

## ROLE OF AMINO ACIDS IN PROTECTING *PECTINOPHORA GOSSYPIELLA* (SAUND.) AGAINST STRONG PROTEIN-DENATURING ACTIVITY OF PHENOLIC COMPOUNDS IN COTTON PLANTS

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### **Abstract**

To test if the generality of glycine secretion in the pink bollworm larvae related to the concentration of phenolic compounds (PCs), a feeding experiment with two concentrations of selected two PCs (pyrocatechol; Pyro & resorcinol; Res) was performed. Then, we determined and compared the lysine and glycine contents in the total body homogenate of treated and untreated 4<sup>th</sup> instar larvae. Results showed that the lysine and glycine contents recorded were 2.29 & 1.47 mg/100mg of control larval body weight. Lysine content increased at low PCs concentration by 5.68 & 7.42% for Pyro and Res, respectively. On the other hand, its concentration decreased at high PCs concentration by 0.87 & 0.44%, respectively. On the other hand, glycine content generally decreased (except at low concentration of Pyro; 8.84%) by 4.76, 4.08 & 12.24%. Also, it could be noted that the generality of glycine secretion related to the concentration of PCs; whereas the less in glycine content was more notable in high concentration of PCs. And, the loss in glycine content was more than that for lysine.

Also, studies extend to other amino acids (AA) and the results indicated that it could be grouped AA in 3 groups:

1. A high AA concentration (more than 3 mg/100mg): GLU and ASP acid.
2. A moderate AA concentration (more than 2 mg/100mg): VAL, LEU, LYS, ARG, ALA and TYR.
3. A low AA concentration (less than 2 mg/100mg): THR, ILE, PHE, HIS, CYS, SER, GLY and PRO.

In the control PBW, glutamic acid and aspartic acid were present in high concentration (4.24 & 3.19 mg/100mg, respectively). As same manner with glycine; the losses in GLU and ASP contents were more than that for LYS. So, it could be used by PBW to overcome PCs toxicity. In addition, generally, at low PCs concentration, AA content increased except in the case of VAL and CYS. On contrast, at high PCs concentration, AA content decreased except in the case of MET, PHE, HIS and TYR. Commonly, it could be noted that Val, Cys, Gly and Ala had a higher percent of reduction. So, it means that other free amino acids may be used by some herbivores as counteractive agents against the denaturing activity of PCs.

### **INTRODUCTION**

The pink bollworm (PBW), *Pectinophora gossypiella* (Saund.) supposed to be a major cotton pest. Plants have established several chemical defenses against insects. Cotton plants produce numerous secondary plant chemicals including phenolic compounds (PCs) (Perveen *et al.*, 2001). Cotton PCs induce a reduction in viability of dietary protein in lepidopterous insects by making covalent bonds with the amino

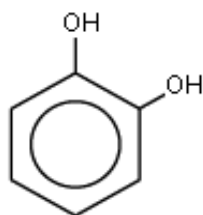
residue of lysine side chain in proteins, so it reduces the amount of lysine in proteins (Appel, 1993). To overcome these compounds, insects have evolved various detoxification mechanisms. In 1996 Konno *et al.* started series of studies about the chemical methods which used by insects to resist compounds which causing protein denaturing like PCs. They observed a high concentration of free glycine in the digestive juice and midgut of the silkworm, *Bombyx mori*, and several other Lepidoptera larvae. In 1997 Konno *et al.* throw *In vitro* studies found that free glycine totally inhibits the denaturing activity of the privet leaves. Pierpoint (1969) mentioned that the amino residue of glycine and lysine reacts with PCs in the same manner. The glycine amino residue competes with the lysine amino residue, and hence inhibits the PCs denaturing activity (Konno *et al.*, 2010). Also, according to their theory, not just free glycine but either other amino acids (GABA,  $\beta$ -alanine and alanine) are able to inhibit PCs denaturing activity, indicating that amino residues are responsible for the inhibition. Previously in 2014 we found that the amount of free glycine in PBW appears to have a clear association with the PCs detoxifying activity. Also, we noticed unexpected presence of some other amino acids (AA) like: glutamic acid. In the current study, we tried to covered some remaining questions. Firstly, we tried to study if the glycine secretion generality related to the concentration of PCs. Secondly, we were concerned about figuring out if glycine is the only AA which has a role against the PCs defense. Thirdly, we asked what amino acids structure is important for the PCs activity inhibition.

