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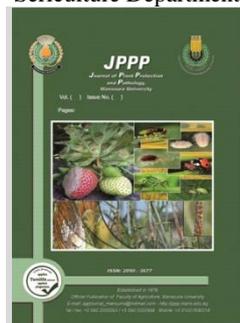
Influence of Royal Jelly and Palm Pollen on Biological, Technological and Physiological Characters of Silkworm, *Bombyx mori* L.

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ABSTRACT

The present study was carried out during the spring season of 2021 to evaluate some biological technological and physiological parameters of the silkworm, *Bombyx mori* L. fed on mulberry leaves *Morus alba* supplemented with royal jelly and palm pollen. Royal jelly at 2, 4 % and palm pollen at 50, 75 % increased significantly the larval weight, silk gland weight, cocoon weight, cocoon shell weight, pupal weight, the fecundity of female moth and improved the silk filament characters. Palm pollen at 75 and 50 % enhanced all physiological characters. Results established potential enhancement in most biological, cocoon and silk production by enriching mulberry leaves with both royal jelly and palm pollen.

Keywords: *Bombyx mori* L., nutritional supplements royal jelly, palm pollen.

INTRODUCTION

The mulberry silkworm, *Bombyx mori* L. (Bombycidae : Lepidoptera) is considered one of the most important economic insects. The silkworm larvae is considered the main source of natural silk, in addition to its high medicinal value and are usually used to reduced blood pressure nerve disorder and heart problems. Moreover, pupal are being employed to excreta vitamins whereas, the male moths are utilized for making medicinal veins and the excreta forms an important part of the fish and poultry – feeding (Fenemore and Prakash, 1992).

Treating silkworm larvae with nutrient supplement is good in improving the qualities of silk filament which can be used for yield enhancement in sericulture industry (Borgohain, 2015).

Recently, modern techniques of silkworm rearing is requested to increase the production of raw silk in Egypt to meet with the higher demands for industrial purpose. Legay (1958) reported that silk production is dependent on the larval nutrition. Therefore, the nutritive value of mulberry leaves plays an important role on larval growth that leads to produce good quality of cocoons and to improve cocoon characteristics (Sengupta *et al.*, 1992).

Many authors such as El – Karaksy (1979) , El-Sayed, (1999) and Gad (2006) studied the effects of royal jelly, honey, propolis as supplementary nutrients to the mulberry and castor leaves on the biological parameters of *B. mori* and *Ph. ricini*. Royal jelly is rich in nutrients and energy, it contains 12 – 13 % protein, 12 – 15 % carbohydrates and 5 – 6 % beneficial lipids, B – complex vitamins including high amount of fatty acid, 10 – hydroxy – 2 – decanoic acid (10 – HAD), which is involved in growth, regulation and immunity (Vitek, 1995). Also, date palm pollen is known to contain a variety of compounds including moisture (28.8 %), ash

(4.57 %), crude fiber (1.37 %), crude fat (20.74 %), crude protein (31.11 %), carbohydrate (13.41 %), vitamins (A, E and C), minerals and amino acids (Hassan, 2011).

Therefore, the present investigation was carried out to evaluate the effects of supplementing mulberry leaves with royal jelly and palm pollen on some biological, productivity aspects and physiological characters of the mulberry silkworm (*Bombyx mori* L.).

MATERIALS AND METHODS

The present study was carried out at the laboratory of Apiculture and Sericulture, Plant Prot. Dept., Fac. Agric., Zagazig University, Egypt during spring (season 2021) to evaluate the effect of two food additives i.e. palm pollen and royal jelly on *Bombyx mori* L. larvae.

1- Insect Source and Rearing

Silkworm eggs of commercial Bulgarian hybrid (H1* KK* G2*V2) were obtained from Sericulture Research Department, Plant Protect. Res. Inst., A.R.C, Dokki, Giza and maintained in rearing room at (25 ± 2 ° C. , 70 ± 5 % R.H. and 14 : 10 hr., light : dark) according to the technique of Krishnaswami (1978).

Mulberry leaves (Balady variety) were collected twice daily, i.e., at 8 am and 4 pm, then washed and left to dry under room conditions. *B. mori* larvae were fed on mulberry leaves 4 times / day which reared on plastic trays (42 x 30 x 10 cm) with 30 larvae / tray. Rearing trays, tools were previously disinfected one week prior onset of the experiment using 3 % formalin (Helaly, Walaa 2012).

The larval bed was changed daily using cleaning net for removing the remained dried food and faces.

