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Toxicity and acute macromolecular abnormalities induced by some plant extracts against the Cowpea aphid; *Aphis craccivora*, Koch."

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ABSTRACT

The Cowpea aphid; *Aphis craccivora*, adult female was treated for five days, under laboratory conditions, with acetonic crude plant extracts to evaluate their acute effects (during 72hr post treatment) and efficiency as natural insecticides. The used plant extracts were Lupine (*Lupinus termis*), Lemon grass (*Cymbopogon citratus*), Moringa (*Moringa oleifera*) and Chillipepper (*Capsicum baccatum*). The results indicated that Lupine was the most efficient followed by Lemon grass. Bioassay testes using leaf dipping technique indicated that LC_{50} of Lupine, Lemon grass, Moringa and Chillipepper was 2.6, 5.2, 13.2, and 38.68gm %, respectively. Lupine and lemon grass extracts were chosen to test their acute effects on aphid adult after 24 and 72 hr post treatment. Treatment of aphids by LC_{50} of Lupine and lemon grass crude extracts led proteins to be significantly reduced after 24 hr as compared to control. The decrease was continued after 72 hr post treatment. This means that plant extracts exert their effect after relatively short time. Carbohydrates as main energy source were, more or less, affected by the same manner as proteins, while there was no reduction in triacelyglycerols content. On the other hand, treatment activated defensive enzymes like estereses and phenoloxidases. Lupine affected the main metabolites, especially proteins and its metabolizing enzymes more than Lemon grass. Besides, reaction of defensive enzymes was more higher in aphids treated with lemon grass than that treated with lupine. This might explains why lupine was more efficient than lemon grass.

keywords; *Aphis craccivora,* (Koch.), plant extracts, Lupine, Lemon grass, main metabolites, defensive enzymes.

INTRODUCTION

The cowpea aphid, *Aphis craccivora*, Koch (Homoptera: Aphididae) is one of the most serious sucking insect pest infested Legumes crops in Egypt,(Negugi *et al.,* 1985 and Attia *et al.,* 1986). They feed by sucking plant sap causing leaf curl, wilting, stunting in shoot growth, reducing final yield as well as a general decline in plant vigor. Some aphids are also vectors of plant diseases as soybean mosaic virus (SMV) (Halbert *et al.*, 2008). The cowpea aphid, *A. craccivora* is a good example of aphids causing serious problems to legumes (Abdel-Rahman *et al.*, 2007).

Considering the adverse effect of synthetic insecticides use for pest control has serious drawbacks (Ignacimuthu, 2002) such as the development of resistant strains, toxic residues, workers' safety and increasing costs(Hasan, 2014). Hence, pest control strategies for the future need reviewing and safer alternatives may need to be sought. In recent years, there have been concerted international efforts at developing non-toxic, safe and biodegradable alternatives to synthetic insecticides (Ofuya, 1997). Pest management through alternative insecticides as use research in recent years has been turning more towards selective bio-rational pesticides, generally perceived to be safer than the synthetics and extensive works on the use of plant extracts in pest control have also been documented.

The toxicity of plant extracts has been studied on other target pests such as *Tetranychus urticae* Koch. (Abd-ELmohsin ,2015 and Sakunwarin, 2004), *Phytoseiulus persimilis* A.-H (Abd-ELmohsin ,2015) *Aphis gossypii* Glover (Fadl ,2013), *Anagasta kuehniella* Zeller (Oliveira *et al.* 2011) and *Pisum. Sativum* L. *(*Bakr ,2003).

Compounds of plant origin used in insect pest control are known to affect digestive enzymes and biochemical compounds (Khosravi and Sendi 2013; Yacoub, 2013; Mohamed, 2014) . Nada and Gaffar (2012) reported that insecticidal activity of plant extract affected lipid, protein and carbohydrates.

