

The Effect of Fertilized Mulberry Leaves with Balanced NPK on the Biological, Quantitative and Technological Parameters of Silkworm, *Bombyx mori* L.

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ABSTRACT

The present investigation was carried out at the Department of Plant Protection, Faculty of Agriculture AL-Azhar University. The effect of balanced fertilization of mulberry plantations with NPK on the development and productivity of silkworm was studied. Treated mulberry (*Mours indica*) leaves were offered to two races of silkworm *Bombyx mori* larvae four times per day. Obtained results showed that feeding of larvae on leaves of fertilized trees increased fresh cocoon weight, cocoon shell weight; cocoon shell ratio, cocooning percentage, silk productivity, hatchability, filament length, and filament weight and size,. However such feeding lead to decrease each of total larval duration and larval mortality percentages.

Key words: *Mours indica*, fertilization of mulberry, NPK, Feeding, silkworm *Bombyx mori* L. cocoon indices, silk productivity, Hatchability.

Introduction

Silkworm, *B.mori* L., is a monophagous insect that drives almost all required nutrients for its growth and development from mulberry leaf. Nutrition plays a pivotal role in sericulture. It improves the growth, development, health, feed consumption and conversion of silkworm there by improving the commercial traits. Application of the required nutrient in the required amount of mulberry trees, very essential for the successful silkworm growth and cocoon production. Mulberry leaves which considered as the sole food for silkworm play vital role in the growth and the development of silkworm larvae and in turn the silk production. Leaf quality and quantity not only influence the larval growth and development, but also the cocoon production, The quality of mulberry leaves plays an important role in the success of the sericulture industry and directs its economics (Choudhury *et al.* 1991). Hence, much effort and research have been carried out to improve the quality and quantity of mulberry-leaf production for silkworm rearing and then cocoon production. Leaves of some mulberry varieties appeared to be superior to others (Raman *et al.* 1995). The amount and quality of mulberry leaves affect growth rate, developmental period, body weight, and survival rate of larvae, as well as influencing the subsequent fecundity, longevity, movement and competitive ability of the adults (Parra 1991). The nutritional status of mulberry leaves can be improved by enriching them with extra nutrients in order to increase larval growth and improve cocoon characteristics (Sengupta *et al.* 1992). The present work studied the effects of fortification of mulberry leaves by NPK on the biological, quantitative and technological parameters of mulberry silkworm *B.mori*. All the nutrients play an important role in the growth and metamorphosis of silk moth larvae. The study aimed to evaluate the effect of balanced NPK fertilization on the development and productivity of silkworm (*B.mori*) when the larvae feed on leaves of fertilized trees.

Material and Methods

Field experiments and treatments:

The experiment was carried out in the Department of Plant Protection, Faculty of Agriculture, AL-Azhar Univ, Nasr City, Cairo, Egypt. The study aimed to test the impact of silkworm feeding with NPK fertilized mulberry leaves on certain biological and technological aspects of silkworm. 100 grams of NPK fertilizer contained N 50g, P 30g K 20g was added to each tree. While the untreated without fertilization.

Silkworm rearing technique:

The rearing of Silkworm and experimental technique were carried out under laboratory conditions (25 ± 3 °C and 75 ± 5 R.H) according to the technique of Krishnaswami (1978) during the spring season of 2014. Eggs were incubated at 24°C and 80% RH. The larvae were covered with plastic sheets and surrounded by witted spongy strips to offer suitable conditions for young larval instars (1st - 3rd instars). Plastic sheets and spongy strips were removed during 4th and 5th larval instar. The new hatched larvae were fed on (*M.indica*) mulberry leaves till pupation. After each moulting, cleaning nets with holes (3mm x 3mm) were used for removing the dried leaves and feces during young larval instars. Cleaning nets, holes of (2cm x 2cm) were used during 4th and 5th larval instars rearing. At the end of the 5th larval stadium, the larvae stop feeding and start to spin cocoon. Collapsible frames were used as montages for cocoon spinning. After ten days of spinning, cocoons were collected. The

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emerged adult mated for 3hr, and the mated males were hardly separated and the females were left to lay eggs, female moths were placed on a special paper for laying eggs and covered with a metallic funnel. During development, larvae need large amounts of mulberry leaves costing effort and money, and hence the duration of the larval and moulting period was measured for each tray. During each of the fourth and fifth larval instars, the daily number of dead larvae was recorded from each tray, and larval mortality rate calculated as a percentage of the total number of alive larvae at the beginning of the 4th instar.

