BIOLOGICA NYSSANA
1 (1-2) • December 2010: 95-98 10th SFSES • 17-20 June 2010, Vlasina lake

Original Article

Antimicrobial activity of *Satureja* L. essential oils against phytopathogenic bacteria *Erwinia amylovora*

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***Abstract*:**
Mihajilov-Krstev, T., Radnović, D., Kitić, D.: ***Antimicrobial activity of Satureja L. essential oils against phytopathogenic bacteria Erwinia amylovora. Biologica Nyssana, 1 (1-2), December 2010: 95-98.***

In this paper, antimicrobial activity of *S. kitaibelii* Wierzb. ex Heuff., *S. montana* ssp. *montana* L., *S. adamovicii* Šilić and *S. fukarekii* Šilić has been investigated. During the testing, two methods were used: disc-diffusion and broth micro-dilution. The results showed high sensitivity of this bacteria to all four essential oils. The essential oil of *S. montana* ssp. *montana* exhibited the largest inhibition zone (25 mm), while *S. adamovicii* showed the highest inhibitory and bactericidal activity (MIC=MBC=0.09 $\mu l ml^{-1}$). All investigated oils showed the same values for MIC and MBC, which means that the oils posses bactericidal effect at very low concentrations and they could be used as non-harmful source of bactericides.

Key words: Genus *Satureja* L., essential oils, antimicrobial activity, *Erwinia amylovora*

Introduction

Erwinia is a genus of *Enterobacteriaceae* bacteria containing mostly plant pathogenic species which was named for the first phytobacteriologist, Erwin Smith. A well-known member of this genus is the species *E. amylovara*, which causes fireblight on apple, pear, and other Rosaceous crops. This species produce enzymes that hydrolyze pectin between individual plant cells. This causes the cells to separate, a disease plant pathologists term plant rot. Fireblight is a systemic disease. The term "fireblight" describes the appearance of the disease, which can make affected areas appear blackened, shrunken and cracked, as though scorched by fire. Sprays of the antibiotics, streptomycin or terramycin can prevent new infections (Falkenstein et al., 1998).

During the last several decades, natural products with antimicrobial effect are investigated in order to eliminate the use of synthetic antibiotics which cause the resistance of microorganisms and can exhibit side effects to human health. Aromatic plants are known for a very long time and they are used in phytotherapy and food preservation (Burt, 2004). Among the aromatic plant species, genus *Satureja* L. occupies a special position. Essential oils of *Satureja* L. species showed very significant antimicrobial activity against various species of bacteria and fungi (Ciani et al., 2000; Azaz et al., 2002; Sahin et al., 2003; Chorianopoulos et al., 2004; Bežić et al., 2005; Skočibušić & Bežić, 2004, 2006; Adiguzel et al., 2007; Razzaghi-Abyaneh et al., 2008).

In this paper, disc-diffusion and broth microdilution method were used to investigate antibacterial effect of essential oils of four species of *Satureja* L. genus against phytopathogenic bacteria *E. amylovora*. According to our knowledge, these are the first investigations of essential oil's effect against this bacterium. Considering these

facts, the results of the present study can find application in the pomology as harmless and natural bactericidal agents.

Material and methods

Plant Material

The aerial parts of wild growing plant materials of eight *Satureja* species were collected during the beginning of the flowering stage in Serbia (*S. kitaibelii* Wierzb. ex Heuff.), Montenegro (*S. monatana* L.) and F.Y.R.O.M. (*S. fukarekii* Šilić and *S. adamovicii* Šilić). Voucher specimens were deposited in the herbarium at the Faculty of Natural Sceince and Mathematics of the University of Novi Sad.

Extraction of the essential oils

Air-drying of the plant was performed in a shady place at room temperature for 10 days. Dried aerial parts (100 g) of plants, were cut and subjected to the hydro-distillation for 3 h, using Clevenger-type apparatus. The resulting essential oils were dried over anhydrous sodium sulfate and stored at 4°C.

