ISSN 1392-3196
Zemdirbyste-Agriculture, vol. 95, No. 3 (2008), p. 440–446
UDK 633.491:631.524.86

EFFECTIVITY OF PLANT ESSENTIAL OILS AGAINST *CLAVIBACTER MICHIGANENSIS, IN VITRO*

Dagmar POUVOVA[1], Blanka KOKOSKOVA[2], Roman PAVELA[2], Pavel RYSANEK[1]

[1]Czech University of Agriculture Prague
Prague 6, Czech Republic

[2]Crop Research Institute
Prague 6, Czech Republic
E-mail: bkokoskova@vurv.cz

Abstract

Ring rot of potato, caused by *Clavibacter michiganensis* subsp. *sepedonicus (Cms),* and bacterial wilt of lucerne, caused by *Clavibacter michiganensis* subsp. *insidiosus (Cmi)* belong to important bacterial diseases. Effectiveness of essential oils from 34 aromatic plants was examined against *Cms* and *Cmi* in laboratory tests. Essential oils from *Origanum vulgare, O. compactum, Eugenia caryophyllata, Artemisia absinthium, Thymus vulgaris, Abies siberica, Ocimum basilicum* and *Citrus aurantifolius* were the most effective against both bacteria. Further promising essences, *Mentha arvensis* and *Citrus limonum* were effective against *Cms* and *Tagetes bipinata* and *Lavandula latifolia* were effective against *Cmi.*

Key words: *Clavibacter michiganensis* subsp. *sepedonicus*, *Clavibacter michiganensis* subsp. *insidiosus*, antibacterial activity, essential oils.

Introduction

Clavibacter michiganensis subsp. *sepedonicus* (*Cms*) causes bacterial ring rot of potato. *Cms* is listed as an A2 quarantine pest by EPPO in many countries all over the world /OEPP/EPPO, 1978/. *Cms* has a narrow host range, which seems to include mainly potato, eggplant and tomato /Larson, 1944/. In the early stages of symptom development on growing potato plants, leaves start to wilt and are slightly rolled at the margins. The inter-vessel spaces of the leaves become light green to pale yellow. As the disease progresses, the leaflets and whole leaves become necrotic, finally the whole plant can collapse /De Boer, Slack, 1984; Romanenko et al., 2002/.

Clavibacter michiganensis subsp. *insidiosus* (*Cmi*) causes bacterial wilt of lucerne, which is the main host of pathogen /Close, Mulcock, 1972/. The pathogen origins from North America and has been spread to many other countries /OEPP/EPPO, 1990/. The bacterium is primarily a wilt pathogen and, following entry through wounds is found in the vascular tissues of the stems and pods, where it produces an extracellular polysaccharide gum, the substance responsible for the wilting /Erwin, 1990/.

Control of plant bacterial diseases remains difficult due to the limited availability of bactericides. Only a few chemical products are available, and their use is hampered by limited efficacy in the field but mainly for their potential negative effects either in the environment or with human and animal health. The use of antibiotics in plant protection is limited because of the possibility to select pathogen populations resistant to bactericides and the potential transfer of resistant genes to animal and human pathogenic bacteria /Iacobellis et al., 2005/.

The use of copper compounds, which are widely used for the control of plant bacterial diseases has been limited in the European Union countries by rule 473/2002 due to their impact on the environment /Iacobellis et al., 2005/. Combination of plant extracts or etheric oils from plants with copper and other chemical compounds can increase their effectiveness /Zeller, 2005/. Some essential oils proved strong antifungal /Wang et al., 2005/, insecticide /Isman, 2000/ and antibacterial activity /Burt, 2004; Iacobellis et al., 2005; Kokošková, Pavela, 2007/.

Materials and Methods

Bacterial Cultures

Bacterial strains used in this study were reference cultures of *Cms* NCPPB 3467 and *Cmi* RIPC 12/5/98. Cultures were grown on the nutrient medium according to Snieszko and Bonde (1943) at 23^{o} C.

Plant material

In our experiments, the essential oils obtained from various plants were purchased from the Essential Oil University (Charleston, IN, USA). The essential oils were obtained by steam or hydrodistillation of botanicals.