Therefore, the present work was carried out as an attempt to investigate certain alternative agents to be incorporated into IPM control of *A. craccivora* by determining toxicity of some plant extracts.

MATERIALS AND METHODS

The Cowpea aphid; *Aphis. Craccivora,* Koch. adult female was treated for five days, under laboratory conditions, with acetonic crude plant extracts to evaluate their acute effects (during 72hr post treatment) and efficiency as natural insecticides. The used plant extract were Lupine (*Lupinus termis*), Lemon grass (*Cymbopogon citratus*), Moringa (*Moringa oleifera*) and Chillipepper (*Capsicum baccatum*).

Tested insect:

The Laboratory strain of *A. craccivora* was obtained from a colony cultured in laboratories of sucking insects department. Plant Protection Research Institute, ARC, Dokki, Giza, Egypt. The colony was reared in the laboratory on faba bean leaves in plastic pots (50X50X200 cm) and maintained under constant conditions(continuous illumination using two fluorescent bulbs (40 watt) at 25 ± 1°C). After four days growing faba bean were infested by aphid individuals which transferred from infested faba bean plant to a new ones for feeding.

Extractions:

The tested plants were lupine(*L. termis*) , lemon grass(*C. citratus*), moringa (*M. oleifera*) and chiipepper (C. *baccatum*) were purchased from local market. The plants used in the present experiments are illustrated in Table(1).

Table(1). The plants which are used in the present experiments:

Common name	Scientific name	Arabic name	part used
Lupine	*Lupinus termis*	الترمس	Seed
Lemon grass	*Cymbopogon citratus*	حشيشة الليمون	Herb
Moringa	*Moringa oleifera*	المورينجا	Leaves
Chillipepper	*Capsicum baccatum*	الشطة	Fruit

The tested dried plants were grinded in electric mill ,then 250 gm of plant powder were soaked in (750 ml) of absolute acetone for 72 hours in dark colored bottles provided with tight stoppers. The solvent was evaporated under pressure using (rotary evaporator). The resultant crude extracts were stored in glass containers in refrigerator for biological and chemical tests.

Bioassays:

For bioassay test, series of aqueous concentrations of Lupine , Lemon grass , Moringa and Chiipepper crude extracts were prepared in 0.1 % Tween 80 (added as surfactant). Leaf dipping method was used to tested the toxicity. Leaves of faba bean were dipped in the tested material solutions for 10 second then left to dry at room temperature, then ten aphid adults were added to each leaf .Treated leaves were put separately in ten plastic boxes (7×7×3.5 cm). Faba bean leaves were dipped in 0.1 % Tween distilled water 80 and used as control . Each treatment had ten replicates (ten individuals in each of which). The uniformed aged adult aphid (one old day) were maintained under laboratory conditions. Percentage mortality was assessed daily after treatment till the fifth day. The mortality percentage was corrected according to Abbott's formula (Abbott, 1925).

LC_{50}, LC_{90} and slope values were calculated according to (Finney ,1971), using "Ldp line" software by (Bakr 2000). The relative efficiency of the tested pesticide was determined according to (Sun, 1950) as follow:

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

Where:

(A) =is the highest effective compound

(B)= is the lowest effective compound

Biochemicals studies:

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 aphids. The aphid adults (0.2gm) were collected by using fine camel hair brush after one and three days post treatment, and kept in small bottles and kept in freezer till analysis operation time.

Apparatus :

Insects were homogenized for biochemical analysis in a chilled glass Teflon tissue homogenizer (ST – 2 Mechanic-Preczyina , Poland). After homogenation , supernatants were kept in a deep freezer at -20ºC till use for biochemical assays . Double beam ultraviolet / visible spectrophotometer (spectronic 1201 , Milton Roy Co. ,USA) was used to measure absorbance of colored substances or metabolic compounds .

