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A comparative analysis of estimation of nutrients and moisture content in larval haemolymph of the mutant strains of Indian tasar silkworm *Antheraea mylitta D.*

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Abstract:- This research article is an effort to study the estimation of protein, carbohydrate, lipid and moisture contents in the larval haemolymph of three mutant strains viz; Daba–blue, Daba–yellow and Daba–almond of tasar silk worm, *Antheraea mylitta* D along with its Control Daba-green. The results are indicative of the fact that the relative percentage of protein, carbohydrate, lipid and moisture in the larval bodies of three mutant strains of *Antheraea mylitta* along with its control present significant variations during the seed crop and commercial crop season. The variation in protein, carbohydrate and lipid contents is greatly influenced by the ecological conditions and more particularly the genetic makeup of the insects. The relative percentage of said biochemical contents except moisture percentage have been found evidently higher in the larval body of Am-blue than the Am-yellow and Am-almond mutant strains of *Antheraea mylitta*. The percentage of moisture in the larval bodies of control has been found higher as compared to three mutant strains of *Antheraea mylitta*. The percentage of biochemical contents such as protein, carbohydrate, lipid and moisture in the larval bodies of mutant strains and also control have been found higher during the commercial crop season than the seed crop season. The variations caused by mutation in genetic architecture became the potent factor for biochemical manifestations subsequently influencing the metabolic functions of tasar worms under the relative actions of genetic variability.

Key words: *Antheraea mylitta,* Ecotype, Carbohydrate, Protein, Lipids, Moisture, Mutant Strains

INTRODUCTION

Antheraea mylitta is a species of moth in the family Saturniidae of order Lepidoptera. It is commonly known as Indian Tropical tasar silkworm or Vanya silkworm. It is actually one of a number of tasar silkworms; species that produce Tasar silk. This species is native to India. This species is mainly confined to the states of Jharkhand, Madhya Pradesh, Orissa, Bihar and Maharashtra touching the fringes of Andhra Pradesh, Karntaka, West Bengal and Assam. This species has at least 40 identified ecotypes, adapted to varied ecological conditions. About a dozen of ecotypes are used for silk production. These ecotypes are uni, bi and trivoltine. The different ecotypes of *Antheraea mylitta* in spite of having the same chromosomal number differ among themselves in their quantitative and qualitative characters.

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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* D. 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). This insect feeds mainly on *Terminalia* trees and on *Shorea robusta*. The secondary food plants are Bahera, Kumbhi, Sidha, sagwan and kachnar.

The investigation in relation to protein contents have been carried out among insects with greater details. It has been found that proteins are essentially required for different biological behavior among insects and have been found to be the building block of life. The proteins are by and large responsible for growth and development of organs and systems and also for regulating physiological and biochemical processes among insects. Tripathi (1988)[1] has menioned that the protein content decreases significantly in the second and third instar and again increases in the fifth instar larvae of *A. mylitta*. Sinha (1982)[2] while working on different ecotypes of Indian tasar silkworm has found marked variation in the protein contents. Jolly and Sen (1972)[3] have mentioned that the haemolymph of tasar silkworm is guided by the dietary variations. They have also mentioned increase and decrease of protein contents at different levels of life cycles under the influence of different factors. Sen and Sinha (1985)[4] mention that the variations in the protein content in the host plants of tasar insects largely depend upon soil and climatic conditions and also on species. Kohli, *et al.* (1969)[5] has mentioned that the deficiency in protein content adversely affects the fertility and fecundity among tasar silk insects.

The role of carbohydrates in relation to behavioural activities among insects has been variously discussed by a number of investigators. It has been found that the presence of carbohydrates in the body of insects is essentially required for the carbohydrate metabolism and for the energy releasing processes. However, in majority of insects the amount of free glucose remain very scanty in *Antheraea pernyi,* it was present throughout the life cycle and has undergone steady increase in level from the egg to the pupa, there after decline in development to the adult (Egorova and Smolin, 1962)[6]. The presence of carbohydrates and its quantitative changes during development have been mentioned in *Bombyx mori* (Dutriev, 1961 and Yamashita, 1965).[7,8]

The importance of lipids in the life cycle of insects has been described as an essential source of energy for maintaining various metabolic functions. However, there exists a wide variation in the lipid content of insects of different orders on even within the same species. Gilbert, *et al.* (1961)[9] asserts that the lipid content in individual larvae in American silkmoth increases with the age and size but the rate of increase presents variation in the various strains when compared to the increases in non-lipid constituents. The fresh larvae have been found to contain greater amount of lipid and subsequently increases as the larvae grow. Sinha, *et al.* (1976)[10] while working on quantative variation in the lipid and fatty acids content during different stages of development of Indian tasar silkworm has found that the percentage of lipid and fatty acids content remain significantly greater in male moth followed by pupa, eggs and newly hatched larvae. They have further reported that there exists marked variation in relation to lipid content in two different sexes.

