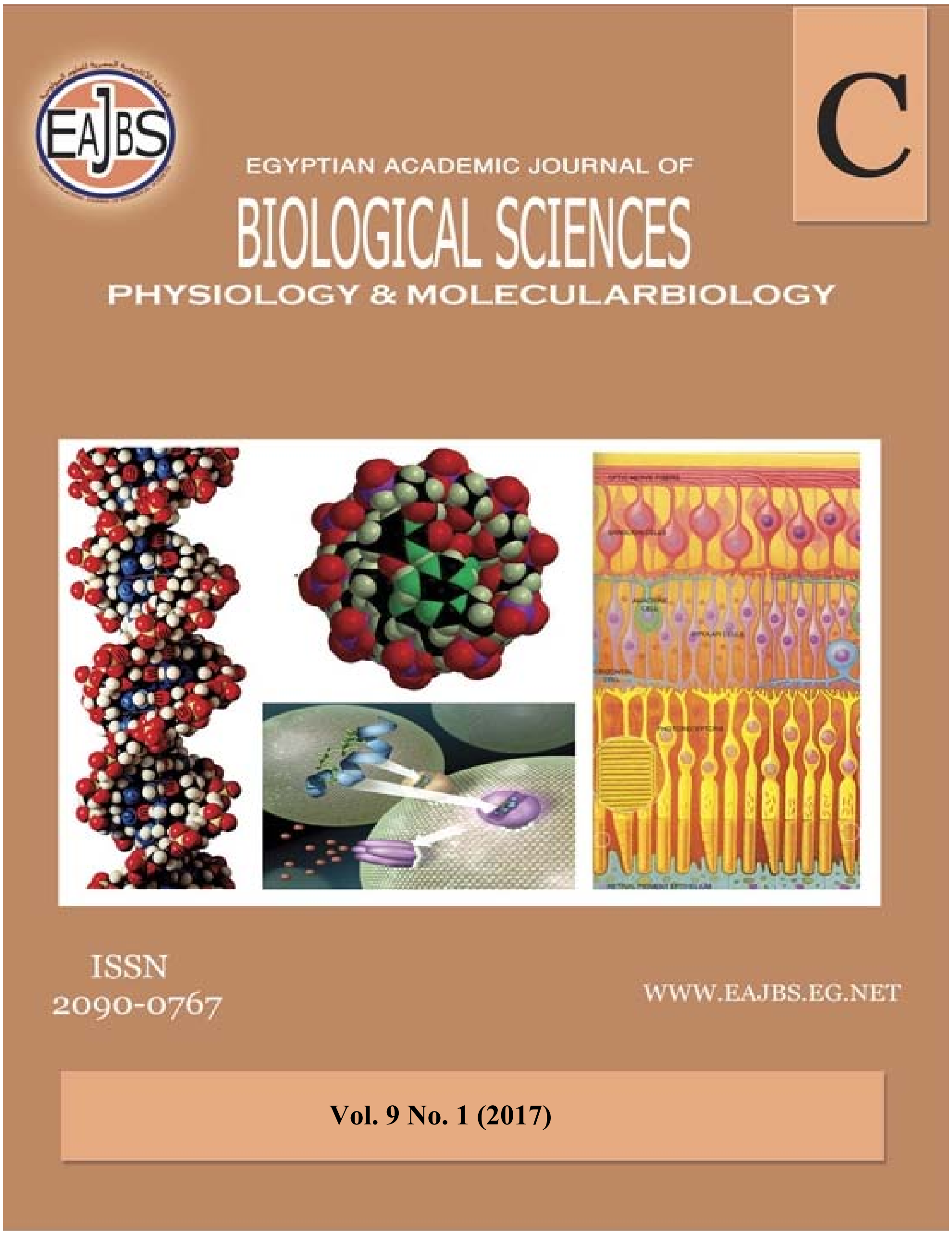

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Effect of Some Essential Oils and Natural Botanical Extracts on Food Consumption and Some Physiological Aspects of Mulberry Silkworm, *Bombyx mori* L.