1- Experimental Design

Silkworm rearing was performed as usual till the beginning of the fifth larval instar which used in the experiment. Three concentrations of royal jelly (1,2 and 4 %)

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were prepared by dissolving 1, 2 and 4 gm in 100 ml of distilled water. For palm pollen treatment 25, 50 and 75 gm were dissolved in 100 cm of distilled water to get on three concentrations (25, 50 and 75 %). Mulberry leaves were dipped in each concentration of the tested materials for 30 sec. then left to dry under laboratory conditions. Control larvae were fed on mulberry leaves dipped in water. Each treatment was replicated three times. The tested larvae were fed 4 times / daily, one of them on treated leaves and the others on untreated leaves. For mounting process, Chicken egg carton plates were used as montages and were placed along the trays for providing larvae with suitable place for cocoon spinning (Zannoon and Shadia 1994).

2- Biological Parameters

Fresh weights of each of full grown larvae, silk gland, pupa and moth were weighed for each treatment, in addition, larval duration and fecundity were determined.

3- Technological Parameters

For measuring technological traits, weights of fresh cocoon and cocoon shell were measured, then cocoons were dried at oven at 60 ° C. for 8 hours. Dry cocoons were reeled by reeling machine, the length of reeled silk filament was measured and weighed for every cocoon. The size of reeled filament was estimated according to Tanaka's formula (1964):

$$\text{Size (dn)} = \frac{\text{w eight of silk filament (g)}}{\text{Length of filament}} \times 9000$$

4- Physiological Characteristics

Haemolymph samples were obtained by removing one of the thoracic legs of the fifth instar larvae and bending the body to expose sternum at the position of the removed leg. The haemolymph of each treatment was collected in Eppendorf tubes 1.5 ml containing a few crystals of phenyl – thiourea (PTU) to prevent melanization of samples (Mahmoud, 1988). The tubes were kept at –20 °C. The blood samples were centrifuged at 10.000 rpm for 10 min at 5 ° C. Total protein, total carbohydrates, total lipids, proteases, amylase, trehalase, lipase and B- glucosidase were determined.

* Determination of total protein and total carbohydrates:

Total protein and total carbohydrates were determined by the method of Bradford (1976).

* Determination of total lipids:

Total lipids were estimated by the method of Knight *et al.* (1972).

* Determination of trehalase and amylase activity were determined according to the modification of Amin (1998) to the method described by Ishaaya and Swirski (1976).

* Determination of B- glucosidase activity:

B- glucosidase activity was measured by assaying glucose liberated by enzymatic hydrolysis of salicin as described by Lindorth (1988).

* Determination of protease activity:

Protease was determined by Broadway (1995).

* Determination of lipase activity:

Lipase was determined according to Bradford (1976).

5- Statistical Analysis

The obtained data were subjected to statistical analysis of variance using software COSTAT Program and presented as means according to Snedecor and Cochran (1967) methods.

RESULTS AND DISCUSSIONS

The effect of supplementing mulberry leaves with royal jelly and palm pollen which offered to the fifth instar larvae of mulberry silkworm, *Bombyx mori* on certain biological, productivity and physiological characters was studied and the results obtained could be discussed as follow:

1. Weight of mature larvae

Data given in Table (1) clear that the mean weight of full-grown larvae was significantly increased when larvae were fed on mulberry leaves treated with royal jelly at the concentrations of 4 and 2% , recording average weights of 2.97 and 2.63, respectively. Palm pollen at 25, 50 and 75% increased the weight of mature larvae, recoding 2.56, 2.87 and 2.96 g as compared to that of the control (2.213 g). Statistical analysis revealed significant differences between means.

2. Silk gland weight

Results in Table (1) reveal that the mean weights of silk gland differed among

The experimented treatments. Treating mulberry leaves with royal jelly increased the mean weight of silk gland showing (0.516 gm), while feeding larvae with mulberry leaves enriched with palm pollen showing a mean value of 0.439 gm. The lowest value (0.356 gm) was observed in the control treatment. Analysis of data revealed significant differences between means.

Table 1. Effect of royal jelly and palm pollen on some biological characters of mulberry silkworm, *Bombyx mori* L. during 2021 season.

Treatment	Conc.	Weight of full grown larva (gm)	Weight of silk gland (gm)	Larval duration (days)	Weight of moth (gm)		Fecundity (Eggs/female)
					Male	Female	
Royal jelly	1	2.46 ab	0.447 b	8.66 b	0.30 c	0.79 a	331.00 c
	2	2.63 ab	0.497 b	8.33 b	0.35 bc	0.70 b	333.67 c
	4	2.97 a	0.603 a	9.67 a	0.32 c	0.58 c	450.00 ab
	Mean	2.68	0.516	8.89	0.32	0.69	371.56
Palm pollen	25	2.56 ab	0.440 bc	8.33 b	0.45 a	0.69 b	306.00 c
	50	2.87 a	0.510 b	8.33 b	0.42 a	0.61 c	410.00 b
	75	2.96 a	0.367 cd	9.17 ab	0.40 ab	0.58 c	483.33 a
	Mean	2.80	0.439	8.61	0.42	0.62	399.78
Control		2.21 b	0.356 d	8.67 b	0.42 a	0.57 c	314.67 c
F.value		3.415*	11.76*	2.95*	1.06*	1.24**	18.14**
LSD 0.05		0.462	0.076	0.896	0.110	0.151	50.88

- * indicates significant differences between means at 0.05 level of probability.

