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RESEARCH ARTICLE :

Strengthening of sericulture industry through fortification of mulberry leaves to enhance commercial cocoon characteristics of silkworm

N. MALA, FATIMA SADATILLA AND S. HARISH BABU

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SUMMARY : Studies on strengthening of Sericulture though fortification of mulberry leaves with aqueous extracts of medicinal plants on larval growth, development and commercial cocoon traits of silkworm, *Bombyx mori* L. were conducted during 2013-2014. The silkworm ($PMxCSR_2$) reared on mulberry leaves fortified with aqueous extracts of *Aloe vera* and *Tinospora cordifolia* at eight different concentrations, *Aloe vera* at 100 % concentration had effective enhancement of larval weight (3.37g), ERR (96.33%), cocoon weight (2.01g), shell weight (0.375g), pupal weight (1.64g), shell ratio (18.66%), silk productivity (4.83cg day^{-1}), filament length (883.95m) and denier (2.41) besides reduced larval duration (7.76 days) and disease incidence (3.67%) when compared to other treatments and control. The plant extracts in higher concentration yielded beneficial effects rather in lesser concentration. This study helps to improve the quality of silkworm nutrition so as to get sustainable cocoon production

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KEY WORDS :
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Author for correspondence :

N. MALA
Department of Sericulture, University of Agricultural Sciences, G.K.V.K., BENGALURU (KARNATAKA) INDIA

See end of the article for authors' affiliations

BACKGROUND AND OBJECTIVES

Sericulture is a unique field of agriculture where silkworms are reared on an extensive scale to produce the fine material of clothing. Sericulture is deep rooted in the culture and tradition of Indian society and has been considered as an economically viable, labour intensive agro- industry in the rural area. Silk is the most elegant textile in the world with unparalleled grandeur, natural sheen, and inherent affinity for dyes, high absorbance, light weight, soft touch and high durability and known as the "Queen of Textiles" the world over. On the other hand, it stands for livelihood opportunity for millions owing to high employment oriented, low capital intensive and remunerative nature of its production. The very nature of this industry with its rural based on- farm and off-farm activities and enormous employment generation potential has attracted the attention of the planners and policy makers to recognize the industry as among one of the most appropriate avenues for socio-economic development of a largely agrarian economy like India.

India is the second largest producer of

silk in the world. The total production of raw silk in India was 26,467 MT in 2014-15 of which, mulberry raw silk output aggregated to 21,272 MT (74.73%), tasar silk 2,404 MT (8.44%), eri silk 4,633 MT (16.27%) and muga silk 158 MT (0.55%) (*Annual report*, CSB, 2015). Though, India ranks second next only to China in raw silk production, the production of raw silk is insufficient to meet the domestic demand due to low productivity coupled with inferior silk quality. Hence, the country is forced to import raw silk. Under these circumstances, there is a need for a strategic approach to improve production of raw silk both in terms of quality and quantity. The knowledge of nutritional requirements of silkworm is of much importance from the point of cocoon and silk production. Mulberry leaf is sole food of the silkworm on commercial scale. The mulberry leaf consumption, digestion and utilization will have a greater impact on the silkworm growth and development and inturn will reflect on the quality of the cocoon and silk produced.

In recent years, after realizing the importance of mulberry nutrition, it is serving as a tool for quality leaf and sustainable cocoon production (Narayanaswamy and Shankar, 2003). Many attempts have been made either to fortify the leaves with nutrients, antibiotics, juvenile hormones, plant products with JH-mimic principles, anti-juvenile hormones or dusting with botanicals or extrafoliation of mulberry leaf with sugars, vertebrate hormones, feed additives, protein rich flours etc. so as to improve the silkworm rearing, cocoon and silk quality. Among several options, use of plant extracts is appropriate for the current scenario because of their cost effectiveness as they are available locally and are ecofriendly. In the present study, mulberry leaves were fortified with the plant extracts along with biologically active principles from two medicinal plants namely *Aloe vera* and *Tinospora cordifolia* to test their potential in improving the cocoon characters of silkworm.

