Asian Journal of Chemistry Vol. 18, No. 2 (2006), 1455–1461

Antimicrobial Activities of Some Thyme (*Thymus*, *Staureja*, *Origanum* and *Thymbra*) Species against Important Plant Pathogens

SÜLEYMAN KIZIL* and FIKRET UYAR†
Department of Field Crops, Faculty of Agriculture, Dicle University, Diyarbakir-21280, Turkey
Tel: (90)(412)2488509; Fax: (90)(412)2488153; E-mail: kizils@dicle.edu.tr

Different types of plants are used as thyme, such as *Tyhmbra*, *Origanum*, *Satureja*, *Thymus* and *Coridathymus* in various regions of the world. In the present study, essential oil components of *Thymus kotschyanus*, *Satureja hortensis*, *Origanum onites* and *Thymbra spicata* were determined by GC and antimicrobial and antifungal activities of essential oils of these plants against four plant pathogens were evaluated at concentrations of 5, 10 and 15 µg and incubation times of 24, 48 and 72 h. The major components of the essential oils determined by GC were thymol (41.6%) in *Thymus kotschyanus*, carvacrol in *Origanum onites* (40.7%), *Satureja hortensis* (20.6%) and *Thymbra spicata* (81%). Aqueous and hexane extracts of each plant were tested for their antibacterial activity by using agar disk diffusion method. The results indicated that all essential oils exhibit antibacterial activity against *Clavibacter michiganensis* subsp. *michiganensis*, *Pseduomonas syringae* pv. *tomato* and *Macrophomina phaseoli*, except *Xanthomonas campestris* pv. *malvacearum*. In addition, it has been observed that gram +ve bacteria are more sensitive than gram –ve bacteria.

Key Words: Thyme, Essential oil, GC, Plant pathogen, Antimicrobial activity.

INTRODUCTION

Naturally derived compounds and other natural products may have applications in controlling pathogens in plants and foods[1]. Some spices are known to contain essential oils that possess antimicrobial activity, such as carvacrol and thymol in thyme, cumin aldehyde in cumin and linalool in coriander[2–5]. This activity could act as chemical defence against plant pathogenic diseases. It is also suggested that complex oil present a greater barrier to pathogen adaptation than would a more simple mixture of monoterpenes.

Investigations on the evaluation of the biological activities of essential oils of some medicinal plant species revealed that some of them exhibited interesting

†Department of Biology, Faculty of Science and Arts, Dicle University, Diyarbakir-21280, Turkey.

activities such as insecticidal, antibacterial and antifungal, antinoceptive, spasmolytic and antiplasmodial[6].

The flora of Turkey have 23 species of *Origanum*, 2 species of *Thymbra*, 14 species of *Satureja* and 36 species of *Thymus*. They are known and used as thyme and "kekik", which is the name given to those species with a thymol/carvacrol type odour in Turkey and all of these species are well known as folk medicines used in several countries[7,8].

Demand for reduction in the use of pesticides in the agriculture increases interest in the possibility of the application of essential oil to control plant pathogens[1].

C. michiganensis, *P. syringae* pv. *tomato*, *X. campestris* pv. *malvacearum* and *M. phaseoli* are important plant pathogens and showed largely agricultural areas.

Bacterial canker of tomato is caused by *C. michiganensis*. The disease occurs worldwide wherever tomatoes are grown. The bacterium is seed borne but may survive on plant debris in soil for at least one year. *P. syringae* pv. *tomato* produces small, brown spots with yellow margins on tomato leaves. *X. campestris* pv. *malvacearum* is often a very serious disease of cotton. Leaf spots are small, angular and water soaked, becoming dark-brown or reddish purple as they age. Charcoal rot is also caused by *M. phaseoli*. Charcoal rot attacks roots, crowns and lower stems when plants are sown after other susceptible crops[9].

The present study is focussed on the determination of the antimicrobial activity of the essential oils of four plants named as thyme, which is rich in terms of carvacrol and thymol content. In addition, the goal of this research was to search the effect of these plants' essential oils against some plant pathogens (bacteria and fungi) that damaged extensively field crops such as tomato, cotton and sesame and essential oil components of these plants.

EXPERIMENTAL

The plant materials used in the study are given below. *Thymbra spicata* L. var. *spicata*, *Thymus kotschyanus* Boiss. & Hohen., *Satureja hortensis* L. and *Origanum onites* L. were cultivated at the Department of Field Crops, Faculty of Agriculture, Dicle University.

