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Research Article

AN INNOVATIVE, ECONOMIC AND GREEN TECHNIQUE FOR THE GENERATION OF COLOUR SILK BY BOMBYX MORI FED WITH DYE MIXED DIET

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A B S T R A C T

Silk the queen of fibers admired by people all over the world for its marvellous properties. Colour is an attractive epithet of silk that magnifies its popularity and demand. However, the hazardous chemicals required for the dyeing process is a threat to the environment. Therefore, it is interesting to introduce a new generation of coloured silk without losing its intrinsic properties. The intrinsically coloured silk has produced directly from silkworms through feed manipulation with dyes those have incorporated effectively into silk gland. This green process without any chemical pollution offers the development of an insect system that produce coloured silk directly from the mulberry silkworm *Bombyx mori (B. mori)* through the in vivo uptake of vital dyes such as neutral red, eosin, light green and phenol red. Colour appearance observed in the silkworm body, haemolymph, silk gland, cocoon, pupa, moth and egg of certain dye fed groups. The physical and economic traits of the larva and cocoon of all the groups have analyzed and the results imply that, with the skillful utilization of vibrant colour dyes, both a novelty in silk industry and reduction of dye waste pollution could be achieved.

INTRODUCTION

Silkworm silk due to its luster, excellent mechanical properties and biocompatibility is widely used in textile industry and for many biomedical applications. There has been a scientific curiosity on the improvement of intrinsic properties of silk such as toughness, ultraviolet resistance, colour, etc., in the recent years. An increase in the ultraviolet resistant property of *B. mori* silk when animals fed with 1% Titanium dioxide nanoparticles added diet has been reported recently (Lingyue *et al*., 2015). Studies proved the integration of metals like zinc, aluminium and titanium by atomic layer deposition could enhance the toughness of the silk (Lee *et al*., 2009). The increase in mechanical properties makes the silk more apposite for biomedical applications. As colour is a peculiar epithet of silk fabrics, recently researchers have been produced genetically modified fluorescent silk with various colours (Lizuka *et al*., 2013; Royer *et al*., 2005). From the recent studies, it is evident that the in vivo uptake through diet is the easier, economic, and green way to incorporate the additives like metal nanoparticles or colour dyes to silkworm silk. Tansil *et al* registered the production of intrinsically coloured silk by *B. mori* through the uptake of rhodamine B added diet (Tansil *et al*., 2011). Similarly, the generated pink coloured silk using azo dyes and rhodamine dyes were reported recently (Nisal *et al*., 2014; Trivedy *et al*., 2016).

Use of dye added diet for the production of coloured silk is a novel technology that offers variously coloured silk with small amounts of dyes. More over this avoids the conventional dyeing process, which produces large volumes of toxic wastewater as byproduct.

The textile industry is one of the fastest growing industries of the world. It requires a sequence of chemical processes those are hazardous to the environment. In order to meet the global demand of coloured textiles, about 700,000 tons of dyes are required and it lead to the release of a huge quantity of synthetic colourant that comprises various chemicals, heavy metals, carcinogenic amines, etc into the environment especially to water bodies which in turn destroys the aquatic population (Dias, 2015; Samanta, 2009; Robinson, 2001).

From the first half of 20th century there have been a series of trial and error investigations to generate coloured silk using dye added diet (Lombardi, 1920; Edwards, 1921). Some researchers observed that certain dyes pass through the midgut of insects (Roeder, 1953; Zacharack, 1963). These investigations aimed to analyze the toxic effect of colours on the physiology of insects and to understand the retention time of mulberry leaves in the silkworm gut.

In this study, silkworms were fed with dye mixed diet to change the cocoon colour. More over the larval and cocoon characteristics were analyzed to find whether the dye used alter the normal growth and physiology of silkworms.

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MATERIALS AND METHODS

Rearing of Silkworm

The silkworm strain used in this experiment was PM X CSR2, a multivoltine X bivoltine hybrid. The eggs had obtained from the Grainage centre, Sericulture Department in the Regional Deputy Directors Office, Trichy, Tamil Nadu. Rearing was done under standard conditions recommended by Krishnaswami *et al* (Krishnaswami *et al*., 1973). The silkworms were fed with fresh mulberry leaves of V1 variety. The fifth instar larvae divided into five groups (50n in each group) including control. The experimental groups other than control were fed with respective dye added diet.

