

Plant Production and Pathology Research

Available online at http://zjar.journals.ekb.eg http://www.journals.zu.edu.eg/journalDisplay.aspx?Journalld=1&queryType=Master



IMPACT OF Azolla pinnata AND Lemna minor AS ARTIFICIAL DIETS AND NUTRITIONAL ADDITIVES ON MULBERRY SILKWORM, Bombyx mori L.

Enas M. Y. Elyamani¹, Mohammed A.I. Youssif^{2*}, Sherin M. M. Y. Helaly² and Walaa M. M. Helaly²

- 1. Sericulture Dept., Plant Protection Research Institute, Agric. Research Center, Giza, Egypt.
- 2. Plant Prot. Dept., Fac. Agric., Zagazig Univ., Egypt.

Received: 12/02/2025; Accepted: 14/04/2025

ABSTRACT: The mulberry silkworm, *Bombyx mori* L. is considered one of the most important insects due to its production of raw silk, which is used in many industries, as well as in the medical field. In this study, the azolla and water lens (duckweed) were used as protein-rich plant sources to feed the mulberry silkworm larvae through as artificial diet and as a nutritional supplement. It was found that feeding first instar larvae of B. mori on artificial diet containing duckweed powder at a ratio of 1 g per 10 g of T0 reduced significantly the mortality rate, followed by a medium including 2 g of duckweed per 10 g of T0. In contrary, larvae fed on artificial diet containing azolla powder at concentrations of 2 and 4 g per 10 g of T0 resulted in total mortality. Generally, the artificial diet containing the duckweed powder at a concentration of 1 g per 10 g of T0 produced the most favorable results in comparison to other concentrations. Control treatment T0 was more effective than all other treatments. Rearing 5th instar larvae of B. mori on mulberry leaves treating with water extracts of azolla and duckweed at all concentrations caused significant differences in weights of larva, silk gland, cocoon and cocoon shell, meanwhile the control larvae showed the least means. The maximum percentage of 5th larval mortality was observed when larvae were fed on mulberry leaves treated with 4% azolla. Concerning silk filament parameters, it was found that 5th instar silkworm larvae fed on mulberry leaves dipped in azolla 2% water extract recorded the highest silk filament length, weight, and size, while Duckweed 4% treatment showed the lowest means. In conclusion, both azolla and duckweed significantly enhance the nutritional value of mulberry leaves because of their higher protein levels and essential amino acids, which are vital for the proper growth and development of silkworm larva, but further study is required to determine how best to include and balance them..

1958).

Key words: Aquatic plants- Azolla- Duckweed- Silkworm- Protein

INTRODUCTION

Silkworm, *Bombyx mori* L. is one of the well known beneficial lepidopteron insects for the production of raw silk which considered queen of textiles (**Soumya** *et al.* **2017**). Larvae of mulberry silkworms, a monophagous insect that feeds on the mulberry for its whole growth and development (**Manu** *et al.* **2020**). Lately, Egypt has given attention to this industry, therefore many research have tried to improve the silk quality and quantity in different ways.

The term *Azolla* combines two Greek words "Azo" (to dry) and "Allyo" (to kill), reflecting

et al. 2005 and Amalarani et al. 2011).

Silk production depends on the larval nutrition

Nutritional supplements include vitamins,

amino acids, proteins and probiotics which added

to larval feed tend to enhance the nutritional

efficiency and economic traits of silkworm (Singh

and nutritive value of mulberry leaves, which are vital in producing good-quality of cocoons (**Legav**

^{*} Corresponding author: Tel.:+ 201020151153 E-mail address: sherinm82@yahoo.com

plants failure to thrive under dry circumstances (Lumpkin and Plucknet 1982).

Azolla is a floating water fern, widely distributed in aquatic habitats like ponds, canals and paddies in temperate and tropical regions.

Azolla pinnata R.Br. is rich in protein (25-30 %), essential amino acids (7-10 %), minerals (10-15 %) like Ca, P, K, Fe, Cu, Mg.....etc. and vitamins *viz* A, B₁₂ and B- Carotene and growth promotor intermediaries.

In addition, duckweed is a small, invasive, free floating aquatic plant widely described in phytoremediation research (**Del Buono** *et al.* **2022**). Recently, duckweed (*Lemna minor* L.) has been considered as a plant-based ingredient for future foods and as a suitable alternative source of protein.

Lemna minor is characterized by a wide phytochemical composition, including amino acids, sterols, terpenoids, glucosinolates, polyphenols and organic acids (**Zhang** et al. **2023**). This plant contains 20-30 % protein, as well as represents a good source of essential amino acids (**Yahaya** et al. **2022**).

Silkworms have an open circulatory system containing haemolymph. High-quality of silk production based on the silkworm protein content (**Kumar and Kalpana 2009**). The fifth instar haemolymph protein contributes to silk protein biosynthesis in the silk gland. High quality protein rich diets are necessary for producing the best silk (**Shivkumar and Subramanya 2015**).

