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The Effect of Onion Powder (*Allium Sp.*) As An Antibiotic on The Larval Haemolymph of Silkworm and Its Effect on The Economic Parameters of Cocoons as A Food Supplementary

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#### **ABSTRACT**

The study aims to use natural antimicrobial such as onion powder ( *Allium sp.*) which has an effective role as a strong antibiotic to raise the immunity of sensitive silkworm larvae to infection with many diseases.

Three doses of Onion powder were used 5,10 and 15gm, and the following was studied:

The first is: Haemolymph of the fifth instar larvae: where the percentage of dissolved solids, the total number of blood cells and the differentiation number of blood cells were calculated. Results showed that the dose of 10 gm recorded a maximum number in T.S.S. (17.67%), while the dose of 5 gm showed a maximum number of THC (3500 haemocytes /mm³). Also, data recorded a significant difference between doses and control in the five types of cells in the haemolymph. The second: The economic parameters of the cocoons and the weight of silkworm larvae of the fifth age and the effect of using the onion powder on these traits were studied. The results showed that the maximum weight of the larvae was in the dose of 15 gm while the dose of 10 gm was the highly increment in the weight of cocoon, cocoon shell weight and the shell ratio. It concluded that the use of 5 and 10gm from Onion Powder as supplementary feeding on the mulberry leaves enhanced the immunology of larvae without affecting the quality of the economic parameters of the cocoons.

#### INTRODUCTION

Silkworm, *bombyx mori* L. is one of the most sensitive Lepidopteron insects, for continuous domestication silkworm becomes susceptible to diseases. Haemolymph is one of the responsible defenses against pathogens. Silkworms are infected by many pathogen groups such as bacteria, protozoa, fungi and viruses (Rafiq *et al.*, 2020). Haemocytes are an important component of the immune system in insects, because of their involvement in coagulation, phagocytosis and encapsulation (Khan, *et al.*, 2016,). Haemolymph of *Bombyx mori L.* included five types of hemocytes, Prohaemocytes, Plasmatocytes, Granulocytes, Spherulocytes and Oenocytes. (Han *et al.*, 1998, Yamashita and Iwabuchi 2002, Khan, *et al.*, 2016). Ling, *et al.*, (2005) cleared that, Prohaemocytes comprised about (60-70%), Granulocytes (0.5-1%) and Oenocytes (30-40%). Authors used diet as a supplementary feeding to enhance the larvae weight which refers to the good health of

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larvae and studied the effect of the treatments on the haemolymph (Rateb, et al., 2010, Rashwan, 2010, Abdel-Rahman, et al., 2012 Shakl, 2015). Antibiotic food supplementation showed steps on deter bacteria and also increased the economic parameters of silkworms (Sheeba, et al., 2008). The total hemocyte count (T.H.C.) and the differential hemocyte count (D.H.C) were studied by (Abdel-Rahman, et al., 2012) they recommended that palm pollen treatment cleared an increment on the T.H.C. Also, the Total haemocytes count was significantly increased after using the antibiotic treatment Ceftiofur sodium (Rafiq et al., 2020). Onion (Allium sp.) is a natural antibiotic medical plant that produced antioxidants and antimicrobial to protect the cellular from pathogens (Omotola, et al., 2018). Onion is rich in Flavonoids which play an important role in enhancing chemical compounds active against microorganisms (Santas et al., 2010). Also, this strong antimicrobial effect of fresh onion was due to both methyl cysteine, sulfoxide and S-n-propyl cysteine, phenolic acid, organosulfur and anthocyanin (Slimestad et al., 2007). Abdel- Salam et al., (2014) concluded that both onion and garlic had antimicrobial activities against the tested bacteria pathogens. The aim of the study was to use the onion powder on the mulberry leaves as a supplementary feeding to the larvae of silkworms infested with bacteria to enhance the immunology of the haemolymph with special references to the economic parameters.

## MATERIALS AND METHODS

The present study was carried out in the laboratory of Plant Protection Institute, Shandawel, Agricultural Research Station, Agriculture Research Center, Sohag Governorate, during two successive rearing seasons of 2021 and 2022. The silkworm eggs (*Bombyx mori L.*) Imported by Sericulture Dep., Plant Protection Research Institute, Agri. Resch. Center, Ministry of Agriculture and Land, Reclamation, Giza, Egypt.

