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EFFECT OF NUTRITIVE ADDITIVE OF MULBERRY AND ITS IMPACT ON NUTRITIONAL COMPONENTS OF SILKWORM *BOMBYX MORI* L.

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ABSTRACT:
Nutritive additive "greenleaf" as a foliar applicant on V_1 mulberry resulted a maximum increase in the macromolecule content when sprayed with 1% concentration during rainy season. The bivoltine hybrid CSR_2 X CSR_4 and poly - bivoltine crossbreed PM x CSR_2 fed on 1% treated leaves showed an increased enzyme activity and nutritive components in their midgut and haemolymph tissues. The enzyme activity and conversion efficiency of mulberry nutrients was found to be significantly higher in CSR_2 x CSR_4 (bivoltine hybrid) than the PM x CSR_2 (poly- bivoltine crossbreed) when fed on leaves treated with optimum concentration of nutritive additive during rainy season.
KEY WORDS: Nutritive additive, Greenleaf, digestive enzymes, macromolecules, approximate digestibility

[1] INTRODUCTION:

Nutrition plays an important role in the productivity of a crop along with variety, management practices and the environmental factors like season [1]. Nutrients are mainly supplied to plants as soil applicants. An alternative to this is supplementing them as foliar spray for immediate and effective incorporation of it into the system [2]. Plants response to foliar applicants depends on species, concentration, frequency of application etc., Under given conditions with the best management practices the possibilities of increasing the productivity exists through application of foliar nutrients [3]. Efforts are on to tap the genetic potential of mulberry varieties so as to obtain optimum growth and yield of quality leaves. Silkworm *Bombyx mori* being a monophagous insect derives almost all the nutrients essential for its growth from the mulberry leaf itself [4] and convert it into animal biomass. Many scientists have observed that the growth and development of silkworm are greatly influenced by the nutritional content of the mulberry [5]. The concentration of carbohydrates and carbohydrases [6] as well as the midgut protease activity varied among the larvae when fed with different types of leaves [7].

In the present work an effort was made to understand the impact of foliar applicant on mulberry leaf and on silkworm maintained on such treated leaves. We mainly focused on

(a) Seasonal impact on the incorporation of applicants by the mulberry leaf.

(b) Conversion efficiency of mulberry nutrients enhanced by foliar applicant in both crossbreed and hybrid silkworm and finally

(c) To elucidate the role of season and silkworm variety on the nutrient absorption.

[II]MATERIALS AND METHODS:

2.1 Preparation and spraying of foliar applicant:

The commercially available foliar applicant (commercial name "green leaf") was diluted to 0.25%, 0.5% and 1.0% with distilled water and sprayed twice on V_1 mulberry plants. Initial spray was given 15-20 days after bottom pruning and the second spray at the interval of 15 days for the proper incorporation of the applicant during the morning hours of the days in all the three seasons studied. The foliar applicant (Green leaf) contains organic plant nutrients with mineral chelates, organic acids and absorbing agents free from hormones and sterols.

2.2 Silkworm rearing:

Silkworm races namely bivoltine hybrid CSR_2 x CSR_4 and cross breed PM x CSR_2 were brought from private grainage near Bangalore. From the third instar to spinning the silkworms were fed separately on 0.25%, 0.5% and 1.0% greenleaf treated mulberry leaves along with the control batch maintained on leaves sprayed with distilled water. Each batch containing 50 worms were maintained with three replications in different experiments. The worms were fed four times a day up to spinning and were maintained by cellular rearing [8].

2.3 Nutritional component assay of mulberry leaf and the silkworm

The coarse leaves were collected from healthy mulberry plants maintained in treated as well as control plots. The leaves were dried at 65-70^{o}C in oven, powdered and 1% homogenate was prepared in distilled water using a mortar and pestle. The homogenate was centrifuged at 3000 rpm for 10 minutes. The supernatant was collected, diluted appropriately and the quantitative estimation of proteins was done by the method of Lowry, *et al.,* [9]. Reducing sugars and total sugars were estimated by employing the method of Plummer [10].

