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Biocidal effect of Ergosterol-Propyl Ester Isolated From Ruta angustifolia (Pers.) on Spodopetra littoralis (Boisd.)

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ABSTRACT

The toxic effects of some *Ruta angustifolia* extracts as a bio agent to control *Spodoptera littoralis* were investigated. Based on LC_{50} values, the acetonic extract was more effective than hexane and ethyl alcoholic ones. Ergosterol-propyl ester was isolated from the acetonic crud extract by thin layer chromatography and identified using Infra-Red spectrophotometer, Mass Spectrum and evaluated as a fraction for its larvicidal, biological, ultrastructure and biochemical effects on 4th instar *S. littoralis* larvae. The most prominent biological effects were presented as: prolongation in the total larval duration and a decrease in the percentages of survived larvae. The adult emergence percentages, longevity, fecundity, hatchability were significantly decreased compared to controls. Also, some deformation symptoms were recorded in larvae, pupae and adults. The ultrastructure alterations in *S. littoralis* 4th instar larvae were observed in cuticle microfilament in muscle myofilaments compared to control. Biochemical responses of the 4th instar *S. littoralis* larva has a decreas in the activities of both ALT and chitinase and elevation in AST of supernatant *S. littoralis* larvae compared to control.

Keywords: Ergosterol-Propyl Ester, Enzymes, *Ruta angustifolia*, *Spodoptera littoralis*, Ultrastructure.

INTRODUCTION

Cotton leaf worm, *Spodopetra littoralis* (Boisd.) (Lepidoptera: Noctudiae) is a high harmful serious damages to more than 112 plant species belongs to 44 different families. Cotton is the main host plant in Egypt; moreover, the pest damage is extended to be seen on many other crops. The insect attacks a wide variety of vegetables, field crops, fruit orchards and ornamental plants at the different growth stages causing serious damage and yield reduction (Khedr *et al.*, 2002). The massive application of pesticides resulted in foundress of pest resistance to these poisons and adverse effects on environment that include acute and chronic hazards to human and non-target organisms, environmental pollution and upsetting the natural balance. Most botanical insecticides are specific, with a save effect on beneficial natural enemies and provide residue-free food and safe environment. So, using botanical insecticides among integrated pest management programms can greatly reduce the use of synthetic insecticides (Hikal *et al*., 2017).

Ruta species have received a growing attention as a source of pesticide active secondary metabolite such as phenolic, terpenoids, coumarins and alkaloids, which it has biological activities, including antifungal, antioxidant, phytotoxic, and anti-inflammatory activities (Gonzalez-Trujano *et al.*, 2006 and Raghav *et al.*, 2006). *Ruta angustifolia* (Pers.) (Sapindales: Rutaceae) is used for medicinal and culinary purposes since ancient times. It is normally grows in mountainous areas. It is also cultivated as a pot plant in Malaysia and occasionally in Vietnam and Java for medicinal purposes, and commonly used to cure cramps, flatulence and fever. In Indonesia, *R. angustifolia* has been known as traditional medicine for liver disease and jaundice, where it contains coumarin, alkaloid and flavonoid compounds (Wahyuni *et al*., 2014). *Ruta angustifolia* Pers. has been traditionally used for various medicinal purposes. One of the common ethno pharmacological uses includes usage in treatment of cancer by the Chinese community, Malaysia and Singapore (Stella and Richardson 2018).

The current study is an attempt to test a new compound which has an insecticidal activity (ergosterol-propyl ester), isolated from the acetonic extract of *R. angustifolia* on *S. littoralis* to suppress the damage via ultrastructure, biological and biochemical studies.

MATERIALS AND METHOD

Plant Collection

Seeds of *Ruta angustifolia* (Pers.) were from the Department of Horticulture, Faculty of Agriculture-Zagazig University; then planted in a private property (350 m^2) in local gardens at Abu Hammad Sharquia, Egypt. Leaves of *R. angustifolia* (Pers.) were collected at the flowering stage (blossoming periods) during May, 2016.

Preparation of plant material

The freshly collected leaves were washed with distilled water and spread to dry at normal room temperature for 40 days in the shade. Upon drying, the leaves were pounded using mortar and pestle into smaller particles and then blended to powder. The powder was stored in airtight containers and kept under normal room temperature until required.

Extraction procedure

Leaves of *R. angustifolia* were extracted at room temperature successively using serial of solvents (hexane, acetone and ethanol 70%), depending on its polarity. Serial exhaustive extraction were used starting with non-polar solvent to a moderate polar and finally polar solvents hexane, acetone and ethanol 70%)

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respectively. Sample of 1000 gm powder was soaked in 2000 ml of the first solvent (hexane) for 72 hrs. The combined extract was filtered concentrated by rotary evaporator (Model 349/2, Corning Limited). The biomass was dried and subsequently subjected to extraction with another solvent (acetone and ethanol 70%). The same precedent extraction was used. The crude extracts were weighed and kept in deep freezer until use.

