



Studying the effect of some botanical extracts of the Aveloz plant (*Euphorbia tirucalli* L.) on biological and productive characters of the mulberry silkworm (*Bombyx mori* L.)

El-Yamani Enas M. *, Saad M.S.I., Hassan Eman M.

Sericulture Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Egypt

Abstract

The production of high quality and quantity of natural silk depends mainly on the nutritional status of the mulberry leaves fed to silkworms so, the healthy growth of silkworms and subsequently the economic traits are influenced largely by improving the mulberry leaf quality and feed efficiency of silkworm. The present study was an attempt to assess the effect of four extracts of *Euphorbia tirucalli* plant; acetone, petroleum-ether, ethyl alcohol and aqueous extracts on the biological characters, cocoon parameters and silk characters of the mulberry silkworm, *Bombyx mori*; the plant powder was soaked in petroleum-ether, acetone, ethyl alcohol and water serially. Each extract was used with three concentrations (2.5, 1.25 and 0.625 %) added to mulberry leaves. The schedule of application was applied since the beginning of the 5th instar (every day first feed). Most concentrations of botanical extracts of *Euphorbia tirucalli* especially the concentrations (2.5% and 1.25%) increased significantly biological and technological characters of *B. mori* compared with the control group; larvae reared on mulberry leaves enriched with these concentrations of extracts especially acetone and aqueous extracts showed a significant increase in larval weight, cocooning percentage, emergence percentage, fecundity and pupal weight compared with the control, while, no significant differences were observed among means of the treatments in fertility percentage. On the same pattern, cocoon aspects (cocoon weight and cocoon shell weight) and silk characters (filament length, weight and size) enhanced significantly by rearing silkworms on fortified leaves with botanical extracts concentrations, compared with the control. While no significant variation was noticed among the means of silk ratio (%). Conclusively, fortification of mulberry leaves with acetone, petroleum-ether, ethyl alcohol and aqueous extracts of *E. tirucalli* plant as nutritional additives improved the biotechnological characters of mulberry silkworm *B. mori* especially the acetone and aqueous extracts with increasing the concentrations.

Keywords: silkworm, *Bombyx mori*, mulberry leaves, nutritional additives, botanical extracts, *Euphorbia tirucalli*.

*Corresponding author: Enas El-Yamani M.,
E-mail address: enaselyamani3@gmail.com

1. Introduction

The silkworm *Bombyx mori* is a distinctive monophagous insect and derives almost all the nutrients required for its growth and development from the mulberry leaf itself (Nasreen *et al.*, 1999). Nutrition of silkworm is the solitary factor which improves the growth, development, health, feed consumption and conversion of silkworm thereby improving the quality and quantity of silkworm cocoon production and productivity (Laskar and Datta, 2000; Waktole and Bhaskar, 2015). The healthy growth of the silkworm and ultimately the economic traits are influenced by the nutritional status of the leaves fed to silkworms (Krishnaswami *et al.*, 1973; Ravikumar, 1988). Plants are the richest source of organic chemicals on world which influenced the life and behavior of different insects (Rajashekaragouda *et al.*, 1997). Recently, many attempts have been made to secure mulberry leaves with botanical extracts to improve the mulberry leaf quality and feed efficiency of silkworm, which in turn help to increase cocoon production and silk quality. It has been shown that feeding of mulberry leaves to silkworm, treated with medicinal botanical plant extracts has varied influences on growth, development and reproduction (Hipparagi *et al.*, 2001; Muruges and Mahalingam, 2005). Previous studies have confirmed the effect of many extracts on various metabolic activities resulting in accelerated of silk cocoon formation and spinning (Shivakumar *et al.*, 1995) and (Murugan *et al.*, 1998) and increase in larval, cocoon as well as shell weight (Sridevi *et al.*, 2003; Pardesh and Bajad, 2014a). Some plant extracts are feeding stimulants and improve the nutritional

