Influence of Two Insect Growth Regulators on Chitinase Activity

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Received on: 18/11/2015 *Accepted: 31/12/2015*

ABSTRACT

Toxicity of two insect growth regulators (IGRs) (lufenuron and hexaflumuron) against two larval instars of cotton leafworm *Spodoptera littoralis*, laboratory and field strains were determined. Chitinase activity in the two strains also was investigated. Results revealed that, 2nd instar larvae were more sensitive than 4th instar larvae to both insecticides. The sensitivity of chitinase activity was measured by I_{50} values. The I_{50} values of lufenuron were 0.31, and 0.64 µM for lab and field strains of *S. littoralis* 2nd larvae respectively, while I_{50} values were 0.44, and 0.75 µM for lab and field strains of *S. littoralis* 4th larvae respectively. The hexaflumuron were 0.57, and 0.76 µM for lab and field strains of *S. littoralis* 2nd larvae respectively, the I_{50} values were 0.65, and 0.81µM for lab and field strains of *S. littoralis* 4th larvae respectively. Also, chitinase enzyme kinetic parameters, as Michaelies-Menten Kinetics (K_m and V_{max}) values and the inhibition constant (K_i) were determined. The obtained data proved that lufenuron and hexaflumuron compounds are competitive inhibitors of chitinase activity. Results indicated that, the IGRs have shown high potentiality against larvae of *S. littoralis*, so, these IGRs may be recommended for *S. littoralis* larvae control, it could be concluded that the use of IGRs instead of conventional hazardous insecticides; may avoid increasing selection pressure of *S. littoralis* populations to conventional insecticides, hazard effects on human health, environmental components and natural enemies, IGRs may play an important role in future insect pest management programs.

Key words: Chitinase- *S. littoralis* – insect growth regulators (IGRs).

INTRODUCTION

The development of multiple insecticide resistance in field strain of the *Spodoptera littoralis* to several insecticides has been recorded by several investigators. Due to severe applications of insecticides for the control of *S. littoralis* larval instars, which are the most destructive stages of the insect on cotton and vegetable crops, the larval stages have become extremely tolerant to the action of pesticides (Ware 2000 and Temerak 2002). So the need to develop novel alternatives or functional combinations of pest control techniques is emphatically a product of this decade and many sources for alternative pesticides were found such as insect growth regulators (IGRs) compounds which are considered nowadays one of the mainly component of IPM program. Term IGRs describe a new class of bio-rational compounds, this group are active against larvae of many lepidopterous species (Fisk & Wright 1992; Schneider *et al*., 2003, and Sandeep & Bhamare 2006).

Therefor the present work was conducted to study the efficiency of two IGRs (lufenuron and hexaflumuron) upon the 2nd and 4th larval instar of *S. littoralis*, and describe the development of biochemical assay system for measuring the sensitivity of chitinase enzyme to two IGRs (lufenuron and hexaflumuron), in laboratory and field strains.

MATERIALS AND METHODS

1. Test insects:

Susceptible laboratory strain of cotton leafworm, *Spodoptera littoralis* was provided by central lab of pesticides, Agricultural Research Center (ARC) Cairo, Egypt which was reared for several years on artificial diet under standard laboratory conditions of 27 ± 2 ºC and 65-70 % RH.

Field strain of cotton leafworm, *Spodoptera littoralis* egg masses were collected from cotton fields at Abeis area Alexandria, governorate Egypt. The 2nd and 4th larval instars were chosen for bioassay and biochemical assessment.

2. Test insecticides:

Lufenuron (Match, 5% EC), and hexaflumuron (consult, 5% EC), were supplied by Syngenta.

3. Bioassay tests:

3.1. Toxicity of the tested IGRs against *S. littoralis*:

Lufenuron and hexaflumuron were bioassayed against the 2nd and 4th larvae of *S. littoralis*. The castor leaves were dipped in different concentrations of the tested IGRs. Lufenuron and hexaflumuron concentrations were prepared in distilled water. Treated and control leaves plants were air-dried for 3 hrs, the treated leaves were placed in clean glass container at the laboratory conditions of (27 ± 2 ºC) and 65-70 % RH, ten larvae (lab and field strains) were used for each test with three replicate at least. Number of alive and dead larvae per replicate was

counted 24, and 48 hr, after treatment. Concentrations-mortality percentage were calculated and corrected for natural death according to Abbott equation (Abbott, 1925). LC_{50} values were calculated and statisticaly and analysed by using the probit-analysis method of Finney (1971).

