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Effect of plant extracted oils on biological aspects and silk production of mulberry silkworm *Bombyx mori* (Lepidoptera: Bombycidae)

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Abstract:

The effects of feeding silkworm larvae *Bombyx mori* L. (Lepidoptera: : Bombycidae) (Egypt hybrid, Giza) on mulberry leaves supplemented with three plant extracted oils; sesame oil (*Seassamum indicum* L.), olive oil (*Olea europaea*.) and nigella sativa oil (*Nigella sativa* L.) at three concentrations (0.5, 1.0 and 2.0 %) for one time (At the beginning of the fourth instar) and two times (At the beginning of fourth and fifth instars) on some biological and technological characters were studied. Sesame oil (0.5%) decreased the grown larval mortality and increased the cocooning percentage, the moth emergency rate and cocoon shell weight, but 1.0% concentration increased fecundity, cocoon weight and silk filament size. Olive oil (2%) increased the content silk ratio while nigella sativa oil (0.5%) caused more length and weight of reelable filament silk.

Introduction

The mulberry silkworm, *Bombyx mori* L. (Bombycidae, Lepidoptera) is one of the most economically important insects not only on the national level but also internationally. The production of high quality and quantity of natural silk depends mainly on larval feeding (Parra, 1991). The mulberry silkworm, *B. mori* . is reared successfully as the main source of natural silk. Recently, considerable attention has been given to improve rearing techniques of silkworms to increase the production of raw silk in Egypt to meet with the higher demands for industrial purpose. Furthermore, developing and improving the practical and applicable techniques for increasing the productivity of silkworm i.e. silk and eggs production is necessary. So, the nutritional studies on the silkworm, *B. mori* L. are of much importance pertaining to its productivity and the nutritional value of local indigenous plants, wild herbs and edible seeds rich in protein are of great significance. Therefore, it has been reported recently that better production of cocoon crops and eggs is possible when mulberry leaves are supplemented with certain nutritional materials (Singh *et al*., 1993; Zannoon, 1994; Ashour, 1997; El-Sayed *et al.*, 1998 and Mesbah *et al.*, 2000).

The present investigation studied the effects of some plant extracted oils as

nutritional additives on biological aspects and the effects on quantitative characteristics of cocoons and silk filament of mulberry silkworm *B.mori* .

Materials and methods

Biological and technological studies were done to determine the effects of addition some plant extracted oils.

1.Materials:

1.1. Mulberry leaves, *Morus alba* variety balady.

1.2. The mulberry silkworm, *B. mori* (Egypt hybrid, Giza) were obtained from the Sericulture Research Department, Plant Protection Research Institute, Agriculture Research Center, Ministry of Agriculture and Land Reclamation in Giza, Egypt.

2.Plant extracted oils:

2.1. Sesame oil (*Seassamum indicum*, L.)

2.2. Olive oil (*Olea europaea*.)

2.3. Nigella sativa oil (*Nigella sativa* L.)

The procedures of plant extraction were produced at the Food Technology Research Institute, Agriculture Research Center, Giza. Three concentrations were used for each oil (0.5%, 1% and 2%) prepared by (Harvey and John, 1898).

2.Methodes:

2.1. Rearing technique:

Rearing mulberry silkworm was carried out under controlled laboratory conditions of 26 ± 2 °c and 70 ± 5 % RH. Rearing procedures were achieved according to Krishnaswami (1978) rearing technique. Mulberry leaves were dipped in each concentration of each used material for one minute and left to dry and then fed to mulberry silkworm larvae. The control leaves were dipped in distilled water. Each concentration was offered to two groups of silkworm larvae, the first group was fed with treated leaves only one time at the beginning of the 4th larval instar, while the second group was fed two times at the beginning of the 4th and 5th larval instars. Using three replicates (50 larvae) for each concentration.