Rearing technique of the cotton leafworm culture, *S. littoralis* (Boisd.)

A laboratory strain of cotton leaf worm, *S. littoralis* was reared away from any insecticidal contamination at the division of Cotton Leafworm Department, Branch of Plant Protection Research Institute at Zagazig, Sharquia Governorate, under constant condition 27±2ºC and 70±5% R.H. to obtain insect culture used in the present investigation according to El-Defrawi *et al.,* (1964).

Application technique for toxic effects of some *R. angustifolia* extracts against the 4th instar larvae of *S. littoralis*

The toxic effects of tested concentrations against newly molted 4th instar larvae of *S. littoralis* were measured using the leaf dip technique. Larvae were starved for 4 hrs before treatment (Merdan 1968).

Mortality percentages were recorded after 72 hrs for all tested plant extracts and corrected according to Abbott's formula (1925). The lethal concentration (LC) value was evaluated and a toxicity line was illustrated using log-Probit software program Ldp Line® model "Ehabsoft" (Bakr 2000) Toxicity Index and Relative Potency calculated according to Sun equation´s (1950):

Toxicity index=

$$\frac{\text{LC50 or LC90 of the efficient compound}}{\text{LC50 or LC90 of the other compound}} \times 100$$

Separation and purification of isolated compound from acetonic extracts using preparative TLC

The most toxic extract (acetonic extract of *R. angustifolia*) was separated using preparative (Thin Layer Chromatography) TLC (20× 20) cm prepared according to the method of Kirchner (1967) with 1mm thick. The previous plates were air dried for several hours and activated at 105°C for 60 min. The concentrated fractions were applied to the chromate-plates as bands with a simple syringe and developed with chloroform, petroleum ether with ratio (3:2), as a solvent system. After the development and drying, bands were detected with UV light (254 and 365 nm). The adsorbent containing the bands was scraped off with a razor blade andextracted with pure acetone. Acetone extract of *R. angustifolia* yielded eight main bands on TLC.

Toxicity tests of the eight isolated compounds against *S. littoralis* 4th instar larvae

Toxicity experiment was conducted to estimate the LC_{50}. Four concentrations (1, 2, 3 and 5%) were used against the 4th instar larvae of *S. littoralis*. The mortality rates were recorded after 72 hrs of treatments and corrected to the positive control values to avoid any effect that may be caused by the solvent used in each treatment. Identification of empirical formula and structure of isolated compound

The toxic compound of *R. angustifolia* was conducted to Infra-Red Spectrometric analysis (IR) and Mass Spectrometric analysis (MS) to determine the chemical structure of the isolated compound.

Biological attributes of ergosterol-propyl ester compound against *S. littoralis*

The previous procedure of leaf dipping technique was also applied to assess the biological activity of LC_{50} of ergosterol-propyl ester compared to negative and positive control against newly molted the 4th instar larvae of *S. littoralis* laboratory strain at 27±2°C, 70±5% relative humidity, with a 14:10 light: dark cycle. Larvae were starved for 4-6 hours before treatment (Merdan, 1968).

Leaf disks (3 cm2) of the fresh castor bean leaves, *R. communis* with a cork borer, were dipped in LC_{50} for 10 sec. Control disks were dipped in distilled water (negative control) and other dipped in acetone solvent (positive control). The treated leaf disks were left to dry then offered to the larvae at the rate of (10 disk/10 larvae) of *S. littoralis*.

Ten replicates were made for ergosterol-propyl ester and controls (5 larvae / replicate). Filter paper was put upon the bottom to absorb any excess moisture. The larvae were allowed to feed on treated disks for 48 hrs then on untreated leaves until pupation. Fresh castor leaves were provided to the larvae daily. The accumulated feces and debits were removed out daily.

The following measurements were recorded: larval duration and mortality percentages of larvae; adult emergence percentage; deformation in larvae, pupae and adults. Adult longevity, pre, ovi and post-position periods, number of eggs laid/female (fecundity), and hatchability percentages of deposited eggs were also recorded.

Ultrastructure studies

Ultra structure sections were made for the cuticle and muscles for 4th instars larvae after three days of treating with LC_{50} of ergosterol-propyl ester in comparison with positive control. Stained sections examined with a JEOL 1010 Transmission Electron Microscope at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.

