**International Journal of Zoology and Applied Biosciences** **ISSN: 2455-9571**
**Volume 9, Issue 4, pp: 41-47, 2024**
**https://doi.org/10.55126/ijzab.2024.v09.i04.007** **http://www.ijzab.com**

**Research Article**

# CONNECT THE MULBERRY LEAF QUALITY AND THE SILKWORM COCOON TRAITS THROUGH CORRELATION AND REGRESSION ANALYSIS

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**Article History:** Received 27th May 2024; Accepted 22nd June 2024; Published 17th July 2024

**ABSTRACT**

An experiment was conducted to study the impact on mulberry leaf quality and silkworm growth performance by the application of growth promoter. In the irrigated mulberry field experiment was carried out in Randomized Block Design layout with four replications and five treatments. Result showed that higher quality of mulberry leaves was obtained on Effective Microorganisms (EM) treated plots compared to others because beneficial microorganism in the EM enhanced the plant growth metabolism to increase their growth. As for correlation analysis, all leaf quality traits were highly significantly positive correlated with silkworm cocoon traits. In simple linear regression analysis, the mulberry leaf quality traits were significantly influenced the silkworm cocoon traits except leaf protein with cocoon weight (Adj $R^2$ = - 0.30337) which has negative relationship between them. Highly close relationship was noticed on total carbohydrate content on larval weight (Adj $R^2$ = 0.93655) followed by total chlorophyll content on shell weight (Adj $R^2$ = 0.90569), silk gland weight (Adj $R^2$ = 0.87275), ERR (Adj $R^2$ = 0.84312) and total carbohydrate content on shell weight (Adj $R^2$ = 0.84606). Thus, the total chlorophyll and carbohydrate content might be a good trait and mostly responsible for producing good quality cocoons.

**Keywords:** Correlation coefficient, Growth promoter, Linear regression, Mulberry and Silk gland weight.

## INTRODUCTION

According to Central Silk Board estimates, sericulture employs approximately 9.18 million persons in rural and semi-urban areas in India. In the recent days, silk cocoon production has gained prime importance in Indian agriculture as it provides profitable occupation in rural and semi urban sector. Although it is considered as a subsidiary occupation, technological innovations and technological innovation have made possible to achieve an intensive scale capable of generating adequate and also continuous income to farmers. Mulberry is a fast growing, deep-rooted perennial plant which is cultivated exclusive for its leaves by sericulture farmers (Vijayan *et al*., 2012). The quality of mulberry silk mainly dependent upon the quality of mulberry leaves which influences the larval growth and cocoon quality (Singhal *et al*., 1999; Nagaraju, 2002). So, instantly improve the mulberry leaf quality by usage of growth promoters as foliar spray, thereby enhance the cocoon and silk quality. Hence it is essential to search for growth promoters which has low cost and eco-friendly source for sustainable sericulture. Some of the growth promoter's *viz.*, jeevamirtham, seriboost, poshan, vermiwash, panchagavya, consortium of beneficial microorganisms, dasakavya and amirthakarasial are utilized for increasing crop yield and enhance the silk quality (Geetha and Murugan, 2017). Therefore, it is deemed necessary to test best growth promoter for improving the mulberry leaf quality and to know the influence of mulberry leaf quality on silkworm cocoon parameters. Thus, with a view to finding out possibilities of outlets for improvement of qualitative traits of mulberry and cocoon by different growth promoter and to identify the relationship between mulberry leaf quality and silkworm

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growth performance, the present investigation was undertaken.

## MATERIAL AND METHODS

The study was conducted at pre-established irrigated V1 mulberry garden at Department of Sericulture, Forest College and Research Institute, Mettupalayam (Figure 1). The experiment was laid out in Randomized block Design (RBD) with four replications and five treatments as 1 % Effective Microorganism (EM), 3 % Panchagavya, 0.25 % Seriboost, 2 % Vermiwash and control. Before starting of experiment, the experimental field was initiated with bottom pruning and recommended cultivation practices for irrigated mulberry was followed as suggested by Dandin *et al.* (2003). Here, the experiment field had plot size of 40 sq. m/plot/replication and having a spacing of 90X90 cm were maintained for the experiment. The different treatments were applied thrice at 15days interval as foliar spray using knapsack sprayer during early hours of the day and first spray was initiated on 25 DAP of mulberry plants. Irrigation water applied as weekly twice uniformly in all plots.