intake, growth, disease resistance/ tolerance of silkworm ultimately improving the cocoon traits (Bhaskar *et al.*, 2004; Mahesha *et al.*, 1999; Shubha, 2005). *Euphorbia tirucalli* L. commonly known as aveloz, is a plant indigenous to Africa and phytochemical screening of *E. tirucalli* reveals it, as a valuable medicinal plant with numerous medicinal properties. Many new drugs can be developed from *E. tirucalli* to control numerous diseases with no side effects as it is from natural source (Cataluna and Rates, 2015; Gupta *et al.*, 2013; Priya and Rao, 2011). Moreover, aveloz has been reported to have antiviral and antimicrobial properties (Jurberg *et al.*, 1985), *E. tirucalli* is reported to possess flavonoids, diterpenes, tannins, steroids and alkaloids as major phytochemical compounds and possess also terpenes, alcohol eufol, alfaeuforbol, taraxasterol, *E. tirucallol*, cycloeuphornol, n-hexacosanol, terpenic alcohol and trigliane (Fauconneau *et al.*, 1997). The present study was therefore, undertaken to determine the effect of four extracts of *E. tirucalli* plant; acetone, petroleum-ether, ethyl alcohol and aqueous extracts on the biological characters, cocoon parameters and silk characters of mulberry silkworm, *B. mori*.

2. Materials and methods

This work was carried in the laboratories of Sericulture Research Department of Sharkkiya Governorate, Plant Protection Research Institute, Agriculture Research Center, Egypt.

2.1 Insect culture and rearing conditions

Diseases free laying eggs of H1 x KK x

G2 x V2 silkworm hybrid were procured from the Sericulture Research Department, Plant Protection Research Institute (PPRI), Agricultural Research Center, Giza, Egypt. After hatching larvae were reared in the laboratory at $25\pm 2^\circ\text{C}$ and $75\pm 5\%$ relative humidity (Krishnaswami, 1978). The newly hatched larvae were fed on fresh clean mulberry leaves until the 5th instar, which stage was used in our bioassays. 100 worms per replications were maintained.

2.2 Mulberry variety

Mulberry leaves variety; *Morus alba* var. Balady (native) was used as a source of feed.

2.3 Disinfection of rearing room for silkworm rearing

Rearing rooms, boxes and tools were thoroughly washed, treated with a disinfective solution (formalin 3%) and left to dry before using. The whole boxes were kept clean and the paper sheets covered the bottom of the boxes were changed frequently.

2.4 Collection and preparation of plant extracts

The plant under investigation was originally collected from Zagazieg city, Sharkia governorate. Mature fresh leaves of *Euphorbia tirucalli* plant were collected, chopped into small pieces, placed in an oven, and dried at 50°C . The leaves were then pulverized into plant powder using a small grinder. The plant powder was weighed and soaked in

petroleum-ether, acetone, ethyl alcohol and water serially. The extracts were filtered and evaporated at 50°C using avocum rotary evaporator separately (Su and Horvat, 1981). The dry extracts saved in refrigerator at 18°C until used. *Euphorbia tirucalli* plant extracts; acetone, petroleum-ether, ethyl alcohol and aqueous extracts, each extract was used with three concentrations (2.5, 1.25 and 0.625 %).

2.5 Schedule of application

Mulberry leaves were washed, let to dry and were sprayed with plant extracts by using a small atomizers and shade dried then fed to the silkworms starting from V instar onwards. The schedule of application was as follows: V instar (every day first feed). The control leaves were dipped in distilled water. For spinning purposes, the mature larvae were handily transferred to the mounches for cocoon building. Once the cocoon formation was completed, the larvae developed to pupae inside the cocoons. The resulted cocoons were harvested 7 days later, and then the cocoon numbers were counted to calculate the cocooning percentage for each replicate. Half number of the resulted cocoons of each replicate was oven dried at 80°C and used to study the technological characters, while the other half of cocoons was used for the biological studies.

