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EFFECT OF DATURA STRAMONIUM AND LEGEUM SPARTUM EXTRACTS ON SOME PRODUCTIVITY CHARACTERS OF THE MULBERRY SILKWORM, *BOMBYX MORI* L.

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ABSTRACT: The present study was carried out to evaluate the probable effect of datura, *Datura stramonium* and halfa, *Legeum spatum* extracts on some productivity characters of the mulberry silkworm, *Bombyx mori* L. Summarized data are as follows:

- **Applying ethanolic and chloroform extracts of *D. stramonium* during 4th instar resulted in producing heavier fresh cocoon weight (0.910 and 0.941g), respectively. The same trend was also recorded for the cocoons resulted from 5th instar larvae treated with chloroform extracts of the two plants. Other extracts were nearly similar to the control (0.850g).**
- **Feeding 5th instar larvae on chloroform extracts of datura and halfa, as well as chlorpyrifos methyl increased significantly pupal weight.**
- **Applying chloroform extract of *D. stramonium* during the 5th instar and chlorpyrifos methyl treated larvae increased significantly cocoon shell weight.**
- **The highest silk ratio was detected for 4th instar larvae treated with chloroform extract of *L. spartum* (19.0%) and for 5th instar with ethanolic extracts of *D. stramonium* (18.29%).**
- **Significant elongation in the reelable silk filament length was realized with all the tested extracts, as it ranged 778.21 – 884.59 m for 4th instar treated and 700.65-757.67 m for 5th instar treated**

larvae compared to 622.98 and 704.14m, recorded for the control of 4th and 5th instars, respectively.

Treating 4th instar larvae with the extracts of the two plants in chloroform caused significant increase in the filament weight.

Key words: *Bombyx mori* , denier, cocooning, pupation, fecundity, reelable filament.

INTRODUCTION

Insects attack to field crops cause serious losses in their productivity. In some seasons insect invasion became epidemic. Hence, the chemical control is obligatory. The wide use of these chemicals cannot pass without consequences. Of course the ecosystem would suffer many complicated problems (pollution, resistant strains) (Hutton *et al.*, 1995; Begum and Shiranandappa, 2003; Moftah and El-Awami, 2004; Radwan *et al.*, 2004; Vedhamathi and Srivastava, 2005 and Yousif-Khalil *et al.*, 2008).

Botanicals (plant oils and extracts) nowadays are said to be safe, less expensive and available alternatives, being commonly used in the IPM in a large scale. However, field application of such extracts by spraying may generate some difficulties to farm animals, green-used vegetables and useful insects such as insect pollinators, predators, parasitoids and silkworms, also may suffer great damage and losses due to the direct or indirect contact and by ingestion (Narayanaswamy *et al.*, 2000; Nripendra-Laskar and Madhuri-Datta, 2000)

Silkworms, in particular, feed on mulberry leaves collected daily from mulberry trees found around and between treated field crops and within the villages. Doubtless the drafts of the sprayed pesticides and plant extracts may pollute mulberry trees in the vicinity. Such polluted mulberry leaves could kill or at least harm the breeds of *Bombyx mori* (Nath and Kumar, 1999; Bohidar and Choubey, 2005). Therefore, the present study aimed to evaluate the effect of *Datura stramonium* and *Legeum spartum* extracts on some productivity characters of the mulberry silkworm, *Bombyx mori* L.

MATERIALS AND METHODS

The present work was performed in the laboratories of

Plant Protection Dept., Fac. Agric., Zagazig Univ., and Plant Protection Research Institute, Sharkia Branch, during 2007.

Preparation of Datura, *Datura Stramonium* and Halfa, *Legeum Spartum* Extracts

The two plants were extracted in a polar and a nonpolar solvents, i.e. ethanol and chloroform. One kg of each of the two plant leaves were cut in small pieces then immersed in the solvent (1.5 l) in tightly closed jars for 24 hours, being shaked several times. Thereafter, a filtration process was taken place to separate the supernatant containing the extract components. The supernatant was left in open until the complete evaporation of the solvent under laboratory conditions (Abd-El-Monem *et al.*, 1995). The sediment was collected, powdered then kept in vials until needed to prepare the tested concentrations.

