Antibacterial activity of essential oils extracted from some medicinal plants, carvacrol and thymol on *Xanthomonas axonopodis* pv. *vesicatoria* (Doidge) Dye causes bacterial spot disease on pepper and tomato

R. Kotan[1,2*], F. Dadasoglu[3], S. Kordali[3], A. Cakır[4], N. Dikbas[1,5] and R. Cakmakcı[1,6]

[1]Biotechnology Research and Application Centre, Atatürk University, TR-25240 Erzurum, Turkey
[2]Oltu Vocational Training School, Nutrition Technology Programme, Atatürk University, TR-25400 Oltu/Erzurum, Turkey
[3]Faculty of Agriculture, Department of Plant Protection, Atatürk University, TR-25240 Erzurum, Turkey
[4]Kazım Karabekir Education Faculty, Department of Chemistry, Ataturk University, Campus, 25240-Erzurum, Turkey
[5]Narman Vocational Training School, Nutrition Technology Programme, Atatürk University, TR-25530-Narman /Erzurum, Turkey
[6]İspir Vocational Training School, Organic Agriculture Programme, Atatürk University, TR-25900 İspir/Erzurum, Turkey

Kotan, R., Dadasoglu, F., Kordali, S., Cakır, A., Dikbas, N. and Cakmakcı, R. (2007). Antibacterial activity of essential oils extracted from some medicinal plants, carvacrol and thymol on *Xanthomonas axonopodis* pv. *vesicatoria* (Doidge) Dye causes bacterial spot disease on pepper and tomato. Journal of Agricultural Technology 3(2): 299-306.

The inhibitory effect of 24 different essential oils in addition to pure carvacrol and thymol, which are the main compounds of some plant species, were evaluated on a *Xanthomonas axonopodis* pv. *vesicatoria* the cause of bacterial spot disease on pepper and tomato. The disc diffusion method with a minor modification was used for testing inhibitory activity. The minimum inhibitory concentration (MIC) values were determined by using the modified agar-well diffusion method at concentrations from 3,125 and 800 µl/ml. The pathogen was inhibited by the whole tested plant oils and pure compounds. The pure carvacrol and thymol showed the highest inhibition zone (85 mm), and MIC value was 3.125 µl/ml on thePetri plate. This zone values was the higher than inhibition zone of Streptocycline used as positive control. Of the 24 plant samples, *Thymus canoviridis*, *Satureja hortensis*, *Melissa officinalis inodora*, *Helichrysum plicatum*, *Thymus haussknechtii*, *Thymus sipyleus* and *Thymus sipyleus rosulans* essential oils was the most active showing an inhibition zone of 22-46.3 mm and a MIC of 25-200 µl/ml. This study indicated that these seven oils, carvacrol and thymol can be used as a seed disinfectant for management of bacterial spot disease.

Key words: antibacterial, bacterial spot, essential oil, pepper, tomato, *Xanthomonas axonopodis* pv. *vesicatoria*

[*] Corresponding author: Kotan, R.; e-mail: rkotan@atauni.edu.tr

Introduction

Xanthomonas axonopodis pv. *vesicatoria* (Doidge) Dye causes bacterial spot disease on pepper (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.). It is an important disease in many production areas of the world. Bacterial pathogens and their control are a serious problem in agriculture practice. Management strategies include the use of disease-free seed and seedlings, resistant cultivars, and copper sprays. However, these strategies are not always effective, especially when environmental conditions are optimal for disease or inoculums levels are high (Sahin and Miller, 1996). Spraying with antibiotics and copper compounds, usually suggested to control bacterial diseases, have never been satisfactory. Furthermore, antibiotics are forbidden in many countries because of their general toxicity; exert a negative impact on both yield and the environment.

As an alternative strategy to prevent the spread of plant diseases, natural compounds of plants can be a source of new pesticides (Elkovich, 1988). There are some studies related to different plant species which have more or less antagonistic activity against plant pathogenic bacteria *Xanthomonas* sp. (Satish *et al*., 1999; Basım and Basım, 2003; Nguefack *et al*., 2005; Kızıl and Uyar, 2006; Mohana and Raveesha, 2006; Vasinauskiené *et al*., 2006).

