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Article

Effectiveness of *Calotropis procera* (Apocynaceae) leaf extract and its fractionations against *Tetranychus urticae* (Acari: Tetranychidae)

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ABSTRACT

In this study, toxicity of crude leaf extract of *Calotropis procera* (Aiton) (Apocynaceae) was assessed against *Tetranychus urticae* Koch (Acari: Tetranychidae) in laboratory conditions using the spraying method. The LC_{50} values after 24 hours of treatment were calculated as 3608.73 and 2277.02 mg/L for female and male mites respectively. These amounts after 48 hours of treatment were 2456.98 and 1671.2 mg/L. Results showed that the incubation period, total immature and life cycle durations of *T. urticae* were prolonged under the influence of the *C. procera* extract. The fecundity and hatchability percentages were significantly lower (18.2 eggs/females and 94.38%) compared to control (44.73 eggs/females and 99.47%), respectively. The reduction rate of *T. urticae* at different stages after 1, 3, 7, and 14 days of spraying showed that *C. procera* had a positive impact on mite population reduction that recorded 94.84 and 100% for females and males 14 days post-treatment. The methanolic extract of *C. procera* leaves was separated into 18 fractions. The 10th fraction exhibited 67, and 73% mortality, 48 and 72 hours post-treatment, respectively. Seventeen substances were identified in this fraction by using the GC-MS. These findings suggest that the leaf extract of *C. procera* which contains different novel compounds has the potential to be used for controlling *T. urticae* in the future.

KEYWORDS: Apple of Sodom, botanical acaricide, GC-MS analysis, identification components, two-spotted spider mite.

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INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the most significant and common pests in Egypt that attacks various crops, fruits, and vegetables (Senbill *et al*. 2023). Spider mites reduce chlorophyll levels, and suck plant sap, causing leaves to turn yellow. In situations of severe infestation by *T. urticae*, the yield is lower in amount and quality (Park and Lee 2002; Meck *et al*. 2013).

The two-spotted spider mite has been controlled using a variety of pesticides. However, the widespread use of synthetic pesticides has made a number of issues worse, including the emergence of pest resistance to most commonly used compounds, the toxic effects on pests' natural enemies, environmental pollution, harm to human health, and quality restrictions placed on fresh produce. Apple of Sodom of *Calotropis procera* (Aiton) (Apocynaceae) has been used widely in traditional

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medicine (Silva *et al*. 2010; Al Sulaibi *et al*. 2020). In addition, extracts of this plant have shown remarkable nematicidal, molluscidal and insecticidal activities (Al Sulaibi *et al*. 2020).

The efficacy of *C. procera* leaves which were extracted either by chloroform or methanol solvent to bioassay their effects by using direct spray technique against *T. urticae* adult females was evaluated. The LC_{50} was (10112.47 and 4916.25 mg/L) in case of extraction with chloroform and (70136.01 and 3060.98 mg/L) in case of extraction with methanol, 24 and 48 hours post-treatment respectively (Elkady *et al*. 2022).

The *C. procera* leaf extract demonstrated a repellent and toxic impact on the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Abbas *et al*. 2012). Also, it was reported to have potential efficacy against cattle ticks, *Rhipicephalus microplus* (Canestrini) (Ixodida: Ixodidae) under in vivo conditions (Nithya *et al*. 2015). The larvicidal activities of leaf and latex extract from *C. procera* against mosquito larvae of *Anopheles stephensi* Liston (Diptera: Culicidae), *Culex quinquefasciatus* Say (Diptera: Culicidae) and *Aedes aegypti* (L.) (Diptera: Culicidae) were confirmed by Singh *et al.* (2005), Elimam *et al*. (2009), and Shahi *et al*. (2010).

Integrated Pest Management (IPM) programs should not concentrate on controlling *T. urticae* with simply synthetic conventional pesticides (Ay 2005; Saad *et al*. 2006; Afify *et al*. 2011). Today, botanical pesticides have become promising in controlling pests due to their many advantages over chemical pesticides, such as a lower residue problems (Bhamat *et al*. 2022).

Consequently, there is an urgent need to find novel environmentally friendly products. Several secondary metabolites such as oils, alkaloids, terpenes, organic acids flavonoids, amines, and phenolic are produced in different varieties of plant, that protect them from pests' attacks (Divekar *et al*. 2022). Therefore, the aim of this study was to evaluate the efficiency of the *C. procera* leaf extract in controlling *T. urticae*, as well as to identify its bio-active metabolites that could be used in the future to develop an alternative botanical acaricide.