4. Biochemical studies:

4.1. Chitinase preparation and activity assay:

Chitinase was prepared from *Spodoptera littoralis* 2nd and 4th instars larvae (lab and field strains) according to the method of Deul *et al*., (1978). Larvae homogenate was prepared in 10^3 M Clelands, reagent (dithiotheritol, DTT) (v/w=2), centrifuged at 12.000 g for 15 min. Then an equal volume of saturated ammonium sulfate solution was slowly added to the supernatant. After stirring for 1 hr, the suspension was centrifuged at 10.000 g for 10 min. The precipitate was washed with half-saturated ammonium sulfate solution and recentrifuged. Then it was suspended in a small volume of water, followed by dialysis for 20 hr at 0-2 °C.

The chitinase activity measurements were done according to the method reported by Reissig *et al*., (1955), which modified by Andrew *et al*., (1982), using sodium acetate buffer instead of tris-HCl buffer and wave-leangth of 416 nm instead of 544 nm. 25 µl of chitin (20mg/ml), 100 µl of enzyme preparation were used and 225 µl of sodium acetate (pH 4.5) in total volume 350 µl. The enzyme substrate mixture was incubated at 35 °C for 60 min, then the reaction was stopped by adding 100 µl of 0.8 M borate buffer (pH 10.0) followed by determination of n-acetylglucoseamine by method of Reissig *et al*., (1955) by adding 1.5 ml of p-dimethyl amino benzaldhyde (DMAB, reagent). The samples were incubated in shaker water bath at 35 °C for 20 min and were measured spectrophotometrically at λ412 nm.

The protein content in prepared homogenates of *S. littoralis* was assayed by the method of Lowery *et al*. (1951) at λ750 nm using Bovine Serum Albumin (BSA) as a standard protein.

4.2. *In vivo* inhibition of chitinase activity

The inhibition percentage of chitinase activity was determined in the 2nd and 4th instars larvae previously feed on leaves treated with the concentration of LC_{50} values of each of the tested insecticides (lufenuron and hexaflumuron). 10 µl of the enzyme preparation was incubated with the substrate for 30 min, the enzyme-substrate mixture was used to measure the remaining activity. The percent inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{V-Vi}{V} \times 100$$

Where:-

(V) is the specific activity in larvae feed on treated castor leaves.

(Vi) is the specific activity in larvae feed on non treated castor leaves.

4.2. *In vitro* inhibition of chitinase activity

The inhibitor of chitinase activity was evaluated to determine enzyme kinetic parameters, the method of Dixon and Webb (1964) was adopted to draw the Dixon-plots by plotting 1/V versus concentrations of the inhibitor (lufenuron and hexaflumuron) at two concentrations of the substrate, chitin (the substrate of chitinase) concentrations of 3.0 and 5.0 mM. Estimation of I_{50} value was carried out by preincubating the enzyme with the inhibitor for 30 min, using the following concentrations 0.1; 1; 5; 10; 50, and 100 µM. K_i (the inhibition constant) values for each inhibitor were estimated from Dixon-plot. Michaelies-Menten Kinetics (K_m and V_{max}) values were calculated by a linear regression of 6 point on each Lineweaver and Burk Plot (1934).

RESULTS AND DISCUSSION

Toxicity of IGRs against *S. littoralis* larvae:

The toxicity of the lufenuron and hexaflumuron in terms of LC_{50} are given in table (1) for 2nd and 4th larvae of *S. littoralis*. LC_{50} values were 0.31 and 0.55 ppm for lufenuron and hexaflumuron respectively against 2nd instar larvae of *S. littoralis* after 24 hr for lab strain, while for field strain LC_{50} values were 0.54 and 0.76 ppm for the two IGRs respectively. Also LC_{50} values were 0.052 and 0.068 ppm after 48 hr for lab strain, while for field strain LC_{50} values were 0.068 and 0.095 ppm for two IGRs, respectively. LC_{50} values were 0.44 and 0.78 ppm for lufenuron and hexaflumuron respectively against 4th instar larvae of *S. littoralis* after 24 hr for lab strain, for field strain LC_{50} values were 0.63 and 0.97 ppm for the two IGRs respectively. LC_{50} values were 0.061 and 0.077ppm after 48 hr for lab strain respectively, while for field strain LC_{50} values were 0.080 and 0.096 ppm for two IGRs respectively.

Table 1: Toxicity of IGRs on *S. littoralis* larvae.

