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### Estimation of the Chitin Deposition in the Integument of the Mulberry Silkworm, *Bombyx mori* Larvae by using the Acetone Extracts of Moringa, Grape and Mulberry and its effect on Technological Characteristics



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#### ABSTRACT

Effect of acetone extracts of grape branches, grape leaves, moringa and mulberry leaves on chitin deposition in mulberry silkworm larvae were studied. As well as the effect of those extracts on the characteristics of cocoons and silk threads were studied. Two concentrations were used (20 and 40%) of the extracts and sprayed on the mulberry leaves offered to larvae at the second day of fifth larval instar. Moringa leaves extract caused the most significant reduction in chitin deposition, especially after 120 h. While grape leaves extract gave increase in chitin deposition at all times in both tested concentration. In addition, the grape sticks extract gave the same effect in concentration 20% but, chitin reduction noticed in concentration 40% after 120 h. The results indicated that applying acetone extract of grape branches and moringa leaves at 20 % and grape leaf extract at 40 % to mulberry leaves recorded the highest fresh cocoon weight and cocoon shell weight. Grape leaves extract at 20 % and grape branches extract at 40 % increased the silk ratio. The highest length of silk threads obtained from silkworm larvae fed mulberry leaves treated with grape branches at 20 and 40 %. The acetone extracts of grape leaves, grape branches and mulberry leaves at concentration 20 % increased the silk filament weight and size. Conclusion: using acetone extracts of grape leaves and branches and moringa leaves in terms of juvenoids is a potent tool for improving silkworm performance and cocoon characters, which resulted in the production of high raw silk yield.

**Keywords:** Silkworm, *Bombyx mori*, chitin deposition, grape, moringa, mulberry leaves.

#### INTRODUCTION

Sericulture means the cultivation of mulberry plants and rearing of silkworm larvae for cocoon production. It acts as an effective implement for rural modernization benefiting the economically, socially weaker sectors of the community.

Enhancement in the rearing methods by the use of plant extractives will support the shell percentage of the cocoon. The compounds used for feeding the larval instars through mulberry leaves and for topical application should be safe, cheap and plant derivative. This is because the plant derivative products exert applicable influence in the production of qualitative cocoons and silk filaments. The attempts should be concerned with the use of active principles of plant extractives, which deserve either Juvenile Hormone Analogues (JHA) or Moulting Hormone Analogues (MHA) or both. In insects, steroid hormones play pivotal roles in the coordinated regulation of many developmental and physiological events, such as molting, metamorphosis, and diapause (Thummel 2001, Fielenbach and Antebi 2008 and Niwa and Niwa 2014). The juvenile hormone (JH) and juvenile hormone analogs or juvenoids are well known to prolong the larval life; improve the physiological status of larval body of insects, exhibit potent activity through massive turnover, alteration of constituency of metabolites like proteins, lipids, carbohydrates, amino acids, fatty acids & chitin too and therefore, they have been tried for qualitative improvement of silk (Khyade 2004). The plants on earth are the richest source of metabolites

including juvenile hormone analogs for leaf-eating insects like a silkworm, *Bombyx mori* (Khyade and Slama 2015).

Mulberry (*Morus* spp.) is used in silkworm (*B. mori*) rearing and cocoon production for silk industries. The ethanol extracts of mulberry leaves showed the presence of flavonoids, tannins, terpenoids, saponins, and alkaloids in a significant amount (Toyinbo *et al.*, 2012; Yadav and Agarwala 2011 and Ramos *et al.*, 2016).

*Moringa oleifera* (Moringa) has a notable range of medicinal uses and high nutritional value. Different parts of this plant contain a profile of important minerals, proteins, vitamins,  $\beta$  carotene, amino acids and various phenolic and has a remarkable effect of food, medication and industrial determinations (Khalafalla *et al.*, 2010; Adebayo *et al.*, 2011 and Moyo *et al.*, 2011).