- ** indicates highly significant differences between means at 0.01 level of probability.

- Means in each column followed by similar letters are statistically insignificant at 0.05 level of probability.

3. Larval duration

Results obtained in Table (1), indicate that the highest mean duration period (9.67 days) was recorded for treating mulberry leaves with royal jelly at 4 %, whereas the lowest mean one (8.33 days) was occurred with both of royal jelly 2 % and palm pollen at 25 and 50%. Control larvae recorded (8.67 days). The differences between means were significant.

4. Weight of moth

a- Male moth

Data presented in Table (1) reveal that larvae provided with mulberry leaves supplemented with palm pollen 25% developed into male moths heavier than that of the control, recording 0.45 gm compared to 0.42 gm for control male moth. The lowest mean weight (0.30 gm) was noticed with treating mulberry leaves with royal jelly 1%. The other treatments showed weight range of 0.32 - 0.42 gm. Analysis of data revealed significant differences between means.

b- Female moth

Data given in Table (1) clear that the addition of royal jelly at 1 and 2 % increased significantly the weight of female moth as compared to that of the control, recording 0.79 and 0.70 gm / female moth, respectively compared to 0.57 gm for control. Meanwhile, royal jelly 4 % and palm pollen 75% resulted in similar weight as that of the control. Other treatments were intermediates.

Regardless of the concentration of food additive, it is obvious that the highest mean weight of newly emerged male adults (0.43 gm) was induced with palm pollen, whereas the lowest one (0.32 gm) was observed with royal jelly (Table 1). In case of females, the highest mean weight of newly emerged adults (0.69 gm) occurred with royal jelly, while the lowest value (0.62 gm) was recorded with palm pollen.

5- Fecundity (number of laid eggs / female)

Data in Table (1) show that the highest significant mean number of eggs laid / female (483.33) was recorded for moths resulted from larvae fed on mulberry leaves treated with palm pollen 75%. The lowest mean number of eggs /

female (306.00 eggs) was recorded when mulberry leaves were enriched with palm pollen 25% .

Generally, it is obvious that the highest mean number of deposited eggs per female (399.78 eggs) was recorded with palm pollen, regardless of concentration followed by royal jelly (371.56 eggs). The differences between means were highly significant.

It was found that royal jelly increased larval weight, silk gland weight and female moth weight followed by the palm pollen which also increased the male moth weight and fecundity of female as compared to the control. Obtained results are in partial accordance with those of Nguku *et al* (2007) who found that larval, pupal and cocoon weights of mulberry silkworm were significantly higher when adding royal jelly as nutritional additives on mulberry leaves than that of the control. The weights of *B. mori* larvae fed on royal jelly treated mulberry leaves were heavier than those of larvae fed on untreated leaves at any day within both 4th and 5th larval instars Gomma (2010). Obtained results are in agreement with the finding of Gad (2013) who stated that the supplementation of honey bee products caused significant effects on all the studied biological and physiological parameters of the mulberry silkworm. Also, Zannoon (1994) reported that, all concentration of bee honey increased the mature larval weight, silk gland weight and the number of deposited eggs per female. The same trend was also recorded by El-Sayed *et al* (2013) who indicated that, the treatments of mixtures of honey bee with palm pollen significantly increased the biological parameters as compared with that of the control especially the number of eggs produced by female moths and gave higher values in the egg production. The rapid growth and developmental as well as increased number of eggs deposited by female moth can be noticed by addition of royal jelly to the normal mulberry leaves (Saikatsu *et al.*,1989).

6- Weight of cocoon

a- Male cocoon

The results arranged in Table (2) clear that the heaviest fresh cocoon weight was recorded when mulberry leaves were supplemented with royal jelly at the concentration of 4 %

Table 2. Effect of royal jelly and palm pollen on some biological and technological characters of mulberry silkworm, *Bombyx mori* L. during 2021 season.

