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Effect of a Soybean Powder Additive on some Characteristics of Bulgarian Silkworm (*Bombyx mori* L.) Hybrid.

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ABSTRACT:

The present study aimed to assess the influence of soybean powder as a protein source added to mulberry leaves at three concentrations (1, 1.5, and 2 g / L water) on the biological and economic parameters of the silkworm, *Bombyx mori*. The experiments began from the beginning of the 4th larval instar and extended to egg hatching that the emerged adult laid during spring season 2023. The obtained results showed non-significant difference between the three concentrations of soybean on silkworm characters. The weight of 4th and 5th larval instar was higher when larvae fed on 1.5 g and 1g of soybean respectively. The duration of 5th larval instar was shorter when larvae fed on soybean 1g. Additionally, soybean 1.5 g gave the highest cocoon shell weight, while, 2g soybean gave the highest values of silk ratio and cocoon %. Furthermore, hatching percentages were high at soybean concentrations of 1.5 and 2 g, respectively. From the results obtained, it can be concluded that soybean with a concentration of 2g is the best concentration, as it gave the highest number of cocoons and silk ratio.

INTRODUCTION

The mulberry silkworm, *Bombyx mori* L. (Bombycidae: Lepidoptera), is regarded as one of the most significant economic insects. In addition to its significant therapeutic potential, silkworm larvae are thought to be the primary source of natural silk and are typically used to treat cardiac issues, blood pressure, and nerve disorders. Furthermore, male moths are used to create therapeutic veins and the excreta are a significant component of fish and poultry feed, the silkworm pupae are used to excrete vitamins (**Fenemore and Prakash 1992**). According to **Borgohain (2015)**, treating silkworm larvae with nutrient supplements improves the quality of silk filament, which can be utilized to increase yield in the sericulture sector. In order to meet the increased demand for raw silk for industrial purposes, Egypt has recently been seeking to enhance its production of silkworms using modern procedures. **Legay (1958)** noted that the larval diet affects the production of silk. By improving each of the quantity and quality of silk cocoon production, soy protein is a rich source of dietary protein and a daily supplement that is known to improve larval growth and boost the economic characteristics of *B. mori* (**Ito, 1980 and Kamaraj *et al*. 2017**). Mulberry leaves, which are high in protein, are the source of over 70% of the protein contained in silk filaments. Additionally, the most crucial enzyme for converting mulberry protein to silk protein is the protease enzyme found in the silkworm *B. mori* L.'s midgut; the activity of this enzyme varies between breeds and hybrids (**Narayanan *et al.* 1967 & Kumar and Kalpana 2009).** The aim of this research is to study the effect of adding soybean powder on certain biological aspects and cocoon yield of the silkworm, *B. mori* L.

Materials and Methods:

During the spring of 2023, all studies were carried out in the Plant Protection Department laboratory, Faculty of Agriculture, Damietta University to examine the impact of soybean additives (1g, 1.5g, and 2g/L) on specific biological characteristics of the silkworm *Bombyx mori* L. For this, the aim of this work to impact the effect of glutamine

powder on the biological and economical characteristics of the mulberry silkworm larvae.

1. Silkworm hybrid:

The Sericulture Research Department of the Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt, provided eggs of the Bulgarian silkworm, *B. mori*, L., hybrid (HoxKKxG2xV2).

2. Mulberry leaves:

The mulberry *Morus alba* L. (Moraceae) leaves were collected, cleaned and treated with three concentrations of soybean solution (1g, 1.5g and 2g/L water) as food additives then introduced as feed to larvae of silkworm, *B. mori*.

3. Rearing room:

A 3% formalin solution was used to sterilize the stands and rearing equipment. The bottom and cover are made of paraffin paper that measures (45×30×12cm).

4. Rearing technique:

B. mori was reared under fluctuated temperature and relative humidity (28.0+ 2.0 °C and 70 ± 5% R.H.) in the laboratory.

Mulberry leaves were soaked for ten minutes in a different concentration of soybean solution, (1 g, 1.5 g, and 2 g/L), and then allowed to air dry. The larvae were then fed mulberry four times a day. To estimate the economic and biological characteristics of the *B. mori* under various treatments, mulberry leaves with various treatments and leaves without food additives (control) were used. The excrement and dried food were removed with a cleaning net.

