

## **TOXICOLOGICAL AND BIOCHEMICAL EFFECTS OF *CALOTROPIS PROCERA* LEAF EXTRACTS ON THE COTTON LEAF WORM, *SPODOPTERA LITTORALIS* (BOIS).**

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### **ABSTRACT**

In a trial to explore new alternatives to chemical insecticides whose negative side effects on humans and the environment are a serious concern, natural alternatives are being addressed to discover new, cost-effective, and environmentally friendly compounds. Therefore, the present study aimed to evaluate the toxicity and biochemical effects of *Calotropis procera* solvent leaf extracts (acetone, ethanol and hexane) against the 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). The acetone extract was more toxic than ethanol and hexane extracts in both the 2<sup>nd</sup> and 4<sup>th</sup> instar larvae with LC<sub>50</sub> values: 5.733, 7.96 and 11.18% for 2<sup>nd</sup> instar larvae and 7.507, 8.44 and 11.827% for 4<sup>th</sup> instar larvae, respectively after 48h. of feeding on treated leaves, it was observed that 2<sup>nd</sup> instar larvae were more sensitive than 4<sup>th</sup> instar larvae for all extracts. The biochemical effects on *S. littoralis* 2<sup>nd</sup> and 4<sup>th</sup> instar larvae were determined using the LC<sub>50</sub> of each extract. All extracts exhibited decreasing activities for all determined enzymes: Aspartate Transaminase (AST), Alanine Transaminase (ALT), Acetylcholinesterase (AChE), Glutathione S-Transferase (GST) and total protein in both 2<sup>nd</sup> and 4<sup>th</sup> instar larvae. The results showed that the overall effects of *C. procera* leaf extracts on some biochemical components in *S. littoralis* larvae can facilitate the development of natural products as insecticides that can be employed in integrated pest management strategies.

**Keywords:** *Calotropis procera*, *Spodoptera littoralis*, toxicity, enzyme activities and integrated pest management.

### **INTRODUCTION**

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is a devastating pest insect with a high propensity for reproduction. It is a highly polyphagous pest that significantly damages many economically significant

crops. Resulting in severe reductions in the commercial yield and significant losses in both the quantity and quality of the attacked crops (Ismail *et al.*, 2020). Between 300 and 500 eggs are laid by female. It is the most harmful pest to a variety of crops, including cotton, tomatoes, and potatoes (Senrunga *et al.*, 2014). Chemical insecticides are currently the most efficient way to control this pest, but it is difficult to eradicate since it has become resistant to them (Ghulam *et al.*, 2018). In addition to insect resistance, ongoing application use of these chemical pesticides can result in a number of risks, including environmental contamination, an adverse impact on beneficial insects, and hazardous effects on people, plants, and animals. There is a lot of focus on promoting the use of safer insecticides like plant extracts in order to reduce the riskiness of chemical pesticides (El-Sayed & Yousef, 2021). Almost 2000 plants showed insecticidal characteristics against various insects. Plant secondary metabolites, which have long been recognized for their therapeutic uses and pharmacological qualities, are what give them their potent insecticidal powers (Fowsiya *et al.*, 2020). According to Chowański *et al.* (2016), secondary plant metabolites have toxic effects that can be seen at both fatal and sublethal doses. They can be extracted from plant by solvents of different polarity starting from a nonpolar alkane-based solvents (e. g. n-heptane, n-hexane, cyclohexane) to intermediate solvents (e. g. dichloromethane, ethyl acetate) and then to polar solvents (alcohols and water) (Gori *et al.*, 2021). Maceration was suitable for secondary plant metabolites extractions, with ethanol and acetone being the best solvents (Lezoul *et al.*, 2020). *Calotropis procera*, also known as "Akado" locally, is a well-known Indian medicinal plant and a member of the Asclepiadaceae family, this plant's bloom, terminal leaf pairs, roots with root bark, and latex are all significant medicinal components (Erdman & Erdman, 1981). The main pharmaceutical companies have made using plants as a source of new pharmaceuticals a priority, and they are now conducting considerable research on plant materials to bring new treatments in the medical practice, the chemical compounds cardenolides, steroids,