Complete hatching was tacked and larvae were reared on the mulberry leaves (*Morus nigra*), under the laboratory condition as the standard methods of rearing according to (Krishnaswami, 1978, Jolly, 1986 and Ghazy, 2014). Three doses of onion powder were used (5,10 and 15 gm)\_The larvae in the 5<sup>th</sup> instar were fed 4 times/day. The sick larvae in early infection with bacteria were collected in three carton boxes (20.5×19.5×6.5cm), each containing 50 silkworm larvae, and 150 larvae/ dose. Control larvae were fed with untreated mulberry leaves.

#### **Measurements:**

# **Hematological Studies:**

# 1-Percentage of Total Soluble Solids (T.S.S.):

From three larvae of the 5<sup>th</sup> instar in each replicate, simple methods adopted by (Hussien, 1978) were used to determination of Total Soluble Solids (%), The amount of T.S.S. was determined using a digital refractometer (Plate 1) (Model No: NR-101 code No: 5901013. S/N: P2069 Barcelona, Spain) according to (A.O.A.C., 1995) (Arnold and Hinks, 1976).



Plate (1): The manual refractometer

## 2-Total Counts of Haemocytes (T.H.C.):

Haemolymph was taken from the abdominal legs with sanitized needle/edge. The exuded haemolymph was collected in a white blood pipette. For the purpose of calculation, the total number of haemocytes per mm<sup>3</sup> (Plate 2) haemolymph was achieved as described by (Jones, 1979 and Ganie, *et al.*, 2015). The (THC) formula suggested by Jones (1962):

Hamocytes in five 1 mm2  $\times$  Dilution  $\times$  Depth factor of chamber

 $FHC = \frac{\text{No. of squares counted}}{\text{No. of squares counted}}$ 



Plate (2): The haemocytometer champers and the square counted

# **3-Differential Haemocytes Count (DHC):**

Giemsa staining was used to the haemocytes slide (**Plate3- B**) (diluted 1:9 in distilled water) for 15 min, then washed with distilled water. After air drying, the slides were covered with coverslips with a drop of Canada balsam and observed with light microscopy. The slides were prepared to count the haemocytes adapted to the method of (Jones, 1967).

Three larvae were collected from each dose, and from each larva, 100 cells were randomly counted in bright field microscopy at  $\times$  40 magnification. Optika Vision Light Microscope was used to count and photographed the cells (Plate3-A).



**Plate (3):** Optika light microscope (A) and the slid staining. (B)

## Fresh Weight of The Fifth Instars Larvae and The Economic Parameters:

Two reading from the 3<sup>rd</sup> day from the 5<sup>th</sup> instar and 8<sup>th</sup> days were recorded. The percent changes from control were calculated. In Nine days old of the 5<sup>th</sup> instar three larvae were collected to determine the silk gland from each replicate (gm). From each replicates cocoons were collected randomly and weighted (gm) then the pupae were extracted from a cocoon and weighted (gm), and the cocoon shell weight was weighed and compared with the control. The cocoon shell ratio for each treatment was calculated according to Tanaka (1964) as follows:

Cocoon shell ratio  $\% = \frac{Wieght\ of\ Cocoon\ shell}{weight\ of\ cocoon} \times 100$ , (in gm), and changing from control

were calculated.

The Percent from control was calculated and illustrated in figures for each of the economic parameters.

## **Statistical Analysis:**

It was carried out to compare the obtained means of studied parameters Test using the statistical computer programmers Mstatc. Duncan (1955).

#### RESULTS AND DISCUSSION

# 1- Hematological Studies:

Data in Table (1) illustrated the effect of feeding mulberry silkworm larvae on Onion powder as an additive on the mean of T.S.S. and T.C.H. during the two successive seasons of 2021 and 2022. Data showed that the three doses of Onion powder affected the haemolymph of silkworm *bombyx mori* by increasing the mean of the total soluble solids (T.S.S.%). Data cleared that there were no significant differences between doses and control. Both doses of 10 and 15 gm showed an increment in the Total soluble solids (17.67 and 17.00%), respectively. While the minimum in Total soluble solids was recorded in the dose of 5gm (12.00 %). This agreement with Abdel – Rahman, *et al.*, (2012), Rashwan (2010), Shakl (2015) and Taha and Shakl (2021) recorded an increment of Total Soluble Solids after treating the mulberry leaves with different additives to enhance the economic parameters.