### Biochemical constituents of foliage

On 70$^{th}$DAP, leaf samples were collected from each plot for biochemical analysis. Collected leaf samples were oven dried at 70$^{o}$ C for one hour, powdered and used for further analysis. Total chlorophyll content in the fresh leaf sample was estimated by acetone method (Manolopoulou *et al*., 2016). The leaf protein and total carbohydrate were analyzed by FCR method (Xing *et al.*, 2014) and Anthrone method (Sadasivam and Manickam, 2005), respectively. The total nitrogen content was estimated as per procedure outline by Karoojee et *al.,* (2021).

### Silkworm Rearing Performance

Leaves were harvested from all plots after 70 DAP and fed to late age worms of bivoltine double hybrid silkworm (CSR2XCSR27) X (CSR6XCSR26)). For each treatment, 50 larvae per replication with three replications were reared and maintained after II moult. The larvae fed three or four times daily with healthy and fresh mulberry leaves. Ambient temperature and relative humidity of the rearing room was recorded using thermo-hygrometer. Cocoon harvesting was carried out after 5$^{th}$ or 6$^{th}$ day of spinning. The rearing performance of each treatment were assessed by the following traits *viz.*, larval weight, silk gland weight, ERR, cocoon weight, shell weight, shell ratio and filament length. Cocoon quality was assessed.

### Data collection and Statistical analysis

Collected data were recorded in Microsoft excel and analyzed by statistical analysis system. Significant difference between values was determined using One-Way ANOVA by SPSS (Ostertagova *et al*., 2013). A correlation and linear regression analysis were also made to find best fit values and linear curve with biochemical constituents of foliage silkworm rearing performance. Regression graphs were presented with the help of statistical software program ‘ORIGIN PRO 8’

## RESULTS AND DISCUSSION

The present study was aimed to find out the effect of foliar application of growth promoters on mulberry leaf quality and silkworm growth performance. There was a positive beneficial impact on mulberry leaf quality which also reflects on silkworm cocoon parameters are presented hereunder. Foliar application of growth promoters on mulberry plants resulted in better leaf quality compared to control (Table 1). Maximum total chlorophyll content (2.81 mg g$^{-1}$), leaf protein (32.45 mg g$^{-1}$), total carbohydrate (35.38 mg g$^{-1}$), total nitrogen content (4. 53 per cent) and moisture content (77.11 per cent) were observed in EM at 1 % treated mulberry plots followed by panchagavya at 3 % treated plots of 2.49 mg g$^{-1}$, 31.90 mg g$^{-1}$, 35.38 mg g$^{-1}$, 3.81 percent and 75.73 percent, respectively. However, lower leaf quality was recorded in control compared to other treatments. The increase in mulberry leaf quality in EM at 1 % treated mulberry leaves might be due to increased photosynthetic activity on treated mulberry leaves (Das *et al.*, 1994). Growth promoters often act as co-factors in enzyme activation and participate in photosynthesis besides plays an important role in the carbohydrate metabolism (Khan *et al*., 2020). The present results were in conformity with findings of Vinoj (2008) who reported increased soluble protein and total carbohydrate content due to foliar application of EM along with biofertilizers on mulberry. Similar views on positive enhancement of quality parameters of EM treated mulberry leaves have been reported by Gnanaselvi (2007). EM treated leaves had large amount of soluble protein and total carbohydrate content due to synthesis of prochlorophyllidae at the time of chlorophyll formation which reflects the synthesis of carbohydrates on leaves (Jing *et al.*, 2018).

