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Chemical Constituents of *Colocasia esculenta* leaves extract in Relation to its Self Defense against the Cotton leafworm, *Spodoptera littoralis* (Boisd.)

Anwaar M. Abaza[1], Yousry M. Ahmed[2], Mohamed G. Abbas[1], Hamdy A. Soliman[3] and Hanaa Kh. Ashour[3]

[1]Plant Protection Research Institute, A.R.C., Dokki, Giza, Egypt.
[2]Plant Protection Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt.
[3]Chemistry Department, Faculty of Science, Suez Canal University, Ismailia, Egypt.

ABSTRACT

Plants evolved elegant and complex chemical systems to defend themselves against the pathogens and herbivores that attempt to consume them. Taro, *Colocasia esculenta* plant has self defense property; it may be containing natural organic substances, allelochemicals, which are biologically active. The anti-feeding and insecticidal activities of petroleum ether extract of *Colocasia esculenta* leaves were studied against the 4th instar larvae of *Spodoptera littoralis* under laboratory conditions. Anti-feeding activity was assessed based on anti-feeding indices under choice and no-choice conditions. The highest anti-feeding activity is reached under no-choice conditions and anti-feeding indices were concentrations dependent; also it was found that the extract exhibits larval toxicity, the toxicity of the extract increased with time post treatment. Petroleum ether extract of *Colocasia esculenta* leaves was subjected to saponification reaction; from the un-saponifiable matter of petroleum ether extract, two phytosterols; namely β-sitosterol and stigmasterol were isolated by column chromatography (CC) and thin layer chromatography (TLC). Their structures were elucidated by ^{1}H-NMR and MS techniques. The presence of these two compounds in *C. esculenta* leaves may be one reason of its self defense. This is the first record on the isolation and identification of chemical constituents from *Colocasia esculenta* leaves.

Key words: *Colocasia esculenta*, allelochemicals, Anti-feeding activity, insecticidal activity, saponification, phytosterol, *Spodoptera littoralis*.

INTRODUCTION

Plants are important sources of natural products with the potential to be developed as commercial active ingredients for pest control, where it is believed that most of the plants that remain un-attacked by insects probably contain anti-feeding compounds (Crombie, 1999; Pavela and Herda, 2007; Rani and Murty, 2009).

Taro, *Colocasia esculenta* (L.), is a member of Araceae family; it is a perennial herb with large leaves which resemble an elephant's ear and bearing one or more underground stems.

Colocasia esculenta plant has self defense property, where it is not be attacked by insects, it may be containing natural organic substances, allelochemicals, which are biologically active. Therefore, its extracts could be applied as natural pesticides, especially insecticides.

Anti-feeding chemicals play a major role in the unsuitability of non-host plants as food for insects, so they have been proposed as an alternative to synthetic insecticides (Belles *et al.*, 1985). It is believed that most of the plants that remain un-attacked by insects probably contain anti-feeding compounds.

Anti-feedants are chemicals that have anti-feeding properties at low concentration, and they act on very specific sensory cells (anti-feedant receptors) in the pest. The neurons associated with these antifeedant receptors either prevent insect feeding (feeding deterrent effect) or cause cessation or slowing of further feeding (feeding suppressant effect), (Chapman, 1974).

Another mode of action of some anti-feedants is through an apparent ability block the function of a herbivore's feeding-stimulant receptors, or an ability to bind directly to its normal feeding cues, such as sugars and amino acids (Mordue and Blackwell, 1993).

During the last two decades many attempts have been made to isolate and identify various naturally occurring biologically active compounds possessing insecticidal properties (Schoonhoven *et al*., 1998; Isman, 2006 and Dayan *et al.,* 2009), among the main advantages reported by the use of natural products from plant origin, their narrow toxicity spectrum and less environmental impacts stand out (Jermy, 1990; Morimoto and Komal, 2000; Harborne, 2001).

The Egyptian cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) is considered as one of the most injurious and widespread pest of different crops in Egyptian agriculture. This phytophagous pest attacks cotton and large number of other field crops and vegetables, ornamental plants and weeds in Egypt as well as Mediterranean and Middle East countries causing heavy damage in different parts of the host plants (Bayoumi *et al*., 1998 and El-Aswad *et al.,* 2003).

