

AMYLASE AND SUCCINATE DEHYDROGENASE ACTIVITY LEVELS IN F₁ PROGENY RAISED FROM ETHYL METHANESULFONATE TREATED SILKWORM, *BOMBYX MORI* L.

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Two races of mulberry silkworm namely, Pure Mysore and NB₄D₂, at fifth instar first day were treated with 2.5, 5 and 10 mM ethyl methanesulfonate by oral injection. The F₁ progeny was obtained by selfing the moths emerged from the EMS treated silkworms and control sets separately. The activity levels of amylase and succinate dehydrogenase in haemolymph and midgut tissues of F₁ progeny at fifth instar was estimated. The results clearly indicated that the silkworm batches obtained from the EMS treated larvae exhibited almost similar pattern with altered levels of enzyme activity when compared to their respective control sets.

Key words : Amylase, *Bombyx mori*, ethyl methanesulfonate, F₁ progeny, succinate dehydrogenase.

INTRODUCTION

Goldschmidt, Troland, Wright and many other investigators have given serious considerations to gene-enzyme hypothesis (Kikkawa, 1953). Research activities concerning different digestive enzymes in the silkworm *Bombyx mori* were initiated after the pioneering works of Matsumura (1934). Of the various enzymes analysed, amylase is the most well worked out because of its association with economic characters of silkworm, *Bombyx mori*. The analysis of enzymes like amylase, succinate dehydrogenase, alkaline phosphatase and alkaline protease may help in the silkworm breeding programme for improvement of cocoon characters and disease resistance (Mahesha, 1997; Lakshmi Kumari, 1995). However, studies combining biochemical aspects with mutagens in general and chemical mutagens in particular are rather scarce. Therefore, the present investigation was carried out in this line.

MATERIALS AND METHODS

Two mulberry silkworm races namely, Pure

Mysore (multivoltine), NB₄D₂ (bivoltine) at the age of fifth instar first day and a well known ethylating agent, ethyl methanesulfonate (EMS) were used for the study.

The silkworm rearing of both parent and F₁ progeny was conducted in the laboratory following the method described by Krishnaswami (1978). The silkworms at the age of fifth instar first day were taken for treatment. Three different concentrations of EMS like 2.5, 5 and 10 mM were selected. Forty µl of final concentration of ethyl methanesulfonate freshly prepared in 0.75 % NaCl solution was administered separately to each worm into the gut by 'oral injection'. The control worms received the same amount of NaCl solution only. For each concentration, 60 worms in triplicate were taken. After treatment, the larvae were allowed to continue development and metamorphose. The F₁ progeny was obtained by selfing the moths emerged from the silkworm batches treated with EMS as well as control sets separately.

About 5-10 F₁ larvae during fifth instar were collected daily at regular interval of 24 h. The abdominal legs were punctured and the haemolymph was collected in pre-chilled eppendorf tubes containing 1 mM thiourea crystals to prevent oxidation. The midgut tissue was obtained from five larvae by dissecting in ice-cold water. The gut contents were removed and the tissue was thoroughly washed in distilled water. A 1% (w/v) homogenate of the midgut tissue was prepared in pre-cooled distilled water using mortar and pestle. Both haemolymph and midgut homogenate samples were centrifuged at 3000 rpm for 10 minutes. The clear supernatant was used for the assay of amylase and succinate dehydrogenase (SDH) activity.

Quantitative analysis of amylase activity was done for haemolymph and midgut tissues following the method of Ishaaya and Swirski (1976). The values were expressed as mg glucose released/ml/min and mg glucose released/g/min for haemolymph and midgut tissue, respectively. Succinate dehydrogenase activity levels were estimated by the method of Nachlas *et al.* (1960). The values were

expressed as μ moles farmazan formed/ml/hour and μ moles farmazan formed/g/hour for haemolymph and midgut tissues, respectively.

The experimental data obtained were subjected to two way ANOVA (Fisher and Yates, 1953) and Duncan Multiple Range Test (DMRT) (Duncan, 1955).