The cocooning percentage was calculated as the number of fresh cocoons produced as a percentage of the total number of alive larvae at the beginning of 5th instar. Six pairs of adult moths from each tray were paired separately in paper cages, and the total numbers of deposited eggs per female were counted as a measure of fecundity. The hatchability percentage was calculated by the total number of hatched eggs per female as a percentage of the total number of eggs laid. For cocoon weight, fifty cocoons from each group were collected randomly soon after pupation, cleaned and weighed separately (g). The silk content ratio (net silk yield) was measured from the weight of the cocoon shell (weighed after removing the internal pupa) as a percentage of the weight of the fresh cocoon (Tanaka 1964). For the size of a reelable filament (denier), fifty cocoons from each group were collected, oven dried and reeled by an individual reeling machine, and the reelable filament length and weight recorded; the size of reeled silk filaments was calculated (Tanaka 1964) as the weight of the silk filament (g) divided by the length (m) and multiplied by 9000 (the result is in denier). Total proteins were estimated in leaves and larvae according to Chapman and Pratt (1961) and Koch and McMeekin (1924). Chlorophyll (a, b) and carotenoids were estimated by using the method of Lichtenhaler and Wellburn (1983). Biological and technological data were statistically analyzed (Little & Hills 1975) using ANOVA implemented by the Costat software program. The effect of NPK treatments on different biological and technological parameters of silkworm *B.mori*, races (Giza B and J444) fed on treated mulberry leaves were compared with the untreated trees.

Results and Discussion

Effect of fertilization on biological parameters of (B. mori).

Larval duration (day).

Data in table (1) show that the duration of silkworm larvae fed on mulberry leaves treated by NPK fertilization recorded 22.282 & 22.359 days for the two tested races (Giza B and J444) during spring season 2014 respectively, While feeding on untreated mulberry leaves lead to slightly prolongation (22.295 & 22.391 days) with no significant differences. These results are supported by the finding of Greiss *et al.* (2001) who reported that beside the main NPK fertilizers at 300:150 :120 and 15 Ton of FMY/ha, adding 2ry and micronutrients to the mulberry field, lead to both regimes decreased larval duration by 48 hours and did not affect the single cocoon weight, shell weight and shell ratio. However (Saad, and Nabil (2012) & Greiss and Petkov (2001) & Potdar *et al* (1997), and Sannappa *et al* (2003) clear that the shortening of the larval stage by 48 hr. compared to the untreated was observed when larvae fed on mulberry leaves plantation with NPK and micronutrients.

Table 1. Effect of fertilization on some biological parameters of *B.mori* L.

| Silkworm races | Treatments | Larval duration (day) | Weight means of larvae (g) | | | Larval Mortality (%) | |
|----------------|------------|-----------------------|----------------------------|--------------------------------------|------------------------------|------------------------|------------------------|
| | | | Fourth instar | Fifth instar larvae | | 4 th instar | 5 th instar |
| | | | | Immature (at the beginning of stage) | Mature (at the end of stage) | | |
| Giza B | NPK | 22.282 | 2.366 | 0.723 | 2.066 | 5.4 | 5 |
| | untreated | 22.295 | 1.927 | 0.718 | 2.039 | 6.4 | 8.3 |
| | P | 0.998 ns | 0.009** | 0.072 ns | 0.582ns | 0.56 | 0.59 |
| | L.S.D | 2.774 | 0.413 | 0.025 | 0.039 | 2.56 | 1.32 |
| J444 | NPK | 22.359 | 2.24 | 0.72 | 2.062 | 7.4 | 7 |
| | untreated | 22.391 | 1.871 | 0.705 | 2.029 | 9.4 | 11.4 |
| | P | 0.998 ns | 0.008 ** | 0.025 | 0.038 | 0.57 | 0.49 |
| | L.S.D | 2.774 | 0.585 ns | 0.035 | 0.056 | 3.63 | 1.87 |

Larval weight (g).

Fourth and fifth larval instar.

The highest weight means of ten larvae (fourth instar larvae) recorded 2.366 and 2.240 g for the two races Giza B and J444, respectively, when they fed on mulberry leaves treated by NPK fertilization. While the lowest ones recorded 1.927 and 1.871 g were obtained in untreated for the two races Giza B and J444 respectively, with significant differences between NPK fertilization treatments and untreated (P=0.008). As shown in table (1) one larvae of the fifth instar (at the beginning of stadium) recorded the highest mean weight

0.723 and 0.720 g for Giza B and J444 larvae fed on mulberry leaves treated by NPK fertilization respectively, while they were 0.718 and 0.705g in untreated respectively.