The purpose of this study is to investigate the anti-feeding and insecticidal activity of petroleum ether extract of *C. esculenta* leaves against the cotton leafworm, *Spodoptera littoralis*. Isolation and purification of some bioactive organic plant constituents using column chromatography and thin layer chromatography.

* *Corresponding author: nora.abaza@yahool.com*

Structure elucidation and characterization of the isolated products using different spectral methods such as ^{1}H-NMR and MS techniques.

MATERIALS AND METHODS

Plant material

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.

Extraction and Fractionation

Leaves powder (700 g) 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 40ºC by using rotary evaporator to get the crude extract as a dark green residue .

Methanolic crude extract was fractionated by dissolving in a mixture of MeOH / H_2O (1:1), transferred in a separator funnel and re-extracted by petroleum ether (40-60ºC). The extract was dried over anhydrous sodium sulphate and concentrated under vacuum, then kept in a refrigerator for further investigation.

Instrumentation

NMR: ^{1}H-NMR were recorded at 300 MHz (at Cairo University, Nuclear Magnetic Resonance Laboratory), chemical shifts are given in δ (ppm) relative to TMS as internal standard material. MS Mass Spectra were recorded on a Ssimadzu GC/MS UB 1000 EX instrument (at Cairo University, Micro Analytical Center). Normal Column Chromatography CC was performed on silica gel Merck grain size 0.2-0.063 mm; TLC Thin Layer Chromatography performed on silica gel Merck GF 254 pre-coated plates 20 x 20 cm on aluminum sheets and polyamide plates (0.25 mm, Merk).

Tested insect

Spodoptera littoralis were obtained from laboratory susceptible strain which was not exposed to insecticidal contamination and maintained for more than 10 generations under laboratory conditions was obtained as egg masses from Plant Protection Research Institute, Dokki, Giza. The colony and experimental procedures were carried out under room conditions of 25±2°C, 75±5% R.H and 16:8 (L: D) light: dark.

Preparation of the tested concentrations

Considering the petroleum ether 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. Stock solution of tested extract was made prior to use.Fore more homogenation of the first dilution, 0.3% of triton x-100 was added to the diluted solution. Successive dilutions were carried out for the later using water to obtain serial of the tested concentrations.

Anti-feeding activity

Each experimental unit consisted of plastic Petri dish (9 cm diameter). Damp filter paper was placed on the bottom of each dish to avoid early drying of the leaf discs.

Fresh castor leaf (*Ricinus communis* L.) discs of 4 cm diameter were punched using cork borer. Every disc treated with pet. ether extract or the corresponding carrier solvent, once the solvent was evaporated; two starved larvae were deposited using forceps into the center of each disc and allowed to feed.

The consumption of leaf discs were expressed as dry weight (mg). The remaining of leaf discs were collected, dried at 40°C and weighed.for estimation the initial dry weight at the beginning of each bioassay; 5 groups of 8 leaf discs were dried and weight (Nelson *et al.*, 2009; Pavela and Vrchotová, 2013).

Anti-feeding activity of tested extract was assayed by using choice and no-choice test as follows:

Discs were dipped in 100 µl of 0.125, 0.25, 0.5 and 1% concentrations of the extract individually. Discs to which only the same amount of carrier solvent had been applied were used as control. In choice test; only alternating leaf discs were treated with the test concentration (C= control leaf disc; T= treated leaf disc), whereas in the no- choice test; all leaf discs were treated with the test concentration (T= treated leaf disc), a control variant (Cv= control leaf disc), with only the carrier solvent was established in every bioassay as a reference to stop the experiment. When approximately 50% of the control leaf discs (C) in choice test, and 75% (Cv) in no-choice was been eaten. Larvae were removed from the petri dishes. Five replications were maintained for each treatment in every test (Nelson *et al.*, 2009; Pavela and Vrchotova, 2013).

From choice test data; it could be calculated:

Feeding deterrence index (FDI) = 100 [(C-T) / (C+T)], where C and T are the control and treated leaf weights consumed by the insect (Sadek, 2003; Pavela, 2010) This index evaluates the potential of a substance to induce the cessation on feeding when tasted by an insect, and whether continues feeding in an alternative source of food, due to the activation of anti-feeding receptors.