RESOURCES AND METHODS

The experiment was conducted at the Department of Sericulture, University of Agricultural Sciences, Bengaluru.

Silkworm rearing :

The worms were reared by feeding three times a day (8.30 A.M., 12.30 P.M. and 5.30 P.M.) with tender mulberry leaves. Bed cleaning was done once, twice and thrice during I, II, and III instars, respectively, whereas daily once during IV and V instars. During rearing, optimum spacing was provided according to the age of silkworm, *i.e.*, after each bed cleaning. Lime powder was dusted on silkworms before settling for each moult so as to keep the bed dry and facilitate easy moulting. Hundred worms were separated and maintained in trays for different treatments @ 3 replications per plant extract. The treatments were imposed for the first feed after every moult and also imposed for the first feed on 4th day of fifth instar. The remaining feeds were provided with normal mulberry leaves. Control (feeding without plant extracts) was also maintained.

Preparation of aqueous extracts of the plants used for the study :

Preparation of aqueous extract of Aloe vera :

The extracts from *Aloe vera* were prepared as per procedure adopted by Krishnaprasad *et al.* (1979). The required quantity of fresh green leaves of *Aloe vera* was collected from "Sanjeevini vatika" (Herbal Garden), Division of Horticulture, UAS, Bengaluru and surface sterilized with 70 % ethyl alcohol and then washed with sterile distilled water and slit open longitudinally, the white gel was scooped with sterile stainless steel knife and homogenized in a domestic mixi and filtered through a sterile stainless steel tea strainer and refrigerated as stock solution (100 % gel) for further use. The extract was squeezed through double layered muslin cloth and the extract collected was used as stock solution. From this stock solution the required concentrations (25, 50, 75 and 100%) were prepared by using distilled water.

Preparation of aqueous extract of Tinospora cordifolia :

The shoots (leaves and stem) of *Tinospora cordifolia* were brought to the laboratory from "Sanjeevini vatika" (Herbal Garden), Division of Horticulture, UAS, Bengaluru and cut at a length of three feet from tip downwards and surface sterilized with 70 % ethyl alcohol and then washed with sterile distilled water. The excess water was allowed to evaporate under fan. Known quantity (100g) of leaves and stem of this material was crushed in known quantity of distilled water (100ml) on weight by volume basis (1:1 proportion) using an electrically operated mixer. The crushed material was

filtered through a muslin cloth and the filtrate was maintained as stock solution (100%). From this stock solution the required concentrations (2, 4, 6 and 8%) were prepared by using distilled water (Patil *et al*., 1997 and Mane, 1998). Every time fresh extract was prepared and used. The respective concentrations thus prepared were smeared using a sterile cotton wad on both the sides of mulberry leaves and shade dried before feeding to silkworms. The control batch was maintained without any treatment.

OBSERVATIONS AND ANALYSIS

This study showed that fortification of mulberry leaves with plant extracts namely, *Aloe vera* and *Tinospora cordifolia* were found beneficial in improving the rearing and commercial cocoon characteristics of PM x CSR_2 silkworm breed when compared to control. The rearing and cocoon characters studied in the present work are as follows.

Effect of plant extracts on rearing parameters :

Fifth instar larval duration (days) :

Among *Aloe vera* gel and *Tinospora cordifolia* shoot extracts at different concentrations tested, the fifth instar larval duration under study was minimum in 100 % *Aloe vera* (7.76 days) followed by 75 % *Aloe vera* (7.81 days), 50 % *Aloe vera* (7.87 days), 25 % *Aloe vera* (7.93 days), 8 % of *Tinospora cordifolia* (7.94 days) and the effect was same with both 4 and 6 % of *Tinospora cordifolia* (7.97 days). Whereas, a longer fifth instar larval duration was recorded in control (8.04 days) (Table 1). The present findings are in accordance with Murari *et al*. (2008) who reported that the application of *C. sativus* extract to fifth instar larvae significantly reduced the larval duration (6.89 days) compared to other treatments.

