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SEASONAL IMPACT ON AMYLASE AND INVERTASE ACTIVITY IN SILKWORM *Bombyx mori* (L) REARED ON V1 AND M5 MULBERRY VARIETY

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The suitable and appropriate environmental conditions and also the mulberry leaves with high nutritional content for feeding is the key for the success of sericulture industry.The rearing of silkworms for getting better yield of raw silk there is need to study the environmental impact on the enzyme activities which will directly affect the food consumption and utilization. Hence, in the present study the impact of seasons viz summer, winter, and rainy on Amylase and Invertase activity from the midgut,fatbodies,haemolymph and silkgland of silkworm *Bombyxmori* L. was analysed. A comparative study was done by feeding the larvae with two different varieties of mulberry viz V1 and M5. Among the seasons,insummer,significant increase in the amylase activityobserved in all test tissues whilein winter invertase activity increasedin all test tissues of silkworm larvae fed on both V1 and M5 mulberry leaves.

Keywords: Silkworm; *Bombyx mori* L; seasons; mulberry; amylase; invertase.

1. INTRODUCTION

Sericulture is an agro-based industry in India from economic point of view. Hence, it becomes necessary to find out most favorable environmental condition and better variety of mulberry leaves to boost this industry. The mulberry, eri, temperate and tropical tasar, muga silk are the varieties of silk produced by the Indian silk Industry. Among these, mulberry silk production is higher and widely used in the India.

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Mulberry is cultivated in the tropical and subtropical countries of world. In India the cultivation of mulberry is mostly done in Tamil Nadu, Andhra Pradesh, Jammu and Kashmir, Karnataka, Uttar Pradesh and West Bengal due to favourable climatic conditions [1]. The leaves of mulberry are the only source of nutrition like protein, carbohydrates, vitamins, mineralsetc for the growth and development of silkworms [2-3]. The quality of mulberry leaves and environmental conditions influence the dietary efficiency and growth of silkworm [4].

Effect of seasons on larval growth, development and cocoon characters of silkworm was studied by Das and Vijayaraghavan [5]. The trehalase, invertase and amylase activites were studied in the black scale, *Saiscetaioleae* [6]. The analysis of amylase and invertase activities in the larvae of Egyptian cottonworm, *Spodopteralittoralis* were carried out earlier [7]. The seasons with change in climatic conditions influence the metabolism of the silkworm in various aspects and so it becomes important to study the effect of seasons. Hence, present study was conducted to study the impact of seasons on the amylase and invertase activities in the silkworm *Bombyx mori* L fed on V1 and M5 mulberry variety.

2. MATERIALS AND METHODS

Multivoltine silkworm race of *Bombyx mori* (L.) pure Mysore was selected for the study.

Leaves two varieties of mulberry V_1 and M_5 were used to feed the silkworm *B. mori*.

2.1 Photoperiod Observation

The photoperiodic change of 18 hrs light: 6 hrs dark and 18 hrs dark and 6 hrs light was achieved by using locally available card sheet box and table lamp. The larvae fed with M5 and V1 mulberry varieties in rearing tray were covered with the card sheet box for the exposure of dark condition and the lamp was focused on larvae from about 2 feet long distance for the light. This was carried out in winter season [8].

Control Group: Silkworm larvaes were reared by maintained standard condition.

Tissues used: Mid gut, silk gland fat bodies and haemolymph.

Preparation of homogenates: These homogenates were centrifuged at 3000 rpm for 15 minutes. The supernatants were used as assay samples for the estimations.

Estimation of enzymes: The carbohydratases like amylase and invertase were determined from mid gut, silk gland, fat bodies and haemolymph of silkworm *Bombyx mori* by using DNSA (Dinitro salicylic acid) method [6]..

