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Exploring The Biological Effects of some Phytochemicals from *Zygophyllum simplex* L. and *Trigonella foenum-graecum* L. on Desert Locust *Schistocerca gregaria* (Forskal)

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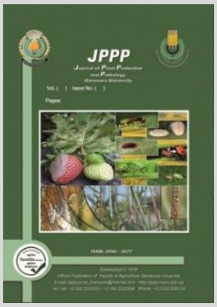

ABSTRACT

Effects of n-hexane, acetone and methanol extracts of *Zygophyllum simplex* L. and *Trigonella foenum-graecum* L. on *Schistocerca gregaria* (Forskal) 5th nymphal instar were tested. Only n-hexane extracts of both plants showed that, biological toxic effects to *S. gregaria* nymphs. Mortalities percentages of 1000 ppm of *Z. simplex* and *T. foenum-graecum* n-hexane extracts to desert locust nymphs were 76.67 and 70.00 % after 8 days post treatment. Toxicity results indicated that, LD_{50}s of *Z. simplex* and *T. foenum-graecum* extracts were 2.04 and 3.16 µg/nymph, respectively. Also total carbohydrates, lipids, and proteins in haemolymph of treated nymphs with 250 ppm, were reduced at days 2, 4, and 6 days post treatment. Thirty four and thirty eight phytochemical compounds were detected in n-hexane extracts of *Z. simplex* and *T. foenum-graecum*, respectively. Seventeen compounds were present in both plant extract.

Keywords: Plant extract, N-hexane, acetone, methanol, IGR, biopesticides, safe control agent.

INTRODUCTION

Locust and grasshoppers has the ability to increase it is population rapidly when the appropriate environmental factors are available, subsequently led to outbreaks in wide area, on other hand synthetic pesticides yet is the major tool for locust and grasshoppers control, while the heavy use of pesticides in locust control rise the concern of the environmental attentions (Lomer *et, al.*, 2000). The main aim of control operations is to prevent locust and grasshoppers outbreaks such tactic relay on using synthetic pesticides, therefore plant extract may provide excellent solution for locust and grasshoppers control, also safe to the environment (Meinzingen 1997). Plant Kingdome contain huge source of chemicals, many of these chemicals have biological effects against insects and pathogens (Isman and Akhtar 2007 and Walia *et, al.*, 2014). Phytochemical biopesticides considered as safe control agents when compared with conventional insecticides, (Walia *et, al.*, 2017). *Zygophyllum simplex* L. is widespread wiled plant in desert locust *Schistocerca gregaria* breading area, usually used as shelter for *S. gregaria* nymphs (Bashir *et, al.*, 2000), while forcing *S. gregaria* nymphs to feed on *Z. simplex* Caused high mortality (Waloff, 1963), and affect it's maturation, development, and fecundity (Jackson *et, al.*, 1978). Fenugreek *Trigonella foenum-graecum* L., one of the most widely used medicinal plants in Africa, Asia and Europe, a lot of research showed its pharmaceutical uses as: antidiabetic, antihyperlipidemic, antiobesity, anticancer, anti-inflammatory, and antioxidant (Venkata *et, al.*, 2017), in spite of that, laboratory observation indicated that fenugreek was not preferred as food for locust and grasshoppers (unpublished data). Therefore the present study investigated the chance of that *Z. simplex* and *T. foenum-graecum* may contain toxic phytochemicals to desert locust *S. gregaria* and possible identification of such chemicals.

MATERIALS AND METHODS

Tested insects

Desert locust *S. gregaria* 5th nymphal instar 1-2 days after molting were obtained from stock colony of Locust and Grasshopper Research Department (LGRD), PPRI, ARC, Egypt. These colony maintained for several generations under crowded conditions according to Hunter-Jones (1961) and fortified with insects collected from field whenever it possible.

