EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES ZOOLOGY

B

ISSN
2090-0759

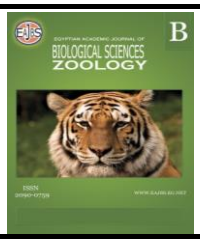
WWW.EAJBS.EG.NET

Vol. 12 No. 1 (2020)

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 12(1) pp: 9-23(2020)*

Egypt. Acad. J. Biolog. Sci., 12(1):9-23 (2020)

Egyptian Academic Journal of Biological Sciences
B. Zoology
ISSN: 2090 – 0759
www.eajbsz.journals.ekb.eg



Effectiveness of Some Plant Extracts against the Two-Spotted Spider Mite, *Tetranychus urticae* Koch Under Laboratory And Greenhouse Conditions (Acari: Tetranychidae).

Heba M. Emam[1] and Mariam T. S. Ibrahim[2]
1- Department of Plant Protection, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt
Email: Hebasoliman@agr.asu.edu.eg
2- Department of Biochemistry, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt
E.Mail: Hebasoliman@agr.asu.edu.eg / Mariam_ibrahim@agr.asu.edu.eg

ARTICLE INFO
Article History
Received:4/1/2020
Accepted:29/1/2020

Keywords:
Tetranychus urticae, plant extracts, Phenolic compounds, flavonoids, *Zygophyllum album, Limoniastrum guyonianum, Calotropis procera*, *Thymelaea hirsuta*, *Dodonaea viscosa*.

ABSTRACT

The present study was carried out to investigate the effect of five plant extracts, *Limoniastrum guyonianum* Del., *Zygophyllum album* L., *Calotropis procera* (Ait.), *Thymelaea hirsuta* (L.) Endl and *Dodonaea viscosa* L. at the concentrations 0.01 and 0.005 %. The toxic effect of these concentrations was tested against adult females of the Two-spotted spider mite *Tetranychus urticae* Koch (TSSM) 24, 48 and 72 h after treatment under laboratory conditions. The results showed that, after 72 h, the highest mortality 93.33% was recorded by treatment with seeds of *C. procera* followed by leaves of *D. viscosa* 86.67% at the concentration of 0.01%. At the concentration 0.005%, the highest mortality was 66.67% by *C. procera* (seed extract)**.** Under greenhouse conditions, after 72 h at 0.01 % and 0.005 % concentrations were giving a highly percent reduction of hatchability for eggs at *C. procera* (seeds extract) with an average 95 % and 79.49 % respectively. Also, of larva stages was recorded highly percent mortality at 0.01% and 0.005 % concentration of *C. procera* (seeds extract) 91.67 % and 70% respectively. In nymphs, the highest mortality percentage with *C. procera* (seeds) at the same concentrations after 72 h were recorded 93.75% and 71.43 % respectively. In adult females was found the highest mortality percentage in *C. procera* (seeds extract) at 0.01% and 0.005 % concentration after 72 h was recorded 91.67% and 76.92 %. Analytical studies proved that, major amounts of phenolic compounds were detected in *T. hirsuta* catechin, gallic and chlorogenic acids at the concentrations 1609.24, 1066.78 and 839.67 µg/g., respectively. In *C. procera* (seeds extract) it was found that phenolic compounds were lower than *T. hirsuta*, while coumaric acid has been only identified in *C. procera* seeds (21.08 µg/ g. plant).

In *D. viscosa*, phenolic compounds were represented by chlorogenic acid (267.41 µg/ g plant) and gallic acid (84.43 µg/ g plant), while catechin was not detected. The highest mortality percentage of *T. urticae* treated with seeds extracts may be due to the appearance of coumaric acid, which was found in seeds more than other extracts.

INTRODUCTION

Tetranychus urticae Koch (Acari: Tetranychidae) is one of the most polyphagous spider mite species and is a major pest in many cropping systems worldwide (Nauen *et al*. 2001). *Tetranychus urticae* can increase its population very rapidly on the suitable host plants and climate conditions. Its outbreaks in agricultural ecosystems, particularly in greenhouses where mite populations can reach very high densities, (Tsagkarakou *et al*. 1999 and Derbalah 1999). Biological control programs of the Two-spotted spider mites recorded a varying degree of success (Helle and Sabelis 1985) Acaricides have been and still so far widely used for mite control in greenhouses, orchards and many other cropping systems (Van *et al*. 2005). Mite high reproductive potential and extremely short life cycle, combined with the frequent applications of chemical acaricides are usually required to maintain mite populations below economic thresholds, facilitate the development of resistance (Stumpf *et al*. 2001). Plant extracts are one of several non-chemical control options that have recently received great attention. There are several reports on botanical acaricides Neem (Sundaram and Sloane 1995; Martinez-Villar *et al.* 2005) *Calotropis porcera* (Ait.) (Asclepiadecae), and *Nerium oleander* L. (Apocynacae) (Islam *et al*. 2008) tansy *(Tanacetum vulgare* L.) and wormwood *(Artemisia absinthium* L.) extracts (Chiasson *et al*. 2001) and garlic oil (Boyd and Alverson 2000), *Satoreja hortensis* L. (Aslan *et al*. 2004) were found effective against the Two-spotted spider mite adults. Phenolic compounds are the main secondary metabolites in plants. These phenolic substances, or polyphenols, contain numerous varieties of compounds ex: simple flavonoids, phenolic acids, complex flavonoids and colored anthocyanins (Babbar *et al*. 2014). Plant phenolic compounds not only act as antioxidants, structural polymers (lignin), attractants (flavonoids and carotenoids), UV screens (flavonoids), signal compounds (salicylic acid and flavonoids) but they are also defense response chemicals (tannins and chlorogenic acid). These substances are produced by plants that act as agents protecting the plant from pathogens and pests, we must first consider whether the compounds are present prior to the time of infection or whether they are synthesized in response to damage.