In recently, a lot of studies related to antimicrobial activities of extracts or essential oils of plants in Turkey have also been made, followed by the genus *Satureja* (Güllüce *et al*., 2003; Sahin *et al*., 2003); and *Thymus* (Sokmen *et al*., 2004b; Ozturk and Ercisli, 2005; Tepe *et al*., 2005; Kızıl and Uyar 2006); *Achillea* (Sokmen et al., 2004a; Barıs *et al*., 2006); *Artemisia* (Kordali *et al.*, 2005) and *Salvia* (Tepe *et al*., 2006). But, it is also known that antimicrobial effects or biological activities of essential oils and extracts of medicinal plants may be subjected to a change, based on the variations in the chemical composition of an essential oil that may be observed due to the origin, the locality, the environmental conditions, and the stage of development of the collected plant material (Güllüce *et al*., 2003).

In the present study, a total of 24 different plant essential oils, carvacrol and thymol which are the main compounds of some species were tested for antagonistic activity against *X. axonopodis* pv. *vesicatoria* (Doidge) Dye under in-vitro conditions. The plants were collected from Erzurum province in Turkey in June 2006.

Materials and methods

Pathogenic bacteria

X. axonopodis pv. *vesicatoria* strain RK-442, used in this study, was isolated from tomato exhibiting typical bacterial spot disease. The bacterial strain was identified by using the MIDI system (Microbial Identification System, Inc., Newark, DE, version 5.0) (Paisley, 1995). The bacterium tested was tested for pathogenic on tomato (cv. H-2274) plants. The bacterial culture preserved in Loria Broth and 15% glycerol solution at -80^{o}C for using further studies.

Plant materials

The aerial parts of used plant samples were collected from Erzurum province in eastern Anatolia region of Turkey in July 2006 at the flowering stages, and were dried in shade. The plant samples were identified by Dr. Kaya and Cakmakcı. They have been deposited in the herbarium of Atatürk University, Erzurum (Turkey). The list of tested plant species was given in Table 1.

The isolation of the essential oils

The dried plant samples (500 g) were subjected to hydro distillation using a Clevenger-type apparatus for 4 hours. The oils were extracted with CHC_{l3} and then were dried over anhydrous Na_2SO_4 and stored under N_2 atmosphere at 20^{o}C in a sealed vial until use.

Determination of antibacterial activities

Antibacterial activity assays were carried out by disc diffusion method (Murray *et al*., 1995) with a minor modification. The essential oils, carvacrol and thymol dilisions were sterilized by filtration by 0.45 µm Millipore filters. Bacterial suspension (100 µl) containing 1x10^{8} CFU/ml of bacteria spread by a sterile swab on Triptic Soy Agar (TSA) medium. The discs (6 mm in diameter) were impregnated with 12,5 µl of the essential oils, thymol (1g/ml dimethylsulfoxide-DMSO) or carvacrol (1/1 ml DMSO) solutions, and put in the middle of the inoculated plates. The bacterial cultures were incubated at 27±2^{o}C for 48 h, and then inhibition zones were measured in diameter (mm) around of the discs. Streptocycline was used as positive, and DMSO used as negative control. The assays were performed with three replicates.

Determination of minimal inhibition concentration (MIC)

The minimal inhibition concentration (MIC) values were determined by using the modified agar-well diffusion method (Okeke *et al*., 2001). In the agar-well diffusion technique, a two-fold serial dilutions of the essential oils, thymol and carvacrol, were prepared by diluting 10% DMSO to achieve a decreasing concentration range from 800 µm/ml to 3,125 mµ/ml. Using 100 µl of suspension containing $1x10^8$ CFU/ml of bacteria spread on TSA plates. The discs were impregnated with 12.5 µl of essential oils, thymol, and carvacrol solutions. Then, they were put in the middle of inoculated TSA agar plates. The bacterial cultures were incubated at 27±2 °C for 48 h. The least concentration of each the essential oils showing a clear zone of inhibition were taken as the MIC. DMSO was used as negative control. Streptocycline was used as positive control. The assays were performed with three replicates.

Statistical analysis

In order to determine whether there is a statistically significant difference among the results of obtained from antibacterial effect of tested plant essential oils, variance analyses were carried out using SPSS 10.0 software package. Values of $p<0.05$ were considered as significantly different.

