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Effect of *Moringa oleifera* Seeds Extract on *Tetranychus urticae* (Koch)

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Abstract: Extraction of *M. oleifera* seed using methanol and petroleum ether solvent has an effect on *T. urticae*, Results showed that polar solvent more effective than non-polar where LC_{50} after 5 days of treatment was 0.95 ppm at methanol solvent, where it was 1.74 ppm at petroleum ether solvent after 5 days of treatment. At different values of pH, the results showed that LC_{50} was 7.37 ppm at pH (4), 5.19 ppm at pH (7) while it was 3.19 ppm at pH (9). There are different compounds in seeds of Moringa which have effect on *T. urticae*, the most component on seeds was Oleic acids, Hexadeconic acid, and Octadeconic acid. The chemical constitute of *M. oleifera* with methanolic extract at different pH has a different compounds which responsible for the effect on mites like oleic acid, hexadecanoic acid, and octadecenoic acid.

Keywords: *Tetranychus urticae*, *Moringa oleifera*, pH, GC-MS

INTRODUCTION

The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch is one of the most important pest, responsible for yielding losses to many horticultural, ornamental and agronomic crops. *T. urticae* is a polyphagous which feeding on more than 600 plant species, commonly found on many horticultural and agricultural crops, which can increase daily up to 40% and damaged epidermal cells on the lower leaf surface of strawberry (*Campbell et al.,* 1990; Bolland *et al.,* 1998; Devine *et al.,* 2001). It is polyphagous and attacks the broad range of crops, including soybean, cowpea, and common bean. The mites are a serious pest because they have several generations per season, phytophagous nature, and high reproductive potential, short life cycle and contributed rapid resistance development to many acaricides even after few applications. Also *T. urticae* is the most important agricultural pest, not only because of the damage that it causes, but also because it has a wide host range, infesting many commercial crops such as leafy greens, cotton, green beans, and soybeans (Khanjani, 2005; Van Leeuwen *et al*., 2005; Raworth *et al*., 2002; Gallo *et al.,* 2002). The egg of *T. urticae* is translucent, which hatches into a larva, and two nymph stages follow: a protonymph, and then a deutonymph, which may display quiescent stages. The adults are typically pale green for most of the year, but later generations are red (Krantz and Walter, 2009; Saeidi *et al*., 2010). *T. urticae* causes direct damage in terms of loss of chlorophyll, stunting of growth, stippling, webbing, leaf yellowing, defoliation, leaf burning, reduction in size and quality of fruits, appearance of various types of plant deformities, followed by death, which severely affect the yield and in extreme outbreaks, plant death. Indirect damage may include decreased photosynthesis and transpiration. Due to high reproductive potential and extremely short life cycle, combined with frequent acaricides applications, this mite has developed resistance to almost all conventional pesticides. *T. urticae* causes significant yield losses in many economically important crops, which belong to the subphylum Chelicerata which comprises the second largest group of animals on this planet next to insects. The small size of mites suggests that the number of described mite species is even more underestimated than that of insect species (Ozsisli and Cobanoglu, 2011; Jafari *et al.,* 2012; Grbic *et al*., 2011; Tehri, 2014).

The chemical control is one of the most methods which used in control *Tetranychus urtica*, although these synthetic insecticides are successful in agriculture, this intensive can cause problems such as the resurgence of the target pest, the appearance of new pests and the selection of populations resistant to the active principle or the mechanism of action (Mercês *et al*., 2018). Plant extraction plays an important role in defense mechanisms against the attack of microorganisms which have been demonstrated to inhibit the growth of several phytopathogenic and nonpathogenic fungi (Ang *et al*., 2014).

The seeds of *Moringa oleifera*, have been widely used as a coagulant in terms of the powder extract, and its active components are soluble cationic proteins and peptides with molecular weight ranging from 6 to 16 KDa (Muyibi and Evison, 1995a; Ndabigengesere, *et al*., 1995).

Also *M. oleifera* is one of the most wide spread plant species that grows quickly at low altitudes in the whole tropical belt. It can grow on medium soils having relatively low humidity; its seeds are an organic natural polymer (Ndabigengesere and Narasiah, 1998). Seeds of *M. oleifera* contain coagulant proteins, and are used for water turbidity removal in northeastern Brazilian regions of difficult access to potable water (Gassenschmidt *et al.,* 1995).