The chemical study of the grape, *Vitis vinifera* branches extract led to the isolation of resveratrol,  $\alpha$ -viniferin, balanocarpol, and  $\beta$ -glucopyranosyl 8- balanocarpol, while, the ethanolic extract from leaves, only resveratrol was quantified (Felicio *et al.*, 2001 and Mansour *et al.*, 2013). Flavonoids, vitamins, organic acids, lipids, carbohydrates, enzymes, and terpenes have been isolated from leaves of *V. vinifera* (Gil *et al.*, 2012). Therefore, this work aimed to study the influence of some important plants extract on chitin deposition in the integument of the fifth instar larvae of the silkworm, *B. mori* and its reflection on cocoon production and silk quantity.

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## MATERIALS AND METHODS

This work was carried out in laboratory of Sericulture Research Department of Sharkia Branch, Plant Protection Research Institute, Agriculture Research Center, Egypt.

**Disinfection of silkworm rearing room:** Rearing rooms, boxes and tools were thoroughly washed, treated with formalin (37% formaldehyde) as (3% concentration) and left to dry before using. The whole set of boxes were kept clean and the paper sheets covered the bottom of the boxes were changed frequently.

### Collection and preparation of plant extracts:

The plant under investigation was originally collected from Zagazieg city, Sharkia province. Mulberry leaves (*M. alba*), grape branches and leaves (*V. vinifera*) and moringa leaves (*M. oleifera*) were collected and washed with tap water, then the leaves were air dried. About (250 g) plant material dried at room temperature for five days then transferred to drying oven at 35 °C until weight stabilized. The dried plant materials were crushed separately into powder and were soaked in acetone 1:2 (w/v) at room temperature for three days with daily agitation. The pooled extracts were filtered and evaporated by rotary evaporator at 50°C until dryness to yield a greenish resinous residue (Su and Horvat, 1981). These residues used to prepare test concentrations (20 and 40%) of crude extracts as a percentage (w/v)

### Insect culture and rearing conditions

Eggs of the silkworm, Bulgarian hybrid (H1 x KK x G2 x V2) were obtained from the Sericulture Research Department, Plant Protection Research Institute (PPRI), Agricultural Research Center, Giza, Egypt and maintained in rearing room of silkworm under laboratory conditions (28 ± 2 °C and 70 ± 5% RH) according to the technique of Krishnaswami (1978). The newly hatched larvae were fed on fresh clean mulberry leaves *M. alba* var. Balady (native) until the end of fifth larval instar, which is the stage used in our bioassays.

**Schedule of application:** Clean mulberry leaves were sprayed with plant extracts by using small atomizers and shade dried then fed to the silkworms during the 2nd day of the fifth larval instar and continued feeding it on untreated mulberry leaves until the formation of cocoons.

The larval bed was cleaned daily using cleaning nets for removing the remained dried food and feces. Chicken egg cartons plates were used as montages for cocoon spinning (Zannoon and Shadia 1994). Once the cocoon formation was completed, the larvae developed to pupae inside the cocoons. Five fresh cocoons were taken randomly from each group to study cocoon characteristics such as cocoon weight, cocoon shell weight, and cocoon silk ratio. The cocoon silk ratio was calculated according to Tanaka (1964) formula:

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

For technological measurements, five cocoons of each concentration were dried in an oven at 60 °C for 8 h to be reeled individually. The length of reeled silk filament was measured and weighed for each cocoon. The size of the reeled silk filament (denier) was estimated according to Tanaka (1964) formula:

$$\text{Filament silk Size (dn)} = \frac{\text{Weight of silk filament (g)} \times 9000}{\text{Length of filament (m)}}$$

### Estimation of The chitin content and chitin deposited in body wall

To detect juvenoid activities extracts of tested chitin estimation was proceeded volumetric according to (Khyade, et al., 2006). Newly molted fifth instar larvae were divided into groups contained 100 larvae each two groups fed during the 2nd day of its 5th instar on each plant extract at two concentrations 20 and 40 % beside one group fed on leaves sprayed with acetone as positive control and another fed on leaves sprayed with distilled water as negative control. Three replications were made for each concentration containing fifty larvae each.