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ABSTRACT

The effects of different concentrations of marjoram oil, thyme oil, (1%, 0.5%, and 0.25%) and their botanical extracts (2%, 1%, and 0.5%) as a nutritional additives offered to silkworm larvae during the 4th and 5th larval instars on food consumption, Approximate weight of digested food (A.D), Approximate digestibility (A.D%), efficiency of conversion of food ingested to body substance (E.C.I%), and efficiency of conversion of food digested to body substance (E.C.D%) were studied. Also, to determine their effects on total carbohydrates and total protein in larval haemolymph. Results showed significant variation in most of the studied parameters of oils and botanical extracts different concentrations compared to control; E.C.D% was found maximum with 0.5% marjoram oil (74.995%) and thyme oil (71.465%), in the botanical extracts 2% of both thyme, and marjoram exhibited the highest ratio (63.170% and 60.975%), respectively compared with control which recorded the minimum (38.830%). The efficiency with which the ingested food is converted into body substances (E.C.I%) 0.5% of marjoram and thyme oils showed the highest efficiency of conversion (21.000% and 20.555%), respectively compared to (13.445%) for the control. 2% of both thyme (19.960%) and marjoram extract (18.845%) showed also the highest ECI% while the control recorded the least result. The highest AD% was shown by control group (43.185%) and AD of the control group exhibited the highest among them (3.400 gm). Results revealed a significant variation between the both larval instars in most parameters. The quantity of food consumed in both oils and extracts varied significantly between the both instars with no significant values among different concentrations of them. Total protein exhibited the highest value when feeding larvae on mulberry leaves supplemented with 0.5% of both marjoram and thyme oils (41.067 and 39.867 mg/ml haemolymph), respectively, and 2% of both thyme and marjoram extracts (36.9 and 36.567 mg/ml haemolymph), respectively while the control group showed the minimum value (22.633 mg/ml haemolymph). The same trend was observed for total carbohydrates. It can be concluded that mulberry leaves fortified with different concentrations of marjoram and thyme oils and extracts were proved to be more efficient in rearing mulberry silkworm as it improved food acquisition, high ingestion, high (E.C.I & E.C.D) and low (A.D),also it increased protein and carbohydrates metabolism.

INTRODUCTION

The mulberry silkworm is a beneficial important insect economically since the silk industry depends on it. Growth of silkworm depends on body weight increase and on the biochemical components accumulation of food like protein, amino acids and enzymes, as the same way, the quality and quantity of food intake, digestibility, and the utilization of food affect the growth, grown larval body weights, salvation, and subsequently silk synthesis and production (Biram Saheb *et al*., 2005 and Rath *et al*., 2005).

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Mulberry leaves consist of principle elements including water and dry matter and the later consists of protein, carbohydrate including (soluble carbohydrate and cellulose), fat, inorganic salt, and vitamins. These elements represent the essential nutrients for the physiological functions of the mulberry silkworm (Wa pang and Chen, 1994). Feeding in larval stage is an active and dynamic process and the amount rate and quality of food consumed affect directly on the growth, final weight, and reproductive potential of the insect (Slansky and Scriber, 1985 and Parra, 1991).

Recently, the natural plant products such as essential oils and plant extracts could be used as a nutritional additive to improve the silkworm development and silk production; they are characterized by its beneficial constitutes and their antimicrobial activity with no side effects on it (Shoukry *et al.*, 1998; Tabassum and Vidyasagar, 2013).

Essential oils of Thyme consists of Thymol and carvacrol and other chemical constituents such as flavonoids (e.g. thymonin) are characterized by their antimicrobial activity which depends on the thymol content of the oil, on the other hand, extracts of thyme and its chemical constituents have shown activity against various pathogenic bacteria and fungi in vitro studies (Didry *et al.*, 1993; Bozin*et al.*, 2006 and Figueiredo *et al.*, 2008).

Aromatherapy has been claimed to be effective by using the essential oil of plants. Aromatic leaves of marjoram are used either green or dry for aroma therapeutic purposes; the tops are cut as the plants begin to flower and are dried slowly in the shade. By steam-distilled procedure, an essential oil can be produced from the flowering leaves and tops of marjoram; it has many chemical components such as camphor, borneol and pinene (Cha*et al.*, 2010, learn 2 grow site).

Essential oils in general lead to significant clinical improvements depending on their intended use, although each oil showed consequently other benefits for other symptoms; so in future, researches should explore use of additional essential oils and modes of administration (Johnson *et al.*, 2016).