The fifth larval instar (at the end of stadium), of the two races fed on mulberry leaves treated with NPK fertilization gave the highest larval weights 2.066 and 2.062 g for Giza B and J444 respectively. While they recorded 2.039 and 2.029g in untreated for Giza B and J444 races respectively (Table 1). It is clear that the influence of mulberry treated by NPK fertilization on full-grown larval weight was better than untreated; these results may be due to existence of higher amounts of essential nutrients from NPK fertilization than untreated. These obtained results are in similar with those of (Tzenov & Petkov 1994, Potdar *et al* (1997), Shankar *et al* (1999), Shankar *et al* (2000), Greiss, and Petkov (2001), Greiss *et al* (2001), Rashid *et al* (2002) and Sannappa *et al* (2003), they reported that the fertilization of mulberry tree with different methods has more effect on silkworm larval body weight than untreated and it is important for biological aspects.

Larval mortality% (Fourth and Fifth instar larvae).

Data presented in table (1) showed that larval mortality percentages resulted from fourth instar larvae fed on mulberry leaves treated by NPK fertilization were 5.4% and 7.4% for *B.mori* races Giza B and J444 respectively, while the mortality percentages were 6.4 and 9.4% when larvae fed on untreated mulberry leaves for Giza B and J444 respectively, with non-significant differences. As shown in table (1), the fifth larval instar mortality percentages resulted from larvae fed on mulberry leaves treated with NPK fertilization were 5 and 7% for Giza B and J444 races respectively. While these mortality percentages were 8.3 and 11.4% in untreated for the two races, respectively. Generally the highest mortality percentages occurred when larvae fed on untreated mulberry leaves. While the lowest mortality percentages obtained when larvae fed on mulberry leaves treated by NPK fertilization with non-significant difference. These results are in conformity with those reported by Greiss and Petkov (2001) who reported that the main NPK fertilizers at 300: 150: 120 and 15 Ton of FYM/ha, adding 2ry and micronutrients to the mulberry field, lead to increase of silkworm pupation ratio by 6.81 – 13.67% and increase in cocoon yield per 1 box by 2.34 -4.37 kg (8.06 -15.33%) over the untreated despite of the genetically and geographical origins of the breeds. Both regimes decreased larval duration by 48 hours and did not affect the single cocoon weight, shell weight and shell ratio.

Effect of fertilization on quantitative parameters of (B. mori L).

Cocoon indices (F.C.W& C.S.W& C.S.R %).

The obtained results in table (2) showed that weight of fresh cocoon differed from female to male in both two races, being heavier for female than male. Also, feeding on treated mulberry leaves by NPK fertilization supplements resulted heavier cocoons in both sexes. The weights of female fresh cocoons recorded 1.42, 1.41gm resulted from treated mulberry leaves for the two tested races respectively, Whereas the means were 1.35 and 1.209g in untreated in cases of Giza B and J444 respectively. Male fresh cocoon recorded 1.40 and 1.33g, in larvae fed on treated leaves for Giza B and J444 races respectively. In untreated, the weights were 1.29 and 1.20g respectively. Results of cocoon shell weights had the same trend of the cocoon fresh weights. Supplementation with NPK fertilization led to increasing in cocoon shell of male and female, but the differences were insignificant. The obtained means were 0.20 and 0.19g with NPK fertilization treatments, and 0.17 & 0.13g in untreated for females of Giza B and J444 respectively, while, male shell cocoon weights recorded 0.19 and 0.18g compared to untreated (0.17 and 0.13 g) for the two races respectively.

The cocoon shell ratios for females of Giza B and J44races were 22.82 and 20.33% in NPK treatments & 21.77% and 17.06% in untreated, respectively, while male cocoon shell ratio% recorded 19.40, 18.40% in NPK treatments compared to untreated (17.19% and 16.06 g) for Giza B and J444 races, respectively, with no-significant differences (Table 2).

Pupal weight and pupation ratio%.