tannins, glycosides, phenols, terpenoids, sugars, flavonoids, alkaloids, and saponins are among the many biologically active chemical groups found in *Calotropis procera* (Mali *et al.*, 2019). The Larger Wax Moth, *Galleria mellonella* (L.), (Lepidoptera: Pyralidae) demonstrated significantly reduced Relative Consumption Rate (RCR), Relative Growth Rate (RGR), and efficiency of Conversion of Ingested Food (ECI) when exposed to *C. procera* latex (El-Hefny, 2019). This study aimed to determine the effects of *C. procera* leaf solvent extracts on the toxicological and biochemical aspects of *S. littoralis* larvae.

## MATERIALS AND METHODS

### 1-Instruments and equipment:

- Rotary evaporator: Labconco, Germany.
- Ultraviolet/visible (U/V) spectrophotometer: Spectronic 1201, USA.
- Homogenizer, France.
- Centrifuge / Beckman J2Mc at 8000 rpm.
- All solvents were HPLC grade.
- Whatman No.1 filter papers.

**2- Plant material:** Leaves of *Calotropis procera* were collected from El Wahaat road, Giza, Egypt.

**3- Plant extraction:** The collected plant had been washed with tap water to avoid dust and dirt and completely dried in a shaded place at room temperature ( $25\pm 2^\circ\text{C}$ ) then milled by an electric mill, the powder of the plant was stored in a deep freezer until subjected to extraction process. It was extracted according to Freedman *et al.* (1979), and the extraction was done at room temperature ( $25\pm 2^\circ\text{C}$ ) by simple extraction method (maceration method) using different solvents, Hexane, Ethanol and Acetone.

**4-Insect rearing:** A laboratory strain of the cotton leafworm, *S.littoralis* was obtained from Qaha agricultural station, Qalyubia Governorate, and reared at the Department of pest physiology, Plant Protection Research Institute, Dokki, Giza for several generations without any insecticidal and/or microbial pressure, under constant laboratory conditions at  $25\pm 2^{\circ}\text{C}$  and  $65\pm 5\%$  relative humidity (RH) described by Eldefrawi *et al.* (1964).

**5. Toxicity assay:** Four concentrations 10, 5, 2.5 and 1.25 % were carried out from each crude extract. Newly active 4<sup>th</sup> instar and 2<sup>nd</sup> instar larvae were selected and starved for 4 hours before the experiments. Treatment was conducted by the dipping technique according to Abo El-Ghar *et al.* (1994) where castor leaves were immersed in the prepared concentrations of each solvent extract for the 30s. The leaves were left to dry at room temperature before being offered to both instars' larvae. Larvae were fed on treated leaves for 48h. The treatment comprised 10 larvae for each petri dish with five replicates. The control larvae were fed on castor leaves dipped in water. Larval mortality was recorded after 24, 48 and 72 h. Larvae were considered dead if they become immobile and have shown no detectable response to external stimuli. Mortality data were subjected to probit analysis for calculating  $\text{LC}_{50}$  and  $\text{LC}_{90}$  according to Finney's method (Finney, 1971).

## **6. Biochemical effects**

### **6.1. Preparation of Homogenate Samples:**

2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *S. littoralis* feeding on leaves treated with  $\text{LC}_{50}$  of *C. procera* solvent extracts for 48h. The survived *S. littoralis* larvae were collected and used for various biochemical tests. A known weight of larvae (whole-body) was homogenized in an appropriate amount of distilled water using a mechanical homogenizer, and centrifuged using Beckman J2Mc at 8000 rpm for 15 minutes at  $-2^{\circ}\text{C}$ . After centrifugation, the supernatant fluid was divided into small aliquots (1 ml) and stored at  $-20^{\circ}\text{C}$  until the biochemical analysis.

**6.2. Determination of total protein:** Total protein content was estimated according to Bradford (1976) using bovine serum albumin as a standard.

