Pak. j. sci. ind. res. Ser. B: biol. sci. 2011 **54** (2) 71-75

Effect of Methods of Nutritional Administration on the Development of Silkworm *Bombyx mori* L.

Rashid Mahmood[a], Saima Asad[b], Shazia Raja[a]*, Ghulam Sarwar and Farida Iftikhar[a]

[a]Honeybee Research Institute, National Agricultural Research Centre, Islamabad, Pakistan

[b]PMAS-Arid Agriculture University, Murree Road, Rawalpindi, Pakistan

(received July 2, 2010; revised December 21, 2010; accepted February 7, 2011)

Abstract. The effect of nitrogen in different concentrations (0.1, 0.2, 0.3 and 0.4 %) through soil, foliar application and dipping of mulberry leaves on food consumption, larval development and cocoon weight of silkworm (*Bombyx mori* L.) was studied. Larvae when fed on wet mulberry (*Morus alba laevigata*) leaves dipped in 0.2% N concentration proved the best, as it consumed maximum food (5726.26 mg), gained significantly more weight (3182.30 mg) and produced heavier cocoons (424.65 mg) as compared to all other treatments. Food efficiency ratio (FER: 4.43) and coefficient of utilization (CU: 67.61) were also maximum for the leaves dipped in 0.2% N.

Keywords: *Morus alba laevigata,* larvae, farmyard manure, nitrogen, *Bombyx mori* L, cocoon weight

Introduction

A number of insects secrete silk which is usually used by them for anchorage, entangling their prey or forming a protective sheath. The annual world production of raw silk is about 60, 000 tons including China producing half of the world supply followed by India, Korea and Japan (Ashfaq and Aslam, 2006). Nearly 95% of commercial silk is obtained from the mulberry silkworm, *Bombyx mori* L. (Ganga and Chetty, 1991). It is a beneficial monophagous insect and mulberry is the main natural food for this insect. Nutrition is an important growth regulatory factor in silkworms because the quality of leaves of mulberry plants greatly influences the biology of these worms (Arai and Ito, 1963). Leaf quality is dependent on the quantity, timing, method, and nutrient balance of fertilizer applications (Shankar and Rangaswamy, 1999).

It is for the past few years that silkworm rearing industry has received recognition through greater research on the nutritional aspects of silkworm. The scientists have experimented with supplementation of various nutrients on the mulberry leaves for feeding the silkworm and promoting both silk quality and quantity. Nutritional supplementation and searching alternate food plants have been on way with varying degree of success.

Silkworm nutritionists have always been searching for better food by supplementing the mulberry leaves with different nutrients. Various minerals *viz.,* 0.2% N (Mahmood *et al.,* 2002a), 0.2% N + 0.1% Ca + 0.1% K (Ishtiaq and Akhtar, 1992) have been reported for having positive influence on silkworm survival and yield. Ashfaq *et al.* (1998) found that silkworm larvae when fed on mulberry leaves treated with 0.2 N + 0.05% Cu concentrations consumed more food, gained more larval weight and produced heavier cocoons as compared to the untreated or other treated leaves.

Ashfaq and Sial (2001) also studied the effect of minerals applied through stem injection on the larval development and silk yield of silkworm, *B. mori.* They concluded that there was a significant increase not only in the food consumption, but also in the coefficient of utilization, body weight, body length and ultimately cocoon yield. Three methods of mineral application i.e. stem injection, foliar spray and soil application were tested (Aslam and Ashfaq, 2004) and foliar spray proved the best, as it gave maximum food consumption, coefficient of utilization, larval body weight, gravid cocoon weight and empty cocoon weight as compared to the stem and the soil application.

Rahmathullah *et al.* (2007) concluded that folic acid solution sprayed on mulberry leaves significantly improved larval weight, silk gland weight and growth rate of silkworm. Higher larval and silk gland weight subsequently improved the economic parameters like cocoon weight, shell weight and shell ratio of folic acid treated batches.

*Author for correspondence; E-mail: shazia_raja2002@yahoo.com

Keeping in view, all the above mentioned facts the present study was conducted to determine the effect of nitrogen in different concentrations through soil, foliar application and dipping of mulberry leaves on food consumption, larval development and cocoon weight of silkworm.