Preparation of Modified Diet

The dyes used in this study were eosin, light green, neutral red and phenol red (all from Merck Chemicals). After the trials, the concentration of each dye was fixed and the modified feed was prepared by spraying an appropriate concentration of respective dye on to the mulberry leaves. Initially the dye concentration in food was of 0.05 weight percentage for all the dyes and it was increased then to 0.1 wt %, 0.2 wt % and 0.4 wt% to find whether there is improvement in colour incorporation.

Larval and Cocoon Features

The larval features such as length and weight of fifth instar larvae from each experimental group were recorded every day until the spinning. Cocoon characteristics such as cocoon weight, pupa weight, shell weight of all the groups were also measured. Before spinning, larvae from each group dissected and both the haemolymph and silk gland were observed to confirm the colour accumulation.

Statistical Analysis

Data on larval and cocoon features had been statistically analyzed by One Way Analysis of Variance (ANOVA) by using SPSS version 20. All the data were presented as mean ± standard deviation of mean. The significance level was set $p < 0.05$.

RESULTS AND DISCUSSION

Out of the four dyes used (eosin, light green, neutral red and phenol red) neutral red imparted obvious colour change to the silkworm body within one hour (Fig. 1).

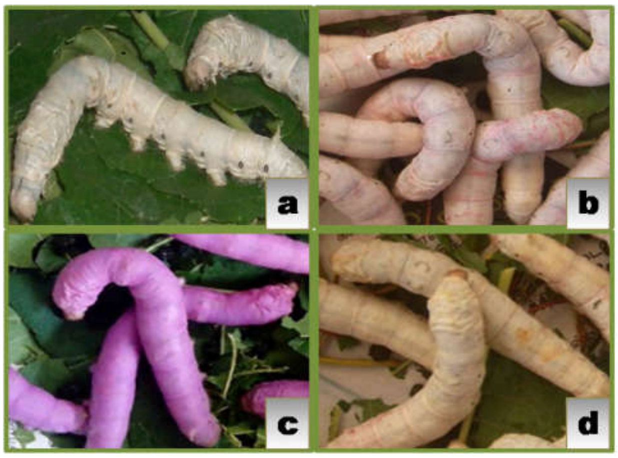

Fig 1 Fifth instar larvae of a) control, b) eosin, c) neutral red and d) phenol red fed groups

This elucidates the efficacy of neutral red to incorporate into the silkworm body. It is in accordance with the earlier studies that reported a colour change of silkworm body on feeding neutral red added diet (Campbell, 1932). Besides that, the silk gland also appeared in a dark red colour. Initially each experimental group fed with 0.05 weight percentage of respective dye sprayed mulberry leaves and only neutral red group produced coloured cocoons. Since there is no colour change for the remaining three experimental groups, increased concentrations of dyes were evaluated (Table.1).

Table 1 Cocoon colour imparted by different concentrations of selected dyes.

Dye con. (wt %)	Eosin	Light green	Neutral red	Phenol red
0.05	No colour change	No colour change	Orange	No colour change
0.1	No colour change	No colour change	mild red	Light yellow
0.2	pink	Greenish yellow	red	Yellow
0.4	Mortality	Mortality	red	Yellow

On the eighth day of V instar, the experimental groups fed with eosin, neutral red and phenol red started to spin intrinsically coloured silk with pink, red and dark yellow colours respectively (Fig. 2). Colour of cocoon generated from light green fed group were pale greenish yellow and were almost similar to that of control cocoons.