2.2. Biological studies:

The following biological aspects under this investigation were studied :

2.2.1. Larval mortality (%)

2.2.2. Larval duration (day)

2.2.3. Cocooning percentage (%)

2.2.4. Adult emergence (%)

2.2.5. Fecundity of female (number of deposited egg/ female).

2.3.Technological studies:

2.3.1. Cocoon indices:

Ten resulted fresh cocoons from each replicate were collected, cleaned, weighed and carefully cut. The pupae and exuviate were removed, and cocoon shells were weighed. Silk content ratio was calculated according to formula by Tanaka (1964).

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

2.3.2. Reelable silk filament parameters:

Another ten cocoons from each replicate of the resulted fresh cocoons were collected; oven dried and reeled by individual reeling machine. The length (m) and weight (mg.) of the dried reelable filament were determined. The sizes of reelable filaments were calculated according to Tanaka (1964) formula:

$$\text{Silk filament size (dn)} = \frac{\text{Weight of silk filament (mg)}}{\text{Length of silk filament (m)}} \times 9000$$

2.4. Statistical analysis:

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

Results and discussion

Effect of fnriching mulberry leaves with some plant extracted oils on biology and silk production of silkworm *Bombyx mori* :

1. Biological aspects:

1.1. Larval mortality:

The 1st concentration (0.5%) in all treatments exhibited the least larval mortality percentage such as, Sesame oil recorded (6.5 and 5.0 %), followed by *Nigella sativa* oil recorded (7 and 6%) if using them one time and two times, respectively.

1.2. Larval duration:

Sesame oil at 1.0% concentration, Olive and *Nigella sativa* oils at 0.5% concentration recorded the shortest larval duration (9 days) when used one time or two times comparing the control which recorded (11 days) (Table,1). These results may be attributed to the richness of sesame oil with many essential nutrients as phosphorous, iron, magnesium, manganese, zinc, vitamin B1 and fatty acids which consider an essential indicator of the nutritional value of the oil, these nutrients have beneficial and very positive effect on larval healthy growth, as documented by (Anilakumar *et al.*, 2010; Hassan, 2012 and Rahman *et al*., 2007). Similarly, More than 200 different chemical compounds have been detected in olive oil including sterols, triterpenic alcohols, fatty acids (oleic acid), triacylglycerols hydrocarbons (squalene and carotenoids), chlorophylls, tocopherols, aliphatic alcohols, and volatile compounds. Furtheremore, it is a source of at least 30 phenolic compounds. These compounds are strong antioxidants and radical scavengers. And have antimicrobial activity against many bacterial strains, which enhanced larval immune system as reported by (PanelKellie and Peter, 2002 and Servili and Montedoro, 2002). These results in accordance with those of Al-Jabre *et al*. (2005) demonstrated that black seed (*Nigella sativa*), its oil and extracts showed a wide spectrum of favorable biological activities act as antimicrobial, immune stimulant, anti-inflammatory and anti-oxidant (Al-Ghamdi, 2001). Also, Morssy (2009) reported that the significantly decreased of larval mortality and the duration of silkworm *Bombyx mori*, as a result to treating larvae with three plant extract oils (lime, clover and jojoba oils) with 3 concentrations (0.5, 1.0 and 2.0%). Moreover, Prasad *et al*. (2001) recorded that the silkworms fed with mulberry leaves supplemented with potato leaf extract recorded the lower larval mortality and larval duration.

Table (1): Effect of some plant extracted oils as nutritional additives on mortality (%) and grown larval duration (day) of *Bombyx mori* .

Treatments	Conc. (%)	Larval mortality (%)			Larval duration (day)		
		4th instar	5th instar		4th instar	5th instar	
			Treated in 4th only	Treated in 4th and 5th		Treated in 4th only	Treated in 4th and 5th
Sesame oil	0.5%	5.00	6.50	5.00	5.00	10.00	9.00
	1%	5.00	7.50	6.00	6.00	9.00	9.00
	2%	6.50	8.50	9.50	7.00	11.00	11.00
	Mean	5.50	7.50	6.83	6.00	10.00	9.67
Olive oil	0.5%	5.00	7.50	6.11	6.00	9.00	9.00
	1%	6.50	8.00	7.83	5.00	9.00	9.00
	2%	7.50	8.50	8.00	7.00	11.00	10.00
	Mean	6.33	8.00	7.31	6.00	9.67	9.33
Nigella sativa oil	0.5%	6.00	7.00	6.00	5.00	9.00	9.00
	1%	7.50	8.50	9.00	6.00	11.00	10.00
	2%	8.50	10.50	12.00	7.00	12.00	12.00
	Mean	7.33	8.66	9.00	6.00	11.00	10.33
Control		10.00	12.00		7.00	11.00	
LSD 5% for concentration		2.137**	2.215**	1.489***	0.851***	1.424***	1.263***
LSD 5% for compound		2.087**	0.0274*	0.0478*	ns	ns	Ns

