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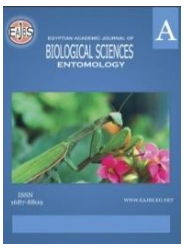

Impact of Adding some Algal Probiotics (*Spirulina Plantalis* and *Azolla Pinnata*) as Food Supplements to Silkworm, *Bombyx mori* L. for Strengthening the Sericulture Industry

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ABSTRACT

Mulberry silkworm is a monophagous insect, so the only way to improve the silk industry is the enrichment of mulberry leaves with supplementary nutrients. In this investigation mulberry leaves treated with algal probiotics (*Spirulina plantalis* and *Azolla Pinnata*) with different concentrations (1, 2, and 3%) from 4th larval instar till spinning to study the impact of these supplements on some biological parameters (larval weight (g.), growth rate%, longevity(hr.) and fecundity (no.)), economical parameters (cocoon weight(g.), cocoon shell weight (g.), shell ratio%, and pupa weight(g.)) and biochemical parameters (total protein, protease, amylase, and invertase). The obtained results indicate that all types of probiotics with different concentrations have a positive impact on all parameters under study but the most effective one was *azolla*$_{2\%}$ that affect positively on the fecundity of females and the shell ratio % of males reduced feeding days in the 5th larval stage that is a very important economical factor.

INTRODUCTION

Mulberry silkworm has an economic importance due to its ability to convert mulberry protein to silk protein. It has attention worldwide, especially countries that are interested in this industry. Lately, Egypt has given attention to this industry so, researchers have tried to improve the silk quality and quantity in different ways.

One of these ways is adding supplements such as proteins, amino acids, botanical extracts and probiotics as a food supplement. Silkworm is a monophagous insect, so the quality of mulberry leaves plays a vital role in the production of silk Stanley and Abraham (2016). Nutritious mulberry leaves are essential for the growth, development, and cocoon production of the larvae Masthan *et al.* (2011). Adding nutritional supplements like vitamins, amino acids, proteins, and probiotics to larval feed can enhance the nutritional efficiency and economic traits of the silkworm Amalarani *et al.* (2011)

Probiotics are live microbial supplements that improve microbial balance and enhance cellular growth Shruti *et al* (2019). Several studies have shown that probiotics have a positive impact on insect health Dillon and Dillon (2004). *Spirulina* is a blue-green microalgae that contains a lot of amino acids, vitamins and $B_{12,}$ Ganeshprabu *et al.* (2011). *Azolla pinnata* is a floating water fern, rich in protein (25-30%) and contains the symbiont

cyanobacterium *Anabaena azollae*, which fixes nitrogen from the atmosphere and also, has essential amino acids (7–10%), minerals (10–15%), vitamins (A, B12, and β-carotene), and growth promoter Shruti *et al.* (2019). After oral administration of *eri* silkworm with mulberry leaves supplemented with *cyanobacteria*, there was an increase in larval and shell weight, as well as commercial cocoon characteristics Kumar *et al.* (2009). The fifth instar silkworms' supplementation with *azolla*, showed positive impacts on cocoon features also, feeding *B. mori* mulberry leaf combined with *spirulina* improved the larval and cocoon characteristics Venkataramana *et al.* (2003).

The factors responsible for the growth and the production of silkworms are the food quality, physiological state of the larvae, enzyme activity and the type of additives Vitthalrao and Sucheta (2012). Silkworm hemolymph plasma almost contains protein particles Marwa (2023). Highly protein mulberry leaves have a positive impact on silk production and are responsible for the activity of the protease enzyme that converts mulberry protein to silk protein Kumar and Kalpana (2009). High quality of silk production depends on the silkworm protein content. The fifth instar hemolymph protein contributes to silk protein biosynthesis in the silk gland. High-quality protein-rich diets are essential for producing the finest silk Shivkumar and Subramanya (2015). Amylase is an important enzyme that concerns carbohydrate metabolism. It was identified with the analysis of digestive fluid or hemolymph Lokesh *et al.* (2012). Invertase enzyme belongs to glycoside hydrolyse family which has different functions in different organisms Cimini *et al.* (2016), Kulshrestha *et al.* (2013). It plays a fundamental role in converting sucrose which is a polysaccharide to glucose and fructose (monosaccharides) Guo *et al* (2018).