2.6 Statistical analysis

All data were analyzed using analysis of variance (ANOVA) and Least Significant Difference Test (LSD) was employed to

compare the treatment means ($P = 0.05$) using the Costat program according to Snedecor and Cochran (1982).

3. Results and Discussion

3.1 Biological characters

The mean values of biological parameters of silkworm *B. mori* reared on mulberry leaves supplemented with different concentrations of botanical extracts of *Euphorbia tirucalli* plant are tabulated in Table (1). Significant increases were noticed in most concentrations compared with the control group.

3.1.1 Larval weight (g)

As shown in Table (1), weights of grown larvae exhibited a significant increase with most concentrations, especially the concentrations: (2.5%) of acetone extract (3.95 gm) followed by (2.5%) of water extract (3.77 gm), then (1.25%) petroleum ether extract and (2.5%) ethyl alcohol extract recorded (3.65 and 3.60 gm) respectively, compared with the control (3.05 gm). The significant increase in larval weight might be attributed to the enhancement of bio-availability of nutrients for digestion and conversion by these plant extracts resulting in healthy growth of the silkworm. Moreover, the plant extracts positively influenced the healthy and vigorous growth of silkworms by stimulating the worms to feed more compared to the control group. These results are in agreement with

Waktole and Bhaskar (2015) who stated that, some plant extracts are feeding stimulants and improve the nutritional intake and growth of silkworm ultimately improving the cocoon traits. Similarly, *E. tirucalli* extracts may possess the compounds with antibacterial and antifungal properties that can be used as antimicrobial agents in the development of new drugs (Ajali *et al.*, 2002; Annapurna *et al.*, 2004). As the same trend, Pardesh and Bajad (2014b) reported that the moderate concentration (2.5%) of *Xanthium indicum* L. extract had growth promoting effect in silkworm, *B. mori*. This is due to the physiological stimulation by plant extracts on silkworm larvae, leading to remarkable larval growth leading to increased food consumption and cocoon weight.

3.1.2 Cocooning percentages (%)

Data represented in table (1) showed that, silkworm larvae fed on mulberry leaves fortified with the concentration 2.5% of acetone extract of *E. tirucalli* plant exhibited significantly the highest value in cocooning % (100%), followed by the concentrations 2.5% of petroleum ether extract and 1.25%, 0.625% of acetone extract which recorded (98.33% for each), then (98%) which recorded for the concentration 2.5% of water extract, compared with (82%) for the control. This improvement in cocooning % could be due to the biochemical constituents of the plant extracts and the influence of the nutrients in the feed supplied which is probably attributed to the stimulatory effect of the botanicals on protein synthesis in the silk gland during larval

period. The present findings are in confirmation with those of Shivakumar *et al.* (1995) and Murugan *et al.* (1998) who observed the effect of many extracts on various metabolic activities resulting in accelerated of silk cocoon formation and spinning.

Table (1): The effect of *Euphorbia tirucalli* plant extracts on the biological characters of mulberry silkworm *B. mori* L.

<i>Euphorbia tirucalli</i> Extracts	Conc.	Larval weight (g)	Cocooning (%)	Emergence (%)	Fecundity (egg/female)	Fertility (%)
Acetone extract	2.5 %	3.95	100	99.83	655	99.97
	1.25 %	3.55	98.33	100	623	100
	0.625%	3.35	98.33	99.57	615	99.5
petroleum-ether extract	2.5 %	3.55	98.33	99.93	601	99.76
	1.25 %	3.65	96	99.87	583	99.5
	0.625%	2.81	87	98.6	551	99.3
Ethyl alcohol Extract	2.5 %	3.60	95	99.9	591	99.67
	1.25 %	2.77	94	98.67	579	98.63
	0.625%	2.94	94	98.37	575	99.17
Water extract	2.5 %	3.77	98	99.93	589	99.9
	1.25 %	3.12	97	99.87	604	99.97
	0.625%	3.39	94	99.97	626	99.47
Control		3.05	82	97.03	505	97.47
LSD _{0.05}		0.389 ***	3.258 ***	0.635 ***	82.746 *	ns