MATERIALS AND METHODS

This study was conducted in the laboratories of Plant Protection Department, Faculty of Agriculture, Fayoum University, Egypt.

Mite colony

In order to create a mite colony, two-spotted spider mites (*Tetranychus urticae*) were collected from infected castor plants, *Ricinus communis* (L.) (Euphorbiaceae). Initially, for the identification of mites, some mite specimens were mounted in Hoyer's medium and identified using appropriate keys of classification (Zaher 1984; Krantz and Walter 2009).

Mite females were moved from collected castor leaves to the upper surface of the copperleaf, *Acalypha wilkesiana* (Euphorbiaceae) using a fine-haired brush, and then left to start laying eggs. The deposited eggs were kept incubated at 20–23 ℃, 50 ± 5% R.H. and 12:12h day:night photoperiod for four months (several generations) before the beginning of experiments. Weekly replacement of acalypha leaves ensured a mite colony had fresh, new leaves.

Preparation of C. procera *extract*

Leaves of *C. procera* were collected from the desert of Fayed (30° 13' 5.304" N, 31° 41' 14.424" E), Ismailia governorate, then dried at room temperature away from direct sunlight for a month. Phonological stage of *C. procera* was fruit maturation. Five-hundred grams of dried leaves were crushed using an electric mixer until it became a powder. It was soaked in methanol (Biotech Company) for 48 hours at room temperature (28 ± 2 ℃). The extract was filtered through Whatman No.1 filter paper followed by evaporation of methanol using rotary vacuum evaporator at 40 RPM and 40 ℃. Fresh stock solution was prepared by mixing two grams of methanol-free extract with 100

mL of water and two drops of Triton X-100 (Oxford lab chem.) to get an emulsion at concentration of 20000 mg/L.

Bioassay experiments

Experiment 1

To determine the *C. procera* leaf extract acute toxicity, different serial concentrations, 1250, 2500, 5000, and 10000 mg/L of the extract were applied in three replications. Adults presented sexual dimorphism, as males are smaller than females (Hoy 2011). Differences in the sex-specific body size and shape were used to determine their sex. In order to obtain uniformly aged male or females of *T. urticae*, individuals that were 1–3 days old were randomly removed from mass rearing arenas and individually placed on to each leaf disc. Thirty adult females or males of *T. urticae* were set in three circular part of castor leaves (2 cm in diameter), 10 individuals/disc, in each Petri dish (9 cm diameter), which placed in a cotton wool pad. One milliliter of each concentration was sprayed using a glass atomizer with one nozzle on the surface of the castor leaf disc containing mites, except the control was sprayed with distilled water and Triton-X100 (Pree *et al*. 1989; Salman *et al*. 2014; Abdel-Rahman and Ahmed 2018). The mortality rate was recorded at 24 and 48 hours post-treatment, the mortality rate was corrected using Abbott's formula (Abbott 1925) and a toxicity line was established according to Finney's analysis (Finney 1971).

Experiment 2

To determine the sublethal effects, the spraying was carried out at only one concentration of the crude extract, the concentration that led to the mortality of 50% of the mites after 48 hours of spraying (LC_{50}), which was calculated from a probit analysis. Females (1 day old) were sprayed with the half lethal concentration (LC_{50}) value. Twenty alive females were placed individually in acalypha leaf discs (2 cm in diameter), and put upon a wet cotton pad in a Petri dish. The females were examined daily until death to calculate their longevity, fecundity and egg hatchability percentage.

Experiment 3

To determine the biological aspects of treated and untreated females, 15 emerged larvae from the deposited eggs were transferred by a camel brush to acalypha leaf discs (2 cm in diameter), which were put on a wet cotton pad in a Petri dish. The larvae were observed daily, and plant leaf discs were replaced by fresh ones when necessary. All developmental periods of *T. urticae* were recorded until it reached adult stage and records. The same steps were repeated with an untreated control (Hassan *et al*. 2005).

