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RESEARCH ARTICLE

Effect of pollen a bee-hive product on some physiological, biochemical and economical characteristics of silkworm, *Bombyx mori* L.

Mohanny, K.M., Safaa I.A.H. Abdelkarim * and Ghada S. Mohamed

Plant Protection Department, Faculty of Agriculture, South Valley University, 83523 Qena, Egypt.

Abstract

Pollen is one of the bee-hive products, which it's rich in protein and a wide range of vitamins. A study was carried out to evaluate the efficiency of pollen as nutritive supplementation on silkworm *Bombyx mori* Land compare its effect with ascorbic acid and an antibiotic (tetracycline). Three concentrations (2, 5 and 10 %) from (pollen, ascorbic acid, and tetracycline) were sprayed on fresh mulberry leaves and fed to silkworms, from the 4th to 5th instar larvae. The study was conducted to find out the physiological and biochemical changes in the haemolymph that occurred in the 5th instar larva. Some physiological parameters such as T.H.S, T.H.C, and D.H.T and six biochemical parameters such as total protein, total carbohydrates, total lipids, ALP, GOT and GPT, have been correlated with some productive traits as larval weights and cocoons parameters. The results showed pollen's ability to improve the physiological and economical characteristics of silkworm. Pollen at a concentration of (5%) had the best results. Further, the results suggest that coadministration of pollen (5%) with mulberry leaves has enhanced the biochemical reaction involved in the silk production in the silkworm.

Keywords: *Bombyx mori*; biochemical;haemolymph; nutritive supplementation; pollen.

Apprivation: T.H.S (Total Haemolymph Solids); T.H.C (Total Haemolymph Count); D.H.T (Differential haemocyte Types): Alkaline phosphatase (ALP); Glutamic Pyruvic Transaminase (GPT) and Glutamic Oxaloacetic Transaminase (GOT)

1. Introduction

Silkworm, *Bombyx mori* L. is beneficial lepidopteron insects for its production of sleek and sensuous silk fiber, often considered as “Queen of textiles”. *B. mori* is a phytophagous lepidopteran insect, and a monophagous feeder on *Morus alba* L. and its growth, development and metabolism depend on its nutritional requirements and environmental conditions (Nirupama, 2015). Silkworm requires specific sugar, amino acid, proteins and vitamins for normal growth and development (Sengupta *et al*., 1972). The quality and quantity of silk improved by fortifying mulberry leaves with nutrients, spraying antibiotics, juvenile plant hormones, plant extracts. These nutrients consequently affect silkworm growth and survival rates, (Hiware, 2006). In recent years, mulberry leaves have been fortified with Honeybees products, Honey, Royal jelly, Propolis and Bee Pollen, which potentially benefit to silkworm due to the bioactive agent in them (Zannoon ,1994). These hive-products are important not only for their nutritional properties but also for their functional and biological properties **(**Nour *et al*., 1997). Antioxidant, anti-inflammatory, antibacterial, antiviral, and anti-ulcerous activities and the

***Corresponding author: Safaa I. A. H. Abdelkarim**
Email: s.ismail@agr.svu.edu.eg
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capacity for inhibiting enzymatic browning are some of these important properties. Honeybee products are fabulously rich in active components such as flavonoids, phenolic acid, terpenes, and enzymes, which have biological functions in preventing some diseases and promoting good health (Pasupuleti *et al*., 2017). Cornara *et al*. (2017) reported that, bee pollen is vitaminic, and contains antioxidant and anti-inflammatory plant phenolics, as well as antiatherosclerotic, antidiabetic, and hypoglycemic flavonoids, unsaturated fatty acids, and sterols. The main chemical compounds of bee pollen include carbohydrates, proteins and amino acids, lipids, and fatty acids, phenolics, enzymes and coenzymes, vitamins and minerals. However, the chemical composition of bee pollen is highly variable, depending on the plant source, geographical region, and climatic conditions, thus profoundly affecting biological properties and therapeutic virtues. A wide range of therapeutic properties have been suggested, furthermore pollen has specific biological, physiological, and pharmacological effects (Robert, 1994; Komosinska-Vassev *et al*., 2015; Denisow and Pietrzyk, 2016).

