

Journal of Plant Protection and Pathology

Journal homepage: www.jppp.mans.edu.eg
Available online at: www.jppp.journals.ekb.eg

The Impact of Fortification of Mulberry Leaves with the Yeast *Saccharomyces cerevisiae* and the Blue Green Algae *Spirulina platensis* on some Quantitative Parameters of Silkworm *Bombyx mori* (L.)

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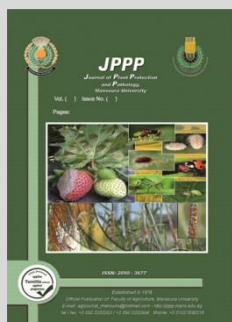
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ABSTRACT

The present investigation was conducted to study the effect of fortification of mulberry leaves with different concentrations of baker's yeast *Saccharomyces cerevisiae* extract and blue-green algae *Spirulina platensis* extract on some quantitative parameters of *Bombyx mori* larvae and cocoons. Statistical analysis of the obtained data revealed that all larval treatments showed a significant increase in the larval and cocoon weights, especially at concentrations 0.5 % of yeast and 0.05% of spirulina. Feeding 5th larval instar with mulberry leaves at concentrations 0.5 % yeast and 0.05% spirulina, lead to increase the larval weight by about 55 % and 30%, respectively, when compared with the control. The same trend was observed in pupal weight, cocoon weight and cocoon shell weight. Larval treatments with yeast extract at concentration 0.5% increased the weights of pupa (0.850 gm), cocoon (0.908 gm) and cocoon shell (0.216 gm) by about 30%, 16% and 27% as compared with controls which were 0.561gm, 0.781gm and 0.170 gm, respectively. On the other hand, all larval trials with spirulina extracts remained at the second grade of implementation on the pupal weight, cocoon weight and shell weight. However, feeding *B. mori* larvae with yeast extract significantly increased the silk ratio and the total haemolymph protein comparing with the spirulina treatments or control ones.

Keywords: *Bombyx mori*; yeast; *saccharomyces cerevisiae*; algae; *spirulina platensis*; cocoon parameters.



INTRODUCTION

Sericulture is one of the most remarkable industries, which includes the exploitation of mulberry leaves (*Morus alba* L.) for rearing silkworm *Bombyx mori* to produce silk. Fortification of mulberry leaves with supplementary nutrients is fundamental prerequisite for improvement of *B. mori* growth and development, hence its appropriate commercial profiteering (Buhroo, *et al.* 2018. and Jaiswal *et al.* 2020).

The mulberry silkworm *B. mori* is a monophagous insect, obtain all the needed nutrients for its development from the mulberry leaves. The goodness of silk produced by the silkworm counts on environmental conditions as well as the quality of yield of mulberry leaves that plays a very influential role in producing cocoons with high quality (Legay, 1958 , Etebari *et al.* 2004 and Samami *et al.* 2019)

Recently, many researchers have made attempts to progress the quality and quantity of mulberry leaf over foliar application on leaf matrix with either use of some natural products (Sudhakar *et al.*, 2000; Hiware, 2006 , Nguku *et al.*, 2007and Kamel *et al.* 2016) or artificial compounds (Shankar and Rangaswamy, 1999; Mamatha *et al.*, 2006; Rahmathulla *et al.*, 2007 and Bhattacharyya *et al.* 2016)

Supplements of nutrition e.g., vitamins, proteins, amino acids and probiotics when added to larval feed tend to elevate nutritional adequacy and economic feature of silkworm (Etebari and Matindoost, 2005; Singh *et al.*, 2005 and Amalarani *et al.*, 2011 and Balasundaram *et al.* 2013)

Probiotics are the live microbial food additions, advantageously affecting the host by enhancement of the

microbial balance and improved the development and the cellular growth. The beneficial role played by the use of probiotics in insects have been reported by several investigators (Fuller, 1993; Dillon and Dillon, 2004). The gut probiotics maximize the digestive utility of feeds, stimulation of nonspecific immune system and detoxification of metabolites (Esaivani *et al.* 2014 and Nishida *et al.* 2017).

