Asian Journal of Chemistry Vol. 21, No. 5 (2009), 3995-4000

Insecticidal Effects of Some Essential Oils Against the Confused Flour Beetle (*Tribolium confusum* du Val) (Col.: Tenebrinoidea) in Stored Wheat

O. SENER*, M. ARSLAN, N. DEMIREL† and I. UREMIS‡
Department of Field Crops, Mustafa Kemal University, 31034 Hatay, Turkey
E-mail: osener9@gmail.com

The essential oils obtained from rosemary (*Rosmarinus officinalis* L.), common thyme (*Thymus vulgaris* L.), common sage (*Salvia officinalis* L.) and oregano (*Origanum syriacum* L.) have been analyzed by GC and GC-MS and tested for their insecticidal properties against the confused flour beetle, *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae) in stored wheat. The essential oil composition varied with the species. The main constituent of oregano, common thyme, common sage and rosemary oils were γ-terpinene, *p*-cymene, β-thujone and 1,8-cineole, respectively. Each essential oil was applied at the concentrations of 2, 4, 8, 16 and 32 μL/100 mL jar on the filler paper attached under the surface of the jar cap to determine mortality. Mortality rate of essential oils increased with the increasing concentrations. Of the 4 essential oils screened, 1,8-cineole from rosemary and common sage exhibited the highest activity, followed by β-thujone from common sage and *p*-cymene from thyme. Therefore, rosemary essential oil could be recommended as a potential source of environment-friendly botanical insecticide in the control of the confused flour beetle.

Key Words: Natural insecticide, γ-Terpinene, *p*-Cymene, β-Thujone, 1,8-Cineole, *Tribolium confusum*, Wheat.

INTRODUCTION

The confused flour beetle, *Tribolium confusum* du Val (Coleoptera: Tenebrionidae), is worldwide insect pests of mills, food warehouses, retail stores and urban homes[1,2]. It causes extensive loss of stored grain products such as flour, cereals, meal, crackers, beans, spices, pasta, cake mix, dried pet food, dried flowers, chocolate, nuts, seeds in tropical and semitropical environments. Infestations of confused flour beetle are mainly controlled either by fumigating with methyl bromide or phosphine, using timed aerosol applications of synergized pyrethrins, or by treating flooring surfaces with a residual insecticide. However, these substances are considered very harmful to both human health and the environment. Therefore, the use of these substances is being reduced[3,4]. The confused flour beetle is also resistant to several fumigants and grain protectants[3,5,6]. Therefore, the use of naturally occurring active compounds is needed for the control of this species.

†Department of Plant Protection, Mustafa Kemal University, 31034 Hatay, Turkey.

Essential oils represent a rich potential source of an alternative and environmentally acceptable pest control agents due to their insecticidal, repellent and/or antifeedant properties. Plants in Labiatae family possess essential oils, which could be utilized for killing or repelling pests. Most of these substances are volatile and can act as fumigants, thus offering the prospect of use against stored-product insects. The insecticidal activities of essential oil of plant origin are well known[7-13]. Human health, environment concern and consumers' demand for residue-free food, necessitate the evaluation of alternative, reduced-risk control methods. Understanding of the plant biochemistry, physiology and chemistry of natural products have shown that the secondary metabolites may be used for pest control to overcome the above problems associated with the synthetic pesticides.

The present study was aimed to investigate the insecticidal properties of essential oils of rosemary (*Rosmarinus officinalis* L.), common thyme (*Thymus vulgaris* L.), common sage (*Salvia officinalis* L.) and oregano (*Origanum syriacum* L on the control of *Tribolium confusum* du Val (Coleoptera: Tenebrionidae).

EXPERIMENTAL

The adults (10 and 40 days old) of confused flour beetle were obtained from laboratory cultures on wheat grains at 26 ºC, 65 % relative humidity and with a photoperiod of 12 h light-dark. To test the fumigant toxicity of the essential oils on the confused flour beetle, 100 mL glass jars with screwed plastic caps were used as exposure chambers. A small piece of filter paper was attached to the under surface of the cap to serve as a diffuser, on which varying doses of essential oils were applied while the control diffuser was left untreated. In each jar, 30 g of wheat grains were put and 10 adults of confused flour beetle were placed and exposed to the various vapours. The insects had no contact with the diffuser and stayed at the bottom of the chamber throughout the experiment. A 72 h exposure time was considered adequate to assess mortality. After exposure, the dead insects were counted. Four replications were performed for each dose and control.