RESULTS AND DISCUSSION

The amylase activity in haemolymph of silkworms of Pure Mysore race (control) increased with the increase in the age during the fifth instar, and reached maximum on sixth day. Again, gradual reduction in the enzyme activity was observed on seventh and eighth day. This pattern of amylase activity was also observed in EMS treated batches with slight alteration, apart from increased rate of activity (Figure 1). The average enzyme activity during fifth instar exhibited by 5 mM set was the highest (0.57 mg/ml/min), followed by 10 mM (0.55 mg/ml/min), control (0.5 mg/ml/min) and 2.5 mM (0.49 mg/ml/min) set. The results of two way ANOVA revealed that the variation between tested batches, between age groups and the interaction effect between tested sets and age groups are all significant at 0.000 level. Further, the DMRT revealed that, 5 mM set has statistically significant ($p < 0.05$) variation in amylase activity from that of the control and 2.5 mM sets. The amylase activity levels in midgut tissue in the control worms of Pure Mysore showed its peak on fifth day. Again, gradual reduction was noticed from sixth day until the end of fifth instar. EMS treated batches also exhibited the same pattern with increased rate of enzyme activity except for 2.5 mM set wherein the highest activity was observed on sixth day (Figure 2). During fifth instar, the average enzyme activity of 2.5 mM set was the highest with 1.1 mg/g/min followed by 5 mM set with 1.05 mg/g/min, 10 mM set with 1.00 mg/g/min and control larvae with 0.95 mg/g/min. The variation between tested sets, between age groups and the interaction effect between tested batches and age groups are all statistically significant at 0.000 level. Further, the DMRT revealed that control sets were significantly ($P < 0.05$) different from worms treated with 2.5 mM EMS. In the case of NB_4D_2 race, the amylase activity levels in haemolymph of control larvae, gradually increased from first day, reached its maximum activity level on third day, and gradually decreased from fourth day up to sixth day. EMS treated batches also exhibited similar pattern with enhanced rate of enzyme activity (Figure 3) of all the batches examined, the average enzyme activity of 2.5 mM set was the highest with 0.52 mg/ml/min, which was followed by the control batch with 0.49 mg/ml/min, 10 mM set with 0.48 mg/ml/min and 5 mM set with 0.45 mg/ml/min. The results of statistical analysis revealed that

the variation between tested batches, between age groups and the interaction effect between treated sets and age groups are all significant at 0.0000 level. Further, the results of DMRT revealed that the 2.5 mM set is significantly different ($P < 0.05$) from 5 mM set. The amylase activity levels in midgut tissue of NB_4D_2 control worms showed gradual increment from first to fourth day. From fifth day, gradual reduction was noticed until the end of last instar. The EMS treated batches also exhibited same pattern of enzyme activity with enhanced rate (Figure 4). Of the tested sets, the average enzyme activity in the worms treated with 10 mM of EMS was the highest (1.40 mg/g/min), followed by 5 mM of (1.22 mg/g/min), 2.5 mM (1.15 mg/g/min) and control worms (1.1 mg/g/min). The results of two way ANOVA revealed that the variation between tested sets, between the age groups and the interaction effect between tested sets and age groups are all significant at 0.000 level. Further, the results of DMRT revealed that the 10 mM set is significantly ($P < 0.05$) different from all the remaining sets. Among the tissues studied, the midgut exhibited more activity than haemolymph of both Pure Mysore and NB_4D_2 races.