This investigation aimed to improve the biological, technological as well as physiological parameters by using each *Azolla pinnata* and *Lemna minor* powders as sources of high protein as an artificial diet or food supplementary.

MATERIALS AND METHODS

Silkworm rearing

The study was conducted at the Laboratories of the Sericulture Department, Plant Protection Research Institute, Agriculture Research Center and the Faculty of Agriculture, Zagazig University, Egypt . Eggs of silkworm, Bombyx mori (H1 x KK x GK3 x V2 hybrid) were obtained from the Sericulture Research Department of Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt. The hatched larvae were reared under laboratory conditions of 28± 2 °C and 70 ±5% RH.

Each of the two tested sources of protein *Azolla pinnata* and *Lemna minor* were collected fresh from Dr. Ahmed Azazy production farm, Hussenia district, Sharkia Governorate, washed thoroughly with clean tap water and distilled water then let to dry under room conditions.

Using each Azolla pinnata and Lemna minor powders as an artificial diet

Artificial diet content and preparation

The 1st and 2nd larval instars were fed on the artificial diets and they completed the rest of the instars fed on fresh mulberry leaves.

Experiment design

The powders of azolla and duckweed plants were used after washing and shade-drying green plants.

The experiment was divided into three groups (T0, T1 and T2). T0 is the basic diet which is illustrated in Table (1). T1 is the diet containing azolla powder and is used in three concentrations (1 gm, 2gm, and 4 gm azolla powder) which was added to 10 gm of the the basic diet T0. T2 is the duckweed powder and is used in three concentrations (1 gm, 2gm and 4 gm duckweed powder) which was added to 10 gm of the basic diet T0.

Potassium sorbate (0.1g.) is a normal organic compound used as an antiseptic (**Miao** et al. **2001**) to all treatments which dissolved in distilled water when cook dry materials. All dry materials found in Table (1) were milled well in the blender and cooked in an autoclave for about 40 min at 105 °C. The diet lets cool under room -temperature and then maintained in a refrigerator at 5 °C until use.

The treatments T1 and T2 concentrations were added to the components of the basic diet separately and cooked as previously described.

Table (1). Composition of the Basic Artificial Diet T0.

	Diet constituents	Amounts(g)
1	Mulberry leaf powder	31.3
2	Soybean powder	31.3
3	Corn powder	31.1
4	Citric acid	2.5
5	Salt mixture	1.5
6	Distilled water	200 ml

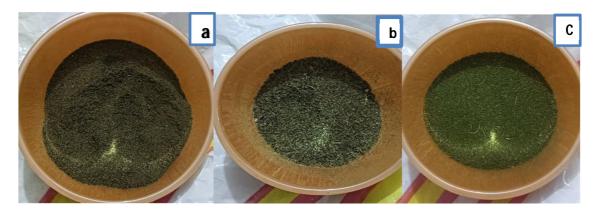


Fig. 1. Ground plants (a) Azolla pinnata R. Br. (b) Lemna minor L. (c) Morus alba L

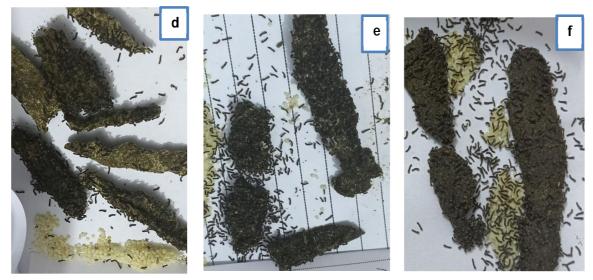


Fig. 2. Media for 1st instar, since (d) T0, (e) T1: 1 gm azolla/10 gm T0 and (f) T2: 1 gm duckweed/10 gm T0

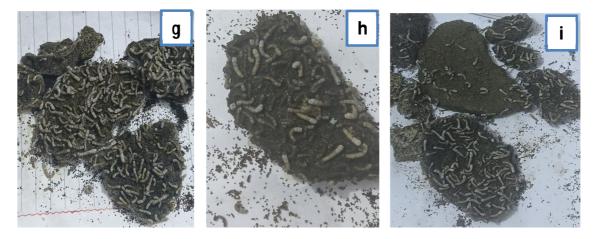


Fig. 3.Media for 2^{nd} instar, since (g) is T0, (h) is T1:1 azolla gm /10 gm T0 and (i) is T2:1 gm duckweed/10gm T0

Each treatment had 0.25 gm of silkworm eggs. The artificial nutrition slices were provided once daily during the 1^{st} and 2^{nd} larval instars. After the 2^{nd} moult, the fresh mulberry leaves were offered to all treatments until the end of the larval stage. The basic diet T0 is considered the control group.

Since the preliminary experiment using artificial diets by adding powdered of azolla and duckweed plants recorded undesirably or ineffective observations for silkworm larvae, especially at higher concentrations, it was noted that the larvae were reluctant to feed. Therefore, the aqueous extracts of both two plants were tested as a nutritional supplement to mulberry leaves for feeding fifth-instar larvae.