The present investigation aims to evaluate the efficiency of polyphenols compounds from some plant extracts to control the two-spotted spider mite *T. urticae*. Therefore, the toxic effect of active compounds of the plant extracts, *Limoniastrum guyonianum* Del., *Zygophyllum album* L., *Calotropis procera* (Ait.), *Thymelea hirsuta* (L.) Endl, and *Dodonaea viscosa* L. against adult females of *T. urticae* in the laboratory and against all mite stages under greenhouse conditions.

MATERIALS AND METHODS

Rearing of the Two-Spotted Spider Mite (TSSM):

1.Source of *T. urticae*:

The Two-spotted spider mite was collected from infested cucumber, tomato, and pepper plants are grown at Faculty of Agricultural, Ain Shams University, Egypt.

2.Mite Colony:

One newly matted adult mite female was transferred by a fine camel hair brush to a sweet potato leaf (1mm), kept on a moist cotton wool pad in Petri dish and left for laying eggs. The deposited eggs were kept under laboratory conditions of 25-27°C, 60% R.H. and 16 L:8 D photoperiod until hatching. The female mite was mounted on a glass slide in Hoyer's, media for identification. The newly hatched larvae were then transferred singly to fresh leaves of sweet potato cutting holding about 8 leaves each was placed in

glass jars containing tap water which was changed every 48 h. The sweet potato cuttings were changed weekly to provide a mite colony with a fresh leaf, Pritam and Clare (1993).

Plant Extracts:

1- Plant Species Choices:

Plant species were collected from Cairo Alexandria Desert Road and Faculty of Agricultural, Ain Shams University. It was placed in the Icebox until transferred to the laboratory and then placed under freezing until use.

2.Preparation of Plant Extracts:

1- Methanolic Extract:

Samples of fresh leaves (10 gm.) from all five plants and (4 gm.) of seeds from only *Calotropis* plant were finely ground using a homogenizer and extracted with 500 ml of 80% methanol. The soak time was 24 h at room temperature. Each mixture was then filtered through Whatman No. 42 filter paper to remove the debris, and the extracts were then evaporated to dryness, and then the residue was dissolved in 25 ml distilled water directly before use.

2- HPLC Conditions: -

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using Eclipse plus C18 column (4.6 mm x 250 mm i.d., 5 μm). The mobile phase consisted of water (A) and 0.02% tri-floro-acetic acid in acetonitrile (B) at a flow rate of 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (80% A); 0–5 min (80% A); 5-8 min (40% A); 8-12 min (50% A); 12-14 min (80% A) and 14-16 min (80% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 μl for each of the sample solutions. The column temperature was maintained at 35 °C.

Acaricidal Test:

1. Laboratory Experiments:

For laboratory evaluation, the five plant extracts were namely *Limoniastrum guyonianum* Del, *Zygophyllum album* L, *Calotropis procera* (Ait), *Thymelea hirsuta* (L.) Endl, and *Dodonaea viscosa* L. Table (1), at the concentrations 0.01 and 0.005 %. Fifteen healthy adult females of the two-spotted spider mite were set in three replicate (5 individuals of female mite /leaf disc) in each petri-dish. Each treatment was replicated three times for each plant extract and concentration in addition to the control test. Each concentration was sprayed using atomizer on the surface of leaf disc which containing mites, except control was sprayed with water. Numbers of live and dead mites were counted 24, 48 and 72h after treatments in addition to control.

2. Greenhouse Experiments:

Seedlings of tomato plants (one-month-old) were singly transplanted in plastic pots (14 cm. in diameter) filled with sterilized sandy and clay soil 1:1 (V/V). Three individuals of adult females *T. urticae* were used for each plant extract and concentration, in addition to control (plant free of mite infestations). There were three replicates for each concentration of five plant extracts in addition to control. Female's mite was transferred using a fine camel hair brush on tomato leaves. Plants were irrigated when needed and fertilized twice a week with soluble fertilizer with N, P, and K (19.19.19). After two weeks from infestation plants and mite's parameters were recorded for all replicates before spraying with plant extracts to find out population density, and then plant extracts were sprayed the next day on plants and control was sprayed with water (Where the effect of solvent on mite infestation), after 24, 48 and72 h mite's stages (eggs, larva, nymphs and adult female) were counted. The percentage mortality of mites

in the laboratory and greenhouse was calculated by using the following formula according to Abbot's (1925).

$$\text{Percentage of mortality} = \frac{\text{Pre-treatment population-post treatment population}}{\text{Pretreatment population}} \times 100$$

Also, the percent reduction in hatchability was calculated according to Kapil *et al*., (2017).

$$\text{No reduction of hatchability} = \frac{\text{No. of un-hatched eggs before treatment} - \text{No. of hatched eggs after treatment Percentage}}{\text{No. of un-hatched eggs before treatment}} \times 100$$

3. Mite Parameters:

Three leaves from each replicate of plant extracts were collected randomly for mite stages counting. All mite stages numbers of eggs, larvae, nymphs, and females were counted in area one in^2 of tomato leaf. Counts were done using the dissecting microscope.

Statistical Analysis:

Data of all results were analyzed with SAS program (Carry, 2004).

RESULTS AND DISCUSSION

Laboratory Experiment:

1- Effect of Plant Extracts on the Mortality Percentage of *Tetranychus urticae* under Laboratory Conditions:

Concentrations of 0.01 and 0.005 % of ethanol plant extracts of *L. guyonianum*, *Z. album*, *C. procera*, *T. hirsuta*, and *D. viscosa*. The toxic effects of all concentrations were tested against adult females of *T. urticae* 24, 48 and 72 h after treatment. According to the results showed in Table (1) and Figures (1&2) it can be stated that the highest mortality percentage after 24 h was 73.33% at C. *procera* seeds extract and *D. viscosa* at concentration 0.01%, but there were no significant differences between them were noticed. The lowest mortality at the same concentrations was obtained when both *L. guyonianum* and *Z. album* were used and there were also, no significant differences were found with a general average of 46.67% on the other hand *T. hirsuta* and *C. procera* (leaf extract) showed significant differences of toxicity with an average mortality of 60.00 and 53.33%. Whereas at the concentration 0.005 % after 24 h, the highest mortality percentage 46.67% also at *C. procera* (seeds extract), however the lowest mortality percentage at the same concentration of *L. guyonianum* and *Z. album* and *C. procera* (leaf extract) were 26.67% and no significant differences between them. After 48 h of treatment at concentrations of 0.01% the highest mortality percentage was recorded at *C. procera* (seeds extract) than *D. viscosa* 80.00%, 73.33% respectively. But the results explained consensus or similarity between mortality percentage at both plant *L. guyonianum* and *C. procera* (leaf extract) together where the mortality percentage reached to 53.33%, whereas at *Z. album* and *T. hirsuta* were recorded 66.66% and no significant differences between them. However, at concentrations, 0.005% was recorded highest mortality of 53.33 % for adult females at *C. procera* (seeds extract) and no significant differences between *Z. album, T. hirsuta* and *D. viscosa*. While the lowest mortality percentage at *L. guyonianum*, and *C. procera* (leaf extract) were 33.33 %.After 72 h of treatment, the results explained, the highest mortality percentage was also, recorded at *C. procera* (seeds extract) 93.33 % followed by of *D. viscosa* 86.67 % at the concentration 0.01%, there are highly significant differences between them and also, the

results showed graduated mortality percentage where recorded 73,33% and 66.66% at *T. hirsuta* and *Z. album* while at *L. guyonianum* and *C. procera* (leaf extract) were 60.00 % and no significant differences between them. On the other side at the concentration 0.005% was recorded the highest mortality percentage 66.67 % at *C. procera* (seeds extract), then of *D. viscosa* 60.00 % but the lowest mortality percentage at *Z. album* 40.00%.

These obtained results are in harmony with Lakhdari *et al*. (2015) whom found that, the extracts of plants *Zygophyllum album* showed a very significant effect on the mite by a mortality rate of 76%. Also, Dürdane *et al*. (2011) illustrated that, all the extracts exhibited significant adult mite mortality as compared to control. *Lolium perenne* L. (flower, leaf), *Anthemis vulgaris* L. (flower) and *Chenopodium album* L. (flower, leaf) extracts had significantly higher mortality rates than azadirachtin (10 g/L) and the synthetic pesticides tested at 5% concentration in adhesive tape and residual film method. Islam *et al*. (2008) revealed that, low concentration of raw flower juice of *Calotropis porcera* (Asclepiadecae) and seed dust of *Nerium oleander* (Apocynacae) significantly delayed the egg to adult development of two-spotted spider mite, *Tetranychus urticae* Koch, for three successive generations under laboratory trial. The flower juice of *C. porcera* was found more effective than the seed dust of N. oleander in delaying the developmental period of TSSM. Rana *et al*. (2015) showed that seed extract was the most effective compared to flower and leaf extracts. It was found that at 5% and 10% concentrations of seed extract, the repellent effects were 92 % and100%, respectively within the first 72 hours. Labanowska (1990) conducted that, spraying a number of chemical pesticides before the bloom of strawberry plant controlled the population of TSSM. So, spraying of flower juice of *C. porcera* or a sprinkling of seed dust *N. oleander* on bean plants may satisfactorily delay the development period of *T. urticae*, which ultimately will control their population without affecting the environment.

Table 1: Plants tested for acaricidal activities against the Two spotted spider mite, *Tetranychus urticae.*

Plant family	Plant species	Parts used	Common name
1-Plombaginaceae	L*imoniastrum guyonianum* Del.	Whole plant	Zita
2-Zygophyllaceae	*Zygophyllum album* L.	Whole plant	Agga
3-Thymelaeaceae	*Thymelaea hirsuta* (L.) Endl.	Whole plant	Mintnan
4- Apocynaceae	*Calotropis procera* (Ait.)	Leaves &seeds	Apple of Sodom
5-Sapindaceae	*Dodonaea viscosa* L.	Leaves	Sticky hopbush

Table 2: Mortality percentage of adult females *Tetranychus urticae* as affected by plant extracts under laboratory conditions.

Plant extracts	Mortality %							
	24 h		48 h		72 h		F. Value	L.S.D
	0.01%	0.005 %	0.01%	0.005 %	0.01%	0.005 %		
L. guyonianum	46.67b	26.67b	53.33b	33.33ab	60.00b	53.33ab	0.10	17.15
Z. album	46.67b	26.67b	66.67ab	40.00a	66.67ab	40.00b	3.34	15.81
T. hirsuta	60.00ab	33.33ab	66.67ab	46.67a	73.33ab	53.33ab	0.52	18.39
C. procera (leaves)	53.33b	26.67b	53.33b	33.33ab	60.00b	46.67ab	0.33	18.39
C. procera (seeds)	73.33a	46.67a	80.00a	53.33a	93.33a	66.67a	0.35	18.39
D. viscosa	73.33a	40.00ab	73.33a	46.67a	86.67ab	60.00ab	0.51	18.39

Means with the same letter within each column are not significantly different from another at the 0.5% level

