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Improving the Silk Industry by Studying the Effect of Several Aqueous Extracts to Enhance the Efficiency of Silkworm *Bombyx mori* L. Production

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ABSTRACT



Mulberry leaves have a high nutritional value for larval silkworm so fortification with plant and animal extracts that contain antioxidant and phytochemical properties and amino acids improve the sericultural industry. Data cleared that, Mulberry leaves that fortified with 2.5% *Aloe Vera* recorded the highest value in all traits significantly. Although, Taurine with a recommended dose showed the high value in all biochemical analyses after *Aloe Vera* the results differed in biological and cocoon traits. Also, *Moringa oliefera* 2% registered high value in biological and cocoon traits after *Aloe vera* and Taurine. From the previous results 2.5% *Aloe vera* is more effective in all parameters than any aqueous extracts understudied.

Keywords: Silkworm, *Aloe Vera, Moringa oleifera*, Taurine, Amylase, Total Protein, Peroxidase, Glutathione- S- Transferase, cocoon traits, and biological parameters.

INTRODUCTION

Silkworm B. mori, It is an economically important pest, It mainly feeds on mulberry leaves and turns protein into silk (Babu, et al, 2009). Nutritional quality food is main responsible factor for growth improvement, and insect body development physiology (Murugan and George, 1992). Fortifying mulberry leaves with herbal extract and feeding of larvae (5th instar), it gave a significant improvement in the levels of proteins and reactions that catalyzed by protease and amylase (Khyade Vitthalrao and Doshi Sucheta, 2012). The plant of Aloe vera, consists of protein 0.013% and water 99.5%, Which is an essential and important factor in the nutrition and quality production of silk material (Murugan et al. 1998). The plant of Aloe vera, L. consists of more than (200) active compounds and nutrients over (75), including amino acids; salicylic acid; saponins; anthraquinones; sugars; lignin; minerals; enzymes and vitamins (Park and Jo, 2006). Phytochemicals extract is an important and very useful factor in improving and producing of the silk yield and its commercial quality (Rajasekaragouda, et al, 1997). The plant of Aloe vera, it considered an herbal tonic very impressive on the parameters growth; pupal and cocoons of silkworm B. mori (Balamurugan and Isaiarasu, 2007). Peptides from plant sources have attention due to their ability to use it as food additives. (Arise et al., 2016b). One plant that has been extensively studied for its numerous bioactivities is Moringa oleifera. Various parts of Moringa oleifera have been reported for their numerous biological activities. Its leaves possess purgative, antimicrobial and hypoglycemic effects (Divi et al., 2012). The Moringa leaves contains highly rates of phytochemical components, including moringinine; moringine; flavonoids; alkaloids; Glycosides; sterols; carotenoids and amino acids that it

works as an effective source of natural antioxidants (Anwar et. al., 2007). Solvent extracts of its seeds have been shown to possess antioxidant and antihypertensive activities (Anwar, et al, 2007). Hagar, 2004, reported that, the amino acid "Taurine" (Tau), who is involved in most biological and good antioxidant processes. Taurine stimulates the secretion of growth hormone (Beckman and Ames 1998). It was hypothesized that aging is accompanied by taurine deficiency, which requires taurine supplementation of the ration Dawson et al, 1999. The enzymes action, it considered factor and very important influential on growth and development of all organisms as they are involved in various biochemical reactions. The growth of the silkworm in size and weight during the larval stage increased due to the activity of various enzymes. Amylase is one such key enzyme responsible for disease resistance and also involved in digestion and metabolism of carbohydrates Umakanth and Devamani (2016). Proteins are the building blocks of an organism; hemolymph serves as a reservoir for nutrients and metabolites during metamorphosis Dash and Kundu (2006) the study of protein concentration in Bombyx mori hemolymph revealed that hemolymph proteins play a major role in metamorphosis.

This investigation aims to study the impact of some aqueous extracts of (*Aloe Vera*, *Moringa oleifera* and Taurine) with different concentrations on some biochemical, biological and cocoon traits.

MATERIALS AND METHODS

The present work was conducted and carried out during spring season of 2017 in Sericultural Research Department of Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza.

A. Materials:

- **1.** Mulberry silkworm: *Bombyx mori* eggs (Bulgarian hybrid) were used.
- **2.** *Aloe vera*: Fresh *Aloe vera* leaves were collected from El Orman Garden in March 2017. They were authenticated and an identification code (UTAV201301) was assigned to the specimen.
- **3.** *Moringa oleifera*: Various parts of Moringa oleifera have been reported for their numerous biological activities. Its leaves possess purgative, antimicrobial and hypoglycemic effects (Divi *et al.*, 2012)

Moringa oleifera seeds have considerably high protein content *Moringa oleifera* L.: extract was obtained from Egyptian Scientific of *Moringa* (ESSM), National Research Center.