Fifth instar larval weight (g) :

It is evident from the results that larval weight was significantly higher (3.37 g) in 100 % of *Aloe vera* followed by 75 % (3.32 g) *Aloe vera*, 50 % (3.28 g) *Aloe vera*, 25 % (3.25 g) *Aloe vera*, 8 % (3.19 g) *Tinospora cordifolia*, 6 % (3.17 g) *Tinospora cordifolia*, 4 % (3.09 g) *Tinospora cordifolia* and 2 % (3.06 g) *Tinospora cordifolia*, respectively. The minimum larval weight was recorded in control (3.02 g) (Table 1) (Fig. 1). The increase in larval weight is due to the enhancement of bio-availability of nutrients for digestibility and phagostimulant properties of various biochemical constituents in *Aloe vera* that are beneficial to insects and also for their better growth and development (Ravi Kumar, 2006).

Effective rate of rearing (ERR) (%) :

Use of *Aloe vera* and *Tinospora cordifolia* extracts at different concentrations to the silkworms increased the survival rate. The maximum ERR was recorded in 100 % (96.33 %) *Aloe vera* followed by 75 % (94.34 %) *Aloe vera*, 50 % (93.66 %) *Aloe vera*, 25 % (93.52 %) *Aloe vera*, 8 % (92.66 %) *Tinospora cordifolia*, 6

Table 1 : Effect of mulberry leaves fortified with phyto extracts on fifth instar larval duration (days), mature larval weight (g), effective rate of rearing (ERR %) and larval mortality (%) of silkworm

Treatments	Fifth instar larval duration (days)	Mature larval weight (g)	Effective rate of rearing (ERR %)	Larval mortality (%)
T_1-25 % *Aloe vera*	7.93	3.25	93.52	6.48
T_2-50 % *Aloe vera*	7.87	3.28	93.66	6.34
T_3-75 % *Aloe vera*	7.81	3.32	94.34	5.66
T_4-100 % *Aloe vera*	7.76	3.37	96.33	3.67
T_5-2 % *Tinospora cordifolia*	8.01	3.06	91.01	8.89
T_6-4 % *Tinospora cordifolia*	7.97	3.09	91.66	8.33
T_7-6 % *Tinospora cordifolia*	7.97	3.17	92.00	8.00
T_8-8 % *Tinospora cordifolia*	7.94	3.19	92.66	7.33
T_9-Control	8.04	3.02	89.46	10.54
F-test	*	*	*	*
S.E.±	0.052	0.022	0.183	0.049
C.D. (P=0.05)	0.156	0.065	0.544	0.147

