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Website: http://www.aun.edu.eg/faculty_agriculture/arabic *E-mail: ajas@aun.edu.eg*

Effects of some Extracts on Growth Characters of Mulberry Silkworm "*Bombyx mori* L."

[*]Rateb, S.H.[1] and Y.A. Abdel-Rahman[2]
[1] Plant Prot. Dept., Fac. of Agric., Assiut Univ.
[2] Plant Prot. Dept., Fac. of Agric., Al-Azhar Univ., Assiut.
[*]Email: salah.hashem@agr.au.edu.eg

Abstract:

Two hybrids of silkworms, imported from Bulgaria, and local hybrid were used during the work. Six alcoholic extracts (*Moringa*, Rosemary, Garlic, *Cestrum durant* "Bostachia", mulberry silkworm, and silk glands, and four aqueous extracts (*Moringa*, Rosemary, Garlic, and *Cestrum durant*, were tested on imported and local hybrid of silkworm. Mulberry leaves were sprayed with (1%) of these extracts and offered to 4th and 5th instar larvae. The following growth characters were detected and calculated: initial, final average and range of 5th instar weight, growth index, silk glands weight, ratio between silk glands and larval weight, pupal weight, fresh and cocoon, shell weight and cocoon shell ratio % was calculated. Using of aqueous extract of Rosemary on imported silkworms leads to an increase in cocoon shell ratio %. Concerning local hybrid this increment was detected in *C. durant*, *Moringa*, and Garlic treatments. All tested aqueous extracts increased cocoon shell ratio % in local hybrid. Generally, in imported hybrid, the only increment in cocoon shell ratio % was in aqueous extracts of Rosemary treatment. However, in local hybrid this increment was found in alcoholic extracts of *Moringa*, Garlic, *C. durant*. This reflects more response of local hybrid as compared with imported one. Also, response to aqueous extracts was more than the alcoholic extracts. Thus, using of local hybrid of silkworm and treatment of mulberry leaves with aqueous extract is considered be available easier and cheaper for feeding of mulberry silkworms, in Egypt.

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Referees: Prof. Moustafa H. Hussein Prof. Samir S. Ebrahim

Introduction:

Different plant parts and plant extracts were used for fortification of mulberry leaves as a food of mulberry silkworm *Bombyx mori* L. by many researchers. Jeyapaul *et al*. (2003) fed silkworm in mulberry leaves which treated with extracts of: *Coffea arabica*, *Alternanthera sessilis* and *Eichharnia Crassipes*, on food consumption and utilization of mulberry leaves. While Eswaran and Sovarkodiyone (2004) used tapioca flour and green leaves of *Amaranthus viridis* .

Bohidar and Choubey (2005) used extracts of 18 different plants for fortification of mulberry leaves. The worms were thrived well and spun cocoon on 15 plant extracts, whereas 3 plant extracts were found to be harmfull.

Hiware and Bhalerao (2008) fed B.mori on the mulberry leaves which treated with theagueous plant extracts of *Phyllanthus miruri* and *Tephrosia purpurea* and found positive results.

Umarani (2009) reported that increasing dose of supplementary diet soyabean is directly proportional to the quality of cocoons.

Vanitha *et al*. (2006) used lgume as additives on mulberry leaves while Mokayes *et al*. (2006) used honeybee products and yeast for the same purpose.

Ascorbic acid, pollen and soyaflour were used by Abdellah (2007) as additives to mulberry leaves. Whereas Nguku *et al*. (2007) used royal jelly. Mahmoud and Ashor, (2008) used the biofertili (Rizobactine) for fortification of mulberry leaves.

Mantri *et al*.(2009) need foxtail millet as dust on mulberry leaves fed to silkworm.

Bentea *et al*. (2011) used minerals and vitamins and Sudha (2013) used iron and Kumar and Michael (2011) tested "Serifeed" as additives to natural food of *B.mori* L.

A review about supplements and additives to mulberry leaves was prepared by Zah and Livin (2011).

This work aims to improve the harvestable silk quantity and having mulberry leaves sprayed or immersed into various plant extracts and various additives to mulberry leaves which used for feeding of silkworm.

Materials and Methods:

This work was conducted in the Laboratory of Silkworm Rearing, Plant Protection Department, Faculty of Agriculture, Assiut University, during 2015.

Local and Bulgarian hybrid of silkworm were supplied from Sericulture Division, Plant Protection Institute, Ministry of Agriculture, Giza, to be used in the experimental work.