Results

Antibacterial activity

A total of 24 different plant essential oils, carvacrol and thymol were tested for antagonistic activity against pathogen. According to the in-vitro test results, all applications showed more or less antagonistic activity against pathogen on Petri plates assays, based on the zone of inhibition (Table 1). The most successful results were obtained from carvacrol, thymol and seven essential oils consisting of *T. canoviridis, S. hortensis, M. officinalis* sub sp. *inodora, H. plicatum, T. haussknechtii, T. sipyleus* and *T. sipyleus* sub sp. *rosulans*. Antibacterial activity of them was highly significant with strong inhibition zone when compared with that of synthetic antibiotics Streptocycline. Mean inhibition zones and minimal inhibitory concentration of these oils changed from 22-46.3 mm and 25-200 µl/ml, respectively. Carvacrol and thymol showed 85 mm of mean inhibition zone and 3.125 µl/ml of a MIC. Positive control streptocycline showed 17.66 mm of mean inhibition zone. Negative control DMSO didn't show any inhibition zone against pathogen. The

inhibition effect of *Thymus sipyleus rosulans*, *Helichrysum plicatum*, *Thymus haussknechtii, Thymus sipyleus, Satureja hortensis, Melissa officinalis inodora, Thymus canoviridis,* Thymol and Carvacrol were stronger that of positive control.

Minimal inhibition concentration results

Minimal inhibition concentration values were given in Table 1. *T. sipyleus, S. hortensis, H. plicatum, A. biebersteini, A. millefolium, A. wilhelmsii* and *A. santonicum* essential oil showed the greatest minimal inhibition concentration (MIC, equal to 25 to 50 µl/ml). *T. aucheranum, T. chilliophyllum, A. absinthium, A. spicigera, S. verticillata, M. officinalis* sub sp. *inodora*, *T. canoviridis, T. haussknechtii, T. sipyleus* sub sp. *rosulans*, *M. perforata* and *S. pratensis* showed also well minimal inhibition concentration (MIC, equal to 100 to 200 µl/ml). Plant inspect responses were variable and depended on the selected plant species oil.

Discussion

It is known that many plant pathogenic bacteria have acquired resistance to synthetic pesticides (White *et al*., 2002). For instance, pathovars of *Xanthomonas campestris* have developed resistance to some antibiotic such as kanamycin, ampicillin, penicillin and streptomycin (Bender *et al*., 1990; Rodriguez *et al*., 1997). In recently, conventionally produced seed have not been allowed for organic farming. Thus, considering the deleterious effects of synthetic pesticides on life supporting systems, there is an urgent need to search for alternative approaches for the management of plant pathogenic microorganisms. There are a lot of reports on the use of several plant by-products on several pathogenic bacteria and fungi, but reports on phytopathogenic bacteria are less.

In this study, significant antibacterial activity was observed in the essential oils of *T. canoviridis, S. hortiensis, M. officinalis* ssp. *inodora, H. pilicatum, T. haussknechtii, T. sipyleus* and *T. sipyleus* ssp. *rosulans*, carvacrol and thymol on inhibition of *X. axonopodis* pv. *vesicatoria*. Furthermore, the antibacterial activity of them was the stronger than that obtained with standard antibiotic. The pathogen suppresses ability of the tested plant oils, carvacrol and thymol varied with plant species. Also, the effect of them on *X. axonopodis* pv. *vesicatoria* depended on the level of concentration. It is suggested that plant oils-pathogen interactions may have played an important role on growth of the pathogen.

Table 1. The main inhibition zone (in millimeter) and minimal inhibition concentration (MIC) of different essential oils of some plants and some important component (thymol and carvacrol) against plant pathogenic bacteria Xanthomonas axonopodis pv. vesicatoria.

