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Interaction of a mixture of selective probiotics and their effects on nutritional indices in *Bombyx mori* larvae fed with vital dye supplemented feed

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Abstract
The silkworm larvae were fed on probiotics fortified mulberry leaves treated with five different kinds of Vital dye as feed supplements and given for silkworm hybrid, CSR-2 Double Hybrid from fourth instar onwards till last day of feeding. The same was carried out with chow feed coloured with vital dyes. Among the probiotics tested, was found to be superior for all the physical and growth parameters such as larval length and weight at total larval duration. The mulberry fortified with probiotics and chow feed fortified are showing remarkable results during their life cycle at larval and cocoon stages. The interaction of feed additives probiotics along with their vital dye supplementation indicated that these combinations bring a superior traits during all larval parameters studied larvae length varies between 2.13-4.02 cms in V instar and 4.15-6.90 cms in V instar larva. Similarly nutritional indices like ingesta varies between 33.41-94.99 in IV and V instar and for digesta 29.01-43.75. Approximate digestability and reference ratio lies between 79.15-84.65; 6.49-7.60 for IV and V instar larva. Highest Efficiency conversion indices (ECI) for larva was 3.05-5.23, for Cocoon it is 2.69 and for shell it is 0.49 Efficiency digestion indices ECD recorded was 3.23-4.83 in larval stages, in Cocoon it was 4.55 and in shell it was 0.83.

Keywords: *Bombyx mori*, Probiotics, vital dyes, larval feeding, growth parameters

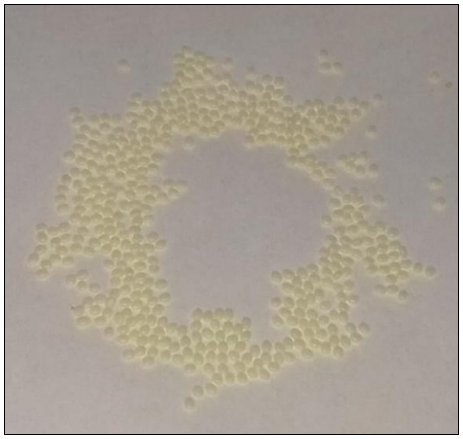
Introduction

Sericulture is an income generating agro-enterprise and a potential sector of the agriculture to raise economic status of the farming community and also earning foreign revenue. Silk the queen of fibers admired by people all over the world for its lustre. The dyeing of silk is a threat to the environment as it is one of the major pollution causing industries of the world. It is interesting to introduce a new generation of silk with its intrinsic properties like colour and luminescence without dyeing. The intrinsically coloured and luminescent silk is produced directly from silkworms through feed manipulation with dyes, which have been incorporated effectively into silk while it is being produced in the silk gland. This green process without any chemical pollution offers the development of an insect system to produce coloured silk directly from silkworm. This paper discloses a green method for the production of coloured cocoons by *Bombyx mori* through the *in vivo* uptake of vital dyes such as Neutral red, Rhodamine-B, Acridine orange, Eosin yellow and Light green, were carried out. Many investigations which involves the enrichment of mulberry leaves by nutrient supplementation was carried out recently (Rouhollah Radjabi, 2010; Ganesh Prabu *et al*., 2012 [6]; Samatha *et al*., 2014,) [15].

There have been a series of trial and error investigations from the 20th century to generate coloured silk using dye added diet (Koyler 1965 [8]; Edwards, 1921 [4], Nisal *et al*., 2014) [11]. Researchers observed that certain dyes pass through the midgut of insects (Roeder 1953 [14]; Zacharack 1963). Silkworm requires a balanced nutrition for a robust growth. Nutrition is an important growth regulatory factor and a physiological need, which depends on the nature of nutritional resources, pattern of acquisition, allocation of incoming resources and the metabolic capacity for stored reserves in the insect which are all critical for fitness (Nirmal Kumar *et al*., 2012) [10]. Nutritional requirement in food consumption have direct impact on the overall genetic traits such as larval growth and cocoon weight, amount of silk production, pupation and reproductive traits (Ganesh Prabu *et al*., 2012) [6]. Enrichment of mulberry leaves by nutrient supplementation is one of the strategies by which cocoon and silk productivity can be increased and the quality can be enhanced (Rouhollah Radjabi, 2010). Many attempts have been made to improve the quality and quantity of silk through enhancing the leaves with nutrients, spraying antibiotics, vitamins, hormones and hormone analogues or plant extracts (Pardeshi *et al*., 2014) [12]. The present 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.