Experiment 4

Population-level effects

To determine the effect of *C. procera* leaf extract on *T. urticae* population levels, white long eggplant, cv. Soma, *Solanum melongena* (L.) (Solanaceae), seedlings were planted in 15 × 20 cm pots in four sets of three pots each. Each pot included a plant that thrived in the lab conditions of 22–28 °C and 60–10% RH and fertilization rate that is recommended. All pots were infested by *T. urticae* adult females after 45 days of seedling growth. After 15 days of infestation, the first group of plants was sprayed with 1 mL for each plant from a mixture of 100 mL water and two drops of Triton-X-100 as control. The other three groups were sprayed with the median lethal concentration value (LC_{50}) of *C. procera* leaf extract using a glass hand sprayer with one nozzle. The number of eggs, immatures, adult females, and adult males was counted one day before spraying and then again 1, 3, 7, and 14 days after spraying by collecting three randomly selected one-inch-square leaves from each set of plants.

Fractionation of the leaf extract by column chromatography

The separation of components of the crude extract was carried out by a glass column filled with silica gel 60–120 mesh (ADWIC Company) according to (Chua *et al*. 2016) with slight modifications.

Gas chromatography mass spectrometry analysis

Gas chromatography mass spectrometry analysis was carried out in food and environmental pollutant analysis laboratory at Fayoum University. Agilent GC model 7890B attached with mass spectrometer 5977A (GC-MS) and fitted with a HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m × 250 µm, film thickness 0.25 µm) was used to identify the crude extract components. One microliters of extract was injected in the GC-MS, split mode with split ratio 1:50 with injection temperature of 250 ℃ and carrier gas (helium 99.9999%) in flow rate 1 ml/min was used. The column oven starts at 45 ℃ for 1 min, then increased by 6 ℃/min to reach 280 ℃ and hold at the temperature degree for 2 min. The total temperature program was 42.17 min. The ion-source temperature of MS was 250 ℃ and the interface temperature, 300 ℃ (Cupido *et al*. 2022) and NIST chemical library (Version 14).

Statistical analysis

All mortality rate data were corrected using Abbott's formula (Abbott 1925) and toxicity line was plotted according to Finney analysis (Finney 1971) and Chi square test. Henderson and Tilton (1955)'s equation was used to calculate the reduction percentages in different mite stages and significant differences among means were compared using multiple test at 5% probability level in different treatments (Tukey 1949). Biological aspects data of *T. urticae* were analyzed using One-Way ANOVA (SPSS Version 21), where p-value is *p-value < 0.05, **p-value < 0.01. In addition, the correlations between female fecundity represented in number of eggs and egg hatchability percentage and mite generations (F0 and F1) were checked statistically according to the Pearson correlation coefficient with p-value < 0.01.

RESULTS

Acute toxicity of the crude extract of C. procera *leaves*

Data in Table 1 shows the slope and the half lethal concentration (LC_{50}) of *C. procera* leaves crude extract against *T. uricae* females according to Finney analysis, where the LC_{50} were 3608.73 and 2277.02 mg/L, after 24 and 48 hours post-treatment, respectively. The LC_{90} values were 8375.4 and 5558.65 mg/L, 24 and 48 hours post-treatment, respectively. Males showed more susceptibility for *C. procera* extract than females, where the LC_{50} values were 2456.98 and 1671.2 mg/L, 24 and 48hr post-treatment, respectively. The slope of the toxicity lines, which resulted from treated male's data are steeper than those from treated females. This indicates that the male mites are more sensitive to *C. procera* leaves extract.

Table 1. The LC_{50}, LC_{90} (mg/l) and slope values after 24 and 48 hours post-treatment of *Calotropis procera* leaves extract against *Tetranychus urticae* females and males.

T. urticae	Time (h)	LC_{50} (mg/l)	LC_{90} (mg/l)	Slope ±SE*	χ^2	df
Female	24	3608.73 CL: (2753.7–4416.3)	8375.4 CL: (6495.4–13630.7)	3.50 ± 0.7	72.87**	3
	48	2277.02 CL: (1752.84–2825.7)	5558.65 CL: (4171.64–9909.38)	3.3 ± 0.67	62.68**	3
Male	24	2456.98 CL:(1789.87–3373.16)	7790.28 CL: (5141.83–18450.15)	2.56 ± 0.53	49.09**	3
	48	1671.2 CL: (969.69–2289.61)	5838.33 CL: (4094.88–11954.64)	2.36 ± 0.53	46.92**	4

* SE = Standard Error, CL = Confidence Limits at 95%, values in parentheses represent lower and upper confidence Limits at 95%, χ^2 = Chi Square test, ** indicate signification Chi Square test at P-value ≤0.05, df = Degree of freedom

Sublethal effects of C. procera *leaf extract on the female fecundity (F0 and F1)*

Treated females by the LC_{50} value (2277.02 mg/L) after 48-hour exposure time lead to a high significant decrease of fecundity (18.20 eggs/female) compared to the control, which deposited 44.73 eggs/female (Table 2). In addition, the egg hatchability percentage was lower in the treatment (94.38%) than the control (99.47%).

