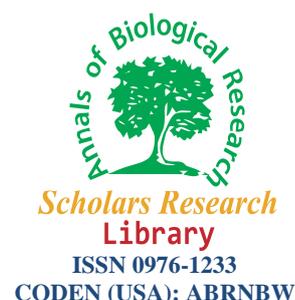




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## Studies on the esterase and its relationship with commercial characters of silkworm *Bombyx mori* L.

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### ABSTRACT

Four pure mulberry silkworm breeds viz., Pure Mysore, Nistari, NB<sub>4</sub>D<sub>2</sub> & CSR<sub>2</sub> and two hybrid (Pure Mysore x CSR<sub>2</sub> and Nistari x NB<sub>4</sub>D<sub>2</sub>) silkworms were selected for the present study. The specific activity of esterase in the haemolymph, midgut and fat body tissues was estimated. The qualitative analysis of esterase was carried out by Native-PAGE. The commercial characters viz., fecundity, larval weight, larval duration, cocoon weight, shell weight, shell ratio, filament length, denier and renditta were selected. The esterase activity levels were subjected for regression analysis against selected commercial characters to know the level and kind of correlation between them. The results of statistical analysis clearly showed that the haemolymph esterase activity levels exhibited positive correlation with denier, filament length, larval duration, shell ratio, cocoon weight and shell weight. The midgut esterase activity levels showed positive correlation with denier, fecundity, filament length, larval weight, shell ratio, cocoon weight and shell weight. Also, the activity levels of fat body esterase indicated positive correlation with denier, fecundity, filament length, larval weight, shell ratio, cocoon weight and shell weight only. The zymograms of esterase also exhibited variation among the selected silkworm breeds.

**Keywords:** *Bombyx mori*, haemolymph, midgut, esterase, commercial characters.

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### INTRODUCTION

By virtue of a long history of rearing for commercial purpose, silkworm has been the subject of research interest, resulting in careful collection, cataloguing and maintenance of various silkworm genetic stocks of considerable scientific and economic interest. A vast array of distinct geographical races and inbred lines are available that represent variations for a number of qualitative and quantitative traits of basic biological and economic interests, such as silk quality, fecundity, pathogen resistance and heat tolerance. Esterases (Acetylcysteine; EC 3.1.1.6) are a group of enzymes which catalyze the hydrolysis of various types of acetyl esters. The study of such polymorphic enzymes is an important tool during the selection for economically important traits. The analysis of enzymes like amylase, succinate dehydrogenase [1,2,3,4], alkaline phosphatase and alkaline protease [5] may help in silkworm breeding programme for cocoon characters and disease resistance. In silkworm, biochemical parameters of amylase in terms of activity and isozyme polymorphism have highlighted to use as a marker in breeding programme [6,7]. In insects, esterase has been implicated in resistance to organophosphate insecticides and their banding patterns on electrophoretic gels have been shown to provide an extremely reliable method for determining biotypes [8]. Esterase zymograms were studied with respect to ontogeny [9] and cocoon shape [10] in *Bombyx mori*. However, correlation studies combining esterase with commercial characters of silkworm *Bombyx mori* are rather scarce. Hence, the present investigation was undertaken.

## MATERIALS AND METHODS

Four pure mulberry silkworm breeds *viz.*, Pure Mysore, Nistari, NB<sub>4</sub>D<sub>2</sub> & CSR<sub>2</sub> and two hybrid (Pure Mysore x CSR<sub>2</sub> and Nistari x NB<sub>4</sub>D<sub>2</sub>) silkworms were selected for the present investigation. The silkworm rearing was conducted in the laboratory following the method described by Krishnaswamy [11,12]. All experimental batches were maintained in triplicate. In each replication 500 larvae were kept after third moult. The economic traits selected for present study included fecundity, weight of fifth instar larva, larval duration, cocoon weight, shell weight, shell ratio, filament length, denier and renditta.

The larvae from first day of fifth instar were collected daily with a regular interval of 24h till the end of fifth instar. The haemolymph was collected, centrifuged at 3000 rpm for 5 minutes in a cooling centrifuge at 5°C [2,13] and preserved in a deep freezer at -20°C as stock and it was used whenever required.

The midgut and fat body tissues were obtained from five silkworms of fifth instar by dissecting the larvae in ice cold water and the gut contents were removed. The tissues were thoroughly washed in distilled water. A 10 % (w/v) homogenate of the tissues were prepared in pre cooled distilled water using mortar and pestle. The homogenate was centrifuged at 3000 rpm for 10 minutes in a cooling centrifuge at 5°C. The clear supernatant was used for the enzyme analysis.

The total soluble protein present in the haemolymph and midgut tissue was estimated by following the method of Lowry *et al.* [14]. Bovine Serum Albumin was used as standard protein.

Quantitative analysis of non specific esterase activity was done in haemolymph and midgut tissues following the method of Valdes and Chambers [15] with following modifications. The reaction mixture contained 40µl of 0.1M sodium phosphate buffer (pH 7.0), 90 µl double glass distilled water and 20 µl 0.025 M paranitrophenylacetate in acetone was incubated at 37 ° C for 5 min. After this pre incubation, appropriately diluted 1 µl haemolymph for haemolymph esterase assay and 10 µl tissue extract (0.5 %) for midgut and fat body esterase assay respectively. Incubation of this mixture was carried out for 1 min at 37°C in a water bath. After 1min, 1 ml of ice cold chloroform was added and the contents were mixed thoroughly, then 1.5ml of 0.2M sodium phosphate buffer (pH 9.0) was added. The contents were shaken vigorously, centrifuged at 2000 rpm for 5 min at 5°C. The supernatant was collected and the optical density was measured at 400 nm setting the spectrophotometer to zero with blank consisted of incubation mixture to which enzyme sample was added after termination of the reaction. The activity of the enzyme was expressed as µmoles of paranitrophenol released /mg protein/min at 37°C. Paranitrophenol was used as standard.

The experimental data were statistically analyzed through SPSS by one way ANOVA, [16], Scheffe's post hoc [17] and linear regression analysis [18] wherever they were applicable.

The qualitative analysis of non specific esterase isozymes was carried out in Native Poly Acrylamide Gel Electrophoresis with the discontinuous buffer system containing 5% stacking and 8% separating gel. The vertical slab gel apparatus was used. The gels, soon after the removal, washed in running distilled water and incubated in the following solution C in a rotary shaker in for 20 min at 37°C in dark. After the appearance of bands the reaction was stopped by the addition of 2-3 ml glacial acetic acid. After the appearance of bands, the gels were scanned, analyzed and photographed in a gel scanner (Vilber Laurmat Bioprofil image analysis system). Solution A was prepared by dissolving 25 mg  $\alpha$  naphthyl acetate and 25 mg  $\beta$  naphthyl acetate in 1 ml of acetone followed by the addition of 1 ml water and 12.5 ml of 0.5 M sodium phosphate buffer pH 5.9. Solution B was prepared by dissolving 25 mg fast blue RR salt in 2 ml of solution A, followed by the addition of 12.5 ml 0.1 M sodium phosphate buffer pH 6.5. Solution C was prepared by mixing solutions A and B.

## RESULTS

The summary of the studied commercial characters are presented in the table1. From the table it is clear that the two bivoltine races are superior for productivity traits whereas multivoltines are superior for viability traits. The hybrids showed average values of their parents. The results of one way ANOVA revealed that the variation in all commercial characters among the experimental batches are all significant at 0.1 % (P<0.001). The specific activity of esterase in haemolymph, midgut and fat body tissue samples is shown in the tables 2, 3 and 4 respectively. The specific activity of esterase in the haemolymph, midgut and fat body tissues were estimated. The activity of esterase in haemolymph, midgut and fat body tissues samples showed significant changes in their activity levels at every 24 hours till the end of fifth instar. The results of one way ANOVA revealed that the variation among the experimental batches are all found to be significant at 0.1 % (P<0.001). The highest esterase activity in haemolymph was

observed in Pure Mysore (8.92  $\mu\text{M}/\text{mg}$  protein/min at 37°C was the average during fifth instar) followed by CSR<sub>2</sub> (8.60  $\mu\text{M}/\text{mg}/\text{min}$  at 37°C), Pure Mysore x CSR<sub>2</sub> (8.53  $\mu\text{M}/\text{mg}/\text{min}$  at 37°C), NB<sub>4</sub>D<sub>2</sub> (7.59  $\mu\text{M}/\text{mg}/\text{min}$  at 37°C), Nistari x NB<sub>4</sub>D<sub>2</sub> (7.33  $\mu\text{M}/\text{mg}/\text{min}$  at 37°C) and Nistari (6.55  $\mu\text{M}/\text{mg}/\text{min}$  at 37°C). In the case of midgut tissue, the highest activity of esterase was observed in NB<sub>4</sub>D<sub>2</sub> (11.43  $\mu\text{M}/\text{mg}/\text{min}$  at 37°C) followed by CSR<sub>2</sub> (11.36  $\mu\text{M}/\text{mg}/\text{min}$  at 37°C), Nistari (10.23  $\mu\text{M}/\text{mg}/\text{min}$  at 37°C), Pure Mysore (9.32  $\mu\text{M}/\text{mg}/\text{min}$  at 37°C), Nistari x NB<sub>4</sub>D<sub>2</sub> (8.72  $\mu\text{M}/\text{mg}/\text{min}$  at 37°C) and Pure Mysore x CSR<sub>2</sub> (8.22  $\mu\text{M}/\text{mg}/\text{min}$  at 37°C). In the case of fat body tissue, the highest activity of esterase was observed in Nistari x NB<sub>4</sub>D<sub>2</sub> (7.76  $\mu\text{M}/\text{mg}/\text{min}$  at 37°C) followed by Pure Mysore x CSR<sub>2</sub> (7.41  $\mu\text{M}/\text{mg}/\text{min}$  at 37°C), NB<sub>4</sub>D<sub>2</sub> (7.24  $\mu\text{M}/\text{mg}/\text{min}$  at 37°C), CSR<sub>2</sub> (7.05  $\mu\text{M}/\text{mg}/\text{min}$  at 37°C), Pure Mysore (6.77  $\mu\text{M}/\text{mg}/\text{min}$  at 37°C) and Nistari (6.70  $\mu\text{M}/\text{mg}/\text{min}$  at 37°C). The results of quantitative analysis were subjected for regression analysis against selected commercial characters to know the correlation coefficient between them. The results of regression analysis between the haemolymph esterase activity levels and commercial characters are presented in figures 1-9. The results of statistical analysis clearly showed that the haemolymph esterase activity levels exhibited positive correlation with denier ( $R^2=0.209$ ), filament length ( $R^2=0.044$ ), larval duration ( $R^2=0.579$ ), shell ratio ( $R^2=0.062$ ), single cocoon weight ( $R^2=0.029$ ) and single shell weight ( $R^2=0.063$ ) only. In the case of midgut tissue, the results of regression analysis are presented in figures 10-18. The esterase activity levels showed positive correlation with denier ( $R^2=0.099$ ), fecundity ( $R^2=0.710$ ), filament length ( $R^2=0.088$ ), larval weight ( $R^2=0.460$ ), shell ratio ( $R^2=0.351$ ), cocoon weight ( $R^2=0.110$ ) and shell weight ( $R^2=0.293$ ) only. Also, in the case of fat body tissue, the results of regression analysis are presented in figures 19-27. The activity levels of fat body esterase showed positive correlation with denier ( $R^2=0.043$ ), fecundity ( $R^2=0.019$ ), filament length ( $R^2=0.381$ ), larval weight ( $R^2=0.143$ ), shell ratio ( $R^2=0.076$ ), cocoon weight ( $R^2=0.296$ ) and shell weight ( $R^2=0.107$ ) only.