Franenkel and Blewett (1946)[11] have mentioned that the insects require poly unsaturated fatty acids, sterols and vitamin E etc. for growth, development and reproduction. They further assert that the insects are unable to synthesize these compounds. Agrawal, *et al.* (1975)[12] have revealed that silkworms which feed on leaves, requires P-citosterol and unsaturated fatty acids particularly linoleic and linolenic acids. However, the growth of the silkworm has been found to improve in the presence of fatty acid sterol combination. Sinha,*et al.* (1971)[13] while working on tasar silkworm have reported the different patterns of lipids and fatty acids at different stages. They have mentioned that the biochemical content in respect of lipid, fatty and sterol show evident variation in relation to consumption of primary and secondary tasar food plants.

The role of moisture or water content in the body of insects has been described by many investigators. The brilliant investigation in relation to the water requirement for different metabolic activities has become a reality.

However, the water content in tasar silkworm along with eri and muga have been reported to show significant increase at fourth and fifth instar stages and subsequently followed by gradual decrease during larval-pupal transformation stage. Jolly (1979)[14] assorted that the larvae cultured on tender leaves carry relatively greater amount of moisture in comparison to the larvae consuming coarse leaves. Pandey (1989)[15] while working on biochemical variations of Indian tasar silkworm has mentioned that the moisture content in the body fluid of tasar larvae show marked variation under two different indoor and outdoor conditions.

The current paper aims to study the estimation of protein, carbohydrate, lipid and moisture contents in the larval haemolymph of three mutant strains viz; Daba–blue, Daba–yellow and Daba–almond of tasar silk worm, *Antheraea mylitta* D along with its Control Daba-green. The biochemical contents in the body of the insects play vital role in their economy. The various metabolic activities are by and large regulated and controlled by the presence and absence of biochemical contents. The various biological behaviours have been found to be influenced by the chemical constituents. The behavior, such as physiological morphological or even the genetic behavior have been found to be regulated and co-ordinated under the influence of the essential biochemical constituents. However, the variation in the biochemical contents among insects is greatly influenced by the different factors of the environment. The principal among them are the food, temperature, light and humidity.

MATERIALS & METHODS

The larval haemolymph of three different mutant strains of *Antheraea mylitta* D in relation to their protein, carbohydrate, lipid and moisture contents were considered for the relative biochemical analysis. The fifth larval and final instars of almost similar age were selected for the analysis of biochemical contents. The larval culture was carried out on *Terminalia arjuna* host plant of tasar worm as per the method suggested by Jolly (1973)[16]. 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. Under given specific method was adopted for the biochemical analysis of mutant strains along with its control.

Estimation of Protein:

For the estimation of protein, the amount of Nitrogen was determined by Duma's method as suggested by Brenad and it was later multiplied by 6.25 to calculate the actual amount of proteins contents in the bodies of different mutant strains. The data in relation to the protein contents in different strains were tabulated carefully at their larval stages for the relative evaluation of protein contents. The data were analysed, correlated and finally presented in the tables.

Estimation of Lipids:

The different samples of three different strains in relation to their stages were collected and prepared within 24 hours, before the laboratory estimation. The samples were washed in distilled water and homogenized in a blender with 20 vol. of chloroform: methanol (2v/v). The different larvae cultured on food plants under laboratory conditions were taken for the estimation of the lipid contents. In order to minimize fatty acid contamination from the gut contents the fresh larvae were considered for the analysis. The biochemical analysis for the lipid contents was made only after the excretion of the myconium as per the suggestion of Jolly and Sen. In this method the dried samples were weighed and placed in a saxhplet with heating mantle, 250ml.capacity for lipid extraction. Petroleum ether was used as solvent. The extraction period was arranged between 24 to 30 hrs. The insoluble residue was dried in an oven (regulated at 65^0 C) for 2 to 3 days till the constant weight were obtained. The difference in weights before and after lipid extraction gave the amount of the lipid in the body. The data so collected were presented in the table.

