Journal of

Entomology

ISSN 1812-5670

Academic
Journals Inc.

www.academicjournals.com

Journal of Entomology 6 (4): 207-214, 2009
ISSN 1812-5670

Effect of Tukra (Mealybug) Infected Mulberry Leaves on the Quantitative Traits of New Polyvoltine Strain of Silkworm, *Bombyx mori* L.

[1]C. Ramesha, [1]S.V. Seshagiri, [1]H. Lakshmi, [2]S.S. Kumari and [1]C.G.P. Rao
[1]Silkworm Breeding and Molecular Genetics Laboratory,
Andhra Pradesh State Sericulture Research and Development Institute,
Kirikera-515 211, Hindupur, Andhra Pradesh, India
[2]Biological Division, Indian Institute of Chemical Technology,
Taranaka, Hyderabad-500 007, Andhra Pradesh, India

Abstract: The influencing effect of tukra infected mulberry leaves on 5th instar polyvoltine silkworm breed of $APM_{1,}$ rearing was studied since, the maximum leaves consuming at this stage of silkworm life cycle and by utilizing non-infected healthy leaves as control. Interestingly in the experimental batch, larval duration was found to be 12 h lesser than the control batch. Mulberry leaves consumption efficiency and economical characters of silkworm like larval weight, pupation rate, cocoon weight, shell weight and reeling parameters were found higher in the experimental batch than the control batch. The result indicated that tukra infected mulberry feed has no adverse effect on silkworm rearing. Moreover, overall improvement was noticed in tukra infected leaves fed batch and sturdily suggest that even mealybug infected mulberry leaves can also be effectively utilized for the silkworm rearing in acute shortage of healthy mulberry leaves.

Key words: Silkworm breed, mealybug, tukra infected leaves, economical characters

INTRODUCTION

The silkworm, *Bombyx mori* L. is an important economic insect and also a tool to convert leaf protein into silk. The industrial and commercial use of silk, the historical and economic importance of production and its application in all over the world finely contributed to the silkworm promotion as a powerful laboratory model for the basic research in biology (Ramesh-Babu *et al*., 2009). The mealybug, *Maconellicoccus* sp., belongs to the family Pseudococcidae, has been reported in India, Bangladesh, Indonesia and identified the species as *Maconellicoccus hirsutus* (Green) (Baskaran *et al*., 1992; Chatarjee and Sarkar, 1993; Bindroo and Dhar, 1996). Its seasonal incidence, transmission and severity of mealybug incidence in mulberry and this dreadful diseases prevalent during the pre-monsoon and summer seasons in sericulture practicing states of India, causing crop loss up to 4500 kg/ha/year (Muralikumaran and Baskaran, 1992a, b). The morphological and anatomical changes such as curling of leaves, thickening and flattening of stems at the growing point observed in tukra infected mulberry (Sriharan *et al*., 1979). Through recent studies with the help of electron microscopic investigation reveals that tukra symptoms are the manifestation

Corresponding Author: C. Ramesha, Silkworm Breeding and Biotechnology Laboratory, Andhra Pradesh State Sericulture Research and Development Institute (APSSRDI), Kirikera-515 211, Hindupur, Andhra Pradesh, India

of mealybug, *Maconellicoccus hirsutus* attack but the exact molecular level interaction is yet to understand and involvement of virus was ruled out. Recently discussed on early diagnosis method for the tukra incidences in mulberry (Sugnana-Kumari *et al*., 2003) and various studies on bio-control of the mealybug by beetle, *Cryptolaemus montrozieri* of an exotic enemy as a part of pest management program (Manjunath *et al*., 1992, 2003; Kishore *et al*., 1995; Chakraborthy *et al*., 1999; Katiyar *et al*., 2000; Masilamani *et al*., 2003).

The studies related to the effect of feeding tukra-infected mulberry leaves on different silkworm breeds and its nutritional conversion efficiency was reported by Thangamani and Vivekanandan (1983), Kumar *et al*. (1992) and Ahmed *et al*. (1999, 2000). The nutritional efficiency during 4th and 5th instars of silkworm is about 80% of the total food consumption compared to the whole larval period (Ueda, 1965; Magadum *et al*., 1996; Rahmathulla *et al*., 2005). In the present study, an effort has been made to examine the nutritional impact of feeding tukra infected leaves on popular polyvoltine silkworm breed (APM_1) during the 5th instar over the healthy leaves fed batch.