S. littoralis strains	LC_{50} (ppm)							
	lufenuron				hexaflumuron			
	24hr		48hr		24hr		48hr	
	2nd	4th	2nd	4th	2nd	4th	2nd	4th
Lab	0.31	0.44	0.052	0.061	0.55	0.78	0.068	0.077
Field	0.54	0.63	0.071	0.080	0.76	0.97	0.095	0.096

According to LC_{50} values it is quite clear that the susceptibility of *S. littoralis* larvae to lufenuron and hexaflumuron decreased by increasing the posttreatment period. Also it was observed that the 2nd instar was more susceptible than the 4th instar. The present results are confirmed by the results of (Fisk & Wright 1992; Toscano *et al*., 2001, and Sandeep & Bhamare 2006).

The *in vivo* inhibition of *S. littoralis* chitinase activity:

The *in vivo* inhibitory effect of the LC_{50} values of tested IGRs against to the *S. littoralis* 2nd and 4th instars lab and field strains larval chitinase are shown in table (2). The data cleared that lufenuron and hexaflumuron concentration exhibited a high percentages of reduction of chitinase activity. The percentages of chitinase inhibition were 88.1, and 74.5 % for lab strain of *S. littoralis* 2nd instar larvae, respectively, while in field strain values were 74.3 and 62.8 % for the two IGRs respectively. Also the values were 73.6, and 63.1 % for lab strain of *S. littoralis* 4th instar larvae, and for field strain the values were 61.9, and 57.4 % for the two IGRs, respectively.

These results show that the tested IGRs act by reducing chitin incorporation in the cuticle of *S. littoralis*, similar results were obtained by Susan *et al*., 1990. Properties of the IGRs were originally recognized through their ability to initiate inappropriately timed and poorly coordinated moulting processes, the resulting perturbation of moulting and metamorphosis leads to death, usually because the insects cannot escape from the exuvie (Ascher & Nemny 1979; Aller & Ramsay, 1988, and Liburd *et al*., 2000). Therefore one may expect that these compounds will be very potent on cotton leafworm and other lepidopterous larvae.

Kinetic parameters of chitinase inhibition:

The kinetic studies were conducted to evaluate the effects of lufenuron and hexaflumuron on chitinase activity in both tested strains of *S. littoralis* 2nd and 4th larvae, table (3) shows the obtained Lineweaver-Burk (L-B) plots for chitinase in lab and field strains and the statistical analysis of the obtained values of K_m (Michaelis-Menten kinetics, constant) and V_{max} (maximum velocity) of the chitinase activity. The K_m values for chitinase were generally higher for field strain than lab strain, the change in K_m values of chitinase between the lab and field strains indicated changes in the affinities.

The present results show that the V_{max} values of chitinase may reflect the physiological importance of the chitinase in the function of the moulting of the *S. littoralis* larvae. The V_{max} values were generally higher in field strains than lab strain, this indicated that the number of active sites on the chitinase of the larvae was increased in the field strain, such change may be followed by decrease in the insect susceptibility which could be altered by field application of the insecticides.

The *in vitro* inhibition of *S. littoralis* chitinase activity:

To characterize more details about the *in vitro* inhibition of chitinase by the inhibitors, the K_i value of each inhibitor was estimated from the graphical method of Dixon and Webb (1964), table (4). The sensitivity of chitinase activity to lufenuron and hexaflumuron were measured by I_{50} values. In the case of lufenuron the I_{50} values were 0.31, and 0.64 µM for lab and field strains of *S. littoralis* 2nd larvae respectively, while I_{50} values were 0.44, and 0.75 µM for lab and field strains of *S. littoralis* 4th larvae respectively. Similarly, in case of the hexaflumuron the I_{50} values were 0.57, and 0.76 µM for lab and field strains of *S. littoralis* 2nd larvae respectively, the I_{50} values were 0.65, and 0.81µM for lab and field strains of *S. littoralis* 4th larvae respectively. The K_i values were 20, and 35 µM for lab and field strains of *S. littoralis* 2nd larvae respectively, in case of lufenuron, while the values were 44, and 50 µM for lab and field strains of *S. littoralis* 4th larvae respectively. Also, in case of hexaflumuron the values were 34, and 51 µM for lab and field strains of *S. littoralis* 2nd larvae respectively, while the values were 52, and 63 µM for lab and field strains of *S. littoralis* 4th larvae, respectively.