- **4.** Taurine: Powder was obtained from the Zoology Department, Faculty of Science, Cairo University.
- **B.** Methods:
- 1. Silkworm Rearing Technique: Silkworm rearing was carried out under laboratory conditions 25±1°c and R.H. 75 \pm 5 %, According to Karishnaswami (1978). The diseased free egg cards were incubated in an incubator at 25°c and 75%R.H till hatching, newly hatched larvae were transferred to rearing trays, using cleaning nets for cleaning the rearing bed. At the beginning of 4th larval instar, larvae were divided into three groups, each group refer to type of aqueous extract understudied, each group divided to three subgroups for three concentrations(1%, 2%, and 2.5%), for Aleo Vera and Moringa Olifera but for Taurine the concentrations applied according to weight of larvae i.e(0.004, 0.002(recommended dose) and 0.001 in 4th instar for 30 larvae but in 5th instar the concentrations differed 0.02, 0. 01 (recommended dose) and 0.005, each concentration with 3 replicates, each replicate contained 30 larvae. Mulberry leaves were sprayed with every extract, and then let to dry about 10 minutes after feeding. Leaves offered to disinfected larvae 4 times every day till cocooning. Groups of control fed on mulberry leaves sprayed with distilled water.
- **2.** Biological parameters: Larval weight was taken at the end of 4th larval instar when stop feeding and in 8th day in fifth larval instar (mature larvae) about 30 larvae from each replicate for all treatments.
- **3.** Cocoon parameters: Three important economic parameters of sericulture, viz., cocoon weight cocoon shell weight and shell ratio were analyzed according to Bohidar *et.al.* (2007).

C. Preparation of aqueous extracts of the plants used for the study.

1. Preparation of aqueous extract of Aloe vera:

The plant of *Aloe vera*, extracts were prepared as a procedure adopted according to (Krishnaprasad *et al*, 1979). The required of fresh green leaves quantity from the plant *Aloe vera* was collected from El Orman Garden and sterilized surface by ethyl alcohol 70 % and washed by distilled water sterile and slit open longitudinally, the white gel was scooped with sterile stainless steel knife and homogenized in a domestic mixer and filtered through a sterile stainless steel tea strainer and refrigerated as stock solution (100 % gel) for further use. The extract was squeezed through a double-layered muslin cloth and the

extract collected was used as a stock solution. From this stock solution, the required concentrations (1%, 2%, and 2.5%) were prepared by using distilled water.

2. Moringa Leaf Extract (MLE) Preparation:

The leaves of *Moringa oleifera*, were brought from National Research Center, Egypt. 1 kg of *Moringa* leaves was air-dried under shade for two weeks and subsequently grounded to reach powder case then mixed with 1-liter ethyl alcohol (80% aq.) using a blender. The extract was purified by filtering twice through (What man No. 1) filter paper. After purification, the extract was subjected to a rotary evaporator to fully evaporate the alcohol and get the crude extract. The concentrations were prepared from the crude extract. 10, 20 and 25 ml from the crude extract were taken and diluted with 990 ml, 980 ml and 975 ml distilled water for reaching the concentration to 1%, 2%, and 2.5% respectively according to (Bashir *et al.*, 2014).

D. Preparation of animal aqueous extraction:

Taurine is supplied by GALL PHARMA, Austria Pharmaceutical form: Taurine 500 mg GPH capsules. Dosage: - Taurine was orally administration 500mg\kg\day Taurine was freshly prepared (dissolved in distilled water) and administered in daily oral dose of 500 mg/kg body. **1. Bioassay:**

Hemolymph was collected from treated larvae at 6th day of 5th larval instar by removal of thoracic leg in eppendorf tubes 1.5_{ml} with small amount of phenyl thiourea crystal (PTU) (Mahmoud, 1988) as an anticoagulant substance. The tubes were kept at -20°c, the blood samples were centrifuged at 1000 rpm for 10 minutes at 5°c. The supernatant was assayed to determine.

2. Determination of amylase activity:

Determined of digestive enzymes conducted according to Amin 1998, modifications, and the described method was conducted according, Ishaaya and Swirski 1976. Totally, the diluted enzyme solution 20µl was putted 10min in an incubator at 30°c with 250µl and starch 1% (soluble potato starch, Lintner grade, Sigma Chemical Co.) in 50mM acetate buffer; PH 5.0 containing 20mM NaCl and 0.1mM CaCl₂. Reaction was stopped by adding 250µl DNS reagent to each tube in boiling water for 5min. Samples were cooled, diluted with 2.5ml H₂O and read at 550nm on Spectronic 1201 (Beckman, 1998). Glucose was used as a standard. Appropriate dilutions of enzyme supernatant were used to obtain a linear production of glucose equivalents. For each test, amylase activity was determined from triplicate analyses of three groups of seedlings. The enzyme activity was expressed as µg glucose released/min/gm fresh weight.