Treatment	Conc	Weight of cocoon(gm) (gm)		Weight of pupa		Weight of shell cocoon (gm)		Silk filament		
		Male	Female	Male	Female	Male	Female	Length (m)	Weight (gm)	Size (dn)
Royal jelly	1	0.92 b	1.157 a	0.767 b	0.990 ab	0.150 c	0.167 ab	501.33 a	0.1660 abc	2.98 b
	2	0.97 b	1.210 a	0.770 b	1.030 ab	0.200 a	0.180 a	610.67 abc	0.1776 abc	2.62 ab
	4	1.10 a	1.183 a	0.913 a	1.003 ab	0.183 ab	0.180 a	680.33 abcd	0.1881 abcd	2.49 ab
	Mean	0.99	1.183	0.817	1.008	0.178	0.176	597.44	0.1772	2.70
Palm pollen	25	0.92 b	1.137 a	0.743 b	0.970 ab	0.180 abc	0.167 ab	853.30 d	0.2245 d	2.37 a
	50	0.94 b	1.233 a	0.763 b	1.070 a	0.183 ab	0.163 ab	746.67 cd	0.1782 abc	2.45 ab
	75	0.92 b	1.237 a	0.750 b	1.080 a	0.170 abc	0.157 ab	779.33 cd	0.2100 cd	2.43 ab
	Mean	0.93	1.202	0.752	1.040	0.178	0.162	793.10	0.2042	2.42
Control		0.85 c	0.990 b	0.700c	0.890 b	0.160 bc	0.103 b	597.44a b	0.1490 a	2.25 ab
F.value		4.18**	3.35*	4.04*	2.01 *	3.15**	12.36*	26.02**	8.40**	3.20**
LSD 0.05		0.112	0.141	0.101	0.142	0.028	0.023	98.2	0.180	0.30

- * indicates significant differences between means at 0.05 level of probability.

- ** indicates highly significant differences between means at 0.01 level of probability.

- Means in each column followed by similar letters are statistically insignificant at 0.05 level of probability.

showing a mean value of 1.10 gm. Meanwhile, the lightest weight of fresh male cocoon was noticed in

control treatment (0.85 gm). The other treatments showed values fluctuated between 0.92 - 0.97 mg.

Generally, the highest mean weight of fresh cocoon (0.99 gm) was recorded by using royal jelly followed by palm pollen (0.93 gm), regardless of concentration. Analysis of data revealed significant differences between means.

b- Female cocoon

Data presented in Table (2) assure that the treatments of royal jelly and palm pollen at all concentrations gave higher values than the control, ranging from 1.137 to 1.237 gm. Statistical analysis of data revealed significant differences between means.

7- Pupal weight

a- Male pupa

As shown in Table (2), it is obvious that feeding *B. mori* larvae on mulberry leaves treated with royal jelly at 4 % resulted in the highest weight of male pupae, recording 0.193 gm. The other concentrations of royal jelly (1 and 2 %) and palm pollen at all concentrations came in the second rank, recording male pupal weight ranged of 0.743 – 0.770 gm pupa. The lowest value (0.700 gm) was observed in the control treatment.

b- Female pupa

Data in Table (2), clear that feeding silkworm larvae on mulberry leaves enriching with palm pollen at 50 and 75% , as well as royal jelly at 2 and 4 % formed the most effective group on the weight of female pupae, recording 1.070, 1.080, 1.030 and 1.003 gm / pupa, successively. The rest concentrations came in the second rank, recording female pupal weight ranged from 0.970 – 0.990 gm / pupa. Control larvae recorded the lowest female pupal weight showing 0.890 gm. The differences between means were significant.

8- Weight of shell cocoon

a- Male cocoon shell

Results in Table (2) reveal that the highest value (0.200 gm) was recorded when larvae were fed on mulberry leaves treated with royal jelly 2 %, while the lowest one (0.150 gm) was observed with the treatment of royal jelly 1 %. The mean shell weight of control was 0.160 gm. It is clear that all palm pollen concentrations as well as royal jelly at 2 and 4 % had positive effects.

b- Female cocoon shell

Data compiled in Table (2) indicate that the highest value (0.180 gm) was obtained when larvae were fed on mulberry leaves treated with royal jelly at 2 and 4 %. Meanwhile, the lowest shell weight of female cocoon was noticed in control (0.103 gm). The other treatments showed median values, ranging from 0.157 – 0.167 gm. Statistical analysis revealed significant differences between means.

The same trend could be observed for the weight of pupa as being affected by the royal jelly addition to larval food. These results in accordance with those Gomaa (2010) demonstrated that the pupal weight and cocoon weight were significantly increased as fourth and fifth instar larvae fed mulberry leaves treated with royal jelly. It was clear that silkworm feeding on Mulberry (Rose variety) treated with palm pollen and royal jelly recorded to the heaviest pupae and cocoon shell of females and males Hamzah et al (2016). Similar results were obtained by Salman et al (2014) revealed that, the palm pollen increased the pupal, cocoons and cocoon

shell weights followed by carrot powder and carrot + pollen, while soya flour, starch and potato powder gave the highest averages of cocoon shell ratios. Moreover, Gad (2013) stated that the supplementation of honey bee products caused significant effects on all the studied biological and physiological parameters of the mulberry silkworm. She found that honey, royal jelly, pollen and their mixture increased significantly cocoon shell weight and fecundity in comparison with control. In the same manner, Xu et al., (1992) found that from 3rd to 5th instar larvae *Bombyx mori* fed on mulberry leaves soaked in extracts of *Brassica campestris* pollen and royal jelly increased the cocoon formation and oviposition.

