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***Research Paper***

# EFFECT OF BOTANICAL EXTRACTS (*Ocimum sanctum & Parthenium hysterophorus*) ON THE INCIDENCE OF MAJOR PEST (TUKRA) IN MULBERRY LEAVES ON EXCRETORY PRODUCTS IN SILKWORM, *Bombyx mori* L.

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**Abstract**

The continuous use of pesticides over a period of time cannot sustain the crop yield and also harmful Effects on soil and environment. Mulberry leaves are the predominant food source for silkworm, *Bombyx mori* rearing. The incidence of Pink mealy bug occurring in mulberry plantation can cause tukra disease that leads to qualitative loss of mulberry plantation. The present study was undertaken to study the effect of *O.sanctum & P.hysterophrus* extracts having potential against the pests and insects as natural botanicals origin by foliar spray. The extracts sprayed to occurring mealy bugs at the early cause of infection to V1 mulberry variety and reared to Silkworm. The total ammonia, urea and uric acid parameters were studied in tissue like haemolymph. The ammonia and uric acid activity gradually increased this increase however was significant at (P>0.05). There were a gradual decrease of urea level from day 3 to day 6, this decrease was however non-significant. Foliar spray of seed kernel extract hold greater promise for control of tukra infested mulberry leaves and did not affect the excretory system in silkworms.

Key words: Tukra, Plant extracts, excretory products and *Bombyx mori L.*

## INTRODUCTION

Catabolism of proteins usually results in the production of some of the unwanted nitrogenous end products like ammonia, urea and uric acid. In animals, two thirds of the total nitrogen eliminated is in the form of ammonia used as uric acid. The remaining one third is composed of various nitrogen compounds including purine bases and xanthine. On the basis of the mode of excretion animals are broadly classified in to ammonotelic, urotelic and uricotelic (Hoar, 1976). Uric acid is the main excretory product in various insect groups, and a variety of adaptations have been observed in insects to dispose the uric acid. There is evidence that uric acid may be stored particularly in the fat bodies (wigglesworth, 1950) and as a chessy mass lining the integument. Where it may stay to serve as nitrogen reserve for further use by the insect (Ludwig, 1954). Anderson and Patton (1955) reported that uric acid is synthesized principally in the fat bodies, whereas it is excreted by way of the malpighiantubules. As a complementary and alternative medicine, the use of herbs have increased in last two decades and one among them is tulsi (*O. sanctum*)called by names like manjari/Krishna tulsi (Sanskrit), Tritlavee (Malayalam), tulshi(Marathi) and tulsi (Telugu) and it is one with reddish leaves. Several studies by Indian scientists suggest the antibacterial and stimulative effect of tulsi and it is described as Dashimani shwasaharini .As there is no work pertaining to its impact on silkworm, the present work had been undertaken. *P. hysterophorus* can be used to increase crop production at minimal expenses and to diminish the current reliance on synthetic agrochemicals that degrade the environmental quality. The allelochemicals can be exploited as herbicides, insecticides, nematicides, fungicides and growth regulator. Pesticidal potential has been established in terms of ovicidal and anti-fleedant effects (Datta and Saxena 2001). The allelochemicals also provide defence against herbivorous predators. Another species of *Parthenium* i.e., *P. argentatum* A. Gray (common name Guayule) is known as very useful possible petroplants. Stem of this plant yields a rubber, which can be substituted for that of *Hevea* for articles, such as tyres, foot wear, belting and hose, A two-years old plants produced alteast 10% rubber by dry weight and can be increased with chemical stimulants at the early stages upto 300%. Leaf yields an essential oil (Sastry and Kavathekar, 1990).

Conversion of ammonia to urea is also one of the important mechanisms of ammonia detoxification in insects (Brown, 1958). Urea production has an additional function in the production of arginine and ornithine. Garcia et al., (1956) suggested that the presence of this cycle in silkworm B.mori based on the identification of arginine, ornithine and urea. Hayashi (1961) also reported the formation of urea from arginine in silkworms. Kilby and Neville (1957) found that homogenates would catalyze transaminations between α-ketoglutarate and many amino acids. All the transaminases are present in the mitochondrial fraction, and the alanine / glutamate and asparate / glutamate also occur in the soluble fraction of fat body in all homogenates (Venkatarami Reddy et al., 1992b).