1.3. Percentage of cocooning:

Adding of sesame oil (0.5%) caused the highest percent of cocooning being 88.42 and 92.10% when mulberry leaves were treated one time and two times, respectively in comparison 61.11% for the control (Table,2).

1.4. Emergence percentage (%):

Sesame and *Nigella sativa* oils at 0.5% concentration of each and olive oil at 1.0% concentration caused significant increasing of adult emergence percent (85 and 90%) if used one time and two times, respectively, comparing 70% for control group (Table,2).

1.5. Female fecundity (No. of eggs/female):

The tested oils cleared that sesame and olive oil at 1.0% concentration exhibited the highest number of deposited eggs per female recording 374 eggs/female followed by *Nigella sativa* oil at 0.5% concentration recording 360 eggs/female when used two times, while control group recorded 303 eggs/female (Table,2).

The improvement of these biological characters under study might be owing to the presence of high levels of flavonoids, tannins and alkaloids in sesame oil characterized by a high level of antioxidant activity and attributed to the antibacterial, antifungal, and antiviral properties of *S. indicum* (Rice-Evans *et al*., 1995 and Bankole *et al*., 2007). Likewise, natural Phytochemicals (phenols and triterpenes) present in olive oil as important bioactive molecules against diseases, exert different biological activity, including antioxidant, anti-inflammatory and antiviral effects and used as defense against microbial and fungal invasion (Hollman and Katan, 1999 and Eastwood, 1999). In harmony with the fore mentioned results, El-Sayed (1999) reported that the mixture of honey and Black Cumin (*Nigella sativa*) seeds increased silk production and number of deposited eggs/female. Also, Mahmoud

et al. (2012) evaluated three types of honey; Carob [*Ceratonia siliqua*], Seder [*Ziziphus* sp.] and Black Cumin honey [*Nigella sativa*] which contains all the good qualities and benefits of black cumin seeds, compared to the untreated control. The evaluated types of honey to more or less extent increased the egg productivity. In the same manner, Xu *et al.* (1992) found that from 3rd- to 5th instar larvae *Bombyx mori* fed on mulberry leaves soaked in extracts of *Brassica campestris* pollen and royal jelly increased the cocoon formation and oviposition. Shoukry *et al.* (1998) recorded that mulberry leaves supplemented with two volatile oils Ploughman's oil and Jasmine oil on silkworm *B. mori*, increased percentage of cocoon production compared with the control.

Table (2): Effect of some plant extracted oils as nutritional additives on cocooning %, emergence % and fecundity of mulberry silkworm *Bombyx mori* .

Treatments	Conc.	Cocooning %		Emergence %		Fecundity (No.egg/female)	
		Treated in 4th instar only	Treated in 4th and 5th instars	Treated in 4th instar only	Treated in 4th and 5th instars	Treated in 4th instar only	Treated in 4th and 5th instars
Sesame oil	0.5%	88.42	92.10	85.00	90.00	341	352.3
	1%	84.73	87.36	85.00	90.00	332	374
	2%	71.66	77.54	80.00	80.00	321	328.3
	Mean	81.60	85.66	83.33	86.67	331.3	342.5
Olive oil	0.5%	85.79	89.47	80.00	85.00	338.6	345.6
	1%	89.83	91.44	85.00	90.00	361.3	374.3
	2%	74.05	75.13	80.00	80.00	325	337
	Mean	83.22	85.34	81.67	85.00	341.7	352.3
Nigella sativa oil	0.5%	86.70	90.42	85.00	90.00	348.3	360.6
	1%	77.29	80.54	80.00	75.00	334	342.3
	2%	69.39	74.86	75.00	70.00	312	310
	Mean	77.79	81.94	80.00	78.33	331.4	337.6
Control		61.11		70.00		303	
LSD 5% for concentrations		1.703***	4.101***	6.140***	4.877***	1.616***	1.525***
LSD 5% for compounds		13.990*	13.226**	6.149**	12.190*	26.157*	32.582*

