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Insecticidal Activity of Some Plant Extracts Against Some Sap-Sucking Insects under Laboratory Conditions

[1]Hala M. Abou-Yousef, [2]Sayeda F. Farghaly and [3]Howida M. Torkey

[1] Department of Bioassay, Central Agricultural Pesticides Laboratory, Dokki, Giza, Egypt
[2] Department of Pest Rearing, Central Agricultural Pesticides Laboratory, Dokki, Giza, Egypt
[3] Department of Pesticides Formulations, Central Agricultural Pesticides Laboratory, Dokki, Giza, Egypt

Abstract: The toxic effect of *Ammi majus* (Laceflower) and *Zinigiber officinale* (ginger) either as crude extracts or as formulations were tested under laboratory conditions against nymphs of *Bemisia tabaci* (Gennadius) and *Aphis craccivora* (global). LC_{50} for formulation of laceflower for first, second, third and fourth stage of *Bemisia tabaci* were 196.20, 329.50, 553.94 and 1056.19 ppm, respectively, while LC_{50} of crude were 310.71, 555.47, 1066.97 and 1275.48 ppm, respectively. For formulation of ginger LC50 were 366.33, 418.74, 897.73 and 1696.70 ppm, respectively but for crude, LC50 were 541.20, 585.96, 1536.74 and 1755.81 ppm, respectively. For Aphis *craccivora,* LC_{50} of crude and formulation for laceflower were 585.96 and 1755.81 ppm, respectively and LC50 were 1066.97 and 2305.46 ppm, respectively for ginger. The results revealed that formulated plant extracts were more toxic than crude extracts and showed good level of efficiency against *Bemisia tabaci* and *Aphis craccivora*. The crude extracts were analyzed by GC-MS technique and AChE assay for h insects in order to determine the major compounds to may know the observed differences in mortality.

Key words: Toxic effect · Laceflower · Ginger · Leaf composition · Mass spectra · AChE assay

INTRODUCTION

Bemisia tabaci and *Aphis craccivora* are serious pests infesting cotton and more than 80 host plants. The continues and unwise use of insecticides to control these pests lead to adverse effects on naturally biological control agents, hazards to man and his animals by environmental pollution, adverse effects on non target organisms and the development of resistance [1, 2]. Great efforts and a lot of money spent yearly to get several synthetic pesticides, which soon become more commonly used for controlling the different pests. Pesticides produced from natural products have been recently attracting the attention of many scientists to avoid the problems caused by synthetic compounds they are deeply interested in their chemical constituents and biological properties.

The objectives of the present study were aimed to investigate the toxic effect of some plant extracts against some sap-sucking insects and their effects on some biochemical parameters.

MATERIALS AND METHODS

Preparation of Plant Extracts: Two plants were used for this study; *Ammi majus* (laceflower) and *Zingiber officinale* (ginger). Leaves of *Ammi majus* and rhizome of *Zingiber officinale* were collected and identified by Flora and plant Classification Research Department, Agricultural Research Centre, Egypt. They were washed with tap water then dried separately at room temperature. The plants were grounded separately in electric mill. Samples of ground plants were extracted in the solvent (methanol) for 24 hours. The combined extract was filtered over anhydrous sodium sulphate. The solvent was then evaporated under vacuum using a rotatory evaporator at 30°C. The extracted solutions were left away for complete dryness to obtain the crude extracts.

Formulation: The formulative extracts were prepared weight / volume (w/ v.) in the form of emulsifiable concentrate according to the procedure of Knowiles [3].

Corresponding Author: Sayeda F. Farghaly, Departmentp of Pest Rearing, Central Agricultural Pesticides Laboratory, Agricultural Research Center, Dokki, Giza, Egypt.

Test Insects: The efficiency of laceflower and ginger and their extracts was evaluated against aphids, *A. craccivora* and whitefly *B. tabaci*. For laboratory tests, samples of bean seedlings infested with *A. craccivora* and samples of cotton seedlings infested with *B. tabaci* which obtained from Syngenta Co. and maintained without insecticide selection pressure for more than seven years in the laboratory of central Agricultural pesticides laboratory, Department rearing of standard insects. The infesting seedlings with insects placed at 27±2°C and 18:6 L: D as described by Coudriet *et al.* [4].