Twenty larvae from each group were selected randomly at zero time of treatments and after 48; 72; 96 and 120 hours and anesthetized with chloroform soaked cotton pad then were dissected in saline. The abdominal fat bodies and visceral organs were removed carefully followed by removing trachea and adhering fat bodies the part remained was designated as integument. The integument of each larva was blotted and weighed on electronic balance. The integument piece of individual larva was placed in separate test tube with 50 ml of 30 % potassium hydroxide (KOH) solution.

All the test tubes were placed in a water bath to boiling for thirty minutes. The KOH-treated integuments were subsequently washed in distilled water twice, then in 96% ethanol twice, finally washed with ether. The residual pieces of integument were weighed accurately. Weights have corresponded to the chitin contents (mg/g) of body weight. Subtraction of initial quantity from final quantity give the quantity of chitin deposited in body wall of the fifth instar larvae for 48-120 h. (mg/g).

### Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA); means were compared using Duncan's test ( $\leq 0.05$ ) according to Snedecor and Cochran (1982) using Costat V.6.311 (2005) Software.

## RESULTS AND DISCUSSION

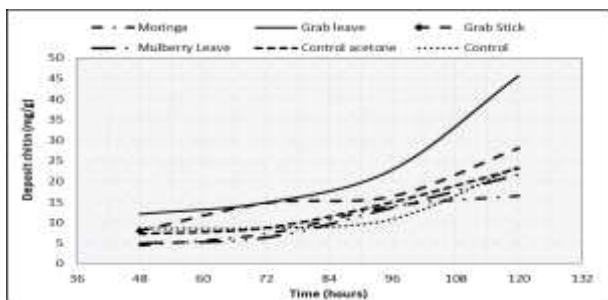
### Estimation of the chitin content and chitin deposited in body wall:

Moringa leaves extract resulted in the most significant reduction in chitin deposition especially after 120 h resulting in 16.43 and 13.92 mg/g with 20 and 40 % extract, respectively Table (1) and Figs. (1 and 2).

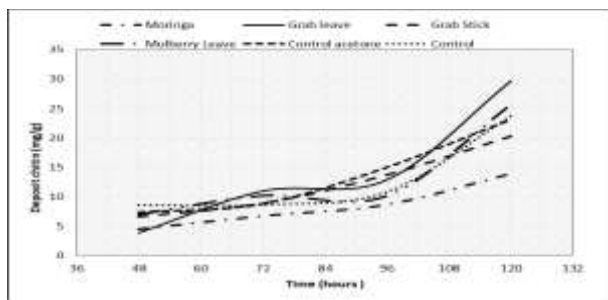
**Table1. Juvenoid activity of acetone extracts of moringa leaves, grape leaves & branches and mulberry leaves on changes in integument deposit chitin.**

	Conc. %	Body wall deposit Chitin (mg/g.)			
		48h	72h	96h	120h
Moringa leaves	20	4.41	7.22	13.94	16.43 <sub>cd</sub>
	40	4.43	6.69	8.63	13.9 <sub>d</sub>
Grape leaves	20	12.08	14.83	22.84	45.66 <sub>a</sub>
	40	3.86	10.95	12.86	29.68 <sub>b</sub>
Grape branch	20	7.99	14.73	16.35	28.07 <sub>b</sub>
	40	6.58	8.86	13.71	20.32 <sub>bcd</sub>
Mulberry leaves	20	4.98	6.38	13.72	21.75 <sub>bcd</sub>
	40	6.97	10.17	9.13	25.62 <sub>bc</sub>
Control		7.34	8.80	15.00	23.16 <sub>bcd</sub>
Control acetone		8.60	8.68	10.84	23.69 <sub>bcd</sub>
LSD <sub>0.05</sub>		8.712	8.96	9.70	6.58
P<0.05		ns	ns	ns	<0.0001 ***

\*\*\* denote highly significant differences at 0.001, ns denote no significant differences at 0.001



**Fig. 1. Daily chitin deposit in fifth larval integument (mg/g) using 20 % concentration of acetone plant extracts.**



**Fig. 2. Daily chitin deposit in fifth larval integument (mg/g) using 40 % concentration of acetone plant extracts.**

The grape branches extract gave a slight reduction in chitin deposition at the concentration 40 % after 120 h reached (20.32 mg/g) and at concentration 20 % the chitin deposition was increased recording 28.07 mg/g. after 120 h.

