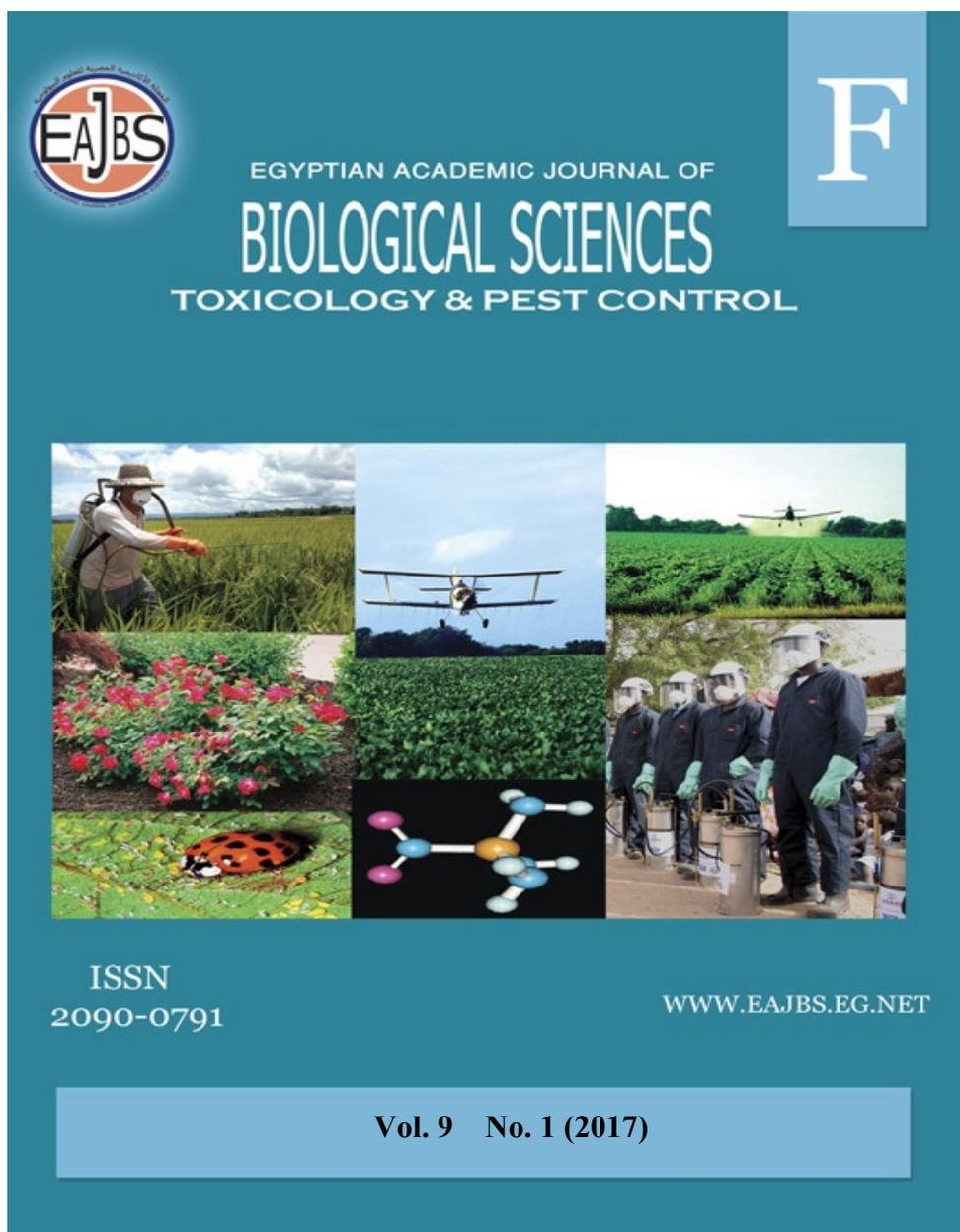


**Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.**



The journal of Toxicology and pest control is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers related to the interaction between insects and their environment.

The goal of the journal is to advance the scientific understanding of mechanisms of toxicity. Emphasis will be placed on toxic effects observed at relevant exposures, which have direct impact on safety evaluation and risk assessment. The journal therefore welcomes papers on biology ranging from molecular and cell biology, biochemistry and physiology to ecology and environment, also systematics, microbiology, toxicology, hydrobiology, radiobiology and biotechnology.

www.eajbs.eg.net



Secondary Metabolites of *Colocasia esculenta* Extract as Green Insecticide Against the Cotton Leaf Worm, *Spodoptera littoralis* (Boisd.)

Anwaar M. Abaza¹ and Mona M. Gaber²

1- Plant Protection Research Institute, A.R.C., Dokki, Giza, Egypt;

2- Plant Protection Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt.

ARTICLE INFO

Article History

Received: 20/2/2017

Accepted: 29/3/2017

Key words:

Colocasia esculenta

Spodoptera littoralis

Insect growth regulator

Natural product

ABSTRACT

A petroleum ether extract of *Colocasia esculenta* leaves yielded two sterols which were identified as; β -sitosterol **I**, Stigmasterol **II**, these secondary metabolites showed insecticidal, antifeeding and insect growth regulatory activities against the 4th instar larvae of *Spodoptera littoralis*. So, the efficacy of this extract and two insect growth regulators belonging to different groups; methoxyfenozide (an ecdysone agonist) and lufenuron (chitin synthesis inhibitor) was assayed against the 4th instar larvae of *S. littoralis* under laboratory conditions. *C. esculenta* extract, methoxyfenozide and lufenuron have sub-lethal concentrations LC₂₅ (17.8 gm/liter, 5.07 mg/liter and 0.80 mg/liter), respectively. It was observed that in case of both *C. esculenta* and methoxyfenozide treatments, the larvae undergo a precocious molting where promoted apolysis process but die due to failure in completing the molting process, at time shorter than the control ones. Ecdysis and sclerotization were incomplete and the pupa that emerged showed many deformities. The joint action of mixing *C. esculenta* extract at two concentrations (LC₁₀ and LC₂₅) with methoxyfenozide and lufenuron at two concentrations (LC₁₀ and LC₂₅) can be evaluated. All the mixtures of *C. esculenta* and methoxyfenozide have synergistic activity against treated larvae, while all the mixtures of *C. esculenta* and lufenuron have antagonistic activity. Biochemical characterization of enzyme activities related to molting process and cuticle synthesis showed that *C. esculenta* extract treatment caused significant disturbance in enzyme balances either decrease or increase comparing to control ones, the reason behind deformations and mortality may be as a result of change in chitinase, phenoloxidase and protease enzymes. These plant sterols **I**, **II** may be considered to be efficient insect growth regulators, as well as having activity similar to phytosteroids, as was evidenced by their significant inhibition of molting processes.

INTRODUCTION

Green chemistry is the design of chemical products and processes that reduce or eliminate the use and generation of hazardous substances (Anastas *et al.*, 2000). The move toward green chemistry processes and the continuing need for developing new crop protection tools with novel modes of action makes discovery and commercialization of natural products as green pesticides an attractive and profitable pursuit that is commanding attention.

