Egypt. J. Agric. Res., 72 (3), 1994.

BIOCHEMICAL EFFECT OF STORED MULBERRY LEAVES ON SOME ENZYMES ACTIVITIES IN THE HAEMOLYMPH OF *BOMBYX MORI*

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(Manuscript received 29 November 1992)

Abstract

The biochemical effect of stored mulberry leaves used in feeding *Bombyx mori* larvae on the haemolymph enzymes was investigated. Nine enzymes were dertermined namely; acid and alkaline phosphatase, alpha and beta esterases, G.O.T, G.P.T., amylase, trehalase and invertase.

The haemolymph enzymes revealed clear differences between larvae fed on fresh and stored leaves. Acid phosphatase, GPT and amylase activities decreased, whereas trehalase activity increased as affected by mulberry leaves storage. On the other hand . alkaline phosphatase. alpha and beta esterases , GOT. and Invertase activities increased firstly then decreased thereafter.

INTRODUCTION

It is necessary to preserve mulberry leaves for later use without affecting its quality . Leaves are thus stored in a cool place in polythene or wet gummy sheets to maintain low temperature and high humidity (Lee 1990).

Fraise (1953) reported that feeding *Bombyx mori* on mulberry leaves was considerably reduced when leaves were not fresh. The protein content determined in the resulting silk was proportional to the protein percentage in the different mulberry leaves (Gaaboub *et al.*, 1977).

The present work aimed at investigating the effects of mulberry leaf storage on haemolymph enzymes of the 4th instar larvae of *Bombyx mori.*

MATERIALS AND METHODS

Experiments were conducted on the Korean hybrid 156x 156 of silkworm 4th instar larvae which were fed on fresh mulberry leaves as a control and on stored leaves in refrigerator at 7-10^{o}C for 1-5 days.

Sample preparation for biochemical assay

For biochemical analysis, twenty healthy full mature 4th instar larvae of the silkworm *Bombyx mori* were collected from the different treatments . Then the larvae were starved for 4 hours and the haemolymph was collected. Haemolymph samples were received in small test tubes surrounded by crushed ice then centrifuged at 3200 rpm for 20 minutes. The supernatants were used for enzyme activity determination.

Sample preparation for biochemical assay

1. Acid and alkaline phosphatases (Ac-Pase and Alk - Pase) were determined by the method described by Powell and Smith (1954)

2. Alpha and Beta esterases (a and B- est.) were determined by the method described by Van Asperen (1962).

3 . Glutamate oxaloacetate transaminase (G.O.T.) and glutamate pyruvate transaminases (G.P.T.) were determined by the mehtod described by Reitman and Frankel (1957).

4 . Amylase, trehalase and invertase were determined by the method described by Ishaaya and Swiriski (1976).

RESULTS AND DISCUSSION

1 . Acid and alkaline phosphatase

From the data in Table 1 it could be observed that acid phosphate activity decreased gradually in the haemolymph of larvae fed on stored leaves as compared with those fed on fresh laeves, the activity reached its minimum value at the 4th

Table 1. Effect of mulberry leaves storage on acid and alkaline phosphatase activity in haemolymph of 4th larval instar of *Bombyx mori*

Days of storage	Ac-Pase	Fresh leaves %	Alk - Pase	Fresh leaves %
Fresh	3.5483	100.00	0.3942	100.00
1	2.4192	68.18	0.6988	177.29
2	2.2794	64.23	1.4766	374.58
3	2.4397	38.38	0.7526	190.91
4	2.1685	59.65	0.3681	93.39
5	3.2258	90.91	1.7203	436.41

Activity = mg phenol /min/ml haemolymph
Ac-Pase = Acid phosphatase.
Alk-Pase = Alkaline phosphatase.

Table 2. Effect of mulberry leaves storage on the activity of alpha and beta esterases in haemolymph of 4th larval instar of *Bombyx mori*

Days of storage	a - E	Fresh leaves %	B - E	Fresh leaves %
Fresh	16.10	100.0	20.76	100.0
1	18.10	112.0	25.86	124.0
2	18.54	115.0	12.69	61.0
3	16.86	104.0	20.13	96.0
4	10.66	66.0	12.98	62.0
5	10.63	66.0	11.83	56.0

Activity = ug phenol /min/ml haemolymph
a - E = alpha esterase.
B - E = Beta esterase.

day and then increased again on the 5th day but was still less than that of the control.