From combined data of no-choice test and the control used to stop the experiment, it could be calculated: Feeding inhibition index (FII) = 100 [(Cv - T) / Cv], this index evaluates the potential of a substance to inhibit the feeding when there is no-alternative source of food. It helps to evaluate the potential feeding inhibition that might be produced in no-treated foliar discs as a consequence of previous feeding in treated discs, that gives an idea of possible toxicity effects (Bentley *et al.*, 1984).

Insecticidal activity

Laboratory experiments were conducted to study the toxic activity of pet. ether extract against the 4th instar larvae of *S. littoralis*, the experiments were carried out using leaf dipping technique as described by Sadek (2003). The larvae were allowed to feed on treated leaves only for 48 h, then on untreated leaves till death or pupation occurred.Three replicates of 10 larvae/each were used for each concentration in addition to control. Mortality of larvae was recorded daily and accumulative larval mortality was calculated till pupation and corrected by Abbott's formula (1925).

Statistical analysis

Data were subjected to statistical analysis to evaluate efficiency of tested extract against the 4th instar larvae of *S. littoralis*. mortality percentages were corrected according to Abbott's formula and then subjected to probit analysis. The toxicity lines were drawn on log concentration-probit paper and statistically analyzed according to Finney's method (1997). The LC_{50}, LC_{90} and slope values and their 95% confidence limits of tested extract were also estimated.

Abbott's formula:

Corrected larval mortality percentage =

$$T - C / 100 - C$$

Where: T: Larval mortality percentage in treatment

C: Larval mortality percentage in control.

Separation of petroleum ether fraction

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; hydrolysis with 10% alcoholic sodium hydroxide over water bath under reflux for 30 min., cooling, then diluted with water and extracted with diethyl ether afforded the unsaponifiable fraction.

The unsaponifiable matter was subjected to column chromatography using silica gel as adsorbent. Elution was carried out using pet. ether, followed by gradual addition of methylene chloride in increasing amount, till reaching methylene chloride 100%.

The sub-fractions (100 ml each) were collected and combined after monitoring by TLC on silica gel plates using toluene: acetone (90: 10) as solvent system. The spots were detected using UV (254 and 366 nm) before and after spraying with sulphoric acid reagent and heating in oven at 110º C. The sub-fractions (8-12) were reacted positively with sulphoric acid reagent. These fractions were eluted using pet. ether: ethyl acetate (80: 20) as solvent system and subjected to TLC, It afforded a mixture of two compounds I and II of R_f = 0.42, which gave deep violet colour on spraying with p-anisaldhyde-sulphoric acid reagent. It yielded a colourless crystals on evaporation to dryness.

RESULTS

Anti-feeding activity

Anti-feeding activity of pet. ether extract of *C. esculenta* leaves was assessed based on anti-feeding indices, where higher anti-feeding index normally indicated decreasing rate of feeding. it was observed that the anti-feeding activity varied depending on concentrations. The highest anti-feeding activity was under no-choice conditions; where no-choice test seems to be more sensitive than choice test, it was observed that the anti-feeding activity increased as the concentration of extract increased. Based on FII values, it was found that anti-feeding activity of petroleum ether extract at conc. 0.125 & 0.25 & 0.5 & 1% in no-choice test were 25.96 & 49.62 & 60.62 & 79.30 %.While, based on FDI values, the anti-feeding activity of petroleum ether extract at conc. 0.125 & 0.25 & 0.5 & 1% in choice test were 13.60 & 26.26 & 35.95 & 58.83%, respectively. (Table 1).

Table (1): Anti-feeding activity of pet. ether extract of *Colocasia esculenta* leaves against the 4th instar larvae of *S. littoralis* after choice and no-choice test.

Extract	Concentrations %	Choice	No-choice
		FDI %	FII %
Petroleum ether	0.125	13.60	25.96
	0.25	26.26	49.62
	0.5	35.95	60.62
	1	58.83	79.30

In the choice test Feeding deterrence index (FDI) = 100[(C-T) / (C+T)], where C and T are the control and treated leaf weight consumed by the insect. In the no-choice test, Feeding inhibition index (FII) = 100[(Cv-T) / Cv], (T= treated leaf disc), a control variant (Cv=control leaf disc), with only the carrier solvent was established in every bioassay as a reference to stop the experiment.

