracts (68.20, 67.63 and 72.29 %), respectively.

Kim *et al*. (2005) reported the repellent index of methanol extract of plant materials at 1% against *T. urticae*1, 2 and 3 days after exposure. These plant extracts showed high repellency activity at 1 day after treatment before declining on 2 and day 3 after exposure.

Effect of extracted oils on biological aspects of mulberry silkworm *Bombyxmori*:

Larval mortality percentage (%): Statistical analysis of data indicated that, there were highly significant differences in larval mortality between means of different treatments. The *C.macrocarpa* volatile oil with all concentrations and 0.5 % *L. sativum* fixed oil decreased the mortality percentage to zero. All investigated oils decreased the mortality percentage compared to the control group which recorded 0.6667 % (Table 2).

Table 2:Effect of extracted oils on larval mortality, larval weight and cocooning percentage% of mulberry silkworm *B. mori*.

Oils	**Conc.%**	**Larval mortality**	**Larval Weight**	**Cocooning%**
Trigonella foenumgraecum	1	0.1667	4.47	100.00
	0.5	0.00	4.07	100.00
	0.25	0.3333	4.65	98.33
	Mean	0.1667	4.40	99.44
Lepidium sativum	1	0.6667	4.69	94.67
	0.5	0.500	4.50	97.67
	0.25	0.3333	4.91	100.00
	Mean	0.500	4.70	97.67
Cupressus macrocarpa	1	0.00	4.74	100.00
	0.5	0.00	4.11	100.00
	0.25	0.00	4.43	100.00
	Mean	0.00	4.43	100.00
Control		0.6667	3.89	98.33
$LSD_{0.05}$		0.4398**	0.6191**	1.8132*

Larval weight (g.): Analysis of data indicated that, enriching mulberry leaves with the tested botanical oils increased larval weight of mature larvae as compared to the control. The concentrations 0.25 and 1 % of *L. sativum* and 1 % *C. macrocarpa* and 0.25 % *T. foenum-graecum*oils increasedthe larval weights and recorded (4.91, 4.69, 4.70, 4.65 g/larva, respectively). While, the mean body weights of mature larvae for control group recorded 3.89 g/ larva (Table 2).

Cocooning percentage: Statistical analysis indicated that, there is a highly significant difference in percent cocooning between concentrations of the botanical oils. All tested concentrations of *C. macrocarpa* and 1, 0.5 %*T. foenum-graecum* oils exhibited the highest cocooning percentage 100 %. It was found that, 1 % *L. sativum* oil decreased the cocooning percentages to 94.666 % compared to 98.333 % for the control group. The botanical oils under investigation decreased the larval mortality percentage, increased the larval body weight and increased the cocooning percentage (Table 2).

The obtained results are in agreement with the finding of Shoukry *et*

al. (1998), Morssy Ghada(2009) and Zannoon *et al.* (2013) who indicated that, the botanical oils improved the cocooning percentages, larval weight and reduced larval mortality.

Cocoon indices:

Fresh cocoon weight (g): As shown in Table (3), there are highly significant differences (p<0.01) in cocoon weights between different concentrations of the three oils. *L. sativum* and *T. foenum-graecum*oils at 0.25 % exhibited highest means of fresh cocoon weights recording (2.0017 and 1.8933 g) followed by (1.8700, 1.8500 and 1.8050 g) for the treatment by 0.5 % *T. foenum-graecum* oil and 1, 0.5 % *C. macrocarpa* oil, respectively. Whereas, the least fresh cocoon weight recorded 1.4400 g for the control group.

Table 3: Effect of extracted oils on Cocoon weight (gm), Cocoon shell weight (gm) and Cocoon shell ratio % of mulberry silkworm *B. mori*:-

Oils	Conc. %	Cocoon Weight (gm)	Cocoon Shell Weight	Cocoon shell Ratio(%)
Trigonella foenum-graecum	1	1.4950	0.36833	24.637
	0.5	1.8700	0.39167	20.945
	0.25	1.8933	0.41000	21.655
	Mean	1.7528	0.39000	22.412
Lepidium sativum	1	1.6400	0.36500	22.256
	0.5	1.7267	0.41000	23.745
	0.25	2.0017	0.40333	20.149
	Mean	1.7894	0.39444	22.043
Cupressus macrocarpa	1	1.8500	0.39667	21.442
	0.5	1.8050	0.40333	22.345
	0.25	1.7383	0.3950	22.723
	Mean	1.7978	0.3933	22.170
Control		1.4400	0.32000	22.222
$LSD_{0.05}$		0.2642**	0.0455*	3.0244*

*=Significant difference where P<0.05** = Highly significant difference where P< 0.01 ns= No significant difference where P>0.05

Cocoon shell weight (g): The means of cocoons shell weights ranged between 0.4100 – 0.3650 g when grown silkworm, *B. mori* larvae fed on mulberry leaves enriched with different treatments compared with 0.3200g for the control group. All tested concentrations increased cocoons shell weight comparing to the control.

