

COMPARATIVE TOXICITY OF SOME PLANT EXTRACTS ON EGGS AND ADULT FEMALES OF *Tetranychus urticae* KOCH (ACARI: TETRANYCHIDAE).

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ABSTRACT

The present study was conducted in the laboratory to assess the effects of two plant extracts (*Jatropha curcas* & *Euphorbia lathyris*) with four solvents (chlorophorm, ethyl acetone, acetone and methanol) on eggs and adult females of *Tetranychus urticae* Koch (Acari : Tetranychidae) at laboratory condition ($27\pm 2^{\circ}\text{C}$ and $65\pm 5\%$ RH.). The results revealed that, the *Jatropha curcas* and *Euphorbia lathyris* gave high effect at concentrations 2.4 and 1.2% after 24, 48 and 72h. for adult females of *T. urticae*. While at concentration, 0.6% gave good results after 72 h. for the two extracts. Also, the concentration of 0.3% was not effective, except for *J. curcas* when used the acetone as a solvent. The acetone was the best solvent followed by chlorophorm and ethyl acetate. But, the solvent of methanol was not effective for the two plant extracts. The extract of *J. curcas* was more effective on eggs of *T. urticae* than the extract of *E. lathyris*; and the concentration of 2.4% was the best for its effect on eggs. However, the concentrations of 0.6 and 0.3% were slightly effective on eggs of *T. urticae*.

INTRODUCTION

The two-spotted spider mite *Tetranychus urticae* Koch is widely spread throughout the Mediterranean area. *T. urticae* is considered one of the most important pests. It is responsible for yield losses in many horticultural, ornamental and agricultural system mainly annual crops and vegetables, (Helle & Sabelis, 1985). This study was conducted to determine efficiency of plant extracts extracted from two different plants (*Jatropha curcas* & *Euphorbia lathyris*) against this mite. Bioassays were tested by four different concentrations (2.4, 1.6, 0.6 and 0.3%) to determine the effects of varying concentrations; and the plants were extracted by four solvents (acetone, acetate ethyl, chlorophorm and methanol). The extracts gave high mortality at high concentrations (2.4 and 1.2%) for eggs and adult females. All of solvents were efficacious, except solvent of methanol was slightly effective as a solvent. The conventional pesticides have become an indispensable tool in controlling some pests economically, rapidly and effectively; extensive use of insecticides may lead to a number of undesirable side effects including the development of insect resistance and resurgence of primary and secondary pests outbreaks. Also, they can have adverse effects on nontarget organisms and general environmental contamination (Georghiou, 1987; Metcalfe, 1989 and Ditrach, 1962). The other problems with synthetic insecticides are environmental pollution and insect resistance. Many researches are

experimenting and developing alternative plant extracts as pesticides to be used against pest insects and mites. The seed kernel extract of neem known as azadirachtin, has been most thoroughly tested, and it has been extracted in larger quantities than the other components of neem (Schmutterer, *et al*; 1981 and Schmutterer & Zebitz, 1984). High rates of mortality have been found on the two-spotted mites fed on the leaves treated with *Azadirachta indica* extract. In addition, the same extract significantly reduced the reproductive capacity of mites and the survival of the progeny of treated females greatly diminished in comparison the control (Miranova and Khorkhordin, 1996). Many investigators studied the effect of plant extracts on the biology of mites (Afifi & Hafez, 1988, Barakat & Shreef, 1984, El-Halawany *et al*, 1988 and El-Kabbany, 1980). In addition, Nassar, *et al*. (1995) found that, the biological aspects of *T. urticae* were more affected by plant extracts of durantana and lantana in the laboratory studies. Therefore, the present study threw light on the efficacy of some plant extracts on eggs and adult females of *Tetranychus urticae* Koch under normal conditions.

MATERIALS AND METHODS

Rearing technique of mite:

The two-spotted spider mite *Tetranychus urticae* Kock was collected from eggplant (*Solanum melongena*) at the farm of faculty of Agriculture Al-Azhar University, Assuit branch. A pure culture of the two-spotted spider mite were maintained on kidney beans plants (*Phaseolus vulgaris*) planted in pots 25 cm. diameter in sunny place.