5. Experimental design:

The larvae of *B. mori* were separated into four groups at the start of the fourth instar. The first 3 groups were fed mulberry leaves with soybean, 1, 1.5, and 2g/L (250 larvae per group), while the 4th group (250 larvae) was fed mulberry leaves as a control during the fourth and fifth larval instars without any additives. Weighing was done three times at the beginning, middle, and end of the fourth and fifth larval instars.

The cocoons were spun on cartons of chicken eggs. After ten days, the cocoons were harvested. To measure the cocoon indices, fresh cocoons from each treatment were collected **(Zannoon and Omera 1994)**.

Male and female adults, pupae, fresh cocoon, and cocoon shell were weighed for each treatment.

The biological experiments were conducted on a different group of cocoons. Each couple was put inside a paper box to mate and deposit eggs after emerging.

6. Studied parameters:

6.1. Larval stage:

6.1.1. Duration of 4th and 5th larval instars (day).

6.1.2. Weight of larvae at the start, (middle and the end of the 4th and 5th instar) before molting (g).

6.1.3. Percentage of larval mortality.

According to **Megalla (1984),** the following formula was used to determine the mortality rate for the fourth and fifth larval instars:

Percentage of mortality = No. dead larvae / Total No. larvae ×100

6.2. Cocoon characters:

6.2.1. Weight of a fresh cocoon (g)

6.2.2. Weight of cocoon shell (g).

6.2.3. Cocoon percentage %.

Cocoon percentage % = *No. cocoon harvest / No. larvae retained ×100.*

6.2.4. Silk ratio (%) **Tanaka (1964).**

Silk ratio = *Weight of cocoon shell / Weight of fresh cocoon ×100.*

6.3. Pupal stage:

6.3.1. Wight of pupae (g).

6.3.2. Pupae percentage.

6.4. Adult stage:

6.4.1. Fecundity, (total number of eggs /female).

6.4.2. Fertility (%), (total number of fertile eggs /female).

6.4.3. Hatchability percentage (%) was estimated according to the following formula of **Lea (1996).**

Hatchability % = Number of hatched larvae / Number of fertilized eggs × 100

7. Statistical analysis:

Collected data were subjected to statistical analysis such as student single way ANOVA to find out the significant difference between control and experimental groups **Sigmaplot 15 (2024).**

Result:

Larval characters:

A- Duration of the fourth and fifth larval instar:

Data in Table (1) and Fig (1) showed the effect of different concentrations of soybean treatments on the 4th and 5th larval duration of *B. mori* during spring rearing season of year 2023. The results showed that. The 4th larval instar arranged 7-8 days on the three concentrations of soybean 1, 1.5 and 2g while, the 4th larval instar in control recorded longer period and arranged from 8 to 9 days to inter in the 5th larval instar. The 5th larval instar of *B.*

mori showed shorter time in the case of 1g (10-11 days) than the other concentrations 1.5 and 2g, it was required 11-12 days to start in spinning the cocoon. While, the 5th larval instar in control recorded longer period and arranged from 13 to 14 days to start in spinning the cocoon.

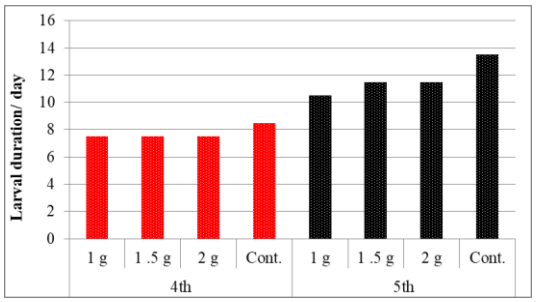

Fig. (1) Effect of different concentration of soybean on duration of the 4th and 5thlarval instars (day) of *B. mori* during spring 2023.

B-Larval weight:

Data in Table (1) and Fig (2) showed the effect of different concentrations of soybean treatments on the 4th and 5th larval instar weight of *B. mori* during spring rearing season of year 2023.