Effect of growth promoters as foliar application on mulberry was witnessed even in the performance of silkworm (Table 2). Higher larval weight (4.07 g), silk gland weight (794.00 mg), ERR (91.13 %), cocoon weight (1.57 g), shell weight (0.367 g), shell ratio (24.45 %) and filament length (1335.81 m) were obtained in the silkworm larval batches fed with EM at 1% treated mulberry leaves compared to control of 3.36 g, 684.30 mg, 81.15 per cent, 1.39 g, 0.286 g, 20.53 and 1199.84 m respectively. Superior leaf quality particularly total chlorophyll and carbohydrate content in treated mulberry leaves which in made the larvae healthy and resulting in increased larval and silk gland weight (Ramarethinam and Krishnachandra, 2007). The present finding similar with filament length (956.38 m) was significantly increased by foliar application of nitrogen fixing bacteria on mulberry (Sudhakar *et al.,* 2000). It was strengthened by the Samuthiravelu *et al.* (2012) who observed the positive effect on single cocoon weight, shell weight and shell ratio due the foliar application of organic promoters on mulberry leaves. Similar trend was also observed by Doss *et al.* (2011) who reported that organic promoters treated mulberry leaves when fed to silkworm larvae resulted in higher larval weight and ERR over control. Present results showed that leaf quality traits were significantly strong correlation with silkworm cocoon parameters (Table 3). Here, total chlorophyll content had significantly positive correlation with all observed silkworm and cocoon traits. These correlation coefficients of larval weight, silk gland weight, ERR, cocoon weight, shell weight, shell ratio and filament length of r = 0.9814*, r = 0.9510*, r = 0.9393*, r = 0.7299*, r = 0.9639*, r = 0.9770*, r = and r = 0.9043*, respectively which indicating a dependency of silkworm growth and cocoon traits on total chlorophyll content of mulberry leaves. Similarly, remaining other leaf quality traits as leaf protein, total carbohydrate, total nitrogen and moisture content had also significantly positive correlation with silkworm growth performance. So, these results represent the silkworm and cocoon traits were highly depends upon mulberry leaf quality. These results are in accordance with those of Waktole Sori and Wosene Gebreselassie (2015) have also opined that good quality of mulberry leaves had greater influence for better production of good quality cocoons. Similarly, Alebiosu *et al.* 2013 reported that the positive correlation coefficients of the cocoon shell weight and cocoon yield with the protein content in mulberry leaves.

**Table 1**. Influence of growth promoters on quality of mulberry leaves.

| Growth promoters | Total Chlorophyll (mg $g^{-1}$) | Leaf protein (mg $g^{-1}$) | Total carbohydrate (mg $g^{-1}$) | Total Nitrogen content (%) | Moisture content (%) |
|---|---|---|---|---|---|
| EM @ 1% | 2.81[a] | 32.45[a] | 40.74[a] | 4.53[a] | 77.11[a] |
| Panchagavya @ 3% | 2.49[b] | 31.90[a] | 35.38[b] | 3.81[b] | 75.73[ab] |
| Seriboost @ 0.25% | 2.11[d] | 29.15[b] | 30.30[c] | 3.72[b] | 73.76[bc] |
| Vermiwash @ 2% | 2.44[c] | 30.41[ab] | 31.61[c] | 3.80[b] | 74.19[bc] |
| Control | 1.73[e] | 29.06[c] | 29.18[c] | 3.01[c] | 72.11[c] |
| SEd | 0.009 | 1.12 | 1.52 | 0.06 | 1.29 |
| CD(P=0.05) | 0.02** | 2.63** | 3.25** | 0.14** | 2.76** |

** Highly significant. Each value is the mean of four replications, Mean followed by same alphabets are on par with each other.