Biochemical tests

Preparation of samples for biochemical assay

Healthy samples of the laboratory 4th instar *S. littoralis* larvae, that were treated with LC_{50} of the ergosterol-propyl-ester, comparing to controls (-ve and +ve). All groups were group homogenized in distilled water (50mg/1mg) using chilled glass Teflon tissue homogenized (ST-2 Mechanic precisian, pland) surrounded with crushed ice jacket for three minutes. The homogenate samples were centrifuged at 8000 rpm for

15 minutes. The deposits were used to determine total lipids. Supernatants were referred as enzyme extracts. All the samples were transferred to cleaned screw-capped tubes and stored frozen at -20 C until using for the biochemical assays. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and chitinase were also determined.

Determination of transaminase enzymes (AST and ALT) activities

Enzymes are given a code number, according to (Moss 1992) such as EC 2.6.1.1 (aspartate aminotransferase (AST/ GOT) and EC 2.6.1.2 (alanine aminotransferase (ALT/ GPT) enzyme activities. AST and ALT enzyme activities were determined calorimetrically according to the method of (Reitman and Frankle 1957).

Determination of chitinase activity

The chitinase (EC 3.2.1.14) assay was carried out according to the method described by Monreal and Reese (1969) with modifications by Shindia *et al*., (2001) and El-Sayed (2008).

Statistical analysis

The obtained data were subjected to analysis of variance (ANOVA), using the software package Costat® statistical software (2005) a product of Cohort Software, Monterey, California, USA. The significance of variation treatments was evaluated by Duncan´s multiple rang test ($P < 0.05$) (Snedecor and Cochran 1980).

RESULTS

Toxic effects of some *R. angustifolia* extracts against the 4th instar *S. littoralis* larvae

The toxic effects of different tested extracts of *R. angustifolia* against the 4th instar larvae of *S. littoralis* were assessed after 72 hrs. of treatments. The LC_{50} and LC_{90} values were (8.77 and 63.95%) followed by hexane extract gave10.94 and 67.00%, respectively. Meanwhile, ethyl alcohol extract gave 17.95 and 82.81%. The 4th instar larvae of S. littoralis was high susceptible for *R. angustifolia* acetone extract than other organic solvent extracts.

TLC technique for fractionation and purification

Thin layer chromatography (TLC) was used for the separation and purification of crude acetonic extract. Acetone extract of *R. angustifolia* yielded eight main bands on TLC by developing system consisted of chloroform, petroleum ether at rate of (3:2), respectively. The compounds were extracted and purified.

Identification of the most effective isolated compound of ergosterol-propyl ester

Isolated compound was identified using Infra-Red spectrophotometer (IR) and Mass Spectrum (MS) with the help of mass bank of North American. Identifications were made by comparison to spectra in the Wiley 275.1 and NIST 98 (National Institute of Standard and Technology, Gaithersburg, MD, USA) libraries.The pure compound is present in the form of amber yellow resin. It displays that Rf. 0.175 on TLC using solvent system chloroform, petroleum ether with ratio (3:2), respectively.

The IR spectrum of the compound shows a broad peak at, 3440.09 cm-1 stretching for bounded hydroxyl group absorption and 1728.87cm-1 stretching for carboxylic group (C=O). It also, exhibit absorption at 2966.95, 2925.48 and 2876.31 cm-1 indicating the aliphatic structure (C-C) and weak peak at 1642.09 cm-1 indicated for (C=C) structure (Figure 1).The MS spectrum in (Figure 2), manifestation the following fragments expressed as m/z (% relative abundance) respectively 421 (46), 375 (52), 363 (100), 317(60), 305 (72), 259 (16), 247 (28), 175 (15), 116 (31), 113 (14) and 59 (89%) m/z.

Confirmation of structure and mass similarity the comparative library using mass bank-data base found that structure of compound is ergosterol with propyl ester (in the side chain) which are illustrated as follows, the parent ion at 452 m/z when, the loss of CH_4 followed by carbon atom showed peaks at (433 and 421) m/z. the loss formic acid confirmed by the peak at 375 m/z due to basic structure of steroid.

Toxic effect of ergosterol-propyl ester against the 4th instar *S. littoralis* larvae

The LC_{50} and LC_{90} values were 18.83 and 148.9% for the 4th instar larvae of *S. littoralis* treated with ergosterol-propyl estar.