Contrarily, grape leaves extract showed a notable increase at all times intervals and reached 45.66 and 29.68 mg/g deposit chitin in both tested concentration (20 and 40 %) after 120 h.

Nil reduction can be noticed with mulberry leaf extracts in both concentrations. Mulberry extract is similar to control in composition and effects naturally *B. mori* depended on it as the only nutritional source.

Current findings as chitin ( mg/ g) deposited in the integument of the fifth larval instar after 48; 72; 96 and 120 h of the fourth molt were recorded as 7.34, 8.79, 15.00 and 23.16 mg/g, respectively, in untreated control group. While the acetone control group recorded 8.60, 8.68, 10.84 and 23.69 mg/ g of deposit chitin in the tested times intervals, respectively. No significant differences had been detected between the control groups that led to ignoring the effect of acetone in other words; acetone had no effect on chitin deposition. One day feeding with mulberry leaf supported with 20 % grape leaves and branches, mulberry leaves provided to 5<sup>th</sup> instar larvae of silkworm, *B. mori* resulted in changes in chitin deposition in the integument during the last instar. (Khyade, *et al.*, 2012) found nearer finding with *V. vinifera* when the lower concentrations of acetone extractives were exhibited non-significant reduction in the chitin content of larval integument and the significant reduction was found in the group treated with higher conc. acetone extractives. It is well known that *B. mori* and other phytophagous insects derive their juvenoid nutrients through the plant material available for them (Khyade, *et al.*, 2018).

Therefore, the current results proved that moringa leaves have a juvenoid activity on silkworm larvae that may attributed to its terpenes and terpenoid contents, 9.1% of total volatile oil in leaves (Mukunzi *et al.*, 2011 and

Mahmood *et al.*, 2010). It can be said that, the terpenes mimic the actions of natural “Insect Juvenile Hormone”. Moreover, we found that the grapes extract of branches had juvenoid activity at high concentration as previously reported by (Khyade, *et al.*, 2012 and 2015) which may appeared with raising the tested concentration.

Khyade (2004) reported that the rate of chitin deposition during an early age (up to 48 h) seems to be non-significant because of the titer of juvenile hormone in the haemolymph is maintained at a significant detectable level. Thereafter, the juvenile hormone in haemolymph is decreased rapidly, for the reason of acceleration of rate activity of esterase normally.

#### **Cocoons characteristics:**

##### **Fresh cocoon weight:**

Acetone extracts of grape branches and moringa leaves at 20 % and grape leaves at 40 % when added to mulberry leaves recorded the highest fresh cocoon weight 1.63, 1.59 and 1.54 g followed by mulberry leaves extract at 20 % recorded 1.47 g as compared to 1.38 g for control. Larvae of the silkworm when fed mulberry leaves treated with acetone only spin cocoons recorded the least fresh cocoon weight 1.29 g Table (2). There were highly significant differences in fresh cocoon weight among different treatments and used concentrations when mulberry silkworm, *B. mori* fed on mulberry leaves treated with the plant extracts.

##### **Cocoon shell weight:**

The data in Table (2) showed that weights of cocoon shells take the same trend as cocoon weight where the extracts of grape branches and moringa leaves at 20 % and grape leaves extract at 40 % recorded the highest cocoon shell weight 0.34, 0.330 and 0.33 g, respectively. Analysis of data showed that the differences among means of treatments and differences among concentrations were highly significant. Also, acetone treated control recorded the least cocoon shell weight 0.25g as compared to 0.28 g for normal control.