Female pupal weights resulted from larvae of *B.mori* races (Giza B and J444) fed on treated mulberry leaves were 0.75 and 0.69g respectively whereas, the means were 0.61and 0.55g in untreated respectively. Male pupal weights of (Giza B and J444) recorded 0.73 and 0.65g for larvae fed on treated and 0.55& 0.52g in untreated, respectively, with non-significant differences (Table 2).The female pupation ratios%were 96.20 & 92.37% for larvae of *B.mori* races Giza B and J444 respectively fed on treated mulberry leaves. While pupation ratio% of male was 88.60 & 87.50 for the two races respectively fed on treated mulberry leaves. The pupation ratios were 90.92% & 86.50% in untreated for female of Giza B and J444 respectively, while they were 85.50 & 84.70% for male for Giza B and J444 respectively. It is clear that, the highest general mean of pupation ratio% for the two races were obtained when larvae fed on mulberry leaves treated by NPK fertilization. There were significant differences between means (Tables 2).Greiss and Petkov (2001) reported that the main NPK fertilizers at 300: 150: 120 and 15 Ton of FYM/ha, adding 2ry and micronutrients to the mulberry field, lead to increase of silkworm pupation ratio by 6.81 – 13.67% and increase in cocoon yield per 1 box by 2.34 -4.37 kg (8.06 -15.33%) over the untreated despite of the genetically and geographical origins of the breeds. Both

regimes decreased larval duration by 48 hours and did not affect the single cocoon weight, shell weight and shell ratio%.

Table 2. Cocoon indices (F.C.W, C.S.W and C.S.R %), pupa weight, fecundity and hatchability of two races of *B.mori* L. fed on treated and untreated mulberry leaves.

| Silkworm races | Cocoon indices | | | | | | Pupa weight | | Pupation Ratio | | cocooning percentage | | Silk prod (cg/days) | | Fecund | Hatch |
|----------------|----------------|------|----------|------|----------|-------|-------------|------|----------------|-------|----------------------|-------|---------------------|------|---------|--------|
| | F.C.W | | C.S.W | | C.S.R% | | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | | |
| | ♀ | ♂ | ♀ | ♂ | ♀ | ♂ | | | | | | | | | | |
| Giza A | 1.42 | 1.40 | 0.20 | 0.19 | 22.82 | 19.40 | 0.75 | 0.73 | 96.20 | 88.60 | 80.60 | 79.00 | 2.19 | 1.81 | 138.07 | 87.70 |
| Untreated | 1.35 | 1.29 | 0.17 | 0.17 | 21.77 | 17.19 | 0.61 | 0.55 | 90.92 | 85.50 | 73.00 | 72.00 | 1.76 | 1.54 | 114.60 | 76.80 |
| p | 0.603 ns | | 0.058 ns | | 0.498 ns | | 0.798 ns | | 0.002 *** | | 0.0018 *** | | 0.206 ns | | 0.0057* | 0.022* |
| L.S.D | 0.155 | | 0.592 | | 1.523 | | 0.731 | | 0.698 | | 0.829 | | 0.902 | | 23.72 | 0.99 |
| Giza B | 1.41 | 1.33 | 0.19 | 0.18 | 20.33 | 18.40 | 0.69 | 0.65 | 92.37 | 87.50 | 77.40 | 74.00 | 2.17 | 1.70 | 129.00 | 80.70 |
| Untreated | 1.20 | 1.20 | 0.13 | 0.13 | 17.06 | 16.06 | 0.55 | 0.52 | 86.50 | 84.70 | 70.00 | 70.00 | 1.71 | 1.44 | 104.60 | 71.80 |
| p | 0.453 ns | | 0.267 ns | | 0.674 ns | | 0.493 ns | | 0.006** | | 0.713 ns | | 0.331 ns | | 0.005** | 0.023 |
| L.S.D | 0.128 | | 0.423 | | 1.379 | | 0.7055 | | 0.182 | | 1.5373 | | 0.091 | | 22.61 | 0.996 |

F.C.W = Fresh Cocoon Weight C.S.W = Cocoon Shell Weight C.S.R = Cocoon Shell Ratio Fecund = Fecundity Hatch = Hatchability

Cocooning Percentage.

Cocooning Percentages of *B.mori* larvae fed on mulberry leaves treated with NPK fertilization were 80.60% & 77.40% for female and 79.00% & 74.00% for male in the two races Giza B and J444 races respectively. While the mean percentages in untreated leaves were 73.00% & 70.00% for female and 72.00% & 70.00% for male in two races, respectively. The differences between treated & untreated were highly significant with the Giza B race, while they were insignificant with the J444 race.

Silk productivity (cg/days).

Silk productivity (cg/days) were (2.19 & 2.17%) for Female and (1.81, 1.70% cg/days) for male larvae of *B.mori* races Giza B and J444 fed on mulberry leaves treated by NPK fertilization respectively, while they were 1.76, 1.71% for Female and 1.54, 1.44 cg/days for male larvae of Giza B and J444 in untreated, respectively. The differences were in-significant.

Fecundity & fertility.

