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Natural Extracts and Their Chemical Constituents in Relation to Toxicity Against Whitefly (*Bemisia tabaci*) and Aphid (*Aphis craccivora*)

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Abstract: Experiments were carried out to determine the toxicity of leaf extracts of *Mentha microphylla* (Mint) and *Citrullus colocynthis* (Colothyn) either as crude extracts or as formulations. The efficacy of crude and formulation were tested against nymphes of whitefly, *Bemisia tabaci* and aphid, *Aphis craccivora*. $LC_{50's}$ of crude for Colothyn for first, second, third and pupae were 594.47, 558.00, 4437.90 and 4979.86 ppm respectively, while LC_{50} of formulation were 303.57, 430.77, 368.27 and 917.78 ppm respectively. For crude Mint $LC_{50's}$ were 309.53, 504.20, 653.89 and 1443.91 ppm respectively. But for formulation $LC_{50's}$ were 170.70, 178.69, 288.21 and 947.79 ppm respectively. Also the previous plant extracts were tested against *Aphis craccivora* $LC_{50's}$ of crude and formulation for Colothyn was 621.94 and 123.68 ppm respectively. LC_{50} of mint was 509.29 and 392.54 ppm for crude and formulation respectively. Generally, results obtained indicated that the formulated plant extracts are more toxic than crude extracts. The differences in chemical composition of the crude leaf extracts that may explain the observed differences in mortality. The study indicated that major chemical compounds were (phemanthrene ester, hexadecanoic acid methyl ester and octadecanoic acid methyl ester).GC/MS analysis revealed that the chemical components of the extracts explained the observed mortality which could reduce reliance on synthetic pesticides. Further study is needed to investigate performance of plant extracts components as new insecticides.

Key words: Mint, Colothyn, whitefly, aphid, GC/MS analysis.

INTRODUCTION

The development of resistance to existing conventional pesticides and the increasing public concern over environmental pollution and health hazards created by synthetic pesticides generate a great need for new types of pest control agent’s advantage with higher activity against the target pests, and lower impact on humans and environmental quality.

The sweetpotato whitefly, *Bemisia tabaci* (Gennadius) and aphid, *Aphis craccivora* (global) pests in greenhouses on many crops. Whiteflies and aphid cause severe yield reductions and reduce crop quality because of the development of black molds on honeydew. Development of resistance to known pesticide groups exacerbated the whitefly and aphid problem in many crops.

The use of plant products for pest control may impart a selective advantage to plants by inhibiting, repulsing, and even killing non-adapted organisms that feed upon, or compete with the plant. Repellence, which involves pushing pests away from growing plants, has three advantages:

1- Reduction reliance of synthetic pesticides.
2- Reduce chance for pesticide adverse environmental impacts.
3- Reduce pesticide residues on crops reaching the consumers

Along the past few years, several Egyptian wild plants were screened for their content of biological activity against insects. Pesticidal activity of Egyptian plants extracts and oils were investigated by Saleh *et al*., 1986 (a,b); Salem, 1995: Amer *et al.* 2001 and Momen *et a*l., 2001. Certain toxicotogical and biological effects of plant extracts and oils on the whitefly, *B. tabaci* were studied by Coudriet *et al.,* 1985; and Radwan *et a*l., 2002.

Several active chemical constituents of this plant were recorded for examples: piperitenone, plegone, piperitenone oxide, menthone, L,8-cineole were determined by (Sartoratto *et al*., 2004 and Ibrahim *et al*.,

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2006). Three decanoic acid methyl esters (pentadecanoic acid methyl ester, hexadecanoic acid methyl ester, and octadecanoic acid methyl ester were determined by (Duke. 1992, Antonious *et al.*, 2007, Maggi *et al.*, 2009).