The haemolymph was collected, by cutting the caudal horn, from 15-20 larvae of V instar 4th day in a haemolymph tube containing a pinch of thiourea to prevent oxidation and diluted 10 times in ice-cold distilled water. A 10% homogenate (w/v) of the midgut tissue was prepared in ice-cold distilled water, using a tissue homogenizer fitted with a teflon coated pestle. The tissue homogenate/haemolymph samples were centrifuged at 3000 rpm for 10 minutes in a refrigerated centrifuge (Remi). The supernatant was used for the estimation of total proteins and carbohydrates. The quantitative estimation of proteins was done in haemolymph and midgut tissues by Lowry *et al.,* [9] method, the reducing sugar by dinitro-salicylic acid (DNS) method [11] and total sugar by Anthrone method [12]. The % assimilation rate was recorded following gravimetric method [13].

2.4 Digestive enzymes assay in midgut of silkworm

A 10% homogenate (w/v) of the midgut tissue was prepared as above. The supernatant was used for the assay of amylase, invertase and protease. Quantitative analysis of amylase activity was carried out following the method of Noelting and Bernfeld [14], using the 3, 5, dinitro salicylic acid reagent as modified by Ishaaya and Swirski [15]. Analysis of invertase activity was done according to the methods of Bernfeld [16] and protease activity was carried out according to the procedure of Eguchi and Iwamoto [17] with slight modification.

2.5 Statastical analysis: The experimental results were subjected to ANOVA and significant of the values were indicated at 5%. [18].

[III] RESULTS:

The major nutritive component of V_1 mulberry leaf is shown in table -1. Total protein in the mulberry leaves shows significant increase in

1% foliar applicant treatment compared to that of control and other concentrations. Rainy season seems to induce high protein content (table 1). Similarly trend was also observed in total sugars and reducing sugars. The 1% treatment of nutritive additive in rainy season is most effective in maximum incorporation of all nutritive components to the mulberry leaves.

The digestive enzymes in phytophagous insects play a major role in converting plant nutrients to animal nutrients. In the present experiments the various enzyme activity is high on the 4^{th} day of V instar as shown in the insert of fig 1 and 2. Hence the enzymic activity of the 4^{th} day is considered in further experiments. Significant increase was found in midgut protease, amylase and invertase on feeding the leaves treated with nutritive additive with increase in concentration. Of the three seasons considered in the present study, the larvae fed on 1% foliar additive treated mulberry leaves during rainy season significantly increased the enzymic activity when compared to winter and summer in both the breeds of silkworms.

Larval midgut and haemolymph proteins and carbohydrates of the two breeds in different seasons are in presented in table 2 and 3. The protein content is high in midgut as well as in haemolymph when fed on 1% foliar applicant treated mulberry leaves during rainy season in both the breeds of silkworms. Similar trend was observed in total sugar and reducing sugar also. Among PMxCSR_2 and CSR_2xCSR_4 the later one showed a better conversion.

Assimilation rate of 4^{th} day of V instar larvae in both the breeds and influence of season are presented in table 4. Seasons have different influence on the assimilation in two breeds studied. Rainy season and 1% foliar applicant treated mulberry leaves fed larvae showed maximum assimilation rate than the worms fed on other treatment leaves during other season.

[IV] DISCUSSION

Mulberry on treatment with 1% foliar applicant resulted in significant increase in major nutrients quantity of mulberry leaf especially in rainy season. These results are on par with the results of Mishra *et al.,* [19] by usage of foliar applicant invariably proved the efficiency of greenleaf in increasing the protein content and carbohydrates of mulberry leaves. Similar results were also reported by Etebari *et al.,* [2], that foliar application had enriched mulberry leaf with macromolecules.

