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Chemical Compositions and Insecticidal Activities of the Essential Oils from Several Medicinal Plants Against the Cotton Whitefly, *Bemisia tabaci*

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Chemical compositions and insecticidal activities of plant essential oils obtained from medicinal plants, thyme (*Thymbra spicata* subsp. *spicata*), rosemary (*Rosmarinus officinalis*), fennel (*Foeniculum vulgare*) and laurel (*Laurus nobilis*), were investigated against adults of cotton whitefly (*Bemisia tabaci* Gen.). Volatile phase effects of different concentrations of the essential oils used were used to determine insecticidal activities. Major compounds found in essential oils of thyme, rosemary, fennel and laurel were carvacrol (70.9 %), borneol (20.4 %), *trans*-anethole (82.8 %) and 1,8-cineole (35.5 %), respectively. Laboratory bioassay results indicated that all essential oils caused adult mortality of whitefly at different concentrations that are not phytotoxic to the host plant. All essential oils showed insecticidal activities in a dose-dependent manner. Essential oil of thyme had a marked insecticidal activity against whitefly adults. Adult viability was totally affected by thyme, laurel, fennel and rosemary at the concentrations of 5.0, 20.0, 30.0 and 30.0 μg mL^{-1} air, respectively. Estimated mean lethal concentrations (LC_{50}) of the essential oils of thyme, laurel, fennel and rosemary were 0.44, 1.82, 7.06 and 2.86 μg mL^{-1} air, respectively. The results of the present study concluded that plant essential oils could be useful in promoting research aiming at the development of new agent for pest control from the plants with medicinal values.

Key Words: *Bemisia tabaci*, Biological control, Essential oil, Insectical, Biopesticide.

INTRODUCTION

The cotton whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) is a major pest of economically important crops worldwide[1,2]. *Bemisia tabaci* damages crops by feeding on phloem sap and the large amounts of sticky honeydew produced can lower the rate of leaf photosynthesis. Most of the important emerging virus diseases are whitefly vectored with resulting yield reductions and economic losses being in the region of hundreds of millions of dollars annually in the affected regions[3,4]. This pest is proving increasingly difficult to control due to continual development

of insecticide resistance[5-7], which, in combination with increasing public awareness regarding effects of chemical insecticides on the environment[8], results in a requirement for non-chemical methods of control to be devised.

The use of biologically based compounds in plant extracts or essential oils may be an alternative to currently used insecticides to control insects, because they virtually constitute a rich source of bioactive chemicals such as phenols, flavonoids, quinones, tannins, alkaloids, saponins and sterols[9]. Several compounds have insecticidal properties[10]. Moreover, essential oils have a broad spectrum of insecticidal activity due to the presence of several modes of action, including repellent and antifeedant activities, inhibition of molting and respiration, reduction in growth and fecundity, cuticle disruption and effect on the invertebrate octopamine pathway[10-12]. Essential oils derived from plants may also have minimal direct and/or indirect effects on natural enemies[13,14].

In this study, we assessed chemical compositions and insecticidal activities of essential oils vapours derived from aromatic plants *Thymbra spicata* L subsp. *spicata*, *Rosmarinus officinalis* L., *Foeniculum vulgare* Mill. and *Laurus nobilis* L., against the adults of cotton whitefly, *B. tabaci*.

EXPERIMENTAL

The plants used in this study were identified by Dr. I. Uremis. A voucher specimen has been deposited in the herbarium of the Plant Protection Department, Mustafa Kemal University (No. TssA1, RoS3, FvN2 and LnKs2). For the extraction of essential oils, plants were collected from Samandag (36º16' N; 35º48' E, 38 m) and Narlica (36º14' N; 36º13' E, 104 m) districts situated in the eastern Mediterranean region of Turkey. Leaves of thyme, rosemary and laurel were used for extraction of the essential oils and in the case of fennel, seeds were used for essential oil extraction. Three different air-dried plant material lots were used separately for extraction. For each lot, air-dried plant material (200 g) was placed in a 5 L round-bottom distillation flask and 3 L double distilled water added. The essential oils were obtained by steam distillation using Clevenger-type apparatus (Ildam, Ankara) for 3 h. The oils were separated, dried over anhydrous sodium sulphate and stored in an amber bottle at 4 ºC until used.