Table 2. Sublethal effects of *Calotropis procera* leaf extract on *T. urticae* fecundity and egg hatchability% (Mean ± SE) in F0 and F1.

Generation	Treatments	Fecundity (No. eggs) Mean ± SE	Hatchability% Mean ± SE
F0	Treated females	18.20 ± 2.16	94.38 ± 1.38
	Control	44.73 ± 2.64	99.47 ± 0.3
	P–value	0.000**	0.001**
F1	Treated females	22.73 ± 1.6	93.84 ± 0.75
	Control	53.87 ± 2.9	98.59 ± 0.5
	P–value	0.000**	0.000**
Correlation	R	0.2	−0.089

**p-value < 0.01, correlation between two generations is significant at the 0.01 level (1-tailed).

In the generation F1, the fecundity of treated *T. urticae* female was significantly decreased. The number of eggs was 22.73 eggs compared with 53.87 in the control. Data revealed significant decrease in hatchability percentage, where it was recorded to be 93.84% compared with 98.59% in control.

Also, data analysis revealed that there was a positive correlation and no significant difference between the fecundity of F0 and F1, while the correlation between hatchability percentage of F0 and F1 was negative but not a significant difference.

Biological activity of C. procera *leaf extract on* T. urticae

Influence of *C. procera* leaf extract on developmental stages of *T. urticae* indicated that all periods were prolonged by the treatment except longevity that causes a reduction in the number of generation/years (Table 3). Treatment significantly affected incubation period, total immatures, and life cycle which were recorded 3.6, 6.0, and 9.6 days respectively, compared with 2.8, 4.53, and 7.33 days in the control, while female longevity and life span had no significant differences, recording 9.33 and 18.93 days respectively, compared with 10.67 and 17.93 days in the control. Data of oviposition period did not show any significant effect, where it was 7.2 days against 8.47 days in control.

Table 3. Sublethal effects of leaf extract on *Tetranychus urticae* eggs deposited by treated females (F1).

Treatment	Mean ± SE (day)					
	Incubation Period	Total Immatures	Life cycle	Longevity	Oviposition Period	Life span
2277.02 mg/l (LC_{50})	3.6 ± 0.13	6.0 ± 0.28	9.6 ± 0.31	9.33 ± 0.49	7.2 ± 0.46	18.93 ± 0.7
Untreated females	2.8 ± 0.11	4.53 ± 0.36	7.33 ± 0.39	10.67 ± 0.45	8.47 ± 0.5	17.93 ± 0.7
P-value	0.000**	0.003**	0.000**	0.054	0.07	0.31

**p-value < 0.01.

Reduction mite populations

Effect of LC_{50} on mite population was more potent in females than the other stages, as it gave an average reduction of 91.02%, compared with 77.87, 79.73, and 71.89% respectively for eggs, immatures, and males (Table 4). It was highly effective and caused the highest reduction percentage on eggs after three days of treatment (93.14%) with no significant differences after seven days (89.75%). Reduction percentage after one and 14 days (58.14 and 70.48%) were significantly different from the previous findings.

Table 4. Reduction populations' percentage of *Tetranychus urticae* stages after treatment by the median lethal concentration of *Calotropis procera* leaf extract on white eggplant under laboratory conditions.

***T. urticae* Stages**	**Populations (Mean ± SE)**				**Average ± SE**
	1 Day	**3 Days**	**7 Days**	**14 Days**	
Eggs	58.14 ± 4.04 b	93.14 ± 1.78 a	89.75 ± 2.22 a	70.48 ± 6.80 b	77.87 ± 4.67
Immatures	79.54 ± 4.55 a	78.93 ± 0.25 a	91.17 ± 0.86 a	78.26 ± 8.63 a	79.73 ± 3.14
Females	81.98 ± 1.38 b	94.29 ± 2.93 a	92.96 ± 1.10 a	94.84 ± 2.58 a	91.02 ± 1.83
Males	51.58 ± 11.75 b	72.44 ± 4.25 ab	63.54 ± 10.50 ab	100 ± 0.00 a	71.89 ± 6.41

Means have the same letters within the same column been not significantly different at $p < 0.05$ according to Tukey test.

