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N Mala
Department of Sericulture, Forest College & Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Tamil Nadu, India

K Vijila
Professor (Ag. Microbiology), Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
N Mala
Department of Sericulture, Forest College & Research Institute, Tamil Nadu Agricultural University, Mettupalayam, Tamil Nadu, India

Beneficial effects of *Bacillus licheniformis* and *Bacillus niabensis* on growth and economic characteristics of silkworm, *Bombyx mori* L.

N Mala and K Vijila

Abstract
Nutrition of silkworm is a sole factor which almost individually augments quality and quantity of silk. Mulberry leaf is the main source of nutrition for the silkworm. The study was aimed at investigating rearing and economic parameters of the silkworm (CSR_6 x CSR_{26}) x (CSR_2 x CSR_{27}) fed on mulberry leaves fortified with *Bacillus licheniformis strain BMGB42* and *Bacillus niabensis strain BMGB17* individually and in combination at different cell concentrations. 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 besides reduced larval mortality due to disease incidence and finer denier compared to control. The results showed that the *Bacillus licheniformis* can be used as probiotics in commercial silkworm rearing.

Keywords: *Bacillus licheniformis*, *Bacillus niabensis*, economic characteristics, silkworm

Introduction
The silkworm, *Bombyx mori* L. is a typical monophagous insect and mulberry (*Morus* spp.) leaf is its sole food. Man has immensely benefited from the silk produced by silkworms and subsequently researchers have always been trying to unveil the factors that can be manipulated to the benefit of the silkworm rearers (Nair and Kumar, 2004) [18]. Nutrition plays an important role in improving the growth and development of *B. mori*. The silkworm larva consumes all kinds of nutrients from the mulberry leaves to build its body and spin cocoon. The healthy growth of the silkworm and ultimately the economic traits such as, cocoon and grainage parameters are influenced largely by the nutritional status of the leaves fed to worms. Fortification of mulberry leaves to rear the silkworms is a useful modern technique to increase economic value of cocoon (Masthan *et al*., 2011) [16].

In recent years attempts have been made in sericulture with nutrients such as proteins, carbohydrates, amino acids, vitamins, sterols, hormones, antibiotics etc., for better performance and get higher yield with quantity and quality cocoon (Sannappa *et al*., 2002) [21].

Effect of supplementary feed such as 'Serifeed' (Narayanaswamy and Ananthanarayanan, 2006; Ananda kumar and Michael, 2011) [19, 4], Amway protein (Amala *et al*., 2011a) [2], probiotics.

(Singh *et al*., 2005; Masthan *et al*., 2010 and 2011; Amala *et al*., 2011b., Bai and Bai, 2012) [10, 15, 16, 3, 6] and pre and probiotics (Lakshmi Bai and Ramani Bai, 2011) [14] on food consumption and utilization and its effect on quality and quantity of cocoon in silkworm had been well illustrated. Oral administration of foliage of mulberry and eri silkworm supplemented with cyanobacteria, enhanced larval and shell weight subsequently commercial characters of cocoon (Kumar *et al*., 2009; Masthan *et al*., 2011) [11, 16].

Probiotics or bio remediators are gaining more popularity as eco friendly supplementary feed to *B.mori.* The FAO/WHO (2001) [7] defines probiotics as 'Live micro organisms which when administered in adequate amounts confer a health benefit on the host'. In the present investigation an attempt is made to study the effect of different cell concentrations of *Bacillus licheniformis strain BMGB42* and *Bacillus niabensis strain BMGB17* isolated from the gut of silkworm on the growth and economic parameters of *B. mori* L.

Material and Methods

Silkworm Rearing

The disease free laying (DFLs) of double hybrid race (CSR_6 x CSR_{26}) x (CSR_2 x CSR_{27}) 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) [13]. 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 *Bacillus licheniformis strain BMGB42***,** *Bacillus niabensis strain BMGB17* and *Bacillus licheniformis* **+** *Bacillus niabensis* separately at different (10^4, 10^6 and 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.

