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EFFECT OF ASCORBIC ACID ANALOGUES ON GROWTH AND SOME CHEMICAL CONTENTS IN PINK BOLLWORM, *Pectinophora gossypiella* (SAUND.)

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ABSTRACT

The effects of ascorbic acid on development and some biochemical analysis of *Pectinophora gossypiella* (Saund.) were investigated after feeding newly hatched larvae with different concentrations of three ascorbic acid analogues i.e., L-ascorbic acid, D- isoascorbic and D- glucoascorbic acid. The added analogue and the used concentration significantly affected larval growth. Statistically, L-ascorbic acid seemed to be the best analogue, as the whole mean of survived larvae averaged 63.737% comparing with 54.17 and 32.9% for the D-isoascorbic and D-glucoascorbic acid analogues, respectively. All survived larvae succeeded to develop into pupae after feeding with any of the three concentrations of L-ascorbic acid, while, all larvae died or failed to develop into pupae when fed with 2.32 mg of D- isoascorbic or D-glucoascorbic acid analogue / 100g diet, respectively. The significant low percentages of survived larvae or produced pupae are a reflection to the reduction in the values of total protein, tyrosine and chitin in larval body. Statistical analysis of data yielded a significant correlation between the percentages of larvae which succeeded to develop into pupae and the quantity of their total protein (r = 0.8043**), tyrosine (r = 0.82277**) and chitin (r = 0.69874*) in larvae.

Key words: L-ascorbic acid, D- isoascorbic acid, D- glucoascorbic acid, protein, tyrosine, chitin, *Pectinophora gossypiell*

INTRODUCTION

Ascorbic acid (vitamin C) is essential for both nutritive and antioxidant functions in phytophagous insects; however, maintaining sufficient quantities of reduced ascorbate may be problematical for them (Felton and Summers, 1995). Several studies of rearing different phytophagous insect species on artificial diets have demonstrated that ascorbic acid must be incorporated into the diet for their normal growth, develop and reproduction (Dadd 1960& 1963; Ito & Aria 1965; Levinson & Navon 1969; Eid & Moursy 1992; Salem *et al.,* 1992 and Moursy 1995). Low levels of ascorbic acid would reduce the amount of DOPA (dihydroxphenylalanine) formed, and would thus reduce the amount of DOPA which would be converted to melanin. Vanderzant and Richardson (1963) reported that a darkening of the body fluids of *Heliothis virescens* (F.) reared on artificial diet was correlated with the deficient in ascorbic acid. Ludwing and Gallagher (1966) also recorded this observation in ascorbic acid deficient *Periplaneta americana* L. Fading of the cuticle color may be due to a decrease in the degree of sclerotization which imports a dark amber color to the cuticle (Gilmore, 1965).

The present study was undertaken to determine the effect of ascorbic acid analogues on survival, pupation and on the quantity of some chemical contents in the body (cuticular and haemolymph) of the pink bollworm *Pectinophora gossypiella* (Saund.) after feeding on different concentrations of these analogues.

MATERIALS AND METHODS

Rearing technique:

Newly hatched larvae of the pink bollworm, *P. gossypiella* were obtained from a colony maintained in the laboratory where larvae were reared on a modified artificial diet as described previously by Abd El-Hafez *et al.* (1982).

Ascorbic acid analogues:

1. L-ascorbic acid (L- threo- Hex- 2- enonic acid Y- lactone).
2. D- isoascorbic acid (D- erthro- Hey- 2- enonic acid Y- lactone).
3. D- glucoascorbic acid (D- arabino- Hept- 2- enoic acid, gamma-lactone).

Bioassay Techniques:

Three concentrations were prepared in half-descending order for each of the three aforementioned ascorbic acid analogues i.e.; 2.23, 1.16 and 0.58 mg/ 100g artificial diet. The weight of each concentration was homogenately mixed with the artificial diet (100g). Treated diet was placed in 2 x 7cm glass vials (5 gm/ each), and then larvae were transferred individually into the glass vials. Three replicates were evaluated, each containing twenty newly hatched larvae for each concentration tested. For the check, the diet was free of any tested analogue. The vials were plugged with absorbent cotton, incubated at 27 °C $\pm$ 1 & 80 $\pm$ 5% R.H. Mortality among larvae and pupation, total protein, tyrosine and chitin were recorded.

