

EFFECT OF SOME ADDITIVES TO MULBERRY LEAVES ON LARVAL HAEMOLYMPH OF SILKWORM (*Bombyx mori* L.)

Rateb, S.H.; M.H. Hussein; M. O. Mohamed and Heba R. Abdel-Karim
Plant Protection Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.

ABSTRACT

Three levels (5, 10 and 15 gm) of ten powders and three concentrations (0.5%, 1% and 2%) of six liquids were used with mulberry leaves for feeding of 4th and 5th instars of local and imported silkworm. Their effects were studied on total soluble solids % (T.S.S.%) and total counts of haemocytes (T.C.H.)/mm³ haemolymph of 5th instar of silkworm larvae. The used tested powders were: glucose, fructose, sucrose, white or brown, soya, skim milk and their mixture (1:1), soya plus pollen (1:1), yeast and foliar fertilizer, while liquid treatments were: bee honey from Assiut or Sohag, chloral hydrate, foliar fertilizer, potassium iodide and ammonium chloride. Different responses in (T.S.S.%) and (T.C.H.) were noticed between local and imported silkworm, with respect to the effect of tested powder and liquid treatments. In powder treatments, maximum increment in (T.S.S.%) and (T.C.H.), in larval haemolymph of local silkworm was observed in skim milk treatment or its mixture with soya, while less effect was noticed in foliar fertilizer treatment. Sugars and soya plus skim milk showed maximum increment of (T.S.S.%) and (T.C.H.) in imported silkworm. T.S.S.% and T.C.H. in powder or liquid treatments were less in imported silkworm as compared with local ones. Liquid treatments with honey, ammonium chloride and chloral hydrate showed positive effect on (T.S.S.%) and (T.C.H./mm³ haemolymph) in local and imported silkworm. Detailed statistical analysis of obtained data was conducted and differences between strains and treatments were discussed.

INTRODUCTION

Insect haemolymph contains haemocytes suspended in plasma. Haemocytes have essential roles in numerous physiological activities (Wigglesworth, 1959). This fluid reflects physiological or pathological condition of the insect (Kostecki, 1965).

Effects of different factors or treatments on the larval haemolymph of silkworms were studied by many researchers: Radwan, 1978; El-Deeb, 1981; Horrie and Watanbe, 1983; Salem *et al.*, 1985a,b; Abd El-Naby, 1988; Reddy *et al.*, 1991; Thyagaraja *et al.*, 1991; Ashour, 1997; Eid *et al.*, 1999 and Abdellah, 2007.

This work aims to study the effects of ten powder and six liquid treatments of mulberry leaves on total soluble solids (%), and total counts of haemocytes/mm³ in 5th instar larval haemolymph of local and imported silkworm.

MATERIALS AND METHODS

Ten powder treatments were used with mulberry leaves in feeding of 4th and 5th instars of silkworm. They were: glucose, fructose, white or brown sucrose, soya, skim milk and their mixture (1:1), soya plus pollen (1:1), yeast and foliar fertilizer. Three levels, 5, 10 and 15 gm, were used for each treatment.

Six liquid treatments were used with mulberry leaves for feeding of 4th and 5th instars of silkworm. They were: bee honey from Assiut or Sohag, chloral hydrate, potassium iodide, ammonium chloride, and foliar fertilizer. Three concentrations, 0.5%, 1% and 2%, were used for each treatment.

Every tested powder or liquid was replicated three times in three carton boxes, each contain 40 larvae. Feeding with treated leaves was conducted four times/day. Control larvae were fed with untreated mulberry leaves.

A simple method adopted by (Hussein, 1978) using hand refractometer was used for determination of total soluble solids (%) in 5th instar larval haemolymph. Total counts of haemocytes in larval haemolymph was conducted as described by (Predtetschensky *et al.*, 1950).

RESULTS AND DISCUSSION

Data of the effect of tested powder treatments and their (percentages over control) on (T.S.S.%) in larval haemolymph of local and imported silkworm, are summarized in Table (1).

Maximum mean (T.S.S.%) in larval haemolymph of local silkworm was 11.97%, or (39.186%), in soya-plus skim milk (5 gm) treatment. T.S.S.% were more in all treatments, as compared with control.

Maximum mean (T.S.S.%) in imported silkworm was 13.73% (or 56.023%) in brown sucrose (10 gm) treatment. T.S.S.% were more than control in all treatments, except foliar fertilizer treatment.

Highly significant differences in T.S.S.% were detected between all treated powder treatments and control, except soya + pollen and foliar fertilizer treatments. No significant differences between tested sugars, and brown sucrose was superior.