The digestive enzymes such as amylase, invertase and protease in silkworms (PM x CSR_2 and CSR_2 x CSR_4 larvae) is maximum when reared during rainy season on leaves treated with optimum concentration treated foliar applicant. These findings are in confirmative with Ishaaya *et al.,* [15]. Their findings indicate the larvae of *Spodoptera littoralis* are fed on leaves with additional factor acts as a stimulant for digestive enzymes probably through hormonal mechanism. Chatterjee [20] also reported that, the enzyme activity was found to be higher in the later stage of larval development and the dietary intake has a role to play. CSR_2 x CSR_4 bivoltine hybrid have more digestive enzymic activity than PM x CSR_2. This could be due to the genetic makeup of the two breeds of silkworm. Similar suggestions were put forward for the increased protease enzymic activity in the midgut of bivoltine larva when compared to multivoltine [21]

There is an increase in protein and carbohydrate content in the haemolymph and midgut of the silkworm fed on 1% foliar applicant treated mulberry leaf in the present study. Rainy season influence the larvae of Bivoltine hybrid CSR2 XCSR4 which showed a higher macromolecule content than the crossbreed larvae PM xCSR2. Similar reports

were reported by Satish [22] on PMxNB4D2 crossbreed fed on nutritive additive treated M5 mulbery leaves even though they lack in seasonal account.

Conversion efficiency of mulberry nutrients by bivoltine hybrid CSR_2xCSR_4 is higher than the crossbreed PMxCSR2 during rainy season when fed on leaves treated with 1% foliar applicant treated leaves. Similar results are shown by Remadevi *et al.,* [23] who reported that ingestion and related nutritional parameters varied from race to race in multivoltine breeds of mulberry silkworm. The notable result of the present study is improvement using a nutritive additive is always beneficial for crop, however choosing a right season and specific breed is essential for conversion efficiency.

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Tables and Figure:

Composition	Season	0.25%	0.50%	1.00%
Total protein	Summer	7.11	12.78	20.32
	Rainy	13.96	21.05	23.47
	Winter	8.28	14.78	21.55
Total sugar	Summer	0.08	0.15	0.36
	Rainy	0.13	0.23	0.46
	Winter	0.10	0.21	0.38
Reducing sugar	Summer	3.24	10.86	20.53
	Rainy	5.16	13.23	29.84
	Winter	4.88	12.94	21.77

Table 1: Δ change in total protein, total sugar and reducing sugar present in V_1 mulberry leaves treated with foliar applicant of different concentrations like 0.25%, 0.5% and 1% in various seasons (unit-mg/g dry weight).

Tissue used	Macromolecules	Season	0.25%	0.50%	1.00%
Haemolymph	Total protein	Summer	1.92	3.96	5.00
		Rainy	3.27	5.13	7.03
		Winter	2.29	4.34	5.29
	Total sugar	Summer	0.53	0.74	0.94
		Rainy	0.54	0.73	0.93
		Winter	0.55	0.75	0.94
	Reducing sugar	Summer	0.37	0.61	1.17
		Rainy	0.86	1.26	1.84
		Winter	0.4	1.09	1.37
Midgut	Total protein	Summer	2.6	3.8	4.5
		Rainy	3.5	4.6	5.7
		Winter	2.9	4.1	4.9
	Total sugar	Summer	0.26	0.68	1.29
		Rainy	0.51	0.86	1.61
		Winter	0.4	0.73	1.37
	Reducing sugar	Summer	1.88	2.18	2.43
		Rainy	1.98	2.63	3.41
		Winter	1.92	2.27	3.06

Table 2: Δ change in the amount of macromolecules especially total protein, total sugar and reducing sugar in haemolymph and midgut of $PMxCSR_2$ (cross breed) silkworms fed on foliar applicant treated (0.25%, 0.5% and 1 %) treated mulberry leaves during different seasons. (unit-mg/ml).