* indicates significance of value at P > 0.05

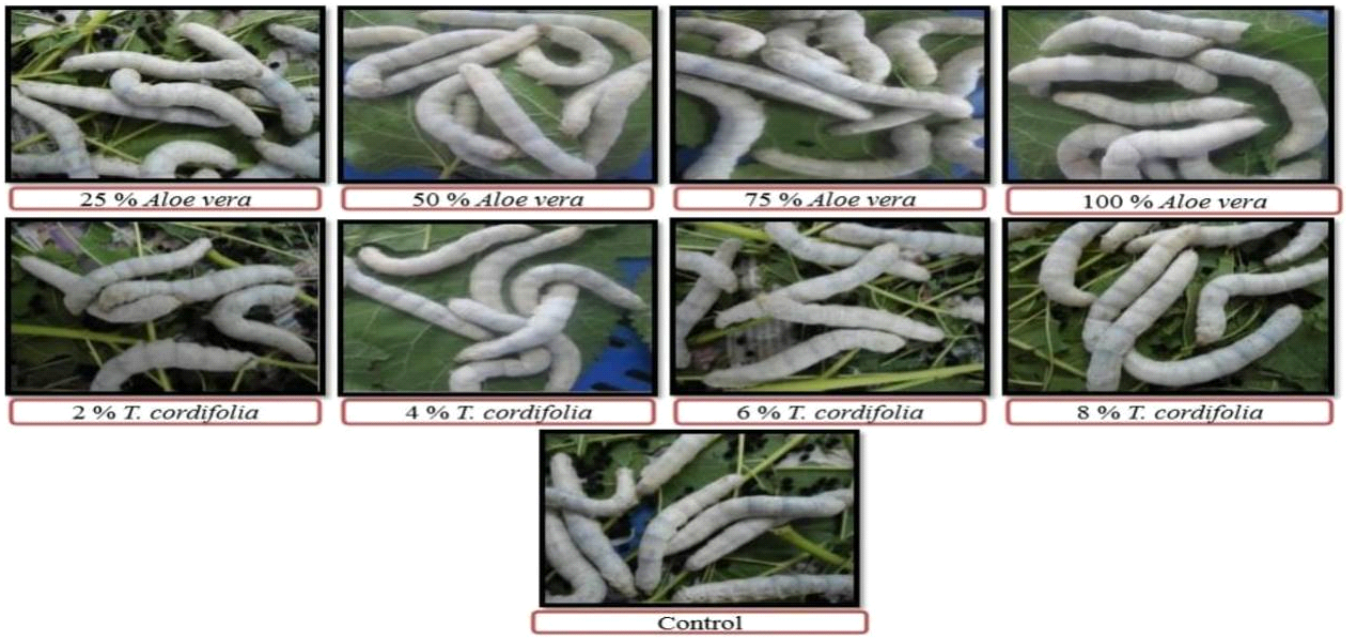

Fig. 1 : Effect of feeding different concentrations of *Aloe vera* and *Tinospora cordifolia* to PM x CSR_2 silkworm of larva

% (92.00 %) *Tinospora cordifolia,* 4 % (91.66 %) *Tinospora cordifolia* and 2 % (91.01 %) *Tinospora cordifolia,* respectively. Whereas, minimum ERR was observed in control (89.46 %) (Table1). The increased ERR observed in the present study may be due to reduction in disease incidence and also the presence of biochemical constituents in phyto extracts that may have contributed to the phagostimulant properties that resulted in increased intake, digestion assimilation of nutrients and inturn the silkworm survival rate. These results are in close conformity with Harish Babu *et al.* (2011), who reported that administration of *Aloe vera* extract (100% gel) to silkworm increased the larval survival (96.37%) by reducing the disease incidence compared to control (86.50%).

Larval mortality (%) :

Use of *Aloe vera* and *Tinospora cordifolia* extracts had significant influence in reducing the incidence of mortality, where 100 % (3.67 %) *Aloe vera* recorded lower mortality followed by 75 % (5.66 %) *Aloe vera,* 50 % (6.34 %) *Aloe vera,* 25 % (6.48 %) *Aloe vera,* 8 % (7.33 %) *Tinospora cordifolia,* 6 % (8.00 %) *Tinospora cordifolia,* 4 % (8.33 %) *Tinospora cordifolia* and 2 % (8.89 %) *Tinospora cordifolia* and the same was maximum (10.54 %) in control (Table 1). The reduced mortality could be due to the suppression of disease causing pathogens by the antiseptic and antifungal properties of *Aloe vera* plant gel as reported by Fatima *et al.* (2008).

Effect of plant extracts on cocoon parameters :

Cocoon weight (g) :

Cocoon weight showed significant differences after the worms were fed with mulberry leaves fortified with *Aloe vera* and *Tinospora cordifolia* extracts at different concentrations. 100 % concentration of *Aloe vera* exhibited increase in the cocoon weight registering 2.01g and the next best ones were 75 % (1.96 g) and 50 % (1.92 g) of *Aloe vera* followed by 25 % (1.89 g) *Aloe vera,* 8 % (1.87 %) *Tinospora cordifolia,* 6 % (1.84 %) *Tinospora cordifolia,* 4 % (1.82 %) *Tinospora cordifolia* and 2 % (1.80 %) *Tinospora cordifolia*. The lowest cocoon weight was noticed in control (1.63 g) (Table 2) (Fig 2). It could be due to phyto extracts containing many biochemical factors that contribute to improvement in digestibility and immunity resulting in the enhancement of cocoon weight. Sujatha and Rao (2003) studied that the application of *C. longa* stem extract on fourth instar larvae resulted in higher cocoon weight.