A need of the hour is to develop a green method to reduce dye-house waste pollution. The production of coloured cocoons from *Bombyx mori* is another vital area without any chemical pollution and the development of an insect system to produce coloured silk has significant potential applications in sericulture research (Tansil *et al*, 2011) [16]. Hajime Mori and colleagues at the Kyoto Institute of Technology in Kyoto, Japan reported that they have developed a technique to produce genetically altered, green fluorescent silk fibers that are spun by the silkworm. A group of researchers succeeded in producing coloured cocoons using *Bombyx mori* larvae fed with green colour dye (Trivedy, *et al*, 2016) [18]. *Hybrid* silkworms increase the quality silk production and raise the return of the farmers to a higher level. A new hybrid with higher survival, higher cocoon weight, higher shell weight, higher cocoon filament length and higher silk percentage than the wild silkworms. With the skillful utilization of hybrid varieties, use of vibrant colour dyes and improved variety of food supplementation a novelty in silk industry could be

reached (Tansil *et al*, 2012) [17]. The present investigation is an attempt to manifest the above. In this study, silkworms were fed with dye mixed diet fortified with a few selected probiotics to change the cocoon colour and silk quality. More over the larval and cocoon characteristics were analyzed to find whether the dye used alter the normal growth and physiology of silkworms.



V1- Victory 1

CSR2 – Double Hybrid EGGS

Ingesta = Dry weight of given leaf – Dry weight of leftover 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

Efficiency conversion of ingesta to larva (%) (ECI of larva) = Maximum dry weight of larva/dry weight of ingesta X 100

Efficiency conversion of ingesta to cocoon (%) (ECI to cocoon) = Dry weight of cocoon/dry weight of ingesta X 100

Efficiency conversion of ingesta to shell (%) (ECI to shell) = Dry weight of shell/dry weight of ingesta X 100

Efficiency conversion of digesta to larva (%) (ECD) to larva = Maximum dry weight of larva/dry weight of digesta X 100

Efficiency conversion of digesta to cocoon (%) (ECD to cocoon) = Dry weight of cocoon/dry weight of digesta X 100

Efficiency conversion of digesta to shell (%) (ECD to shell) = Dry weight of shell/dry weight of digesta X 100

Silkworm larvae: The egg of Wild and hybrid variety like CSR2 Double Hybrid strains were collected from the government grainage centre, at Manikandam, Tiruchirappalli. These were grown in rearing room under hygienic conditions. Rearing was carried out in a climatized room where temperature, relative humidity and photoperiod were controlled.Temperature was set at 30±1°C, relative humidity (R.H.) 70.5% during the fourth instar, and 65.5% during the fifth instar. Regarding feeding, diet was given at regular intervels with weighed amount daily to larvae in the fourth and fifth instar stage based on the previous experiments carried out to establish their food needs.