Mounting and Harvesting

The mature larvae were picked up from the rearing bed and mounted on chandrike for cocoon spinning. The cocoons were harvested on fifth day after mounting. During the period of investigation the following observations were recorded.

Mature larval weight (g)

Ten larvae were randomly selected from each treatment, replication-wise during fifth day of fifth instar and weighed; average single larval weight was computed.

Larval mortality (%)

The per cent of dead worms to the total number of worms reared was recorded at the time of bed cleaning.

Effective rate of rearing (ERR) (%)

The number of cocoons harvested to the number of worms brushed and expressed in percentage.

Cocoon weight (g)

Average weight of ten cocoon samples was calculated.

Shell weight (g)

The weight recorded after removing floss layer, pupa and exuvium of larva was expressed as shell weight.

Shell ratio (%)

Calculated as shell weight to the cocoon weight and expressed in percentage.

Filament length (m)

Five cocoons from each treatment were taken and reeled individually on a single cocoon reeler (epprouvette). Total number of revolutions was recorded and converted to in meter using the following formula:

$$L = R \times 1.125$$

Where,

L= Total length of the filament in meter/cocoon.

R= Number of revolutions recorded by epprouvette.

1.125 = Circumference of epprouvette.

Denier: The raw silk filament reeled with the epprouvette was dried in oven at 70-80ºC. The number or count in the denier system is weight in grams per 9000 meters of fibre.

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

Statistical analysis

All the data were subjected to analysis of variance (ANOVA) using complete randomized design.

Results

Mature larval weight (g)

The weight of larvae on fifth day of fifth instar exhibited significant differences, when *Bacillus niabensis* and *Bacillus licheniformis* treated mulberry leaves in different cell concentrations individually and in combination were administered (Table 1). The larval weight was significantly maximum when silkworms fed with mulberry leaves fortified with *Bacillus licheniformis* (4.488 g) followed by *Bacillus niabensis* + *Bacillus licheniformis* (4.316 g) at 10^6cfu/ml bacterial cell concentration. The minimum larval weight was recorded in control (3.640 g). All the bacterial treated batches performed better than control. *Bacillus niabensis* performed better in combination with *Bacillus licheniformis* than individual supplementation for all growth and economic parameters.

Larval Mortality (%)

The administration of *Bacillus niabensis* and *Bacillus licheniformis* treated mulberry leaves to different stages of silkworm (*Bombyx mori* L.) registered significant differences in reducing larval mortality in silkworm hybrid rearing. The batches treated with *Bacillus licheniformis* at 10^6cfu/ml bacterial cell concentration recorded minimum of 5.650 per cent mortality followed by *Bacillus niabensis* + *Bacillus licheniformis* (5.935 %) at 10^6cfu/ml bacterial cell concentration and the same was maximum (12.531 %) in control batches fed only with mulberry leaves (Table 1).

Effective Rate of Rearing (ERR) (%)

Feeding silkworms with mulberry leaves fortified with *Bacillus niabensis* and *Bacillus licheniformis* increased the effective rate of rearing significantly. The maximum effective rate of rearing was recorded in *Bacillus licheniformis* (94.339 %) followed by *Bacillus niabensis* + *Bacillus licheniformis* (94.062 %) at 10^6cfu/ml bacterial cell concentration supplemented batches. Whereas, the minimum effective rate of rearing was recorded in control (87.465 %) (Table 1). All the bacterial treated batches performed better than control for all rearing parameters.

Cocoon Weight (g)

Silkworm diet supplementation with different bacteria proved to be effective in improving the cocoon traits. The weight of cocoons spun by the larvae fed on mulberry leaves fortified with *Bacillus licheniformis* exhibited increase in the cocoon weight registering 2.011 g at 10^6cfu/ml bacterial cell concentration. The lowest cocoon weight was noticed in control (1.670 g) (Table 2).

Pupal Weight (g)

The pupal weight was maximum in *Bacillus licheniformis* (1.628 g) at 10^6cfu/ml bacterial cell concentration and the

minimum pupal weight was recorded in control (1.380 g) (Table 2).