9- Technological traits of silk filament

The effects of feeding mulberry silkworm larvae on mulberry leaves enriched with the tested additives on the length (m), weight (gm) and size (dn) of resulted reelable silk filament were evaluated.

a-The length

Obtained results clear that the effect of the two tested nutritional additives on the measured mean length of silk filament was significant. The longest mean reeled silk filament (853.30 m / cocoon) was recorded for palm pollen 25% . In addition, palm pollen at 50 and 75% came in the second class, recording a mean range of 746.67 – 779.33 m. Royal jelly at 2 and 4 % occupied the third category, recording silk filament length from 610.67 – 680.33 meters.

On the other hand, it was found that royal jelly at 1 % was the lonely treatment which gave shorter length than the control, showing an average of 501.33 m. Analysis of data revealed that the differences between means were highly significant.

Generally, obtained results indicate that the length of reeled silk filament varied according to the tested food additives, recording means of 597.44 and 793.10 m / cocoon for royal jelly and palm pollen, respectively compared to 597.44 m / cocoon for the control, regardless of the concentrations.

a- The weight

As seen in Table (2), the mean silk filament weight of cocoon of *B. mori* influenced significantly by the use of two nutritional additives, and all treatments gave heavier filament weight than that of the control.

The heaviest mean weight of silk filament (0.2445 g) was obtained when larvae were fed on mulberry leaves treated with palm pollen at 25% comparing with control (0.1490 g). The following effective treatments were palm pollen 75% which gave mean weight of 0.2100 g. The least mean filament weight was observed when mulberry leaves fed to the larvae treated with royal jelly at 1 %, recording 0.1660 g. The differences between means were highly significant.

Regardless of the concentration of the tested additives, the highest mean weight of reeled silk filament (0.2042 g) was recorded with palm pollen, while the least one (0.1722 g) was observed with royal jelly.

b- The size (dn)

Statistical analysis of the data compiled in Table (2) show that mean silk filament size was affected significantly by using the tested nutritional additives. The effective treatments were royal jelly at 1 and 2 % which

gave mean thickness of 2.98 and 2.62 dn, respectively, as compared with control (2.25 dn). Statistical analysis revealed significant differences between means.

As shown in Table (2), the tested food additives could be arranged dissentingly according to the thickness of reeled silk filament as follow: palm pollen (2.42) and royal jelly (2.70), regardless of the concentration. According to Kamimura and Kiuchi (1998) the increase in silk filament length is the most important commercial character in the improvement of natural silk yield. Our results revealed that palm pollen and royal jelly at high concentrations increased the filament characters. The same results were reported by Nguku (2007) who found that the filament obtained from cocoons produced by larvae fed on royal jelly was significantly longer than the filament obtained from the control group. Also, the mean filament weight obtained from the control group was less compared to that of the royal jelly group. In connection Zannoon (1994) found that, various solutions of bee honey gave heavier and longer filaments of reeled cocoons.

10- Physiological characters

a- Total protein content

As shown in Table (3), the protein content of fifth instar larvae was significantly affected by enriching the mulberry leaves with the two tested food additives. Maximum protein content observed in in palm pollen at 50 and 75% were 22.10 and 22.57 µg / ml compared with control (15.75 µg / ml). Statistical analysis revealed highly significant differences between means.

b- Total carbohydrates

Data presented in Table (3) clear that the highest carbohydrates content was recorded on supplementation of palm pollen 75% , recording 4.023 µg / ml, compared to 3.510 µg / ml for control larvae. The other treatments showed values fluctuating from 1.647 to 3.817 µg / ml. The differences between means were highly significant.

Table 3. Effect of royal jelly and palm pollen on some physiological characteristics in silk worm, Bombyx mori (L.)

Treatment	Conc.	Physiological characteristics		
		Total protein (µg/ml)	Total carbohydrates (µg/ml)	Total lipids (µg/ml)
Royal Jelly	1	15.85 c	1.647 d	1.41 c
	2	16.80 c	2.750 c	1.417 c
	4	17.21 c	3.800 a	2.42 b
	Mean	16.62	2.732	1.749
Palm pollen	25	20.73 b	3.317 bc	2.24 b
	50	22.10 ab	3.817 a	2.70 a
	75	22.57 a	4.023 a	2.80 a
	Mean	21.80	3.719	2.58
Control		15.75 c	3.510 b	2.30 b
F.value		44.13 **	75.784 **	49.55 **
LSD 0.05		1.36	0.288	0.242

* indicates significant differences between means at 0.05 level of probability

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

- Means in each column followed by similar letters are statistically insignificant at 0.05 level of probability.

c- Total lipids

Data in Table (3) indicate that the total lipids content of the fifth instar larvae was significantly affected by the addition of the tested nutrients. The highest mean (2.80 µg / ml) was recorded with palm pollen at 75%, while it was 2.30 µg / ml in control. The lowest value (1.410 µg / ml) was observed when larvae were fed on mulberry leaves treated with royal jelly 1%. Analysis of data revealed that the differences between means were highly significant.

c- Protease

As shown in Table (4), the protease activity in the midgut of silkworm was significantly differed among the

Table 4. Effect of royal jelly and palm pollen on some physiological characteristics in silk worm, Bombyx mori (L.)