Pupal weight (g) :

The pupal weight was significantly enhanced when *Aloe vera* and *Tinospora codifolia* fortified mulberry

Table 2 : Effect of mulberry leaves fortified with phyto extracts on cocoon weight (g), pupal weight (g), shell weight (g) and shell ratio (%) of silkworm

Treatments	Cocoon weight (g)	Pupal weight (g)	Shell weight (g)	Shell ratio (%)
T_1-25 % *Aloe vera*	1.89	1.56	0.340	17.98
T_2-50 % *Aloe vera*	1.92	1.58	0.346	18.02
T_3-75 % *Aloe vera*	1.96	1.61	0.360	18.37
T_4-100 % *Aloe vera*	2.01	1.64	0.375	18.66
T_5-2 % *Tinospora cordifolia*	1.80	1.47	0.325	18.06
T_6-4 % *Tinospora cordifolia*	1.82	1.49	0.330	18.13
T_7-6 % *Tinospora cordifolia*	1.84	1.51	0.335	18.21
T_8-8 % *Tinospora cordifolia*	1.87	1.54	0.336	17.96
T_9-Control	1.63	1.36	0.265	16.26
F-test	*	*	*	*
S.E.±	0.011	0.052	0.005	0.113
C.D. (P=0.05)	0.034	0.154	0.016	0.335

*indicates significance of value at P > 0.05

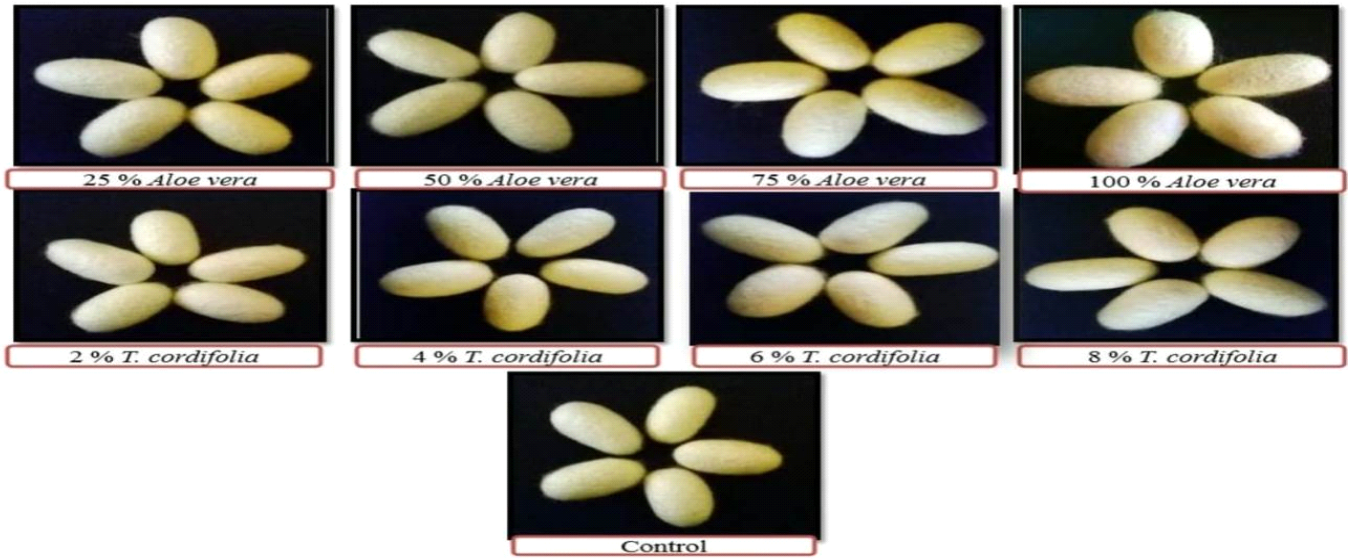

Fig. 2 : Effect of feeding different concentrations of *Aloe vera* and *Tinospora cordifolia* to PM CSR_2 silkworm on cocoon