## MATERIALS AND METHODS

### Maintenance of Silkworms

For the present investigation, the popular south Indian cross breeds (CB) silkworms PMxNB4D2 of Bivoltine breeds of Mulberry silkworms variety, *Bombyx mori* (L) was used as test materials.

The disease free laying (DFLS,) of this cross breed PMxNB4D2 (Bivoltine hybrid) were produced under field conditions and brought to the laboratory.

### Maintenance of Seed Kernel Extract Sprayed Tukra Infested Mulberry Leaves:

Mulberry crop was maintained by following standard agronomic practices. Treatments were imposed on 15th day of pruning in each plot, five plants were randomly selected and the population of pink mealy bug was counted. In each plant, population was counted on three leaves (top, middle and bottom). The total number leaves per plant were also counted and the population was expressed as number per leaf. Observations were made just before spraying (pretreatment count), 3, 5 and 7 days after spraying. The following seed kernel extract with naturally existing insecticidal properties were selected for preparation of plant extract *O.sanctum & P. hysterophorus.*

### Preparation of aqueous plant extract:

Plants having insecticidal properties like *Ocimum sanctum* and *Parthenium hysterophorus* were taken from the department of Botany, University College of sciences, Acharya Nagarjuna University, Guntur, AndhraPradesh. The leaves of plants were collected, washed thoroughly with distilled water the fresh leaves were homogenate with the help of mechanical device. Further 200 gm of crude selected plants were subjected to extraction through soxhlet apparatus with 500 ml methanol solvent for 24 hrs. After 24 hrs given extract was filtered and filtrate was evaporated completely. Evaporated extract material was dissolved in distilled water and diluted to 2.5 % concentration and used for spray at the identified plot with earlier infection of mealy bug in mulberry plants. Botanical extracts sprayed to tukra leaves of various concentrations were fed to third instar larvae with four feeding per day. The feeding was maintained up to the earlier end of cocoon stage of the silkworm.

**Studies of Excretory Products in Silkworm Fed with botanical extracts-Sprayed Mulberry Leaves:**

A bioassay was conducted to find out the effect of feeding healthy and botanical-Sprayed leaves on silkworm hybrid, PMxNB4D2. Leaves were collected from plots from 0, 2, 5, 7, 10, 15 and 20 days after spray and were fed to fifth instar silkworm. The haemolymph was drawn out from the larvae by puncturing the proleg. The haemolymph was collected in small ice cooled test tubes rinsed with phenylthiourea solution (1% w/v). All the results obtained in this investigation were subjected to statistical analysis. The standard deviation was calculated and‘t’ values were derived between the control and experimental. The levels of significance were noted from the standard‘t’ values and represented in the respective histogram.

## RESULTS

**Ammonia level as a function fed with botanical sprayed mulberry leaves:**

From the data presented in (Table 1) and (figure 1) it has observed that ammonia levels in the haemolymph of silkworm when fed with botanical extracts sprayed mulberry leaves fed to by silkworms, the ammonia level increased from day 3 to day 6 relative to the respective controls. Especially between day 5 and 6 were statistically significant. The decrease of ammonia level at day 3 and day6 in PMxNB$_4$D$_2$ hybrid by fed with tukra infested mulberry fed to hybrid silkworm was statically insignificant ($P>0.05$).Highest increase in haemolymph ammonia level was observed at day 5 and day 6 in the silkworm at botanical extracts sprayed fed larvae

**Urea level as a function fed with botanical extracts sprayed mulberry leaves:**

From the data presented in (table.2& fig.2) it is noticed that the urea level in the haemolymph of the hybrid silkworm fed with botanical extracts sprayed mulberry leaves decrease gradually from day 4 to day 6 relative to respective controls. This decrease was less at tukra fed larvae at day 3 to day 6 and the differences between one day 5 to day 6, were statically significant ($P<0.05$). The decrease in the urea level was non significant ($P< 0.05$) when campared with control.

**Uric acid level as a function fed with botanical extract sprayed mulberry leaves:**

From the data presented in (table.3 & fig.3) it is seen that the uric acid level in the haemolymph of the hybrid silkworms fed with botanical extracts sprayed mulberry leaves gradually increased from day 3 to day 6, relative to the respective controls. The increase of uric acid level from 4 to day 6 at botanical sprayed mulberry leaves fed to the silkworm larva was non-significant, but significance was observed in the rest. A decrease in uric acid when fed with tukra leaves were observed at day 3(P<0.05) and rest of the days were Non significant (P<0.01).