Silkworm Rearing Technique

The imported silkworm Chinese hybrid (9F7X) (*Bombyx mori*) eggs were incubated until hatching then the larvae were arised, as usual upto the end of the third larval instar. Newly moulted fourth and fifth instar larvae were offered mulberry leaves dipped in 10, 5 and 2.5 % dilutions of *Datura stramonium* and *Legeum spartum* extracts for 48 hrs (= 4 feeds/day). Thereafter, the survived larvae were offered clean untreated mulberry leaves up to the end of their larval stage. Control larvae were fed on mulberry leaves dipped in the tap water. Three replicates of 50 silkworm larvae each were considered for each concentration for both extracts as well as the control. Another set of 4th and 5th instar larvae were fed on mulberry leaves dipped in a concentration equivalent to $^1/_{512}$ of the recommended field rate of chlorpyrifos methyl (Reldan). The following productivity parameters were recorded.

Measurements

Cocoon indices

The following parameters were measured:

a) Fresh cocoon weight.

b) Cocoon shell weight.

c) Silk content % = b/a × 100 (Tanaka, 1964).

d) d) Pupal weight

Reeled silk filament parameters

The weight (mg) and length (m) of reeled silk filament were

measured and recorded. The size of the reeled filament (denier) was estimated according to Tanaka (1964) formula:

Size (dn) = weight of silk filament/length of filament (m) × 9000

Data obtained were statistically analyzed according to Snedecor and Cochran (1967) methods using software Costat program.

RESULTS AND DISCUSSION

The effect of feeding fourth and fifth instar larvae on mulberry leaves dipped in three concentrations of ethanolic and chloroform extracts of *D. stramonium* and *L. spartum* for two days at the beginning of each instar on some productivity characters was evaluated. Data obtained, in details, are as follow.

Cocoon Indices

Cocoon weight

The mean weight of fresh cocoon spun by silkworm larvae fed during their 4th instar on mulberry leaves dipped in the extracts of *D. stramonium* and *L. spartum* recorded 0.910 and 0.853 g with the ethanolic extracts and 0.941 and 0.812 g for chloroformic extracts of the two plants, respectively regardless of the concentration. The weight of the cocoons spun by chlorpyrifos methyl treated larvae attained 0.740 g compared to 0.850 g for control cocoons. The differences between treatments, concentrations and control were mostly significant (Table 1).

The same trend was observed in case of the weight of cocoons spun by treated *Bombyx mori* larvae during their 5th instar with the tested extracts and the insecticide chlorpyrifos methyl. However, the differences between the concentrations within each extract were mostly significant. Where the higher concentrations were significantly higher than the lower ones, meanwhile data of the insecticide (chlorpyifos methyl) were the inverse Table 2. In connection, Saad (2001) found that the mean weight of fresh cocoon ranged between 1.048 – 1.392 g working on Japanese mulberry varieties and on selected silkworm breeds. Also, Yousif-Khalil *et al.* (2004) recorded fresh cocoon weight of 1.155 g for male and 1.248 g for female cocoons of the same Chinese hybrid (9F7X). The variation between obtained data and that in the literature could be

Table 1. Cocoon indices and weight of pupa of *Bombyx mori* resulted from larvae fed during their 4th instar on mulberry leaves treated with the extracts of *Datura stramonium* and *Legeum spartum*