Shell Weight (g)

The mulberry leaves fortified with bacteria at different cell concentrations increased the shell weight significantly. The increased shell weight ranged from 0.290 to 0.383g. The best treatment was *Bacillus licheniformis* (0.383 g) at 10^6cfu/ml bacterial cell concentration. The lowest shell weight was recorded in control (0.290 g) (Table 3).

Shell Ratio (%)

Supplementation of *Bacillus licheniformis* (19.045 %) at 10^6cfu/ml bacterial cell concentration revealed significant difference with respect to shell ratio. The shell ratio ranged from 17.365 (control) to 19.045 per cent with *Bacillus licheniformis* treated lots (Table 3).

Silk Productivity (cg/day)

The highest silk productivity was recorded in *Bacillus licheniformis* (4.758 cg/day) followed by *Bacillus niabensis* + *Bacillus licheniformis* (4.734 cg/day) at 10^6cfu/ml bacterial cell concentration. The lowest silk productivity was recorded in control (3.580 cg/day) (Table 4).

Silk Filament Length (m)

The administration of mulberry leaves fortified with different cell concentrations of *Bacillus niabensis* and *Bacillus licheniformis* to silkworm exhibited significant differences with respect to single cocoon filament length. Significantly longer filament length was recorded in *Bacillus licheniformis* (1170.469 m) at 10^6cfu/ml bacterial cell concentration. While, the shortest silk filament length was recorded in control (917.412 m) (Table 4).

Denier

Silkworms fed with mulberry leaves treated with *Bacillus licheniformis* showed significant effect on denier. Significantly denier was higher in control (2.718) and lowest being recorded in *Bacillus licheniformis* (2.381) at 10^6cfu/ml bacterial cell concentration (Table 4). Silkworm larvae recorded better values for cocoon-silk parameters when fed with mulberry leaves fortified with *Bacillus niabensis* and *Bacillus licheniformis* individually and in combination at different cell concentrations compared to control which is fed only with mulberry leaves

Discussion

Nutritional background of the larval stage significantly influences the status of the resulting larva, pupae, adult and fiber (Fukuda *et al.*, 1963; Takano and Arai, 1978; Aftab Ahmed *et al.*, 1998, Rahmathulla *et al.*, 2002) [8, 24, 20]. In the present study, the growth and cocoon parameters of silkworm significantly increased in all the treated groups compared to control. Highly significant increase in larval weight (4.488 g), ERR (94.339 %), cocoon weight (2.011 g), pupal weight (1.628 g), shell weight (0.383g), shell ratio (19.045%), silk productivity (4.758 cg/day) and filament length (1170.469 m) was registered in *Bacillus licheniformis* at 10^6cfu/ml bacterial cell concentration as against the control. The reduced mortality (5.650%) could be due to the suppression of disease causing pathogens by the antimicrobial property of supplemented bacteria. Various live microorganism (probiotics) have been demonstrated to modify the composition of the micro flora, restore the microbial balance and therefore have the potential to provide health benefits when normal intestinal flora is disturbed due to diarrhea, food toxification etc. Probiotics prevent infections by production of 'bactericins' and production of other antibacterial substances with enhancement of intestinal motility and up gradation of genes mediating immunity (Masthan *et al.*, 2017) [17]. *Litopenaeus vannamei* treated with *Bacillus licheniformis and Lactobacillus rhamnosus* showed significantly higher growth than the control (Swapna *et al*., 2015) [23].