Materials and Methods

These investigations were carried out in the Sericulture Laboratories of Entomology, University of Agriculture, Faisalabad, Pakistan. Eggs of Korean strain, F_1 Hybrid (JAM 108 × JAM 107) of *Bombyx mori* L. were procured from Punjab Forest Department, Lahore, Pakistan. Eggs were placed for hatching during the last week of February in the growth chamber at 28 ± 2 °C and 70-80% relative humidity. Soon after hatching, silkworm larvae were divided into twenty two groups including control (without any treatment). Fifteen plants of Japanese mulberry (*Morus alba laevigata*) of 6-8 years old, were selected from the Entomological Experimental Area, University of Agriculture, Faisalabad and treated with different nitrogen concentrations derived from urea and FYM as below:

Soil/root application

I) Urea in different nitrogen concentrations, 0.1%, 0.2%, 0.3% and 0.4% was applied at 10 days interval through irrigation water to four experimental mulberry plants (T_1, T_2, T_3, and T_4).

II) Well rotten Farm Yard Manure (FYM) was applied at 10 days interval during February and March, at the rate of 2 kg / tree through irrigation water to one sprouted experimental mulberry plant (T_5).

III) Two kg well rotten (FYM) along with urea 46% in different nitrogen concentrations, 0.1, 0.2, 0.3, 0.4% were applied at ten days interval during February and March to soil through irrigation water to four sprouted experimental mulberry trees (T_6, T_7, T_8 and T_9).

Foliar spray. The same concentrations of nitrogen were prepared in tap water and sprayed separately on four mulberry plants after every 10 days with the help of knapsack sprayer (T_{11}, T_{12}, T_{13} and T_{14}).

Dipping method. (a) The leaves plucked from untreated mulberry plants were dipped in the said concentrations of nitrogen, 0.1% (T_{15}), 0.2% (T_{16}), 0.3% (T_{17}) and 0.4% (T_{18}). (b) The leaves plucked from untreated mulberry plants were subjected to nitrogen in the same way as above i.e. 0.1% (T_{19}), 0.2% (T_{20}), 0.3% (T_{21}) and 0.4% (T_{22}) but were dried under shade. Untreated mulberry leaves, (T_{10}) were used as control.

An effective blocking not only yield more precise results than an experimental design of comparable size without blocking but also increases the range of validity of experimental results; therefore, the newly hatched (1980) silkworm larvae were divided into 22 groups) following complete randomized design (CRD) with three replications having 30 larvae each. All the groups of silkworm larvae were fed on the fresh green leaves obtained from the aforementioned treatments. The silkworm larvae of each group were separately reared in cardboard trays size 10 × 10 cm up to the 3rd instar while the 4th and the 5th instar larvae were reared in 10 × 20 cm cardboard trays (Mahmood *et al.,* 2002a; Ashfaq *et al.,* 1998,). The first two instars of silkworm were fed on chopped leaves of mulberry, while the rest of the three instars were fed on whole mulberry leaves. All the 22 groups of silkworm larvae were fed equal quantities of mulberry leaves twice a day, first at 8.00 A.M. and then at 8.00 P.M. and it was continued (for 3 h) till the larvae were fully fed. Special attention was paid to the cleaning of trays to avoid any infection in the worms. The larval weight was recorded on the last day of each instar using electronic balance. The residual leaves and feaces were collected separately and dried in the oven at 100 °C for 24 h. The food consumption was measured by subtracting the dry weight of residual leaves directly from the determined total dry weight of leaves offered to silkworm. Cocoon weight with and without pupae under different treatments was also recorded. The data were analyzed statistically by one way ANOVA and Duncan's multiple range (DMR) test was applied to test the significance of the results (Steel *et al.,* 1997).

The co-efficient of utilization (CU) was calculated using the following formula after EVANS (1939).

$$CU = \frac{\text{Dry wt. of food - Dry wt. of faeces}}{\text{Dry wt. of food consumed}}$$

The overall efficiency of conversion of food (CF) into body matter was calculated by using the following formula:

$$CF = \frac{\text{Body wt.}}{\text{Dry wt. of food consumed}}$$

Weight of dry cocoons with and without pupae was recorded to find out the silk yield in different treatments.