The results showed that, there were significant differences between treatments and control in the beginning 4th larval instar, while no significant differences between treatments and control in the middle and end of the 4th larval instar. The high value of 4th larval weight was recorded when larvae fed on soybean 1.5g and represented with (0.182, 0.320 and 0.547 g.) in the beginning, middle, and end of the 4th larval instar respectively. While, the low value of larval weight was recorded when larvae fed on soybean 1g and represented with (0.175, 0.300 and 0.486 g.) in the beginning, middle, and end of the 4th larval instar respectively. The weight of 4th larval in the case of soybean 2g came intermediate between 1.5 and 1g was (0.175, 0.300 and 0.505 g.) in the beginning, middle, and end of the 4th larval instar respectively. On the other hand the weight of the 4th larval instar when larvae fed on untreated mulberry leaves (control group) showed lowest weight in comparing with all treatments and represented with (0.175, 0.301 and 0.481 g.) in the beginning, middle, and end of the 4th larval instar.

The 5th larval instar weight was recorded high value when larvae fed on soybean 1g and represented with (0.478, 1.590 and 2.026 g.) in the beginning, middle, and end of the 5th larval instar respectively. While, the low value of larval weight was recorded when larvae fed on soybean 2g represented with (0.493, 1.440 and 1.961 g.) in the beginning, middle, and end of the 5th larval instar respectively. While the weight of the larvae when larvae fed on untreated mulberry leaves (control group) was (0.474, 1.472 and 1.981 g.) in the beginning, middle, and end of the 5th larval instar.

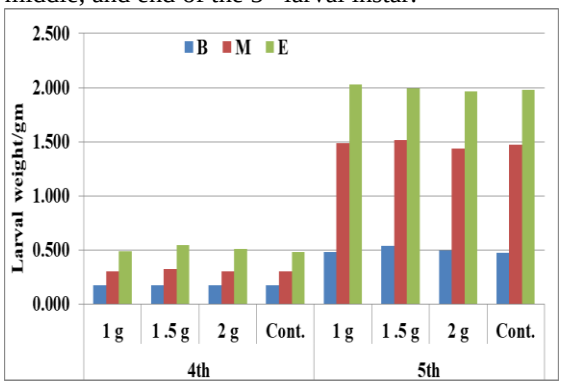

Fig. (2) Effect of different concentration of soybean on weight of the 4th and 5thlarval instars (g) of *B. mori* during spring 2023.

C- Larval mortality percentage:

Data in Table (1) and Fig (3) the effect of different concentrations of soybean treatments on 4th and 5th larval instars' larval mortality percentage of *B. mori* during spring rearing season of year 2023. The results showed that the highest larval mortality percentage was recorded when larvae fed on soybean 1g (21.2%). Whereas, the lowest larval mortality percentage was recorded when larvae fed on soybean 2g (12.8%). In control group the larval mortality percentage was (7.5%).

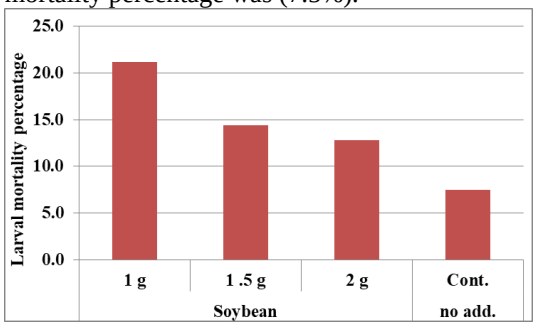

Fig. (3) Effect of different concentration of soybean on larval mortality percentage (%) of *B. mori* during spring 2023.

Table (1) Effect of different concentration of soybean on weight (g), duration (day) and mortality (%) of 4th and 5th larval instars of *B. mori* during spring 2023.

