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Utilization of some volatile and fixed oils in silkworm diets: a novel strategy for maximizing silk production

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ABSTRACT

The purpose of the present research is to utilize natural oils such as citronella oil (CO) (*Cymbopogon nardus*), lemon grass oil (LO) (*Cymbopogon citratus*), the mixture of (fresh and used sunflower, *Helianthus annuus* L. and soybean, *Glycine max* L.) oils combined in a 1:1 ratio and jojoba oil (JO) as nutritional additives for silkworm, *Bombyx mori*. Results reported that CO gave the highest larval weight at all concentrations whereas; the lowest larval weight was recorded with the treatment of mulberry leaves by LO. The highest larval duration was noticed by the larval treatment of sunflower and soybean mixture at 5 % and 2.5 %, respectively whereas; CO treatment recorded the lowest larval duration. The larval mortality showed that jojoba, (*Simmondsia chinensis*) oil at all concentrations decreased the mortality of 5th larval instar. Moreover, the outcomes of biochemical studies explained that all treatments resulted in a decrease in the activity of AST enzyme, whereas the same treatments increased the activity of ALT enzyme. The Acetylcholinesterase activity significantly decreased with tested oils, particularly CO and JO. Furthermore, the activities of chitinase increased in the larvae treated with JO. Finally, the highest Total soluble proteins and Total lipid contents were obtained in the hemolymph of larvae treated with a concentration of 0.06 % LO. The data indicated that jojoba oil (JO) had the most positive impact on the most investigated parameters among the oils tested. In contrast, the used mixture of sunflower oil and soybean oil (UO) exhibited the least effect on the silkworms when compared to the control group.

Keywords: Silkworm, Nutrition, Enzymes, Volatile oils, Fixed oils

INTRODUCTION

Mulberry silkworm *Bombyx mori* (Linnaeus) (Lepidoptera: *Bombycidae*) is a highly significant insect because it produces natural silk threads. China is thought to make the most silk globally, with India coming in second (Elyamani *et al.,* 2017). Essential oils play a significant role in influencing various biological and physiological aspects of the mulberry silkworm *B. mori*. Researchers have shown that different essential oils like basil, lemon, lemongrass, thyme, and spinach oils impact the growth, immunity, cocoon quality, and endocrine system of silkworms (Gupta *et al*. 2019; Khade *et al.,* 2024). Oil crops are well-known for providing protein and calories in human diets. Sunflower (*Helianthus annuus*) is a large crop, with 15-21% protein and 50% oil. It is the world's second largest producer of edible oil, trailing only soybean oil, and is widely recognized as one of the best plant oils for human consumption due to its nutritional worth.

Its seeds include a high quantity of vitamins, minerals, and tocopherols. It has been reported to be rich in minerals like magnesium, iron, copper, calcium, zinc, salt, potassium, phosphorus, selenium, and manganese (Ayeen 1996; Skoric *et al.,* 2008; Skoric 2009; Nandha *et al.,* 2014) Scientists have recently depended on food additives for mulberry leaf to improve nutritional contents including vitamins, minerals, antibiotics, and hormones, (Sallam *et al*., 2018). Jojoba, *Simmondsia chinensis* is native to the southwestern United States and northern Mexico and belongs to the family *Simmondsiaceae*. It is an evergreen shrub that has attracted considerable interest owing to its unique properties (Yermanos, 2018). Jojoba seeds are rich in crude oil up to 50% usually known as jojoba oil (Purcell *et al.*, 2000). Awad *et al.* (2022) said that fatty acid profiles varied significantly depending on the jojoba genotype. Attention is increasingly being paid to the use of oils (fresh and expired) as nutrition for mulberry silkworms to increase silk production.

Edible oils such as sunflower and soybean are subjected to a variety of changes during the frying process. When food oils are fried, they undergo several transformations, including sunflower, soybean, and palm oil. These modifications result in the oil becoming inappropriate for human consumption. They include oxidation, polymerization, and the production of carcinogenic chemicals. There is currently a movement to try and use these materials as valuable products because they pose a severe risk to human health and the environment (Choe and Min 2007; Peiro, *et al.,* 2008; Chen *et al.,* 2013; Doğan, 2016). The re-purposing of

waste materials has sparked widespread attention in terms of cost savings and elimination of environmental disposals. Various used oils have been employed as inexpensive substrates (Lan *et al*. 2015). As a result, numerous studies concentrated on adding botanical extracts with mulberry leaves as a supplement to raise the nutritional content of silkworm meals (Hipparagi *et al*., 2001; Murugesh and Mahalingam, 2005). The study aims to evaluate the impact of different plant oil extracts on larval weight, duration, mortality, total lipid and total soluble protein content, transaminase enzymes, chitinase and acetylcholinesterase activities of the 5th instar larvae of mulberry silkworm *B. mori*.