This investigation has provided new insights into improving the economic parameters of the sericulture industry by adding green probiotics to regulate the biochemical machinery that directly on the production.

MATERIALS AND METHODS

The experiment was carried out at the Sericultural Research Department, Plants Protection Institute, and Agricultural Research Centre in Giza.

A- Preparation of Stock Culture:

Silkworm seeds were imported from India (PM X CSR_2) and then incubated at 27 ± 1°C and 80 ± 5% RH till hatching. Rearing was carried out under hygienic conditions according to Marwa (2020). Newly hatched silkworm reared in the summer season at 29-32 °C and relative humidity 80-90% RH. Silkworms fed on clean mulberry leaves from hatching till the end of the 3^{rd} larval instar.

B- Preparation of Probiotics Concentrations Solution Used for The Study:

Azolla was collected fresh from the Soil, Water and Environment Research Institute, Agriculture Research Centre, brought to the laboratory, washed thoroughly with clean tap water and subsequently with distilled water. *Azolla* was weighed 000 grams for extraction using 1000 ml distilled water. Initially, *Azolla* was grinded with 100 ml of distilled water (out of 1000 ml) and sieved, the residue was mixed with 100 ml of distilled water and grinded and sieved, and the procedure was repeated 4-5 times and finally, the filtrate volume was made up with the remaining distilled water and treated as stock solution. From this stock solution, different concentrations were prepared viz., one, two and three, percent and the same was used for feed supplementation as done by Vijaykumar *et al* (2016).

Spirulina powder was purchased from the Algal Department, National Research Centre, and 500 ml *spirulina* solution of different concentrations *viz*., one, two and three percent.

C- Experimental Design:

At the beginning of the 4th larval instar, the larvae are divided into 6 groups each group consists of three replicates each one consists of 50 larvae. All groups fed on dipped mulberry leaves with aqueous solution prepared probiotics concentrations (1%, 2%, and 3%). keeping in shade for half an hour to remove excessive wetness, the leaves were fed to the larvae from the first day of the fourth instar. One feed of the probiotics-treated leaves and three feeds of the normal mulberry leaf were given to the silkworms on odd days till the spinning of cocoons.

D- Biological Parameters:

4th larval weight (g.), 5th larval weight (g.), 4th overall growth rate (%), 5th overall growth rate (%), longevity (hr.) and Fecundity (no.).

$$\text{Overall Growth Rate \%} = = \frac{\text{final weight} - \text{initial weight}}{\text{Initial weight}} x100$$

E- Cocoon Indices:

Fresh cocoon weight (g.)(C.W), Fresh cocoon shell weight (g.) (C.S.W), Fresh pupa weight (g.) (P.W) and Fresh cocoon shell ratio (%) (C.S.R) were calculated according to Tanaka (1964).

$$\text{Silk ratio (\%)} = \frac{\text{Weight of cocoon shell (g.)}}{\text{Weight of fresh cocoon (g.)}} x100$$

F- Physiological Characters:

Hemolymph was collected from matured larvae of the 5th larval instar by removal of the thoracic leg in 1.5 ml Eppendorf tubes with a small amount of phenyl thiourea crystal (PTU) as an anti-coagulant substance, Mahmoud (1988). The tubes were kept at -20°c, and the blood samples were centrifuged at 10000rpm for 10 minutes at 5°c. the supernatant was assayed to determine.

a- **Total protein,** was determined by the method of Bradford (1976).

b- **Amylase,** was determined by the method of the modifications of Amin (1998) to the method described by Ishaaya and Swirski(1976)**.**

c- **Invertase,** digestive enzymes were determined according to the modifications of Amin (1998) to the method described by Ishaaya and Swirski(1976).

d- **Protease,** proteolytic enzyme was determined according to Tatchell *et al.* (1972) with some modification of measuring the increasing of amino acid according to Lee and Takabashi (1966).