**Table 2.** Impact of growth promoters on silkworm bioassay.

| Growth promoters | Larval weight (g) | Silk gland weight (mg) | ERR (%) | Cocoon weight (g) | Shell weight (g) | Shell ratio (%) | Filament length (m) |
|---|---|---|---|---|---|---|---|
| EM @ 1% | 4.07[a] | 794.00[a] | 91.13[a] | 1.50[b] | 0.393[a] | 24.45[a] | 1335.81[b] |
| Panchagavya @ 3% | 3.71[b] | 786.53[a] | 89.47[a] | 1.57[a] | 0.367[b] | 23.48[a] | 1332.97[a] |
| Seriboost @ 0.25% | 3.55[bc] | 717.90[c] | 85.31[b] | 1.47[b] | 0.315[d] | 21.41[bc] | 1213.03[d] |
| Vermiwash @ 2% | 3.71[b] | 757.90[b] | 85.43[b] | 1.49[b] | 0.342[c] | 22.94[ab] | 1326.65[b] |
| Control | 3.36[c] | 684.3[d] | 81.15[c] | 1.39[c] | 0.286[e] | 20.53[c] | 1199.84[e] |
| SEd | 0.14 | 6.81 | 1.27 | 0.02 | 0.04 | 0.95 | 2.18 |
| CD(P=0.05) | 0.31* | 14.51** | 2.71* | 0.04* | 0.09** | 2.03** | 4.65** |

** Highly significant. Each value is the mean of four replications. Mean followed by same alphabets are on par with each other.

**Table 3**. Correlation coefficients between rearing characters and mulberry leaf quality influenced by growth promoters.

| Attributes | Total chlorophyll | Leaf Protein | Total carbohydrate | Total Nitrogen content | Moisture content |
|---|---|---|---|---|---|
| Larval weight | 0.9184 | 0.7459 | 0.9759 | 0.9561 | 0.2511 |
| Silk gland weight | 0.9510 | 0.9184 | 0.8665 | 0.8330 | 0.6743 |
| ERR | 0.9393 | 0.9510 | 0.7166 | 0.8715 | 0.3530 |
| Cocoon weight | 0.7299 | 0.9393 | 0.5897 | 0.6197 | 0.9072 |
| Shell weight | 0.9639 | 0.7299 | 0.9405 | 0.8996 | 0.5287 |
| Shell ratio | 0.9770 | 0.9639 | 0.9126 | 0.86949 | 0.5515 |

| | | | | | |
|---|---|---|---|---|---|
| Filament length | 0.9043 | 0.9770 | 0.7152 | 0.6616 | 0.6879 |

In this study, simple linear regression equation was estimated with silkworm cocoon traits as dependent variable and mulberry leaf quality as independent variable. There was significant and simple linear relationship between mulberry leaf quality and silkworm cocoon traits as shown in figure 1 – 4. Observed silkworm cocoon traits had a significantly positive linear and positive significant relationship with total chlorophyll content (Figure 1). An adjusted coefficient of determination for larval weight of $R^2$ = 0.7913, silk gland weight of $R^2$ = 0.87275, ERR of $R^2$ = 0. 84312, cocoon weight of $R^2$ = 0.37717, shell weight of $R^2$ = 0.90569 and filament length of $R^2$ = 0.75702. These coefficients were revealed that the variations in total chlorophyll content explained about variation in larval weight of 79 per cent, silk gland weight of 87 per cent, ERR of 84 per cent, cocoon weight of 37 per cent, shell weight of 90 per cent and filament length of 75 per cent. These rates ware represented by the nearest dots to the linear line. The linear regression equations were formed as follows. Larval weight = 2.30 + 0.59 CHL, Silk gland weight = 498.69 + 107.70 CHL, ERR = 65.75 + 8.96 CHL, Cocoon weight = 1.22 + 0.11 CHL, Shell weight = 0.11 + 0.10 CHL, Filament length = 930.47 + 151.63 CHL Where, CHL – Total chlorophyll content; 2.30, 498.69, 65.75, 1.22, 0.11 and 930.47 – Constant; 0.59, 107.70, 65.75, 1.22, 0.11 and 930.47 – regression coefficient (b) indicating the increasing of total chlorophyll content will increase silkworm cocoon traits in these rates.