This work aimed to evaluate how the forced consumption of bee pollen affects the physiological condition and the biochemical's aspect of *B. mori* by evaluating the circulating hemocyte number in haemolymph, silk gland development and the ovary of moth in addition to some economic aspect.

2. Material and methods

The experimental works were carried out in the laboratory of silkworm rearing, Plant Protection Department, Faculty of Agriculture, South Valley University, Qena, Egypt. during the spring season of 2021to evaluate the efficiency of enriching bee pollen for a silkworm diet and compare its effect with nutrition supplement as Ascorbic acid and an antibiotic (tetracycline) with the physiological, biochemical and economical views.

2.1. Insect Source and Rearing

The Bulgarian hybrid (imported) of the mulberry silkworm *B. mori* were chosen for the present study. The eggs were obtained from the Sericulture Research Department, Plant Protection Research Institute (A.R.C) in Giza, Egypt.

The larvae were reared on trays and provided with suitable amounts of fresh leaves of mulberry *Morus alba* (var. Kanva-2).

Rearing of silkworm larvae was carried out under hygrothermal laboratory conditions of approximately (25±2°C and 70±5% R.H). Chicken egg cartons plates were used as montages for cocoon spinning as described by Zannoon and Omera, 1994.

2.2. Experimental design

Experimental rearing was carried out with uniform-size larvae recruited from the stock that had been reared and acclimatized. The 4th instar larvae were reared on trays and provided with mulberry leaves. These larvae were formed into triplicates and placed separately in the rearing trays. Required quantities (2, 5 &10 gm) of pollen, and ascorbic acid were weighed and dissolved in 100 ml distilled water, while for tetracycline the quantities (2, 5 &10 gm) were weighed and dissolved in 1000 ml distilled water. Then, three concentrations of each treatment (2, 5 and 10% concentration) were supplemented with mulberry leaves for feeding 4th and 5th instar larvae (2 cm of concentration /50g of leaves for 50 larvae on one diet). The larvae were fed on mulberry leaves with supplements till pupation, Meanwhile the control treatment fed on mulberry leaves only.

2.3. Criteria of evaluation

2.3.1. Economic parameters

Larvae weight and cocoon parameters were determined.

2.3.2. Physiological investigation

- Silk gland weight were determined from ten 5th instar larvae.

- Weight of ovaries and number of eggs from ten moths were determined.

The Haemolymph samples were obtained from the 5th-day old of 5th instar larvae by puncturing the larval prolog cuticle with a fine hypodermic needle

a- Total Haemolymph Solids (T.H.S.) Hussein (1978) adopted a simple method for determining the T.H.S. % by using a refractometer.

b- Total Hemocytes count (T.H.C) was calculated according to Predetshensky *et al*. (1950).

c- Differential Hemocytes count (D.H.C) was determined according to Arnold and Hinks (1979).

2.3.3. Biochemical investigation

Collected haemolymph samples undergone different biochemical investigations including the following parameters:

a. The protein content of the haemolymph was determined using folic phenol reagent according to the method of Lowry *et al.* (1951).

b. The total carbohydrate content of the haemolymph was determined according to Singh and Sinha (1977).

c. The total lipid content of the haemolymph was determined by the phosphovanillin method of Baronos and Blackstock (1973).

d. Alkaline phosphatase activity (ALP) was measured according to the method of Laufer and Schin (1971).

e. Determination of transaminases activity, the level of both transaminases (glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT)) was determined colorimetrically according to Reitman and Frankel (1957).

2.4. Statistical analysis

Obtained data were analyzed using the factorial design. F-test was estimated for each analysis. The means were compared according to Duncan's Multiple Range Test.

3. Results

In this study to evaluate the efficiency of bee pollen enriched to the mulberry leaves and feed to the 4th and 5th of silkworm larvae the results indicated that:

3.1. The biochemical investigation

3.1.1. Total haemolymph protein (THP)

Total haemolymph protein in the 5th instar larval was highly significantly influenced by the rearing diets, pollen (5%) recorded the highest THP (762.2 mg/100 ml), followed by pollen (10%) and Ascorbic acid (5%) 712.77, 634.7 mg/100 ml, respectively with high significant differences.

3.1.2. Total Lipids %

Significant changes were detected in the total lipid due to the application of pollen, Ascorbic acid and tetracycline. The maximum amount of total lipid was recorded in pollen (10%) (299.47 mg/g), in which pollen with its all concentration were statistically superior over all other treatments (Table 1).