At the beginning of the last century, *saccharomyces cerevisiae* (baker's yeast) has been used in animal feeding as an essential source of well digested protein as well as vitamin B and some important bioelements. Yeast is considered as a plentiful source of a vitamin B complex which plays a vital function in various physiological and biochemical aspects of biological systems (House ,1974), including B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B7 (biotin) and B9 (folic acid) (Moyad 2007; Moyad 2008 and Shurson 2018).

Spirulina platensis is blue-green microalgae belongs to the class of cyanobacteria. It contains various minerals, 18 amino acids, and pivotal vitamins like tocopherol, biotin, thiamine, niacin, riboflavin, pyrodozoic acid, folic acid, beta-carotene and vitamin B12, etc. (Kumar *et al.*, 2009; , Ganeshprabu *et al.*, 2011 and Thangapandiya and Dharanipriya 2019).

The present study was designed to determine the effects of enrichment of mulberry leaves with different concentrations of baker's yeast *S. cerevisiae* and blue-green microalgae *S. platensis* extracts on some biological parameters of *B. mori* i.e. larval weight, pupal weight, cocoon weight, cocoon shell weight and silk ratio. The total

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DOI: 10.21608/jppp.2021.58388.1011

haemolymph protein of the 5th larval instar of *B. mori*. as a physiological parameter was also determined.

MATERIALS AND METHODS

Insect Strain

B. mori larvae (strain: Jingsong × Haoyue) were maintained in the laboratory at Department of Applied Entomology and Zoology and reared on mulberry (*Morus alba*) reared under a 12-h light/12-h dark condition and at 26 °C. Food was served to larvae three times per day.

Treatment of fifth instar larvae with yeast and spirulina extracts

Different concentrations of yeast extract (0.1%, 0.3%, and 0.5%) and blue green algae spirulina extract (0.01%, 0.03%, and 0.05%) were made by dissolving in distilled water and diluting into the proper concentrations.

Experimental groups

Fifth instar larvae of *B. mori* were distributed into 7 experimental groups. Each group consisting of 50 larvae including control (C); 3 groups (Y₁, Y₂, Y₃) for yeast extract treatment and the last 3 groups (S₁, S₂, S₃) for spirulina treatments. Fresh mulberry leaves were drenched with different concentrations of aqueous extracts of yeast and spirulina for 10 minutes. The leaves were dried in the air before delivered to the silkworms (three feeding/day) till the maturation of the fifth instar. The control group larvae were fed on mulberry leaves treated with distilled water. Mortality was registered daily, and the weights of treated larvae and the control were recorded before cocoon spinning.

Sample collection

The haemolymph of treated larvae beside control ones were gathered by producing an aperture within the proleg. Haemolymph that poured from the wound without any pressure was gathered into Eppendorf tubes containing 0.001g phenylthiourea powder for preventing tyrosinase activity.

Total haemolymph protein

The obtained haemolymph were vortexed with a pipette. Three microliters of haemolymph suspension were

Table 1. Effects of enrichment of mulberry leaves with different concentrations of yeast extract and blue green algae spirulina extract on some biological and physiological parameters of silkworm *B. mori*

Parameters (gm)	Control	Yeast treatments			Spirulina treatments		
		0.1%	0.3%	0.5%	0.01%	0.03%	0.05%
Larval weight	1.5228±0.05 e	2.134±0.02 b	2.184±0.03 b	2.355±0.05 a	1.715±0.06 d	1.673±0.04 d	1.984±0.05 c
Pupal weight	0.6519±0.03 d	0.7233±0.04 c	0.75106±0.07 b	0.8503±0.08 a	0.7227±0.06 c	0.7253±0.01 c	0.7515±0.02 b
Cocoon weight	0.7814±0.04 d	0.8553±0.02 b	0.8818±0.03 a	0.9082±0.02 a	0.79408±0.03 d	0.8115±0.04 c	0.8435±0.06 b
Cocoon shell weight	0.1703±0.01 c	0.1945±0.05 a	0.1943±0.06 a	0.2166±0.07 a	0.1718±0.003 c	0.1723±0.008 c	0.1761±0.005 b

-Each value represents the mean ± SE.

- Means followed by the same letters is not significant.

-Probability level at 0.05.