Toxicity test and treatments design of adult females:

To evaluate the effect of the plant extracts on the females of *t. urticae* mite, ten newly emerged adult females were transferred to the upper surface of Kidney beans leaf discs (3cm. diameter). Two leaf discs were kept on moist cotton pad in each Petri-dish (15cm. diameter), each dish was replicated four times, and continuously moistened during the experiment. The disc surface which carrying the adult females was sprayed separately with plant extract using a manual atomizer, and the other one was covered with plastic paper, and the dishes were left at room temperature at $27\pm 2^{\circ}\text{C}$ and 65% ± 5 RH. The un-treated control was sprayed by water and additive solvent Twen80 by rate (0.1 %). Mortality percent was calculated after 24, 48 and 72hrs. of treatments, according to Abbot's formula (1925).

Toxicity test and treatments design of eggs:

To evaluate the effect of the plant extracts on the eggs of *t. urticae* mite, ten adult females were transferred to the upper surface of Kidney beans leaf discs (3cm diameter), and left 24hour to deposited eggs. Two leaf discs were kept on moist cotton pad in each Petri-dish (15-cm. diameter), each dish was replicated four times, and continuously moistened during the experiment. After 24hour the females were removed and the eggs were counted, and then the disc surface which carrying the eggs was sprayed separately with plant extract using a manual atomizer, and the other one was covered with plastic paper, and the dishes were left at room temperature at $27\pm 2^{\circ}\text{C}$ and

65% \pm 5 RH. The un-treated control was sprayed by water and additive solvent Twen80 by rate (0.1 %). Mortality percent was calculated after 6 days of treatments, according to Abbot's formula (1925).

Tested plant oils:

- 1- Jatropha, *Jatropha curcas*
- 2- Greek, *Euphorbia lathyris*

Plant extracted:

Samples:

The plant materials (seeds) of *Jatropha curcas* and *Euphorbia lathyris* were collected from the forests, Faculty of Agriculture, Al-Azhar University, Assuit branch in March 2013. Two hindered gram of the seeds were dried at room temperature for two weeks and grinded after removed the shell of seeds using an electric blender.

Preparation of extracts:

The seeds of *J. curcas* and *E. lathyris* were dried in shade at room temperature, homogenized to coarse powder, and stored in opaque screw tight jar until use. Powdered drug was charged into soxhlet apparatus and extraction was carried out with following solvents successively: 1- Chloroform, 2- Ethyl acetate, 3-Acetone, 4- Methanol. Each time before employing the solvent of higher polarity sample was dried.

Statistical analysis:

Data obtained were statistically analyses according to procedures outlined by Gomez and Gomez (1984). The mean values were compared at 5 % level of Duncan's multiple range tests.

RESULTS

Effect of tested plant extracts on population of *T. urticae*.

From Table (1), it can be observed that, after 24h. from treatment, the plants which extracted by acetone had a significant mortality and the highest effect on females of *T. urticae* at all concentrations. They gave reduction percentages 96.25, 90.00, 42.50 and 16.25% for *Euphorbia lathyris* extract and 95.00, 78.75, 73.75 and 66.25% for extract of *J. curcas* at concentrations 2.4, 1.2, 0.6 and 0.3% respectively. The extract of *J. curcas* was more effective when acetone used as a solvent at high and low concentrations comparative with extract of *E. lathyris*. The solvents of ethyl acetate and chlorophorm were affective only on high concentrations (2.4 and 1.2%). The reduction percentages of *J. curcas* extract at concentrations of 2.4 and 1.2% were 93.75 and 75.00% with solvent of chlorophorm and 96.25 and 57.50% with solvent of ethyl acetate respectively. The reduction percentages of *E. lathyris* extract were near to *J. curcas* extract at the same concentrations and solvents; it were 90.00 and 71.25% with solvent of chlorophorm, 85.00 and 57.50% with solvent of ethyl acetate at concentrations 2.4 and 1.2% respectively. While the concentrations of 0.6 and 0.3% weren't reached 50% mortality with solvents of ethyl, chlorophorm and methanol. On the other hand the methanol solvent was below three solvents, it accomplished 60.00%

reduction with extract of *J. curcas* only at concentrate 2.4%. While the lowest effects were found at concentrations 1.2, 0.6 and 0.3% with the same plant extract. However, *E. lathyris* extract with solvent of methanol had the lowest effect on the females of *T. urticae* at all concentrations. The mortality percentages were 27.50, 20.00 and 16.25% at concentrations 2.4, 1.2 and 0.6% respectively. Statistical analysis showed significant difference between the concentrations and solvents.