Test of antibacterial activity of the essential oils

Thirty-four essential oils, obtained from different aromatic plants, were tested (Table). The antibacterial activity tests were conducted *in vitro* on agar plates contaminated by the target bacterium. ENA II medium (nutrient agar no. 2 – 6.6 g, glucose – 6.6 g, yeast extract – 0.7 g, agar – 15.0 g, sterile water – 1 L, pH – 6.6) was used for the screening essential oils /Kokošková, 1992/. Bacterial strains of *Cms* and *Cmi* were used as 4-day cultures in concentration corresponding to OD~0.5_{620} in all tests. The crude extracts were dropped on the agar surface in a dosage of 1 µl per 6 µl plant grindings. After treatment, the Petri dishes were incubated in a thermostat at 23^{o} C. The diameter of the inhibitory zones was measured after 3 days and then the average of the obtained values was calculated. Index of antimicrobial effectivity (IAE) of each essential oil was calculated for both bacteria. The effectivity of the essential compounds was directly proportional to the size of inhibitory zone. Streptomycin (SIGMA, Germany) in concentration 0.02% was used as control /Kokošková, 1992/.

Calculation and statistical analysis

The index antimicrobial effectivity (IAE) was calculated from the formula:

$$IAE (\%) = [-1*(C-T/C+T)]*100$$

where C is the average percentage zone on the control (streptomycin 0.02%) dish and T is the average zones on the treated dish (i. e. to which essential oils were applied). The IAE indicates whether the effectiveness of the essential oils is lower and/or higher than the control /Kokošková, Pavela, 2007/.

A one-way analysis of variance (ANOVA test) was performed for comparing areas of effectiveness of essential oils compared with streptomycin, followed by a ranking of their averages using Tukey's test.

Table. The plants for isolation of the essential oils and their effect on *Clavibacter michiganensis in vitro*

	Percentage reduction in colony size (cm)	
Plant	*C. michiganensis* subsp. *sepedonicus*	*C. michiganensis* subsp. *insidiosus*
1	2	3
Abies siberica	12.17 ± 2.32***	14.00 ± 0.92***[1]
Acorus calamus	4.17 ± 0.34***	6.33 ± 1.57
Amyris balsamifera	3.00 ± 0.00***	8.83 ± 1.12
Artemisia absinthium	20.67 ± 1.02***	28.50 ± 2.31***
Citrus aurantifolia	10.33 ± 0.44***	14.33 ± 0.87***
Citrus limonum	10.17 ± 0.34 ***	8.67 ± 0.87
Eugenia caryophyllata	22.00 ± 1.09***	20.17 ± 1.35***
Juniperus communis	7.33 ± 0.43	9.17 ± 1.12
Juniperus virginiana	4.33 ± 0.87**	7.33 ± 0.69
Lavandula angustifolia	9.33 ± 1.02**	8.00 ± 0.93
Lavandula latifolia	7.33 ± 1.27	12.33 ± 1.75**
Mellisa officinalis	8.50 ± 1.58	8.50 ± 0.71
Melaleuca quinquenervia	8.00 ± 1.31	7.67 ± 1.02
Mentha arvensis	11.00 ± 0.53***	8.50 ± 0.71
Mentha citrata	6.33 ± 0.43	9.33 ± 0.88
Mentha pulegium	6.33 ± 0.69	11.33 ± 1.02*
Mentha spicata	9.17 ± 0.34**	5.50 ± 0.46**
Nepeta cataria	9.33 ± 1.74	10.50 ± 2.76
Ocimum basilicum	11.17 ± 1.24**	18.00 ± 1.85***
Origanum compactum	24.50 ± 3.05***	20.33 ± 3.57***
Origanum majorana	6.83 ± 0.98	4.33 ± 0.43***
Origanum vulgare	28.83 ± 2.29***	30.17 ± 1.72***
Pelargonium graveolens	6.50 ± 1.03	4.50 ± 0.46***
Pelargonium roseum	5.67 ± 0.87	4.50 ± 0.46***
Pogostemon cablin	6.83 ± 0.98	6.50 ± 0.46*
Rosmarinus officinalis	8.50 ± 1.03	5.50 ± 1.03***
Salvia officinalis	6.67 ± 1.27	7.67 ± 0.43
Salvia sclarea	10.33 ± 1.74	11.00 ± 0.76*

Table continued

1	2	3
Tagetes bipinata	8.33 ± 1.27	13.83 ± 3.69
Thuja occidentalis	7.00 ± 0.92	3.17 ± 0.43***
Thymus matschiana	4.67 ± 0.69*	3.33 ± 0.43***
Thymus vulgaris	15.33 ± 2.24***	13.50 ± 2.31**
Tsuga canadensis	7.17 ± 1.24	5.33 ± 0.43***
Zingiber officinale	7.00 ± 0.00	6.83 ± 0.34**
Streptomycin 0.02 g/ml	7.00 ± 2.32	9.00 ± 1.60

[1]The diameter (cm) of inhibitory zones (mean ± standard error); Asterisks indicate that means are significantly different from control (**P<0.05, **P<0.01, ***P<0.001*).