GC-MS analysis of essential oil: The analysis of the essential oil was performed using a Hewlett-Packard 6890 GC linked to a Hewlett-Packard 5973 mass selective detector equipped with a HP-5 MS (crosslinked 5 % phenyl methyl siloxane) capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The carrier gas was helium, at a ratio of 1.0 mL min^{-1}. The amount of the samples injected was 0.1 μL in split mode (50:1). The oven temperature was initially 50 ºC, increased at a rate of 2 ºC min^{-1} to 90 ºC, 5 ºC min^{-1} to 210 ºC and finally isothermal for 5 min. The injector and detector temperature were maintained at 250-280 ºC, respectively. The quadrupole mass spectrometer was scanned over the range 50-550 amu at 1.53 scan s^{-1}, with an ionizing voltage of 70 eV. The major components of essential oils

were identified on the basis of comparison of their retention times and mass spectra with those of authentic samples or published data[15] and computer matching with Wiley 275.L registry of mass spectral data[16].

Biological material: *B. tabaci* originated from a research colony maintained on cotton (*Gossypium hirsutum* L. cv. Cukurova 1518) plants without any pesticide exposure in a growth chamber set at 26 ºC, 60-65 % R.H. and a 16:8 h L:D photoperiod.

Toxicity tests: Transparent acrylic cups (6 cm height and 3.5 cm diameters which offer 50 mL air space) were used as test chambers for the determination of volatile phase of the essential oils. Two-weeks-old cotton leaves without insects were harvested and individually placed in small vials with distilled water. Each vial containing one leaf then stored in individual transparent acrylic cups covered with lids. Twenty adult insects were introduced in each cup and allowed to settle for 0.5 h before exposure to essential oil. The top of the insect chamber was covered by lids. The essential oils were applied with an micropipette on a filter paper strip (3 cm × 1 cm) attached to the lids. Different concentrations of essential oil were prepared by dissolving the requisite amounts in sterile dimethyl sulfoxide solution. Essential oils were diluted from the concentration of 1500.0-12.5 µg in dimethyl sulfoxide and a 10 µL aliquot of each concentration was added on the inner surface of the lid of test chamber with a micropipette giving concentrations of 0.25, 0.5, 1.0, 2.0, 5.0, 10.0, 15.0, 20.0 and 30.0 µg mL^{-1} air. Insect chambers were sealed immediately with parafilm to prevent loss of essential oils from the chamber. Three replications were made for each concentration. As untreated control, three cups containing only 10 µL of dimethyl sulfoxide were used.

The treated insect-chambers were returned to the incubator set at 25 ºC, 60-65 % R.H. and a photoperiod of 16:8 (L:D) h. Mortality was determined under a dissecting microscope 24 h after treatment. Adult insects were assessed at 0.5 h for mobility and at 2 h for mortality, defined by lack of response to stroking with a paintbrush.

Phytotoxic effects of the essential oils on greenhouse-grown cotton, bean, tomato, pepper, cucumber and eggplant plants as host plant were determined by means of a bioassay. The highest concentration of the essential oils used in the experiment (30 µg mL^{-1}) was dissolved in DMSO-H_2O solution (1 % v/v). These emulsions (10 mL for each plant) were sprayed uniformly with a glass atomizer on the surface of whole plant leaves, fruits and flowers of plants in fruiting stages. The plants in each pot, sprayed uniformly with 10 mL of DMSO-H_2O solution (1 %), were used as negative control groups. Sprayed plants were randomly placed on a greenhouse bench. The differences in the appearance of treated plants compared with healthy controls were considered as the indication of phytotoxicity.

Data analysis: Mortality observations were analyzed using the SPSS program, version 11.5, for ANOVA. Tukey's test was used to compare means. Probit analysis was used to determine lethal concentrations (LC_{50}), by using the SPSS program, version 11.5. Abbott's formula was used to correct mortality in controls.

RESULTS AND DISCUSSION

The chemical compositions of the essential oils used in this study were determined by GC-MS analysis and given in Table-1. The number of compounds and their relative amount found in essential oils varied according to plant species and the particular compound. The number of component identified in *T. spicata*, *L. nobilis*, *F. vulgare* and *R. officinalis* essential oils was 8, 36, 15 and 26, respectively. Based on GC-MS investigations, the major compounds found in essential oils of *T. spicata*, *L. nobilis*, *F. vulgare* and *R. officinalis* were carvacrol (70.9), 1,8-cineole (35.5 %), *trans*-anethole (82.8 %) and borneol (20.4 %), respectively (Table-1).