3.1.3 Emergence percentage (%)

The results as shown in Table (1) indicated that, all concentrations of the botanical extracts of *E. tirucalli* plant under investigation recorded significantly high emergence percentage, such as the concentration 1.25% of acetone extract and 0.625% of water extract (100% and 99.97%) respectively, also the concentration 2.5% of petroleum ether extract, water extract and ethyl alcohol extract recorded (99.93%, 99.93% and 99.9%) respectively, compared with control group (97.03%). These findings are in accordance with Prasad *et al.* (2011) stated that, Acetone, and petroleum ether extracts of *E. tirucalli* were reported to possess antibacterial activity against several bacterial species and antifungal activity. As the same way,

Jyothi *et al.* (2008) and Parekh *et al.* (2005) opined that, aqueous extract of *E. tirucalli* was reported to carry antioxidant activity and Antibacterial activity against *Staphylococcus* and *Bacillus* sp.

3.1.4 Fecundity of female moth (egg\ female) and fertility percentage (%)

As illustrated in Table (1), the different concentrations of botanical extracts showed significant increases in means of fecundity compared with the control, especially the concentration 2.5% of acetone extract recorded (655 egg/female), while the control group recorded (505 egg/female). While no significant variation was observed among means of fertility % within different concentrations of botanical extracts. The results are in confirmity with the findings of Lall and

Meyer (2000) suggested that, the presence of compounds in the acetone extract possessing lipophilic characteristics, these observed antimicrobial properties agree with its use in traditional medicine. Further, Murugan *et al.*, (1998) noticed a strong correlation between the growth of silkworm and the silk production in the silkworm after the treatment of plant extracts. Also, Priyanka *et al.*, (2014) reported that, treatment mulberry leaves with different amount of *Aloe vera* oil then feeding of the larvae of silkworm, *Bombyx mori* (L) resulted in elicitation better response of reproductive ability (Fecundity, Hatchability).

3.2 Cocoon indices

Cocoon indices represented the most important indices in silk production. Cocoon weight, cocoon shell weight, cocoon shell ratio and pupal weight were recorded in Table (2).

3.2.1 Fresh cocoon weight (g)

Obtained data in Table (2) cleared that larvae fed on mulberry leaves treated with different concentrations of the botanical extracts of *E. tirucalli* plant showed a significant increase in fresh cocoon weights, especially the concentrations (2.5 and 1.25 %) of the acetone extract recording (1.786 and 1.734 g, respectively), followed by the concentration (1.25 %) of both water and petroleum ether extracts which recorded (1.698 and 1.620g) respectively, exhibited the highest cocoon weights compared with (1.284 g) for the control group. This

might be due to the fact that, the plant extracts contain many biochemical factors that contributed to the improvement of cocoon weight, and these results in line with the findings of Deshmukh and Khyade (2013) observed that, Aloe tonic treated mulberry leaves influence the cocoons, pupal and growth parameters of *B. mori*.

3.2.2 Cocoon shell weight (g)

Data tabulated in Table (2) cleared that, the fifth instar larvae treated with the tested concentrations of *Euphorbia tirucalli* plant extracts, resulted a significant increase in the cocoon shell weights of the resulted cocoons compared with the control (0.260 g) which considered the least shell weight among treatments. This improvement in shell weight in the different plant extracts may be due to increased nutritional efficiency of food which is utilized by the insect. These results are in line with Itokowa *et al.* (1989) and Wu *et al.* (1991), observed that the Whole *Euphorbia tirucalli* plant has afforded to contain 7.4% citric acid with some malonic, succinic acids, Terpenoids and sterols which represents in plants important sources of vitamins, steroid compounds.