Cocoon shell ratio (%): Analysis of variance revealed highly significant differences (p< 0.01) between the means of cocoons shell ratios of the botanical oils. In addition,1 % *T. foenum-graecum* oil and 0.5 % *L. sativum* oil caused the highest increase in cocoons shell ratio recording (24.637 and 23.745 % respectively,) whereas, 0.25 % *L. sativum* and 0.5 % *T. foenum-graecum* oils manifest the least means of silk content ratios recording (20.945 and 20.149 %) as compared to (22.630%) for control group. Similar observations on cocoons parameters were reported by many authors (Bohidar *et al.* (2004), Lotfy Lamiaa *et al.* (2009), Hassan Eman and Saad (2012) and Zannoon *et al.* (2013).

Technological studies: Silk filament lengths, weights and sizes were recorded in Table (4).

Silk filament length: The concentrations 0.25 % *T. foenum-graecum*, 0.25 % *L. sativum* and 1 % *C. macrocarpa* oil caused the highest increase in filaments length recording (1682.00, 1579.00 and 1537.50 m). While, 1 % *L. sativum* oil and 0.25 % *C. macrocarpa* oil manifested the least means of filament length (1349.83 and 1362.67 m) compared with (1424.83 m) for control. The difference in silk filament length between means of different treatments was highly significant (p< 0.01).

Silk filament weight (g): The obtained results cleared that, the highest means of the silk filaments weights recorded

(0.36667, 0.3350 and 0.3500 g, respectively) for the treatment of mulberry silkworm, *B. mori* with 0.25, 1 % *T. foenum-graecum* and 0.25 % *L. sativum*. It was found that, all tested oil concentrations increased the silk filament weight as compared to the control group which recorded 0.300 g.

Silk filament size (dn): The results in Table (4) showed that, there was no significant difference (P>0.05) in silk filament sizes between means of different oil concentrations when *B. mori* larvae were fed on investigating plants oils. The means of silk filament sizes ranged between 2.1866 – 1.893dn.

Table 4: Effect of extracted oils on silk filament parameters of *B. mori*:

Oils	Conc. %	Silk Filament length (m)	Silk Filament Weight (gm)	Silk Filament Size (dn)
Trigonella foenum-graecum	1	1378.83	0.3350	2.187
	0.5	1438.00	0.3067	1.919
	0.25	1682.00	0.3667	1.972
	Mean	1499.61	0.3361	2.017
Lepidium sativum	1	1349.83	0.3017	2.011
	0.5	1430.17	0.3033	1.909
	0.25	1579.00	0.3500	1.995
	Mean	1453.00	0.3183	1.972
Cupressus macrocarpa	1	1537.50	0.3233	1.893
	0.5	1482.83	0.3267	1.983
	0.25	1362.67	0.3183	2.103
	Mean	1461.00	0.3228	1.988
Control		1424.83	0.3000	1.895
$LSD_{0.05}$		164.05**	0.0442*	0.2601 ns

These results with increasing larval body weight, cocooning percentage, cocoons weights, silk ratios, filament lengths and silk filaments weights and reducing mortality by application of treated mulberry leaves to *B. mori* larvae may be due to immunological, antibacterial, antioxidant activities of *T. foenum-graecum* oil (Yadav Rashmi *et al*., (2011), Ahmadiani *et al*., (2001), Alkofahi *et al.* (1996) and Bhatti *et al.* (1996) which may cure a number of diseases and enhanced the growth and development of the mulberry silkworm. Moreover, *T. foenum-graecum* seeds are well-known for their pungent aromatic properties which stimulate appetite, improved feed intake and protein utilization (Chevallier, 1996) and enhanced food consumption Atefeh (2013).