Plant extraction

Zygophyllum simplex L. were collected from south eastern desert of Egypt through normal survey investigations of desert locust, while Fenugreek *T. foenum-graecum* were planted at LGRD, then collected at the flowering time. The aerial parts of both plants were washed with water then left to dray at room temperature, the dried plants (leaves and stems) were grounded. Three solvents with increasing polarity were used in extraction of phytochemicals. 200 grams of the powder were soaked in n- hexane for 2days with occasional shaking. Then filtered, the filtrate was evaporated to dryness, the powder were weighted, then kept in refrigerator tell further use. The residues were extracted in the same procedure with acetone then methanol.

Phytochemicals toxicity testing

A preliminary trial were conducted to test which extract has a potential bioactive compound against *S. gregaria*, 1000 ppm concentration of each solvent for both tested plants were prepared,10 µl of each concentration

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were placed on 7mm diameter disk of lettuce. One treated disk were introduced for one 24 hrs. starved 5th nymphal instar of *S. gregaria* individually, then allowed to consume the entire disk for 2 hrs. 30 treated nymphs with each solvent of each plant were divided into 3 replicates, and kept together in small plastic cage under same rearing conditions, feed with fresh food. Mortalities and any morphological changes were observed daily and fresh food were introduced. Serial concentrations (500, 250 and 125 ppm) of each n-hexane extracted powder in n-hexane were prepared, thirty nymphs were treated with each concentration as described previously. Thirty untreated nymphs were separated in 3 replicates used as control treatment.

Chemical composition of *S. gregaria* haemolymph

Thirty 5th nymphal instar of *S. gregaria* were treated as previously mentioned with 250 ppm of n-hexane extract of both tested plants *Z. simplex* and *T. foenum-graecum*, 0.5 ml of haemolymph from treated nymphs were withdrawn at days 2, 4, 6 post treatment in small tubes contain traces of phenyl thiouria, the tubes containing the haemolymph were then kept in deep freezing till further determination of total protein, total carbohydrates and total lipids as following : A known volume of the collected haemolymph (0.1ml) was diluted up to 2 ml with saline solution and purified by centrifugation to remove blood cells and pigments. Then the filtrate was collected for haemolymph analysis. Protein content was determined by Biuret reagent according to the method described by Gornall *et al*., (1949)**.** Total carbohydrates were estimated by the method of Trinder (1969). Total lipids were estimated by modified method of Knight *et al*, (1972).

Bio-active phytochemicals identification

One hundred grams of both *Z. simplex* and *T. foenum-graecum* powder were extracted with N-hexane as previous, then dried extract were redissolved in 10 ml of N-hexane. The chemical composition of these samples were performed using Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50°C and then increased by 5°C /min to 250 °C hold for 2 min. increased to the final temperature 300°C by 30°C /min and hold for 2 min. The injector and MS transfer line temperatures were kept at 270, 260°C respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 4 min and diluted samples of 1 µl were injected automatically using Auto sampler AS1300 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 50–650 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 14 mass spectral database.

Statistical analysis

Mortality were corrected according to Schneider-Orelli's formula (Püntener, 1981), while dose mortality responses were calculated using Ldp Line software (http://www.ehabsoft.com/ldpline) according to Finney (1971).

RESULTS AND DISCUSSION

The preliminary trial indicated that, n-hexane extract of both plants showed the most bioactivity against desert locust 5th nymphal instar. Percentages of mortalities by the 8th day after treatment represented by Figure (1), the mortality of Z. *simplex* n-hexane extract was 76.67% and for *T. foenum-graecum* n-hexane extract was 70.00, the great majority of died nymphs resulted from n-hexane extracts of both plants were due to molting failure Figure (2). That finding suggested that n-hexane extracts might contain chitin synthesis inhibitors.