For immatures, the highest reduction percentage was recorded after seven days of treatment (91.17%) with no significant differences with the other reduction percentages after 1, 3 and 14 days.

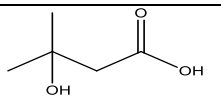
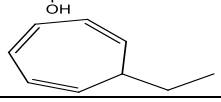
For adult females, the least reduction percentage was recorded after one day of treatment (81.98%), which significantly different from other reduction percentages recorded after 3, 7 and 14 days (94.29, 92.96, 94.84% respectively).

The highest reduction percentage for adult males was recorded after 14 days of treatment (100%) and was significantly different from reduction percentages recorded after 1 day (51.58%). Notably, this plant extract displayed considerable toxic effects on different stages of *T. urticae*.

Components of C. procera *leaf extract*

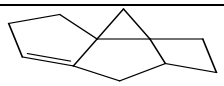
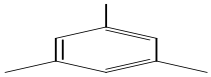
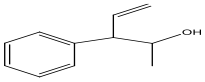
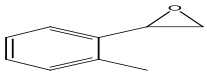
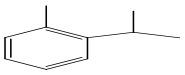
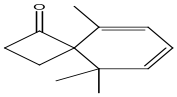
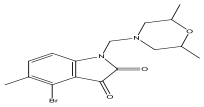
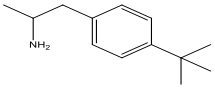
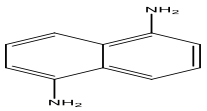
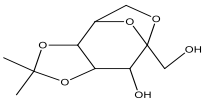
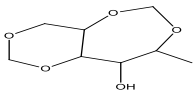
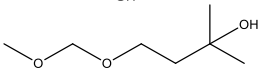
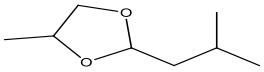
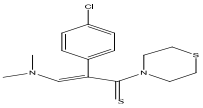
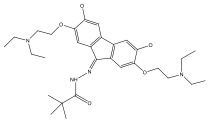
The crude extract of *C. procera* leaf was fractionated to 18 fractions and their bioactivity evaluated on *T. urticae*. Mortality was not noted in all fractions. In fractions 1 to 18, the mortality percentage ranged from 0 to 63 after 48 hours of treatment. After 72 hours, the mortality range for the same fractions was 10 to 73 (Fig. 1). The fractions that had the highest mortality percentage were F6 and F10. The fraction No. 6 (F6) exhibited 50% mortality after 48 and 72 hours of spraying, while fraction ten (F10) showed the highest mortality (67 and 73%) after 48 and 72 hours of spraying, respectively (Fig. 1). Therefore, the current research concentrates on fraction 10, as analyzed by GC-MS to identifying their components. The analytical data resulting from the GC-MS showed 17 different compounds separated from fraction 10 (Table 5, Fig. 2). All chemical compounds were obtained from the mixture of methanol/dichloromethane (60:40), of the leaves extract of *C. procera*.

Table 5. GC/MS profiling of different compounds separated from fraction 10 of *C. procera* extract.

Peak	**Compound Name**	**Retention time (R.F) (min.)**	**Molecular Weight (M.W.)**	***Structure Formula**
1	3-hydroxy-3-methyle butanoic acid	4.8	119.06	
2	7-ethyl-1,3,5-cycloheptariene	7.3	120	

* The chemical structures of the compounds were plotted using ChemDraw software.

Table 5. Continued.