The positive effect of *L. sativium* seeds oil may be due to it contains a considerable amounts of nutrients, protein, carbohydrate, moisture and volatiles, crude fiber, lipid and ash (Solomon *et al* 2016), linolenic and linoleic acids which are the sources of the omega-6 and omega-3 series (Kassie *et al.,*2002 and Elyamani Enas 2014) could be served as a good source of essential fatty acid in food formulation which enhanced the biological aspects of mulberry silkworm and subsequently increased the productive characters of the cocoons and the reeled silk filament.

The useful effects of *Cupressus macrocarpa* oil may be due to the presence of Monoterpene hydrocarbons, sesquiterpene hydrocarbons, α-pinene and δ-3 carene (Emami *et al* 2004 and Christelle *et al.*, 2003) which possess juvenoid activities or the presence of carvacrol (94.4 %) which act as strong anti-fungal (Zhang *et al* 2012).

Effect of extracted oils on Biochemical parameters: The data present in Table (5) highlighted the changes in biochemical parameters astotal soluble protein, alanine transaminase (ALT) and aspartate transaminase (AST) enzymes in the haemolymph of 5^{th} instar larvae of the silkworm after treatments with three

different concentrations for each tested oils.

Total soluble proteins (TSP): Generally, all treatments increase protein concentration in the haemolymph of 5th instar larvae of silkworm. *C. macrocarpa* and *L. sativum* oils exhibited the highest elevation in protein contents (97.82 and 94.81 mg protein /ml) with increasing percent (51.45 and 46.79%) at 1% conc., respectively, followed by the rest of oil concentrations except at 0.25% *C. macrocarpa* oil which recorded a slight reduction percentage (-8.84 %) in protein levels in comparable with soluble protein level in control (64.59mg protein /ml).

Table 5: changes in the effect of total soluble protein (gm./ml.) and activities of transaminase enzymes (AST and ALT, mg pruvate/L./min.) in the haemolymphof 5thlarval instar of silkworm.

Tested oils	Conc.	TSP		AST		ALT	
		Quantity	C%	SA	RA%	SA	RA%
Trigonella foenumgraecum	**0.25**	68.19±7.480**bc**	5.57	18.23±4.138**bc**	-3.03	142.47±6.503**a**	-5.01
	0.50	70.143±5.673**bc**	8.59	22.57±3.517**abc**	20.05	157.06±15.420**a**	4.72
	1.0	83.95±10.433**ab**	29.97	31.12±3.146**a**	65.53	204.32±44.168**a**	36.23
Lepidium Sativum	**0.25**	72.76±3.769bc	12.65	17.92±2.863bc	-4.68	189.03±8.120a	26.04
	0.50	76.69±3.836abc	18.73	21.73±5.844abc	15.59	200.29±37.530a	33.54
	1.0	94.81±5.379a	46.79	30.24±1.494a	60.85	215.26±22.407a	43.53
Cupressus Macrocarpa	**0.25**	58.88±11.283c	-8.84	17.21±1.626c	-8.46	167.95±13.745a	11.98
	0.50	79.58±2.387abc	23.21	25.89±4.254abc	37.71	175.88±18.011a	17.27
	1.0	97.82±12.511a	51.45	27.17±1.907ab	44.52	191.74±74.268a	27.84
Control	-	64.59±0.577bc	----	18.80±0.578abc	----	149.98±0.333a	---
P value		0.0215*		0.0421*		0.7775ns	
$LSD_{0.05}$		21.769		9.716		95.170	

Data expressed as Mean ± S.E. Mean under each variety having different letters in the same column denote a significant different ($p \leq 0.05$).

The effect of treatments on protein metabolism is profound as evidenced by an upsurge in the levels of all the biochemical parameters examined. Since, proteins are the chief organic constituents regulating the biochemical events in the cell (Jyothi*et al*., 2010). Protein is necessary for various biological activities during development, metamorphosis and maintenance of various physiological functions in different tissues (Kumar *et al*., 2011).

It worthy to mention that, our results are in agreement with that obtained by El- Gindi (2000) on *Parasargophaga argyrostoma*, Raju e*t al*. (2012) when evaluated turmeric on protein metabolism of silkworm. Also (Khedr *et al.*, 2013) stated that honey bee and Pharovit iron enhance protein metabolism that very important in silk production.

Alanine transaminase (ALT) activity: All tested concentrations of applied oils showed significant elevation in ALT activity compared to control; the highest concentration recorded the highest increase 43.52, 36.23 and 27.84% with *L. sativum, T. foenumgraecum* and *C. macrocarpa*, respectively.