TABLE-1
CHEMICAL COMPOSITIONS OF ESSENTIAL OILS INVESTIGATED

Compound	Retention time	Essential oils and average peak area* (%)			
		T.s.s	L.n	F.v	R.o
α-Pinene	8.97	0.65	7.48	0.6	10
Camphene	9.66	–	–	–	2.49
Verbenene	9.94	–	–	–	1.93
Sabinene	11.31	–	**15.0**	0.33	–
Myrcene	12.13	1.25	1.01	0.13	1.57
α-Phellandrene	12.65	–	0.7	0.06	0.22
δ-3-Carene	12.96	–	–	–	2.81
α-Terpinene	13.04	1.31	–	–	–
p-Cymene	13.72	**13.77**	–	–	0.14
Limonene	14.29	–	–	**5.79**	–
cis-Ocimene	14.84	–	–	0.37	0.16
1,8-Cineole	15.03	–	**35.5**	–	**17.4**
trans-β-O Cymene	15.74	-	0.2	0.03	–
γ-Terpinene	16.3	**6.98**	1.41	0.14	0.35
Linalool oxide	16.8	–	–	–	0.19
trans-Sabinene hydrate	16.9	–	0.57	-	–
α-Terpinolene	17.3	–	–	–	1.29
Fenchone	17.7	–	–	1.69	–
α-Terpinolene	17.92	–	0.88	–	–
cis-Sabinene hydrate	18.75	–	0.38	–	–
Linalool	19.29	–	1.51	–	6.02
Neo-allo-Ocimene	20.61	–	–	0.27	–
α-Phellandrene epoxide	21.23	–	0.22	–	–
Camphor	22.14	–	–	0.06	**19.5**
Borneol	23.7	–	–	–	**20.4**
3-Cyclohexen-1-ol	24.27	–	4.93	–	–
α-Terpineol	25.26	–	4.63	–	–
4-Allyl anisole	25.49	–	–	**6.56**	–
Verbenon	26.75	–	–	–	4.23
trans-Carveol	27.46	–	–	–	0.58

p-Anisaldehyde	29.32	–	–	0.85	–
4-Thujen-2-α-YL	30.2	–	0.15	–	–
(-)-Bornyl acetate	30.7	–	0.31	–	–
(-)-Bornyl acetate	30.96	–	–	–	0.94
Sabinyl acetate	31.37	–	0.10	–	–
Carvacrol	32.07	**70.93**	–	–	–
trans-Anethole	33.59	–	–	**82.8**	3.45
α-Terpineneyl acetate	35.75	–	**14.20**	–	–
α-Ylangene	35.96	–	–	–	0.32
Eugenol	36.16	–	1.30	–	–
Neryl acetate	36.35	–	0.54	–	–
α-Copaene	36.47	–	–	–	0.61
β-Bourbonene	36.97	–	0.03	–	–
Geranyl acetate	37.38	–	0.07	–	–
Anisyl acetone	37.57	–	–	0.22	–
β-Elemene	37.61	–	0.59	–	–
β- Caryophyllene	39.02	3.30	–	–	1.49
Methyl eugenol	39.17	–	2.94	–	–
Germacrene D	39.54	–	0.05	–	–
(+)-Aromadendrene	40.07	–	–	–	0.08
(-)-Isoledene	40.73	–	0.07	–	–
α-Humulene	41.0	–	–	–	0.32
trans-Cinnamyl aceteta	41.2	–	0.31	–	–
β-Selinene	42.99	–	0.1	–	–
cis-Methyl isoeugenol	44.54	–	0.26	–	–
α-Amorphene	44.73	–	0.19	–	0.46
δ-Cadinene	45.31	–	0.07	–	–
γ-Cadinene	45.87	–	0.12	–	–
γ-Gurjunene	46.03	–	0.07	–	–
cis-α-Bisabolene	46.52	–	0.22	–	–
Elemicin	47.70	–	0.09	–	–
Caryophyllene oxide	48.42	1.10	1.09	–	0.59
Total	–	**99.29**	**97.19**	**99.80**	**97.54**

*Percentages are the mean of three runs and were obtained from electronic integration measurements using selective mass detector. Quantitative data were obtained by relating individual peak areas to the total area of the total ion chromatogram. Calibration factors were neglected. Dash indicates the compound was not found. Bold values indicate the most abundant compounds of the oils; T.s.s. = *T. spicata*, L.n. = *L. nobilis*, F.v. = *F. vulgare*, R.O. = *R. officinalis*.