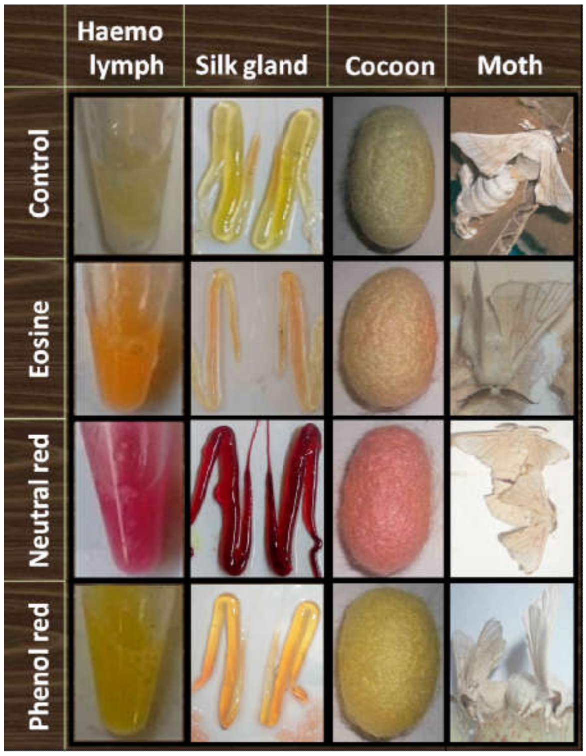

Fig 2 Haemolymph, silk gland, cocoon and moth of control and experimental groups

The results observed for neutral red group demonstrates that the colour intensity of cocoons can be reduced by lowering the dye concentration. However, there is no significant increase in cocoon colour with an elevation in concentration of dye above 0.2 wt %. It is consistent with the studies using rhodamine B, which showed no increase in colour intensity of cocoons above a specific concentration (Tansil *et al*., 2011). Eosin and phenol red groups spun cocoons with noticeable colour change only at a dye concentration of 0.2 wt % and above that growth retardation and mortality was observed.

On dissecting the larvae of each group on the seventh day of V instar before spinning, eosin, neutral red and phenol red groups had coloured haemolymph and silk gland (Fig. 2). It was reported that initially the gut epithelia absorbs carotenoids pigments from mulberry leaves, then haemolymph takes its turn to transfer these pigments into the silk gland resulting yellow haemolymph and yellow silk gland (Tansil at al., 2012). Interestingly, the peritoneal membrane of alimentary canal of neutral red group also possessed a noticeable pink colour. All these together indicate the diffusion of dye through the alimentary canal to the haemolymph and from there to silk gland.

The midgut of silkworm larva has a permeability barrier against compounds with a molecular weight greater than 400 g/mol and compounds with smaller molecular weight have a faster transport rate through the midgut membrane (Hamamoto, 2005). As the molecular weight is an important factor for the passage of molecules, the required range of molecular weight of neutral red (288.78 g/mol) and phenol red (354.38 g/mol) may aided the better colour accumulation in silk gland of those groups and thus the production of bright coloured cocoons. Among the four stains tested light green with higher molecular weight (749.89 g/mol) exhibited less colour incorporation than the remaining three dyes. However, further investigations are required to confirm the factors, which facilitated the passage through the biochemical pathways and accumulation of dyes in the silk gland.

The statistical analysis of the larval and cocoon traits of control and all the experimental groups reveal that there is no significant difference between the groups (Table. 2, 3 and 4).

Table 2 Effect of selected dyes on the weight of V instar larvae

Exp. condition	Day of V instar						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	0.734 ± 0.005	0.848 ± 0.031	1.384 ± 0.147	2.278 ± 0.135	3.314 ± 0.195	3.577 ± 0.171	3.958 ± 0.134
Eosin	0.733 ± 0.002	0.845 ± 0.024	1.314 ± 0.122	2.261 ± 0.117	3.297 ± 0.236	3.535 ± 0.112	3.916 ± 0.196
Light green	0.732 ± 0.003	0.845 ± 0.042	1.265 ± 0.171	2.253 ± 0.136	3.225 ± 0.246	3.406 ± 0.253	3.907 ± 0.279
Neutral red	0.734 ± 0.009	0.856 ± 0.055	1.491 ± 0.368	2.533 ± 0.309	3.319 ± 0.236	3.759 ± 0.284	3.994 ± 0.173
Phenol red	0.735 ± 0.004	0.862 ± 0.044	1.541 ± 0.316	2.557 ± 0.332	3.390 ± 0.321	3.866 ± 0.153	4.042 ± 0.190