The quantity of laid eggs/adult female (fecundity) resulted from larvae of the two *B.mori* races (Giza B and J444) fed on treated mulberry leaves were (138.07 & 129.00) .while they were 114.60, & 104.60 eggs/female for untreated respectively. The lowest quantity of eggs/female occurred when larvae fed on untreated mulberry leaves. Statistically, there was a highly significant difference between the treated and untreated (Table 2). The hatching percentages of deposited eggs of female moth resulted from larvae of *B.mori* races fed on treated mulberry leaves were (87.70 and 80.70% respectively) and significantly higher than untreated (76.80 and 71.80%) for the two races, respectively. Our results are in conformity with those reported by Ray and Senapati (2001) who reported that higher yield potential S1 can be selected for egg production only after improving the leaf quality by fertilizer application at higher levels, especially during the summer which is not favorable for silk production. These resulted found in similar with those of (Tzenov & Petkove (1994) who investigated the nitrogen balanced in food of the silkworm (*B.mori* L.). They found that digested N used for moth and eggs was more effective at early spring rearing. Skankar *et al.* (1999) reported that feeding of leaf obtained by application of micronutrients (Iron, Zinc and Magnesium) recorded significantly higher fecundity.

Technological parameters.

The studied technological parameters include length; weight and size of reelable filaments were studied during spring seasons 2014. Data existed in table (3) show the mean values of length (m), weight (g) and size (denier) of reelable filaments resulted from silkworm races fed on treated mulberry leaves with NPK fertilization and untreated. The data of treated mulberry trees by NPK fertilization gave the highest means for the mentioned technological parameters, (length, weight and size) these means were 303 m, 0.131g and 1.279 denier for Giza B, These parameters were 302.67 (m), 0.128 (g) and 1.312 denier, of reelable filaments for J444, respectively, While the lowest ones, were recorded in untreated, 290.3 m, 0.063 g and 0.737 denier in (Giza B race), and 256.7 m, 0.056 (g) and 0.056 (denier) of reelable filaments for J444.

Chemical analysis.

Chemical analysis was carried out to estimate Chlorophylls a, b and carotenoids by using the method of Lichtenhaler and Wellburn (1983). Total proteins were estimated in leaves and larvae according to Chapman Pratt (1961). The data in table (4) showed that the total protein in treated mulberry leaves was (5.05), it was higher than that of untreated leaves (2.81) and affect consequently, the total protein in larvae of Giza B fed on

treated and untreated leaves 8.37 and 7.54 mg/g respectively. However it was 7.89 and 7.35mg/g for J444 in treated and untreated respectively. The data in tables (4) showed that the Chlorophyll (a and b) and Carotenoids were differed in both treated and untreated mulberry trees. The data in table (4) showed that Chlorophylls a, b and Carotenoids in treated mulberry leaves was (24.924&25.314 and 2.91 respectively), it was higher than that of untreated leaves (24.035 &20.157 and 2.96 respectively, and affect consequently.

Table 3. Effect of fertilization on technological parameters *B.mori* fed on mulberry leaves.

| Silkworm races | Treatments | Technological parameters | | |
|----------------|------------|--------------------------|------------|--------------|
| | | Length(m) | Weights(g) | Size(denier) |
| Giza B | NPK | 303 | 0.131 | 1.279 |
| | untreated | 290.3 | 0.063 | 0.737 |
| | p | 0.012 * | 0.296 ns | 0.628 ns |
| | L.S.D | 3.195 | 0.031 | 0.095 |
| J444 | NPK | 302.67 | 0.128 | 1.312 |
| | untreated | 256.7 | 0.056 | 0.055 |
| | p | 0.013 * | 0.296 ns | 0.637 ns |
| | L.S.D | 4.516 | 0.044 | 0.135 |

Table 4. Amounts of total protein, Chlorophylls a, b and Carotenoids in treated and untreated mulberry leaves and total protein in silkworm larvae.

| Mulberry leaves | | Total protein (mg/g) | | Chlorophylls a ,band Carotenoids (mg/g) in mulberry leaves | | |
|-----------------|--------|----------------------|-----------|--|-----------|--------|
| | | Treatment | Untreated | | | |
| | | 5.05 | 2.81 | Chlorophyll a | Treated | 24.924 |
| | | | | | Untreated | 24.035 |
| Larvae | Giza B | 8.37 | 7.54 | Chlorophyll b | Treated | 25.314 |
| | | | | | Untreated | 20.157 |
| | J444 | 7.89 | 7.35 | Carotenoids | Treated | 2.91 |
| | | | | | Untreated | 2.96 |

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