Extract	*Datura stramonium*		*Legeum spartum*		Chlorpyrifos methyl	Control
Solvent / Concentrations	Ethanol	chloroform	Ethanol	chloroform		
			Cocoon weight (g)			
10 %	0.914	0.983	0.847	0.703	0.740	0.850
5 %	0.953	0.982	0.882	0.898		
2.5 %	0.862	0.858	0.830	0.834		
Mean	0.910	0.941	0.853	0.812		
L.S.D. $_{0.05}$ for conc.	ns	ns	ns	0.129		
L.S.D. $_{0.05}$ for Treat.			0.0969			
			Pupal weight (g)			
10 %	0.762	0.827	0.700	0.444	0.625	0.701
5 %	0.795	0.824	0.731	0.719		
2.5 %	0.721	0.699	0.688	0.684		
Mean	0.760	0.783	0.704	0.618		
L.S.D. $_{0.05}$ for conc.	ns	ns	ns	0.212		
L.S.D. $_{0.05}$ for Treat.			ns			
			Cocoon shell weight (g)			
10 %	0.150	0.155	0.145	0.134	0.133	0.148
5 %	0.157	0.158	0.150	0.178		
2.5 %	0.140	0.158	0.141	0.153		
Mean	0.149	0.157	0.145	0.155		
L.S.D. $_{0.05}$ for conc.	ns	ns	ns	0.022		
L.S.D. $_{0.05}$ for Treat.			ns			
			Silk ratio %			
10 %	16.411	15.768	17.119	19.061	17.970	17.412
5 %	16.479	16.089	17.006	19.822		
2.5 %	16.241	18.415	16.987	18.345		
Mean	16.377	16.684	17.037	19.088		
L.S.D. $_{0.05}$ for conc.	ns	ns	ns	ns		
L.S.D. $_{0.05}$ for Treat.			1.1817			

Table 2. Cocoon indices and weight of pupa of *Bombyx mori* resulted from larvae fed during their 5th instar on mulberry leaves treated with the extracts of *Datura stramonium* and *Legeum spartum*

Extract	*Datura stramonium*		*Legeum spartum*		Chlorpyrifos methyl	Control
Solvent / Concentrations	Ethanol	Chloroform	Ethanol	Chloroform		
Cocoon weight (g)						
10 %	1.017	0.933	0.804	0.879	1.024	0.850
5 %	0.775	0.933	0.846	0.946		
2.5 %	0.717	1.145	0.876	0.848		
Mean	0.836	1.003	0.842	0.891		
L.S.D. $_{0.05}$ for conc.	0.138	0.152	ns	0.146		
L.S.D. $_{0.05}$ for Treat.	ns					
Pupal weight (g)						
10 %	0.837	0.772	0.665	0.741	0.845	0.701
5 %	0.631	0.817	0.695	0.803		
2.5 %	0.581	0.946	0.729	0.716		
Mean	0.683	0.845	0.696	0.753		
L.S.D. $_{0.05}$ for conc.	0.116	0.130	ns	0.123		
L.S.D. $_{0.05}$ for Treat.	0.1249					
Cocoon shell weight (g)						
10 %	0.179	0.160	0.139	0.137	0.178	0.148
5 %	0.143	0.175	0.151	0.142		
2.5 %	0.135	0.198	0.146	0.131		
Mean	0.152	0.177	0.145	0.137		
L.S.D. $_{0.05}$ for conc.	0.023	0.024	ns	ns		
L.S.D. $_{0.05}$ for Treat.	0.0227					
Silk ratio %						
10 %	17.600	17.148	17.288	15.586	17.382	17.412
5 %	18.451	18.756	17.849	15.011		
2.5 %	18.828	17.293	16.667	15.448		
Mean	18.293	17.732	17.268	15.348		
L.S.D. $_{0.05}$ for conc.	1.031	ns	ns	ns		
L.S.D. $_{0.05}$ for Treat.	0.9144					

attributed to the varied rearing conditions and treatments applied.

Pupal weight

Significant differences were noticed in the mean pupal weight between the treatments of the 5th instar larvae. For instance, the mean pupal weight recorded 0.760, 0.704, 0.783, 0.618, 0.625 and 0.701g for the larvae treated during the 4th instar, and 0.683, 0.696, 0.845, 0.753, 0.772 and 0.701 g for *B. mori* larvae treated during the 5th instar with ethanolic and chloroform extracts of *D. stramonium* and *L. spartum*, chlorpyrifos methyl and control, respectively. Generally, significant differences were recorded between some concentrations, especially for those treated during the 5th instar (Tables 1, 2).

