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RESEARCH ARTICLE

STUDIES ON THE GROWTH RATE OF SILKWORM *BOMBYXMORI* (L.)(LEPIDOPTERA: BOMYCIDAE) FED WITH CONTROL AND NATURAL DYE TREATED MR_2 MULBERRY LEAVES IN RELATION TO SILK PRODUCTION

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ABSTRACT

The silkworm, *Bombyxmori* being a monophagous insect, derives all the nutrients required for its growth from the mulberry leaves. The quality of silk produced by the silkworm depends on the quality and yield of mulberry leaf as well as environmental conditions. The larval and pupal parameters of silkworm *Bombyxmori* fed with vegetable dyes treated MR_2 mulberry leaves, the following works have been considered. The vegetable dye was treated on fresh mulberry leaves (*Morus alba L.*) were sprayed by each concentration and were fed to silkworm from 3^{rd} , 4^{th} and ,5^{th} instar for four feeding were recommended. Then, group T_2,T_3,T_4,$T_5$and control T_1 sprayed mulberry leaves with vegetable dye and distilled water, respectively. Silkworm larvae fed on *Morusalba (L.)* (MR_2) leaves sprayed with 1 gram indigo was significantly increased the larvae and cocoon length, width and weight, cocoon shell weight, pupal weight, shell ratio and silk filament length as compared to those fed on control (group T_1) MR_2 mulberry leaves and other groups (T_3,T_4 and T_5). It has been observed from the present study that 1 gram indigo treated (group T_2) leaves fed by silkworms have enhanced the larval and pupal growth and quantity of silk production than control.

INTRODUCTION

The silkworm *Bombyxmori*rearing is a traditional industry, Asia and the life of many people is depended on it. Increase of larval growth and cocoon quality would result better economics for this industry and meet the production needs. Consequently, the enrichment of mulberry leaves by supplement compounds with aim of increasing the production of cocoon is a very important aspect. Many investigations have been done on this topic and various reports have been published (Eteberi 2002; Etebari *et al.*, 2004; Islam *et al.*, 2004; Centhilnayaki 2004; Balasundaram *et al*., 2008 and Ganeshprabu *et al.*, 2012). India has a rich biodiversity and it is not only of the world’s twelve mega diversity countries but also one of the eight major centres of origin and diversification of domesticated taxa. It has approximately 490,000 plant species of which aout 17,5000 are angiosperms; more than 400 domesticated crop species and almost an equal number their wild relatives. India harbus a wealth of useful germ plasm resources and there is no doubt that the plant kingdom is a treasure-house of diverse natural products. One such product from nature is the dye. Dyes are one of the most important uses of the plants. In these times of ‘Holi’ festivals were safe because of the natural dyes were used but not harmful for the human body. But recent times a few cheaper chemical dyes are commonly used broadly in the market as an alternative of natural dyes. Which causes different health hazards like skin allergy, respiratory, kidney and liver diseases. Research has shown that the natural dyes are quite safe and environment friendly (Mohanta and Tiwari 2005). The leaves of *Morus*species are healthy and useful for growth of the silkworm larvae. The feeding of the sole source of the food for silkworm, *Bombyxmori*. (L.) is on mulberry leaves. Nutritional quality of leaves plays a vital role in determining the health and growth of larvae. The feeding of nutritionally enriched leaves showed better growth and development of silkworm larvae as well as directly influence on the quality and quantity of silk production. Nearly, 70% of the silk proteins produced by silkworm are directly derived from the protein of mulberry leaves. The silkworm larvae are highly sensitive and respond sharply to the changes of leaf quality. Supplementary nutrients are when added to normal food increases the nutritional value of the food making it more useful. In recent years, several attempts have been made to fortify leaves with different beneficial nutrients such as carbohydrates, proteins, amino acids, hormones, chemicals and salt and combination of nutrients to improve the quality of cocoon crop. Nutrients play an important role improving the growth and development of the silkworm *B.mori*like other organisms, Legay (1958), has stated that silk production is dependent on the larval nutrition and nutritive value of