The volatile phase effects of different concentrations of essential oils on the mortality of *B. tabaci* adults are shown in Table-2 and Fig. 1. All essential oils were found to cause 100 % adult mortality of *B. tabaci* in a dose-dependent manner. Essential oils of thyme caused the highest insecticidal effects, causing high adult mortalities at the lower concentrations in comparison to other essential oils tested. Adult viability was totally affected by thyme, laurel, fennel and rosemary at the concentrations of 5.0, 20.0, 30.0 and 30.0 μg mL^{-1} air, respectively. The LC_{50} (lethal concentration 50) was the concentration of the essential oil, which kills 50 % of

TABLE-2
EFFECTS OF DIFFERENT CONCENTRATIONS OF VOLATILE PHASES OF ESSENTIAL OILS ON PER CENT MORTALITY OF *B. tabaci* ADULTS

Concentrations (μg mL^{-1} air)	Essential oils			
	T. spicata	*L.nobilis*	*F. vulgare*	*R. officinalis*
0.25	31.4 aD	4.9 bF	0.0 bF	3.7 bF
0.50	52.3 aC	15.5 bE	3.3 cF	9.6 bcF
1.0	81.7 aB	30.7 bD	7.4 cEF	24.2 bE
2.0	94.0 aA	53.6 bC	15.8 cE	49.5 bD
5.0	100.0 a	79.0 bA	34.5 dD	59.5 cD
10.0	100.0 aA	85.8 bA	53.4 dC	73.6 cC
15.0	100.0 aA	96.8 aA	69.4 cB	83.3 bBC
20.0	100.0 aA	100.0 aA	77.8 cB	95.2 bAB
30.0	100.0 aA	100.0 aA	100.0 aA	100.0 aA
LC_{50}	0.44	1.82	7.06	2.86

Mean values (n = 3) followed by the same small or capital letters within the row or column, respectively, are not significantly different according to Tukey's test ($p \leq 0.05$). The estimated lethal concentration (LC_{50}) values (μg mL^{-1}) for each essential oil were estimated by using probit analysis.

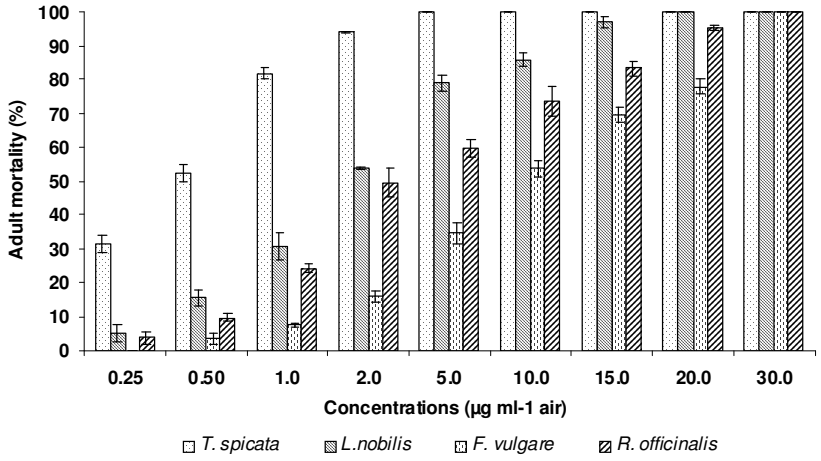

Fig. 1. Per cent mortality of *B. tabaci* adults caused by the different concentrations of essential oils. Bars represent means ± SE; n = 3 replicates with 20 adult per replicate

adult whitefly within 24 h following exposure. The estimated LC_{50} values obtained for each essential oil were calculated by using probit analysis (Table-2). The lowest LC_{50} values were recorded for thyme essential oil (0.44 μg mL^{-1}) was followed by laurel (1.82 μg mL^{-1}), rosemary (2.86 μg mL^{-1}) and fennel (7.06 μg mL^{-1}), respectively. Phytotoxicity tests were performed on foliage, flowers and fruits of host plant species of *B. tabaci*. No sign of phytotoxicity was found among the tested host plants at the highest concentration (30 μg mL^{-1}) used in the experiments.