The zymograms of esterase also exhibited variation among the selected silkworm varieties. A number of qualitative and quantitative variations were observed in the zymograms of haemolymph, midgut and fat body tissue esterase (figures 28-36). In the case of multivoltines, the haemolymph esterase isozymes in Pure Mysore exhibited entirely different pattern of banding when compared to Nistari silkworms. In the case of Pure Mysore larvae, an isozyme fraction with 0.395 R.F. exhibited more intensity during days of fifth instar except 5<sup>th</sup> and 6<sup>th</sup> day. Also, another band with R.F. 0.323 appeared prominently from 1<sup>st</sup> to 6<sup>th</sup> day only and it was completely disappeared on 7<sup>th</sup> and 8<sup>th</sup> day. Also, a band with R.F. 0.105 appeared on 1<sup>st</sup>, 2<sup>nd</sup> and 7<sup>th</sup> day only. However, in the case of Nistari three bands with R.F. 0.404 is prominent in 1<sup>st</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 6<sup>th</sup> day. Also one band with R.F. 0.140 was appeared in 2<sup>nd</sup> and 4<sup>th</sup> day only. Among the bivoltines, the bands with R.F. 0.186 and 0.314 were observed in 5<sup>th</sup> and 6<sup>th</sup> day of NB<sub>4</sub>D<sub>2</sub> silkworms; however a band with R.F.0.201 appeared from 1<sup>st</sup> to 6<sup>th</sup> day in case of CSR<sub>2</sub> silkworms. In addition, two isozyme fractions with R.F. 0.429 and 0.467 were prominent in CSR<sub>2</sub> silkworms. Of the hybrids, Pure Mysore x CSR<sub>2</sub> silkworms showed two prominent bands with R.F. 0.335 and 407. In Nistari x NB<sub>4</sub>D<sub>2</sub> silkworms, a band with R.F. 0.392 exhibited gradual reduction in its intensity as the age advances. In the case of midgut esterase isozyme profiles of Pure Mysore and Nistari silkworms, the banding pattern was almost same. In Pure Mysore silkworms, a band with R.F. 0.431 was present from 2<sup>nd</sup> to 4<sup>th</sup> day only and the same band was absent in case of Nistari silkworms. In the case of bivoltines, two bands with R.F. 0.321 and 0.331 from NB<sub>4</sub>D<sub>2</sub> and CSR<sub>2</sub> silkworms respectively, appeared only during later stages of fifth instar. In the case of hybrids, an isozyme fraction with 0.244 present throughout the fifth instar of Pure Mysore x CSR<sub>2</sub> silkworms, but same fraction is totally absent in case of Nistari x NB<sub>4</sub>D<sub>2</sub> silkworms. In the case of fat body esterase isozyme profiles of Pure Mysore silkworms, two bands with R.F. 0.257 and 0.614 were prominent on 6<sup>th</sup> day only. Also, in Nistari silkworms a band with R.F. 0.515 is prominent on 2<sup>nd</sup> day only. In bivoltine zymogram profiles, NB<sub>4</sub>D<sub>2</sub> showed two bands with R.F. 0.271 and 0.363 were prominent during early ages of fifth instar. In case of CSR<sub>2</sub> silkworms, a band with R.F. 0.246 was prominent on 3<sup>rd</sup> day. Of the hybrids the banding pattern was almost same. However, in Pure Mysore x CSR<sub>2</sub> silkworms a band with R.F. 0.487 was absent on 6<sup>th</sup> and 7<sup>th</sup> day. On the other hand, Nistari x NB<sub>4</sub>D<sub>2</sub> silkworms showed two bands (R.F. 0.422 and 0.586) with more intensity.

**Table 1: Mean values  $\pm$  SD of nine commercial characters in six breeds of silkworm *Bombyx mori***

SILKWORM BREEDS	FECUNDITY (No.)	LARVAL WEIGHT (g)	LARVAL DURATION (h)	COCOON WEIGHT (g)	SHELL WEIGHT (g)	SHELL RATIO (%)	FILAMENT LENGTH (m)	DENIER	RENDITTA
Pure Mysore	467.22 $\pm$ 10.96	2.01 $\pm$ 0.06	660 $\pm$ 10.39	1.02 $\pm$ 0.75	0.12 $\pm$ 0.01	12.57 $\pm$ 0.49	426.44 $\pm$ 19.83	1.77 $\pm$ 0.09	11.77 $\pm$ 0.82
Nistari	485.11 $\pm$ 5.30	2.83 $\pm$ 0.06	564.88 $\pm$ 10.01	1.14 $\pm$ 0.71	0.15 $\pm$ 0.01	13.41 $\pm$ 0.87	435.66 $\pm$ 17.21	1.78 $\pm$ 0.07	13.26 $\pm$ 0.24
CSR <sub>2</sub>	509.10 $\pm$ 16.58	4.07 $\pm$ 0.05	578.88 $\pm$ 6.45	1.81 $\pm$ 0.47	0.43 $\pm$ 0.01	24.02 $\pm$ 0.18	1011.99 $\pm$ 12.34	2.93 $\pm$ 0.22	5.78 $\pm$ 0.23
NB <sub>4</sub> D <sub>2</sub>	520.55 $\pm$ 16.65	4.16 $\pm$ 0.05	576.67 $\pm$ 11.08	1.76 $\pm$ 0.30	0.35 $\pm$ 0.01	20.27 $\pm$ 0.15	1020 $\pm$ 29.96	2.48 $\pm$ 0.06	8.34 $\pm$ 0.47
Pure Mysore X CSR <sub>2</sub>	466.66 $\pm$ 11.52	2.68 $\pm$ 0.07	610 $\pm$ 11.10	1.67 $\pm$ 0.23	0.28 $\pm$ 0.01	17.29 $\pm$ 0.21	910 $\pm$ 18.74	2.75 $\pm$ 0.06	7.64 $\pm$ 0.12
Nistari X NB <sub>4</sub> D <sub>2</sub>	490.77 $\pm$ 6.81	3.46 $\pm$ 0.04	557 $\pm$ 10.21	1.47 $\pm$ 0.22	0.23 $\pm$ 0.01	16.06 $\pm$ 0.85	805.99 $\pm$ 12.36	1.83 $\pm$ 0.02	9.22 $\pm$ 0.85
F	89.775	4210.79	853.92	3570.99	3898.36	1484.63	65.17	311.48	28230.47

Values are the mean  $\pm$  SD of Pre monsoon, Monsoon and post monsoon observations.

The variation between the races is statistically significant at 0.1 % ( $P < 0.001$ ).

**Table 2: Esterase activity levels ( $\mu$  moles of product released/mg protein/min at 37°C) in haemolymph during fifth instar**

SILKWORM BREEDS	1 <sup>st</sup> Day	2 <sup>nd</sup> Day	3 <sup>rd</sup> Day	4 <sup>th</sup> Day	5 <sup>th</sup> Day	6 <sup>th</sup> Day	7 <sup>th</sup> Day	8 <sup>th</sup> day	AVERAGE
Pure Mysore	8.35	8.32 (-0.35)	8.45 (+1.56)	8.56 (+1.30)	9.62 (+12.38)	8.94 (-7.06)	9.73 (+8.83)	9.42 (-3.18)	8.92
Nistari	5.61	6.01 (+7.13)	6.56 (+9.15)	6.17 (-5.94)	6.59 (+6.80)	7.99 (+21.24)	-	-	6.55
CSR <sub>2</sub>	10.35	8.77 (-15.26)	9.48 (+8.09)	7.37 (-22.25)	7.38 (+0.13)	8.26 (+11.92)	-	-	8.60
NB <sub>4</sub> D <sub>2</sub>	8.69	7.49 (-13.80)	8.16 (+8.94)	6.92 (-15.19)	6.73 (-2.74)	7.60 (+12.92)	-	-	7.59
Pure Mysore x CSR <sub>2</sub>	7.75	9.23 (+19.09)	10.06 (+8.99)	7.69 (-23.55)	8.39 (+9.10)	8.17 (-2.62)	8.43 (-3.18)	-	8.53
Nistari x NB <sub>4</sub> D <sub>2</sub>	5.62	6.56 (+16.72)	6.68 (+1.82)	7.08 (+5.98)	8.96 (+26.55)	9.10 (-1.56)	-	-	7.33

The variation between the races is statistically significant at 0.1 % ( $P < 0.001$ ).

Values within parentheses represent per cent change over previous day.

