Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(4): 780-784

Received: 04-05-2019
Accepted: 06-06-2019

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Fortification of mulberry leaves with indigenous probiotic bacteria on larval growth and economic traits of silkworm (*Bombyx mori* L.)

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Abstract
The feeding of nutritionally enriched leaves provide better growth and development of silkworm larvae, as well as directly influence the quality and quantity of silk production. Probiotic are viable, non-pathogenic microorganisms which when administered in adequate amounts, confer a health benefit on the host. Hence the present study was aimed at investigating rearing and economic parameters of the silkworm bivoltine double hybrid (CSR6 x CSR26) x (CSR2 x CSR27) fed on mulberry leaves fortified with *Staphylococcus gallinarum* strain SWGB 7 and *Staphylococcus arlettae* strain SWGB 16. Among the treatments, *Staphylococcus gallinarum* Strain SWGB 7 (10^8 cfu/ml) recorded maximum larval weight (4.12 g), effective rate rearing (96.36 %), cocoon weight (1.97 g), shell weight (0.37 g), pupal weight (1.60 g), shell ratio (18.78 %), silk productivity (4.81 g), filament length (1170.84 m), filament weight (0.31 g) and finer denier (2.38) besides reduced larval mortality (3.64 %) due to disease incidence compared to control. The outcome of the study indicated that, there is profound increase due to probiotic treatment in larval growth and cocoon characters than the control with enhanced immunity and quality silk production.

Keywords: Silkworm, probiotic, *S. gallinarum*, economic characters

Introduction

Silkworm *Bombyx mori* L. is a well-known lepidopteran (Family: Bombycidae), the larval instars of which feed on the leaves of mulberry (*Morus* sp). The growth, development of larva and subsequent cocoon production are greatly influenced by nutritional quality of mulberry leaves. In India, considerable seasonal fluctuations occur in the nutritional value and composition of mulberry leaves depending on factors such as weather, agricultural practices, pest and diseases which have an immense impact on the growth and development of silkworm which in turn results in crop loss (Ito and Niminura, 1966) [11]. Supplementation of mulberry leaves by using different nutrients and feeding to the silkworms are useful modern techniques to increase economic value of cocoons (Masthan *et al.*, 2011). Nutritional studies on silkworms are an essential requisite for its proper commercial exploitation and are sole factors which augment quality and quantity of silk (Laskar and Datta, 2000) [3]. Impact of probiotic (*Lactobacillus, Saccharomyces cerevisiae* and effective microorganisms) treatment on mulberry leaves to modulate the economic parameters of fifth instar larvae of *B. mori* was studied. The *Lactobacillus plantarum* improved the cocoon production of mulberry silkworm *Bombyx mori* (Singh *et al.,* 2005) [20].

In recent years, attempts have been made in sericulture with nutrients such as protein, vitamins, carbohydrates, amino acids, vitamins, hormones and antibiotic etc. for better performance of good quality of cocoons (Sannappa, 2002). Probiotic are the live microbial food supplements that benefits host by improving the microbial balance and enhancing the rapid cellular growth and development (Fuller *et al.*, 1993) [7]. Certain probiotic bacteria inhibit the growth of microbes. *Streptomyces noursei* are probiotic microbes which have been proved for their antibacterial activity and used as good ecofriendly management of silkworm diseases. (Subramanian *et al.*, 2009) [21]. Effect of supplementary feed such as 'Serifeed' (Narayanaswamy and Ananthanarayanan, 2006; Ananda kumar and Michael, 2011) [2], Amway protein (Amala *et al.*, 2011a) [1] improved the growth and development of *Bombyx mori* L. Irianto and Austin (2002) [10] reported that probiotic might produce vitamins and detoxify the compounds in the diets or breakdown the digestible compounds, which may lead to the nutritional improvement and stimulate appetite.

