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Efficacy of insecticidal activity of crude seed extracts of *Sabal palmetto* against *Spodoptera littoralis* (Lepidoptera: Noctuidae)

Demiana, H. Khalil; Rania, S. Ammar and Mona, A. A. Mahmoud

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

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Abstract

Control of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) infestation has been achieved by using chemical insecticides, however, environment-friendly methods without unwanted side effects of these chemicals are becoming very important in modern pest management strategies. Natural products including plant extracts and oils are some of the alternative approaches to pest control. In the present work, insecticidal activities of oleic acid from sabal palmetto extraction were evaluated on *S. littoralis* by means of sublethal concentrations. The extracted oil and its chemical composition were identified using GC-MS (Gas chromatography–Mass spectrometry). The results showed that there were high differences between all treatments and the control in some biological aspects. The larval and pupal duration was prolonged, also pupal morphology was distorted for all treatments when compared to the control and apparent alterations as in the cytoplasm, the nuclei, and chromatin. As well as the histopathology effect of the extraction on 4th instar of cotton leaf worm *S. littoralis* was studied. The use of this extraction is therefore quite promising against this pest.

Introduction

The extensive use of conventional insecticides has resulted in a widespread environmental pollution, toxicity to non-target organisms and negative effects on human health (Damala, 2011 and Gill and Garg, 2014).

Botanical insecticides offer alternative to the use of chemical insecticides because many of them are target specific, biodegradable, and have lower toxicity to mammals (Senthil-Nathan *et al*., 2006a, b, 2007 and Maheswaran and lgnacimuthu, 2013).

The essential oils from various plants are toxic to different insect pests *viz*. *Artemisia judaica* has antifeedant activity against *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) (Abdelgaleil *et al*., 2008), *Nigella sativa* against *Callosobruchus chinensis* Linnaeus · (Coleoptera: Chrysomelidae) (Chaubey, 2008), ginger against *Drosophila melanogaster Meigen* (Diptera: Drosophilidae) (Xu *et al*., 2007).

The Egyptian cotton leafworm *S. littoralis* is a major polyphagous pest in Egypt and is considered one of the

most dangerous pests that attack cotton plants. This pest has at least 7-9 generations during the cotton season as well as infesting more than 29 other crops and vegetables (Magd El-Din and El-Gengaaihi, 2000).

The current study aimed to evaluate for the first time the insecticidal activity of crude seed extracts of *Sabal palmetto* against *S. littoralis*.

Materials and methods

1. Insect rearing:

The cotton leaf worm *S. littoralis* was reared in the laboratory for several generations at room temperature ranging between 25-28 °C and 60-65% relative humidity. The instar larvae were fed on fresh castor leaves *Ricinus communis* L., in a wide glass jar until pupation and adult emergence. The newly emerged adults were mated inside glass jars supplied with 10% sugar solution offered in a piece of cotton tissue soaked in this solution. Each jar was provided with branches of tafla, *Nerium oleander* for egg laying. The eggs were detached daily and surface sterilized by 10% formaldehyde for 2-5 mins and washed with double distilled water and allowed to dry for hatching.

2. Crude extract preparation:

Seeds of *S. palmetto* were dried at room temperature and grounded in an electrical mil. The seeds powdered (50 g) were extracted by soxhlet apparatus using two solvents varied in their polarity following this order: Petroleum ether, acetone, and methanol. The solvent was removed by vacuum evaporation in a rotary evaporator and the deposits were stored at 4 °C for further use.

3. GC–MS analysis of *Sabal palmetto* seeds extract:

The GC–MS (Gas chromatography–Mass spectrometry) analysis was done using a thermo scientific gas chromatograph GC Trace 1300 coupled with a mass spectrometer ISQ 7000 model (Thermo Scientific USA) equipped with thermo TR-50 MS capillary column (30 m in length × 250 µm in diameter × 0.25 µm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system that utilized high energy electrons (70 eV), MS transfer line temperature 300 °C and ion source temperature 300 °C. Pure helium gas (99.995%) was used as the carrier gas with a flow rate of 1 mL/min. The initial temperature was set at 60°C for 2 mins, then increased to 100 °C at a rate of 10 °C/min kept for 5 mins, then with 10 °C/min to 150 °C and kept for 5 min, then with 10 °C/min to 200 °C and kept for 5 mins, then with 10 °C/min to 250 °C and kept for 20 mins. One microliter of the prepared extracts was injected in a splitless mode.

4. Mortality bioassays:

Bioassays were implemented on second larval instars of *S. littoralis* using concentrations of 4000, 5000, 6000 and 7000 ppm of petroleum ether and acetone. Castor leaves were sprayed with different concentrations and air dried. The larvae were allowed to feed on treated leaves for continuous feeding until death or pupation occurred. Control larvae were fed on water treated castor leaves in the same way. A least 10 larvae were used for all the experiments per concentration, and the tests were replicated three times (Total, n=30) and each replicated set contained one control. The percentage mortality was calculated by using the formula:

$$\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of treated larvae}} \times 100$$

To calculate the mean lethal concentration (LC_{50}) the corrected percent mortality data was subjected to probit analysis (Finney, 1971).