Aspartate transaminase (AST) activity: It seems clearly from table (5) that the aspartate transaminase(AST) was highly activated at 0.5 and 1% conc. after treatments with the three oils, the highest significant increase in activity (65.53%) for 1% of *T. foenum-graecum*with the exception of 0.25% conc. which caused a slight reduction in AST activity from the control (18.80mg pruvate/L./min.).

In the present work, the elevation of ALT and AST after application of tested oils to *B. mori* larvae may be attributed to the changes occurring in the quality and quantity of proteins as a consequence of juvenoid action. In insects and other animals transaminase enzymes are known to be the key

enzymes in the formation of nonessential amino acids, in metabolism of waste nitrogenous products and in gluconeogenesis (Kaur*et al*., 1985). A close relation was found in insects between synthesis and transaminases levels whereas; these enzymes provide the building blocks for the protein synthesis. El-Domiaty *et al.* (2003) found a decrease in both AST and ALT activity by treatment of *M. domestica* larvae with *Populusnigra* volatile oil. So that, eventually AST and ALT are important anaplerotic enzymes providing oxaloacetate and pyruvate, respectively, as important precursors of Krebs's cycle; hence, inhibition of these enzymes causes impairment of this cycle that could affect the normal reproduction and growth rate of the treated insects.

Generally, under the effect of oil treatments protein metabolism and enzyme activities were stimulated to increase turnover of silk proteins, greater spinning activity and consequently greater cocoons crop.

CONCLUSION

It could be concluded that, the investigated oils, *C. macrocarpa*, *T. foenum-graecum* and *L. sativum* with experimented concentrations are useful to be applied to mulberry trees infested with *Tetranychus urticae* Koch without any harmful effects on mulberry silkworm, *Bombyx mori* that feed on these trees. These oils increased the productive characters of mulberry silkworm *B. mori* and can be used as nutritional additives to increase the silk yield. Therefore, future investigations are needed to be carried out on the various active components in these oils.

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ARABIC SUMMERY

تقييم الزيتين الثابتين لنباتي حب الرشاد والحلبة والزيت الطيار لنبات سرو الليمون كعامل طارد للعنكبوت الاحمر ذو البقعتين الذي يصيب أشجار التوت وتأثيرها علي دودة الحرير التوتية

إيناس مصطفى اليمنى – هند محمد صبرى – أميرة الدسوقى مصباح

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تم التقييم المعملى للتأثيرات الطاردة للزيتين الثابتين حب الرشاد والحلبة والزيت الطيار لسرو الليمون ضد إناث العنكبوت الأحمر ذو البقعتين الذى يصيب أشجار التوت التى تعتبر الغذاء الوحيد لدودة الحرير التوتية. إتضح من النتائج أن الزيوت الثلاثة لها تأثيرات طاردة على إناث العنكبوت الأحمر ومن الممكن الأستفادة منها للتغلب على تعداد الأكاروسات النباتية مستقبليا.

كما تم دراسة تأثيرالزيوت النباتية الثلاثة على دودة الحرير التوتية فوجد أنها أدت إلي زيادة كلا من وزناليرقات, ووزن الشرانق, وزن قشرة الشرانق, وزن خيوط الحرير مقارنة بالكنترول كما أدت إلي تقليل نسبة الموت. التركيزات الثلاثة لزيت سرو الليمون أدت إلى زيادة نسبة التشرنق.تم تقدير البروتين الكلى الذائب و مستوى نشاط الإنزيمات الناقلة للأمين فى هيموليمف اليرقات محل الدراسة وكان للتركيز ١% لزيتي حب الرشاد و سرو الليمون أعلي محتوي للبروتين الذائب في الهيموليمف .

بالتحليل البيوكيميائيوجد أن التركيز الاعلي للزيوت المستخدمة أدي الي زيادة ملحوظة في نشاط الإنزيمات الناقلة للأمين لذلك فهذة الزيوت أمنة الإستخدام وتعتبر إضافة غذائية لورق التوت لتحسينها للخواص اللإنتاجية لدودة الحرير التوتية ويمكن تطبيقها كعامل طارد للعنكبوت الأحمر من على أشجار التوت والمحاصيل الحقلية المجاورة دون حدوث أى ضرر على أوراق التوت الغذاء الوحيد لدودة الحرير التوتية.