Four species of native plants in Labiatae family, rosemary (*Rosmarinus officinalis* L.), common thyme (*Thymus vulgaris* L.), common sage (*Salvia officinalis* L.) and oregano (*Origanum syriacum* L.), were grown in Telkalis Research Farm of Mustafa Kemal University, Hatay, Turkey in 2007. Essential oil was extracted by water distillation for 3 h from air-dried leaves of each individual species, using a Clevenger-type apparatus. The essential oils obtained from extraction were dried over anhydrous sodium sulfate (Merck, Buenos Aires, Argentina) and stored at 4 ºC in a refrigerator until analysis. The GC analyses were carried out using Hewlett-Packard 6890 GC with FID. A HP-5 MS capillary column (30 m × 0.25 mm i.d. 0.25 µm film thickness) was used. Helium was used as a carrier gas (1.4 mL/min). The column was temperature programmed as follows: 5 min at 45 °C; then at 3 °C/min to 220 °C and held for 10 min. The injector and detector temperatures were to 220 and 250 °C, respectively. Injection was carried out automatic mode. Samples [0.5 µL

of the oil solution in hexane (1:100)] were injected by the splitless technique into helium carrier gas. Peak areas and retention times were measured by electronic integration.

GC/MS analyses of the essential oils were carried out on Hewlett Packard 5970A mass selective detector (MSD), directly coupled to a HP 6890 GC. The column, temperature programme and injection were performed as described above. Injection was carried out automatic mode. Library search was carried out using Wiley Library, WILEY275, NBS75K, NIST98, FLAVOR. EI mass spectra were measured at 70 eV ionization voltage over the mass range 10-400 u. Identification of the compounds was achieved by comparing retention times and mass spectra with those of the standards in the library[14].

All experiments were conducted twice in a completely randomized design with four replications. Analysis of variance was performed for all data using a general linear model procedure[15]. Differences between means were tested through LSD and values of $p < 0.05$ were considered significantly different.

RESULTS AND DISCUSSION

The ANOVA showed that main effects species, dose and species × dose interactions were all significant for mortality at $p = 0.0001$ (Table-1). The toxicities of 2, 4, 8, 16 and 32 µL/100 mL jar doses of rosemary, common thyme, common sage and oregano essential oils on confused flour beetle were determined. Mortality rate was highly significant among the tested plant species. Mortality of the confused flour beetle after 72 h of exposure on 2 µL/100 mL jar essential oils was 1.25, 71.25, 8.75 and 12.5 % for oregano, common thyme, rosemary and common sage, respectively (Fig. 1). At the dose rate of 2 µL/100 mL jar the highest mortality rate was obtained from rosemary essential oil, while the lowest was obtained from oregano essential oil. The mortality on wheat treated with essential oils increased with increasing essential oil doses for the tested essential oils. When oregano essential oil was considered, mortality was quite low at 2, 4 and 8 µL/100 mL jar. However, the mortality rate remarkably increased after 8 µL/100 mL jar and it reached 80 and 90 % at 16 and 32 8 µL/100 mL jar, respectively. At the highest dose, all of the beetles were dead on wheat treated with rosemary, common thyme and common sage essential oils, except for oregano oil. On wheat treated with rosemary essential oil, mortality was significantly higher (71, 90, 90 and 100 %, at 2, 4, 8 and µL/100 mL jar, respectively) than that for the other essential oils.

More than 25 components were identified in each essential oil, but most of them constituted less than 1 % (data not given). Only 6 components detected in the essential oil of oregano in concentrations more than 2 % were myrcene, α-terpinene, γ-terpinene, terpinene-4-ol, β-caryophyllene and *p*-cymene (Table-2). Nine components *i.e.*, 1,8-cineole, linalool, myrcene, α-terpinene, γ-terpinene, terpinene-4-ol, thymol, *p*-cymene and carvacrol were detected for common thyme. Nine components, 1,8-cineole, α-humulene, β-pinene, α-thujone, β-thujone, L-camphor, β-caryophyllene, viridiflorol and *p*-cymene were detected for common sage. Eight components,

TABLE-1
ANALYSES OF VARIANCE FOR THE CONFUSED FLOUR BEETLE TREATED WITH ROSEMARY, COMMON THYME, COMMON SAGE AND OREGANO ESSENTIAL OILS