##### **Silk ratio:**

As shown in Table (2) statistical analysis cleared that, the differences in silk ratio among different treatments and used concentrations were highly significant. Grape leaves extract at 20 % gave the highest silk ratio 23.11 % followed by grape branches; mulberry and moringa leaf extracts at concentrations 40 % recording 22.49, 22.32 and 22.08 %, respectively. Acetone treated control and mulberry leaves extract at a concentration of 20 % decreased the silk ratio to 19.36 % and 19.86 % comparing to 20.37 % for normal control.

These results are in partial accordance with the finding of Saad *et al.*, (2019) who said that the highest cocoon weights were recorded for larvae fed on mulberry leaves treated with the extracts of 3, 2 % basil leaves and the cocoon shell weights increased significantly by treatment with 3, 2 % basil leaves and 3, 1 % black cumin seed extracts. In addition, they said all tested concentrations of black cumin seed extract increased the silk ratios of the resulted cocoons. Rudroju *et al.* (2017) found that seed extract of *Trichosanthes cucumerina* recorded maximum cocoon weight, shell weight, silk ratio and the highest filament length. Similar results were reported by Enas and Zannoon (2017) who found that the highest cocoon weights of *B. mori* were recorded for larvae treated with 2.0, 1.0 and

0.25 % of *Cichorium intybus* extract in comparison to control. Also, Sujatha and Sampath (2015) stated that cocoon parameters of silkworm increased significantly by clove oil treatment. Khyade et al. (2015) stated that the topical application of acetone solution of limonene to the fifth instar larvae of silkworm, *B. mori* were enhanced cocoon weight, shell weight and shell ratio.

**Table 2. Effect of acetone extracts of moringa leaves, grape leaves & branches, and mulberry leaves on silkworm cocoon indices.**

Extracts	Conc. %	Fresh cocoon weight (g)	Cocoon Shell weight (g)	Silk ratio %
Moringa leaves	20	1.59 ab	0.33 abc	20.77 cdef
	40	1.39 de	0.31cd	22.08 abc
Grape leaves	20	1.36 efg	0.31 bcd	23.11a
	40	1.54 bc	0.34 ab	21.71 abcd
Grape branch	20	1.63 a	0.34 a	21.02 bcde
	40	1.41 de	0.32 abc	22.49 ab
Mulberry leaves	20	1.47 cd	0.29 de	19.86 ef
	40	1.30 fg	0.29 de	22.32 abc
Normal Control		1.38 ef	0.28 e	20.37 def
Acetone Control		1.29 g	0.25 f	19.36 f
LSD <sub>0.05</sub>		0.0819	0.0250	1.663
P<0.05		<0.0001***	<0.0001***	<0.0004***

\*\*\* denote highly significant differences at 0.001,

Thulasi and Sivaprasad (2013) stated that ascorbic acid yielded significant gains in cocoon weight and raw silk weight and the lemon juice yielded gain in shell weight, shell protein content. Konala et al., (2013) reported that the feeding of silkworm larvae on mulberry leaves fortified with bovine milk led to a noticeable increase of about 8% for cocoon indices.

In the same trend, Saad et al., (2014) found that the weight of fresh cocoon and cocoon shells were significantly increased with glycine supplementation.

**Technological characters:**

**Silk filament length (m):**

The data tabulated in the Table (3) showed that acetone extract of grape branches at 20 % and 40 % manifest the highest length of silk filament recording 1180.16 m and 1126.32 m followed by 1117.44 m and 1113.28 m for extracts of moringa 40 % and grape leaves 20 %, respectively. It was found that moringa 20 % decreased the length of silk filament to 926.40 m as compared to 1037.44 m for normal control. The differences in silk filament length among different treatments and used concentrations were highly significant.