leaves were fed to the silkworms. The pupal weight was maximum in 100 % (1.64 g) *Aloe vera* followed by 75 % (1.61 g) *Aloe vera*, 50 % (1.58 g) *Aloe vera*, 25 % (1.56 g) *Aloe vera*, 8 % (1.54 g) *Tinospora cordifolia*, 6 % (1.51 g) *Tinospora cordifolia*, 4 % (1.49 g) *Tinospora cordifolia* and 2 % (1.47 g) *Tinospora cordifolia* found at par with each other and the minimum pupal weight was recorded in control (1.36 g) (Table 2) (Fig. 3). The present findings are in agreement with Harish Babu *et al.* (2011) who reported that mulberry leaves administered with *Aloe vera* extract (100% gel) when supplied to the silkworms has resulted in significant increase in pupal weight (1.59g) over control (1.52g).

Shell weight (g) :

Shell weight showed significant increase after the worms were fed with mulberry leaves supplemented with *Aloe vera* and *Tinospora cordifolia* extracts at different concentrations. Where 100 % *Aloe vera* recorded higher shell weight (0.375 g) followed by 75 % (0.360 g) *Aloe vera*, 50 % (0.346 g) *Aloe vera*, 25 % (0.340 g) *Aloe vera*, 8 % (0.336 g) *Tinospora cordifolia*, 6 % (0.335 g) *Tinospora cordifolia*, 4 % (0.330 g) *Tinospora cordifolia* and 2 % (0.325 g) *Tinospora cordifolia* compared to control which recorded lower shell weight of 0.265 g (Table 2). The improvement in shell weight with phyto extracts may be due to increased nutritional

efficiency of food which is utilized for the maximum silk protein content of the cocoon shell. The results are in conformity with the findings of Fatima *et al.* (2008) who reported that maximum shell weight was recorded due to the application of *Aloe vera* gel extract (100% gel) (0.26g) compared to control (0.24g). Mahesha (1999) reported that maximum shell weight due to the application of *P. hysterophorus* and *T. procumbens.*

Shell ratio (%) :

Application of different concentrations of *Aloe vera* and *Tionospora cordifolia* extracts influenced shell ratio significantly. Where 100 % concentration (18.66 %) of *Aloe vera* recorded highest shell ratio followed by 18.37, 18.21, 18.13, 18.06, 18.02, 17.98 and 17.96 % for 75 % *Aloe vera*, 6 % *Tinospora cordifolia*, 4 % *Tinospora cordifolia*, 2 % *Tinospora cordifolia*, 50 per cent % *Aloe vera*, 25 % *Aloe vera* and 8 % *Tinospora cordifolia*, respectively compared to control (16.26 %) (Table 2). The increased shell ratio may be due to increased shell weight which was influenced by the nutrients in the food supplied. This could have probably

Table 3 : Effect of mulberry leaves fortified with phyto extracts on silk productivity (cg/day), filament length (m) and denier of silkworm

Treatments	Silk productivity (cg/day)	Filament length (m)	Denier
T_1-25 % *Aloe vera*	4.28	812.52	2.49
T_2-50 % *Aloe vera*	4.40	848.84	2.47
T_3-75 % *Aloe vera*	4.61	864.56	2.45
T_4-100 % *Aloe vera*	4.83	883.95	2.41
T_5-2 % *Tinospora cordifolia*	4.06	779.14	2.62
T_6-4 % *Tinospora cordifolia*	4.14	782.12	2.60
T_7-6 % *Tinospora cordifolia*	4.20	794.45	2.56
T_8-8 % *Tinospora cordifolia*	4.23	812.20	2.51
T_9-Control	3.30	760.94	2.72
F-test	*	*	*
S.E.±	0.072	1.614	0.045
C.D. (P=0.05)	0.214	4.796	0.131

