Coloured Silk Produced With Neutral Red Containing Diet By Silkworm *Bombyx Mori* L.

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Abstract
Nutritional requirement in food consumption have direct impact on the overall growth of silkworm and also increase the larval, pupae and cocoon weight with amount of silk production. The use of natural dyes for textile fibers has become an alternative to solve issues as environmental pollution and health risks by the larvae fed with neutral red treated mulberry leaves. In the present study the silkworm *Bombyx mori* were fed with the concentration of Neutral red (0.05 and 0.025%) treated mulberry leaves from the 5th instar larvae. Different nutrition of silkworm is sole factor which almost augment quantity and quality of slide fortification of mulberry leaves by nutrient supplementation can be increased the quality most popularity and productivity of silk. This study was to find out the economic parameters such as larval length, larval weight, larval survivability, cocoon weight, shell weight, pupal weight and shell ratio. The observed results were statistically analysed and discussed.

Key words: Dyes, Neutral red, *Bombyx mori*, nutrition, mulberry leaves.

Introduction
The silkworm is a beneficial, eco – friendly arthropteran insect reared commercially and economically for the production of cocoons and silk. Silk is a natural fibre and the fabric production is followed throughout the world. Silkworm silk is a fibrous protein reeled from silkworm cocoons. It is one of the most popular textile fibers used for various applications due to its incredible physical, chemical and mechanical properties. Now a days there are several ways to improve the properties of silk which increases its popularity and demand all the world over. Lingue *et al.*, (2015) reported the colour is an attracting quality of silk, dyeing of silk is unavoidable in the textile industry. However, this dyeing process is a threat to the environment as it affect the water bodies, aquatic population and in turn the human beings as it releases dye waste abundantly in to the environment especially to the water bodies Pongtratic., (2008). The reduction of dye waste pollution by introducing a new technology for the production of colour silk in the silk gland itself before spinning (Anumol Anto *et al.,* 2018). *Anumol et al.,* (2017) reported the change of the larval and cocoon parameters of *B. mori* fed on various vital dyes. Sericulture depends on rearing of silkworms (*Bombyx mori L.*)on mulberry leaves, which is its traditional food.
In this study, our concern is the production of coloured silk using the Neutral red dye and the analysis of larval and cocoon traits to find the effect of the dye on *B. mori* L.

MATERIALS AND METHODS
Experimental animal -*Bombyx mori*
The silkworm, *B. mori* (Lepidoptera) CSR2 strains were used in this study. Eggs of CSR2 obtained from the Grainage centre, Sericulture Department in the Regional Deputy Director's office, Tenkasi, Tamil Nadu.

Silkworm feed
Morus alba is the common mulberry plant and the sole preference of the silkworm larvae. This perennial plant is well suited for the Indian climate. All the essential elements such as protein, carbohydrate, inorganic salts, essential vitamins and minerals, required for the physiological functions of the silkworm are present in it. The V1 mulberry leaves to feed the control animal were collected from the mulberry garden. The control animals fed regularly with mulberry leaves throughout the period. The experimental group fed with the prepared modified diet.

Preparation of dye added diet
The dye selected in this study is basic neutral red (Nice Chemicals), a vital dye. After some trials, the concentration (0.05 and 0.025%) which has no harmful effect on silkworm life cycle was selected. The dye solution sprayed uniformly

on the mulberry leaves and fed the silkworms from the fifth instar. Three groups of fifth instar larvae were made, which include one untreated control group and two neutral red treated group. Each group was reared 50 larvae.

Nutritional parameters of *B. mori*

In order to investigate the effect of the selected dye on the nutritional and dietary efficiency of *B. mori*. To measure the selected dietary parameters, a known quantity of mulberry leaves should be provided to the animals. For that, mulberry leaves were accurately weighed in an electronic balance and fed two times a day to both experimental and control groups of silkworms. From the observed values, the various nutritional parameters are calculated using the following equations described by Waldbauer (1968).

Ingesta = Dry weight of given leaf – Dry weight of left over leaf
Digesta = Dry weight of the ingested food – dry weight of excreta
Approximate digestibility (AD%) = Dry weight of digesta/Dry weight of ingesta X 100
Reference Ratio (RR) = Dry weight of ingesta/Dry weight of excreta

Larval and Cocoon parameters of *B. mori*

Analysis of larval parameters was carried out on fifth instar. Ten larvae from each group were selected randomly and noted the weight and length of the larva individually. The cocoons of each group were separated from the mountage. Ten cocoons from each group were selected randomly. The weight of individual cocoon was recorded. Each cocoon was cut vertically using the blade and weight of pupa was recorded. For knowing the shell weight of individual cocoon, the reading of the weight of pupa was subtracted from weight of respective cocoon. The silk shell percentage or shell ratio was calculated through the use of readings of weight of whole cocoon and weight of silk shell in cocoon. Colour manipulation of larva and cocoons of experimental groups were also observed.