Cocoon shell weight

The mean cocoon shell weight of the cocoons spun by 4th instar *Bombyx mori* larvae fed on treated mulberry leaves with the tested extracts, chlorpyrifos methyl and control cocoons were 0.149, 0.145, 0.157, 0.155, 0.154 and 0.148 g for the larvae treated with ethanolic and chlorformic extracts of *D. stramonium* and *L. spartum*, chloropyrifos methyl and the control cocoons, respectively. The corresponding figures for the cocoon shell of 5th instar treated *B. mori* were 0.152, 0.145, 0.178, 0.137, 0.178 and 0.148 g, regardless of the concentration applied. The concentrations of chloroformic extract of *L. spartum* and chlorpyrifos methyl among the treatments of the 4th instar, ethanolic and chloroformic extracts of *D. stramonium* in the 5th instar only showed significant differences, in this parameter (Tables 1,2).

In this respect, Saad (2001) recorded cocoon shell weight range of 0.178-0.258 (g) according to the silkworm breed and the mulberry leave variety. Also Helaly (2004) working on some Romanian races and hybrids, found that this parameter recorded weight range of 0.152-0.513 (g) for male cocoon, 0.152-0.602 g for female one. The variation found between data is expected and it may be multi-factor dependant parameter (rearing conditions silkworm strain, mulberry variety, treatments applied and calculation and measurement tools and procedure).

Silk ratio

Obtained results clear that chloroformic extract of *L. spartum*

in case of 4th instar larvae treatment and ethanolic extract of *D. stramonium* in 5th instar larvae treatment showed the highest significant mean silk ratio, recording 19.01 and 18.290 %, respectively (Tables 1,2). Similar data were also reported by Saad (2001) and Helaly (2004) who recorded silk ratio range of 17.01-20.36%.

Technological Characters of Reelable Silk Filament

Filament length (m)

Obtained results indicated that the mean silk filament length attained 884.59, 778.21, 810.01, 801.36, 778.21 and 622.98 m for treated larvae during the 4th instar, and 757.67, 755.53, 756.65, 700.65, 866.80 and 704.14 m for the cocoons spun by treated larvae during their 5th instar with ethanolic and chloroform extracts of *D. stramonium* and *L. spartum*, chorpyrifos methyl and untreated (control) larvae, respectively (Tables 3,4).

Analysis of variance clear that the tested compounds induced significant increase in the length of reelable silk filament as compared to that of the control for the larvae treated during the 4th instar only. Moreover, significant differences were detected between the tested concentrations of each compound.

Filament weight (g)

The mean filament weight recorded 0.140, 0.138, 0.149, 0.147, 0.136 and 0.117 g for treated larvae during the 4th instar, and 0.124, 0.129, 0.141, 0.133, 0.157 and 0.117 g for 5th instar larvae treated with ethanolic and chloroform extracts of *D. stramonium* and *L. spartum*, chlorpyrofos-methyl and control, respectively (Tables 3, 4).

Statistical analysis indicated that all the tested extracts and sublethal concentration of chlorpyrifos methyl induced significant increase in this parameter over the control for the silk filament of treated 4th and 5th instar larvae.

Filament size (dn)

As shown in Tables 3 and 4, the mean filament size attained 1.589, 1.653, 1.599, 1.654, 1.712 and 1.690 dn for treated 4th instar larvae, and 1.503, 1.667, 1.537, 1.716, 1.593 and 1.495 dn for 5th instar larvae treated with ethanolic and chloroform extracts of *D. stramonium* and *L. spartum*, chlorpyrifos-methyl and control larvae, respectively. The differences between treatments

Table 3. Reelable silk filament length (m); weight (g) and size (dn) as affected by feeding 4th instar larvae of *Bombyx mori* on treated mulberry leaves on extracts of *Datura stramonium* and *Legeum spartum*