Chitinase plays an essential role during ecdysis. This enzyme is vital to moult in insects, and may also affect gut physiology through their involvement in peritrophic membrane turnover. The exoskeleton of insect might constitute a useful target site for insecticidal chemicals. The obtained changes in enzymes activity between lab and field strains may due to the variation in the protein synthesis as a response to the different treatment (Clarke & Jewess 1990; Smagghe *et al*., 1997; Wilson & Cryan 1997; Dean *et al*., 1999; Merzendorfer & Zimoch, 2003, and Kostyukovsky & Trostanetsky 2006).

Table 2: *In vivo* inhibition of *S. littoralis* larvae chitinase activity by two IGRs (LC_{50}).

	% inhibition of chitinase activity			
S. littoralis	**lufenuron**		**hexaflumuron**	
Strains	**2nd**	**4th**	**2nd**	**4th**
Lab	88.1	73.6	74.5	63.1
Field	74.3	61.9	62.8	57.4

Table 3: Michaelies-Menten Kinetics of the chitinase of larval of *S. littoralis*.

S. littoralis Strains	lufenuron				hexaflumuron			
	K_m mM		V_{max} mM		K_m mM		Vmax mM	
	2nd	4th	2nd	4th	2nd	4th	2nd	4th
Lab	0.33	0.46	6.8	5.2	0.52	0.60	4.7	3.6
Field	0.54	0.65	4.7	3.2	0.64	0.77	2.9	1.8

Table 4: I*n vitro* inhibition of *S. littoralis* larvae chitinase activity by two IGRs.

S. littoralis Strains	Lufenuron				Hexaflumuron			
	I_{50} µM/L/min		K_i µM		I_{50} µM/L/min		K_i µM	
	2nd	4th	2nd	4th	2nd	4th	2nd	4th
Lab	0.31	0.44	20	44	0.57	0.65	34	52
Field	0.64	0.75	35	50	0.76	0.81	51	63

Finally, according to the results presented, lufenuron and hexaflumuron are potentially potent insecticides for controlling *S. littoralis*. These compounds are effective suppressors for the development of the entire life cycle of insects. They act preferentially by interfering with chitin synthesis metabolism (chitin synthesis inhibitors) and with the deposition of chitin in the insect cuticle. Therefore, these compounds could be used in the integrated pest management (IPM) programs, in order to minimize the negative effects of conventional insecticides on the environments and to protect the natural enemies.

1- Yield/ vine:

Data in Table (1) clearly show that spraying clusters of Early sweet grapevines with GA_3 at 10 to 40 ppm or Sitofex at 2.5 to 10 ppm was significantly effective in improving the yield relative to the check treatment. The promotion on the yield was accompanied with increasing concentrations of each plant growth regulator. Using GA_3 at 10 to 40 was significantly preferable than using Sitofex at 2.5 to 10 ppm in improving the yield. A slight and unsignificant promotion on the yield was attributed to increasing concentrations of GA_3 from 20 to 40 ppm and Sitofex from 5 to 10 ppm. The maximum yield was produced on the vines that received one spray of GA_3 at 40 ppm but the best treatment from economical point of view was the application of GA_3 at 20 ppm (since no measurable promotion on the yield was recorded between 20 and 40 ppm of GA_3). Under such promised treatment, yield/ vine reached 13.6 and 14.0 kg during both seasons, respectively. The control vines produced 9.1 and 9.6 kg during 2013 and 2014 seasons, respectively. The percentage of increase on the yield due to application of GA_3 at 20 ppm over the check treatment reached 49.5 and 45.8 % during both seasons, respectively. The beneficial effects of GA_3 on the yield might be attributed to their positive action on increasing cluster weight. The promoting effects of GA_3 on the yield was supported by the results of Dimovska *et al*., (2011) and Abu- Zahra and Salameh (2012) on different grapevine cvs.

The results regarding the beneficial effects of Sitofex on enhancing the yield are in harmony with those obtained by Juan *et al*. (2009); Abdel- Fattah *et al*., (2010) and Al- Obeed (2011).