Antimicrobial screening

The antimicrobial activities of the tested essential oils were evaluated using disc-diffusion and broth micro-well dilution method. All tests were performed in triplicate with two growth controls: ethanol (negative control) and streptomycin (positive control).

Microbial strain. Laboratory control strain *Erwinia amylovora* NCPPB 595.

Disc-diffusion assay. Antimicrobial tests were carried out by disc-diffusion method using 100 µl of suspension (containing 2.0 x 10^8 CFU/ml of bacteria) spread on Mueller-Hinton agar (MHA, Torlak) in sterilized Petri dishes (90 mm in diameter). The discs (6 mm in diameter, HiMedia Laboratories Pvt. Limited) were impregnated with 15 µl of oil dilution (2 %, 5 % and 10 %) and placed on the inoculated agar. The inoculated plates were kept at 4 ^{0}C for 2 h and incubated at 37 ^{0}C for 24h. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test bacterial strain.

Micro-well Dilution Assay. The inocula of the bacterial strain was prepared from overnight broth culture and suspension was adjusted to 0.5 McFarland standard turbidity (corresponding to 10^7-10^8 CFU ml^{-1}) (consensus standard by the NCCLS). Ethanol was used to dissolve the essential oils and then diluted to the highest concentration (500 µl ml^{-1}). A serial doubling dilutions of the oils were prepared in a 96/well microtiter plate over the range of 50.00-0.02µl ml^{-1} in inoculated nutrient broth (the final concentration in each well adjusted to 2.0 x 10^6 CFU ml$^{-1)}$. The plates were incubated for 24 h at 37^{0}C. The microbial growth was determined by absorbance at 620nm using the universal microplate reader (ThermoLabsystems, Multiskan EX, Software for Multiscan ver.2.6.). The highest dilution without growth is the minimum inhibitory concentration – MIC. To determine MBC, broth was taken from each well and inoculated in Mueller Hinton agar (MHA) for 24 h at 37 ^{0}C. The MBC is defined as the lowest concentration of the essential oil at which inoculated microorganism was 99.9 % killed.

Statistical analysis of data. Analysis of variance (ANOVA) was used to determine the significance ($p \leq 0.05$) of the data obtained in all experiments.

Results and discussion

The dominant components of *Satureja* L. essential oils were monoterpenes carvacrol and thymol, p-cymene, limonene and also monoterpenoid alcohols borneol and linallole. Considering sesquiterpenes, the most ambudant were β-caryophyllene, caryophyllene–oxide and spathulenol (Pavlović et al., 1987; Slavkovska et al., 2001; Azaz et al., 2002; Sahin et al., 2003; Chorianopoulos et al., 2004; Bežić et al., 2005; Skočibušić & Bežić, 2004; Adiguzel et al., 2007; Razzaghi-Abyaneh et al., 2008).According to the results of cited authors rich in carvacrol was essential oil of the species *Satureja montana* ssp. *montana* L. (16.1 – 52.4 %) (Pavlović et al., 1987; Slavkovska et al., 2001; Skočibušić et al., 2004). Monoterpenoidal alcohol borneol was present in high content in the *Satureja fukarekii* Šilić (55.0 %) essential oil (Pavlović et al., 1987). Significantly smaller content of borneol was present in the *Satureja kitaibelii* Wierzb. ex Heuff. (9.8 %) oil, where dominant compounds were the precursors of carvacrol p–cymene (20.9 %) and limonene (16.0 %) (Slavkovska et al., 2001). In the oil of the species *Satureja adamovicii*, p-cymol was present (40.0 %) together with 1,8-cineole and limonene (35.0 %) (Pavlović et al., 1987).