Treatment	Weight of larval instar (g)						Larval duration/day		Larval mortality percentage
	Beginning 4th	Mid of 4th	Finally 4th	Beginning 5th	Mid of 5th	Finally 5th	4th instar	5th instar	(%)
Soybean 1g	0.175±0.002 a	0.300±0.004 b	0.486±0.006 c	0.478±0.006 c	1.490±0.031 a	2.026±0.021 a	7-8	10-11	21.2
Soybean 1.5g	0.175±0.002 a	0.320±0.005 a	0.547±0.006a	0.538±0.005 a	1.515±0.023 a	1.991±0.023 a	7-8	11-12	14.4
Soy bean 2 g	0.175±0.002 a	0.300±0.004 b	0.505±0.007 b	0.493±0.005 b	1.440±0.028 a	1.961 ±0.024 a	7-8	11-12	12.8
Control	0.175±0.002 a	0.301±0.005 b	0.481±0.007 c	0.474±0.007 c	1.472±0.027 a	1.981±0.020 a	8-9	13-14	7.5
LSD 5%	0.007	0.01	0.019	0.019	0.085	0.066	-	-	-

According to Duncan's Multiple Rang Test, means that have the same letter in the same column across the various treatments do not differ significantly at the 5% probability level.

2. Cocoon characters:

A-Fresh Cocoon weight:

Data in Table (2) and Fig (4) showed the effect of different concentrations of soybean treatments on the fresh cocoon weight of *B. mori* during spring rearing season of year 2023. The results showed that, there were non-significant differences between treatments, the high value of fresh cocoon weight was recorded when larvae fed on soybean 1.5g (0.795 g.) whereas, the low value of fresh cocoon weight was recorded when larvae fed on soybean 2g (0.771 g.), the fresh cocoon weight in the control group recorded the highest weight in comparing with soybean treatments it was 0.803 g.

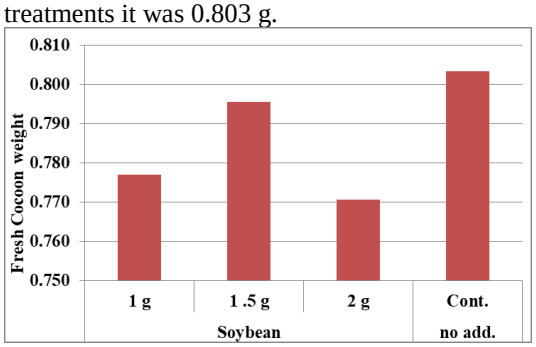

Fig.(4) Effect of different concentration of soybean fresh cocoon weight (g) of *B. mori* during spring 2023.

B-Cocoon shell weight:

Data in Table (2) and Fig (5) showed the effect of different concentrations of soybean treatments on the cocoon shell weight of *B. mori* during spring rearing season of year 2023. The results showed that, there were non-significant differences between treatments. The high value of cocoon shell weight was recorded when larvae fed on soybean 1.5g. (0.155 g.). Whereas, the low value of cocoon weight was recorded when larvae fed on soybean 1g. (0.148 g.) while, the weight of the cocoon shell are equal in control group and soybean 2 g group by value (0.152 g.).

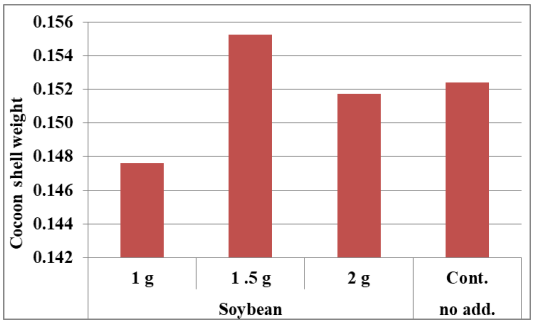

Fig.(5) Effect of different concentration of soybean cocoon shell weight (g) of *B. mori* during spring 2023.

C-Silk ratio (%):

Data in Table (2) and Fig (6) showed the effect of different concentrations of soybean treatments on the silk ratio of *B. mori* during spring rearing season of year 2023. The high value of silk ratio percentage was recorded when larvae fed on soybean 2g (19.68%) whereas, the low value of silk ratio was recorded when larvae fed on soybean 1g (19%) while, silk ratio when larvae fed on untreated mulberry leaves (control group) (18.98%).

Fig.(6) Effect of different concentration of soybean silk ratio (%) of *B. mori* during spring 2023.

D-Cocoon percentage:

Data in Table (2) and Fig (7) showed the effect of different concentrations of soybean treatments on cocoon percentage of *B. mori* during spring rearing season of year 2023. The results showed that highest of cocoon percentage was recorded when larvae fed on soybean 2g. (93.1%). whereas, the lowest cocoon percentage was recorded when larvae fed on soybean 1g. (84.3 %) the cocoon percentage (%) when larvae fed on untreated mulberry leaves (control group) was (72.4 %).