Chemical analysis:

Larvae fed on artificial diet treated with each concentration of each analogue were subjected to the chemical analysis after 15 – 20 days (full grown larvae) as following:

a. Determination of the total crude protein:

The total crude protein was determined in full-grown larvae and pupae by biuret reaction described by Gornall *et al.,* (1949).

b. Determination of tyrosine:

The amino acid tyrosine was quantitatively determined in the haemolymph and the cuticular dry material of full-grown larvae by specific methods described by Kasting & McGinnis (1962) and Contrate & Singer (1956).

c. Determination of chitin:

The presence of chitin was demonstrated in the dry cuticular material of full-grown larvae by the procedure of Van Wisselingh (Klein, 1931) and determined according to the method of Huber (1958) which was modified by Hill *et al.,* (1968).

Analyses of variance were conducted on all data (ANOVA) and when statistical differences existed within a data set, Duncan's multiple range test was used to separate the means (Snedecor & Cochran 1980).

RESULTS AND DISCUSSION

Larval survival and pupation: Data in Table (1) show the percentages of larval survival and pupation of *Pectinophora gossypiella* after feeding with different concentrations of three ascorbic acid analogues i.e., L-ascorbic acid, D- isoascorbic and D- glucoascorbic. The added analogue and the used concentration significantly affected larval growth. Furthermore, the suitable concentration which needed for larvae to develop to pupae differed significantly according to the added analogue. Statistically, L-ascorbic acid proved to be the best analogue, as the whole mean of survived larvae averaged 63.75% comparing with 54.17 and 32.9% for the D- isoascorbic and D- glucoascorbic acid analogues, respectively.

Table (1): Effect of feeding the newly hatched larvae of *Pectinophora gossypiella* with different concentrations of ascorbic acid analogues on larval survival and pupation.

Concentration (Mg/ 100g diet)	Ascorbic acid analogues					
	L- ascorbic		D- isoascorbic		D- glucoascorbic	
	Larval survival (%)	Pupation (%)	Larval survival (%)	Pupation (%)	Larval survival (%)	Pupation (%)
Blank (0.0)	$40.00^{d} \pm 5.00$	100	$40.00^{c} \pm 5.00$	100	$40.00^{b} \pm 5.00$	100
0.58	$71.67^{b} \pm 2.89$	100	$86.67^{a} \pm 2.89$	100	$48.33^{a} \pm 5.77$	0.0
1.16	$86.67^{a} \pm 2.89$	100	$58.33^{b} \pm 2.89$	100	$43.33^{ab} \pm 2.89$	0.0
2.32	$56.65^{c} \pm 7.64$	100	$31.67^{d} \pm 2.89$	0.0	0.0^{c}	-
Mean	$63.75^{A} \pm 18.60$	100	$54.17^{B} \pm 22.24$	70.352	$32.92^{C} \pm 20.39$	33.333
F "value"	48.08***		142.44***		88.79***	
LSD (5%)	9.414		6.657		7.687	

Means followed by the same lower letter at the same column or the same capital letter at the same raw are not significantly different.

All survived larvae succeeded to develop to pupae after feeding with any of the three concentrations of L-ascorbic acid. The highest percentage of larval survival (86.67%) was obtained when 1.16mg of L-ascorbic acid analogue was added to 100g of the larval diet, while it decreased to 71.67 and 56.65% by adding 0.58 mg or 2.23 mg of the same analogue to 100g diet, respectively. In case of D- isoascorbic, 86.67% of larvae survived and developed to pupae when fed with 0.58 mg of this analogue / 100g diet, however larval survival decreased to 58.33 and 31.67% by increasing the added analogue to 1.16 or 2.32mg /100g diet, respectively. Moreover, complete failure of develop to pupae occurred when larvae were fed with the highest concentration (2.32mg/ 100g diet) of D-isoascorbic analogue.