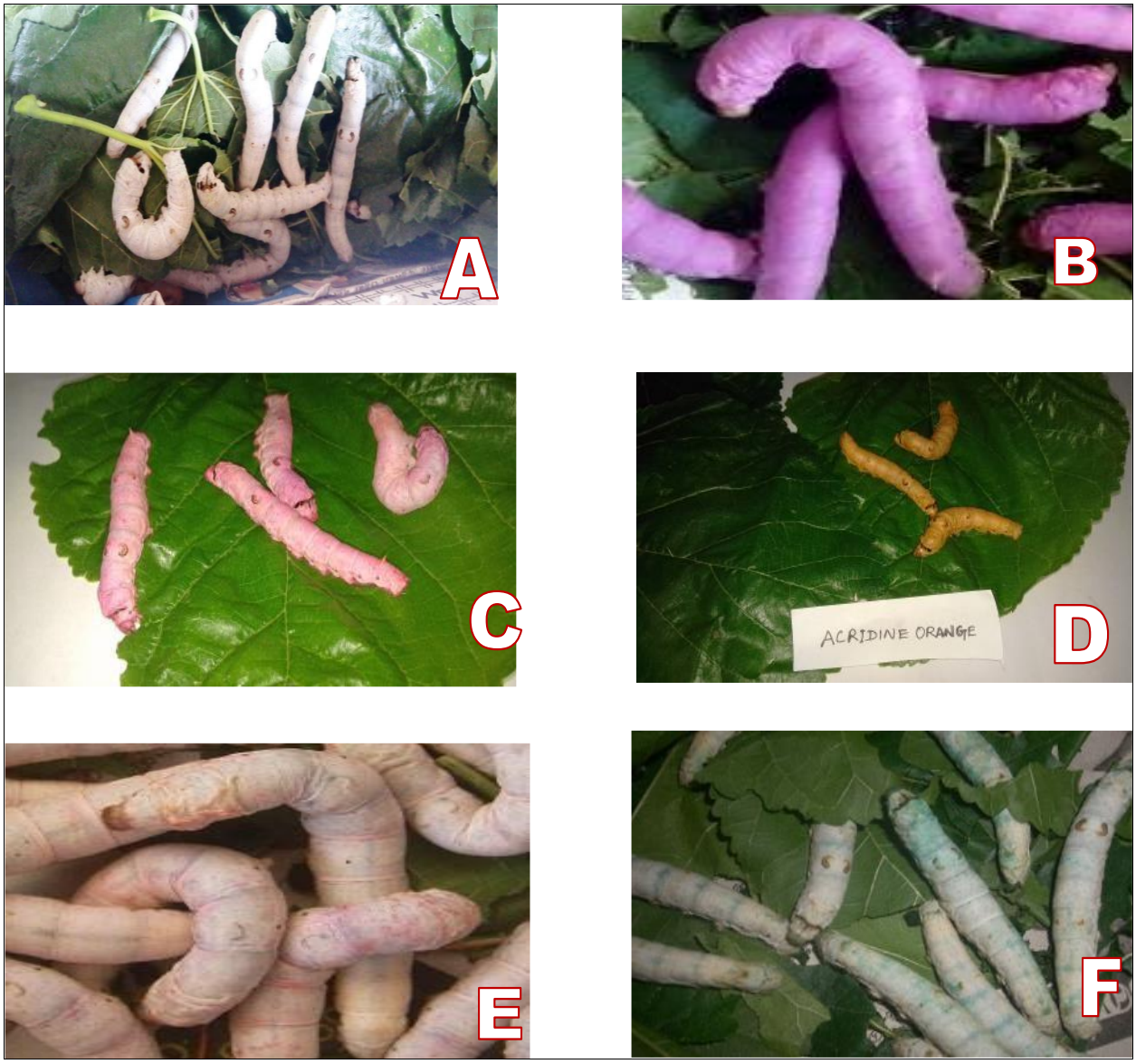
Materials and Methods

Mulberry leaves

The V-1 mulberry plant leaves were washed thoroughly in tap water and shade dried to remove the water drops before feeding. They were fortified with probiotic spray and supplemented with vital dyes before use.

Vital dye supplementation: Neutral red, Rhodamine -B, Acridine orange, Eosine yellow and Light green (Nice, chemicals) are some of the common biological vital dyes used in this study.Vital dyes concentration was standardized after few trials, a concentration of (0.02wt %) which has no harmful effect on silkworm life cycle (Anumol anto *et al*., 2017) [1] The mulberry leaves were fortified with selective mixture of probiotics and vital dyes. All the six experimental groups containing 30 larvae in each group were in their fourth instar stage. were observed to access nutritional indices. The mulberry leaves fortified with selective mixture of probiotics and vital dyes such as Neutral red, Rhodamine B, Acridine orange, Eosine yellow and Light green and a control were fed with treated mulberry leaves. All the silkworms were kept in a plastic package laid with a clean wax paper, and cultured at 30° C. fed every day till cocooning. As colour manipulation and mulberry fortified with probiotics was the focus of this investigation, all the groups thoroughly noticed after the consumption of dye added diet (Anumol Anto *et al* 2017) [2]. The size and morphology of the silkworms were observed. The body weight of the silkworms was determined using an analytical balance and length by using graph paper during the period of study. Along with the observation of colour change, visible effects such as normal growth, larval duration and mortality were also evaluated.

Analysis of nutritional traits: Nutritional indices were studied for fourth and fifth instar larvae of CSR2. Control and five dye added diet fed groups in three replications were reared in separate trays at standard conditions described by Krishnaswami *et al* (1973). The temperature and humidity were maintained at 25^0C and 70% respectively. To measure the selected dietary parameters, a known quantity of mulberry leaves should be provided to the animals, for that, mulberry leaves accurately weighed in an electronic balance and feed three times a day to both experimental and control group of silkworms for the determination of the ingesta, the same quantity of mulberry leaves kept as dummies. From the observed. Values, the various nutritional parameters are calculated using the following equations described by waldbauer, (1968).