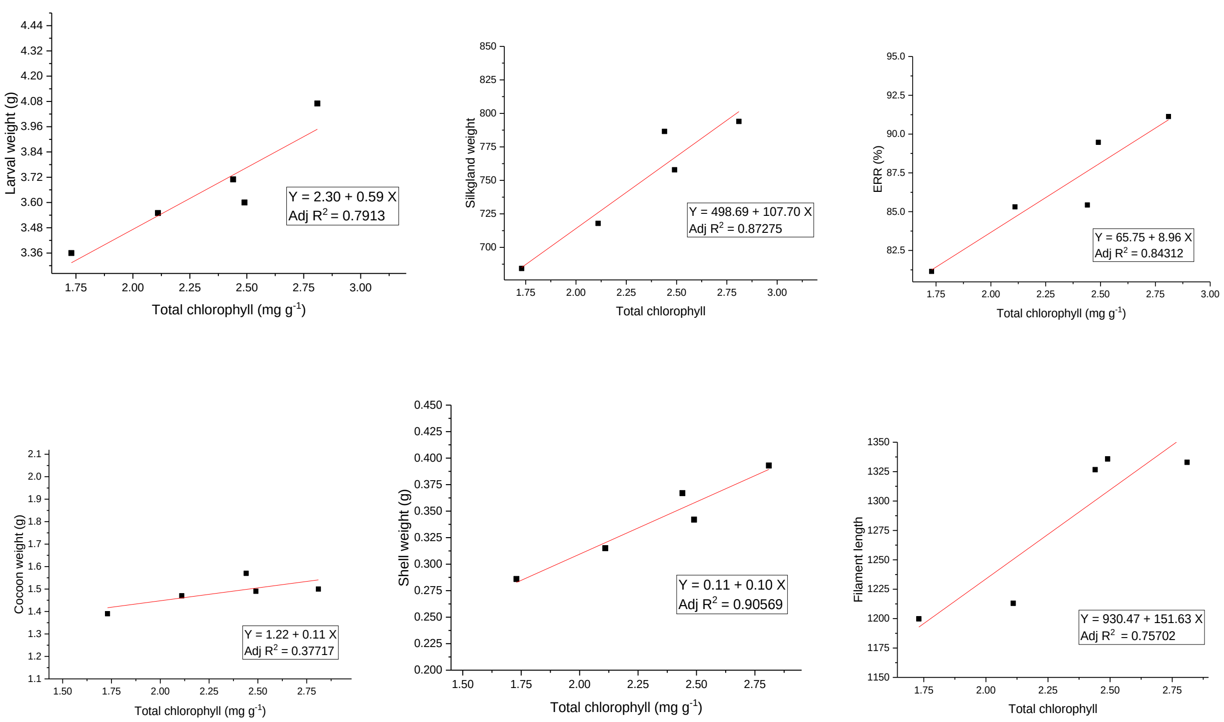



**Figure 1.** Linear regression between mulberry leaf total chlorophyll content and silkworm cocoon traits.

Leaf protein is the main constituent of the mulberry leaf, which contributes main role in the synthesis of silk (Dong et al., 2017). Analysis of simple linear regression between leaf protein and silkworm cocoon traits was significant as shown in figure 2. Leaf protein had a linear and positive linear relationship with silkworm cocoon traits, an adjusted coefficient of determination for larval weight was R2 = 0.273, silk gland weight was R2 = 0.273, ERR was R2 = 0.273, shell weight was R2 = 0.273 and filament length was R2 = 0.273. However, there was a linear and negative linear relationship between leaf protein and cocoon weight that having an adjusted coefficient of determination was – 0.30336. Variation in leaf protein could explain the variation in silkworm cocoon traits as approximately larval weight by 26 per cent, silk gland weight by 0.70 per cent, ERR by 80 per cent, shell weight by 10 per cent and filament length by 8 per cent and these rates are represented by all nearest dots to the linear line. The linear regression equations for variations in leaf protein on silkworm cocoon traits were formed as follows Larval weight = 0.19 + 0.11 LP, Silk gland weight = 284.18 + 15. 16 LP, ERR = 15.14 + 2.33 LP, Cocoon weight = 1.29 + 0.006 LP, Shell weight

= 0.13 + 0.02 LP, Filament length = 521.38 + 24.85 LP, Where, LP – Leaf Protein; 0.19, 284.18, 15.14, 1.29, 0.13 and 521.38 – Constant; 0.11, 15.16, 2.33, 0.006, 0.02 and 24.85 – regression coefficient (b) indicating the increasing of leaf protein will increase silkworm cocoon traits in these rates.