MATERIALS AND METHODS

Materials:

Tested Silkworm: The eggs of the mulberry silkworm *B. mori* (H_1 x KK x GK_3 x V_2 hybrid) were procured from the Sericulture Research Department of Plant Protection Research Institute (PPRI), Agricultural Research Center, Giza, Egypt. The mulberry leaves of *Morus alba* var Rosa (Egyptian native) are used.

Tested volatile oils: Two volatile oils were investigated; citronella oil (CO) (*Cymbopogon nardus*) and lemon grass oil (LO) (*Cymbopogon citratus*). The pure oils used in the tests were acquired from the Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agriculture Research Centre, Giza, Egypt.

Tested fixed oils: Three fixed oils were included in present experiment. The mixer of fresh sunflower (*Helianthus annuus* L.) and soybean (*Glycine max* L.) oils combined in a 1:1 ratio (FO), purchased from Afco Misr Ataqa company, Suez city, Egypt. The used oil (UO) conducted under the following process. We bought the fresh potatoes from the nearby market. The potato batches were deep-fried in sunflower oil and soybean oil combined in a 1:1 ratio for one hour, with 20-minute intervals between each between each frying in the oil mixture. After being filtered and dried over anhydrous sodium sulfate, the oil was kept at 5 °C until analysis. The fresh Jojoba oil (JO) was purchased from Gogreen Company, El-Sadat city, Egypt.

Methods:

Experimental assay:

Silkworm larvae were reared under laboratory conditions of temperature 28 ± 2 °C and relative humidity 70±5% based on the following methods (Sabry *et al*., 2023). The newly hatched larvae were fed on fresh clean mulberry leaves three times daily until the end of the 4th instar. We made a series of concentrations of each oil and then selected the concentrations that made the larvae accept food without any contraindications.

At beginning of the 5th larval instar silkworm larvae divided to two major groups (A) and (B).

(A): represent treatments that were divided into 15 sub-groups.

Five plant oils; two volatile oils which were used each with three concentrations (0.60, 0.30, and 0.15 %), and three fixed oils were used each with three concentrations (5, 2.5, and 1.25%) where mulberry leaves were sprayed with each concentration of the tested oils using atomizers and left for 15 min to dry under room conditions. The treatment schedule is made at 1st, 3rd, and 5th day of the 5th larval instar. Each replicate has one hundred larvae.

(B): represent untreated group (control) repeated three times each one hundred larvae.

Chemical parameters of tested fixed oils: Peroxide value (PV) was determined according to (A.O.A.C., 2016) while acidity was determined according to (PN-ISO 660:2020).

Fatty acid composition of tested fixed oils: Fatty acid composition of (FO) and (UO) samples determined by gas chromatography (GC) according to (PN-ISO 12966-2:2017).

Gas chromatography analysis (GC) of tested volatile oils (CO and LO): The gas chromatography analysis of essential oil samples was performed at the Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. Gas chromatograph Dschrom 6200 with flame ionization detector. The sample size is 1 µl and the temperature ramp is set to increase at a rate of 10°C/min from 70 to 200°C. The detector temperature is 280°C, and the carrier gas is nitrogen. The flow rate is N_2 30 ml/min, H_2 30 ml/min, and air 300 ml/min. The column is BPX-5 with 5% phenyl (equiv.), and the film is polysilkphenylenesiloxane 30m × 0.25 mm ID × 0.25 µm. The composition of the essential oils was determined by comparing the retention duration of the essential oils' basic elements to those of actual samples injected under the identical circumstances. The area of the peak corresponding to each compound was used to compute the relative percentage of each component (Farag and Sakla, 2019).

Biochemical assays:

Ten larvae were used to collect the hemolymph by making a tiny puncture in one of the pro-legs of the fifth instar larvae and the hemolymph dropped into an Eppendorf tube containing 10 µg of phenylthiourea.

The samples were stored in the refrigerator until use. Throughout the testing process, samples were kept on ice and centrifuged for 10 minutes at 2000 rpm after each tube had been inverted multiple times.

Total lipid content: The hemolymph total lipid was quantified using the method established by (Schmit, 1964).

Total soluble protein (T.S.P.) content: **T.S.P.** was assayed according to the method of (Bradford, 1976).

Acetylcholinesterase activity (**AChE**): **AChE** was determined using the method published via (Simpson *et al*., 1964).

Chitinase activity: Chitinase activity was limited utilizing the method published via (Kimura, 1973).

Transaminase enzymes (AST) and (ALT): The enzyme activities of aspartate transaminase (AST) and alanine transaminase (ALT) were determined using the method reported via **(**Reitman and Frankel, 1957).

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) according to **(**Cohort, software 2005).