Table (1) reduction percentages (%) of *T. urticae* (adult females) affected by spraying of two plant extracts after 24h.

Concent.	solvents Treat.	Acetone	Ethel acetate	chlorophorm	methanol	Mean
	<i>E. lathyris</i>	96.25 a	85.00 abc	90.00 ab	27.50 hi	74.69 B
1.2%	<i>J. curcas</i>	78.75 bcd	57.50 f	75.00 cd	16.25 jkl	56.88 C
	<i>E. lathyris</i>	90.00 ab	57.50 f	71.25 def	20.00 ijk	59.69 C
0.6%	<i>J. curcas</i>	73.75 cde	37.50 gh	20.00 ijk	15.00jkl	36.56 D
	<i>E. lathyris</i>	42.50 g	25.00 hi	26.25 hi	16.25 jkl	27.50 E
0.3%	<i>J. curcas</i>	66.25 def	15.00 jkl	6.25 klm	2.50 lm	22.50 E
	<i>E. lathyris</i>	16.25 jkl	05.00 klm	21.25 ij	00.00 m	10.63 F
	Mean	69.84 A	47.34 B	50.47 B	19.69 C	

F. test

A=135.4**

B = 163.2**

AB = 9.539**

Data in Table (2) showed that, the concentration of 2.4% was the highest effect on adult females of *T. urticae* after 48h. The reduction percentages at concentration of 2.4% were 100% with solvents acetone, methyl acetate and chlorophorm, and 73.47% with methanol solvent for extract of *J. curcas*. While the extract of *E. lathyris* achieved reduction percentages 100.00, 92.36, 91.11 and 31.65 with solvents acetone, methyl acetate, chlorophorm and methanol respectively at concentration of 2.4%. The concentration of 1.2% recorded too high mortality; with reduction percentages 94.94, 73.33, 89.87 and 21.67% for *E. lathyris* and 91.14, 74.68, 92.50 and 21.39% for extract of *J. curcas* by solvents acetone, methyl acetate, chlorophorm and methanol respectively. The decreased concentration led to decrease mortality. The concentration of 0.6% gave 85.97% mortality for *J. curcas* extract and 51.90% mortality for *E. lathyris* extract when the acetone used as a solvent. The concentrations of 0.6 and 0.3% were slightly effected on females of *T. urticae* for two plant extracts when methyl acetate, chlorophorm and methanol used as solvents after 48h. from treatment. The effect of concentrations and solvents interaction of *J. curcas* and *E. lathyris* extracts were very significant at P < 0.05.

Table (2) reduction percentages (%) of *T. urticae* (adult females) affected by spraying of two plant extracts after 48h.

Concent.	solvents Treat.	Acetone	Ethyl acetat	chlorophorm	methanol	Mean
	2.4%	<i>J. curcas</i>	100.00 a	100.00 a	100.00 a	73.47 b
<i>E. lathyris</i>		100.00 a	92.36 a	91.11 a	31.65 def	78.78 B
1.2%	<i>J. curcas</i>	91.14 a	74.68 b	92.50 a	21.39 fg	69.93 C
	<i>E. lathyris</i>	94.94 a	73.33 b	89.87 a	21.67 fg	69.95 C
0.6%	<i>J. curcas</i>	85.97 ab	48.10 c	48.20 c	16.25 gh	49.63 D
	<i>E. lathyris</i>	51.90 c	43.47 cd	40.70 cde	16.46 gh	38.13 E
0.3%	<i>J. curcas</i>	73.33 b	17.64 fgh	29.17 efg	03.75 h	30.97 F
	<i>E. lathyris</i>	16.46 gh	05.06 h	22.92 fg	03.61 h	12.01 G
	Mean	76.72 A	56.83 C	64.31 B	23.53 D	