For estimation of amylase and invertase 1 ml of buffer (Carbonate – bicarbonate buffer, pH: 9.2 for amylase and phosphate buffer, pH: 6.6 for invertase) and 1 ml of substrate (1% starch solution) followed by 0.5 ml of assay sample were added in each tube. For blank the assay sample was replaced by distilled water. This mixture was incubated at 30^0C for 20 minutes in water bath. After incubation the enzyme activity was terminated by the addition of 2 ml DNSA solution followed by 2 ml distilled water. Then these tubes were heated in boiling water bath for 5 minutes. After heating the tubes were cooled and the optical density was measured against blank at 540 nm on colorimeter. For each set of experiment three replicates were taken and the experiment was repeated 3 times.

From the optical density the activity of amylase was calculated with the help of standard graph. The values were expressed as μ mole glucose / mg tissue.

2.2 Standard curve for Amylase and Invertase

For this standard graph the stock solution glucose of 1 mg / ml was prepared. From the above standard solution 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml (0, 200, 400, 600, 800 and 1000 μg glucose / ml respectively) were taken in different test tubes. The volume was made to 1 ml by adding appropriate volumes of distilled water. To this, 1 ml buffer was added (Carbonate bicarbonate- pH 9.2). Then this mixture was heated at 30°C for 20 minutes. After incubation 2 ml DNSA (Dinitro salicylic acid) solution followed by distilled water was added to this mixture. These tubes were again heated in boiling water bath for 5 minutes and then they were cooled and the optical density was measured at 540 nm on colorimeter. The graph was plotted for glucose concentration versus optical densities.

3. RESULTS

3.1 Amylase

Perusal of Table 1 revealed the amylase activity in different tissues of silkworm larvae fed on M_5 mulberry variety at different seasons. The amylase activity from midgut, fatbodies, silkgland and haemolymph increased significantly in summer season (0.33, 0.0745 μ mole glucose/mg, 0.235 μ mole glucose/mg and 0.0785 μ mole glucose/ml tissue respectively), while decreased significantly in winter (0.292, 0.0412 µ mole glucose/mg, 0.172 µ mole glucose/mg tissue and 0.0467 µ mole glucose/ml respectively) and rainy season (0.232, 0.0355 µ mole glucose/mg tissue, 0.165 µ mole glucose/mg and 0.0432µ mole glucose/ml tissue respectively) as compared to the control i.e. 0.317, 0.0575 µ mole glucose/mg tissue, 0.2 µ mole glucose/ mg and 0.0482µ mole glucose/ml tissue respectively.

In case of V_1 mulberry variety fed larvae the activity of amylase in midgut, fatbodies, silkgland and haemolymph was decreased in winter (0.453, 0.0695 µ mole glucose/mg tissue, 0.257 µ mole glucose/mg and 0.0667 µ mole glucose/ml tissue respectively) and rainy season (0.347, 0.053 µ mole glucose/mg tissue , 0.225 µ mole glucose/mg and 0.062 µ mole glucose/ml tissue respectively). However, it increased in all tissues in summer season (0.506, 0.098 µ mole glucose/mg tissue, 0.342 µ mole glucose/mg and 0.114 µ mole glucose/ml tissue respectively), as compared to the control (0.46, 0.0632 µ mole glucose/mg tissue, 0.027 µ mole glucose/mg and 0.0622 µ mole glucose/ml tissue respectively) (Table 2).

Table 3 revealed that the amylase activity in midgut, fatbodies, silkgland and haemolymph of M_5 mulberry variety fed larvae increased significantly in the 18L: 6D photoperiod (0.447, 0.075 µ mole glucose/mg tissue , 0.029 µ mole glucose/mg and 0.0845 µ mole glucose/ml tissue respectively) while decreased in 18hrs. dark (0.266, 0.0312 µ mole glucose/mg tissue, 0.142 µ mole glucose/mg and 0.062 µ mole glucose/ml tissue respectively), when compared to the control (0.317, 0.0575 µ mole glucose/mg tissue, 0.2 µmole glucose/mg and 0.0482 µ mole glucose/ml tissue respectively).