Tissue used	Macromolecules	Season	0.25%	0.50%	1.00%
Haemolymph	Total protein	Summer	1.57	1.83	2.31
		Rainy	1.78	2.65	3.37
		Winter	1.7	2.28	2.97
	Total sugar	Summer	0.65	0.77	0.90
		Rainy	0.70	0.93	1.25
		Winter	0.68	0.86	1.02
	Reducing sugar	Summer	1.09	1.54	1.80
		Rainy	1.29	2.54	2.80
		Winter	1.19	1.74	2.00
Midgut	Total protein	Summer	3.12	4.34	6.17
		Rainy	3.86	5.32	6.56
		Winter	3.36	5.07	6.33
	Total sugar	Summer	0.31	0.85	1.36
		Rainy	0.43	1.21	1.84
		Winter	0.37	0.94	1.48
	Reducing sugar	Summer	2.13	2.82	3.16
		Rainy	2.75	3.74	3.84
		Winter	2.31	3.31	3.37

Table 3: Δ change in the amount of macromolecules especially total protein, total sugar and reducing sugar in haemolymph and midgut of CSR_2xCSR_4 (hybrid) silkworms fed on foliar applicant (0.25%, 0.5% and 1 %) treated mulberry leaves during different seasons. (unit-mg/ml)

Race	Season	Control	0.25%	0.50%	1.00%
PM x CSR_2	Summer	58.90 ±0.64	62.29 ±0.72	65.43 ±0.73	68.12 ±0.57
	Rainy	64.25 ±0.73	69.21 ±0.71	72.55 ±0.63	76.37 ±0.77
	Winter	61.60 ±0.61	65.35 ±0.60	68.31 ±0.94	71.36 ±0.79
CSR2 x CSR4	Summer	60.51 ±0.77	64.97 ±0.46	68.78 ±0.56	71.58 ±0.63
	Rainy	66.37 ±0.56	72.67 ±0.83	75.49 ±0.87	78.41 ±0.74
	Winter	62.64 ±0.70	65.46 ±0.73	71.48 ±0.80	74.77 ±0.81

Table 4: Assimilation rate in % in two breeds of silkworm $PMxCSR_2$ (crossbreed) and CSR_2xCSR_4 (hybrid) fed on control and foliar applicant treated (0.25%, 0.5% and 1 %) mulberry leaves during different seasons.