Estimation of Carbohydrates:

The estimations of carbohydrate among different types of mutant strains were calculated mathematically by using the under given equation:

Carbohydrate = (dry weight of insect) – (weight of Protein) + (Lipid) + (ash)

The data so collected were recorded separately for each type of strain and the calculation were made on wet weight to show the percentage composition.

Estimation of Moisture:

The different strains of tasar silkworms were weighed separately and dried in vacuum desiccators at $66^0C \pm 1^0C$ till constant weight was obtained. The difference between the wet weight and dry weight of the sample (Stage of tasar worm) were considered to represent the actual amount of moisture in the body. The differences obtained were further converted in terms of percentage and recorded in the table.

RESULTS

The relative biochemical estimations concerning protein, carbohydrate, lipid and moisture 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 relative percent protein content in the larval haemolymph of three mutant strains of *Antheraea mylitta* and control during the seed crop and commercial crop seasons. Table indicates that the percentage of protein in the larval haemolymph of three mutant strains viz; Am-blue, Am-yellow and Am-almond during the seed crop season is in the tune of 59.7%, 56.8% and 52.21% respectively as compared to control (50.10%). During the commercial crop season it is in tune of 60.15%, 58.39% and 53.45% as compared to control (51-93%) thus showing evident variation in respect of protein content among the mutant strains and control during both the seasons of during.

Table 2 accounts for the relative percent carbohydrate contents in the larval haemolymph of three mutant strains and control during the seed crop and commercial crop seasons. It shows that during the seed crop season percentage of carbohydrate in the larval haemolymph of Am-blue (9.68%), Am-yellow (9.16%) and Am-almond (8.95%) percent variations among themselves but relatively greater than the control (8.10%) during the commercial crop season the percentage of carbohydrate in the larval haemolymph of Am-blue, Am-yellow and Am-almond mutant strain is in the tune of 10.12%, 9.99% and 9.70% respectively as compared to control (8.93%) showing relative superiority over the seed crop season.

Table 3 reveals the percentage of lipid contents in the larval haemolymph of three mutant strains of *Antheraea mylitta*, during the seed crop and commercial crop seasons. During the seed crop season the percentage of lipid in the larval haemolymph of Am-blue, Am-yellow and Am-almond is recorded in the tune of 15.48%, 14.42% and 13.94% respectively and in control it is 12.51%. Likewise during the commercial crop season the percentage of lipid in the larval haemolymph of Am-blue, Am-yellow and Am-almond is recorded in the tune of 15.98%, 14.96% and 14.10% and in control it is 13.76%. it is thus very clear that strains of *Antheraea mylitta* are relatively higher during the commercial crop season as compared to seed crop season. It is further clear that all the three mutant strains of *Antheraea mylitta* differ among themselves in respect of percentage of lipid contents in the larval haemolymph. The percentage of lipid contents in the larval body of Am-blue mutant strains are relatively greater as compared to Am-yellow and thereafter Am-almond. However, the percentage of lipid contents in the larval bodies of all the three mutant strains have been found relatively higher than the control in spite of their relative differences.

Table 4 accounts for the relative percentage of moisture contents in the larval bodies of three mutant strains of *Antheraea mylitta* along with control during both the seasons of rearing. Table indicates that the percentage of moisture in the larval bodies of Am-blue, Am-yellow and Am-almond mutant strains of *Antheraea mylitta* is in the tune of 69.02%, 68.58% and 67.38% respectively as compared to control which is 70.82% during the seed crop season. Likewise during the commercial crop season the percentage of moisture in the larval bodies of Am-blue, Am-yellow and Am-almond mutant strains of *Antheraea mylitta* is recorded in the tune of 70.38%, 69.92% and 68.58% as compared to control which is 71.12%.

Figure 1 and 2 shows an analytical representation of protein, Carbohydrate and lipid content among the mutant strains of *Antheraea mylitta* during seed crop season and commercial crop season respectively. Figure 3 shows a comparative representation of moisture content among the mutant strains of *Antheraea mylitta* during Seed crop season and Commercial crop season.

The results thus indicate that the moisture percentage in the larval bodies of *Antheraea mylitta* is greater in control as compared to mutant strains during both the seed crop and commercial crop season. It is further clear that the moisture percentage in the larval body of Am-blue mutant strains as compared to Am-yellow and Am-yellow and Am-almond strains is higher during both the seasons of estimation of biochemical content.