Perusal of Table 3 shows that in case of V_1 mulberry variety fed larvae the amylase activity in midgut, fatbodies, silkgland and haemolymph decreased significantly in larvae exposed to 18 hrs dark (0.369, 0.066 µ mole glucose/mg tissue, 0.208 µ mole glucose/mg and 0.057 µ mole glucose/ml tissue respectively), and increased when the larvae were exposed to 18 hrs. light i.e. 0.529, 0.094 µ mole glucose/mg tissue, 0.36 µ mole glucose/mg and 0.122 µ mole glucose/ml tissue respectively when compared to that of control (0.46, 0.0632 µ mole glucose/mg tissue, 0.27 µ mole glucose/mg and 0.0622 µ mole glucose/ml tissue respectively).

Table 1. Effect of different seasons on amylase activity in silk worm *Bombyx mori* (L.) reared on M_5 mulberry variety

Sr. No	Tissue	Amylase activity at different seasons			
		Control	Summer	Winter	Rainy
1.	Mid gut	0.317^{a} (±0.0095)	0.33^{a} (±0.0081)	0.292^{a} (± 0.029)	0.232^{a***} (± 0.005)
2.	Fat bodies	0.0575^{a} (±0.0089)	0.0745^{a**} (± 0.0056)	0.0412^{a} (± 0.054)	0.0355^{a**} (± 0.0018)
3.	Haemolymph	0.0482^{b} (±0.0086)	0.0785^{b***} (± 0.0017)	0.0467^{b} ** (± 0.0055)	0.0432^{b} (± 0.0084)
4.	Silkgland	0.2^{a} (±0.014)	0.235^{a} ** (± 0.0057)	0.172^{a} ** (± 0.005)	0.165^{a} ** (± 0.0126)

Figures in parenthesis indicate standard deviation

*** $P < 0.01$*** $P < 0.001$a- µ mole glucose/mg tissue b- µ mole glucose/ml*

Table 2. Effect of different seasons on amylase acitivty in silkworm *Bombyx mori* (L.)V_1 mulberry variety

Sr. No.	Tissue	Amylase activity at different seasons			
		Control	Summer	Winter	Rainy
1.	Mid gut	0.46^{a} (±0.016)	0.506^{a***} (±0.0075)	0453^{a} (± 0.0095)	0.347^{a} *** (± 0.0125)
2.	Fat bodies	0.0632^{a} (± 0.013)	0.098^{a} ** (±0.0067)	0.0695^{a} (± 0.011)	0.053^{a} (±0.0070)
3.	Haemolymph	0.0622^{b} (±0.018)	0.114^{b} ** (±0.0048)	0.0667^{b} (± 0.010)	0.062^{b} (±0.0081)
4.	Silkgland	0.27^{a} (±0.0081)	0.342^{a***} (±0.0095)	0.257^{a} (±0.0125)	0.225^{a***} (± 0.0035)

Figures in parenthesis indicate standard deviation

*** $P < 0.01$*** $P < 0.001$a - µ mole glucose/mg tissueb- µ mole glucose/mg tissue*

3.2 Invertase

The invertase activity in midgut, fatbodies, silkgland and haemolymph of silkworm fed on M_5 mulberry variety leaves are depicted in Table 4. It decreased significantly in summer (0.332, 0.0675 µ mole glucose/mg tissue, 0.257 µ mole glucose/mg and 0.0752 µ mole glucose/ml tissue respectively), and in rainy seasons (0.461, 0.0917 µ mole glucose/mg tissue, 0.330 µ mole glucose/mg and 0.106 µ mole glucose/ml tissue respectively). It increased significantly in winter season (0.746, 0.122 µ mole glucose/mg tissue, 0.136 µ mole glucose/ml and 0.430 µ mole glucose/mg tissue respectively) as compared to the control (0.614, 0.101 µ mole glucose/mg tissue, 0.405 µ mole glucose/mg and 0.114 µ mole glucose/ml tissue respectively).