MATERIALS AND METHODS

Tukra Infected Mulberry Leaves

The V1/S13 varieties of mulberry leaves harvested with typical symptoms of curling of apical leaves, secretion of white powdery substances by mealybug in the infected mulberry garden maintained at APSSRDI campus was utilized for the present study. Healthy mulberry leaves are harvested for the control batch after confirmation of non-infection of tukra (Fig. 1). The both mealybug infected and healthy mulberry leaves were utilized for the experiment was brought from well maintained and irrigated mulberry garden.

Silkworm Rearing

The popular polyvoltine breed, APM_1 was utilized for the present study. Silkworm layings of APM_1 was incubated, brushed on the freshly chopped mulberry leaves and reared under standard rearing conditions for two subsequent trials during the season of February-March and April-May- 2008 (Krishnaswami, 1975). The young larvae (1st-3rd instars) were reared at 27-28°C with 85-90% relative humidity and the late age larvae (4th and 5th instars) were reared at 24-26°C with a relative humidity of 70-80%. From 1st to 4th stages, healthy V1/S13 mulberry leaves variety was fed under standard silkworm rearing conditions. After resumption from 4th moult, 300 larvae were counted and kept in a plastic tray and fed with strictly fresh mealybug infected (tukra) V1/S13 varieties of mulberry leaves (Fig. 2) harvested separately and fed to the experimental batch till spinning. Simultaneously, another batch was maintained as a control by strictly fed with healthy V1/S13 varieties of mulberry leaves (Fig. 3). These batches were maintained in three replications and repeated for two subsequent trials. Proper care was taken during feeding, bed cleaning and spinning under hygienic conditions. Fully ripened worms were collected manually and mounted on plastic collapsible mountage for spinning. The experiment was conducted in the Silkworm Breeding and Molecular Genetic Laboratory (SBMG), Andhra Pradesh State Sericulture and Development Institute (APSSRDI), Kirikera-515 211, Hindupur, Andhra Pradesh, India.

Data Collection on Parameters

Larvae and cocoons were assessed and the data was collected on required parameters viz., 5th instar larval duration, larval weight, yield per 10,000 larvae, pupation rate, cocoon weight, shell weight, shell ratio and single cocoon reeling was carried out by utilizing fifteen

Fig. 1: Symptoms of mulberry infection with mealybug (tukra). (A) Mealy bug infected mulberry garden. (B) Curling of tender shoot and (C) White powdery substance secreted by Mealy bug

Fig. 2: Feeding of mealybug (tukra) infected mulberry leaves to silkworm larvae (experimental batch)

Fig. 3: Feeding of healthy mulberry leaves to silkworm larvae (control batch)

good cocoons with the help of epprouvette (Mono cocoon reeling equipment) for reeling parameters viz., average single cocoon filament length, non-breakable filament length and cocoon denier. These parameters were calculated with the assistance of the following formulae.

$$\text{Cocoon yield/10,000 larvae (wt.)} = \frac{\text{Weight of cocoons obtained (kg)}}{\text{Larvae retained after 3rd moult (300)}} \times 10{,}000$$

$$\text{Cocoon yield/10,000 larvae (No.)} = \frac{\text{Total No. of cocoons obtained}}{\text{Larvae retained after 3rd moult (300)}} \times 10{,}000$$

$$\text{Pupation rate (\%)} = \frac{\text{No. of good cocoons + (No. of double cocoons} \times 2)}{\text{Larvae retained after 3rd moult - Uzi infested cocoons}} \times 100$$

$$\text{Single cocoon weight (g)} = \frac{\text{Weight of 25 male (g) + 25 female cocoons (g)}}{50}$$

$$\text{Single cocoon shell weight (g)} = \frac{\text{Weight of 25 male (g) + 25 female cocoons shells (g)}}{50}$$

$$\text{Cocoon shell ratio (SR\%)} = \frac{\text{Cocoon shell weight (g)}}{\text{Cocoon weight (g)}} \times 100$$

$$\text{Average single cocoon filament length (m)} = \frac{\text{Total No. of revolutions} \times \text{1.125 (m) (circumference of wheel)}}{\text{Total No. of cocoons reeled}}$$

$$\text{Average non-brekable filament length (m)} = \frac{\text{Total filament length (m)}}{\text{Total No. of breaks}}$$

$$\text{Cocoon denier (Filament size) (d)} = \frac{\text{Total filament weight (g)}}{\text{Total length of silk reeled (m)}} \times 9000$$