## MATERIALS AND METHODS

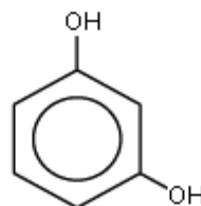
*Insects: Pectinophora gossypiella* were reared on a modified artificial diet according to Abd El-Hafez *et al.* (1982), at the laboratory of Bollworms Department, Plant Protection Research Institute, ARC, Dokki, Giza.

*Tested phenolic compounds:* Two PCs namely; pyrocatechol and resorcinol (Roth: Bestellen sie zum Nulltarif); were used in present study. Figure (1) illustrated the chemical structure of these compounds. According to the results of Ahmed (2007), pyrocatechol and resorcinol proved to be toxic to PBW. For this reason, these compounds were chosen for studying the role of AA as detoxifying agent against cotton PCs in PBW. In order to examine the generality of glycine secretion related to the concentration of PCs, two concentrations of PCs were used:

1. Twice the  $LC_{50}$  (1.718 & 1.46 g/100g diet, for pyrocatechol & resorcinol, respectively).
  2. Half of  $LC_{50}$  (0.4295 & 0.365 g/100g diet, for pyrocatechol & resorcinol, respectively).
- The data for  $LC_{50}$  was taken from Ahmed *et al.* (2014).



**Pyrocatechol (Pyro)**



**Resorcinol (Res)**

**Fig. 1. Chemical structures of the tested phenolic compounds**

**Amino acids analysis:** This part of study was conducted in order to determine free amino acids content in the 4<sup>th</sup> instar larvae of PBW after treatment with tested PCs concentrations. Newly hatched larvae ( $\approx 100$  larvae) of PBW were allowed to feed on artificial diet containing the tested concentrations of the two tested PCs, for 2 hours and then transferred to feed on control diet. Fourteen days after treatment, the survived 4<sup>th</sup> instar larvae were collected and refrigerated (at 5°C) for few minutes and then dried in the oven overnight (at 60°C) for biochemical analysis. The total larval bodies were homogenized to get dry powder. Homogenates were weighted and kept till the biochemical determinations. Quantitative determination of amino acids was carried out at Regional Center for Food and Feed, ARC, Dokki, Giza, by high perform Amino Acid analyzer (Biochrom 30) and EZ chrome manual (software for data collection and processing), according to AOAC (2012).

## RESULTS AND DISCUSSION

### Does glycine secretion relate to the concentration of PCs?

To test if the generality of glycine secretion related to the concentration of PCs, a feeding experiment with two concentrations of each PCs was performed. Then, we determined and compared the lysine and glycine contents in the total body homogenate of treated and untreated 4<sup>th</sup> instar larvae (Table 1).

Lysine and glycine contents recorded 2.29 & 1.47 mg/100mg of control larval body weight. Lysine content increased at low PCs concentration by 5.68 & 7.42% for Pyro and Res, respectively. On the other hand, its concentration decreased at high PCs concentration by 0.87 & 0.44% for Pyro and Res, respectively. On the other hand, glycine content generally decreased (except at low concentration of Pyro; 8.84%) by 4.76, 4.08 & 12.24%. Also, it could be noted that the generality of glycine secretion related to the concentration of PCs; whereas the less in glycine content was more notable in high concentration of PCs. And, the loss in glycine content was more than that for lysine. It could be due to that glycine is assumed to be lost by binding to PCs, but this loss is less detrimental for herbivores than the loss of lysine. The larvae could be expending glycine (nonessential AA), for saving lysine (essential and a more valuable AA) (konno *et al.*, 2010). So, the amount of free glycine in PBW seems to have a clear

association with the detoxification activity of the PCs. These findings approve our early findings (Ahmed *et al.*, 2014), and in agreement with (konno *et al.*, 2009).

Whether glycine is the only amino acid that has a role against the PCs defense? And what amino acids structure is important for the PCs activity inhibition?

To study if not just glycine but also other AA which has amino residues has a role against the PCs defense, we extend the determination to other amino acids (Table 1).

