



**PG & RESEARCH DEPARTMENT OF ZOOLOGY &
DEPARTMENT OF MICROBIOLOGY
MUSLIM ARTS COLLEGE**

[Affiliated to Manonmaniam Sundaranar University]
Thiruvithancode - 629 174, K.K. Dist, Tamilnadu, India.



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CERTIFICATE

This is to certify that Prof./Dr./Mr./Mrs./Ms **M. RAMANI** **BAI** **ASSOCIATE**
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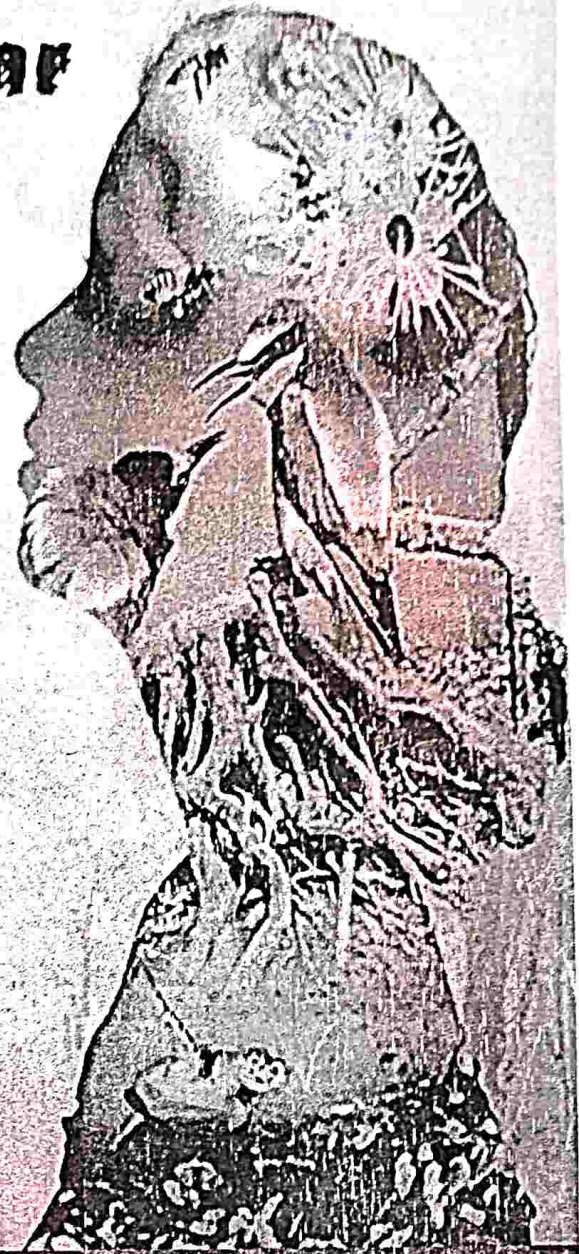
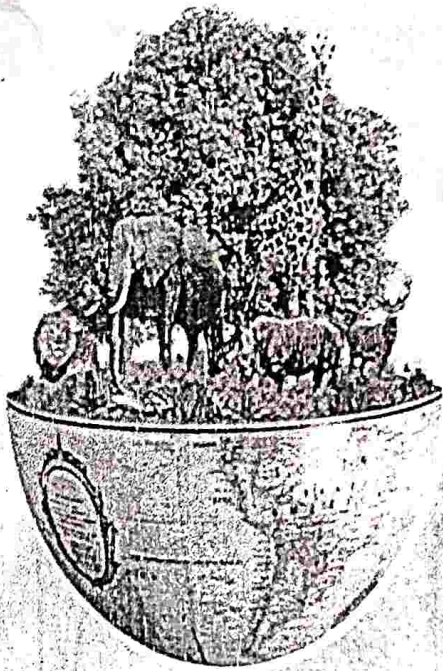
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MUSLIM ARTS COLLEGE

(Affiliated to M.S.University, Tirunelveli)

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EFFECT OF PIPER BETLE EXTRACT ON THE TUKRA INFESTED MULBERRY LEAVES AND BIOCHEMICAL PARAMETERS OF SILKWORM *BOMBYX MORI*.

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ABSTRACT

The pink mealy bug (*Maconellicoccus hirsutus* Green) is small soft bodied mulberry plant sap sucker's insect pests. Which affect both the mulberry leaf quality and silk production. This study investigates the biochemical responses of *Bombyx mori* larva to varying dietary conditions, specifically focusing on the influence of 30% and 50% *Piper betle* extract as well as tukra-infested mulberry leaves when compared to a control group. The haemolymph and fat body compositions of the larvae were analyzed to assess changes in carbohydrate, glycogen, protein, lipid, and trehalose levels. In the haemolymph larvae fed with 30% *P. betle* extract exhibited significant increases in carbohydrate, glycogen, protein, lipid, and trehalose contents, highlighting the nutritive potential of *P. betle* extract. Furthermore, a 30% *P. betle* extract diet increased protein levels in the fat body, while, akin to the haemolymph, tukra-infested mulberry leaves led to reduced levels of all studied biochemical constituents.

