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Effects of the different levels of Tawa-Tawa (*Euphorbia hirta* L.) extract on the growth and cocoon characters of *Bombyx mori* L.

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Key words: *Euphorbia hirta* extract, Growth, Cocoon characters, *Bombyx mori,* Profitability

Abstract

This study was conducted to determine the effects of the different levels of tawa-tawa (*Euphorbia hirta* L.) extract on the growth and cocoon characters of *Bombyx mori* L. This was conducted at Sericulture Research and Development Institute Sapilang, Bacnotan, La Union, from February- to March 2019. A total of seven hundred fifty silkworms (4^{th} Instar Larvae) were used in the study. The silkworms were randomly distributed into five treatments replicated three times following the Complete Randomized Design (CRD). Results show that on the weight of a single cocoon, silkworms fed with mulberry leaves sprayed with 30% Tawa-tawa extract (T3) produced the heaviest cocoon. The result ranged from 16.88% to 19.49% regarding the cocoon shell percentage. Silkworms fed with 30 % tawa-tawa extract (T3) got the lowest cocoon shell percentage of 18.88. Silkworm provided with 10% Tawa-tawa (T1) produced the most extended filament length of 844.23m. On the filament size, the result ranged from 2.54 to 2.63 (denier). The reliability percentage (%) ranged from 81.19% to 83.71%. disease incidence (%), silkworms fed with mulberry leaves with no application of tawa-tawa extract (T_o) had the highest percentage of disease incidence (10.67%), while silkworms fed with 100% tawa-tawa extract (T4) had the lowest disease incidence (4.0%). For Profitability, Treatment 3 (T3) produced the highest or heaviest cocoon yield of 31.71kgs. And a return on investment (ROI) of 79.90%. In conclusion, Silkworm fed with mulberry leaves containing 3% tawa-tawa extract (T_3) produced the heaviest cocoon yield, with an ROI of 79.90% and the lowest cost per unit.

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Introduction

Mulberry (*Morus* sp.) is the only food plant and plays a vital role in the silkworm's growth and development and subsequently in silk's production and productivity. The quality and quantity of leaves influence the growth and development of silkworms, the production of cocoons, and the quality of the raw silk produced.

Indeed, nearly 70% of the silk proteins produced by the mulberry silkworm come from the proteins of the mulberry leaves. Therefore, the silkworm should feed on good quality mulberry leaves of optimal quality for successful cocoon production (Bhaskar *et al.*, 2008). Few plant extracts exhibit the presence of specific growth stimulants and can be used to increase the silk yield in commercial silkworm rearing (Singhk, 2016).

Natural silk is formed from the silkworm, which is used for many purposes. Silk thread has various uses, including parachute making, tire lining, electrical insulation, artificial blood vessels, and surgical thread. The oil can be extracted from the pupa of the silkworm, amino acids, and vitamin B2 and can be used in the pharmaceutical industry. Phytol, an important raw material for producing vitamin E and K and color, can be extracted from the feces of the silkworm. The wastes can also be used to make activated carbon sheets and protein-rich acid-resistant plastic, the silkworm feces are good food for fish, pigs, cows, and sheep. It is super organic compost (Mahmoud, 2016).

The silkworm industry plays an essential and active role in developing national income. It stimulates the rural economy and the general development of agriculture, forestry, animal husbandry, secondary occupations, and fishing (Dandin, 2014).

The mulberry silkworm, *B. mori*, produces silk, which is influenced by many pathogens, including fungi, bacteria, viruses, and protozoa that damage cocoons up to 75% (Saad *et al.*, 2019). Misfortunes happen amid the final stages of silkworm farming that produce impressive vitality and cash losses.

Plant biodiversity provides a potential source of antimicrobial agents (Bushra and Ganga, 2003), which can be used as antiviral, antibacterial, and antifungal (Pereira *et al.*, 2004).

Silkworm diseases such as viral, bacterial, fungal, and protozoal pathogens affect their cocoon production. Bacterial and fungal pathogens independently cause the most significant cocoon loss, so using botanicals is considered an important strategy to control silkworm diseases (Saad *et al.*, 2019).