Laboratory Tests: A series concentrations (0.062 -1 ml) from crude or formulation were added to one ml acetone, then diluted in 10 ml of water, acetone was added to water, in the control treatment.

For Nymphs of *B. Tabaci*: Leaf dipping method was used to experiments of immature stages as described by Prabhaker *et al.* [5]. The infested cotton leaves were dipped for 10 seconds in each concentration and four replicates were made for each concentration. The stages that were treated involving: First stage (2 day old), second stage (3 day old), Third stage (3 day old) and fourth stage (4 days old). The numbers of each stage on each leaf were counted before treatment and after 48 h. from treatment.

For Nymphs of *A. Craccivora*: The slide dipping method was used by placing 10 individual up side down on a double achesive tap fitted to glass slide according to Dittrich [6]. These prepared devices were immersed for 5 seconds in different concentrations. Count of alive and dead was recorded after 2 h of exposure.

Statistical Analysis: Mortality data were corrected according to Abbott [7]. The toxicity index of each insecticide was determined according to Sun [8] as the following equation.

$$\text{Toxicity index} = \frac{\text{LC50 of the effective insecticide}}{\text{LC50 of each insecticide used}} \times 100$$

GC-MS Conditions: 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 split less mode. The solvent delay was 4 min. and the injection size was 1.0 µl. 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°C and the quadruple temperature 150°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°C (3 min) then elevated to 260°C at rate of 8° C/min. the detector and injector temperature were set at 280 and 250°C, respectively. Wiley and pesticides mass spectral data base were used in the identification of the separated peaks.

Under this conditions, retention times (RT) for Ginger Alpha-curcumen, Zingiberene, Capsaicin, Beta-sesquiphellandrene and 3-pyridineacetic acid were 13.05, 13.23, 23.81, 13.68 and 23.30 seconds for mint while it was Methoxsalen ,8-dimethoxypsoralen, 6,7-dihydroxanthotoxin and Palmitic acid were 20.65, 22.87, 19.72 and 19.05 for Laceflower, respectively.

Acetylcholinesterase (AchE) Assay: Acetylcholinesterase (AchE) activity was determined by the method of Ellman *et al.* [9]. In which the hydrolysis of the natural substrate analogue acetylthiocholine iodide (ATChI) was determined calorimetrically by the absorbance of 2-nitro-5-thiobenzoate (TNB) at 405 nm after the reaction of 5, 5 –di-thio-bis (2-nitrobenzoic acid) (DTNB) with the liberated thiocholine. Prior to all assays, 50 nymphs of *B. tabaci* and *A. craccivora* were homogenated in 0.1M phosphate buffer PH 7.5, containing 0.1% Triton X-100. These were centrifuged at3000 g and 4°C for 15 min. the supernatants were incubated with 100 mM DTNB for 30 min at 4°C. Malathion (97%), crude from laceflower and crude from ginger were used as inhibitors to AChE. Dilution was made by using a buffer to get concentration 0.5 ml/l of each inhibitor and add to the substrate. A reaction without the inhibitor was included as a control. All the assay conditions were the same as that used for the above kinetic assays. Progressive inhibition of AChE activity over time was continuously recorded for 5 min.

RESULTS AND DISCUSSION

Physical Properties of Formulative Extracts: The evaluation of the physical properties was carried out according to WHO [10]. The contrasting results obtained by using the tested formulated extracts can be explained on the basis of physico-chemical changes that contributed to decreases in pH and surface tension and increase of conductivity and viscosity are the main causes. The reduced values of pH of formulated solutions lead to more attraction between the extracted particles and surface of treated plants. The decrease in the surface tension of the formulated extracted particles increases their spreading and deposition on the surface of treated plants. The increase of viscosity significantly affected the efficiency of plant extracts there by decreasing the longevity of the target pests. Also, increased electric conductivity of the formulated extracts coupled with increased mortality rate due to increased deposition and penetration of the formulated extracted particles.

Data in Table 1 demonstrated the physicochemical properties of the prepared two formulations as emulsifiable concentrates (20% EC). For spontaneity test, data show that the prepared EC of the *Ammi majus* (Formulation 1) and *Zingiber officinale* (Formulation 2) gave the highest stability percentage in T.W. 78% and 66%, respectively. 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. Also, Table 1 indicated that, the formulations proved satisfactory properties of the sedimentation and cold and heat stability parameters accordance to WHO specifications.