The present study aims to evaluate the effects of different concentrations of the essential oils of marjoram, thyme plants, and their botanical extracts as nutritional additives on: Food utilization (food consumption, Approximate weight of digested food (A.D), Approximate digestibility (A.D%), efficiency of conversion of food ingested to body substance (E.C.I%) and efficiency of conversion of food digested to body substance (E.C.D%). Also, to determine their effects on total carbohydrates and total protein in larval haemolymph.

MATERIALS AND METHODS

The present study was carried out in the laboratory of Sericulture Research, Department of Plant Protection Research Institute, Agriculture Research Center, during the spring season.

Materials:

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

2- Thyme and marjoram oils (Cap. Pharm. Company) were used with 3 concentrations (1%, 0.5%, and 0.25%) Prepared by (Harvey and John 1898).

3- Thyme and marjoram extracts were used with 3 concentrations (2%, 1%, and 0.5%) according to Vedhamathi (2004).

Methods:

Silkworm Rearing Technique:

Rearing of silkworm was carried out under the hygro-thermic conditions 28 ± 1 °C and 75 ± 5% RH, 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 five groups. Two groups for thyme and marjoram oils and two groups for botanical extracts of them. Each group was divided into three subgroups representing three concentrations using 3 replicates for each concentration (30 larvae for each replicate). Weighted fresh mulberry leaves were dipped in each concentration for 5 minutes and left to dry then offered to larvae during the 4th and 5th larval instar to attain full growth and maturity, and the fifth (control group) fed on leaves was treated only with distilled water.

Leaves and faeces were collected and kept. Thus, for each day of feeding period dry matter, ingested, digested, faeces produced and increase in larval weight were recorded and the various nutritional parameters i.e., amount of consumed food, ingested, digested, and converted in to dry matter were calculated and expressed on fresh weight basis using the methods of Waldbauer (1968).

Physiological Assays:

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. Total carbohydrates were extracted and prepared for assay according to Crompton and Birt (1967) and were estimated in acid extract of sample by the phenol-sulphuric acid of Dubois *et al.*, (1956). Total proteins were determined by the method of Bradford (1967).

Statistical Analysis:

The data were statistically compacted using proc ANOVA in SAS (SAS institute 1988).

RESULTS AND DISCUSSION

Utilization of Food:

B. mori like other Lepidopteron insect is highly specialized for rapid growth primarily achieved by a higher rate of food acquisition. In order to understand the feeding process in *B. mori* as influence by mulberry leaves supplemented with essential oils of plants (marjoram and thyme) and their botanical extracts with different concentrations, the amount of food consumed, digestibility and conversion of food must be define.

Food Consumption:

As shown in Tables (1&2), quantity of food consumed by larvae in both groups (oils and extracts) varied significantly between the both instars (4th and 5th instars) while has no significant value among different concentrations of them.

These results on the same line with Sujatha and Sampath (2015) reported that, the quantity of ingested and digested food showed a consistent decrease at different dosages of clove oil, a positive correlation between ingested and digested food was reported. And in agreement with those of Aruna*et al.* (1995) observed that food consumption increases with age in all the five instars of *Antheraea mylitta* silkworm and there was a significant and positive relationship between food consumption and feeding duration, and between growth and food consumption. And also emphasized with Rath (2011) who stated that food consumption and its utilization affect metabolism of enzyme synthesis, storage of nutrients, and other different activities. Parra (1991) observed that a minimum of mulberry leaves consumed by larvae and consequently utilized to reach the maximum extent for body weight should be considered more potential than others.

Approximate Weight of Digested Food (A.D):

The quantity of food utilized during the 4th and 5th larval instars expressed in terms of approximate weights of food digested (A.D); as illustrated in Tables (1&2) it was comparable among the different concentrations in both instars and the control group exhibited significantly the highest value among them (3.400 gm). While 0.5% and 0.25% of marjoram oil recorded (1.845 & 1.980 gm, respectively) and thyme oil (1.910 & 1.990 gm, respectively) showed the least values. In both botanical extract groups, no significant value among different concentrations was observed.

Approximate Digestibility (A.D %):

The approximate digestibility (A. D%) was computed for all concentrations of treatments and recorded. In Tables (1&2), the highest value was shown by control group (43.185%). On the other hand, the least A.D% values exhibited significantly with the concentrations 0.5% and 0.25% of oils (25.010 & 29.150% for majoram and 26.905 & 29.450% for thyme oil), respectively. The same trend was noticed with the concentrations 2% and 1% of both botanical extracts which recorded the least A.D% values (31.310 & 36.370% for majoram and 29.600 & 34.580% for thyme extract), respectively, compared with the control. The 5th instar in both groups; oils and extracts revealed significantly the least values (28.483 & 32.781%, respectively) compared with the 4th instar (35.516 & 40.244%, respectively).