The present study was carried out to evaluate the impact of *E. hirta* L. (Tawa-Tawa) on the growth of silkworms as well as its cocoon characters. The study will contribute knowledge on the possibility of using botanical extracts like Tawa-Tawa as a sustainable method to improve silkworm cocoon production. Further, this study will provide baseline data on developing plant-based supplements for silkworms.

This study was conducted to determine the effects of the different levels of Tawa-Tawa (*E. hirta* L.) extract on the growth and cocoon characters of *Bombyx mori* L.

Materials and methods

Theoretical framework

Bombyx mori, or silkworm, is a poikilothermic insect that is the primary source of silk production (Tahmathulla *et al.*, 2004). Silkworms can only eat mulberry leaves on a commercial scale. The nutritional level of the leaf, which differs among mulberry kinds, has a significant impact on the larvae's growth and development, as well as the final economic features like cocoon output, shell weight, and silk %. In terms of cocoon and silk production, understanding the nutritional requirements of the silkworm is critical. Temperature, humidity, light, air, feed quality, and amount all impact its growth and development (Tahmathulla *et al.*, 2004). The amount of food eaten and digested by silkworms directly affected their performance, mating success, and reproduction (Ruth *et al.*, 2019). The digestibility and metabolic activity of larvae are affected by dietary deficiencies or a nutritionally imbalanced diet (Ruth *et al.*, 2019). Therefore, mulberry feeding trials with different levels of Tawa-tawa (*Euphorbia hirta* L.) extract are conducted to select and recommend the suitable levels of the section that would yield the maximum efficiency and profitability. Silkworm races' rearing performance is measured in larval weight and improved economic features such as cocoon weight, shell weight, and silk percentage (Gangawar, 2010; Seidavi, 2011 cited in Ruth *et al.*, 2019). The importance of the cocoon and the significance of the shell are the most critical factors in determining productivity (Gaviria *et al.*, 2006, cited in Ruth *et al.*, 2019).

Research design

Seven hundred fifty silkworms (4th instar larvae) were used in the study. The silkworms were randomly distributed into five treatments replicated three times following the Complete Randomized Design (CRD). There were fifty silkworms per experimental unit. The treatments used were the as follows:

T_0	No Application
T_1	1.0 % Tawa-tawa extract
T_2	2.0 %Tawa-tawa extract
T_3	3.0% Tawa-tawa extract
T_4	100% Tawa-tawa extract

Materials and procedure

Ten days before rearing, the rearing house, rearing implements/materials, and surroundings were thoroughly cleaned. Five days before rearing, all implements/materials were placed inside the rearing house and were sprayed with hypochlorite solution (35g hypochlorite) dissolved in 4 liters of water. Two days before rearing, windows and ventilators were opened in the rearing house.

Young silkworms were fed with young, tender, and gloss freshly picked mulberry leaves. Plucking method is used to harvest from the top of the plant early in the morning and late in the afternoon.

Grown silkworms (4th – 5th instar larvae) were given mature and freshly picked leaves from 50-69 days old shoot or branch.

Feeding was done daily at 5 am, 10 am, 3 pm, and 6 pm. The researcher sprayed the Tawa-tawa extract while feeding the grown silkworm.

Preparation of tawa-tawa leaf extract

The fresh tawa-tawa plants (*E. hirta* L.) were gathered around the surrounding Sericulture Research and Development Institute. The roots were cut and washed with tap water and allowed to stand for 10 minutes to remove the remaining water.

The researchers cut a kilogram of fresh tawa-Tawa (*E. hirta* L.) plants into pieces and blended them using an electric blender**.** After agreeing, the mixture was squeezed using a fine cloth. The liquid extract was placed in a sterilized bottle and stored in a refrigerator.