Treatments	Inhibition zone (mm)*	MIC (µl/ml)
Positive control (Streptocycline)	17,66±0,57 ^{ef}	NT
Negative control (DMSO)	0,00±0,00 ^{a}	-
Salvia verticillata L.	7,66±0,57 ^{b}	100
Teucrium chamaedrys L.	9,00±0,00 ^{bc}	800
Artemisia absinthium L.	9,00±0,00 ^{bc}	100
Salvia pratensis L.	9,00±1,00 ^{bc}	200
Artemisia dracunculus L.	9,66±0,57 ^{bc}	NT
Salvia candidissima VAHL	9,66±0,57 ^{bc}	NT
Teucrium polium L.	9,66±0,57 ^{bc}	600
Achillea biebersteini AFAN	10,00±1,00 ^{bc}	50
Artemisia spicigera C. KOCH	10,00±1,00 ^{bc}	100
Tanacetum aucheranum (DC.) SCHULTZ BIP.	10,00±1,00 ^{bc}	100
Achillea millefolium L.	11,33±0,57 ^{c}	50
Matricaria perforate L.	11,66±0,57 c	200
Galium verum L.	14,66±4,50 d	600
Achillea wilhelmsii C. KOCH	15,00±1,00 ^{de}	50
Tanacetum chilliophyllum FISCH. ET MEY	15,33±1,52 $^{d-f}$	100
Artemisia santonicum L.	15,33±2,51 $^{d-f}$	50
Eryngium thorifolium BOISS.	18,00±1,00 ^{f}	400
Thymus sipyleus rosulans BOISS	22,00±3,00 ^{g}	200
Helichrysum plicatum DC	33,00±0,00 ^{h}	25
Thymus haussknechtii VELEN	32,66±0,57 ^{h}	200
Thymus sipyleus BOISS	31,33±3,51 ^{h}	25
Satureja hortensis L.	41,66±0,57 ^{ı}	25
Melissa officinalis inodora L.	40,66±2,08 ^{ı}	200
Thymus canoviridis JALAS	46,33±2,51 ^{j}	200
Thymol	85,00±0,00 ^{k}	3,125
Carvacrol	85,00±0,00 ^{k}	3,125

*Data in columns with different letters are statistically different according to Duncan's multiple range test at p=0.05. Data given are mean of three replicates ± standard error p=0.05. - : Not effective, NT: Not tested

Thymus species were more effective than other plant species. We think that this related to main compounds of essential oils obtained from *Thymus* species, in which found to be rich monoterpene phenols, especially carvacrol and thymol (Hüsnü Can Baser, 2002; Yılmaz *et al*., 2004; Tepe *et al*., 2005).

There are some studies related to *Satureja hortensis* extract or essential oil (Güllüce *et al*., 2003; Kızıl and Uyar, 2006) which have antagonistic activity against *Xanthomonas* pv. But, according to our knowledge, this is the first study that the rest of tested essential oils have inhibitory activity against *X. axonopodis* pv. *vesicatoria*.

Application of the plant essential oils as a seed disinfectant is an inexpensive and effective technique, and its easy adaptability will give additional advantages leading to acceptances of this technology by farmers. In conclusion, our results show that this plant oils especially *T. canoviridis, S. hortensis, M. officinalis* sub sp. *inodora*, *H. plicatum, T. haussknechtii, T. sipyleus* and *T. sipyleus* sub sp. *rosulans* plants oils, carvacrol and thymol can be used as a seed disinfectant and as potential control agents for management of bacterial spot disease.

Acknowledgement

This study was supported by TUBITAK (The Scientific and Technical Research Council of Turkey) under the project TOVAG-107 O 525.

References

Barıs, O., Güllüce, M., Sahin, F., Ozer, H., Kılıc, H., Ozkan, H., Sökmen, M. and Ozbek, T. (2006). Biological activities of the essential oil and methanol extract of Achillea biebersteini Afan. Afan. (Asteraceae). Türkish Journal of Biology 30: 65-73.

Basım, E. and Basım, H. (2003). Antibacterial activity of Rosa damascene essential oil. Phytotherapy 74: 394-396.

Bender, C.L., Malvick, D.K., Conway, K.E., George, S. and Pratt, P. (1990). Characterization of pXV10A, a copper resistance plasmid in Xanthomonas campestris pv. vesicatoria. Applied and Environmental Microbiology 56: 170-175.

Elkovich, S.D. (1988). Terpenoids from the genus Artemisia as potential pesticides. In Cutler, H. G. (ed.): Natural products and their potential role in agriculture: ACS Symposium Series 380. American Chemical Society, Washington DC, pp. 250-261.

Güllüce, M., Sökmen, M., Daferera, D., Agar, G., Özkan, H., Kartal, N., Polissiou, M., Sökmen, A. and Sahin, F. (2003). In-vitro antibacterial, antifungal and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of Satureja hortensis L. Journal of Agriculture and Food Chemistry 51(14): 3958 -3965.

Hüsnü Can Baser, K. (2002). Aromatic biodiversity among the flowering plant taxa of Turkey. Pure Applied Chemistry 74 (4): 527–545.

Kızıl, S. and Uyar, F. (2006). Antimicrobial activities of some thyme (Thymus, Satureja, Origanum and Thymbra) species against important plant pathogens. Asian Journal of Chemistry 18 (2): 1455-1461.