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mulberry leaves play a very effective role in producing good quality cocoons. Seki and Oshikane (1959) have observed better growth and development of silkworm larvae as well as good quality cocoons when fed on nutritionally enriched leaves. Akhtar and Asghar, (1972) have found that vitamins and minerals and salt played an important role in the nutrition of silkworm. The present study has been aimed to find out feed efficacy of natural dyes treated MR_2mulberry leaves with regard to food utilization by larvae and ultimate impact on the cocoon parameters of silkworm so as to spot out the most nutritive are for bivoltine silkworm in Tamilnadu climatic conditions. This work is related to the studies on the growth rate of *B.mori* fed with control and natural dye treated MR_2 mulberry leaves. Therefore, this study has been carried out to know the impact of natural dye treated *B. mori.*

MATERIALS AND METHODS

The eggs of silkworm *B.mori* LN $B_{4,}$ D2 (Local Bivoltine) race were collected from farmers training centre at Jayankondampatinam, Tamilnadu, India. The eggs were placed in ambient temperature of 25±27^{o}c and relative humidity of 70to 80% in an incubator for hatching. After hatching, larvae were isolated from stock culture. The larvae were divided in to 5 experimental groups including controls (Distilled water control), each group consisting of 6 larvae. The larvae were reared in card board boxes measuring 22x5x15 cms covered with polythene sheet and placed in iron stand with ant wells. The larvae were subjected to the following treatments. Vegetable dyes treated mulberry leaves fed of *B.mori* (L). Indigo dye was diluted in distilled water. Fresh mulberry leaves were sprayed by each concentration and then dried in air for 10 minutes. The supplementary leaves were fed to silkworms for five feedings / day. Group T_1 larvae received mulberry leaves sprayed with distilled water and served as control group T_2 larvae received 1 gram (indigo) dye sprayed mulberry leaves. Group T_3 larvae larvaereceived 1gram (Desert) dye sprayed mulberry leaves. Group T_4 larvae received 1gram (Rhine-m) dye sprayed mulberry leaves. Group T_5 larvae received 1gram (sahara) dye sprayed mulberry leaves, respectively, and they were maintained up to cocoon, 3rd,4rd and 5th instar larvae length, width and weight, cocoon length, width and weigh were determined for all groups.

Preparation of natural dye

Natural dye powder were purchased from natural dye manufactures in Tamilnadu, India and to prepare the experimental dose for 1 gram concentration.

Mulberry *(M.alba)* MR_2 variety

This is one of the variety of mulberries selected from Jayamkondapattinam sericulture farm. Branches are simple vertical, grayish leaves are darkly green, unlobed, elliptic, palmate veined and leathers / smooth/wrinkled. It has good agronomy characters like light rootig ability (80%) natural dyes were diluted to add 100ml distilled water in 1gm dye in each group concentration, respectively. Fresh MR_2 mulberry leaves were soaked in each concentration for 15 minutes and then were dried in air for 10 minutes. The treated leaves were used for feeding the 3rd, 4th and 5th instars larvae of silkworm *B.mori*. They were maintained upto cocoon stage.

Experimental group

There are 5 experimental groups 3rd,4th and 5thinstars of *B.mori* larvae fed with the following treated MR_2 mulberry leaves group T_1 larvae fed with distilled water treated mulberry leaves. It serve as a control group T_2 larvae fed with 1gram indigo. Natural dye only indigo treated mulberry leaves group T_3, T_4, T_5 larvae fed were to silkworm. They were maintained up to cocoon stage.