**Table 3: Esterase activity levels ( $\mu$  moles of product released /mg protein/min at 37°C) in midgut tissue during fifth instar**

SILKWORM BREEDS	1 <sup>st</sup> Day	2 <sup>nd</sup> Day	3 <sup>rd</sup> Day	4 <sup>th</sup> Day	5 <sup>th</sup> Day	6 <sup>th</sup> Day	7 <sup>th</sup> Day	8 <sup>th</sup> day	AVERAGE
Pure Mysore	8.10	7.93 (-2.09)	8.04 (+1.38)	7.79 (-3.10)	9.40 (+20.66)	10.89 (+15.85)	11.75 (+7.89)	10.67 (-9.19)	9.32
Nistari	9.07	9.99 (+10.14)	9.92 (-0.7)	10.28 (+3.62)	11.27 (+9.63)	10.88 (+3.46)	-	-	10.23
CSR <sub>2</sub>	9.95	10.45 (+5.02)	11.09 (+6.12)	10.88 (-1.89)	13.02 (+19.66)	12.81 (-1.61)	-	-	11.36
NB <sub>4</sub> D <sub>2</sub>	9.67	12.10 (+25.12)	12.01 (-0.74)	11.53 (-3.99)	10.68 (-7.37)	12.61 (+18.07)	-	-	11.43
Pure Mysore x CSR <sub>2</sub>	6.73	6.86 (+1.93)	7.67 (+11.80)	7.38 (-3.78)	8.72 (+18.15)	9.99 (+14.56)	10.22 (+2.30)	-	8.22
Nistari x NB <sub>4</sub> D <sub>2</sub>	7.81	8.37 (+7.17)	8.03 (-4.06)	8.39 (+4.48)	8.99 (+7.15)	10.73 (+19.35)	-	-	8.72

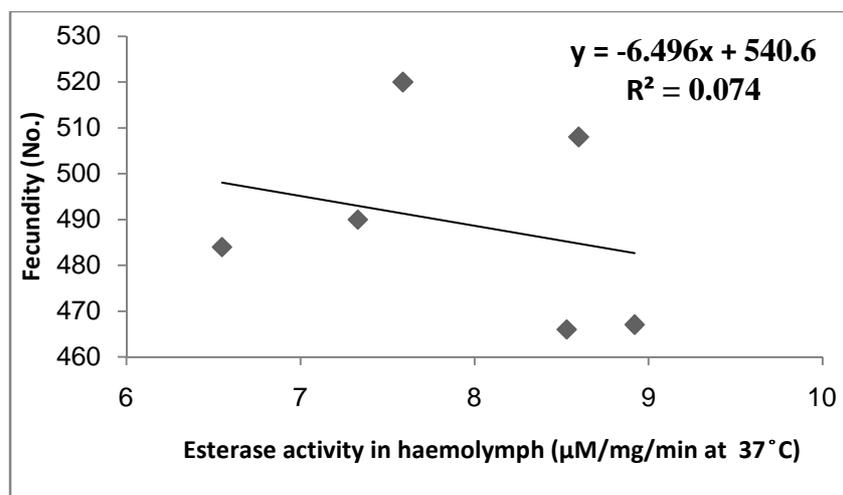
The variation between the races is statistically significant at 0.1 % ( $P < 0.001$ ).

Values within parentheses represent per cent change over previous day.

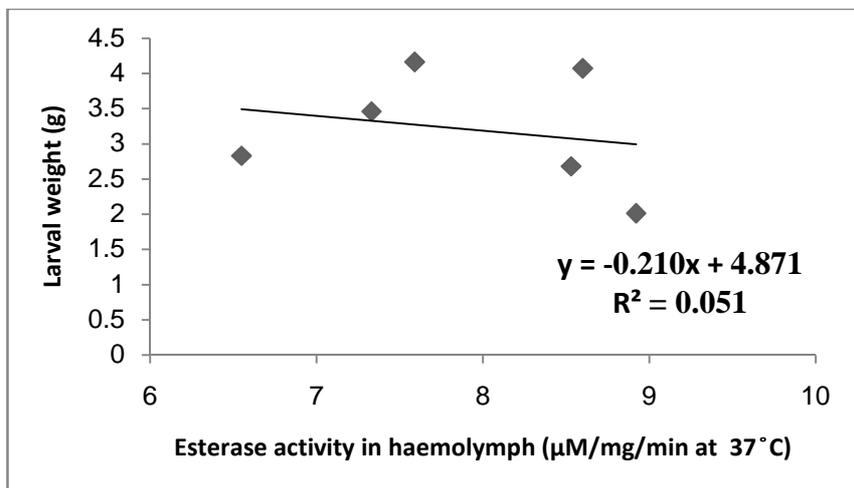
**Table 4: Esterase activity levels ( $\mu$  moles of product released /mg protein/min at 37°C) in fat body during fifth instar**

SILKWORM BREEDS	1 <sup>st</sup> Day	2 <sup>nd</sup> Day	3 <sup>rd</sup> Day	4 <sup>th</sup> Day	5 <sup>th</sup> Day	6 <sup>th</sup> Day	7 <sup>th</sup> Day	8 <sup>th</sup> day	AVERAGE
Pure Mysore	5.06	6.44 (+27.27)	7.25 (+12.57)	6.21 (-14.34)	6.01 (-3.22)	6.86 (+14.14)	8.01 (+16.76)	8.35 (+4.24)	6.77
Nistari	4.37	4.87 (+11.44)	7.50 (+54.00)	7.46 (-0.53)	8.04 (+7.77)	7.99 (-0.62)	-	-	6.70
CSR <sub>2</sub>	5.76	6.17 (+7.11)	6.41 (+3.88)	6.55 (+2.18)	8.21 (+25.34)	9.25 (+12.66)	-	-	7.05
NB <sub>4</sub> D <sub>2</sub>	4.87	5.59 (+14.87)	6.75 (+20.75)	7.84 (+16.14)	8.67 (+10.58)	9.73 (+12.22)	-	-	7.24
Pure Mysore x CSR <sub>2</sub>	6.02	6.58 (+9.30)	7.72 (+17.32)	6.61 (-14.37)	7.77 (+17.54)	8.23 (+5.92)	8.97 (+8.99)	-	7.41
Nistari x NB <sub>4</sub> D <sub>2</sub>	5.87	6.81 (+16.01)	7.71 (+13.21)	8.51 (+10.37)	8.11 (+4.70)	9.55 (+17.55)	-	-	7.76

The variation between the races is statistically significant at 0.1 % ( $P < 0.001$ ).  
Values within parentheses represent per cent change over previous day.



**Figure 1: Correlation between haemolymph esterase activity level and fecundity**



**Figure 2: Correlation between haemolymph esterase activity level and larval weight**

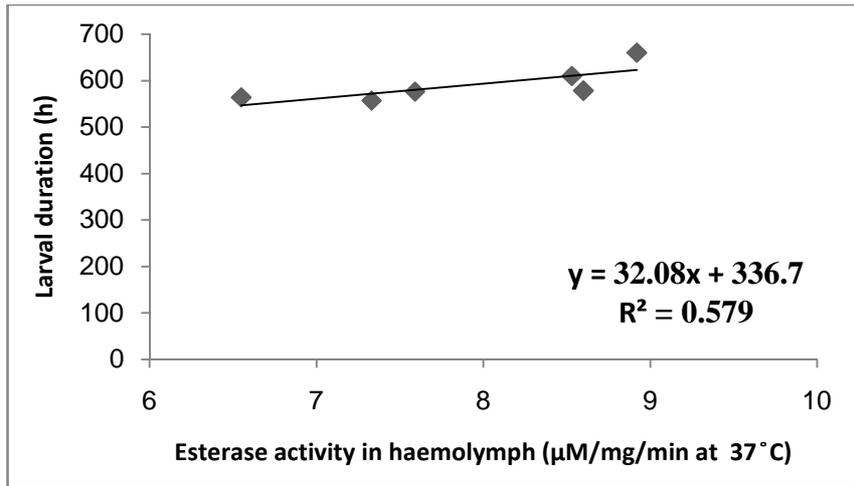


Figure 3: Correlation between haemolymph esterase activity level and larval duration

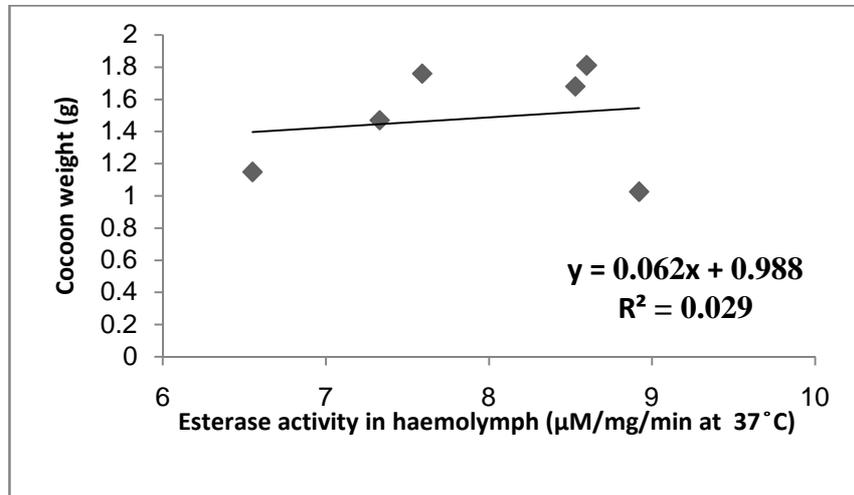


Figure 4: Correlation between haemolymph esterase activity level and cocoon weight

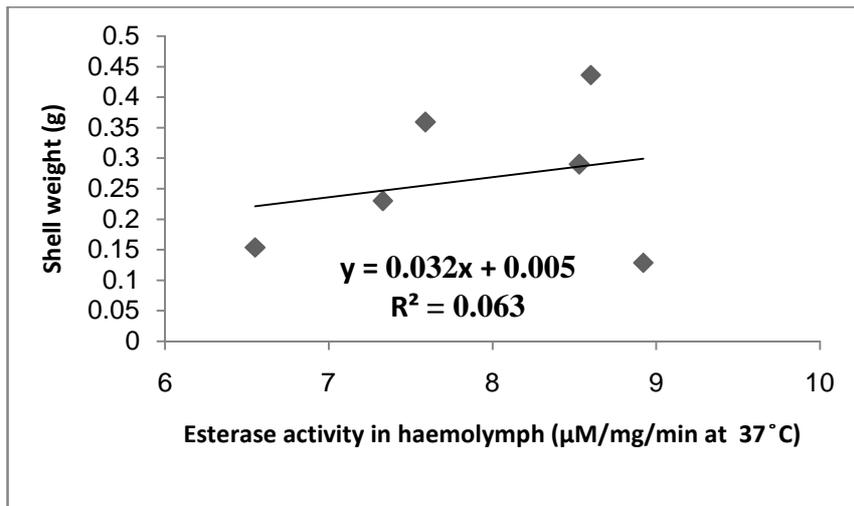


Figure 5: Correlation between haemolymph esterase activity level and shell weight