**Table: 1** Estimation of Ammonia in haemolymph (mg/100ml) the hybrid race of multi X bivoltine (PMxNB4D2) of silkworm, *Bombyx mori* when fed with tukra and botanical extracts sprayed mulberry leaves at days of V^th instar larvae. Each value is a mean of six replicants and Percent change over control is given in parenthesis.

| Race/breed | Name of the tissue | | Days of Vth instar | | | |
|---|---|---|---|---|---|---|
| | | | 3 | 4 | 5 | 6 |
| PMxNB4D2 | Haemolymph | Control | 6.140 | 6.280 | 6.350 | 6.410 |
| | | S.D.± | 0.2400 | 0.2300 | 0.2900 | 0.3200 |
| | | Sprayedbatch | 6.440 | 6.480 | 6.550 | 6.660 |
| | | S.D.± | 0.3300 | 0.3500 | 0.3800 | 0.4100 |
| | | % | 4.880 | 3.180 | 3.140 | 3.900 |
| | | ‘t’ test | N.S | N.S | N.S | N.S |
| | | Tukrafedbatch | 6.360 | 6.430 | 6.500 | 6.600 |
| | | S.D.± | 0.3500 | 0.2500 | 0.2800 | 0.3000 |
| | | % | 3.580 | 2.380 | 2.300 | 2.960 |
| | | ‘t’ test | N.S | N.S | N.S | N.S |

S.D.±: Standard deviation P: level of significance. N.S: Non Significant

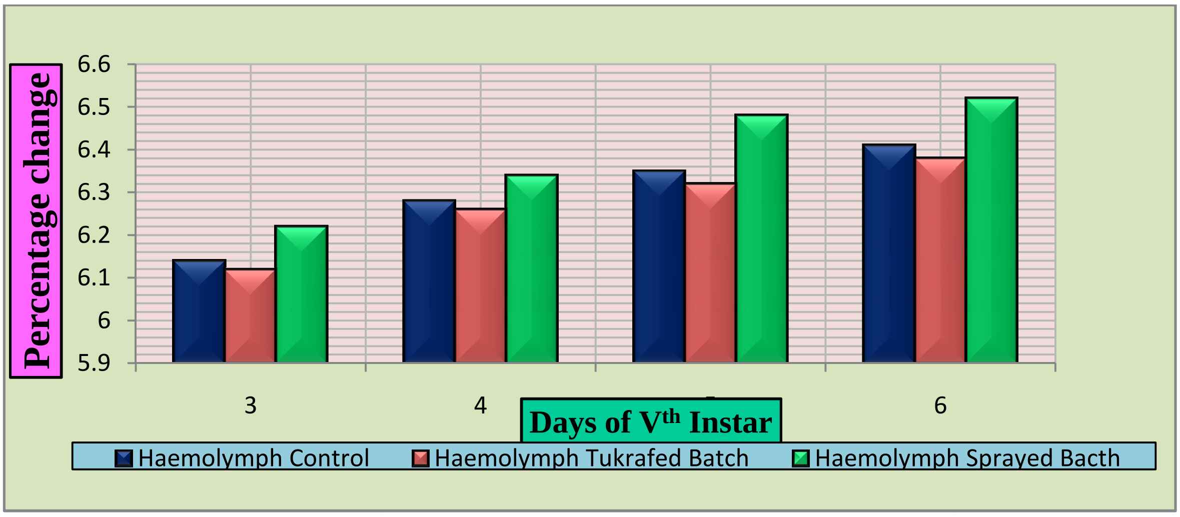


**Fig: 1** Percent change over control in Ammonia of haemolymph of PMxNB4D2 hybrid silkworm at different days of V^th instar larvae fed with tukra and botanical extract sprayed mulberry leaves.