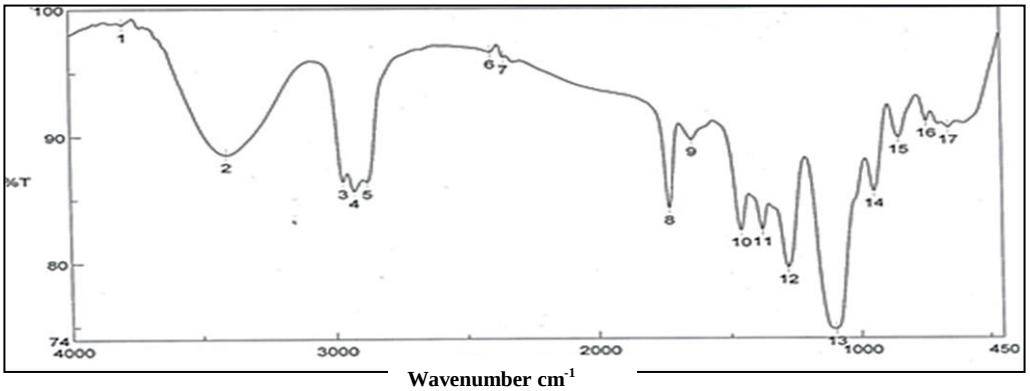

Figure (1): Infra-Red spectrophotometer of ergosterol-propyl ester.

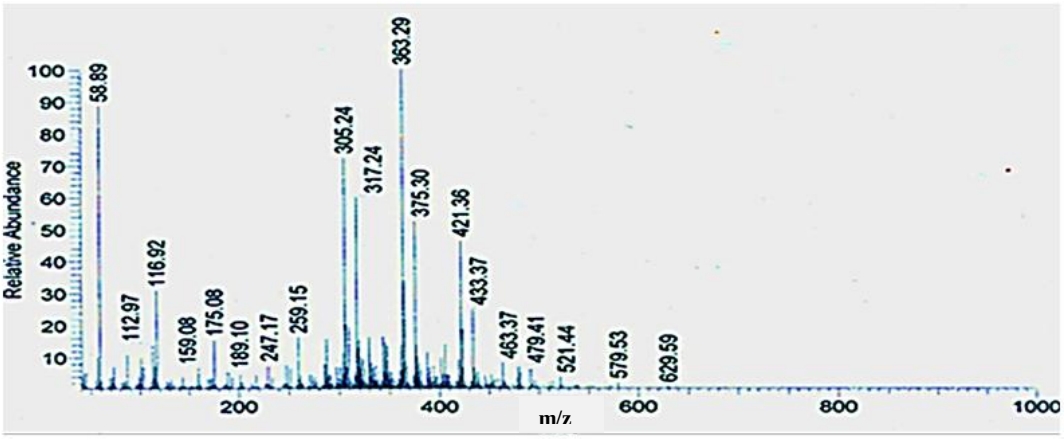

Figure (2): Mass Spectrum of ergosterol-propyl ester.

Impact of ergosterol-propyl estar on some biological attributes of *S. littoralis*

Effect on larval stage

Data concerning all the larval stage affected with the tested isolated compound (ergosterol-propyl ester) is tabulated in Table (1). It is clear that all treatments caused highly significant retardation in the total larval duration in different larval instars (13.46±0.35days) Controls (-ve and +ve) recorded 11.51±0.12 and 11.56±0.09 days, respectively (F 2, 6 =25.05, *P*< 0.001). The larval mortality percentages was recorded (34.0%) for ergosterol-propyl ester, while (-ve and +ve) cont-rols did not give any mortality Table (1).

Effect on adult stage

Highly significant differences on longevity of males were noticed between treatment and control. Data in Table (2) show that, the tested treatment seemed to decrease male longevity than control. Ergosterol-propyl ester give highly significant decr-eased in longevity 8.83±0.12 days, while controls (-ve and +ve) lived 9.36±0.06 and 9.40±0.05 days, respectively (F (2, 6 =13.56, *P* < 0.005).

Table (1): Biological aspects of *S. littoralis* larvae after treated with the isolated compound.

Treatments	Larval stage/days ± S.D.					larval mortality %
	4th instar	5th instar	6th instar	Pre-pupa	Total larval duration	
Control (-ve)	2.56±0.09 [b]	2.96±0.07 [b]	4.56±0.07 [b]	1.43±0.07	11.51±0.12 [b]	0
Control (+ve)	2.6±0.06 [b]	3±0.06 [b]	4.53±0.07 [b]	1.43±0.03	11.56±0.09 [b]	0
Ergosterol-propyl ester.	3.03±0.09 [a]	3.7±0.12 [a]	5.06±0.09 [a]	1.66±0.09	13.46±0.35 [a]	34
L.S.D0.05	0.274	0.29	0.257	0.23	0.765	
P	**0.0104**	**0.0013**	**0.0039**	**0.076[ns]**	**0.0012**	
F	10.764	24.368	16.066	4.083	25.053	

Control (-ve), (treated with H2O; Control (+ve), treated with acetone; Treatment at the level of LC50.
Data expressed as Mean ± Standard Error (SE); Treatment at the level of LC_{50}.
Means under each variety sharing the same letter are not significantly different at *P*< 0.05.