The concept of "Green pesticides" refers to all types of nature-oriented and beneficial pest control materials that can contribute to reduce the pest population and increase food production. They are safe and ecofriendly. They are more compatible with the environment components than synthetic pesticides (Isman and Machial, 2006).

Plants produce a great variety of secondary metabolites (allelochemicals), include terpenoids, alkaloids, steroids, flavonoids, tannins and saponins are often important for mediating interactions between plants and their biotic environment (Berenbaum, 2002; Kessler and Baldwin, 2002). They can be models of active defense against phytophagous insects and pathogens. Interest in the application of plant secondary metabolites for insect pest management has led us to isolate and identify biologically active, environmentally friendly and biodegradable natural compounds.

Research on the site of action of secondary metabolites responsible for insect control indicates that many terpenoid and steroid compounds are involved in insecticidal, anti-feeding and insect growth regulation activities. These compounds are important enzymatic and metabolic inhibitors on phytophagous insects (Céspedes *et al.*, 2000, 2001; Kubo *et al.*, 2003; Panzuto *et al.*, 2002).

Our field observation in Egypt indicated that Taro (*Colocasia esculenta*) is very resistant to insect attack especially to the lepidopteran cotton leafworm, *Spodoptera littoralis*, which has its importance as one of the most destructive phytophagous lepidopterous pests in Egypt because it causes various ravages not only for cotton plants but also for other field crops and vegetables. *Colocasia esculenta* (L.), a member of the Araceae family, is an ancient crop grown throughout the humid tropics for its edible corms and leaves as well as for

its traditional uses. *C. esculenta* plant has self-defense against insect predators.

The petroleum ether extract of *C. esculenta* leaves has a potent insecticidal, insect growth regulating and antifeeding activities against *S. littoralis*, where we demonstrated that treated larvae fed on the extract revealed a developmental disruption in which the larvae died during pharate conditions after initiation of molting without completion of morphogenesis (Abaza *et al.*, 2015).

Insect molting and metamorphosis are regulated by ecdysterone, a steroid hormone secreted, cyclically, into the hemolymph by the prothoracic gland (Sehnal, 1989). Ecdysterone acts primarily on gene transcription by activating a nuclear receptor heterodimer consisting of the ecdysteroid receptor (Yao *et al.*, 1993). Control of some pests depends on some techniques that based on natural compounds which inhibit their growth and development.

Insect Growth Regulators (IGRs) disrupt the normal activity of the endocrine system of insects affecting development, metamorphosis and reproduction (Oberlander, *et al.*, 1997). IGRs can be grouped according to their mode of action into:

(i) Chitin synthesis inhibitors (CSIs): such as lufenuron that acts on the incorporation of N-acetyl glucosamine monomer into chitin in the integument, where chitin is a major component of the insect exoskeleton. Insects treated with CSIs become unable to synthesize a new cuticle, and therefore unable to successfully molt into the next stage (Nakagawa and Matsumura, 1993; Taunz and Uygun 2004).

(ii) Non-steroidal ecdysteroid agonists: such as methoxyfenozide (diacylhydrazines) mimic the action of molting hormone, 20-hydroxyecdysone (20E), exert their toxicity by binding to the nuclear ecdysteroid receptors (EcR) in insect cells, thus induce a precocious

and incompletes molt insect. It is highly selective to lepidopterous larvae (Dahadialla *et al.*, 1998; Palli and Retnakaran, 2001).

The aim of this work was to correlate the secondary metabolites of *Colcasi aesculenta* extract with insect growth regulators having different modes of action using *S. littoralis* as model system for the study of pest insects. We study the efficacy of secondary metabolites of *C. esculenta* extract, tested IGRs and their mixtures on growth regulatory and biochemical parameters of *S. littoralis*.

Our data indicate that is possible to correlate the growth regulatory and biochemical parameters with chemical structure of steroids from *C. esculenta*, these data help us to estimate the mechanism of action of these *C. esculenta* steroids.

MATERIALS AND METHODS

Extraction and fractionation

Fresh green leaves were collected, cleaned and dried under room temperature. The dried leaves were homogenized to fine powder by using electric machine. The leaves powder packed in paper bags and stored in air tight container until use. Leaves powder was extracted by continuous hot extraction process using soxhelt apparatus with methanol (MeOH), after extraction the resulting extract was evaporated to its 1/3 volume under vacuum at 40°C by using rotary evaporator to get the crude extract as a dark green residue.

The methanolic extract was diluted with water, transferred to a separator funnel and re-extracted by petroleum ether (40-60°C). The extract was dried over anhydrous sodium sulphate and concentrated under vacuum, then kept in a refrigerator for further investigation.

Insect Growth regulators (IGRs)

1- Molting hormone agonist:
Methoxyfenozide (Runner[®] 24%

SC), (3-methoxy-2-methylbenzoic acid 2-(3,5-dimethylbenzoyl)-2-(1,1-dimethylethyl) hydrazide), a trademark of Dow Agro Sciences.

2- Chitin synthesis inhibitor: lufenuron (Match[®] 5% EC), (*RS*)-1-[2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxy) phenyl]-2-(2,6-difluorobenzoyl) urea, a trademark of Syngenta, Swaziland.

Preparation of the *C. esculenta* extract and IGRs concentrations

For obtaining the lethal concentrations of the tested extract and IGRs:

For petroleum ether extract; considering the crude extract as 100%, a known weight of the crude was added to a least volume of the solvent to obtain stock solution as a readymade. All the extract concentrations were formulated as emulsions (in distilled water) containing 0.3% triton x-100 for more homogeneity. The emulsions were used immediately after preparation.

For the tested IGRs; a series of concentrations (in distilled water) of each IGR was prepared on the basis of active ingredient (a.i.) by diluting its commercial formulation (Runner[®], 24% SC) and (Match[®], 5% EC)

The concentrations used were selected after preliminary bioassays with a wide range of concentrations to determine the range needed. After the proper range was obtained, at least five serially diluted concentrations covering the range 10-90% mortality were prepared.

Insect

S. littoralis strain used in this study is a laboratory susceptible strain reared in the plant protection research institute, Dokki, Giza, Egypt according to El-Defrawi *et al.*, (1964). The culture was maintained in climatic chamber under optimum conditions 25±2°C, 75±5% R.H and (16L:8D) light: dark photoperiod, where reared on fresh castor bean leaves

until the 4th instar larvae which used in this study.