Gibson and Robert froid (1995) [8] emphasized that prebiotic along with intestinal tract and in turn improve the host intestinal balance. According to Charles (2004) [4], lower animals do not have well developed humoral immunity and under such circumstances vaccine development may not be of much use and in these lower animals immunostimulation could be achieved easily through probiotic. Thus, an attempt was made to study the effect of potent indigenous probiotic candidate strains *viz., Staphylococcus gallinarum* SWGB 6 and *Staphylococcus arlettae* BMGB 17 isolated from the silkworm gut for larval growth and economic parameters of *Bombyx mori* L.

Materials and Methods

Silkworm Rearing

Silkworm rearing experiments were carried out in the Department of Sericulture, Forest College and Research Institute, TNAU, Mettupalayam, which is located at 11.19° North Latitude and 77.56° East Longitude at an altitude of 300 m above mean sea level. The disease free laying (DFLs) of bivoltine double hybrid race (CSR6 x CSR26) x (CSR2 x CSR27) were procured from Silkworm Seed Production Centre, Coimbatore. The larvae were reared from first to fifth instar under hygienic conditions with optimum temperature (25-28ºC) and relative humidity (75-85%) in rearing room (Krishnaswami, 1978). The mulberry leaves of V1 variety were fed 3-4 times a day from first to third instar and were divided into different treatment groups. The worms were reared on mulberry leaves sprayed with *Staphylococcus gallinarum* strain SWGB 7 and *Staphylococcus arlettae* strain SWGB 16 separately at 10^8 cfu/ml bacterial cell concentrations on both the sides of mulberry leaves and shade dried before feeding to silkworms. A control batch was maintained feeding only with mulberry leaves. The treatment was given for the first feed on the first and third day of 4^{th} and 5^{th} instar respectively. The remaining feed was given only with mulberry leaves. Fifty worms of 4^{th} instar were separated and maintained in trays for different treatments at five replications per treatment.

Silkworm larval and economic parameters

The worms were carefully monitored every day and from the first day of V instar moulting, the larval weight was determined everyday till the worms started to spin. Then the energetic in silkworm like cocoon weight, pupal weight, shell weight, shell ratio, silk productivity, filament length & weight and denier were recorded.

Larval weight (g)

For recording mature larval weight (g), ten larvae were randomly selected from each treatment, replication-wise during fifth day of fifth instar.

Larval mortality (%)

The per cent of dead worms to the total number of worms reared was recorded and larval mortality (%) was worked out.

Effective rate of rearing (%)

Effective rate of rearing (%) was arrived by recording the number of cocoons harvested to the number of worms brushed.

Cocoon weight (g)

Cocoon weight (g) was calculated by taking ten randomly selected cocoons from all groups and weighed using an electronic balance. Weight of each cocoon from each group was recorded separately.

Pupal weight (g)

Pupal weight (g) was weighed using an electronic balance, after removing the floss. The cocoons were cut open and the pupae were taken out without causing any damage to them.

Shell weight (g)

Shell weight (g) was calculated by taking randomly selected 10 cocoons and cut open with the help of a blade and the shell weight was taken accurately.
Shell ratio (%)
Calculated as shell weight to the cocoon weight and expressed in percentage.

$$\text{Shell ratio (\%)} = \frac{\text{Shell weight}}{\text{Cocoon weight}} \text{X } 100$$

Silk filament length

Five cocoons from each replication were reeled on *epprouvette* and the filament length was determined by the following formula and expressed as metres.

L = R x 1.125

Where,
L = total length of the silk filament
R = number of revolutions recorded
1.125 = circumference of *epprouvette* in meter

Denier

The thickness of the silk was calculated by using the following formula,

$$\text{Denier} = \frac{\text{Weight of the silk filament (g)}}{\text{Length of the silk filament (m)}} \text{ x } 9000$$

Silk productivity (cg/day)

The silk productivity was calculated replication wise by adopting the formula,

$$\text{Silk productivity (cg / day)} = \frac{\text{Weight of cocoon shell (cg)}}{\text{Fifth instar duration (days)}}$$

Statistical analysis

The data were subjected to Analysis of Variance (ANOVA). Duncan's multiple range test (DMRT) (Duncan, 1955) was applied to separate the mean values of analyzed data (Gomez and Gomez, 1984).

