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TOXICITY AND BIOCHEMICAL EFFECT OF SOME PLANT EXTRACTS AGAINST THE TWO-SPOTTED SPIDER MITE *TETRANYCHUS URTICAE* (KOCH). (ACARI: TETRANYCHIDAE)

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Abstract

The two spotted spider mite *Tetranychus urticae* Koch is a very destructive pest causing considerable loss to crops. Adult females were treated for seven days, under laboratory conditions, with three acetone crude extracts to evaluate their acute effects (during 7days after treatment) and it –s efficacy as natural pesticides. The used plant extracts were lupine (*Lupinus termis*), lemongrass (*Cymbopogon citratus*) and Chilli pepper (*Capsicum baccatum*). The results indicated that lupine exhibited the highest toxic action against adult females followed by lemongrass and chili pepper. Bioassay testes using leaf dipping technique indicated that LC_{50} of lupine, lemon grass and chili pepper was 1.44, 4.71 and 5.255 gm%, respectively. Lupine and lemongrass extracts were selected to test their acute effects against *T. urticae* adult females after 24, 48 and 72 hrs post treatment. Treatment of mites by LC_{50} of lupine, lemongrass crude extracts led proteins to be significantly reduced after 24 hrs as compared with control. The decrease was continued after 72 hrs post treatment. Carbohydrates as the main energy source were, more or less, affected by the same manner as proteins, while there was slightly reduction in lipids.

Key words: *Tetranychus urticae* (Koch.), plant extracts, Lupine, Lemon grass, Chili pepper, Proteins, Carbohydrates, Lipids.

Introduction

Two-spotted spider mite (TSSM), *Tetranychus urticae*, is widely distributed worldwide and is a common pest of many plant species in greenhouses, nurseries, orchards and field crops (Jeppson *et al.,* 1975). The mite causes plant damages by piercing the plant cells and sucking out the contents. The damaged cells appear as yellowish white spots (chlorophyll is destroyed) on the upper surface of leaf. As population increases, the whole leaf eventually turns yellow. Nymphs and adults produce webs and if the population is high the plant can be completely covered with webs. At this point, using miticides is necessary to control the TSSM. But the intensive use of synthetic pesticides in the recent years did not meet the criteria of integrated pest management programs (IPM). There is a growing concern globally, over the continuous use of synthetic chemicals on food crops because of their potential effects on human health and the environment. Mite resistance is another problem as a result of continuous use of synthetic pesticides.

Spider mites are the common mites attacking economic plants and the two-spotted spider mite *Tetranychus urticae* Koch. is one of the main pests of green houses as well as on field grown crops (Hassan, 2003). A number of vegetable crops such as tomatoes, squash, eggplant, cucumber are also subjected to the two spotted spider mite infestation and damage.

The toxicity of plant extracts has been studied against the *T. urticae* (Abd-Elmohsin, 2015 and Sakunwarin 2004), *phytoseiulus persimilis* A-H (Abd-Elmohsin, 2015), *Aphis gossypii* Glover (Fadl, 2013) and *Pisum sativum* L. (Bakr, 2003).

Components of plant extracts used as pesticide control are known to affect biochemical compounds (Khosravi and Sendi 2013; Yacoub, 2013; Mohamed, 2014).

So, this present work is conducted to make proof that plant extracts can be a good agent to use in IPM program control of *T. urticae*.(Koch).

Materials and Methods

Rearing of *T. urticae* Koch

The original colony of the two spotted spider mite *T. urticae* in this work was supplied from Acarology Laboratory Dept. of plant protection Research Institute,

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It was reared a test mite for several generation at 25 ± 1°C away from any pesticide contamination. The mite was maintained on detached mulberry leaves with the lower surface up wards placed on moist cotton wool pads in fiber-dishes (20 cm in diameter). The cotton pads were moisted daily to avoid disc dryness, and the prevent mite escape Mulberry leaves were changed by fresh one from time to time when necessary (Hassan 2009).

Extractions

The tested plants were lupine (*L. termis*), lemon grass (*C. citratus*) and chilli pepper (*C. baccatum*) were purchased from local market.

The plants used in the present experiments are illustrated in Table 1.

Table 1: List of used plants in this work.

Common name	Scientific name	Arabic name	Parts used
Lupine	*Lupinus termis*		Seeds
Lemon grass	*Cymbopogon citratus*		Herb
Chilli pepper	*Capsicum baccatum*		Fruits

The tested plant were dried and grinded in electric mill, then 250 gm of each material powder were soaked in (750 ml) of absolute acetone for 72 hours in dark colored bottles with tight stoppers. Using (rotary evaporator) to release the solvent under pressure.

The crude extracts were kept in glass bottles in refrigerator for biological and chemical tests.