Substitution the D- glucoascorbic acid analogue in the pink bollworm diet hampered larval growth and pupation, as all larvae died when fed on diet containing the highest concentration (2.23-mg/ 100g diet) of this analogue. When larvae were fed with the lower concentrations (1.16 and 0.58 mg/ 100-g diet) of the same compound, 48.3 and 43.3% reached full-grown but the all failed to develop into pupae. While, 40% of pink bollworm larvae succeeded to develop into pupae when deprived of dietary ascorbic acid.

Total of crude protein in larvae and pupae: Regarding the results in Table (2), it could be noted that the values of total protein in both larvae or pupae were differed significantly according to the added analogue and the used concentration in larval diet. When larvae were deprived of ascorbic acid, the values of total protein in the larval and pupal bodies were 17.677 and 37.037μg/ mg wt, respectively. Regardless the used concentration, L-ascorbic acid is significantly the best analogue, as the whole mean of the total protein quantity in the larval body averaged 23.772 μg comparing with 14.731μg and 12.927 μg for the D- isoascorbic and D- glucoascorbic acid analogues, respectively. While these values averaged 49.978 and 33.807μg in pupae when larvae were fed with different concentrations of L-ascorbic acid and D- isoascorbic analogues, respectively (no pupae were obtained after feeding on D- glucoascorbic acid analogues). Moreover, the highest values of protein in larvae and pupae (34.091& 68.182μg) were obtained when larvae were fed with 1.16mg L-ascorbic acid in their diet. These values decreased to 23.541 & 55.676 μg and 19.781 & 38.72μg by adding 0.58mg or 2.32mg of the same analogue to 100g of the larval diet, respectively.

As for feeding with D- isoascorbic analogue, the total protein in the larval body varied insignificantly when larvae were deprived of ascorbic acid (17.677μg) or when D- isoascorbic was added at 0.58 mg (18.098μg) or 1.16mg (14.308μg). Moreover, the total protein in the larval body declined to 8.841μg when larvae were fed with the highest concentration of D-isoascorbic analogue (2.32mg/ 100g diet). Also, the total protein in pupae decreased from 37.037μg when larvae were deprived ascorbic acid to 35.346 and 29.040μg when larvae were fed with this analogue at a level of 0.58 or 1.16mg/ 100g of their diet, respectively.

Adding D- glucoascorbic analogue to larval diet reduced significantly the total protein in their body, as it averaged 10.582μg and 10.522μg at the concentrations of 0.58 and 2.32mg/ 100g of larval diet, respectively.

Table (2): Effect of feeding the newly hatched larvae of *Pectinophora gossypiella* with different concentrations of ascorbic acid analogues on total quantity of crude protein (µg/mg wt) in larvae and pupae.

Concentration (Mg/ 100g diet)	Ascorbic acid analogues			
	L- ascorbic	**D- isoascorbic**	**D- glucoascorbic**	**Mean**
Larvae				
Blank (0.0)	17.677 [b] ± 2.186	17.677 [a] ± 2.186	17.677 [a] ± 2.186	17.676 [b] ± 1.894
0.58	23.541 [b] ± 4.042	18.098 [a] ± 2.631	10.582 [b] ± 2.527	17.407 [a] ± 6.258
1.16	34.091 [a] ± 3.788	14.308 [a] ± 2.630	10.522 [b] ± 4.059	19.640 [a] ± 11.38
2.32	19.781 b ± 2.629	8.841 [b] ± 2.185	-	14.311 [b] ± 6.370
Mean	23.772 [A] ± 7.158	14.731 [B] ± 4.384	12.927 [B] ± 4.427	17.527 ± 7.255
"F" value	15.066**	4.0628*	5.507*	(Conc. x analogues) 12.035***
L.S.D. (5%)	6.1286	9.2105	6.0651	Analogues = 2.532 Conc. = 2.924
Pupae				
Blank (0.0)	37.037 [c] ± 2.628	37.037 [a] ± 2.628	37.037 ± 2.276	37.037 [b] ± 2.276
0.58	55.976 [b] ± 2.628	35.346 [a] ± 2.194	-	45.661 [a] ± 11.66
1.16	68.182 [a] ± 3.788	29.040 [b] ± 2.187	-	48.611 [a] ±21.616
2.32	38.720 [c] ± 0.728	-	-	38.720 [b] ± 0.728
Mean	49.978[A] ± 13.625	33.807 [C] ± 4.177	37.037 [B] ± 2.276	42.296 ±12.572
"F" value	82.235***	9.686*		(Conc. x analogues) 28.73***
L.S.D. (5%)	5.0427	4.6857		Analogues = 2.6211 Conc. = 3.0266