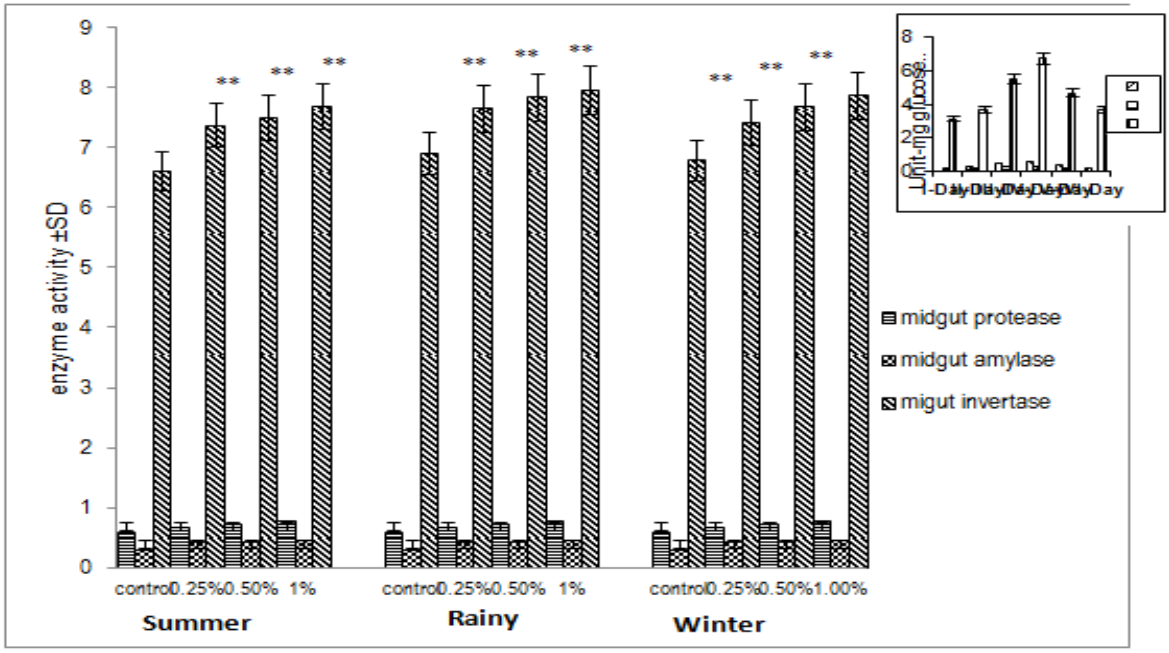

Fig: 1. The graph depicts the enzymic activity of digestive enzymes of 4th day, V instar PMxCSR2 (Crossbreed) silkworm larvae fed on control and (0.25%, 0.5% and1%) foliar applicant treated mulberry leaves. Midgut protease activity is expressed in µ mol tyrosine liberated / min/ g tissue, midgut amylase activity is expressed in mg glucose liberated/ min/ g tissue and midgut invertase is expressed in mg glucose liberated /min / g tissue.

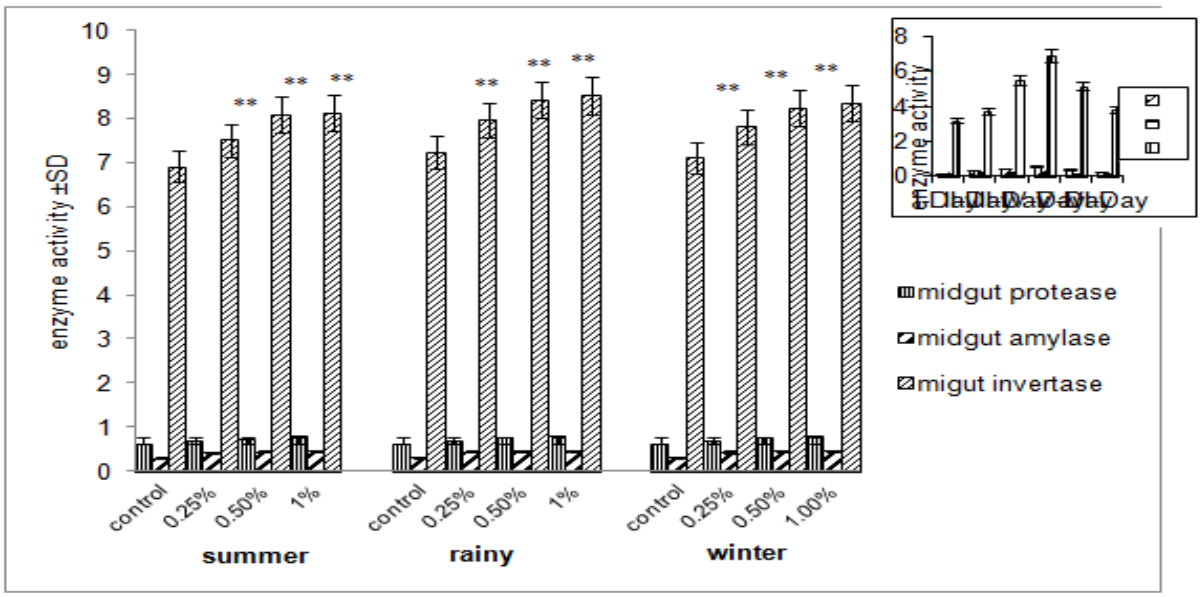

Fig: 2. The graph depicts the enzymic activity of digestive enzymes of 4th day, V instar CSR2xCSR4 (hybrid) silkworm larvae fed on control and (0.25%, 0.5% and1%) foliar applicant treated mulberry leaves. Midgut protease activity is expressed in µ mol tyrosine liberated / min/ g tissue, midgut amylase activity in mg glucose liberated/ min / g tissue and midgut invertase activity in mg glucose liberated /min / g tissue.