The improvement in cocoon and shell weight with treated larva in the present study may be due to increased nutritional efficiency of food which is utilized for the maximum silk protein content of the cocoon shell. Enrichment of mulberry leaves by pre and probiotics could increase leaf quality, which reflect on the quantitative performance of cocoon significantly (Kumari Sethu Lakshmi Bai and Ramani Bai, 2015) [12]. According to Irianto and Austin (2002) [9] probiotics may produce vitamins and detoxify the compounds in the diets or breakdown the digestible compounds, which may lead to the nutritional improvement. The present results are correlated with the previous observations where the specific dose of *Spirulina* with 300 ppm concentration (Venkatesh kumar *et al*., 2009) [25], *Spirulina* and yeast with 300 ppm concentration (Masthan *et al.*, 2011) [16] and 1:1 ratio of probiotic and a neutraceutical combination (Bai and Bai, 2012) [6] as feed supplementation to *B.mori* found to be effective in increasing larval weight, cocoon weight, shell weight, pupal weight, shell ratio and silk filament length. Similar results were obtained when eri silkworm *Samia cynthia ricini*, Boisduval were treated with *Spirulina* (Jaya prakash *et al*., 2005) [10] and probiotic Darolac (Anitha *et al*., 2015) [5] recorded maximum larval, cocoon, pupal and shell weight, silk ratio % and ERR% at 2% concentration when compared with control. Singh *et al*., (2005) [10, 22] observed improvement in larval body weight, cocoon weight, shell weight and pupation percentage of silkworm larvae when fed on mulberry leaves treated with a commercial probiotic formulation containing *Lactobacillus plantarum.*

Table 1: Effect on larval rearing characteristics of silkworm fed with fortified mulberry leaves

Treatments	Larval wt. (g)			Larval mortality (%)			ERR (%)		
	Bacterial cell concentration (cfu/ml)			Bacterial cell concentration (cfu/ml)			Bacterial cell concentration (cfu/ml)		
	10^4	10^6	10^8	10^4	10^6	10^8	10^4	10^6	10^8
Bacillus niabensis	4.009 (±0.035)	3.812 (±0.043)	3.798 (±0.033)	7.984 (±0.070)	8.330 (±0.073)	8.882 (±0.078)	91.993 (±0.807)	91.650 (±0.776)	90.904 (±0.078)
Bacillus licheniformis	4.190 (±0.047)	4.488 (±0.058)	4.075 (±0.025)	7.089 (±0.062)	5.650 (±0.049)	7.795 (±0.068)	92.910 (±0.715)	94.339 (±0.049)	92.198 (±0.068)
Bacillus niabensis + *Bacillus licheniformis*	4.249 (±0.045)	4.316 (±0.029)	4.214 (±0.036)	6.208 (±0.054)	5.935 (±0.052)	6.390 (±0.056)	93.791 (±0.523)	94.062 (±0.052)	93.598 (±0.056)
Control (only	3.640	3.640	3.640	12.531	12.531	12.531	87.465	87.465	87.465

mulberry)	(±0.031)	(±0.031)	(±0.031)	(±0.101)	(±0.101)	(±0.101)	(±0.101)	(±0.101)	(±0.101)
	SEd		CD (0.05%)	SEd		CD (0.05%)	SEd		CD (0.05%)
Treatments	0.0128		0.0258	0.0283		0.0570	0.2933		0.5898
Cell concentration	0.0111		0.0224	0.0245		0.0494	0.2540		0.5108
Treatments x Cell concentration	0.0222		0.0448	0.0491		0.0988	0.5081		1.0221

Figures in the parenthesis represents data mean ± standard deviation

Table 2: Effect on cocoon and pupal weights of silkworm fed with fortified mulberry leaves

Treatments	Cocoon wt (g)			Pupal wt (g)		
	Bacterial cell concentration (cfu/ml)			Bacterial cell concentration (cfu/ml)		
	10^4	10^6	10^8	10^4	10^6	10^8
Bacillus niabensis	1.923 (±0.026)	1.919 (±0.015)	1.810 (±0.016)	1.559 (±0.013)	1.560 (±0.032)	1.454 (±0.020)
Bacillus licheniformis	1.951 (±0.019)	2.011 (±0.016)	1.945 (±0.021)	1.581 (±0.023)	1.628 (±0.014)	1.583 (±0.013)
Bacillus niabensis + *Bacillus licheniformis*	2.001 (±0.023)	2.006 (±0.037)	1.982 (±0.029)	1.622 (±0.014)	1.625 (±0.028)	1.607 (±0.013)
Control (only mulberry)	1.670 (±0.014)	1.670 (±0.014)	1.670 (±0.014)	1.380 (±0.012)	1.380 (±0.012)	1.380 (±0.012)
	SEd		CD (0.05%)	SEd		CD (0.05%)
Treatments	0.0060		0.0121	0.0049		0.0098
Cell concentration	0.0052		0.0105	0.0042		0.0085
Treatments x Cell concentration	0.0104		0.0210	0.0084		0.0170