3.1.3. Total Carbohydrate

Larvae reared on Ascorbic acid (2%) had the highest rates of carbohydrate with an average (523.03 mg/g) and pollen (5%) (509.5 mg/g), followed by pollen (2%) (480.63 mg/g) which the least carbohydrate rate recorded on Tetracycline (388.2 mg/g). Rearing larvae on certain different diets significantly influenced on the carbohydrate proportion.

3.1.4. Transaminases or aminotransferases enzymes (ALP, GOT and GOT)

The performed chemical analysis in Table (1) showed a significant effect of treatments on the estimated transaminases or aminotransferases enzymes, Glutamic Pyruvic Transaminase (GPT) and Glutamic Oxaloacetic Transaminase (GOT) in addition to Alkaline phosphatase (ALP), in haemolymph of 5th instar silkworm larvae, when larvae reared on Ascorbic acid (2%), ALP mean showed the highest values (21.44 mg/µl) than the others. Maximum GOT and GPT means was recorded in pollen (10%).

Table 1. performances of pollen compared with supplement or antibiotics on some biochemical characteristics of mulberry silkworm.

Treatments	Concs. %	Total Protein (µg /ml) *	Total carbohydrates (µg /ml) *	Total lipids (µg /ml) *	ALP (µg phosphate /min/ml)*	GOT (µg oxaloacetate/ min/ml)*	GPT (µg pyruvate/min/ml) *
Pollen	2%	562.63 ±7.03d	480.63 ±5.51c	299.47±6.24a	18.78±.58b	241.69 ±8.19b	112.23±6.11a
	5%	762.2 ±7.5a	509.5 ±6.51b	277.4 ±5.12b	18.55 ±.58b	199.11 ±6.51c	100.7 ±2.08c
	10%	712.77 ±7.24b	456.07 ±6d	270.6 ±1.8b	18.77±.58b	268.72 ±12.06a	124.31±4.16a
Ascorbic acid	2%	547.27 ±2.7e	523.03 ±4.58a	200.9 ±10.03ef	21.44±1.53a	177.69 ±3d	60.31 ±8.5g
	5%	572.17 ±4.6d	429.97 ±6.56e	241 ±4.2d	17.37 ±.58b	186.213 ±3d	86.88 ±5.29d
	10%	497 ±5.5f	436.47 ±5.51e	198.83 ±7.55ef	15.81 ±1.15c	211.12±5.69c	65.79 ±3.51fg
Tetracycline	0.02%	426.3 ±5.24h	416.63 ±3.61f	188.47 ±8.2f	15.47 ±.58c	205.21 ±6.81c	75.91 ±5e
	0.05%	540.63 ±3.91e	417.13 ±8.96f	210.33 ±11.2e	18.38 ±.58b	179.75±7.09d	90.51 ±5.13d
	0.010%	634.7 ±5.48c	388.2 ±2.52g	257.1 ±7.2c	20.55 ±.58a	93.55 ±4.73	70.66 ±1.15ef
Control		440.23 ±8.71g	409.4 ±9.29f	229.27 ±6.26d	15.047 ±1c	236.43 ±6.03b	74.45±1.5e

-Values are mean of observations; ± slandered deviation

** Means within the same column followed by the same letter do not differ significantly at 5% level of probability.*

3.2. Physiological investigation

3.2.1. Silk gland weight

Significant changes were detected in silk gland weight in Table (2) pollen with all concentration recorded the highest mean, which pollen (5%) being the best of them. Different diets had a great impact on the silk gland weight of larvae.

3.2.2. Fecundity of female moth

When larvae reared on pollen (2%), Ovarioles weight showed the highest values (0.61 mg) than the others. The ovarioles weight was significantly changed by larval nutrition's on different diets (Table 2). On the other hand, a number of eggs led by female moth from pollen (10) treatment had the highest value (421 eggs/moth), then pollen (5) (355 eggs/moth), while the least values were on Ascorbic acid (176 eggs/moth). The untreated treatments (control) was (220 eggs/moth). The rearing diets directly affected on the fecundity of female moth in both ovaries' weights and number of eggs per month.