Insecticidal activity

The toxic action as initial lethal effect of investigated extract to the 4th instar larvae of *S. littoralis* was evaluated under lab-conditions. The main criteria of the toxicity regression lines; LC_{50}, LC_{90}, and slope values were used as parameters in evaluation the insecticidal activity of tested extract. The insecticidal activity of petroleum ether extract was investigated by feeding of newly molted 4th instar larvae for 48 hrs on castor bean leaves treated with 0.125, 0.25, 0.5 and 1% of the extract followed by feeding on untreated leaves till pupation under laboratory conditions.

It was observed that the toxicity increased with time post treatment, when pupation occurred serious abnormalities were observed, where ecdysis and sclerotization were incomplete. (Table 2).

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Table (2): Insecticidal activity of pet. ether extract of *C. esculenta* leaves against the 4th instar larvae of S. littoralis

Extract	LC_{50} (%) and confidence limits	Slope	LC_{90} (%) and confidence limits
Petroleum ether	0.029 (0.001 – 0.075)	0.718 ± 0.213	1.784 (0.861 – 21.352)

The extract show insecticidal activity against the 4th instar larvae of *S. littoralis*, so the extract will be studied in details. Two compounds I and II were isolated from un-saponifiable fraction by using thin layer chromatography as colourless crystals, R_f = 0.42. They gave deep violet colour upon spraying with p-anisaldhyde-sulphoric acid reagent indicating it's steroidal or triterpenoidal nature.

The ^{1}H-NMR spectrum of compounds I and II

, δ_H 0.83 (3H, *d*, Me-26), δ_H 0.81 (3H, *d*, Me-27) and δ_H 0.85 (3H, *t*,

The ^{1}H-NMR spectrum revealed the presence of six methyl groups in the up field region showing that the compound is aliphatic and may be steroidal or triterpenoidal compound. The spectrum indicating the presence of a multiplet at δ_H 3.52 ppm for one proton H-3 indicating its steroidal nature. An olefinic proton appeared as broad singlet at δ_H 5.34 ppm (distorted doublet (*br s*), which was assigned for H-6 suggesting the presence of a Δ^5- 3β- hydroxysterol. The spectrum indicated the presence of two tertiary methyl proton signals at δ_H 0.691 and δ_H 1.01 ppm, corresponding to (Me-18, Me-19), respectively. The side chain signals appeared at δ_H 0.92 (3H, *d*, Me-21)Me-29) and δ_H 1.87 (1H, *m*, H-25), suggesting that the sterol has a Stigmast-5-en-3β-ol skeleton. Based on all spectral data, compound I is *β- sitosterol*. (Table 3).

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

The mass spectral analysis of compound I

The mass spectrum of compound I, showed the presence of ion peak M^+ at m/z 414 (82%) corresponding to the molecular formula $C_{29}H_{50}O$. The spectrum also showed an ion peak at m/z 396 (54%) due to expulsion of water molecule. Another ion peak at m/z 381 (40%) due to further expulsion of methyl group, while the fragment peak at m/z 329 (77%) is due to the formula $C_{23}H_{37}O$, 303 (73%) is due to $C_{21}H_{35}O$ and the fragment peak at m/z 231 (47%) is due to $C_{16}H_{23}O$. In addition, an ion peak at m/z 273 (45%) due to expulsion of the side chain $C_{10}H_{21}$ from M^+ ion, ion peak at m/z 255 (60%) due to further expulsion of one molecule of H_2O and the ion peak 213 (91%) is due to the formula $C_{16}H_{21}$. The fragmentation pattern was in agreement with the reported spectrum in WILEY and NIST libraries that obtained by GC/ MS; identified for *β-sitosterol*.