RESULTS

1- Chemical analysis of the tested oils:

Chemical properties of fixed oils such as acidity percentage and peroxide value of sunflower and soybean oils combined in a 1:1 ratio (FO and UO) were described as follows:

Chemical properties of FO and UO were studied Table (1). Acidity percentage and PV determinations are one of the best jointers of oil harm. The results indicated that UO had a greater acidity percentage and peroxide value than FO. The acidity and peroxide values of oils were (0.05 & 4.19%) and (6.32 & 19.17 Meq.O_2/Kg) for FO and UO, respectively.

GC Analyses of sunflower and soybean fixed oils combined in a 1:1 ratio (FO and UO):

Analysis of fatty acids components of oils FO and UO in Table (2) reported that the presence of unsaturated fatty acids as $C_{16:1}$ (0.10 & 0.09 %), $C_{18:1}$ (24.90 & 25.25%), $C_{18:2}$ (53.03 & 53.31%), $C_{18:3}$ (5.54 & 5.44%), for FO and UO, respectively. Whilst, the saturated fatty acids such as $C_{16:0}$ (11.28 & 10.49 %) and $C_{18:0}$ (4.15 & 4.21%), for fresh and used, respectively were offered in high percentages.

Gas chromatography analysis (GC) of the tested volatile oils:

Figures (1 and 2) indicate the primary components and percentages of the assessed essential oils after gas chromatography investigation. *C. nardus* oil (CO) contains citronellal (35.20%), geranial (26.18%), and β-Pinene (20.24%) as the main ingredients (Fig. 1). The primary components of *C. citratus* (LO) were geranial (36.22%), neral (28.56%), and myrcene (17.01%) (Fig. 2).

Table 1. Acidity % and peroxide value of sunflower oil and soybean oil combined in a 1:1 ratio (FO and UO):

Tested oils	Chemical properties	
	Acidity % (as oleic acid)	Peroxide value (Meq.O_2/Kg)
Fresh oils mixture	0.05	6.32
Used oils mixture	4.19	19.17

Table 2. Fatty acids composition of sunflower oil and soybean oil combined in a 1:1 ratio (FO and UO).

Fatty acids	FO	UO
Myristic acid $C_{14:0}$	0.10	0.08
Palmitic acid $C_{16:0}$	11.28	10.49
Palmitoleic acid $C_{16:1}$	0.10	0.09
Margaric acid $C_{17:0}$	0.08	0.08
Myristoleic acid $C_{17:1}$	0.04	0.04
Stearic acid $C_{18:0}$	4.15	4.21
Oleic acid $C_{18:1}$	24.90	25.25
Linoleic acid $C_{18:2}$	53.03	53.31
Linolenic acid $C_{18:3}$	5.54	5.44
Arachidic acid $C_{20:0}$	0.29	0.33
Eicosenic acid $C_{20:1}$	0.17	0.21
Behenic acid $C_{22:0}$	0.31	0.35

2. Biological parameters:

Larval weight /g. of 5th instar:

As shown in Table (3) the Citronella oil at 0.15, 0.30 and 0.60 % concentrations gave the highest larval weight were 3.35, 3.34, and 3.69 g. respectively with highly significant deference between means. The lowest larval weight was 2.67 g. for lemon grass oil treatment at 0.15 %.

Larval duration of 5th instar:

Duration of the fifth larval instars recorded highly significant differences between means. The concentration of 0.15 % of Citronella oil recorded the lowest mean larval duration of 9.10 days, while the highest means were obtained from the treatments of UO at 5 % and 2.5 % and recorded 10.07 and 9.63 days (Table 3).

Larval mortality percentage of 5th instar:

Larval mortality showed highly significant differences among means hence the treatments of mulberry leaves with jojoba oil at all concentrations were decrease the larval mortality and no mortality was recorded at a 1.25 % concentration (Table 3). The highest mortality were recorded of UO at concentrations 5, 2.5, and 1.25 % of UO (6.33, 5.67, and 5.33 %, respectively) compared to 4.0 % for the control group.