Toxicological studies

a. Insecticidal activity:

Leaf dipping bioassay method was used to determine the lethal concentrations. Castor bean leaves were dipped for 30 seconds in each concentration of the extract and IGRs then left to dry. The treated leaves were offered to newly molted 4th instar larvae of *S. littoralis* for 48 hrs then replaced by untreated ones. Accumulative mortality percentages were recorded 7 days post treatment and corrected according Abbott's formula (1925). From the corrected mortality percentages the

corresponding toxicity lines (LC-P lines) were estimated in addition to determine LC₁₀, LC₂₅ and LC₅₀ values and their confidence limits, slope values of tested compounds were also estimated.

b. Joint action activity:

The combination between tested secondary metabolites and tested IGRs was used at two concentrations (LC₁₀ and LC₂₅) for each tested compound. The efficacy of tested mixtures was experimented against the 4th instar larvae of *S. littoralis* as mentioned above and expressed as Co-toxicity factor which has been calculated according to the equation given by Mansour *et al.* (1966) as follows:

$$\text{Co-toxicity factor} = \frac{\text{Observed mortality \%} - \text{Expected mortality \%}}{\text{Expected mortality \%}} \times 100$$

This factor was used to differentiate the results into three categories; a positive factor (+20) or more is considered potentiating or synergism, a negative factor of (-20) or more means antagonism, and intermediate values more than (-20) and less than (+20) indicate only additive effect.

Biochemical studies

a. Biochemical experiment:

Aqueous solutions of methoxyfenozide and lufenuron at the determined LC₂₅ concentration were prepared. An emulsion solution of petroleum ether extract of *C. esculenta* at the concentration LC₁₀ was prepared. Castor bean leaves were dipped for 30 seconds in the mentioned solutions, then left to dry for 1 hr in room. The treated leaves were offered to newly molted 4th instar larvae of *S. littoralis* for 48 hrs then replaced by untreated ones for another 48 hrs. Survived and healthy larvae were collected in clean jars then starved for 4 hrs.

Aqueous solution of a mixture of methoxyfenozide at LC₂₅ concentration and *C. esculenta* extract at LC₁₀ concentration was prepared. Also aqueous solution of a mixture of lufenuron at LC₂₅ concentration mixed with *C. esculenta* extract at LC₁₀ concentration was prepared. Castor bean leaves were dipped for 30 seconds in the mentioned solutions, then left to dry for 1 hr in room. The treated leaves were offered to newly molted 4th instar larvae of *S. littoralis* for 48 hrs then replaced by untreated ones for another 48 hrs. Survived and healthy larvae were collected in clean jars then starved for 4 hrs.

b. Preparation of homogenate samples for biochemical analysis:

The collected larvae were homogenized in distilled water at 500 r.p.m. using a chilled glass Teflon tissue homogenizer (ST-2 Mechanic-Preczyjna, Poland) surrounded with a jacket of crushed ice for 3 minutes. Homogenates were collected in cold tube (on ice) and centrifuged at 8000 r.p.m. for 15 min. at

5° C in a refrigerated centrifuge (BECKMAN GS-6R Centrifuge) to remove haemocyte. After the centrifugation, the supernatant fluid was divided into small aliquots (0.5 ml) and stored at -20° C until analysis. Three replicates were carried out for each biochemical determination. Double beam ultraviolet / visible spectrophotometer (Spectronic 1201, Milton Roy Co., USA) was used to measure absorbance of colored substances or metabolic compounds.

c. Enzymes characterization:

- Phenoloxidase was assayed according to a modification of Ishaaya (1971).
- Chitinase was assayed using 3,5-dinitrosalicylic acid reagent to determine the free aldehydic groups of hexoamones liberated on chitin digestion according to the method described by Ishaaya and Casida (1974).
- protease was assayed according to the method described by Tachell *et al.*, (1972) with some modifications by measuring the increase in free amino acids split from substrate protein (albumin), during one hour incubation at 30 °C.

RESULTS AND DISCUSSION

The present paper specifically deals with the efficacy of steroids of petroleum ether extract of *C. esculenta* leaves against growth, insecticidal and biochemical parameters of *S. littoralis*.

The isolation and structure elucidation of secondary metabolites from *C. esculenta* leaves was firstly recorded by Abaza (2014). We have previously demonstrated that the petroleum ether extract rich in terpenoid and steroid allelochemicals.

The pet ether fraction was evaporated to its 1/3 volume, where a dark green residue was obtained. The residue was subjected to saponification reaction using alcoholic aqueous sodium hydroxide, in order to obtain the unsaponifiable and saponifiable fractions.

The unsaponifiable fraction was subjected to column chromatography (CC) which was performed on silica gel Merck grain size 0.2-0.063 mm as adsorbent and eluted using pet. ether / methylene chloride. Twelve fractions were obtained by increasing the polarity. The sub-fractions (8-12) were eluted using pet. ether: ethyl acetate (80: 20) as solvent system and subjected to thin layer chromatography (TLC) which was performed on silica gel Merck GF 254 pre-coated plates 20×20 cm on aluminum sheets.

It yielded a mixture of two compounds **P-I** and **P-II** of ($R_f = 0.42$) as a colourless crystals on evaporation to dryness which gave deep violet color on spraying with *p-anisaldehyde-sulphoric acid* reagent.

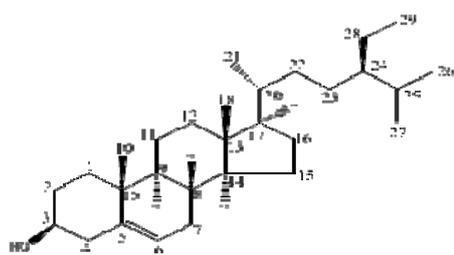
Their structures were elucidated by ¹H-NMR which was recorded at 300 MHz, chemical shifts are given in δ (ppm) relative to TMS as internal standard material and mass spectra (MS) which was recorded on Shimadzu GC/MS UB 1000EX instrument.

The spectrum indicated the presence of two tertiary methyl proton signals at δ 0.691 and δ 1.01 ppm, corresponding to (Me-18, Me-19), respectively. The side chain signals appeared at δ 0.92 (3H, d, Me-21), δ 0.83 (3H, d, Me-26), δ 0.81 (3H, d, Me-27) and δ 0.85 (3H, t, Me-29) and δ 1.87 (1H, m, H-25), suggesting that the sterol has a Stigmast-5-en-3 β -ol skeleton. Based on all spectral data, compound P-I is β -sitosterol.

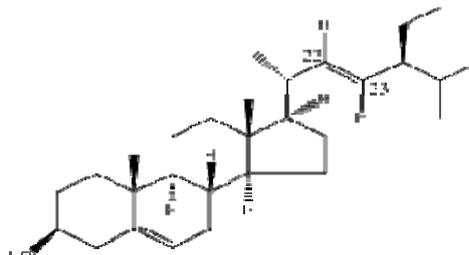
Also the ¹H-NMR spectrum of compound P-II is identical with the spectrum of compound P-I in addition to the presence double bond which proved by the presence of two olefinic protons that appeared as double of doublet at δ 4.98 and 5.12 ppm each (dd, H-22 and H-23) suggesting the presence of a (22E)-stigmast-5,22-dien-3 β -hydroxyl sterol. Thus, all the previous data support that the compound P-II is Stigmast-5, 22-

dien-3-ol which is known as *Stigmasterol*. This was the first record on the isolation and structure elucidation of chemical constituents from *C. esculenta* leaves (Abaza, 2014).