Results and Discussion

The silkworm larvae consumed significantly more quantities of wet mulberry leaves treated with different nitrogen concentrations. Leaves in wet condition with 0.2% N (T_{16}) presented the maximum consumption while control (T_{10}) showed minimum food consumption (Table 1). The next best treatment was mulberry leaves dipped in different nitrogen concentrations, dried and fed to silkworms. The mulberry leaves treated with the aforesaid four nitrogen concentrations, affected the food consumption and this supports the contention that food with high moisture contents enhances food consumption (Table 1). Our results tally with those of El-Shaarway *et al.* (1975) who concluded that the larvae preferred red mulberry variety having significantly higher values of N as compared with the green one.

Liaw and Shikata (1980) also supported the present findings as they found younger leaves rich in nitrogen to be more attractive than the old ones containing comparatively low nitrogen contents. Thus it is concluded that silkworm larvae of different instars consumed significantly more food in wet condition with 0.2% and 0.3% N concentrations. The next best N concentrations were 0.4% or 0.1%, however, mulberry leaves treated with the aforesaid four N concentrations through dipping method but fed in dry conditions reduced the consumption and the same was true in the rest of the treatments. Thus food with high moisture content enhances food consumption in all the instars.

Further, the consumption of nitrogen treated leaves in all the treatments was significantly higher as compared to the untreated leaves. This indicates the preference of the larvae for the N treated leaves. These results are in agreement with the findings of Mahmood *et al.* (2002a, 2002b, 2001) and Akram *et al.* (2002). However, the present results are at variance of those of Mahmood *et*

Table 1. Effect of nitrogen application methods on the development of silkworm

Treatments	Methods of application	Average food consumption (mg)	Average larval weight (mg)	Average cocoon weight (mg)	Average food efficiency ratio (FER)	Average coefficient of utilization (CU %)
T_1	0.1%N Soil apply	4331.70^{bc}	1614.43^{efgh}	200.22^{c}	3.49	61.42
T_2	0.2%N Soil apply	4367.52^{bc}	1670.09^{de}	210.26^{c}	3.58	61.35
T_3	0.3%N Soil apply	4326.12^{bc}	1570.64^{ghi}	196.90^{c}	3.46	61.04
T_4	0.4%N Soil apply	4306.77^{c}	1555.08^{hij}	185.37^{c}	3.48	59.98
T_5	2kg F Y M	4294.89^{c}	1479.23^{k}	198.99^{c}	3.44	60.71
T_6	2kg FYM +0.1% N	4372.56^{bc}	1570.48^{ghi}	211.73^{c}	3.52	61.79
T_7	2kg FYM +0.2% N	4404.33^{bc}	1629.52^{efg}	222.41^{c}	3.54	62.65
T_8	2kg FYM +0.3%N	4347.99^{bc}	1549.83^{hij}	207.75^{c}	3.50	60.85
T_9	2kg FYM+ 0.4% N	4341.51^{bc}	1524.54^{ijk}	204.88^{c}	3.49	60.67
T_{10}	Check (untreated)	2304.09^{d}	1033.92^{n}	137.75^{d}	3.16	54.36
T_{11}	0.1%N Foliar spray	4397.85^{bc}	1614.43^{efgh}	228.03^{c}	3.55	62.41
T_{12}	0.2%N Foliar spray	4443.93^{bc}	1670.09^{de}	250.77^{c}	3.57	62.25
T_{13}	0.3%N Foliar spray	4384.91^{bc}	1570.64^{ghi}	218.59^{c}	3.45	62.50
T_{14}	0.4%N Foliar spray	4358.25^{bc}	1555.08^{hij}	206.67^{c}	3.44	61.62
T_{15}	0.1%N wet leaves	5626.26^{a}	3114.68^{b}	404.72^{a}	4.39	65.34
T_{16}	0.2%N wet leaves	5726.26^{a}	3182.30^{a}	424.65^{a}	4.43	67.61
T_{17}	0.3%N wet leaves	5623.65^{a}	3049.64^{c}	395.71^{a}	4.28	64.88
T_{18}	0.4%N wet leaves	5622.93^{a}	3004.53^{c}	383.73^{a}	4.22	64.31
T_{19}	0.1%N dry leaves	4445.46^{bc}	1637.10^{ef}	272.65^{b}	3.57	62.05
T_{20}	0.2%N dry leaves	4505.30^{b}	1702.73^{d}	275.57^{b}	3.64	61.58
T_{21}	0.3%N dry leaves	4442.58^{bc}	1604.56^{fgh}	271.74^{b}	3.42	62.70
T_{22}	0.4%N dry leaves	4395.42^{bc}	1575.89^{fghi}	271.13^{b}	3.45	62.24