Lectins are proteins of nonimmune origin which bind specifically and reversibly to carbohydrates (Peumans and Van Damme, 1995).

M. oleifera seeds contain bioactive molecules including lectins, proteins of non-immune origin that possess carbohydrate binding sites able to interact reversibly and specifically with sugars through hydrogen bonding, hydrophobic interactions and Van der Waals forces. These proteins also known for their ability to agglutinate erythrocytes (Kennedy *et al*., 1995; Weis and Drickamer, 1996).

The aim of this study was to evaluate the potential of *M. oleifera* seeds extract at *T. urticae*.

MATERIALS AND METHODS

1. *Tetranychus urticae* rearing:

For establishing a colony of *T. urticae* Koch in the laboratory, the technique of (Guirguis *et al.,* 1977) was followed. The individuals of the mite were collected from infested leaves of castor trees grown on the farm of Ismailia Agricultural Research Station. The colony was kept in cheese cloth cage 60 × 60 × 60 cm under laboratory conditions, 25±2ºC, 65±5 relative and 12 hrs daily illuminations by using fluorescent tubes of 40-60 watt. The colony was kept away from any contamination for six months.

2. Moringa oleifera:

Plant collection

Moringa seeds were collected from a farm in Agricultural researcher station in Ismailia. The seeds were sun-dried to reduce the moisture content. Once the seeds were properly dried, the seeds were broken to get rid of hard shell. The obtained kernels were well grinded until it be fine powder. The solvent (Methanol, Petroleum ether) were used.

Preparation of Samples

The seeds were dehusked and dried through the same process, using an electric blender and both were stored in 40°C temperature in refrigerator in well labeled air tight containers for analysis. About 300 ml of distilled methanol, ethyl acetate and water each of the solvent was measured and added to the 100g of the blended mixture of husk and seeds first, also with seed only in a stoppered glass container. The mixture was left for three days for extracting and then finally filtered. The methanol, ethyl acetate and aqueous extracts for dehusked seeds and whole seed were concentrated on the water bath and stored for subsequent.

The plant extract at different pH level of polar solvent

There pH levels of the obtained extracts were modified (acidic - alkaline - neutral) using pH meter. To modified pH of plant extracts, the following steps were followed: Stock solution A: 0.2 M monobasic sodium phosphate monohydrate (27.6 g/L). Stock solution B: 0.2 M dibasic sodium phosphate (28.4 g/L). Mixing an appropriate volume (ml) of A and B.

As shown in the Table below and diluting to a total volume of 200 ml, to make a 0.1 M phosphate buffer of the required pH at room temperature. To make a 1 M phosphate buffer starting with 2 M of A and B stock, (Sambrook and Russell, 2001).

pH	0.2 M monobasic sodium phosphate	0.2 M dibasic sodium phosphate
4.5	94.0	6.0
6.5	68.5	31.5
7.0	39.0	61.0
8.5	6.0	94

Identification of natural compound by using Gas chromatography–mass spectrometry (GC-MS) analysis

The chemical composition of samples were performed using Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m × 0.25 mm × 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.

RESULTS AND DISCUSSION

Effect of methanol extract

Data in Table (1) showed that the values of LC_{25} were 0.97, 0.49 and 0.33 ppm after 3, 4, and 5 days. Depending on the value of LC_{50}, it was observed that after five days of treatment was the most potential one with value 0.95 ppm. According the slope value, the steepest one is after 5 days with high value 1.50 ppm and the flattest one is after 4 days with lowest value 1.24 ppm.

Effect of petroleum ether extract of moringa seeds on *T. urticae*

Data in Table (2) showed that Value of LC_{25} was 0.49 ppm after five days of treatment and 2.09 ppm after three days of treatment. Data showed that the values of LC_{50} were 5.71, 4.74, and 1.74 ppm after three, four, and five days respectively. While the value of LC_{90} were 38.63, 212.2, and 18.68 ppm after 3, 4, and 5 days of treatment respectively. According to slope value the steepest one is after three days of (1.54 ppm) treatment, and the most flat one is after four days (0.77 ppm).

pH value for the most efficacy natural additives on *T. urticae*

Data in Table (3) showed that acid pH (6.5), values are 2.30, 5.01, and 22.0 ppm at LC_{25}, LC_{50}, and LC_{90} respectively. At pH (5.8) values of LC_{25}, LC_{50}, and LC_{90} were 2.79, 7.37 and 46.57 ppm respectively. At neutral pH we found that the slope value was 1.99 ppm and the LC_{25} was 1.60 ppm, LC_{50} was 5.19 ppm and LC_{90} was 48.15ppm.