RESULTS

Larval parameter

Table 1 shows that morphometric data of control MR_2 mulberry leaves and Vegetable dye treated MR_2 mulberry leaves fed 3rd instar of *B. mori* larvae length, width and weight. The mean value of control (group T_1) were (1.5202±0.1292cm, 0.3353±0.9832cm and 0.0884±0.0054gm), respectively. The mean value of 1g vegetable dye indigo treated (group T_2) were (1.8034±0.1543cm, 0.3653±0.3162cm and 0.1132±0.0153gm), respectively. The mean value of 1g vegetable dye desert treated (group T_3) were (1.7532±0.1425cm, 0.3430±0.0983cm and 0.1025± 0.0068gm), respectively. The mean value of 1g vegetable dye Rhine –m treated (group T_4) were (1.7428±0.1415cm, 0.3342±0.2000cm and 0.1028±0.0053gm), respectively. The mean value of 1g vegetable dye saharatreated (group T_5) were (1.6538±0.1328cm, 0.3240±0.2065cm and 0.1018±0.0048gm), respectively. In these five observations, 1g vegetable dye indigo (group T_2) treated 3rd instar larvae length, width and weight was significantly increased than control (T_1) and other three groups ($T_{3,}T_4$ and T_5).

Table 1. Morphometric data of various concentration of vegetable dye treated with MR_2 mulberry leaves on the 3rd instars larvae length, width and weight of *Bombyxmori*

Group	Length	Width	Weight
Control (C) (Distilled water)	15202±0.1292^a	0.3353±0.9832^a	0.0884±0.0054^a
MR_2 Mulberry + 1 g (Indigo) (T_1)	1.8034±0.1543^b	0.3653±0.3162^b	0.1132±0.0153^b
MR_2 Mulberry + 1 g (Desert) (T_2)	1.7532±0.1425^{ab}	0.3430±0.0983^b	0.1025±0.0068^{ab}
MR_2 Mulberry + 1 g (Rhine-m) (T_3)	1.7428±0.1415^{ab}	0.3342±0.2000^a	0.1028±0.0053^{ab}
MR_2 Mulberry + 1 g (Sahara) (T_4)	1.6538±0.1328^a	0.3240±0.2065^a	0.1018±0.0048^a

Values are Mean ± Standard Deviation of six observations. Values in the same column with different superscript letters (a, b & c) differs significantly at $P<0.05$ (DMRT)

Table 2 shows that morphometric data of control MR_2 mulberry leaves and Vegetable dye treated MR_2 mulberry leaves fed 3rd instar of *B. mori* larvae length, width and weight. The mean value of control (group T_1) were (5.0462±0.1721cm, 0.4532±0.1329cm and 0.3132±0.0120m), respectively. The mean value of 1g vegetable dye indigo treated (group T_2) were (5.9152±0.2650cm, 0.6052±0.1366cm and 0.5030±0.0213gm), respectively. The mean value of 1g vegetable dye desert treated (group T_3) were (5.4540±0.1960cm, 0.5821±0.2258cm and 0.4740± 0.0192m), respectively. The mean value of 1g vegetable dye Rhinetreated (group T_4) were

(5.2640±0.1952cm, 0.5752±0.0836cm and 0.4650±0.0182gm), respectively. The mean value of 1g vegetable dye saharatreated (group T_5) were (5.1460±0.1882cm, 0.5632±0.4082cm and 0.4440±0.0153gm), respectively. In these five observations, 1g vegetable dye indigo (group T_2) treated 3rd instar larvae length, width and weight was significantly increased than control (T_1) and other three groups (T_3, T_4 and T_5).

Table 2. Morphometric data of various concentration of vegetable dyes treated with MR_2 mulerry leaves on the 4th instars larvae length, width and weight of *Bombyxmori*

Group	Length	Width	Weight
Control (C) (Distilled water)	5.0462±0.1721^a	0.4532±0.1329^a	0.3132±0.0120^a
MR_2 Mulberry + 1 g (Indigo) (T_1)	5.9152±0.2650^b	0.6052±0.1366^c	0.5030±0.0213^b
MR_2 Mulberry + 1 g (Desert) (T_2)	5.4540±0.1960^{ab}	0.5821±0.2258^b	0.4740±0.0192^{ab}
MR_2 Mulberry + 1 g (Rhine-m) (T_3)	5.2640±0.1952^{ab}	0.5752±0.0836^{bc}	0.4650±0.0182^{ab}
MR_2 Mulberry + 1 g (Sahara) (T_4)	5.1460±0.1882^a	0.5632±0.4082^{ab}	0.4440±0.0153^a