3. Total proteins:

Determined of total proteins were conducted with Bradford method (Bradford, 1976). Prepared of protein reagent was done by using dissolving 100mg (Coomassie Brilliant Blue G-250) in 50ml 95% ethanol. To this solution 100ml, 85% W/V phosphoric acid was added and the solution resulting was diluted to volume 1 liter. Solution sample 50µl, or, for preparation of standard curve 50µl serial concentrations containing 10-100µg bovine serum albumin were pipetted into the test tubes. The volume in test tube was adjusted to 1ml with phosphate buffer (0.1M, pH 6.6). Protein reagent (5mM) was added to test tube and content were mixed either by inversion or vortexing. At 595nm, the absorbance was measured after 2min and before 1hr against blank prepared from phosphate buffer (1ml) and protein reagent (5ml).

4. GST:

Glutathione S-transferase (GST) catalyzes the conjugation of reduced glutathione (GSH) with 1-chloro 2,4dinitrobenzene (CDNB) via the -SH group of glutathione. The conjugate, S-(2,4-dinitro-phenyl)-L-glutathione could be detected and described according to (Habig et al, 1974 method). Reaction mixture consisted of 1ml potassium salt of phosphate buffer (pH6.5), 100µl GSH and 200µl larval homogenate. Reaction started with addition 25µl substrate CDNB solution. Concentration of GSH and CDNB was adjusted to be 5mM and 1mM, respectively. Enzyme and reagents were incubated at 30°C for 5min. Increment in absorbance at 340nm was recorded against a blank included everything except enzyme to determine nanomole substrate conjugated/min/larva by using a molar extinction coefficient of 9.6/mM/cm.

5. Quantitative Determination of Peroxidase:

According to procedure given by Hammerschmidt et al, 1982, the peroxidase activity was determined. To a spectrophotometer sample cuvette, 1.5ml of pyrogallol 0.05M and 100 μ l enzyme extract were added. The readings were adjusted to zero at 420nm. To initiate the reaction, 100 μ l of hydrogen peroxide 1% was added to sample cuvette. Enzyme activity was expressed as a change in absorbance/min/g. sample.

Statistical analysis: Obtained data were analyzed using Proc ANOVA in SAS (Anonymous. 2003). Means separation was conducted using LSD in the same statistical program.

RESULTS AND DISCUSSION

• Biological parameters:

Data obtained in Tables, the 4th and 5th larval weight significantly higher in 2.5% of *Aleo vera* gel that recorded (0.904, 3.952_{gm}) respectively, followed by 2% of *Moringa oliefera* extract that recommended (0.878, 3.740_{gm}) but control recorded the least value in two instars(0.707, 3.038_{gm}) as shown in Table (1). These results are in agreement with (Rajasekaragouda *et al.* 1997), who noticed that, effect of water and ether extracts of plants such as, *Tribulus terrestris* and *Psoralea corylifolia* on promoting growth. Also, (Subburathinam and Krishnan, 1998) concluded that the Soybean meal accelerates larval growth significantly. Sundarraj *et al.* (2000) observed high significance larval

Table 2. Effect of different extracts on some cocoon traits.

weight when reared on leaves fortified with soybean flour. Rajeswary and Isaiarasu (2004) recommended that, the fortification of flower, leaf and pod extract with 1% concentration of *Moringa oleifera* effect on the response of grown larvae. Also, Verma and Atwal (1968) observed the slightly increased in the weight of larvae during feeding with leaves sprayed with distilled water alone.

Kanafy *et. al.*, (2007), reported that, the nutrition plays an important role in improving of growth and development silkworm *Bombyx mori* L., and Sarker (1993), mentioned that, the growth of silkworm larvae improved significantly when feeding them with mulberry leaves fortified with different nutrients.

Table 1. Effect of different extracts on the larval weight of silkworm *Bombyx mori* L.

T	Sub	4 th larval	5 th Larval	
Treatments	treatments	Weight	Weight	
	1%	0.787 ed	3.490 d	
Aloe vera	2%	0.823 dc	3.759 cbd	
	2.5%	0.904 a	3.952 a	
Moringa oleifera	1%	0.867 bac	3.362 cb	
	2%	0.878 ba	3.740 b	
	2.5%	0.813 c	3.615 cb	
Taurine	T1	0.756 e	3.362 d	
	T2 (Recommended dose)	0.840 bc	3.618 cb	
	T3	0.756 e	3.577 cbd	
Control		0.707 f	3.038 e	
F value		16.42	7.77	
P value		0.0001	0.0001	
L.S.D		0.040	0.206	

Means with the same letter are not significant.