Table 2. performances of pollen compared with supplement or antibiotics on some physiological characteristics of mulberry silkworm**.**

Treatments	Concs.	Silk gland weight (g) *	Fecundity parameters	
			Ovarioles weight (g) *	No.eggs*
Pollen	2%	0.648 ±.05 ab	0.61 ±0.04 a	302 ±69.66 bc
	5%	0.73 ±0.16 a	0.52 ±0.04 d	355 ±54.1 ab
	10%	0.645 ±0.03 ab	0.577 ±0.01 ab	421 ±28.04 a
Ascorbic acid	2%	0.599 ±0.08 bcd	0.557 ±0.06 ab	229 ±18.04 cd
	5%	0.591 ±0.05 bcd	0.51 ±0.03 bc	176 ±3.51 d
	10%	0.577 ±0.03 bcd	0.463 ±0.02 cd	218 ±85.25 cd
Tetracycline	.02%	0.613 ±0.04 abc	0.477 ±0.09 cd	237 ±109.26 cd
	.05%	0.503 ±0.02 cd	0.303 ±0.01 f	231 ±50.29 cd
	.010%	0.480 ±0.07 d	0.400 ±0.03 de	200 ±14.93 cd
Control		0.466 ±0.05 d	0.333 ±0.03 ef	220 ±42.62 cd

-Values are mean of observations; ±slandered deviation

** Means within the same column followed by the same letter do not differ significantly at 5% level of probability.*

3.2.3. Effect of different diets on the hemocytic parameters

Some characteristics of the silkworm haemolymph have been studied, where pollen was used at a concentration of 2%, 5% & 10%, and their effect was compared with the same concentrations of ascorbic acid and tetracycline. On the one hand, and control on the other. The results showed the bee pollen ability to improve the silkworm's physiological characteristics when feeding the mulberry leaves enriched with them.

3.2.4. Total Haemolymph Solids THS %

The maximum total haemolymph solids THS% was recorded after fed larvae on pollen (10%) (3.83%); pollen (2%) (12.8%) and pollen (5%) (12.4%) diets; while those on Ascorbic acid (5%) diet had the lowest percentage (9.17%).

3.2.5. Total Haemolymph Count THC

Total number of hemocytes in the haemolymph of whole grown larvae changed significantly related to different diets (Table 3). However, larvae fed on a natural diet (only mulberry leaves), pollen and the other treatments had the lowest THC.

Table 3. Performance of pollen compared with supplement or antibiotics on haemolymph of silkworm.

Treatments	Concs.	T.H.S	T.H.C*	Hemocytes type (%)					
				PRs	PLs *	GRs	SPH *	ONs	Ads
Pollen	2%	12.8 ±2.89a	8133 ±1418.92ab	27.31	43.99	17.11	4.08	5.87	1.39
	5%	12.4 ±4.04a	6400 ±800bc	31.11	47.81	9.66	3.10	6.98	1.33
	10%	13.83 ±2a	6067 ±1101.51bc	43.87	36.37	12.50	2.01	4.17	1.06
Ascorbic acid	2%	10.33 ±1.73a	6667 ±832.67bc	38.70	52.14	3.62	3.29	2.17	0.07
	5%	9.17 ±0.58a	5067 ±702.38c	16.76	68.34	6.89	4.40	3.13	0.47
	10%	12.27 ±2.31a	5733 ±1616.58c	28.90	54.01	4.09	2.54	9.53	0.93
Tetracycline	0.02%	9.233 ±2.08a	8067±1101.51ab	27.45	61.88	3.12	0.29	7.25	0.00
	0.05%	11.067 ±2a	6333 ±1527.53bc	25.93	63.32	3.13	0.79	6.64	0.19
	0.010%	11.37 ±3.21a	6867 ±1026.32bc	27.07	60.85	3.55	1.49	6.27	0.77
Control		9.333 ±1.15a	9000 ±1000a	17.23	64.88	5.78	2.94	8.75	2.50