I- Rearing of silkworm:

The temperature during the incubation of silkworm eggs was ranged between 23 and 25°C, while the relative humidity was ranged between 85 and 90%. Complete hatching took place, after few days of incubation. New leaves, or tender strips of mulberry leaves, were put over the hatching larvae, which crawled up the leaves, then these were removed with silkworms to the rearing place.

Normal method of silkworm rearing was carried out, till the beginning of 4th instar, or beginning of tested treatments. The mulberry leaves for feeding of silkworm were

cut early morning, then covered with wet clothes to protect than from loss of water. The leaves were cleaned and given to the first and second larval instars as strips or buds. Afterwards, the whole leaves was distributed in a usual manner four times/ day, till the beginning of fourth instar. Regular cleaning of rearing beds were carried out by removing uneaten leaves and faeces, to avoid disease infection.

II- Treatments:

Six alcoholic extracts and four aqueous extracts were sprayed on mulberry leaves and used in feeding of 4th and 5th instars larvae of local and imported hybrids. They were": alcoholic extracts of: *Moringa*, Rosemary, Garlic, *Cestrum Durant* "Bostashia", silkworm extract, and silk glands extracts and aqueous extracts of *Moringa*, Rosemary, Garlic and "Bostashia".

For preparing of alcoholic plant extracts: The dried leaves were crowded, extracted using 70% ethyl alcohol in a rate of 10 gms/100 ml alcohol, in a water bath at 45°C for 48 hrs. Then filtered and the filtrate was cooled, then completed to 100 ml using alcohol 70%. For larvae and silk glands extracts: 10 larvae and 10 silk glands of 5th instar were soaked in 100 ml ethyl alcohol 70% for one week, filtered and the filtrate was kept in refriginator till used.

All these treatments were used at concentration of 1% with water.

Every tested treatment was replicated three times in three carton boxes (20.5 x 19.5 x 6.5 cm), each contain fifty silkworms larvae and 150 larvae/treatment. Feeding with treated leaves was conducted four times/day. Control larvae were fed with untreated mulberry leaves. Ten treatments were used during the work.

III- Criteria for evaluation:

1- Fresh weight of silkworms larvae:

Twenty larvae of fifth instar from each replicate were weighed (in gms), with a total of 60 larvae/treatment. Control larvae were also weighed. Total and mean weight/larvae (gm.) was calculated.

2- Fresh weight of silk glands:

Fifteen nine days old 5th instar larvae, from each treatment were used for determination of silk glands weight (in gms). Control silk glands were also weighed (in gms). Total and mean weight (in gms) were calculated.

3- Fresh weight of cocoons:

From each replicate and treatments, good cocoons were collected, cut, opened, and pupae were weighed. The whole cocoon was weighed (in gms). Total and mean weight/cocoon (in gms), were calculated.

4- Pupal weight:

From each replicate and treatment, pupae were weighed (in gms). Total and mean weight/pupa (in gms) were estimated.

5- Cocoon shell weight:

The previous cocoons were carefully opened and pupae were removed and cleaned from excuviae, then weighed. Total and mean weight/shell (in gms) were calculated.

6- Cocoon shell ratio (%):

Cocoon shell ratio for each treatment was calculated according to Tanaka (1964) as follows:

$$\text{Cocoon shell ratio \%} = \frac{\text{Weight of cocoon shell}}{\text{Weight of cocoon}} \times 100$$

Total and mean weight/shell (in gms.) were calculated.

7- Growth Index:

Twenty five 5th instar larvae of one, three, five and seven day old were weighed (in gms) and the following formula was used for calculation of the Growth Index:

$$\text{Growth Index} = \frac{\text{Final weight of the larvae (gms)} - \text{Initial weight of the larvae (gms)}}{\text{Initial weight of the larvae (gms)}}$$

VI- Statistical analysis:

Obtained data were analysed using the factorial design. F-test was estimated for each analysis. The means were compared according to Duncan's Multiple Range Test.

Results and Discussion:

Data of the effect of alcoholic and aqueous extracts which added to mulberry leaves on growth characters of imported and local hybrids of silkworm are summarized in Tables (1-4) and growth rate pattern of 5th instar larvae was graphically illustrated in Figures (1-4).

I- Effect of tested treatments on imported hybrid (Tables 1 and 2; Figs. 1 and 3)

Table 1 show the effect of tested alcoholic extracts on some growth parameters of imported hybrid. An increase of initial weight of 5th instar larvae was noticed in "Bostashia", Garlic, extract of larvae and silk glands. While, in the final weight of 5th instar larvae, this increment was in *Moringa*, Rosemary and " Bostashia" treatments with maximum range of weight in *Moringa*, followed by Rosemary treatment.