T.S.S.% in two strains together was significantly more than control in the following powder treatments: white, or brown sucrose, fructose, glucose and soya.

Data in Table (2) show the effect of examined liquid treatments on mean (T.S.S.%) in larval haemolymph of local and imported silkworm and their percentages over control.

Maximum mean of (T.S.S.%) in haemolymph of local silkworm was 11.15% or (29.651%) and 10.62%, or (23.488%), in Sohag and Assiut bee honey (0.5%) treatments. Figures of (T.S.S.%) in liquid treatment were less than those in powder treatments of local silkworm.

Maximum mean of (T.S.S.%) in haemolymph of imported silkworm was 10.21%, or (16.023%), and 10.18%, or (15.682%), in ammonium chloride (1%) and chloral hydrate (1%) treatments. Figures of (T.S.S.%) in liquid treatments were less than powder treatments, in both of local or imported silkworm.

No significant differences in T.S.S.% in liquid treatments, were noticed between tested treatments, and between them and control.

Data in Table (3) show the effect of powder treatments on total counts of haemocytes (T.C.H./mm³) of 5th larval instar haemolymph of local and imported silkworm and their percentages over control.

Maximum mean number of haemocytes was found in soya plus skim milk treatment (15 and 5 gm). The same was noticed with respect to (T.S.S.%) in Table (1). The following treatments were less than control: glucose, fructose, brown sugar, yeast and foliar fertilizer (Table 3).

Maximum mean number of haemocytes was noticed in glucose (15 gm) treatment. T.C.H./mm³ haemolymph in imported silkworm were less than those in local silkworm.

No significant differences in T.C.H. were noticed between all powder treatments and control, except in soya-plus skim milk treatment, in which T.C.H. was significantly increased over control. No significant differences in T.C.H. between different tested powder levels inside treatments.

Table 1: Effect of powder treatments on mean T.S.S.%, larval haemolymph, and (percentages over control), of local and imported silk worm.

Treatments	Local strain				Imported strain				Grand mean
	Dose (gm)			General mean	Dose (gm)			General mean	
	5	10	15		5	10	15		
1- Glucose	9.42	10.24	10.11	9.923	12.710	11.730	12.310	12.250	11.09 ABC
	(9.535)	(19.024)	(4.658)	(8.960)	(47.277)	(33.295)	(21.520)	(33.341)	
	EF								
2- Fructose	10.82	9.77	10.66	10.417	11.440	12.930	13.000	12.457	11.44 AB
	(25.814)	(7.837)	(10.352)	(19.875)	(32.561)	(46.932)	(28.332)	(35.594)	
	DEF							AB	
3- Sucrose, White	10.95	9.36	11.06	10.457	12.310	13.670	12.420	12.800	11.63 A
	(27.326)	(3.311)	(14.493)	(14.824)	(42.642)	(55.341)	(22.606)	(39.327)	
	DEF							A	
4- Sucrose, Brown	10.33	10.51	11.71	10.850	11.550	13.730	12.580	12.620	11.73 A
	(20.116)	(16.004)	(21.222)	(15.845)	(33.835)	(56.023)	(24.186)	(37.368)	
	CDE							A	
5- Soya Flour	10.77	9.22	10.00	9.997	10.670	11.590	12.620	11.623	10.81 ABC
	(25.233)	(1.766)	(3.520)	(9.773)	(23.638)	(24.432)	(24.580)	(21.291)	
	EF							ABCD	
6- Skim Milk	10.97	11.77	10.24	10.993	9.490	10.490	9.840	9.940	10.47 BC
	(27.588)	(29.912)	(6.004)	(20.709)	(9.965)	(19.205)	(-)	(8.196)	
	BCDE							EF	
7- Soya + Skim Milk (1:1)	11.97	10.26	9.48	10.570	9.670	9.220	10.87	9.920	10.24 C
	(39.186)	(13.245)	(-)	(16.065)	(12.051)	(4.773)	(7.305)	(7.979)	
	DEF							EF	
8- Soya + Pollen (1:1)	9.910	9.960	10.060	9.977	10.800	9.840	9.80	10.147	10.06
	(15.233)	(9.934)	(4.141)	(9.773)	(25.145)	(11.818)	(-)	(10.450)	
	EF							DEF	
9- Yeast	10.660	9.870	9.88	10.137	10.53	10.440	10.54	10.503	10.34 BC
	(23.953)	(8.940)	(2.277)	(11.310)	(22.016)	(18.636)	(4.047)	(14.325)	
	DEF							DEF	
10- Foliar Fertilizer	10.430	10.280	9.64	10.117	8.57	8.120	7.20	7.963	9.039
	(21.279)	(13.466)	(-)	(11.090)	(-)	(-)	(-)	(-)	
	DEF							G	
Control	8.600	9.060	9.660	9.107	8.630	8.800	10.130	9.187	9.147 D
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	
	FG							FG	

Means followed by the same letter are not significantly different at 5% level of significance.