Using each Azolla pinnata R.Br. and Lemna minor L. Powders as Food Supplementary

Preparation of Azolla and Duckweed Solutions for Water Extract Treatments

One, two and four grams of the tested floated plants were soaked in 100 ml water for 30 minutes and then filtered. The filtrate water volume was used in this experiment. Mulberry leaves dipped in the prepared solution (1, 2 and 4 %) for 15 minutes.

Experimental Design

Larvae of mulberry silkworm, *Bombyx mori* fed on fresh clean mulberry leaves from hatching till the end of 4th instar 4 times /day. At the beginning of the 5th larval instar, the larvae were divided into 7 groups, each group consisting of three replicates with 150 larvae. Larvae fed on mulberry leaves treated with aqueous extract of 1, 2 and 4 % *Azolla* & 1, 2 and 4 % *Lemna* twice a day as well as two feeds on normal mulberry leaves until they reached the spinning stage. The control larvae fed on fresh clean mulberry leaves 4 times / day.

Biological Parameters

The different parameters were calculated

Weight of mature larva (g)

Five mature larvae were taken randomly per replication per treatment and weighed on a digital balance to record the weight.

Weight of silk gland (g)

Five larvae per treatment were dissected, silk gland removed and put on filter paper then weighed by the digital balance.

Cocoon, pupa and cocoon shell weights

Ten cocoons (5 male and 5 female) were picked randomly from each treatment and weighed, cut by a cutter to get out the pupae, then the pupae and cocoon shells were weighed and recorded separately, (Islam et al. 2020).

Technological Parameters

Filament Length, Weight and Size

Five cocoons from each treatment were taken and dried in oven at 60° C for eight hours, then reeled by aid of reeling machine. Length and weight of silk filament of each cocoon were recorded. With regard of silk filament size, it was calculated by formula of **Tanaka 1964** as follows:

$$Filament size = \frac{\text{Weight of filament}}{\text{Length of filament}} \quad X \quad 9000$$

Physiological Parameters

Five healthy larvae on the 7^{th} day of the 5^{th} larval instar were taken from each treatment for measuring physiological parameters. haemolymph was collected by cutting one of the prolegs with scissors and the blood was collected by an insulin syringe of 2 ml. The collected blood was transferred in sterilized, clean eppendorf tubes containing 0.002 g of phenylthiourea crystals as an anti-coagulant substance (Mahmoud 1988). The tubes were kept at -20 °C., then the haemolymph samples were centrifuged at 1000 rpm for 10 minutes at 5 °C. to determine ALS and AST enzymes. As well as a part of haemolymph was taken to study the effect of each Azolla and Lemna on complete blood count. The counting of the free haemocytes, was done by using haemocytometer with improved double Naubauer ruling. The mixed fluid was drawn into the chamber and the cells were counted under the light microscope.

RESULTS AND DISCUSSION

Effect of Artificial Nutrition of Diets Containing Azolla and Duckweed on The Larval Weights and Mortality Percentages

According to the results presented in Table (2), the use of duckweed powder treatment with 1 g / 10 g of T0 significantly reduced mortality rate, recording 19.33%, followed by the

application of medium contained the duckweed 2 g / 10 g of T0, which resulted in a mortality rate of 26.33%. In contrast, when using an artificial feeding media supplemented with azolla powder at concentrations of 2 and 4/10 g of T0, total mortality (100%) was observed among the larvae offered this diet, as the larvae showed no attraction to this food source. Furthermore, if larvae were manually placed on this artificial medium, they did not consume it, leading to total mortality within two to three days after hatching.

Silkworms have a specialized olfactory receptor that is specifically adapted to sense the jasmine-scented compound cis-jasmone, which plays a vital role in helping them find their main food source, mulberry leaves **Cell Press**, (2009).

The larval mortality by the addition of azolla powder to the artificial media at concentrations of 2 and 4/10 g of T0 may be due to the strong distinctive odor of azolla **Loreto** *et al.* (2006), **Fons** *et al.* (2018) and **Liu** *et al.* (2021), this odor probably masked the distinct scent of mulberry leaves, resulting in an anti-feeding response in the larvae that could ultimately lead to their death. This scent is essential because it serves as the main attractant, making mulberry the exclusive host plant for silkworm larvae. Therefore, we must use small concentrations of both plants if we want to obtain the best results by feeding mulberry silkworms an artificial feed containing the ground of the duckweed and azolla.

Regarding the weight of larvae at the end of the second instar, as indicated in Table 2, results show that artificial diet using duckweed powder at a concentration of 1 g / 10 g of T0 yielded the best results compared to other concentrations. However, it remains that using the control treatment T0 was superior to all other treatments. Additionally, using azolla powder in artificial feeding, particularly at concentrations of 2 and 4 g / 10 g of T0, resulted in 100% mortality among the larvae, therefore, no larvae remained for weighing at the end of the second or fifth instars. The findings demonstrated that azolla and duckweed powder were ineffective as artificial food when added at concentrations of 1, 2, and 4 grams per 10 grams of the recommended T0, even though they are rich in proteins and amino acids. Consequently, the study focused on using their aqueous extract as a food supplement by immersing mulberry leaves in these water extracts.