-Values are mean of observations; ±slandered deviation

** Means within the same column followed by the same letter do not differ significantly at 5% level of probability.*

3.2.6. Differential haemocytes Types DHC %

Hemocytes types were identified in larval haemolymph, in all examined diets. From the index of Gupta (1979) and Jones (1962), these types were classified as (prohemocytes (PR), plasmatocytes (PL), granulocytes (GR), oenocytoids (OE) and spherulocytes (SP) (Figure 1). The hemocytes percentages were significantly varied among the treatments. Regardless of the tested diets PLs type was the most frequently recorded cell type in haemolymph than the other types, followed by PRs and the lowest type was ADs cells (Table 3). Under natural feeding condition of larvae on pollen (10%) diet, the PRs % had the highest rates (43.87%) from the total counts, but the lowest were on ascorbic acid (5%) (16.76%) and control (17.23%). In contrast, ascorbic acid (5%) (68.34%) and the control (64.88%) treatments significantly recorded the highest PL %. In addition, larvae from pollen (2%) diet showed superiority in percentages of GR (17.11%) but decreased gradually on the remaining diets. The highest % of SP (4.40%) was from ascorbic acid (5%) then followed by pollen (2%) (8.74%). The ADs % had the highest rates (2.5%) in untreated treatments (control). Larval nutrition on different diets showed significant variations in the percentages of PLs & SPH hemocytes types.

3.3. The Economic parameters

3.3.1. 5th instar larvae weight

The highest mean weights of ten larvae (5th instar larvae) were recorded in pollen with all concentrations, which the superior for pollen (5%). All treatments had increased significantly from the control. Table (4)

3.3.2. Cocoon parameters

The highest mean of fresh cocoon weight, pupa weight, shell weight and shell ratio % was recorded in pollen with all concentrations, which the superiority for pollen (5%), especially in shell ratio%. Furthermore, all treatments had increased significantly compared to the control.

Table 4. Performance of pollen compared with supplement or antibiotic on some economic charactrestics of mulberry silkworm.

Treatments	Concs.	Larvae weight (g)*	Cocoon weight(g) *	pupal weight (g) *	Cocoon shell weight(g) *	Shell Ratio % *
Pollen	2%	3.99 ±0.11bc	1.37 ±0.03b	1.11 ±0.02b	0.26 ±.007ab	18.98 ±.12ab
	5%	4.49 ±0 .19a	1.41 ±0.09b	1.14 ±0.08b	0.27 ±0.03a	19.22 ±1.41a
	10%	4.34 ±0.21ab	1.49±0.03a	1.21 ±0.02a	0.27 ±0.01a	18.34 ±.11ab
Ascorbic acid	2%	3.7 ±0 .22cd	1.19 ±0.05d	0.98 ±0.04cd	0.2 ±0.004de	17.25 ±1.71abc
	5%	3.47±0.38de	1.2 ±0.03cd	0.97 ±0.02d	0.22 ±0.01cd	18.74 ±.51ab
	10%	3.28 ±0.18ef	1.25 ±0.03cd	1.02 ±0.03cd	0.23 ±0.004bc	18.67 ±0.39ab
Tetracycline	0.02%	3.3 ± 0.2ef	1.26 ±0.04a	1.04 ±0.04c	0.23 ±0.01cd	17.85 ±1.09abc
	0.05%	3.26 ± 0.15ef	1.02 ±0.02c	0.85 ±0.02e	0.17 ±0.002f	17 ±0.18bc
	0.010%	3.15 ± 0.2ef	1.07 ±0.04c	0.88 ±0.03e	0.18 ±0.02ef	16.98 ±1.26bc
Control		2.91 ± 0.29f	0.99 ±0.01 c	0.83 ±0.02e	0.16 ±0.02f	16 ±1.81c

-*Values are mean of observations;* ±*slandered deviation*

** Means within the same column followed by the same letter do not differ significantly at 5% level of probability.*

4. Discussion

4.1. performances of pollen compared with supplement or antibiotics on some biochemical characteristics of mulberry silkworm

The increase in protein content indicated that the digestive activities are high during the early part of 5th instars larvae development, which results in an increased accumulation of proteins that are then transported to other tissues through the haemolymph for further physiological activities in the healthy larva. The enhancement caused by pollen may be due to its constituents of protein, carbohydrates and beneficial lipids. Also, including high amount of *B complex* vitamins which is involved in growth, regulation and immunity **(**Pal, 2003). Also, Aruna and Murugesh (2021**)** indicated that oral administration of honey and protein sources to silkworm significantly changed metabolic processes, which significantly increased the carbohydrate and lipids in haemolymph of silkworm, also increased the activity of enzymes (GPT) and (GOT), which play an essential role in protein metabolism.