Values are Mean ± Standard Deviation of six observations. Values in the same column with different superscript letters (a, b & c) differs significantly at P<0.05 (DMRT)

Table 3 shows that morphometric data of control MR_2 mulberry leaves and Vegetable dye treated MR_2 mulberry leaves fed 3rd instar of *B. mori* larvae length, width and weight. The mean value of control (group T_1) were (5.4175±0.1833cm, 1.0554±0.0836cm and 2.6360±0.1020gm), respectively. The mean value of 1g vegetable dye indigo treated (group T_2) were (6.1450±0.2767cm, 1.1025±0.2160cm and 3.3553±0.2269gm), respectively. The mean value of 1g vegetable dye desert treated (group T_3) were (6.0240±0.2287cm, 1.0705±0.0983cm and 3.2804± 0.1502gm), respectively. The mean value of 1g vegetable dye Rhine–m treated (group T_4) were (5.8765±0.2210cm, 1.0608±0.1169cm and 3.0220±0.1228gm), respectively. The mean value of 1g vegetable dye saharatreated (group T_5) were (5.5126±0.2112cm, 1.0509±0.2345cm and 3.0112±0.1122gm), respectively. In these five observations, 1g vegetable dye indigo (group T_2) treated 3rd instar larvae length, width and weight was significantly increased than control (T_1) and other three groups (T_3, T_4 and T_5).

Table 3. Morphometric data of various concentration of vegetable dyes treated with MR_2 mulerry leaves on the 5th instars larvae length, width and weight of*Bombyxmori*

Group	Length	Width	Weight
Control (C) (Distilled water)	5.4175±0.1833^a	1.0554±0.0836^a	2.6360±0.1020^a
MR_2 Mulberry + 1 g (Indigo) (T_1)	6.1450±0.2767^b	1.1025±0.2160^c	3.3553±0.2269^b
MR_2 Mulberry + 1 g (Desert) (T_2)	6.0240±0.2287^{ab}	1.0705±0.0983^{ab}	3.2804±0.1502^{ab}
MR_2 Mulberry + 1 g (Rhine-m) (T_3)	5.8765±0.2210^{ab}	1.0608±0.1169^b	3.0220±0.1228^{ab}
MR_2 Mulberry + 1 g (Sahara) (T_4)	5.5126±0.2112^a	1.0509±0.2345^a	3.0112±0.1122^a

Values are Mean ± Standard Deviation of six observations. Values in the same column with different superscript letters (a, b & c) differs significantly at P<0.05 (DMRT)

Cocoon Parameters

Table 4 shows the morphometric data of mean length, width and weight of the cocoon of *B.mori* fed with Vegetable dye treated MR_2 leaves were found to be more than that of the larvae fed with control MR_2 leaves. The length, width and weight of the T_1 larvae produced cocoon were found to be about (3.0810±0.1542cm, 2.2133±0.2190cm and 1.4140±0.1507gm), respectively. The length, width and weight of the T_2 larvae produced cocoon were found to be about (3.5562±0.2532cm, 2.3056±0.1472cm and 2.0112±0.3110gm), respectively. The length, width and weight of the T_3 larvae produced cocoon were found to be about (3.3700±0.2325cm, 2.2542±0.3076cm and 1.8750±0.2433gm), respectively. The length, width and weight of the T_4 larvae produced cocoon were found to be about (3.2850±0.2150cm, 2.2520±0.2316cm and 1.7475±0.2100gm), respectively. The length, width and weight of the T_5 larvae produced cocoon were found to be about (3.1950±0.2014cm, 2.2520±0.0894cm and 1.6423±0.2020gm), respectively. In these five observations, the 1g vegetable dye indigo (group T_2) treated larvae produced cocoon length, width and weight were significantly increased than control (T_1) and other three groups (T_3, T_4 and T_5).

