



Research Article

IMPACT OF PROBIOTIC *SACCHAROMYCES CEREVISIAE* ON THE ENZYMATIC PROFILE AND THE ECONOMIC PARAMETERS OF SILKWORM *BOMBYX MORI* L

C. Esaivani^{1,*}, K. Vasanthi¹, R. Bharathi¹, K. Chairman²

¹Department of Zoology, Sri Parasakthi College for Women, Courtallam, M.S. University, Tirunelveli, Tamilnadu, India

²Department of Microbiology, Kamarajar Arts College, Surandai, M.S. University, Tirunelveli, Tamilnadu, India

Correspondence should be addressed to C. Esaivani

Received 25 November 2014; Accepted 5 December 2014; Published 31 December 2014

Copyright: © 2014 C. Esaivani et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

There are many factors that influence the production of silk among which the activity of enzyme plays a significant role in enhancing the digestibility of silkworm larva this in turn influences the growth, development and resistance to disease in silkworm and subsequently enable the silkworm to produce good qualified cocoon and silk. In recent years research interest is currently directed towards the activities of digestive enzymes like amylase, succinate dehydrogenase, alkaline phosphatase and alkaline protease which will help in silkworm breeding programme for improvement of cocoon characters and disease resistance. Hence in the present investigation an attempt is made to study the impact fortification of mulberry leaf with probiotic microorganism *saccharomyces cerevisiae* on the enzymatic profile and the quantitative economic parameters of silkworm *Bombyx mori*. The results indicate that there is profound increase in the activity of the amylase and invertase in the digestive juice of the probiotic treated worms than the control with enhanced immunity and quality silk production.

KEYWORDS: Sericulture, *Bombyx mori*, *Saccharomyces cerevisiae*, Enzymatic assay, economic parameters

INTRODUCTION

Sericulture or silk farming is the rearing of silkworms for the production of raw silk although there are several commercial species of silkworms, *Bombyx mori* is the most widely used and intensively studied. According to Chinese records, the discovery of silk production from *B. mori* occurred about 2700 BC, making the start of the history of silk. In integrated farming system sericulture is an important component, which is an agro-based rural industry, with tremendous potential for employment generation in rural areas. It is the biggest village industry after handloom and khadi, providing full or partial employment to about 6.5 million people in India (Pankaj et al., 2005).

In the world, India ranks second in raw silk production next to China. Karnataka state alone produces bulk of Indian raw silk (Govindan and Devaiah, 1995). During 2003-2004 the total annual production of raw silk in India was 15.74 thousand tones, of which mulberry raw silk contributed to about 13.97 thousand tones. However, the demand for raw silk production is increased than the current production.

Silkworms are the larva of a moth (*Bombyx mori*) native to Asia that spins a cocoon of fine, strong, lustrous fiber that is the source of commercial silk. *Bombyx mori* is the common mulberry silkworm that can be easily domesticated. It is a monophagous lepidopteran feeding on mulberry leaves (*Morus alba*). The quality of leaves provided to the worms for feeding has been considered as the prime factor governing the production of good crop cocoon. The leaves of superior quality enhance the chances of reaping good cocoon crop (Ravi Kumar, 1998).

Silkworm is a poikilotherm, it cannot regulate its body temperature and is susceptible to several diseases (Prasad, 1999). Diseases in silkworm and mulberry plants caused by pathogens reduce the quality and quantity of silk production which in turn affects national economy. In national countries, the loss due to diseases in sericulture is to the line of 10 percent of the total crop loss (Tokoyama, 1963; Aruga and Tanda, 1971), while in less developed countries like India the loss is to an extent of 30-40 percent (Janakiraman, 1961 and Nanavathy, 1965).