G- Analysis of Proximate Compositions of The Primary Phytochemicals of Algae:

Powders of *spirulina*, and *azolla* were subjected to proximate composition analysis (crude protein, etheric extract, total ash, moisture and total carbohydrate) following the methodology of Castell and Tiews (1980) as given in AOAC (1995).

H-Preparation of Solvent Extracts of Algae:

The powdered samples of *spirulina*, and *azolla* (75g each) were taken and packed in Whatmann No. 1 filter paper and put into the soxhlet apparatus. The extracts were successively soaked with 300 ml (1:4 w/v) of a polar solvent, methanol (99.9% purity, Changshu Yangyuan Chemicals, China), and a non-polar solvent, petroleum ether (99.98% purity, SRL Pvt. Ltd. India) individually and separately for 6-9 h each (30 to 36 cycle) until a clear colorless solution was obtained. The extracts were filtered by using a double-layered muslin cloth and concentrated at 40-50 °C using a rotary vacuum evaporator (ROTAVAP). The extracts obtained were vacuum-dried under 40°C and used for further investigation. The extracts obtained appeared as dark green, gummy solid.

RESULTS

A- Biological Parameters:

Feeding quality is an important factor affecting all biological parameters, larval weight is an indicator for healthy larvae and good cocoons produced. Table (1), showed weights of the larvae not affected by the additives during the 4th instar, the maximum results recorded for *Spirulina*1%, *Spirulina*2%, *Spirulina*3% and Control on par (0.530 g., 0.519 g., 0.524 g. and 0.505 g.) respectively and the lowest weight recorded (0.441 g.) for *Azolla*1%. But the results differed during the 5th larval instar where, the maximum weight recorded for the larvae that fed on *Azolla*3%, *Azolla*2% and *Spirulina*1% significantly, was 3.63g., 3.52 and 3.62 g. respectively and the control value was the minimum (2.64g.).

Regarding the overall growth rate, the results have the same direction of the larval weights, during 4th instar. The highest values recorded for *Spirulina*1%, *Spirulina*2%, *Spirulina*3% and Control without any significance between each other were, 443.5, 431, 439 and 416 % respectively, and *Azolla*1% recorded the lowest value (354%). Significant results were obtained during the 5th larval instar, the maximum results for overall growth weight were 674.5 and 647 % for *Azolla*2% and *Azolla*3%, respectively and the minimum value was (387.5 %) for the control.

In the larval duration or longevity, the data take another direction, whereas the control has significantly the highest result (768 hr.) representing 32 days but the larvae fed on *Azolla* with all concentrations recorded the lowest values (696 hr.) representing 29 days.

Fecundity is an indicator of the effect of protein on larvae, Table (1) showed that larvae fed on *Azolla*2% recorded maximum significant value (708 eggs), followed by other treatments that were 587, 581, 569, 553 and 549 for (*Azolla*3%, *Spirulina*1%, *Spirulina*3%, *Spirulina*2% and *Azolla*1%) respectively, without any significance between each other. But the lowest value was (464 eggs) for the control.

Table (1): Effect of *Azolla pinnata* and *Spirulina plantalis* as a probiotic on the biological characteristics of the silkworm larvae *Bombyx mori* L.