**Table: 2** Estimation of Urea in haemolymph (mg/100ml) the hybrid race of multi X bivoltine (PMxNB4D2) of silkworm, *Bombyx mori* when fed with tukra and botanical extracts sprayed mulberry leaves at days of V$^{th}$ instar larvae. Each value is a mean of six replicants and Percent change over control is given in parenthesis.

| Race/breed | Name of the tissue | | Days of V$^{th}$ instar | | | |
|---|---|---|---|---|---|---|
| | | | 3 | 4 | 5 | 6 |
| PMxNB4D2 | Haemolymph | Control<br>S.D.± | 0.108<br>0.0043 | 0.120<br>0.0045 | 0.194<br>0.0055 | 0.224<br>0.0060 |
| | | Sprayedbatch<br>S.D.±<br>%<br>'t' test | 0.118<br>0.0048<br>13.600<br>P<0.001 | 0.138<br>0.0049<br>-1.660<br>N.S | 0.180<br>0.0053<br>-6.200<br>P<0.01 | 0.220<br>0.0055<br>-8.800<br>N.S |
| | | Tukrafedbatch<br>S.D.±<br>%<br>'t' test | 0.098<br>0.0053<br>-7.400<br>P<0.05 | 0.100<br>0.0058<br>-16.330<br>P<0.001 | 0.140<br>0.0062<br>-20.830<br>P<0.001 | 0.170<br>0.0049<br>-26.810<br>P<0.001 |

S.D.±: Standard deviation P: level of significance N.S: Non Significant

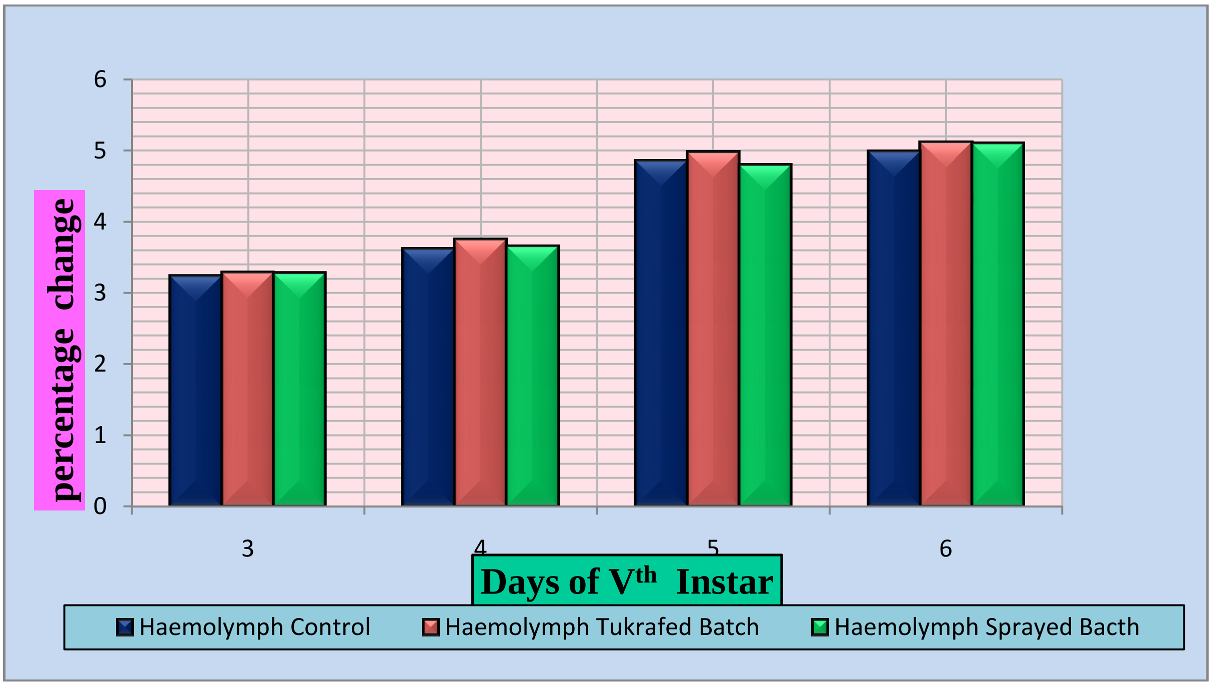


**Fig: 2** Percent change over control in Urea of haemolymph of PMxNB4D2 hybrid silkworm at different days of V$^{th}$ instar larvae fed with tukra and botanical extract sprayed mulberry leaves.