3.2.3 Cocoon shell ratio (%)

According to the results obtained in Table (2), most concentrations of the botanical extracts of *E. tirucalli* plant under investigation exhibited high silk ratio than control group with no significant variation among them.

Table (2): The effect of *Euphorbia tirucalli* plant extracts on the cocoon parameters of mulberry silkworm *B. mori* L.

<i>Euphorbia tirucalli</i> Extracts	Conc.	Fresh cocoon weight (g)	Cocoon shell weight (g)	Silk ratio (%)	Pupal weight (g)
Acetone extract	2.5 %	1.786	0.366	20.5	1.395
	1.25 %	1.734	0.382	22.08	1.354
	0.625%	1.698	0.368	21.66	1.324
petroleum-ether extract	2.5 %	1.576	0.368	23.34	1.202
	1.25 %	1.620	0.350	21.62	1.265
	0.625%	1.452	0.324	22.30	1.126
Ethyl alcohol Extract	2.5 %	1.582	0.344	21.71	1.230
	1.25 %	1.434	0.334	23.27	1.100
	0.625%	1.478	0.264	17.83	1.206
Water extract	2.5 %	1.594	0.372	23.37	1.232
	1.25 %	1.698	0.336	19.67	1.352
	0.625%	1.650	0.356	21.52	1.288
Control		1.284	0.260	20.19	1.024
LSD _{0.05}		0.301*	0.061**	ns	0.272*

3.2.4 Pupal weight (g)

The results in Table (2) showed that, all treatments were effective significantly in increasing the pupal weights; the concentration (2.5 %) of acetone extract recorded the highest pupal weight (1.395 g) among all the treatments, while the least pupal weight recorded by the control group (1.024 g). The significant improvement in cocoon characters (cocoon weight, pupal weight, shell weight and cocoon shell ratio) could be due to improved appetite of silkworms becoming stronger and tolerant to diseases and increased nutritional efficiency of feed which is utilized by worms. The present findings are in accordance with the findings of Priya and Rao (2011) stated that, phytochemical screening of *E. tirucalli* reveals it as a valuable medicinal plant with numerous medicinal properties. It is reported to possess flavonoids, diterpenes, tannins, steroids and alkaloids as major phytochemical compounds and possess

also terpenes, alcohol eufol, alfaeuforbol, taraxasterol, *E. tirucallol*, cyclo euphornol, n-hexacosanol, terpenic alcohol and trigliane (Fauconneau *et al.*, 1997). Thus, the broad-spectrum antimicrobial activity exhibited by *E. tirucalli* may be attributed to the various active constituents present in it, which either due to their individual or combined action, exhibit antimicrobial activity (Bhuvaneshwar *et al.*, 2010).

3.3 Technological aspects

According to the technological aspects which is very important from the view of industry, results of this work revealed a significant increase in the filament characters as a result of rearing silkworm *B. mori* on mulberry leaves supplemented with different concentrations of the botanical extracts of *Euphorbia tirucalli* plant as in Table (3).

3.3.1 Silk filament length (m)

Data tabulated in Table (3) cleared that all

tested concentrations of *E. tirucalli* plant extracts revealed a significant increase in silk filament lengths. The highest filament lengths were recorded (1297 and 1249 m) for the concentration (2.5%) of acetone and ethyl alcohol extracts followed by the concentration (0.625 %) of petroleum-ether extract (1218 m), compared with the control group (895 m). This might be due to more shell content of cocoon in respective treatments. This has been further confirmed by Bhuvaneshwar *et al.* (2010) reported that, the alcoholic extracts of leaves of *E. tirucalli* possess antimicrobial activity against clinical and lab isolates several kinds of bacteria and fungi. Furthermore, Padma and Ramani (2015) noticed that, the larval weight and all the economic characters increased due to application of 2% ethanolic extract of *Ocimum sanctum* along with mulberry leaves and concluded that *Ocimum sanctum* extract has growth promoting effect on silkworm, which helps to enhance the commercial qualities of silk and can be used in sericulture for yield improvement. Similarly, Pardeshi and Bajad (2014a) reported that, the nutritional supplementation with plant *Amaranthus hybridus* extract at the concentration of 0.5 %, 1.0 %, 1.5 % and 2.0 % may have beneficial effect on the growth of the silkworm *B. mori* and also increased the larval weight, cocoon weight, pupal weight, shell ratio and filament length by enhancing feed efficacy than control.