In other side of alkaline pH, it observed that the value of LC_{25} is 1.48 ppm, LC_{50} is 3.69 ppm, and LC_{90} is 19.97 ppm and the slope value is 1.73 ppm at pH (7.4). At pH (8.5) results recorded that values were 1.52, 3.19, and 12.97 ppm for LC_{25}, LC_{50}, and LC_{90} respectively. Slope record 2.10 ppm.

Table (1): Effect of methanol extract of moringa seeds on *T. urticae*

Time (days)	Toxicity			Slope
	LC_{25}	LC_{50}	LC_{90}	
3	0.97	3.11	8.49	1.33
4	0.49	1.74	18.68	1.24
5	0.33	0.95	6.76	1.50

Table (2): Effect of petroleum ether extract of moringa seeds on *T. urticae*

Time (days)	Toxicity			
	LC_{25}	LC_{50}	LC_{90}	Slope
3	2.09	5.71	38.63	1.54
4	0.64	4.74	212.2	0.77
5	0.49	1.74	18.68	1.24

Table (3): Effect of distillated water extract with different value of ph on *T. urticae*

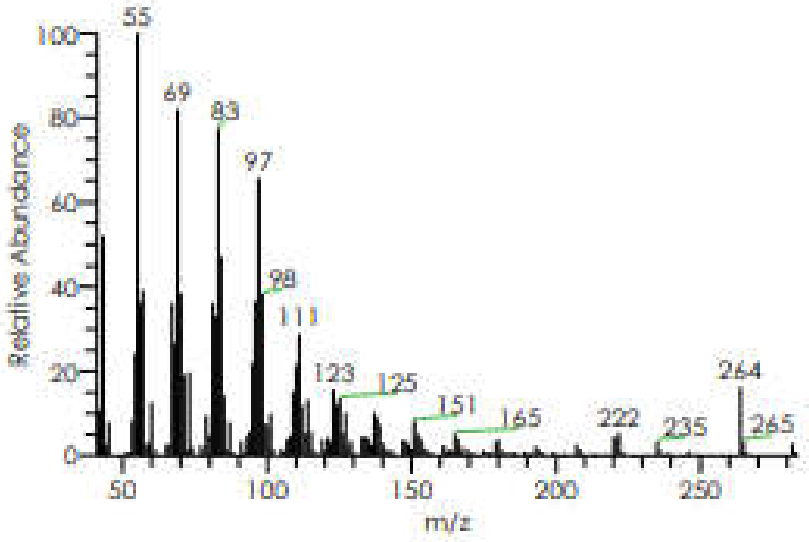
pH	*Toxicity (ppm)*			
	LC_{25}	LC_{50}	LC_{90}	Slope
4.5	2.79	7.37	46.57	1.60
6.5	2.30	5.01	22.0	1.99
7	1.60	5.19	48.15	1.32
7.4	1.48	3.69	19.97	1.73
8.5	1.52	3.19	12.97	2.10

Chemical constituents of *M. oleifera* seeds by using GC/MS technique

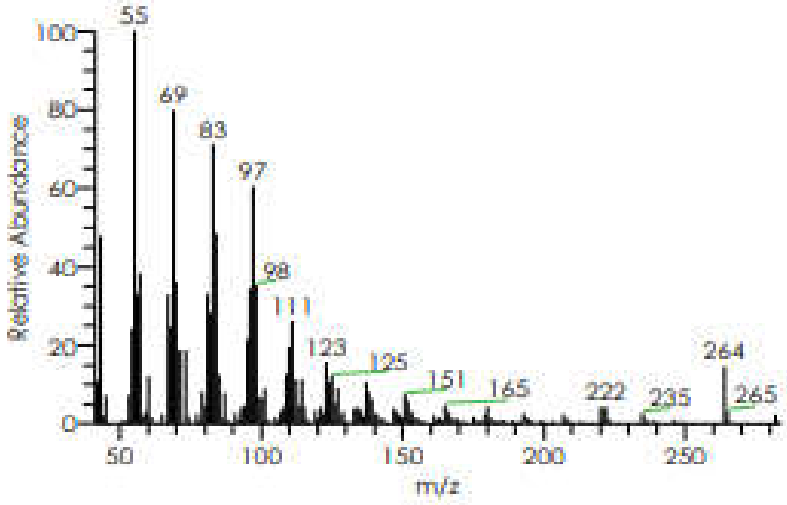
Data in Table (4) represent the most important compounds present in the seeds are fatty acid octadecenoic acid unsaturated and it is called oleic acid. At pH (9) the area of compound was 92.91 at Rt 23.08 min, but it was 33.54 at Rt 23.03 min at pH (4). The second compound was hexadeconate which have area 23.09 at Rt 19.57 min in pH (9), and it have area 23.96 at Rt 19.90 min in pH (4).