The results are in partial accordance with Sujatha and Sampath (2015) who stated that, approximate digestibility is the percentage of ingested leaf which is digested in the midgut showed a gradual decrease with clove oil concentrations comparing with control, it depends on ingestion levels and activity of digestive enzymes in the silkworm mid gut region and these two factors support high growth rates and larval weights. Slansky and Sciber (1985) explained that A.D can vary with a negative manner as a function of consumption rate; when a larva increases its consumption rate, the rate of passage of food through the gut of silkworm may be increased allowing a reduction in A.D, while a prolongation of food in the gut may lead to an increase in A.D.

Efficiency of Conversion of Food Ingested to Body Substance (E.C.I %):

The (E.C.I%) efficiency of conversion of ingested dry matter to body substance varied significantly among the different concentrations of treatments; as shown in Tables (1&2), the concentration 0.5% of marjoram and thyme oils showed the highest efficiency of conversion (21.000% & 20.555%), respectively compared to control (13.445%) and the concentration 2% in both thyme (19.960%) and marjoram extract (18.845%) showed also the highest ECI% while the control recorded the least result. The 4th instar exhibited significantly the highest ECI% in both groups; oils and extracts (19.969 & 18.095%, respectively) compared with the 5th instar (16.924 & 15.554%, respectively).

By the same way, Panizzi and Parra (1991) reported that AD is inversely correlated with E.C.I%, since 5th instar larvae consumed the food in an indiscriminate manner, including leaf nurtures. Thus a smaller amount of food is utilized for energy and a large part of it is incorporated into body tissue increasing the E.C.I%

Efficiency of Conversion of Food Digested to Body Substance (E.C.D %):

As illustrated in Tables (1&2), the efficiency of conversion of digested food into body matter (E.C.D%) was found maximum with the concentration 0.5% of marjoram oil (74.995%) and thyme oil (71.465%) and the minimum was in control group (38.830%), in the botanical extracts the concentration 2% in both thyme and marjoram exhibited the highest ratio (63.170% & 60.975%), respectively, compared with control. The 5th instar revealed significantly the highest E.C.D% (55.253%) compared with the 4th instar (47.015%) in botanical extract treatment group. While, no significant variation was noticed between the 4th and 5th larval instars in oils treatments group and this is maybe due to the nature of the composition of these essential oils and the increase in the age of larvae.

These results are in agreement with Sujatha and Sampath (2015) who observed that a significant increase in ECL and ECD percentage in treated larvae with essential clove oil as a result of better conversion of ingested and digested food material into body matter in larvae. Scriber and Penny (1979) found that E.C.I% value of Bombycidae larvae was much dependent on the food plants, and Soohoo and Frankel (1966) observed that when the food is ingested by the larvae more slowly, the more efficiently this food is digested and thus transformed into body substances, indicating an inverse relationship between consumption rate, E.C.I% and E.C.D%.

Table 1: Effect of essential oils of marjoram and thyme plants as nutritional additives on food utilization of the *B. mori* larvae.

treatments	Conc.	Consumed food	AD	AD%	ECI%	ECD%
Marjoram oil	1%	7.125[a]	2.355[ab]	29.725[bc]	18.950[a]	63.905[b]
	0.5%	7.795[a]	1.845 [b]	25.010[c]	21.000[a]	74.995[a]
	0.25%	7.570[a]	1.980 [b]	29.150[bc]	19.650[a]	68.420[ab]
Thyme oil	1%	6.970[a]	2.450 [ab]	31.425[b]	19.120[a]	62.790[b]
	0.5%	7.575[a]	1.910 [b]	26.905[bc]	20.555[a]	71.465[ab]
	0.25%	7.440[a]	1.990 [b]	29.450[bc]	19.660[a]	64.890[b]
Control+tween		5.890[a]	3.280 [a]	41.145[a]	15.190[b]	41.865[c]
Control		5.755[a]	3.400 [a]	43.185[a]	13.445[b]	38.830[c]
L.S.D.		-	1.203	6.223	3.254	9.106
Pr		0.6074	0.0598	<.0001	0.0002	<.0001
Instars	4th	1.568 [b]	0.903 [b]	35.516[a]	19.969[a]	59.618[a]
	5th	12.463 [a]	3.900 [a]	28.483[b]	16.924[b]	62.173[a]
L.S.D.		1.2655	0.601	3.1115	1.627	-
Pr		<.0001	<.0001	<.0001	0.0005	0.2633