Peak	Compound Name	Retention time (R.F) (min.)	Molecular Weight (M.W.)	*Structure Formula
3	2,3,4,5,6,7-hexahydro-3a,6-Methano-3aH-indene	8.6	134	
4	Mesitylene	8.9	120	
5	β-ethenyl-α-methyl-Benzeneethanol	9.3	162	
6	2-Tolyloxirane	9.7	134	
7	o-Cymene	9.8	134	
8	Spiro[3.5]nona-5,7-dien-1-one, 5,9,9-trimethyl	9.9	176	
9	1H-Indole-2,3-dione, 4-bromo-1-[(2,6-dimethyl-4-morpholinyl)methyl]-5-methyl	13	366	
10	4-tert-butylamphetamine	13.3	189	
11	1,5-Diaminonaphthalene	14.6	159.09	
12	3,4-O-Acetone sedoheptulosan	19.6	234	
13	1,3:2,5-Dimethylene-l-rhamnitol	19.7	190	
14	1-(Methoxymethoxy)-3-methyl-3-hydroxybutane	20.9	148	
15	iso-Valeraldehyde propyleneglycol acetal	25	143	
16	iso- 3-Dimethylamino-2-(4-chlorphenyl)-thioacrylic acid, thiomorpholide	32.7	326	
17	9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy] fluorene	36.7	576	

* The chemical structures of the compounds

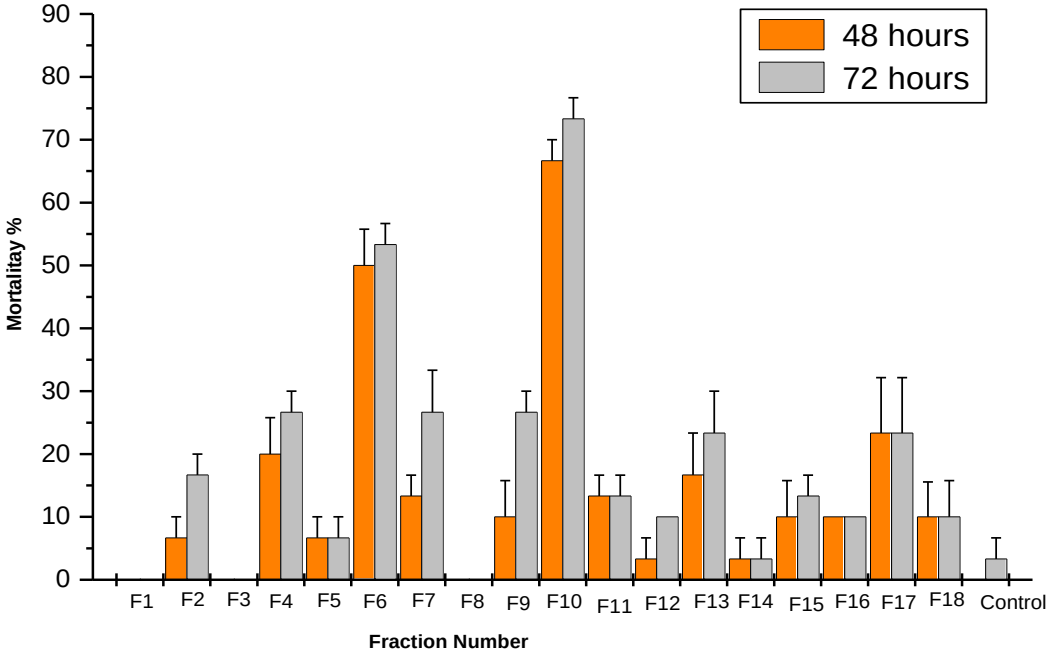


Figure 1. The mortality percent of the separated fractions (from 1 to 18) in *T. urticae* 48- and 72-hours post-treatment.