Longevity of female moth that includes (pre oviposition, oviposition and post oviposition period) were tabulated in Table (2). Ergosterol-propyl ester compound was caused non-significant reduction in female longevity estimated by 8.03±0.03 days, comp-ared to -ve control that recorded 8.06±0.03 days.

The total number of eggs laid/female moth (Fecundity) resulted from larvae treated with all tested compound revealed highly significant decrease clarified in Table (2). The reduction in the number of eggs/female treated with ergosterol-propyl estar was 1653.1±50.23 egg laid/female, while for controls (-ve and +ve) recorded 2016.7±27.06 and 1989.1±32.63 egg laid/female, respectively.

Data in Table (2) indicate that there was a reduction in hatchability percentages by using ergosterol-propyl ester compound compared to control (-ve and +ve) which give 98.0±44 and 97.3±44%, respectively. Ergo-serol-propyl ester compound showed highly signi-ficant reduction in hatchability percentages and reco-rded 82.9±1.04% (F (2, 27) =145.2, *P* < 0.001).

Deformations of larvae, pupae and adult malformations

Based on the external morphological characters of deformed larvae, pupae and malformations of adult were illustrated in Figure 3 and 4. Ten percent

Table (2): Biological aspects of *S. littoralis* adults resulted from treated 4th instar larvae with isolated compound**.**

Treatments	Emergence %	Longevity / days ± SD					Fecundity	Hatchability %
		Male	Female at different period					
			Pre-oviposition	Oviposition	Post-oviposition	Female		
Control† (-ve)	98	9.36±0.06 [a]	2	4.06	2	8.06±0.03	2016.7±27.06 [a]	98±0.44 [a]
Control (+ve)	98	9.4±0.05 [a]	2	3.92	2.04	7.96±0.08	1989.1±32.63 [a]	97.3±0.44 [a]
Ergosterol-propyl ester	90	8.83±0.12 [b]	2.1	3.67	2.26	8.03±0.03	1653.1±50.23 [b]	82.9±1.04 [b]
L.S.D$_{0.05}$		0.297				0.199	110.127	2.051
P		**0.005**				**0.500 [ns]**	**0.001**	**0.001**
F		13.56				0.777	28.448	145.286

†Control (-ve), (treated with H_2O; Control (+ve), treated with acetone; Treatment at the level of LC_{50}.
Data expressed as Mean ± Standard Error (SE);
Means with the same letter for each column are not significantly different at $P< 0.05$.

of deformed larvae were appeared after treatment with ergosterol-propyl ester and six percent of forms deformations are appeared on pupae. Meanwhile, malformed adults recorded 4%.

Figure (3): Illustrated normal larval, pupal and adult stages of *S. littoralis.* (A), the 4th larval instar; (B), Pupae,(C), Adult moth male; (D), Adult female.

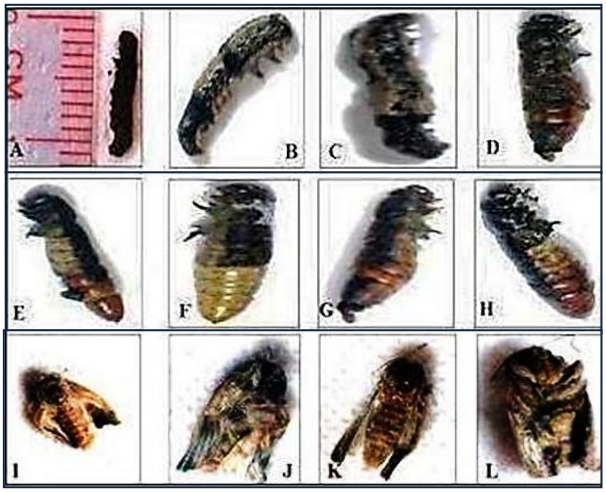

Figure (4): Illustrated larval, pupal and adult stage of *S. littoralis* after treatment with Ergosterol propyl-ester. (A): Dwarf larvae; (B, and C), Unable to discarding the old cuticle; (D, and E), Larval - Pupal intermediates; (F, G, and H), Pupal - Larval intermediates; (I, J, K, and L), illustrated adult malformation.

Ultrastructure studies on *S. littoralis* larvae

Ultrastructure studies showed that LC_{50} of the isolated compound ergosterol propyl-ester caused pathological changes in the cuticle and muscles in the 4th *S. littoralis* larval compared with (+ve control).