To produce the mixture used in spraying the mulberry leaves, the desired extract of tawa-tawa was added with the different levels of water. To achieve ultra-sensitivity, pure water is essential. It provides more control over the type of dissolved substances and the solute's exact concentration, leading to higher experimental reproducibility. The mixture was sprayed with the corresponding levels to the mulberry leaves from the 4th instar (2nd day) larvae up to the 5th instar larvae.

Data analysis

The data was recorded, tabulated, computed, and statistically analyzed using One-ANOVA Complete Randomized Design (CRD) for the cocoon characters of *Bombyx mori L.* Significant result was subjected to Tukey's Honest Significant Difference (HSD) Test, and profitability analysis was computed using ROI method.

Results and discussion

Length of matured silkworm (cm)

Table 1 shows the length of matured *B. mori* fed with different levels of tawa-tawa extract. Based on the results of the study, the mean length of silkworm larvae ranges from 5.03 cm- 5.82cm. The enrichment of mulberry leaves with tawa-tawa extracts increased larval weight.

Table 1. Mean length of matured silkworm as affected by different levels of tawa-tawa extract (cm)

Treatment	Mean **
T0 – No Application	5.03 b
T1 -1.0% Tawa-tawa extract	5.71 ab
T2 –2.0% Tawa-tawa extract	5.73 ab
T3- 3.0% Tawa-tawa extract	5.67 ab
T4 -100% Tawa-tawa cextract	5.82 a

Means followed by the same letter are not significantly different from each other at .05 level (HSD)

Analysis of Variance revealed a highly significant result. Comparison among treatment means (HSD) revealed that silkworms fed with mulberry leaves sprayed with 100% Tawa-tawa extract (T4) had produced the longest matured silkworm of 5.82 cm, which were comparable to silkworms fed with 3.0% tawa-tawa extract (T3), 2.0% tawa-tawa extract (T2) and 1.0% tawa-tawa extract (T1) but significantly different to other treatments. The result agrees with the discoveries of Gobena and Bhaskar (2015) that the feeding of leaves fortified with plant extracts led to better larval growth and development which were ultimately reflected in the economic traits of the silkworm.

Table 2. Mean weight of matured silkworm as affected by different levels of tawa-tawa extract (g)

Treatment	Mean **
T0 – No Application	2.13 b
T1 -1.0% Tawa-tawa extract	2.81 a
T2 – 2.0% Tawa-tawa extract	2.85 a
T3- 3.0% Tawa-tawa extract	2.56 a
T4 - 100% Tawa-tawa extract	2.81 a

Means followed by the same letter are not significantly different from each other at .05 level (HSD)

Weight of matured silkworm larvae (g)

Results revealed that silkworms fed with mulberry leaf sprayed with 2.0% tawa-tawa extract (T2) got the heaviest mean weight of 2.85g while silkworms fed without tawa-tawa extract (T0) got the lightest mean weight of 2.13g (Table 2).

Analysis of Variance revealed a highly significant result. The result of the study showed that the addition of tawa-tawa extract had significantly affected the weight of matured silkworm. The result could be attributed with the presence of compounds found in *Euphorbia hirta* like flavanoids, trepenoids, alkanes and amino acids (Kumar *et al.*, 2010).

Comparison among treatment means shows that silkworm fed with mulberry leaves sprayed with tawa-tawa extract (T1, T2, T3, T4) is significantly heavier than those silkworm fed with leaves without tawa-tawa extract (T0).

The result is inconformity with the findings of Murugesh (2002), that there is a significant increase in larval weight when using *P. coryleifolia* and *P. niruri* which could be attributed to a better bioavailability of nutrients for digestion and conversion by this plant extracts, resulting in robust growth of the silk worm. In addition, plant extracts positively influenced the healthy and vigorous growth of silkworms by stimulating worms to feed more than control lots. Many researchers have reported an increase in larval weight following the supplementation of mulberry leaves with aqueous extracts and / or a powder formulation of medicinal botanical plants.

Weight of single cocoon (g)

The cocoon weight is an important commercial feature used to roughly determine the amount of raw silk that can be obtained. The weight of the shell provides a better measure, but cannot be determined in commercial cultures because it requires damage to the cocoon (Gaviria *et al.*, 2006).