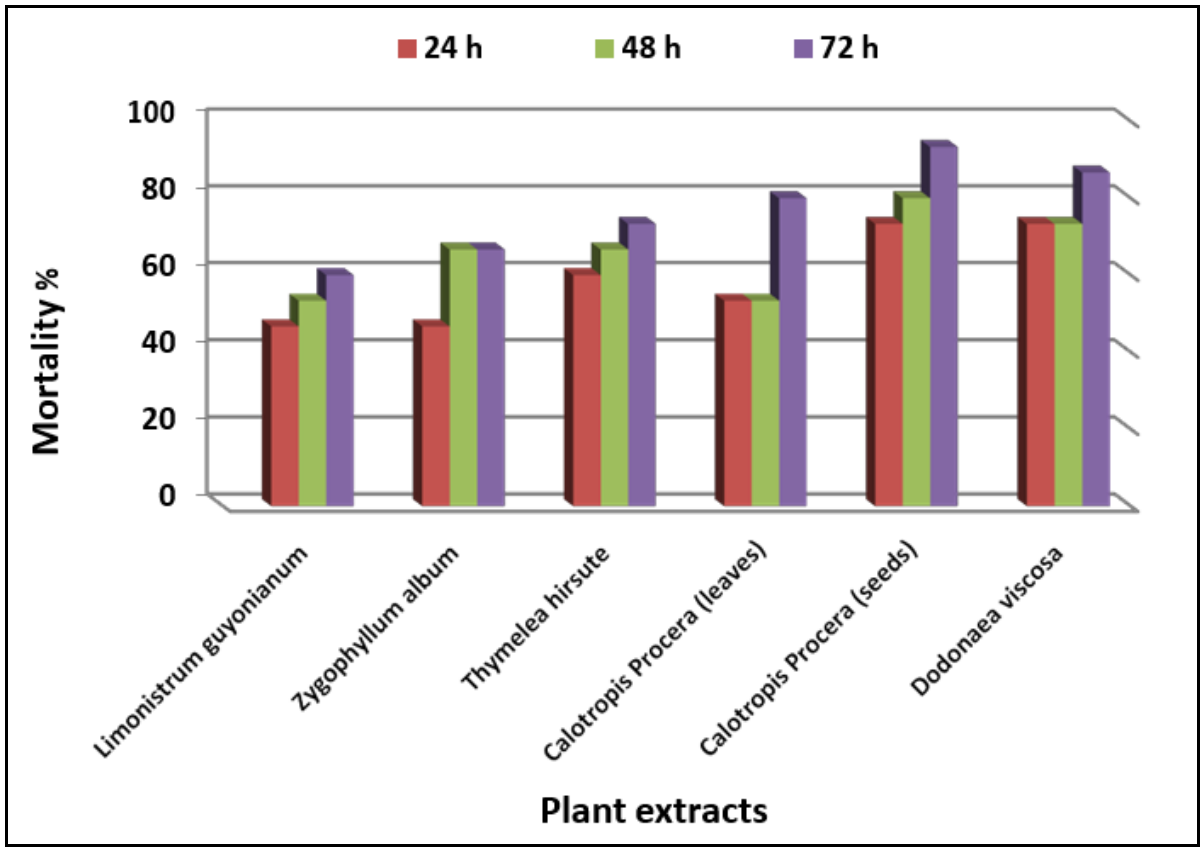


Fig. 1. Mortality percentages of adult females *Tetranychus urticae* as affected by plant extracts at concentration 0.01% under laboratory conditions.

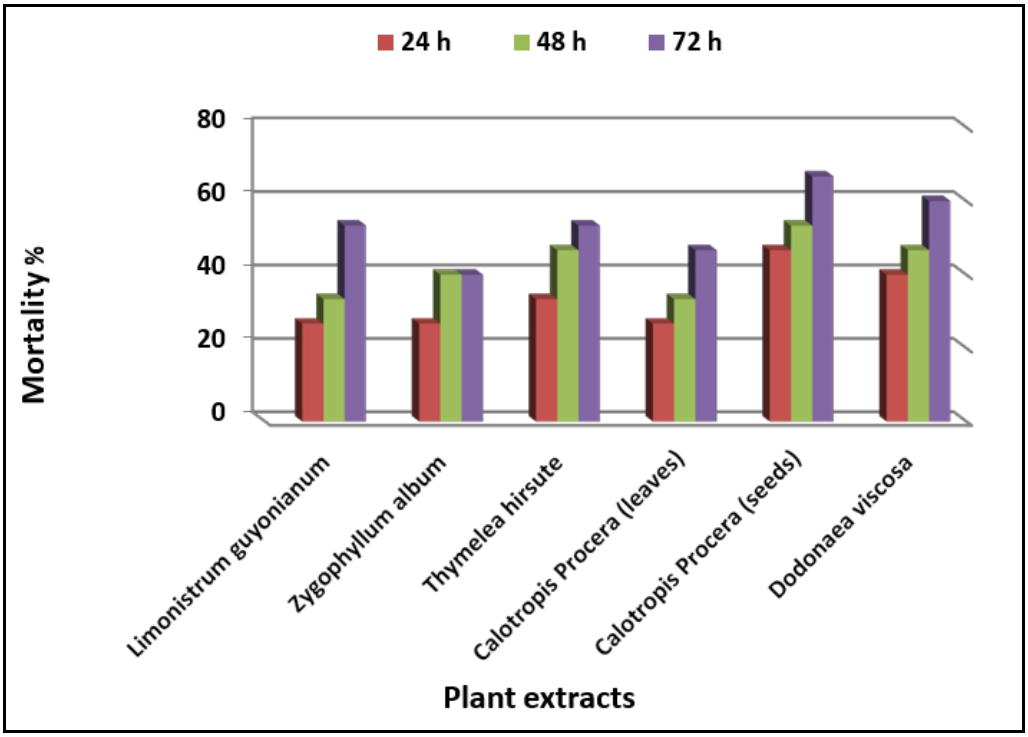


Fig. 2. Mortality percentages of adult females *Tetranychus urticae* as affected by plant extracts at concentration 0.005% under laboratory conditions.

Greenhouse Experiment:

Plant extracts above and concentrations were tested to evaluate their poisonous effect against all stages of mite *T. urticae* (eggs, larva, Nymphs, and Adult females) after 24,48 and 72 h under greenhouse conditions. The obtained results have been illustrated in Tables 3-7**.** Data in Table (3) showed that effect of plant extracts on percent reduction in hatchability for *T. urticae* eggs after 24 h, the highest percent reduction in hatchability for eggs was recorded 78.26 % at *C. procera* (seeds extract) at 0.01 % concentration followed by *D. viscosa* observes percent reduction 60.49 % at the same concentration, but the lowest percent reduction of *C. procera* (leaf extract) with an average 44.12 % compared with the other plant extracts, and there were significant differences between all plant extracts. On the other hand, at 0.005 % concentration was recorded highly percent reduction in hatchability for eggs with an average 58.97 % also, on *C. procera* (seeds extract) followed by *D. viscosa* 40.83 % also, there were significant differences between plant extracts. However, after 48 and 72h at 0.01 % concentration was gave highly percent reduction of hatchability for eggs at *C. procera* (seeds extract) with an average 86.96 % and 95 % respectively, then *D. viscosa* and *T. hirsuta* with an average 79.01 % and 72.22% after 48 h and 87.04% and 83.33% after 72 h comparing with other plant extracts. At concentration 0.005 % was recorded highly percent reduction of hatchability at *C. procera* (seeds extract) with an average 79.49 % and 71.80 % after 72 and 48 h respectively, but the lowest percent reduction of hatchability after 24,48 and 27 h were 25, 35 and 40 % at *Z. album* respectively. These obtained results are in harmony with Hamdy *et al.* (2009) revealed that, peppermint; thyme, caraway, and clove extracts were the most effective against TSSM, followed by chenopodium and visnaga extracts and then eucalyptus and sweet basil which showed relatively the least miticidal activity. Radhakrishnan and Prabhakaran (2014) illustrated that, the extracts were evaluated for adulticide and ovicidal activity at two concentrations viz., 2.5 and 5.0%. Among the plants, the aqueous extracts of *A.catharitica* and

C.bonariensis showed100.0 % and 80.0 % adult mortality respectively at 5% concentration after 96 h of observation.

Table 3: Percent reduction in eggs hatchability of *Tetranychus urticae* as affected by plant extracts on under greenhouse condition.

Plant extracts	Percentage reduction in hatchability (%)						F. Value	L.S.D
	24 h		48 h		72 h			
	0.01%	0.005 %	0.01%	0.005 %	0.01%	0.005 %		
L. guyonianum	53.49 c	26.74e	67.44 d	48.83 e	77.91 d	54.65 d	4.28	30.7
Z. album	47.06 e	25 f	58.82 f	35 f	70.59 e	40 e	9.91	22.48
T. hirsuta	50 d	33.13 c	72.22 c	54.16 ab	83.33 c	66.67 c	1.54	38.44
C. procera (leaves)	44.12 f	30 d	64.70 e	53.33 c	76.47 d	56.67 c	1.43	35.09
C. procera (seeds)	78.26 a	58.97 a	86.96 a	71.80 a	95 a	79.49 a	4.69	21.36
D. viscosa	60.49 b	40.83 b	79.01 b	55 b	87.04 b	72.50 b	2.58	33.51

Means with the same letter within each column are not significantly different from another at the 0.5% level

The Average data on percent mortality of larva after 24, 48 and 72 h of treatment are presented in Table (4). The highest percent mortality of larva 50%, 66.67%, and 91.67 %, respectively was observed at concentration 0.01% of *C. procera* (seeds extract). Followed by *D. viscosa* and *T. hirsuta* with an average 42.86 %, 38.89% after 24 h, 61.90 %, 61.11% after 48 h and 85.71 %, 83.33 % after 72 h. The lowest percent mortality of larva 18.10 %, 36.36 and 54.54 % at *Z. album*. On the other side at 0.005 % concentration after 24,48 and 72h also, *C. procera* (seeds extract) was recorded highly percent mortality of larva 40%, 50%, and 70% respectively, but the lowest percent mortality was illustrated on *Z. album* 15.38%, 23.08%, and 30.77% respectively, and there were significant differences between all plant extracts.

Table 4: Mortality percentage of larva *Tetranychus urticae* as affected by plant extracts under greenhouse conditions.

Plant extracts	Mortality of Larva (%)						F. Value	L.S.D
	24 h		48 h		72 h			
	0.01%	0.005 %	0.01%	0.005 %	0.01%	0.005 %		
L. guyonianum	31.81e	18.19 f	45.45 e	27.28 f	68.18 f	40.90 e	2.48	34.69
Z. album	18.10 f	15.38 g	36.36 f	23.08 g	54.54 g	30.77 f	1.35	31.7
T. hirsuta	38.89 c	26.92 c	61.11 c	38.61 c	83.33 c	46.15 d	2.91	38.86
C. procera (leaves)	33.13 d	20.69 e	50 d	34.48 e	75 e	41.38 e	2.28	37.75
C. procera (seeds)	50 a	40 a	66.67 a	50 a	91.67 a	70 a	1.16	41.59
D. viscosa	42.86 b	30.77 b	61.90 c	46.15 b	85.71 b	53.85 c	1.98	39.23

Means with the same letter within each column are not significantly different from another at the 0.5% level

Mortality percentage of nymphs of *T. urticae* was found in Table (5) to be the highest with *C. procera* (seeds) at 0.01% concentration after 24,48 and 72 h of treatment 75%, 87.50 %, and 93.75% respectively, while the lowest mortality percentage of nymphs also with *Z. album* with an average 20.83 %, 29.16 % and 41.67 % respectively. At 0.005% concentration also, C. *procera* (seeds) was recorded high mortality percentage 42.86 %, 57.14 %, and 71.43 % respectively, but the lowest mortality percentage at *Z. album* 16.67%, 20.83%, and 25% respectively, and there were significant differences between all plant extracts.

Table 5: Mortality percentage of nymphs *Tetranychus urticae* as affected by plant extracts under greenhouse conditions.

Plant extracts	Mortality of nymphs (%)						F. Value	L.S.D
	24 h		48 h		72 h			
	0.01%	0.005 %	0.01%	0.005 %	0.01%	0.005 %		
L. guyonianum	27.28 f	21.43 e	36.36 f	28.57 f	63.64 f	35.71 f	1.41	32.42
Z. album	20.83 g	16.67 g	29.16 g	20.83 g	41.67 g	25 g	2.23	18.09
T. hirsuta	47.61 c	33.33 c	66.67 c	42.86 c	85.71 c	47.62 d	4.65	32.69
C. procera (leaves)	33.33 e	27.28 d	50 e	36.36 e	83.33 d	45.45 e	1.51	43.33
C. procera (seeds)	75 a	42.86 a	87.50 a	57.14 a	93.75 a	71.43 a	8.12	27.54
D. viscosa	62.50 b	38.46 b	75 b	46.15 b	87.50 b	53.85 c	11.59	23.53

Means with the same letter within each column are not significantly different from another at the 0.5% level.

