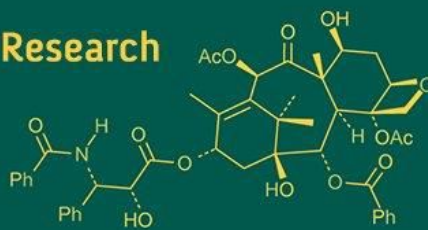


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Effect of probiotics on biological traits of mulberry silkworm

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Abstract

The present investigation entitled “effect of probiotics on biology of mulberry silkworm (*Bombyx mori* L.)” was undertaken with the intention of identification, assessing, and validating appropriate probiotics with significantly better economic traits. It was under taken to know currently available, widely recognized probiotics with the goal of choosing superior. The hybrid was reared on mulberry variety V-1. The variety V-1 of mulberry which was already planted at Sericulture Research Unit, VNMKV, Parbhani. The significantly highest hatching per cent was observed in T₅ Baker’s yeast 1.5% (96.16 per cent) over the rest of the treatments followed by T₄ Baker’s yeast 1% (95.16 per cent) and T₆ Baker’s yeast 2% (95.16 per cent) were at par with each other. Significantly highest 10 mature larval weight was shown by T₈ Spirulina + Baker’s yeast 1.5% which recorded larval weight (40.33 g) followed by T₆ Baker’s yeast 2% (39.05 g) and T₅ Baker’s yeast 1.5% (39.04 g) which were at par with each other. Regarding larval duration, it was revealed that T₅ Baker’s yeast 1.5% had shown significantly shortest larval duration (576.67 h) and found superior to T₁₀ Control (596.00 h) followed by T₄ Baker’s yeast 1% (578.17 h) and T₆ Baker’s yeast 2% (579.17 h) which were at par with each other.

Keywords: Silkworm, biological traits, Spirulina, Baker’s yeast, probiotics, mulberry

Introduction

Silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) is an important economic insect is being used to convert mulberry leaf protein into silk. The use of silkworm for the production of natural silk in the form of cocoons has been exploited worldwide since its discovery during 2700 BC. Sericulture industry plays a big role in both of the global and rural economics of India. Particularly, this industry is suitable for rural population due to its low investment requirement and giving higher returns. This industry generates income and employment opportunities for rural people of Maharashtra, in spite of being a non-traditional sericulture state, produces both Mulberry and Tasar silk. The state has expertise in bivoltine sericulture and ranks first in non-traditional states in India, making it a promising region for silk manufacturing. Area of sericulture in Maharashtra during the year 2023-24 is 19005.75 acres with production of cocoon with 4,919.37 metric tons.

The world’s leading silk producing countries are China, India and Korea. The silk goods export earnings decreased over the years in our country due to global recession. Other notable silk-producing countries include Uzbekistan, Brazil, Japan, the Republic of Korea, Thailand, Vietnam and Iran. In the fiscal year 2021, India witnessed the highest production of mulberry silk (raw silk), with nearly 24 thousand metric tons, followed by Vanya or wild silk with over nine metric tons. The southern state of Karnataka held the leading position as the producer of raw silk during that period (Anonymous, 2022) ^[1]. India is a unique country in world which produces all four known varieties of silk, viz, Mulberry silk (*Bombyx mori* L.), Eri silk (*Philosoma ricini* D.), Tasar silk (*Antheraea mylitta* J.) and Muga silk (*Antheraea assama* Ww.). India is the second largest producer of raw silk after China and the biggest consumer of silk and silk fibrics. India has a distinct advantage of practicing sericulture, yielding 4-6 crops throughout the year as a result of its tropical climate situation. World silk production during year 2022-23 was 91,221 MT in which China stand first with 50,000 MT (51.81%) followed by India with 36,582 MT (40.10%).

