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A comparative analysis of estimation of free amino acids in larval haemolymph of the mutant strains of *Antheraea mylitta* Drury

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Abstract: This research article is an effort to study the relative biochemical estimation of free amino acids in the larval bodies of three mutant strains viz: Daba-blue, Daba-yellow and Daba-almond of tasar silk worm, *Antheraea mylitta*. Results obtained are indicative of the fact that the Am-blue mutant strains of *Antheraea mylitta* carry greater number of free amino acid as compared to Am-yellow and Am-almond mutant strains of *A. mylitta.* The concentration of free amino acids during commercial crop season in all the mutant strains have been found relatively greater than the seed crop season. The number and concentration of free amino acids have been found to present variation in relation to genetic makeup, dietary and ecological conditions.

Key words:- Ecotype, Free Amino Acid, Haemolymph, Mutant Strains

INTRODUCTION

Antheraea mylitta is an indigenous traditional tasar producing insect, which exists in the forms of nearly 20 ecotypes distributed all over the ecological zones of tropical tasar belts in our Country. The different ecotypes of *Antheraea mylitta* in spite of having the same chromosomal number differ among themselves in their quantitative and qualitative characters. These ecotypes are uni, bi and trivoltine. Different local names have been assigned to each group by the tribals of this area eg. Daba, Raily, Model, Naila, Bogari etc. Among these, the Daba ecotypes of *Antheraea mylitta* Drury are distributed to the different localities of tasar belts particularly in the states of Bihar, Jharkhand and Madhya Pradesh. The tribals usually reared this ecotype by preparing the eggs in captivity and rear the larvae outdoor on foliages of *Terminalia arjuna, Terminalia tomentosa* and *Shorea robusta* during Seed Crop (July-August) and Commercial Crop Seasons (September- October).

Agarwal and Jolly (1974)[1] while working on biochemistry of tasar silkworm have mentioned that free amino acids in the larval haemolymph vary with the different concentrations of diet. Drilhon, *et al.* (1951)[2] have reported that the concentrations of free amino acids in the larval haemolymph has a tendency to show increase or decrease according to physiological conditions at different stages of their life cycle. Sharma and Pandey (1990)[3] while working on the variations of free amino acids contents have found that there exists a great variation in the number of amino acids among different ecotypes of tasar silkworm. Florkin (1959)[4] has reported qualitative and quantitative changes in the free amino acids contents at different stages

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in the life cycle of insects. Chen (1962)[5] has reported similar pattern of amino acid variations in Calliphora.

Chen (1960)[6] in *Drosophilla* shows maximum increase of amino acids at 72 hours after oviposition and steady dropping after 96 hours, corresponding to the period of most intensive growth as determined by wet weight if the values were calculated percent body weight. The pattern of free amino acids in *Drosophilla* was quite similar to the larvae of mosquito *Culex pipiens*.[7]

MATERIALS AND METHODS

The larval haemolymph of three different mutant strains of *Antheraea mylitta* Drury, in relation to their free amino acid was considered for the relative biochemical analysis. The fifth larval and final instars of almost similar age were selected for the analysis of biochemical content of free amino acids. The larval culture was carried out on *Terminalia arjuna* host plant of tasar worm as per the method suggested by Jolly (1973)[8]. During larval collection cautions were taken for obtaining the disease free larvae. The pathological analysis in respect of disease free larvae was carried out before taking out the larval haemolymph under aseptic condition.

The estimations of free amino acids of larval haemolymph of three different mutant strains of *Antheraea mylitta* was carried out by paper chromatography method. In this method the larval haemolymph of three different mutant strains were collected separately and deproteinised with ethanol (70%v/v) and there after centrifuged for ten minutes at 300 rpm. The protein free clear supernatant was evaporated to dryness on a water bath. Fats and lipids were removed by extracting the residue with ether (1ml) and residue was further dissolved in iso-propanol (1.5ml, 10% v/v) and all the extract were prepared in the same manner and used for two dimensional paper partition chromatography for the comparative biochemical analysis of larval haemolymph of three different mutant strains. 1ml haemolymph was used at every stage of analysis. Analar chemicals were used for the experiment.

Paper Chromatography Method: 10 ml of the extract was applied in each case on whatmann no. 1 filter paper (20x20 cm). The chromatography were first run butanol: acetic acid: water (4:1:5, v/v) in ascending manner and after overnight thorough drying the second run was made with phenol water (4:4, v/v). The chromatograms were dried at room temperature.