Data in Table (6) was showed significant differences in percentage mortality of adult females of *T. urticae* during both concentrations of plant extracts of the investigation after 24, 48 and 72 h. The highest mortality percentage of adult females was found at *C. procera* (seeds extract) followed by *D. viscosa* and *T. hirsuta* at concentration 0.01% was recorded 50%, 75% and 91.67%, 46.67%, 71.43% and 78.57% and 42.86%, 66.67% and 73.33 %, respectively. The lowest mortality percentage at *Z. album* 23.08 %, 30.77%, and 46.15% respectively. Also, at C. *procera* (seeds) at 0.005% concentration was recorded highly mortality percentage of adult females followed by *D. viscosa* and *T. hirsuta* 38.46 %, 69.23% and 76.92 %, 25%,55.56% and 75% and 22.23 %, 50% and 66.67 % respectively, and there were significant differences between all plant extracts.

Table 6: Mortality percentage of adult females *Tetranychus urticae* as affected by plant extracts under greenhouse conditions.

Plant extracts	Mortality of adult females (%)						F. Value	L.S.D
	24 h		48 h		72 h			
	0.01%	0.005 %	0.01%	0.005 %	0.01%	0.005 %		
L. guyonianum	33.13 e	8.33 e	41.67 f	25 f	50 f	41.67 f	2.37	29.95
Z. album	23.08 g	7.14 f	30.77 g	21.43 g	46.15 e	28.57 g	2.38	25.7
T. hirsuta	42.86 c	22.23 c	66.67 c	50 c	73.33 c	66.67 c	0.82	43.23
C. procera (leaves)	35.29 d	13.33 d	58.82 d	46.67 d	64.70 g	53.33 e	0.98	42.46
C. procera (seeds)	50 a	38.46 a	75 a	69.23 a	91.67 a	76.92 a	0.4	46.85
D. viscosa	46.67 b	25 b	71.43 b	55.56 b	78.57 b	75 b	0.62	50.23

Means with the same letter within each column are not significantly different from another at the 0.5% level

Determination of Phenolic Compounds and Flavonoids by HPLC:

Phenolic compounds and flavonoids are playing a major role as bioactive compounds present in plants. In the present study, we tried to throw a light upon their role as natural protection from the Two-spotted spider mite. Because the enormous diversity of polyphenols in different plant methanolic extracts, phenolic, and flavonoid composition were quantified by HPLC in the central laboratory, National Research Center. Data obtained in Tables (7, 8 and 9). The phenolic and flavonoid compounds have been identified in methanolic extracts at *T. hirsuta*, *D. viscosa* and *C. procera* (seeds). polyphenols contents were arranged at *T. hirsuta* > *D. viscosa* > *C.procera* (seeds). The highest compounds content in three extracts were Phenolic compounds but not flavonoids.

Major amounts of phenolic compounds were detected, Catechin, Gallic acid, and chlorogenic acid. Whereas, Catechin, Gallic acid and Chlorogenic acid in *T. hirsute* were 1609.24, 1066.78 and 839.67 µg/g plant respectively. *C. procera* (seeds extract). phenolic compounds were lower than *T. hirsuta* (catechin =772.55 µg/g plant, gallic acid= 303.77 µg/g plant and chlorogenic acid =114.88 µg/g plant), While coumaric acid has been identified at *C. procera* (seeds extract) only (21.08 µg/ g plant). *D. viscosa* phenolic compounds were chlorogenic acid (267.41 µg/ g plant) and gallic acid (84.43 µg/ g plant), while catechin was not detected.

Flavonoids contents at *T. hirsuta* were highest concentration followed by *D. viscosa* and *C. procera* (seeds extract) showed that, Naringenin and quercetin were major amounts at *T. hirsuta* with concentration 265.58 and 254.06 µg/g plant respectively, quercetin also was a major amount at *D. viscosa* (256.74 µg/g plant). As for the *C. procera* (seeds extract) two flavonoids only were detected, propyl gallate (44.82 µg/g plant) and cinnamic acid (11.12 µg/g plant).

Gallic and chlorogenic acids were constituents of hydrolysable tannins, which were defined as esters of gallic and chlorogenic acids; on the other hand, condensed tannins are oligomers and polymers formed by the polycondensation of two or more flavonoid units and have a great biological significance for their strong interactions with metallic ions and macromolecules, such as polysaccharides, besides presenting the ability to form soluble complexes with alkaloids, gelatin, and various proteins. This ability to interact

with proteins makes tannins very toxic to insects, fungi, and bacteria **(Simões *et al.* 2001).** In the three extracts, when concentrations of polyphenols increased the mortality decreased, which is the contrast with our expectations. This is in agreement with **Emanuele *et al.* (2014)** whom showed that the (TSSM) was found on strawberry fruits which reached with polyphenolic compound without any effects on mite, and suggested that TSSM infestation decreases fruit quality and that the biological control of TSSM using a predatory mite is a suitable alternative to organic production since the presence of predatory mite does not affect fruit quality and development. **Leszczynski *et al*. (1988)** who found that, no statistically significant correlations between leaf total phenol concentrations (including soluble and insoluble total phenols) and mite, and negatively correlated with p-coumaric acid also. Although he could not show that the hop leaf total phenols affected the mites, total phenols have been shown to have a negative effect on TSSM with peppermint **(Larson and Berry 1984).**

The difference between *Calotropis procera* seeds extract and the other two extracts in our study were coumaric acid, the high percentage of mortality in TSSM with seeds ext. maybe due to the appearance of coumaric acid in seeds extract. Dahrowski and Rodriquez (1972) suggested that, the concentrations of p-coumaric acid as low as 10^{-5} and 10^{-6} M reduced the feeding rate of TSSM and higher concentrations prevented feeding for 12 to 24 h. Although *Thymelea hirsuta* extract has a high concentration of polyphenols their effect on TSSM mortality was weak, that is due to "active" phenolics occur mostly as aglycones but most of the phenolic aglycones in plants are glycosylated and must be hydrolyzed or oxidized for maximum effectiveness (Levin, 1971).