The present work aims to determine the insecticidal effect of different crude and formulated extracts of Mint and Colothyn against the different immature stage of whitefly *B. tabaci* and aphid *Aphis craccivora* and determination the chemical components which most effective in the plants

MATERIAL AND METHODS

***Preparation of Plant Extracts*:**

Leaves of *Mentha microphylla* (mint) and fruits of *Citrullus colocynthis* (Colothyn) were collected and cleaned from the dust then dried under room temperature. The fruits and leaves were grounded in electric mill separately. A powder of grounded plants were soaked in solvent (methanol) for 24 hours and shacked for 1 hour. The combined extract was filtered over anhydrous sodium sulphate. The solvent was then evaporated using a rotatory evaporator at 40 ºC. The crude extract was transferred quantitatively to a clean and weighted flask, and then kept in the refrigerator until used for toxicological investigation.

***Preparation of Crude Extract as Suitable Formulation*:**

Crude extracts were prepared as emulsifiable concentrate contained 20 (Wt./V.) crude extract. To prepare 100 ml of EC, 20 grams of crude extract weighted then dissolved in 27 ml of co-solvent (butanol) and added to 50 ml solvent xylene, 3 gram of emulsifier was added to the previously mixture and transferred quantitatively to 100 ml. glass stopper.

The locally prepared plant extracts EC's should be agree with the world specifications, therefore, spontaneity and emulsion stability tests were carried out by adding 5 ml EC's to 95 ml soft and hard water presents in 100 ml graduated cylinders and storage the formulations at 0ºC for one day and at 54±1ºC for days according to WHO (1979) specifications. Therefore, surface tension of spray solution was measured using DUNOÜY Tensiometer, while pH value was measured using pH meter and conductivity and salinity were measured using conductimeter.

3-*Insects*:

Laboratory susceptible strains (s) of *Bemisia tabaci* and *Aphis craccivora* were obtained from syngenta Co. and maintained without insecticide selection pressure for more than six years in the laboratory of Central Agricultural Pesticides laboratory, department of standard rearing insects.

Strain of *B. tabaci* was reared on cotton plants, while *A. craccivora* was reared on bean plants. All strains were confined with plants at 27 ± 2 ºC and 18:6 L:D as described by Coudriet *et al.* (1985).

4-*Bioassay*:

a) Experiments involving immature stages of *B. tabaci* were carried out according to (Prabhaker *et al.* 1985) by introducing 50 adults into small cages that were clamped on the underside of egg plant leaves. The adults were removed after 48h. With an aspirator the infested plants were maintained in environmental chambers under conditions of 27 ± 2 ºC, 60% RH and a photoperiod of 16:8 L: D until the appropriate stage was ready of treatment. The stages that were treated consisted of: First stages larvae (2 day old), second stage larvae (4 day old), third stages (4 day old) and pupae (6 day old). The numbers of each stage on each leaf were counted before treatment.

Effects of the Colothyn and Mint on each immature stage were determined by dipping infested leaves in different concentration 5000, 2500, 1250 and 750 ppm. Mortality counts were made after 48h. Larvae and pupae that were dry and detached from the leaf when probed were considered dead.

For *Aphis craccivora*. All compounds were dissolved in 7ml of water and 3ml of acetone, several concentrations were prepared for each toxicant, and then 10 newly nymphs were placed on slide. The slide with nymphy were dipped in the toxicant. Mortality count was made after 2h after treatments. Control treatment with 7ml of water and 3ml of acetone.

***Statistical Analysis*:**

Mortality data were corrected according to Abbott (1925). The toxicity index of each insecticide was determined according to Sun (1950) as the following equation:

$$\text{Toxicity index} = \frac{LC_{50} \text{ of the effective insecticide}}{LC_{50} \text{ of each insecticide used}} \times 100$$

5-*GC-MS Condittions:*

GC-MS analysis was performed with an Aglient 6890 gas chromatograph equipped with an Aglient mass spectrometric detector, with a direct capillary interface and fused silica capillary column HP—5MS (30 m X 0.32 mm i.d. X 0.25 pm film thickness). Pesticide samples were injected under the following conditions:

Helium was used as carrier gas at approximately 1.0 ml/min., pulsed splitless mode. The solvent delay was 4 min. and the injection size was 1.0 ul. The mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 e.v. scanning from m/z 50 to 700. The ion source temperature was 230 ^{O}C and the quadruple temperature 150^{O}C. The electron multiplier voltage (EM voltage) was maintained 1050 v above auto tune. The instrument was manually tuned using perfluorotributyl amine (PFTBA). The GC temperature program was started at 80 ^{O}C (3 min) then elevated to 260 ^{O}C at rate of 8 ^{O}C/min. the detector and injector temperature were set at 280 and 250 ^{O}C, respectively. Wiley and pesticides mass spectral data base was used in the identification of the separated peaks.