Bioassay

Prepare for bioassay tests several of aqueous concentrations of lupine, lemon grass and chili pepper crude extracts were made in 0.1% Tween 80 (added as surfactant). Leaf dipping method was used to tested the toxicity. Leaves of mulberry discs were dipped in the tested solutions for 10 seconds then left to dry at room temperature. Then 20 adult females were placed on a single leaf-disc of mulberry (2.5 cm in diameter) and were kept on moist cotton wool in fiber dishes, each dish contained 5 discs as replicate.

Discs were treated with 3 concentrations of the plant extracts. The treated adult females of mite, *T. urticae* and control were incubated at 25±1°C. Percentage mortality was determined daily after treatment till the seventh day. The mortality percentages were corrected according to Abbott's formula (Abbott, 1925). LC_{25}, LC_{50}, LC_{90} and slope values were calculated according to (Finney, 1971) using "Ldp line" software by (Bakr 2000).

The relative efficacy of the tested pesticides were determined according to (Sun, 1950) as follow:

$$\text{Toxicity index} = \frac{LC_{50} of\ the\ compound\ (A)}{LC_{50}\ of\ the\ compound\ (B)} \times 100$$

Where:

(A) = is the highest effective compound

(B) = is the lowest effective compound

Biochemical and physiological analysis

Spider mites are minute so the number of mites needed in order to study these changes were 1000 adult females placed in a 1.5 micro tube.

Characterization of biochemical and physiological changes of adult females after treatment:

Lupine and lemon grass crude extracts (the efficient extracts) were chosen to test the acute effect of their LC_{50-} values on some biochemical component of the treated mites.

Preparation of mites for analysis

(Amin, 1998) describe the preparation of mites. They were homogenized in distilled water (50 mg/1mL). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 2°C in refrigerated centrifuge. The deposits were discarded and the supernatants, which is referred as enzyme activity when stored at 5°C.

Determination of total proteins

Total proteins determined by the method of Braford (1976) protein reagent was prepared by dissolving 100 mg of coomassie Billiant blue G-250 in 50 mL 95% ethanol. To this solution 100 mL of phosphoric acid (85% w/v) were added.

The resulting solution, (50 mL) or for preparation of standard curve 50 mL of serial concentrations containing 10 to 100 mg boving serum albumin were pipetted into test tubes the absorbance at 595nm was measured after 2min and before 1hr against blank prepared from 1mL of phosphate buffer and 5mL protein reagent.

Determination of total carbohydrates

Total carbohydrates were estimated in acid extract of sample females of *T. urticae* by the phenol-sulfuric acid reaction of Dubois *et al.,* (1956). Total carbohydrates were extracted and prepared for assay according to Crompton and Birt (1967). Sample of mites were homogenized in 0.3 $HCLO_4$ (5mL) at 0°C for 1 min. in soluble matter was removed by centrifugation for 3 min at 2000 r.p.m. and washed twice in ice-cold $HCLO_4$

(4mL) by re-dispersion and centrifugation. Hundred micro-liters of the acid extract were added into a colorimetric tube to 0.5 mL of phenol then 5mL of concentrated sulfuric acid were added rapidly with shaking. The tubes were allowed to stand 10 min., then they were shaken and placed for 10-20 min in 25 to 30°C before readings.

The absorbance of characteristic yellow-orange color is measured at 490 nm against blank. Total carbohydrates expressed as: Mg glucose/1000 individuals.

Determination of total lipids

Total lipids were estimated by the method of Knight *et al.,* (1972) using phosphovanillin reagent prepared by dissolving of 0.6 mg pure vanillin in 10mL ethanol and completed to 100 mL with distilled water, then 400 mL conc. Phosphoric acid were added 20 mL of sample were added to conc. Sulphuric acid (5 mL) in a test tube and heated in a boiling water bath for 10 min. after cooling to room temperature, the digest was added to phosphovanillin reagent (6 mL). After 45 min, the developed color was measured at 525nm against reagent blank. Optical density was compared to that of a reference standard and results expressed as Mg lipids/1000 individuals.

Statistics

The results were analyzed by one-way analysis of variance (ANOVA) using SAS for regression analysis (SAS Institute, 2006). When the ANOVA statistics were significant ($P < 0.01$), means were compared by the Duncan's multiple range test.

Results

Toxicological effect

Table 2: The effect of crude plant extracts against adult females of *T. urticae.*

Plant extract	LC_{50}	r	Slope	LC_{90}
Lupine	1.4419	0.9583	1.6203	8.9106
Lemon grass	4.7122	0.999	1.8732	22.7714
Chilli pepper	5.2557	0.9991	1.5859	33.7891

Plant extracts are promising alternative natural products for the control of spider mite, *T. urticae* Koch. These extracts facilitate the handling and its application, besides they can be an option of lower cost in relation to the using chemical control.