The succinate dehydrogenase activity in the haemolymph of control larvae of Pure Mysore race showed a gradual increment from first to fifth day. From sixth day onwards till the end of fifth instar, gradual decrement in enzyme activity was noticed. In the case of EMS treated batches, the pattern of enzyme activity was almost the same (Figure 5). Of all the batches tested, the average activity of the succinate dehydrogenase was more in 10 mM set with 2.29 μ moles/ml/hour followed by control larvae with 2.19 μ moles/ml/hour, 2.5 mM batch with 2.17 μ moles/ml/hour and 5 mM set with 1.95 μ moles/ml/hour. The variation between tested sets, between age groups and the interaction effect between experimental sets and age groups are all found to be significant at 0.000 level. In the case of midgut tissue, the SDH activity levels gradually increased from first to fifth day. From sixth day onwards till the end of fifth instar, gradual decrement in enzyme activity was noticed. The EMS treated batches also exhibited the same pattern of enzyme activity with enhanced rate (Figure 6). Of all the batches examined, the average activity of SDH in 2.5 mM set was the highest with 64.65 μ moles/g/hour/followed by 5 mM set with 61.31 μ moles/g/hour, 10 mM batch with 54.98 μ moles/g/hour and control set with 52.92 μ moles/g/hour. The SDH activity levels in haemolymph of the control larvae of NB_4D_2 , significantly increased till the end of fifth instar. The EMS treated batches also exhibited similar pattern with slight alteration apart from enhanced rate of enzyme activity (Figure 7). Of all the batches examined, silkworms of 5 mM set had the highest activity of 1.87 μ moles/ml/hour followed by 10 mM set with 1.76 μ moles/ml/hour,

control larvae of 1.66 $\mu\text{moles/ml/hour}$ and 2.5 mM batch of 1.52 $\mu\text{moles/ml/hour}$. The variation among tested batches, between the age groups and the interaction effect between tested sets and age groups are all significant at

0.000 level. Further, the DMRT revealed that worms treated with 2.5 mM had significant variation in enzyme activity at 0.05 level from that of 5 mM batch. In the case of midgut tissue, the succinate dehydrogenase activity was

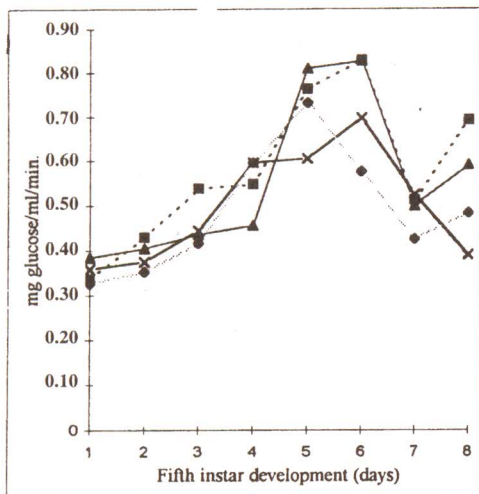


Figure 1: Amylase activity levels in haemolymph of F_1 progeny raised from EMS treated Pure Mysore larvae.

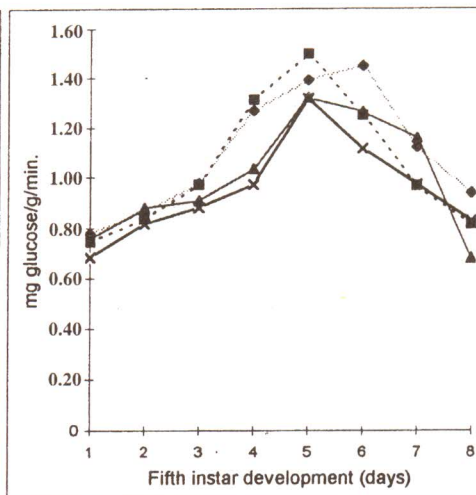


Figure 2: Amylase activity levels in midgut of F_1 progeny raised from EMS treated Pure Mysore larvae.

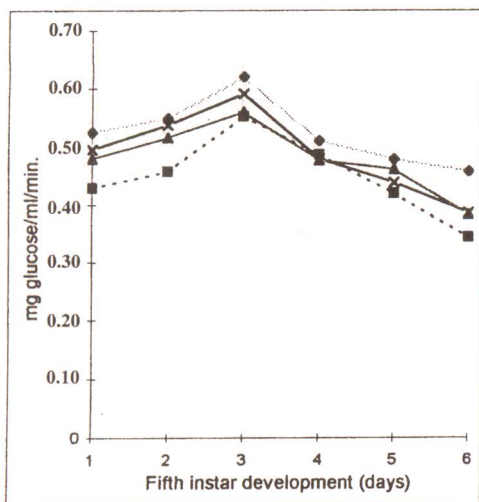


Figure 3: Amylase activity levels in haemolymph of F_1 progeny raised from EMS treated NB_4D_2 larvae.