Using Azolla pinnata R.Br. and Lemna minor L. Water Extracts as Food Supplementary

The impact of feeding the fifth instar larvae of *Bombyx mori* L. on mulberry leaves treated with different concentrations of azolla and duckweed as nutritional additives was studied. The obtained results can be presented and discussed as follows:

Biological Parameters

Data given in Table (3), revealed that the mean weight of 5th instar larvae recorded 3.636, 3.430, 3.342, 3.388, 3.350, 3.304, 3.304 and 2.990 gm when larvae were fed on mulberry leaves treated with water extract of azolla 1, 2, 4 %, duckweed 1, 2, 4 % and control, respectively. Generally, treating mulberry leaves with two water extract plants had better 5th instar larval weight in comparison with control. Statistical analysis of data revealed highly significant differences between means.

These results are in agreement with those of **Vijaykumar** *et al.* (2016) who reported the silkworm hybrid PM x CSR2 reared on mulberry leaves supplemented with 50 percent azolla concentration gave higher larval weight. **Shruti** *et al.* (2019a), reported that adding azolla to mulberry leaves increased body weight. Also, **Moustafa, Marwa** (2024) reported that all tested types of pro-biotic with different concentrations have a positive impact on all biological parameters.

Regarding to the silk gland weight, it was found that feeding 5th instar larvae on mulberry leaves supplementary with duckweed 1% concentration resulted in the highest weight of silk gland recording 1.0067 gm, followed by duckweed 2% (0.9767 gm). Meanwhile, control larvae represented the lowest mean (0.8166 gm) (Table 3). Analysis of data revealed insignificant differences between means.

The nutrients supplemented through mulberry leaves in terms of probiotics might have contribute to additional growth of silkworm, thereby increasing the body weight and silk gland weight, this may be due to stimulation effect on protein synthesizing machinery of silkworm and silk gland cells (Shruti, et al. 2019b).

Table 2. Effect of artificial nutrition of diets containing azolla and duckweed on mortalit	y and
the 2^{nd} larval weight of <i>Bombyx mori</i> L.	

Treatments	Amount	Larval mortality %	Weight of 2 nd instar g/10 larvae	Weight of 5 th instar g/ larvae
	1 gm.	55.67	0.112	2.594
T1 (Azolla)	2 gm.	100		
	4 gm.	100		
	1 gm.	19.33	0.224	2.632
T2 (Duckweed)	2 gm.	26.33	0.164	2.476
	4 gm.	49.67	0.124	2.144
T0 Basic diet (control)		12.33	0.266	2.708
$\mathrm{LSD}_{0.05}$		5.758 **	0.0263**	0.0891**
P		0.000	0.000	0.000

^{**} indicates highly significant differences between means at 0.01 level of probability.

Table 3. Effect of water extracts of both azolla and duckweed as nutritional additives on some biological aspects of mulberry silkworm *Bombyx mori* L.

Treatments	Conc.	5 th instar weight	Silk gland weight	Pupal weight (gm)		Larval mortality
		(gm)	(gm)	Female	Male	%
T1 (Azolla)	1 %	3.636	0.8667	1.134	0.968	2.22
	2 %	3.430	0.9067	1.128	0.846	0.00
	4 %	3.342	0.8667	1.138	0.858	4.44
T2 (Duckweed)	1 %	3.388	1.0067	1.130	0.874	0.00
	2 %	3.350	0.9767	1.118	0.866	2.22
	4 %	3.304	0.8333	1.028	0.858	2.22
Control		2.990	0.8166	0.994	0.802	0.00
$LSD_{0.05}$		0.2634**	Ns	0.078**	ns	ns
P		0.0027	0.292	0.001	0.5157	0.1095

^{**} indicates highly significant differences between means at 0.01 level of probability.

These results are in agreement with those of **Kumar and Balasubramanian** (2014) who observed *Spirulina* supplementation at higher concentration at five percent had better response on silk gland weight (617.43 mg) when compared to one percent (485.47 mg). Also, **Khazaei** et al. (2019), mentioned that lentil seeds are a good source of endophagous amino acids which considered the vital component of silk gland.

The maximum percentage of 5th larval mortality (4.44%) was noticed when *B. mori*

larvae were fed on mulberry leaves treated with 4% azolla concentration, whereas, the minimum percentage (0.00%) recorded with each of azolla 2%, duckweed 1% and control treatments. The other treatments showed the intermediate mean (2.22%)(Table 3).

Concerning the pupal weight, it was found that supplementary mulberry leaves with azolla 4% and 1% resulted in the highest means of female and male weight, 1.138 and 0.968 gm, respectively. On the other hand, control treatment represented the lowest means of female and male weight, 0.994 and 0.820 gm,

ns indicates no significant differences between means at 0.01 level of probability.

successively. The differences between means are highly significant in female pupal weight and insignificant in male pupal weight.

Generally, the variation in the response of probiotics may be attributed to supplementation of nutrients which in turn might have played role on metabolic process in silkworm. These results agree with **Moustafa, Marwa** (2024) who reported that all tested types of probiotics with different concentrations have a positive effect on pupal weight.