Under this conditions, retention times (R_t) for Phenanthrene, Hexadecanoic acid and Octadecadienoic acid were 17.43, 19.05 and 12.13 seconds for mint while it was 17.45, 19.05 and 21.05 for Colothyn respectively.

RESULTS AND DISCUSSION

1- Effect of Physico-chemical Properties:

Data in table (1) demonstrated the physicochemical properties of the prepared two formulations as emulsifiable concentrates (20% EC). For spontaneity test, data showed that the prepared EC of the mint extract (Formulation 2) gave the highest percentage (63%) while the Colothyn formulated extract (Formulation 1) gave the highest percentage (58 %) in T.W.

On the other hand, the emulsion stability of the two prepared formulations showed no oily separation or creamy layer and no foams when mixed with water for use. The table also indicates that, the formulations proved satisfactory properties of the sedimentation and cold and heat stability parameters accordance with WHO specifications.

For pH test, data in table (1) showed that the prepared EC of the Mint extract gave the low value of pH (6.77) than Colothyn 7.50 in T.W. respectively. As reported by Tawfik and El-Sisi (1991) and El-Sisi *et al.* (1989), the reduced values of pH of formulated solutions lead to more attraction between the extracted particles and surface of treated plants. On the other hand, there is a particular concern about the intrinsic effect of the formulated extracts on pH and water contents of the target insects. Such change, however, leads to a particular change on the respiratory rate (Simkiss and Mason, 1983 and Stadnichenko *et al.*, 1986) that leads to a passive loss of ions that leads to more bioavailability and finally mortality increases dramatically. Initially before death, the tested pests sailed completely to feed or grow normally and became incapable to move, which suggest the previously mentioned explanations.

The lowest values of surface tension of Mint and Colothyn formulated extracts were 31 and 35 dyne/cm in S.W., respectively. As recorded by El-Hariry and El-Sisi (1990), the decrease in the surface tension of the formulated extracted particles increases their spreading and deposition on the surface of treated plants.

The maximum effect of viscosity of Colothyn and Mint formulated extract occurred in T.W. which was 10.57 and 11.65 m poise, respectively. In another experiment, Bodi *et al.* (1976) postulated that, the increase of viscosity significantly affected the efficiency of botanical extracts thereby decreasing the longevity of the target pests.

The salinity of the two prepared EC gave zero in all types of water. The conductivity of the prepared EC of the Colothyn and Mint extract gave the highest values (875 and 892 m MHOS in H.W., respectively). Also, according to El-Attal *et al.* (1984) increased electric conductivity of the formulated extracts was coupled with increased mortality rate due to increased deposition and penetration of the formulated extracted particles.

There are a correlation between the physico-chemical properties of spray solution and retention on treated surface then pesticidal efficiency as adopted by Furmidge (1962) who proved that wetting, spreading then insecticidal efficiency increased as surface tension of spray solution decreased and pH value as mentioned by Tawfik and El-Sisi (1991) who proved that retention and insecticidal efficacy increased by decreasing pH value and increasing conductivity.

Table 1: The physical-chemical properties of the formulated plant extracts.