Table 3 presents the single cocoon weight as affected by different levels of tawa-tawa extract. Silkworms fed with 3.0% tawa-tawa extract (T3) produced the heaviest cocoon weight (1.64g) while silkworms fed without tawa-tawa extract (T0) produced the lightest cocoon weight (1.39g). Analysis of Variance showed a significant difference which means that the different treatment affected the weight of cocoon. Comparison among treatment means showed that silkworm fed with 3.0% tawa-tawa extract produced the heaviest single cocoon weight with a mean of 1.64g, which were comparable with silkworms fed with 2.0% (T2) and 100% tawa-tawa extract (T4) but significantly different to other treatments. Results agrees with the findings of Rajashekaragouda *et al.* (1997), that the significant improvement of the cocoon traits could be due to the improvement of the appetite of the silkworms which become stronger and more tolerant to diseases, the biochemical components of the plant extracts, the increased nutritional efficiency of the feed that is used by the worms, all the influence of nutrients in the feed supplied which is probably attributed to the stimulating effect of plants on protein synthesis in the silk gland during the larval period.

Table 3. Mean of single cocoon weight as affected by different level of tawa-tawa extract (g)

Treatment	Mean *
T0 – No Application	1.49b
T1 -1.0% Tawa-tawa extract	1.45b
T2 – 2.0% Tawa-tawa extract	1.53ab
T3- 3.0% Tawa-tawaextract	1.64a
T4 - 100% Tawa-tawa extract	1.55ab

Means followed by the same letter are not significantly different from each other at .05 level (HSD)

Previously, it was reported that enrichment of mulberry leaves by some vitamins could increase the cocoon yield. Nirwani and Kaliwal, (1996) have determined that folic acid causes a significant increase in economic parameters such as the weight of the female and male cocoon.

Cocoon shell percentage (%)

The mean cocoon shell percentage (%) as affected by different levels of tawa-tawa extract is presented in Table 4. Silkworms fed with no application (T0) of tawa-tawa extract got the highest (19.49%) cocoon shell percentage, while silkworms fed with 3.0% tawa-tawa extract (T3) got the lowest (16.88%) cocoon shell percentage.

Table 4. Mean cocoon shell percentage as affected by different levels of Tawa-tawa extract (%)

Treatment	Mean *
T0 – No Application	19.49 a
T1 -1.0% Tawa-tawa extract	18.69 ab
T2 – 2.0% Tawa-tawa extract	19.24 a
T3- 3.0% Tawa-tawa extract	16.88 b
T4 - 100% Tawa-tawa extract	18.44 ab

Means followed by the same letter are not significantly different from each other at .05 level (HSD)

Analysis of Variance showed a significant difference. Comparison among treatment means showed that silkworms fed without tawa-tawa extract (T0) got the highest cocoon shell percentage with a mean of 19.49%, which were comparable with 10 ml tawa-tawa extract (T1), 2.0% tawa-tawa extract (T2), and 100% tawa-tawa extract (T4) but significantly different to other treatments. However, silkworms fed with 3.0% tawa-tawa extract (T3) had the lowest cocoon shell percentage of 16.88% which were comparable to silkworms fed with 1.0% tawa-tawa extract (T1), and 100% tawa-tawa extract (T4). Based on the result of the study of Murugesh and Mahalingam reported that *T. terrestis* leaf extract improved the cocoon characters of B. mori.

Filament length (m)

Mulberry leaves have been supplemented with various nutrients for feeding the silkworm to promote the quality and quantity of silk. The integration and fortification of mulberry leaves are a recent technique in sericulture research (Murugan *et. al.*, 1998).

The mean filament length as affected by the different levels of tawa-tawa extract is presented in Table 5. Results of the study, revealed that silkworms fed with mulberry leaves sprayed with 1.0% tawa-tawa extract (T1) produced the longest filament length with a mean of 844.23m while silkworms fed with mulberry leaves without tawa-tawa extract (T0) had the shortest filament length with a mean of 782. 30m.