The diseases of silkworm are broadly categorized into two types namely infectious and non-infectious. Infectious diseases are those caused by viruses, bacteria, fungi, protozoa and similar pathogenic microorganisms which enter and harm the body of the silkworm. These diseases can be transmitted from infected larvae to healthy ones. Those that due to damage from arthropods, agricultural chemicals and mechanical injuries and that cannot be spread from sick larvae to normal ones are termed non-infectious diseases. Four silkworm diseases are very common in India viz, grasserie, flacherie, muscardine and pebrine. Among these diseases the main diseases reported in the study area was flacherie wide fluctuation in temperature and humidity with poor quality mulberry leaves are the major predisposed factors to flacherie.

In recent years attempts have been made in sericulture with nutrient such as protein, vitamin, carbohydrates, amino acids, vitamins, hormones and antibiotic etc for better performance of good quality of cocoons Sannappa (2002). In addition to mulberry leaves feed supplements are also given to silkworm to enhance economic characteristics (Jayapaul *et al.*, 2003, Sheeba *et al.*, 2006).

Characteristics of probiotic microorganisms

Probiotics are the live microbial food supplements beneficially affecting host by improving the microbial balance and enhanced rapid cellular growth and development (Fuller *et al.*, 1993). The gut probiotics are involved in the digestive utilization of feeds and detoxification of metabolite, stimulation of non-specific immune system. They also promote the production of vitamins and increase host resistance and compete with pathogenic bacteria by producing organic and antibiotic substance. The *Lactobacillus plantarum* is a probiotic which improves the cocoon production of mulberry silkworm *Bombyx mori* (Singh *et al.*, 2005). Certain probiotic bacteria inhibit the growth of microbes. *Streptomyces noursei* are probiotic microbes which prove the antibacterial activity and good eco-friendly management of silkworm diseases (Subramanian *et al.*, 2009). 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 immune-stimulation could be achieved easily through probiotics (Charles 2004). Impact of probiotics (*Lactobacillus*, *Saccharomyces cerevisiae* and effective microorganisms) treatment on mulberry leaves to modulate the economic parameters of v instar larvae of *B. mori* were studied (Jayapaul *et al.*, 2004).

Amala *et al.*, 2011) had stated that *Saccharomyces cerevisiae* serves as an immunomodulating 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. Yeast improves the protein content and commercial production.

The highly specialized proteins are called enzymes. Enzymes are the reaction catalyst of any biological system. The digestibility of silkworm larva depends upon the activity of an enzyme called amylase. Amylase is one of the most important enzyme which helps in digestion of starch in silkworm. It is the key enzyme involved in digestion and carbohydrate metabolism in insect. Of the various enzymes analyzed amylase is well worked out because of its close association with the economic parameters of silkworm.

According to (Martignoni, 1964) pathogens induce several biochemical and physiological alterations in insect tissues and also they tend to induce shift in metabolic profiles. (Bhosale and kallapur, 1990). Since enzymes play a significant role in food digestion which in turn influences the growth, development and resistance to disease in silkworm and subsequently enable the silkworm to have a better survival under field condition. In recent years probiotic microorganisms are successfully used to enhance prawn, dairy, poultry production etc. However these nutrients supplements has been tried in sericulture to find out the impacts of probiotics on the changes in economic parameters, disease resistance potential and molecular nature of protein components in silkworm. Voluminous literature has been documented in this area and some of them are worth to mention (Sengupta *et al.*, 1972; Mathavan *et al.*, 1984b; Jeyapaul *et al.*, 2003a and 2003b and Sheeba *et al.*, 2007). The available literature reveals that the biochemical mutagen and biochemical formulations promote the level of enzyme activity which ultimately enhances the quality of the traits. However the literature regarding the influence of probiotic *Saccharomyces cerevisiae* on the activity of the digestive enzyme in silkworm is not available. Hence in the present study an attempt has been made to observe the impact of *Saccharomyces cerevisiae* on the digestive enzyme activity of silkworm *B. mori* and its influence on the economic parameters of silkworm.