Tests	Formulation 1 (20%EC) *Citrullus colocynthis* (Colothyn) Type of water			Formulation 2 (20%EC) *Mentha microphylla* (Mint) Type of water		
	T.W.	H.W.	S.W.	T.W.	H.W.	S.W.
Spotanious stability	58%	50%	47%	63%	51%	55%
Foams	----------	----------	----------	----------	--------	--------
Emulsion stability	passed	passed	passed	passed	passed	passed
Heat test	passed	passed	passed	passed	passed	passed
Cold test	passed	passed	passed	passed	passed	passed
pH	7.50	7.17	7.36	6.77	6.43	6.76
Salinity	0	0	0	0	0	0
Conductivity (m MHOS)	287	875	195	310	892	183
Surface tension (dyne/cm)	33	30	35	29	25	31
Viscosity (m poise)	10.57	10.26	10.39	11.65	11.03	11.48

T.W.: tap water.
H.W.: hard water.
S.W.: soft water.

2-*effect of Extracts on Whitefly and Aphids:*

A) Nymphs of Bemisia Tabaci:

All tested treatments were effective on first nymph table (2) formulation of Mint was more toxic with LC_{50} 170.70 ppm. But formulation of Colothyn was less toxic with LC_{50} 303.58 ppm. In contrast the pupae were the least susceptible stage to all test treatments. Formulation of Colothyn was more effect than formulation of Mint, where LC_{50} were 917.78, 947.80 ppm respectively. In general formulations of two crude extracts were effective on all nymphs, but the crude extracts were less toxic.

Crude extract and formulation of mint were the most effective treatments against all nymphs than colothyn.

Table 2: Toxic effect of plants extracts against Lab-strain of whitefly *Bemisia tabaci*

Treatments	First nymphs			Second nymphs			Third nymphs			Pupae		
	Slope	LC_{50} ppm	T.I %	Slope	LC_{50} ppm	T.I %	Slope	LC_{50} ppm	T.I %	Slope	LC_{50}ppm	T.I %
A	1.04	170.70	100	1.04	178.69	100	1.70	288.21	100	1.45	947.79	96
B	1.45	303.57	56	2.15	430.77	41	2.28	368.27	78	4.21	917.78	100
C	0.69	309.53	55	0.92	504.20	35	1.25	653.89	44	035	1443.91	63
D	1.77	594.47	28	2.08	558.00	32	1.02	4437.90	6	1.15	4979.86	18

A= Formulation of *Mentha microphylla*
B= Formulation of *Citrullus colocynthis*
C= Crude of *Mentha microphylla*
D= Crude of *Citrullus colocynthis*

b) Adults of Aphis craccivora:

Formulated extract of Colothyn as shown in table (3) was extremely toxic with LC_{50} 123.68 ppm but its crude extract was 621.95 ppm also, formulation of Mint was effective than the extract.

Table 3: Toxic effect of plants extracts against Lab-strain of aphid Aphis *craccivora*

Treatments	Slope	LC_{50} ppm	T.I %
1	1.26	123.68	100
2	1.12	392.54	31
3	1.23	621.94	19
4	1.43	509.29	24

T.I= Toxicity index
1= Formulation of *Citrullus colocynthis*
2= Formulation of *Mentha microphylla*
3= Crude of *Citrullus colocynthis*
4= Crude of *Mentha microphylla*

Results obtained about the toxicity of plant extracts of mint and colothyn against the tested insects are agree with Magd El-Din and El-Gengaihi (2000) who found that, ethyl alcohol and chloroform extracts of Colothyn gave moderate mortalities, which ranged between 60 and 20% after 7 and 22 days at concentration (5%) against 2^{nd} instar larvae of *Spodoptera littoralis*. Also, agree with Soliman *et al*., (2005) who reported that, LC_{50} of hexane, diethyl ether, ethyl acetate, acetone and ethanol extract of Colothyn were 1.558, 48.156, 3.325, 3.740 and 2.935 ppm respectively against apterous adults of *Aphis gossypii.*

Ibrahim *et al*., (2006) found that essential oil from leaves and flowers of Mint have toxic against nematodes example *Meloidogyne incognita*.

The results suggested that extracts of Mint and Colothyn can be explored for developing natural products for use as biodegradable alternatives to synthetic insecticides.