Finally, plants extract had many groups of compounds with polyphenols, for that we will study these groups of compounds in the future and evaluate their effects on TSSM. Because compounds are present chemical control of mites is often costly, and the excessive use of pesticides damage the environment and harms human health, biological control by natural plant extracts are worth study.

Table 7: HPLC analysis of Polyphenols concentrations in methanol extract of *Thymelaea hirsuta.*

Thymelaea hirsuta Extract (1 gm. / 50 ml.)			
Polyphenoles	**Area**	**Conc. (µg / ml)**	**Conc. (µg / 50 ml = µg / g plant)**
Gallic acid	307.35	21.34	1066
Chlorogenic acid	266.33	16.79	839.
Catechin	194.16	32.18	1609
Caffeine	0.00	0.00	0.00
Coffeic acid	38.88	1.28	63.8
Syringic acid	29.66	1.11	55.3
Rutin	0.00	0.00	0.00
Pyro catechol	0.00	0.00	0.00
Ellagic acid	0.00	0.00	0.00
Coumaric acid	0.00	0.00	0.00
Vanillin	0.00	0.00	0.00
Ferulic acid	0.00	0.00	0.00
Naringenin	131.76	5.31	265.
Propyl Gallate	200.37	4.80	239.
4`.7-DihydroxyisoFlavone	106.18	2.63	131.
Querectin	63.62	5.08	254.
Cinnamic acid	93.37	0.78	39.1

Table 8: HPLC analysis of Polyphenols concentrations in methanol extract of *Calotropis procera* (seeds).

Calotropis procera (seeds) Extract (1 gm. / 50 ml.)			
Polyphenoles	**Area**	**Conc. (µg / ml)**	**Conc. (µg / 50 ml = µg / g plant)**
Gallic acid	87.52	6.08	303.
Chlorogenic acid	36.44	2.30	114.
Catechin	93.21	15.45	772.
Caffeine	0.00	0.00	0.00
Coffeic acid	18.19	0.60	29.8
Syringic acid	0.00	0.00	0.00
Rutin	0.00	0.00	0.00
Pyro catechol	0.00	0.00	0.00
Ellagic acid	0.00	0.00	0.00
Coumaric acid	19.10	0.42	21.0
Vanillin	0.00	0.00	0.00
Ferulic acid	0.00	0.00	0.00
Naringenin	0.00	0.00	0.00
Propyl Gallate	37.42	0.90	44.8
4`.7-DihydroxyisoFlavone	0.00	0.00	0.00
Querectin	0.00	0.00	0.00
Cinnamic acid	26.51	0.22	11.1

Table 9: HPLC analysis of Polyphenols concentrations in methanol extract of *Dodonaea viscosa*

***Dodonaea viscosa* Extract (1 gm. / 50 ml.)**			
Polyphenoles	**Area**	**Conc. (µg / ml)**	**Conc. (µg / 50 ml = µg / g plant)**
Gallic acid	30.53	1.69	84.43
Chlorogenic acid	91.15	5.35	267.41
Catechin	0.00	0.00	0.00
Caffeine	0.00	0.00	0.00
Coffeic acid	0.00	0.00	0.00
Syringic acid	0.00	0.00	0.00
Rutin	42.71	5.18	258.95
Ellagic acid	0.00	0.00	0.00
Coumaric acid	0.00	0.00	0.00
Vanillin	34.28	0.97	48.42
Ferulic acid	0.00	0.00	0.00
Naringenin	29.48	1.25	62.55
Propyl Gallate	27.44	1.05	52.51
4`.7-DihydroxyisoFlavone	64.08	1.30	64.90
Querectin	80.00	5.13	256.74
Cinnamic acid	89.54	0.64	32.23

Conclusion

From the present study, it can be concluded that, after 72 h, the highest mortality 93.33% was recorded by treatment with seeds of *C. procera* followed by leaves of *D. viscosa* 86.67% at the concentration 0.01% for adult females under laboratory conditions. While under greenhouse conditions the highest reduction percentage of hatchability for eggs and mortality percentage of other tested stages of larva, nymphs and adult females of *T. urticae* was implemented by using *C. procera* (seeds extract) followed by *D. viscosa*, *T. hirsuta*, *C. procera* (leaf extract) and *L. guyonianum* but the lowest percentage of hatchability for eggs and percentage mortality with *Zygophyllum album* in both concentrations 0.01 and 0.005 %. The highest mortality percentage of TSSM with seeds extract may be due to the appearance of coumaric acid in seeds extract, it recorded the highest level compared to other extracts.

REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. J. Econ. Entomol.; 18: 265-267.

Aslan, I.; H. Ozbek; O. Calmasur and F. Sahin (2004). Toxicity of essential oilvapours to two greenhouse pests, *Tetranychus urticae* Koch and *Bemesia tabaci* Genn. Ind. Crop Prod. 19(2): 167-173.

Babbar, N.; H.S. Oberoi; S.K. Sandhu and V.K. Bhargav (2014). Influence of different solvents in extraction of phenolic compounds from vegetable residues and their evaluation as natural sources of antioxidants. J. Food Sci. Technol., 51, 2568–2575.

Boyd, D.W.; and D.R. Alverson (2000). Repellency effects of garlic extracts on two spotted spider mite, *Tetranychus urticae* Koch. J. Entomol. Sci.35: 86_90.

Carry, N.C. (2004). Statistical analysis system, SAS user's guide: Statistics. SAS

Institute.

Chiasson, H.; A. Betanger; N. Bostanian ;C. Vincent and A. Poliquin (2001).Acaricidal properties of *Artemisia absinthium* and *Tanacetum vulgare*(Asteraceae) essential oils obtained by different methods of extraction. J. Econ. Entomol. 94: 167-171.