Table 2: Effect of botanical extracts of marjoram and thyme plants as nutritional additives on food utilization of the *B. mori* larvae.

treatments	Conc.	Consumed food	AD	AD%	ECI%	ECD%
Marjoram extract	2%	6.910[a]	2.390 [a]	31.310[de]	18.845[ab]	60.975[a]
	1%	6.285[a]	2.995 [a]	36.370[bc]	17.000[abc]	51.815[bc]
	0.5%	6.050[a]	3.145 [a]	38.370[abc]	16.180[bc]	48.705[c]
Thyme extract	2%	7.260[a]	2.220 [a]	29.600[e]	19.960[a]	63.170[a]
	1%	6.425[a]	2.810 [a]	34.580[cd]	17.395[ab]	54.375[b]
	0.5%	6.115[a]	3.080 [a]	37.540[bc]	16.580[abc]	49.335[c]
Control		5.755[a]	3.400 [a]	43.185[a]	13.445[c]	38.830[d]
L.S.D.		-	-	4.9679	3.7191	4.4797
Pr		0.6746	0.7139	<.0001	0.0358	<.0001
Instars	4th	1.096[b]	1.165 [b]	40.244[a]	18.095[a]	47.015[b]
	5th	11.576[a]	4.665 [a]	32.781[b]	15.554 [b]	55.253[a]
L.S.D.		0.8836	0.7433	2.484	1.8595	2.2399
Pr		<.0001	<.0001	<.0001	0.0087	<.0001

Charai *et al.* (1996) explained that dried whole marjoram plant and its essential oil and water extract of leaves have demonstrated antimicrobial effect and essential oil was more active against bacteria and yeasts than water extract. By the same way, Erenler *et al.* (2016) stated that the parts of marjoram that are used in medicine are dried leaves, leaves extract, and essential oil. Marjoram (*Origanumm ajorana*) leaves have been claimed to have antimicrobial properties.

The Physiological Aspects:

Based on the retrospective studies mentioned in review of this work, that claimed several beneficial effects of marjoram and thyme as nutritional additives in rearing silkworm, the physiological functions of silkworm were investigated to assess the effects of these additives on the growth of silkworm larvae.

Total Protein:

As shown in Tables (3&4), data exhibited a significant increase of total protein means of larvae fed on mulberry leaves supplemented with both marjoram and thyme oils and their botanical extracts with different concentrations than control (22.633 mg/ml haemolymph) especially the concentration 0.5% of both marjoram and thyme oils (41.067 & 39.867 mg/ml haemolymph), respectively, and the concentration 2% of both thyme and marjoram extracts (36.9 & 36.567 mg/ml haemolymph), respectively.

Table 3: Effect of essential oils of marjoram and thyme plants as nutritional additives on total protein and total carbohydrates of *B. mori* larval haemolymph.

Treatments	Conc.	Total carbohydrates	Total protein
Marjoram oil	1%	6.257±0.13[c]	35.800±0.76[c]
	0.5%	7.990±0.08[a]	41.067± 0.75[a]
	o.25%	6.703±0.16[b]	39.567±0.58[ab]
Mean		6.983	38.811
Thyme oil	1%	5.963±0.14[c]	35.467±0.33[c]
	0.5%	6.793±0.10[b]	39.867±0.50[ab]
	o.25%	6.693±0.12[b]	38.333± 0.71[b]
Mean		6.482	37.887
Control + tween		4.763±0.14[d]	25.833± 0.59[d]
control		4.320± 0.23[e]	22.633± 0.59[e]
LSD 0.05 (conc.)		0.4345	1.8484
Pr.		<.0001	<.0001

Table 4: Effect of botanical extracts of marjoram and thyme plants as nutritional additives on total protein and total carbohydrates of *B. mori* larval haemolymph.