Finally, azolla and duckweed affected positively the biological parameters, it may be due to the fact that, azolla is a rich source of protein (25-30%), essential amino acids (7-10%) and minerals (10.15%) **Vijaykumar** *et al.* (2016).

Technological Parameters

Data in Table (4) showed the impact of different concentrations of *A. pinnata* and *L. minor* on characteristics of the silkworm. It is obvious that treating mulberry leaves by 1% azolla gave the highest means of female and male cocoon weight, 1.462 and 1.216 gm, respectively. Control larvae represented the lowest means for female and male cocoon weight, 1.264 and 1.088 gm, successively. Analysis of data revealed highly significant differences for female cocoons weight and insignificant in male ones.

For cocoon shell weight, data in Table (4), cleared that the mean female shell weight recorded 0.330, 0.276, 0.304, 0.268, 0.288, 0.250 and 0.256 gm for *B. mori* larvae fed on mulberry leaves treated with azolla 1, 2, 4 %, duckweed 1, 2, 4 % and control, respectively. The differences between means were highly significant. In case of male shell weight, it was found that treating mulberry leaves with duckweed 2%, resulted in the highest weight (0.302 gm), meanwhile, duckweed 4% gave the lowest mean (0.246 gm), with no significant differences between means. Generally, it was found that females ones recorded higher weights for cocoon and cocoon shell weights than male ones.

Concerning silk filament indices, data presented in table (4), cleared that 5th instar silkworm larvae fed on mulberry leaves dipping in azolla 2%, represented the highest means of silk filament length, weight and size, showing, 1112.56 m, 0.332 gm and 2.692 dn, respectively.

On the other hand, duckweed 4% gave the lowest silk filament length and weight, 926.24 m and 0.236 gm, successively. While control larvae resulted in the lowest silk filament size (2.244 dn). The differences between means were insignificant in silk filament length, highly significant and significant for silk filament weight and size, respectively.

These findings are in harmony with those of **Sujatha** *et al.* (2015) who reported that certain concentrations of algal extract improved the economic characteristics of silkworm larvae. **Vijakumar** *et al.*(2016) reported that azolla has proteinaceous source can enhance cocoon weight, shell weight and silk production. Also, **Shruti** *et al.* (2019a) found that cocoon weight and cocoon shell weight increased by adding *Azolla pinata* 5% compared with control. Adding 3% of probiotics increased the cocoon traits of silkworm larvae (**Pachiappan** *et al.* 2021).

Physiological Parameters

Haemolymph is the only extracellular fluid containing the products needed for every physiological exertion of the insect body. Therefore, changes in the composition of haemolymph reflect the physiological and biochemical metamorphosis taking place in the insect tissues (Ponmurugan and Karthikeyan 2017). Biochemical analysis of silkworm haemolymph showed fluctuations in all biochemical analysis of the larvae fed on enriched mulberry leaves with different concentrations of the two plant extracts.

Data in Table (5) indicated that the content of total protein differs according to food supplementary type which increased significantly in tested treatments comparing with control, recording 41.3, 31.9, 17.5, 28.5, 12.4, 11,3 mg/ml for larvae fed on mulberry leaves dipped in solutions of azolla 4, 1, 2 % and duckweed 2, 4, 1%, respectively, compared with 8.4 mg/ml in control.

Azolla is known for its high protein content, ranging from 25% to 35% on a dry weight basis (Kamalasanana et al. 2002). This protein is crucial for the growth and development of silkworm larvae, as proteins are essential for various physiological functions, including silk production.

Table 4. Effect of water extracts of both azolla and duckweed as nutritional additives on some technological aspects of mulberry silkworm, *Bombyx mori* L.

Treatments	Conc.	Cocoon wt.(gm)		Shell wt.(gm)		Filament length	Filament wt.(gm)	Filament size (dn.)
		Female	Male	Female	Male	(m.)	······································	Size (uiii)
T1(Azolla)	1 %	1.462	1.216	0.330	0.256	1039.72	0.306	2.662
	2 %	1.430	1.158	0.276	0.266	1112.56	0.332	2.692
	4 %.	1.454	1.192	0.304	0.288	1111.12	0.294	2.376
T2	1 %	1.420	1.158	0.268	0.282	1001.12	0.278	2.506
(Duckweed)	2 %	1.428	1.150	0.288	0.302	1019.72	0.260	2.284
	4 %	1.296	1.136	0.250	0.246	926.240	0.236	2.300
Control		1.264	1.088	0.256	0.264	1024.00	0.250	2.244
$LSD_{0.05}$		0.0867	ns	0.0315**	ns	ns	0.0473**	0.334*
P		0.0001	0.0906	0.0002	0.091	0.0609	0.0036	0.026

^{**} indicates highly significant differences between means at 0.01 level of probability.

Table 5. Effect of water extracts of both azolla and duckweed as nutritional additives on some physiological aspects of mulberry silkworm, *Bombyx mori* L.