Results

The mulberry leaves fortified with SWGB 7 *Staphylococcus gallinarum* strain and SWGB 16 *Staphylococcus arlettae* strain at 10^8 cfu ml^{-1} of bacterial cell concentration when fed to silkworms gained a positive influence on the rearing parameters over the control batch.

Larval characters

Significant differences were recorded on the weight of larvae on 5^{th} day of 5^{th} instar when mulberry leaves were fortified with *S. gallinarum* which recorded maximum larval weight (4.38 g), minimum larval mortality (3.64 %) and the highest effective rate of rearing (96.36 %) than control batch shown in Table 1.

Cocoon parameters

Silkworms fed with bacterial fortified mulberry leaves were effective in improving the intestinal microbial balance thereby increasing the cocoon characters (Table 2).

Silkworms exhibited considerable differences when fed with bacterial fortification with *S. gallinarum*. The highest cocoon weight (2.014 g); pupal weight (1.605 g); shell weight (0.409 g) and shell ratio (20.30 %) was recorded in *S. gallinarum* than control.

Silk characters

The highest silk productivity (5.62 cg g^{-1}); silk filament length (1270.43 m) and silk filament (0.34 g) was recorded when fortified with *S. gallinarum* than control (Table 3).

Denier

Silkworms fed with mulberry leaves treated with *S. gallinarum* showed significant effect on denier. Significantly denier was the highest in control (2.64) and the lowest was recorded in *S. gallinarum* (2.40). Silkworm larvae recorded better values for cocoon-silk parameters when fed with mulberry leaves fortified with *S. gallinarum* and *S. arlettae* individually at 10^8 cfu/ml bacterial cell concentrations compared to control which was fed only with mulberry leaves.

Discussion

Nutritional contributions and the symbiotic benefits offered by insect gut-dwelling bacteria (Dillon, 2004) [5] and (Yuan *et al.*, 2006) [24] which can substantially modify and promote the health and silk production capacity of *B. mori*. The digestive system is home to many types of bacteria. They help to keep intestines healthy and assist digesting food. They are also believed to help the immune system. In probiotic therapy, live microbial feed supplements are improving the intestinal microbial balance of host. In the present investigation, the growth and cocoon parameters of silkworm significantly increased in all the treated groups compared to control. The significant increase were recorded in larval weight (4.12 g), ERR (96.36 %), cocoon weight (1.97 g), pupal weight (1.60 g), shell weight (0.37 g), shell ratio (18.78 %), silk productivity (4.81 cg/day), filament length (1170.84 m), filament weight (0.31 g) and denier (2.38) was registered in *Staphylococcus gallinarum* at 10^8 cfu/ml at bacterial cell concentration. The reduced mortality (3.64 %) was recorded which could be due to the suppression of disease causing pathogens by the antimicrobial property of supplemented bacteria. The present results were in accordance with the results reported by Mala and Vijila (2018) [14], among different treatments, *Bacillus licheniformis* followed by *Bacillus licheniformis* + *Bacillus niabensis* (10^6cfu/ml) recorded maximum larval weight, effective rate rearing, cocoon weight, shell weight, pupal weight, shell ratio, silk productivity and filament length. Probiotic prevent infections by production of bacteriocins and other antibacterial substances with enhancement of intestinal motility and up gradation of genes mediating immunity (Masthan *et al.*, 2017) [15]. Probiotic can also stimulate the body's innate defense mechanisms, as with the increased production of the antimicrobial peptide defenses in intestines.

The present findings also confirmed with results by Venkatesh Kumar *et al.* (2009) reported that Blue green algae (*Spirulina*) at 300 ppm concentration recorded highest cocoon weight (1.083 g), shell weight (24.500 g), pupal weight (0.828 g), shell % (22.640) and filament length (866.605 m). Silkworm *B. mori* reared on mulberry leaves supplemented with minerals, oral protein supplementation, cereal flours, medicinal extracts, plant growth hormones (Sunder Raj *et al.*, 2000; Singh, 1997) are reported to have beneficial effects on economic parameters. (Bai and Bai, 2012) as feed supplementation to *B. mori* were found to be effective in increasing larval weight, cocoon weight, shell weight, pupal weight, shell ratio and silk filament length. According to Singh *et al.* (2005) [20], probiotic *Lactobacillus* supplementation improved the cocoon production of mulberry silkworm *Bombyx mori*. Masthan *et al.* (2017) [15] revealed that blue green algae spirulina and *Saccharomyces cerevisiae* yield better fibroin content indicating the good quality silk when compared to *Lactobacillus acidophilus* and *Lactobacillus sporogens*.