Table 1: Table showing relative variation in percent of protein content among the mutant strains of *Antheraea mylitta* during seed crop season and commercial crop season

Sl. No.	Strains of *Antheraea mylitta*	Seed Crop Season	Commercial crop season	C.D at 0.1 % level for seasons
1	Am-blue	59.7	60.15	*
2	Am-yellow	56.8	58.39	*
3	Am-almond	52.21	53.45	*
4	Am-green (Control)	50.10	51.93	*
C. D at 0.1 % level for characters		**	**	-

Am – ***Antheraea mylitta*** ** - Highly Significant * - Significant

Table 2: Table showing relative variation in percent of carbohydrate content among the mutant strains of *Antheraea mylitta* during seed crop season and commercial crop season

Sl. No.	Strains of *Antheraea mylitta*	Seed Crop Season	Commercial crop season	C.D at 0.1 % level for seasons
1	Am-blue	9.68	10.12	*
2	Am-yellow	9.16	9.99	*
3	Am-almond	8.95	9.70	*
4	Am-green (Control)	8.10	8.93	*
C. D at 0.1 % level for characters		**	**	

Am – ***Antheraea mylitta*** ** - Highly Significant * - Significant

Table 3: Table showing relative variation in percent of lipid content among the mutant strains of *Antheraea mylitta* during seed crop season and commercial crop season

Sl. No.	Strains of *Antheraea mylitta*	Seed Crop Season	Commercial crop season	C.D at 0.1 % level for seasons
1	Am-blue	15.48	15.98	*
2	Am-yellow	14.42	14.96	*
3	Am-almond	13.94	14.10	*
4	Am-green (Control)	12.51	13.76	*
C. D at 0.1 % level for characters		**	*	-

Am – ***Antheraea mylitta*** ** - Highly Significant * - Significant

Table 4: Table showing relative variation in percent of moisture content among the mutant strains of *Antheraea mylitta* during seed crop season and commercial crop season

Sl. No.	Strains of *Antheraea mylitta*	Seed Crop Season	Commercial crop season	C.D at 0.1 % level for seasons
1	Am-blue	60.02	70.38	*
2	Am-yellow	68.58	69.92	*
3	Am-almond	67.38	68.58	*
4	Am-green (Control)	70.82	71.12	*
C. D at 0.1 % level for characters		**	**	-

Am – ***Antheraea mylitta*** ** - Highly Significant * - Significant

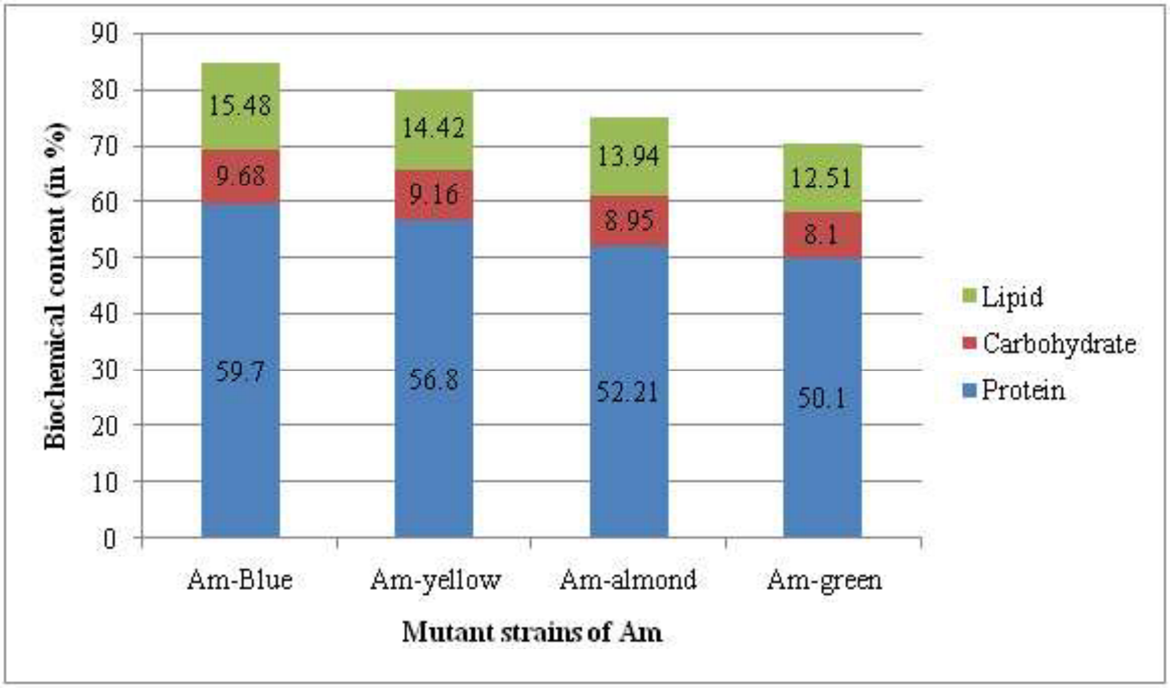

Fig 1: Bar diagram showing percent protein, Carbohydrate and lipid content among the mutant strains of *Antheraea mylitta* during seed crop season