MATERIALS AND METHODS

The disease free laying of LXCSR2 race purchased from the sericulture farm V.M. Chattiram, Tirunelveli district Tamilnadu was used for the present study.

Silkworm Rearing

In the present investigation rearing operations were carried out according to (Krishnaswami *et al.*, 1978). Silkworms were reared under standard recommended condition at $26 \pm 2^\circ$ C temperature, 75% relative humidity. They were fed with MR₂ variety of Mulberry leaves maintained under irrigated condition.

Preparation of probiotic bacteria

In the present experiment, *Saccharomyces cerevisiae* (yeast) was given as probiotic to the silkworm through feed supplementation. Commercially available yeast was purchased from the local bakery. The probiotic stock solution was prepared. From the stock solution, different concentration was prepared (1%, 3%, 5%) for the treatment.

Silkworms were fed with untreated leaves until the end of III instar stage. Freshly moulted IV instar larvae were recruited and were divided into four groups for the treatment each group consisted of 30 larvae, one group served as control and the others were used for experimental trails with 1%, 3% and 5% of yeast. The freshly plucked mulberry leaves were washed with tap water and cleaned. The clean leaves were then completely dipped in different dosages of *S. cerevisiae*. The leaves were dipped in such a way that both the dorsal and ventral side of the leaves contains the probiotics and the treated leaves were allowed to dry in air for 15 minutes. The probiotic enriched leaves were fed to the IV instar stage from the first day up to V instar stage. The leaves of the control worms were dipped in water and dried before feeding.

Three replications were maintained for each treatment. The faecal matter and unfed leaves were removed from the trays daily. The quantitative analysis of enzymes such as amylase and invertase were observed. (Saito, 1963). The observation on cocoon quantitative parameters such as cocoon weight, pupal weight, shell weight, shell percentage, filament length, reelability, denier were also determined (Waldbauer, 1968).

Enzyme Assay

The guts of the V instar larvae were analyzed for enzymatic activity one day prior to spinning. The silkworms were kept in freezer at 4°C for 10-15 minutes before dissection. The integument was cut away and the exposed gut was dissected longitudinal and rinsed thoroughly with ice cold phosphate buffer saline at P^H7.4. (Saito, 1963). The gut contents were removed and the tissue was washed again with ice-cold phosphate buffer. The gut was then homogenized with pre-cooled phosphate buffer using mortar and pestle. The homogenized samples were then centrifuged at 3000 rpm for 10 minutes. Finally the clear supernatant was used for the enzymatic analysis of amylase and invertase.

Quantitative Analysis

A. Quantitative assay of amylase

The activity of amylase was measured at P^H 7.4 with temperature of 37°C. Four test tubes were taken and to this 1500ml of starch substrate solution and 50ml of sample, extract was added to each test tube. The samples were then incubated at 37°C for 80min. It was then kept in environmental chamber for 5 to 10 minutes and to this 2ml of 3-5 dintro salicylic acids (DNS) reagent were added and the content was heated in water bath for 10 minutes. Then after cooling to room temperature the OD was taken at 575nm using Colorimetry. The estimation was done in replicates and the average value was taken as the OD value (Bernfeld, 1955 and Baker, 1991).

B. Quantitative assay of invertase

The enzymatic assay of invertase was measured at P^H 7.4 with the temperature of 37°C. Four test tubes were taken and to this 2500ml of sucrose solution and 250ml of sample was added to each test tube and 2ml of phosphate buffer was added to the above and incubated at 37°C for 1 hour. Then immediately 500ml of 4% NaOH along with 500ml of DNS reagent was added and kept in boiling water bath for 10 minutes. Then after cooling OD was read at 500nm using Colorimetry. The replication was done in triplicates to get the final OD value. (Jeyaraman, 1981).

The enzyme levels were calculated using the formula followed by Bernard and Prosser (1973).

Enzyme calculation = OD of unknown x std. concentration x Dilution factor x 1 OD of known mg protein.