CSR 2: Double Hybrid mulberry silkworm bombyx mori fed with vital dye fortified leaves A- Control, B- Netural Red, C- Rhodamine-B, D- Eosin Yellow, E- Acridine Orange, f- Light Green

Measurement of larval growth: The mean weight of the thirty randomly selected fourth and fifth instar larvae from each replication were recorded everyday using an electronic balance. Along with that the length of the same larvae were measured manually by marking the two proximities of larva and then the distance between the two points measured (Fujia Chen, *et al*, 2012) [5].

From the observed values, the data on the biomass of the larva and cocoon was obtained for the nutrigenetic traits and the standard gravimetric procedure. The data subjected to statistical analysis using Two-way Anova (SPSS Version 16**).**

Results

Bombyx mori is a monophagous insect solely depends on the proteins of mulberry plant. Digested leaves are converted into fibroin and sericin in their silk gland for synthesis of silk. The experiments reveled the data for the Nutritional efficacy of mulberry leaves fortified with probiotics and coloured with vital dyes. Silkworm nutritional indices are calculated from the consumption of the feed and the conversion of the components for their growth and metabolism. The rate of Ingesta, Digesta, Approximate Digestibility and Refernce Ratio for the fourth and fifth instar larvae fed with fortified with probiotics. Intake of mulberry leaves in IV and V instar larva gradually increases from day 1 to day 5. In fourth instar larva ingesta varies from 12.33-33.41, whereas in fifth instar larva it ranges between35.95-94.99 (Table-1 and 2). Intake was higher than the control in dye supplemented group. In digesta also exhibited similar trend with 11.03-29.01 in fourth instar larva and 30.11-58.63 in fifth instar larva (Table-3, 4) Approximate digestability of fourth instar gradually increases 84.41-94.41 in fourth instar larva, while in fifth instar larva it was 45.0-86.5.

Table 1: Ingesta of *Bombyx mori* (CSR2) Double Hybrid IV th & Vth instar larva fed with (Mulberry leaves coated with vital dyes(Mean ±standard deviation)

S.no	Experiments	Day 1	Day 2	Day 3	Day 4	S.no	Experiments	Day 1	Day 2	Day 3	Day 4	Day-5	Day-6
1	Control	13.95 ± 0.05	23.73 ± 0.03	21.71±0.01	30.67±0.03	1	Control	35.95±0.05	62.41±0.02	83.11±0.02	82.13±0.03	85.71±0.02	74.63±..040
2	Neutral red	13.62 ± 0.02	23.49 ± 0.00	19.15±0.05	29.85±0.05	2	Neutral red	36.53±0.03	59.33±0.04	80.29±0.05	87.59±0.00	87.79±0.00	81.43±..034
3	Rhodamine-B	12.33 ± 0.04	23.23 ± 0.00	23.62±0.02	30.09±0.00	3	Rhodamine-B	37.23±0.03	60.55±0.05	84.92±0.02	85.25±0.05	87.71±0.01	92.99±..005
4	Acridine orange	13.23 ± 0.04	22.14 ± 0.04	21.95±0.05	33.41±0.02	4	Acridine orange	36.15±0.05	63.25±0.05	87.49±0.00	88.14±0.04	94.99±0.00	93.40±..011
5	Eosin yellow	12.95 ± 0.05	19.55 ± 0.05	22.83±0.02	32.11±0.02	5	Eosin yellow	37.59±0.00	61.33±0.03	68.64±0.04	74.23±0.03	75.99±0.00	83.43±..040
6	Light green	13.43 ± 0.03	21.62 ± 0.02	23.03±0.00	30.35±0.05	6	Light green	36.43±0.03	63.75±0.05	89.01±0.10	86.21±0.02	93.89±0.00	73.61±..023

Table 3: Digesta of Bombyx mori (CSR2) Double Hybrid IVth & Vth instar larva fed with mulberry leaf coated with vital dyes and fortified with probiotics.