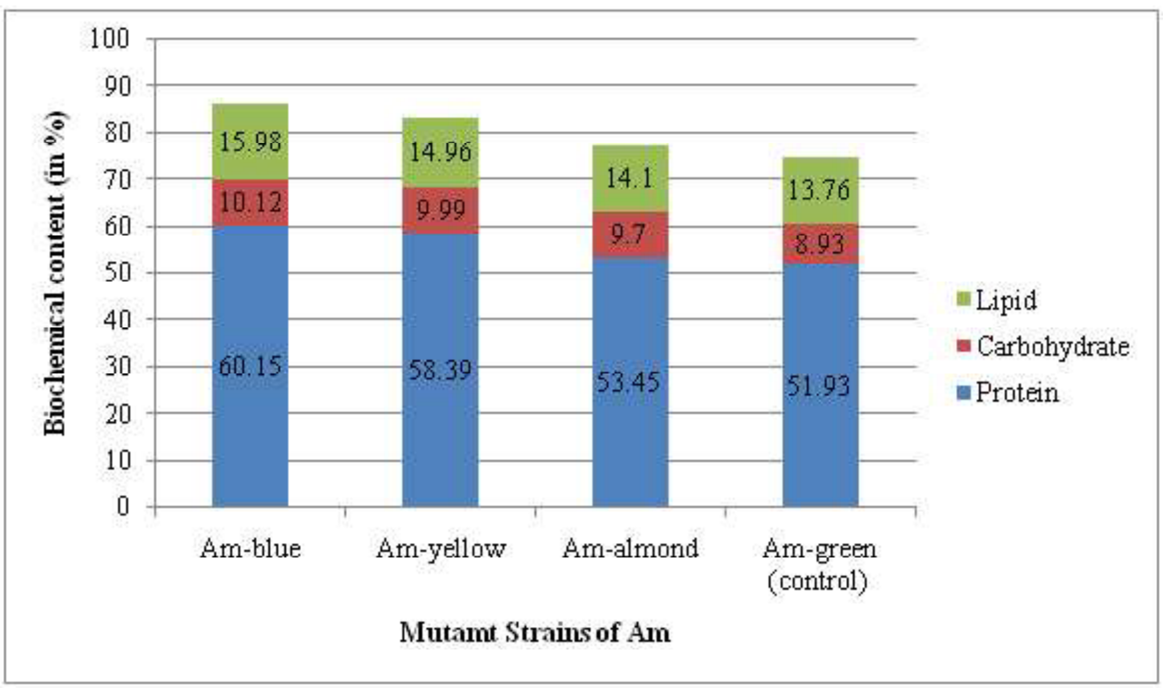

Fig 2: Bar diagram showing percent protein, Carbohydrate and lipid content among the mutant strains of *Antheraea mylitta* during Commercial crop season