** p > 0.05*

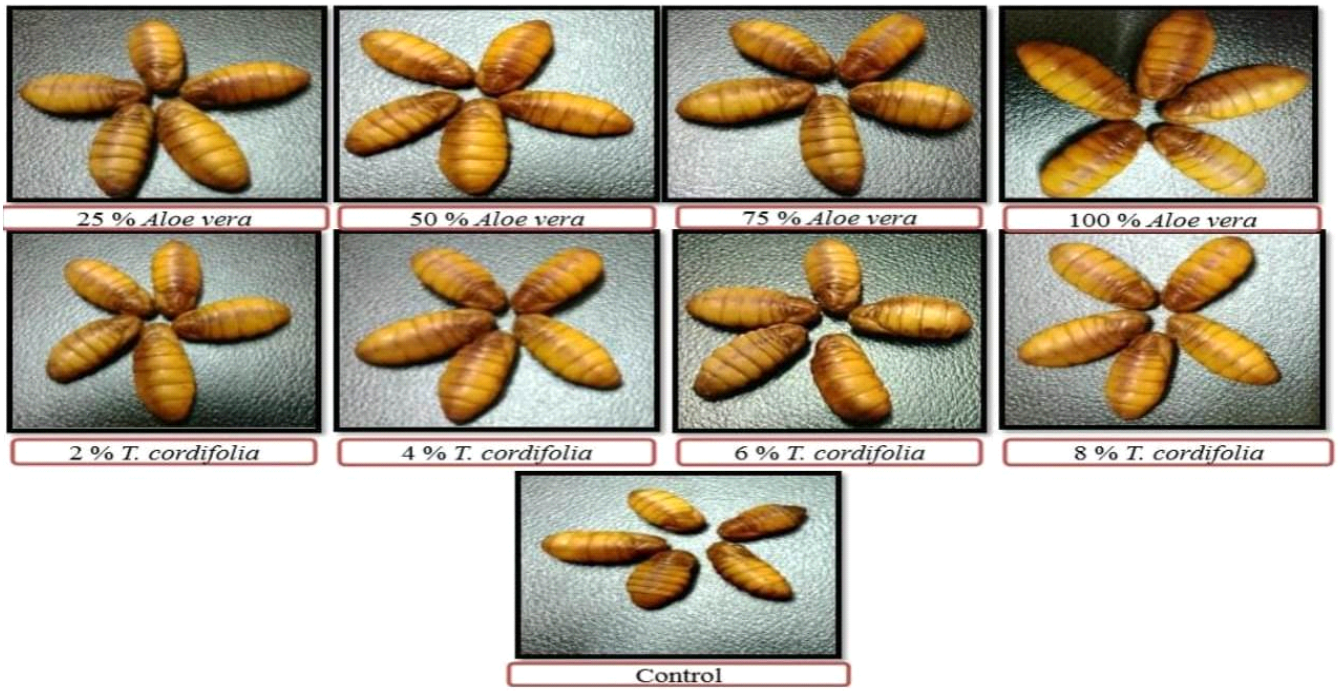

Fig. 3 : Effect of feeding different concentrations of *Aloe vera* and *Tinospora cordifolia* to PM CSR_2 silkworm on pupa

attributed to the stimulatory effect of these plant extracts on protein synthesis in the silk gland during larval period. These observations are comparable to those of Harish Babu *et al.* (2011) who reported that, the administration of *Aloe vera* extract (100% gel) to silkworm by smearing to mulberry leaf showed increased shell ratio (17.60 %) significantly over control (17.06 %).