RESULTS

Silkworm Rearing Performance

Fifth instar larval duration of experimental batch was found to be shorter by 12:00 h (Table 1). Fully grown silkworm (5th instar of 5th day after 2nd feed) average larval weight was observed to be higher in experimental batch (33.8 g) than control (31.3 g) larvae. The cocoon yield per 10,000 larvae was higher by number (9194) as well as by weight (12.54 kg) over control batch (9188) and (12.18 kg), respectively. Average pupation rate was found to exhibit slightly more in experimental batch (91.77%) than the control batch (90.81%).

Economical Characteristics of Cocoon

The important economic characteristic of higher average single cocoon weight was 1.364 g and average single cocoon shell weight of 0.233 g with an 17.09% of silk ratio in experimental batch recorded. Slightly lower average on single cocoon weight was recorded 1.326 g and average single cocoon shell weight of 0.214 g with an average of 16.15% silk ratio in control batch was revealed (Table 1).

Table 1: Silkworm rearing performances of mealybug infected leaves fed batch over control batch

Season	5th instar larval duration (h)	10 larval weight (g)	Yield/10,000 larvae No.	Yield/10,000 larvae Wt. (kg)	Pupation rate (%)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
Mealybug infected mulberry leave fed batch (experimental batch)								
Feb.-March	144	34.8	9278	13.05	93.20	1.407	0.237	16.84
Apri-May	132	32.7	9110	12.03	90.33	1.321	0.229	17.34
Mean	138	33.8	9194	12.54	91.77	1.364	0.233	17.09
SD	8	1.5	119	0.72	2.03	0.061	0.006	0.35
Healthy mulberry leave fed batch (control batch)								
Feb.-March	156	32.2	9270	12.52	92.30	1.351	0.209	15.47
Apri-May	144	30.3	9105	11.84	89.31	1.301	0.219	16.83
Mean	150	31.3	9188	12.18	90.81	1.326	0.214	16.15
SD	8	1.3	117	0.48	2.11	0.035	0.007	0.96

Table 2: Cocoon reeling parameters of mealybug infected leaves fed batch over control batch

Season	Average filament length (m)	Non broken filament length (m)	Cocoon denier (d)
Mealybug infected mulberry leave fed batch (experimental batch)			
Feb.-March	753	579	2.3
Apri-May	698	536	2.1
Mean	726	558	2.2
SD	39	30	0.1
Healthy mulberry leave fed batch (control batch)			
Feb.-March	614	541	1.9
April-May	587	518	1.9
Mean	601	530	1.9
SD	19	16	0.0

Mono Cocoon Reeling

In reeling parameters the average single cocoon filament length in experimental batch (726 m) was higher than that of control batch (601 m). In relation to the non-breakable filament length was also higher in experimental batch (558 m) and lesser in control batch (530 m). Further, the significant difference between experimental batch (2.2 d) over that of control batch (1.9 d) was observed with regard to single cocoon denier i.e., size/thickness of silk filament (Table 2).