Toxic Effect of Extracts on *B. Tabaci* and *a. Craccivora*:
Nymphs of *B. Tabaci*: Data in Table 2 showed that the formulation of Laceflower was more toxic than formulation of Ginger. While LC_{50} of Laceflower was 196.20, 329.50, 553.94 and 1056.19 ppm, but LC_{50} of Ginger was 366.33, 418.73, 897.73 and 1696.7 ppm, for first, second, third and fourth stage of *B. tabaci*, respectively. In contrast, crude of two extracts were less toxic than of two formulations. Zhang *et al.* [11] evaluated whether ginger oil would protect tomato seedlings from whitefly *B. argentifolii* settling and oviposition, repellency increased with increasing ginger oil concentration when leaf disks were dipped in ginger oil, but not when ginger oil was sprayed onto the leaf disks, higher quantities of monoterpenes and sesquiterpenes were deposited on leaf disks according to gas chromatographic quantification. Schuster *et al.* [12] evaluated twenty two trademarked products and 42 other products on setting of *B. tabaci* adults in the laboratory bioassay. Non-choice bioassay using leaf disks of tomato, ginger oil and limonene reduced oviposition in at least one screen house trail but did reduce transmission of TYLCV, where Ultra-fine oil and Olive oil reduced oviposition and transmission of TYLCV in the screen house trails.

Nymphs of *Aphis craccivora*: Data in Table 3 indicated that, LC_{50} for formulation of Laceflower was 585.96 ppm, but LC_{50} for formulation of Ginger was 1066.97 ppm. While LC_{50} for crude of two extracts were very low. Xu *et al.* [13] tested different concentrations of ginger essential oil and time-dosage-mortality model was used to simulate their cumulative mortality within 96h. The results indicate that higher concentration of ginger essential oil had high acute toxicity to *Drosophila melanogaster* adults, 1% and 10% ginger essential oil; the cumulative mortality of h female and male adults was up to 100%, the LC_{50} of ginger essential oil decreased gradually with increasing time.

Chemical Constituents: The chemical constituents of the crude Laceflower and Ginger (which may explain the toxic effect for *B. tabaci* and *A. craccivora*) were analyzed by GC-MS.

Laceflower Extract: Table 4 indicated that, the main compounds were, Methoxsalen (18.50%), 8-dimethoxy-psoralen (12.67%), 6,7-dihydroxanthotoxin (11.70%), Palmitic acid (10.81%). These findings are in agreement with Duke [14] and WHO [10] which reported that, the major constituents are furanocoumarins, the principal compounds being xanthotoxin (methoxsalen, 8-methoxypsoralen (8-MOP) ammoidin; up to 1.15%), imperatorin (ammidin; up to 0.75%) and bergapten (heraclin, majudin, 5-methoxypsoralen (5-MOP), up to 1.88%), other coumarins of significance are marmesin (up to 0.25%), isoimperatorin (0.01%) heraclenin (0.07) and isopimpinellin (0.01%).

Ginger Extract: Data in Table 5 explained that, there are many components such as: Alpha-cucumen (15.38%), Zingiberene (13.72%), Capsaicin (11.62%), beta-sesquiphellandrene (10.32%), 3-pyridineacetic acid.

Table 1: Physical properties of the most promising formulated plant extracts

	Formulative 1 (20% EC) *Ammi majus*			Formulative2 (20% EC) *Zingiber officinale*		
	Type of water			Type of water		
Tests	Tap water	Hard water	Soft water	Tap water	Hard water	Soft water
Spotanious stability	78%	75%	69%	66%	57%	59%
Foams	–––	––	––	––	––	––
Emulsion stability	pass	pass	pass	pass	Pass	pass
Sedmentation	nµl	nµl	nµl	nµl	nµl	nµl
pH	6.64	6.31	6.24	7.77	7.59	7.29
Salinity	0	0	0	0	0	0
Conductivity (µ MHOS)	340	980	196	293	860	188
Surface tension (dyne/cm)	31	26	38	36	29	45
Viscosity (m poise)	11.49	11.23	11.54	10.39	10.64	10.73
Heat test	pass	pass	pass	pass	Pass	pass
Cold test	pass	pass	pass	pass	pass	pass