**6.3. Determination of Transaminases Enzymes:** Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) activities were assayed according to the method of Reitman and Frankel (1957). AST transfers amino group from L-aspartate to  $\alpha$ -ketoacid ( $\alpha$ -ketoglutaric acid), forming a new amino acid (L-glutamate) and a new ketoacid (oxaloacetic acid). While, ALT transfers the amino group from D, L alanine to  $\alpha$ -keto acid ( $\alpha$ -ketoglutaric acid) producing a new amino acid (L-glutamate) and a new keto acid (pyruvic acid). Oxaloacetate or pyruvate reacts with 2, 4-dinitrophenylhydrazine, producing oxaloacetate or pyruvate hydrazone which gives a brown color in an alkaline medium, and this color could be spectrophotometrically measured at 520 nm.

**6.4 Determination of detoxification enzymes:**

**6.4.1 Glutathione S-Transferase (GST) activity:** Glutathione S-transferase (GST) catalyzes the conjugation of reduced glutathione (GSH) with 1-chloro 2, 4-dinitrobenzene (CDNB) via the -SH group of glutathione. The conjugate, S-(2, 4-dinitro-phenyl)-L-glutathione could be detected spectrophotometrically at 340 nm. as described by Habig *et al.* (1974).

**6.4.2. Acetylcholinesterase (AChE) activity:** AChE (Acetylcholinesterase) activity was measured according to the method described by Simpson *et al.* (1964) using acetylcholine bromide (AchBr) as substrate, the decrease in AchBr resulting from hydrolysis by AChE was read at 515 nm.

**7. Statistical analysis:**

The average mortality data were subjected to Probit analysis for calculating LC<sub>50</sub> and LC<sub>90</sub> for all crude extracts and the data were statistically analysed by means of the analysis of variance (ANOVA) (Tukey's test) by using the software Statistical Package for Social Sciences (SPSS) version 27.0 for windows; significance level was

set at  $P < 0.05$  (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp, n.d.).

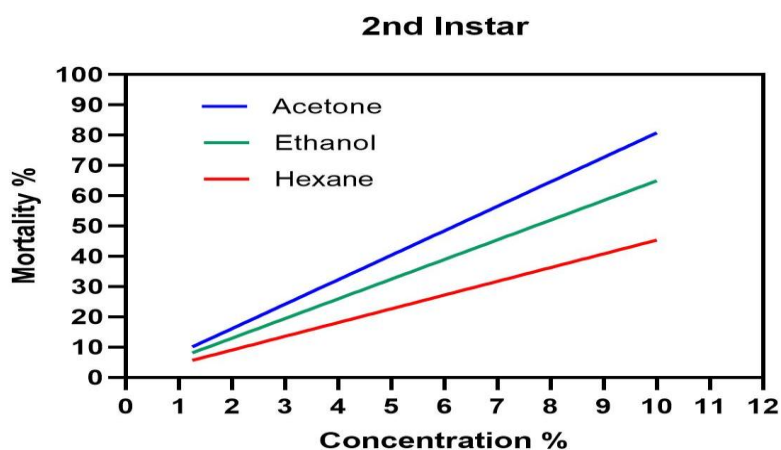
## RESULTS AND DISCUSSION

### 1. Toxicity assay:

Table (1) and figure (1) Showed that the  $LC_{50}$  values of the *C. procera* leaf acetone extract to the 2<sup>nd</sup> instar larvae of *S. littoralis* were 8.571 %, 5.733 % and 4.366%, and for ethanol extract were 10.48 %, 7.96 % and 5.36 %, where for hexane extract were 16.04 %, 11.185% and 9.96% after 24, 48 and 72h. after treatments, respectively. Table (2) and figure (2) illustrated that the  $LC_{50}$  of the *C. procera* acetone extract to the 4<sup>th</sup> instar larvae of *S. littoralis* were 12.33%, 7.5% and 5.36% and for ethanol extract were 15.3%, 8.44% and 6.32% where for hexane extract were 17.426%, 11.827% and 11.2% after 24, 48 and 72h. after treatments, respectively. The results showed also, that the acetone extract was more toxic than ethanol and hexane extracts in both 2<sup>nd</sup> and 4<sup>th</sup> instar larvae. These results agreed with Nilamsari et al. (2022) who found that secondary metabolites of ethanol extract of *Calotropis gigantea* were more toxic than those of hexane extract against *Spodoptera exigua*. In addition, results showed that the toxicity of *C. procera* leaf solvent extracts to 2<sup>nd</sup> instar larvae was more sensitive than the 4<sup>th</sup> instar larvae, this agreed with Ismail (2020) who reported that the toxicity of EOs decreased with the advancement of larval instars of *S. littoralis*.