Treatments	Conc.	Serum total protein mg/ml	Serum ALT (SGPT) mg/ml	Serum AST (SGOT) mg/ml	Total haemocytic count. Cells/mm ³
T1(Azolla)	1 %	31.9	21	95	204735
	2 %	17.5	51	172	221891
	4 %	41.3	69	371	362540
T2	1 %	11.3	39	194	295631
(Duckweed)	2 %	28.5	85	314	349467
	4 %	12.4	211	405	354231
Control		8.4	173	305	225628

Regarding ALT enzyme, it was increased only in the haemolymph of 5th instars larvae reared on mulberry leaves treated with duckweed 4% solution (211 mg/ml) than control (173 mg/ml), meanwhile the rest treatments showed less means than the control. Concerning AST enzyme, it was noticed that feeding 5th instar larvae of *B. mori* on mulberry leaves enriching with azolla 4% and duckweed 2, 4% solution resulted in the highest means of 371, 314 and 405 mg/ml, successively than the control (305 mg/ ml).

Data compiled in Table (5) revealed that the total haemocytic count (THC) during 5th larval stage of *B. mori* increased gradually by

increasing the concentration of azolla and duckweed which mulberry leaves treated with its solution. Generally, 4% of the tested solution resulted in the highest THC showing 362540 and 354231 cells/mm³ for azolla and duckweed, respectively compared to control 225628 cells/mm³.

Generally, Heamocytes plays an important role in the survival of insect species and are of economic and medical importance (**Siddiqui and Al-Khalifa**, **2014**). These results are in harmony with those of **Wago and Ichikawa** (1979) and **Kerenhap** *et al.* (2007) who reported that gradual increase of THC in larvae of *B.mori* by using different organic manures.

ns indicates no significant differences between means at 0.01 level of probability.

It has been demonstrated that adding water extracts from duckweed and azolla to mulberry leaves increases the weight and overall growth rate of silkworms, improving the properties of their cocoons. When fed to mulberry leaves, they have a rich profile of essential amino acids that are critical for the weight, growth, and cocoon properties of the larva (Muzamil et al. 2023). Silkworms' performance in terms of weight gain and silk production can be improved by the presence of bioactive compounds in aquatic plants, which can serve as growth boosters and increase the nutritional value of their diet (Moustafa, Marwa 2024).

Conclusion:

Azolla and duckweed, aquatic plants with high protein content, could improve silkworm diets, leading to increased silk production and quality. However, further research is needed to optimize their inclusion, balance with other components. Overall, exploring the potential of azolla and duckweed as sustainable and cost-effective protein sources for silkworms warrants further investigation.

REFERENCES

- Amalarani, G., Padmalatha, C. and Ranjith, S.A. (2011). Probiotic supplementations to improve commercial characteristics, disease resistance and protein in the silkworm *Bombyx mori* L. ISPROMS, 4(2): 1994-2108.
- Cell Press. (2009). Why Silkworms Find Mulberries Attractive. *Science Daily*.
- Cui, W.-Z.; Wang, Y.-W. and Mu, Z.-M. (2001). Gustation electrophysiological response to vitamin C of silkworm. Acta Entomol. Sinica 27: 92-95.
- Del Buono, D., Bartucca, M. L., Ballerini, E., Senizza, B., Lucini, L. and Trevisan, M. (2022). Physiological and biochemical effects of an aqueous extract of *Lemna minor* L as a potential biostimulant for maize. Journal of Plant Growth Regulation, 41: 3009 3018.

- Fons, F., Froissard, D., Morel, S., Bessière, J.M., Buatois, B. and Sol, V. (2018) Pteridaceae fragrant resource and bioactive potential: a mini-review of aroma compounds. Natural Product Communications, 13(5), 651–655.
- Halawa, F. Samah; El-Dash, A. A; El-Defrawy, B., Saad, Mahmoud S. I. and Abdelaa, A.A.A. (2023). Biological and technological aspects of silkworms *Bombyx mori* 1. affected by artificial nutrition in comparison with natural nutrition. Menoufia Journal of Plant Protection, Volume 8, Issue 6, P.P. 96-106.
- Islam, T., Khan, I.L., Ganie, N.A., Sahaf, K. A., Jan, N. and Gora, M. M. (2020). Impact of egg albumen (egg white) fortified mulberry leaf on rearing and some cocoon parameters of silkworm, *Bombyx mori* L. (CSR6 X CSR26) X (CSR2 X CSR27) double hybride. International Journal of Chemical Studies, 8(4): 267 271.
- Kamalasanana P., Premalatha, S. and Rajamony, S. (2002). Azolla A sustainable feed substitute for livestock. Leisa India, March, pp. 15-17.
- Kerenhap, W., Thiagarajan, V. and Kumar, V. (2007). Biochemical and bioassay studies on the influence of different organic manures on the growth of Mulberry Variety V-1 and silkworm, *Bombyx mori* L. Casp J. Envi Sci. 5(1), 51-56.
- Khazaei, H., Subed, M., Nickerson, M., Martinez Villaluenga, C., Frias, J. and Vandenberg, A. (2019). Seed protein of Lentils: Current Status, Progress and Food Applications. Foods, 8 (391): 1 23.
- Kumar, K. and Balasubramanian, U. (2014). Supplementary effect of *Spirulina* on lipids and enzymes in silk gland of silkworm, *Bombyx mori* L. JEZS, 2 (4): 279-282.
- Kumar, M.N.A. and Kalpana, G.V. (2009). Studies on the seasonal variation in the protease activity in different developmental stages of