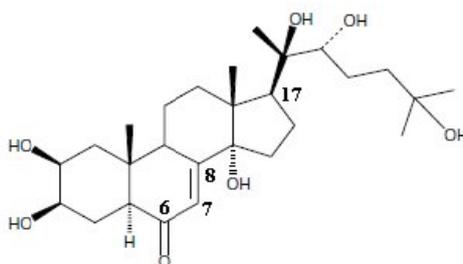
The presence of these two steroidal compounds in *C. esculenta* leaves is one reason of its self-defense and insect growth regulatory activity against *S. littoralis*.



I

 β - Sitosterol

II

Stigmasterol

III

20- hydroxy ecdysone (20E)

Toxicological studies

a. Insecticidal activity

In our screening program which is designed to discover the mechanism of action of *C. esculenta* secondary metabolites. It was found that *C. esculenta* showed disturbance of insect molting. Based on this information, we have compared the efficacy of these secondary metabolites with commercial IGRs having different modes of action.

Data presented in Table (1) demonstrated the LC_{10} , LC_{25} , LC_{50} values and their confidence limits, and slope values of *C. esculenta* secondary metabolites and tested IGRs against the 4th instar larvae of *S. littoralis* under laboratory conditions using leaf dipping

technique, where the larvae fed on treated castor bean leaves for 48 hrs then transferred to untreated ones.

By using the LC-P program the results showed that the toxicity of either *C. esculenta* secondary metabolites, methoxyfenozide and lufenuron increased with time post treatment according to descending order of either LC_{10} , LC_{25} or LC_{50} values.

The results indicated that the slope values of *C. esculenta* metabolites, methoxyfenozide and lufenuron are 0.73 ± 0.19 , 0.86 ± 0.08 and 0.78 ± 0.17 , respectively, these values were relatively close. This indicates some sort of parallelism in the conc.-response relationship of tested compounds.

Table 1: Toxicity data *C. esculenta* secondary metabolites and two IGRs against the 4th instar larvae of *S. littoralis* under laboratory conditions

Tested compound	LC ₁₀ (Lower-Upper)	LC ₂₅ (Lower-Upper)	LC ₅₀ (Lower-Upper)	Slope ± SE
<i>C. esculenta</i> secondary metabolites	0.25 (0.01-0.75) gm/100ml	1.78 (0.86-3.79) gm/100ml	12.67 (6.32-19.46) gm/100ml	0.73 ± 0.19
Ecdysone agonist (Methoxyfenozide)	0.62 (0.38-0.97) mg/liter	5.07 (3.48-7.53) mg/liter	21.75 (15.85-25.49) mg/liter	0.86 ± 0.08
Chitin synthesis inhibitor (Lufenuron)	0.13 (0.09-0.26) mg/liter	0.80 (0.48-1.97) mg/liter	5.78 (2.65-7.32) mg/liter	0.78 ± 0.17

It was observed that in case of both *C. esculenta* and methoxyfenozide treatments, the larvae undergo a precocious molting where initiate molting process but die due to failure in completing the molting process, at time shorter than the control ones. Also the weight gain of treated larvae would be decreased as a result of feeding cessation. When pupation occurred, serious abnormalities were observed. Ecdysis and sclerotization were incomplete and the pupa that emerged showed many deformities. These results suggest that sterols of *C. esculenta* extract have effects on ecdysone receptors as methoxyfenozide treatment. Dhadialla *et al.*, 2005 found that tebufenozide (acts as ecdysone agonist and belongs to the same group of methoxyfenozide) has similar hormonal activity with the natural hormones in the insects and particularly in Lepidoptera of which the principal effect is to cause incomplete and lethal molts.

In case of lufenuron treatment, the larvae become inactive, appeared to be wet and paralyzed, dark color and viscous excretions comes out from the body, the larvae fail to ecdyse due to inhibition of synthesis of new cuticle. These symptoms are in agreement with Khedr (2002) who manifested the symptoms of death due to the exposure of *S. littoralis* larvae to the tested IGRs

(lufenuron, flufenoxuron and chlorfluazuon).

Research on the site and mechanism of action of secondary metabolites responsible for insect control indicates that many terpenoids and steroids are involved in insecticidal and insect growth regulation activities, these allelochemicals are important enzymatic and metabolic inhibitors (Hammond and Kubo, 1999; Kubo and Kinst-Hori, 1999; Cespedes *et al.*, 2000, Calderon *et al.*, 2001 and Panzuto *et al.*, 2002).

b. Joint action Studies

Insecticide mixtures are usually applied in the field to enhance the spectrum of the control when multiple pests are attacking simultaneously. They are also recommended to increase the efficacy of the control of a single pest to delay the development of insecticide resistance in a pest species. When two compounds are mixed, they can be either synergistic or additive or antagonistic in insect species. These effects can be varied on different insect species and class of insecticide depending upon their physiology and the mechanism (s) of resistance developed. If a mixture is synergistic (potentiating), it is useful tool in enhancing control efficacy and combating insecticide resistance. In this case, there may be a potential for reducing the application rate of one or both components of the mixture. If a mixture is antagonistic, it should not be

used, because it will reduce the efficiency of pest control and aggravate the resistance problem (Swelam and Sayed, 2006).

In this topic; mixing of chemical insecticides (IGRs) with natural products (*C. esculenta* metabolites) in the same formula to reduce the amount and costs

of insecticides and also reduce the human and environment hazards. Thus, the joint action of mixing *C. esculenta* secondary metabolites at two concentrations (LC₁₀ and LC₂₅) with methoxyfenozide (ecdysone agonist) and lufenuron (CSI) at two concentrations (LC₁₀ and LC₂₅) can be evaluated.

Table 2: The joint action between *C. esculenta* secondary metabolites and tested IGRs against the 4th instar larvae of *S. littoralis*.

Tested compounds	Expected mortality %		Observed mortality %		Co-toxicity factor	
	LC ₁₀	LC ₂₅	LC ₁₀	LC ₂₅	LC ₁₀	LC ₂₅
<i>C. esculenta</i> extract						
Ecdysteroid agonist (Methoxyfenozide)						
LC ₁₀	20	35	33.33	46.67	66.64	33.34
LC ₂₅	35	50	80	68.89	128.57	37.78
(chitin synthesis inhibitor) Lufenuron						
LC ₁₀	20	35	10	13.33	- 50	- 61.91
LC ₂₅	35	50	3	36.67	- 91.42	- 26.66

The results in Table (2) showed that all the mixtures of *C. esculenta* and methoxyfenozide increased mortality percentages especially those of *C. esculenta* at LC₁₀ with methoxyfenozide at LC₁₀ and LC₂₅, the observed mortality rates increased from 20% and 35% to be 33.33% and 80% respectively with co-toxicity factor 66.64 and 128.57, respectively (S, synergistic activity). These results are compatible with the results of Smagghe *et al.*, (1999) who found that the combination of either methoxyfenozide or tebufenozide with pirproxybutoxide (PB) on *S. littoralis* synergized the toxicity of ecdysone agonist (methoxyfenozide). Adel and Sehnal (2000) found that the effect of methoxyfenozide was highly increased when it was combined with neem seed extracts.