Results indicated that it could be grouped AA in 3 groups:

1. A high AA concentration (more than 3 mg/100mg): GLU and ASP acid.
2. A moderate AA concentration (more than 2 mg/100mg): VAL, LEU, LYS, ARG, ALA and TYR.
3. A low AA concentration (less than 2 mg/100mg): THR, ILE, PHE, HIS, CYS, SER, GLY and PRO.

**Table 1. Concentration of free amino acids (mg/100mg) in the total body homogenate of *P. gossypiella* 4<sup>th</sup> instar larvae.**

Amino acids	Control	After treatment with				%Change after Treatment With*				
		Pyrocatechol		Resorcinol		Pyrocatechol		Resorcinol		
		1	2	1	2	1	2	1	2	
Essential	Threonine (THR)	1.66	1.79	1.60	1.71	1.63	7.83	-3.61	3.01	-1.81
	Valine (VAL)	2.28	2.18	2.02	1.94	1.78	<u>-4.39</u>	-11.40	<u>-14.91</u>	-21.93
	Methionine (MET)	1.03	1.15	1.12	1.11	1.16	11.65	<u>8.74</u>	7.77	<u>12.62</u>
	Isoleucine (ILE)	1.58	1.68	1.5	1.6	1.44	6.33	-5.06	1.27	-8.86
	Leucine (LEU)	2.55	2.64	2.43	2.62	2.47	3.53	-4.71	2.75	-3.14
	Phenylalanine (PHE)	1.82	1.98	1.86	1.99	1.88	8.79	<u>2.20</u>	9.34	<u>3.30</u>
	Lysine (LYS)	2.29	2.42	2.27	2.46	2.28	5.68	-0.87	7.42	-0.44
Semi-essential	Histidine (HIS)	1.16	1.25	1.21	1.28	1.24	7.76	<u>4.31</u>	10.34	<u>6.90</u>
	Arginine (ARG)	2.26	2.48	2.22	2.29	2.2	9.73	-1.77	1.33	-2.65
	Cystine (CYS)	0.55	0.51	0.46	0.48	0.48	<u>-7.27</u>	-16.36	<u>-12.73</u>	-12.73
Nonessential	Aspartic acid (ASP)	3.19	3.24	3	3.47	3.06	1.57	-5.96	8.78	-4.08
	Serine (SER)	1.37	1.47	1.28	1.41	1.33	7.30	-6.57	2.92	-2.92
	Glutamic acid (GLU)	4.24	4.27	3.84	4.29	3.91	0.71	-9.43	1.18	-7.78
	Glycine (GLY)	1.47	1.6	1.4	1.41	1.29	8.84	-4.76	-4.08	-12.24
	Alanine (ALA)	2.28	2.65	2.11	1.81	1.65	16.23	-7.46	-20.61	-27.63
	Tyrosin (TYR)	2.53	2.87	2.77	2.65	2.77	13.44	<u>9.49</u>	4.74	<u>9.49</u>
	Proline (PRO)	1.82	1.95	1.66	1.88	1.63	7.14	-8.79	3.30	-10.44

$$* \%Change = \frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$$

In the control PBW, Glutamic acid and aspartic acid were present in high concentration (4.24 & 3.19 mg/100mg, respectively). As same manner with glycine; the losses in GLU and ASP contents were more than that for LYS. So, it could be used by PBW to overcome PCs toxicity. Amino acids used to overcome PCs toxicity must be non-essential amino acids (insects can synthesize by themselves), like glycine, or derivatives, such as GABA ( $\gamma$ -amino butyric acid) and  $\beta$ -alanine, where it could be simply formed from glutamate and aspartate, respectively (Konno *et al.*, 2010). They found that GABA and  $\beta$ -alanine inhibited the lysine-decreasing activity as effectively as glycine.

Generally, at low PCs concentration, AA content increased except in the case of VAL and CYS (underlined data; Table 1). On contrast, at high PCs concentration, AA content decreased except in the case of MET, PHE, HIS and TYR (dotted underlined data). Commonly, it could be noted that Val, Cys, Gly and Ala had a higher percent of reduction. So, it means that other free amino acids may be used by some herbivores as counteractive agents against the denaturing activity of oxidized PCs as mentioned previously by konno *et al.* (2010). They also indicated that amino residues are responsible for the inhibition, and the inhibitory activity differed significantly among the amino acids. While glycine, GABA, and  $\beta$ -alanine inhibited lysine-declining activity approximately completely at lower concentrations, alanine did not do this even at 5 times higher concentrations. It could be attributed to the structure similarity between glycine,  $\beta$ -alanine and GABA, where they have amino residues at the molecules ends, versus alanine that does not. They also indicated that these AA appear to be the only AA that are at the same time economically and efficiently, and therefore the benefits of production overreached the costs.