Significant increment in average larval weight (2.990±0.102 gms) was detected in Garlic treatment, followed by *Moringa* treatment, and general increase in larval average weight was found in all treatments.

A general decrease in the ratio between larval weight and silk gland weight was noticed in all treatments.

An increase in fresh cocoon weight (gms) was observed in "*Moringa*" treatment. No significant differences were detected in pupal weight (gms) in all tested treatments. While, a signficiant increase in weight of cocoon shell (gms) was noticed after treatment with *Moringa*.

Non-significant decrease in cocoon shell ratio % was observed in all tested treatment with alcoholic extract on imported hybrid. Growth rate pattern was illustrated in Figure 1.

Table 2 show the effect of tested aqueous extracts on some growth characters of imported hybrid of silkworm.

Maximum increase in final and range of larval weight was noticed in *Moringa* treatment and followed by Rosmary.

Maximum growth index (Fig. 3) was noticed in Rosemary and *Moringa* treatments.

Maximum average of silk glands weight (gms) was noticed when mulberry leaves were sprayed with Rosemary aqueous extract.

General increase in larval average weight was detected in all treatments and significant differences were found in *Moringa*, followed by Rosemary and Garlic.

General decrease in the ratio between larval and glands weight was found. The same was noticed with alcoholic extract. A general and non-significant increase in fresh cocoon

weight and pupal were noticed in all tested treatments.

Signficiant increase in cocoon shell weight (gms) was noticed in *Moringa* and Rosemary treatments. The only increase in cocoon shell ratio % was detected with Rosemary aqueous extract on the imported hybrid.

Effect of plant extracts was studied in India by Jeyapaul *et al*. (2003). Eswaran and Savarkodiyone (2004) used 1% aqueous extract tapioca flour and *Amaranthus* leaves for feeding of silkworms.

II- Effect of tested treatments on local hybrid: (Tables 3 and 4, Figs. 2 and 4):

Table (3) show the effect of alcoholic extracts on some growth characters of local hybrid of silkworm.

Maximum initial weight of 5th instar larvae was observed in *Moringa* treatment, while in the final weight and growth index (Fig. 2) were noticed in Rosemary and larval alcoholic extract.

Significant increase in average larval weight was found between all treatments, except Garlic, and control readings.

A general decrease in the ratio between glands weight and larval weight. The same was noticed with imported hybrid.

General increase in pupal weight was noticed in all treatments, except with Rosemary. The maximum one was in larvae and glands extracts.

Significant increase in cocoon shell weight (gms) was detected in: Rosemary, "Bostashia", larval and glands extract.

An increase in cocoon shell ratio % in *Moringa*, Garlic, "Bostashia", with maximum increase in the last one.

More response to alcoholic extracts was found in local hybrids, compared with the imported one.

Table (4) show the effect of tested aqueous extracts on local hybrid of silkworm.

General increase in initial weight was noticed in all treatments, except Rosemary, with maximum increase in *Moringa* treatment. While, the final and range of larval weight and growth index (Fig. 4), and glands weight were noticed in "Bostashia" treatment.

A decrease in the ratio between glands and larval weights was noticed. The same was observed with imported hybrid.

Significant increase in fresh cocoon weight was detected with Rosemary and "Bostashia" treatments. Pupal weight was increased in all tested treatments, except in Garlic.

General increment in cocoon shell weight (gms) and cocoon shell ratio % and maximum increase in cocoon shell weight and cocoon shell ratio % was observed in "Bostashia" treatment.

Table (1): Effect of tested alcoholic extracts on some growth characters of imported hybrid of the mulberry silkworm *B. mori* .