On the contrary, all the mixtures of *C. esculenta* and lufenuron decreased the observed mortality rates sharply especially when *C. esculenta* at LC₁₀ mixed with lufenuron at LC₂₅, the observed mortality decreased from 35% to be 3% with co-toxicity factor (- 91.42 An, antagonistic activity).

Consequently, the present topic suggests that the use of *C. esculenta* secondary metabolites and methoxyfenozide mixture would minimize the amount of insect growth regulator (ecdysone agonists) applied.

Biochemical impacts

Based on data obtained from toxicological studies we study the effect of the most effective sublethal concentration of *C. esculenta* secondary metabolites LC₁₀ and the sublethal concentrations LC₂₅ of methoxyfenozide and lufenuron. Also based on the joint action studies we select the most effective mixtures; a mixture of lufenuron at LC₂₅ concentration and *C. esculenta* metabolites at LC₁₀ concentration, and a mixture of methoxyfenozide at LC₂₅ concentration and *C. esculenta* metabolites at LC₁₀ concentration.

We study the effect of these treatments on some enzyme activities related to molting process and cuticle synthesis; phenoloxidase, chitinase and protease of the 4th instar larvae of *S. littoralis*. Such examinations were undertaken as an attempt to interpret the

primary mechanism of action of tested compounds as well as illustrate the molting disturbance which observed in treated larvae.

Insect cuticles form an exoskeleton that exhibits only a limited capacity to keep pace with body growth because it is a more or less rigid structure due to the presence of chitin and sclerotized proteins. To allow growth and development, insects are therefore periodically forced to replace their old cuticle with a new and looser one during molting (ecdysis).

The nascent, non-sclerotized integument underneath the old cuticle is strongly furrowed and can only expand when molting is complete.

Ecdysis is initiated by apolysis, the process that separates epidermal cells from the old cuticle by molting fluid secretion and ecdysial membrane formation. The molting fluid contains proteases and chitinases, enzymes that digest the main constituents of the old endocuticle (Reynolds and Samuels, 1996). Shortly before ecdysis, the molting fluid, which has accumulated in the apolysial space, is reabsorbed, allowing there cycling of old cuticle components. Formation of the new cuticle starts after the ecdysial space

opens as a result of the secretion of cuticle proteins and chitin fibers through the apical membranes of epidermal cells. Initially, patches of cuticullin, forming later on the outer epicuticle, are secreted, and followed by an unsclerotized, chitinous cuticle referred to as procuticle.

Afterwards, formation of the epicuticle seals the epidermis and protects it against the digestive enzymes of the molting fluid. Before sclerotization is completed, the insects expand their new cuticle and shed their old envelope, now called exuvia, by performing distinct motor programs and increasing body pressure (Carlson and Bentley, 1977).

During a molt, ecdysteroid levels first rise to stimulate onset of apolysis and cuticle synthesis, but then must fall to facilitate release of eclosion hormone (EH) (Truman *et al.*, 1983) and the ecdysis-triggering hormone (ETH) (Zitnan *et al.*, 1996, 1999). These last substances act in concert to trigger insect ecdysis during the final stages of the molt. Sterols may disrupt ecdysteroid metabolism resulting in inhibition of emergence behavior, or may, alternatively, act to inhibit the release of ETH (Hesterlee and Morton, 1996).

Table 3: Enzyme activities in haemolymph of the 4th instar larvae of *S. littoralis* after treatment with *C. esculenta* metabolites, methoxyfenozide and lufenuron.

Treatments	Enzyme activity \pm S. E					
	Chitinase $\mu\text{g N-acetyl glucosamine}$ $/\text{min./g b. wt}$		Phenoloxidase O. D. unit $\times 10^3 / \text{min./}$ g b. wt		Protease $\mu\text{g alanin} / \text{min./g b. wt}$	
	Activity	Change %	Activity	Change %	Activity	Change %
<i>C. esculenta</i> metabolites LC ₁₀	348.67 \pm 3.75	- 15.71	49.96 \pm 2.12	357.09	124 \pm 8.02	- 72.05
Methoxyfenozide LC ₂₅	361.33 \pm 11.97	- 12.65	12.82 \pm 0.88	17.29	170.67 \pm 8.74	- 61.53
<i>C. esculenta</i> LC ₁₀ + Methoxyfenozide LC ₂₅	321.67 \pm 3.84	- 22.23	51.67 \pm 2.89	372.73	277.66 \pm 8.25	- 37.41
Lufenuron LC ₂₅	514 \pm 12.22	24.25	13.93 \pm 0.61	27.44	426.66 \pm 9.27	- 3.83
<i>C. esculenta</i> LC ₁₀ + lufenuron LC ₂₅	385.33 \pm 4.91	- 6.84	29.76 \pm 1.14	172.27	391.66 \pm 8.83	- 11.72
Control	413.66 \pm 5.84		10.93 \pm 0.61		443.67 \pm 8.41	

Chitinase enzyme

Chitin, a high molecular weight polymer of *N*-acetyl-D-glucosamine, a major component not only of the cuticle of insects and arthropods, but also of other parts of the body, such as the lining of the fore- and hind-gut and the tracheae. One characteristic of insects is that they are enclosed within a relatively inextensible exoskeleton which must be cast at intervals to permit growth. To assist shedding, the old cuticle prior to its discard is thinned by a molting fluid which has long been assumed to possess chitinolytic and proteolytic activity.

There are two types of chitinolytic enzymes, one exhibiting an endo-cleaving mechanism known as chitinase or endo-chitinase, [poly- β -1.4-(2 acetamido-2-deoxy)-D-glucoside glycanohydrolase] initiates primary breakdown of chitin by hydrolyzing chitin polymer to (GlcNac₂). Which is subsequently hydrolyzed by other enzyme which exhibiting exo-cleaving mechanism and known as chitobiase [β -N-acetyl glucosaminidase] (Koga *et al.*, 1983).

Chitinolytic enzymes have been demonstrated in the molting fluid which appears in the space between the old and the new cuticles during ecdysis and secreted by the hypodermis (Kimura, 1973), and in the integument (Koga *et al.*, 1983), together they degrade chitin via chitobiase to *N*-acetyl-D-glucosamine.

Data in Table (3) showed that all treatments caused decreasing in chitinase enzyme activity relative to control ones except lufenuron treatment which caused increasing in chitinase activity with (24.25 %) relative to control ones. The enzyme activity is decreased sharply in mixture of *C. esculenta* metabolites and methoxyfenozide treatment (-22.23 %), followed by *C. esculenta* treatment (-5.17%) and methoxyfenozide treatment (-12.65 %) relative to control ones. This

result means that decreasing of chitinase activity has a relationship with increasing deformation and toxicity of *C. esculenta* metabolites and methoxyfenozide treatments.