Cocoon traits:

Data obtained in Table 2., assured that, Cocoon weight, shell weight and shell ratio of larvae fed on 2.5% of *Aleo vera* as food additives showed positive impact significantly followed by *Moringa oleifera* with two concentrations 2 & 1% with no significant variance between them in male and female but control recorded the least value in all traits. The results arranged as follow (1.946, 1.912 and 1.88 mg) in female (1.50, 1.490 and 1.458 mg) in male for C.W. For C.S.W (0.446, 0.428 and 0.422 mg) in female and (0.4, 0.390 and 0.378 mg) in male. In S.R% the results arranged as the previous arrangement as follow(22.89, 22.38 and 22.43 %) in Female and (26.71, 26.15 and 25.72%) in male. It could be due to the existence of biochemical factors in the phytochemical extract that improves cocoon traits.

treatments	Sub treatment -	Female		Male			
		C.W mg	C.S.W mg	C.S.R%	C.W mg	C.S.W mg	C.S.R%
	1%	1.852 abc	0.398 bc	21.49 bac	1.432 a	0.370 ba	25.34 bac
Aleo Vera	2%	1.764 bc	0.372 dc	21.11 bdc	1.426 ab	0.350 bc	24.50 bc
	2.5%	1.946 a	0.446 a	22.89a	1.50 a	0.4 a	26.71 a
	1%	1.88 ab	0.422 ba	22.43ba	1.458 a	0.378 ba	25.72 bac
Moringa oleifera	2%	1.912 ab	0.428 ba	22.38 ba	1.490 a	0.390 a	26.15 ba
	2.5%	1.788 bc	0.376 dc	21.03 becd	1.440 a	0.370 ba	24.65 bc
	T1	1.704 dc	0.354 de	20.76 edc	1.426 ab	0.350 bc	24.50 bc
Taurine	T2	1.556 ed	0.310 f	19.93 ed	1.244 c	0.30 d	24.12 c
	T3	1.566 ed	0.318 fe	20.29 edc	1.334 bc	0.324 dc	24.31 bc
С		1.458 e	0.286 f	19.61 e	1.248 c	0.294 d	32.89 c
F value		11.46	15.83	5.80	9.07	11.67	2.36
P value		0.0001	0.0001	0.0001	0.0001	0.0001	0.030
L.S.D		0.141	0.038	1.313	0.080	0.030	1.752

Means with the same letter are not different.

These results are in line with Sujatha and Rao (2003) who studied the application of C. longa stem extract on fourth instar larvae that resulted in higher cocoon weight. Subburathginam et al. (1990) showed that the fortification of mulberry leaves with calcium chloride increase the cocoon characters as cocoon weight, shell weight cocoon, shell ratio, and silk proteins. Ganga and Gowri (1990) concluded that the dietary addition of wheat and rice flours increased some growth and cocoon parameters. Also, Quader et al., (1992) found that the nutritional value of mulberry leaves was directly reflected on cocoon characters of B. Mori that may explain the difference in cocoon characteristic among treatments. Moreover, Bentea et al., (2011) reported that the use of some (mineral and vitamin additives) improves the quality parameters of the cocoon. Also, Lokesh and Anantha Narayana (2011) found that when larvae treated with different concentrations of vitamins C and B that increased the food consumption and conversion leading to increased growth of silkworms.

• Biochemical Parameters:

Total Protein:

Metabolism of protein, it considered a very important to silkworm physiology, its important role for chemical characteristics determination of silk proteins. The level of hemolymph protein in this study increases significantly in larvae fed on mulberry leaves enriched with 2.5% Aleo vera followed by the recommended dose of Taurine and 2% of Moringa Oleifera with no significant variance between the last two as follow(36.66, 33.23 and 32.23 mg/ml). But in all cases, larvae fed on mulberry leaves without any addition recorded the least value. A similar trend of data was demonstrated by Manoharan (1997) who found that the addition of hydrolyzed soybean increases the total hemolymph proteins of the B. Mori fifth instar larvae. Also, the importance of vitamins in silkworm nutrition such as nicotinic acid, thiamin, riboflavin, and niacin, was reported by Govindan et al. (1998). Quraiza et al., (2008), protein contents of silk gland, muscles and fat body, increased significantly when larvae fed with ascorbic acid 1 and 2%. Increase in protein concentration in silkworm body after fourth moult is due to the regular feeding activity krishnaswami, et al. (1978).