The *Bombyx mori* L., commonly known as the Mulberry silkworm, exclusively feeds on the leaves of the Mulberry tree (*Morus* spp.) for its nutritional needs and produces natural

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protein-based silk. During its larval stage, the silkworm typically reaches a weight of around 4-5 grams, depending on factors such as the type of mulberry leaf, environmental conditions, and the specific race or hybrid of the silkworm. It consumes approximately 15-20 grams of leaves throughout its various larval stages, with the last two stages accounting for 80-85% of the total consumption. While the rate of food consumption increases with each larval stage, the conversion of food into body weight decreases. These nutrients which are very easy to digest protein (Bili protein), carbohydrates (mucopolysaccharides, rhamnose and glycogen), 50 different minerals and trace minerals, β carotene, chlorophyll, GLA (γ -linolenic acid), Ω -3 fatty acid, and many other nutrients found in Spirulina. They can also act as a food source for intestinal flora such as *Lactobacillus* and *Bifidus*.

In recent years attempts have been made to supplement nutrient, such as proteins, carbohydrates, amino acids, vitamins sterols, hormones, antibiotics, plant extracts etc., for better performance of silkworm. Mulberry leaf supplemented with Spirulina as a feed to *B. mori* was found to be effective in enhancing the larval and cocoon characters. *Saccharomyces cerevisiae* serves as an immune-modulating agent in silkworm *Bombyx mori*. When the probiotic *S. cerevisiae* was used for the treatment there was a considerable increase on the energy budget and the commercial characteristics of *B. mori* and also there was an increase in the level of protein content in treated worms. Although the mulberry leaves are complete diet of silkworm, supplementation of extra nutrients along with mulberry leaves resulted in higher yield.

Material and Methods

Investigation was carried out to know the "Effect of probiotics on biology and economic traits of mulberry silkworm (*Bombyx mori* L.)." The experiment was conducted at Sericulture Research Unit, VNMKV, Parbhani during the month July- August 2022. Methods involved and materials used are described as below.

Rearing Technique

The recent technology of silkworm rearing adopted in this present investigation was described by Krishnaswamy (1987). The rearing of silkworm hybrids was undertaken with the use of already established mulberry plantation of V-1 variety at Sericulture Research Unit, VNMKV, Parbhani.

The rearing house and rearing appliances were disinfected with Sanitech (ClO₂) solution (500 ppm ClO₂+ 0.5% slaked lime) to make them aseptic before rearing. Paper sheets of disease free layings (dfls) of silkworm hybrids were procured from the multivoltine and bivoltine breeding laboratories of Central Sericultural Research and Training Institute, Mysore and incubated at 25 + 1 °C and 75 +5 per cent relative humidity. The egg sheets were spread out in a single layer in rearing trays and covered with paraffin paper. Wet newspapers were kept all around the egg sheets to ensure the humidity requirement for incubation. The trays containing egg sheets were stored in cool place in rearing house. On attaining the blue egg growth stage, the egg sheets were placed in plastic incubation tray and covered by black piece of cloth called as black boxing and left undisturbed for 48 hours for uniform growth of embryo, after which the eggs were exposed to bright tube light for one hour for uniform hatching.

The newly hatched worms were fed with chopped pieces of fresh tender mulberry leaves of variety V-1. The leaves were chopped into small pieces of 0.5 sq.cm.x 1.5 sq.cm. size and spread over newly hatched young ones which crawled on leaves and started feeding from cut edges. The size of chopped leaves was regulated according to the condition and size of worms. Feeding was given four times in a day. Timing of the feeding was fixed at 7 hours, 11 hours, 16 hours and 20 hours in a day. The cleaning of trays was done once in a day probably in the morning. The silkworm moults for four times during its larval growth phase (Five instars). During moulting period, the worms were not provided any food and were not disturbed when worms showed moulting signs. After moulting worms were disinfected with disinfectant (Vijetha powder) to avoid pathogen infection. More quantity food of full matured mulberry leaves were increased during the fourth and fifth instar stage because they eat voraciously during this instar.

After full development, the ripe worms were identified as they looked translucent with creamy colour and ceased to eat and crawled towards periphery of trays and tried to spin the cocoon, such worms were handpicked and kept on the mountages. Larvae spun the cocoons within 48 to 72 hours. The pupa remains inside the cocoon till emergence. The harvesting of cocoons was made on fifth day of release of worms from mountages.