RESULTS

The findings of the present study implies that when the probiotic microorganism *Saccharomyces cerevisiae* was used as a supplementary diet there was a profound increase in the enzymatic activity of the amylase and invertase in the treated larvae compared to the untreated worms. (Table 1 & Table 2) which is very much essential for better food utilization with increase in the larval stage. During the period of study the untreated larvae were noticed to be more susceptible to bacterial infection (Flacherie). The symptoms observed in the susceptible larvae were in agreement with the earlier report. (loss of appetite, sluggishness, wriggling movement, vomiting brown fluid, excretion of soft faeces, lifting of heads, paralysis and sudden collapse (Singh *et al.*, 1994, Savithri and Murali Mohan, 2003).

According to Gururaj *et al.*, (1999) pathogenic microbial infection on *B. mori* induces a shift in metabolic profiles and the activities of enzymes like amylase, invertase, trehalase and protease. Bacterial flacherie inflicts abnormal multiplication of bacteria in the larval gut lumen as reported by Ratnasen *et al.*, (2003) and interfered with gut physiology causing poor food intake. The poor food intake, bio-chemical changes, bacterial toxins and histopathological changes in gut epithelium had affected the energy budget in the worms, which in turn resulted in poor cocoon parameters. When compared to control the activity of amylase and invertase was high in the digestive juice of probiotics treated larvae. The OD values of amylase and invertase in *S. cerevisiae* treated worms were found to be increased when compared to control.

Since enzymes serve as a reaction catalyst of any biological system. There is need to direct the research towards the activities of digestive enzymes in silkworm as the analysis of enzymes like amylase, succinate dehydrogenase, alkaline phosphatase and alkaline protease will help in silkworm breeding programme for improvement of cocoon characters and disease resistance (Mahesha, 1997; Lakshmi kumara, 1995). Amala *et al.*, (2011) had reported that by administering yeast the immunity of the silkworm was enhanced. Hence in accordance with the earlier report the probiotic yeast utilized in the present study enabled the treated larvae to

develop resistance against the infection with increased enzymatic activity which in turn enhanced economic and nutritional parameter of silkworm.

Commercial characteristics

The commercial characteristics of the silkworms were enhanced by the probiotic supplement, when compared to the worms that was not provided with supplements.

A. Cocoon weight

Table 3 shows the data of control and *Saccharomyces cerevisiae* treated MR₂ mulberry leaves fed Vinstar *B.mori* larvae produced cocoons weight. The mean weight of the cocoon was maximum (35.12 ± 0.19) in 5% of yeast treatment. It was followed by 3% of treatment of yeast (31.65 ± 0.94). The cocoon weight of the control was only (29.17 ± 0.2).

B. Pupal weight

The mean weight of the pupa was maximum in 5% of yeast treatment (16.69 ± 0.12), it was followed by 3% treatment of yeast (14.19 ± 0.32) and the pupal weight of the control was only (10.17 ± 0.55).

C. Shell weight

The shell weight of the control larvae was (19.78 ± 0.004), 1% yeast treated larvae was (19.43 ± 0.019), 3% yeast treated larvae was (25.08 ± 0.014) and 5% yeast treated larvae was (29.27 ± 0.01) respectively. In these four observations the 5% yeast treated larvae shell weight was significantly increased then the other treatments.

D. Shell ratio

The shell ratio is an important commercial characteristic of *B.mori* when compared to control the shell ratio had increased 2.6%, 12.9% and 16.5% in 1%, 3% and 5% treatment of yeast respectively. This was in accordance with the results of previous workers.

E. Silk filament length

The filament length was maximum (924.2 ± 164) in 5% yeast treated worms and it showed an increase of 6.09% when compared to the control. Silkworm larvae treated with 3% of yeast showed a filament length of (890 ± 18.6) and it was 2.11% more than the control. This suggests that the supplements have an ability to increase the length of the silk filament.