Analysis of Variance showed significant results. Comparison of treatment means (HSD) showed that silkworms fed with 1.0% tawa-tawa extract (T1) were comparable to those silkworms fed with mulberry leaves sprayed with 2.0% tawa-tawa extract (T2), 3.0% tawa-tawa extract (T3) and 100% tawa-tawa extract (T4), but significantly better compared to those silkworms fed with no application (T0) of tawa-tawa.Similar results were observed by Sujatha (2003) studied the effect of Eucalyptus globules leaf extract (0.1, 1.0, 2.0 and 5.0%) when fed to silkworm along with mulberry leaf. The economic nature such as the length of the filament showed an improvement in the concentration of 15 leaves of eucalyptus leaf extract. Sujatha *et al.*, (2003) reported the effects of the *Azadirachta indica* and Vitex negundo leaf when supplemented with the feeding of the silkworm mulberry. There was a significant improvement in filament length with a 2.0% concentration of Vitex leaf extracts.

Table 5. Mean filament length as affected by different levels of Tawa-tawa extract (m)

Treatment	Mean *
T0 – No Application	782.30 b
T1 -1.0% Tawa-tawa extract	844.23 a
T2 – 2.0% Tawa-tawa extract	819.40 ab
T3- 3.0% Tawa-tawa extract	798.03 ab
T4 - 100% Tawa-tawa extract	840.80 ab

Means followed by the same letter are not significantly different from each other at .05 level (HSD)

Filament size (Denier)

The mean denier size as affected by different levels of tawa-tawa extract is shown in Table 6. The filament size ranges from 2.57- 2. 63 (Denier). Analysis of Variance revealed an insignificant result, which showed that the experimental silkworms as affected by different levels of tawa-tawa extract have not significantly influenced the filament size of the cocoons. The present findings contradicts with the findings of Sridevi, *et al.*, (2004) were conduct an experiment was conducted to investigate the effect of extracts (at 0.1 and 0.5 %) of medicinal plants (*Tagetes erecta, Withania somnifera, Tinospora cordifolia, Leptadenia reticulata, Terminilia arjuna and Adhatoda vasica)* on cocoon and reeling parameters of silkworm, *Bombyx mori* L. (PM x CSR2). The extract treated mulberry leaves fed to silkworm resulted in the improvement of denier, of (PM x CSR2). The treatment with extract of *W. somnifera* at 0.1 percent concentration recorded the minimum denier (2.21) compared to the control. The treatments with the extracts of *Tagetes erecta and Adhatoda vasica* recorded the maximum denier (Sunil and Jaiba, 2016).

Table 6. Mean filament size (denier) as affected by different levels of Tawa-tawa extract

Treatment	Mean
T0 – No Application	2.62
T1 -1.0% Tawa-tawa extract	2.62
T2 – 2.0% Tawa-tawa extract	2.54
T3- 3.0% Tawa-tawa extract	2.63
T4 - 100% Tawa-tawa extract	2.57

Reelability percentage (%)

The mean reelability percentage as affected by the different levels of tawa-tawa extract is presented in Table 7. Results of the study reveals that the reelability percentages ranges from 81.19% - 83.71%. Analysis of Variance reveals insignificant results. This implies that the silkworm fed with mulberry sprayed with tawa-tawa extract were as efficient as those silkworms fed without tawa-tawa extract (T0). The results agrees with the finding of Hiware (2006) shows that silkworm *Bombyx mori* L. larvae were fed on mulberry leaves treated with Nux vomica mother tincture. The impact on denier was investigated. The results were the number of breakages per filament during reeling in both the groups was only two and there was no significant different between treatments.