S.no	Experiments	Day 1	Day 2	Day 3	Day 4	S.no	Experiment	Day 1	Day 2	Day 3	Day 4	Day-5	Day-6
1	Control	11.82±0.02	19.61±0.01	17.19±0.00	29.01±0.02	1	Control	30.11±0.02	53.00±0.01	65.02±0.02	38.65±0.05	59.23±0.03	43.75±0.5
2	Neutral red	11.85±0.05	19.60±0.01	13.11±0.02	27.21±0.02	2	Neutral red	30.95±0.05	51.31±0.02	69.73±0.04	49.59±0.00	66.60±0.01	45.40±0.0
3	Rhodamine-B	11.03±0.04	19.75±0.05	18.60±0.01	27.31±0.01	3	Rhodamine-B	31.23±0.03	51.83±0.04	69.60±0.01	43.23±0.04	59.01±0.01	58.00±0.1
4	Acridine orange	11.62±0.02	18.32±0.02	17.60±0.01	28.69±0.11	4	Acridine orange	31.32±0.00	53.24±0.04	70.92±0.02	41.65±0.05	69.80±0.01	58.63±0.4
5	Eosin yellow	11.43±0.04	16.82±0.02	17.63±0.04	28.20±0.01	5	Eosin yellow	31.29±0.00	53.31±0.01	58.13±0.04	58.03±0.04	64.33±0.04	56.01±0.1
6	Light green	11.73±0.04	18.63±0.03	18.04±0.04	28.69±0.00	6	Light green	31.03±0.04	53.92±0.02	69.90±0.01	38.61±0.01	68.63±0.04	45.84±0.4

Table 5: Approximate digestibility and of *Bombyx mori* (CSR2) Double Hybrid IV instar larva fed with (Mulberry leaves coated fortified with Probiotics with Vital dyes)

S.no	Experiment	Day 1	Day 2	Day 3	Day 4	S.no	Experiment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1	Control	84.71±0.02	82.57±0.06	79.14±0.04	94.51±0.06	1	Control	83.70±0.05	84.88±0.04	78.19±0.04	47.60±0.05	69.03±0.07	58.58±0.04
2	Neutral red	87.36±0.31	83.38±0.07	68.40±0.05	91.10±0.05	2	Neutral red	84.68±0.04	86.43±0.04	86.77±0.05	56.55±0.00	75.81±0.05	55.70±0.05
3	Rhodamine-B	89.39±0.06	84.68±0.29	78.56±0.15	90.70±0.05	3	Rhodamine-B	83.83±0.05	85.54±0.04	81.90±0.05	50.65±0.05	67.22±0.04	62.32±0.04
4	Acridine orange	87.79±0.04	82.69±0.04	80.14±0.04	85.83±0.04	4	Acridine orange	86.58±0.05	84.12±0.05	81.01±0.04	47.20±0.05	73.39±0.10	62.72±0.04
5	Eosin yellow	82.22±0.04	85.97±0.06	77.17±0.05	87.77±0.05	5	Eosin yellow	83.18±0.05	86.89±0.04	84.63±0.04	78.12±0.04	84.60±0.05	67.02±0.04
6	Light green	87.29±0.04	86.12±0.04	78.29±0.04	94.47±0.02	6	Light green	85.13±0.04	84.53±0.05	78.52±0.03	45.00±0.51	73.05±0.05	62.19±0.10

Table 7 Reference ratio of IVth & Vth instar larva and of *Bombyx mori* (CSR2) Double Hybrid fed with (Mulberry leaves coated fortified with Probiotics with Vital dyes)

S.no	Experiment	Day 1	Day 2	Day 3	Day 4	S.no	Experiment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1	Control	1.173±0.00	1.203±0.00	1.253±0.00	1.046±0.00	1	Control	1.183±0.00	1.166±0.00	1.266±0.00	2.113±0.00	1.436±0.00	1.69.00
2	Neutral red	1.136±0.00	1.186±0.00	1.45±0.00	1.016±0.05	2	Neutral red	1.173±0.00	1.146±0.00	1.143±0.00	1.756±0.00	1.306±0.00	1.78.00
3	Rhodamine-B	1.106±0.00	1.66±0.00	1.256±0.00	1.093±0.00	3	Rhodamine-B	1.183±0.00	1.156±0.00	1.213±0.00	1.966±0.00	1.476±0.00	1.59.00
4	Acridine orange	1.126±0.00	1.196±0.00	1.236±0.00	1.153±0.00	4	Acridine orange	1.143±0.00	1.176±0.00	1.223±0.00	2.106±0.00	1.353±0.00	1.58.00
5	Eosin yellow	1.123±0.00	1.53±0.00	1.283±0.00	1.126±0.00	5	Eosin yellow	1.193±0.00	1.143±0.00	1.173±0.00	1.266±0.00	1.173±0.00	1.47.00
6	Light green	1.133±0.00	1.300±0.25	1.266±0.00	1.045±0.00	6	Light green	1.163±0.00	1.173±0.00	1.263±0.00	2.22±0.00	1.356±0.00	1.59.00