Amylase:

The large biological molecules responsible for thousands of biochemical reactions that sustain life are Enzymes. Without Enzymes, metabolism would neither progress through the same steps nor be fast enough to serve the needs of the cell. Amylase is one of the key enzymes involved in digestion and carbohydrate metabolism in insects Daone et al., (1975); Buonocore et al., (1976) and Horie&Watanabe, (1980). From the previous results, there was increasing in the activity of Amylase Enzyme in all treatments over control significantly. The activity recorded (32.53, 23.6 and 23.06 glucose/min/ml) for 2.5% Aleo vera, Taurine (recommended dose) and 2% Moringa oleifera respectively, with the significant variance between Aleo vera and other treatments. These results are in agreement with Christopher and Mathavan 1985, who concluded that rational food consumption by a lepidopteran larva was correlated directly with the activities of amylase and invertase. The amylase activity increased with an increase in age during the fifth instar in EMS treated batches of silkworm with 1.1 mg/g/min, (Mahesha and Honnaiah, 2002).

Glutathione- S- Transferase:

Glutathione-S-transferases are a group of detoxification enzymes mainly localized in the cytosol that catalyze the conjugation of reduced glutathione Habig et al. (1974). The results in Table (3) cleared that, there was a significant difference between treatments, 2.5% of Aleo vera recorded the highest value (14.55 Ml mole) significantly followed by Taurine with a recommended dose (11.4 Ml mole) and 2% Aleo Vera(11.2 Mi mole) with no significant difference between them. Otherwise expected larvae fed on mulberry leaves fortified with Moringa Oleifera extract registered the least GST activity in all concentrations as follows (4.1, 4.1, and 3.6 Ml mole). Earlier reports suggested that the ingestion of pro-oxidant rich food increases the glutathione-S-transferase activity Peri- Mataruga et al., (1997). A higher level of phenolic content was observed in T.tomentosa during October to December Sharan et al., (2005), which coincides with the feeding period of diapausing larval generations. We can explain the observed difference to the consumption of higher phenolic compounds and pure amino acids by silkworms during that period. **Peroxidases:**

By checking the previous Table (3), results revealed that, the activity of peroxidases enzyme increased in larvae fed on mulberry leaves with 2.5% of *Aleo vera* followed by Taurine (recommended dose) and 2% *Moringa Oleifera* with a significant variance between them and all treatments (12.66,9.46 and 9.20 O.Dmin/ml) respectively.

Table 3. Effects of different extracts on biochemical parameters.

	Sub	Total protein	Amylase	Peroxidase	G.S.T Ml mole sub	
Treatments	treatments	mg∖ml	Ug glucose\min\ml	O.D\min\ml	conjugated\min\ml	
	1%	28.76 d	22.3 bcd	7.53 e	8.63 d	
Aloe Vera	2%	31.5 c	20.90 cebd	8.06 e	11.2 cb	
	2.5%	36.66 a	32.53 a	12.66 a	14.55 a	
	1%	25.86 e	18.5 cebd	9.03 cbd	4.1 f	
Moringa Oleifera	2%	32.23 bc	23.06 b	9.20 cb	4 gf	
	2.5%	28.76 d	22.8 cb	8.26 ed	3.06 g	
	0.0004	27.46 d	15.7 ed	8.36 ced	10.3 c	
Taurine	0.0002	33.23 b	23.6 b	9.46 b	11.4 b	
	0.0001	32.03 f	15.2 e	6.36 f	5.13 e	
Control		22.33 f	17.33 cebd	6.56 f	8.46 d	
F value		71.44	5.44	39.13	134.36	
P value		0.0001	0.0008	0.0001	0.0001	
L.S.D		1.586	6.602	0.839	0.982	

Means with the same letters are not significant.

All organisms possess mechanisms to maintain homeostasis which are essential for survival. Among these mechanisms is the antioxidant enzyme mechanism which plays a vital role in larval development and survival. These results are in line with Wood et al.(1986); Kotze & Rose (1987); Kostarpoulos & Papadopoulos (1998); Kostarpoulos et al. (2001), who reported although there are few relations between antioxidant defenses in insects to their larval development, majority of studies are focused antioxidant enzymes as POD; GST and catalase. Relatively relation between components of antioxidant defense system and extent of oxidative injury caused during development (Aslanturk et al. 2011). The foliar consumption constituents by larvae, which differ in quantity for larval stages, will alter the antioxidant and pro-oxidant balance of larval tissues. Antioxidant and pro-oxidant balance has been reported to affect larval development and aging in (Drosophila Klichko et al. 2004).

CONCLUSION

It was well known that feeding plays a vital role in larval development especially by adding aqueous extracts in leaves. Based on the present observations and above information it may be concluded that *Aleo vera* is more an important additive than *Moringa oleifera* for larval development, economic parameters and physiological state in all concentrations and the benefits increase by increasing the concentration. Although Taurine recorded high value in all enzymes understudied but not effect on biological and cocoon traits maybe there is an indirect effect. The presence of Taurine as a pure amino acid raised the level of antioxidant enzyme system in silkworm larva that in turn preserves larval health.