**Table: 3** Estimation of Uric acid in haemolymph (mg/100ml) the hybrid race of multi X bivoltine (PMxNB4D2) of silkworm, *Bombyx mori*. When fed with tukra and botanical extracts sprayed mulberry leaves at days of Vth instar larvae. Each value is a mean of six replicants. Percent change over control is given in parenthesis.

| Race/ breed | Name of the tissue | | Days of Vth instar | | | |
|---|---|---|---|---|---|---|
| | | | 3 | 4 | 5 | 6 |
| PMxNB4D2 | Haemolymph | Control<br>S.D.± | 3.240<br>0.1200 | 3.620<br>0.1500 | 4.860<br>0.2000 | 4.990<br>0.2100 |
| | | Sprayedbatch<br>S.D.±<br>%<br>'t' test | 3.280<br>0.1800<br>1.440<br>N.S | 3.657<br>0.1100<br>1.630<br>N.S | 4.800<br>0.2300<br>1.000<br>N.S | 5.100<br>0.2300<br>2.600<br>N.S |
| | | Tukrafedbatch<br>S.D.±<br>%<br>'t' test | 3.290<br>0.1400<br>0.650<br>N.S | 3.750<br>0.1800<br>0.820<br>N.S | 4.980<br>0.1700<br>1.320<br>N.S | 5.120<br>0.2200<br>2.300<br>N.S |

S.D.±: Standard deviation P: level of significance. N.S: Non Significant

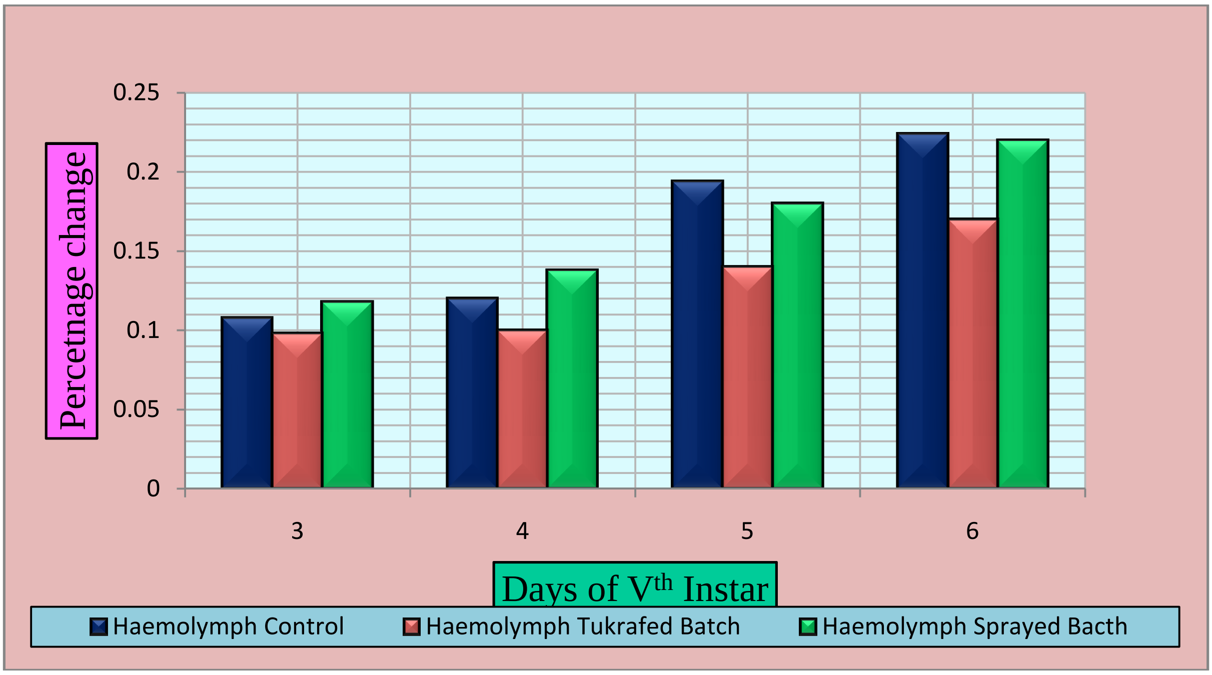


**Fig: 3** Percent change over control in the Uric acid of haemolymph of PMxNB4D2 hybrid silkworm at different days of Vth instar larvae fed with tukra and botanical extract sprayed mulberry leaves.