Growth characters / Treatments	Initial weight of the 5th instar larvae (g)	Final average larval weight (g)	Range	Growth index	**	Average gland weight (g)	±SEM	*	Average larval weight (g)	±SEM	*	Gland weight ratio of larval weight %	*	Fresh cocoon weight (g)	NS	Pupal weight (g)	NS	Cocoon shell weight (g)	*	Cocoon shell ratio %	NS
1-*Moringa*	0.5852	3.5045	2.9192	4.9881	A	0.7600	0.018215	AB	2.9887	0.083489	A	25.43%	CD	1.3489	A	1.0588	A	0.2901	A	21.50%	A
2-Rosemary	0.6336	3.4639	2.8302	4.4668	B	0.5594	0.045322	C	2.5352	0.088111	B	22.06%	D	1.2318	A	0.9612	A	0.2706	C	21.97%	A
3-Garlic	0.6352	3.1271	2.4919	3.9233	D	0.6583	0.020715	BC	2.9903	0.102091	A	22.01%	D	1.2632	A	1.0052	A	0.2581	D	20.43%	A
4-*Cestrum durant*	0.6360	3.2996	2.6636	4.1878	BCD	0.7052	0.027161	AB	2.7463	0.082495	AB	25.68%	CD	1.3223	A	1.0365	A	0.2858	AB	21.61%	A
5-Larval extract	0.6284	3.1572	2.5288	4.0242	CD	0.7947	0.044213	A	2.7452	0.074461	AB	28.95%	BC	1.2623	A	0.9941	A	0.2682	C	21.25%	A
6-Gland extract	0.6543	3.1831	2.5288	3.8649	D	0.8255	0.019312	A	2.6353	0.078838	AB	31.33%	AB	1.2239	A	2.2923	A	0.2688	C	21.96%	A
7-Control	0.6033	3.2162	2.6129	4.3312	BCD	0.8399	0.032585	A	2.3365	0.093864	B	35.95%	A	1.2672	A	0.9864	A	0.2807	B	22.15%	A
LSD value at																					
0.05 alpha level				0.2690		0.1258			0.3774			4.662		0.1412		0.1314		0.0082		1.983	
0.01 alpha level				0.3672																	

Table (2): Effect of tested aqueous extracts on some growth characters of imported hybrid of the mulberry silkworm *B. mori* .

Growth characters / Treatments	Initial weight of the 5th instar larvae (g)	Final average larval weight (g)	Range	Growth index	**	Average gland weight (g)	±SEM	NS	Average larval weight (g)	±SEM	*	Gland weight ratio of larval weight %	*	Fresh cocoon weight (g)	NS	Pupal weight (g)	NS	Cocoon shell weight (g)	*	Cocoon shell ratio %	NS
1-*Moringa*	0.6450	3.4466	2.8016	4.344	AB	0.7769	0.025558	A	3.0552	0.082737	A	25.43%	B	1.3271	A	1.0382	A	0.2890	A	21.77%	AB
2-Rosemary	0.5850	3.2484	2.6634	4.552	AB	0.8108	0.046701	A	3.0450	0.101914	A	26.63%	AB	1.2775	A	0.9892	A	0.2883	A	22.57%	A
3-Garlic	0.6766	3.1784	2.5018	3.697	C	0.6360	0.022211	A	3.0190	0.108585	A	21.07%	B	1.3082	A	1.0255	A	0.2827	AB	21.61%	AB
4- *Cestrum durant*	0.6192	3.0893	2.4701	3.989	BC	0.6387	0.042284	A	2.4725	0.062920	B	25.83%	B	1.2824	A	1.0206	A	0.2618	C	20.42%	B
5-Control	0.6305	3.2367	2.6062	4.134	AB	0.7847	0.039229	A	2.3750	0.058915	B	33.04%	A	1.2622	A	0.9844	A	0.2778	B	22.01%	AB
LSD value at																					
0.05 alpha level				0.2855		0.1786			0.3949			0.7120		0.1275		0.1248		0.0082		2.0000	
0.01 alpha level				0.4155																	

Table (3): Effect of tested alcoholic extracts on some growth characters of local hybrid of the mulberry silkworm *B. mori*.