Treatment parameters	4th larval weight (g.)	5th larval weight (g.)	4th overall growth rate (%)	5th overall growth rate (%)	Longevity (hr.)	Fecundity (n.)
Azolla 1%	0.441 c	2.89 c	354 b	558 b	696 c	549 b
Azolla 2%	0.473 b	3.52 ba	383 ba	647 a	696 c	708 a
Azolla 3%	0.470 bc	3.63 a	380 ba	674.5 a	696 c	587 b
Spirulina1%	0.530 a	3.62 a	443.5 a	584.5 b	702 b	581 b
Spirulina2%	0.519 a	3.42 b	431 a	563.5 b	702 b	553 b
Spirulina3%	0.524 a	3.55 a	439 a	576.5 b	702 b	569 b
Control	0.505 a	2.46 d	417 ba	387.5 c	768 a	464 c
P	<.0001	<.0001	<0.05	<.0001	<.0001	<.0001
L.S. D	0.03	0.125	0.656	0.487	446E-9	64.24

Means with the same letter is not significant

B- Cocoon Indices:

Table (2), shows the impact of different concentrations of *Azolla pinnata* and *Spirulina* plantalis on cocoon characteristics of the silkworm, it was obvious that in females the maximum values of cocoon weight for the larvae that fed on *Azolla*3%, *Azolla*2% and *Spirulina*3% were 1.75 g., 1.67g. and 1.70 g. respectively, with no significance observed between each other but varied significantly from the untreated larvae that recorded the lowest value (1.29 g.).There was a significant rise in cocoon shell weight and

pupa weight in *Azolla*$_{3\%}$, *Azolla*$_{2\%}$ and S*pirulina*$_{3\%}$ (o.296 g., 0.277 g., and 0.284 g.) and (1.46 g., 1.39 g., and 1.41 g.), respectively. But the results concerning to cocoon shell ratio showed no significance between all treatments that were (16.92, 16.78, 16.66, 16.61, 16.56, 16.13 and 15.81 %) for (*Azolla*$_{3\%}$, *Azolla*$_{1\%}$, Spirulina3%, *Spirulina*$_{1\%}$, *Azolla*$_{2\%}$, control, and *Spirulina*$_{2\%}$), respectively.

In the males, the results showed no significant variance in all criteria under study between the following treatments (*Azolla*$_{3\%}$, *Spirulina*$_{1\%}$, *Spirulina*$_{3\%}$, *Azolla*$_{2\%}$, and *Spirulina*$_{2\%}$), the data of cocoon weight was recorded as follows (1.39 g., 1.38 g., 1.35 g., 1.32 g., and 1.32 g) respectively, in cocoon shell weight, (0.271 g., 0.272 g., 0.255g., 0.260 g., and 0.262 g.) respectively and in cocoon shell ratio% the results were 19.33%, 19.68%, 19.35%, 19.65%, and 19.30%\ respectively. But in pupae weight, the arrangement differed (1.11 g., 1.10 g., 1.03 g., 1.05 g., 1.04 g., and 1.04) for (*Azolla*$_{3\%}$, *Spirulina*$_{1\%}$, *Spirulina*$_{3\%}$, *Azolla*$_{2\%}$, *Spirulina*$_{2\%}$, and *Azolla*$_{1\%}$). Also, the control recorded the lowest values significantly for the previous criteria which were 1.18g., 0.200 g., 17.07%, and 1 g. for cocoon weight, cocoon shell weight, shell ratio% and pupa weight respectively.

Table 2: Effect of the Impact of *Azolla pinnata* and *Spirulina platensis* as a probiotic on the cocoon indices of the silkworm larvae *Bombyx* mori L.