Extract	*Datura stramonium*		*Legeum spartum*		Chlorpyrifos methyl	Control
Solvent / Concentrations	Ethanol	Chloroform	Ethanol	Chloroform		
	Filament length (m)					
10 %	801.38	819.32	805.40	789.48	714.88	622.98
5 %	765.60	829.44	733.28	801.96		
2.5 %	846.80	781.28	795.96	812.64		
Mean	804.59	810.01	778.21	801.36		
L.S.D. $_{0.05}$ for conc.	65.023	66.441	84.427	50.312		
L.S.D. $_{0.05}$ for Treat.	45.771					
	Filament weight (g)					
10 %	0.144	0.149	0.142	0.143	0.136	0.117
5 %	0.131	0.149	0.142	0.144		
2.5 %	0.146	0.148	0.130	0.155		
Mean	0.140	0.149	0.138	0.147		
L.S.D. $_{0.05}$ for conc.	ns	0.021	ns	0.015		
L.S.D. $_{0.05}$ for Treat.	0.0091					
	Filament size (dn.)					
10 %	1.617	1.637	1.586	1.630	1.712	1.690
5 %	1.599	1.617	1.743	1.616		
2.5 %	1.552	1.705	1.470	1.717		
Mean	1.589	1.653	1.599	1.654		
L.S.D. $_{0.05}$ for conc.	ns	ns	ns	ns		
L.S.D. $_{0.05}$ for Treat.	ns					

Table 4. Reelable silk filament length (m); weight (g) and size (dn) as affected by feeding 5th instar larvae of *Bombyx mori* on treated mulberry leaves on extracts of *Datura stramonium* and *Legeum spartum*.

Extract	*Datura stramonium*		*Legeum spartum*			
Solvent / Concentrations	Ethanol	Chloroform	Ethanol	Chloroform	Chlorpyrifos methyl	Control
	Filament length (m)					
10 %	612.36	741.92	789.12	733.56	866.80	704.14
5 %	949.00	711.92	770.52	659.76		
2.5 %	711.64	816.12	706.96	708.64		
Mean	757.67	756.65	755.53	700.65		
L.S.D. $_{0.05}$ for conc.	64.479	38.315	66.879	73.222		
L.S.D. $_{0.05}$ for Treat.	ns					
	Filament weight (g)					
10 %	0.120	0.130	0.133	0.135	0.157	0.117
5 %	0.139	0.138	0.148	0.129		
2.5 %	0.113	0.154	0.107	0.136		
Mean	0.124	0.141	0.129	0.133		
L.S.D. $_{0.05}$ for conc.	0.010	0.008	0.007	0.015		
L.S.D. $_{0.05}$ for Treat.	0.0202					
	Filament size (dn.)					
10 %	1.763	1.576	1.517	1.656	1.593	1.495
5 %	1.318	1.727	1.729	1.767		
2.5 %	1.429	1.698	1.362	1.727		
Mean	1.503	1.667	1.537	1.716		
L.S.D. $_{0.05}$ for conc.	0.156	0.089	0.123	0.142		
L.S.D. $_{0.05}$ for Treat.	ns					

were insignificant, however, significant differences were noticed between concentrations of the tested materials, especially in filaments spun by the 5th instar treated larvae.

It is obvious that the tested extracts and the sub-lethal concentration of chlorpyrifos-methyl cause noticeable improvement the cocoon and realable filament characters. These materials caused satisfactory control against the cotton leafworm, therefore it was expected to gain some losses in silkworm rearing, the inverse was true, it is a surprise but after reviewing the literature the surprise disappeared. The literature involving previous studies of the effect of some plant extracts and even insecticides at lower concentrations that induced considerable improvement in the biology and productivity characters of *B. mori*.

The causatives of the current improvement could be attributed to many factors. For instance, Raghavaiah and Jayaramaiah (1987) reported that *D. stramonium* extract showed moderate inhibitory effect against the fungus *Beauveria bassiana* the pathogen of the white muscardine disease of *Bombyx mori*. In addition, Sugum and Vijayakumar (2004) reported that the extract of some plants possessed antiviral effects against grasserie disease of *Bombyx mori* (a viral disease). Nataraju *et al.*, (1989) went so far when attributed the positive effect of plant extracts to their content of insect hormones that can be used to increase silk yield in commercial silk yield rearing. Similarly, Tenguria and Goyal (2006) attributed the positive effect of *Oscimum sanctum* extract to silkworm to its effect as a phytoecdysone. Moreover, Padwelatha *et al.* (2005) stated that plant extract, treatments to silkworm rearing increased food consumption and efficiency and increased nucleic acid in silk glands. Therefore, Prasad *et al.* (2000) and Bohidar *et al.* (2004) used the extracts of some solanaceous and mulvaceous plants (leaves) to enhance cocoon production in *Bombyx mori*. Concerning the insecticides, Nath *et al.* (1997) and Nath and Kumar (1999) stated that the sub-lethal doses of op insecticides caused a slight increase in food intake of *Bombyx mori* larvae.