Table (3): ^{1}H-NMR of compounds I and II recorded in $CDCl_3$

H atom	δ value (ppm), multiplicity	δ value (ppm), multiplicity
	P-I	**P-II**
3	3.52 (1H, *m*)	3.52 (1H, *m*)
6	5.34 (1H, *br s*)	5.34 (1H, *br s*)
Me-18	0.69 (3H, *s*)	0.69 (3H, *s*)
Me-19	1.01 (3H, *s*)	1.01 (3H, *s*)
20	1.48 (1H, *m*)	1.48 (1H, *m*)
Me-21	0.92 (3H, *d*)	0.92 (3H, *d*)
22	1.01 (2H, *m*)	4.98 (1H, *dd*)
23	1.26 (2H, *m*)	5.12 (1H, *dd*)
25	1.87 (1H, *m*)	1.87 (1H, *m*)
Me-26	0.83 (3H, *d*)	0.83 (3H, *d*)
Me-27	0.81 (3H, *d*)	0.81 (3H, *d*)
Me-29	0.85 (3H, *t*)	0.85 (3H, *t*)

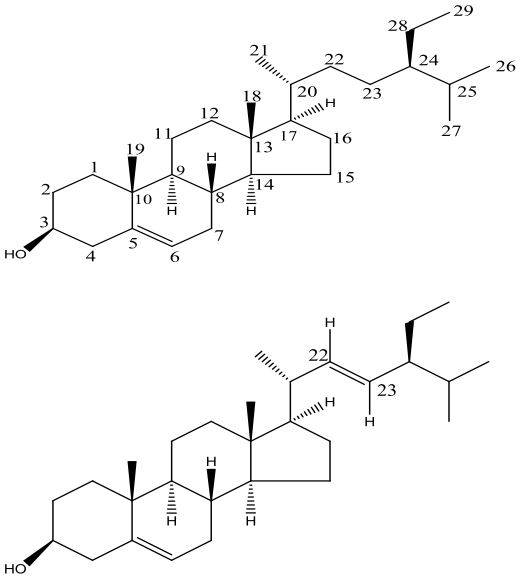
The mass spectral analysis of compound II

The mass spectrum of compound II, showed M^+ at m/z 412 (47%) corresponding to the molecular formula

$C_{29}H_{48}O$. The spectrum also showed an ion peak at m/z 394 (10%) due to expulsion of water molecule from M^+, ion peak at m/z 379 (9%) due to further expulsion of methyl group and ion peak at m/z 351 (22%) due to further expulsion of C_2H_4 group. The fragment peaks at m/z 255 (64%) is due to the formula $C_{19}H_{27}$, at m/z 213 (36%) due to the formula $C_{16}H_{21}$ and the ion peak at m/z 199 (20%) is due to $C_{15}H_{19}$. the base peak at m/z 55

(100%) is due to the formula C_4H_7. In addition, an ion peak at m/z 171 (33%) is due to the formula $C_{13}H_{15}$, and at m/z 159 (68%) is due to $C_{12}H_{15}$ and 145 (61%) corresponding to the formula $C_{11}H_{13}$. the fragmentation pattern was in agreement with the reported spectrum in WILEY and NIST libraries that obtained by GC/ MS; identified for *stigmasterol*.

Based on all spectral data the compound I is stigmast-5-en-3β-ol which is known as *β- sitosterol*. The compound II is stigmast-5, 22-dien-3-ol which is known as *stigmasterol.*



DISCUSSION

In lepidopteran larvae, terpenoids and steroids block the stimulatory effects of glucose, sucarose and inositol on chemosensory receptors cells located of the insect mouth parts and they could also act on receptors in other ways (Greshenzon and Croteau, 1991) and (Gershenzon and Dudareva, 2007). It was found that steroids, terpenoids, phenols, coumarins have anti-feeding and growth inhibiting effects on *S. littoralis* larvae (Pavela, 2007).

The obtained results from insecticidal activity of pet. ether extract are parallel with anti-feeding activity where the extract recorded maximum FII index (94.39%) in no-choice at conc. 1% . These results are in agreement with Bentley *et al.*, (1984); they showed that the calculated FII index from no-choice test is an indication of possible toxicity effect.

The ^{1}H-NMR and mass spectra of compounds I and II revealed that the two compounds which were isolated and identified together from unsaponifiable fraction of pet. ether fraction are *β-sitosterol* (I) and *Stigmasterol* (II) which were isolated and characterized previously from the aerial parts of *Ferulago bernardii* by Khalighi *et al*., (2006). Also, Singh *et al*., (2013) isolated and characterized β-sitosterol and stigmasterol compounds together from the pet. ether extract of the aerial parts of *Cassia* species; *C. renigera*, *C. pumila* and *C. nodosa*.