Phenoloxidase enzyme

The results in Table (3) showed that all treatments caused elevation in phenoloxidase enzyme activity relative to control ones. The maximum elevation percentage was observed for the mixture of methoxyfenozide and *C. esculenta* metabolites that record (372.73%) relative to control ones, followed by *C. esculenta* treatment alone that gave (357.09%) relative to control ones, also mixture of lufenuron and *C. esculenta* metabolites recorded elevation in enzyme activity with (172.27%) relative to control.

Sclerotization of insect cuticle is assumed to be caused by the introduction of covalent cross-links between the protein molecules in the cuticle. The cross-links are formed from a low molecular weight diphenol which after oxidation to a reactive intermediate can react with amino and phenolic groups on the proteins. *N*-acetyldopamine is the diphenol used as sclerotizing agent by many insect species (Karlson and Sekeris, 1962) and it can either be oxidized in the ring to give an ortho-quinone, which is a highly reactive compound (Hackman, 1974) or it can be oxidized to another intermediate where the β -position of the side chain of the carbon atom which is closest to the ring is activated and connected to the proteins (Anderson and Barrett, 1971; Anderson, 1974). Such process is an essential step for the survival of all insects (Becker *et al.*, 2012).

Protease enzyme

Data showed that the activity of protease decreased in all treatments compared to control ones. The enzyme activity decreased sharply in *C. esculenta*

treatment (- 72.05 %) followed by methoxyfenozide treatment (- 61.53%). While lufenuron caused increasing in protease activity (24.25%) but the mixture of lufenuron and *C. esculenta* treatments caused decreasing in enzyme activity (- 6.84%) relative to control ones. This decreasing in enzyme activity may due to decrease of feeding larvae. The enzyme production is clearly related to feeding behavior, amount of food that passes through the alimentary canal (Chapman, 1985). A correlation between the rise in edysteroid titer, and the increase in activity of chitin-degrading enzymes and cuticle tanning enzymes has been shown in various arthropods (Rees, 1977; Koga *et al.*, 1991).

These results confirmed that *C. esculenta* metabolites treatment caused significant disturbance in enzyme balances either decrease or increase comparing to control ones, the reason behind deformations and mortality may be as a result of change in chitinase, phenoloxidase and protease enzymes activity, where these enzymes played a known role in ecdysis and metamorphosis. Sabry and Khedr (2014) found that methoxyfenozide caused significant elevation in the activities of chitinase, protease, phenoloxidase and trahalase enzymes in *S. littoralis*.

Based on these results, β -*Sitosterol* (**I**) and *Stigmasterol* (**II**) of *C. esculenta* extract may be considered to be efficient insect growth regulators (IGRs), as well as having activity similar to phytosteroids, as was evidenced by their significant inhibition of molting processes.

This finding suggests that hydroxyl groups play an important role in both insecticidal and IGR activities of these compounds. These compounds **I**, **II** contain a small and relatively lipophilic group at C-17, hydroxyl group at C-3, and double bond between C-5 and C-6, whereas β -ecdysone (20-hydroxy ecdysone) (**20 E**) **III** has this same

functionality between C-7 and C-8 and a carbonyl group (α,β -unsaturated) at C-6. These results confirm previous finding on structure activity relationships (SAR) for ecdysteroid, namely that the growth inhibitory activity of the respective natural product depends on number of hydroxyl groups and the presence of a moderate bulky group at C-17 (Dinan, 2001).

In summary, the activity of *C. esculenta* metabolites is comparable to that of the known insect growth regulator, methoxyfenozide (ecdysone agonist). The insecticidal activity of *C. esculenta* metabolites may due to a synergistic effect shown by ecdysone like activity of the extract in the tested parameters used in this investigation

The preceding experimental observations suggest that acute toxicity and growth inhibition of our compounds may due to inhibition of protease and phenoloxidase that bind to these steroids. This target has been demonstrated for other compounds of natural origin (Kubo *et al.*, 1986; Carrizo *et al.*, 1998; Tamayo *et al.*, 2000).

The site and mode of action of these metabolites are being investigated and probably correspond to a combination of antifeeding action, midgut phenol oxidase, protease and cuticle synthesis inhibition, as well as molting sclerotization toxicity, as has been found for other natural compounds (Kubo *et al.*, 2000 and 2003; Kubo 2000; Taybi *et al.*, 2003; Berghiche *et al.*, 2003). Thus, the effects of *C. esculenta* metabolites, and a mixture of methoxyfenozide and *C. esculenta* metabolites on reducing insect growth, modifying apolysis during molting and producing high mortality in *S. littoralis* larvae were more powerful than methoxyfenozide alone. The *C. esculenta* sterols **I**, **II** may be considered to be efficient insect growth regulators, as well as having activity similar to phytosteroids, as was evidenced by their

significant inhibition of molting processes.

REFERENCES

- Abaza, A. M. (2014): Chemical and biological studies of some natural extracts to control the cotton leafworm, *Spodoptera littoralis*. Ph.D., *Fac. Sci., Suez Canal Univ., Egypt*.
- Abaza A.M.; Y.M. Ahmed; M.G. Abbas and H.Kh. Ashour (2015): Chemical constituents of *Colocasia esculenta* leaves extract in relation to its self defense against the cotton leaf worm, *Spodoptera littoralis* (Boisd.). *Egypt. Soc. Environ. Sci.*, 13 (1): 1-7.
- Abbott, W.S. (1925): A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, 18: 265-267.
- Adel, M. M. and F. Sehnal (2000): Azadirachtin potentiates the action of ecdysteroid agonist RH-2485 in *Spodoptera littoralis*. *J. Insect Physiol.*, 46: 267-270.
- Anastas, P.T.; Heine, L. G.; Williamson, T. C. (2000): Green Syntheses Chemical and Processes: Introduction. In *Green Chemical Syntheses and Processes*; Anastas, P. T., Heine, L. G., Williamson, T. C., Eds.; American Chemical Society: Washington, DC, 2000; Chapter 1.
- Anderson, S.O. (1974): Evidence for two mechanisms of sclerotization in insect cuticle. *Nature. Land.*, 251: 507
- Anderson, S.O. and F.M. Barrett (1971). the isolation of ketocatechols from insect cuticle and their possible role in sclerotization. *J. Insect. Physiol.*, 17: 69-83.
- Becker, J.M.; M. Sugumaran and E. L. Cooper (2012): Phylogenetic perspectives on the vertebrate immune system (vol. 484). *Springer science & Business Media*.
- Berenbaum, M.R. (2002): Postgenomic chemical ecology: from genetic code to ecological interactions. *J. Chem. Ecol.*, 28 (5): 873-896.
- Berghiche, H.; G. Smagghe and N. Soltani (2003): In vitro effects of RH-0345 and KK-42 on ecdysteroids level and cuticle synthesis by pupal integument of mealworms. *Commun. Agric. Appl. Biol. Sci.*, 68 (1): 43-48.
- Calderon, J.S.; C.L. Cespedes; R. Rosas; F. Gomez-Garibay; J.R. Salazar; L. Lina; E. Aranda and I. Kubo (2001): Acetyl cholinesterase and insect growth inhibitory activities of *Gutierrezia microcephala* on fall armyworm *Spodoptera frugiperda*. *J.E. Smith. Z. Naturforschung*. 56: 382-394.
- Carlson, J.R. and D. Bentley (1977): Ecdysis: neural orchestration of a complex behavioral performance. *Science*. 195: 1006-1008.
- Cespedes, C.L.; M. Martinez-vazquez; J. S. Calderon; J.R. Salazar and E. Aranda (2000): Growth inhibitory effects of fall armyworm *Spodoptera Frugiperda* of some limonoids isolated from *Cedrela* spp. (Meliaceae). *J. Agric. Food Chem.* 48: 1903-1908
- Cespedes, C.L.; M. Martinez-Vazquez; J. S. Calderon; J.R. Salazar and E. Aranda (2001): Insect growth regulatory activity of some extracts and compounds from *Partheniumar gentatumon* fall armyworm, *Spodoptera Frugiperda*. *Z. Naturforsch.* c 56: 95-105.
- Chapman, R. F. (1985): Structure of the digestive system. In: G.A. Kerkut and L.I. Gilbert (editors): *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. Vol. 4. Pergamon Press, Oxford, 165-211.
- Carrizo, F.R.; M.E. Sosa; L. S. Favier; F. Penna; E. Guerreiro; O. S.