F. test

A=153.4**

B = 209.3**

AB = 9.790**

Obtained results are presented in Table (3) explained that, there are large differences between the concentrations and solvents. After 72h. from spraying the concentration of 0.6% became more effective on females of *T. urticae*; it gave reduction percentages 92.36, 56.53, 65.42 and 21.67% for *J. curcas* extract and 58.97, 61.56, 52.56 and 24.36% for *E. lathyris* extract with solvent acetone, ethyl acetate, chlorophorm and methanol respectively. However, the concentration of 0.3% recorded 83.33% reduction for *J. curcas* extract when acetone used as a solvent; while the other three solvents gave low reduction with same extract and concentration. The extract of *E. lathyris* was the lowest reduction for *T. urticae* with the four solvents. On the other hand, the concentrations of 2.4 and 1.2% were the highest reduction for both two extracts. The extract of *J. curcas* gave reduction percentages 100, 100, 100 and 82.08% at concentration of 2.4% and 96.11, 88.46, 96.15 and 30.77 at concentration of 1.2% with solvents acetone, acetate ethyl, chlorophorm and methanol. While the extract of *E. lathyris* gave reduction percentages 100, 97.50, 93.61 and 38.46% at concentration of 2.4% and 97.50, 84.86, 96.25 and 25.64% at concentration of 1.2% when acetone, ethyl acetate, chlorophorm and methanol used as solvents respectively. In general, mortality, increased as the concentration of plant extracts and exposure period increased. All tested solvents were very affective except solvent of methanol was slightly affected on adult females of *T. urticae*.

Table (3) reduction percentages (%) of *T. urticae* (adult females) affected by spraying of two plant extracts after 72h.

Concent.	solvents Treat.	Acetone	Ethyl acetate	chlorophorm	methanol	Mean
	<i>E. lathyris</i>	100.00 a	97.50 a	93.61 abc	38.46 ef	82.39 B
1.2%	<i>J. curcas</i>	96.11 abc	88.46 abc	96.15 abc	30.77 efg	77.87 B
	<i>E. lathyris</i>	97.50 ab	84.86 abc	96.25 abc	25.64 efg	76.06 B
0.6%	<i>J. curcas</i>	92.36 abc	56.53 d	65.42 d	21.67 g	85.99 C
	<i>E. lathyris</i>	58.97 d	61.56 d	52.56 d	24.36 fg	49.36 D
0.3%	<i>J. curcas</i>	83.33 dc	38.46 e	33.33 efg	07.69 h	40.70 E
	<i>E. lathyris</i>	23.33 fg	08.75 h	32.50 efg	06.25 h	17.71 F
	Mean	81.45 A	67.01 B	71.23 B	28.83 C	

F. test

A=125.4**

B = 198.7**

AB = 8.987**

Effect of tested plant extracts on eggs of *T. urticae*.

Table (4) indicated that, after 6days from spraying on eggs, only concentration of 2.4% was effective for *E. lathyris* extract; while the concentrations of 2.4 and 1.2% were effective for extract of *J. curcas*. The mortality percentages of eggs were 84.87, 88.12 and 85.69% for extract of *J. curcas* at concentration of 2.4% and 78.14, 56.72 and 33.37% at concentration of 1.2% with solvents acetone, ethyl acetate and chlorophorm respectively. While the extract of *E. lathyris* gave 46.01, 57.68 and 84.14 at concentration of 2.4% when acetone, ethyl acetate and chlorophorm used as solvents respectively. The solvent of methanol and concentrations of 0.6 and 0.3% were slightly effective on mortality of eggs of *T. urticae* for the two tested plant extracts. Statistical analysis showed highly significant difference among the concentrations, solvents and plant extracts.

Table (4) reduction percentages (%) of *T. urticae* (eggs) affected by spraying of two plant extracts after 6days.

Concent.	solvents Treat.	Acetone	Ethyl acetate	chlorophorm	methanol	Mean
	<i>E. lathyris</i>	46.01 e	57.68 d	84.14 abc	07.09 nop	48.73 B
1.2%	<i>J. curcas</i>	78.41 bc	56.72 d	33.37 f	09.58 nop	44.52 B
	<i>E. lathyris</i>	29.80 ghi	21.79 ijk	33.74 f	00.31p	21.41 C
0.6%	<i>J. curcas</i>	30.68fg	29.93 fg	18.63jkl	06.72 nop	21.49 C
	<i>E. lathyris</i>	09.00 nop	19.50 jkl	16.00 klm	00.51op	11.25 D
0.3%	<i>J. curcas</i>	20.03jkl	06.63 nop	12.93 klm	03.66	10.81 D
	<i>E. lathyris</i>	05.99 nop	12.09 lmn	11.79 lmn	.0017 p	07.49 D
	Mean	38.10 A	36.56 A	35.78A	04.87 B	

F. test

A=135.4**

B = 163.2**

AB = 9.539**

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فعالية تأثير بعض المستخلصات النباتية وطرق استخلاصها على العنكبوت الأحمر ذو البقعتين.