Treatment	Conc.	Physiological characteristics				
		Protease (µg/ml)	Amylase (µg/ml)	Trehalase (µg/ml)	Lipase (µg/ml)	B- glucosidase (µg/ml)
Royal jelly	1	386.00 e	30.13 d	22.13 d	4.11 d	9.43 e
	2	459.67 d	32.40 d	31.13 c	4.68 d	12.77 d
	4	531.00 cd	45.73 c	34.07 c	7.21 c	18.63 b
	Mean	458.89	36.09	29.11	5.33	13.61
Palm pollen	25	771.33 b	42.33 c	38.13 b	10.47 b	14.90 c
	50	1018.33 a	50.47 b	42.77 a	13.93 a	20.03 b
	75	1058.33 a	54.93 a	43.17 a	14.37 a	22.00 a
	Mean	949.33	49.24	41.36	12.92	18.97
Control		551.67 c	45.93 c	38.67 b	8.13 c	20.00 b
F.value		115.57**	42.79 **	63.09**	122.6 **	67.96 **
LSD 0.05		76.37	4.20	2.82	1.128	1.702

-* indicates significant differences between means at 0.05 level of probability.

- ** indicates highly significant differences between means at 0.01 level of probability.

- Means in each column followed by similar letters are statistically insignificant at 0.05 level of probability.

treatments. Among all of the treatment groups, palm pollen group recorded the highest protease activity compared with control group, recording 771.33, 1018.33 and 1058.33 µg/ml at 25,50 and 75%, respectively compared to 551.67 µg/ml in the control. The differences between means were highly significant.

d- Amylase

The data presented in Table (4) showed that the highest amylase activity (54.93 µg/ml) was recorded with palm pollen at 75%, while it was 45.93 µg/ml in control. Statistical analysis revealed highly significant differences between means.

Generally, it is obvious that the highest mean of amylase activity (49.24 µg/ml) was recorded with palm

pollen, followed by control (45.93 µg/ml) and royal jelly (36.09 µg/ml), regardless of the concentration.

f- Trehalase

According to the results presented in Table (4), the trehalase activity was significantly affected by feeding larvae on mulberry leaves supplemented with the tested food additives. The highest trehalase activity (43.17 µg/ml) was recorded with palm pollen at 75% compared with the control (38.67µg/ml). The lowest value (22.13 µg/ml) was detected with royal jelly at 1 %. Analysis of data revealed highly significant differences between means.

g- Lipase

Data in Table (4) indicate that lipase activity of fifth instar larvae activity was significantly affected by the addition of the two tested nutrients. The highest value (14.37 µg/ml) was recorded when larvae were fed on mulberry leaves treated with palm pollen at 75%, while the lowest one (4.11 µg/ml) was observed with the treatment of royal jelly 1 %. The mean of lipase activity for control was 8.13 µg/ml. Statistical analysis revealed highly significant differences between means.

h- B- glucosidase

According to the results presented in Table (4), B- glucosidase activity was significant affected by feeding larvae on mulberry leaves supplemented with the tested food additives. The lowest B- glucosidase activity (9.43 µg/ml) was recorded with royal jelly 1%. The highest value (22.00 µg/ml) was observed with palm pollen 75 % compared to 20.00 µg/ml for control. The differences between means were highly significant.

It can be investigated that the artificial foods such as, royal jelly, dietary proteins, amino acid and vitamin B3 are effective in increasing the protein and lipid content as well as improve the growth and development of silkworm larvae Borgohain, (2015).

The chemical composition and nutritional value of palm pollen grains was found to be crude fat (20.74%), crude protein (31.11%), carbohydrate (13.41%) and a logical amount of vitamins A, E and C. Also, the presence of good source of minerals such as Zn, Se, B, mo, Cu, Ni, Co and the major amino acids leucine and lysin make the Palm pollen a good source as nutritional additives (Hassan 2011). These compounds are strong antioxidants, radical scavengers and have antimicrobial activity against many bacterial strains, which enhanced larval immune system as reported by (PanelKellie and Peter, 2002 and Servili and Montedoro, 2002). Also, El-Kholy *et al* (2019) stated that the Egyptian date palm pollen grains evaluation revealed its rich content of protein and carbohydrate, high content of Fe, Zn and Mg and unsaturated fatty acids ω-3, ω-6 and ω-9. The FTIR analyses indicated the presence of soluble amides (proteins), polysaccharides (fibers) and phenolic compounds especially catechin which were pronounced in DPP antioxidant potentials.