Table 9 Efficiency Conversion Indices of *Bombyx mori* (CSR2) Double Hybrid IV instar larva (Mulberry leaves fortified with Probiotics and Vital dyes)

S.no	Experiment	Day 1	Day 2	Day 3	Day 4	S.no	Experiment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1	Control	1.63±0.00	1.80±0.00	2.84±0.00	3.18±0.00	1	Control	3.13±0.00	2.10±0.00	3.07±0.00	4.78±0.00	4.76±0.00	4.66±0.00
2	Neutral red	1.67±0.00	1.69±0.00	3.22±0.00	2.98±0.01	2	Neutral red	3.19±0.00	2.09±0.00	3.26±0.00	3.95±0.00	4.64±0.00	4.97±0.00
3	Rhodamine-B	1.85±0.00	1.84±0.00	2.61±0.00	3.24±0.00	3	Rhodamine-B	3.02±0.00	2.08±0.00	3.11±0.00	4.22±0.00	4.69±0.00	4.35±0.00
4	Acridine orange	1.95±0.00	2.06±0.00	3.36±0.00	3.16±0.00	4	Acridine orange	3.14±0.00	2.31±0.00	2.90±0.00	4.21±0.00	4.35±0.00	4.20±0.00
5	Eosin yellow	1.92±0.00	2.29±0.00	3.14±0.00	2.88±0.00	5	Eosin yellow	3.04±0.00	2.07±0.00	3.74±0.00	4.43±0.00	5.39±0.00	4.17±0.00
6	Light green	1.85±0.00	2.07±0.00	3.11±0.00	3.05±0.00	6	Light green	3.58±0.00	2.13±0.00	3.01±0.00	4.53±0.00	4.36±0.00	5.24±0.00

Table 11: Efficiency Digestion Indices of *Bombyx mori* (CSR2) Double Hybrid in the IVth & Vth instar stages (Mulberry leaves fortified with Probiotics and Vital dyes) %

S.no	Experiment	Day 1	Day 2	Day 3	Day 4	S.no	Experiment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1	Control	1.93±0.00	2.18±0.00	3.59±0.00	3.36±0.00	1	Control	3.74±0.00	2.48±0.00	3.92±0.00	10.14±0.05	6.92±0.04	7.96±0.00
2	Neutral red	1.93±0.00	2.03±0.00	5.63±0.00	3.26±0.00	2	Neutral red	3.77±0.00	2.42±0.00	3.76±0.00	6.97±0.00	6.13±0.00	8.93±0.00
3	Rhodamine-B	2.07±0.00	2.16±0.00	3.32±0.00	3.57±0.00	3	Rhodamine-B	3.60±0.00	2.44±0.00	3.79±0.00	8.34±0.00	6.98±0.00	5.66±0.00
4	Acridine orange	2.22±0.00	2.50±0.00	4.19±0.00	3.68±0.00	4	Acridine orange	3.62±0.00	2.75±0.00	3.58±0.00	8.92±0.00	5.93±0.00	6.71±0.00
5	Eosin yellow	2.17±0.00	2.66±0.00	4.07±0.00	3.28±0.00	5	Eosin yellow	3.66±0.00	2.39±0.00	4.42±0.00	5.69±0.00	6.37±0.00	6.22±0.00
6	Light green	2.12±0.00	2.40±0.00	3.98±0.00	3.23±0.00	6	Light green	4.21±0.00	2.53±0.00	3.83±0.00	10.10±0.05	5.97±0.00	8.43±0.00

Table 13 Relative Growth Rate (RGR) of *Bombyx mori* (CSR2) Double Hybrid in the IVth & Vth instar stages (Mulberry leaves fortified with Probiotics and Vital dyes)