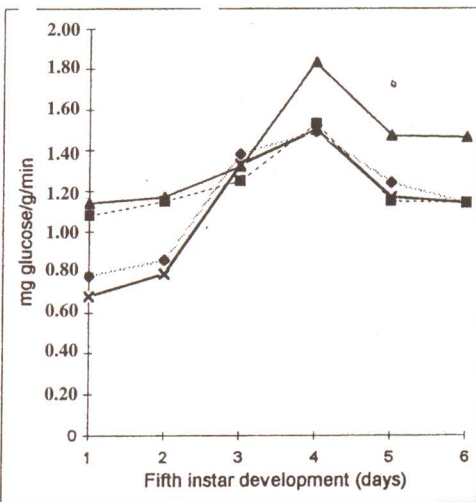


Figure 4: Amylase activity levels in midgut of F_1 progeny raised from EMS treated NB_4D_2 larvae.

Control—x—; 2.5 mM—♦—; 5 mM—■—; 10 mM—▲—

the highest on first day and it decreased gradually up to fifth day. Again, on sixth day, there was a significant increment in the SDH activity when compared to the preceding days that is, from third to fifth day. However, it was significantly less than that of first day's activity. In the case of EMS treated batches, the 2.5 mM set followed the pattern of control set, whereas, 5 mM and 10 mM sets

exhibited their peak activity on the first day and gradual reduction was observed throughout the fifth instar (Figure 8). Of all the batches analyzed, the average enzyme activity during fifth instar was found to be the highest in 5 mM set (60.95 μ moles/g/hour) followed by 2.5 mM (58.92 μ moles/g/hour), control (58.09 μ moles/g/hour) and 10 mM (53.57 μ moles/g/hour) batches.

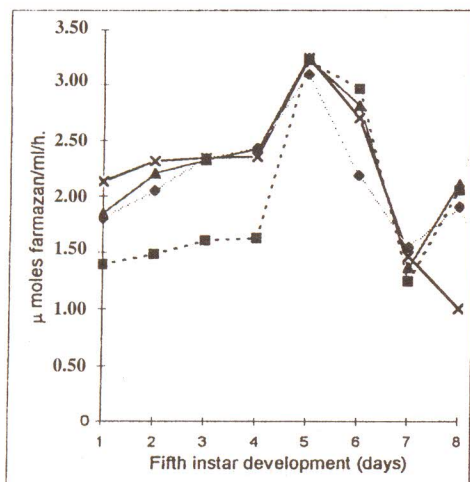


Figure 5: Succinate dehydrogenase activity levels in haemolymph of F_1 progeny raised from EMS treated Pure Mysore larvae.

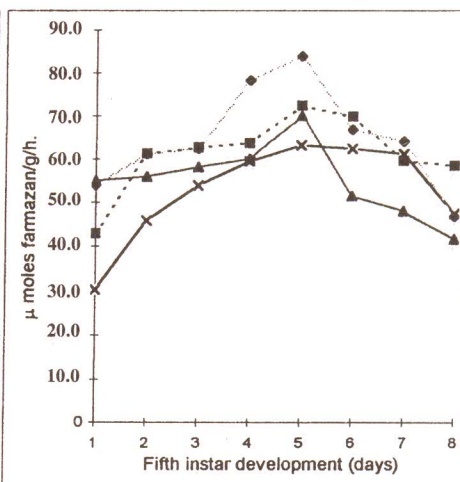


Figure 6: Succinate dehydrogenase activity levels in midgut of F_1 progeny raised from EMS treated Pure Mysore larvae.