Means followed by the same lower letter at the same column or the same capital letter at the same raw are not significantly different.

Tyrosine and chitin quantity in larvae: Data in Table (3) show major differences in tyrosine and chitin quantity as a result of feeding pink bollworm larvae with three concentration of the three aforementioned ascorbic acid analogues. The highest mean value of the two compounds (0.0.3837 & 0.0343 µg) existed in larvae fed on L-ascorbic acid while the least mean values (0.1147 & 0.0147µg) were obtained in larvae fed on D-glucoascorbic analogue. Statistically, the values of the two compounds in larvae fed on either D- isoascorbic or D-glucoascorbic analogues were equal or less than those in larvae deprived of ascorbic acid in there food. The tyrosine values ranged between 0.281µg in larvae fed on diet free of ascorbic acid to 0.015 µg when those larvae were fed on 1.16 mg of D-glucoascorbic acid. As for chitin, these values ranged between 0.0193µg and 0.0117µg, respectively.

Concerning the obtained data, the significant low percentages of survived larvae and produced pupae are a reflection to the reduction in the values of total protein, tyrosine and chitin in larval body (Fig, 1). Statistical analysis of data yielded a significant correlation between the percentages of larvae which succeeded to develop into pupae and the quantity of their total protein (r = 0.8043**), tyrosine (r = 0.82277**) and chitin (r = 0.69874*). On the other hand, the high percentages of pupae which obtained from larvae fed on the moderate concentration of L- ascorbic acid is confirmed with the results of Navon *et al.* (1983) and Navon *et al*. (1985). They indicated that L-ascorbic acid had an important function on the metabolism of protein and in stimulating the amino acids to incorporate into protein. Also, the significant low values of tyrosine and chitin in larvae fed on diet containing D-isoascorbic and D- glucoascorbic acids instead of L- ascorbic acid may be explained according to Venson and Law (1971) who stated that L-ascorbic acid activating the conversion of phenyalanine to tyrosine. Furthermore, Navon *et al.* (1983) reported that the abnormal sclerotization in the L-ascorbic acid deficient insects could be linked with the low value of tyrosine.

The present results may suggest that both of D- isoascorbic and D-glucoascorbic acids had harmful effect on pink bollworm development and revealed a reduction in total protein, tyrosine and chitin in the survived larvae. On contrary, L- ascorbic acid activating larval growth and regulating the quantity of total protein, tyrosine and chitin in the survived larvae when added to larval diet with a limited weight (1.16 mg/ 100g diet).

Table (3): Effect of feeding the newly hatched larvae of *P. gossypiella* with different concentrations of ascorbic acid analogues on tyrosine and chitin quantity (mean ± SD, µg/mg wt) in larvae.