- Giordano and C. E. Tonn (1998): Growth inhibitory activities of benzofuran and chromene derivatives toward *Tenebrio molitor*. *J. Nat. Prod.*, 61: 1209-1211.
- Dhadialla T.S.; G.R. Carlson and D.P. Lee (1998): New insecticides with ecdysteroidal and juvenile hormone activity. *Annu. Rev. Entomol.*, 43: 545-569.
- Dhadialla, T.S.; A. Retnakaran and G. Smagge (2005): Insect growth and development disrupting insecticides. In: Gilbert, L. I.; Costas I. and Gill, S. (Eds.) *Comparative Insect Molecular Science*, vol. 6. Elsevier, Oxford, UK, pp. 55-116.
- Dinan, L. (2001): Phytosteroids: Biological aspects. *Phytochem.*, 57: 325-339.
- El-Defrawi, M.E.; A. Topozada; N. Mansour and M. Zeid (1964): Toxicological studies on the Egyptian cotton leafworm, *Prodenialitura* (F.) I. Susceptibility of different larval instars of *prodenia* to insecticides. *J. Econ. Entomol.*, 57: 591-593.
- Hackman, R.H. (1974): Chemistry of the insect cuticle. In the physiology of insecta (Ed. By Rockestein M.) 6: 215-270. Academic press, New York.
- Hammond, D.G. and I. Kubo (1999): Structure-activity relationship of alkanols as mosquito larvicides with novel findings regarding their mode of action. *Bioorg. Med. Chem.*, 7:271-278.
- Hesterlee, S. and D.B. Morton (1996): Insect physiology: the emerging story of ecdysis. *Curr. Biol.*, 6 (6): 648-50.
- Ishaaya, I. (1971): Observations on the phenoloxidase system in the armored scales, *Aonidiellaaurantii* and *Chrysomphalus aonidium*. *Comp. Biochem. Physiol.*, 39B: 935-94.
- Ishaaya, I. and J. E. Casida (1974): Dietary TH-6040 alters composition and enzyme activity of housefly larval cuticle. *Pestic. Biochem. Physiol.*, 4: 484-490.
- Isman, M. B. and C.M. Machial (2006): pesticides based on plant essential oils: from traditional practice to commercialization. In M. Rai and M.C. Carpinella (eds.), *Naturally Bioactive Compounds*, Elsevier, BV, PP 29-44.
- Karlson, P. and C.E. Sekeris (1962): N-acetyldopamine as sclerotizing agent of the insect cuticle. *Nature Lond.*, 183-184.
- Kessler, A. and I.T. Baldwin (2002): plant responses to insect herbivory: the emerging molecular analysis. *Ann. Rev. Plant Biol.*, 53: 299-328.
- Kimura, S. (1973): Chitinolytic enzymes in the larval development of the silkworm, *Bombyx mori.*, *Appl. Ent. Zool.*, 8: 234-236.
- Khedr, M.M. (2002): Effect of some plant extracts and insect growth regulators applied to control cotton leafworm on honeybees, *Apis mellifera*, L., *M. Sc. Thesis, Fac. Agric. Zagazig Univ., Egypt*, pp. 204.
- Koga, D.; T. Funakoshi; H. Fujimoto; E. Kuwano; M. Eto and A. Ide (1991): Effects of 20-hydroxyecdysone and KK-42 on chitinase and B-N-acetylglucosamine during larval-pupal transformation of *Bombyx mori*. *Insect Biochem.*, 21: 277-284.
- Koga, D.; Jilka, J. and Kramer, K. J. (1983): Insect endochitinases: glycoproteins from moulting fluid, integument and pupal haemolymph of *Manducasexta*L. *Insect Biochem.* 13: 295-305.
- Kubo, I. (2000): Tyrosinase inhibitors from plants. *Rev. Latinoam. Quim.* 28: 7-20.