Silk productivity (cg day^{-1}) :

Silk productivity is a function of cocoon shell weight and fifth instar duration (days). These two were influenced to greater extent by rearing environment, quality and quantity of food, etc. Treatment with *Aloe vera* and *Tinospora cordifolia* extracts at different concentrations registered significant results with respect to silk productivity. The higher silk productivity was encountered in 100 % *Aloe vera* (4.83 cg day^{-1}) followed by 75 % *Aloe vera* (4.61 cg day^{-1}), 50 % *Aloe vera* (4.40 cg day^{-1}), 25 % *Aloe vera* (4.28 cg day^{-1}), 8 % *Tinospora cordifolia* (4.23 cg day^{-1}), 6 % *Tinospora cordifolia* (4.20 cg day^{-1}), 4 % *Tinospora cordifolia* (4.14 cg day^{-1}) and 2 % *Tinospora cordifolia* (4.06 cg day^{-1}). The lowest silk productivity was recorded in control (3.30 cg day^{-1}) (Table 3). The improvement in silk productivity might be due to the useful biochemical constituents present in these phyto extracts supplied along with mulberry leaf. The results fall in line with the findings of Harish Babu *et al.* (2011), who reported that mulberry leaves administered with *Aloe vera* extract (100% gel) when supplied to the silkworms has resulted in increased silk productivity (4.45 cg day^{-1}) over control (3.95 cg day^{-1}).

Silk filament length (m) :

The longest silk filament length was obtained in case of 100 % of *Aloe vera* (883.95 m) and it was followed by 75, 50 and 25 % (864.56, 848.84 and 812.52 m) of *Aloe vera* and 8, 6, 4 and 2 % of *Tinospora cordifolia* (812.20, 794.45, 782.12 and 779.14 m), respectively. While, the shortest silk filament length was recorded in control (760.94 m) (Table 3). Longest silk filament length recorded might be due to increased effect on larval, cocoon and shell weight ultimately leading to long silk filament length revealing that phyto extracts might have stimulated the metabolic activities of silkworm. Patil *et al.* (1997) who reported longest filament of 873 m due to supplementation of 20 % *Parthenium* leaf extract. Bhaskar *et al.* (2004) also recorded longest silk filament length in *W. somnifera* leaf extract followed by *S. androgynous* and *P. niruri* compared to control.

Denier :

Feeding of mulberry leaf smeared with *Aloe vera* and *Tinospora cordifolia* extracts to the larvae of *B. mori* produced significant results with respect to denier. Finer denier was recorded in 100 % *Aloe vera* (2.41) followed by 75 % (2.45) *Aloe vera*, 50 % (2.47) *Aloe vera*, 25 % (2.49) *Aloe vera*, 8 % (2.51) *Tinospora cordifolia*, 6 % (2.56) *Tinospora cordifolia*, 4 % (2.60) *Tinospora cordifolia* and 2 % (2.62) *Tinospora cordifolia*, respectively. While, the control recorded coarser denier (2.72) (Table 3). Improvement in denier might be attributed to decreased filament weight per unit length of silk filament. The present results are in agreement with that of Savitha and Bhaskar (2007) where leaf extract of *P. niruri* sprayed on worms and inoculated with 10^{-3} and 10^{-5} spore dilution of *B. bassiana* recorded highest silk productivity (3.51 and 3.76 cg day^{-1}), longest filament length (724 and 847 m) and improved filament denier (1.92 and 2.00) followed by *A. vasica*.

Conclusion :

It can be concluded from the study that, fortification of mulberry leaves with plant extracts were found beneficial in improving the rearing performance and all commercial cocoon characters of silkworm. Among the two plant extracts tested, *Tinospora cordifolia* found best next only to *Aloe vera*. Among different concentrations of plant extracts tested, 100 per cent *Aloe vera* registered increased results when compared to other treatments. This paves the way for strengthening the sericulture industry in means of quality and quantity through nutritional fortification.

Authors' affiliations :

FATIMA SADATILLA AND S. HARISH BABU, Department of Sericulture, University of Agricultural Sciences, G.K.V.K., BENGALURU (KARNATAKA) INDIA

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