Table 2: Effect of liquid treatments on mean T.S.S.%, larval haemolymph and (percentages over control), of local and imported silk worm.

Treatments	Local strain				Imported strain				Grand mean
	Concentration (%)			General mean	Concentration (%)			General mean	
	0.5	1.0	2.0		0.5	1.0	2.0		
11- Honey, Assiut	10.62	9.02	9.68	9.773	9.57	9.41	9.47	9.483	9.627 AB
	(23.488)	(-)	(0.207)	(7.313)	(10.636)	(6.932)	(-)	(3.222)	
				ABC				ABC	
12- Honey, Sohag	11.15	9.60	9.17	9.973	10.08	6.82	8.04	8.313	9.143 AB
	(29.651)	(5.960)	(-)	(9.509)	(16.802)	(-)	(-)	(-)	
				AB				BC	
13- Chloral Hydrate	10.60	8.88	9.57	9.683	9.33	10.18	9.07	9.527	9.604 AB
	(23.256)	(-)	(-)	(6.325)	(8.111)	(15.682)	(-)	(3.701)	
				ABC				ABC	
14- Foliar fertilizer	10.22	10.53	9.86	10.203	7.64	9.80	9.14	8.527	9.367 AB
	(18.837)	(16.225)	(2.070)	(12.035)	(-)	(11.364)	(-)	(-)	
				A				ABC	
15- Potassium iodide	9.28	9.35	9.23	8.973	8.47	8.00	7.93	8.133	8.552 B
	(-)	(3.201)	(-)	(-)	(-)	(-)	(-)	(-)	
				ABC				C	
16- Ammonium chloride	10.43	10.28	9.64	10.117	9.40	10.21	10.02	9.877	9.997 A
	(21.279)	(13.466)	(-)	(11.090)	(8.922)	(16.023)	(-)	(7.511)	
				A				AB	
Control	8.60	9.06	9.66	9.107	8.63	8.80	10.13	9.187	9.147 AB
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	
				ABC				ABC	

Means followed by the same letter are not significantly different at 5% level of significance.

Data in Table (4) show the effect of tested liquid treatments on (T.C.H.) in local and imported silkworm, and calculated percentages over control readings.

Maximum mean of (T.C.H.) in local silkworm was observed in chloral hydrate (0.5%) treatment. Sohag honey and ammonium chloride treatments were less than control readings, while Assiut honey, foliar fertilizer, potassium iodide and chloral hydrate readings, were more than control.

Maximum mean of (T.C.H.) in imported silkworm was found in ammonium chloride (0.5% and 2%) treatments. T.C.H. in imported silkworm were less than those of local silkworm, in both of powder or liquid treatments.

No significant differences in T.C.H. were found between liquid treatments and control. Significant differences was noticed between T.C.H. in control larvae of local and imported silkworm.

In powder treatments, highly significant differences were detected between strains and between and within treatments, while in liquid treatments, highly significant differences were noticed between strains, and not between or within treatments.

Table 3: Effect of powder treatments on 5th instar near total counts of haemocytes/mm³ haemolymph of local and imported silk worm and (percentages over control).