In conclusion, PBW could develop molecular strategies based on the secretion of AA like glycine, glutamic acid, and aspartic acid as a counteracting, to feed on the cotton plants which defense by accumulating PCs.

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## دور الأحماض الأمينية في حماية دودة اللوز القرنفلية من التأثير المتبطل للبروتين الحادث بفعل المركبات الفينولية المتواجدة في نبات القطن

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1. قسم بحوث ديدان اللوز - معهد بحوث وقاية النباتات - مركز البحوث الزراعية

تحتوي نباتات القطن على مركبات فينولية والتي تُعد من مرسبات البروتين، وقد وُجد أنها تُحدث تأثيرها من خلال الارتباط مع الحمض الأميني الليسين (حمض أميني ضروري) مما يؤدي لخفض كمية الليسين في البروتين. وقد أُثبتت الدراسات السابقة أن عديد من حرشفيات الأجنحة تتميز بوجود تركيز عالي من الحمض الأميني الجليسين (حمض أميني غير ضروري) وأنها تستخدم هذا الحمض في مقاومة الفعل المرسب للبروتين الحادث بفعل المركبات الفينولية، وذلك لأن الجليسين يرتبط مع مرسبات البروتين بدلا من الليسين كوسيلة للحفاظ على محتواها من الحامض الأميني الليسين. وقد وجد في دراسات سابقة وجود علاقة بين وجود الجليسين في دودة اللوز القرنفلية ومقاومة فعل المركبات الفينولية، بالإضافة إلى أنه لوحظ وجود تركيز مرتفع من أحماض أمينية أخرى كالجوتاميك. وعلى هذا فقد اهتم البحث الحالي باستكمال بعض النقاط والتي منها:

1. هل هناك علاقة بين إفراز الجليسين وتركيز المركبات الفينولية؟
  2. هل الجليسين هو الحمض الأميني الوحيد الذي له دور في مقاومة المركبات الفينولية؟
  3. هل هناك تركيب محدد للأحماض الأمينية التي تساهم في مقاومة الأحماض الأمينية؟
- من الدراسات السابقة تم اختيار تركيزين لإثنين من المركبات الفينولية (البيروكاتيكول والريزورسينول) التي ثبتت سميتها لدودة اللوز القرنفلية وتم قياس محتوى كل من الجليسين والليسين، فكانت الكميات المسجلة هي 2.29 و 1.47% في حالة الحشرات غير المعاملة. ارتفعت نسبة الليسين مع استخدام التركيز المنخفض من المركبات الفينولية بمقدار 5.68 و 7.42% للبيروكاتيكول والريزورسينول، على التوالي. في حين حدث انخفاض في نسبته مع التركيز المرتفع بمقدار 0.87 و 0.44% على التوالي. وعلى الجانب الآخر فقد انخفض تركيز الجليسين بصفة عامة، كما أن الإنخفاض الحادث في تركيز الجليسين كان أعلى من ذلك الحادث في تركيز الليسين، مما يؤكد على دور الجليسين في مقاومة فعل المركبات الفينولية مهما كان تركيزها.

كذلك أُثبتت الدراسة وجود تركيزات مرتفعة من أحماض الجلوتاميك (4.24%) والإسبارتيك (3.19%)، وأن الفقد الحادث لمحتواها أكبر من الفقد الحادث لمحتوى الليسين. هذا بالإضافة إلى أن التركيزات المنخفضة من المركبات الفينولية أدت بصفة عامة إلى زيادة تركيز الأحماض الأمينية فيما عدا الأحماض الأمينية الفالين والسيستين، بينما في التركيزات المرتفعة حدث إنخفاض لمحتوى الأحماض الأمينية فيما عدا الميثيونين، الفيناييل ألانين، الهستيدين والتايروزين، وكان أعلى معدل خفض للأحماض الأمينية الفالين، السيستين، الجليسين، والفالين مما يشير إلى إمكانية مشاركة أحماض أخرى غير الجليسين في مقاومة فعل المركبات الفينولية.