F. Reelability

Significant change in reelability was observed in treated worms.

G. Denier

There was not much difference in the denier value of worms treated with 1% dilution of yeast. However 3% and 5% of yeast treatment showed an increase of 6.03% and 11.9% than the control.

CONCLUSION

The present study is an embodiment of adaptability of the silkworm to the nutrient feed supplement *Saccharomyces cerevisiae* and their influence on the enzymatic profiles and the commercial characteristics. The commercial characteristic of the silkworm such as cocoon characters (cocoon weight, pupal weight, shell weight, shell ratio, silk characters (filament length, denier & reelability), were enhanced by *S.cerevisiae*. There was significant variation in enzymatic profiles of the control and experimental worms fed with mulberry leaves enriched with various concentrations of yeast. Among the concentrations used 5% of yeast treatment was found to be significant in increasing the activity of the enzymes, the fundamental basis of silkworm disease control is the constant implementation of the “prevention first” policy. As an earnest step in prevention, the present study insists the upgradation of the immunity of the insect with improved enzymatic activity rather than bathing the insects with harmful chemicals.

Table 1: Enzymatic activity of amylase in the midgut of the fifth instar larvae of control and *Saccharomyces cerevisiae* treated silkworms

Treatment	Different dosages	Amylase activity
<i>Saccharomyces Cerevisiae</i>	Control	62.4
	1%	62.8
	3%	69.6
	5%	75.7

Figure 1: Enzymatic activity of amylase in the midgut of the fifth instar larvae of control and *Saccharomyces cerevisiae* treated silkworms

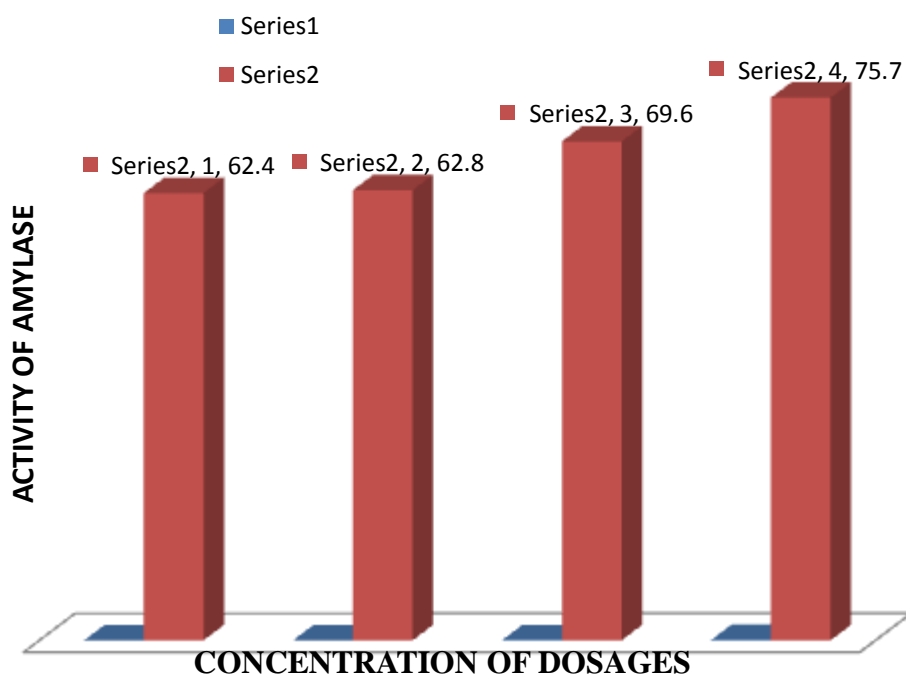


Table 2: Enzymatic activity of Invertase in the midgut of the fifth instar larvae of control and *Saccharomyces cerevisiae* treated worms