Table 1: Treatment details

Treatment 1	Application of spirulina at the rate of 1%.
Treatment 2	Application of spirulina at the rate of 1.5%
Treatment 3	Application of spirulina at the rate of 2%
Treatment 4	Application of baker's yeast at the rate of 1%
Treatment 5	Application of baker's yeast at the rate of 1.5%
Treatment 6	Application of baker's yeast at the rate of 2%
Treatment 7	Application of spirulina + baker's yeast at the rate of 1%
Treatment 8	Application of spirulina + baker's yeast at the rate of 1.5%
Treatment 9	Application of spirulina + baker's yeast at the rate of 2%
Treatment 10	Control.

Results and Discussion

Effect of probiotics on the 10 mature larval weight (g) of mulberry silkworm

The data on effect of probiotics on the 5th instar larval weight (g) of bivoltine silkworm (*B. mori* L.) are presented in Table 1, the larval weight varied in the range of 31.40 g to 40.33 g.

Significantly highest 10 mature larval weight was shown by T₈ Spirulina + Baker's yeast 1.5% which recorded larval weight (40.33 g) followed by T₆ Baker's yeast 2% (39.05 g)

and T₅ Baker's yeast 1.5% (39.04 g) which were at par with each other. T₁₀ Control showed lowest larval weight of 31.40 g.

Table 1: Effect of probiotics on the 10 mature larval weight (g) of mulberry silkworm

Sr. No.	Treatments	Larval weight (g)			Total	Mean (g)
		R-I	R-II	R-III		
T ₁	Spirulina 1%	34.91	35.57	35.68	106.16	35.39
T ₂	Spirulina 1.5%	35.9	34.36	30.33	100.59	33.53
T ₃	Spirulina 2%	37.99	38.15	36.46	112.6	37.53
T ₄	Baker's yeast 1%	37.29	36.86	36.48	110.63	36.88
T ₅	Baker's yeast 1.5%	41.16	39.2	36.76	117.12	39.04
T ₆	Baker's yeast 2%	41.02	38.54	37.59	117.15	39.05
T ₇	Spirulina + Baker's yeast 1%	36.08	34.54	36.95	107.57	35.86
T ₈	Spirulina + Baker's yeast 1.5%	40.19	41.22	39.59	121	40.33
T ₉	Spirulina + Baker's yeast 2%	34.12	34.58	33.77	102.47	34.16
T ₁₀	Control	32.26	30.57	31.38	94.21	31.40
	SE $\pm$					0.724
	CD at 5%					2.169
	CV (%)					3.455

Revealed that fifth instar larval weight a day before spinning was significantly highest in mulberry supplemented with azolla (32.79 g) followed by soy milk, yeast and spirulina ranged from 28.40 to 30.37 g and the same was significantly lowest in absolute control (26.10 g). The present results are in conformity with the previous workers. Moreover, the quality of food consumption has direct relevance on weight of larva and supplementation with probiotics perhaps increased the haemolymph protein and amino acid content in the silkworm larvae resulting in better silkworm growth as observed by Horie and Watanabe (1983).

Effect of probiotics on the larval duration (h) of mulberry silkworm

The data on effect of probiotics in Table No. 2, with respect to larval duration indicated that larval duration varied in the

range of 576.67 to 596.00 h. Regarding larval duration, it was revealed that T₅ Baker's yeast 1.5% had shown significantly shortest larval duration (576.67 h) and found superior to T₁₀ Control (596.00 h) followed by T₄ Baker's yeast 1% (578.17 h) and T₆ Baker's yeast 2% (579.17 h) which were at par with each other. Mathavan *et al.* (1984) reported that final instar of *Bombyx mori* L. fed on mulberry leaves supplemented with *Spirulina fusiformis* (Worohichin), a single cell protein (SCP) required only six days to attain spinning stage as against the control (nine days). Roger and Chen (1984) reported that larvae reared on diets of powdered blue-green algae, *Spirulina platensis* (Nordst) with 30 to 40 per cent algal powder had shorter fourth instar larval duration. The present results are in conformity with the previous workers.