Preparation of insects for analysis :

The insects were prepared as described by **Amin** (1998). They were homogenized in distilled water (50 mg /1 ml). Homogenates were centrifuged at 8000 r.p.m. for 15 min at 2 ºC in a refrigerated centrifuge . The deposits were discarded and the supernatants, which is referred as enzyme extract, can be stored at least one week without appreciable loss of activity when stored at 5^0C .

Determination of total carbohydrates:

Total carbohydrates were estimated in acid extract of sample by the phenol-sulphuric acid reaction of Dubois *et al.*,(1956).

Total carbohydrates were extracted and prepared for assay according to Crompton and Birt (1967).

Determination of total proteins:

Total proteins were determined by the method of Bradford (I976). Protein reagent was prepared by dissolving 100mg of Coomassie Brilliant blue G-250 in 50ml 95% ethanol.

Triglycerides determination:

Triglycerides were assayed using Stanbio kit (Stanbio Laboratory ,Inc. 2930 East Houston street , San Antonio ,Texas 78202) . Triglycerides area generally determined by a combination of hydrolysis to glycerol and free fatty acids and measurement of the amount of glycerol released

Transaminases determination:

Glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were determined colorimetrically according to the method of Reitman and Frankle (1957).

Defensive enzymes assay:

Phenoloxidase activity was determined according to a modification of Ishaaya(I971), in a reaction mixture consisting of 0.5ml phosphate buffer(0.1 M,pH 7),200µl enzyme solution and 200µl catechol solution(2%). Prior to the initiation of the reaction , the substrate and other ingredients of the reaction mixture were separatel Non specific **Esterases***:*

Alpha esterases (α-esterases)and beta esterases(β-esterases)were determined according to Van Asperen(I962).

Statistics :

The results were analyzed by one – way analysis of variance (ANOVA) using costat statistical software (cohort software , Berkeley). When the ANOVA statistics were significant ($P < 0.01$), means were compared by the Duncan's multiple range test.

RESULTS

Toxicological effect :

Obtained results in Table (2) Showed that *A. craccivora* were affected by the tested plant extracts. The LC_{50} values were 2.6, 5.2 , 13.2 and 38.68 gm % while LC_{90} values were 18.67, 15.14 ,77.24 and 567.76 gm % for lupine, lemon grass, Moringa and Chillipepper, respectively, 5 days after treatment. It also proved that lupine and lemon grass were the most

efficient plant extracts against *A. craccivora* than other extracts. Fig. (1) cleared that the concentration - mortality regression lines of lupine, lemon grass, Moringa and Chillipepper .

Table (2) Toxicity of four crude plant extracts against apterous adult females *A. craccivora*

Plant extract	LC_{50} (Limits)	Index	Slope	LC_{90}
Lupine	2.6 (1.65 - 3.33)	100	1.49	18.67
Lemone grass	5.2 (4.6 - 5.9)	50	2.76	15.14
Moringa	13.2 (10.44 - 23.25)	19.6	1.67	77.24
Chilipepper	38.68 (18.1 - 76.2)	6.87	1.09	567.76

Effect on the main metabolites :

The present results in Table (3) indicated that the effect of lupine and lemon grass crude extracts on the main metabolites of treated *A. craccivora*. Treatment of aphids by LC_{50} of lupine and lemon grass extracts led total protein to be significantly decreased. It was 6.1,7.5 and 9.4 mg/g.b.wt for lupine ,lemon grass-treated aphids and control, respectively, after 24 hr post treatment. It decreased by 35.2 and 21.3 % for insects treated by lupine and lemon grass, respectively as compared to control. The results revealed that proteins were more decreased by lupine than lemon grass, and the decrease was continued reaching to 52.7 % decrease as compared to control after 72 hr post treatment with lupine .

Carbohydrates as the main energy source were more or less, affected by the same manner as proteins. The results cleared that total carbohydrates content in aphids was significantly decreased by the plant extracts treatment. It was 5 and 7.2 mg /g.b.wt after 24 and 72 hr post treatment with lupine, respectively, while it was 5.2 and 8.5 mg/g.b.wt for that treated with lemon grass, respectively. Control insects had carbohydrates content equaled to 6.6 and 10.3 mg/g.b.wt, respectively.