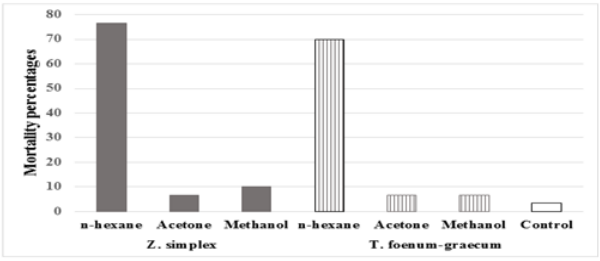

Fig. 1. Mortality percentages of *Schistocerca gregaria* 5th nymphal instar after 8 days post treatment with 1000 ppm of tested extracts and control.

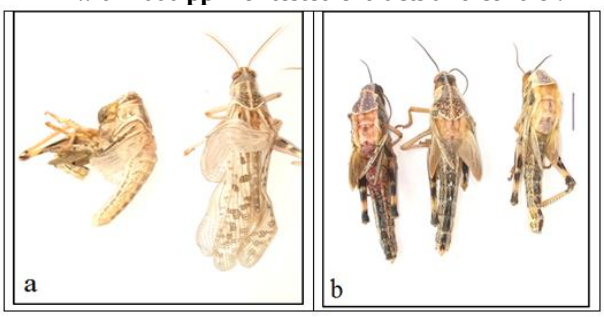

Fig. 2. Molting failure of *Schistocerca gregaria* 5th nymphal instar after treatment with 1000 ppm of n-hexane extracts of *Trigonella foenum-graecum* (a) and *Zygophyllum simplex* (b). Bar = 1cm.

Figure (3) and Table (1) illustrated the toxicities of n-hexane extracts of *Z. simplex* and *T. foenum-graecum* against *S. gregaria* 5th nymphal instar, it is clear that, *Z. simplex* was slightly toxic than *T. foenum-graecum*, where LD_{50} values were 2.04 and 3.16, LD_{25} values were 0.45 and 0.58 and LD_{90} values were 35.63 and 80.39 µg/nymph.

Figures 4, 5, and 6 demonstrate that, n-hexane extract of tested plants reduced the total carbohydrates, lipids and proteins levels in the haemolymph of treated 5th nymphal instar of *S. gregaria*, these reduction effects were increased by the time post treatment increase. The level of total carbohydrates in untreated *S. gregaria* nymphs at days 2, 4, and 6 post treatment were 4.33, 4.29, and 4.37 while in *Z. simplex* treated nymphs were 3.65, 2.47 and 2.45 and in *T. foenum-graecum* treated nymphs were 3.45, 2.56, and 2.35 g/100 ml respectively. Furthermore total lipids levels at same periods were 3.97, 3.89, and 3.85 in untreated nymphs, 3.45, 2.34, and 1.86 in *Z. simplex* treated nymphs and 3.22, 1.99, and 1.66 g/100 ml respectively. Total proteins were 2.34, 2.44, and 2.5, in untreated nymphs 2.13, 4.85, and 1.23, in treated nymphs

with *Z. simplex* and in treated nymphs with *T. foenum-graecum* were 2.06, 1.73, and 1.12 g/100 ml respectively.

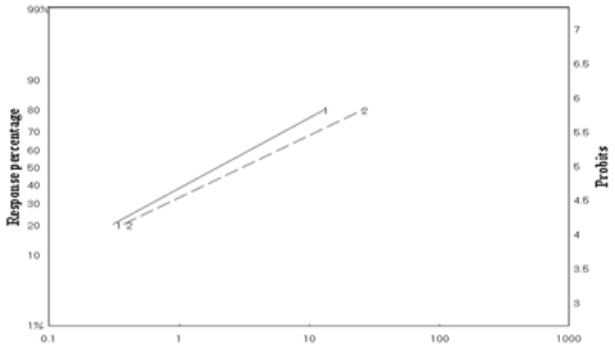

Fig. 3. Dose mortality response of *Schistocerca gregaria* treated 5th nymphal instar with n-hexane extract of *Zygophyllum simplex* (1) and *Trigonella foenum-graecum* (2), after 8 days post treatment.