Table 4. Morphometric data of various concentration of vegetable dyes treated with MR_2 mulberry leaves on the Cocoon length, width and weight of *Bombyxmori*

Group	Length	Width	Weight
Control (C) (Distilled water)	3.0810±0.1542^a	2.2133±0.2190^a	1.4140±0.1507^a
MR_2 Mulberry + 1 g (Indigo) (T_1)	3.5562I±0.2532^b	2.3056±0.1472^b	2.0112±0.3110^b
MR_2 Mulberry + 1 g (Desert) (T_2)	3.3700±0.2325^{ab}	2.2542±0.3076^{ab}	1.8750±0.2433^{ab}
MR_2 Mulberry + 1 g (Rhine-m) (T_3)	3.2850±0.2150^{ab}	2.2520±0.2316^{ab}	1.7475±0.2100^{ab}
MR_2 Mulberry + 1 g (Sahara) (T_4)	3.1950±0.2014^a	2.2520±0.0894^{ab}	1.6423±0.2020^a

Values are Mean ± Standard Deviation of six observations. Values in the same column with different superscript letters (a, b & c) differs significantly at P<0.05 (DMRT)

Pupal Parameters

Table 5 shows the morphometric data of control MR_2 mulberry leaves and Vegetable dye treated MR_2 mulberry leaves were found to be more than that of larvae produced, cocoon shell and pupal weight. The mean value of control (T_1) were (0.3550±0.0125gm and 1.0244±0.1246gm), respectively. The mean value of 1g vegetable dye indigo treated group(T_2) were (0.4445±0.0212gm and 1.5885±0.0945gm), respectively. The mean value of 1g vegetable dye desert treated group(T_3) were (0.4170±0.0182gm and 1.4780±0.0865gm), respectively. The mean value of 1g vegetable dye Rhine –m treated group (T_4) were (0.4025±0.0162gm and 1.3454±0.0855gm), respectively. The mean value of 1g vegetable dye saharatreated (group T_5) were (0.4010±0.0135gm, and 1.3250±0.0789gm), respectively. In these five observations, 1g vegetable dye indigo (group T_2) treated larvae produced cocoon shell and pupal weight was significantly increased than control (T_1) and other three groups (T_3, T_4 and T_5).

Silk Traits

Table 6 shows that the morphometric data of control MR_2 mulberry leaves and vegetable dyetreated MR_2 mulberry leaves fed *B.mori* larvae produced cocoon shell ratio (%) and silk filament length (meters). The mean value of control (group T_1) were (12.6555 ± 0.1525% and 751 ± 1.1mtrs), respectively.

Table 5. Morphometric data of control and vegetables dyes treated *Bombyxmori* larvae producedcocoon shell and pupal weight

Group	Cocoon shell weight (gm) (Mean ± S.D)	Pupal weight (gm) (Mean ± S.D)
Control (C) (Distilled water)	0.3550±0.0125^{a}	1.1244±0.0246^{a}
MR_2 Mulberry + 1 g (Indigo) (T_1)	0.4445±0.0212^{b}	1.5885±0.0945^{b}
MR_2 Mulberry + 1 g (Desert) (T_2)	0.4170±0.0182^{ab}	1.4780±0.0865^{ab}
MR_2 Mulberry + 1 g (Rhine-m) (T_3)	0.4025±0.0162^{ab}	1.3454±0.0855^{ab}
MR_2 Mulberry + 1 g (Sahara) (T_4)	0.4010±0.0135^{a}	1.3250±0.0789^{a}

Values are Mean ± Standard Deviation of six observations. Values in the same column with different superscript letters (a, b & c) differs significantly at P<0.05 (DMRT)