The values sharing similar letters are non-significant at P<0.01.

al. (1987) which might be due to the varietal difference, feeding time and silkworm strain. On overall basis, 0.2% N concentrations was found to be the optimum concentration affecting the food consumption. In general, silkworm larvae of all the five instars when fed on wet mulberry leaves treated with 0.1% 0.2%, 0.3%and 0.4%, N gained significantly more weight as compared with all the other treatments, but among these concentrations, 0.2% N proved to be the best one (Table 1). The next best treatment was mulberry leaves, also in wet condition but with 0.1% N. However, mulberry leaves treated with the aforesaid four N concentrations either through dip or spray method, but were fed in dry condition showed reduction in larval weight and the same was true for the rest of the treatments. This indicates that the silkworm larvae prefer food with high moisture and N contents which consequently increases larval weight. The present findings are in line with those of Thapa and Ghimire (2005), Kawada and Horie, (2002) and Akram *et al.* (2002) who observed that feeding silkworm larvae on the top or tender leaves (rich in N) gave better performance than feeding on coarse leaves (low N) as the latter gave poor larval development. Our findings are strongly supported by Hanif and Islam (1987) who found that feeding of both young mulberry leaves and leaves treated with 0.2% N concentration significantly increased the larval weight, as compared to those fed on old, or untreated leaves or leaves treated with 0.1% and 0.4% N concentrations.

Overall 0.2% N concentration was found to be the optimum concentration affecting the larval weight studies. It was observed that food with higher N i.e. 0.3% and 0.4% affected larval weight negatively as also observed by Mahmood *et al.* (2002a, 2002b) The silkworm larvae of each instar when fed on wet leaves treated with 0.1% and 0.2% concentrations produced significantly heavier cocoons as compared to all the other concentrations and methods of application (Table 1). This also indicates that N combination with high moisture content of leaves is important in the production of heavier cocoons and consequently the higher cocoon weight.

The present findings are in line with those of Krishanswami *et al.* (1971) who reported that application of N at the rate of 900 kg/ha of mulberry plantation significantly improved cocoon weight. The results were also confirmed by Mitsuo and Takeshi (2002), who found that feeding silkworm with tender leaves (high N), resulted in the increase in cocoon weight than feeding on coarse leaves (less N).

The CF and CU are presented in Table 1 which shows that leaves dipped in 0.2% N produced the highest ratios i.e. 4.43, 67.61 followed by 4.39 and 61.62 with 0.3% N. These findings are almost in agreement with those of Akram *et al.* (2002) who recorded coefficient of utilization varying between 55.30- 60.33% however, at variance with the findings of Ali and Younas (1970) who reported 24% and 75% CU in their studies.

Body weight and body length are two parameters of silkworm life which are ultimately dependent upon better food quality, rate of consumption and co-efficient of utilization. During the present study, it was observed that in case of maximum food consumption and co-efficient of utilization, the performance of larvae in terms of body weight was also maximum which was only due to the better food. The net outcome of the present studies is reflected in the form of better cocoon which again confirmed the outcome of previous workers. Keeping in view all the results presented in the paper it can be safely said that the individuals fed with 0.2% N by dipping method resulted not only in better food consumption, coefficient of utilization, and larval development but also produced heavier cocoons.

Acknowledgement

We acknowledge all the staff members of Department of Entomology, University of Agriculture for collection and feeding of silkworm's larvae.

References

Akram, W., Zafar, U., Lee, J. 2002. Silkworm growth and silk yield on selected supplemented/un-supplemented mulberry varieties. *Entomological Research,* **32:** 201-204.

Ali, R., Younas, M. 1970. Rearing of silkworm on alternate host plant. *Pakistan Journal of Agricultural Science,* **7:** 155-160.

Arai, N., Ito, T. 1963. Food value of mulberry leaves for the silkworm *Bombyx mori* L. determined by means of artificial diets. II. Comparison between soft and hard leaves. *Bulletin of Sericulture Export Statistics* Tokyo, **18:** 1-16.

Ashfaq, M., Aslam, M. 2006 Renewal of sericulture industry. *Middle East Journal of Scientific Research,* **1:** 58-59.