Cuticle layer

In control cuticle, normal parallel running chitin microfilaments associate with proteins forming sheets called laminae, to accord exoskeleton elasticity and constitute the pro-cuticle that lies between the protein rich epi-cuticle and the apical plasma membrane. However, the epi-cuticle is often no laminae and is distinguishable in oblique sections, Plate (1A). In case of ergosterol-propyl ester application, the epi-cuticle and the pro-cuticle cannot be distinguished and chitin microfilament is absent and loses its layered organization, Plate (1B).

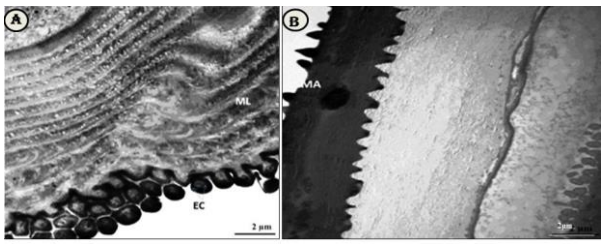

Plate (1): Electron micrographs (EM) illustrating transverse section in the cuticle of Spodoptera littoralis 4th instar larvae [1200X].(A), Control +ve (using acetone only); (B), Treated with ergosterol-propyl ester.[EC, Epicuticle; MA, Microfilaments Absent; ML, Microfilaments lammina].

Muscles

The transverse section in muscle cells clarified that, in normal muscle of untreated larvae there are typically greater number of mitocondria and they tend to be distributed in association with the contractile filaments,

Plate (2A). In case of ergosterol-propyl ester treatment the mitochondria became smaller in size, more spherical and more separated from the myofilmentes segementaly and the latter more separated from each other leaving wide spaces in beetwen, Plate (2B).

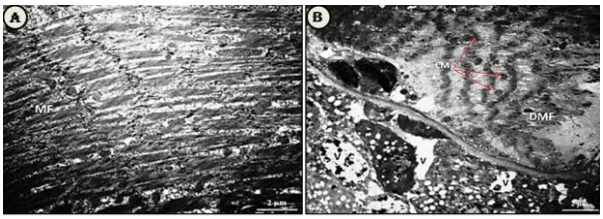

Plate (2): EM. illustrating transverse section in muscle of *S. littoralis* 4th instar larvae [1200X].(A), Cont. +ve of the 4th larvae of *S.* [CM, Condensed Mitochondria; DMF, Disentgration Muscle fiber; MF, Muscle Fiber; V, Vacuoles].

Biochemical response of 4th instar larva of *S. littoralis* to ergosterol-propyl ester

The changes in some enzymatic activities like transaminase, chitinase of the 4th instar larvae of *S. littoralis* as responses with LC_{50} of ergosterol-propyl ester after 72 hrs post-treatments.

Alanine aminotransferase (ALT)

Ergosterol-propyl ester compound caused insignificant reduction in ALT enzyme activity as compared to controls Table (3). Ergosterol-propyl ester recorded 33.72±1.84µg Pyr. /g.b.w./min. compared to controls (-ve and +ve) which recorded 37.99±0.32 and 37.96±0.41 µg Pyr./g.b.w./min, respectively. In table (3) F (2,6) =4.917, $P < 0.054^{ns}$.

Aspartate aminotransferase (AST)

Data indicats that the ergosterol-propyl ester compound caused a significant increase in AST activities as compared to -ve and +ve controls. This decrease recorded 20.63±0.37mg.Pyr./g.b.w./min, compared to controls (-ve and +ve) which recorded 19.79±0.07 and 19.49±0.17 µg. Pyr. /g.b.w. /min, respectively Table (3), (F (2, 6) =6.02, $P < 0.036$).

Chitinase enzyme

The statistical analysis shows highly significant reduced in the chitinase activities by ergosterol-propyl ester compound (57.14 ±0.08 µg.N-acet. /g.b.w. /min.) as compared to for -ve and +ve controls (65.98 ±0.11 and 64.98±0.15 µg.N-acet./g.b.w./min.), respectively. In table (3), (F (2, 6)=15.13, $P < 0.001$).

Table (3): Changes in the transaminase enzymatic activities and chitinase of the 4th instar larva of *S. littoralis* treated with ergosterol-propyl ester.

Treatments	Transaminase enzymes(ALT) (µg. Pyr. / g.b.w. /min)	Transaminase enzymes(AST) (µg. Pyr./g.b.w. /min)	Chitinase (µg. N-acet./g.b.w. /min)
Control† (-ve)	37.99±0.32	19.79±0.07 [b]	65.98±0.11 [a]
Control (+ve)	37.96±0.41	19.49±0.17 [b]	64.98±0.15 [b]
Ergosterolp ropyl ester	33.72±1.84	20.63±0.37 [a]	57.14±0.08 [c]
L.S.D $_{0.05}$	3.835	0.838	0.43
P	0.0544 [ns]	0.0368	0.001
F	4.917	6.023	15.13

† Control (-ve), (treated with H2O; Control (+ve), treated with acetone; AlltTreatment at the level of LC_{50}.
Data expressed as Mean ± Standard Error (SE);.
Means with the same letter under each variety sharing are not significantly different at $P< 0.05$.