2- Harvesting date:

It is clear from the data in Table (1) that all GA_3 and Sitofex treatments had significantly delayed on the harvesting date of Early Sweet grapevines rather than the control treatment. The degree of delayness on harvesting date was correlated to the increase of the concentrations of both GA_3 and Sitofex. Using GA_3 significantly delayed harvesting date comparing with using Sitofex. Increasing concentrations of GA_3 from 20 to 40 ppm and Sitofex form 5 to 10 ppm failed to show significant delay on harvesting date. A considerable advancement on harvesting date was observed on untreated vines the great delay on harvesting date was observed on the vines that received GA_3 at 40 ppm during both seasons. GA_3 and Sitofex were shown by many authors to retard the release of ethylene and the disappearance of pigments such as chlorophylls and carotenoids and onest of maturity start. Also they were responsible for prolonging pre-maturity stages Nickell (1985). These results regarding the delaying effect of GA_3 and Sitofex on harvesting date were in harmony with those obtained by Wassel *et al*., (2007), Kassem *et al*., (2011), Abu- Zahra and Salameh (2012) and Refaat *et al*. (2012).

3- Cluster weight and dimensions:

It is evident from the data in Table (1) that treating clusters with GA_3 at 10 to 40 ppm or Sitofex at 2.5 to 10 ppm was significantly accompanied with enhancing weight, length and width of cluster relative to the control treatment.

The promotion was significantly associated with increasing concentrations of GA_3 and Sitofex. Using GA_3 was significantly favourable than using Sitofex in this respect. The maximum values were recorded on the vines that received one spray of GA_3 at 40 ppm. Meaningless promotion was detected with increasing concentrations of GA_3 from 20 to 40 ppm

and Sitofex from 5 to 10 ppm. The untreated vines produced the minimum values during both seasons. The positive action of GA_3 on cluster weight and dimensions might be attributed to its essential role on stimulating cell division and enlargement of cells, the water absorption and the biosynthesis of proteins which will lead to increase berry weight. Dimovska *et al*., (2011); Abu- Zahra and Salameh, (2012) and Dimovska *et al*., (2014).

The previous essential role of CPPU on cluster weight was attributed to its higher content of cytokinin when applied to plants (Nickell, 1985).

4- Shot berries %:

Data in Table (2) obviously reveal that percentage of shot berries in the clusters of Early Sweet grapevines was significantly controlled with spraying GA_3 at 10 to 40 ppm or Sitofex at 2.5 to 10 ppm relative to the check treatment. Using GA_3 was preferable than using Sitofex in reducing the percentages of shot berries. There was a gradual reduction on the percentage of shot berries with increasing concentrations of GA_3 and Sitofex. There was a slight reduction on such unfavourable phenomenon with increasing concentrations of GA_3 form 20 to 40 ppm and Sitofex from 5 to 10 ppm. The minimum values of shot berries (7.3 and 6.9 % during both seasons, respectively) were recorded on the clusters harvested from vines treated with GA_3 at 40 ppm. The maximum values of shot berries (12.0 & 12.5 %) during both seasons were recorded on the untreated vines during both seasons. The reducing effect of GA_3 on shot berries might be attributed to its important role on enhancing cell division and the biosynthesis of proteins Nickell, (1985). These results were supported by the results of wassel *et al*. (2007) and Abu Zahra and Salameh (2012).

5- Fruit quality:

Data in Tables (2, 3 & 4) clearly show that spraying clusters with GA3 at 10 to 40 ppm or Sitofex at 2.5 to 10 ppm significantly was accompanied with enhancing weight, longitudinal and equatorial of berry, total acidity%, proteins % and percentages of P, K and Mg and T.S.S. %, reducing sugars %, T.S.S. / acid and total carotenoids relative to the check treatment. The effect either increase or decrease was associated with increasing concentrations of each auxin. Using GA_3 significantly changed these parameters than using Sitofex. A slight effect was recorded on these quality parameters with increasing concentrations of GA_3 from 20 to 40 ppm and Sitofex from 5 to 10 ppm. From economical point of view, the best results with regard to fruit quality were observed due to treating clusters with GA_3 at 20 ppm. Untreated vines produced unfavourable effects on fruit quality. These results were true during both seasons. The effect of GA_3 on increasing berry weight and dimensions might be attributed to its effect in promoting cell division and enlargement of cells, water uptake and the biosynthesis of proteins Nickell (1985). These results were in concordance with those obtained by Williams and Ayars (2005) and Dimovska *et al*., (2014).

The higher content of Sitofex from cytokinins surly reflected on enhancing cell division and the elongation of berries Nickell (1985). These results were in agreement with those obtained by Abu-Zahra (2013) and Retamales *et al*. (2015).

CONCLUSION

Treating Early Sweet grapevines once when the average berries reached 6mm with GA_3 at 20 ppm was responsible for promoting yield and fruit quality.