Keywords *Maconellicoccus hirsutus*, *B. mori*, *Piper betle*, Biochemical, haemolymph, fatbody.

INTRODUCTION

Silkworm nutrition is of great applied value which involves chemical and physiological activities transforming food into body structure. Insect nutrition primarily deals with biochemical substances that are necessary to activate various metabolic processes resulting in growth and development (Sannappa, 2019). Major biomolecules such as proteins, carbohydrates and lipids play an important role in biochemical process underlying growth and development on insects (Ito and Horie, 1959). The changes in biochemical constituents in silkworm when fed with infested mulberry leaves reducing the chemical constituents like sugar and amino acids, (Siddaramaiah and Hegde 1990). The pest *M. hirsutus* is not only reduces the yield but also altered the biochemical components in mulberry leaves which are obviously nutritionally interfering, leads to crop failure (Manjunath et al., 2003). Moreover, chemical control of disease leads to environmental pollution as well as bio degradation in soil leads to toxicity (Purohit et al., 1978). Plants are the richest source of organic chemicals on the earth and phytochemicals have been reported to influence the life and behavior of different insects (Gouda et al., 1997). The role of plant products having potential growth promoting properties particularly on silkworm gaining importance in recent years (Murugan et al., 1998). The present study explores to assess the *P. betle* extract sprayed to tukra infested mulberry leaves fed to silkworm and to analyses the biochemical changes in the tissues of *B. mori*.

MATERIALS AND METHODS

2.1 Maintenance of silkworm rearin

Fresh diseases free layings (DFL_s) of PM_x CSR₂ multivoltine race was purchased from Government sericulture farm, Nannagaram, Tirunelveli district, Tamilnadu, India. All the rearing operations were carried out according to Krishnaswami et al. (1973). The larvae were divided in to control and experimental groups (7) with 5 replications of 100 larvae each fed with same quantity and quality of mulberry leaves.

2.2 Collection of plant material and preparation of extracts

P. betle with medicinal properties was used in this work. The leaves were collected, washed thoroughly with distilled water and allowed to dry in the shade for about 3 to 4 days, and powdered. Further 50 gm powdered, thus obtained was subjected to extraction through Soxhlet apparatus with 500 ml methanol solvent for 24 hrs. After 24 hrs, the extract was filtered and the filter was evaporated completely. Evaporated extract material dissolved in distilled water and prepared 10%, 30%, 50%, 70% and 90% for further experiment. Tukra infested mulberry leaves at earlier stage were identified and sprayed with different concentrations and fed to III, IV and V instar larvae, four feedings per day (served as treated). The silkworm larvae fed normal mulberry leaves (served as control).

2.3 Collection of haemolymph and removal of fat body from *B. mori*

Haemolymph was collected separately from few *B. mori* larvae (Mahesha et al., 2000) and centrifuged at 3000rpm for 10 minutes. The supernatant was removed and kept in -20^o C for the determination of carbohydrate, glycogen, protein, lipid and trehalose. The fatbody was dissected out from the fifth instar on the sixth day in 0.9 percent saline following the method of Yamoka et al. (1971).

Table 1.1 Biochemical contents in the haemolymph of *B. mori* larvae fed with *M. hirsutus* infested leaves fortified with different concentrations of *P. betle* plant extract.

Concentrations (%)	Haemolymph (mg/l)				
	Carbohydrate	Glycogen	Protein	Lipid	Trehalose
Control	27.45±1.39	1.51±0.04	15.51±1.54	4.3±0.19	6.47±0.05
Tukra infested mulberry leaves	21.67±1.78 (-21.03)	1.45±0.03 (-3.97)	11.93±1.03 (-23.05)	3.34±0.15 (-22.32)	4.74±0.08 (-26.72)
10	21.29±0.83 (44.40)	1.15±0.02 (1.76)	22.47±1.82 (62.23)	2.89±0.04 (26.74)	4.26±0.06 (32.71)
30	27.37±1.49 (85.63)	1.28±0.03 (13.27)	26.89±1.31 (94.15)	3.79±0.11 (66.21)	4.39±0.06 (36.76)
50	22.83±0.97 (54.85)	1.20±0.02 (6.19)	25.37±1.88 (83.17)	3.26±0.17 (42.97)	4.06±0.06 (26.48)
70	17.70±0.73 (20.06)	1.18±0.02 (4.42)	19.30±0.77 (39.34)	2.66±0.07 (16.66)	3.84±0.03 (19.62)
90	16.92±0.03 (14.78)	1.14±0.03 (0.88)	15.99±0.57 (15.45)	2.48±0.08 (8.77)	3.62±0.05 (12.77)

Note: per cent deviation over control values in parentheses

Table 1.2 Biochemical contents in the fatbody of *B. mori* larvae fed with *M. hirsutus* infested leaves fortified with different concentrations of *P. betle* plant extract.