Volatile compounds from plants, especially essential oils, have antimicrobial activity against a variety of food borne, human and plant pathogens and pest[10,17]. Botanical insecticides have long been recommended as attractive alternatives to synthetic chemical insecticides for pest management because these chemicals pose little threat to the environment or to human health[13]. Pyrethrum and neem are well established commercially, pesticides based on plant essential oils have recently entered the marketplace. In this study, we have evaluated insecticidal effect of essential oils on *B. tabaci*. Present results clearly confirm that essential oils from medicinal plants belonging to different plant families such as thyme, laurel, fennel and rosemary possess insecticidal activity against the *B. tabaci* adults.

In previous studies, insecticidal efficacy of essential oils, derived from taxonomically different medicinal plants species such as *Micromeria fruticosa*, *Nepeta racemosa*, *Origanum vulgare*, *Artemisia absinthium*, *A. herba-alba*, *A. monosperma*, *Vanillosmopsis pohlii*, *Satureja hortensis*, *Ocimum basilicum*, *Thymus vulgaris*, *Hyptis martiusii*, *Lavandula officinalis*, *L. angustifolia*, *Majorana hortensis*, *Mentha spicata*, *M. piperita*, *Crozophera plicata*, *Cymbopogon martinii*, *C. winterianus*, *C. citratus*, *Pongamia glabra* and *Azadirachta indica* were investigated against *B. tabaci* and *B. argentifolii* species[18-26]. To our best of knowledge, this is the first study demonstrating that the essential oils of *T. spicata*, *L. nobilis*, *F. vulgare* and *R. officinalis* possess insecticidal activities against *B. tabaci*.

The number of compounds and their relative amount found in plant essential oils varied according to plant species and the particular compound. The major compounds found in essential oils of thyme, laurel, fennel and rosemary were carvacrol (70.9), 1,8-cineole (35.5 %), *trans*-anethole (82.8 %) and borneol (20.4 %), respectively. These compounds have previously been reported to have antimicrobial activity against a variety of insects, mites, weeds and plant pathogens[17,27].

Major components of essential oils such as monoterpenoids, anethole, carvacrol, 1,8-cineole, *p*-cymene, menthol, γ-terpinen, terpinen-4-ol and thymol were investigated against three major greenhouse pests, females and eggs of the carmine spider mite, females of the cotton aphid *Aphis gossypii* and second instar larvae of the western flower thrips *Frankliniella occidentalis*[28]. These individual components possess antifeeding and oviposition deterant effect against three major greenhouse pests. Their results suggested that essential oils containing anethole, carvacrol and thymol may be recommended as toxic and/or reproduction-deterring fumigants against greenhouse pests without causing any phytotoxicity. The essential oil of *Hyptis martiusii* leaves and 1,8-cineole showed pronounced insecticidal effect against *Aedes aegypti* larvae and whitefly *B. argentifolii*[18]. The essential oil of heartwood and the pure sesquiterpene α-bisabolol were tested against *B. argentifolii* and pronounced insecticidal effects were observed[21]. Carvone, the major constituent of spearmint oil, was reported to possess 100 % fumigation toxicity against *B. tabaci*[20]. In addition to insecticidal activities, essential oils derived from medicinal plants possess antifungal, herbicidal and acaricidal actvities against plant pathogenic fungi, weeds and mites[29-31].

Spearmint, *M. spicata*, thyme (*Thymus* spp.) and *R. officinalis* have been shown to inhibit settling of the green peach aphid. The effect of the essential oils on aphid mortality was attributed primarily to starvation and to oral and fumigant toxicity[32]. In this aspect, insecticidal activities of essential oils used in our study on adult whitefly mortalities could be due to the fumigant toxicities of the major components of essential oils.

In conclusion, natural insecticides are desirable alternative to synthetic pesticides because they have low toxicity in mammals, little environmental effect and wide public acceptance. For the development of a successful integrated pest management (IPM) system, however, simultaneous use of insecticides and biocontrol agents may be required. In the current work, essential oils from medicinal plants growing in the region can offer good control of *B. tabaci* adult. The essential oils tested in this study could be considered as potential alternatives for synthetic insecticide with modification as their structures could lead to the development of new classes of insecticide compounds. However, further studies need to be conducted to evaluate the cost and efficacy of these essential oils on wide range of pests in commercial greenhouses.

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