On the other hand, lemon grass had no effect on triacylglycerols content, while treatment with lupine led to significant increase in this type of lipids. The increase in treated insects was 160% more than control after 24 hr , and it was only 64 % after 72 hr .

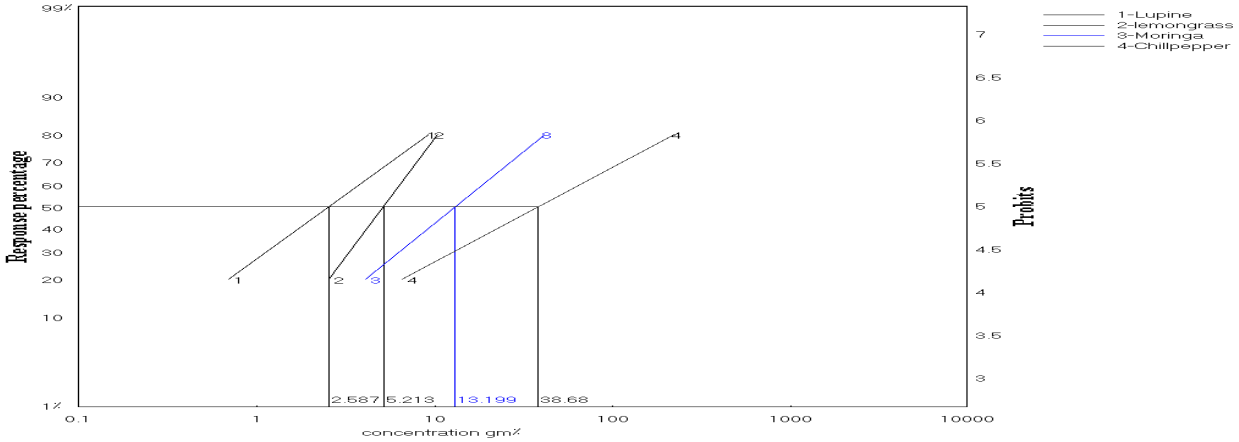

Fig.(1)Concentration- mortality regression lines of adult females *A. craccivora* treated with acetone extracts of four different crude plants extract for five days.

Table(3)Main metabolites content (mg/g.b.wt) of *A. craccivora* treated with LC_{50} of lupine and lemon grass crude extracts.

plant extract	Mean ± SD					
	Protein		Carbohydrate		Triacylglycerols	
	24hr.	72hr.	24hr.	72hr.	24hr.	72hr.
Lupine	6.1± 0.2 e	3.6± 0.5 c	5 ± 0.14 c	7.2± 0.3 c	16.9±0.89 a	27.6±1.9 a
lemon grass	7.5 ± 0.4 cd	6.1± 0.3 b	5.2 ±0.2 c	8.5± 0.3 b	6.36±0.55 c	17.6±0.81 bc
Control	9.4 ± 0.5 a	7.6 ± 0.3 a	6.6 ± 0.4 b	10.3 ± 0.5 a	6.5±0.45 c	16.8±1 bc

Means bearing different letters, within column, are significantly different (p< 0.01).

Enzymes related to protein metabolism :

The obtained results in Table (4) revealed that the effect LC_{50} values of lupine and lemon grass crude extracts on GOTand GPT activity of *A. craccivora.*

Transaminases was dramatically reduced after 72 hr post treatment with lupine and lemon grass . GOT activity was only 18 and 20.2 % as compared to control for lupine and lemon grass , respectively. GPT activity was 933,655 and 7476 U x 10^3 /g.b.wt for lupine ,lemon grass-treated insects ,and control, respectively at the same time . Treatment slightly altered transaminases during the first 24 hr post treatment .