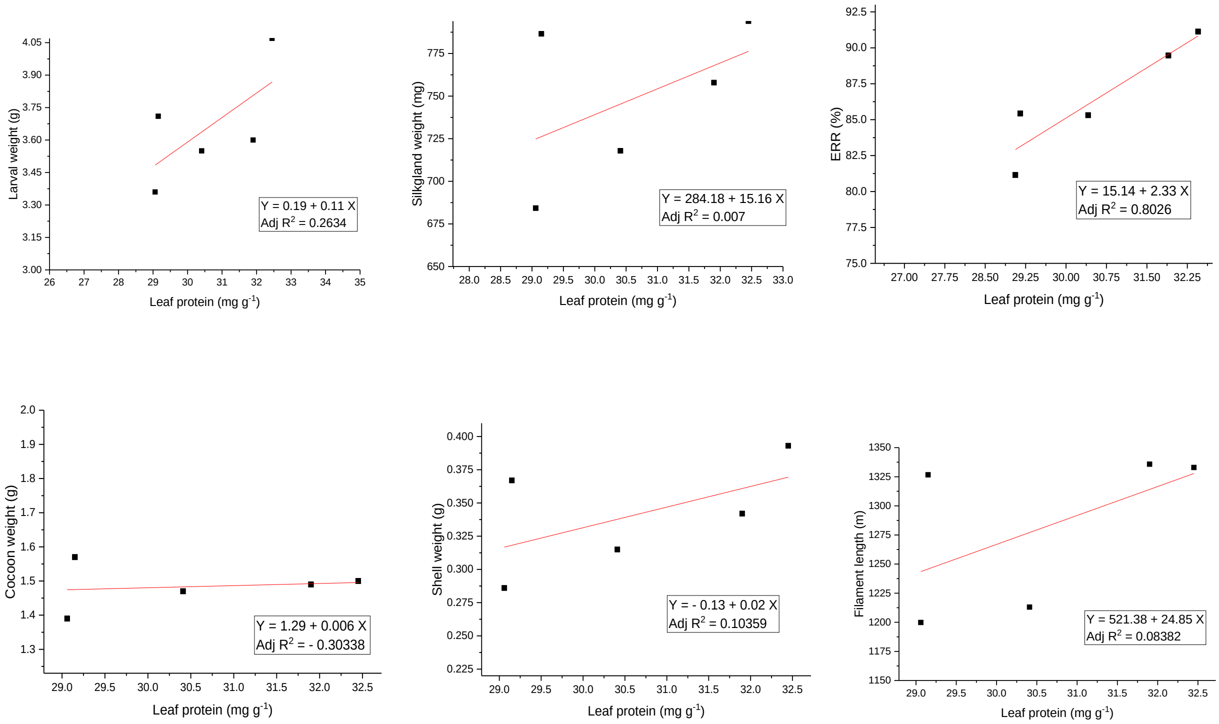
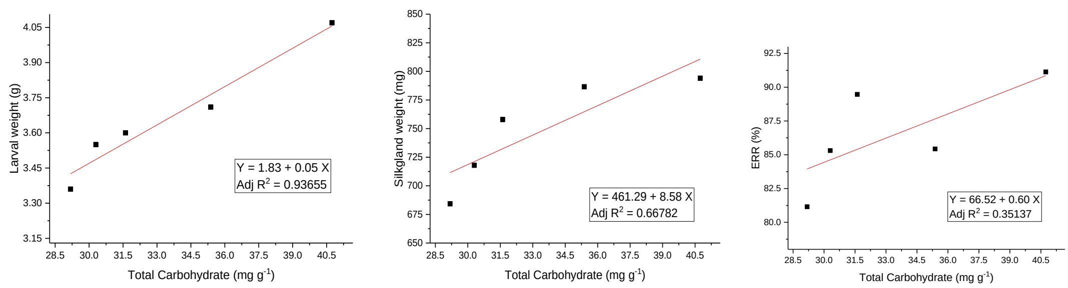