In case of V_1 mulberry variety fed larvae at different seasons the invertase activity from midgut, fatbodies, silkgland and haemolymph increased significantly in winter (0.577, 0.100 µ mole glucose/mg tissue, 00.404 µ mole glucose/mg and 124 µ mole glucose/ml tissue respectively), while decreased in summer (0.260, 0.044 µ mole glucose/mg tissue, 0.210 µ mole glucose/mg and 0.07 µ mole glucose/ml respectively) and rainy season (0.426, 0.076 µ mole glucose/mg tissue, 0.3 µ mole glucose/mg and 0.090 µ mole glucose/ml tissue respectively), compared to control (0.538, 0.083 µ mole glucose/mg tissue, 0.378 µ mole glucose/mg and 0.108 µ mole glucose/ml tissue respectively) (Table 4.).

4. DISCUSSION

The digestive carbohydrates in insect hydrolyze polysaccharides, oligosaccharides and disaccharides to their constituent monosaccharides. These enzymes are secreted mainly by the salivary glands and epithelium of the mid gut. Among carbohydrates amylase is the key enzyme. It is important for the survival of silkworm (Chatterjee et.al 1993). Silkworm needs specific nutrient components such as vital sugars, amino acids, proteins, and vitamins for its optimal growth and development [9,10]. Lowly nutrition diets will directly affect the primary biochemical and physiological metabolism in insects, and in turn alter the detoxification system leading to amplifiedsusceptibility to diseases [11].

Table 3. Effect of different seasons on invertase activity in silk worm *Bombyx mori* (L.) reared on M_5 mulberry variety

Sr. No.	Tissue	Invertase activity at different seasons			
		Control	Summer	Winter	Rainy
1.	Mid gut	0.614^{a} (± 0.011)	0.332^{a}*** (± 0.011)	0.746^{a} ** (± 0.076)	0.461^{a} *** (± 0.016)
2.	Fat bodies	0.101^{a} (± 0.0068)	0.0675^{a}*** (± 0.011)	0.122^{a} ** (± 0.0090)	0.0917^{a} (± 0.0062)
3.	Haemolymph	0.114^{b} (± 0.0062)	0.0752^{b}*** (±0.0040)	0.136^{b} ** (± 0.0047)	0.106^{b} (± 0.0036)
4.	Silkgland	0.405^{a} (± 0.0036)	0.257^{a}*** (±0.0106)	0.430^{a} *** (± 0.0057)	0.330^{a} *** (± 0.0076)

Figures in parenthesis indicate standard deviation

*** P <0.01*** P < 0.001a - µ mole glucose/mg tissueb - µ mole glucose/ml*

Table 4. Effect of different seasons on invertaseacitivty in silkworm *Bombyxmori* (L.) reared on V_1 mulberry variety

Sr. No.	Tissue	Invertase activity at different seasons			
		Control	Summer	Winter	Rainy
1.	Mid gut	0.538^{a} (± 0.0076)	0.260^{a}*** (±0.0101)	0.577^{a} ** (± 0.017)	0.426^{a} *** (± 0.0043)
2.	Fat bodies	0.083^{a} (± 0.0080)	0.044^{a}*** (±0.0058)	0.100^{a} * (± 0.0062)	0.076^{a} (± 0.0105)
3.	Haemolymph	0.108^{b} (±0.0063)	0.07^{b} *** (± 0.0067)	0.124^{b} * (± 0.0098)	0.090^{b} ** (± 0.0031)
4.	Silkgland	0.378^{a} (±0.0132)	0.210^{a}*** (±0.0042)	0.404^{a} ** (± 0.0041)	0.3^{a} *** (± 0.0127)