REFERENCES

Abbott, W. S. (**1925**). A method for computing the effectiveness of an insecticide. J. Econ. Entomol. **18**: 265-267.

Aller, H. F., and Ramsay, J. R. (**1988**). Rh-5849-A novel insect growth regulator with a new mode of action. Proceedings of the Brighton Crop Protection Conference, Pests and Diseases. **2**: 511-518.

Andrew, C. C.; Mayer, R. T., and De-loach, J. R. (**1982**). Purification and characterization of chitinase from the stable fly *Stomoxys calciteans*. Arch. Biochem. Biophysiol. **216**: 314- 321.

Ascher, K. R. S., and Nemny, N. E. (**1979**). Toxcicity of chitin synthesis inhibitors diflubenzuron and its dichlorobenzoyl analogue to *Spodoptera littoralis* larvae, Pestic. Sci. **7**: 1-9.

Clarke, B. S., and Jewess, P. J. (**1990**). The inhibition of chitin synthesis in *Spodoptera littoralis* larvae by flufenoxuron, teflubenzuron and diflubenzuron. Pestic. Sci. **28**: 377-388.

Dean, S. R.; Meola, R. W.; Meola, S. M., Sittertz-Bhatkar, H., and Schenker, R. (**1999**). Mode of action of lufenuron in adult *Ctenocephalides felis* (Siphonaptera: Pulicidae). J. Med. Entomol. **36**: 486-492.

Deul, D. H.; De-Jong, B. J., and Kortenbach, J. A. M. (**1978**). Inhibition of chitin synthesis by two 1-(2,6-disubstitut benzoyl)-3-phenylurea insecticides. Pestic Biochem. and Physiol. **8**: 98-105.

Dixon, M., and Weeb, E. C. (**1964**). Enzymes. Academic press. Inc., New York. 2nd Edition. 328-330.

Finney, D. J. (**1971**). Probit analysis, 3rd edn, Cambridge Univ. Press, Cambridge, England. pp: 318.

Fisk, T., and Wright, D. J. (**1992**). Speed of action and toxicity of acylurea insect growth

regulators against *Spodoptera exempta* (Walk.) and *Spodoptera littoralis* (Boisd.) larvae: Effect of intomult age. Pestic. Since. **35**: 331-337.

Kostyukovsky, M., and Trostanetsky, A. (**2006**). The effect of a new chitin synthesis inhibitor, novaluron, on various developmental stages of *Tribolium castaneum* (Herbst). J. Stored Prod. Res. **42**: 136-148.

Liburd, E. O.; Funderburk, J. E., and Olson, S. M. (**2000**). Effect of biological and chemical insecticides on *Spodoptera* species (Lep. Noctuidae) and marketable yield of tomatoes. J. of Applied Entomol. **124**: 19-25.

Lineweaver, H., and Burk, D. (**1934**). The determination of enzyme dissociation constants. J. Amer. Chem. Soc. **56**: 658.

Lowery, O. H.; Rosebrough, N. J.; Farry, A. L., and Randall, R. J. (**1951**). Protein measurements with folin phenol reagent. J. Bio. Chem. **193**: 265-271.

Merzendorfer, H., and Zimoch, L. (**2003**). Chitin metabolism in insects: structure, function and regulation of chitin synthases and chitinases. The J. Exp. Biol. **206**: 4393-4412.

Reissig, J. L.; Strominger, J. L., and Leloir, L. F. (**1955**). A modified colorimetric method for the estimation of N-acetylamine sugers. J. Biol. Chem. **217**: 959-966.

Sandeep, D. Gaikwad, and Bhamare, K. V. (**2006**). Efficacy of newer insect growth regulators and insecticides against cotton leafworm. J. Plant Sci. **1**: 104-106.

Schneider, M.; Smagghe, G., and Vifiuela, E. (**2003**). Susceptibility of *Hyposoter didymator* (Hymenoptera: Ichenumonidae) adults to several IGRs pesticides and spinosad by different exposure methods. IBOC/wprs Bull. **26**: 111-122.

Smagghe, G.; Auda, M.; Laecke, K., and Van Degheele, D. (**1997**). Significance of penetration and metabolism on topical toxicity of diflubenzuron in *Spodoptera littoralis* and *Spodoptera exigua*. Entomol. Exp. Et Applicata. **82**: 255-260.