**Table (1):** LC<sub>50</sub> and LC<sub>90</sub> values of *C. procera* solvent extracts against 2<sup>nd</sup> instar larvae of *S. littoralis* after 24, 48 and 72h after treatment.

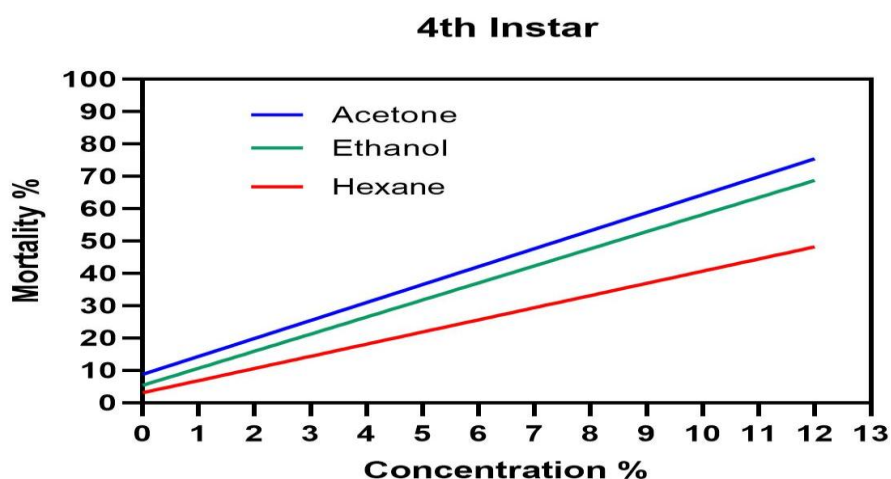
Time (hours)	LC <sub>50</sub> (%)	95% fiducial limit		LC <sub>90</sub> (%)	95% fiducial limit		Slope	χ <sup>2</sup>
		Lower	Upper		Lower	Upper		
<b>Ethanol Extract</b>								
24 h	10.48	8.405	15.08	20.639	15.761	32.868	0.13	3.641
48 h	7.96	6.423	10.747	17.833	13.887	26.978	0.12	5.136
72 h	5.36	4.384	6.518	12.214	10.229	15.734	0.18	6.16
<b>Hexane Extract</b>								
24 h	16.042	11.695	32.743	28.627	19.717	64.635	0.1	0.798
48 h	11.185	9.644	18.424	21.639	16.338	35.526	0.12	1.579
72 h	9.96	7.783	15.222	21.64	16.014	37.539	0.1	2.234
<b>Acetone Extract</b>								
24 h	8.571	7.071	11.215	17.382	13.848	24.916	0.14	1.686
48 h	5.733	4.477	7.379	14.784	11.791	21.131	0.13	5.266
72 h	4.366	3.262	5.502	11.896	9.784	15.876	0.15	4.098



**Figure (1):** Toxicity lines of *S. littoralis* 2<sup>nd</sup> instar larvae for the determination of LC<sub>50</sub> of *C. procera* leaf extracts.

**Table (2):** LC<sub>50</sub> and LC<sub>90</sub> values of *C. procera* solvent extracts against 4<sup>th</sup> instar larvae of *S. littoralis* after 24, 48 and 72h after treatment.