Table 1. Toxic effects of n-hexane extract of *Zygophyllum simplex* and *Trigonella foenum-graecum* on *Schistocerca gregaria* 5th nymphal instar.

Tasted plants	LD_{25} *	LD_{50} *	LD_{90} *	Slope
Zygophyllum simplex	0.45	2.04 ns	35.63	1.032
Trigonella foenum-graecum	0.58	3.16 ns	80.39	0.912

*** Microgram plant extract per nymph.**
ns= there were no significant differences between tested extracts based on LD_{50}.

Data presented in Table (2) revealed that, phytochemicals component of n-hexane extract of *Z. simplex* and *T. foenum-graecum* arranged according to their percentages of occurrence, 34 chemical compounds were identified by GC/MS analysis in *Z. simplex* extract and 38 chemical compounds in *T. foenum-graecum* extract. There were 17 chemical compounds present in both plant extract, these compounds illustrated in Table (3) and arranged according to their occurrence in *Z. simplex*.

Table 2. phytochemicals components in n-hexane extract of *Zygophyllum simplex* and *Trigonella foenum-graecum*

No.	*Zygophyllum simplex*	Area %	*Trigonella foenum-graecum*	Area %
1	CELIDONIOL, DEOXY-	49.35	Oleic Acid	16.27
2	Heptacosane	11.06	Carbonic acid, eicosyl vinyl ester	13.25
3	Oleic Acid	8.03	Phytol	12.53
4	PENTATRIACONTANE	5.12	2-Pentadecanone, 6,10,14-trimethyl-	5.01
5	Diisooctyl phthalate	3.7	1-DODECANAMINE,N,N-DIMETHYL	4.25
6	n-Hexadecanoic acid	2.44	1,3,5-TRIAZINE-2,4-DIAMINE,6-CHLORO-N-ETHYL	3.61
7	Dodecane,1-cyclopentyl-4-(3-cyclopentylpropyl)-	1.52	HEXADECANOIC ACID	3.4
8	Glycidyl oleate	1.42	9-EICOSYNE	3.36
9	1,2-Benzenedicarboxylic acid,bis(8-methylnonyl) ester	1.29	Heptacosane	2.74
10	HENEICOSANE	1.16	á-Sitosterol	2.48
11	1-DODECANAMINE,N,N-DIMETHYL	1.05	Diisooctyl phthalate	2.43
12	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	1.01	Glycidyl oleate	2.33
13	2-Pentadecanone, 6,10,14-trimethyl-	1.01	DOCOSANE	2.21
14	2,2-DIDEUTERO OCTADECANAL	0.94	9-OCTADECENOIC ACID (Z)-	1.83
5	9,12-Octadecadienoyl chloride,(Z,Z)-	0.94	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1.77
16	9-OCTADECENOIC ACID	0.79	9,12-Octadecadienoyl chloride,(Z,Z)-	1.71
17	DOTRIACONTANE	0.79	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	1.44
18	2-HEXADECEN-1-OL,3,7,11,15-TETRAMETHYL-,[R-[R*,R*-(E)]]-	0.74	1-TETRADECANOL	1.3
19	ISOCHIAPIN B	0.71	Hexacosylamine, N,N-dimethyl-	1.22
20	Tetradecane	0.71	Neophytadiene	1.21
21	NONADECANE	0.68	Dibutyl phthalate	0.8
22	5-METHYL-1,5-HEXANEDIOL	0.57	1-CHLOROOCTADECANE	0.76
23	1-CHLOROOCTADECANE	0.53	4,6,6-TRIMETHYL-2-(3-METHYLBUTA-1,3-DIENYL)-3-OXA-TRICYCLO[5.1.0.0 2,4]OCTANE	0.75
24	3-BUTEN-2-OL,4-(2,6,6-TRIMETHYL-1-CYCLOHEXEN-1-YL)-	0.52	HEXADECANOIC ACID, METHYLESTER	0.66
25	9-OCTADECENOIC ACID (Z)-	0.49	ETHANOL,2-(9-OCTADECENYLOXY)-, (Z)-	0.66
26	Cholestan-3-ol, 2-methylene-,(3á,5à)-	0.49	9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1-(hydroxymethyl)ethylester	0.66
27	4,8,12,16-Tetramethylheptadecan-4-olide	0.45	3-BUTEN-2-OL,4-(2,6,6-TRIMETHYL-1-CYCLOHEXEN-1-YL)-	0.65
28	Undecanal, 2-methyl-	0.43	1-Heptatriacotanol	0.63
29	Dibutyl phthalate	0.42	ETHYL(9Z,12Z)-9,12-OCTADECADIENOATE #	0.58
30	9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1-(hydroxymethyl)ethyleste	0.39	ISOCHIAPIN B	0.57
31	Hexadecanoic acid, methyl ester	0.32	Cholestan-3-ol, 2-methylene-,(3á,5à)-	0.54
32	Tetradecane, 2,6,10-trimethyl-	0.32	Hexadecanoic acid, ethyl ester	0.52
33	(9E,12E)-9,12-OCTADECADIENOYL CHLORIDE #	0.3	7-Methyl-Z-tetradecen-1-ol acetate	0.5
34	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.3	2,2,3,3,4,4 HEXADEUTEROOCTADECANAL	0.4
35			Nonadecane	0.37
36			DOTRIACONTANE	0.37
37			(3R*,4S*)-3-(2-NITRO-4-METHOXYPHENYL)-4-(4-HYDROXYPHENYL)HEXANE	0.32
38			Benzene, (1-methyldodecyl)-	0.31