2-Chemical Constituents of the Two Crude Extracts:

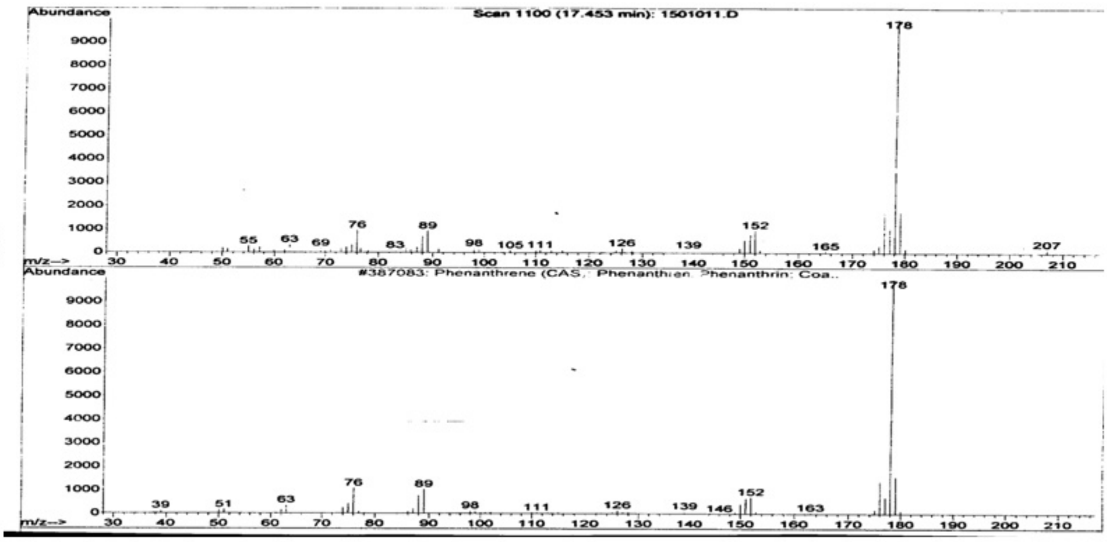
Results of using GC/MS, for investigating the contents and their percentages of Mint and Colothyn extracts that may explain the observed differences in toxicity to two insects are shown in table 4 and fig. 1**.** Data indicated that there are three decanoic acid methyl esters (pentadecanoic acid methyl ester, hexadecanoic acid methyl ester, and octadecanoic acid methyl ester) predominated extracts.

Table 4: Concentration of contents detected in two extracts.

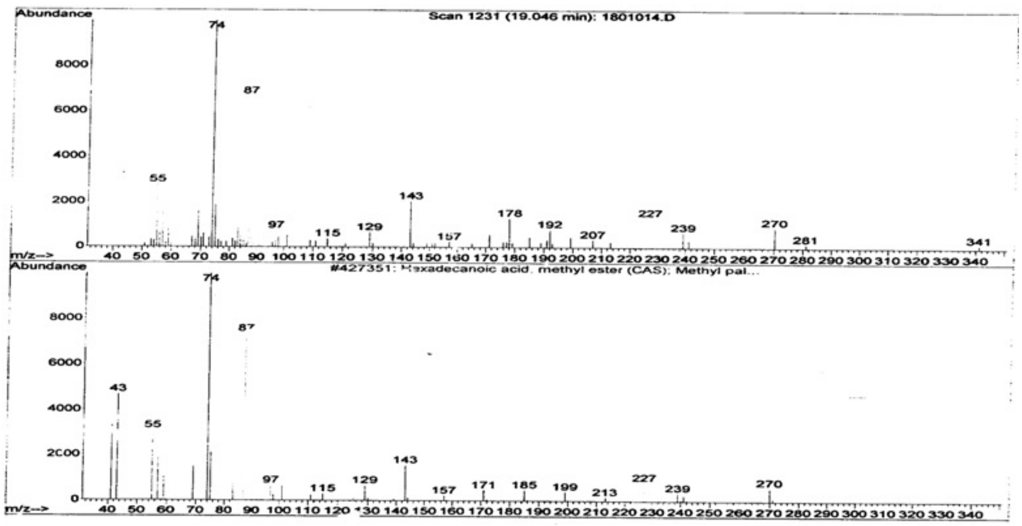
Plants	Phenanthrene		Hexadecanoic acid		Octadecadienoic acid	
	Concentration	R.T. time	Concentration	R.T. time	Concentration	R.T. time
Mentha microphylla	47.67%	17.43	15.87%	19.05	2.52%	12.13
Citrullus colocynthis	6.31%	17.45	17.23%	19.05	54.17	21.05