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REFERENCES

- Amin, T.R. (1998). Biochemical and physiological studies of some insect growth regulators on the cotton leafworm, *Spodoptera littoralis* (Boisd.) .Ph.D. thesis, Faculty of science, Cairo Univ.
- Anonymous. 2003. SAS Statistics and graphics guide, release 9.1. SAS Institute, Cary, North Carolina 27513, USA.
- Anwar F., Latif S., Ashraf M. and Gilani A.H. (2007). Moringa oleifera: A food plant with multiple medicinal uses. *Phytother Res.* 21(1):17–25.
- Arise R.O, Yekeen AA, Ekun OE. and Olatomiwa OJ (2016b). Angiotensin-I converting enzyme-inhibitory, antiradical and hydrogen peroxide scavenging properties of *Citrullus lanatus* seed protein hydrolysates. *Ceylon J. Sci.* 45: 39–52.
- Aslanturk A., Kalender S., Uzunhisarcikli M., and Kalender Y. (2011). Effects of methidathion on antioxidant enzyme activities and malonaldehyde level in midgut tissues of Lymantria dispar (Lepidoptera) larvae. Journal of Entomological Research Society 13:27–38.
- Babu, K. R., S. Ramakrishna, Y. H. K. Reddy, G. Lakshmi, N. V. Naidu, S. S. Basha and Bhaskar, M. (2009). Metabolic alterations and molecular mechanism in silkworm larvae during viral infection: A review. *Afr. J. Biotechnol.* 8, 899-907.

- Balamurugan R and Isaiarasu L (2007). Effect of the herbal tonic "Iogen" on the growth and cocoon parameters of mulberry silkworm, *Bombyx mori L. Proceedings* of the National Seminar on Applied Zoology, ANJA College, Sivakasi.
- Bashir, K.A., J.A. Bawa and I. Mohammed, 2014. Efficacy of leaf extract of drumstick tree (*Moringa oleifera* L.) on the growth of local tomato (*Lycopersicon esculentum*) Journal of Pharmacy and Biological Sciences, 9(4): 74-79.
- Beckman, K.B. and Ames, B.N.(1998). Physiol. Rev., 78, No. 2, 547-581.
- Bentea, M., L.A. Marghitas, and A.Sara, (2011). The effect of some additives on bio productive performances of silkworm *Bombyx mori* L., Animal Science, and Biotechnologies, 44(1): 9-12.
- Bohidar, K., Sahoo, B.S. and Singh, D.K. (2007).Effect of different varieties of mulberry leaves on economic parameters of the silkworm *Bombyx mori L.* under Orissa climate. *Bull. Ind. Acad. Seric.*, 11: 60-64.
- Bradford, M.M.(1976). A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal.Biochem., 72:248-254.
- Buonocore, V., Poerio, E., Silano, V. and Tomasi, M. (1976): Physical and catalytic properties of alpha-amylase from *Tenebriomolitor* L. larvae. *Biochem. J.*, 153: 621-625.
- Christopher MS, Mathavan S. (1985): Regulation of digestive enzyme activity in the larva of Catopsilia crocale (Lepidoptera). J Insect Physiol. 31:217-21.
- Daone, W.W., Abraham, I., Kolar, M.M., Martenson, R.E. and Deibler, G.E. (1975): Purified Drosophila amylase isoenzyme. In: *IsoenzymeIV*(ed.C.L Martet), Academ.ic Press Inc., New York. pp. 585-607.
- Dash R Mukherjee S and Kundu SC (**2006**). Purification and biochemical characterization of a 70- KDa sericin from tropical tasar silkworm Antheraea mylitta. *Int J Bio macromol* (3);255-258.
- Dawson R. Jr., S. Liu, B. Eppler, and T. Patterson (1999). *Mech. Ageing Dev.*, 107, No. 1, 73-91.
- Divi SM, Bellamkonda R. and Dasireddy SK (2012). Evaluation of antidiabetic and anti hyperlipedemic potential of aqueous extract of Moringa oleifera in fructose-fed insulin resistant and STZ induced diabetic wistar rats: a comparative study, Asian J. Pharm. Clin. Res. 5: 67–72.
- Ganga, G. and G. Gowri, 1990. Flour diet to the silkworm to optimize cocoon characters. Indian silk,13: 13-14.
- Govindan, R., S.B. Magadum and T.K. Narayanaswami, (1998). Effect of supplementing vitamins B2, B6 and C to Eri-silkworm, Samia Cynthia ricini Boisd on economic traits. Agric. Sci., (1): 80–82.
- Habig, W.H.; M.J.Pabst and W.B.Jakoby(I974). Glutathione S-transferase., the first enzymatic step in mercapturic acid formation. J. Biol. chem., 249:7130-7139.
- Hagar H.H. (2004) The protective effect of taurine against cyclosporine A-induced oxidative stress and hepatotoxicity in rats. Toxicol Lett 151(2):335–343.
- Hammerschmidt, R.; F.Nuckles and J. Kuc (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to Colletotrchum lagenarium. Physiol.Plant Pathol., 20:73-82.
- Horie, Y. and Watanabe, H. (1980): Recent advances in sericulture. *Annu. Rev. Ent.*, 25: 49-71.