Table 2: Effect of probiotics on the larval duration (h) of mulberry silkworm

Sr. No.	Treatments	Larval duration (h)			Total	Mean (h)
		R-I	R-II	R-III		
T ₁	Spirulina 1%	592.5	593	591	1776.5	592.17
T ₂	Spirulina 1.5%	590	591.5	593.5	1775	591.67
T ₃	Spirulina 2%	585.5	587	588	1760.5	586.83
T ₄	Baker's yeast 1%	577	579	578.5	1734.5	578.17
T ₅	Baker's yeast 1.5%	576.5	577.5	576	1730	576.67
T ₆	Baker's yeast 2%	581	579	577.5	1737.5	579.17
T ₇	Spirulina + Baker's yeast 1%	583	582.5	584	1749.5	583.17
T ₈	Spirulina + Baker's yeast 1.5%	586.5	585	584.5	1756	585.33
T ₉	Spirulina + Baker's yeast 2%	591	590.5	594	1775.5	591.83
T ₁₀	Control	596.5	594.5	597	1788	596.00
	SE $\pm$					0.789
	CD at 5%					2.364
	CV (%)					0.233

Effect of probiotics on effective rate of rearing (%) of mulberry silkworm

The data on effect of probiotics in Table No. 3 with respect to effective rate of rearing% indicated that it is varied in the range of 88.33 to 92.66 per cent. Regarding effective rate of rearing, it was revealed that T₄ baker's yeast 1% had shown significantly highest effective rate of rearing (92.66) and

found superior to T₁₀ control (88.33). T₁ Spirulina 1%, T₆ Baker's yeast 2%, T₇ Spirulina + Baker's yeast 1%, T₈ Spirulina + Baker's yeast 1.5%, (90.66) and T₂ Spirulina 1.5%, T₃ Spirulina 2% (91.33) which were at par with each other found Dorlac at two per cent had higher ERR in eri silkworms (95.00%) when compared to control (85.00%).

Table 3: Effect of probiotics on effective rate of rearing (%) of mulberry silkworm

Sr. No.	Treatments	Effective Rate of Rearing (%)			Total	Mean (%)
		R-I	R-II	R-III		
T ₁	Spirulina 1%	91	90	91	272	90.66 (72.18)
T ₂	Spirulina 1.5%	92	91	91	274	91.33 (72.85)
T ₃	Spirulina 2%	91	91	92	274	91.33 (72.85)
T ₄	Baker's yeast 1%	93	93	92	278	92.66 (74.26)
T ₅	Baker's yeast 1.5%	92	92	91	275	91.66 (73.19)
T ₆	Baker's yeast 2%	91	90	91	272	90.66 (72.18)
T ₇	Spirulina + Baker's yeast 1%	90	91	91	272	90.66 (72.18)
T ₈	Spirulina + Baker's yeast 1.5%	91	90	91	272	90.66 (72.18)
T ₉	Spirulina + Baker's yeast 2%	90	89	89	268	89.33 (70.91)
T ₁₀	Control	88	88	89	265	88.33 (70.00)
	SE $\pm$					0.328
	CD at 5%					0.981
	CV (%)					0.626

Note: Figures in parenthesis are angular transformed values.

Shruti *et al.* (2019) reported that significantly lowest ERR was registered in water control (87.67%) followed by *Spirulina* (89.07%). Hassan (2020) 2.5% spirulina increased ERR% for the same hybrids (97% & 96.9%) respectively. The present results are in conformity with the previous workers. The enhanced ERR observed in the present study may be due to less incidence of diseases in the treated silkworms and this might be due to effect of feed additives containing protein, carbohydrates and minerals which act as building blocks of tissues making them robust and so imparted healthiness to larvae. The lowest ERR in Control might be due to reduced survival rate.

Effect of probiotics on hatching (%) of mulberry silkworm

The data on effect of probiotics on Table No. 4. revealed that 90.50 to 96.16 per cent egg hatching of mulberry silkworm hybrids were recorded. The significantly highest hatching per cent was observed in T₅ Baker's yeast 1.5% (96.16 per cent) over the rest of the treatments followed by T₄ Baker's yeast 1% (95.16 per cent) and T₆ Baker's yeast 2% (95.16 per cent) were at par with each other. The treatment T₈ Spirulina + Baker's yeast 1.5% (90.50 per cent) showed lower hatching per cent than T₁₀ control treatment (92.33 per cent).