5. Specimen treatment and preparation:

Larvae were treated as fourth instar with LC50 of *S. palmetto* extraction at a concentration of 4000 ppm according to Abd El-Kareem *et al.* (2010). Treated larvae were dissected at 4th instar and prepared for Transmission Electron Microscopy.

Preparation and ultra-scan micrograph were carried out at the faculty of medicine at Alexandria university, Egypt. The midgut from the larvae was dissected and immediately fixed in 2.5% glutaraldehyde at 4°C, for 3 days. Then washed in 0.1M buffer, fixed in 2% osmium tetroxide in 0.2 M buffer solution for one hour then rinsed in 0.2 M buffer. specimens were dehydrated by ethanol series dehydration. Then added to propylene oxide and transferred to eponate epoxy. Finally, the specimens were embedded in labeled capsules with freshly prepared resin and polymerized at 60°C for 24 hrs. The pH was 7.4.

6. Section preparation:

Sections of the resin embedded specimens were obtained using an ultra-cut E microtome. The slide sections were stained with uranyl acetate and lead citrate and examined with an electron microscope.

Results and discussion

1. GC-MS Analysis of *Sabal palmetto* seeds extract:

The GC-MS analysis revealed that nine major compounds were present in *S. palmetto* seeds extract (Table 1). The retention times, names of compounds, peak area percentages and molecular weights were noted. Analysis of *S. palmetto* seeds extracts indicated nine chief compounds with significant matches to oleic acid (13.27%) and 12-Methyl-E, E-2,13-octadecadien-1-ol (4.47%) peak area. Besides, dodecane, tridecane, tetradecane, tetrapentacantane, 1, 54-dibromo, heptacosane and Undecane were existed.

Table (1): Chemical composition of *Sabal palmetto.*

No	Retention times	Compound name	Peak area (%)	Molecular formula
1	7.05	Undecane	1.85	$C_{11}H_{24}$
2	9.47	Dodecane	3.10	$C_{12}H_{26}$
3	9.47	Tridecane	3.10	$C_{13}H_{28}$
4	9.47	Tetradecane	3.10	$C_{14}H_{30}$
5	32.70	2-piperidinone, N-[4-bromo-n-butyl]	1.86	$C_9H_{16}BrNO$
6	32.70	Heptacosane	1.86	$C_{27}H_{56}$
7	43.79	Oleic acid	13.27	$C_{18}H_{34}O_2$
8	35.04	12-Methyl-E,E-2,13-octadecadien-1-ol	4.47	$C_{19}H_{36}O_2$
9	37.12	Tetrapentacantane,1,54-dibromo	2.35	$C_{54}H_{108}BR2$

2. Insecticidal activity of *Sabal palmetto* extracts against *Spodoptera littoralis* under laboratory conditions:

The toxicity of two solvent extracts obtained from *S. palmetto* was evaluated against the 4th instar larvae of *S. littoralis* from laboratory strain using leaf dipping technique. The toxicity data was recorded at 3,7,10 and 14 days post treatment and LC_{50} values were presented in Table (2). Data indicated on 3-day- LC_{50} values, the petroleum ether extract was toxic against *S. littoralis* recording LC_{50}= 7400.9 ppm, acetone extract hasn´t significant toxicity against S. *littoralis*. At 7days post treatment, petroleum ether extract has toxic effect recording LC_{50} values = 6004.9 ppm, acetone extract hasn´t significant toxicity. At 10 and 14 days post treatment, the petroleum ether

extract was more toxic than acetone extract recording low LC_{50} 5284 and 4554 ppm, respectively comparable with LC_{50} values of acetone extract = 6820.7 and 6178.2 ppm, respectively.

Table (2): LC50, slope values and stander error of *Sabal palmetto* extracts of petroleum ether and acetone against 4th instar larvae of *Spodoptera littoralis* at different days post treatment.

Solvent	Days after treatment	LC_{50} (ppm)	95% Confidence limits (%)		Slope ±SE	χ^2
			Upper	Lower		
Petroleum ether	3	7400.9	8007.3	6681.2	4.05±1.2	2.08
	7	6004.9	6742.9	5541.6	3.6±0.77	5.02
	10	5284	5673	4899.5	4.2±0.72	4.22
	14	4554	4882.7	4109.3	4.6±0.74	5.92
Acetone	3	----	----	----	----	----
	7	----	----	----	----	----
	10	6820	7608	6421.21	0.27±1.3	0.01
	14	6178.2	6646.6	5829.6	5.8±1.25	0.01

3. Morphogenetic effect:

Treatment of the second instar of *S. littoralis* larva with petroleum ether extract at concentration 4000 ppm induced noticeable changes in insect development stages and prepupa failed to cast the old cuticle with complete blackening of the body leading to death (Figure 2) in comparison with the control larvae molt normally to pupal stage (Figure 1) .

Figure (1): Control pupa.

Figure (2): Malformed prepupa failed to cast the old cuticle with complete blackening of the body leading to death.