Table (4): The most chemical constituents of *M. oleifera* seeds by using GC/MS technique

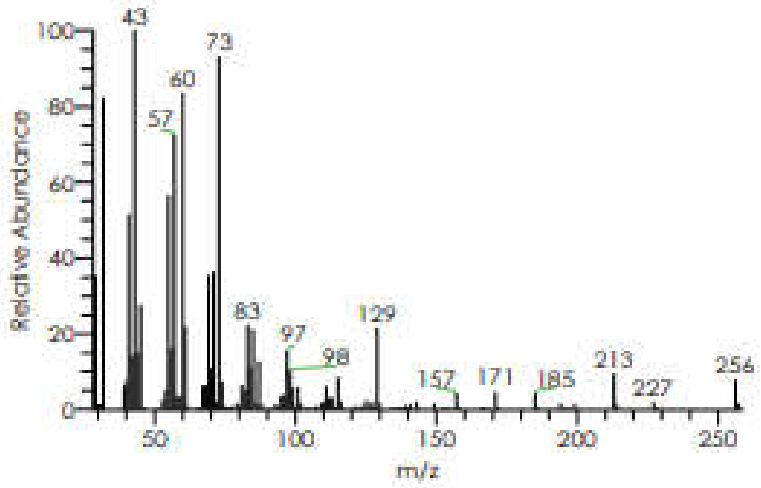
Component name	pH	Rt (min)	Area	Formula
Oleic acid	4	23.03	33.54	$C_{18}H_{34}O_2$
	7	23.07	14.30	
	9	23.08	92.91	
Hexadeconic acid	4	19.90	23.96	$C_{16}H_{32}O_2$
	7	19.60	3.93	
	9	19.57	23.09	
Octadeconic acid	4	23.03	35.67	$C_{21}H_{40}O_4$
	7	22.37	2.28	
	9	22.33	50.94	



Oleic acid



Octadeconic acid



Hexadeconic acid

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تأثير مستخلص بذور المورينجا علي العنكبوت الأحمر ذو البقعتين

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تم استخلاص بذور المورينجا باستخدام نوعان من المذيبات وهما الميثانول والبيترواليم ايثر، وقد أظهرت النتائج فاعليه مستخلص الميثانول عن مذيب البيترواليم ايثر حيث أوضحت النتائج أن التركيز القاتل لـ ٥٠% من الأفراد كان ٠.٩٥ (جزء في المليون) وذلك بعد ٥ أيام من المعاملة بينما كانت ١.٧٤ (جزء في المليون) عند استخدام مذيب البيترواليم ايثر بعد ٥ أيام من المعاملة. وقد تم عمل الاستخلاص في درجات مختلفة من الأس الهيدروجيني وقد أظهرت النتائج أنه في الوسط الحامضي كان التركيز القاتل لـ ٥٠% من الأفراد ٧.٣٧ (جزء في المليون) بينما كان ٥.١٩ (جزء في المليون) في الوسط المتعادل وكان في الوسط القلوي ٣.١٩ (جزء في المليون). ونتيجة لفاعليه مستخلص المورينجا ضد أفراد العنكبوت الأحمر ذو البقعتين فقد تم التعرف علي أهم المركبات المتواجدة في الأوساط المختلفة وكانت أهم المركبات التي ظهرت في التحليل عن طريق GCMS وهم حامض الأوليك، وحامض الهيكساديكونيك وأيضا حامض الاوكتاديكونيك.

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