Treatment Parameters	♀				♂			
	C.W	**C.S.W**	**P.W**	**C.S.R%**	**C.W**	**C.S.W**	**P.W**	**C.S.R%**
Azolla 1%	1.56 c	0.263 bc	1.31 dc	16.78 a	1.29 b	0.231 b	1.04 ba	17.83 bc
Azolla 2%	1.67 ba	0.277 ba	1.39 bac	16.56 a	1.32 ba	0.260 a	1.05 ab	19.65 a
Azolla 3%	1.75 a	0.296 a	1.46 a	16.92 a	1.39 a	0.271 a	1.11 a	19.33 ba
Spirulina 1%	1.62 bc	0.271 b	1.33 bdc	16.61 a	1.38 a	0.272 a	1.10 a	19.68 a
Spirulina 2%	1.54c	0.244 c	1.27 d	15.81 a	1.32 ba	0.262 a	1.04 ba	19.30 ba
Spirulina 3%	1.70 ba	0.284 ab	1.41 ba	16.66 a	1.35 ba	0.255 ba	1.03 a	19.35 ba
Control	1.29 d	0.208 d	1.08 e	16.13 a	1.18 c	0.200 c	1.00 b	17.07 c
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
L.S. D	0.095	0.024	0.083	1.16	0.07	0.02	0.07	1.72
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
L.S. D	0.095	0.024	0.083	1.16	0.07	0.02	0.07	1.72

C.w: Cocoon weight C.S.W: Cocoon shell weight P.W: Pupa weight C.S.R%: Cocoon shell ratio
Means with the same letter is not significant

B- Biochemical Analysis:

Pooled analysis of silkworm heamolymph showed fluctuations in all biochemical analyses of the larvae fed on enriched mulberry leaves with different concentrations of algal probiotics under study.

Total Protein:

The larvae fed on fortified mulberry leaves with *Azolla*$_{2\%}$ recorded the maximum results, which was a significance of 73. 8 mg/ml. Followed by *Azolla* $_{1\%}$, *Azolla* $_{3\%}$ and *Spirulina* $_{3\%}$ on par were 60.26, 60.30, and 62.26 mg/ml..The lowest value was 38.86 mg/ml, for the control.

Amylase:

The results of the amylase enzyme have the same direction as the total protein data, whereas the maximum significant data for larvae fed on *Azolla*$_{2\%}$ (90.96 u glucose/min/ml) followed by *Azolla*$_{3\%}$ (33u glucose/min/ml) and the lowest result were 11.3, 11.8 and 12.06 for *Spirulina*$_{2\%}$, *Spirulina*$_{3\%}$, and the control without any variance between each other.

Invertase:

The maximum results in invertase enzyme were recorded for *Azolla*$_{2\%}$, *Azolla* $_{3\%}$,

Spirulina $_{1\%}$, and *Spirulina*$_{2\%}$ significantly were 63.53, 29.76, 27.33, and 25.56 ug glucose/min/ml respectively. Followed by the control (20.03 ug glucose/min/ml) and the lowest value was 16.96 ug glucose/min/ml for *Spirulina* $_{3\%}$ significantly.

Protease:

The data of protease enzyme have the maximum significant value (188.66 ug alanin/min/ml) for *Spirulina*$_{3\%}$. Followed by the control which was 142.33 ug alanin/ min/ ml and the lowest data were 66.70, 74.43, and 72 ug alanin/min/ml significantly for *Azolla*$_{1\%}$, *Azolla*$_{2\%,}$ *and Azolla*$_{3\%}$ respectively,

Table 3: Effect of the Impact of *Azolla pinnata* and *Spirulina platensis* on some biochemical analysis of the silkworm larvae *Bombyx mori* L.

Treatment parameters	Total protein (mg/ml)	Amylase (u glucose/min/ml)	Invertase (ug glucose/min/ml)	Protease (ug alanin/min/ml)
Azolla$_{1\%}$	60.26 b	21.70 d	22.73 bcd	66.70 d
Azolla $_{2\%}$	73.8 a	40.96 a	63.53 a	74.43 d
Azolla $_{3\%}$	64.30 b	33 b	29.76 ab	72 d
Spirulina $_{1\%}$	52.43 c	26.06 c	27.33 ab	101.33 c
Spirulina $_{2\%}$	55.46 c	12.06 e	25.56 ab	102 c
Spirulina $_{3\%}$	62.26 b	11.800 e	16.96 d	188.66 a
Control	38.86 d	11.300 e	20.03 b	142.33 b
P	<.0001	<.0001	<.001	<.0001
L.S. D	4.398	2.99	8.04	12.26