Concentration (Mg/ 100g diet)	Ascorbic acid analogues			
	L- ascorbic	D- isoascorbic	D- glucoascorbic	Mean
Tyrosine				
Blank (0.0)	0.281^{d} ± 0.0137	0.281^{a} ± 0.0137	0.281^{a} ± 0.0137	0.281 ± 0.0119
0.58	0.428^{b} ± 0.0155	0.2343^{ab} ± 0.0511	0.048^{b} ± 0.0121	0.2367 ± 0.1667
1.16	0.466^{a} ± 0.0137	0.1993^{b} ± 0.0119	0.015^{c} ± 0.0052	0.2268 ± 0.1966
2.32	0.360^{c} ± 0.0250	0.1433^{c} ± 0.0205	-	0.2518 ± 0.1206
Mean	0.3837^{A} ± 0.0750	0.2145^{B} ± 0.0580	0.1147^{C} ± 0.1259	0.2488 ± 0.1392
"F" value	63.579***	12.025**	521.215***	(Conc. X analogues) 70.676***
L.S.D. (5%)	0.03323	0.0546	0.02198	Analogues = 0.01847Conc. = 0.0213
Chitin				
Blank (0.0)	0.0193^{b} ± 0.0025	0.0193^{a} ± 0.0025	0.0193^{a} ± 0.0025	0.0193 ± 0.0022
0.58	0.0417^{a} ± 0.0029	0.0223^{a} ± 0.0023	0.013^{b} ± 0.001	0.0257 ± 0.0128
1.16	0.040^{a} ± 0.0036	0.0117^{b} ± 0.0006	0.0117 ± 0.0011	0.0211 ± 0.0143
2.32	0.0363^{a} 0.0015	0.0143^{b} 0.0015	-	0.0253 ± 0.0121
Mean	0.0343^{A} ± 0.0096	0.0169^{B} ± 0.0046	0.0147^{C} ± 0.0038	0.0226 ± 0.0111
"F" value	41.985***	19.372***	17.423**	(Conc. x analogues) 33.2886***
L.S.D. (5%)	0.0052	0.0036	0.0096	Analogues = 0.0019 Conc. = 0.0022

Means followed by the same lower letter at the same column or the same capital letter at the same raw are not significantly different.

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REFERENCES

Abd El-Hafez, Alia; A. G. Metwally; and M.R.A. Saleh (1982). Rearing pink bollworm *Pectinophora gossypiella* (Saund.) on kidney bean diet in Egypt. (Lepidoptera - Gelechiidae). Res. Bull., Fac. of Agric., Zagazig Univ., April, (576) 10 pp.

Contrat, F.H. and B. Singer (1956). A new technique for analysis of histidine, tyrosine, methionine and arginine in protein hydrolysates. Archives of Biochem. And Biophys., 65: 296-301.

Dadd, R.H. (1960). Some effects of dietary ascorbic acid on locusts*. Proc. R.* Soc. B., 153: 128-143.

Dadd, R.H. (1963). Feeding behaviour and nutrition in grasshoppers and locusts. Adv. Insect Physiol., 1: 47-109.

Eid, M.A.A. and Eman B. Moursy (1992). Ascorbic acid analogue as a new type of male-sterilizing agent in moths of the pink bollworm *Pectinophora gossypiella* (Saund.) J. Agric. Sci., Mansoura Univ., 17: 911 –918.

Felton, G.W. and C.B. Summers (1995). Antioxidant systems in insects. Archives-of-insect-biochemistry-and-physiology (USA), 29(2): 187-197.

Gilmore, D. (1965). The metabolism of insects. W. H. Freeman Co. San Francisco, Calif., 195p

Gornall, A.G.; C.J. Bardawill and M.M. David (1949). Determination of serum protein by means of the biuret reaction. J. Biol. Chem., 177: 751-766.

Hill, L.; A.J. Luntz and P.A. Steel (1968). The relationship between somatic growth, ovarian growth and feeding activity in the adult desert locust. J. Insect Physiol., 14: 1-20.

Huber, J. (1985). Untersuchugen zur physiologie insekte-ntotender pilze. Arch. Mikrobiol, 29: 257-265.

Ito, T. and N. Arai (1965). Nutrition of the silkworm *Bombyx mori* L. LX. Further studies on the nutritive effects of ascorbic acid. Bull. Seric. Exp. Stn, Japan, 20: 1-19.

Kasting, R. and A. J. McGinnis (1962). Nutrition of the pale western cutworm, *Agrotis orthogonia* Morr (Lipid, Noctuidae). J. Insect Physiol., 8: 96-103.

Klein, G. (1931). Handbuch der pflanzenanalyse, II: Sringer-Verlag, Vienna.

Levinson, H.Z. and A. Navon (1969). Ascorbic acid and unsaturated fatty acids in the nutrition of the Egyptian cotton leafworm, Prodenia litura. J. insect Physiol., 15: 591-595.