Figures in the parenthesis represents data mean ± standard deviation

Table 3: Effect on shell weight and shell ratio of silkworm fed with fortified mulberry leaves

Treatments	Shell wt (g)			Shell ratio (%)		
	Bacterial cell concentration (cfu/ml)			Bacterial cell concentration (cfu/ml)		
	10^4	10^6	10^8	10^4	10^6	10^8
Bacillus niabensis	0.364 (±0.004)	0.359 (±0.012)	0.356 (±0.006)	18.928 (±0.158)	18.707 (±0.146)	18.563 (±0.155)
Bacillus licheniformis	0.370 (±0.010)	0.383 (±0.019)	0.362 (±0.002)	18.964 (±0.141)	19.045 (±0.160)	18.611 (±0.156)
Bacillus niabensis + *Bacillus licheniformis*	0.379 (±0.003)	0.381 (±0.013)	0.370 (±0.009)	18.940 (±0.145)	18.993 (±0.139)	18.668 (±0.150)
Control (only mulberry)	0.290 (±0.002)	0.290 (±0.002)	0.290 (±0.002)	17.365 (±0.139)	17.365 (±0.139)	17.365 (±0.139)
	SEd		CD (0.05%)	SEd		CD (0.05%)
Treatments	0.0011		0.0022	0.0592		0.1190
Cell concentration	0.0009		0.0019	0.0512		0.1030
Treatments x Cell concentration	0.0019		0.0039	0.1025		0.2061

Figures in the parenthesis represents data mean ± standard deviation

Table 4: Effect on silk characteristics of silkworm fed with fortified mulberry leaves

Treatments	Silk productivity (cg/day)			Filament length (m)			Denier		
	Bacterial cell concentration (cfu/ml)			Bacterial cell concentration (cfu/ml)			Bacterial cell concentration (cfu/ml)		
	10^4	10^6	10^8	10^4	10^6	10^8	10^4	10^6	10^8
Bacillus niabensis	4.452 (±0.049)	4.376 (±0.038)	4.325 (±0.037)	942.850 (±9.189)	940.113 (±8.122)	935.130 (±8.103)	2.467 (±0.033)	2.471 (±0.025)	2.454 (±0.023)
Bacillus licheniformis	4.533 (±0.039)	4.758 (±0.041)	4.431 (±0.038)	970.124 (±7.406)	1170.469 (±6.518)	958.931 (±8.242)	2.453 (±0.037)	2.381 (±0.022)	2.442 (±0.021)
Bacillus niabensis + *Bacillus licheniformis*	4.689 (±0.033)	4.734 (±0.051)	4.620 (±0.040)	982.343 (±8.448)	1038.614 (±7.494)	976.970 (±8.435)	2.436 (±0.050)	2.404 (±0.042)	2.430 (±0.023)
Control (only mulberry)	3.580 (±0.029)	3.580 (±0.029)	3.580 (±0.029)	917.412 (±0.557)	917.412 (±0.557)	917.412 (±0.557)	2.718 (±0.023)	2.718 (±0.023)	2.718 (±0.023)
	SEd		CD (0.05%)	SEd		CD (0.05%)	SEd		CD (0.05%)
Treatments	0.0138		0.0278	3.1245		6.2826	0.0080		0.0161
Cell concentration	0.0120		0.0241	2.7059		5.4408	0.0069		0.0139
Treatments x Cell concentration	0.0240		0.0482	5.4118		10.8817	0.0139		0.0279

Figures in the parenthesis represents data mean ± standard deviation

Conclusion

The live microbial culture of *Bacillus licheniformis* beneficially affect the silkworm by enhancing larval biomass and healthiness which inturn reflects on the qualitative and quantitative improvement of cocoon characters. The results showed that the *Bacillus licheniformis* can be used as probiotics in commercial silkworm rearing.

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