Growth characters / Treatments	Initial weight of the 5th instar larvae (g)	Final average larval weight (g)	Range	Growth index	**	Average gland weight (g)	±SEM	*	Average larval weight (g)	±SEM	*	Gland weight ratio of larval weight %	*	Fresh cocoon weight (g)	*	Pupal weight (g)	*	Cocoon shell weight (g)	*	Cocoon shell ratio %	NS
1-*Moringa*	0.7956	2.7975	2.0019	2.5163	C	0.5098	0.036814	CD	2.76895	0.150489	BCD	18.41%	C	1.1195	B	0.8798	B	0.2397	BCD	21.41%	AB
2-Rosemary	0.7219	3.3017	2.5797	3.5733	AB	0.5837	0.040914	BC	3.10637	0.098094	AB	18.79%	BC	1.3057	A	1.0470	A	0.2587	AB	19.81%	AB
3-Garlic	0.7289	2.7539	2.0251	2.7783	BC	0.4519	0.028527	D	2.42492	0.091686	D	18.64%	BC	1.0870	B	0.8577	B	0.2293	CD	21.09%	AB
4- *Cestrum durant*	0.7482	3.1131	2.3648	3.1606	ABC	0.6057	0.035774	BC	2.96184	0.064606	ABC	20.45%	BC	1.1765	B	0.9168	B	0.2597	AB	22.07%	A
5-Larval extract	0.6425	3.2283	2.5858	4.0249	A	0.7275	0.038620	A	3.27963	0.102058	A	22.18%	AB	1.3687	A	1.0961	A	0.2726	A	19.91%	AB
6-Gland extract	0.6985	2.9348	2.2364	3.2018	ABC	0.7093	0.039683	A	3.16727	0.089030	A	22.39%	AB	1.2799	A	2.4719	A	0.2500	ABC	19.53%	B
7-Control	0.7016	3.1638	2.4622	3.5095	AB	0.6661	0.021253	AB	2.71277	0.086456	CD	24.55%	A	1.0868	B	0.8660	B	0.2208	D	20.31%	AB
LSD value at																					
0.05 alpha level				0.5765		0.0974			0.2602			3.5120		0.0998		0.0964		0.0258		2.1880	
0.01 alpha level				0.8082																	

Table (4): Effect of tested aqueous extracts on some growth characters of local hybrid of the mulberry silkworm *B. mori* .

Growth characters / Treatments	Initial weight of the 5th instar larvae (g)	Final average larval weight (g)	Range	Growth index	*	Average gland weight (g)	±SEM	*	Average larval weight (g)	±SEM	*	Gland weight ratio of larval weight %	NS	Fresh cocoon weight (g)	*	Pupal weight (g)	NS	Cocoon shell weight (g)	NS	Cocoon shell ratio %	NS
1-*Moringa*	0.7991	3.0327	2.2337	2.795	B	0.5715	0.047003	BC	2.8265	0.112154	B	20.22%	A	1.2185	AB	0.9621	A	0.2564	AB	21.04%	A
2-Rosemary	0.6888	3.1004	2.4116	3.501	A	0.5630	0.026273	BC	2.8310	0.099760	B	19.89%	A	1.2480	A	0.9848	A	0.2632	AB	21.09%	A
3-Garlic	0.7497	2.7927	2.0429	2.725	B	0.4741	0.024057	C	2.4019	0.074076	C	19.74%	A	1.1075	B	0.8731	A	0.2344	AB	21.16%	A
4- *Cestrum durant*	0.7792	3.5337	2.7545	3.535	A	0.7112	0.026289	A	3.4129	0.074367	A	20.84%	A	1.2374	A	0.9633	A	0.2741	A	22.15%	A
5-Control	0.7359	3.2314	2.4955	3.391	A	0.6384	0.054552	AB	3.0054	0.124711	B	21.24%	A	1.1111	B	0.8862	A	0.2249	B	20.24%	A
LSD value at																					
0.05 alpha level				0.5087		0.1331			0.3261			5.6490		0.1104		0.1073		0.0368		3.0280	
0.01 alpha level																					