Table 3. Joint phytochemicals components in n-hexane extract of *Zygophyllum simplex* and *Trigonella foenum-graecum*

No.		Z %	T %
1	Heptacosane	11.06	2.74
2	Oleic Acid	8.03	16.27
3	Glycidyl oleate	1.42	2.33
4	1-DODECANAMINE,N,N-DIMETHYL	1.05	4.25
5	2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	1.01	1.44
6	2-Pentadecanone, 6,10,14-trimethyl-	1.01	5.01
7	9,12-Octadecadienoyl chloride,(Z,Z)-	0.94	1.71
8	DOTRIACONTANE	0.79	0.37
9	ISOCHIAPIN B	0.71	0.57
10	Nonadecane	0.68	0.37
11	1-CHLOROOCTADECANE	0.53	0.76
12	3-BUTEN-2-OL,4-(2,6,6-TRIMETHYL-1-CYCLOHEXEN-1-YL)-	0.52	0.65
13	9-OCTADECENOIC ACID (Z)-	0.49	1.83
14	Dibutyl phthalate	0.42	0.8
5	9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1-(hydroxymethyl)ethylester	0.39	0.66
16	Hexadecanoic acid, ethyl ester	0.32	0.52
17	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.3	1.77

Z= Zygophyllum simplex* and T= *Trigonella foenum-graecum

Plants developed through it is historic battle against insects many phytotoxic compounds, such phytochemicals act as insecticides, repellants, antifeedants or insect growth regulator (Saxena 1982 and Dimetry 2014), in the present study tested plant extract exhibited IGR mode of action (slow acting and molting failure). Molting process involve juvenile hormone and ecdysone, as well a number of relevant peptide hormones (Palli and Cusson 2007), therefore n-hexane extract may contains one or more phytochemicals that interfere with the complex molting process. Also such compounds may inhibit the synthesis of haemolymph carbohydrates, lipids, and proteins due to hormonal imbalance.