Table 4: Effect of probiotics on hatching (%) of mulberry silkworm

Sr. No.	Treatments	Egg hatching (%)			Total	Mean (%)
		R-I	R-II	R-III		
T ₁	Spirulina 1%	91.5	91	93	275.5	91.83 (73.38)
T ₂	Spirulina 1.5%	92	93.5	91	276.5	92.16 (73.75)
T ₃	Spirulina 2%	90	91.5	93	274.5	91.50 (73.06)
T ₄	Baker's yeast 1%	94.5	96	95	285.5	95.16 (77.29)
T ₅	Baker's yeast 1.5%	95	97	96.5	288.5	96.16 (78.74)
T ₆	Baker's yeast 2%	96.5	95	94	285.5	95.16 (77.34)
T ₇	Spirulina + Baker's yeast 1%	92	91.5	93	276.5	92.16 (73.73)
T ₈	Spirulina + Baker's yeast 1.5%	90	91	90.5	271.5	90.50 (72.02)
T ₉	Spirulina + Baker's yeast 2%	91.5	93	91	275.5	91.83 (73.38)
T ₁₀	Control	93	92	92	277	92.33 (73.90)
	SE $\pm$					0.598
	CD at 5%					1.791
	CV (%)					1.115

Note: Figures in parenthesis are angular transformed values.

Effect of probiotics on pupal duration (Days) of mulberry silkworm

The data on effect of probiotics on pupal duration were recorded and presented in Table No.5, revealed that pupal duration varies in the range of 11.58 to 12.75 days. Minimum pupal duration was observed in T₂ Spirulina

1.5%, T₃ Spirulina 2%, T₇ Spirulina + Baker's yeast 1% and T₁₀ control (11.58 days). While maximum pupal duration was observed in T₁ Spirulina 1% (12.75 days). No significant difference observed. The literature on effect of probiotics on pupal duration of silkworm is not available so results could not be discussed.

Table 5: Effect of probiotics on pupal duration (days) of mulberry silkworm

Sr. No.	Treatments	Pupal duration (days)			Total	Mean (days)
		R-I	R-II	R-III		
T ₁	Spirulina 1%	13.5	12.75	12	38.25	12.75
T ₂	Spirulina 1.5%	11.25	11.5	12	34.75	11.58
T ₃	Spirulina 2%	12.5	11	11.25	34.75	11.58
T ₄	Baker's yeast 1%	11.25	12.25	11.5	35	11.67
T ₅	Baker's yeast 1.5%	12	13.25	12.5	37.75	12.58
T ₆	Baker's yeast 2%	11.25	12.75	12	36	12.00

T ₇	Spirulina + Baker's yeast 1%	11.5	12	11.25	34.75	11.58
T ₈	Spirulina + Baker's yeast 1.5%	12.75	11.5	12	36.25	12.08
T ₉	Spirulina + Baker's yeast 2%	13.5	11.25	11.5	36.25	12.08
T ₁₀	Control	11.25	12	11.5	34.75	11.58
	SE $\pm$					0.408
	CD at 5%					N/A
	CV (%)					5.908

Summary and Conclusion

The present investigation entitled "Effect of Probiotics on the Biology of Mulberry Silkworm" was undertaken with the intention of identification, assessing, and validating appropriate probiotics with significantly better economic traits. It was under taken to know currently available, widely recognized probiotics with the goal of choosing superior Hatching percentage (%): The significantly highest hatching per cent was observed in T₅ Baker's yeast 1.5% (96.16 per cent) over the rest of the treatments followed by T₄ Baker's yeast 1% (95.16 per cent). Ten Larval weight (g): Significantly highest 10 mature larval weight was shown by T₈ Spirulina + Baker's yeast 1.5% which recorded larval weight (40.33 g) followed by T₆ Baker's yeast 2% (39.05 g). Larval duration (Days): T₅ Baker's yeast 1.5% had shown significantly shortest larval duration (576.67 h) followed by T₄ Baker's yeast 1% (578.17 h). Effective rate of rearing (%): T₄ baker's yeast 1% had shown significantly highest effective rate of rearing (92.66) and found superior to T₁₀ control (88.33). Pupal duration (Days): Minimum pupal duration was observed in T₂ Spirulina 1.5%, T₃ Spirulina 2%, T₇ Spirulina + Baker's yeast 1% and T₁₀ control (11.58 days).

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