4.2. Performances of pollen compared with supplement or antibiotics on some physiological characteristics of mulberry silkworm

From obvious results, using pollen feeding with mulberry leaves can be improve the silkworm's physiological and economical characteristics. Fortification of mulberry leaves by bee pollen can be increased the productivity of silk and fecundity of moth. These results agreement with Attia *et al*. (2011) which showed that the administration of bee pollen prolongs life span, promotes weight gain, increases plasma hemoglobin levels, and provides tissues with vitamin C and Mg. These virtues may be related to a complex of active substances, including amino acids, vitamins like

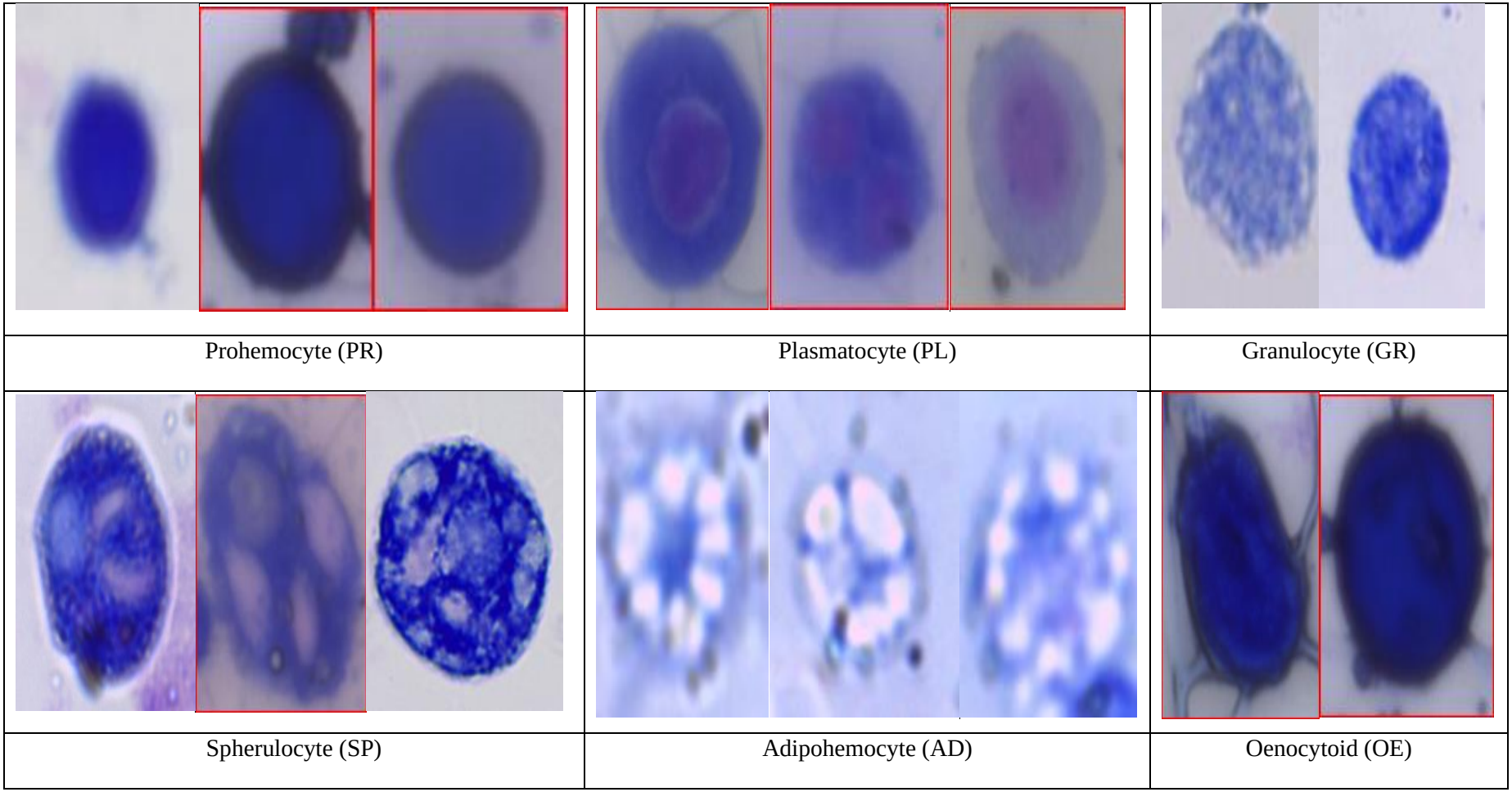

Figure 1. The six main hemocytes types in haemolymph of silkworm (*Bombyx mori* L.)