Means with the same letter is not significant

C- Chemical Composition of Algal Probiotics:

The results of the approximate analysis of dried *Azolla pinnata* and *Spirulina plantalis* are represented in Table (4). it was obvious that the chemical composition values of *Azolla* were (9.27, 90, 4, 25.8, 23, 17.34, and 19.5 %) for moisture content, dry matter, crude fat, crude fiber, crude protein, total ash, and carbohydrate, respectively. But in *Spirulina plantalis* the values were (9.62, 77.54, 2, 1, 61.77, 7.15 and 23.38 %), respectively.

Table 4: chemical composition of *Azolla pinnata* and *Spirulina Plantalis*

Proximate composition (% on dry wt. basis)	*Azolla pinnata*	*Spirulina plantalis*
Moisture content	9.27	9.62
Dry matter	90	77.54
crude fat	4	2
Crude fiber	25.8	1
Crude protein	23	61.77
Total ash	17.34	7.15
Carbohydrate	19.5	23.38

DISCUSSION

Friendly microorganisms (probiotics) are a very important factor in maintaining a healthy intestine and raising immunity. Although silkworm gut contains different types of microorganisms adding algal probiotics supplements positively on all characters.

A- Biological Parameters:

The previous results indicate that adding probiotics during the 4th larval instar affect negatively on larval weight and 4th overall growth rate of silkworms. These findings are in line with Saad *et al.* (2014) who concluded that full-grown larvae were affected significantly by adding the supplements. Marwa (2023) stated that *Echinacae* botanical extract affects negatively the larvae during the 4th instar. However, the results changed during the 5th larval instar and the additives affect positively on 5th larva weight, 5th overall growth rate, longevity and fecundity. These results are in agreement with Mala *et al.* (2017) who supplemented mulberry leaves with *Alo vera* that gave a positive impact on silkworm growth. Shruti *et al.* (2019) reported that adding *Azolla* and *Spirulina* to mulberry leaves increased body weight. Shinde *et al.* (2014) silk industry was affected positively by mulberry variety and nutritive additives. Also, Masthan *et al.* (2017) reported the positive impact of *Spirulina* on silkworm larval weight. The overall growth rate increased by adding the honey bee to the mulberry leaves Sharma *et al* (2023).

Longevity or larval duration is an important economic factor in sericulture, the reduction of larval duration saved the consumed mulberry leaves. In this investigation adding *Azolla pinnata or Spirulina* reduced the larval duration. Mulberry leaves enriched with soya bean reduced the larval duration Horie and Watanabe (1983).

Larval duration is reduced by adding *Azolla* to mulberry leaves during the spring season. Furthermore, increasing larval duration depends on the additives and the type of probiotics Shruti *et al.* (2019). Silkworms fed on mulberry leaves smeared with *Spirulina fusiforma* during the 5th instar consumed 6 days otherwise untreated group took 9 days Mathavan *et al.* (1984).

As shown previously, moths that emerged from the larvae fed on mulberry leaves sprayed with 2% *azolla pinnata* and laid the maximum number of eggs significantly. This result was confirmed with the female pupal weight that recorded the highest weight in this investigation. These results are in line with Singh and Prasad (1987)who referred to the positive correlation between pupal weight and fecundity. There was a linear correlation between *spirulina* concentrations and the number of laid eggs Kumar and Bakiya (2023). The number of eggs laid increased as a result of adding soya bean to the feeding of mulberry silkworm Sawsan (2020).