Origanum seeds were provided from Agriculture Faculty of Ankara University, and *T. spicata* L. var. *spicata*, *T. kotschyanus*, *S. hortensis* seeds were collected from the districts of Dicle, Karacadağ and Ergani, Diyarbakir, Turkey between July and September 1997–2001. Seeds of *Origanum*, *Thymbra*, *Satureja* and *Thymus* were first sown in greenhouse in December 2002; later, when seedlings reached at 10–15 cm, they were transferred to the field in April 2002. Except *Satureja*, in the first year, leaves were not harvested, but in the second year; the harvest was performed at the flowering period in June 2003. Fresh leaves were dried in a shadow and airy place for one week. Voucher specimens were stored in the warehouse until the analyses were performed. In the study, plant materials were grounded in a spice mil before used.

Isolation of the essential oils: Dry aerial materials of four plants were pulverized; essential oil of plants was extracted by Clevenger-type apparatus for 4 h. Results were recorded as mL/100 g (%).

GC conditions: Samples of essential oils were dissolved in hexane (1/100) and 1 μL was injected on the following GC column. Supelcowax-10, fused silica capillary column (30 m length × 0.25 mm i.d. × 0.25 μm film thickness). Flow rate and temperature programming are as shown below. Initial column temperature was 75°C, programmed to 200°C at 2.5°C min^{-1} and held for 5 min at 200°C. GC was performed with a Varian 3400 gas chromatograph fitted with flame ionization detector and an effluent splitter with a split ratio of 30 : 1; carrier gas nitrogen, flow rate 1 mL min^{-1}, injector temperature 250°C, detector temperature 300°C.

Micro-organisms: The micro-organisms used in the study were obtained from the Department of Plant Production, Faculty of Agriculture, Dicle University. *Clavibacter michiganensis* subsp. *michiganensis* (gram +ve), *Pseduomonas syringae* pv. *tomato* (gram –ve), *Xanthomonas campestris* pv. *malvacearum* (gram –ve) and *Macrophomina phaseoli* were used in the study.

Antibiotic discs: In the study, the disk diffusion method was used to determine the antimicrobial activity[10]. The essential oil solutions were prepared in hexane. By pipetting 5, 10 and 15 μL volumes of stock essential oils, 10 μg discs were prepared. The discs used as a control were absorbed with 10 μL hexane.

Antibacterial activity: Test organisms were inoculated on to the specific LB (Lauria Broth) media and incubated at 37°C for 2–6 h. The suspension was adjusted to a 0.5 McFalland turbidity standard (1×10^8 CFU/mL) by using a spectrophotometer. Of the bacteria suspension prepared from LB, 15 mL was suspended on agar (Mueller Hinton). paper discs with a diameter of 6 mm containing 5, 10 and 15 μg of essential oil samples to be assayed were put on to agar. Plates were incubated at 37°C for 24, 48 and 72 h and the inhibition zones of the microbial produced by different essential oils were measured as mm. All the tests used against microorganisms were conducted three times and the results were expressed as average values.

RESULTS AND DISCUSSION

The essential oil components identified are given in Table-1. It is seen that main components of *T. kotschyanus* essential oil are thymol (41.6%), carvacrol (15.1%), myrcene (11.8%), limonene (8.8%) and α-pinene (7.3%); for *O. onites*, they were carvacrol (40.7%), α-pinene (16.6%) and camphene (13%); for *S. hortensis*, they were determined as carvacrol (20.6%), *p*-cymene (19.1%), thymol (16%), camphene (6.8%), γ-terpinene (5%) and α-pinene (4.5%), and *T. spicata* essential oil contains as major component carvacrol (81%), *p*-cymene (7.6%) and α-pinene (2.5%).

About 17 compounds identified were in common for four oils. Carvacrol was the major compound of the essential oils of *S. hortensis*, *O. onites* and *T. spicata*, representing 20.6, 40.7 and 81.0% of the total oils, respectively. Regarding the previously reported content of *Satureja*, *Origanum*, *Thymbra* and *Thymus* essen-

tial oils, it is interesting to point out that there are important quantative differences, suggesting that the environmental factors may strongly influence its chemical composition[11, 12].