R.T= Retention time

(A)



(B)



(C)

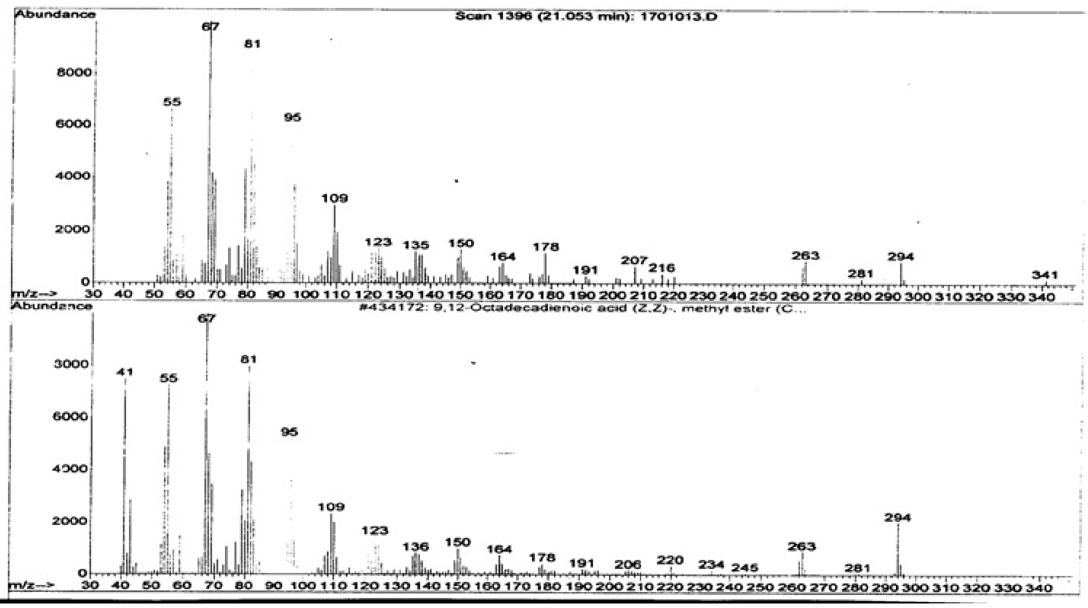


Fig. 1: Gas Chromatography-Mass Spectrometry of (A) Phenanthrene ($C_{14}H_{10}$), (b) hexadecanoic acid methyl ester ($C_{17}H_{34}O_2$) (c) octadecanoic acid methyl ester ($C_{19}H_{34}O_2$) detected in the *Mentha microphylla* and *citrullus colocynthis* indicating molecular ions of m/z 256 and 270, respectively along with other characteristic fragment ions.

Results obtained are agree with Duke (1992), Antonious *et al*., (2007), Maggi *et al*., (2009), who proved the toxicity of three decanoic acid esters studied, pure standards of the three decanoic acid methyl esters (pentadecanoic acid methyl ester, hexadecanoic acid methyl ester, and octadecanoic acid methyl ester) as insecticide against cabbage hooper, *Trichopulsia ni* larvae. Results indicated that pentadecanoic acid methyl ester was the most effective (74% mortality) compared to hexadecanoic and octadecanoic acid methyl ester. In contrast, Ibrahim *et al*., (2006) showed that, the majour components of essential oil of Mint were piperitenone (54.2%), plegone (10.7%), piperitenone oxide (11.3 %), menthone (3.3%), L,8-cineole (2.8%).

Sartoratto *et al*., (2004) reported that, linalool (51.0%), carvone (23.42%) and 3-octanol (10.1%) were identified from *Menthe pipenita L*. and piperitenone oxide (94.8%).

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