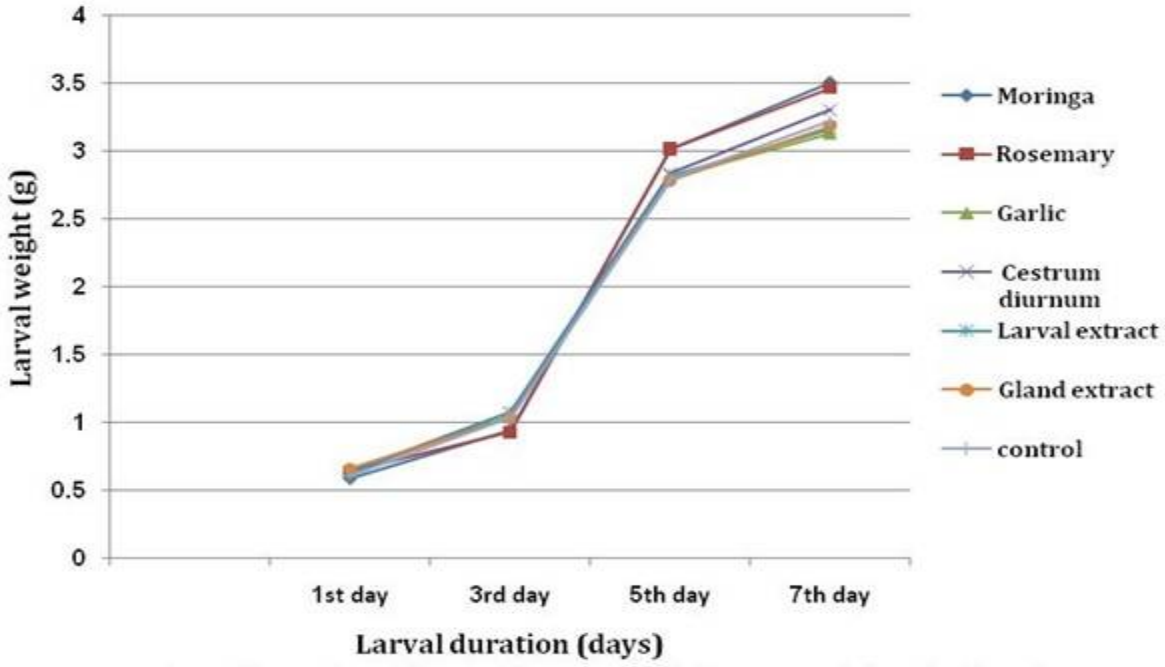

Fig. (1): The effect of mulberry leaves enrichment with ethylic extract on larval weight in the 5th instar of the imported silkworm strain.

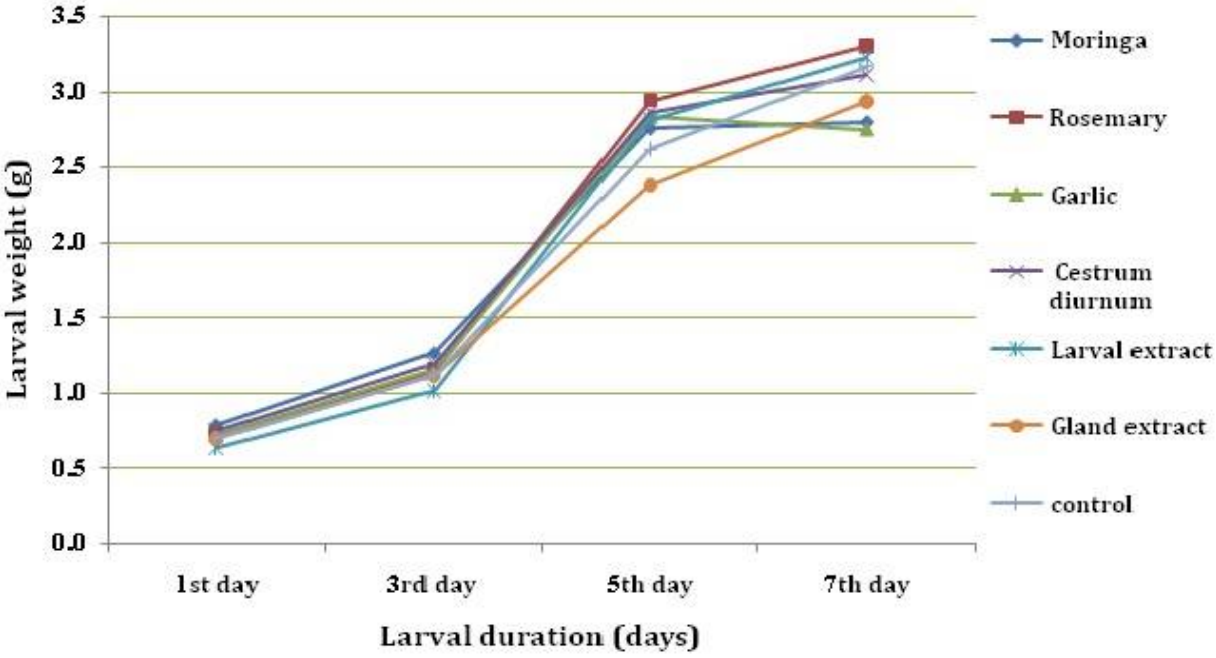

Fig. (2): The effect of mulberry leaves enrichment with ethylic extract on larval weight in the 5th instar of the local silkworm strain.