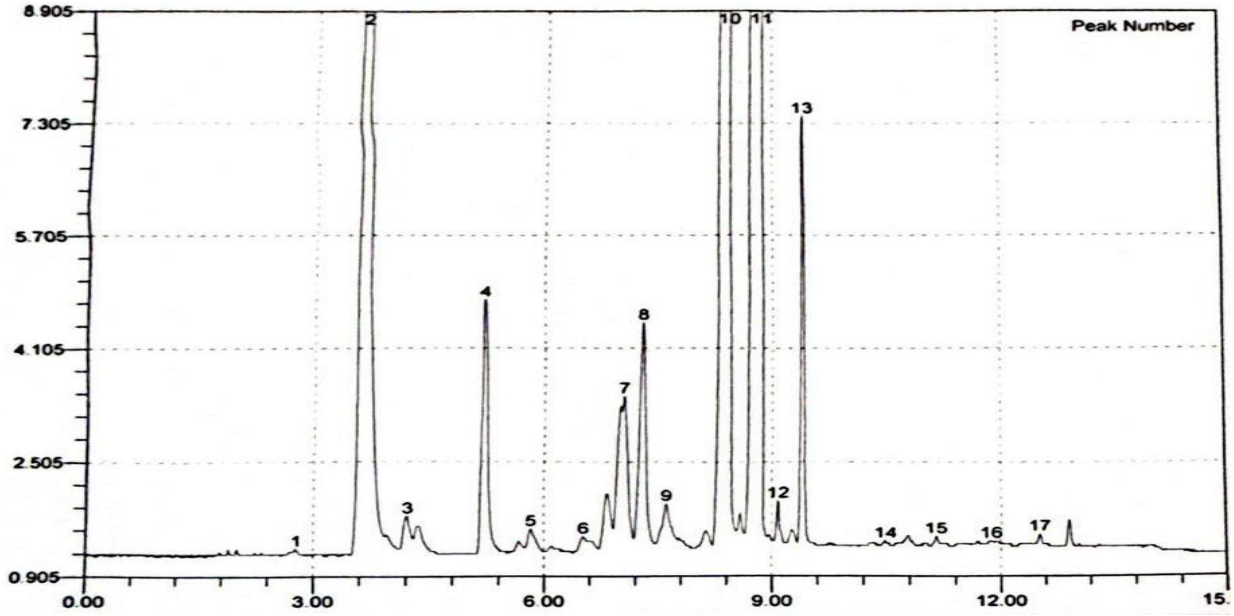

Fig. 1. Chemical composition of citronella oil (CO) (*C. nardus*) essential oil.

1= α-Pinene (Area%, 0.58) , 2 = β –Pinene (Area%, 20.24), 3= P-Cymene (Area%, 0.85), 4 = Limonene (Area%, 2.87), 7 = α-Terpineol (Area%,3.90) , 8 = Linalool (Area%,2.93), 9 = Citronellol (Area%, 1.09), 10 = Geranial (Area%, 26.18), 11 = Citronellal (Area%, 35.20),13 = Geranyl acetate (Area%, 3.19), 15 = β – Caryophyllene (Area%, 0.47), 5,6,12,14,16,17 = Unknown.

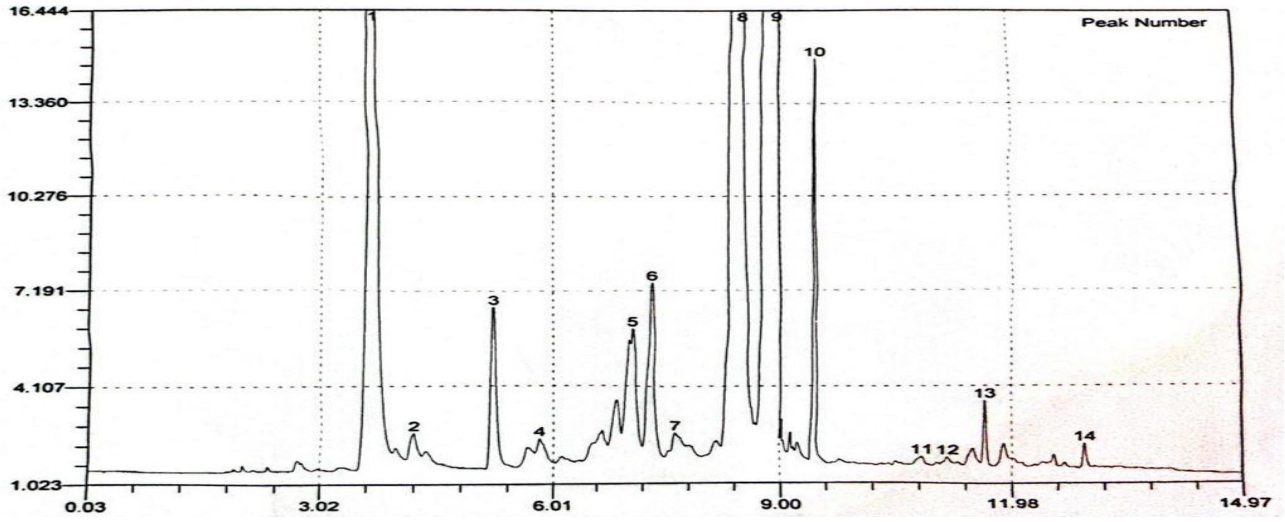

Fig. 2. Chemical composition of lemon grass oil (LO) (*C. citratus*) essential oil.
1= Myrcene (Area%, 17.01), 3= P-cymene ((Area%, 1.93), 5 = Limonene (Area%, 5.70), 6 = Linalool (Area%, 2.92), 8 = Neral (Area%, 28.56), 9 = Geranial (Area%, 36.22), 10 = Geranyl acetate (Area%, 3.10), 2,4,7,11,12,13,14= Unknown.