tocopherol, niacin, thiamine, biotin, folic acid, polyphenols, carotenoids, phytosterols and minerals (Denisow and Pietrzyk, 2016). All these important compounds act as feeding stimulants which enhanced the feeding behavior of larvae and improve the metabolic rate of larvae as a result of more food consumption by larvae and more free amino acids production in all body systems which consequently followed by increasing the proteins in silk gland then improving the fecundity of moth. In agreement with our finding Abd El- Rahman (2012) indicated that a maximum increase of silk gland weight (g) was shown in palm pollen as additives to mulberry leaves. Also, Hassan (2019) who indicated that feeding larvae with some mixture as a source of protein improved ovary weight of moths. Also, Helaly *et al*. (2021) investigated that royal jelly and palm pollen significantly increased the larval weight, silk gland weight, the fecundity of female moth and enhanced all physiological characteristics of *B. mori*. Moreover, Ito (1978) mentioned that the deficiency in the silkworm diet can be enriched with fortification of mulberry leaves with medicinal and botanical extracts having secondary metabolites which alter the silkworm physiology.

4.3. Performance of pollen compared with supplement or antibiotic on some economic characteristics of mulberry silkworm

The overall performance of silkworm in response to influence of pollen treatments observed in the present study and the evaluation index values worked out showed that the parameters observed were increased in the set that received of treatments. Since protein is major and chief constituents of silk thus, the treatment of larvae with pollen as an additive material to mulberry leaves, found that using of pollen showed a maximum weight of the silk gland compared with other treatments or control**.** Pollen may be increased the nutritional value of mulberry leaves, which directly reflects the improvement in larval growth and cocoon characters. Therefore, in a related study, mulberry leaves were enriched with various concentrations of extract (*Vigna unguiculata*) that significantly increased pupal weight, silk length, and silk weight of larvae which was observed for the high protein content of the extract (Manjula *et al*., 2011). Also, El-Shewy and Elgizawy (2017) have disclosed the positive impacts on the biological and physiological parameters upon feeding silkworm on alternative food. Also, it produced healthy cocoons and increased eggs production (Horie *et al*., 1982). Hassan *et al*. (2020) indicated that the improvement of the growth and cocoon characteristics of the *B. mori* when larvae were supplemented with some plants powder mix may be attributed to the medicinal properties of plant and increased the dietary protein in nutrition feed which allow the improvement of bio-productive parameters. The results showed the superiority of pollen with agreement with Kamel *et al*. (2016**)** was conducted on the effects of enriching mulberry leaves with protein supplements on some biological and economic parameters. Leaves supplemented with pollen increases fresh cocoon weight and cocoon shell weight. This feeding also led to shorter larval duration and decreased larval mortality percentages. Attia *et al*., (2011) & Tikhonov *et al*., (2006) proves that pollen caused faster weight gains than a normal diet, also pollen has a high nutritional value and a property of fast supplementing the nutritional deficiencies. The components playing the vital role in the process are dispensable amino acids, vitamins, and bioelements.

5. Conclusion

The results have showed through a comparative study that, the bee pollen can influence the larvae weights, cocoon characters and fecundity of moths. Thus, may be revered to its contain from protein, vitamins, and bioelements considered to be important in the productivity of the silkworm. Pollen at a concentration of (5%) had the best results. Furthermore, the results suggest that coadministration of Pollen (5%) with mulberry

leaves has enhanced the biochemical reaction involved in the silk production in the silkworm.

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Institutional Review Board Statement
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Data Availability Statement
Data presented in this study are available on fair request from the respective author.
Ethics Approval and Consent to Participate
Not applicable
Consent for Publication
Not applicable.
Conflicts of Interest
The authors disclosed no conflict of interest starting from the conduct of the study, data analysis, and writing until the publication of this research work.

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