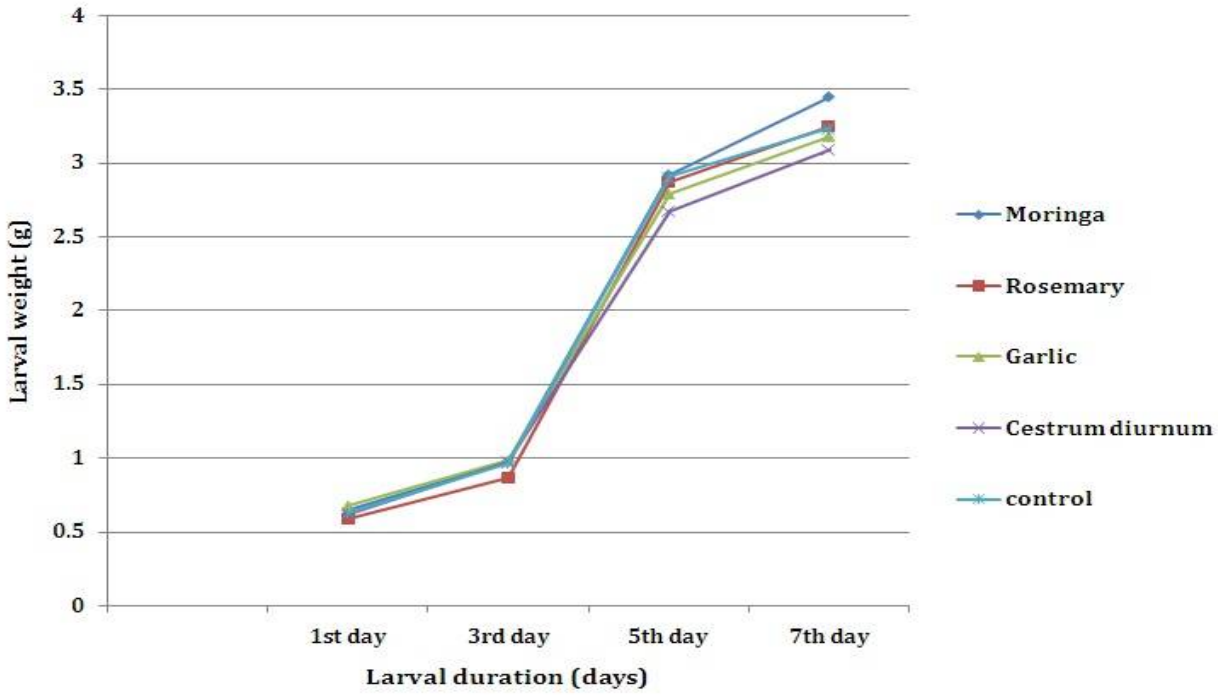

Fig. (3) : The effect of mulberry leaves enrichment with aqueous extract on larval weight in the 5th instar of the imported silkworm strain.