- Ishaaya, I. and E.Swirski(1976). Trehalase, invertase and amylase activities in the black scale, Saissetia oleae, and their relation to host adaptability. J.Insect physiol.,16:1025-1029.
- Kanafi, R.R., Ebadi, R., Mirhosseini, SZ., Seidavi, AR., Zolfaghari, M. and Eteban, K. 2007. A review on nutritive effect of mulberry leaves enrichment with vitamins on economic traits and biological parameters of silkworm, Bombyx mori L. Indian Sericulture Journal, 4:86 - 91.
- Khyade Vitthalrao and Doshi Sucheta, S. 2012. Protein contents and activity of enzymes in the mod gut homogenate of fifth instar larvae of silkworm, Bombyx mori (L) (Race: PM x CSR2) fed with herbal drug(Kho-go) treated mulberry leaves. Res. J. Recent Sci. 1(2): 49-55.
- Klichko VI, Radyuk SN, and Orr WC. (2004). Profiling catalase gene expression in Drosophila melanogaster during development and aging. Archives of Insect Biochemistry and Physiology 56:34-50.
- Krishnaprasad, K.S., Siddaramaiah, A.L. and Lingaraju, S. (1979). Possible control of muscardine disease of silkworms by using a few plant extracts. Curr. Res., 8(5): 79-80.
- Krishnaswami, S., 1978. New technology of silkworm rearing. Bulletine. No.2 Central Silk Board, India, :1-23
- Lokesh, G. and S. R. Anantha Narayena, (2011). Changes in the protein profile of silkworm Bombyx mori L. (Lepidoptera: Bombycidae) in response to the chemical mutagen .1. J.S.N., 2 (3): 559-563.
- Mahesha, H.B., and Honnaiah, S. (2002). Amylase and succinate dehydrogenase activity levels in F1 progeny raised from ethyl methanesulfonate treated silkworm, Bombyx mori L. Indian J. Seric. 41 (1): 24-28.
- Mahmoud, Souad M. (1988). Activation of Silk Secretion by Silkworms, Philosamia ricini and Bombyx mori L. After applying antibiotics. Ph.D. Thesis, Faculty of Agriculture, Cairo Univ.
- Manoharan, T., (1997). A study of hydrolyzed soybean protein. Supplementation on the reproductive system of mulberry silkworm, Bombyx mori L. Ph.D. Thesis Jamal Mohamed College, Bharathidasan University, Tiruchirappalli, India.
- Murugan K, Jeyabalan D, Senthikumar N, Senthilnathan S and Siaprakasham N (1998). Growth promoting effect of plant products on silkworm - A Biotechnological Approach. Journal of Scientific and Industrial Research. 57: 740-745.
- Murugan, K., and George, A. (Sr.). 1992. Feedings and nutritional influence on growth and reproduction of Daphnia near (L). Insect Physiol. 38, 961-969.
- Park YI and Jo TH (2006). Perspective of Industrial Application of Aloe vera. In: New perspective on Aloe (Eds.) Park, Y.I. and S.K. Lee. Springer Verlag, New York .: 109 - 200.