Susan, A. Lee; Barry, S. Clarke; Donald, W. Jenner, and Francis, A. Williamson. (**1990**). Cytochemical demonstration of the effects of the acylureas flufenoxuron and diflubenzuron on the incorporation of chitin into insect cuticle. Pestic. Sci. **28**: 367-375.

Temerak, S. A. (**2002**). History records of field cotton leafworm (*Spodoptera littoralis*) resistance to conventional insecticides as influenced by the resistance programs in Egypt. Resistant Pest Management Newsletter. **12**: 7-10.

Toscano, N.; Prabhaker, N.; Castle, S. J., and Henneberrt, T. J. (**2001**). Interregional differences in baseline toxicity of *Bemisia argentifolii* (Homoptera: Aleyrodidae) to the two insect growth regulators, buprofezin and pyriproxyfen. J. Econ. Entomol. **94**: 1538-1546.

Ware, G. W. (**2000**). The Pesticide Book, 5th Ed. Thomson Publications, Fresno, California.

Wilson, T. G., and Cryan, R. J. (**1997**). Lufenuron, a chitin synthesis inhibitor interrupts development of *Drosphila melanogaster*. J. Exp. Zool. **278**: 37-44.

Abdel- Fattah, M.E.; Amen, K.A.; Alaa, A.B. and Eman, A.A. (**2010**). Effect of berry thinning, CPPU spraying and pinching on cluster and berry quality of two grapevine cultivars. Assiut J. of Agric. Sci., **40(4)**: 92-107.

Abu- Zahra, T.R. (**2013**). Effect of plant hormones application methods on fruit quality of Superior seedless grape. Bioscience Biotechnology Research Asia Vol. **10(2)**: 527-531.

Abu- Zahra, T.R. and Salameh, N. (**2012**). Influence of Gibebrellic acid and cane girdling on berry size of Black Magic grape cultivar . Middle East Journal of Scientific Research **11(6)**: 718-722.

Al- Obeed, R.S. (**2011**). Enhancing the shelf life and storage ability of Flame seedless grapevine by agrochemicals preharvest foliar applications. Middle East Journal of Scientific Research **8 (2)**: 319-327.

Association of Official Agricultural Chemists (A.O.A.C.) (2000). Official Methods of Analysis (A.O.A.C), 12th Ed., Benjamin Franklin Station, Washington D.C., U.S.A. pp. 490-510.

Dimovska, V.; Ivanova, V.; Ilieva, F. and Sofijanova, E. (**2011**). Influence of bioregulator gibberellic acid on some technological characteristics of cluster and berry from some seedless grape varieties. Journal of Agric. Science and technology B1 1074-1058.

Dimovska, V.; Petropulos, V.I.; Salamovska, A. and Ilieva, F. (**2014**). Flame seedless grape variety (*Vitis vinifera* L.) and different concentration of gibberellic acid (GA3). Bulgarian Journal of Agric. Sci., **20** (No.1), 137-142.

Dokoozlian, N.K. (**2001**). Gibberellic acid applied at bloom reduces fruiit set and improves size of "Crimson seedless" Table grapes. Hort.science **36(4)**: 706-709.

Guiseppe; F.; Andream, M.; Guiseppe, N. Carmela, P. Angela, M.; Isabella, C. Piero, M.,

Mariangela, V. and Vito, G. (**2014**). Girdling, Gibberellic acid, and forchlorfenuron effect yield, quality and metabolic profile of table grape cv. Italia. Am. J. Enol. Vitic. 65.3.

Hiscox, A. and Isralstam B. (**1979**). Method for the extraction of chlorophylls from leaf tissue without maceration. Can. J. Bot. **57**: 1332-1334.

Juan, P.Z.; Bernardo, A.L. and Paulina, N. (**2009**). Preharvest applications of growth regulators and their effect on postharvest quality of table grapes during cold storage. Postharvest Biology and technology **51**. 183-192.

Kassem, H.A.; Al Obeed, R.S. and Soliman, S. S. (**2011**). Improving yield, quality and profitability of Flame seedless grapevine grown under arid environmental by growth regulators preharvest applications. Middle East Journal of Scientific research **8 (1)**: 165-172.

Lane, J. H. and Eynon, L. (**1965**). Determination of reducing sugars by means of Fehlings solution with methylene blue as indicator A.O.AC. Washington D.C.U.S.A. pp. 490-510.

Leopold, A. C. (**1964**). Plant growth and development. pp. 133-143.TATA McGraw – Hill publishing Comp. LTD. Bombay New Delhi.