Ashfaq, M., Sial, A.A. 2001. Larval development and silk yield of *Bombyx mori* L. fed on leaves from

mulberry plants treated with mineral nutrients through stem injection. *Pakistan Entomologist,* **23:** 103-106.

Ashfaq, M., Ahmad, S., Arif, J., Ahmad, A. 1998. Effect of feeding minerals supplement treated mulberry leaves on the larval development of silkworm, *Bombyx mori* L. and silk yield. *Pakistan Entomologist,* **20:** 89-91.

Aslam, M., Ashfaq, M. 2004. Influence of methods of nutritional administration on the developmental and productive aspects of mulberry silkworm *Bombyx mori* L. *Pakistan Entomologist,* **26:** 43-45.

El-Shaarawy, M.F. Gomaa, A.A., El-Garhy, A.T. 1975. Chemical determination and utilization of dietary constituents of two castor-bean varieties by larvae of the Eri silkworm. *Attacus ricini* Biosd. *Zeitschrife für Angewandte Entomologie,* **78:** 171-176.

Ganga, G., Chetty, J.S. 1991. *An Introduction to Sericulture,* pp. 91-96, Oxford and IBM Publishing Company Private Limited, New Delhi, India.

Hanif, M. A., Islam, B.N. 1987. Nutrient contents of mulberry leaves and their effect on silk production by *Bombyx mori* L. *Bangladesh Journal of Zoology,* **15:** 131-136.

Ishtiaq, A., Akhtar, M. 1992. Effect of feeding nitrogen, calcium and potassium treated mulberry leaves on the larval development and silk yield of silkworm, *Bombyx mori* L. *Pakistan Entomologist,* **14:** 74-76.

Krishnaswami, S., Kumararaj, S., Vijayaraghavan, K., Kasiviswanathan, K. 1971. Silkworm feeding trials for evaluating the quality of mulberry leaves as influenced by variety, spacing and nitrogen fertilization. *Indian Journal of Sericulture,* **10:** 79-89.

Kuwada, M., Horie, T. 2002. Effects on silkworm larvae attractiveness of mulberry leaves grown at different fertilizer application rates. *Journal of Sericultural Science of Japan*, **71:** 75-81.

Liaw, G.J., Shikata, M. 1980. Mulberry's attractiveness to newly hatched silkworm, *Bombyx mori* L. *NTU Phytopathologist and Entomologist,* **7:** 37-43.

Mahmood, R., Tahir, J., Khan, M.I. 2002a. Impact of feeding mulberry leaves with foliar spray of Nitrogen on the larval development and silk yield of silkworm. *Indus Journal of Plant Sciences,* **4:** 349-353.

Mahmood, R., Tahir, J., Khan, M.I. 2002b. Effects of nitrogen (farm yard manure + urea) treated mulberry trees on the larval development and cocoon weight of silkworm, *Bombyx mori* L. *Asian Journal of Plant Sciences,* **2:** 93-94.

Mahmood, R., Tahir. J., Khan, M.I. 2001. Effects of food from mulberry treated with urea "N" on the larval development and cocoon weight of silkworm, *Bombyx mori* L. *Journal of Research (Science),* **12:** 123-129.

Mahmood, K., Ahmad, M., Gilani, A.H. 1987. Effect of feeding leaves of *Morus alba* and *Morus leavigata* on larval growth and silk yield of silkworm *Bombyx mori* L. *Pakistan Journal of Zoology,* **19:** 239-243.

Rahmathulla, V.K., Das, P., Ramesh, M., Rajan, R.K. 2007. Growth rate pattern and economic traits of silkworm, *Bombyx mori* L. under the influence of folic acid administration. *Journal of Applied Sciences and Environmental Management,* **11:** 81-84.

Shankar, M.A., Rangaswamy, B.T. 1999. Effect of applied nitrogen and potassium on mulberry leaf yield and quality in relation to silkworm cocoon characters. *Better Crops International,* **13:** 20-21.

Steel, R.G.D., Torie, J.H., Dickey, D.A. 1997. *Principles and Procedures of Statistics: A Biometrical Approach*. 3rd edition, Mc Graw-Hill Book Co. Inc., New York, USA.

Thapa, R.B., Ghimire, N.P. 2005. Performance of mulberry silkworm (*Bombyx mori* L.) under leaf and shoot feeding methods. *Journal of the Institute of Agricultural and Animal Sciences,* **26:** 83-86.