Total Haemocytes Count was illustrated in Table (1), Data showed that the dose of 5 gm recorded highly significant differences between other doses and control (3500 haemocytes /mm³) while the minimum numbers were recorded in the control (1583 haemocytes /mm³). Also, a dose of 10 gm showed an increased number (2167 haemocytes /mm³). This agreement with Abdel – Rahman, *et al.*, (2012) recorded that THC /mm³ haemolymph increased after being treated with the dose of 5 gm of guava. Reddy, *et al.*, (2012) studied the effect of some antibiotic substances such as Cecropin on the THC of a silkworm, they concluded that the total haemocytes count increased by the antibiotic. Rajitha, *et al.* (2013) studied the changes in THC Day after day on silkworms infested with *Beauvaria bassiana*, they showed that THC increased gradually to deter the infection. Shakl (2015) concluded that potato powder increased the total haemocytes count thus may be due to the antioxidant component of the treatment.

**Table 1:** Effect of feeding mulberry silkworm larvae on Onion powder additive on the mean of T.S.S. and T.H.C during the two successive seasons of 2021 and 2022.

Doses (gm)	Larval haemolymph			
	T.S.S. %	T.H.C. (haemocytes /mm <sup>3</sup> )		
5	12.00A	3500A		
10	17.67A	2167B		
15	17.00A	1792B		
Control	13.67A	1583B		

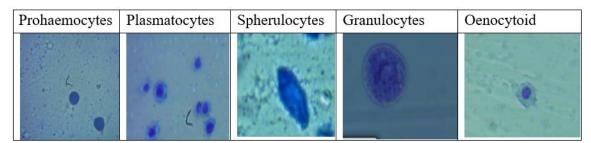
Means for each column followed by the same letters do not differ significantly at a 5% level of significance.

Data in Table (2) showed the effect of feeding mulberry silkworm larvae on an Onion powder diet on the Differential Haemocytes Count (DHC%) during the two successive seasons of 2021 and 2022. Data cleared that, there were significant differences between doses and control. A dose of 5 gm showed an increased number of Plasmatocytes, Prohaemocytes and Spherulocytes (39.33, 42.67 and 4.00%), respectively. Author increments were in a dose of 10 gm in Plasmatocytes, Spherulocytes and Oenocytes (39.00,

2.66 and 1.66%). While the maximum numbers of Granulocytes were recorded in the doses of 15 and 5gm. (6.00 and 5.66%), respectively. The minimum DHC was recorded in the control. it was (28.33, 20.33, 1.33, 1.33 and 0.66%) in Pl., Pro., Sp., Gr., and Oenocytes, respectively. Yamashita and Iwabuchi (2001) and Nataraju *et al.*, (2005) explained the importance of Prohaemocytes which are divided into other types of hemocytes, Also the gradual decrease in Prohaemocytes may be du to divided into Granulocytes and Spherulocytes as a mechanism to defend against the infection with the pathogen. Krishnan *et al.*, (2000), Anandkumar and Sandhymichael (2011), reported that an increase in both Plasmatocytes and Granulocytes were the main ones responsible for combating invading bacteria in *bombyx more*.

Haemocytes plays an essential role in the immunology system in insect, Phagocytosis, and encapsulation may be due to Plasmatocytes and Granulocytes mechanisms. Rajitha, *et al.*, (2013) reported that studying both Total Haemocytes Count and Differential Haemocytes count were the way to recognize the mechanism of cellular defense in silkworm infested with fungi.

Plate (4) showed the haemocytes observed in a light microscope and the difference between haemocytes types. Prohaemocytes were small cells with high numbers, Plasmatocytes, appeared in different shapes and could be divided into Gr and Sp., cells contained a large nuclear with a little cytoplasm. Spherulocytes, it's a spindle cell, Granulocytes, a big cell with a grain surface. Oenocytoid, its content is a large cytoplasm with a little nuclear. A whole discerption of the haemocyte's types according to (Khosravi, et al., 2021).