2. Technological studies:

2.1. Cocoon indices:

2.1.1. Fresh cocoon weight (g):

The weight of fresh cocoon was significantly higher with Sesame oil at 1.0% (1.400 and 1.416 g) when used one and two times compared to control cocoon 1.105 g. (Table, 3).

2.1.2. Shell cocoon weight (g):

All the tested treatments induced significant increase over the control especially; Olive oil (1.0%) recorded the best means recording 0.222 and 0.236 g when used one and two times. while Sesame oil (0.5%) recorded 0.240 g when used two times, comparing with control 0.150 g. (Table, 3).

2.1.3. Cocoon shell ratio (%):

The olive oil (2.0%) recorded (18.695 and 18.898%) ratio of silk content if using it one and two times, respectively. Meanwhile, Sesame oil at o.5% recorded 17.868 and 18.320 g, when used one time and two times, respectively (Table, 3).

Table (3): Effect of some plant extracted oils as nutritional additives on cocoon indices of *Bombyx mori* .

Treatment	Conc. (%)	Fresh cocoon weight (g)		Shell cocoon weight (g)		Silk ratio (%)	
		Treated in 4th instar only	Treated in 4th and 5th instars	Treated in 4th instar only	Treated in 4th and 5th instars	Treated in 4th instar only	Treated in 4th and 5th instars
Sesame oil	0.5%	1.220	1.310	0.218	0.240	17.868	18.320
	1%	1.400	1.416	0.210	0.216	15.000	15.254
	2%	1.200	1.140	0.174	0.172	14.500	15.087
	mean	1.273	1.288	0.200	0.209	15.789	16.220
Olive oil	0.5%	1.285	1.302	0.200	0.215	15.564	16.513
	1%	1.265	1.322	0.222	0.236	17.549	17.851
	2%	1.150	1.180	0.215	0.223	18.695	18.898
	mean	1.233	1.268	0.212	0.224	17.269	17.754
Nigella sativa oil	0.5%	1.250	1.305	0.208	0.230	16.640	17.624
	1%	1.186	1.196	0.185	0.191	15.598	15.969
	2%	1.167	1.173	0.177	0.179	15.167	15.260
	mean	1.201	1.224	0.190	0.200	15.801	16.284
Control		1.105		0.150		13.537	
LSD 5% for concentration		0.152*	0.144**	0.0262***	0.0222***	1.524***	1.324***
LSD 5% for compound		ns	Ns	0.0287**	0.0422*	2.379*	2.346*

2.2. Reeled silk filament parameters:

2.2.1. Silk filament length (m):

Nigella sativa oil at 0.5% exhibited the highest length of reeled silk filament of cocoon recording (1145.5 and 1180.6 m) when used one and two times respectively. Followed by sesame oil at 1% concentration recorded (1100 and 1125.2 m) respectively, when used one and two times. While the control recorded 845.8 m (Table,4).

2.2.2. Silk filament weight (g):

The heaviest weight of reeled silk filament (0.223 and 0.224 g) was recorded for *Nigella sativa* oil at 0.5% for adding one and two times, respectively. While, the least weight of reeled silk filament (0.130 g) for control (Table,4).

2.2.3. Silk filament size (dn):

All the plant oils means induced highly significant increase over the control especially with sesame oil 1.781 dn. with using two times (Table,4).