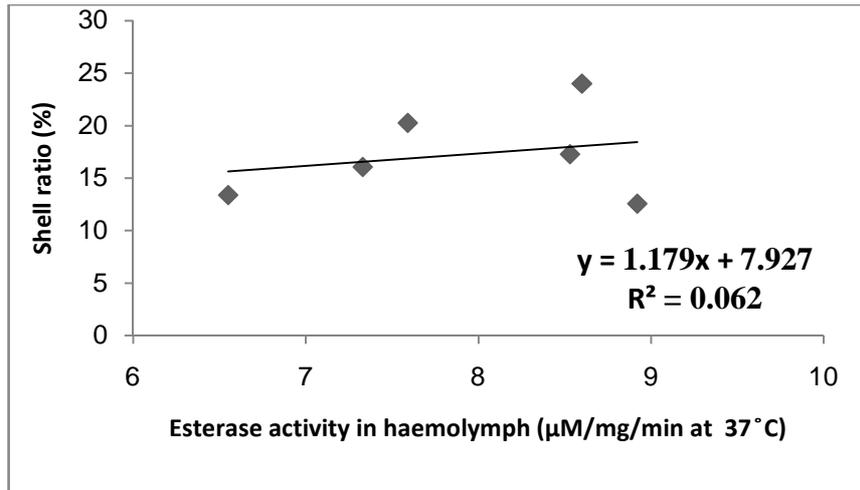


Figure 6: Correlation between haemolymph esterase activity level and shell ratio

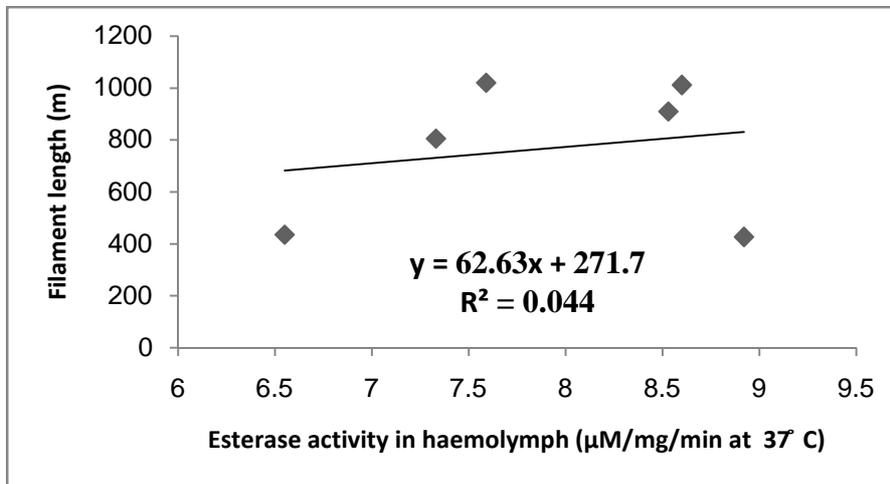


Figure 7: Correlation between haemolymph esterase activity level and filament length

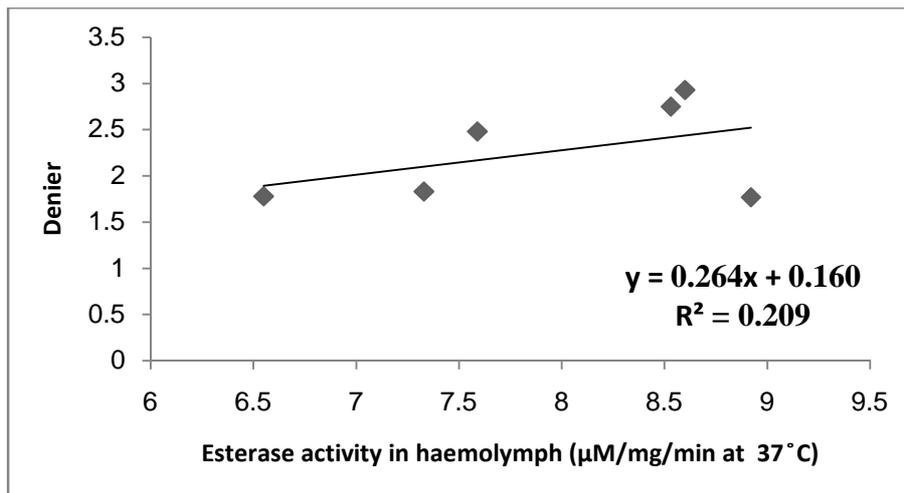


Figure 8: Correlation between haemolymph esterase activity level and denier

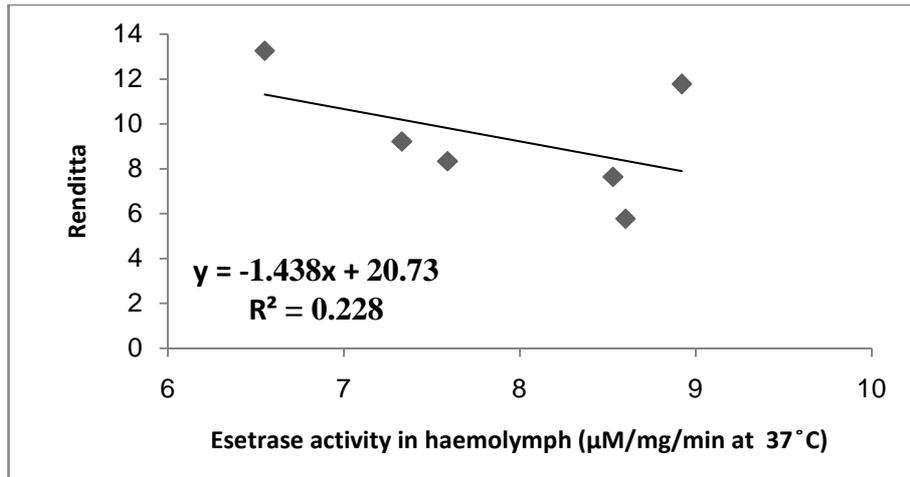


Figure 9: Correlation between haemolymph esterase activity level and Renditta

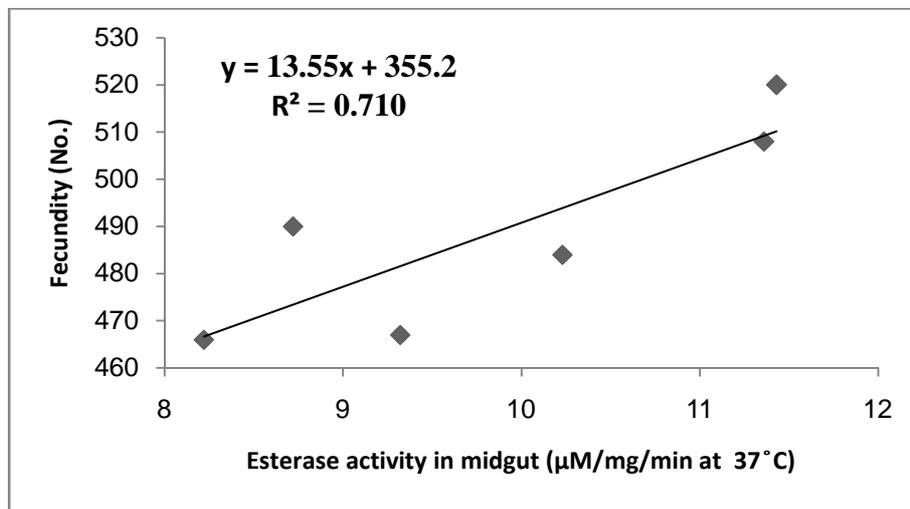


Figure 10: Correlation between midgut esterase activity level and fecundity

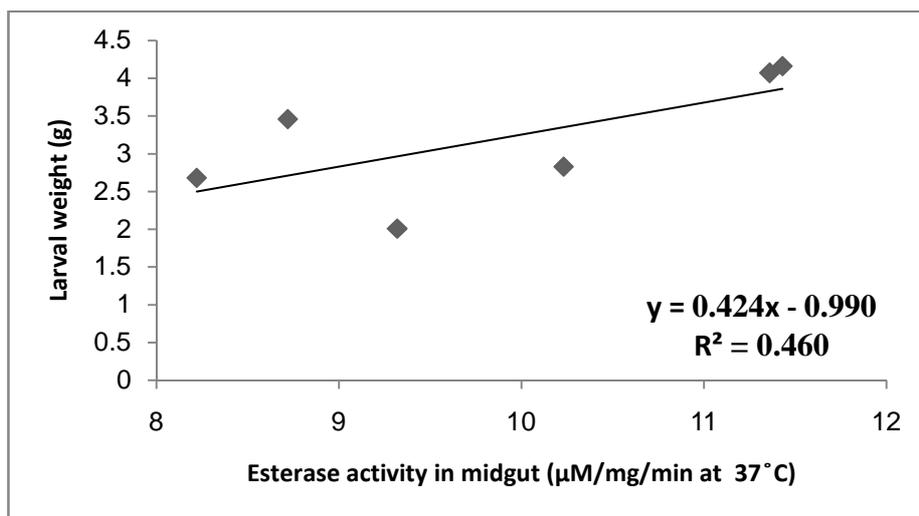


Figure 11: Correlation between midgut esterase activity level and larval weight

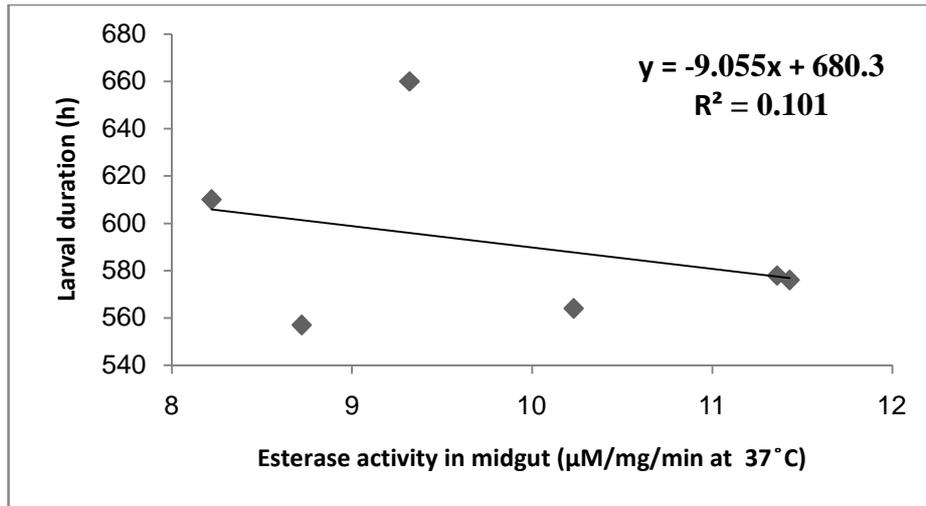


Figure 12: Correlation between midgut esterase activity level and larval duration