Ludwing, D. and M.R. Gallagher (1966). Vitamin synthesis by symbionts in the fat body of the cockroach, *Perplaneta americana* (1) J.N.Y. Entomol. Soc., 74: 134-139.

Moursy, B. Eman (1995). Influence of D-isoascorbic acid on growth and reproduction of the pea aphid, *Aphis craccivora* Koch (Homoptera: Aphididae). Bull. Ent. Soc. Egypt, Econ. Ser., 22: 55-64.

Navon, A.; J. Nesbitt; W. Henzel and H. Lipke (1985). Effect of ascorbic acid defficiency on growth and cuticle composition in *on Manduca sexta* and *Spodoptera littoralis.* Insect Biochem., 15: 285-291.

Navon, A.; J. Nesbitt; W. Henzel; K. Mulligan; J.A. Mullen; M. Sugumaran and H. Lipke (1983). The effect of D- isoascorbic acid on spermatophore composition in *Spodoptera littoralis*. Insect Biochem., 13: 247-250.

Salem, M.S.: E. B. Moursy; F.M. El-Borollosy and S. A. Mohamed (1992). Influence of feeding with Liso-ascorbic acid and Diso-ascorbic analogues on the reproductive potentiality of cotton bollworms *Pectinophora gossypiella* (Saund.) and *E. insulana* (Boisd.). J. Agric. Sci., Mansoura Univ., 17: 585-593.

Snedecor, G. W. and W. G. Cochran (1980). Statistical methods,2nd ed. The Iowa State University Press, Ames, Iowa, pp. 318. Insect Biochem., 15: 285-291.

Vanderzant, S.E. and D.C. Richardson (1963). Ascorbic acid in the nutrition of plant feeding insects. Science, 140: 989-991.

Venson, S.B. and P.K. Law (1971). Cuticular composition and DDT-resistance in thetobacco budworm. J. Econ. Entomol., 64: 1387-1390.

تأثير التغذية على بعض مشابهات حمض الأسكوربيك على النمو وبعض المكونات الكيميائية في دودة اللوز القرنفلية ***Pectinophora gossypiella*** **(Saund.)**

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تم دراسة تأثير حامض الأسكوربيك (فيتامين س) على النمو وبعض المكونات البيوكيميائية في دودة اللوز القرنفلية، وذلك بعد تغذية اليرقات حديثة الفقس على بيئة صناعية مضاف إليها تركيزات مختلفة لثلاث مشابهات من هذا الحمض وهي: L-ascorbic acid, D- isoascorbic and D- glucoascorbic acid. وقد أثر نوع المشابه والتركيز المستخدم تأثيرا معنويا على نمو اليرقات. وأوضح التحليل الإحصائي أن المشابه L-ascorbic acid هو أفضل الأنواع الثلاثة حيث بلغ المتوسط العام لنسبة البقاء في اليرقات التي تم تغذيتها على التركيزات المختلفة من هذا الحمض 63.73% مقارنة بالنسبة 54.17% و 32.9% في حالة التغذية على نفس التركيزات منD- isoascorbic و D- glucoascorbic acid على التوالي. وكذلك نجحت جميع اليرقات التي اكتمل نموها بعد التغذية على أي من التركيزات المختلفة من المشابه L-ascorbic acid في التحول إلى طور العذراء، بينما فشلت جميع اليرقات في التحول إلى طور العذراء عندما غذيت على بيئة مضاف إليها أي من المشابهين D- isoascorbic أو D- glucoascorbic acid بمعدل 2.32 مج / 100جرام من البيئة الغذائية.

هذا ويعتبر الانخفاض المعنوي في نسبة البقاء بين اليرقات أو العذارى الناتجة ماهو إلا انعكاس لانخفاض كمية البروتين والتيروسين والشيتين في جسم اليرقات. فقد أظهر التحليل الإحصائي وجود ارتباط معنوي بين نسب اليرقات التي نجحت في التحول إلى طور العذراء وكمية كل من البروتين (معامل الارتباط= 0.8043) والتيروسين (معامل الارتباط= 0.82277) والشيتين (معامل الارتباط= 0.69874) في جسمها.