Treatments	Local strain				Imported strain				Grand mean
	Dose (gm)			General mean	Dose (gm)			General mean	
	5	10	15		5	10	15		
1- Glucose	985	1200	725	961	1050	500	1958	1169	1065 AB
	(23.125)	(2.128)	(-)	(-)	(366.667)	(81.818)	(213.280)	(211.733)	
2- Fructose	875	1050	700	875	925	608	942	825	1013 AB
	(9.375)	(-)	(-)	(-)	(311.111)	(121.091)	(50.720)	(120.000)	
3- Sucrose, White	1217	825	867	970	575	933	550	685	827.8 B
	(52.125)	(-)	(-)	(0.310)	(155.556)	(239.273)	(-)	(82.667)	
4- Sucrose, Brown	1042	767	783	864	1625	558	550	911	887.5 B
	(30.250)	(-)	(-)	(-)	(622.222)	(102.909)	(-)	(142.933)	
5- Soya Flour	900	1592	1283	1258	842	600	1008	817	1038 AB
	(12.500)	(35.489)	(38.703)	(30.093)	(274.222)	(118.182)	(61.280)	(117.867)	
6- Skim Milk	1308	1733	1150	1397	475	433	1083	664	1031 AB
	(63.500)	(47.489)	(24.324)	(44.467)	(111.111)	(57.455)	(73.280)	(77.067)	
7- Soya + Skim Milk (1:1)	1858	1550	2225	1878	658	1042	675	792	1335 A
	(132.250)	(31.915)	(140.541)	(94.209)	(192.444)	(278.909)	(8.000)	(111.200)	
8- Soya + Pollen (1:1)	608	1342	1475	1142	800	458	517	592	866.7 B
	(-)	(14.213)	(59.459)	(18.097)	(255.556)	(66.545)	(-)	(57.867)	
9- Yeast	842	1383	558	928	500	858	525	628	777.8 B
	(5.250)	(17.702)	(-)	(-)	(122.222)	(212.000)	(-)	(67.467)	
10- Foliar Fertilizer	917	1033	733	894	692	433	283	469	681.9 B
	(14.625)	(-)	(-)	(-)	(207.556)	(57.435)	(-)	(25.067)	
Control	800	1175	925	967	225	275	625	375	670.8 B
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	

Means followed by the same letter are not significantly different at 5% level of significance.

Table 4: Effect of liquid treatments on 5th instar mean total counts of haemocytes/mm³ haemolymph of local and imported silk worm and (percentages over control).

Treatments	Local strain				Imported strain				Grand mean
	Concentration (%)			General mean	Concentration (%)			General mean	
	0.5	1.0	2.0		0.5	1.0	2.0		
11- Honey, Assiut	1142	875	1583	1200	525	442	708	558	879.2 A
	(42.750)	(-)	(71.135)	(24.095)	(133.333)	(60.727)	(132.800)	(48.800)	
12- Honey, Sohag	550	800	900	750	350	642	450	480	615.3 AB
	(-)	(-)	(-)	(-)	(55.556)	(133.455)	(-)	(28.000)	
13- Chloral Hydrate	558.33	533	742	611	733	333	425	497	554.2 B
	(109.375)	(-)	(-)	(1.655)	(225.778)	(21.091)	(-)	(32.533)	
14- Foliar fertilizer	667	1592	1058	1106	608	367	325	433	769.4 AB
	(-)	(35.489)	(14.378)	(14.374)	(170.222)	(33.455)	(-)	(15.467)	
15- Potassium Iodide	1025	1200	775	1000	608	250	1008	622	811.1 AB
	(28.125)	(2.128)	(-)	(3.413)	(170.222)	(-)	(61.280)	(65.867)	
16- Ammonium chloride	917	1033	733	894	1025	508	758	764	829.2 AB
	(14.625)	(-)	(-)	(-)	(355.556)	(84.727)	(21.280)	(103.733)	
Control	800	1175	925	967	225	275	625	375	670.8 AB
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	

Means followed by the same letter are not significantly different at 5% level of significance.

REFERENCES

- Abdellah, E.E. (2007): Studies on some factors affecting growth and productivity of mulberry silkworms (*Bombyx mori* L.). M.Sc. Thesis, Assiut Univ., 138 pp.
- Abd El-Nabi, S.M.M. (1988): Activation of silk secretion in silkworms *Philosamia ricini* and *Bombyx mori* after applying antibiotics. Ph.D., Cairo Univ., 124 pp.
- Ashour, A.T. (1997): Effect of feeding and hormonal treatments on some biological and physiological parameters of silkworms *Bombyx mori* and *Philosamia ricini*. Ph.D., Cairo Univ., 110 pp.
- Eid, M.A.A., Souad M. Mahmoud and S.A.S. El-Maasarawy (1999): Effect of antibiotics on the haemocytes of the silkworms *Philosamia ricini* and *Bombyx mori* L. XVIIIth ISC Congress 12-16 October 1999, 403.
- El-Deeb, A.S. (1981): Biological and physiological studies on *Philosamia ricini* Boisid. M.Sc. Thesis, Faculty of Science, Alexandria University.
- Horie, Y. and K. Watanabe (1983): Effect of various kinds of dietary protein and supplementation with limiting amino acids on growth, haemolymph components and uric acid excretion in the silkworm, *Bombyx mori*. J. Insect Physiol. Vol. 29 (2): 157-198.
- Hussein, M.H. (1978): Haematological studies on some lepidopterous larvae. 4th Conf. Pest Control, NC. 357-365.