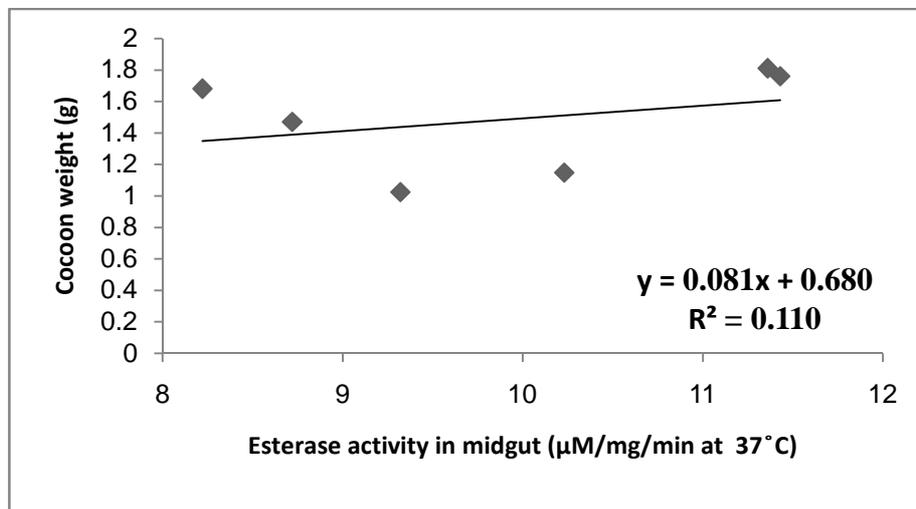


Figure 13: Correlation between midgut esterase activity level and cocoon weight

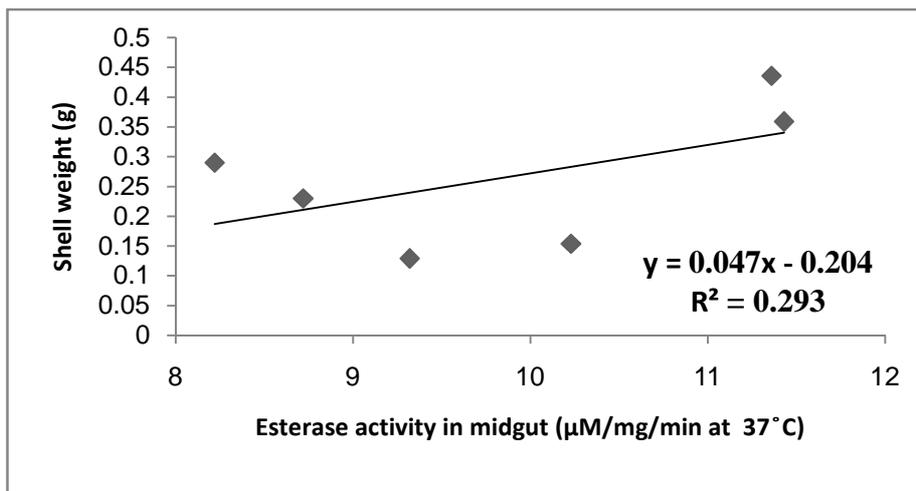


Figure 14: Correlation between midgut esterase activity level and shell weight

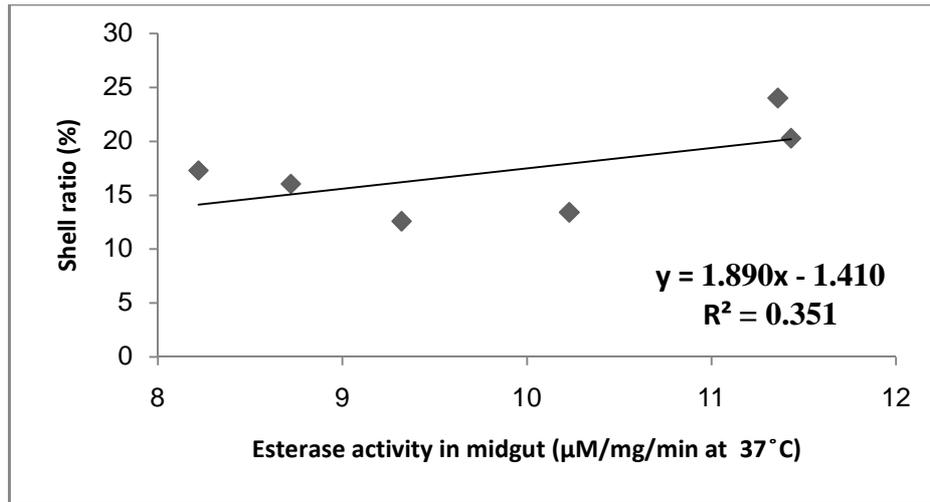


Figure 15: Correlation between midgut esterase activity level and shell ratio

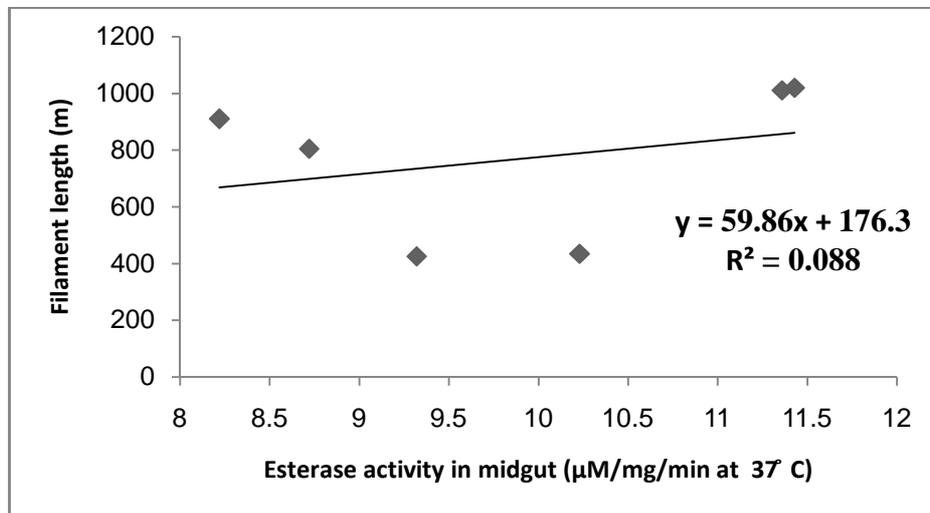


Figure 16: Correlation between midgut esterase activity level and filament length