Kordali, S., Cakir, A., Mavi, A., Kilic, H. and Yildirim, A. (2005). Screening of chemical composition and antifungal and antioxidant activities of the essential oils from three Turkish Artemisia species. Journal of Agriculture and Food Chemistry 53 (5): 1408-1416.

Mohana, D.C. and Raveesha, K.A. (2006). Anti-bacterial activity of Caesalpinia coriaria (Jacq.) Willd. against plant pathogenic Xanthomonas pathovars: an eco-friendly approach. Journal of Agricultural Technology 2 (2): 317-327.

Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C. and Yolke, R.H. (1995). Manual of Clinical Microbiology, vol. 6th ed. ASM, Washington, DC.

Nguefack, J., Somda, I., Mortensen, C.N. and Amvam Zollo, P.H. (2005). Evaluation of five essential oils from aromatic plants of Cameroon for controlling seed-borne bacteria of rice (Oryza sativa L.). Seed Science and Technology 33 (2): 397-407.

Okeke, M.I., Iroegbu, C.U., Eze, E.N., Okoli, A.S. and Esimone, C.O. (2001). Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity. Journal of Ethnopharmacology 78: 119-127.

Ozturk, S. and Ercisli, S. (2005). Broad-spectrum antibacterial properties of Thymus fallax. Pharmaceutical Biology 43 (7): 609-613.

Paisley, R. (1995). MIS whole cell fatty acid analysis by gas chromatography. MIDI, Inc., Newark, DE, 5.

Rodriguez, H., Aguilar, L. and Lao, M. (1997). Variations in Xanthan production by antibiotic-resistant mutants of Xanthomonas campestris. Applied Microbiology and Biotechnology 48: 626-629.

Sahin, F. and Miller, S.A. (1996). Characterization of Ohio strains of Xanthomonas campestris pv. vesicatoria, causal agent of bacterial spot of pepper. Plant Disease 80: 773-778.

Sahin, F., Karaman, İ., Güllüce, M., Ogütcü, H., Sengül, M., Adıgüzel, A., Oztürk, S. and Kotan, R. (2003). Evaluation of antimicrobial activities of Satureja hortensis L. Journal of Ethnopharmacology 87: 61-65.

Satish, S., Raveesha, K.A. and Janardhana, G.R. (1999). Antibacterial activity of plant extracts on phytopathogenic Xanthomonas campestris pathovars. Letters in Applied Microbiology 28: 145-147.

Sokmen, A., Sokmen, M., Daferera, D., Polissiou, M., Candan, F., Unlu, M. and Akpulat, A. (2004a). The in-vitro antioxidant and antimicrobial activities of the essential oil and methanol extracts of Achillea biebersteini Afan. (Asteraceae). Phytotherapy Research 18 (6): 451-456.

Sokmen, A., Gulluce, M., Akpulat, H.A., Daferera, D., Tepe, B., Polissiou, M., Somken, M. and Sahin, F. (2004b). The in-vitro antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic Thymus spathulifolius. Food Control 15: 627-634.

Tepe, B., Sokmen, M., Akpulat, A.H. and Sokmen, A. (2006). Screening of THA antioxidant potentials of six Salvia species from Turkey. Food Chemistry 95: 200-204.

Tepe, B., Sokmen, M., Akpulat, H.A., Daferera, D., Polissiou, M. and Sokmen, A. (2005). Anti-oxidative activity of the essential oils of Thymus sipyleus subsp sipyleus var. sipyleus and Thymus sipyleus subsp sipyleus var. rosulans. Journal of Food Engineering 66 (4): 447-454.

Vasinauskiené, M., Radušiené, J., Zitikaité, I. and Survilienė, E. (2006). Antibacterial activities of essential oils from aromatic and medicinal plants against growth of phytopathogenic bacteria. Agronomy Research 4: 437-440.

White, D.G., Zhao, S., Simjee, S., Wagner, D.D. and McDermott, P.F. (2002). Antimicrobial resistance of food-borne pathogens. Microbes and Infection 4: 405-412.

Yılmaz, G., Telci, I., Kandemir, N. and Kaya, N. (2004). Essential oil contents compositions of Thymus sipyleus growing wild in Central Turkey. Asian Journal of Chemistry 16 (2), 841-847.

(Received 5 March 2007; accepted 30 October 2007)