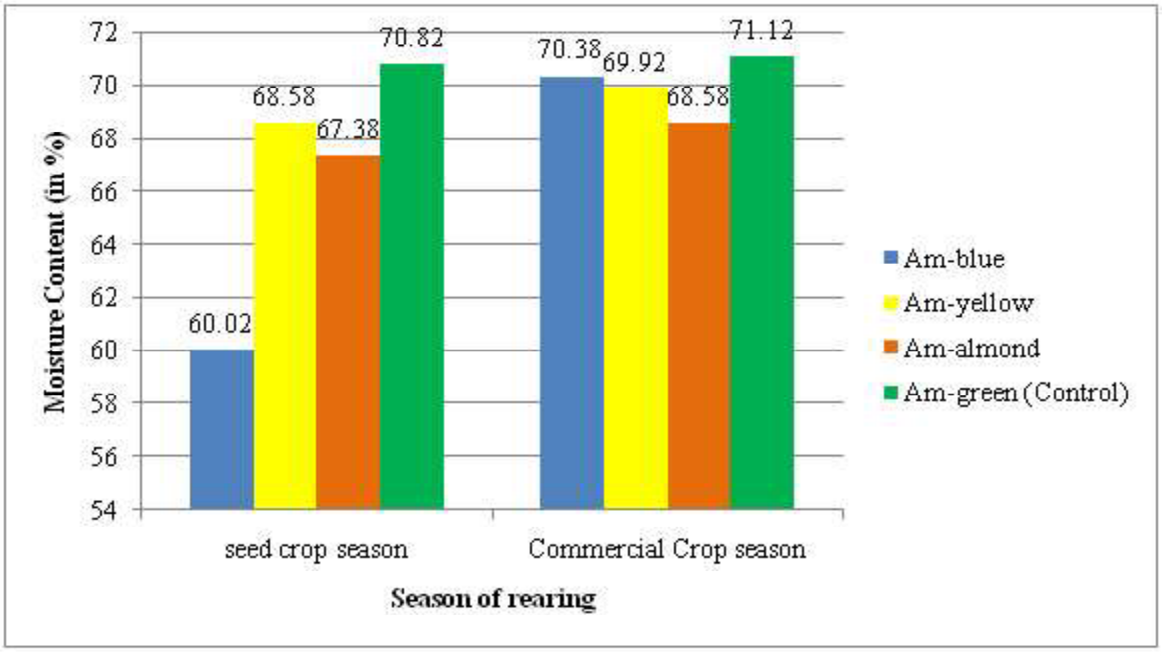

Fig 3: Bar diagram showing percent moisture content among the mutant strains of *Antheraea mylitta* during Seed crop season and Commercial crop season

DISCUSSION AND CONCLUSION

A comparative analysis in respect of percentage of biochemical contents in the larval bodies of all the three mutant strains along with control during the seed crop and commercial crop seasons reveals the following facts:

- The relative percentage of protein, carbohydrate, lipid and moisture in the larval bodies of three mutant strains of *Antheraea mylitta* along with control present significant variations during the seed crop and commercial crop season.
- The relative percentage of said biochemical contents except moisture percentage have been found evidently higher in the larval body of Am-blue than the Am-yellow and Am-almond mutant strains of *Antheraea mylitta*.
- The percentage of moisture in the larval bodies of control has been found higher as compared to three mutant strains of *Antheraea mylitta*.
- The percentage of biochemical contents such as protein, carbohydrate, lipid and moisture in the larval bodies of mutant strains and also control have been found higher during the commercial crop season than the seed crop season, which shows significant impacts of two different seasons on the biochemical makeup of mutant strains of *Antheraea mylitta* and their control.
- The significance of above said results obtained is confirmed by statistical analysis.

The notable investigations carried by Bosch (1972)[17], and Agarwal *et al.* (1957)[12] mention that the protein, carbohydrate and lipid play an important role in maintaining the metabolic activities in insects. The variation in the protein, Carbohydrate and lipid contents is greatly influenced by the ecological conditions and genetic makeup of insects. This observation is similar to observation of Roy *et al.* (2011)[18] and Sharma *et al.* (2013)[19] in relation to behavioural and phenotypic performances of *Antheraea mylitta*. Verma (1992)[20] while working on different ecotypes of *Antheraea mylitta* has found evident variation in the protein, lipid and carbohydrate contents during seed crop seasons and Commercial Crop seasons.

The results obtained appear to be the outcome of genetic variability among the three mutant strains of *A. mylitta* D. on account of relative differences in their physio–genetic makeup. Chakraborty *et al.* (2015)[21] mentioned that although there is a large degree of phenotypic variation among the different ecoraces of *Antheraea mylitta,* genetically they are not different and the phenotypic differences may largely be a result of their respective ecology. It further appears that the Am–blue mutant strain as compared to Am–yellow and thereafter Am–almond is more robust in its genetic architecture as such it has shown relatively better performances in various biochemical parameters. Results obtained are indicative of the fact all the three mutant strains of *A. mylitta* D. in spite of relative differences have registered their supremacy over the control (Daba–green) on account of desired beneficial mutation in relation to their biochemical performances. It is therefore proper to assume that the biochemical variations in respect of protein, Carbohydrate and lipid contents in the larval haemolymph of mutant strains are the logical outcome of different genetic makeup. Thus the evolution of three distinct mutant strains of tropical tasar silkworm is in the larger interest of tasar culture.

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