Results and Discussion

Of all thirty-four plant essential oils examined in this study, the oils from *Origanum vulgare*, *O. compactum*, *Eugenia caryophyllata* and *Artemisia absinthium* were the most efficient against both bacteria *Cms* and *Cmi*. Some authors reported similar results concerning various plant pathogenic bacteria /Dorman, Deans, 2000; Daferera et al., 2003; Iacobellis et al., 2005/. Species *Clavibacter michiganensis* proved sensitivity to all essential oils tested in our study.

The strongest inhibitory effect against *Cms* was found in six essential oils from *Origanum vulgare*, *Origanum compactum*, *Eugenia caryophyllata*, *Artemisia absinthium*, *Thymus vulgaris* and *Abies siberica*. Resultant value IAE was higher by 60.7% to 26.7% compared to the standard (Figure). The strongest inhibitory effect against *Cmi* was found only in five essential oils from *Origanum vulgare, Artemisia absinthium*, *Eugenia caryophyllata*, *Origanum compactum* and *Ocimum basilicum* respectively, resultant value of IAE was higher by 53.7% to 32.9% than standard (Figure). Similar results concerning effectivity of plant essential oils from *Origanum* sp. /Soylu et al., 2005/ and *Origanum* sp., *Thymus* sp. and *Eugenia caryophyllata* against *Clavibacter michiganensis* subsp. *michiganensis* were reported by other authors /Kokošková et al., 2006/.

Antimicrobial activity against *Cms* was significantly higher than standard in essential oils from *Ocimum basilicum*, *Mentha arvensis*, *Salvia sclarea*, *Citrus aurantifolia*, *Citrus limonum*, *Lavandula angustifolia*, *Nepeta cataria* and *Mentha spicata*, *Rosmarinus officinalis*, *Mellisa officinalis*, *Tagetes bipinata* and *Melaleuca quinquenervia*. Resultant values of IAE were included to interval 22.5–5.8% (Figure). Essential oils from *Citrus aurantifolia*, *Abies siberica*, *Tagetes bipinata*, *Thymus vulgaris*, *Lavandula latifolia*, *Mentha pulegium* and *Nepeta cataria* shown resultant values of IAE in interval 22.7–5.6% (Figure). The higher inhibitory effect of the essential oils from *Abies siberica* found in this study is in contrast with results obtained by other authors /Kokošková et al., 2006/. The inhibitory effect against *Cms* and *Cmi* comparable with standard was proved in essential oils from *Lavandula latifolia*, *Tsuga canadensis, Zingiber officinale* and *Juniperus communis*, and *Juniperus communis* and *Mentha citrata* respectively (Figure).