Treatment	Different dosages	Invertase activity
<i>Saccharomyces Cerevisiae</i>	Control	34.4
	1%	34.6
	3%	38.7
	5%	45.2



Figure 2:Enzymatic activity of Invertase in the midgut of the fifth instar larvae of control and *Saccharomyces cerevisiae* treated worms

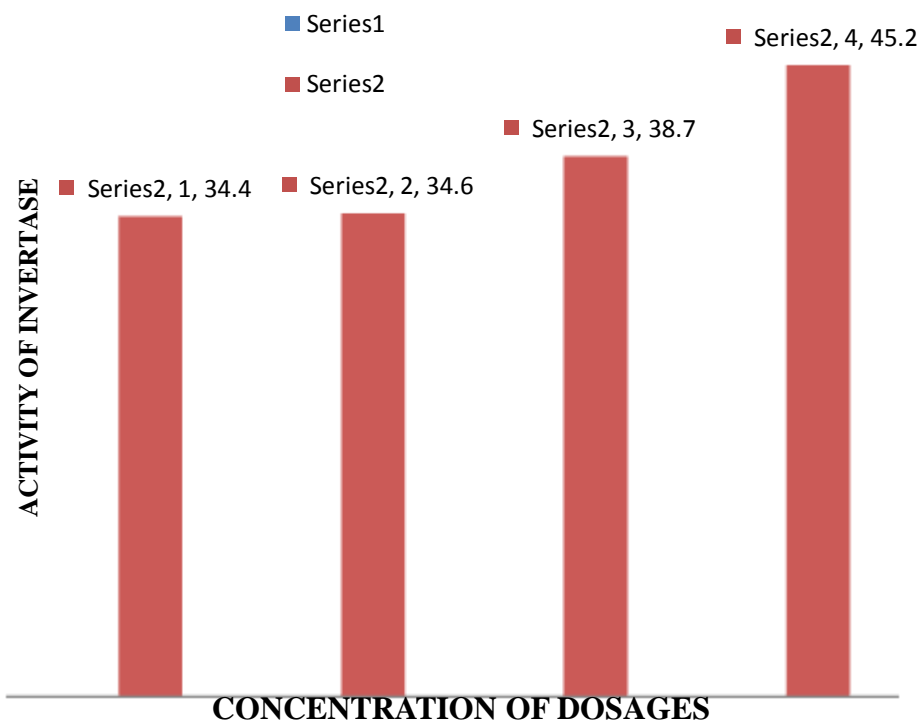


Table 3: Economic parameters data of control and different concentrations of *Saccharomyces Cerevisiae* treated MR2 mulberry leaves fed larvae produced cocoon (x+SD)Percentage change over control is given in parenthesis

Probiotic Treatment	Different dosages	Cocoon weight (gm)	Pupal weight (gm)	Shell weight (gm)	Shell ratio (%)	Filament length (meters)	Reelability (%)	Denier (%)
<i>Saccharomyces cerevisiae</i>	Control	29.17 ± 1.02	10.17 ±0.55	19.78 ±0.08	15.01	881.4 ± 17.5	40	23.43 ± 1.01
	1%	28.19 ± 1.89	10.15 ±0.62	19.43 ±0.09	15.25 (2.6%)	880.6 ± 17.9	40.4	22.13 ± 2.42
	3%	31.65 ± 0.94	14.19 ±0.32	25.08 ±0.04	16.96 (12.9%)	890.8 ± 16.4 (2.11%)	44	25.24 ± 0.09 (6.03%)
	5%	35.12 ± 0.19	16.69 ±0.12	29.27 ±0.001	17.48 (16.5%)	924.7 ± 12.6 (6.09%)	50	26.19 ± 0.01 (11.9%)