DISCUSSION

The tukra infected mulberry leaves with symptoms shown in the Fig. 1, mealybug infected mulberry garden (Fig. 1A), curling of apical leaves (Fig. 1B), secretion of white powdery substances (Kumar *et al*., 1997) (Fig. 1C), shortening and flattening of internodes, brittleness of leaves, stunted growth, etc. The productive polyvoltine breed, APM_1 developed by Andhra Pradesh State Sericulture Research and Development Institute (APSSRDI), Hindupur, A.P., India, utilized as female component in the preparation of commercial cross breed Swarnandhra (Chandrashekharaiah and Babu, 2003). This was chosen for the study to generate necessary information on impact of tukra infected leaves on new polyvoltine breed commercialized in Southern part of India. As tukra incidence is the common problem found in mulberry gardens of India during pre-monsoon and summer which is very prevalent in the southern regions. Certain studies were carried out on tukra disease, its seasonal incidence and its transmission between mulberry plants (Sriharan *et al*., 1979; Muralikumaran and Baskaran, 1992a, b; Baskaran *et al*., 1992). The studies revealed that the mealybug infection causes decline in the quantity of mulberry leaves and leads to the serious concern to the sericulture industry particularly to Southern states of India (Baskaran *et al*., 1992; Manjunath *et al*., 1993). Very scanty information is available on the effect of feeding mealybug infected mulberry leaves to the silkworm and its nutritional efficiency (Kumar *et al*., 1992; Ahmed *et al*., 1999, 2000). Cellular level understanding through electron microscopic studies revealed that occurrence of tukra disease is not due to viral transfection but due to the attack of insect (Babu *et al*., 2003) and a novel method for early diagnosis of tukra infection in mulberry has also been discussed (Sugnana-Kumari *et al*., 2003). Changes of nutritional components viz., proteins, sugar, phenols and total chlorophyll etc., in mealybug infected mulberry leaves has also been reported by Umesh Kumar *et al*. (1990) and Chatterjee and Sarkar (1993). Some researchers objected the need of effective utilization of mealybug infected mulberry leaves for commercial silkworm rearing (Thangamani and Vivekanandan, 1983). As the control of mealybug, application of chemical pesticides are not advised since they harm the silkworms and the biological control agents like *Coccinellid*, *Anagyrus kamali* (Moursi) (Hymenoptera: Encyritidae), *Cryptomonotrouzieri muls*, Dechlovos etc. (Chakraborthy *et al*., 1999; Katiyar *et al*., 2000; Manjunath *et al*., 1992, 1993, 2003; Masilamani *et al*., 2003; Kishore *et al*., 1995) are recommended but yet to be practiced commercially.

The present study was undertaken to study the impact of tukra (mealybug) infected mulberry leaves on 5th instar silkworm larvae and its economically important characters with cocoon reeling parameters. Since, the maximum leaves consumption and conversion efficiency was more in the silkworm during the 5th instar (Rahmathulla *et al*., 2005). February-March and April-May seasons were chosen for the study since this particular dreadful disease incidence on mulberry is prevalent during these months causing severe crop loss (Muralikumaran and Baskaran, 1992b; Baskaran *et al*., 1992). The 5th stage larval duration found to be less by 12 h. in the experimental batch may be due to rich nutrition aspects in tukra infected mulberry leaves led to the early maturation of eating silkworm larvae than that of control batch. Reduction in larval duration is an advantage to sericulture farmer since it reduces the labour cost of the farmer and without reduction of economically important quantitative traits of silkworm in experimental batch. This may be due to high moisture content found in the mealybug infected mulberry leaves than that of normal mulberry leaves (Ahamed *et al*., 2000; Umesh-Kumar *et al*., 1990; Thangamani and Vivekanandan, 1983). Fully grown larval body weight was also found higher in experimental batch suggesting that the nutrient values are not affected in tukra infected mulberry leaves and in conformation with the observations of Ahamed *et al*. (2000). It is also evident that of tukra infected mulberry leaves has a positive influence on the food and water utilization pattern with respect of most industrial important economical characters such as cocoon and shell weight in the silkworm (Ahamed *et al*., 1999, 2000; Kumar *et al*., 1992). As the silkworm is monophagous in nature and derives all nutrients essential for its biological activities from the mulberry leaves only. It is also observed that feeding of tukra infected mulberry leaves to the silkworm shown positive impact on the important economical characters viz., larval duration, cocoon and shell weight with filament length, non-brekable filament length and cocoon denier over the control batch. The most important traits of silkworm in particular to seed farms/farmers is the pupation rate, observed with an average of 91.77% in experimental batch over that of control batch 90.81%, a slight more positive result was noticed. It also revealed that the longer cocoon filament length (726 m) and thicker cocoon filament denier (2.2 d) in mealybug infected leaves fed batch than that of control bath is clearly indicated the presence of more protein in mealy bug infected leaves and its proper assimilation in silkworm. The exact reasons for unaffected these quantitative traits are yet to be studied.