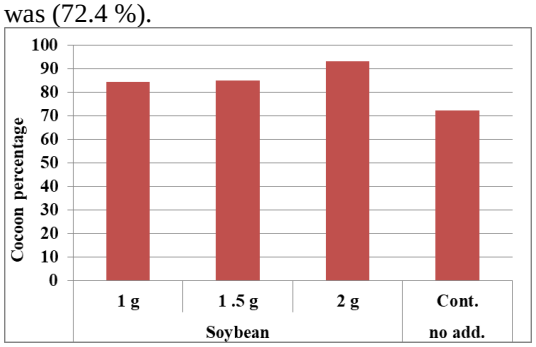

Fig.(7) Effect of different concentration of soybean cocoon percentage (%) of *B. mori* during spring 2023.

3. Pupal stage:

A- Pupae weight:

Data in Table (2) and Fig (8) showed the effect of different concentrations of soybean treatments on the pupae weight of *B. mori* during spring rearing season of year 2023. The results showed that, there were non-significant differences between treatments. The high value of pupae weight was recorded when larvae fed on soybean 1.5g (0.632g.). Whereas, the low value of pupae weight was recorded when larvae fed on soybean 2g. (0.609 g.) the pupae weight when larvae fed on untreated mulberry leaves (control group) was (0.642g.).

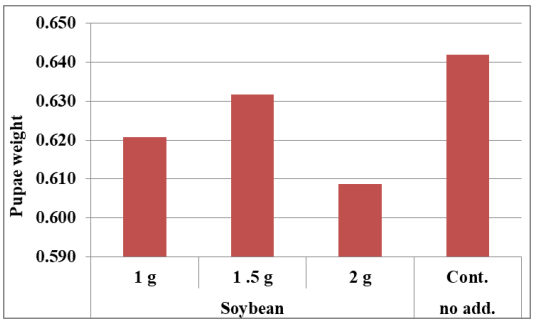

Fig. (8) Effect of different concentration of soybean pupae weight (g) of *B. mori* during spring 2023.

B-Pupation percentage:

Data in Table (2) and Fig (9) showed the effect of different concentrations of soybean treatments on pupation percentage of *B. mori* during spring rearing season of year 2023 were (100%) ,while the pupation percentage when larvae fed on untreated mulberry leaves (control group) was (93.28 %).

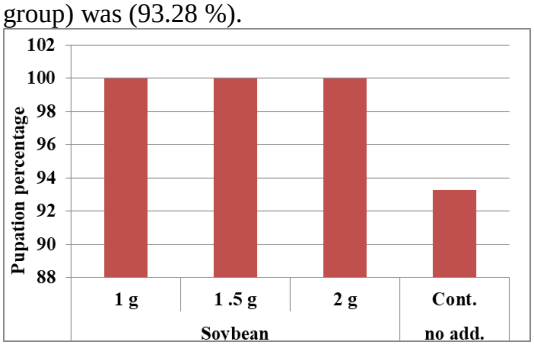

Fig.(9) Effect of different concentration of soybean pupation percentage (%) of *B. mori* during spring 2023.

4. Female fecundity:

Data in Table (2) and Fig (10) showed the effect of different concentrations of soybean treatments on number of eggs per female of *B. mori* during spring rearing season of year 2023. The results showed that higher average number of eggs/female was observed when larvae fed on soybean 1g. (560.6 eggs/ female). Whereas, lower average number of eggs/ female were observed when larvae fed on soybean 2g. (283 eggs/ female). While the number of eggs per female when larvae fed on 1.5g soybean and untreated mulberry leaves (control group) came intermediate between 1g and 2g it was represented with (325 and 330.8 eggs/ female) respectively.