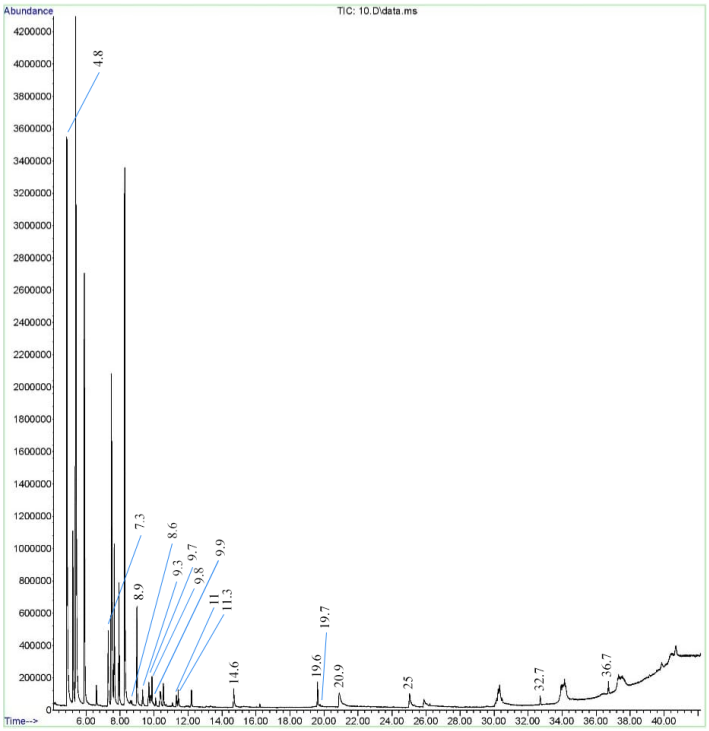


Figure 2. Gas chromatography (GC/MS) profiling of the chemical compounds separated from fraction ten of methanolic extract leaf *C. procera*.

DISCUSSION

The detection of novel bioactive compounds to control pests is currently needed as most pests have developed resistance to many synthetic pesticides. This research is an attempt to find new natural chemicals to be used as acaricides. We have demonstrated the toxicity of crude extract of *C. procera* leaves on *T. urticae*. In addition to possessing strong biological activity, the extract affected the reproductive ability of females and the rate of egg hatching from treated females at the mean lethal concentration. Interestingly, this extract has a prolonged effect, where it reduced fecundity of *T. urticae* females emerging from eggs deposited by treated females. In a similar report by (Emam and Ibrahim 2020), the toxicity of the *C. procera* seed extract against *T. urticae* was confirmed, where the mortality percentage was 93.33% after 72 hours at 0.01% concentration. Also, the female treatment with seeds and leaves extracts of *C. procera* reduced the percentage of hatchability for eggs. Likewise, other studies showed that the latex of *C. procera* causes 100% mortality of third-instar larvae of *A. aegypti* and also prevents egg hatching (Ramos *et al*. 2006).

Toxicity of plants may be due to effect of their different phytochemicals, including terpenoids, alkaloids, saponins, tannins, cardinolides, procerursenyl acetate, proceranol, and phytosterol (Verma *et al*. 2013; Waheed *et al*. 2016). *Calotropis procera* possesses insecticidal, nematicidal, and molluscidal properties (Al Sulaibi *et al*. 2020). Numerous previous publications referred to efficacy of *C. procera* against various pests such: *T. castaneum, A. stephensi*, *C. quinquefasciatus, A. aegypti*, and *R. microplus* (Singh *et al*. 2005; Elimam *et al*. 2009; Shahi *et al*. 2010; Abbas *et al*. 2012; Nithya *et al*. 2015).

The mechanism of *C. procera* against pest is still unknown, where further studies are warranted. Nevertheless, it has been reported that extracts of this plant can inhibit α-amylase and α-glucosidase enzymes (Kazeem *et al*. 2016). An alpha-amylase enzyme is used as the main source of energy for arthropod metabolic processes (Hubert *et al*. 2005). In fact, plant amylase inhibitors may be a useful potential strategy for pest management in the future.

In current study, several compounds have been separated from leaf methanolic extract of *C. procera* and identified using GC-MS. Previous studies revealed that some of these isolated compounds have proven biological activities. We isolated and identified o-Cymene from the leaves of *C. procera*. We hypothesize that they contribute to the toxicity of the crude extract against *T. uritcae*. There are two cymene isomers (p-Cymene and o-Cymene) with the same structure, but different in the position of methyl and isopropyl on benzene ring. In a related previous research by (Feng *et al*. 2021), it was demonstrated these two cymene isomers efficacy against *Liposcelis bostrychophila* Badonnel (Psocodea: Liposcelididae) and *T. castaneum* as insecticides and repellent. Additionally, they demonstrated that the o-Cymene is more toxic than m-cymene in fumigant and contact methods. In the same context, the p-cymene-8-ol and o-Cymene components have been reported by (You *et al*. 2015), where they were separated from *Clausena anisum-olens* (Blanco) (Rutaceae) by silica gel column chromatography. The p-cymene-8-ol demonstrated high contact toxicity and repellency against *Lasioderma serricorne* (Fabricius) (Coleoptera: Ptinidae) and *L. bostrychophila* adults.