Madhuri and Prasad (1999) proved the double faces of plant extracts, where the lower concentrations act as growth

promoter, inducing an increase in the majority of biological and technological parameters of *B. mori*. On the contrary, the higher concentrations induced anti-feedant action and growth retardant effect.

In addition, Narayanaswamy *et al.* (2000) reported ovividal a of busil and *Ipomoea* extracts against eggs of *Exorista bombycic*. Moreover, Bohidar and Choubey (2005) stated that some plant extracts are harmful, others showed better results than the rest, but not as good as in the control.

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تأثير مستخلصات الداتورة والحلفا على بعض الصفات الإنتاجية لدودة الحرير التوتية *Bombyx mori* L.

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أجريت هذه الدراسة لتقييم التأثير المحتمل لمستخلصات نباتي الداتورا والحلفا في الايثانول و الكلوروفورم بتركيزات مختلفة (١٠، ٥، ٢,٥ %) على بعض الصفات الإنتاجية لدودة الحرير التوتيه – ويمكن تلخيص النتائج المتحصل عليها كما يلي:

- أحدث مستخلص الداتورا فى المذيبين (متوسط تركيزات الايثانول والكلورفورم) زيادة معنوية فى وزن الشرانق الطازجة حيث سجلا ٠,٩١٠ و ٠,٩٤١ جرام/شرنقة لكلا المستخلصين على الترتيب فى حين كان تأثير مستخلص الحلفا فى ذات المذيبين مشابها تقريبا للكنترول (٠,٨٥٠ جرام/شرنقة كنترول)
- أدت تغذية يرقات العمر الخامس على أوراق توت سبق غمرها في مستخلص الداتورا والحلفا فى الكلوروفورم وكذا التركيز المعادل لـ ١/١٠ من التركيز الموصى به لمبيد كلوربيرويفوس مثيل إلى حدوث زيادة معنوية في وزن العذراء.
- أدت معاملة يرقات العمر الخامس بمستخلص الداتورا فى الكلورفورم والكلوربيرويفوس الى حدوث زيادة معنوية فى وزن قشرة الشرنقة في حين كانت الشرانق الناتجة من اليرقات المعاملة بمبيد كلوربيرويفوس ميثايل الأقل معنويا فى وزن القشرة.
- سجلت أعلا نسبه حرير (١٩,٨٢٢%) ، ٢٢,٨٦٠ لمعاملات العمر الرابع بمستخلص الحلفا في الايثانول أما بالنسبة لمعاملات العمر الخامس فكان المستخلص الكحولي للداتورا هو الأكثر فاعلية (١٨,٨٢٨%).
- تم تسجيل زيادة معنوية في طول الخيط الحريري لكل المعاملات تقريبا حيث تراوح بين ٧٧٨,٢١ – ٨٨٤,٥٩ متر لمعاملات العمر الرابع و٧٠٠,٦٥– ٧٥٧,٦٧ متر لمعاملات العمر الخامس مقارنة بـ ٦٢٢,٩٨ و ٧٠٤,١٤ متر لشرانق كنترول العمرين الرابع والخامس على الترتيب.
- أدت معاملة اليرقات في عمرها الرابع بمستخلصات النباتين المختبرين في الكلوروفورم إلى حدوث زيادة معنوية في وزن الخيط حيث تراوح ٠,١٣٨ – ٠,١٤٩ جرام مقارنة بـ ٠,١١٧ جرام لخيط شرانق الكنترول.
- لم تؤثر المعاملات المختبرة معنويا على حجم الخيط الحريري.