Time (hours)	LC <sub>50</sub> (%)	95% fiducial limit		LC <sub>90</sub> (%)	95% fiducial limit		Slope	χ <sup>2</sup>
		Lower	Upper		Lower	Upper		
<b>Ethanol Extract</b>								
24 h	15.3	11.27	29.802	27.784	19.333	59.983	0.1	1.283
48 h	8.44	6.853	11.418	18.132	14.154	27.257	0.13	3.224
72 h	6.32	5.255	7.766	13.694	11.338	18.056	0.17	3.146
<b>Hexane Extract</b>								
24 h	17.426	12.339	40.794	30.775	20.571	79.733	0.09	1.04
48 h	11.827	9.329	17.953	22.384	16.783	37.494	0.12	0.987
72 h	11.2	8.594	18.765	23.833	17.142	45.23	0.1	3.37
<b>Acetone Extract</b>								
24 h	12.332	9.588	19.597	23.469	17.299	41.312	0.11	1.075
48 h	7.507	6.081	9.908	16.947	13.35	24.921	0.13	3.026
72 h	5.343	4.195	6.734	13.538	10.997	18.562	0.14	3.299



**Figure (2):** Toxicity lines of *S. littoralis* 4<sup>th</sup> instar larvae for the determination of LC<sub>50</sub> of *C. procera* leaf extracts.



## 2. Biochemical activities

### 2.1 Biochemical effects on 2<sup>nd</sup> instar larvae of *S. littoralis*

Results in Table (3) indicated that the total protein was significantly decreased ( $P < 0.05$ ) for all extracts, which recorded 1.93, 2.26 and 2.27 mg/g body weight for acetone, ethanol and hexane, respectively compared with control (3.18 mg/gm. body wt.) for 2<sup>nd</sup> instar larvae. The results also revealed that there was a significant decrease in AST activity (18.68, 22.30 and 27.713  $\mu\text{g}$  oxaloacetate/min/ml) for acetone, ethanol and hexane, respectively compared with the control (31.1633  $\mu\text{g}$  oxaloacetate/min/ml.) For ALT activity there was a significant decrease in acetone, ethanol and hexane extracts (10.7600, 14.2767 and 17.976 oxaloacetate/min/ml, respectively) comparing with control (25.41  $\mu\text{g}$  oxaloacetate/min/ml.). There was a very significant decrease in the activities of both GST and AChE enzymes ( $P < 0.01$ ), for GST were 107.13, 136.43 and 145.5167  $\mu\text{L}/\text{min}/\text{ml}$  compared with the control (170.40  $\mu\text{L}/\text{min}/\text{ml}$ ) and for AChE were 13.88, 87.66 and 103.77  $\mu\text{mol}/\text{min}/\text{mg}$  protein compared with control (188.05  $\mu\text{mol}/\text{min}/\text{mg}$ ) protein for acetone, ethanol and hexane extracts, respectively.

**Table (3):** Biochemical effects of *C. procera* leaf solvent extracts on 2<sup>nd</sup> instar larvae of *S. littoralis*.

TREATMENTS	TOTAL PROTEIN MEAN $\pm$ SE MG/G. BODY WT.	ALT MEAN $\pm$ SE $\mu\text{G}$ OXALOACETATE/ MIN/ML.	AST MEAN $\pm$ SE $\mu\text{G}$ OXALOACETATE /MIN/ML.	GST MEAN $\pm$ SE $\mu\text{L}/\text{MIN}/\text{ML}$	ACHE MEAN $\pm$ SE $\mu\text{MOL}/\text{MIN}/\text{MG}$ PROTEIN
Acetone extract	1.93 $\pm$ 0.13 <sup>b</sup>	10.76 $\pm$ 0.17 <sup>d</sup>	18.68 $\pm$ 0.255 <sup>d</sup>	107.13 $\pm$ 4.8 <sup>c</sup>	13.88 $\pm$ 1.8 <sup>d</sup>
Ethanol extract	2.26 $\pm$ 0.08 <sup>b</sup>	14.276 $\pm$ 0.75 <sup>c</sup>	22.30 $\pm$ 0.486 <sup>c</sup>	136.43 $\pm$ 2.2 <sup>b</sup>	87.66 $\pm$ 6.2 <sup>c</sup>
Hexane extract	2.27 $\pm$ 0.07 <sup>b</sup>	17.977 $\pm$ 0.088 <sup>b</sup>	27.71 $\pm$ 0.415 <sup>b</sup>	145.52 $\pm$ 3.5 <sup>b</sup>	103.77 $\pm$ 2.0 <sup>b</sup>
Control	3.18 $\pm$ 0.14 <sup>a</sup>	25.41 $\pm$ 0.68 <sup>a</sup>	31.16 $\pm$ 0.337 <sup>a</sup>	170.40 $\pm$ 4.2 <sup>a</sup>	188.05 $\pm$ 4.2 <sup>a</sup>

Means followed by different letters within the same column are significantly different (Tukey's test,  $p < 0.05$ ).