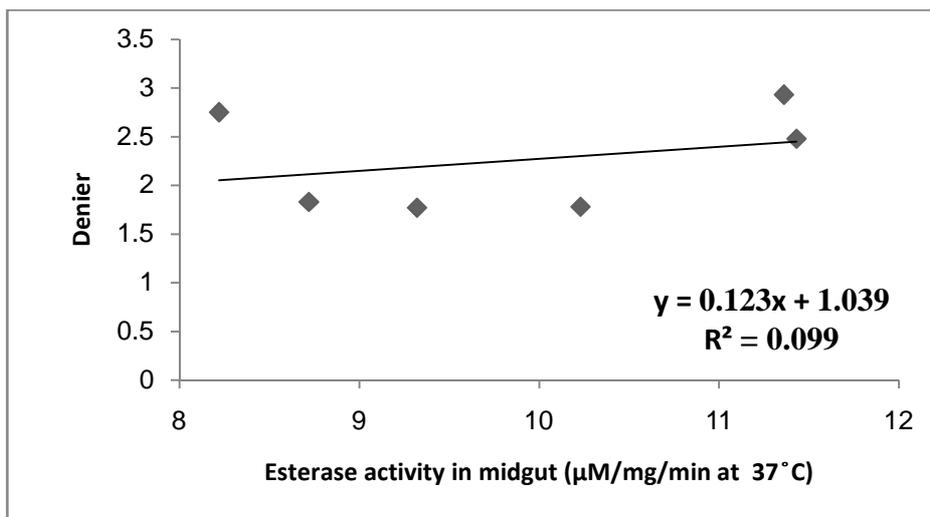


Figure 17: Correlation between midgut esterase activity level and denier

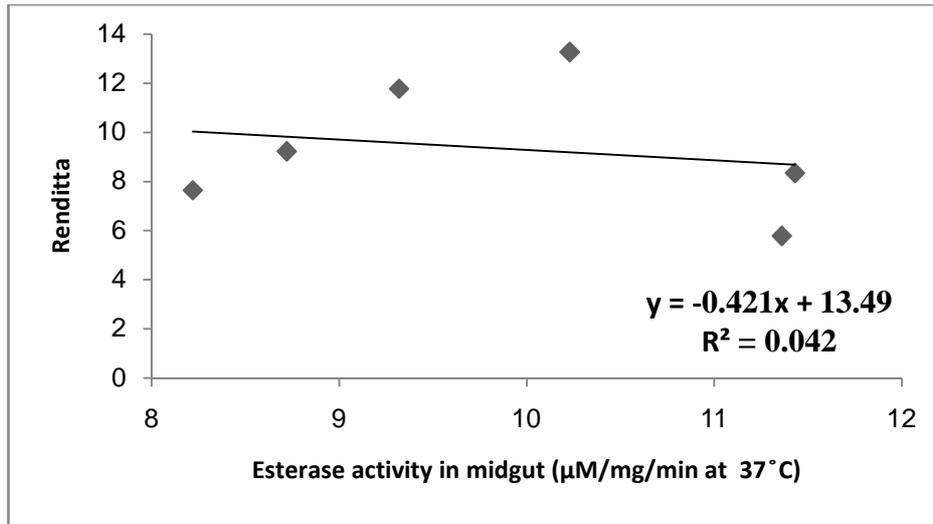


Figure 18: Correlation between midgut esterase activity level and renditta

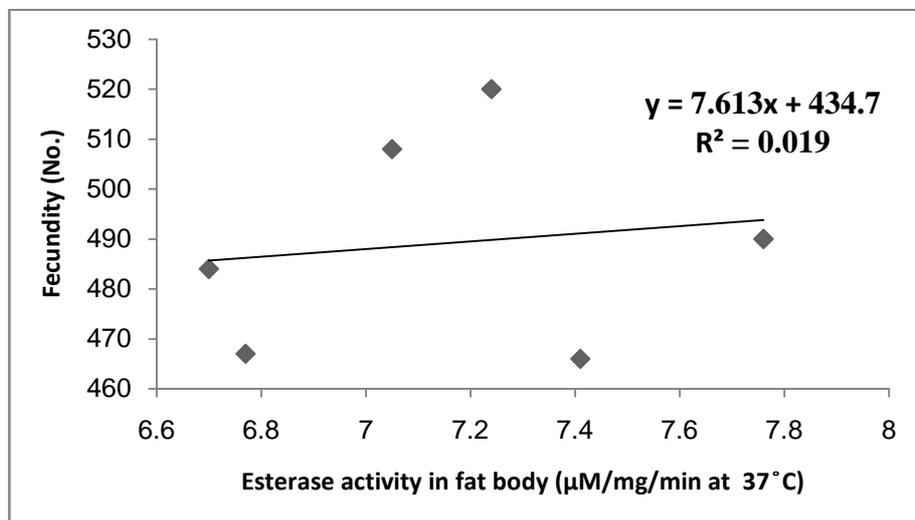


Figure 19: Correlation between fat body esterase activity level and fecundity

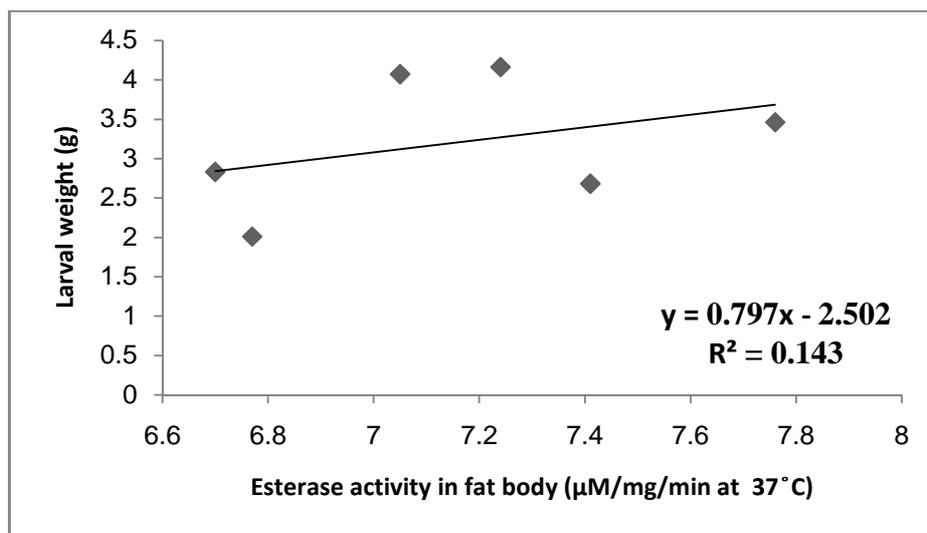


Figure 20: Correlation between fat body esterase activity level and larval weight

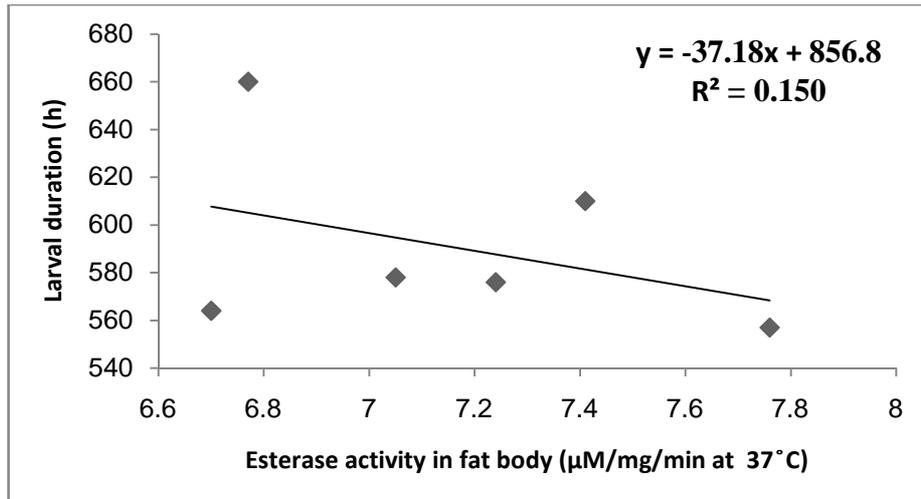


Figure 21: Correlation between fat body esterase activity level and larval duration

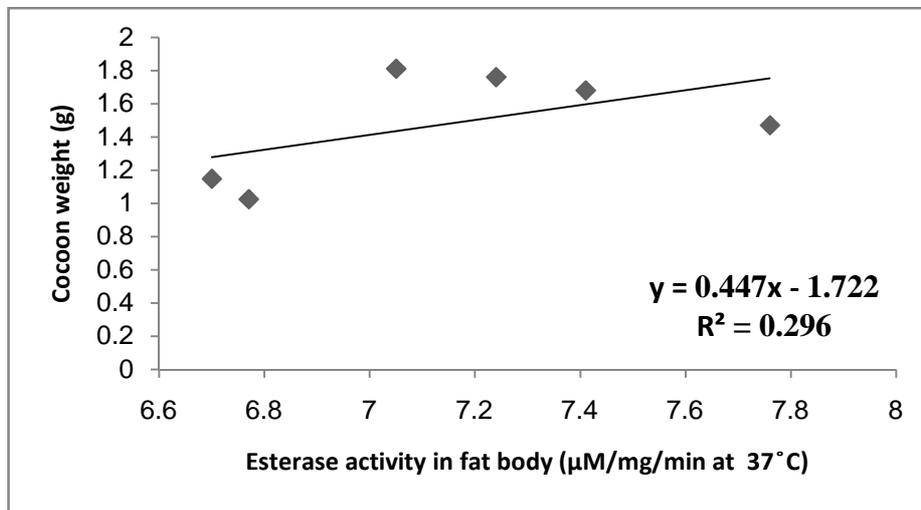


Figure 22: Correlation between fat body esterase activity level and cocoon weight

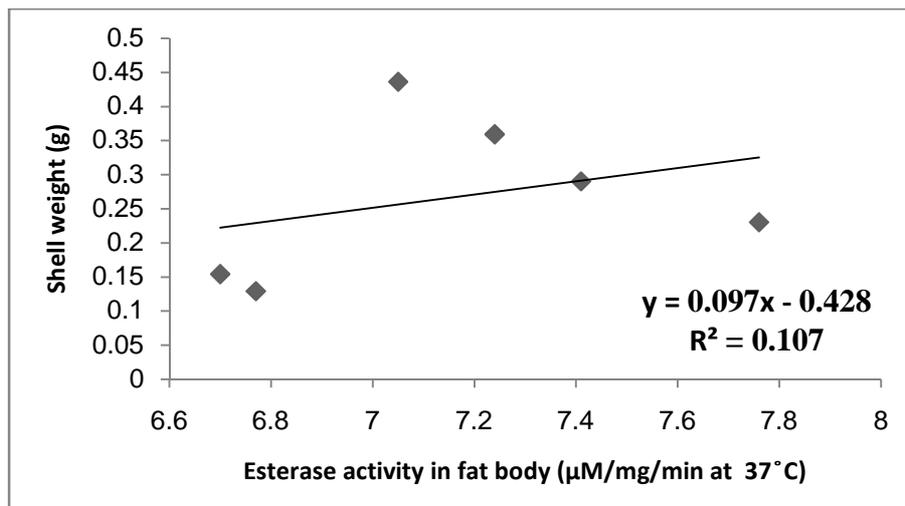


Figure 23: Correlation between fat body esterase activity level and shell weight

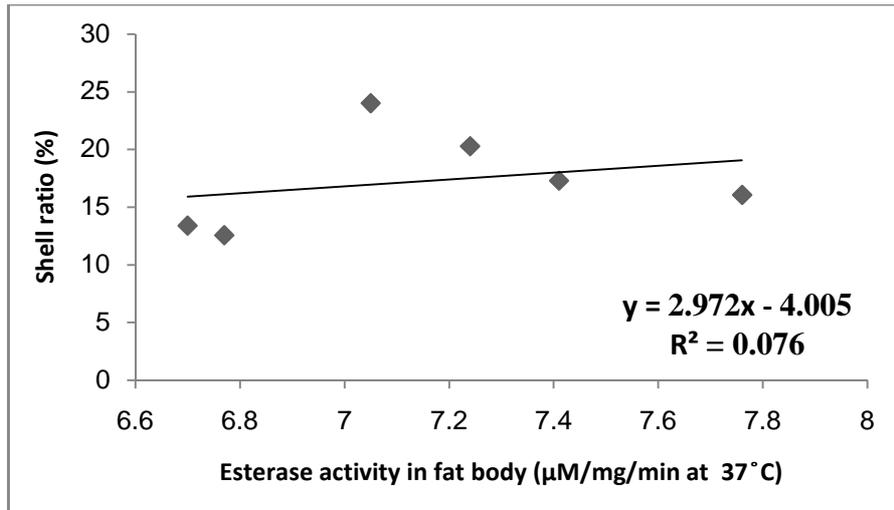


Figure 24: Correlation between fat body esterase activity level and shell ratio

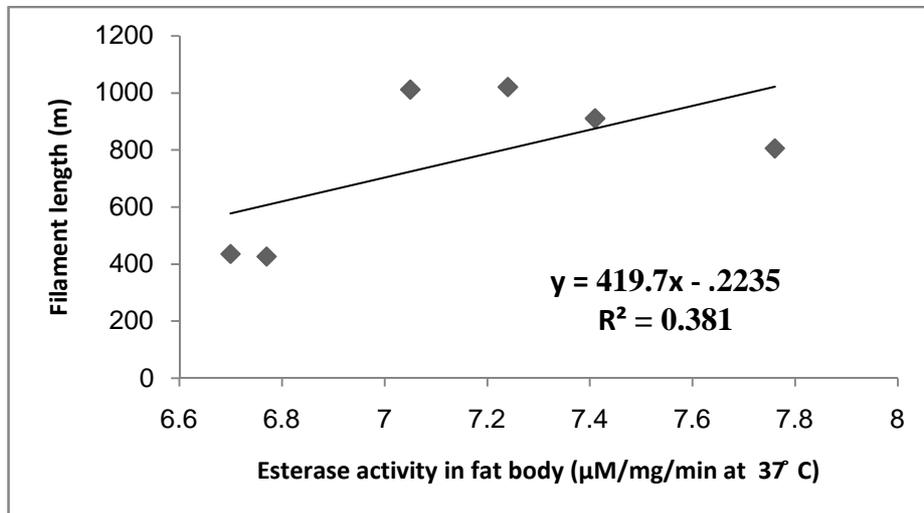


Figure 25: Correlation between fat body esterase activity level and filament length