Conclusion

Nutrition is the single most factors that influence the growth and development of *B. mori*. Fortification of mulberry leaves with complementary compounds were increased the larval growth and post cocoon characteristics. The present investigation revealed that two strains *viz.*, *Staphylococcus gallinarum* and *Staphylococcus arlettae* significantly promoted the larval growth which in turn was reflected on qualitative and quantitative improvement in cocoon characters. Based on the above observations, it could be concluded that the novel probiotic strains SWGB 7 *Staphylococcus gallinarum* and SWGB 16 *Staphylococcus arlettae* could serve as a potential probiotic candidate for silkworm growth and development.

Table 1: Effect of bacterial fortification on larval characters

Treatments	Bacterial cell concentration (10^8 cfu/ml)		
	Larval weight (g)	**Larval mortality (%)**	**ERR (%)**
S. gallinarum	4.12 (±0.032)^a	3.64 (±0.007)^c	96.36 (±1.095)^a
S. arlettae	3.89 (±0.019)^b	4.45 (±0.018)^b	95.55 (±0.202)^b
Control (mulberry alone)	3.60 (±0.018)^c	10.15 (±0.128)^a	89.85 (±0.493)^c
SEd	0.0336	0.1060	0.9866
CD (0.05%)	0.0732	0.2309	2.1495

ERR- Effective Rate of Rearing Values represent data mean ± standard deviation Values not sharing a common superscript letter differ significantly at $P < 0.05$ (DMRT)

Table 2: Effect of bacterial fortification on cocoon weight, pupal weight, shell weight and shell ratio

Treatments	Bacterial cell concentration (10^8 cfu/ml)			
	Cocoon weight (g)	**Pupal weight (g)**	**Shell weight (g)**	**Shell ratio (%)**
S. gallinarum	1.974 (±0.005)[a]	1.601 (±0.018)[a]	0.374 (±0.003)[a]	18.78 (±0.156)[a]
S. arlettae	1.932 (±0.013)[b]	1.579 (±0.010)[b]	0.353 (±0.002)[b]	18.27 (±0.144)[b]
Control (mulberry alone)	1.674 (±0.022)[c]	1.380 (±0.009)[c]	0.294 (±0.004)[c]	17.56 (±0.071)[c]
SEd	0,0211	0.0201	0.0047	0.1679
CD (0.05%)	0.0460	0.0362	0.0102	0.3658

Values represent data mean ± standard deviation
Values not sharing a common superscript letter differ significantly at $P < 0.05$ (DMRT)

Table 3: Effect of bacterial fortification on silk characteristics

Treatments	Bacterial cell concentration (10^8 cfu/ml)			
	Silk productivity (cg/g)	**Filament length (m)**	**Silk filament weight (g)**	**Denier**
S. gallinarum	4.812 (±0.055)[a]	1170.843 (±6.291)[a]	0.31 (±0.004)[a]	2.38 (±0.004)[b]
S. arlettae	4.778 (±0.007)[b]	1038.783 (±1.733)[b]	0.29 (±0.004)[b]	2.50 (±0.020)[a]
Control (mulberry alone)	3.560 (±0.035)[c]	960.432 (±0.483)[c]	0.27 (±0.003)[c]	2.53 (±0.028)[a]
SEd	0.1679	5.3423	0.0043	0.0287
CD (0.05%)	0.3658	11.6401	0.0100	0.0626

Values represent data mean ± standard deviation
Values not sharing a common superscript letter differ significantly at $P<0.05$ (DMRT)

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