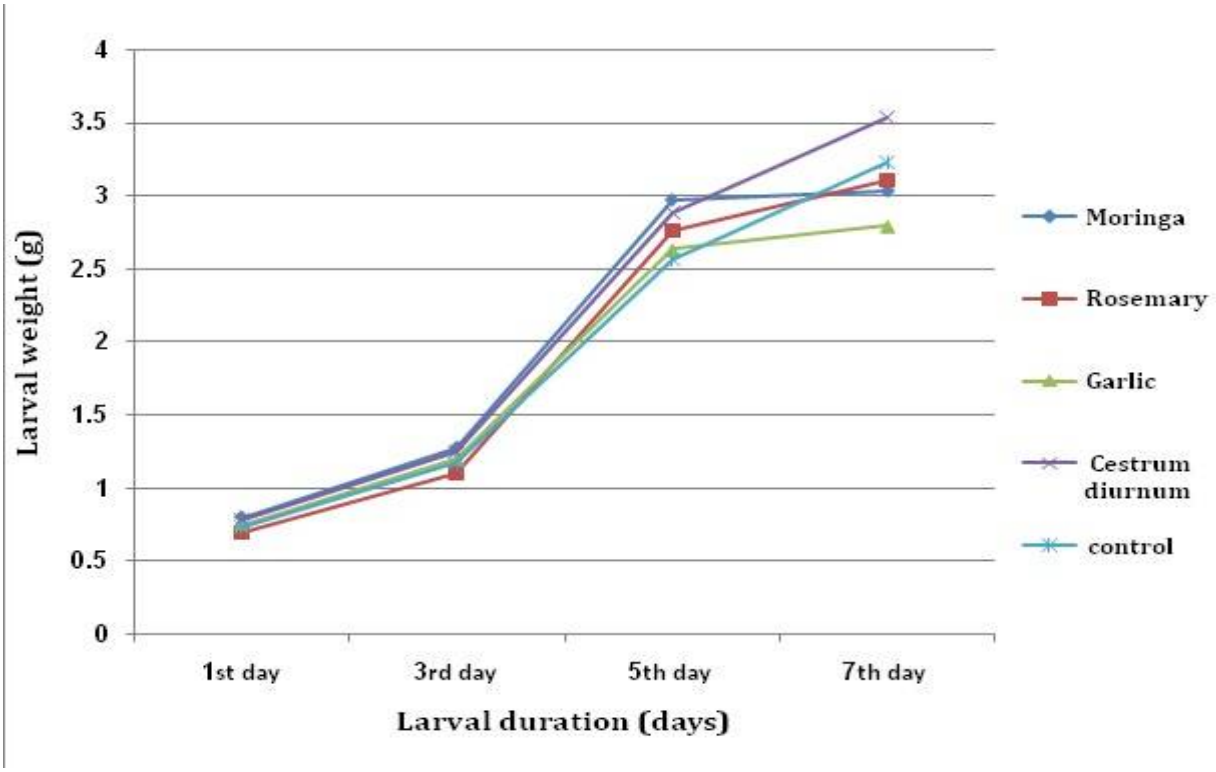

Fig. (4): The effect of mulberry leaves enrichment with aqueous extract on larval weight in the 5th instar of the local silkworm strain.

Rajeswari and Isaiarasu (2004) found that extracts of *Moringa oliefera* (1% w/v) elicited a responses in the final instar larvae. The same was noticed in our work.

Hiware and Bhalerao (2008) used aqueous extracts of two medicinal plants and found interesting and pesticide results with respect to many parameters.

From the above mentioned results, it can be concluded that, the only increment of cocoon shell ratio %, in imported hybrid was in aqueous extract of Rosemary. While, in local hybrid this increment was noticed with alcoholic extracts of *Moringa*, Garlic, "Bostashia", and their aqueous extracts, in addition to Rosemary or in seven of tested treatments. This reflects more positive response of local hybrid as compared with imported one. Also, using of aqueous extracts is of special merits.

Taking in consideration, that aqueous extraction and using of local hybrid is more available easy and cheapest. Thus, it can be advised to use aqueous extracts and local hybrid of silkworm in Egypt.

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تأثير بعض المستخلصات علي صفات النمو في دودة الحرير التوتية (بومبيكس موراي)

صلاح حفني راتب[١]، يحي عبد الفتاح عبد الرحمن[٢]

[١] قسم وقاية النبات ، كلية الزراعة ، جامعة أسيوط

[٢] قسم وقاية النبات ، كلية الزراعة، جامعة الأزهر، أسيوط

الملخص:

تم استخدام هجينين لدودة القز أحدهما مستورد من بلغاريا والثاني هو السلالة المحلية. كما تم اختبار ستة مستخلصات كحولية (مورينجا ، روزماري ، ثوم ، بستاشيا، مستخلص يرقات دودة القز ومستخلص غدد الحرير لدودة القز). كما تم أيضاً إختبار ٤ مستخلصات مائية (مورينجا ، روزماري ، ثوم و "بستاشيا" وذلك برشها علي أوراق التوت المقدمة لتغذية دودة القز وذلك بتركيز (١%).

تم قياس وحساب صفات النمو التالية: الوزن المبدئي والنهائي والمدي ، متوسط الوزن ليرقات العمر الخامس وكذلك معامل النمو، وزن غدد الحرير ونسبتها إلي وزن اليرقات، وزن العذاري، والشرانق الطازجة وغلافها وحساب معامل غلاف الشرنقة %. أدي استخدام المستخلص المائي للروزماري علي الهجين المستورد، أدي إلي زيادة في معامل غلاف الشرنقة %. أما بالنسبة للهجين المحلي لوحظت هذه الزيادة في حالة "البستاشيا" ، المورنجا والثوم. أدت كل المستخلصات المائية إلي زيادة معامل غلاف الشرنقة % وذلك في الهجين المحلي.

عموماً، كانت الزيادة الوحيدة في معامل غلاف الشرنقة % للهجين المستورد كانت مع المستخلص المائي للروزماري، بينما بالنسبة للمحلية فكانت مع المستخلصات الكحولية للمورينجا، الثوم ، البستاشيا وكذلك في المستخلصات المائية لها وهذا يعكس استجابة أفضل للهجين المحلي عن المستورد وكذلك استجابة أفضل للمستخلصات المائية بالمقارنة بالكحولية.

لذا ينصح باستخدام الهجين المحلي لدودة القز مع معاملة أوراق التوت بالمستخلصات المائية وليست الكحولية حيث أنها متوافرة وسهلة ورخيصة في الاستخلاص وذلك تحت الظروف المصرية.