**Table 3. Effect of acetone extracts of moringa leaves, grape leaves & branches, and mulberry leaves on silkworm filament characters.**

Extracts	Conc. %	Silk Filament length (m)	Silk Filament weight (g)	Silk Filament Size (dn.)
Moringa leaves	20	926.40 f	0.18e	1.75 cd
	40	1117.44 ab	0.22 bcd	1.74 cd
Grape leaves	20	1113.28 ab	0.26a	2.07 a
	40	1011.90 e	0.21d	1.83 bcd
Grape branches	20	1126.32 ab	0.24 a	1.95 ab
	40	1180.16 a	0.24 ab	1.8b cd
Mulberry leaves	20	1104.88 bc	0.23 abc	1.91 abc
	40	1094.72 bcd	0.21 d	1.72 d
Normal Control		1037.44 de	0.21 bcd	1.86 bcd
Action Control		1041.68 cde	0.21 cd	1.83 bcd
LSD <sub>0.05</sub>		66.958	0.0228	0.1881
P<0.05		<0.0001***	<0.0001***	0.0149 *

\* denote significant differences at 0.05

\*\*\* denote highly significant differences at 0.001

**Silk filament weight (g):**

Statistical analysis of filament weight data takes the same trend as silk filament length where the differences among means of treatments and concentrations were high significant (Table 2). The results cleared that, the cocoons obtained from silkworm larvae fed on mulberry leaves treated with grape leaves & branches extracts at concentration 20 % manifest the highest silk filament weight and recorded 0.26 and 0.24 g followed by 0.24 and 0.23 g for silk filament reeled from cocoons span from larvae treated by 40 % grape branches and 20 % mulberry leaves extracts.

**Silk Filament Size (dn.):**

As shown in Table (3) it was found that the acetone extracts of grape leaves and grape branches and mulberry leaves at concentration 20 % exhibited the highest filament size recorded 2.07, 1.95 and 1.91 dn., respectively. The difference between means was significant where the mulberry leaves acetone extractive 40 % was recorded the least value of the filament size 1.72 dn.

Similar results were confirmed by Saad et al., (2019) who indicated that the application of 3 % mulberry leaves, sweet basil leaves and black cumin seeds extracts to mulberry silkworm manifest the longest filament length. Enas and Zannoon (2017) found that all tested concentrations of *C. intybus* aqueous extract increased silk filament length. Moreover, Soumya (2011) proved that the two botanicals (*Eucalyptus* sp and *M. olifera*) when dusted at 100 % every day on silkworm enhanced larval weight, cocoon weight, shell weight, pupal weight, shell ratio, silk filament length, denier. Gad (2013) reported that the improvement of mulberry leaves with some concentrations of honeybee leading to an increase in the silk filament characters.

Hassan and Saad (2012) found that mulberry leaves supplemented with wheat oil when feed to fourth larval instar enhanced significantly all biotechnological characters. In the same trend, Saad et al., (2014) found that the economical parameters were enhanced by treatment mulberry silkworm with glycine 0.1 %. Acetone extract of limonene at 48; 54; 60 and 66 h after the 4th molt were exhibited most significant improvement in the silk filament Khyade et al. (2015).

From the previous result, the acetone extract of grape branches and leaves at concentrations 20 and 40 % and moringa leaves extract at concentration 20 % were recorded the highest cocoon weight and shell weight. In addition, silk ratios were increased by application of these plant extracts on silkworm larvae in comparison with control.

This enhancement by adding the extracts of grape leaves and branches may be due to the presence of resveratrol, ε-viniferin and balanocarpol (Felicio et al., 2001 & Bombardelli and Morazzoni, 1995). Resveratrol and its derivative exhibited high antifungal activity and its antioxidant potential may play a role in the inhibition of diseases besides its anti-inflammatory and anticancer properties (Fremont, 2000).