## 2.2 Biochemical effects on 4<sup>th</sup> instar larvae of *S. littoralis*:

The data summarized in Table (4) revealed that there was a significant decrease in total protein for all extracts (2.23, 2.54 and 2.37 mg/g body wt.) compared with control (4.22 mg/g. body wt.) for acetone, ethanol and hexane, respectively. While there were no significant differences within the extracts (all take the same letter).

The results cleared that there was a significant decrease in AST for acetone, ethanol and hexane extract (21.076, 27.68 and 31.91 $\mu$ g oxaloacetate/min/ml, respectively) when compared with the control 35.89  $\mu$ g oxaloacetate/min/ml. For ALT there was a significant decrease in acetone, ethanol and hexane extracts (12.1, 21.08 and 25.94 oxaloacetate/min/ml, respectively) compared with control 29.88  $\mu$ g oxaloacetate/min/ml. There was a very significant decrease in the activities of both GST and AChE enzymes ( $P < 0.01$ ), for GST were 145.5, 146.16 and 196.83 $\mu$ L/min/mL for acetone, ethanol and hexane extract, respectively compared with control 233.23  $\mu$ L/min/ml and for AChE were (110.28, 441.57 and 461.67  $\mu$ mol/min/mg protein) for acetone, ethanol and hexane extracts, respectively compared with control 514.86  $\mu$ mol/min/mg protein.

**Table (4):** Biochemical effects of *C. procera* leave solvent extracts on 4<sup>th</sup> instar larvae of *S. littoralis*.

TREATMENTS	TOTAL PROTEIN MEAN $\pm$ SE MG/GM. BODY WT.	ALT MEAN $\pm$ SE $\mu$ G OXALOACETATE/MIN/ML.	AST MEAN $\pm$ SE $\mu$ G OXALOACETATE/MIN/ML.	GST MEAN $\pm$ SE $\mu$ L/MIN/ML	ACHE MEAN $\pm$ SE $\mu$ MOL/MIN/MG PROTEIN
Acetone extract	2.23 $\pm$ 0.07 <sup>b</sup>	12.1 $\pm$ 1.10 <sup>d</sup>	21.076 $\pm$ 0.682 <sup>d</sup>	145.5 $\pm$ 5.40 <sup>c</sup>	110.28 $\pm$ 2.8 <sup>c</sup>
Ethanol extract	2.54 $\pm$ 0.2 <sup>b</sup>	21.08 $\pm$ 0.24 <sup>c</sup>	27.68 $\pm$ 0.32 <sup>c</sup>	146.16 $\pm$ 6.49 <sup>c</sup>	441.57 $\pm$ 5.05 <sup>b</sup>
Hexane extract	2.37 $\pm$ 0.13 <sup>b</sup>	25.94 $\pm$ 0.08 <sup>b</sup>	31.91 $\pm$ 0.2 <sup>b</sup>	196.83 $\pm$ 0.66 <sup>b</sup>	461.67 $\pm$ 7.7 <sup>b</sup>
Control	4.22 $\pm$ 0.34 <sup>a</sup>	29.88 $\pm$ 0.35 <sup>a</sup>	35.89 $\pm$ 0.16 <sup>a</sup>	233.23 $\pm$ 4.1 <sup>a</sup>	514.86 $\pm$ 4.4 <sup>a</sup>

Means followed by different letters within the same column are significantly different (Tukey's test,  $p < 0.05$ ).