The mean value of 1g vegetable dye indigo treated group (T_2) were (17.1645±0.2375% and 890±6.5mtrs), respectively. The mean value of 1g vegetable dye desert treated group (T_3) were (15.4623±0.2329% and 880±2.5mtrs), respectively. The mean value of 1g vegetable dye Rhine –m treated group (T_4) were (14.2452±0.2178% and 829±5.3mtrs), respectively. The mean value of 1g vegetable dye saharatreated (group T_5) were (13.2255±0.2168%, and 767±1.2mtrs), respectively. In these five observations, 1g vegetable dye indigo (group T_2) treated larvae produced cocoon shell ratio (%) and silk filament length (mtrs) was significantly increased than control (T_1) and other three groups ($T_{3,}T_4$ and T_5).

Table 6. Morphometric data of control and vegetables dyes treated *Bombyxmori* larvae produced cocoon shell and pupal weight

Group	Shell ratio (%) (Mean ± S.D)	Silk filament (m) (Mean ± S.D)
Control (C) (Distilled water)	12.6555±0.1525^{a}	785.1855±16.5525^{a}
MR_2 Mulberry + 1 g (Indigo) (T_1)	17.1645±0.2375^{b}	856.5784±24.1555^{b}
MR_2 Mulberry + 1 g (Desert) (T_2)	15.4623±0.2629^{ab}	842.7124±21.2372^{ab}
MR_2 Mulberry + 1 g (Rhine-m) (T_3)	14.2452±0.2178^{ab}	822.5114±19.5325^{ab}
MR_2 Mulberry + 1 g (Sahara) (T_4)	13.2255±0.2168^{a}	810.6524±18.5245^{a}

Values are Mean ± Standard Deviation of six observations. Values in the same column with different superscript letters (a, b & c) differs significantly at P<0.05 (DMRT)

DISCUSSION

In the present study, the larval and cocoon length, width and weight were significantly increased in some groups. Many researchers showed that the larval characters improve by different concentrations of complementary compounds such as ascorpic acid, folic acid, thiamin, Vitamin B complex etc., (Sarker *et al*., 1995; Nirwani and Kaliwal, 1996, 1998; Etaberi *et al*., 2004; Balasundaram *et al*., 2008). Muniandy *et al*., 1995 have showed that multi-vitamins and mineral compounds could increase the food intake, growth and conversion efficiency of silkworm In the present study, it has been observed that silkworms fed by the natural dye have enhanced the larval length, width and weight and cocoon characters were concomitantly increased from 3rd to 5th instars ,suggested that natural dye which were stimulate silkworm to feed more amount of nutrient intake than the control. This work is corroborated with Nirwani and Kaliwal (1996), suggested that this enhancement in larval and cocoon length, width and weight related to phago stimulation of folic acid. Several authors have also reported these effects about ascorbic acid (Dobzshenok, 1974; Ito, 1978; Singh and Reddy, 1981; Kl-karkasy and Idriss, 1990). Since most of this multi-vitamin a compound is consists of ascorbic acid, it could be thought that the increase of larval weight is due to an enhancement of feeding activity. Therefore, natural dye can improve the food digestibility and increase the larval, cocoon and pupal parameters. The enrichment of mulberry leaves with natural dye increase larval and cocoon length, width and weight increase in these insects was related to metabolism other than proteins. It is assumed that fortification of diet supports the metabolism of carbohydrates and lipids, in conclusion, natural dye could increase some biological characteristics in silkworm, but this enhancement could economically improve the Sericulture goals. Natural dyes of which are less toxic, less polluting, less health hazards, non-carcinogenic and non-poisonous. Added to this, they are harmonizing colours, gentle, soft and subtle, and create a restful effect. Above all, they are environment friendly and can be recycled after use. In the present study, the treatment of natural dye at the concentration of 1 gram indigo natural dye may have beneficial effects on the growth of the silkworm larval and pupal width and weight and also increased the quantity of silk production by enhancing the feed efficacy than control. So this supplementation could be prescribed to the farmers to get more quantity of silk.

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