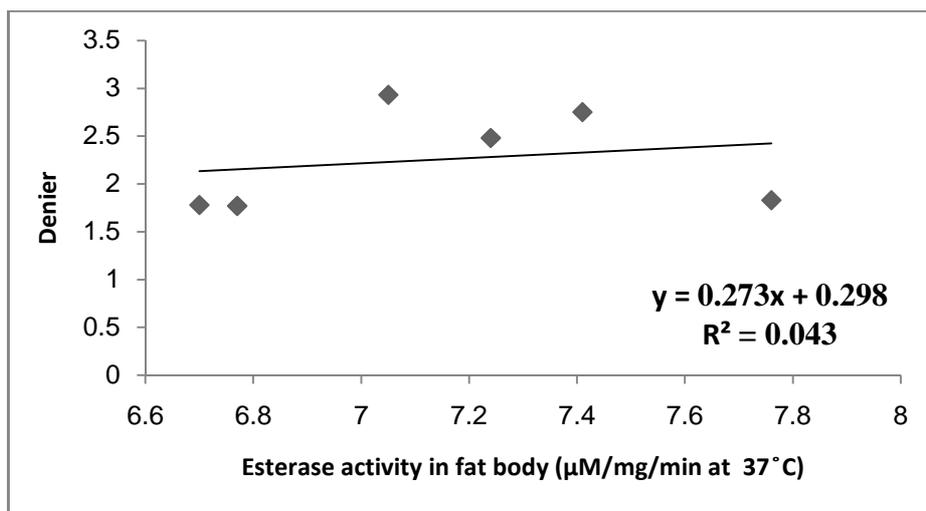


Figure 26: Correlation between fat body esterase activity level and denier

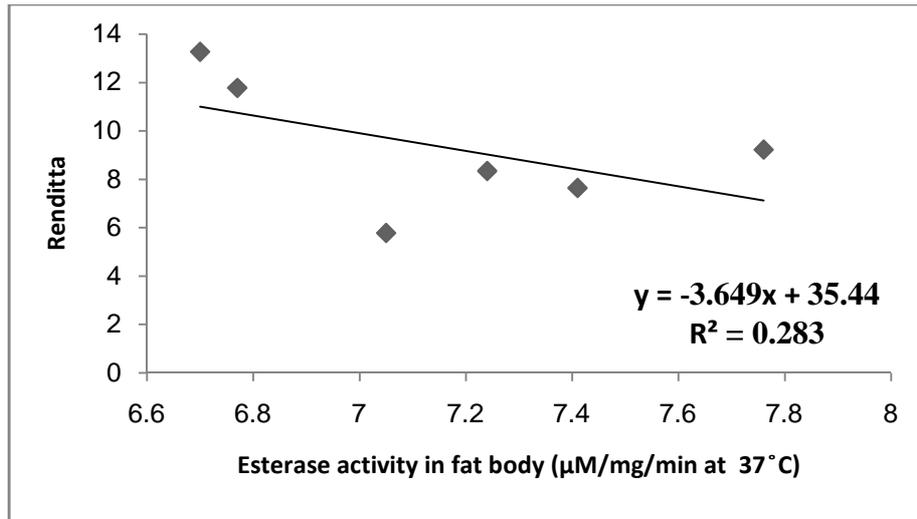


Figure 27: Correlation between fat body esterase activity level and Renditta

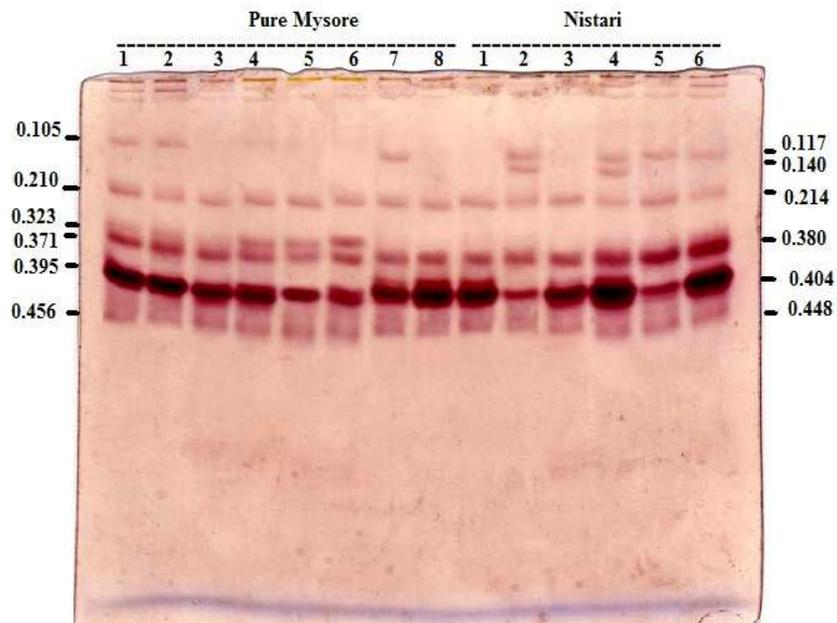


Figure 28: Native PAGE analysis of haemolymph esterase of Pure Mysore and Nistari silkworms. Lanes: 1-8 days in fifth instar.

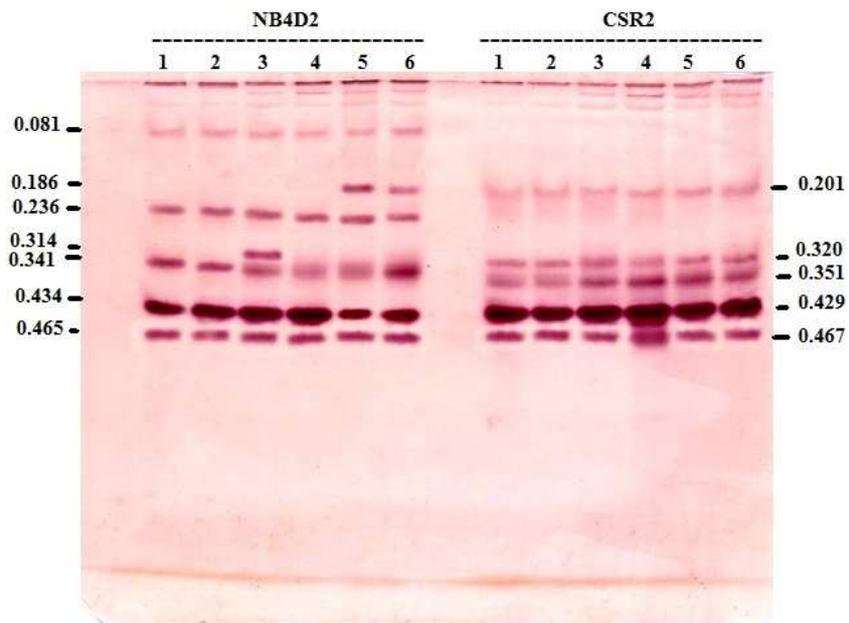


Figure 29: Native PAGE analysis of haemolymph esterase of NB<sub>4</sub>D<sub>2</sub> and CSR<sub>2</sub> silkworms. Lanes: 1-6 days in fifth instar.

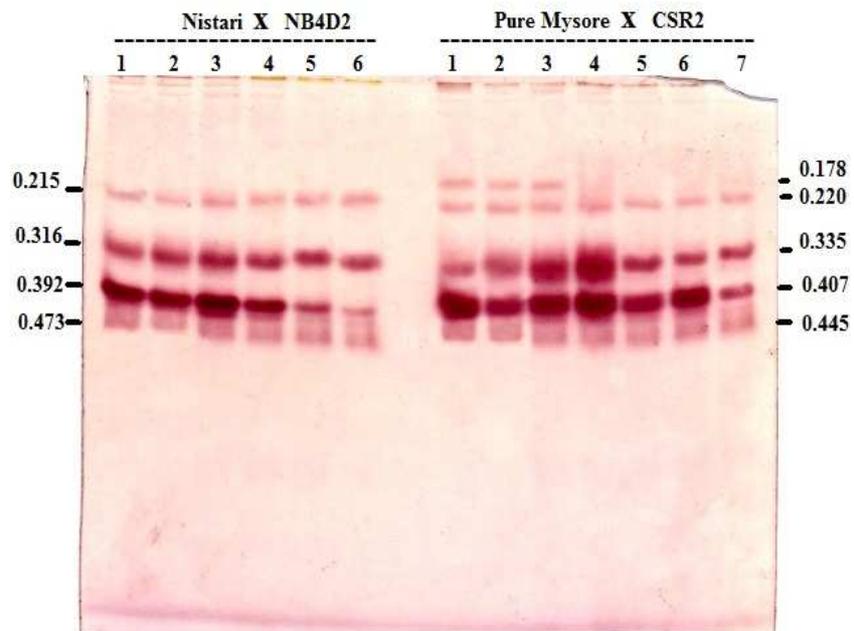


Figure 30: Native PAGE analysis of haemolymph esterase of Nistari x NB<sub>4</sub>D<sub>2</sub> and Pure Mysore x CSR<sub>2</sub> silkworms. Lanes: 1-7 days in fifth instar.

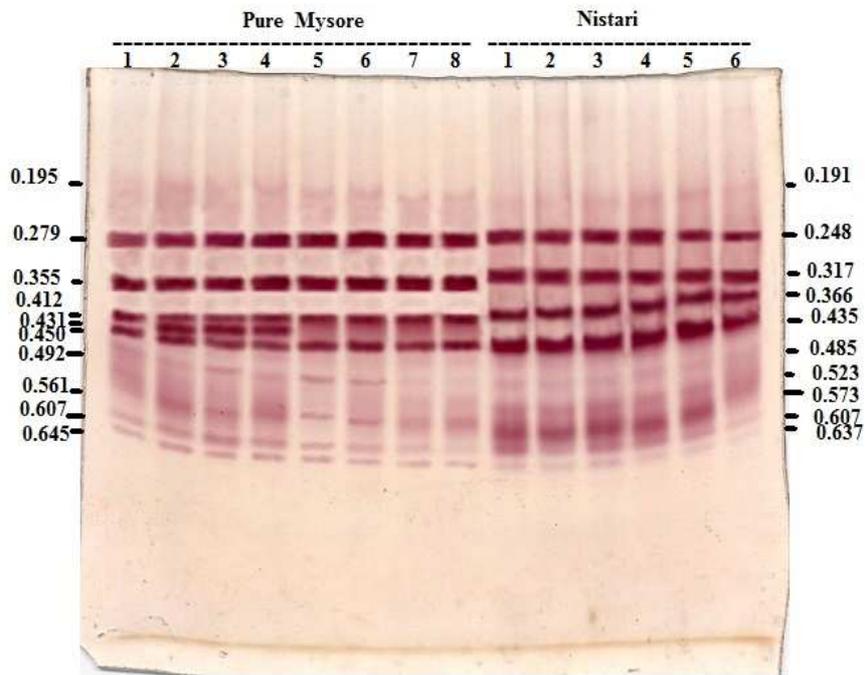


Figure 31: Native PAGE analysis of midgut esterase of Pure Mysore and Nistari silkworms. Lanes: 1-8 days in fifth instar.