The results of disc-diffusion testing showed that all tested oils (0.75 µl/disc) ehibited very significant antimicrobial activity against *E. amylovora*. The oils showed higher activity than the

tested reference antibiotic Streptomycin (38 mm, 30 µl of the active substance/disc). Especially high activity showed the oil isolated from *S. montana* ssp. *montana* with inhibition zone of 25 mm **(Fig. 1).**

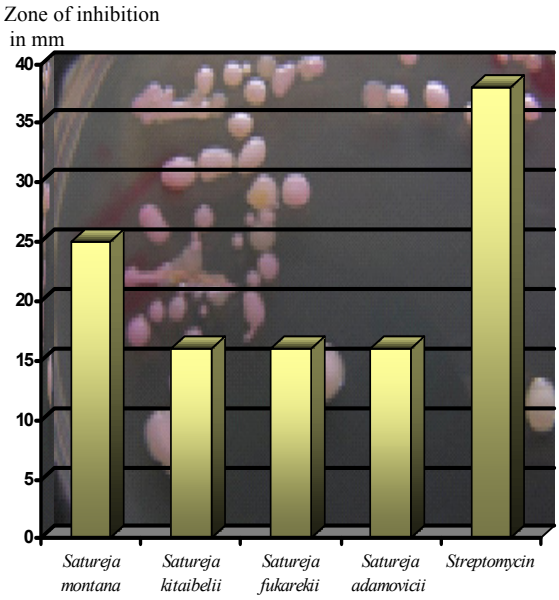

Fig.1. Mean diameter of inhibition zone for *Satureja* essential oils and Streptomycin (30 µg/disc) against *Erwinia amylovora,* obtained by disc-diffusion method

The results of broth micro-well dilution method confirmed high essential oil activity of four *Satureja* L. species (**Fig. 2**). Their MIC/MBC values were in the range from 0.09 – 0.18 µl ml^{-1}, which is very good considering the values of the reference antibiotic, Streptomycin (MIC/MBC = 8.0/16.0 µl ml^{-1}).

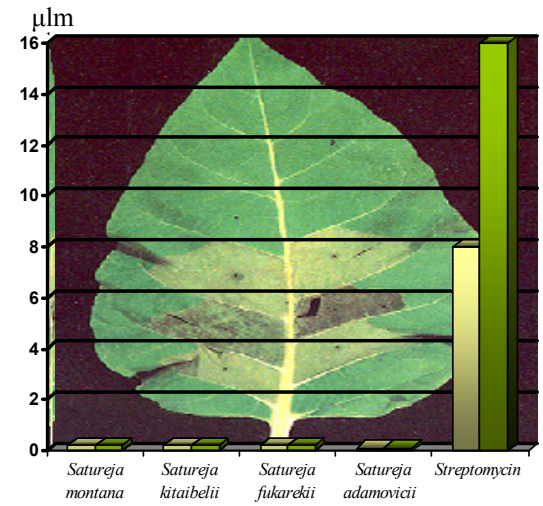

Fig. 2. The MIC/MBC values (µl ml^{-1}) of the four *Satureja* essential oils and streptomycin against *Erwinia amylovora* by the broth microdilution method

Phenolic compound carvacrol, monoterpene limonene and alcoholes linalool and borneol are mostly responsible for the obtained high antimicrobial activity. The most possible mechanism is sinergistic effect of sesquiterpenes like β-caryophyllene and caryophyllene–oxide with other active compounds. Beside this, it is well-known that some compounds do not possess antimicrobial activity, but can enhance the activity of some other antimicrobial compounds by incorporating into the membrane bilayer and enabling the passage of the active compounds. One of these components is p-cymene, which is constituent of allmost all investigated species of the genus *Satureja* L. (Lamber et al., 2001; Arfa Ben et al., 2006).

Conclusion

Essential oils of four species from the genus *Satureja* L. showed high antibacterial activity against *E. amylowora*. That can be explained by high content of monoterpenes and sesquiterpenes, which are well known and many times proved as antimicrobials. These results demonstrated that essential oils of the investigated species could be used as a natural potential antimicrobial agents in prevention and treatment of "fireblight".

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