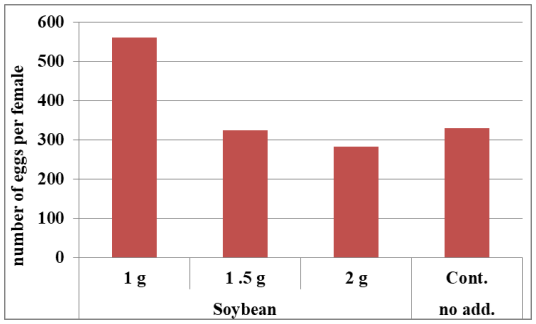

Fig. (10) Effect of different concentration of soybean number of eggs per female (Female fecundity) of *B. mori* during spring 2023.

5-Eggs fertility:

Data in Table (2) and Fig (11) showed the effect of different concentrations of soybean treatments on egg fertility percentage of *B. mori* during spring rearing season of year 2023. The results showed that the highest fertility of eggs was registered by using soybean 1.5g. (97.03%) whereas, the lowest fertility of eggs was registered by using soybean 2g. (90.2%) while, the eggs fertility percentage when larvae fed on untreated mulberry leaves (control group) was 96.8%.

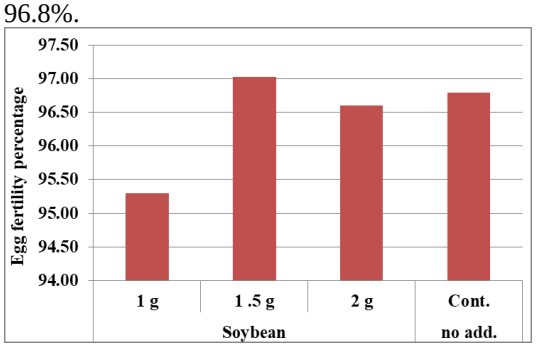

Fig.(11) Effect of different concentration of soybean egg fertility (%) of *B. mori* during spring 2023.

6-Hatchability percentage:

Data in Table (2) and Fig (12) showed the effect of different concentrations of soybean treatments on hatchability percentage of *B. mori* during spring rearing season of year 2023. The results showed that the highest hatchability was registered by using soybean 1.5g. (100%) whereas, the lowest hatchability was registered by using soybean 1g. (78.9%). In spite of increase in eggs fertility percentage when larvae fed on untreated mulberry leaves (control group) the hatchability percentage were in the last category it was 52.7%.

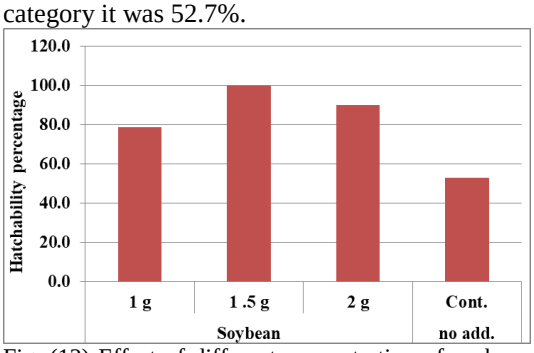

Fig. (12) Effect of different concentration of soybean hatchability percentage (%) of *B. mori* during spring 2023.

Table (2) Effect of different concentration of soybean on cocoon, pupae and eggs of *B. mori* during spring 2023.

Treatment	Fresh cocoon weight	Cocoon shell weight	Pupae weight	Silk ratio%	Cocoon percentage (%)	Pupation percentage (%)	No. of egg (egg/female)	Egg fertility (%)	Hatchability (%)
Soybean 1 g	0.777±0.016 a	0.148±0.003 a	0.621±0.014 a	19	84.3	100	560.6±3.287 a	95.3	78.9
Soybean 1.5 g	0.795±0.017 a	0.155±0.004 a	0.632±0.014 a	19.53	85.05	100	325±2.096 b	97.03	100
Soy bean 2 g	0.771±0.012 a	0.152±0.003 a	0.609±0.011 a	19.68	93.1	100	283±2.731 b	96.6	90.2
control	0.803±0.017 a	0.152±0.004 a	0.642±0.015 a	18.98	72.4	93.28	330.8±4.167 b	96.8	52.7
LSD 5%	0.043	0.009	0.037	-	-	-	-	-	-

According to Duncan's Multiple Rang Test, means that have the same letter in the same column across the various treatments do not differ significantly at the 5% probability level.