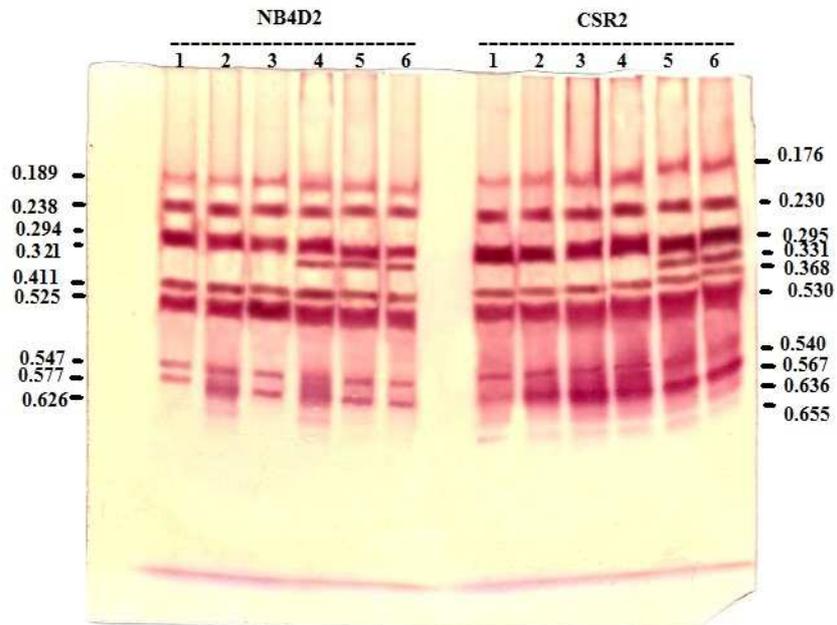


Figure 32: Native PAGE analysis of midgut esterase of NB<sub>4</sub>D<sub>2</sub> and CSR<sub>2</sub> silkworms. Lanes: 1-6 days in fifth instar.

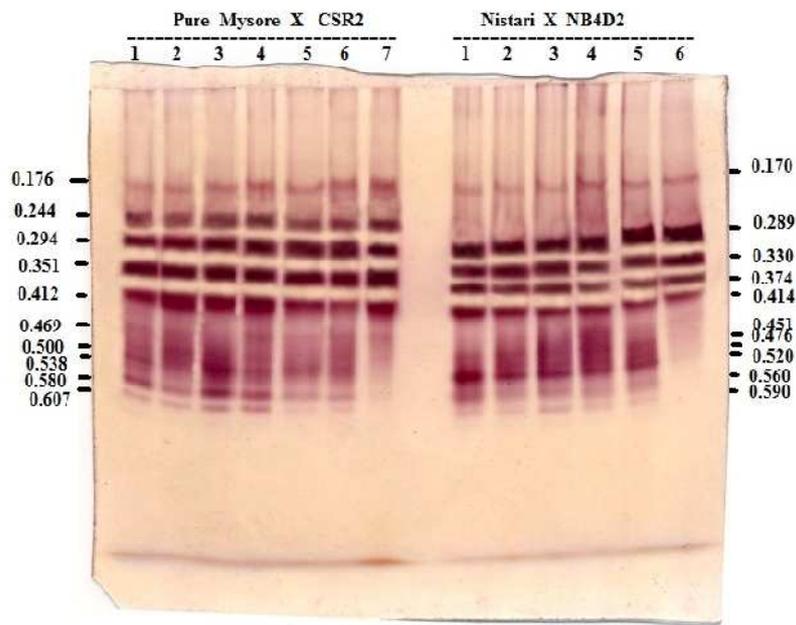


Figure 33: Native PAGE analysis of midgut esterase of Nistari x NB<sub>4</sub>D<sub>2</sub> and Pure Mysore x CSR<sub>2</sub> silkworms. Lanes: 1-7 days in fifth instar.

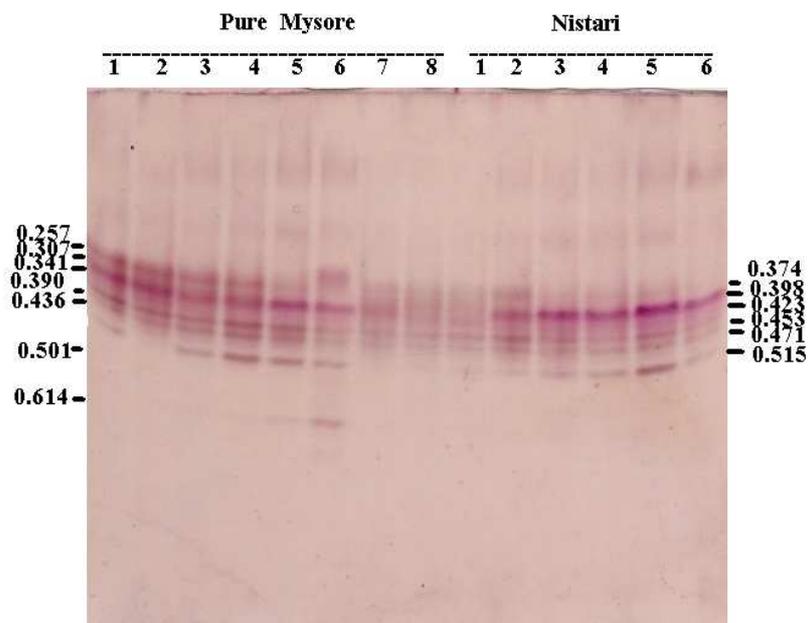


Figure 34: Native PAGE analysis of fat body esterase of Pure Mysore and Nistari silkworms. Lanes: 1-8 days in fifth instar.

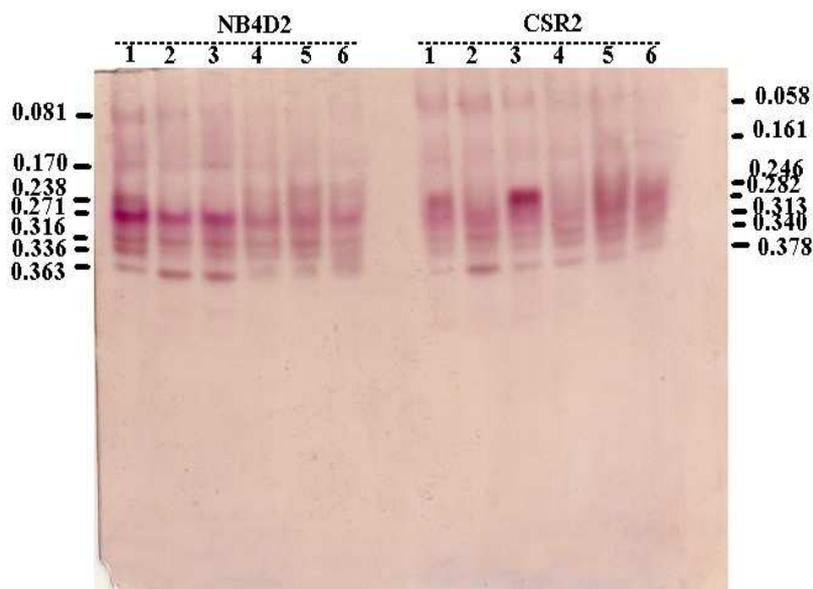


Figure 35: Native PAGE analysis of fat body esterase of NB<sub>4</sub>D<sub>2</sub> and CSR<sub>2</sub> silkworms. Lanes: 1-6 days in fifth instar.

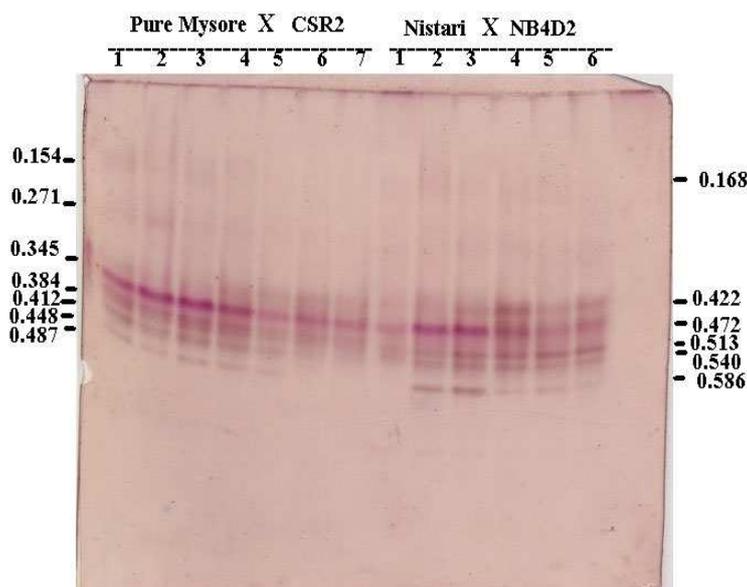


Figure 36: Native PAGE analysis of fat body esterase of Nistari x NB<sub>4</sub>D<sub>2</sub> and Pure Mysore x CSR<sub>2</sub> silkworms. Lanes: 1-7 days in fifth instar.

### DISCUSSION

Quantitative analysis of esterase activity levels clearly indicated two types of correlations *viz.*, positive or negative correlation between enzyme activity levels with commercial characters. In the group of nonspecific esterases from different tissues, a genetically determined polymorphism has been ascertained [19,20,21]. Various authors reported different number of esterase fractions in the gut spectrum of different breeds of *B. mori* L. [22,23,24,25]. Esterase isozyme exhibited higher level of polymorphism in vertebrates and invertebrates [26,27]. Gillespie and Kojima [28] reported a relationship between level of polymorphism and metabolic enzymes such as esterase. The differences in fractions of esterase may be due to the degree of genetic heterogeneity.

Observation on the esterase isozyme pattern has revealed that the banding pattern differs between pure races, between hybrids and between pure races and hybrids. The zymograms indicated the variation in R.F. and volume/intensity of the bands among the experimental silkworm breeds. The qualitative analysis of esterase indicated six types of changes *i.e.*, the intensity of the bands either more or less, besides, some of the bands

either present or absent. Some of the bands increased or decreased in their intensity as the age advances in addition to altered R.F. value. Presence or absence of protein bands indicates either the non production or utilization or degradation of protein contents [13] when the studies are restricted within a particular breed. However, when the studies are concentrated between the breeds, it directly targets the genetic material as they are exactly determined by the genetic material of the organism. The variations in the activity levels and isozyme pattern clearly showed differences between the races. Therefore, by studying the silkworm esterase with commercial characters, it is possible to have a clear picture about the kind and degree of correlation between them. An understanding of such correlations will help us to identify and exploit the marker molecule during the evolution of new breeds of silkworm *Bombyx mori* with improved commercial characters.

### CONCLUSSION

The present results clearly indicated that the haemolymph esterase activity levels showed highly positive correlation with larval duration and moderately positive correlation with denier. The midgut esterase activity levels indicated highly positive correlation with fecundity and larval weight only. On the other hand midgut esterase exhibited moderately positive correlation with shell weight and shell ratio. The fat body esterase showed moderately positive correlation with cocoon weight, filament length and renditta. In view of the above results, the esterase may be used as marker molecules during the evolution of new breeds of silkworm *Bombyx mori* with better economic characters.

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