The enhancement caused by royal jelly may be due to its constituents of protein, carbohydrates and beneficial lipids. Also, B complex vitamins including high amount of fatty acid, 10-hydroxy-2-decanoic acid (10-HDA), which is involved in growth, regulation and immunity (Vitek, 1995). It contains acetylcholine, which

is important requirement in the diet of silkworm larvae for normal molting as well as growth (Keizo *et al.*,1965). All of these important compounds act as feeding stimulants which enhanced the feeding behavior of larvae and improved 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 in cocoon yield.

REFERENCES

- Amin, T.R. (1998). Biochemical and physiological studies of some insect growth regulators on the cotton leafworm, *Spodoptera littoralis* (Boisd). Ph.D.Thesis, Fac. Sci., Cairo Univ., Egypt.
- Borgohain, A. (2015). Nutritional supplement and its effect on mulberry silkworm *Bombyx mori* L. Int. J. Innovative Research in Science, Engineering, and Technology, 4(8), 6961-6962.
- Broadford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein – dye binding. Anal. Biochem., 72 (1-2): 248-254.
- Broadway, R.M. (1995). Are insects resistant to plant proteinase inhibitors. J. Insect Physiol.,41(2): 107-116.
- El – Karakasy (1979). Studies on silkworm in Egypt. Attempts for enhancing silk production by means of certain food. Ph. D. Thesis, Fac.of Agric. Alex. University.
- El – Sayed, Nagda A. A. (1999). Evaluation of six mixtures of food additives on some bio physiological and reproductivity parameters of the mulberry silkworm *Bombyx mori* L. Monofia J. Agric. Res., 24 (6) : 1971 – 1986.
- El-Kholy W.M. ; T.N. Soliman and A.M.G. Darwish (2019). Evaluation of date palm pollen (*Phoenix dactylifera* L.) encapsulation, impact on the nutritional and functional properties of fortified yoghurt. PLoS ONE 14(10): e0222789.
- El-Sayed, N.; H.A. M. El-Seehy ; M. A. M. EL-Kady and O. M. El-Gamel (2013). Effect of Food Additives on the Productivity of Silk Worm *Bombyx mori* L. Alexandria Science Exchange Journal, 34(April-June), 228-233.
- Fenemore, R.G. and A. Prakash (1992). Applied Entomolgy willey eastern limited, New Delhi, India, 115 pp.
- Gad, Abir, A. M. (2006). Effects of honey bee products on some biological and physiological parameters of mulberry silkworm, *Bombyx mori*. The Egypt. Sci. Mag. 3(2): 27 – 32.
- Gad, Abir, A. M. (2013). Biological and physiological effects of some honey bee products and its mixtures as nutritional additives on two strains of the mulberry silkworm *Bombyx mori*. Alexandria Journal Agriculture Researcher, 58(1), 47-52.
- Gomaa, H. A. (2010). Biological activity and enhancement of silk yield produced by *Bombyx mori* l. larvae fed on mulberry leaves fortified with royal jelly. J. Plant Prot. and Path., Mansoura Univ., Vol. 1 (10): 837 – 844.