Table 2: Toxic effect of plants extracts against Lab-strain of nymphs of whitefly *B. tabaci*

	First stage			Second stage			Third stage			Pupae		
Treatments	Slope	LC_{50}ppm	T.I %	Slope	LC_{50}ppm	T.I %	Slope	LC_{50}ppm	T.I %	Slope	LC_{50}ppm	T.I %
Formulation of Laceflower	1. 40	196.20	100	1.75	329.50	100	1.65	553.94	100	2.5	1056.19	100
Crude of Laceflower	0.94	310.71	63	0.81	555.47	59	1.29	1066.97	51	3.74	1275.48	82
Formulation of Ginger	2.32	366.33	53	1.84	418.74	78	3.19	897.74	36	1.12	1696.70	62
Crude of Ginger	1.92	541.20	36	2.08	585.96	56	0.76	1536.74	61	6.24	1755.81	60

T.I= Toxicity index

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

Treatments	Slope	LC_{50} ppm	Toxicity index%
Formulation of Laceflower	1.29	585.96	25
Crude of Laceflower	2.73	1755.81	33
Formulation of Ginger	1.29	1066.97	100
Crude of Ginger	8.20	2305.46	54

Table 4: Identification and concentration compounds of Laceflower

Compounds	Total concentration %	Retention time R.T. (min)
Methoxsalen	18.50	20.65
8-dimethoxypsoralen	12.67	22.87
6,7-dihydroxanthotoxin	11.70	19.72
Palmitic acid	10.81	19.05

Table 5: Identification and concentration compounds of Ginger

Compounds	Total concentration %	Retention time R.T. (min)
Alpha-curcumen	15.38	13.05
Zingiberene	13.72	13.23
Capsaicin	11.62	23.81
Beta-sesquiphellandrene	10.32	13.68
3-pyridineacetic acid	9.38	23.30

Table 6: Specific activity of AChE in adult of *A. craccivora* and nymphs of *B. tabaci*

				AChE With malathionnmol/min/mg protein at concentration of 1/2000		
No.	Sample name	Total proteinmg/ml	AChEnmol/min/mg protein	Malathion	Ginger	Laceflower
1	Nymph of aphis	5.5	7.42	0.000	2.967	0.000
2	Third nymph of *B. tabac*i	1.06	15.4	0.000	5.132	5.132
3	Fourth nymph of *B. tabac*i	1.8	7.55	0.000	3.022	0.000

These results agree with Duke [14] and Agarwal *et al.* [15] ,they found that cucumene is the major constituent and Zingiberene- rich fraction was obtained from its diethyl ether extract, the test compounds exhibited moderate insect growth regulatory (IGR) and antifeedant activity against *Spilosoma oblique* and significant antifungal activity against *Rhizoctonia solani*. Antonious and Snyder [16] tested correlation analysis between cucumene, Zinigiberene and time mite spent in the ring of the repellency bioassay. They indicated that none of the correlation were significant, but were best correlated with mortality on toxicity testing. The results of this investigation indicate the potential usefulness of two extracts and their formulations for control of B. *tabaci* and *A. craccivora*. These materials could play an important role in the integrated pest management of these pests due to their distinct modes of action to conventional insecticides and their possible selective characteristics. Additional research is needed to compare results from leaf-dip bioassays with achieve different degrees of coverage and to test the effects of these materials on natural enemies of *B. tabaci* and *A. craccivora*.

Inhibition of AChE Activity: Specific activity of AChE before and after adding the inhibitors malathion, crude of laceflower and ginger were presented in Table 6. Data indicated that, the level of AChE activity of aphis with inhibitors were lower than without inhibitors, similarity AChE activity of fourth nymph for *B. tabac*i . In contrast AChE (third nymph of *B. tabac*i) with all inhibitors was similar. In this study, AChE activity was more sensitive to inhibition by extract of laceflower than extract of ginger; this is agreement with above toxic effect of extracts. These considerations may open new possibilities to develop highly efficient combined biological products on the basis of entomopathogenic fungi and inhibitors of detoxification enzymes which could cause to decrease environmental risk posed by synthesized pesticides [17].

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