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اجريت هذه الدراسة لمعرفة مدى فعالية المستخلصات النباتية في خفض أعداد العنكبوت الأحمر *Tetranychus urticae* Koch لذلك فقد تم استخلاص نوعين من بذور النباتات وهما نبات الجتروفا *Jatropha curcas* ونبات حبة الملوك *Euphorbia lathyris* باستخدام أربع أنواع من المذيبات الكيماوية وهي الأستون وخلات الايثايل والكلوروفورم والميثانول. ثم تم اختبار هذه المستخلصات على الإناث الكاملة والبيض باستخدام أربع تركيزات مختلفة وهي ٢.٤%، ١.٢%، ٠.٦%، ٠.٣% على درجة حرارة الغرفة، وتم أخذ النتائج بعد ٢٤، ٤٨، ٧٢ ساعة وقد أسفرت النتائج عن الآتي.

أولاً التأثير على الإناث الكاملة:

١- حقق كلا المستخلصين نسبة خفض عالية للإناث الكاملة وصلت إلى ١٠٠% بعد ٤٨، ٧٢ ساعة حيث كانت نسبة الخفض بعد ٧٢ ساعة من المعاملة لمستخلص الجتروفا باستخدام مذيب الأستون ١٠٠%، ٩٦.١١%، ٩٢.٣٦%، ٨٣.٣٣%، على التركيزات ٢.٤%، ١.٢%، ٠.٦%، ٠.٣% على الترتيب؛ أما نتائج مستخلص حبة الملوك باستخدام نفس المذيبات ونفس التركيزات المذكورة سابقاً فكانت ١٠٠%، ٩٧.٥٠%، ٥٨.٩٧%، ٢٣.٣٣% على الترتيب.

٢- حقق مستخلص الجتروفا نتائج أفضل من مستخلص حبة الملوك على التركيز المنخفض وهو ٠.٣%.

٣- كان مذيب الأستون هو أفضل المذيبات الأربع مع كلا النباتين يليه مذيب الكلوروفورم ثم خلات الايثايل، أما مذيب الميثانول فقد كانت نسب الخفض التي حققها أقل ما يمكن.

٤- كان التركيزان ١.٤% و ١.٢% هما الأفضل لأنهما حققا أعلى نسب خفض مع كلا المستخلصين.

ثانياً التأثير على البيض:

كان بيض العنكبوت الأحمر أقل تأثراً بالمستخلصات من الأطوار الكاملة لذا كانت النتائج كالتالي.

١- تفوق مستخلص الجتروفا عن مستخلص حبة الملوك في التأثير على البيض حيث أعطى نسب خفض وصلت إلى ٨٨.٠١٢%، ٨٤.٨٧%، ٧٥.٦٩%، ٢.٤% وبإستخدام المذيبات ألتيه خلات الايثايل و الأستون و الكلوروفورم على الترتيب.

٢- حسب نتائج التحليل الاحصائي فقد حقق مستخلص حبة الملوك نتائج أقل معنوية من مستخلص الجتروفا حيث كانت نسب الموت في البيض ٨٤.١٤%، ٥٧.٦٨%، ٤٦.٠١%، ٢.٤% وبإستخدام مذيبات الكلوروفورم و خلات الايثايل ثم الأستون على الترتيب.

٣- كان التركيز ٢.٤% هو الأفضل في استخدام المستخلصات على البيض حيث أعطى مستخلص حبة الملوك نتائج ضعيفة جداً باستخدام التركيزات الأقل.

٤- اختلفت كفاءة المذيبات بالنسبة للنباتات المستخدمة ، فكان مذيب خلات الايثايل هو الأفضل حسب النتائج بالنسبة للجتروفا ؛ وكان الكلوروفورم هو الأفضل في استخلاص نبات حبة الملوك.

٥- استخلاص النباتين باستخدام مذيب الميثانول لم يحقق نتائج تذكر إحصائياً.