TABLE-1
CHEMICAL COMPOSITION OF ESSENTIAL OILS OF DIFFERENT PLANTS USED AS THYME (% TOTAL PEAK AREA)

Components	*Thymus kotschyanus*	*Satureja hortensis*	*Origanum onites*	*Tyhmbra spicata*
α-Pinene	7.3	4.5	16.6	2.50
Camphene	—	6.8	13.0	1.80
Myrcene	11.8	16.1	—	0.58
Limonene	8.8	—	6.8	0.55
γ-Terpinene	6.4	5.0	4.7	—
1,8-Cineole	—	2.9	2.9	2.08
p-Cymene	3.0	19.1	5.9	7.60
α-Terpineol	3.1	0.4	1.9	0.40
Linalool	0.6	0.4	1.2	—
β-Caryophyllen	0.3	0.7	0.6	—
Camphor	0.5	0.6	1.1	0.08
Borneol	0.1	0.3	0.8	—
Geranyl acetate	—	0.3	0.3	—
Carvone	1.0	0.8	1.9	2.80
Thymol	41.6	16.0	0.9	0.40
Carvacrol	15.1	20.6	40.7	81.00
Oil yield (% mL/100 g)	0.8	2.2	3.3	1.1

Determination of the antimicrobial activity of essential oils by paper disc diffusion method

The antimicrobial activities of different essential oils against microorganisms examined in the present study were assessed by the presence or absence of inhibition zones and zone diameter. The essential oils from the four plants were inhibitory to the growth of all the bacteria under test and these findings are summarized in Table-2.

It has been shown that essential oils from different plants possessed a wide range of antibacterial spectrum. Because they inhibited the growth, the diameter of inhibition varied from 10–46 mm, depending upon the susceptibility of the tested organism. In the study, among bacteria, the most resistant is *X. malvacearum*. In addition, essential oils of *Thymbra*, *Savory*, *Oregano* and *Thymus* were ineffective against *X. malvacearum*.

All essential oils at 5 µg decreased the inhibition zone against bacteria than 10 and 15 µg. Lower essential oil amount produced lower inhibition zone than the higher one. *Oregano* and *Savory* essential oils showed high inhibition zone against *C. michiganensis* subsp. *michiganensis* at 5 µg (34 and 30 mm) and *Thymbra* oil showed high inhibition zone on *M. phaseoli* at 5 µg as 32 mm.

At 10 µg, the highest inhibition zones are obtained from oregano and savory oils against *C. michiganensis* as 46 and 32 mm, respectively and at 15 µg, the highest inhibition zones were obtained from *Satureja* and *Thymus* essential oils against *C. michiganensis* as 34 and 30 mm, respectively. Inhibition zones were found to be gradually increased considerably when the concentration rate increased. Kizil *et al.*[13] have reported that, for cumin and coriander, essential oils at 25 µg concentration amount inhibited bacteria growing than 50 µg concentration. Therefore, it can be said that the quantity of oil is important for inhibition effects. Low concentration of essential oils were quite sufficient to prevent the growth of tested *C. michiganensis* (gram-positive), *P. syringae* pv. *tomato* (gram-negative) and *M. phaseoli* (fungi). Furthermore, gram-positive bacteria were more sensitive to essential oils than gram-negative[14].

TABLE-2
INHIBITION ZONES OF DIFFERENT ESSENTIAL OILS AT DIFFERENT CONCENTRATIONS (5, 10 AND 15 µg) AND INCUBATION TIMES (24, 48 AND 72 h)

Essential oils	Rate (µg)	*Clavibacter michiganensis*			*Pseudomonas syringae*			*Xanthomonas malvacearum*			*Macrophomina phaseoli*		
		24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Thymbra spicata	5	10	10	10	10	10	10	R	R	R	32	32	32
	10	12	12	12	12	12	12	R	R	R	34	34	34
	15	22	22	22	20	20	20	R	R	R	38	38	38
Thymus kotschyanus	5	10	10	10	12	12	12	R	R	R	16	16	16
	10	18	20	18	14	14	14	R	R	R	14	14	14
	15	30	30	30	16	16	16	R	R	R	18	18	18
Origanum onites	5	34	34	10	10	8	8	R	R	R	28	28	28
	10	46	46	R	12	12	12	R	R	R	30	30	30
	15	R	R	R	12	18	18	R	R	R	32	32	32
Satureja hortensis	5	30	30	30	10	10	10	R	R	R	R	R	R
	10	32	32	32	16	12	12	R	R	R	10	10	10
	15	34	34	34	32	32	32	R	R	R	14	14	14

Antimicrobial activities of different essential oils against three bacteria and a fungus at different incubation times are shown in Table-2. For all the essential oils included in this biological investigation, the antibacterial and antifungal activity was also observed after 24, 48 and 72 h of incubation. Different incubation times did not show a positive effect on tested bacteria and fungi. Inhibition zones of 24, 48 and 72 h incubation times were similar to each other.

It can be said that 24 h incubation time is sufficient for microorganisms. Similar results were also reported by Cimanga *et al.*[6].

It has earlier been reported that the culture medium, the technique of testing, the botanical source of the plant, the age of the plant, the state of plant material used (dried or fresh), the quantity of the oil used for the test and the isolation technique are some factors implicated in the great variation of the activity of the essential oil[1, 15].