S.no	Experiment	Day 1	Day 2	Day 3	Day 4	S.no	Experiment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1	Control	1.85±0.05	1.92±0.05	2.11±0.05	1.46±0.05	1	Control	0.550±0.00	0.590±0.05	0.953±0.05	1.016±0.06	2.000±0.04	1.470±0.05
2	Neutral red	1.75±0.05	1.87±0.05	1.60±0.05	1.29±0.05	2	Neutral red	0.460±0.05	0.860±0.05	1.130±0.05	0.673±0.05	1.890±0.05	1.060±0.05
3	Rhodamine-B	2.24±0.05	1.68±0.28	1.22±0.05	1.43±0.00	3	Rhodamine-B	0.360±0.05	0.750±0.05	0.850±0.05	0.830±0.05	1.900±0.05	0.370±0.05
4	Acridine orange	1.95±0.06	2.15±0.05	1.54±0.05	0.93±0.05	4	Acridine orange	0.52±0.05	0.520±0.05	0.700±0.05	0.770±0.05	1.560±0.05	0.340±0.05
5	Eosin yellow	2.05±0.05	2.59±0.05	1.38±0.05	1.13±0.05	5	Eosin yellow	0.310±0.05	0.690±0.05	1.820±0.05	1.490±0.05	2.440±0.00	0.940±0.05
6	Light green	1.91±0.05	2.24±0.05	1.34±0.05	1.36±0.03	6	Light green	0.480±0.05	0.480±0.05	0.600±0.05	0.770±0.05	1.610±0.05	1.530±0.05

Table 15: Efficiency conversion of Ingesta, Digestia of Cocoon and shell Indices of *Bombyx mori* (CSR2) Double Hybrid

S.no	Experiment	ECI of Cocoon & Shell		ECD of Cocoon & Shell	
		Cocoon	Shell	Cocoon	Shell
1	Control	15.45±0.05	2.83±0.05	22.05±0.05	4.09±0.05
2	Neutral red	15.02±0.26	2.87±0.05	21.12±0.05	4.00±0.05
3	Rhodamine-B	14.70±0.03	2.66±0.05	20.87±0.05	3.82±0.05
4	Acridine orange	15.53±0.05	2.82±0.05	20.76±0.05	3.78±0.05
5	Eosin Yellow	14.41±0.05	2.50±0.05	20.84±0.05	3.71±0.05
6	Light green	15.20±0.05	2.74±0.05	20.92±0.06	3.70±0.10

Table 16: Length of *Bombyx mori* (CSR2) Double Hybrid in the IVth & Vth instar larva (Mulberry leaves fortified with Probiotics and Vital dyes

S.no	Experiment	Day 1	Day 2	Day 3	Day 4	S.no	Experiment	Day 1	Day 2	Day 3	Day 4	Day-5	Day-6
1	Control	2.13±0.01	2.68±0.04	3.07±0.04	4.22±0.01	1	Control	4.15±0.05	4.46±0.05	5.08±0.05	5.67±0.04	6.28±0.03	6.89±0.01
2	Neutral red	2.60±0.01	3.12±0.02	3.63±0.01	4.02±0.01	2	Neutral red	4.14±0.06	4.45±0.08	5.07±0.01	5.65±0.00	6.25±0.01	6.88±0.05
3	Rhodamine-B	2.12±0.05	2.66±0.05	3.53±0.02	4.08±0.00	3	Rhodamine-B	4.15±0.05	4.46±0.05	5.08±0.05	5.67±0.04	6.28±0.03	6.89±0.01
4	Acridine orange	2.13±0.01	2.66±0.05	3.06±0.04	4.30±0.01	4	Acridine orange	4.16±0.07	4.46±0.06	5.09±0.05	5.67±0.05	6.29±0.04	6.90±0.02
5	Eosin yellow	2.12±0.01	2.66±0.05	3.07±0.04	4.00±0.01	5	Eosin yellow	4.14±0.05	4.45±0.05	5.08±0.05	5.67±0.04	6.28±0.03	6.89±0.02
6	Light green	2.60±0.01	3.12±0.02	3.63±0.01	4.02±0.01	6	Light green	4.15±0.06	4.46±0.05	5.08±0.05	5.68±0.04	6.29±0.04	6.89±0.01

Table 18: Weight of *Bombyx mori* (CSR2) Double Hybrid in the IVth & Vth instar larva (Mulberry leaves fortified with Probiotics and Vital dyes