**Plate.** (4): Giemsa-stained circulating haemocytes from *Bombyx mori* larvae under light microscopy

Table 2: Effect of feeding mulber	ry silkworm larv	vae on Onion p	powder additive	on the
Differential cells				

	Haemocytes						
Doses	Plasmatocytes	Prohaemocytes	Spherulocytes	Granulocytes	Oenocytes		
gm							
5	39.33±11.5	42.67±10.5	4.00±0.7	5.66±0.4	1.33±0.4		
	A	A	A	A	A		
10	39.00±2.9	23.67	2.66±0.9	5.00±0.8	1.66±0.5		
	A	± 4.5AB	AB	A	A		
15	34.67±11.1	30.00±8.16	2.33±1.25	$6.00\pm0.0$	1.33±0.9		
	A	AB	AB	A	A		
Control	28.33±6.2	20.33±3.6	1.33±0.4	1.33±0.4	0.66±0.4		
	A	В	В	В	A		

Means for each column followed by the same letters do not differ significantly at a 5% level of significance.

## 2- Fresh Weight of The Fifth Instars Larvae and The Economic Prametares:

The larval weight during the 5<sup>th</sup> instar of silkworm was recorded on the 3<sup>rd</sup> day and the 8<sup>th</sup> day after feeding with Onion powder as a supplementary feeding on mulberry

leaves during the two successive seasons of 2021 and 2022 were illustrated in Table (3). Data showed that. The three doses of onion powder recorded significant differences compared to the control. The maximum weight on the 3<sup>rd</sup> and 8<sup>th</sup> days of the fifth instar larva was recorded in the dose of 15gm(2.550 and 2.970 gm), respectively. Also, the dose of 5 gm showed an increment in the two readings recorded 3<sup>rd</sup> and 8<sup>th</sup> days (2.480 and 2.793gm), respectively. The minimum weight of larvae was in the control in the two readings weight.

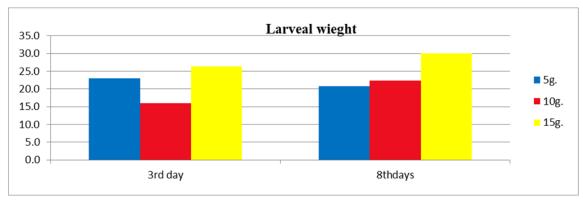
Variable effects of the three doses were recorded in the economic parameters compared to the control. the weight of cocoon, shell, silk gland and shell ratio% were the maximum weight in the dose of 10 gm (1.122, 0.203, 0.658 gm and18.12%), respectively. Whereas the pupal showed a maximum weight in the dose of 5gm(0.927gm). No significant differences were detected between doses and control. Data cleared those sick larvae fed on onion powder as a supplementary feeding on the mulberry leaves revealed a slight increase in the economic parameters, and also enhanced the larval weight compared to the control. This may be due to components of onion which is rich in flavonoids. The authors studied the effect of different natural substance additives on the mulberry leaves to enhance the larval weight and the economic parameters. Rashwan (2010) used honey as a diet on the mulberry leaves, and data cleared that, honey was revealed to increase the larval weight and the economic parameters. Also, the palm pollen additives which are rich in flavonoids led to an increment in the larvae weight, cocoon, shell ratio and silk gland(gm) (Salman *et al.*, 2014) and (Shakl 2015).

**Table 3:** Effect of Onion powder additive on the larval weight (gm) of the 5<sup>th</sup> larval instar and economic parameters of silkworm during two successive seasons of 2021and 2022.

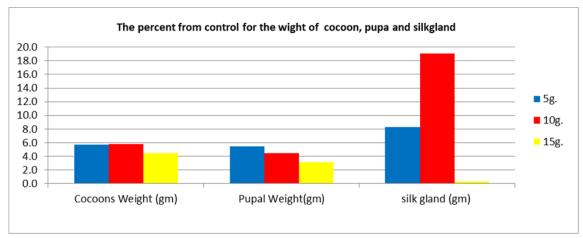
Doses	Larva	al weight	Economic parameters				
gm			Cocoons	Pupal	Shell	Shell	Silk
	3rd days	8th days	Weight	Weight	Weight	Ratio	Gland.
		-	(gm)	(gm)	(gm)	(%)	(gm)
5	2.480A	2.759A	1.121A	0.927A	0.193A	17.24A	0.599A
10	2.340AB	2.793A	1.122A	0.919A	0.203A	18.12A	0.658A
15	2.550A	2.970A	1.108A	0.907A	0.200A	18.08A	0.555A
Control	2.018B	2.250B	1.061A	0.9133A	0.147A	13.51A	0.553A

Means for each column followed by the same letters do not differ significantly at a 5% level of significance.