Application of moringa acetone extracts on mulberry silkworm exhibit numerous improvements in cocoon weight, cocoon shell weight with concentration 20% and increased the silk filament length when applied at concentration 40 %. This enhancement may be due to the presence of a considerable amount of vitamin A, β-carotene, vitamin C, vitamin E, thiamine, riboflavin, alkaloids,

niacin, flavonoids, saponins and phenolic acids in *M. oleifera* leaves (Leone et al 2015, Panda et al., 2013, Sahakitpichan et al., 2011, Förster et al., 2015 and Dinkova-Kostova and Kostov, 2012). All these bioactive compounds have pharmacological properties and exhibit various biological properties: antibacterial and anti-HIV replication activity (Kancheva and Kasaikina, 2013; Shih et al., 2011) so its application plays an important role in health promoting and prevention the diseases of silkworm larvae affects the cocoon weight, shell weight, shell ratio, and silk filament length.

Use of juvenoids in the rearing of silkworm larvae are positively reflected into the retention of larval features long enough enabling the larvae to consume maximum quantity of mulberry leaves and to synthesize paramount silk to be used for qualitative improvement of silk cocoon Mamatha, et al., 2008; Khyade, 2004 and Khyade and Sarawade, 2013).

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## تقدير ترسيب الكيتين في جدار الجسم ليرقات دودة الحرير التوتية، *Bombyx mori* باستخدام مستخلص الأسيون المورينغا والعنب والتوت وتأثيرها على الخصائص التكنولوجية.

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يهدف البحث لدراسة تأثير تغذية يرقات العمر الخامس لدودة الحرير التوتية على أوراق توت معاملة بمستخلص الأسيون لفروع وأوراق العنب وأوراق المورينغا وأوراق التوت من حيث ترسيب الكيتين في جليد اليرقات. وكذلك على الشرائق من حيث وزن الشرنقة الطازجة، ووزن قشرتها ونسبة الحرير فيها وطول خيوط الحرير، ووزن الخيوط، وحجم الخيوط. تغذية يرقات العمر الخامس على أوراق التوت المرشوش بالتركيزين 20 أو 40% من كل مستخلص خلال اليوم الثاني بعد الإنسلاخ الرابع نتج عنه تغيرات في ترسيب الكيتين أثناء العمر اليرقي الأخير. أسفر مستخلص أوراق المورينغا عن أهم انخفاض في ترسيب الكيتين خاصة بعد 120 ساعة من المعاملة. بينما أظهر مستخلص أوراق العنب زيادة ملحوظة في ترسيب الكيتين بجميع القترات الزمنية مع كل تركيز تم اختياره. بالإضافة إلى ذلك أعطى مستخلص الأفرع للعنب نفس التأثير في التركيز المنخفض لكنه أظهر انخفاضاً في ترسيب الكيتين في حالة التركيز العالي بعد 120 ساعة. أشارت النتائج إلى أن إضافة المستخلص الأسيوني لأفرع العنب وأوراق المورينغا بنسبة 20% ومستخلص أوراق العنب بنسبة 40% إلى أوراق التوت سجلت أعلى وزن للشرائق الطازجة ووزن قشرة الشرنقة. مستخلص أوراق العنب بتركيز 20% وفروع العنب بتركيز 40% زيادة نسبة الحرير. فيما يتعلق بصفات الخيط الحريري، فإن الشرائق الحريرية التي تم الحصول عليها من يرقات دودة القز التي عولمت بمستخلص أفرع العنب بتركيز 20 و 40% تعطي أعلى قيم لطول خيوط الحرير، كما أن معظم التركيزات المستخدمة زاد من طول الخيط الحريري. ويمكن التوصل إلى أن مستخلصات الأسيون من أوراق العنب وفروع العنب وأوراق التوت بتركيز 20% زادت من وزن خيوط الحرير وحجمها. مما سبق نجد أن استخدام المستخلص الأسيوني لأوراق العنب وأفرع العنب وأوراق المورينغا من ناحية مشابهات هرمون الشباب هو أداة قوية لتحسين أداء دودة القز وصفات الشرنقة، مما أدى إلى إنتاج عائد أعلى من الحرير الخام.