- Kostecki, R. (1965): Investigation on the haemocytes and haemolymph of honey bees. J. Apicult. Res. 4 (1): 49-54.
- Predtetschensky, V.E., V.M. Parovska and L.T. Margolina (1950): Goso Uzd. Medgez. Moskva (In Russian).
- Radwan, M.A.S. (1978): The effect of castor plant fertilizers on the biochemical and properties of silk of the eri-worm. M.Sc. Cairo Univ., 103 pp.
- Reddy, K.V.R.; O.R.R. Deri and K.V. Benchamin (1991): Impact of uzi fly parasitisation on the body growth, silk gland tissue somatic index and haemolymph properties of silkworm. Indian J. Seric., 30 (2): 113-120.
- Salem, M.A., M.A. Eid and S.A.S. El-Maasarawy (1985a): Blood pattern in the Eri-silkworm, *Philosamia ricini* Boisd. under varied conditions (Lepidoptea, Saturniidae). Proc. 1st Conf. Pl. Prot. Res. Ins. Vol. II: 173-191.
- Salem, M.S., M.A. Eid and S.A.S. El-Maasarawy (1985b): Quantitative analyses of blood pattern in the Eri-silkworm, *Philosamia ricini* Boisd., under varied conditions (Lepidoptea: Saturniidae). Proc. 1st Conf. Pl. Prot. Res. Ins. Vol. II: 193-209.
- Thyagaraja, B.S., T.J. Kelly, E.P. Maslev and A.B. Brokovec (1991): Thyroxine-induced haemolymph protein and ecdystroid increase in the silkworm, *Bombyx mori*: Effect on larval growth and silk production. J. Insect. Physiol. 37 (2): 153-159.
- Wigglesworth, V.B. (1959): Insect blood cells. Ann. Rev. Entomol. 4: 1-16.

تأثير بعض الإضافات لأوراق التوت على هيموليمف يرقات دودة القز صلاح حنفى راتب، مصطفى حسن حسين، محمد عمر محمد و هبة رشوان عبد الكريم قسم وقاية النبات - كلية الزراعة - جامعة أسيوط - جمهورية مصر العربية

تم اختبار ثلاثة جرعات (5، 10 و 15 جم) من عشرة مساحيق وثلاثة تركيزات (0.5، 1 و 2%) من ستة سوائل وذلك بإضافتها لأوراق التوت المستخدمة لتغذية العمرين الرابع والخامس لدودة القز المحلية والمستوردة وذلك من ناحية تأثيرها على النسبة المئوية للمواد الصلبة الكلية والعدد الكلى لخلايا الدم في م³ هيموليمف ليرقات العمر الخامس لدودة القز. كانت المساحيق المختبرة هي: جلوكوز، فركتوز، سكروز أبيض أو بنى، صويا، لبن فرز ومخلوطه مع الصويا (1:1)، صويا + حبوب لقاح (1:1)، خميرة وسماد ورقي، بينما كانت السوائل المختبرة كما يلي: عسل النحل من أسيوط أو سوهاج، كلورال هيدرات، سماد ورقي، يوديد بوتاسيوم وكلوريد أمونيوم. لوحظت إستجابة مختلفة فى محتوى المواد الصلبة الكلية (%) والعدد الكلى لخلايا الدم ما بين السلالة المحلية والمستوردة من دودة القز، وذلك بعد المعاملة بالمساحيق والسوائل المستخدمة. بالنسبة لمعاملات المساحيق كانت أعلى زيادة لصفات الهيموليمف المدروسة فى السلالة المحلية وذلك فى معاملة لبن الفرز وكذلك مخلوطه مع الصويا، بينما لوحظ أقل تأثير فى معاملة السماد الورقي. أظهرت السكريات المستخدمة والصويا مع لبن الفرز أعلى تأثير بالنسبة للسلالة المستوردة. كانت نسبة المواد الصلبة الذاتية الكلية وأعداد خلايا الدم فى معاملات المساحيق أو السوائل أقل فى السلالة المستوردة عن السلالة المحلية. كان لمعاملات العسل وكلوريد الأمونيوم والكلورال هيدرات والمستخدمة كسوائل، كان لها تأثير موجب على الصفات المدروسة للهيموليمف فى السلالة المحلية أو المستوردة لدودة القز. تم إجراء تحليل إحصائى تفصيلى وتمت مقارنة ومناقشة النتائج الخاصة بالمعاملات والسلالتين موضع الاختبار.

قام بتحكيم البحث

كلية الزراعة - جامعة المنصورة
كلية الزراعة - جامعة القاهرة

أ.د/ هالة أحمد كامل الصيرفي
أ.د/ محمود السيد نور