3.3.2 Silk filament weight (g)

Results represented in Table (3) showed that, there are high significant differences

in silk filament weights among different concentrations under investigation; most tested concentrations revealed a significant increase in the weights of reeled silk filament compared with control (0.243 g). The higher weights were recorded for the concentration 2.5 % of acetone extract (0.380 g) followed by the concentration 0.625 % of aqueous extract (0.338 g).

3.3.3 Silk filament size (dn)

Statistic analysis of data revealed highly significant differences in silk filament size among different concentrations treatments. The highest means of filament size were recorded (2.872, 2.637 dn) for the treatment of mulberry leaves fortified with *Euphorbia tirucalli* acetone extract at concentration 1.25%, 2.5%, and water extract at 2.5 % and 1.25 % (2.547 and 2.548 dn) respectively, compared with control which recorded (2.443 dn). While, the least mean of silk filament size was recorded (1.874 dn) for the treatment with 0.625 % ethyl alcohol extract of *Euphorbia tirucalli* plant. The improvement in the quality of the silk filament might be due to bio-availability of certain nutrients to the worms from the plant extracts, which might have enhanced the protein synthesis, particularly the fibroin synthesis in the posterior silk gland, which in turn might have influenced the filament characters. Similar kind of results has been obtained by Rajashekaragouda *et al.* (1997) reported that, the plant extracts phytochemicals could benefit sericulture by improving the silk yield of *B. mori* and commercial silk production. Further,

Saravanan *et al.* (2011) observed the mulberry leaves at different supplementation of Cowpeas (*Vigna unguiculata*) aqueous extract with concentrations enhanced the quality and quantity of silk in *Bombyx mori*.

Table (3): The effect of *Euphorbia tirucalli* plant extracts on the silk filament characters of mulberry silkworm *B. mori* L.

<i>Euphorbia tirucalli</i> Extracts	Conc.	Filament length (m)	Filament weight (g)	Filament size (dn)
Acetone extract	2.5 %	1297	0.380	2.637
	1.25 %	1028	0.328	2.872
	0.625%	1064	0.273	2.309
petroleum-ether extract	2.5 %	1169	0.308	2.371
	1.25 %	1165	0.298	2.302
	0.625%	1218	0.280	2.069
Ethyl alcohol Extract	2.5 %	1249	0.328	2.363
	1.25 %	1144	0.268	2.108
	0.625%	1071	0.223	1.874
Water extract	2.5 %	1060	0.300	2.547
	1.25 %	1088	0.308	2.548
	0.625%	1198	0.338	2.539
Control		895	0.243	2.443
LSD _{0.05}		166.94*	0.056***	0.406**

Conclusively, *Euphorbia tirucalli* L. is a valuable medicinal plant with numerous medicinal properties. And the four medicinal botanical extracts of *E. tirucalli* (acetone, petroleum-ether, ethyl alcohol and aqueous extracts) under investigation may possess the compounds with antibacterial (Ajali *et al.*, 2002), antifungal (Annapurna *et al.*, 2004) properties and carry antioxidant activity (Parekh *et al.*, 2005). So, according to the above results, it could be recommended that, fortification of mulberry leaves with botanical extracts of *E. tirucalli* as nutritional additives, especially acetone and aqueous extracts with increasing the concentrations were proved to be more efficient in rearing silkworm as it improved healthy growth, hence increased the viability of larvae and the biological characters ultimately enhancement of the cocoon productivity and the silk

production.

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