Discussion:

These results were in agreement with those obtained by **Mahmoud (2013)** who mentioned that rearing mulberry silkworm *Bombyx mori* L. larvae on different protein diets (mushroom, soybean and a mixture of mushroom & soybean) using semi-synthetic food. The results showed that the larvae fed on semi-synthetic food containing soybean gave the highest value in (the weights of the fifth instar larvae, silk gland, pupae, cocoon, cocoon shell and the number of eggs laid by the adult). It gave the highest duration for the 5th instar larvae and the lowest mortality rate, while the larvae fed on mushroom food gave low weights. **Hassan (2020)** showed that the effect of soybean and spirulina as protein sources at different concentrations (2.5, 5 and 10% w/v) on the biological, economic and physiological characteristics of three hybrids of mulberry silkworm *Bombyx mori*; Two imported hybrids (Bulgarian and Chinese) and a local Egyptian hybrid (used as a control) were used. These treatments were applied from the beginning of the 5th larval instar until spinning. The results showed that the imported hybrid larvae showed a significant increase in the weight of larvae, pupae and the percentage of silk compared to the local hybrid, especially the larvae that were reared on a concentration of 5% soybean and then spirulina at a concentration of 2.5%.

Additionally, during the fourth and fifth instars of *B. mori* larvae, **Hamzah *et al.* (2016)** assessed the biological and economic parameters of a silkworm hybrid (HoxKKxG2xV2) that was fed mulberry leaves (*Morus alba*, Rose variety) supplemented with four different types of protein sources (pollen grains, royal jelly, amino acid, and bee honey). They also investigated the effects of these supplements on the larval duration, as well as the weight and ratio of cocoon, shell, and pupal development. The data obtained demonstrated that the fresh cocoon weight, cocoon shell weight, and shell/cocoon ratio increased when larvae were fed on leaves enriched with pollen grains, royal jelly, amino acids, and bee honey. Additionally, such feeding resulted in lower larval mortality rates and shorter larval duration.

CONCLUSION

Adding different concentrations of soybean to mulberry leaves to feed 4th and 5th instar larvae of silkworm (*Bombyx mori* L.) positively affected both biological and economic aspects, where soybean concentration (2 g/L) gave the best results as it gave the highest number of cocoons and silk ratio.

FUNDING:

This research did not receive any funding.

CONFLICTS OF INTEREST:

The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTION:

Ata, T. E.; H. A. El-Kady; Nada, A. El-Kady and Dina, M. Fathy wrote the manuscript. All authors checked and confirmed the final revised manuscript

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الملخص العربي

تأثير اضافة مسحوق فول الصويا على بعض صفات هجين دودة القز البلغارية *Bombyx mori* L.

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هدفت الدراسة الحالية إلى تقييم تأثير مسحوق فول الصويا كمصدر بروتيني مضاف إلى أوراق التوت بثلاثة تراكيز مختلفة (1، 1.5، و2 جم/لتر ماء) على النواحى البيولوجية والاقتصادية لدودة القز *Bombyx mori* . بدأت التجارب من بداية العمر اليرقي الرابع وامتدت إلى فقس البيض الذي وضعته الحشرة البالغة خلال موسم ربيع 2023. وأظهرت النتائج المتحصل عليها عدم وجود فرق معنوي بين التراكيز الثلاثة لفول الصويا على صفات دودة القز. كان وزن العمر اليرقي الرابع والخامس أعلى عندما تتغذى اليرقات على 1.5 جرام و1 جرام من فول الصويا على التوالي. كانت مدة العمر اليرقي الخامس أقصر عندما تتغذى اليرقات على 1 جرام فول صويا. بالإضافة إلى ذلك، أعطى فول الصويا بتركيز 1.5 جرام أعلى وزن للشرنقة، بينما أعطى 2 جرام فول الصويا أعلى نسبة حرير ونسبة شرانق. علاوة على ذلك، كانت نسب الفقس عالية عند تركيزات فول الصويا 1.5 و 2 جرام على التوالي. من النتائج المتحصل عليها يمكن استنتاج أن فول الصويا بتركيز 2 جرام هو أفضل تركيز حيث أعطى أعلى عدد شرانق ونسبة حرير.