Table 3. Effect of some oils on selected biological parameters of *B. mori* .

Oils	Conc.	Larval weight (g)	The 5th instar duration (day)	Mortality (%)
Citronella oil (CO)	0.15%	3.35^{b}	9.10^{g}	4.67^{cd}
	0.30%	3.34^{b}	9.50^{bc}	4.33^{de}
	0.60%	3.69^{a}	9.60^{bc}	5.33^{bc}
Lemon grass oil (LO)	0.15%	2.67^{e}	9.57^{bc}	1.67^{g}
	0.30%	2.93^{de}	9.40^{ef}	3.67^{de}
	0.60%	3.01^{cd}	9.23^{fg}	3.33^{ef}
Fresh oils mixture (FO)	1.25%	2.78^{de}	9.40^{ef}	3.67^{de}
	2.50%	3.07^{bc}	9.27^{fg}	4.33^{de}
	5%	2.82^{de}	9.57^{bc}	4.67^{cd}
Used oils mixture (UO)	1.25%	2.86^{de}	9.57^{bc}	5.33^{bc}
	2.50%	3.07^{bc}	9.63^{b}	5.67^{ab}
	5%	2.73^{de}	10.07^{a}	6.33^{a}
Jojoba oil (JO)	1.25%	3.07^{bc}	9.40^{ef}	0.0^{h}
	2.50%	2.86^{de}	9.33^{fg}	2.0^{g}
	5%	2.77^{de}	9.47^{de}	2.33^{fg}
Control	_____	2.84^{de}	9.27^{fg}	4.0^{de}
P		0.0001**	0.0001**	0.0001**
L.S.D.$_{0.05}$		0.34	0.21	1.13

** indicates highly significant differences between means at 0.01 level of probability.
Means in each column followed by similar letters are statistically insignificant at 0.05 level of probability.

3. Biochemical studies of the 5th instar larvae hemolymph:

Data in Table (4) represent the activity of the AST enzyme and reported that treatments by FO and JO decreased the larval AST enzyme activity which were recorded (10.45, 12.61, and 13.09 µg/ml) and (9.60, 10.75, and 11.35 µg/ml) at concentrations 1.25, 2.5, and 5%, respectively. While CO and UO increased enzyme activity that were (10.99, 11.23, and 16.22 µg/ml) at concentrations 0.15, 0.30 and 0.60 % and (13.57, 15.73, and 16.28 µg/ml) at concentrations 1.25, 2.5, and 5%, respectively compared to the control that was 15.40 µg/ml using spraying technique of *B. mori* larvae diets. In contrast to all treatments, there were increases in the activity of ALT enzyme (Table 4). The activity of AChE was represented in (Table 4) and showed that significant decreases in activity of AChE of the hemolymph when larvae fed on the leaves treated with the three tested concentrations of tested oils especially CO and JO were (5.62, 6.30, and 7.30 U/ml) and (3.27, 4.70, and 5.69 U/ml), respectively in comparison with control (7.86 U/ml). As showed in Table (4) the activity of chitinase significantly decreased in the larvae fed on mulberry leaves treated with tested oils at three concentrations except 5 % JO which was increased its activity recorded 115.88 U/ml in comparison with control (81.42 U/ml). Also, the highest contents of T. S. P. were (52.88, 56.26 and 87.93 mg/ml) in larvae treated with LO at concentrations 0.15, 0.30 and 0.60 %, respectively while the lowest contents were recorded 37.30 mg/ml for 0.60 % CO (Table 4).

Finally, the highest increase in Total lipid content was obtained with the treatments 0.60 % LO and recorded 54.62 mg/ml. (Table 4) reported the lowest total lipids contents by treatments of 1.25 % JO, 5 % UO and 1.25 % FO were (5.04, 6.86 and 8.96 mg/ml) compared to 42.23 mg/ml for control.

Table 4. Effect of some oils on some biochemical parameters of *B. mori*.