4. Histopathology effect of the extraction on 4th instar of cotton leaf worm *Spodoptera littoralis*:

The histopathological effect showed normal midgut ultrastructure of untreated larvae of the cotton leafworm as shown in Figure (3). The midgut epithelial ultrastructure of untreated larvae of *S. littoralis* and normal lining epithelium of the midgut consists of columnar cells resting on a basement membrane with an oval centrally located nucleus bound by a well defined nuclear envelope and patches of varying densities of nuclear chromatin are clear while Figure (4) presented treatment undifferentiated epithelial tissue and separation space between epithelium (Ept) and peritrophic membrane (PM).

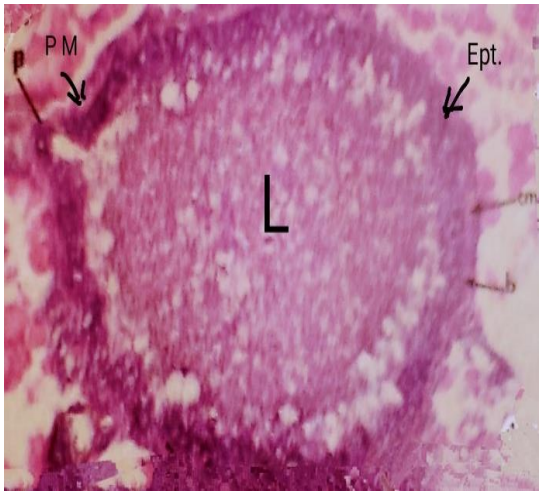

Figure (3): Transmission electron micrograph of the midgut of 4th instar non treated larva of *Spodoptera littoralis.*

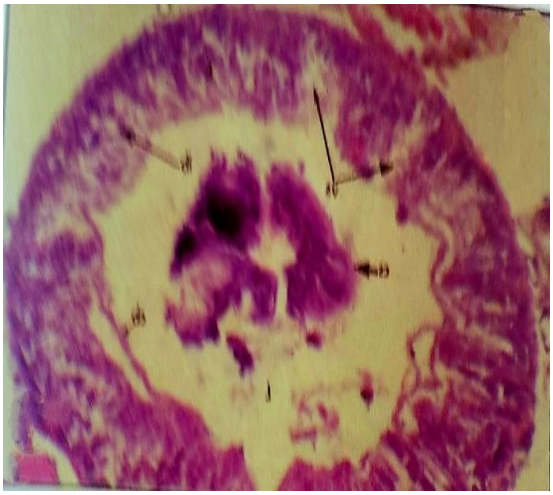

Figure (4): Transmission electron micrograph of the midgut of 4th instar treated larvae of *Spodoptera littoralis.*

Plants are considered source of bioactive chemical compounds, botanical insecticides may present attractive alternatives to currently used synthetic chemical insecticides for pest management (Pino *et al*., 2013; Miresmailli and Isman, 2014 and Pavela, 2016).

In this paper, compounds in seed extracts of *S. palmetto* had larvicidal activity against *S. littoralis*. Similar to previous results, Leatemia and Isman (2004) evaluated the efficacy of crude seed extracts of *Annona squamosa* and found at a concentration of 0.5% (w/v), an aqueous emulsion of ethanolic seed extract was 2.5 fold more effective than 1% rotenone. Abdelaziz *et al*. (2012) reported that seed extracts of *R. communis* achieved high mortality (58%) against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) at concentration of 77500 mg/l. The results obtained were in agreement with the work of Gonçalves-Gervá and Vendramim (2007), they found that *A. indica* seeds extract made high larval mortality of *T. absoluta* under laboratory conditions. Gurunathan *et al.* (2016) evaluated larvicidal activity of oleic acid, eicosyl ester in comparison to that of the crude methanol extract of aerial parts of *Thalictrum javanicum* and recorded the predicted biological activity of oleic acid, eicosyl ester unveiled the plausible larvicidal activity by the presence of certain inhibitors of chitins and ecdysone-20 monooxygenase. Chitinase enzyme is essential for insect growth and morphogenesis and it is found in molting fluid that digest the main constituent of the endocuticle (Reynolds and Samuels, 1996). Ecdysone-20 monooxygenase is found to be critical to all stages of insect development (Drummond and Smith, 2012).

Morphological abnormalities were recorded in *S. littoralis* when treated with petroleum ether extract of S. *palmetto* at concentration 4000 ppm. The results are similar to that obtained by Mahmoud (2002), who demonstrated that pupal and adult malformations percent as result of the larval treatment of 4th instar larvae of *A. ipsilon* with the *Artemisia maritima*, *Tipuana tipu*, *Lantana camara* and *Cassia fistula* extracts, the effect was more pronounced with both *A. maritime* and *T. tipu*, where the highest

pupal and adult malformations were recorded by the larval with the two extracts , while the other *C. fistula* and *L. camara* extracts had the least effect one. Bakr *et al*. (2006) showed sever malformation when 4th larval instar of *S. littoralis* treated with rice bran extract. Amal *et al.* (2020) found camphor oil treatment gave a shrank or incomplete pupal stage, where, pupae could not complete or develop a normal pupal cuticle.

Concluded of what was experienced is that the seeds of *Sabal palmetto* have insecticidal properties and can be included as potent elements in pest control management programs.

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