The significant decrease in total protein that recorded in 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *S. littoralis* agreed with that of Khatter and Abuldahab (2012) who recorded that the total protein of normal larvae of the house fly, *Musca domestica* vicina (Diptera: Muscidae) was higher than that treated with *Calotropis procera* extracts. Aprialty *et al.* (2021) recorded that the plant from the Asclepiadaceae family Contained steroid compounds ( $\beta$ -sitosterolacetate) that can cause growth inhibition of *Spodoptera frugiperda*, steroid compounds in some plant extracts will inhibit protein by blocking sterol carrier proteins. Mordue and Goldsworthy (1973) mentioned that AST and ALT are key enzymes in the formation of non-essential amino acids, gluconeogenesis, metabolism of the nitrogen compound, and associated with protein metabolism. Hence, the reduction in total protein content may be related to the reduction in the activities of both AST and ALT which were resulted in the present study. The reduction in those enzymes might be due to the decrease in total protein as the enzymes are protein in nature (Mitlin *et al.*, 1977).

Detoxification enzymes, AChE and GST in insects is generally demonstrated as the enzymatic defense against xenobiotic compounds and play significant roles in maintaining their normal physiological function (Kumrungsee *et al.*, 2022). Matthew and Adaramoye (2014) studied the methanolic extract of *C. procera* Calotoxin and found that calotoxin from *C. procera* was a great inhibitor of AChE. AChE is primarily responsible for the termination of cholinergic neurotransmission at synapses in insects (Fournier & Mutero, 1994). Therefore, AChE is known to be the target of many organophosphate- and carbamate-based insecticides (Carlier *et al.*, 2008). The present findings agree with Yousef *et al.* (2016) who recorded that the leaf extracts of *C. procera* may be utilized as the probable candidates for the development of bioinsecticides to control the cotton pink bollworm.

## CONCLUSION

The results of this research showed toxic and biochemical effects of some leaf extracts of *C. procera* against cotton leafworm *S. littoralis*, which caused a decrease in AST, ALT, GST, AChE and total protein, which caused high mortality percent, and this encourages the possibility of using these extracts in integrated control programs for this pest, taking into account the work of further studies on this plant to shed light on its secondary effects on the environment and beneficial organisms.

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## التأثيرات السمية والبيوكيميائية لمستخلصات أوراق نبات العشار على دودة أوراق القطن

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### المستخلص

في تجربة لاستكشاف بدائل جديدة للمبيدات الحشرية الكيميائية التي تشكل آثارها الجانبية السلبية على الإنسان والبيئة نتائج خطيرة، حيث أصبحت البدائل الطبيعية مصدر اهتمام الباحثين لاكتشاف مركبات جديدة وفعالة وقليلة التكلفة وصديقة للبيئة. لذلك يهدف هذا البحث إلى تقييم السمية والتأثيرات الكيميائية الحيوية لمستخلصات أوراق نبات العشار بواسطة بعض المذيبات (الأسيتون والإيثانول والهكسان) ضد يرقات العمر الثاني والرابع من دودة ورق القطن. كان مستخلص الأسيتون أكثر سمية من مستخلصي الإيثانول والهكسان في كل من يرقات العمر الثاني والرابع وكانت قيم التركيزات نصف المميتة 5.733% و 7.96% و 11.18% ليرقات العمر الثاني و 7.507% و 8.44% و 11.827% ليرقات العمر الرابع على التوالي بعد 48 ساعة من التغذية على الأوراق المعاملة بالمستخلصات، كما وجد أن يرقات العمر الثاني كانت أكثر حساسية من يرقات العمر الرابع لجميع المستخلصات، كما تم دراسة التأثيرات البيوكيميائية للمستخلصات على كل من العمر الثاني والرابع للحشرة باستخدام التركيز المميت النصف لكل مستخلص، وقد أظهرت النتائج أن جميع المستخلصات أدت إلى انخفاض في نشاط بعض الإنزيمات المسؤولة عن الوظائف الحيوية وكذلك انخفاض

نسبة البروتين الكلي في كل من العمر الثاني والرابع للحشرة. أظهرت نتائج هذا البحث أنه يمكن استخدام مستخلص نبات العشار كبديل للمبيدات التقليدية واستخدامه في برامج مكافحة المتكاملة لهذه الآفة. **الكلمات المفتاحية:** نبات العشار، دودة ورق القطن، السمية، أنشطة الانزيمات، الإدارة المتكاملة للآفات.