Oils	Conc.	AST µg/ml	ALT µg/ml	AChE U/ml	Chitinase U/ml	T.S.P. mg/ml	Total Lipid mg/ml
Citronella oil (CO)	0.15%	10.99^{c}	60.18^{fg}	5.62^{ef}	62.94^{cd}	65.98^{b}	10.92^{bc}
	0.30%	11.23^{c}	67.97^{fg}	6.30^{ef}	34.97^{d}	50.37^{cd}	11.20^{bc}
	0.60%	16.22^{a}	109.37^{cd}	7.30^{de}	34.47^{d}	37.30^{e}	20.59^{bc}
Lemon grass oil (LO)	0.15%	16.34^{a}	141.63^{b}	8.69^{a}	76.42^{b}	52.88^{cd}	19.89^{bc}
	0.30%	15.31^{ab}	123.35^{bc}	8.55^{ab}	51.95^{cd}	56.26^{bc}	23.67^{b}
	0.60%	10.15^{c}	26.04^{h}	4.98^{ef}	39.96^{d}	87.93^{a}	54.62^{a}
Fresh oils mixture (FO)	1.25%	10.45^{c}	210.45^{a}	6.09^{ef}	44.96^{cd}	55.37^{cd}	8.96^{bc}
	2.50%	12.61^{bc}	139.75^{b}	5.57^{ef}	61.94^{cd}	51.74^{cd}	11.34^{bc}
	5%	13.09^{bc}	42.70^{gh}	4.46^{ef}	72.93^{bc}	51.07^{cd}	11.76^{bc}
Used oils mixture (UO)	1.25%	13.57^{bc}	186.27^{a}	7.85^{bc}	59.94^{cd}	56.26^{bc}	20.17^{bc}
	2.50%	15.73^{a}	88.13^{ef}	7.68^{cd}	54.45^{cd}	52.74^{cd}	13.45^{bc}
	5%	16.28^{a}	67.17^{fg}	5.80^{ef}	43.96^{cd}	43.15^{de}	6.86^{bc}
Jojoba oil (JO)	1.25%	9.60^{c}	74.16^{ef}	3.27^{f}	39.46^{d}	46.89^{de}	5.04^{c}
	2.50%	10.75^{c}	95.66^{de}	4.70^{ef}	59.94^{cd}	48.59^{de}	14.57^{bc}
	5%	11.35^{bc}	107.49^{cd}	5.69^{ef}	115.88^{a}	53.83^{cd}	22.27^{b}
Control	_____	15.40^{a}	19.99^{h}	7.86^{bc}	81.42^{b}	53.02^{cd}	42.23^{a}
P		0.0017**	0.0001**	0.0267*	0.0001**	0.0001**	0.0001**
L.S.D.$_{0.05}$		4.04	28.86	3.08	30.10	12.81	16.87

* indicates significant differences between means at 0.01 level of probability.
** indicates highly significant differences between means at 0.01 level of probability.
Means in each column followed by similar letters are statistically insignificant at 0.05 level of probability.

DISSCUSSION

1- Chemical analysis of the tested oils:

Chemical properties of sunflower and soybean mixture used (UO) and fresh (FO) indicated that UO had a greater acidity percentage and peroxide value than FO which agreed with those gained by (Kaleem *et al*., 2015; Farag and Sabry, 2017). Analysis of fatty acids components of fixed oils FO and UO reported the presence of unsaturated fatty acids as $C_{16:1,}$ $C_{18:1}$, $C_{18:2}$ and, $C_{18:3\ and}$ saturated fatty acids as $C_{16:0}$ and $C_{18:0}$, in high percentages. Similar observations were mentioned by (Marinova *et al.,* 2012) who found $C_{16:0}$ (7.4 & 9.8%), $C_{18:0}$ (4.1 & 3.4%), $C_{18:1}$ (25.6 & 25.1%), $C_{18:2}$ (62.7 & 55.6%), and $C_{18:3}$ (0.0 & 5.6%) in sunflower and soybean oils, respectively. Linoleic acid content is widely employed as a measure of the degree of oil deterioration, because the polyunsaturated linoleyl chain is particularly vulnerable to oxidization (Farag and Sabry, 2017). (Quinchia *et al.,* 2010) showed that the fatty acids components of sunflower oil were $C_{16:0}$; 6.18%, $C_{18:0}$; 3.41%, $C_{18:1}$; 25.60 %, $C_{18:2}$; 64.80% and $C_{18:3}$; trace.

After gas chromatography analysis the components and percentages of the tested volatile oils indicated the presence of citronellal (35.20%), geranial (26.18%), and β-Pinene (20.24%) as the main constituents of *C. nardus* oil (CO) analysis. Similar results were obtained by Misrahanum *et al.,* (2022) reported that the *C. nardus* essential oil contains 26.06% citronellal, 26.314% citronellol, and 17.90% geraniol.

These results are agreed with the finding of Trindade and his team showed that the major component of C*. nardus* oil was citronellal that range from (22.2 to 37.8%) (Trindade, *et al.,* 2015 and Kaur *et al.,* 2021)

The essential ingredient and percentages of *C. citratus* oil (LO) were geranial (36.22 %), neral (28.56%), and myrcene (17.01 %). Earlier similar data have been obtained via various researchers who reported that major components of *C. citratus oil* were geranial, neral and myrcene (Masamba *et al.,* 2003 and Mohamed *et al.,* 2012).