Statistical analysis

All the data were presented as mean ± standard deviation. The significance was calculated at 5% level (values are significant when $P< 0.05$).

RESULTS

The experimental group silkworm fed with neutral red sprayed mulberry leaves were observed for feeding and morphological changes. The weight and length of the V^{th} instar of the control and the experimental groups, the colour of the body, silk gland, pupa and cocoon were studied. The larvae, pupae, cocoon and the emerging moth were maintaining the imparted colour throughout. To extend the study, the adult silk moth laid colour eggs.

A comparison between control and neutral red fed group has shown in Plate 3 - 5. The duration of the larval instars was same for both the groups and spinning started on the eighth day of V^{th} instar. The experimental group fed with the dye had spun intrinsically coloured silk with pink colour. In the initial trial, the experimental group fed with 0.05 wt % of dye sprayed mulberry leaves spun cocoons with pink colour. In order to analyse the effect of high concentration on colour incorporation, increased concentrations of dye were evaluated.

Table 1: Larva, silk gland and cocoon colour imparted by control and different concentrations of neutral red fed group of *B. mori.*

Concentration (%)	Larval colour	Silk Gland colour	Cocoon colour
Control	Milky white	Yellow	White
0.025	Pale Pink	Pale Pink	Pale Pink
0.05	Pink	Pink	Pink

Table 2: Larval and cocoon parameters of control and different concentrations of neutral red fed group.

Concentration (%)	Control	0.025	0.05
Larval Weight (gm)	4.46 ± 0.18	4.7 ± 0.12 (5.38)	4.42 ± 0.68 (-0.90)
Larval Length (cm)	10.80 ± 1.23	10.88 ± 1.15 (0.74)	10.8 ± 1.10 (-0.55)
Larval Survivability (%)	89 ± 0.5	92 ± 0.7 (3.37)	88 ± 0.9 (-1.12)
Cocoon Weight (gm)	2.63± 0.17	2.68 ± 0.34 (1.90)	2.48 ± 0.15 (-5.70)
Pupa Weight (gm)	2.17 ± 0.30	2.21 ± 0.26 (1.84)	2.03 ± 0.21 (-6.45)
Shell Weight (gm)	0.46 ± 0.01	0.47 ± 0.03 (2.17)	0.45 ± 0.17 (-2.17)
Shell Ratio (%)	20.46± 2.50	20.69 ± 2.01 (1.12)	20.05 ± 2.42 (-2.00)

a) Larval Weight (gm):
In fifth instar larval weight (gm) was recorded in table 2. In control, the larval weight was 4.46 ± 0.18 gm. In experimental group, the larval weight 4.7 ± 0.12 gm and 4.42 ± 0.68 gm were recorded on the exposure of 0.05 and 0.025 % of neutral red respectively.

b) Larval Length (cm):
In fifth instar larval length (cm) was recorded in table 1. In control, the larval length was 10.80 ± 1.23 cm. In experimental group, the larval length 10.88 ± 1.15 cm and 10.74 ± 1.10 cm were observed on the exposure of 0.05 and 0.025% of neutral red respectively.

c) Larval Survivability (%):
In fifth instar larval survivability (%) was recorded in table 1. In control, the larval survivability was 89 ± 0.5 %. In experimental group, the larval survivability 92 ± 0.7 % and 88 ± 0.9 % were calculated on the exposure of 0.05 and 0.025 % of neutral red respectively.

d) Cocoon Weight (mg):
In B. mori the cocoon weight (gm) was recorded in table 2. In control, the cocoon weight was 2.63 ± 0.17 mg. In experimental group, the cocoon weight 2.68 ± 0.34 mg and 2.48 ± 0.15 mg were recorded on the exposure of 0.05 and 0.025 % of neutral red respectively.

e) Pupal Weight (mg):
In fifth instar pupal weight (mg) was recorded in table 2. In control, the pupal weight was 2.17 ± 0.30 mg. In experimental group, the larval length 2.21 ± 0.26 mg and 2.03 ± 0.21 mg were observed on the exposure of 0.05 and 0.025 % of neutral red respectively.

f) Shell Weight (mg):
In fifth instar shell weight (mg) was recorded in table 2. In control, the shell weight was 0.46 ± 0.01 mg. In experimental group, the shell weight 0.47 ± 0.03 mg and 0.45 ± 0.17 mg were obtained on the exposure of 0.05 and 0.025 % of neutral red respectively.

g) Shell Ratio (%):
In fifth instar shell ratio (%) was recorded in table 2. In control, the shell ratio was 20.46 ± 2.50%. In experimental group, the shell ratio 20.69 ± 2.01 % and 20.05 ± 2.42% were noted on the exposure of 0.05 and 0.025 % of neutral red respectively.