Stigmasterol, the active constituent of *Cacalia tangutica* was found to be toxic to *Spodoptera litura* and its action was more marked in comparison to the other active constituents of the plant (Kaur *et al*., 2011)

This is the first record on the isolation and structure elucidation of chemical constituents from *Colocasia esculenta* leaves. The presence of these two compounds in *C. esculenta* plant may be one reason of its self defense.

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REFERENCES

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

BAYOUMI, E., F.R. BALANA, A.K. SOBEIHA, AND M.K. HUSSEIN. 1998. The biological activity of some chitin synthesis inhibitors against the cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Bol. San. Veg. Plagas*., **24:** 499-506.

BELLES, X., F. CAMPS, J. COLL AND M. D. PIULACHS. 1985. Insect antifeedant activity of clerodane diterpenoids against larvae of *Spodoptera littoralis* (Boisd.), *J. Chem. Ecol.*, **11:** 1439-1445.

BENTLEY, M., D. LEONARD, W. STODDARD AND L. ZALKOW. 1984. Pyrrolizidine alkaloids as larval feeding deterrents for spruce budworm *Choristoneura fumiferana* (Lepidoptera: Tortricidae). *Ann. Entomol. Soc. Am*., **77:** 393-397.

CHAPMAN, R.F. 1974. The chemical inhibition of feeding by phytophagous insects: a review. *Bull. Entomol. Reeserch*., **64**: 339-363.

CROMBIE, L. 1999. Natural product chemistry and its part in the defense against insects and fungi in agriculture. *Pesticide Science*. **55**(8): 761- 774.

DAYAN, F. E., C. L. CANTRELL AND S. O. DUKE. 2009. Natural products in crop protection. Bioorgan. *Med. Chem*. **17:** 4022-4034.

EL-ASWAD, A. F., S. A. M. ABDELGALEIL, AND M. NAKATANI. 2003. Feeding deterrent and growth inhibitory properties of limonoids from *Khaya*

senegalensis against the cotton leafworm, *Spodoptera littoralis. Pest. Manag. Sci.,* **60**: 199-203.

FINNEY, D. J. 1997. Probit analysis, Statistical treatment of the sigmoid response curve, 2nd Ed., Cambridge Univ. Press, London, UK.

GRESHENZON, J., AND R. CROTEAU. 1991. Terpenoids. In: Rosenthal, G., Brerenbaum. M. (Eds.), Herbivores: their interactions with secondary plant metabolites. vol. I, 2nd ed., Academic press, San Diego, pp. 165- 209.

HARBORNE, J. 2001. Twenty-five years of chemical ecology. *Nat. Prod. Rep*. **18**: 361-379.

ISMAN, M. 2006. Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol*. **51**: 45- 66.

JERMY, T. 1990. Prospects of anti feeding approach to pest control. A critical review. *J. Chem. Ecol*., **16**: 3151-3160.

KAUR, N., J. CHAUDHARY, A. JAIN, AND L. KISHORE. 2011. Stigmasterol: a comprehensive review, *Int. J. Pharm. Sci. Res.,* **2**(29): 2259-2265.

KHALIGHI, S. F., A. HADJIAKHOONDI, A. SHAFIEE, V. MOZAFFARIAN, A. R. SHAHVERDI, AND S. H. R. ALAVI. 2006. Phytochemical analysis of *ferulogo bernardii* Tomk & M. Pimen. *DARU. J. Phram. Sci*., **14** (4): 214-221.

MORDUE, A. J. AND A. BLACKWELL. 1993. *Azadirachtin*: an update. *Journal of Insect Physiology.* **39**: 903-924.

MORIMOTO, M. AND K. KOMAL. 2000. Plant secondary metabolites of plant defense systems recent Res. Dev. *Phytochem*., **4:** 99-114.

NELSON, Z., B. FLOR, V. ELISA, AND M. PILAR. 2009. Anti feeding and growth inhibitory effects of extracts of drimanes and *Drimys Winteri* stem bark against *Spodoptera littoralis* (Lep., Noctuidae). *Ind. Crops prod* **30:** 119- 125.