DISCUSSION

Based on LC_{50} values, the type of solvent used for extraction seems to be considerably effect the toxicological activity of *R. angustifolia*. The acetonic extract was more effective than hexane and ethyl alcoholic extracts. These results were in agreement with Hafez (2001) who tested petroleum ether and acetonic extracts of Iberis amara seeds and Antholyza aehtiopica scale leaves against *S. littoralis* and Nassar *et al*., (2015) when studied the toxic effects of acetonic, hexane and alcoholic extracts of the aerial parts of Ipomea carnea against the 4th instar larvae of *S. littoralis*.

Ergosterol-propyl ester was isolated and purified from the acetonic extract of *R. angustifolia* using thin layer chromatography and identified by Infra-Red spectrophotometer and Mass Spectrum. Fragmentation data were found typical for compounds with ergosterol propyl ester (Zaretskii, 1976 and El Shafeiy 2011). Furthermore, many scientists had been isolated ergosterol derivatives which naturally produced in some plants and fungi. Rocha et al., (2014) isolated five steroids from the stems of Rauia nodosa (Rutaceae) sistostenone, stigmastenone, sitosterol, stigmasterol and ergosterol peroxide, one coumarin, O-geranylosthenol and three alkaloids, N-methylflindersine , zantobungeanine and veprissine.

Toxicity experiment shows that ergosterol-propyl ester compound is effective against 4th instar larvae of S. after 72 hrs of treatment.The toxicity of erogosterol-propyl ester may be attributed with their different activities.

The presented data revealed that the biological

activities on successive stages of *S. littoralis* were severely affected by varying degree due to feeding newly molted 4th instar larvae with LC_{50} of the ergosterol-propyl ester compared to (-ve and +ve) controls. As a general trend, the statistical analysis revealed that, there are no significant difference between (-ve and +ve) controls against the 4th *S. littoralis* larvae in all tested biological parameters. Thus, the effects against *S. littoralis* larvae may attribute to the active compound in the acetone extract of *R. angustifolia* leaves.

Many authors revealed the same biological results when tested many plant extracts against *S. littoralis* larvae. Likewise, EL-Kholy *et al*., (2014) who recorded that the increased in the larval mortality after feeding the 2nd instar larvae of *S. littoralis* on crud extracts from leaves of three plants (i.e. Damsissa, Camphor and Datura). The effect of ethanolic extract of Zingiber officinali at concentrations (2, 4 and 6 %) on the 3rd and 6th larval stage of *S.* l*ittoralis*. Increase the mortality percentages and prolonged durations of larval stages Ali *et al*., (2017). Also, Salama (2015) the acetonic extract of I. carnea and chlorpyrifos against *S. littoralis*. Reduese adult emergence percentages and fecundity after treated with I. carnea extract.

Fourty-eight hours of feeding on castor bean oil leaves treated with LC_{50} of ergosterol-propyl ester compared to controls resulteing larvae not able to complete the molting process and subsequently died. Salama (2015) reached to the same conclusion when tested two different concentrations of I. carnea against 4th instar larvae of *S.* l*ittoralis*. The reason of deformations and malformations may be as a result of reduction or elevation in transaminase enzymes. The aminotransferases, especially ALT is one of the components of oxidative metabolism of protein which in certain insect is utilized during the initial periods of flights.

In the current study, ultrastructure studies were done using TEM to encourage the role of the new isolated compound ergosterol-propyl ester in damage of cuticle and muscles. The tested compounds were characterized by a mode of action on the cuticle similar to that's of Juvenile Hormones compound and their activity have characteristics resembling to Insect Growth Regulator. Khedr *et al*., (2015) evaluated the ultrastructural changes in the 4th larval instar cuticle of *S. littoralis* as a result of application with the acetonic extract of Ipomea carnea compared to traditional insecticide (chlorpyrifos) such as cuticle being edges and separation of endocuticle from epicuticle.

Also, the extracted component application on larvae caused alterations in myofilaments striation which may be due to mitochondrial deformations and lead to block the energy supply to muscle needed for mechanical activities. Many researchers are coincides with these results when applied extracted components on *S. littoralis* larvae, Sabry (2009) and Sakr and Roshdy (2015) evaluated the histological damage in muscles of *S. littoralis* larvae caused by Hyptis brevipes methanol extract.