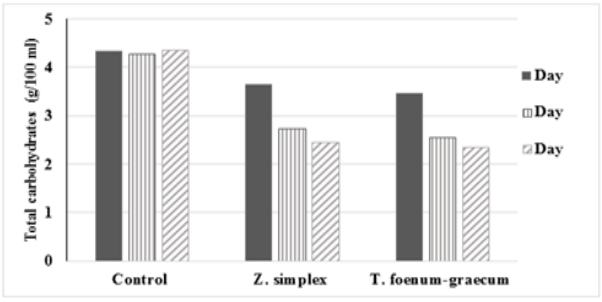

Fig. 4. Effect of n-hexane extracts on total carbohydrates contents of treated *Schistocerca gregaria* 5th nymphal instar at days 2, 4, and 6 post treatments.

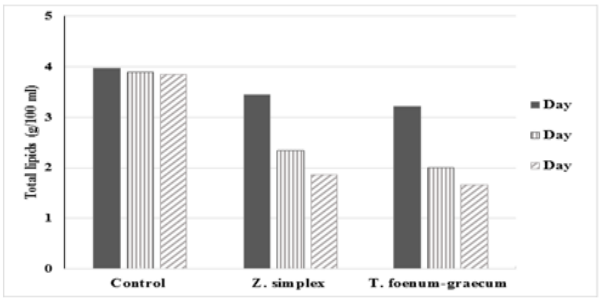

Fig. 5. Effect of n-hexane extracts on total lipids contents of treated *Schistocerca gregaria* 5th nymphal instar at days 2, 4, and 6 post treatment.

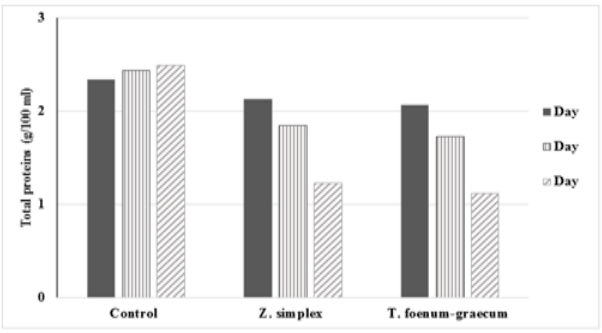

Fig. 6. Effect of n-hexane extracts on total proteins contents of treated *Schistocerca gregaria* 5th nymphal instar at days 2, 4, and 6 post treatment.

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استكشاف الآثار البيولوجية لبعض المواد الكيميائية النباتية من القرمل والحلبة على الجراد الصحراوي

جمال محمد محمود عبداللطيف و جيهان على محمد

قسم بحوث الجراد و النطاط - معهد بحوث وقاية النباتات – مركز البحوث الزراعية – مصر

تم دراسة تأثير مستخلصات ان-هكسان و الاسيتون و الميثانول لنباتى .L *Zygophyllum simplex* (القرمل) و *Trigonella foenum-graecum* L (الحلبه) على حوريات العمر الخامس للجراد الصحراوى. مستخلص ال ان-هكسان كان المستخلص الوحيد الذى كان له تأثيرات سامه بيولوجيه على حوريات العمر الخامس للجراد الصحراوى. و بلغت نسبة الموت نتيجه لمعاملة حوريات العمر الخامس للجراد الصحراوى بهذا المستخلص لنباتى القرمل و الحلبة بتركيز 1000 جزء فى المليون 76.6 و 70.00 % على التوالى. نتائج السمية اظهرت ان الجرعه القاتله لنصف الأفراد من تلك المستخلصات كانت 2.04 و 3.16 ميكرو جرام لكل حورية. ايضا محتوى الدم من الكربوهيدرات و اللبيدات و البروتينات الكليه انخفضت نتيجه لمعامله الحوريات بتركيز 250 جزء فى المليون من تلك المستخلصات . وقد تم تعريف 34 و 38 مركب من مستخلصات القرمل والحلبه على التوالى من هذة المركبات كان هناك 17 مركب موجود فى مستخلص النباتين.