Significant at $P < 0.05$

REFERENCES

- [1] Aruga, H. and Tarada, Y. (1971) the cytoplasmic polyhedrosis virus of the silkworm, University of Tokyo Press, Japan pp : 234.
- [2] Baker, J.E. (1991) Purification and partial characterization of amylase allozymes from the lesser grain borer *Rhyzoperthadominica*. *Insect Biochem*, 21, pp : 303-311.
- [3] Bernard, E.A. and prosser.(1973) Comparative biochemistry and physiology of digestion. In *Comparative Animal Physiology* (Edt, by Prosser , C.L.) 3rd ed. Chapter 4,pp :133-163. Saunders,Philedephia.
- [4] Bernfeld, P. (1955)Amylase , a and b. In *methods in Enzymology* (Edited by colowick S.P. and Kaplan, N.O) Vol. 1, pp: 149-158.

- [5] Bhosale, S.H. and Kallapur, V.L. (1990) Fuel reserves in the silkworm *Bombyx mori* larvae infested with polyhedral bodies. *India J. Seric.*, 29(1) : 83-87.
- [6] Buonocore, V., Poerio, E., Silano, V. and Tomasi, M. (1976) Physical and catalytic properties of α -amylase from *Terebrimolitor* L. larvae. *Biochem. J.* 153, pp : 621-625.
- [7] Charles, T.J.B., (2004). Probiotics in agriculture. *Advanced Biotech.*, January pp27-30.
- [8] Daone, W.W., Abraham, I., Kolar, M.M., Martenson, R.E. and Deibler, G.E. (1975) Purified *Drosophila* α -amylase isozyme. In isozyme IV (Edited by market C.L.), pp: 585-607. Academic press, New York.
- [9] Fuller., (1993). Probiotics foods. current use and future developments *Int. Food Ingrid.*, 3:23- 26.
- [10] Govindan R and Devaiah MC (1995). Aspergillosis of silkworm. *Silkworm Pathology Technical Bull No.1*. Dept. of Seri. UAS Bangalore, p:68.
- [11] Gururaj, C.S., Sekarappa, B.M. and Sarangi, S.K (1999). Effect of BM NPV Infection on the digestive enzyme activity in the silkworm. *Indian. J.Seric.*, 38 (2):102-106.
- [12] Horie, Y. and Watanabe, H. (1980) Recent advances in sericulture. *Ann. Rev. Ent.* 25: 49-71.
- [13] Janakiraman, A.T. (1961) Disease affecting the Indian silkworm, races. *J. silkworm* 13: 91- 101.
- [14] Jeyaraman, J. (1981) Laboratory manual in Biochemistry, Willey Limited, New Delhi.
- [15] Krishnaswami S. (1978). *Sericulture Manual Vol. 2: Silkworm rearing*. FAO Bulletin, Rome. Srirampura, Mysore. 16-18 November ABSTRAC NO.MMSPP/0-7 P.87.
- [16] Lakshmi Kumari, B. (1995) Effects of gamma radiation on the cytological, physiological and biochemical aspects of silkworm *Bombyx Mori* L. Ph.D. Thesis, Bangalore University, Bangalore, India.
- [17] Laskar, N. and Datta, M. 2000. Effect of Alfalfa tonic and its organic ingredients on growth and development of silkworm *Bombyx mori* L. *Race Nistari. Environment and Ecology*. 18(3): 591-596.
- [18] Mahesh, H.B. (1997) Cytogenetic and biochemical studies in few races of *Bombyx mori* L., Treated with cytoplasmic ployhedrosis virus and a mutagen. Ph.D Thesis, University of Mysore, Mysore, India.
- [19] Martignoni, M.E. (1964) Pathophysiology in the insect. *Annu. Rev. Entmol.*, 9: 179-206.
- [20] Nanavathy, M.M. (1965) *Silkworm from Grob to Glamour*. Paramount Publishing House, Bombay, pp: 227.