Development of spots: The chromatograms were sprayed with ninhydrin solution in acetone (0.25% w/v) sprayed chromatograms were heated in an air oven at 100°C for 15 minutes. The identification of amino acids was made as follows:

a) Comparing R.F values with known standards, running under the same experimental conditions

b) Using various specific spray reagents as given below:

i. Sakaguchi- reagents for arginine and other monosubstituted guanine derivatives (Sakaguchi, 1925)[9]
ii. Isatin for proline and hydroxyl proline as described by Hackman and Lazarus (1956)[10]
iii. Ehrlich's reagent for tryptophan, cytruline and urea (Smith, 1953)[11]
iv. Folin's reagent for amino butyric acid and their isomers (Pant and Agarwal, 1963)[12]

The results of the experiment in relation to variations of different amino acids contents were tabulated and presented in tables in the forms of traces (+ -), present (+), absent (-) and higher concentration (++) for both the seasons. Data obtained were recorded in the tables.

RESULTS

The relative biochemical estimations concerning free amino acids contents in the larval bodies of three mutant strains of *Antheraea mylitta,* namely Am- blue, Am- yellow and Am- almond along with control have been carried out during the seed crop and commercial crop seasons and results so obtained are recorded in tables 1 to 5.

Table 1 reveals the number and concentration of free amino acids in the larval haemolymph of Am - blue mutant strain of *Antheraea mylitta* during the seed crop and commercial crop season. As per table the total number of free amino acid (F.A.A.) is 17 in seed crop seasons and 19 in commercial crop seasons. In seed crop season, the concentration and argine, asparagin, cystic acid and glutamine have been found in traces. The leucin and glycine are altogether absent apart from this other amino acids have shown normal concentration. During the commercial crop season, the concentration of aspartic acid, beta alanine, glutamic acid, lysine, methionine-sulphoxide and serine have been recorded in abundance showing greater concentration. Glycine and histidine are in traces.

Apart from this all other amino acids have shown normal concentration in the larval haemolymph of the Am-blue mutant strain of *Antheraea mylitta*. It is worthwhile to mention that Am-blue mutant strain carry all the 19 free amino acids in its biochemical concentration during the commercial crop season.

Table 2 further accounts for the number and concentration of free amino acids (F.A.A.) in the larval haemolymph of Am-yellow mutant strain of *Antheraea mylitta* during the seed crop and commercial crop seasons.As per the table the total number of free amino acids is recorded 16 during the seed crop season in which glutamine, methionine-sulphoxide and valine are totally absent. The cystic acid, glycine, lysine and theronine have been found in traces and apart from this remaining free amino acids have shown normal concentration. During the commercial crop season the total number of free amino acids is recorded 17 in which leucin and methionine-sulphoxide are altogether absent and asparagines, lysine and serine have been recorded in traces.

Table 3 indicates the number and concentration of free amino acids (F.A.A) in the larval haemolymph of Am-almond mutant strain of *Antheraea mylitta* during both the seasons of rearing. As per table during the seed crop season the total number of free amino acids in the larval haemolymph of Am-almond mutant strain is recorded 15 out of which 19 in which cystine, glutamine, lysine and valin are totally absent and cystic acid, alpha9alanine, arginine, leucin and proline have been recorded in traces. During the commercial crop season the total number of free amino acids in the larval haemolymph of Am-almond mutant strain has been recorded 16 and the free amino acids namely cystine, serine and valine are altogether absent. Aspartic, cystic acid and threonine are recorded in traces. Apart from this remaining free amino acids have shown normal concentration.

Table 4 accounts for the number and concentration of free amino acids in Am-green (control) in its larval haemolymph during the seed crop and commercial crop seasons. Table clearly shows that during the seed crop season the total number of free amino acids (F.A.A.) is only 15 out of 19in which Beta-alanine, glutamic acid, leucin and tyrosine have been found missing. The concentration of cystic acid, glutamic acid, methionine-sulphoxide, proline, threonine and valine are recorded in traces. The concentration of gelycine and histidine is recorded in abundance showing greater concentration of free amino acids. During the commercial crop season the total number of free amino acids in the larval haemolymph is 16 out of 19 in which Beta-alanine, glycine and lysine are totally missing. The cystic acid, histidine, lysine, threonine and tyrosine have shown their concentration in traces. Arganine, asparagines and methionine-sulphoxide have been found in abundance in Am-green control of *Antheraea mylitta*. The rearing amino acids have shown their normal presence in the larval haemolymph of control.

A comparative picture in respect of number and concentration of free amino acids in the larval haemolymph of three mutant strains along with control during the seed crop and the commercial crop seasons has been summed up in table 5 and Figure 1.