Likewise, the chemical 2-Tolyloxirane was isolated from secondary metabolites of fungus *Trichoderma virens* (6011) (Hypocreales: Hypocreaceae) as a volatile organic compound. It was separated using n-butanol solvent by GC-MS. There is potential of using 2-tolyloxirane as a natural product in pest control (Tabarestani *et al*. 2016). Similar with this research, (Shilov *et al*. 2022) have documented antimicrobial activity of 3-hydroxy-3-methyle butanoic acid component as a monocarboxylic acid. This component was separated from roots extract of *Onosma gmelinii* L. (Boraginales: Boraginaceae) by chromatography/mass spectrometry (GC-MS). There are new chemical compounds in this study which are not recorded in any previous studies including β-ethenyl-α-methyl-Benzeneethanol, 1H-Indole-2,3-dione, 4-bromo-1-[(2,6-dimethyl-4-morpholinyl) methyl]-5-methyl, iso-Valeraldehyde propyleneglycol acetal and iso-3-Dimethylamino-2-(4-chlorphenyl)-

thioacrylic acid, thiomorpholide. These components separated from the leaf extract of *C. procera* can be used to develop formulations as novel natural pesticides for pest control.

CONCLUSION

The current study documented the acaricidal potential of *C. percora* in toxicity experiments. This plant is widespread in Egypt as well as all over the year. It suppresses the population of *T. urticae* through its effect on different biological aspects. We can conclude that, the extract of *C. percora* had a potent toxic activity against *T. uritcae* and affected females' ability for reproduction. Additionally, the extract was having a prolonged effect on *T. uritcae* female life cycle. The fractions which exhibited toxicity against *T. uritcae* can be subjected to purification process to get pure effective substance. The pure isolated compound can be used to create formulations, which could be utilized in the integrated management strategy for *T. uritcae*. On the other hand, more research is required to ensure that this extract has no negative effects on human health and the environment.

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اثربخشی عصارهٔ برگ *Calotropis procera* (Apocynaceae) و اجزای آن علیه *Tetranychus urticae* (Acari: Tetranychidae)

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چکیده

در این مطالعه، سمیت عصاره خام برگ گیاه *Calotropis procera* (Aiton) (Apocynaceae) علیه *Tetranychus urticae* Koch (Acari: Tetranychidae) در شرایط آزمایشگاهی با استفاده از روش پاشش مورد بررسی قرار گرفت. مقادیر LC50 پس از ٢٤ ساعت تیمار به ترتیب ٣٦٠٨/٧٣ و ٢٢٧٧/٠٢ میلی‌گرم در لیتر به ترتیب برای کنه‌های ماده و نر محاسبه شد. این مقادیر پس از ٤٨ ساعت تیمار ٢٤٥٦/٩٨ و ١٦٧١/٢ میلی‌گرم در لیتر بود. نتایج نشان داد که دوره‌های تفریخ، نابالغ کل و دورهٔ زندگی *T. urticae* تحت تأثیر عصاره *C. procera* طولانی شد. درصد باروری و تفریخ به‌ترتیب کمتر (١٨/٢ تخم به ازای هر ماده و ٩٤/٣٨ درصد) نسبت به شاهد (٤٤/٧٣ تخم به ازای هر ماده و ٩٩/٤٧ درصد) ثبت شد. میزان کاهش تعداد *T. urticae* در مراحل مختلف ١، ٣، ٧ و ١٤ روز پس از پاشش نشان داد که *C. procera* تأثیر مثبتی بر کاهش جمعیت کنه داشته است به طوری که ١٤ روز پس از تیمار به ترتیب برای ماده‌ها و نرها ٩٤/٨٤ و ١٠٠ درصد را ثبت کرد. عصاره متانولی برگ *C. procera* به ١٨ جزء جدا شد. جزء دهم به ترتیب ٦٧ و ٧٣ درصد مرگ و میر را ٤٨ و ٧٢ ساعت پس از تیمار نشان داد. هفده ماده در این بخش با استفاده از GC-MS شناسایی شد. این یافته‌ها نشان می‌دهد که عصاره برگ *C. procera* که حاوی ترکیبات جدید مختلف است، پتانسیل استفاده برای کنترل *T. urticae* در آینده را دارد.

واژگان کلیدی: استبرق، کنه‌کش گیاهی، تجزیه و تحلیل GC-MS، شناسایی اجزاء، کنهٔ تارتن دو لکه‌ای.

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