- Peri-Mataruga V, Blagojevi D, Spasi MB, Ivanovi J, Jankovi-Hladni M. (1997). Effect of the host plant on the antioxidative defense in the midgut of Lymantria dispar L. caterpillars of different population origins. Journal of Insect Physiology 43: 101–106.
- Quader, M.A., M.A. Qaiyyum, A. A.Sarker, M.A. Rab, and S.U. Ahmed, 1992. Varietal response to NPK Fertilizers in combination with foliar spray of urea on leaf yield and leaf quality of Mulberry. Bulletin of sericulture Research, 3:64-66.
- Quraiza, M.T.F., C.R.Rathic, P.T.Muthu, S.M. Das, and M.R.Bai,(2008). A study on the effect of ascorbic acid in modifying tissue protein content and economic traits of Bombyx mori L. Journal of Basic and Applied Biology; 2(4): 32-35.
- Rajasekaragouda R, Gopalan M, Jeyaraj and Natarajan N (1997). Field performance of plant extracts on mulberry silkworm, Bombyx mori L. Entomon., 22 (3 &4): 235 - 238.
- Rajeswary K, and Isaiarasu L, (2004): Influence of leaf, flower and pod extracts of Moringa oleifera on the growth and reproductive parameters of Bombyx mori L. Entomon 29 (4):331-338.
- Sarker. A. 1993. Effects of feeding different races of silkworm (Bombyx mori .L) with leaves varying in ploidy level. Sericologia, 33 (1): 25-34.
- Sharan SK, Mishra PK, Kumar D, Mukherjee SN, Sen A, Roy GC, Suryanarayana N. (2005). Studies on the interaction and performance of Antheraea mylitta Drury on its host plants with special reference to Shorea robusta Roxburhii. Central Tasar Research and Training Institute Annual Report: 55-59.
- Subburathinam, K. M. and Krishnan, M. (1998). Role of soyabean protein in silkworm nutrition, Indian Silk,37:9 - 10.
- Subburathinam, K. M., V.Kabila and J. Sulochanachetty, 1990. Mineral spray can increase cocoon quality. Indian silk, 28 (12):35-36.
- Sujatha, K. and Rao, A.P. (2003). Effect of Curcuma longa (turmeric) on the commercial characters of multivoltine silkworm, Bombyx mori L. J. Exptl. Zool., 6 (1): 109-111
- Sundarraj, S., Nangia, N., Chinnaswamy, K.P. and Sannappa, B. (2000). Influence of protein supplement on performance of PMxNB4D2 silkworm breed. *Mysore* Journal of Agricultural Science, 34: 302 - 307.
- Umakanth R.S. and Devamani (2016). Quantitative Estimation Of Amylase Activity In Larva Mutants Of The Silkworm, Bombyx mori L.
- Verma, A.N. and A.S. Atwal, 1968. Effect of temperature and food on the development and silk production of Bombyx mori L. Beitr. Entomol., 18(1-2): 249-258.

تحسين صناعة الحرير بدراسة تأثير مستخلصات مائية متعدة لتعزيز كفائة إنتاجية دودة الحرير التوتية Bombyx mori L. مروى نبيل مصطفى

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تمثل أوراق التوت قيمة غذائية عالية ليرقات دودة الحرير التوتية L. وBombyx mori L. ولذلك إضافة بعض المستخلصات المائية النباتية و الحيوانية التي تحتوي علي خصائص مضادة للأكسدة و أيضا أحماض أمينية تعمل علي تحسين صناعة الحرير . الهدف من هذا البحث هو دراسة تأثير تركيزات مختلفة لبعض المستخلصات المائية من (نبات الصبار المورينجا , التوريين) علي الخصائص البيولوجية و البيوكيميائية وأيضا خصائص الشرائق. حيث وجد أن البرقات التي تمت يغيا بأوراق (نبات الصبار المورينجا , التوريين) علي الخصائص البيولوجية و البيوكيميائية وأيضا خصائص الشرائق. حيث وجد أن البرقات التي تمت تغذيتها بأوراق التوت المضاف إليها (نبات الصبار الموريين الصبار سجلت أعلي النتائج في كل الخصائص موضع الدراسة وكانت الزيادة معنوية عن بقي المعاملات التوت المضاف إليها التوريين (الجرعة الموصي بها أظهرت زيادة في نشاط كل الإنزيمات موضع الدراسة بعد الصبار إلا أنها مترثر علي الخصائص البيولوجية و الشرائق . أيضا البرقات التي تمت تغذيتها بأميرت زيادة في نشاط كل الإنزيمات موضع الدراسة بعد الصبار إلا أنها مترثر الشرائق . أيضا البرقات التي تمت تغذيتها بأوراق التوت المضاف البيها ٢٪ من مستخلص المورينجا أظهرت زيادة في ألم و النه التربية . إن المساد البيولوجية و خصائص البيولوجية و منات من الزير وعات وصب الدراسة بعد الصبار إلا أنها مترثر علي الخصائص البيولوجية وخصائص الشرائق . أيضا البرقات التي تعذينها بأوراق التوت المضاف اليها ٢٪ من مستخلص المورينجا أظهرت زيادة في الموريين الحراب المائين في حمائص البيولوجية وخصائص النه التي أيضا البرقات التي تعذينها بأوراق التوت المضاف اليها ٢٪ من منتقل المورينجا أظهرت زيادة في الحصائص البيولوجية والتي في في الخصائص البيوكيميائية أظهرت زيادة في نشاط بعض الإنزيمات بعد الصبار والتوريين . ونستنتج من النتائج السابقة مستخلص الصبار ذو التركيز ٢,٥٪ هو الأكثر فعالية وتأثيرً على كل الخصائص موضع الدر اسة .