S.no	Experiment	Day 1	Day 2	Day 3	Day 4	S.no	Experiment	Day 1	Day 2	Day 3	Day 4	Day-5	Day-6
1	Control	0.23±0.00	0.43±0.00	0.62±0.01	0.98±0.00	1	Control	1.13±0.00	1.32±0.00	2.56±0.01	3.94±0.03	4.09±0.00	3.49±0.02
2	Neutral red	0.23±0.03	0.40±0.06	0.74±0.00	0.89±0.01	2	Neutral red	1.17±0.00	1.25±0.0	2.63±0.02	3.47±0.03	4.09±0.00	4.06±0.03
3	Rhodamine-B	0.23±0.00	0.43±0.00	0.62±0.01	0.98±0.00	3	Rhodamine-B	1.13±0.00	1.27±0.00	2.65±0.01	3.61±0.02	4.13±0.00	4.06±0.03
4	Acridine orange	0.26±0.00	0.46±0.00	0.74±0.00	1.06±0.00	4	Acridine orange	1.14±0.00	1.47±0.00	2.55±0.03	3.72±0.04	4.15±0.00	3.94±0.05
5	Eosin yellow	0.25±0.00	0.45±0.00	0.72±0.00	0.93±0.01	5	Eosin yellow	1.15±0.00	1.28±0.01	2.58±0.01	3.30±0.02	4.11±0.00	3.49±0.02
6	Light green	0.25±0.00	0.45±0.00	0.72±0.00	0.93±0.01	6	Light green	1.31±0.00	1.37±0.01	2.69±0.02	3.92±0.02	4.11±0.00	3.87±0.03

Efficiency Conversion ingesta (ECI) of fourth & fifth instar larva recorded gradual increase as day Progresses with maximum of 3.23 % in Rhodamine-B treated group and 5.23% in light green treated group.(Table-9,10) Similar trend was observed in energy conversion of digesta (ECD) with 3.71 % observed in 3.71 on fourth of iv instar and 8.93 % on sixth day of v instar larva Higher concentration of ECI Percentage in Cocoon and shell in light green treated group, while ECD of Cocoon and shell control recorded maximum followed by treated groups. (Table -11, 12). Similar trend was observed in relative growth rate (Table- 13, 14).

Physical parameters of larva such as the length and weight reflect the general health of the larva. The result obtained only slight variation in dye treated group when compared to that of control animals. In fourth instar larva length varies between 2.12 – 4.30 cms and for fifth instar larva it lies between 4.14 – 6.90 cms (Table 17, 19). Weight of fourth and fifth instar larva recorded maximum weight of 1.06 gms and 4.06 respectively similar to nutritional indicas dye treated group recorded maximum length and weight. This indicates dye treatment supplemented with probiotic supplementation aagmented the uptake of feed clearly indicated in all dye treated groups.

Discussion

The silkworm fed with sprayed probiotic mulberry leaves fortified with dye from the fourth instar stage. Out of the five dyes used (Rhodamine B. Eosin yellow, Light green, Neutral red and Acridine orange) Rhodamine b and Acridine orange fed larvae showed a promising colour change along with improved nutritional indices like ingesta, digesta, approximate digestibility, reference ratio, efficiency of conversion of ingesta and efficiency of convertion of digesta. Their physiological parameters such as length and weight of the fourth and fifth instar larvae also have improved results when fortified with probiotics. This elucidates the efficacy of Neutral red to incorporate into the silkworm body (Anumole Anto, *et al*, 2017) [2]. It is in accordance with the earlier studies by Campbell (1932) [3] who reported a colour change of silkworm body on feeding neutral red added diet. Besides that, the silk gland also appeared in a dark red colour. Colour changes are observed in the silkworm body, haemolymph, silk gland, cocoon, pupa, moth and egg of the dye fed groups. The duration of life cycle, physical traits of the larva and cocoon were analyzed and the results showed no significant difference between the control and dye added diet fed groups. The findings suggested that, with the skillful utilization of vibrant colour dyes, a novelty in silk industry,to reduce produces large volumes of toxic wastewater as waste pollution.

A comparison between control and all dye fed groups has shown in tables. On the eighth day of V instar, the experimental groups fed with Eosin yellow, Acridine orange, Neutral red and Rhodamine B started to spin intrinsically coloured silk with yellow, orange, red and pink, Colours respectively. Colour of cocoon generated from light green fed group were pale greenish yellow and were almost similar to that of control cocoons. Initially each experimental group fed with 0.05 wt% of respective dye sprayed mulberry leaves fortified with probiotics fed group produced coloured cocoon

Conclusion

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, the investigations aimed to analyse the toxic effect of colours on the physiology of insects and biocompatibility. As colour is a peculiar epithet of silk fabrics, *in vivo* uptake through diet is the easier, economic, and green way to incorporate the colour dyes to silkworm silk. 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.

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