The important problems which are encountered while effective utilization of mealybug infected mulberry leaves for silkworm rearing is attack of black small ants to the infected and storing infected leaves. Moreover, it was impressively observed that of tukra infected mulberry leaves could be utilized more effectively for any kind of silkworm rearing as suggested by Thangamani and Vivekandan (1983) and even for new popular breed of APM_1

in acute shortage of healthy mulberry leaves or by mixing with healthy mulberry leaves as potential utilization of entire mulberry leaves produced from the garden.

CONCLUSION

If mulberry garden is infected with tukra disease, the sericulture farmer or the rearer need not be discouraged. In the present study revealed that the tukra infected mulberry leaves may be effectively utilized even for newly popularized pure polyvoltine breed (APM_1) for silkworm rearing since it is not affecting the quantitative traits of the silkworm and obtained an encouraging results in experimental over the control batch. It may be due to the presence or over expression of more nutrients by the stimulation of the pathogen in tukra infected leaf than that of healthy mulberry leaf and emphasis for further research may be given on actual biochemical/biotechnological process.

ACKNOWLEDGMENT

The authors wish to thank the former director and colleagues of the Moriculture Division as well as other staff of APSSRDI, Kirikera for their encouraging support in the present investigation and preparation of the manuscript.

REFERENCES

Ahmed, C.A.A., M.V. Chandrakala and V.G. Maribashetty, 1999. Effect of feeding mealy bug affected mulberry leaves (tukra) on nutritional efficiency and cocoon yield in the new bivoltine silkworm, *Bombyx mori* L. Entomon, 24: 265-274.

Ahmed, C.A.A., M.V. Chandrakala, V.G. Maribashetty, C.S. Gururaj and R. Raghuraman, 2000. Influence of feeding mealy bug infected mulberry leaves on food and water utilization and cocoon yield in a bivoltine race of silkworm, *Bombyx mori*. J. Exp. Zool., 3: 49-54.

Babu, A.M., V. Kumar, K. Sathya-Prasad and B.K. Kariappa, 2003. Anatomical alternations in the leaf and stem of mulberry associated with tukra symptoms. Proceedings of the National Conference on Tropical Sericulture for Global Competitiveness, 2003, CSRTI, Mysore, India, pp: 54-54.

Baskaran, P., K. Ramanujam, C. Muralikumaran and N.V. Radhkrishan, 1992. Incidence and severity of mealy bug-associated mulberry leaf curl (Tukra) in Tamilnadu. Proceedings of the National Conference on Mulberry sericulture Research, 1992, CSRTI, Mysore, India, pp : 49-49.

Bindroo, B.B. and A. Dhar, 1996. Correlation of tukra disease in mulberry (*Morus* spp.). Geobios, 23: 215-218.

Chakraborthy, N., M.V. Santha-Kumar and S.K. Sen, 1999. Studies on the feeding potential of a Coccinellid predator, *Nephus* sp. On pink mealy bug (*Maconellicoccus hirsutus* (green)). Sericologia, 39: 193-203.

Chandrashekharaiah and M.R. Babu, 2003. Silkworm breeding in India during the last five decades and what next? Proceedings of the Mulberry Silkworm Breeders Summit, 2003, APSSRDI, Hindupur, India, pp: 6-13.

Chatarjee, K.K. and A. Sarkar, 1993. Mealy bug infestation in mulberry. Indian Silk, 32: 19-20.

Katiyar, R.L., V. Kumar, D. Manjunath, A.K. Sen, M.A. Shekar and R.K. Datta, 2000. Biology of anagyrus kamali (Moursi) (Hymenoptera: Encyritidae) - A parasitoid of the Mealybug, *Maconellicoccus hirsutus* (green) with a note on its incidence. Int. J. Ind. Entomol., 1: 143-148.

Kishore, R., V. Kumar, P. Kumar and R.K. Datta, 1995. Dechlovos for effective conservation of bio-control agent of mealy bug. Indian Silk, 33: 9-10.

Krishnaswami, S., 1975. New technology of Silkworm rearing. CSRTI Mysore Bull., 2: 23-23.

Kumar, P., R. Kishore, M.K.R. Noamani and K. Sengupta, 1992. Effect of feeding tukra affected mulberry leaves on silkworm rearing performance. Indian J. Seric., 31: 27-29.