Pupal weight

The present results showed that all concentrations of the yeast extract have a positive effect on the pupal weight compared with control. The maximum and significant pupal weight were obtained at 0.5% concentration group (0.8503gm), followed by 0.3% yeast group (0.7510gm). Also, spirulina concentrations significantly increased the pupal weight. The maximum increase was observed at concentration 0.05% to be 0.7515gm, followed by concentrations 0.03% and 0.01% to be 0.7253 gm and 0.7227 gm, respectively, while control was 0.6519gm.

Cocoon weight

As shown in table 1, the analysis of variance revealed highly significant differences in the cocoon weight between larval treatments with yeast extract and the control. The high

used for total protein assessment. The resultant supernatant containing plasma was diluted with distilled water (1:500). The Lowry method (1951) for measuring the total protein concentration in haemolymph were used using spectrophotometer (Cambridge, UK) at 750 nm wavelength. Bovine serum albumin (BSA, Merck) were used for preparing a standard curve. Protein assessment were repeated 3 times for each experimental and control groups.

characters of the cocoon

After spinning of cocoons by all the groups of the silkworm, the cocoon quantitative parameters like single cocoon weight, shell weight, pupal weight and silk ratio [expressed as a percentage using the following equation: (Shell weight (g) / Cocoon weight (g) x 100)] of randomly chosen 15 cocoon were determined

Statistical analysis

The obtained data were subjected to statistical analysis of variance for recognize significant differences among the treatments using standard method under MS Excel software. Significant tests were carried out using Dunnett's comparison method

RESULTS AND DISCUSSION

Biological Parameters:

The effects of feeding mulberry leave enriched with different concentrations of yeast and spirulina extracts on the weights of larvae, pupae, cocoon and shell of *B. mori* are presented in Table 1.

Larval Weight:

The records obtained proved that feeding 5th instar larvae of *B. mori* on mulberry leaves enriched with 0.5% yeast extract increased the percentages of larval weight (2.355 gm) by about 55 %, followed by 0.3% (2.184 gm) and 0.1% (2.134 gm) when compared with the control one (1.522 gm). On the other hand, feeding 5th larval instar of *B. mori* on mulberry leaves enriched with 0.05% spirulina extract leads to increase the larval weight to be 1.98 gm, followed by 0.01% (1.715gm) and 0.03% (1.673gm) with no significant differences, while control was 1.52 gm.

value of cocoon weight (0.908 gm) was recorded at concentration 0.5%, followed by 0.881gm and 0.855 gm at concentrations 0.3% and 0.1%, respectively. On the other hand, spirulina at concentration 0.05% caused a significantly increase in the cocoon weight to be (0.8435gm) followed by the concentration 0.03% (0.811 gm). There was no significant difference in cocoon weight between larvae fed with mulberry leaves treated with 0.01% spirulina (0.794 gm) and control (0.781 gm). However, the present results are in agreement with the findings of Amalarani *et al.* (2011) who stated that using *S. cerevisiae* as an immune modulating factor in silkworm *B. mori*, increase the energy budget and the traits of *B. mori*. Masthan, *et al.*, (2011) reported that 300 ppm concentration of spirulina and yeast as food supplement to silkworm contain the ultimate amount of vitamins and

fundamental amino acids which define the specificity for different metabolic operations in silkworm and found to effectively enhances single cocoon weight, pupal weight, and silk filament length. The same records were recorded by Asaf and Mahavishnu (2018), who reported that the total body weight, and the weight of cocoon were maximum in silkworm larvae when fed on mulberry leaves treated with spirulina. Moreover, Kumar and Balasubramanian, (2014) mentioned that the activation of tissue metabolism by spirulina seems to be a main factor for the enhancement of silk gland biological parameters in silkworms.

Shell weight

Data presented in Table 1 revealed that feeding larvae of *B. mori* with mulberry leaves treated with yeast extract significantly increased the shell weight regardless of its concentrations comparing with the control. It was found that yeast extract at concentrations 0.1%, 0.3% and 0.5%, implemented average shell weight to be 0.1945gm, 0.1943gm and 0.2116gm respectively, with no significant differences. On the other hand, there is no significant difference among the concentrations 0.01% , 0.03% of larval treatments with spirulina and control. However, larval treatments with spirulina at concentration 0.05% had an intermediate effect on the average shell weight. Generally, the present results were agreed with Esaivani *et al.*, (2014) who stated that pupal weight, cocoon weight, shell weight, shell ratio and silk traits of *B. mori* were enhanced by using probiotic *S. cerevisiae* which found to be considerable in elevation the activity of the enzymes; the substantial keystone of silkworm disease control (Masthan, *et al.*, 2011, Rahul *et al.* 2017 and Masthan, *et al.*, 2017).