B- Cocoon Indices:

Cocoon weight is an important economic indicator in sericulture that refers to the approximate quantity of raw silk output. As shown previously feeding enhanced with probiotics (*Azolla pinnata*, and *Spirulina plantalis)* had a positive impact on cocoon parameters. Our results conform with Venkataramana *et al.* (2003) who reported the positive impact of adding *Spirulina* to the feeding of silkworm larvae during 5th instar on cocoon characters. Feeding silkworm larvae on mulberry leaves smeared with *Spirulina* and thyroxine during the 5th instar affected positively on the cocoon parameters (cocoon weight, shell weight, and shell ratio) Maqbool *et al*. (2023). Vijaykumar *et al.* (2016) referred to the increase in cocoon weight, and shell weight resulting when added protein source to silkworm larvae feeding. Cocoon weight and cocoon shell increased by adding *Azolla pinata* 50% over control Shruti *et al.* (2019). Also, our results are comparable with Sujatha *et al.* (2015) who reported that certain concentrations of algal extract improved the biological and economic characteristics of silkworm larvae. Adding 3% of probiotics increased the cocoon parameters of silkworm larvae Pachiappan *et al.* (2021). Sekar *et al.* (2016) reported that larvae fed on mulberry leaves coated with *Lactobacillus Casei* improved cocoon parameters.

C- Biochemical Analysis:

Previous findings of the present investigation imply that adding algal probiotics

as a food supplementary has a good impact on enzymatic activity that increases total protein, amylase, invertase and protease enzymes in the treated larvae compared to untreated larvae. Protein is an aid in the development, growth and silk production our investigation recorded the highest activity for larvae fed on 2% of *Azolla pinnata.* This results in agreement with Wani *et al.* (2021) hemolymph protein content varied according to the rate of protein metabolism. Larvae fed on mulberry leaves coated with different concentrations of *chlorella pyrinoidosa* increased heamolymph protein Dyanaselin (2021). Hemolymph protein increased at 2% of probiotics normagut in silkworm larvae Anisha *et al.* (2022). Although *Spirulina plantalis* had a high protein content of about 61% from its dry weight of protein its effect was less than *Azolla* in all biological economic and biochemical analyses. These results conform with Marwa (2024) who referred to the negative effect of high doses of egg albumen on silkworm production.

Amylase enzyme plays a vital role in complex carbohydrate digestion so increasing the activity does not mean an increase in the production directly but supports the larvae with energy to complete its life cycle. An increase in hemolymph amylase activity results from increasing metabolism that raises enzyme secretion Lokes *et al.* (2012). Pachiappan *et al.* (2021) concluded that amylase and invertase activity increased by increasing the concentration of *Bifidobacterium longum* in silkworm feeding.

About the protease enzyme, the increase in the activity in the larva fed on mulberry leaves enriched with *spirulina* resulted from its acting as an inducer for releasing and activating enzyme. Our findings are consistent with Bhuvaneswari *et al.* (2023) who reported the importance of *spirulina* for the activation of protease enzyme. Enzyme activity varied due to the food chemical constituted Kumari *et al.* (1997). *Spirulina* is an important factor in protein digestion by raising protease activity Selin *et al.* (2018). In this investigation although larvae fed on mulberry leaves smeared with 3% of *Spirulina* showed high activity of protease enzyme but recorded lower protein content and biological parameters than *Azolla pinnata*. These results are in line with Ruth *et al.* (2019) who referred to the larvae fed on Johart mulberry leaves recorded a high increase of protease enzyme but larvae fed on TR10 mulberry leaves recorded high protein content that resulted from the ability of feeding to facilitate the reaction of enzyme on its substrate.

CONCLUSION:

Adding algal probiotics (*Azolla* or *Spirulina*) to the feeding of silkworms had a positive impact on growth and enzyme activity. Although the most effective concentrations in all criteria under study were *Azolla* (2% and 3%), and *Spirulina* (1% and 3%) the most recommended one was $Azolla_{2\%.}$ It affects significantly the fecundity in the females and the shell ratio in the males. Also, reduced larval duration than *Spirulina.*

Declarations:

Ethical Approval: Ethical Approval is not applicable.

Competing interests: The authors declare no conflict of interest.

Funding: No funding was received.

Availability of Data and Materials: All datasets analysed and described during the present study are available from the corresponding author upon reasonable request.

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