Source	df	ms	F	p
Replication	3	308.3333	1.73	0.1664
Repeat	1	302.5000	1.70	0.1960
Error 1	3	224.1667	–	–
Species	3	19698.3333	110.39	<0.0001
Repeat x species	3	1067.5000	5.98	0.0009
Error 2	18	206.8056	–	–
Dose	4	36880.6250	206.69	<0.0001
Repeat × Dose	4	96.2500	0.54	0.7071
Species × Dose	12	2572.2917	14.42	<0.0001
Repeat × Species × Dose	12	294.5833	1.65	0.0904

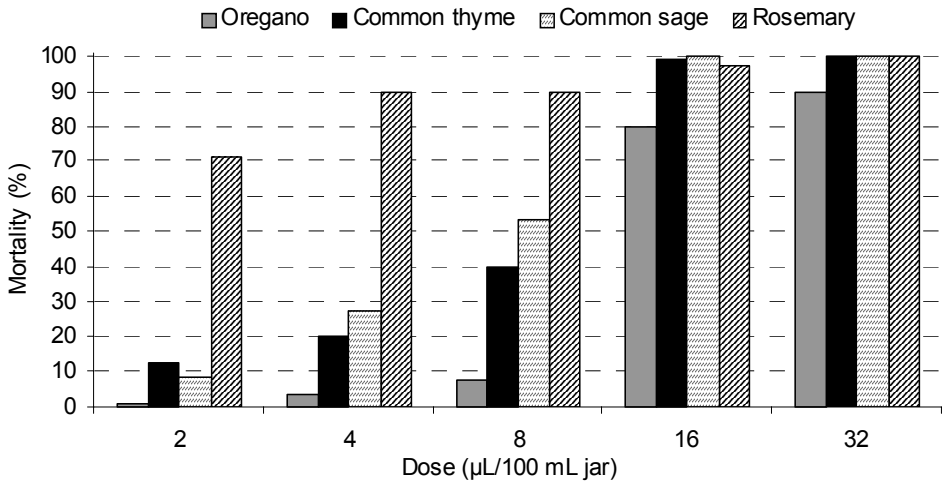

Fig. 1. Mean mortality of confused flour beetle exposed for 72 h on wheat treated with rosemary, common thyme, common sage and oregano essential oil at the dose rates of 2, 4, 8, 16 and 32 µL/100 mL jar

1,8-cineole, linalool, endobornyl acetate, terpinene-4-ol, *p*-cymene, borneol, camphor and 1,3-dimethylbicyclo were detected for rosemary. The active ingredient for insecticidal activity in oregano, common thyme, common sage and rosemary, were γ-terpinene, *p*-cymene, β-thujone and 1,8-cineole, respectively.

Essential oils are generally composed of complex mixtures of monoterpenoids, biogenetically related phenols and sesquiterpenes. Essential oils demonstrate a wide range of bioactivities such as direct toxicity to insects, feeding deterrence, repellence and attraction. Numerous studies have assessed the ability of plant essential oils and their components to protect stored crop products from insect pests using essential oils as fumigants and repellents. Essential oils cause physiological changes to insects

TABLE-2
CHEMICAL COMPOSITION OF OREGANO, COMMON THYME, COMMON SAGE AND ROSEMARY ESSENTIAL OILS

Components	Oregano	Common thyme	Common sage	Rosemary
1,8-Cineole	–	2.40	22.83	21.45
Linalool	–	4.60	–	5.88
Endobornyl acetate	–	–	–	2.44
Methyl eugenol	–	–	–	1.02
α-Humulene	–	–	2.82	–
Myrcene	6.00	2.11	1.96	1.90
Sabinene	1.19	–	–	–
α-Terpinene	8.86	2.62	–	1.00
γ-Terpinene	36.17	10.07	–	1.07
Terpinene-4-ol	3.59	2.53	0.93	3.12
Thymyl methylether	–	1.31	–	–
Carvacrol methyl ether	–	1.17	–	–
Thymol	–	17.88	–	–
β-Pinene	–	–	2.63	0.92
α-Thujone	–	–	4.29	–
β-Thujone	–	–	25.05	–
L-Camphor	–	–	18.38	–
β-Caryophyllene	4.52	1.71	3.83	0.94
Viridiflorol	–	–	4.51	–
α-Phellandrene	1.01	–	–	–
Limonene	1.30	–	–	–
p-Cymene	30.25	40.00	2.03	3.08
trans-Sabinene hyrate	1.52	–	–	–
Carvacrol	–	3.79	–	–
Borneol	–	–	1.30	8.58
Verbenene	–	–	–	1.79
Camphor	–	–	–	19.70
6,6-Trimethylbicyclo	–	–	–	1.91
1,3-Dimethylbicyclo	–	–	–	8.24
Nopol	–	–	–	1.27

by membrane disruption and some how they affect the nervous system of the insects. The detrimental effects of tested essential oils could be due to one or two of these factors. However, the exact mechanism of the mortality caused by essential oils has not been determined.