2-Biological characters of mulberry silkworm, *B. mori*:

All concentrations of the Citronella oil increased the larval weight of 5th instar whilst lemon grass oil treatment at 0.15 % decreased the weight of tested larvae. Citronella oil is a plant that is frequently used as an analgesic, antipyretic, and antimicrobial activities. So, the improvement in larval weights and larval longevity by using tested concentrations of citronella may be due to its contents of citronellal, geranial, and β –Pinene. The aromatic characters of citronellal may help reduce stress in silkworms, promoting better growth and development. This finding is the same results for basil oil treatment at 2000 ppm recorded the highest significant 5th instar larval weight, the lowest significant mortality percentage and 5th instar larval duration, were 2.226 g, 0.787 g and 5.09 % respectively (Pasha and Soliman, 2022).

The inclusion of jojoba oil in mulberry silkworm food resulted in lower larval mortality, with no deaths observed at 1.25% concentration compared to a 4.00% mortality rate in the control group. This decrease in mortality with jojoba oil treatments may be attributed to the presence of vitamin D and its derivatives, including α, γ, and δ tocopherol, as well as other fat-soluble vitamins like vitamin A as suggested by (Belhadj, *et al.,* 2018). Jojoba oil has been shown to have a positive effect on the growth and development of *B. mori*. Studies have shown that treating silkworm larvae with jojoba oil can result in decreased larval mortality and a

shorter larval duration. Additionally, jojoba oil has been shown to improve silk production in silkworms. Because it contains approximately 98% wax (mostly wax esters, with a small amount of free fatty acids, alcohols, sterols, along with a small number of triglyceride esters, flavonoids, phenolic, and vitamins, jojoba oil is regarded as one of the best oils (Gad *et al.,* 2021). Abou-Zeid *et al.* (2021) discovered that the remarkable oxidative stability of crude jojoba oil is due to the presence of natural antioxidants.

Furthermore, citronella and lemongrass oils, which are non-toxic and well-known for their ability to repel insects, have been employed. To optimize the advantages of the low concentrations of these two oils as a nutritional supplement for the mulberry silkworm larvae, they were applied at low concentrations that did not prevent the larvae from feeding. Tannins, saponins, terpenoids, and flavonoids are among the phytochemicals found in lemon grass and citronella oils (Shendurse *et al.,* 2021). The antioxidant, antibacterial, and antifungal properties and their positive impacts are explained by these phytochemicals found in citronella and lemon grass oils **(**Nakahara, *et al.,* 2003; Balakrishnan *et al.,* 2015 and Mukarram *et al.* 2021). To understand the phytoconstituents of the oils, more investigation could be needed. Waste oils are typically less expensive than fresh one, for using them as an economical choice for feeding silkworms. However, the treatment of mulberry leaves with the Used oil (mixture of sunflower and soybean oils 1:1) decreased the larval weight, increased larval mortality and elongated the duration of fifth larval instar especially at the highest concentrations. And used oils are usually more oxidized, which can result in the development of harmful compounds that may jeopardize the health of the silkworms. To reduce these risks, it is essential to thoroughly filter and purify the waste oils before using them as feed additives. Ensuring that the oils are free from harmful contaminants and stored properly to enhance their safety for silkworms.

3- Biochemical studies:

Undoubtedly, the physiological state of silkworm affected by food additives especially essential oils in addition the usage of edible oil and used oils as a new technique for recycling waste. Results demonstrated a highly significant between the three concentrations of tested oils (CO, LO, FO, UO and JO) and vitality parameters such as AST, ALT, AChE, Chitinase, T.S.P. and Total lipid. The immune response is considered a crucial process due to the production of reactive oxygen species (ROS), which may lead to cell death and tissue destruction (Tak and Isman, 2016). This was evident in larvae treated with used oil and Citronella oil, which showed a high mortality rate. In contrast, larvae fed on Jojoba oil exhibited decreased activity of AChE and Chitinase, enzymes important for enhancing immunity and reducing mortality, corresponding to the biological results. Larvae fed on Jojoba with 1.25% concentration recorded 0% mortality that go in line with Gad *et al.* (2021) who referred to the ability of Jojoba oil to decrease the mortality of mice due to possess anti-inflammatory that resulted from its thermal stability which prevent oxidation. Also, it possesses inhibitory effect on the *Bacillus spp.* growth that confirmed the present results for the control which had high enzyme activities and mortality percent (Ranzato *et al.,* 2011). Supported by (Tak and Isman, 2016) who reported that essential oils have a direct effect on insect physiological functions. One of the most significant and vital processes is immunological responses, which trigger cell death and tissue damage. (El-Ashram *et al.* 2022) showed that lemon oil caused low inhibition of AChE activity in silkworm *B. mori.* Sabry *et al.* (2023) reported that *T. distichum* essential oil produced biochemical changes in treated *B. mori*, leading to decreased activity of acetylcholinesterase compared to control. Also, they mentioned that the most dominant compound in oil was α-Pinene. Although Lemon grass oil increased the activity of total protein and Lipids but decrease the larval weight compared to the other treatments that are conformity by (Wani *et al.,* 2021) who concluded that the amount of hemolymph protein may vary due to differences in how proteins are made and used on the seventh day of the fifth instar stage. All larval parameters improved at certain level of total protein activity, increases the activity of total protein led to the toxicity (Moustafa, 2024). In addition, Chowdhury *et al*., (2007) mentioned that sunflower oil contains appreciable quantities of vitamin E, sterols, squalene and other aliphatic hydrocarbons, terpene and methyl ketones. Also (Macri *et al.,* 2024) studied the effect of high concentration of fried oils during the growth period of mice may lead to high lipid toxicity, and the build-up of reactive oxygen species that was on line of our results concerned of ACHE, Chitenase, ALT, AST, and Lipids. Elsetiha *et al.* (2019) confirmed our results, rabbits fed on 1% of sunflower oil was appropriate for the activity of AST and ALT enzymes.