- [21] Pankaj, U., Raundal., Harshal, E., Patil., Pramod, M. and Chaudhari. (2005) *Sericulture: an agro – based rural industry*, *Agrobios*, 4(4): pp: 48-49.
- [22] Prasad, N.R. (1999) *Silkworm disease management and its limitations*. *Indian silk*, Vol. 31 (10): pp: 7-9.
- [23] Ratna Sen, Nataraju B., Selvakumar, T., Chandrasekharan, K. and Thiagarajan, V., (2003). Relationship between the susceptibility of silkworm, *Bombyx mori* to denso nucleosis and infectious flacherie virus infection, *Ind. J. Seric.*, 42(1): 59-60.
- [24] Ravikumar, C. (1988) western Ghats as a bivoltine region prospects, challenges and strategies for its development *Indian silk*, 26(9): 39-54.
- [25] Saito, S. (1963) Trehalose in the body fluid of the silkworm, *Bombyx Mori* L. *J. Insect Physiol.*, 9: 509-519.
- [26] Sannapa, B., MJ. Ramesh and D. Chandrappa, (2002). Influence of castor genotype on consumption indices of eri silkworm *sumia Cynthia ricini*. *Bioduval. Enviorn. Eco.*, 20: 960-964.
- [27] Satapathy, S. (2003) Studies on bio-energetic of tasar silkworm, *Antheraeamyitta D.* during diapauses. *Indian J. Seric.*, 42(1): 32-34.
- [28] Savithri, G. and Murali Mohan, P. (2003a) Pathogenicity of the bacterium *Bacillus coagulans* in silkworm *Bombyx mori* L. *India J. Seric.* 42(1): 4-8
- [29] Savithri, G. and Murali Mohan, P. (2003b) *In vitro* screening of antibiotics against *Bacillus coagulans*. *Indian J. Seric.*, 42(1) 25-28.
- [30] Savithri, G. and Murali Mohan, P. (2003). Pathogenicity of the bacterium *Bacillus coagulans* In silkworm *Bombyx mori* L. *Indian J. Seric.* 42(1): 4-8.
- [31] Sengupta, K., Singh, B.D. and Mustafi, J.C. (1972) Nutrition of silkworm *Bombyx mori* L. 1. studies on enrichment of mulberry with various sugars, proteins, amino acids and vitamins for vigorous growth of worms and increased cocoon production. *Indian J. Seric.*, 11(1): 11-19.
- [32] Sheeba Rajakumari, D.V. Padamalatha, C., Jeyapaul. C., Sam Manohar doss, and S., Ranjit Singh, A.J.A. (2005) Leucine and valine on the Economic characters of silkworm *Bombyx mori* L. (Bombycidae : Lepidoptera). *Journal of Theoretical and Experimental Biology* 1(4): xx.xx.
- [33] Singh, B.D., Balavenkatasubbaiah, M., Sharma, S.D and Baig, M. (1994). Selection of resistance to pathogenic bacterium in laboratory populations of the silkworm, *Bombyx mori* L., *Sericologia*, 34: 103-108.
- [34] Singh, K.K., R.M. Chauhan, A.B. Pande, S.B. Gokhale and N.G. Hegde, (2005). Effect of Use of *Lactobacillus plantarum* as a Probiotics to improve Cocoon Production of Mulberry Silkworm, *Bombyx mori* (L.). *J. Basic Appl. Sci.* Vol. (1), June-Nov.: 1-8.
- [35] Singh, K.K., Chauhan, R.M., Pande, A.B., Gokhale, S.B. and Hegde, N.G. 2005. Effect of use of *Lactobacillus plantarum* as a probiotics to improve cocoon production of mulberry silkworm, *Bombyx mori* (L.). *J. Basic Appl. Sci.*, June-November: 1-8.
- [36] Venkataramana, P., Srinivasa Rao, T.V.S., Sreenivasa Reddy, P. and Suryanarayan, N. (2003) Effects of spirulina on the larval and cocoon characters of the silkworm, *Bombyx mori* L. *Proc. Nat. Acad. Sci. India*, 73B(1): 89-94.