Kumar, V., S.K. Tewari and R.K. Datta, 1997. Dermal pores and wax secretion in mealybug *Maconellicoccus hirsutus* (Hemiptera, Pseudococcidae). A pest of mulberry. Italian J. Zool., 64: 307-311.

Magadum, S.B., O.K. Ramadevi, N. Shivashankar and R.K. Datta, 1996. Nutritional indices in some bivoltine breed of silkworm, *Bombyx mori* L. Indian J. Seric., 35: 95-98.

Manjunath, D., R. Kishore, K.S. Prasad, V. Kumar, P. Kumar and R.K. Datta, 1992. Biology of mulberry mealy bug and predatory potential of its biocontrol agent. Proceedings of the National Conference on Mulberry sericulture Research, 1992, CSRTI, Mysore, India, pp: 50-50.

Manjunath, D., P. Kumar, R. Kishor, K.S. Prasad, K.C. Narayanaswamy and R.K. Dutta, 1993. Towards understanding tukra and its management. Indian Silk, 32: 6-9.

Manjunath, D., K.S. Prasad, and D.K. Sidde-Gowda, 2003. Ecological approach for the management of mealy bug, *Maconellicoccus hirsutus* causing tukra in mulberry. Proceedings of the National Conference on Tropical Sericulture for Global Competitiveness, 2003, CSRTI, Mysore, India, pp: 41-41.

Masilamani, S., S.M.H. Qadri, C.A. Mary -lora and N. Dhahira-Beevi, 2003. A critical appraisal of the lady bird predator, *Cryptolaemus montrouzieri* Muls. against tukra mealy bug on mulberry. Proceedings of the National Symposium on Biological Control of Insect Pests, 2003, Loyola College, Chennai, India, pp: 7-8.

Muralikumaran, C. and P. Baskaran, 1992a. Disease transmission studies pertaining to mealy bug, *Maconellicoccus hirsutus* (Green) associated tukra. Proceedings of the National Conference on Mulberry Sericulture Research, 1992, CSRTI, Mysore, India, pp: 51-51.

Muralikumaran, C. and P. Baskaran, 1992b. Seasonal incidence of mealy bug associated tukra disease in mulberry, *Morus alba* L. in Chidambaram taluk, Tamilnadu. Proceedings of the National Conference on Mulberry sericulture Research, 1992, CSRTI, Mysore, India, pp: 52-52.

Rahmathulla, V.K., H.Z. Haque Rufaie, M.T. Himanthraj, G.S. Vindhya, R.K. Rajan, 2005. Food ingestion, assimilation and conversion efficiency of mulberry silkworm, *Bombyx mori* L. Int. J. Ind. Entmon., 11: 1-12.

Ramesh-Babu, K., S. Ramakrishna, Y. Harish-Kumar-Reddy, G. Lakshmi, N.V. Naidu, S. Sadak-Basha, M. Bhaskar, 2009. Metabolic alterations and molecular mechanism in silkworm larvae during viral infection: A review. Afr. J. Biotechnol., 8: 899-907.

Sriharan, T.P., M.V. Samson and S. Krishnaswami, 1979. Studies on the tukra disease (*Maconellicoccus hirsutus*) of mulberry. Indian J. Seric., 18: 78-80.

Sugnana-Kumari, S., S. Misra, C. Rajagopal-Reddy, Chandrashekariah and U. Suryanarayana-Murthy, 2003. A novel method for early diagnosis of tukra incidence in mulberry. Proceedings of the National Symposium on Bioresources, Biotechnology and Bioenterprise, 2003, Osmania Universty, Hyderabad, India, pp: 71-71.

Thangamani, R. and M. Vivekanandan, 1983. Effective utilization of tukra diseased mulberry leaves as silkworm, *Bombyx mori* L. feed. Sericologia, 23: 213-223.

Ueda, S., 1965. Changes in some quantitative factors and their mutual relationships concerning the growth and development in the fifth larval instar of the silkworm, *Bombyx mori* L. Bull. Seric. Exp. Stn. Jap., 19: 331-341.

Umesh-Kumar, N.N., M.P. Sree-Muthegowda, G. Bhoraiah, 1990. Changes in proteins, sugars, phenols and total chlorophyll content of mulberry plants affected by tukra. Indian J. Seric., 29: 93-100.