- productive and robust silkworm breeds and their hybrids of *Bombyx mori* L. National Journal of Life Sciences, 6 (1): 113 119.
- Legay, J.M. (1958). Recent advances in silkworm nutrition. Annual Review of Entomol., 3: 75 86.
- Liu, B., Zhang, L., Rusalepp, L., Kaurilind, E., Sulaiman, H.Y. and Püssa, T. (2021). Heat priming improved heat tolerance of photosynthesis, enhanced terpenoid and benzenoid emission and phenolics accumulation in Achillea millefolium . Plant, Cell and Environment, 44(7): 2365–2385.
- Loreto, F., Barta, C., Brilli, F. and Nogues, I. (2006). On the induction of volatile organic compound emissions by plants as consequence of wounding or fluctuations of light and temperature. Plant, Cell and Environment, 29(9): 1820–1828.
- Lumpkin, T.A. and Plucknett, D.L. (1982). Azolla as a green manure: Use and management in crop production. Westview Tropical Agriculture Series No. 5. Westview Press, Bloder, Colorado. 230 pp.
- Mahmoud, S.M. (1988). Activation of silk secretion by silkworms, *Philosamia ricini* and *Bombyx mori* L. after applying antibiotics. Ph. D. Thesis, Faculty of Agriculture, Cairo Univ.
- Manu, H.N., Prashanth, J., Chaitra, S. and Jagadeeshkumar, T.S. (2020). Evaluation of economic traits of selected FC1XFC2 and FC4XFC3 double hybrids treated with different doses of cholesterol during IV and V instar silkworm *Bombyx mori* L. J. Pharmacogn Phytochem, 9 (3): 129-133.
- Miao, Y. G., Liun, J., Yungen, M. and Liun, J.A. (2001). Artificial diet rearing for the silkworm. III-physical properties of diet. Indian Acad. of seric., 5(1): 6-8.
- Moustafa, Marwa N. (2024). Impact of adding some algal probiotics (*Spirulina plantalis* and

- Azolla pinnata) as food supplements to silkworm, *Bombyx mori* L. for strengthening the sericulture industry. Egyptian Academic Journal of Biological Sciences, 17 (2): 13 -24.
- Muzamil, A., Tahir, H. M., Bhatti, Ali, A., Munir, M. F., Ijaz,F., Adnan, F., Khan, M., Abdul Qayyum, H. A. K. (2023). Effect of amino acid fortified mulberry leaves on economic and biological traits of *Bombyx mori* L. *Heliyon*, 9(10), e21053. https://doi.org/10.1016/j.heliyon.2023.e21053.
- Pachiappan, P., Swathiga, G., Alagesan, T., Umapathy, G. and Chzhan, K. (2021). Impact of probiotics on enzymatic activity and economic traits of double hybrid silkworm, *Bombyx mori* L. The Pharma Innovation Journal, 10 (11): 1022 1026.
- Ponmurugen, M. and Karthikeyan, A. (2017). A comparative study on the biochemical composition of haemolymph of the fifth instar larvae of a multivoltine breed and a bivoltine breed of the mulberry silkworm, *Bombyx mori* L. Int. J. Zool. Appl. Biosci, 2(6): 292-298.
- Shivkumar, G. and Subramanya, G. (2015). Quantitative estimation of haemolymph protein during different days of fifth instar larvae in bivltines, multivolines and mutants of the silkworm, *Bombyx mori*. Global Journal of Bioscience and Biotechnology, 4 (3): 239 241.
- Shruti, A. J., Hadimani, D.K., Sreenivas, A.G. and Beladhadi, R.V. (2019a). Effect of probiotic feed supplements to mulberry silkworm, *Bombyx mori* L. for larval growth and development parameters. International Journal of Chemical Studies, 7 (3): 3914 3919.
- Shruti, A. J., Hadimani, D.K., Sreenivas, A.G. and Beladhadi, R.V. (2019b). Economics of probiotic feed supplementation to mulberry silkworm. Journal of Pharmacognosy and Phytochemistry, 8 (6): 749 753.