- Kubo, I. and I. Kinst-Hori (1999): Flavonols from saffron flowers: tyrosinase inhibitory activity and inhibition mechanism. *J. Agric. Food. Chem.*, 47(10): 4121-4125.
- Kubo, I.; I. Kinst-Hori; S.K. Chauduri; Y. Kubo; Y. Sa'nchez and T. Ogura (2000): Flavonols from Heterotheca inuloides: tyrosinase inhibitory activity and structural criteria. *Bioorg. Med. Chem.*, 8: 1749-1755.
- Kubo, I.; I. Kinst-Hori; K.I. Nihei; F. Soria; M. Takasaki; J.S. Caldero'n and C.L. Ce'spedes (2003): Tyrosinase inhibitors from Galls of *Rhus javanica* leaves and their effects on insects. *Z. Naturforsch.*, c (58): 719-725.
- Kubo, I.; A. Matsumoto; T. Matsumoto and J.A. Klocke (1986): New insect ecdysis inhibitory limonoid-acetylazadirachtinol isolated from *Azadirachtaindica* (Meliaceae) oil. *Tetrahedron*, 42: 489-496.
- Mansour, F.A.; M.E. El-Deerawi; A. Tobbozada and M. Zeid (1966): Toxicological studies on the Egyptian cotton leaf worm *Prodenia litura*. VI: Potentiation and antagonism of organophosphorus and carbamate insecticides. *J. Econ. Entomol.*, 59: 307-311.
- Nakagawa, Y. and F. Mastsmura (1993): Effect of diflubenzuron on the incorporation of UDP-N-acetyl - [3H] Glucosamine (UDP-[3H] NAGA) to chitin in permeabilized and isolated integuments from the newly molted American Cockroach *Periplaneta Americana*. Comparative Biochemistry and Physiology part C: *Toxicol. Pharmacol.*, 106: 705-710.
- Oberlander, H.; D.L. Silhacek; E. Shaaya and I. Ishaaya (1997): Current status and future perspectives of the use of insect growth regulators for the control of stored product pests. *J. Stored Prod. Res.*, 33: 1-6.
- Palli, R. and A. Retnakaran (2001). Ecdysteroid and juvenile hormone receptors: properties and importance in developing novel insecticides, pp 107-132. In Ishaaya (ed.), Biochemical sites for insecticides action and resistance. Springer, Berlin, Germany.
- Panzuto, M.; Y. Mauffette and P.J. Albert (2002): Developmental, gustatory and behavioral responses of leaf roller larvae, *Choristoneura rosaceana*, to tannic acid and glucose. *J. Chem. Ecol.*, 28: 145-160.
- Rees, H. H. (1977): Insect biochemistry, pp 27 (Ed.) Chapman and Hall, London.
- Reynolds, S. E. and R. I. Samuels (1996): Physiology and biochemistry of insect moulting fluid. *Adv. Insec. Physiol.*, 26: 157-232.
- Sabry, H.M. and M.A. Khedr (2014): Biochemical and histological variations induced by IGRs in *Spodoptera littorals* (Boisd.). *Glob. J. Environ. Sci. Toxicol.*, 1 (2): 163-178.
- Sehna, F. (1989): Homonal role of ecdysteroids in insect larvae and during metamorphosis. In Koolman j (ed) Ecdysone: from chemistry to mode of action thieme. *Stuttgart*, 262-270.
- Smagghe, G.; B. Carton; W. Wesemael; I. Ishaaya and L. Tirry (1999): Ecdysone agonists – mechanisms and application on *Spodoptera littoralis*. *Pestic. Sci.*, 55: 343.
- Swelam, E. S. and M.A. Sayed (2006): Joint action of methomyl, carbaryl, esfenvalerate and profenofos and its latent on the cotton leafworm, *Spodoptera littoralis*. *J. Pest. Con. Environ. Sci.*, 14: 317-331.
- Tachell, R.J.; S.F. Arman and F.N. Boctor (1972): Biochemical and

- physiological studies of certain Ticks. (Ixodoidea). *Z. Parsitenk* 9:345-350.
- Tamayo, M.C.; M. Rufat; J.M. Bravo and B. San Segundo (2000): Accumulation of a maize proteinase inhibitor in response to wounding and insect feeding, and characterization of its activity toward digestive proteinases of *Spodoptera littoralis* larvae. *Planta*, 211: 62-71.
- Taunz, H. and N. Uygun (2004): Insect growth regulators for insect pest control. *Turk. J. Agric.*, 28: 377-387.
- Tay'bi, F.; G. Smagghe; L. Amrani and N. Soltani-Mazouni (2003): Effect of ecdysone agonist RH-0345 on reproduction of mealworm, *Tenebrio molitor*. *Comp. Biochem. Physiol.*, 135c (3): 257-26.
- Truman, J. W.; D. B. Rountree; S. E. Reiss and L. M. Schwartz (1983): Ecdysteroids regulate the release and action of eclosion hormone in the tobacco hornworm, *Manduca sexta* (L.). *J. Insect Physiol.*, 29: 895-900.
- Yao, T. P.; B. M. Forman; Z. Jiang; L. Cherbas; J. D. Chen; M. Mckeown; P. Cherbas and R. M. Evans (1993): Functional ecdysone receptor is the product of EcR and ultraspiracle genes, *Natura* 366: 476-479.
- Zitnan, D; T.G. Kingan; J.L. Hermesman and M.E. Adams (1996): Identification of ecdysis triggering hormone from an epitracheal endocrine system. *Science*. 271: 88-91.
- Zitnan, D; L.S. Roses; I. Zitnanova; J. L. Hermesman; S.S. Gill and M.E. Adams (1999): Steroidinduction of a peptide hormone gene leads to orchestration of a defined behavioral sequence. *Neuron.*, 23: 523-535.

ARABIC SUMMARY

مركبات عضوية طبيعية لمستخلص نبات القلقاس كمبيد أخضر وطبيعي لمكافحة دودة ورق القطن، اسبودوبتيراليتوراليس

أنوار محمد أباطة^١ - منى محمد جابر^٢

١ - معهد بحوث وقاية النباتات - مركز البحوث الزراعية - مصر،

٢ - قسم وقاية النباتات - كلية الزراعة - الاسماعيلية - جامعة قناة السويس - مصر

تم الفصل و التعرف على مركبين استيروديين (بيتا- سيتوستيرول) I و (ستيجما ستيرول) II من مستخلص الاثير البترولي لنبات القلقاس، حيث أظهرت هذه المركبات تأثيرها السام و كمانعات للتغذية وأظهرت ايضاً تأثيرها على النمو اليرقي ليرقات العمر الرابع لدودة ورق القطن. تم اختبار كفاءة المستخلص واثان من منظمات النمو، الميثوكسي فينوزيد (مشابه هرمون الانسلاخ) و الليوفينورون (مثبط تكوين الكيتين) ضد يرقات العمر الرابع لدودة ورق القطن معملياً، حيث أظهرت النتائج ان التركيز القاتل ل ٢٥% من تعداد الافراد المختبرة لمستخلص القلقاس (١٧.٨ جم/لتر)، الميثوكسيفينوزيد (٥.٠٧ مجم/لتر)، الليوفينورون (٠.٨٠ مجم/لتر). حيث كانت اليرقات المعاملة بكلا من مستخلص القلقاس و الميثوكسي فينوزيد تبدأ في الانسلاخ في وقت اسرع من اليرقات الغير معاملة ثم تفشل في اتمام عملية الانسلاخ. تم تقييم التأثيرات المشتركة لمستخلص نبات القلقاس بتركيزات (LC₁₀ & LC₂₅) مع كل من مبيد الميثوكسي فينوزيد و الليوفينورون بتركيزات (LC₁₀ & LC₂₅)، حيث أظهرت النتائج ان مستخلص القلقاس له تأثيراً منشطاً لمبيد الميثوكسي فينوزيد، بينما مبيد له تأثيراً مثبطاً لمبيد الليوفينورون ضد اليرقات المعاملة. التحليل البيوكيميائي لنشاط الانزيمات المرتبطة بعملية الانسلاخ و تكوين الكيوتيكول أظهرت ان مستخلص نبات القلقاس قد أحدث خللاً في نشاط بعض الانزيمات. تدل هذه النتائج ان سبب التشوهات و السمية في اليرقات المعاملة ترجع الى حدوث خلل في نشاط انزيم الكيتينيز، الفينول اوكسيديز، البروتيز. لذلك تعتبر المركبات الاستيرودية I، II منظمات نمو ضد دودة ورق القطن لتأثيرها الواضح على عملية الانسلاخ كما يحدث تماماً في حالة المعاملة بمبيد الميثوكسي الفينوزيد.