It is also known that the composition of essential oils and their antimicrobial effects depend on plant species and regional conditions, such as altitude and climatic conditions. Some researchers reported that there is a relationship between the chemical structures of the most abundant compounds in the tested essential oils and the antimicrobial activity. Essential oils rich in phenolic compounds such as cavracrol and thymol are widely reported to possess high levels of antimicrobial activity, which has been confirmed and extended in the previous studies[7, 16]. The antimicrobial activities of *Origanum*, *Satureja*, *Thymus* and *Thymbra* may possibly be due to the presence of carvacrol and thymol[17, 18] as the most prevalent compounds.

Some researchers also reported that inhibition zones of oregano and thyme essential oil increased against bacteria when concentration rate was increased[19].

It appears that there is a relationship between the chemical structures of the most abundant compounds in the tested essential oil and antimicrobial activity.

Further research is needed in order to obtain information regarding the practical effectiveness of essential oils to protect the plants, seeds or the plant products without phytotoxic effects, but our study gives support for the application of certain essential oils to control plant pathogens such as *P. syringae* pv. *tomato*, *X. campestris* pv. *malvacearum* and *M. phaseoli* or to eliminate the *C. michiganensis* subsp. *michiganensis* under specific application conditions.

The results of the present study revealed that the antibacterial activity of essential oils of plants used as thyme and containing carvacrol and thymol varied greatly depending upon the plant species as well as the origin of microorganisms. In the study, the data show that gram-negative bacteria were more resistant to various essential oils than gram-positive. Our results suggest that the use of some thyme plants' essential oils as antimicrobial agents may be exploitable to prevent the deterioration of seeds by some bacteria and fungi.

REFERENCES

1. D.J. Daferera, B.N. Ziogas and M.G. Polissiou, *Crop Protection*, **22**, 39 (2003).
2. P.C. Hsieh, J.L. Mau and S.H. Huang, *Food Microbiol.*, **18**, 35 (2001).
3. S.G. Deans, K.P. Svoboda, M. Gundidza and E.Y. Brechany, *Acta Hort.*, **306**, 229 (1992).
4. R. Piccaglia, M. Marotti, E. Giovanelli, S.G. Deans and E. Eaglesham, *Ind. Crop Prod.*, **2**, 47 (1993).
5. C.F. Bagamboula, J. Uyttendaele and L. Debevere, *Food Microbiol.*, **21**, 33 (2004).
6. K. Cimanga, K. Kambu, L. Tona, S. Apers, T. De Bruyne, N. Hermans, J. Totté, L. Pieters and A.J. Vlietinck, *J. Ethnopharmacol.*, **79**, 213 (2002).
7. H. Baydar, O. Sağdiç, G. Özkan and T. Karadogan, *Food Control*, **15**, 169 (2004).

8. J. Jalas, *Thymus* L. in: P.H. Davis (Ed.), Flora of Turkey and the East Aegean Islands, Vol. 7, pp. 349–382, Edinburgh-London (1982).
9. R.A. Leliott and D.E. Stead, Methods for the Diagnosis of Bacterial Diseases of Plants (Methods in Plant Pathology), Blackwell Scientific Publications, London (1987).
10. E. Bagci and M. Digrak, *Turk. J. Biol.*, **21**, 273 (1997).
11. L. Hornok, in: L. Hornok (Ed.), The Cultivation of Medicinal Plants: Cultivation and Processing of Medicinal Plants, Budapest, p. 336 (1992).
12. M. Ünlü, D. Daferera, E. Dönmez, P. Moschos, B. Tepe and A. Sökmen, *J. Ethnopharmacol.*, **83**, 117 (2002).
13. S. Kizil and Sögüt, *Turkish J. Field Crops*, **7**, 1 (2002).
14. N. Nakatani, *Dev. Food Sci.*, **34**, 251 (1994).
15. P.J. Delaquis, K. Stanich, B. Girard and G. Mazza, *Int. J. Food Microbiol.*, **74**, 101 (2002).
16. S. Karaman, M. Digrak, U. Ravid and A. Ilçim, *J. Ethnopharmacol.*, **76**,183 (2001).
17. S.G. Deans and K.P. Svoboda, *Flavour Fragr. J.*, **5**, 187 (1990).
18. R.S. Farag, Z.Y. Daw, F.M. Hewedi and G.S.A. El-Baroty, *J. Food Prot.*, **52**, 665 (1989).
19. O. Sagdiç, A. Kusçu, M. Özcan and S. Özçelik, *Food Microbiol.*, **19**, 473 (2002).

(*Received*: 16 September 2005; *Accepted*: 31 December 2005) AJC-4592