Reaction of defensive enzymes: The data in Table (5) indicated that the LC_{50} effect of lupine and lemon grass crude extract on defensive enzymes of treated *A. craccivora*. The results revealed that treatment of aphids with LC_{50} of lupine and lemon grass extracts activated defensive enzymes such as esterases and phenoloxidases . In general , esterases titre was more higher in the case of treatment with lemon grass than that of lupine, and the activation was continued after 72 hr, except lupine . Alpha esterases activity was 109 , 148 and 78 ug α-naphthol/min/g.b.wt after 24 hr post treatment with lupine and lemon grass , and control , respectively . After 72 hr , the activity was 157,193 and 159 ,respectively . The same trend was observed for beta esterases and phenoloxidases .

Table (4)) Transaminases activity (U x 10^3 /g.b.wt) of *A. craccivora* treated with LC_{50} of lupine and lemon grass crude extracts.

plant extract	Mean ± SD			
	GOT		GPT	
	24hr.	72hr.	24hr.	72hr.
Lupine	1153 ± 50 c	636 ± 35.5 bc	4126 ± 196 d	933 ± 62 b
lemon grass	3126± 115a	701 ± 17 b	7684 ± 283 a	655 ± 34 bc
Control	3027 ± 141 ab	3456 ± 189 a	6936 ±270 b	7476 ±456 a

Means bearing different letters, within column, are significantly different (p< 0.01).

Table (5) Defensive enzymes of *A. craccivora* treated with LC_{50} of lupine and lemon grass crude extracts.

plant extract	Mean ± SD					
	Phenoloxidases(O.D. units x 10^3/min/g.b.wt)		Alpha esterases (ug α naphthol/min/g.b.wt)		Beta esterases(ug β-naphthol/min/g.b.wt)	
	24hr.	72hr.	24hr.	72hr.	24hr.	72hr.
Lupin	164± 6 b	238 ± 10.4 b	109± 3.6 cd	157 ± 4.9 c	75 ± 3.6 ab	72 ±4.4 e
lemon grass	201± 10.5 a	326± 18.2 a	148± 2.5 b	193 ± 6.2 b	62.3± 4.2 cd	117± 3 c
Control	107 ± 3.8 d	104 ±5 c	78 ± 4.9 e	159 ± 4.7 c	53.3± 3.8 de	65 ± 4.2 e

Means bearing different letters, within column, are significantly different (p< 0.01).

DISCUSSION

The results of our study clearly indicated that lupine and lemon grass crude extracts were the most efficient extracts against adult cowpea aphid. Furthermore, the two crude extracts had physiological effect on main metabolites and defensive enzyme system at LC_{50} values findings are in partial conformity with Sakunwarin (2004) He found that ethanol crude extracts from 7 plant species: Sugar apple, Neem, Citronella grass, Longan ,Cubé root, Chinaberry and Sweet oleander were able to reduce the survival rate when compared to the control against. *T. truncatus* . While Shehawy (2007) Tested the insecticidal activity of Ethanolic and Acetonic extracts of some plant extract against five species of aphids*; A. craccivora*, *A. gossypii*, *Myzus persicae* (Sulz.) and *Rhopalosiphum padi* (Linnaeus) in the laboratory. It was found that all extracts of these plants have insecticidal effect against all species of aphids according to the concentration as well as solvent used. He indicated that the acetonic extracts of Lupine was the most potent against all species of aphids followed by Fenugreek seeds, Black pepper, Ginger rhizomes, then Demsesa . Fadl (2013) Determined the insecticidal activity of Lupine extract, Olive oil, Marjoram oil, Anise oil and Orange oil against two strains of *A. gossypii* and *Rhopalosiphum maidis* (Fitch). All botanical oils had toxic effect against tested aphid, but LC_{50} differed according to the strain.