The enhancement in cocoon and silk filament characters may be referred to haemolymph protein improvement, as a result to rearing larvae with the nutritional oil extracts under investigation, as documented by Mbaebie *et al.* (2010) reported that *S. indicum* is a good source of protein, carbohydrate, minerals and crude fibre. Similarly, Crews *et al*. (2006) stated that, Sesame oil contains significant amounts of the lignans sesamin and sesamolin compounds, which have beneficial effects on serum lipid levels and give sesame oil a marked antioxidant activity, because of the responsibility of the lignans for the great stability of sesame oil to oxidation. In the same way, *N. sativa* seeds contain fixed oil, proteins, alkaloids, saponins, and essential oil. The biological effects of *N. sativa* are attributed to its various characterized constituents, as documented by (Ali and Blunden, 2003).

Murugappan *et al.* (1996) mentioned that feeding larvae with mulberry leaves soaked in 1% jaggery solution which improved the cocoon

characters (cocoon weight by 45% and shell weight by 30%). Also, Shoukry *et al.* (1998) recorded that the cocoon production, the weight of shell cocoon, silk content ratio and cocoon weight were increased when mulberry leaves were supplemented with two volatile oils Ploughman's oil and Jasmine oil. And Prasad *et al*. (2001) recorded that the silkworms fed with mulberry leaves supplemented with potato leaf extract recorded the highest cocoon weight, shell ratio and shell weight.

Zannoon (1994) found that, different solutions of bee honey (i.e. citrus honey, clover honey and cotton honey), mixture of equal volumes of the three types of honey plus pollen grains offered to silkworm larvae *Bombyx mori* as nutritional additives gave longer and heavier filament of reeled cocoons with no change of its size., Kuntamalla and Rao (2004) found that the leaf extract of neem (1.0%) added to mulberry leaves offered to silkworm caused highest filament length and silk ratio. In connection, Khalil *et al.* (2006) stated that addition of various concentrations of anise extract showed that the higher concentration gave the best values in terms length of filament and weight of the cocoons. But, Kumar *et al.* (2009) cited that blue green algae spirulina (100 ppm, 200 ppm and 300 ppm foliar spray) caused that Silk filament length is significantly higher at 300 ppm concentration compared to control.

Feeding silkworm larvae two times at the beginning of fourth and fifth instars on mulberry leaves dipped in Sesame oil (0.5 % or 1.0 %) decreased the grown larval mortality and increased the cocooning percentage, the moth emergency rate, cocoon shell weight and filament size.

Table (4): Effect of some plant extracted oils as nutritional additives on reeled filament characters of the *Bombyx mori*.

Treatment	Conc. (%)	Silk filament length (m)		Silk filament weight (g)		Silk filament size (dn)	
		Treated in 4^{th} instar only	Treated in 4^{th} & 5^{th} instars	Treated in 4^{th} instar only	Treated in 4^{th} &5^{th} instars	Treated in 4^{th} instar only	Treated in 4^{th} &5^{th} instars
Sesame oil	0.5%	996.6	1021.3	0.188	0.212	1.697	1.867
	1%	1100.0	1125.2	0.179	0.190	1.464	1.519
	2%	1008.4	1015.6	0.217	0.221	1.935	1.958
	mean	1035	1054.0	0.194	0.207	1.698	1.781
Olive oil	0.5%	921.5	956.5	0.161	0.177	1.572	1.663
	1%	1045.5	1100.1	0.205	0.218	1.764	1.782
	2%	912.0	922.0	0.183	0.188	1.805	1.835
	mean	959.6	992.8	0.183	0.194	1.713	1.760
Nigella sativa oil	0.5%	1145.5	1180.6	0.223	0.224	1.751	1.707
	1%	1016.3	1043.5	0.195	0.204	1.726	1.758
	2%	1007.8	1020.5	0.185	0.191	1.651	1.683
	mean	1056.5	1081.5	0.201	0.206	1.709	1.716
Control		845.8		0.130		1.383	
LSD 5% for concentration		145.174*	146.141**	.0211***	.0227***	0.212**	0.247**
LSD 5% for compound		114.164*	133.871*	0.033**	.0295***	0.255*	0.235*

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