Silk ratio

Such as the shell weight, the results indicated that silk ratio had the same trend; it increased significantly by feeding *B. mori* larvae on mulberry leaves enriched with yeast comparing with the control without any significant differences among its concentrations. As shown in Fig. 1 silk ratio at concentration of 0.5% yeast was found to be the highest (23.8%) and significantly increased the shell ratio by about 9.6% over control. Also, at concentration of 0.1% yeast, the silk ratio increased by about 4.6% over control. On the other hand, all spirulina treatments were significantly less effective than yeast and almost had the same effect of control without any significant differences.

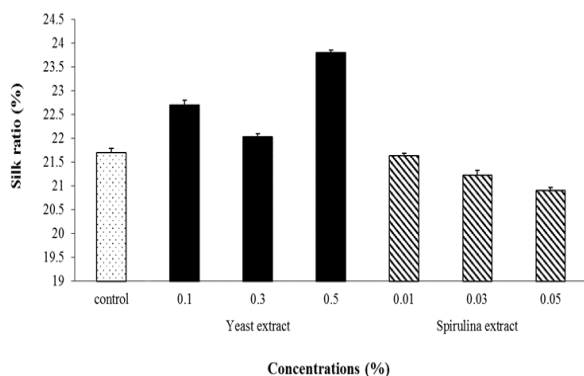


Figure 1. Effect of feeding *B. mori* larvae with different concentrations of yeast extract (0.1%, 0.3%, 0.5%) and spirulina extract (0.01%, 0.03%, 0.05%) on the silk ratio.

Physiological Parameter:

Total haemolymph protein

As shown in Fig. 2, feeding *B. mori* larvae with yeast extract significantly elevated the total haemolymph protein comparing with spirulina treatments or control. 0.5 % yeast extract significantly increased the total haemolymph protein to be 85.7mg/ml. Furthermore, larval treatments with 0.1% and 0.3 % yeast extract increased the total haemolymph protein to be in respect 77 mg/ml and 77.3 mg/ml while the control was 63.7 mg/ml. In contrast, spirulina treatment at concentrations 0.03% and 0.05% slightly increased the total haemolymph protein to be 68 mg/ml and 69.3 mg/ml, respectively.

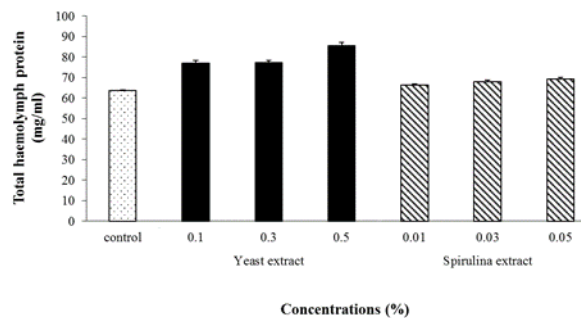


Figure 2. Effect of feeding *B. mori* larvae with different concentrations of yeast extract (0.1%, 0.3%, 0.5%) and spirulina extract (0.01%, 0.03%, 0.05%) on the total haemolymph protein.

Similar findings have been reported by Horie and Watanabe, (1983) and Amalarani *et al.*, (2011) who reported that supplementation with probiotic like *S. cerevisiae* perhaps raise the content of haemolymph protein and amino acid in the silkworm larvae, resulting in improved growth of silkworm. On the other hand, Sangamithirai *et al.* (2014) attributed the positive effects of spirulina on *B. mori* due to better quality of spirulina treated mulberry leaves concerning the higher content of protein, carbohydrate, and moisture content which ultimately resulted in the production of a higher and better quality cocoon. However, the altitude in total protein content suggesting an increase in constitutional and effective standards of organization in the body of larvae exposed to yeast and spirulina, which are a rich protein sources (Venkataramana *et al.*, 2003 and Jach *et al.*, 2015), that leads -as the present data refers- to a noticeable increase in the cocoon and shell weight. The elevated protein suggested the creation of a positive balance of nitrogen and efficiency of transformation of dietary nitrogen into the cocoon shell and pretext for good cocoon and shell weight production .