Table 7. Mean reelability percentage as affected by different levels of Tawa-tawa extract (%)

Treatment	Mean
T0 – No Application	83.71
T1 -1.0% Tawa-tawa extract	83.33
T2 – 2.0% Tawa-tawa extract	83.71
T3- 3.0% Tawa-tawaextract	81.57
T4 - 100% Tawa-tawa extract	81.19

Disease incidence (%)

The mean disease incidence as affected by different levels of tawa-tawa extract is presented in Table 8. Results reveal that silkworm fed with mulberry leaves sprayed with 100% tawa-tawa extract (T4) got the lowest disease incidence of 4.0% while silkworm fed with mulberry leaves without tawa-tawa extract (T0) got the highest disease incidence of 10.67%. Analysis of Variance reveals a highly significant result. The result of the study reveals that the addition of 100% tawa-tawa extract (T4) had significantly lowered the disease incidence in silkworm as manifested by the decrease in the occurrence of diseases during the study.

Table 8. Mean disease incidence as affected by different levels of Tawa-tawa crude extract (%)

Treatment	Mean **
T0 – No Application	10.67a
T1 -1.0% Tawa-tawa extract	6.00b
T2 – 2.0% Tawa-tawa extract	4.67b
T3- 3.0% Tawa-tawaextract	4.67b
T4 - 100% Tawa-tawa extract	4.00b

Means followed by the same letter are not significantly different from each other at .05 level (HSD)

Comparison among treatment means shows that silkworms fed with no application of tawa-tawa extract (T0) had significantly got the highest percentage of disease incidence. On the other hand, silkworms fed with the different experimental levels of tawa-tawa extract had a comparable result. This indicates that the addition of tawa-tawa extract in the mulberry leaves had greatly reduced the disease incidence. The present results are consistent with the findings of Krishnaprasad *et al.* (2001) who reported that the mulberry leaf supplemented with potato leaf extract once during the 3rd and 4th instar and twice during the 5th instar registered a lower larval mortality. Similar results were observed by Subha *et al.* (2005), demonstrating that the effect of medicinal plant extracts on the larval and pupal mortality of *Bombyx mori* L. was conducted in the experiment.

The results revealed that the botanical extract *Psoralea coryleifolia* fed the larvae recorded lower larval and pupal mortality (3.00 and 3.08%) compared to other medicinal botanical extracts.

Profitability analysis

Table 9 shows the result of profitability analysis on the effect of Tawa-Tawa *(Euphorbia hirta* L.*)* extract on the growth and cocoon characters of *Bombyx mori* L. Treatment 3 (T3) produced the highest or heaviest cocoon yield of 31.71kgs., with a total gross income of P11, 098.50, net income amounting to P4,929.25, and a return on investment (ROI) of 79.90%, but registered the lowest break-even cost (BEC) of P194.55. Profitability analysis in the production of cocoons showed that the higher the cocoon produced, the lower the breakeven cost, higher net income would be noticed and higher profit would be attained.

Table 9. Profitability analysis of Tawa-Tawa

Treatment	Total production cost	Unit produced (In kgs)	Breakeven cost	Selling price	Gross income	Net income	ROI (In %)
T0	6,169.25	26.87	229.60	350.00	9,404.50	3,235.25	52.44
T1	6,159.25	28.23	218.18	350.00	9,880.50	3,721.25	60.42
T2	6,169.25	29.58	208.56	350.00	10,353.00	4,183.75	67.82
T3	6,169.25	31.71	194.55	350.00	11,098.50	4,929.25	79.90
T4	6,169.25	29.76	207.30	350.00	10,416.00	4,246.75	68.84

Conclusion

Based on the result of the study, the following conclusions were derived by the researchers:

1. The varying levels of the tawa-tawa extract significantly influence the length and weight of matured silkworm larvae, single cocoon weight, coon shell percentage, filament length, and disease incidence except for the filament size (denier) and reliability percentage (%).
2. Silkworm fed with mulberry leaves containing 3% tawa-tawa extract (T_3) produced the heaviest cocoon yield, with an ROI of 79.90% and the lowest cost per unit.

Recommendations

Based on the result of the study, the researchers recommend that tawa-tawa extract from 1.0% to 100% can be used to increase the growth and cocoon parameters of a silkworm.

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