Dabrowski, Z. T., and J. G. Rodriquez (1972). Gustatory responses of *Tetranychzus urticae* Koch to phenolic compounds of strawberry foliage. Zesz. Probl. Postep. Nauk Roln, (129), 69-78.

Derbalah, A. S. H. (1999). Integrated pest management of spider mites.M.Sc. Thesis, Fac. Kafr El-Sheikh, Tanta Univ., pp.158.

Dürdane Y.; K. l. Izzet and A. Gökçe (2011). Acaricidal effects of different plant parts extracts on two-spotted spider mite (*Tetranychus urticae* Koch). African Journal of Biotechnology Vol. 10(55), pp. 11745- 11750 .

Emanuele, L.; A. S. Raul; J. F. Noeli and F. V. d. S. Claucia (2014).
Physicochemical and nutritional alterations induced by two-spotted spider mite infestation on strawberry plants. Electronic Journal of Biotechnology, 17: 193-198.

Hamdy, A.; F. A. Ayad and A. H. El-sebae (2009). Structure Activity Relationship For the Acaricidal Potency of Plant Extracts and Their Main Terpinoides on Two-Spotted Mite *Tetranychus urticae* (Acari:Tetranychidae). https://www.researchgate.net/ publication/216073934_Acaricidal_Activity of Plant Extracts and ThereMain Terpenoids on the two Spotted Spider Mite Tetranychus Urticae Acari Tetranychidae.

Helle, W. and M. W. Sabelis (1985). Spider mites: Their biology, natural enemies and control. Elsevier, Amsterdam, Volume 1 Part A. 406 pp..

Isman, M.B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol. 51: 45- 66.

Islam, M. T.; M. M. Haque; N. Naher and S. Parween (2008). Effect of Plant Materials on Developmental Periods of Two Spotted Spider Mite, *Tetranychus urticae* Koch (ACARI: Tetranychidae). J. bio. sci. 16: 121-124.

Kapil, K. B.; G. K. Saikia; M. K. Deka; B. Phukan and S. C. Barua (2017).Evaluation of indigenous biopesticides against Red Spider Mite, *Oligonychus coffeae* (Nietner) in tea. Journal of Entomology and Zoology Studies 2017; 5(2): 731-735.

Labanowska, B. H. (1990) Effectiveness of some new generation acaricides in the control of two-spotted spider mite (*Tetranychus urticae* Koch) on strawberries. Fruit Science Report (Poland) 17(3): 137-147.

Larson, K. C. and R. E. Berry (1984). Influence of peppermint phenolics and monoterpencs on two spotted spider mite (Acari: Tetranychidae). Environ. Entomol. 13, 282-285.

Lakhdari, W.; A. Dehliz; F. Acheuk; A. Soud ; H .Hammi; R. Mlik and B. D. Mitiche (2015). Acaricidal Activity of Aqueous Extracts against the mite of date palm *Oligonychus afrasiaticus* Meg (Acari: Tetranychidae). Journal of Medicinal Plants Studies. 3(6): 113-117.

Levin, D. A. (1971). Plant phenolics: an ecological perspective. Am. Nat. 105, 157- 181.

Leszczynski, B.; L. C. Wright; W.W. Cone and S. T. Kenny (1988). Hop leaf phenolics and resistance to the two spotted spider mite. J. Agric. Enlomol. 5(4): 257-266.

Martinez-Villar, E.; F. S. de-Cabezon ; F. Moreno-Grijalba ;V. Marco and I. Perez-Moreno (2005). Effects of azadirachtin on the Two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). Exp. Appl. Acarol. 35: 215-222.

Nauen, R.; N. Stumpf ; C.P.W. Elbert; A. Zebitz and V.Winkler (2001). Acaricide toxicity and resistance in larvae of different strains of *Tetranychus urticae* and *Panonychus ulmi* (Acari: Tetranychidae). Pest manag. Sci. 57: 253-261.

Pritam, S. and G. K. Clare (1993). A method for continuous production of diapausing two-spotted mite in the laboratory. The Horticulture and Food Research Institute of New

Zealand, vol.16.

Radhakrishnan, B. and P. Prabhakaran (2014). Biocidal activity of certain indigenous plant extracts against red spider mite, *Oligonychus coffe*ae (Nietner) infesting tea. J. Biopest 7(1):29-34.

Rana, A. ;M. Soysal and E. Hassan (2015). Toxic and repellent effects of *Prunus laurocerasus* L. (Rosaceae) extracts against *Tetranychus urticae* Koch(Acari: Tetranychidae). Türk. entomol. derg., 39 (4): 367-380.

Simões, C.M.O.; E.P. Schenkel; G. Gosmann; J.C.P. Mello; L.A. Mentz and P.R. Petrovick (2001). Farmacognosia: da planta ao medicamento. Editora da Universidade Federal do Rio Grande do Sul, Porto Alegre, Brasil.

Stumpf, N.; C.P.W. Zebitz ;W. Kraus; G.D. Moores, and R. Nauen (2001).Resistance to organophosphates and biochemical genotyping of acetylcholinestrases in *Tetranychus uritcae* (Acari: Tetranychidae). Pestic. Biochem. Physiol. 69:131.

Sundaram, K.M.S. and L. Sloane (1995). Effects of pure and formulated azadirachtin, a neembased biopesticide, on the phytophagous spider mite, *Tetranychus urticae*. J. Environ. Sci. Health, Part B (Pestic. Food Contam. Agric. Wastes), 30: 801-814.

Tsagkarakou, A.; M. Navajas; J. Lagnel ; J. Gutierrez and N. Pasteur (1999).Genetic variabitity in *Tetranychus urticae* (Acari:Tetranychidae) from Geece: insecticide resistance and isozymes. J. Econ. Entomol. 89(6):1354-1358.

Van, L. W. T.; S. P. Van and L. Tirry (2005). Comparative acaricide susceptibility and detoxifying enzyme activities in field-collected resistant and susceptible strains of *Tetranychus urticae*. Pest Manag. Sci., 61(5): 499- 507.