Obtained results in Table 1 showed that *T. urticae* were affected by the tested plant extracts. The LC_{50}– s could be arranged in the following exponentially order: 1.44, 4.71 and 5.255 gm% while, LC_{90} values were 8.91, 22.77 and 33.78 gm% for the tested extracts; lupine, lemon grass and chili pepper, respectively, 7 days after treatment. It also an evidence the lupine exhibited the highest toxic action against adult females of *T. urticae* while, chili pepper was the lowest toxic action one.

Fig. 1. Cleared that the concentration mortality regression lines of lupine lemon grass and chili pepper.

The effect of tested plant extracts on the principle metabolites

The results in Table 2 revealed that the effect of lupine crude extraction on the main metabolites of treated *T. urticae* adult females. The mites were treated by LC_{50} of lupine extract showed that total protein significantly decreased. The obtained results were 130, 110 and 88 Mg/1000 individuals respectively, after 24, 48, 72 hrs.

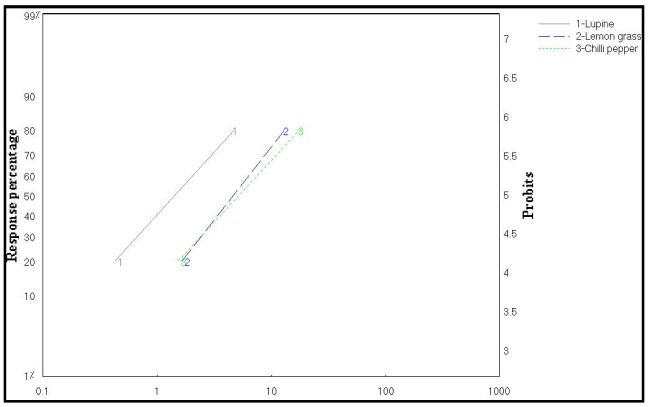

Fig. 1: Concentration –mortality regression lines of adult females *T. urticae* treated with acetone extracts of three different crude plant extract for 7 days.

Table 2: The effect of lupine and lemon grass (LC_{50}) on total proteins, total carbohydrates and total lipids contents in treated adult females of *T. urticae*.

Plant extract	Means ± SD								
	Total Proteins			Total Carbohydrates			Total Lipid		
	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
Lupine	130± 8.54b	110.33±8.14c	88±3.00c	92 ±2.00b	84±4.00 b	72.33±2.52c	62.66±2.52 b	53±2.52b	42.66±1.53c
lemon grass	136±4.36 b	128.00±3.00b	125±2.52b	95.66± 4.04 ab	88.33± 1.53ab	85.66±2.08b	68±1.00 a	57± 1.15 b	48.66± 22.52b
Control	151±1.00 a	133.66 ±1.00a	135.66±1.53a	99±1.53 a	93.±1.53a	90.66 ±1.53a	68.33±0.58 a	67.66±1.15 a	66±1.53a
LSD	7.7378	4.8483	4.848	5.3277	5.8057	3.2625	2.7458	4.9389	5.1154

Values represent of letters separated groups SD, ≤ 0.01 high significant.

These results were decreased by .16, 17, 34% after treatment compared with the untreated samples. carbohydrates is the main energy source was also decreased after treatment it is clear that was 72 Mg/ 1000 individuals after 72 hr post treatment compared with 90 Mg/1000 individuals at untreated.

Also data in table 2 revealed that the mean total lipid were 62, 53 and 42 Mg/1000 individuals after 24, 48 and 72 hrs respectively.

Compared with the results of untreated adult females that were 68 , 67and62 Mg/1000 individuals respectively.

Discussion

The obtained results of bioassay demonstrate that lupine seeds extract in this study showed a great effect on females *T. urticae*. The extract caused biological activities and physiological damage.

Geng *et al.,* (2014) reported that the garlic-straw extracts (20, 10, 5, 2.5 and 1.25 g/L) were tested against adult females of *T. urticae* in the laboratory and the 20 g/L concentration caused 76.5% Mortality 48 hr after treatment and that was similar to obtained data.

The highest reduction of density of *Tuta absolute* was recorded by treatment of garlic extract followed by lemon grass extract. Lemon grass extract significantly increased L-ascrobic acid (Vitamin C) contents in tomato and garlic extract recorded the highest values of total phenolic compounds and total flavonoids. That was reported by Hussein Nehal *et al.,* (2015).

These results also agree with Manzoor *et al.,* (2011). They concluded that lemongrass and Datura were tested against three stored grain pests *Oryzaephilus surinomensis Tribolium castaneum* and *Callosobruchus chinensis*.

Datura extract showed maximum mortality of (21.43%) in *Tribolium castaneum* and lemongrass showed maximum repellency against *Callosobruchus chinensis*.

Fadl (2013) determined the insecticidal activity of Lupine extract, olive oil, Marjoram oil, Anise oil and orange oil gainst two strains of *Aphis gossypii* and *Rhopalosiphum maidis* (Fitch). Mead (2012) mentioned that the toxic effect of *Chymbopogon citratus* essential oil against *T. urticae* was undoubtedly due to citral (geranial and neral).

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