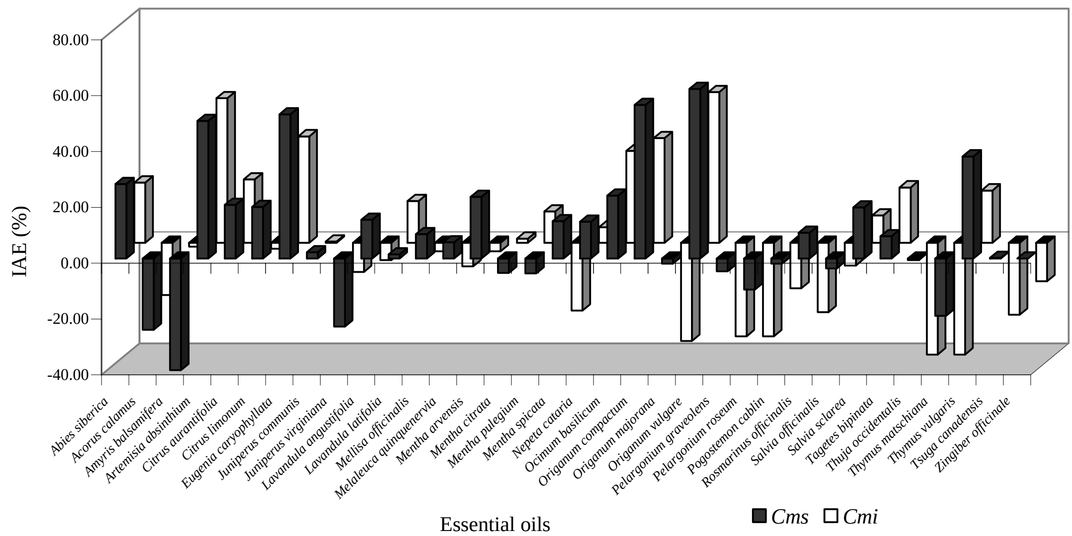

Figure. The antimicrobial activity of essential oils against *Clavibacter michiganensis* subsp. *sepedonicus* (*Cms*) and *Clavibacter michiganensis* subsp. *insidiosus* (*Cmi*). Effectivity of essential oils was compared with standard (streptomycin in concentration 0.02 %). IAE (%) indicates whether effectivity of essential oils is higher or lower than standard

Essential oils from some plant species (e. g. *Origanum* sp. and *Thymus* sp.) are rich in phenolic compounds, which are believed to be responsible for their marked antimicrobial activity /Zaika, Kissinger, 1981/. Phenolic compounds are capable of dissolving within the bacterial membrane and thus penetrating inside the cell, where they interact with cellular metabolic mechanisms /Juven et al., 1972; Oussalah et al., 2006/.

Essential oils from *Acorus calamus*, *Amyris balsamifera*, *Juniperus virginiana*, *Mentha citrata*, *M. pulegium*, *Origanum majorana*, *Pelargonium roseum*, *P. graveolens*, *Pogostemon cablin*, *Salvia officinalis* and *Thuja occidentalis* showed an inhibitory effect against *Cms*, but lower than standard. The weak antimicrobial efficiency of *Origanum majorana* essential oils tested in this study contrasts with results obtained by Vagi et al. (2005). Essential oils from *Acorus calamus*, *Amyris balsamifera*, *Citrus limonum*, *Lavandula angustifolia*, *Juniperus virginiana*, *Mellisa officinalis*, *Melaleuca quinquenervia*, *Mentha arvensis*, *M. spicata*, *Origanum majorana*, *Pelargonium roseum*, *P. graveolens*, *Pogostemon cablin*, *Rosmarinus officinalis*, *Salvia officinalis*, *Thuja occidentalis*, *Thymus vulgaris* and *Tsuga canadensis* showed an inhibitory effect against *Cms*, but lower than standard (Figure). However, comparison of the data obtained in this study with previously published results is not straightforward, considering that the composition of plant oils and extracts vary according to environmental conditions and plant species /Sivropoulou et al., 1995/.

Conclusion

Based on results of our tests, plant essential oils and their chemical compounds appear to be prospective for their possible use in new bactericidal preparations for plant protection in the future. However, further experiments are needed to obtain information regarding the economic aspects and antibacterial activities of essential oils *in vivo* without phytotoxic effects on seed germination. Research on the chemical composition of the essential oils used and antibacterial activities against different of plant pathogenic bacteria are currently under investigation.

Acknowledgements

The work was supported by the Ministry of Agriculture of CR, project No.0002700603 and by the Ministry of Education, project No. MSM6046070901.

Received 2008-06-06
Accepted 2008-08-12

REFERENCES

1. Burt S. Essential oils: their antibacterial properties and potential applications in foods – a review // International Journal of Food Microbiology. – 2004, vol. 94, p. 223–253

2. Close R., Mulcock A. P. Bacterial wilt, *Corynebacterium insidiosum*, of lucerne in New Zealand // New Zealand Journal of Agricultural Research. – 1972, vol. 15, p. 141–148

3. Daferera J. D., Ziogas N. B., Polissiou G. M. The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis* // Crop Protection. – 2003, vol. 22, p. 39–44

4. De Boer S. H., Slack S. A. Current status and prospects for detecting and controlling bacterial ring rot of potatoes in North America // Plant Disease. – 1984, vol. 68, p. 841–844