Several studies showed that some Chitinases could be assumed as markers for diagnosis, prognosis, activity, and severity of a disease and therefore can be helpful in the choice of treatment.

CONCLUSION

The purpose of this study was to determine how utilizing some volatile and fixed oils for enhancing the silkworms' nutrition to enhance the silkworm performance. Data reported that the highest effect of oils was JO treatments with the lowest concentration for most biological and biochemical parameters. Small amounts of the examined volatile oils can also be used to benefit from their constituents, which have shown promise as

antioxidants, anti-fungal, and anti-bacterial agents. Adding low quantities of citronella oil has been demonstrated to make the larvae heavier. Furthermore, when compared to the control, a lesser concentration of lemongrass oil can reduce the larval mortality rate. Recycling waste oils minimizes environmental waste and encourages a more sustainable method of resource management. Despite being used, these oils retain important nutrients that can aid in the growth and development of silkworms. However, it is important to ensure that the waste oils are properly cleaned and free from harmful contaminants before being used as feed additives.

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إستخدام بعض الزيوت المتطايرة والثابتة في غذاء دودة القز: استراتيجية جديدة لتعظيم إنتاج الحرير

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الغرض من البحث الحالي هو استخدام الزيوت الطبيعية مثل زيت السترونيلا (*Cymbopogon nardus*)، زيت حشيشة الليمون (*Cymbopogon citratus*)، خليط من زيتي عباد الشمس (*Helianthus annuus* L.) وفول الصويا (*Glycine max* L.) الممزوجة بنسبة 1:1 وزيت الجوجوبا كإضافات غذائية ليرقة دودة القز (*Bombyx mori*) أفادت النتائج بأن زيت السترونيلا بجميع تركيزاته أعطى أعلى وزن لليرقات في حين تم تسجيل أقل وزن لليرقات عند معاملة أوراق التوت بزيت حشيشة الليمون. عند اضافة خليط من زيتي عباد الشمس والصويا والذي تم استخدامة في القلي مسبقا (زيت مستعمل) أدي الي زيادة طول العمر اليرقي الخامس خصوصا التركيزين 5% و2.5% على التوالي، في حين سجلت معاملة اليرقات بزيت السترونيلا أقصر مدة ليرقات العمر الخامس. أظهرتالنتائج ان زيت الجوجوبا (*Simmondsia chinensis*) عند جميع التركيزات قللت من نسبة موت اليرقات في العمر الخامس. علاوة على ذلك، أوضحت نتائج الدراسات البيوكيميائية أن جميع المعاملات أدت إلى انخفاض في نشاط إنزيم AST، في حين أن نفس المعاملات أدت زيادة نشاط إنزيم ALT وانخفض نشاط إنزيم الأستيل كولين بشكل كبير مع كل التركيزات للزيوت المختبرة وخاصة زيت السترونيلا وزيت الجوجوبا. علاوة على ذلك، زاد نشاط انزيم الكيتينيزفي اليرقات المعالجة بزيت الجوجوبا. أخيرًا تم الحصول على أعلى محتوى من البروتيناتالذائبة والدهون الكلية في الهيموليمف لليرقات التي أعطيت ورق التوت المعامل بتركيز 0.06% من زيت الجوجوبا. وتشير البيانات إلى أن زيت الجوجوبا كان له التأثير الإيجابي الأكبر على أغلب الصفات المختبرة. على النقيض من ذلك أظهرت النتائج أن معاملة اليرقات ب الخليط لزيتي عباد الشمس وفول الصويا المستعمل أقل تأثير على ديدان القز مقارنة بالمجموعة الضابطة.

الكلمات المفتاحية: دودة القز، التغذية، الإنزيمات، الزيوت المتطايرة، الزيوت الثابتة