**DISCUSSION**

The tukra infected mulberry leaves with symptoms of minute mealy bugs, infected mulberry garden curling of apical leaves (Kumar *et al*., 1997). The mulberry infested with *M.hirsutus* (green) is a major pest of mulberry in southern parts of India and has become regular pest of mulberry in Andhra Pradesh and Tamil Nadu and other

southern states especially during warmer. It has been reported that most of the mulberry varieties were susceptible for the mealy bug, *M.hirsutus* (green) attack (Mukhopadhy *et.al,* 2006). Leaf curling with mealy bug is the symptoms of tukra infested mulberry and to find out whether spray of the aqueous seed kernel extracts of *Azadirachta indica* extract was sprayed to tukra incindence at earlier of V1 mulberry variety and fed to Silkworm (PMxNB4D2 Bivoltine hybrid). As the control of mealy bug, application of chemical pesticides are not advised since they harm the silkworms and recently non-chemicals avenues like botanicals acted as an efficient alternative for the pesticides in mulberry garden . Mukhopadhyay *et al.,* (2008; 2009) reported that when silkworms fed with botanical sprayed of infested mulberry leaves after observing the waiting period and feeding to silkworms there was no impact on the economic parameters of cocoons. Neem leaves extract demonstrated a strong ability against the development of many disease causing fungi through its addition to the soil or by its direct application (Locke, 1965, Tewari, 1991). Some extracts from neem plant have been shown to be toxic to fungal pathogens such as *Poria monticolad* infecting wood (Dhyani *et al.,* 2004). The impacts of mealy bug infestation in mulberry (malformed, curled leaves) were assessed for their nutritive value and relative ability to support the growth and development of *Bombyx mori* of this cross breed PMxNB4D2 (Vamseedhar *et al.,* 1999). Neem extracts do not normally kill pests right away rather they repel them or affect their growth and its repellent and pesticidproperties are broad spectrum in nature (Ganguli, 2002). Neem seed kernel extract maybe can induce plant defens reactions and useful in management of leaf stipe disease (Barley and Cao *et al.,* 2004). Extracts of neem are being extensively used to control pests (Michereff *et al.,* 2008).
Ramarathinam and Sangeetha (2002) reported that the mealy bugs, M.hirsutus (green) infected mulberry leaves when fed to silkworms the metabolities of prenylalamine ammonia lyase, showed a marked increase in infected leaves in lthe adjoining of leaves when compared with healthy leaves fed to silkworm larvae. The operation of urea ornithine cycle in insects in less significant, hence the urea level is low in the haemolymph of the silkworm larvae. The further decrease in the level of urea in haemolymph with lack of nutrition or pathophysiological condition and created in tissues of silkworm indicates that the little conversion of ammonia to urea might been suppressed gradually, there by a rise in ammonia level is observed. The main basic mechanism exists in insects to detoxfy ammonia is its conversion in to uric acid, and hence uric acid is primary excretory product of nitrogen and purine metabolism in *B.mori*. It has to be excreted out as and when it is formed, so that a lower level should be maintained in the haemolymph which will in any way affects the normal growth of the larvae. From the results obtained in the present study it appears that part of the ammonia formed in tissues on active transdeamination has been converted into uric acid, but it has not been excreted out there by resulted in urecomia, the accumulation of uric acid in the haemolymph, due to the pest stress condition or lack of nutritive value to silkworm. The increase in ammonia and uric acid levels in haemolymph causes acid base imbalance; also the ammonia is toxic to the central nervous systems and effects neuronal transmission. The results are in agreement with the reports of Raghaviah *et al., (*1988) and Ambika (1990) showed that the silkworm changes in biochemical functions when fed with fungal and pest's contaminated mulberry leaves fungal and pest varied to environmental factors. Similarly, enhanced uric acid level in the larval of neodiprion swainei was reported under different patho-physiological conditions.

The urea ornithine cycle in insect is less significant. Hence the urea level is low in the haemolymph of silkworm due the lack of nutrition (or) pathophysiological condition created in tissues of silkworm indicates the little conversion of ammonia to urea might be suppressed gradually (or) on active transamination in to uric acid by a rise in ammonia level is observed (Smirnof, 1971). The progressive reduction of urea levels suggest the physiological stability up to some extent created is the tissues of silkworm when fed with tukra affected leaves, hence instead of discarding tukra leaves the farmers can make use tukra leaves.

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