Generally, it can be said that the nutrients supplemented through mulberry leaves in terms of certain probiotics might lead to the additional growth of silkworm thereby enhancement the body weight and this might be due to the stimulatory effect on machinery for protein synthesizing of the silkworm.

The differences in the effects on larval and cocoon characteristics between yeast and spirulina treatments obtained from the present study could be interpreted according to the difference between the two microorganisms in protein and vitamin contents as well as the balance in nutritional supplements in each one (Narayanaswamy and Ananthanarayana, 2006 and Ramesh *et al.*, 2018).

CONCLUSION

It could be concluded that mulberry leaves enriched with various concentrations of the two probiotics namely the yeast *S. cerevisiae* and the blue-green algae *S. platensis* might have beneficial and significant effects on the development of *B. mori* larvae and to promote the qualities of cocoon characters which can be used for yield enhancement in the sericulture industry. Larval trials with the yeast gave better results when compared with spirulina ones. It is recommended that 0.5 % concentration of an aqueous solution of the yeast as feed to silkworm is found to be effective and increases single larval weight, single cocoon weight, single shell weight, pupal weight, silk ratio, and total haemolymph protein. This supplementation could be recommended to the sericulture farmers in terms of cocoon crop productivity.

ACKNOWLEDGMENT

The author is grateful to Prof. Dr. Abir A. Gad. Department of Applied Entomology and Zoology, Faculty of Agriculture, (El-Shatby), Alexandria University, Egypt, for her valuable helps in experimental design, statistical analysis, beneficial advice, and comments.

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تأثير دعم اوراق التوت بالخميرة *Saccharomyces cerevisiae* والطحلب الاخضر المزرقي *Spirulina platensis* على بعض الخصائص الكمية لدودة الحرير التوتية *Bombyx mori*

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تعتبر تربية دودة الحرير التوتية واحدة من أهم الصناعات التي تعتمد بشكل أساسي على التغذية على أوراق التوت لإنتاج الحرير. لذا فإن إثراء أوراق التوت بالمغذيات التكميلية عامل أساسي لتحسين نمو وتطور ديدان الحرير التوتية، ومن ثم الاستثمار التجاري المناسب لها بشكل جيد. أجريت هذه الدراسة لتحديد تأثير إضافة بعض المركبات الحيوية مثل خميرة الخبز وطحلب السبيرولينا لأوراق التوت على بعض الخصائص البيولوجية والفسيولوجية لدودة الحرير التوتية كوزن اليرقات، وزن العذاري، وزن الشرائق، وزن قشرة الشرائق والنسبة المئوية للحرير وكذلك المحتوى الكلي للبروتين في الدم. أوضحت الدراسة أن وزن اليرقات والعذاري لدودة الحرير قد تم تحسينها بشكل معنوي عند التغذية على أوراق التوت المعاملة بالخميرة بتركيز 0.5% و الطحلب بتركيز 0.05% حيث أدت المعاملة بتلك التركيزات لإرتفاع ملحوظ في وزن اليرقات بما يعادل حوالي 55% و 30% على الترتيب مقارنة بالكنترول. لوحظت نفس النتائج في أوزان العذاري و الشرائق وقشرة الشرائق حيث أحدثت المعاملة بتركيز 0.5% من الخميرة زيادة في أوزان العذاري و الشرائق وقشرة الشرائق بحوالي 30% و 16% و 27% على الترتيب. من ناحية أخرى احتلت المعاملات اليرقية بالسبيرولينا المرتبة الثانية في تأثيرها على أوزان العذاري و الشرائق وقشرة الشرائق. كما وجد أن تغذية دودة الحرير التوتية على مستخلص الخميرة قد زاد معنوياً كل من النسبة المئوية للمؤوية للحرير وكذلك المحتوى الكلي للبروتين الدم مقارنة بمعاملات السبيرولينا أو الكنترول.