Biochemical parameters in organisms exposed to toxicants considered as sensitive index to monitor disturbances occurred inside the organism. Biochemical parameters are important diagnostic tools to evaluate the effects of stressors. The amino transferases enzymes constitute a group of enzymes, which catalyze the interconversion of amino acids and α-ketoacids by transfer of amino groups (Kachmar 1970).They have an important role in linking amino acids and carbohydrate metabolism, being an essential group of enzymes in the gluconeogenesis pathway. AST and ALT in insects are the most active transaminase enzymes (Carbetree and Newssholom 1970). The aminotransferase, especially ALT is one of the components of oxidative metabolism of protein, which in certain insects is utilized during the initial periods of flights (Bursell 1963). Data obtained revealed a decrease of ALT and an increase in AST activities in the supernatant of *S. littoralis* in all treatments compared to (-ve and +ve) controls. The greater and continuous release of AST might be due to the necessity of enhancing domination of aspartic acid for the process of gluconeogenesis especially under conditions of impaired carbohydrate metabolism. These results were strengthened by El-lakwah (2018) who studied the effect of Ocimum sanctum leaves extract against the 2nd and the 4th instar larvae of *S. littoralis*. On the other hand, Salama (2015) found significant elevation in ALT and AST enzymes in 4th instar larvae of *S. littoralis* treated with LC_{50} of the acetonic extract of Ipomea carnea.

Chitinase are generally found in organisms that either needs to reshape their own chitin or dissolve and digest the chitin of insect's chitin. Data indicate that all the treatments caused a decrease in chitinase activities as compared to (-ve and +ve controls). Additionally, Taira *et al*., (2002) reported that the roles of chitinase are usually part of the digestive tract. Insects and crustaceans, chitinase are associated with the need for partial degradation of old cuticle. Chitinase have been implicated in plant resistance against fungal pathogens because of their inducible nature and antifungal activities in vitro. These results were in agreement with; Sabry (2018) evaluated the efficacy of the Taxodium distichum (L.) ethanol extract fruits and the chitin synthesis inhibiting insecticide, lufenuron on the 4th instar of larvae *S. littoralis*.

CONCLUSION

The presented data showed the importance of naturally origin compounds especially those derived from plants. It also indicates ergosterol-propyl ester that isolated from the acetonic extract of *Ruta angustifolia* has the possibility to play an important role as larvicidal action against *S. littoralis*.These findings are useful and important to inegrate these compounds into IPM programs for control *S. littoralis*. further studies are necessary to evaluate the cost-efficiency of this compound on wide range of pests as alternative biocide in pest control.

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التأثيرات الحيوية لمركب الايرجوستيرول بروبيل ايستر المستخلص من نبات السذاب المصري على دودة ورق القطن (سبودوبترا ليتوراليس)

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الملخص العربـــــي

إستهدفت الدراسة تقييم التأثير السام لبعض مستخلصات نبات السذاب المصري كنوع من المكافحة الحيوية لدودة ورق القطن طبقاً لمعدلات التركيز القاتل لنسبة (50 %) لليرقات المعاملة كان المستخلص الأسيتوني هو الأكثر فاعلية عن كُلاً من مُستخلصي الهيكسان والكحول الإيثيلي. ولقد تم فصل مركب الإيرجوستيرول- بروبيل استر باستخدام طريقة الفصل بألواح السيليكا (Thin Layer Chromatography) وتعريفه بإستخدام قياس الطيف الكتلي (Mass Spectrometric analysis) تم تحليله باستخدام مطيافية الأشعة تحت الحمراء (Spectrometric analysis) (Infra-Red). وبحساب معدلات التركيز القاتلة لنسبة (50 %) من اليرقات المعاملة للمركب ودراسة التأثيرات البيولوجية وعلى التركيب الدقيق وكذلك دراسة الإستجابة البيوكيميائية على يرقات دودة ورق القطن. ودلة النتائج على المعامله حدوث إطالة للعُمر اليرقي و نقص في النسب المئوية لليرقات الحية، حدوث إنخفاض في نسب خروج الفراشات, وخصوبة الحشرة الكاملة ونسبة الفقس للبيض. كذلك ظهورتشوهات في كلا من اليرقات والعذارى والطور البالغ الذي أدى الى عدم إستطاعة هذه اليرقات إتمام عملية الإنسلاخ و بالتالي موتها. بالإضافة إلى ظهور العديد من التغيرات في التركيب التشريحي للالياف الدقيقة المكونة لطبقة الجليد والعضلات مقارنة بالعينة الضابطة. كما حدثت بعض التغيرات البيوكيميائية متمثلة في نقص نشاط الإنزيم الناقل لمجموعة الأمين الألانين (ALT) وانزيم الكيتينيز وزيادة في نشاط الإنزيم الناقل لمجموعة الأمين الإسبارتات (AST).