**Figure 2.** Linear regression between mulberry leaf protein and silkworm cocoon traits.

There was a significant linear positive relationship between total carbohydrate content in the mulberry leaves and silkworm cocoon traits as presented in figure 3. An adjusted coefficient of determination for larval weight of $R^2$ = 0.93655, silk gland weight of $R^2$ = 0. 66782, ERR of $R^2$ = 0.35137, cocoon weight of $R^2$ = 0.13045, shell weight of $R^2$ = 0.84606 and filament length of $R^2$ = 0.3487. These coefficients indicated that about 93 per cent, 66 per cent, 35 per cent, 13 percent, 84 per cent and 34 per cent of variation in the total carbohydrate content could be explained by the variation in larval weight, silk gland weight, ERR, cocoon weight, shell weight and filament length respectively that are represented by the nearest dots to the linear regression line in figure 3. The following linear regression equation for relationship between total carbohydrate content and silkworm cocoon traits were formed. Larval weight = 1.83 + 0.05 CHO, Silk gland weight = 461.29 + 8.58 CHO. ERR = 66.52 + 0.60 CHO, Cocoon weight = 1.21 + 0.008 CHO, Shell weight = 0.06 + 0.008 CHO, Filament length = 931.09 + 10.48 CHO, Where, CHO – Total carbohydrate content; 1.83, 461.29, 66.52, 1.21, 0.06 and 931.09 – Constant; 0.05, 8.58, 0.60, 0.008, 0.008 and 10.48 – regression coefficient (b) indicating the increasing of total carbohydrate content will increase silkworm cocoon traits in these rates.



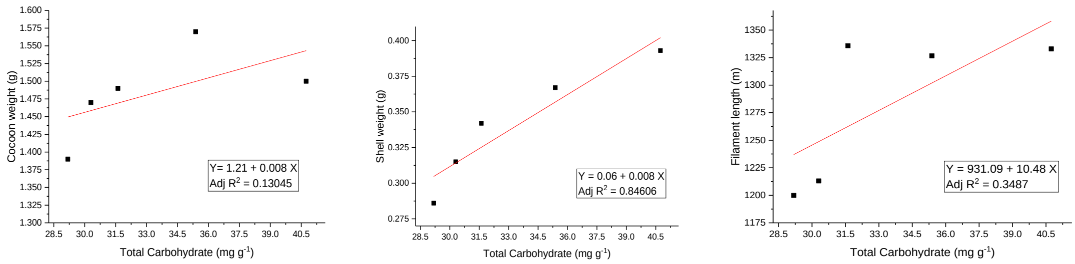



**Figure 3.** Linear regression between mulberry leaf total carbohydrate content and silkworm cocoon traits.

Simple linear regression between total nitrogen content in the mulberry leaves and silkworm cocoon traits was significant with positive adjusted coefficient of determination for larval weight of $R^2$ = 0.88573, silk gland weight of $R^2$ = 0.59203, ERR of $R^2$ = 0.67936, cocoon weight of $R^2$ = 0.17885, shell weight of $R^2$ = 0.74574 and filament length of $R^2$ = 0.2503. Variation in the total nitrogen content explained about variation in the larval weight of 88 percent, silk gland weight of 59 percent, ERR of 67 percent, cocoon weight of 17 percent, shell weight of 74 percent and filament length of 25 percent. These were represented the nearest dots to the linear regression line shown in figure 4. The linear regression equations were formed as follows. Larval weight = 1.90 + 0.47 N, Silk gland weight = 476.45 + 71.99 N, ERR = 62.56 + 6.34 N Cocoon weight = 1.20 + 0.07 N, Shell weight = 0.08 + 0.07 N, Filament length = 962.17 + 84.66 N Where, N – Total Nitrogen content; 1.90, 476.45, 66.56, 1.20, 0.08 and 962.17 – Constant; 0.47, 71.99, 6.34, 0.07, 0.07 and 84.66 – regression coefficient (b) indicating the increasing of total nitrogen content will increase silkworm cocoon traits in these rates.