Values are mean ± standard deviation of ten observations All the results are non-significant ($p > 0.05$)

Table 3 Length of V instar larvae fed with selected dyes

Exp. condition	Day of V instar						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	3.830 ± 0.200	4.360 ± 0.227	4.720 ± 0.270	5.740 ± 0.324	6.320 ± 0.388	6.750 ± 0.190	6.970 ± 0.125
Eosin	3.810 ± 0.228	4.170 ± 0.306	4.700 ± 0.200	5.690 ± 0.251	6.280 ±0.361	6.620 ± 0.230	6.950 ± 0.143
Light green	3.770 ± 0.231	3.950 ± 0.184	4.610 ± 0.218	5.670 ± 0.200	6.270 ±0.302	6.410 ± 0.446	6.830 ± 0.306
Neutral red	3.850 ± 0.184	4.370 ± 0.177	4.800 ± 0.211	5.820 ± 0.199	6.380 ± 0.339	6.750 ± 0.425	6.990 ± 0.160
Phenol red	3.850 ± 0.283	4.430 ± 0.283	4.850 ± 0.172	5.820 ± 0.175	6.770 ± 0.226	6.820 ± 0.239	7.01 ± 0.152

Values are mean ± standard deviation of ten observations All the results are non-significant ($p > 0.05$)

Table 4 Economical parameters of control and experimental groups

Exp.condition	Cocoon weight	Pupa weight	Shell weight	Shell ratio
Control	1.609 ± 0.282	1.310 ± 0.243	0.289 ± 0.047	18.081 ± 2.037
Eosin	1.417 ± 0.123	1.162 ± 0.123	0.255 ± 0.023	18.074 ± 1.972
Light green	1.503 ± 0.146	1.228 ± 0.135	0.267 ± 0.022	17.830 ± 1.644
Neutral red	1.561 ± 0.176	1.270 ± 0.163	0.282 ± 0.024	18.204 ± 1.833
Phenol red	1.507 ± 0.185	1.228 ± 0.165	0.269 ± 0.030	17.974 ± 1.745

Values are mean ± standard deviation of ten observations
All the results are non-significant ($p > 0.05$)

From this, it is evident that the dyes have no harmful effect on the physical and economical parameters of *B. mori*. It is in agree with the recent study which showed no significant difference of cocoon characters between control and rhodamine B added diet fed group of silkworms (Trivedi *et al*., 2016). Among the five groups including the control, the phenol red mixed diet group has the maximum weight (4.042 ± 0.190 cm) on the seventh day of V instar followed by neutral red (3.994 ± 0.173 cm), control (3.958 ± 0.134 cm), eosin (3.916 ± 0.196 cm) and light green (3.907 ± 0.279 cm) diet groups respectively. Lengths of these groups on the same day were 7.01 ± 0.152 cm, 6.99 ± 0.160 cm, 6.97 ± 0.125 cm, 6.95 ± 0.143 cm and 6.83 ± 0.306 cm respectively. Though the maximum cocoon, pupa and shell weight were recorded for control group, highest shell ratio was observed for neutral red group (18.204%). Next to it was the control group (18.081%) followed by eosin (18.074%), phenol red (17.974%) and light green (17.830%).

CONCLUSION

This paper deals with the two issues in the present scenario, viz. the reduction of textile dye pollution and in vivo production of coloured silk by *B. mori* fed by a dye added diet. As the title denotes this technique has no harmful effect for the insect and the environment. It provides a cost effective method for dyeing silk, which avoids hazardous chemicals. The process has no unfavourable effects on the silkworm, as there is no significant difference in the observed traits of normal and neutral red fed group silkworms. It was observed that neutral red, eosin and phenol red modified the cocoon colour noticeably. As the dyes have no significant impact on silkworm other than changing colour, the selected dyes in appropriate concentration can be suggested for the production of coloured silk. This proves it is a promising technology for producing various vital coloured silk in the near future. Thus, it can be recommended for large-scale production of coloured cocoons. Further characterization studies are required to confirm whether the colour incorporated into the fibroin of silk.

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