5. Dorman H. J. D., Deans S. G. Antimicrobial agents from plants: antibacterial activity of plant volatile oils // Journal of Applied Microbiology. – 2000, vol. 88, p. 308–316

6. Erwin D. C. Bacterial wilt. In: *Compendium of alfalfa diseases* // American Phytopathological Society. – St. Paul, 1990, edition 2, p. 5–6

7. Iacobellis N. S., Lo Cantore P., Capasso F., Senatore F. Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils // Journal of Agriculture Food Chemistry. – 2005, vol. 53, p. 57–61

8. Isman M. S. Plant essential for pest and disease management // Crop Protection. – 2000, vol. 19, p. 603–608

9. Juven B., Henis J., Jakoby B. Studies on the mechanism of the antimicrobial action of oleuropein // Journal of Applied Bacteriology. – 1972, vol. 35, p. 559–567

10. Kokošková B., Pavela R., Vokálková M., Ryšánek P. Effectivity of essential oils against *Clavibacter michiganensis* subsp. *michiganensis*, the causal agent of bacterial tanker of tomato: book of abstracts // Czech University of Live Sciences Prague: Czech and Slovak Plant Protection Conference, 12–14 September, 2006, Prague, Czech Republic. – 2006, XVII, p. 197–198

11. Kokošková B., Pavela R. Effectiveness of plant essential oils on the growth of *Erwinia amylovora*, the causal agent of fire blight disease // Pest Technology. – 2007, vol. 1, p. 76–80

12. Larson R. H. The ring rot bacterium in relation to tomato and eggplant // Journal of Agricultural Research. – 1944, vol. 68, p. 309–326

13. OEPP/EPPO. Data sheets on quarantine organism No. 51, *Corynebacterium sepedonicum* // Bulletin OEPP/EPPO Bulletin. – 1978, vol. 8 (2)

14. OEPP/EPPO. Specific quarantine requirements // EPPO *Technical Documents*. – 1990, vol. 1008

15. Oussalah M., Caillet S., Saucier L., Lacroix M. Antimicrobial effects of selected plants essential oils on the growth of a *Pseudomonas putida* strain isolated from meat // Meat Science. – 2006, vol. 73, p. 236–244

16. Romanenko A. S., Lomovatskaya L. A., Graskova I. A. Necrotic lesions as unusual symptoms of ring rot in the potato leaves // Russian Journal of Plant Physiology. – 2002, vol. 49, p. 690–695

17. Sivropoulou A., Papanikolaou E., Nikolaou C. et al. Antimicrobial and cytotoxic activities of *Origanum* essential oils // Journal of Agricultural and Food Chemistry. – 1996, vol. 44, p. 1202–1205

18. Snieszko S. F., Bonde R. Studies on the morphology, physiology, serology, longevity, and pathogenicity of *Corynebacterium sepedonicum* // Phytopathology. – 1943, vol. 33, p. 1032–1044

19. Soylu S., Soylu E. M., Baysal O., Zeller W. Antibacterial activities of the essential oils from medicinal plants against the growth of *Clavibacter michiganensis* subsp. *michiganensis* // Biocontrol of Bacterial Plant Diseases, 1st Symposium, 23–26 October, 2005, Darmstadt, Germany. – 2005, vol. 408, p. 82–85

20. Vági E., Simándi B., Suhajda Á., Héthelyi É. Essential oils composition and antimicrobial activity of Origanum majorana L. extracts obtained with ethyl alcohol and supercritical carbon dioxide // Food Research International. – 2005, vol. 38, p. 51–57

21. Wang S. W., Chen P. F., Chány S. T. Antifungal activities of essential oils and their constituents from indigenous cinnamon (*Cinnamomus osmophloeum*) leaves against wood decay fungi // Bioresource Technology. – 2005, vol. 96, p. 813–818

22. Zaika L. L., Kissinger J. C. Inhibitory and stimulatory effects of oregano on *Lactobacillus plantarum* and *Pediococcus cerevisiae* // Journal of Food Science. – 1981, vol. 46, p. 1205–1210

23. Zeller W. Status of biocontrol methods against fire blight // International Conference on: Biological and pro-ecological methods for control of disease in orchards and small fruit plantations, 29–31 August, 2005, Skierniewice, Poland. – 2005, vol. 2, p. 21