Table 1: Table showing free amino acid content in the larval haemolymph of Am-blue mutant strain of *A. mylitta* during seed crop season and Commercial crop season

Sl. No.	Free amino acid	Seed Crop Season	Commercial Crop season
1	Alpha-alanine	+	++
2	Arginine	+-	+
3	Asparagine	+-	+
4	Aspartic acid	++	++
5	Beta-alanine	+	++
6	Cystic acid	+-	+
7	Cystine	+	+
8	Glutamic acid	++	++
9	Glutamine	+-	+
10	Glycine	-	+-
11	Histidine	+	+-
12	Leucin	-	+
13	Lysine	+-	++
14	Methoinine-sulphate	+	++
15	Proline	+	++
16	Serine	+	+
17	Threonine	++	+
18	Tyrosine	+	+
19	Valine		

++Abundant, +Present, +-Trace, -Absent, Am-*Anthearea mylitta*

Table 2: Table showing free amino acid content in the larval haemolymph of Am-yellow mutant strain of *A. mylitta* during seed crop season and Commercial crop season

Sl. No.	Free amino acid	Seed Crop Season	Commercial Crop season
1	Alpha-alanine	+	++
2	Arginine	+	+
3	Asparagine	+	+-
4	Aspartic acid	++	++
5	Beta-alanine	+	++
6	Cystic acid	+-	++
7	Cystine	+	+
8	Glutamic acid	++	++
9	Glutamine	-	+
10	Glycine	+-	+
11	Histidine	+	+
12	Leucin	+	-
13	Lysine	+-	+-
14	Methoinine-sulphate	-	-
15	Proline	+	++
16	Serine	+	+-
17	Threonine	+-	+
18	Tyrosine	++	++
19	Valine	-	+

Table 3: Table showing free amino acid content in the larval haemolymph of Am-almond mutant strain of *A. mylitta* during seed crop season and Commercial crop season

Sl. No.	Free amino acid	Seed Crop Season	Commercial Crop season
1	Alpha-alanine	+-	+
2	Arginine	+-	+
3	Asparagine	+	++
4	Aspartic acid	+	+-
5	Beta-alanine	+	+
6	Cystic acid	+-	+-
7	Cystine	-	-
8	Glutamic acid	+-	+
9	Glutamine	-	+-
10	Glycine	+	+
11	Histidine	+	+
12	Leucin	+-	+
13	Lysine	-	+
14	Methoinine-sulphate	+	+
15	Proline	+-	+
16	Serine	+	-
17	Threonine	+	+-
18	Tyrosine	++	+
19	Valine	-	-

Table 4: Table showing free amino acid content in the larval haemolymph of Am-green (Control) mutant strain of *A. mylitta* during seed crop season and Commercial crop season

Sl. No.	Free amino acid	Seed Crop Season	Commercial Crop season
1	Alpha-alanine	+	+
2	Arginine	+	+
3	Asparagine	+	++
4	Aspartic acid	+	+
5	Beta-alanine	-	-
6	Cystic acid	+-	+-
7	Cystine	+	+
8	Glutamic acid	+-	+
9	Glutamine	-	+
10	Glycine	++	-
11	Histidine	++	+-
12	Leucin	-	+
13	Lysine	+	-
14	Methoinine-sulphate	+-	++
15	Proline	+-	+
16	Serine	+	+
17	Threonine	+-	+-
18	Tyrosine	-	+-
19	Valine	+-	+

Table 5: Table showing a comparative picture concerning the total number of free amino acid in the larval haemolymph of three mutant strains and its control of *A. mylitta* during seed crop season and Commercial Crop season

Sl. No.	Strains of *Anthearea mylitta*	Seed Crop Season	Commercial Crop season
1	Am-Blue	17	19
2	Am-yellow	16	17
3	Am-almond	15	16
4	Am-green (Control)	15	16

++Abundant, +Present, +-Trace, -Absent, Am-*Anthearea mylitta*

DISCUSSION

A comparative analysis of estimation of free amino acids in various mutant strains of *Antheraea mylitta* reveals the under given results

- All the three mutant strains of *Antheraea mylitta*, namely Am-blue, Am-yellow and Am-almond on account of their genetic diversities present evident variations in respect of number and concentration