Concentrations (%)	Fat body (mg/g)				
	Carbohydrate	Glycogen	Protein	Lipid	Trehalose
Control	7.26±0.09	7.18±0.07	8.18±0.13	13.56±0.07	1.15±0.04
Tukra Infested mulberry leaves	6.69±0.07 (-7.84)	6.52±0.07 (-9.18)	7.85±0.07 (-4.03)	12.79±0.08 (-5.67)	0.68±0.04 (-40.86)
10	8.23±0.07 (13.35)	8.19±0.03 (14.05)	8.71±0.07 (6.47)	13.64±0.04 (0.58)	1.20±0.03 (4.34)
30	9.11±0.05 (25.47)	8.38±0.07 (16.70)	8.83±0.04 (7.94)	14.27±0.06 (5.23)	1.25±0.02 (8.69)
50	9.42±0.07 (29.74)	9.24±0.07 (28.67)	8.78±0.04 (7.33)	14.71±0.06 (8.47)	1.35±0.03 (17.39)
70	8.13±0.06 (11.97)	8.67±0.05 (20.70)	8.76±0.02 (7.08)	13.81±0.06 (1.84)	1.31±0.02 (13.91)
90	7.41±0.06 (2.06)	7.37±0.04 (2.64)	8.47±0.08 (3.54)	13.54±0.05 (-0.14)	1.24±0.03 (7.82)

Note: per cent deviation over control values in parentheses

RESULTS

The result shows that the carbohydrate (27.37±1.49mg/g), glycogen (1.28±0.03mg/g), protein (26.89±1.31mg/g), lipid (3.79±0.11mg/g) and trehalose (4.39±0.06mg/g) contents in the haemolymph were increased in the larvae fed with 30% *P. betle* extract, whereas all the biochemical constituents were decreased when the larvae fed with tukra infested mulberry leaves, when compared to control (Table 1.1).

The results shows that the carbohydrate (9.42±0.07mg/g), glycogen (9.24±0.07mg/g), lipid (14.27±0.06mg/g) and trehalose (1.35±0.03mg/g) contents in the fat body were increased in the larvae fed with 50% *P. betle* extract. On the other hand, protein (8.83±0.04mg/g) content was increased at 30%, whereas all the biochemical constituents were decreased when the larvae fed with tukra infested mulberry leaves, when compared to control (Table 1.2).

DISCUSSION

It is well recognized that the nutritional components of mulberry leaves have a significant impact on both the quality and output of silk. Silkworms require specific biochemical components such as sugar, carbohydrates, proteins, amino acids, fatty acids, sterols, and vitamins to maintain their growth and development. These chemical substances are also required for silk gland growth, metamorphosis, egg production, and silk synthesis (Ito, 1978). According to Narayanaswamy et al. (1999), the pest-infested mulberry leaves are quantitatively and qualitatively very poor. The mealybug, *M. hirsutus*, has been implicated in the transmission of tukra (Babu et al., 1994). Silkworm larvae fed tukra-infested mulberry leaves revealed minor decreases in biochemical parameters such as protein and amino acids (Zaman et al. 1996). In the current study, biochemical indicators such as carbohydrate, glycogen, protein, lipid, and trehalose in the silk gland, haemolymph, and fat body indicated a negative trend when larvae were fed tukra-infested mulberry leaves.

Natural botanicals are currently being used to treat plant and animal ailments as well as pest control. These are used in silkworm raising to prevent illnesses and improve the biochemical contents of silkworm. In the present study the biochemical constituents such as carbohydrates, glycogen, trehalose, protein, and lipids in haemolymph, and fat body showed positive trend, when the larvae of larvae fed tukra-infested mulberry leaves enriched with different concentrations of *P. betle* extract. Horie et al. (1968) discovered that silkworms used carbohydrates as energy sources and for the synthesis of lipids and amino acids. Accumulation of lipid content in different stage of silkworm growth on mulberry leaves raised from organic based nutrients. Naik (2012) discovered that the

trehalose content in the haemolymph and fat body of silkworms fed botanical extract-treated tukra-infested mulberry leaves boosted activity compared to the haemolymph control. The active levels gradually increased in comparison to the corresponding control fat bodies.

CONCLUSION

These results suggest that the presence of *P. betle* extract, especially at 30% and 50% concentrations, has a significant impact on the biochemical composition of both the haemolymph and fat body in the larvae. The presence of *P. betle* extract appears to promote an increase in carbohydrate, glycogen, protein, lipid, and trehalose levels. On the other hand, when larvae were fed with tukra-infested mulberry leaves, there was a decrease in these biochemical constituents. These findings indicate the potential nutritional benefits of *P. betle* extract for the larvae and suggest that it can positively influence their biochemical profiles.

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