Discussion

The silkworm *Bombyx mori*, being a monophagous insect, draws all its nutrition from mulberry leaves. Therefore, the growth and development of the silkworm depends on the quantity and quality of neutral red treated leaves provided to them Prashanth Kumar and Umakanth (2017).

In the present study the silkworms were fed with neutral dye treated mulberry leaves to change the larva, silk gland and cocoon colour. It is consistent with Anumol *et al*., (2017) the earlier studies that identified a colour change of silkworm body on feeding neutral red added diet. It is in accordance with the earlier studies by Campbell (1932) who proved a colour change of silkworm body on feeding dye added diet. Dandin *et al*, (2010) investigated a Chinese group produced pink coloured cocoons using Rhodamine B and a group in Singapore (2011) also analysed pink colour cocoon production using Rhodamine B (Tansil *et al.,* 2011 & 2012).

In the present investigation, the larval weight is significantly increased in the 0.025 Neutral red concentration. Our work is, supported by Meeramaideen., *et al.,* (2017) have reported that feeding mulberry leaves, supplemented with distilled water alone slightly increased the weights of larva, pupa and cocoon shell. Pakhale *et al*., (2014) analysed the larval weight was ranges from 33.77-40.67g. Murugesh *et al.,* (2020) who identified the fortification permanaganate and feeding them to silkworm larva resulted in increased larval weight.

The results of the present investigation explained that the larval length is significantly increased in the 0.025 neutral red concentration. This result is strengthened by the findings of Gokul (2015), who recorded higher fifth instar larval weight, when the Silkworm larvae were provided with mulberry leaves sprayed with different minerals, is also in line with the present observation Ramesh *et al*., (2018) has suggested that the enhancement in larval weight was related to phasgostimulation of ascorbic acid. Soliman (2021), findout that the feeding 5th instar larve of *B.mori* on mulberry leaves enriched with 0.5% yeast extract increased the percentages of larval weight.

The weight of the cocoon is significantly increased in the 0.025 Neutral red concentration. This findings are in accordance with the studies of Shivkumar *et al.,* (2020) analysed the amino acids treated mulberry leaf fed silkworm larvae shows higher in terms of body weight, cocoon weight, shell weight and yield/100 DfLS(kg) when compared with control Sivaprasad *et al.,* (2012) reported that supplementation of zinc chloride at higher concentrations enhanced the cocoon yield and quality. These findings are in parity with the present observations. Asaf and Nlahavishnu (2018), who reported that the total body weight, and the weight of cocoon were maximum in silkworm larva when fed on mulberry leaves treated with spirulina. Maximum in silkworm larvae when fed on mulberry leaves treated with spirulina.

The present result showed that the pupal weight is significantly increased in the 0.025 neutral red concentration. The same records were recorded by Masthan *et al.,* (2011) reported that 300 ppm concentration of spirulina and yeast as food supplement to silkworm contain the ultimate amount of vitamins and fundamental amino acids which define the specificity for different metabolic effectively enhances single cocoon weight, pupal weight and silk filament length.

In the present study the shell weight is significantly increased in the 0.025 neutral red concentration. The results are in agreement with the earlier findings of *Ashfaq et al.,* (2010). Who have identified that application of zinc chloride either indicidually or in combination increased the shell weight significantly. Esaivani *et al.,* (2014) who find out the pupal weight, cocoon weight, shell weight, shell ratio and silk traits of *B.mori* were enhanced by using probiotic *S.cerevisiae* which found to be considerable in elevation the activity of the enzymes.
Investigation revealed that the shell ratio is significantly increased in the 0.025 neutral red concentration. The result is strengthened by the findings of Kavitha *et al.,* (2012) who have analysed that application of zinc chloride either individually or in combination increased the shell ratio significantly.

Conclusion

In the present study to be concluded that growth and economic parameters of *Bombyx mori.* L was comparatively more when the silkworm fed with neutral red treated mulberry leaves than the control.

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