- Siddiqui, M. I. and Al-Khalifa, M. S. (2014). In vitro cultivation of hemocytes of *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae). Can. J. Zool., 49: 1355–1358.
- Singh, K.I., Chauhan, R.M., Pande, A.B., Gokhale, S.B. and Hegde, N.G. (2005). Effect of use of *Lactobacillus plantarum* as a probiotics to improve cocoon production of mulberry silkworm, *Bombyx mori* L. J. Basic. Appl. Sci., 1: 1-8.
- Soumya, M., Harinatha, A.R., Nageswari, G. and Venkatappa, B. (2017). Silkworm (*Bombyx mori*) and its constituents: A fascinating insect in science and research. Journal of Entomology and Zoology Studies, 5 (5): 1701-1705.
- Sujatha, K., Sathish, J. and Anitha, J. (2015). Effect of medicinal botanical (*Ocimum sanctum*), Family, Labiateae on commercial parameters of the silkworm, *Bombyx mori*, L. International Journal of Multidisciplinary and Current research, 3: 76 78.
- Tanaka, Y. (1964). Sericology central silk board, Bombay (95). B Method Marine Drive: 216-220.

- Vijaykumar, A., Patil, G.M. and Kavita, M.B. (2016). Effective concentration of *Azolla* supplementation to grown up silkworms. Adv. In Life Sci. 5 (10): 4029 4038.
- Wago, H. and Ichikawa, Y. (1979). Haemocytic reactions to foreign cells in the silkworm, *Bombyx mori*, during post-embryonic development. Appl. Ent. Zool., 14: 36-43.
- Yahaya, N., Hamdan, N. H., Zabidi, A. R., Mohammad, A. M., Suhaimi, M.K.H. and Johari, M.A.A.M. (2022). Duckweed as a future food: Evidence from metabolite profile, nutritional and microbial analyses. Future Foods, 5, Article 100128. https://doi. Org/10.1016/j.fufo.2022.10028.
- Zhang, L., Rocchetti, G., Zengin, G., Del Buono, D., Treisan, M. and Lucini, L. (2023). The combination of untargeted metabolomics with response surface methodology to optimize the functional potential of common duckweed (*Lemna minor* L.). Antioxidants, 12, 313. https://doi. Org/10.3390/antioxi1202031

تأثير كل من الأزولا وعدس الماء كبيئات صناعية وإضافات غذائية على دودة الحرير التوتية Bombyx mori L.

 2 ایناس مصطفی یوسف الیمانی 1 ، محمد أحمد ابراهیم یوسف 2 ، شیرین مجاهد محمد یوسف هلالی 2 و ولاء مجاهد محمد یوسف هلالی

1- قسم بحوث الحرير - معهد بحوث وقاية النباتات – مركز البحوث الزراعية- الجيزة- مصر

2 - قسم وقاية النبات – كلية الزراعة – جامعة الزقازيق – مصر

تعتبر ديدان الحرير التوتية واحدة من أهم الحشرات وذلك لإنتاجها الحرير الخام والذي يستعمل في كثير من الصناعات إضافة إلى المجال الطبي. تم استخدام كلاً من الأزولا وعدس الماء كمصدر نباتي غنى بالبروتين من خلال تغذية يرقات ديدان القز على بيئات صناعية و كإضافات غذائية. وجد أنه بتربية يرقات العمر الأول لديدان الحرير التوتية على بيئات صناعية تحتوى على مسحوق عدس الماء بنسبة 1 جم 1 (1 جم من البيئة الأساسية قالت معنوياً نسبة الموت متبوعة بالبيئة الصناعية المحتوية على 2 جم من عدس الماء 1 (1 جم من البيئة الأساسية. على العكس فقد نتج موت جميع اليرقات التي تم تربيتها على بيئات صناعية تحتوى على مسحوق الأزولا بتركيزات 1 جم 1 (1 جم من البيئة الأساسية. وبصفة عامة فقد وجد أن البيئات الصناعية التي تحتوي على مسحوق عدس الماء بتركيزات 1 جم 1 (1 جم من البيئة الأساسية نتج عنه أفضل النتائج مقارنة بالتركيزات الأخرى. كانت معاملة المقارنة أفضل تأثيراً عن المعاملات المائية الأخرى. لوحظ أن تربية يرقات 1 المستخلصات المائية وقشرة الشرنقة، في حين أظهرت معاملة المقارنة أقل القيم. لوحظت أعلى نسبة موت ليرقات العمر الخامس بتغذيتها على أوراق توت معاملة بالمستخلص المائي للأزولا بتركيز 1.)

بخصوص قياسات الخيط الحريري، فقد وجد أن تغذية يرقات العمر الخامس لديدان الحرير على أوراق توت مغمورة في المستخلص المائي للأزولا بتركيز 2 % قد سجل أعلى قيم لطول، وزن وحجم الخيط الحريري، في حين أظهرت المعاملة بعدس الماء 4 % أقل القيم .

وبوجه عام فقد وجد أن كلاً من الأزولا وعدس الماء عظما معنوياً القيمة الغذائية لأوراق التوت وذلك بسبب محتواها العالي من البروتين والأحماض الأمينية والتي تعتبر هامة لنمو وتطور ديدان الحرير والتي تتطلب دراسة مستقبلية لتحديد كيفية التوازن بينهما.

أستاذ الحشرات الاقتصادية المتفرغ - كلية الزراعة - جامعة الزقازيق. أستاذ الحشرات الاقتصادية - كلية الزراعة - جامعة سوهاج.