Treatments	Conc.	Total carbohydrates	Total protein
Marjoram extract	2%	6.380±0.08 [a]	36.567±0.61[ab]
	1%	5.840±0.12 [b]	32.100±0.67[c]
	o.5%	5.203±0.14 [c]	29.067±0.66[d]
Mean		5.808	32.578
Thyme extract	2%	6.393±0.15 [a]	36.900±0.64 [a]
	1%	5.927±0.12 [b]	34.833±0.66 [b]
	o.5%	5.787±0.15 [b]	30.433±0.56[cd]
Mean		6.036	34.055
control		4.320±0.23 [d]	22.633 ± 0.59[e]
LSD 0.05 (conc.)		0.4405	1.8632
Pr.		<.0001	<.0001

These results on the same line with Eman *et al.* (2014) who observed that mulberry leaves fortified with essential oils as *Eucalyptus oil* with different concentrations were improved protein enzymes activities and total protein of the larval haemolymph, hence, the viability of larvae increased proving the efficiency of essential oils in rearing silkworm. And in agreement with those of Morssy (2009 and 2012) who evaluated the effects of mulberry leaves supplemented with some botanical oils on silkworm *Bombyx mori*. The results proved that oils exhibited the highest activity of protein enzymes and increasing of total protein and total carbohydrates.

Bari *et al*. (1985) stated that proteins are the most important organic nitrogenous compound in the food of silkworm larvae which are involved partially in all the structure and function of the cells, and larvae digests and absorbs over 60% of the ingested nitrogen and 65% of this nitrogen is utilized by the 5th instar larvae for the synthesis of silk proteins.

In addition, Nagata and Kobayashi (1990) observed that, the nutritional richness in the diet influenced the accumulation of storage proteins in the haemolymph of the silkworm larvae i.e., the quantity of storage protein in the silkworm larvae fed on low protein were less than the standard diet but the larvae fed on optimal level of protein show a higher levels of storage protein.

Total Carbohydrates:

In parallel direction, means of total carbohydrates in Tables 3 & 4 cleared that larvae fed on mulberry leaves supplemented with both marjoram and thyme oils and their botanical extracts with different concentrations recorded a significant higher means of total carbohydrates than control group (4.320 mg/ml haemolymph); 0.5% of both marjoram and thyme oils (7.990 & 6.793 mg/ml haemolymph), respectively, and the concentration 2% of both thyme and marjoram extracts (6.393 & 6.380 mg/ml haemolymph), respectively.

The physiological results of this study are supported by the work of Sumathi (2008), who found that the plant extracts with mulberry leaves as supplementary diet to the silkworm, *Bombyx mori* has showed significant elevation of protein, carbohydrate, and lipid in digestive tissues in the experimental groups compared the control. And, Prakash (2010) studied that the effect of supplementation of aqueous tamarind seed coat extract with different concentrations on the food utilization pattern of the silkworm, *Bombyx mori*. Results showed that the supplementation of carbohydrate rich tamarind seed coat extract, having carbohydrate, protein and lipid contents, their respective rates were increased when fed with mulberry leaves soaked in increasing concentrations of tamarind seed coat extract.

And as reported by Sinha *et al*. (2005), carbohydrates are utilized by silkworm larvae as an energy source and utilized for synthesis of both lipids and amino acids and the degree of fat body glycogen and trehalosein haemolymph is also dependent on the carbohydrate content in the diet of silkworm.

Similar results were obtained by Sayed (2002) who reported that protein and carbohydrate percentages of the leaves are highly and positively correlated with the activity processes of silkworm.

In parallel direction, results explained by Guerra-Boone *et al.* (2015) reported that both thyme (*Thymus vulgaris*) and marjoram (*Origanum majorana*) essential oils contained thymol; the chemical characterization and antimicrobial activity assays revealed that oils with a higher percentage of aliphatic and oxygenated monoterpenes, mainly phenolic monoterpenes such as thymol, showed antimicrobial activity. And the antibacterial activity of thyme oil is associated with the presence of thymol and carvacrol in its consistents, and such activity is not related to its concentration, but is suggested its interaction with other compounds present in the oils (Rota *et al*., 2008). As the same trend, Ramos *et al.* (2011) reported that *cis*-Sabinene hydrate in essential oil of marjoram have been claimed to be responsible for the antibacterial effect of this oil.

Conclusively, in the present study, the food supplementations with essential oils of marjoram and thyme plants and their botanical extracts with different concentrations produced a remarkable increase in larval growth owing to increase in food utilization and also improved larval physiological characteristics.