Seventeen plants extract were evaluated against the two-spotted spider mite *T. urticae* Koch. According to their LC_{50} and LC_{90} values, the best acaricidal activity of the tested seventeen crude plant extracts against *T. urticae* adults were Chilli pepper, lemongrass, Fennel, Coriander and Dill while against *T. urticae* eggs were Chilli pepper and Fennel(Abd-ELmohsin ,2015).

These results also agree with Medhini *et al.* (2012) .They Concluded that the plant extract reduced the total protein and carbohydrate in heamolymph *Spodoptera litura* (Fabricius) laravea. Khosravi and Sendi (2013)Stated that compounds of plant origin used in insect pest control are known to affect digestive enzymes and biochemical compounds. The neem insecticide exhibited a significant antifeedant activity when used at the highest concentration. Neem was found to affect digestive enzyme activities of treated mulberry pyralid larvae *Glyphodes pyloalis* (Walker). Biochemical compounds in the hemolymph, such as protein, lipid, and glucose decreased compared with the control. Also Yacoub (2013) Found that certain plant extracts such as lantana and lemon grass leaves have a clear effect on the carbohydrates hydrolysis enzymes as amylase and trehalse *of S. littoralis*. These finding are disagree with Srour (2014) Found that methanolic Alphonso leaf extract did not affect total protein, carbohydrate and lipid content in treated cotton leaf worm larvae.

Fadl (2013) Found that the oils of Marjoram, Anise oil and Lupine extract had bioactivity on *R. maidis* and *A. gossypii* collected from Fayoum and El-Behayra Governorates. The treatment with plant oil, inhibited the bioactivity of α-esterase and β-esterase by different ranges according to the extract.

Methanolic Alphonso leaf extract significantly reduced activity of amylase, invertase and trehalase and significantly elevated activities of non-specific esterases, acetylcholinesterase (AChE), glutathione-S-transferase (GST) and peroxidase after 24 hours in treated cotton leaf worm larvae Srour (2014).

Lupine affected the main metabolites, especially proteins and its metabolizing enzymes more than Lemon grass. Besides, reaction of defensive enzymes was more higher in aphids treated with lemon grass than that treated with lupine. This might explains why lupine was more efficient than lemon grass.

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التأثير السام والبيوكيميائى لبعض المستخلصات النباتية على آفة من البقوليات

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تم قياس فعالية مستخلصات نبات الترمس وحشيشة الليمون والمورينجا والشطة على الطور البالغ من آفة المن وذلك لتقييم تأثيرها السمي والبيوكيميائي.وقد اظهرت النتائج ان معاملة المن لمدة ٥ أيام بالتركيز النصف قاتل (ت ق.٥٠) من المستخلص المحضر بواسطة مذيب الأسيتون ان مستخلصى الترمس وحشيشه الليمون هما الاكثر فاعلية على الحشرة من باقي النباتات .وكان ت ق.٥٠ مساويا ٢,٦ و٥,٢ و ١٣,٢ و ٣٨,٨٦ جرام % لمستخلص الترمس وحشيشه الليمون والمورينجا والشطة على التوالى .وقد وجد ان ا لتغيرات في جسم الحشرة على المستوى البيوكيميائى تبدا بعد ٢٤ ساعة من المعاملة بواسطة ت ق.٥٠ من مستخلصي الترمس وحشيشه الليمون وهذا يعتبر وقت قليل نسبيا بالنسبة للمعاملة بالنباتات . وبصفة عامة قد اثر الترمس معنويا على مستوى مكونات الايض الرئيسية وخاصة البروتين وأنزيماته وذلك أكثر من حشيشه الليمون .وعلى العكس من ذلك فقد ادت المعاملة بحشيشة الليمون إلى ارتفاع مستوى الإنزيمات الدفاعية مثل الاستيريزات فى الحشرات المعاملة وهذا قد يفسر لماذا الترمس أكثر فاعلية على الآفة .