Adults of the confused flour beetle were exposed to various concentrations of essential oils of oregano, common thyme, common sage and rosemary. When the essential oils were applied at 2, 4, 8, 16 and 32 µL/100 mL jar mortality of the confused flour beetle started at 2 µL/100 mL jar and increased with the increasing concentrations. Regardless of the plant species, the effect of essential oils is dose-dependent as in the case of synthetic insecticides.

The insecticidal effects of the essential oils can be attributed to their major components[10,16,17]. In the current study, γ-terpinene from oregano, *p*-cymene from common thyme, β-thujone from common sage and 1,8-cineole from rosemary have been identified as the main volatile components. Differences in insecticidal activity of essential oils have been ascribed to differences in major active ingredients (γ-terpinene, *p*-cymene, β-thujone and 1,8-cineole). The present study confirms the findings of various researchers[13,18].

Essential oils have inhibitory effects on seed germination. Therefore, care must be taken before planting essential oil exposed seeds due to lose of germination rate. We tested the inhibitory effects of four essential oils at the concentrations of 2, 4, 8, 16 and 32 μL/100 mL jar on wheat germination. However, any differences between control and essential oil exposed seeds were found (data not given). The toxic effects of essential oils are most probably related to the humidity level of the seeds. Since the humidity of wheat seed was 12 %. Therefore, it could be concluded that essential oils has inhibitory effects on germinating seeds, not on dry seeds.

Present study indicated that essential oils can be used successfully as an insecticide against the confused flour beetle. However, some essential oils are more toxic to the confused flour beetle than the others. Because of its volatile property and broad spectrum functions, 1,8-cineole from rosemary showed strong toxic potential to be used as a biofumigant for the control of the confused flour beetle in stores. However, further studies are needed to be conducted to evaluate the cost and efficacy of these essential oils on the confused flour beetle in commercial stores.

REFERENCES

1. A.D. Aitken, Insect Travellers, I: Coleoptera, Technical Bulletin 31, HMSO, London (1975).
2. D. Rees, Insects of Stored Products, CSIRO Publishing, Collingwood, Vic., Australia (2004).
3. F.H. Arthur, *J. Stored Prod. Res.*, **32**, 293 (1996).
4. C.H. Bell, *Crop Protection*, **19**, 563 (2000).
5. F.H. Arthur and J.L. Zettler, *J. Stored Prod. Res.*, **28**, 55 (1992).
6. J.L. Zettler and F.H. Arthur, *J. Econ. Entomol.*, **90**, 1157 (1997).
7. K.N. Don-Pedro, *Pesticide Sci.*, **47**, 213 (1996).
8. E. Shaaya, M. Kostjucovski, J. Eilberk and C. Sukprakarn, *J. Stored Prod. Res.*, **33**, 7 (1997).
9. S. Keane and F.M. Ryan, *Insect Biochem. Molecul. Biol.*, **29**, 1097 (1999).
10. I. Tunc, B.M. Berger, F. Erler and F. Dagli, *J. Stored Prod. Res.*, **36**, 161 (2000).
11. D.P. Papachristos and D.C. Stamopoulos, *J. Stored Prod. Res.*, **38**, 365 (2002).
12. S. Lee, C.J. Peterson and J.R. Coats, *J. Stored Prod. Res.*, **39**, 77 (2003).
13. G.K. Ketoh, H.K. Koumaglo and I.A. Glitho, *J. Stored Prod. Res.*, **41**, 363 (2005).
14. R.P. Adams, Identification of Essential Oil Components by Gas Chromatography Mass Spectroscopy, Allured Publishing Corporation, Carol Stream, IL, USA (1995).
15. SAS Institute, SAS/Stat Software: Changes and Enhancements Through Release 6.12. SAS Inst., Cary, NC (1997).
16. W.I. Choi, S.G. Lee, H.M. Park and Y.J. Ahn, *J. Econ. Entomol.*, **97**, 553 (2004).
17. O. Calmasur, I. Arslan and F. Sahin, *Ind. Crops Prod.*, **23**, 140 (2006).
18. D.C. Stamopoulos, P. Damos and G. Karagianidou, *J. Stored Prod. Res.*, **43**, 571 (2007).

(*Received*: 27 September 2008; *Accepted*: 23 February 2009) AJC-7277