- Hamzah, M. Kamel; F.G. Abo-Laban, and M. M. Nabil (2016). The effect of mulberry leaves enrichment with different nutritional supplements on biological aspects and economic traits of silkworm, *Bombyx mori* (L.). *Annals of Agric. Sci., Moshtohor*. 54(4): 977-982.
- Hassan, H.M.M. (2011). Chemical composition and nutritional value of palm pollen grains. *Global Journal of Biotechnology & Biochemistry*. 6 : 01 - 07.
- Helaly, Walaa, M.M. (2012). Studies on some factors affecting the performance of the mulberry silkworm, *Bombyx mori* L. Ph.D. Thesis, Fac. Agric., Zagazig Univ., Egypt, 180 pp.
- Kamimura, M. and M. Kiuchi (1998). Effect of Juvenile Hormone Analog Fenoxycarb on 5th stadium larvae of the silkworm, *Bombyx mori* L. *Appl. Entomol. & Zool*. 33 (2): 333-338.
- Keizo, H. ; K. Masaru and H. Yasuji (1965). Acetylcholine as a growth factor in early larval development of silkworm. *Nature* 205. 620 - 621.
- Knight, J.A.; A. Anderson and J.M. Rawle (1972). Chemical basis of the sulfo - phosphor - vanillin reaction for estimating total serum lipids. *Clinical Chem.*, 18 (3) : 199-202.
- Krishnaswami, S. (1978). New technology of silkworm rearing. *Central Sericulture Resarches and Training, Institute Mysore Bull* 2:1 - 10 .
- Legay, J.M. (1958). Recent advances in silkworm nutrition. *Ann. Revi. of Entomol.*, 3: 75 - 86.
- Lindorth, R.I.(1988). Hydrolysis of phenolic glycosidase in *Papilo glaucus* subspecies. *Insect Biochem.*, 18 : 789-792.
- 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 University Egypt.
- Nguku, E. K. ; M. Mulie and S. K. Raina (2007). Larvae, cocoon and post-cocoon characteristics of *Bombyx mori* L.(lepidoptera: bombycidae) fed on mulberry leaves fortified with Kenyan royal jelly. *J. Appl. Sci. Environ. Manage.* Vol. 11(4) 85 - 89
- PanelKellie, L. and J. Peter (2002). Major phenolic compounds in olive oil: metabolism and health effects. *The Journal of Nutritional Biochemistry*,13(11): 636-644.
- Saikatsu, S. ; K. Ikeno ; Y. Hanada and T. Ikeno (1989). Physiologically active substances in the oral excreta produced by honey bee-effects of royal jelly on silkworm. *Ohu Daigaku Shigakushi* 16 (3):113 - 116
- Salman, A.M.A.; M.H. Hussein; N.S. Omran and Shimaa, Y. E. Shaki (2014). Influence of Certain Additives to Mulberry Leaves to Enhance the Economic Parameters of Silkworm, *Bombyx mori* L. (Lepidoptera : Bombycidae). *Middle East Journal of Agriculture Research*, 3(4): 962-967.
- Sengupta, K. ; B. D. Singh and J.C. Mustafi (1992). Role of vitamins in silkworm nutrition. *Indian J. of Seri*. 11 (1) : 11 - 19.
- Servili, M. and Montedoro, G. (2002). Contribution of phenolic compounds to virgin olive oil quality. *Eur. J. Lipid Sci. Technol.*,104:602-613.
- Snedecor, G.W. and W.G. Cochran (1967). *Stastical methods*. Iowa State University Press, Amer Iowa.
- Tanaka, Y. (1964). *Central Silk Board, Bombay* (95). B. Medgoot Marine Drive : 216 - 220.
- Vitek, J. (1995). Effect of royal jelly on serum lipids in experimental animals and humans with atherosclerosis. *Experientia* 51:9-10 927-35.
- Xu, L. G. ; L. P. Chen ; S. P. You ; W. Y. Wang ; J. R. Li ; L.S. Yao and Schmidt, J. O. (1992): Stated that the effects of Pollen and royal jelly on growth, development and cocooning of silkworms *Entomological. Knowledge*, 29 (4):223-226.
- Zannoon, A.A (1994). Effect of some nutritional additives on certain biological and productivity characters of mulberry silkworm, *Bombyx mori* L. *J. Product. and Dev.*, 2 (1): 19-24.
- Zannoon, A.A. and M.O. Shadia (1994). Efficiency of certain natural materials a mountags for mulberry silkworm, *Bombyx mori* L. *Egypt J. Appl. Sci*, 9 (8) : 691 - 696.

تأثير غذاء ملكات النحل وحبوب لقاح النخيل على الصفات البيولوجية ، التكنولوجيا والفسيلوجية لدودة الحرير *Bombyx mori* L. التوتية

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تم إجراء هذا البحث خلال موسم ربيع 2021 وذلك لتقييم بعض الصفات البيولوجية، التكنولوجيا والفسيلوجية لديدان الحرير التوتية وذلك عند تغذيتها على أوراق توت معاملة بغذاء الملكات وحبوب لقاح النخيل. نتج عن معاملة أوراق التوت بكل من غذاء الملكات بتركيز 2، 4 وحبوب لقاح النخيل بتركيز 50 ، 75 % فقد زادت كل من أوزان اليرقة، غدة الحرير ، الشرنقة الطازجة، قشرة الشرنقة، العذراء وخصوبة الأنثى بالإضافة إلى تحسين صفات الخيط. أيضاً معاملة أوراق التوت بحبوب لقاح النخيل بتركيز 75 و 50 % قد عظم من جميع الصفات الفسيلوجية المختبرة. وبصفة عامة فقد أظهرت النتائج تحسين في معظم الصفات البيولوجية (الشرنقة والخيط) وذلك عن طريق معاملة أوراق التوت بكل من الغذاء الملكي وحبوب لقاح النخيل، بالإضافة إلى زيادة كل من محتوى البروتين ، الكربوهيدرات، اللبيدات وأيضاً نشاط الإنزيمات والتي يمكن الإستفادة منها لتحسين جودة الشرائق في مجال تربية ديدان الحرير .