Figures in parenthesis indicate standard deviation

** P < 0.05** P < 0.01*** P <0.001a - µ mole glucose/mg tissue b - µ mole glucose/ml*

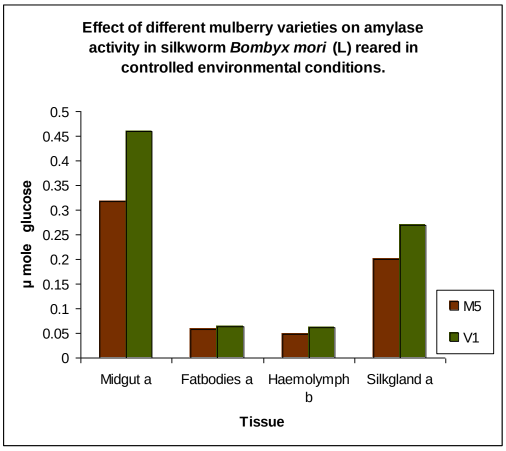

Fig. 1. a -µ mole glucose / mg tissue
b-µ mole glucose / ml

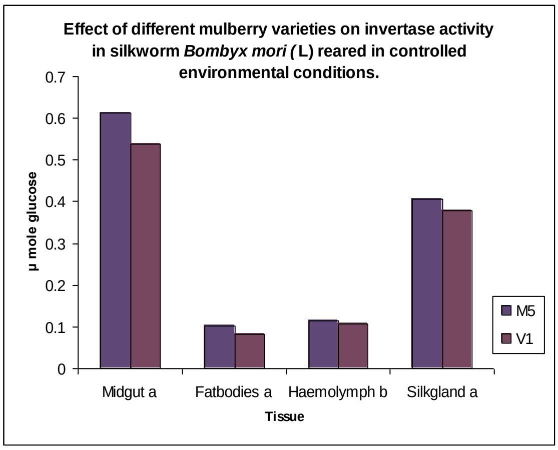

Fig. 2. a - µ mole glucose / mg tissue
b - µ mole glucose / ml

In the present investigation, amylase activity was studied in midgut, fatbodies, haemolymph and silkgland of silkworm larvae. Amylase activity was more in case of midgut, haemolymph and silkgland in V_1 mulberry variety fed larvae than M_5 mulberry variety fed larvae at controlled environmental conditions, while it was at par in case of fatbodies. (Figs 1 and 2). The amylase activity improved with increase in age during the fifth instar in EMS treated batches of silkworm with 1.1 mg/g/min [12] Proceedings of the 107th indian science congress bangalore, section of agriculture and forestry sciences).

The activity of amylase increased significantly in summer season while decreased in winter and rainy seasons in all test tissues of the larvae reared on M_5 and V_1 mulberry variety. Here also it was observed that increase in amylase activity in summer was significant in case of V_1 mulberry variety fed larvae than in case of M_5 mulberry variety fed larvae. Increased amylase activity in summer season is beneficial for the survival of the silkworm. Reverse picture was found in case of invertase activity. When maintained at controlled conditions invertase activity was observed more in case of M_5 mulberry variety fed larvae than V_1 mulberry variety fed larvae. Throughout spring, the amylase activity levels in midgut digestive juice showed its peak on the fifth day of fifth instar larvae of silkworms [13]. From the 1^{st} to 2^{nd} day gradual increase and on 3^{rd} to 4^{th} day gradual decrease in enzyme activity in multivoltine breeds were detected.

Invertase activity increased in winter season in all test tissues of silkworm larvae fed on M_5 and V_1 mulberry varieties. While decreased in both summer and rainy season the increase in the invertase activity may be due to higher sucrose content of mulberry leaves in the winter season.

The mulberry silkworm is one of the most excellent models to study insect physiology and biochemistry. When the exposure of light is increased to 18 hours carried out in winter season the larvae shows tremendous response in growth pattern. The optimal light regime for the culture of miiuy croaker during the early life stage was 18L:6D [14].

5. CONCLUSION

From the present work it can be concluded that there is possibilities of acquire positive seasonal impact and increased photoperiod impact in improving physiological parameters like enzyme activity in silkworms. The V1 mulberry variety, winter season and 18hours light all these factors showed improved enzymatic activities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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