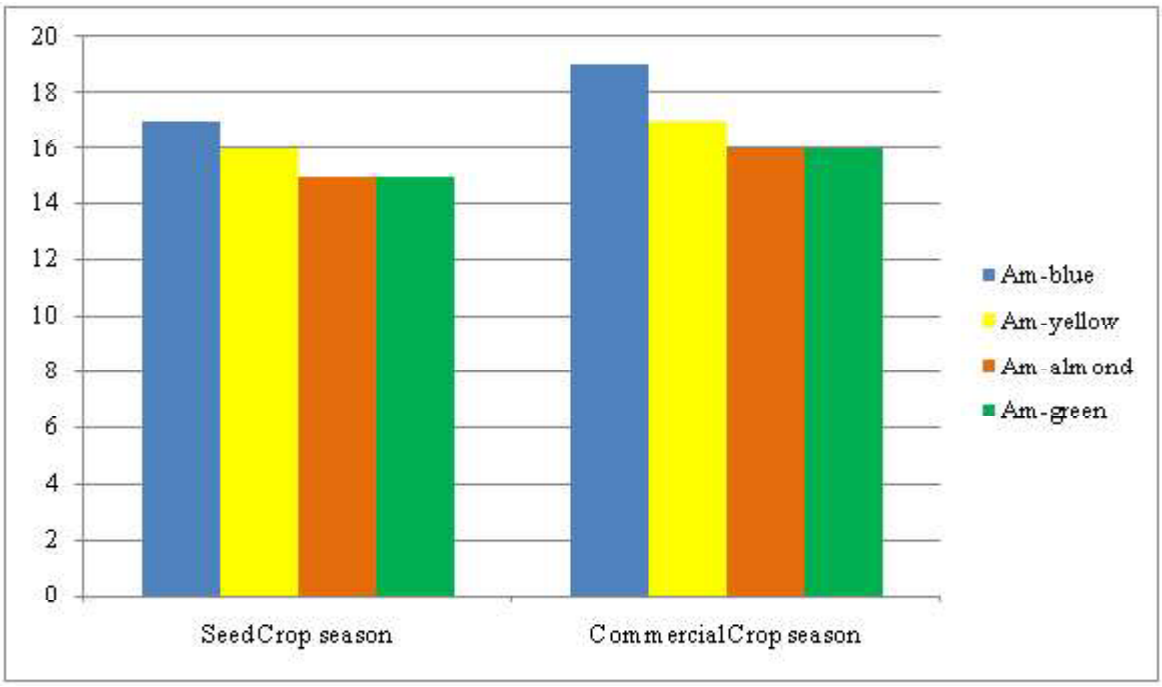

Figure 1: Bar Diagram showing a comparative picture concerning the total number of free amino acid in the larval haemolymph of three mutant strains and its control of *A. mylitta* during seed crop season and Commercial Crop season

of free amino acids in their larval haemolymph during the seed crop and commercial crop seasons.

- Among the three mutant strains the number and concentration of free amino acids are relatively greater in the larval haemolymph of Am-blue followed by Am-yellow and Am-almond respectively.
- The number and concentration of free amino acids among all the three mutant strains of *Antheraea mylitta* in spite of their relative differences are evidently and significantly greater and higher than the control during both the seasons of estimations.
- On account of seasonal differences the number and concentration of free amino acids in the larval haemolymph of all the three mutant strains and control have been found relatively greater during commercial crop season than the seed crop season.

The number and concentration of free amino acids have been found to present variation in relation to genetic makeup, dietary and ecological conditions.[13] Agarwal *et al* (1975)[14] reported that the healthy tasar larvae of *Antheraea mylitta* on account of genetic robustness carry greater number of free amino acids than the unhealthy larvae with poor genetic vigour. Auclar while working on *Galleria melonella* has found evident variation in the free amino acid concentration in relation to ecological factors and dietary variation. Pandey (1989)[15] while working on different ecotypes of *Antheraea mylitta* has mentioned that the ecotypic differentiation on account of genetic divergence is the most effective factors for the variation in free amino acid content at the different stages of life cycle of *Antheraea mylitta.* Sinha has reported that the tasar larvae reared on mature leaves carry greater number of free amino acids as compared to tender and coarse leaves. Verma (1992)[16] while working on different ecotypes of *Antheraea mylitta* maintains that the physiogeneic factors are very much responsible for evident variation in the free amino acid content among the different ecotypes of *Antheraea mylitta.*

CONCLUSION

In the light of aforesaid information, it is logical to assume that the relative differences in amino acids among the three mutant strains owing to their different

physiogeneic constitution is a natural outcome and very much inconformity with the works of earlier investigators. The greater concentration of free amino acids during commercial crop season at the larval, pupal and adult stages of life cycle among three mutant strains are certainly associated with diverse nutritional and ecological conditions. Since the nutritional and ecological condition during Commercial crop season are more favourable to the mutant strains than the seed crop season, thus, the differences in the free amino acids content in relation to two different seasons appear to be fully justified.

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