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ARABIC SUMMERY

تأثير بعض الزيوت الضرورية والمستخلصات النباتية الطبيعية على الغذاء المستهلك وبعض القياسات الفسيولوجية لدودة الحرير التوتية بومبيكس موراى

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الهدف من هذه الدراسة هو تقييم تأثير تركيزات مختلفة من زيت البردقوش و الزعتر (٠,٢٥% ، ٠,٥% ، ١%) وتركيزات مختلفة من مستخلص نبات البردقوش و الزعتر (٠,٥% ،١% ، ٢%) كإضافات غذائية لورق التوت قدمت لدودة الحرير التوتية *Bombyx mori* أثناء العمر الرابع و الخامس علي بعض عوامل كمية الغذاء المستهلكة مثل AD, AD%, E.C.I%, E.C.D%

و أيضا دراسة تأثيرها علي القياسات الفسيولوجية مثل المحتوى الكلى للكربوهيدرات و البروتين فى الدم. و قد أظهرت النتائج وجود فروق معنوية بين التركيزات المختلفة للزيوت و المستخلصات مقارنة بمجموعة الكنترول فى معظم القياسات فى العمرين. حيث سجلت E.C.D% أعلى قيمة لها في تركيز ٠,٥% من زيت البردقوش (٧٤,٩٩٥%) و زيت الزعتر(٧١,٤٦٥%)، وكان التركيز ٢% من المستخلص النباتى لكل من الزعتر والبردقوش هو الأعلي في القيمة حيث كانت القيم كالتالي (٦٣,١٧٠% &٦٠,٩٧٥%) علي التوالي مقارنة بالكنترول الذى سجل أقل قيمة (٣٨,٨٣٠%). وقد وجد إختلاف معنوي بين التركيزات فى كفاءة الغذاء المتحول إلي وزن E.C.I% و قد سجل تركيز ٠.٥% فى كل من زيت البردقوش و الزعتر أعلى قيمة معنوية (٢١ % & ٢٠,٥٥٥%) علي التوالي مقارنة بالكنترول (١٣,٤٤٥%) و سجل تركيز ٢% فى المستخلصات النباتية للزعتر والبردقوش أعلى قيمة (١٩,٩٦٠% & ١٨,٨٤٥%) علي التوالي مقارنة بالكنترول الذى سجل أقل قيمة. وقد تم حساب A.D% لكل التركيزات فى جميع المعاملات و أظهر الكنترول أعلي نتيجة معنوية (٤٣,١٨٥%) و أيضاً سجل الكنترول أعلى قيمة A.D (٣,٤٠٠جم) مقارنة بالتركيزات المختلفة للمعاملات. أوضحت الدراسة أيضاً وجود إختلاف معنوى بين كمية الغذاء المستهلكة بين العمرين و عدم معنوية هذا الإختلاف بين التركيزات . أشارت النتائج أيضاً إلى وجود فروق معنوية بين العمرين الرابع والخامس فى معظم القياسات.

وبالنسبة لنتائج القياسات الفسيولوجية سجلت أعلي قيمة معنوية للمحتوى الكلى للبروتين في اليرقات التي تغذت علي أوراق التوت المضاف إليها تركيز٠,٥% من كل من زيت البردقوش و الزعتر (41.067&39.867 mg/ml haemolymph)علي التوالي و ٢% لكل من مستخلص الزعتر و البردقوش (36.9&36.567 mg/ml haemolymph) على التوالى بينما مجموعة الكنترول أظهرت أقل قيمة للبروتين (22.633 mg/ml haemolymph).وقد لوحظ نفس الأتجاه في نتائج المحتوى الكلى للكربوهيدرات . مما سبق نستخلص أن تغذية ديدان الحرير التوتية بأوراق التوت المدعمة بتركيزات مختلفة لزيت البردقوش و الزعتر وأيضا المستخلصات النباتية منهما أدت إلى تحسين كفاءة اليرقة للأستفادة من الغذاء من حيث زيادة الوزن المستهلك و كفاءة تحويل الغذاء المتناول و المهضوم، كما أدت إلى زيادة فى المحتوى الكلى للكربوهيدرات وكذلك زيادة فى المحتوى الكلى للبروتين مما يؤدى إلى تنشيط عملية تكوين الحرير.