Marzouk, H.A. and Kassem, H.A (**2011**). Improving yield, quality and shelfe life of Thompson seedless grapevine by preharvest foliar application. Scientia Horticulruea **130**: 425-430.

Mead, R.; Currnow, R. N. and Harted, A. M. (**1993**). Statistical Biology. 2"d Ed. Methods in Agriculture and Experimental and Hall, London pp. 10-20.

Nickell, L.G. (**1985**). New plant growth regulator increase grape size. Proc. Plant growth reg. Soc. of Am. 12. 1-7.

Refaat, S.S.E.; Ghada, Sh.Sh. and Ola, A.A.(**2012**). Effect of foliar spraying with gibberllic acid and/ or sitofex on bud behaviour, vegetative growth, yield and cluster quality of Thompson seedless grapevines. Journal of American Science, **8 (5)**: 99: 21- 34.

Retamales, J.; Bangerth, F. Cooper, T. and Callejas, R. (**2015**). Effect of CPPU and GA3 on fruit quality of Sultanina table grape. Ishs Acta Hoerticulturae 394: plant Bioregulators in Horticulture.

Wassel, A.H.; Abdel Hameed, M.; Gobara, A and attia, M. (**2007**). Effect of some micronuitrients, gibberellic acid and ascorbic acid on growth, yield and quality of white Banaty seedless grapevines. African Crop Science Conference Proceeding Vol. **8** p. 547-553.

Weaver, R. J.(**1976**). Grape Growing . A Wiley Interscience Puplication John Wiley & Davis. New York. London. Sydney. Tronto. Pp 160-175.

Wilde, S. A.; Corey, R. B.; Lyer, I. G. and Voigt, G. K. (**1985**). Soil and Plant Analysis for Tree Culture. 3rd Oxford & IBH publishing Co., New Delhi, pp. 1 – 218.

Williams, L.E. and Ayars, J.E. (**2005**). Water use of Thompson seedless grapevines as affected by the application of Gibberellic acid (GA_3) and trunk girdling practices to increasing Berry size. Agriculture and Forest Meterology, **129**: 85-94

الملخص العربى

تأثير أثنين من منظمات النمو الحشرية على نشاط أنزيم الكيتينيز.

~~تاثير رش حامض الجبريليك والسيتوفكس فى تحسين المحصول وجودة حبات العنب الإيرلى سويت فى منطقة المنيا- مصر~~

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تم تقدير سمية أثنين من منظمات النمو الحشرية اليفيرون والهيكسافلوميرون للعمر اليرقى الثانى والرابع لسلالتين من دودة ورق القطن أحداهما حقلية والآخرى معملية وتم دراسة تأثيرالمركبين على نشاط أنزيم الكيتينيز للعمريين لسلالتين الحقلية والمعملية تحت الدراسة. وأضحت النتائج أن يرقات العمر الثانى أكثر حساسية للمركبين مقارنة بيرقات العمر الرابع. وقد تم دراسة المقدرة التثبيطية للمركبين على نشاط أنزيم الكيتينيز وبعض الثوابت الخاصة بأنزيم الكيتينيز مثل ثابت ميخائيل (K_m) وأقصى نشاط نوعى (V_{max}) وقيم الـ I_{50} للمعاملة *in vitro* كانت ٠,٣١ و ٠,٦٤ ميكرومولر بالنسبة ليرقات العمر الثانى للسلالة المعملية والحقلية بعد ٢٤ ساعة من المعاملة باليفيرون على التوالى، أما ليرقات العمر الرابع فكانت ٠,٤٤ و ٠,٧٥ ميكرومولر على التوالى. أما بعد ٢٤ ساعة من المعاملة بالهيكسافلوميرون ليرقات العمر الثانى كانت ٠,٥٧ و ٠,٧٦ ميكرومولر للسلالة المعملية والحقلية على التوالى، بينما ليرقات العمر الرابع كانت ٠,٥٦ و ٠,٨١ ميكرومولر على التوالى، وأيضاً تقدير قيم ثابت التثبيط K_i وقد أظهر المركبين تثبيط تنافسى على نشاط أنزيم الكيتنيز. ومن هذه النتائج نجد أن عند أستخدام منظمات النمو الحشرية فى مكافحة دودة ورق القطن فإنه يمكن تقليل التأثيرات السلبية على صحة الأنسان والبيئة والأعداء الطبيعية وتفادى زيادة مقاومة هذه الآفة لفعل المبيدات التقليدية.

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