PAVELA, R. 2007. Possibilities of botanical insecticide exploitation in plant protection. *Pest Technol* **1:** 47-52.

PAVELA, R. 2010. Antifeedant activity of plants extracts on *Leptinotarsa decemlineata* Say. and *Spodoptera llittoralis* (Boisd.). *Industrial Crops and products* **32**: 213 - 219.

PAVELA, R., AND G. HERDA. 2007. Effect of pongam oil on adults of the greenhouse whitefly *Trialeurodes vaporariorum* (Homoptera: Trialeurodidae). *Entomol. Gener* **30:** 193-201.

PAVELA, R. AND N. VRCHTOVA. 2013. Insecticidal effect of furanocoumarins from fruits of *angelica archangelica* L. against larvae *Spodoptera littoralis* Boisd. *Industrial Crops and products*, **43**: 33 - 39.

RANI, A.S. AND U. S. MURTY. 2009. Anti feeding activity of *Splanthes acmella* flower head extract against *Spodoptera litura* (Fabricius). *J. Entomol. Res* **33:** 55- 57.

SADEK, M. 2003. anti-feeding and toxic activity of *Adhatoda vasica* leaf extract against *Spodoptera littoralis* (Lep Noctuidae). *J. Appl. Entomol* **127:** 396-404.

SCHOONHOVEN, L., T. JERMY AND J. VAN LOON. 1998. Insect-plant biology. Chapman & Hall. Londres.

SINGH, D., S. K. SHARMA, A. BANSAL, P. SHARMA, AND A. HASHMI. 2013. A comparative study of isolated phytosterols from different plant parts of selected *Cassia* species Asian. *J. Res. Pharm. Sci.* **3**(3): 117-121.

المكونات الكيميائية لمستخلص أوراق نبات القلقاس وعلاقتها بخاصية الدفاع الذاتية ضد دودة ورق القطــن

[١]أنوار محمد أباظة ، [٢] يسرى محمد أحمد ، [١]محمد جمعة عباس، [٣]حمدى عبد العظيم سليمان [٣]هناء خليل عاشور

[١]معهد بحوث وقاية النباتات- مركز البحوث الزراعية- مصر. [٢] قسم وقاية النباتات- كلية الزراعة- الاسماعيلية- جامعة قناة السويس- مصر. [٣]قسم الكيمياء- كلية العلوم- الاسماعيلية- جامعة قناة السويس- مصر.

الملخص العربى

تطلق النباتات مركبات كيميائية معقدة لتدافع عن نفسها ضد مسببات الأمراض والآفات التى تحاول مهاجمتها. يتميز نبات القلقاس بخاصية الدفاع الذاتية حيث انه لا يصاب بالآفات و مسببات الأمراض، و ذلك ربما لإحتوائه على مركبات عضوية طبيعية لها نشاط بيولوجى. لذلك تم دراسة تأثير مستخلص الاثير البترولى لأوراق نبات القلقاس كمانع للتغذية ودراسة تأثيره السام على يرقات العمر الرابع لدودة ورق القطن تحت الظروف المعملية. حيث تم دراسة تأثير منع التغذية للمستخلص عن طريق الاختبار الاختيارى واللا اختيارى، حيث وجد مؤشرات منع التغذية تعتمد على تركيز المستخلص. اظهرت النتائج ايضا ان المستخلص حقق سمية ضد اليرقات حيث تزداد هذه السمية بمرور الوقت بعد المعاملة. تم اجراء عملية التصبن على مستخلص الاثير البترولى لاوراق نبات القلقاس وفصل الجزء غير المتصبن، تم الفصل و التعرف على مركبين استيرويدين (بيتا– سيتوستيرول) و (ستيجماستيرول) عن طريق طرق الكروماتوجافى العمودى ، كروماتوجرافى الطبقة الرقيقة ، طيف الرنين النووى المغناطيسى للهيدروجين و طيف الكتلة. وجود هذه المركبات فى مستخلص الاثير البترولى ربما يكون من احد اسباب خاصية الدفاع الذاتية لاوراق نبات القلقاس.