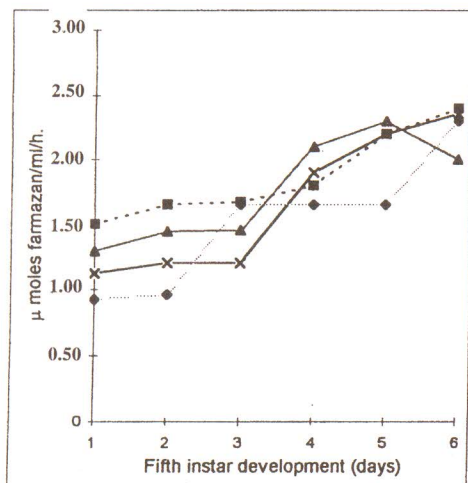


Figure 7: Succinate dehydrogenase activity levels in haemolymph of F_1 progeny raised from EMS treated NB_4D_2 larvae.

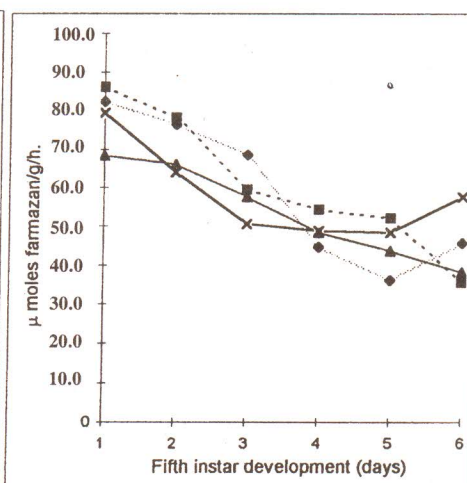


Figure 8: Succinate dehydrogenase activity levels in midgut of F_1 progeny raised from EMS treated NB_4D_2 larvae.

Control- —x— ; 2.5 mM- —●— ; 5 mM- —■— ; 10 mM- —▲— :

In the present investigation, variation in the amylase and succinate dehydrogenase activity levels might be either due to one of the two possibilities. Firstly, the genotype of the silkworm larvae might be altered as the EMS is a potent mutagen, which causes all sorts of mutations in a wide variety of organisms *i.e.*, from viruses to mammals (Sega, 1984). Secondly, it might be due to transmission of some influencing factor generated by the EMS treatment, from parents to progeny. This alteration and/or transmission might enhance the levels of utilization of exogenous food material and its efficient conversion (Mahesha, 1997). Apart from this, Hirata and Yosuo (1974) found that the silkworm strains which have more amylase activity had better cocoon weight, shell weight and shell percentage. Lakshmi Kumari, (1995) treated the Pure Mysore silkworm larvae with different doses of gamma rays. The silkworm batches treated with lower doses (500 roentgen) of gamma rays exhibited enhanced rate of amylase and protease activity, in haemolymph as well as midgut tissue. Further, 500 roentgen treated larvae were heavier and produced better cocoons with more shell weight, shell percentage and filament length when compared with other experimental sets. Mahesha (1997), reported that the silkworms treated with lower doses of EMS exhibited low level of blood glucose, more protein content with altered protein fractions (Mahesha *et al.*, 2000) and increased rate of digestive as well as oxidizing enzymes in both blood as well as midgut tissue. Such alterations in the biomolecules of silkworm larvae might help in the utilization of more food material and efficient conversion of digested food material, ultimately leading to superior economic traits. Further, the silkworm larvae of both Pure Mysore and NB₄D₂ races, treated with 2.5 and 5 mM EMS exhibited improvement in commercial characters like fecundity, cocoon yield, cocoon weight, shell weight and filament length when compared to their respective controls as well as 10 mM sets. Lakshmi Kumari *et al.* (1997) also opined that the high activity of the amylase and protease in haemolymph and midgut tissue might be due to a greater utilization of exogenous proteins resulting in the production of more silk. Therefore, by studying the amylase and succinate dehydrogenase enzymes, it is possible to have a clear picture of the metabolism of the insect after the treatment with a mutagen. The variations in the activity levels of enzymes during present investigations clearly indicated both synthesis as well as utility of specific proteins. An understanding of such biochemical processes will help us to identify the productive proteins during the evolution of the new races of silkworm, *Bombyx mori*.

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