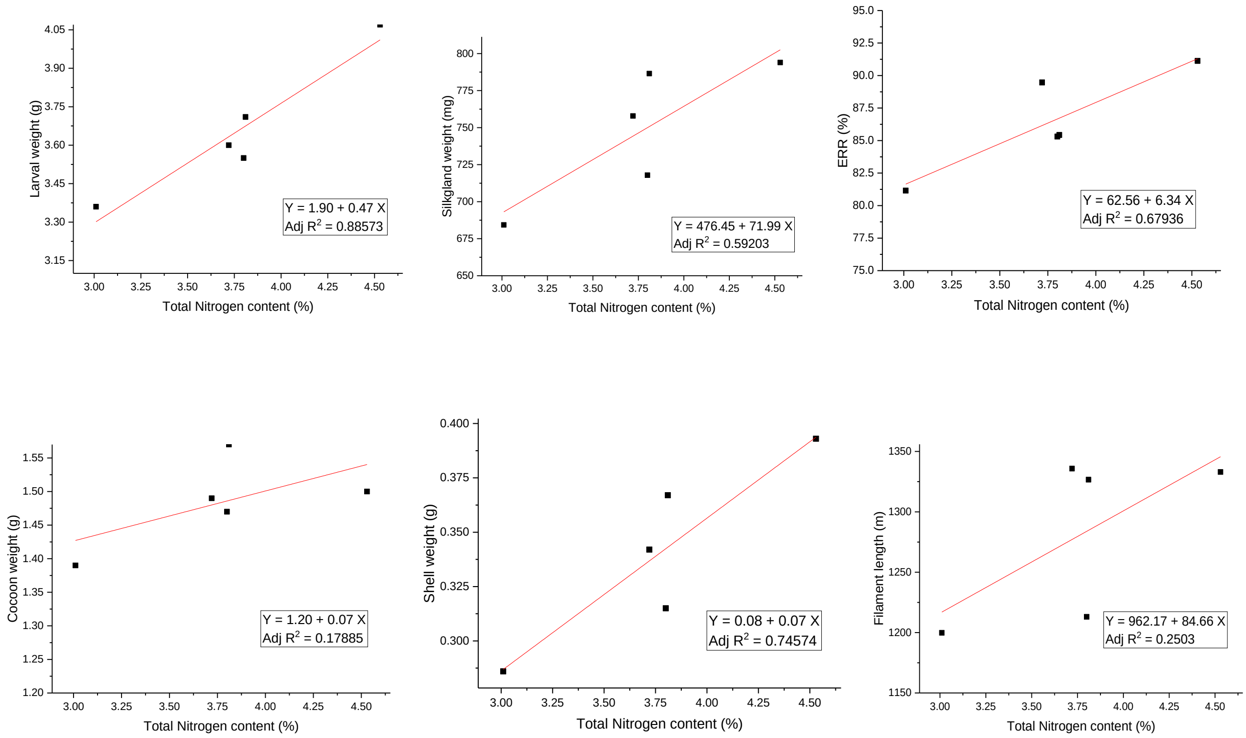



**Figure 4.** Linear regression between mulberry leaf total nitrogen content and silkworm cocoon traits.

## CONCLUSION

Application of growth promoters improves mulberry leaf quality realizing better silkworm growth performance without causing any damage to ecosystem. Among different growth promoters, EM at 1 per cent was found to be better growth promoter in terms biochemical constituents of foliage together with rearing performances This can be more suitable for increasing leaf quality instantly as per of requirement of producing a good quality silk. And, there was significantly strong correlation between leaf quality traits and silkworm rearing performances. From the results of simple linear regression analysis, the most promising traits were total chlorophyll content and total carbohydrate content which was responsible for producing good quality cocoons.

## ACKNOWLEDGMENT

The authors express sincere thanks to the head of the Department of Sericulture, Tamil Nadu Agricultural University, Coimbatore (District), Tamil Nadu for the facilities provided to carry out this research work.

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