2. Alpha and Beta esterases

Table 2 shows the activity of a and B-esterases in the 4th larval instar of *B. mori* as fed on fresh and stored mulberry leaves. It is clear that esterases (a and B) increased at one-day storage , after two days a -esterases slightly increased , then decreased to reach its minimum on the 4th and 5th days. As for B-esterase, its activity dropped after two days but increased again after three days then decreased gradually to reach its minimum on the 5th day. As a general trend, esterase activity had a minimum value on the 5th day, meaning the storage of mulberry leaves had led to the redution of esterase activity after 5days of storage.

3. Transaminases

Tansaminase activity (Table 3) showed that G.O.T. and G.P.T. activity in larvae fed on stored leaves clearly decreased as compared with those fed on fresh leaves.

4 . Carbohydrates

As shown in Table 4, the activities of amylase and invertase in the haemolymph of larvae fed on stored mulberry leaves for one day slightly differed as compared with those fed on fresh leaves (99.08, 103.20%), and then gradually decreased nutil the 5th day at which the activities increased again . As for trehalase. the increment on the first day was very great (542%), then decreased on 2nd and 5th days , but was elevated again on the 4th and 5th days. Nevertheless, the activity was still less than that of fresh leaves.

From the above mentioned data it could be observed that there are clear differences in enzyme activity in silkworm larvae fed on stored leaves as compared with those fed on fresh leaves. Trehalase increased on the first day to more than five folds of its normal value . Since trehalose is the main sugar in insect heamolymph, it could be suggested that the changes in enzyme activity may refer to metabolic changes in the blood biochemical utilization of sugar contained in the stored consumed leaves. This suggestion coincides with Fraise (1953) who reported that in every meal the silkworm ingested the greatest quantity of food during the first half hour, then feeding was considerably reduced especially when leaves were not needed fresh. More investigations are needed to clarify the effect of storage on the biochemical components of mulbery leaves.

Table 3. Effect of mulberry leaves storage on GOT and GPT activity in haemolymph of 4th larval instar of *Bombyx mori*

Days of storage	GOT	Fresh leaves %	GPT	Fresh leaves %
Fresh	0.1882	100.00	0.3007	100.00
1	0.2037	08.00	0.0815	27.09
2	0.0962	57.12	0.1365	45.42
3	0.6987	37.11	0.1202	40.00
4	0.0432	22.94	0.0979	32.58
5	0.0329	17.52	0.1280	42.58

Activity = u mole pyruvic /min/ml haemolymph
GOT = Glutamate - oxaloacetate transminase.
GPT = Glutamate - pyruvate transminase.

Table 4. Effect of mulberry leaves storage on amylase, trehalase and invertase activities in haemolymph of 4th larval instar of *Bombyx mori*

Days of storage	Amylase	Fresh leaves %	Tre.	Fresh leaves %	Inv.	Fresh leaves %
Fresh	296.26	100.00	277.89	100.00	2150.24	100.00
1	295.29	99.68	1181.20	542.11	2219.05	103.26
2	195.72	66.06	214.07	98.25	2099.40	97.63
3	271.41	91.61	118.50	54.39	1948.02	90.60
4	199.90	67.37	173.43	79.59	1780.07	82.78
5	338.30	114.19	206.42	94.75	3.224.40	149.96

Activity = ug glucose /min/ml haemolymph
Tre. = Trehalase/
Inv. = Invertase.

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تأثير التغذية بأوراق التوت الطازجة المخزنة علي بعض الإنزيمات في هيموليمف يرقات دودة الحرير

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معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي

في هذا البحث تمت دراسة التأثير الكيماوي الحيوي للتغذية بأوراق التوت الطازجة والمخزونه علي حرارة تتراوح من ٧-١٠ درجة مئوية لمدة خمسة أيام علي مستويات بعض انزيمات هيموليمف يرقات دودة الحرير مثل انزيمات الفوسفاتيز الحامضي ، والفوسفاتيز القلوي، والألفا استيريز ، البيتا استيريز، والجلوتاميك اكسالوترانس أمينيز ، والجلوتاميك بيروفيك ترانس أمينيز ، والأميليز ، والتريهاليز، والأنفرتيز.

وقد أظهرت النتائج فروقا معنوية واضحة في مستوي نشاط هذه الإنزيمات بين اليرقات المغذاه علي أوراق طازجة وتلك المغذاه علي أوراق مخزنة.