Data in Figures (1,2 and 3) showed the effect of onion powder as a supplementary feeding on the percent changes from control during the two successive seasons of 2021 and 2022. Data in Figure (1), showed that maximum percent change from control was recorded in the dose of 15gm in both fresh larval weight 3<sup>rd</sup> and 8<sup>th</sup> das (26.4 and 30.1%), respectively. Figure (2) showed that the dose of 5 gm showed a high percentage from control in both cocoons and pupal weight (5.7 and 5.4%), respectively. While a dose of 10 gm showed a high increment from the control it was (19.0%, 93.5 85.1 29.3%) in silk gland(gm), cocoon shell weight, shell ratio and T.S.S., respectively. Total Hemocyte count showed a maximum percent from control in the dose of 5 gm (57.9%) (Fig.3.) This agrees with Rateb *et al.*, (2010), Rashwan (2010), Salman, *et al.*, (2014), Shakl, (2015), and Taha and Shakl(2021), concluded that the different treatments additives to the mulberry leaves revealed to be affected by the increased percentages over the control in all the parameters of silkworm.



**Fig. (1):** The percentage changes from control in the larval weight of the 5<sup>th</sup> instar fed with Onion powder during the two successive seasons of 2021 and 2022.



**Fig. (2):** The percentage changes from control in the weight of cocoon, pupal and silk gland fed with Onion powder during the two successive seasons of 2021 and 2022.

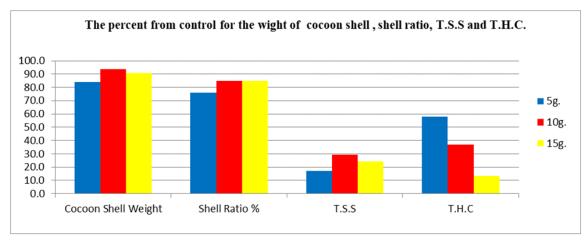


Fig. (3): The percentage changes from control in the economic parameters and larvae haemolymph fed with Onion powder during the two successive seasons of 2021 and 2022.

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#### **ARABIC SUMMARY**

# تأثير مسحوق البصل كمضاد حيوى على هيموليمف الدم ليرقات دودة الحرير وتأثيرها على الصفات الاقتصادية للشرائق كإضافات غذائية

شيماء يوسف عيسى شقل قسم وقاية النبات — كلية الزراعة والموارد الطبيعية — جامعة أسوان.

تهدف الدراسة الى استخدام مضادات حيوية طبيعية كمسحوق البصل الذي له دور فعال كمضاد حيوى قوى وذلك لرفع مناعة يرقات دودة الحرير الحساسة جدا للاصابة بالعديد من الامراض.

تم أستخدام ثلاث جرعات من مسحوق البصل وهي 15,10,5 جم وتم دراسة الاتي:

اولا: هيموليمف الدم ليرقات العمر الخامس حيث تم حساب كلا من العد الكلى لخلايا الدم، نسبة المواد الصلبة الذائبة و العد التفريقي لخلايا الدم اظهرت النتائج وجود فروق معنوية بين التركيزات الثلاثة مقارنة بالكنترول

كما لوحظ اعلى نسبة للمواد الصلبة الذائبة عند التركيز 10 جم (17.67%) ، بينما اظهرت الجرعة 5جم اعلى نسبة في اجمالي العد الكلي لخلايا الدم ( 3500 خلايا /مم²).

اظهرت النتائج وجود فروق معنوية بين الثلاث جرعات وخمسة انواع من خلايا الدم مقارنة بالكنترول ثانيا: تم دراسة الصفات الاقتصادية للشرائق ووزن يرقات الحرير للعمر الخامس ومدى تاثير استخدام البصل على هذة الصفات. اظهرت النتائج ان التركيز 15 جم كان الاعلى في وزن يرقات العمر الخامس بينما التركيز 10 جم كان الاعلى في نسبة وزن الشرائق، وزن غلاف الشريقة ومعامل غلاف الشرئقة.

ويمكن التوصية باستخدام الجرعات 5و 10 جم من مسحوق البصل كاضافات غذائية على اوراق التوت لرفع الكفاءة المناعية للبرقات دون التاثير على جودة الصفات الاقتصادية للشرانق