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A preliminary study of antibacterial activity of thirty essential oils against several important plant pathogenic bacteria

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SUMMARY

Numerous scientific research studies all over the world have addressed the problem of agriculture in the 21st century as being particularly sensitive to climate change, which has caused phytopathogenic bacteria to spread. Therefore, there is a clear and urgent need to contain this kind of risk in agricultural production (both conventional and organic farming). The objective of this study was to determine the antibacterial activity of 30 essential oils (EOs) against three harmful plant pathogenic bacteria of agricultural importance, *Erwinia amylovora*, *Xanthomonas campestris* pv. *campestris* and *Pseudomonas syringae* pv. *syringae*. The study included *in vitro* testing, using an agar-diffusion assay. The EOs of Ceylon cinnamon (leaf and bark), oregano, clove bud and palmarosa revealed antibacterial activity against the test bacteria, and the maximum mean inhibition zone diameters of 35 mm was found against *E. amylovora* and *X. campestris* pv. *campestris* (highly sensitive reaction), while it was smaller in the case of *P. syringae* pv. *syringae*, from 18.25–26.25 mm (sensitive to very sensitive reaction). Maximum diameter of the zone of inhibition (35 mm) was obtained using basil and peppermint against *E. amylovora*, and rosemary, blue gum and camphor tree against *X. campestris* pv. *campestris*. Not a single EO inhibited *P. syringae* pv. *syringae* with the resulting total diameter zone of 35 mm, and this test bacteria was resultingly classified as the least susceptible bacterium of the three tested. EOs of lemongrass, aniseed, ylang ylang, silver fir, lemon, dwarf mountain pine, bay laurel and scots pine caused sensitive reaction of the tested bacteria. Peppermint, black cumin, Indian frankincense, bergamot orange, common juniper, bitter orange and neem produced variable reactions from total to weakly or no inhibition at all. Weakly activity was found in niaouli and Atlas cedar. Eastern red cedar, patchouli, Indian sandalwood and ginger caused no reaction of any of the test bacteria. The results offer a basis for further work based on *in vivo* testing for the purpose of developing “natural pesticides” for control of phytopathogenic bacteria, thus giving a significant contribution to reducing yield losses in agriculture and sustainable development.

Keywords: Essential oils; Plant pathogenic bacteria; Bactericides

INTRODUCTION

Bacteria acting as significant plant pathogens include the following genera: *Erwinia*, *Pectobacterium*, *Pantoea*, *Agrobacterium*, *Pseudomonas*, *Ralstonia*, *Burkholderia*, *Acidovorax*, *Xanthomonas*, *Clavibacter*, *Streptomyces*, *Xylella*, *Spiroplasma* and *Phytoplasma* (Kannan et al., 2015). They cause a number of plant diseases, such as leaf spot, blight, necrosis, canker, wilt, rot, galls and tumours, dwarfing, discoloration of plants parts, etc. Of them all, *Pseudomonas syringae* pathovars, *Xanthomonas campestris* pathovars and *Erwinia amylovora* have been included on a list of ten most scientifically and economically important bacterial pathogens based on their pathogenesis, economic impact and molecular aspects (Mansfield et al., 2012). Each of these bacteria is able to cause multiple diseases that are damaging and/or cause complete yield loss. Their impact on global agriculture is increasing (Kannan et al., 2015).

Control of plant pathogenic bacteria is limited due to a lack of efficient bactericides, and restricted use of antibiotics and copper compounds in E.U. countries as a result of their negative impact on the environment (Bajpai et al., 2011). Therefore, search for novel alternative crop protectants is becoming more and more important. Recently, a wide range of essential oils (EOs) have been extensively studied for their antibacterial activity against many plant pathogenic bacteria (Deans & Ritchie, 1987; Vasinauskiene et al., 2006; Dadasoglu et al., 2011; Kokoskova et al., 2011; Hossein Nezhad et al., 2012; Badawy & Abdelgaleil, 2014; Gormez et al., 2013, 2015; Gakuubi et al., 2016; Todorović et al., 2016; Popović et al., 2017), usually using a direct-contact antimicrobial assay (Bajpai et al., 2011). Essential oils as products of plant secondary metabolism are the most interesting substances possessing antimicrobial activity (Deans & Ritchie, 1987). Their hydrophobic nature, which allows them to penetrate microbial cells and so disrupt the cell membrane structure and impair cell functions, is well known (Lopez-Romero et al., 2015). The effectiveness of EOs varies from one type to another, and from one to another target bacterium, depending on their structure (Gram-positive and Gram-negative bacteria) (Swamy et al., 2016). The lower susceptibility found in Gram-negative bacteria could be due to distinctive properties of their external lipopolysaccharide wall that surrounds the peptidoglycan cell wall (Bajpai et al., 2011).

Essential oils, known also as volatile or ethereal oils, are extracts of various aromatic plants or organs, such as the flower, bud, seed, leaf, twig, bark, fruit or root, and prepared by steam distillation (Burt, 2004;

Bakkali et al., 2008; Bajpai et al., 2011). Essential oils are present in over 2000 plant varieties from about 60 families, including *Apiaceae*, *Asteraceae*, *Cupressaceae*, *Hypericaceae*, *Lamiaceae*, *Lauraceae*, *Fabaceae*, *Liliaceae*, *Myrtaceae*, *Pinaceae*, *Piperaceae*, *Rosaceae*, *Rutaceae*, *Santalaceae*, *Zingiberaceae* and *Zygophyllaceae* (Thormar, 2010; Gakuubi et al., 2016). They are generally composed of a mixture of phenols, flavonoids, quinons, tannins, alkaloids, saponins and sterols (Dorman & Deans, 2000; Isman, 2000; Burt, 2004; Pichersky et al., 2006; Bakkali et al., 2008; Bajpai et al., 2011; Akhtar et al., 2014). These substances are a rich source of bioactive chemicals that may provide an alternative to the current use of synthetic pesticides. As natural bio-pesticides, EOs may prove effective, selective, biodegradable, non-toxic or less toxic products to the environment, as well as in food and agriculture industries (Bajpai et al., 2011), and potentially suitable for use in integrated pest management programs (Soylu et al., 2006). Some biopesticides, such as azadirachtin, are derived from seeds of the neem tree (*Azadirachta indica*) and have been commercialized as botanical pesticides (Isman, 2000; Soyly et al., 2006). According to Isman (2000), EO-based pesticides cannot replace pesticides in crop protection, but should serve in situations when full operator safety and environmental protection are required.

The objective of this study was to assess the antibacterial activity of EOs of 30 different plants against three economically significant phytopathogenic bacteria: *Erwinia amylovora* (fire blight), *Xanthomonas campestris* pv. *campestris* (black rot) and *Pseudomonas syringae* pv. *syringae* (bacterial canker and leaf spot).

MATERIALS AND METHODS

Collection of essential oils

Thirty EOs, listed in Table 1, were screened in this experiment for antimicrobial activity against plant pathogenic bacteria.

Plant pathogenic bacterial strains

Three important plant pathogenic bacteria: *Erwinia amylovora* (strain Ea1, originating from apple), *Xanthomonas campestris* pv. *campestris* (strain Xc40, originating from cabbage) and *Pseudomonas syringae* pv. *syringae* (strain Ps105, originating from chard) were used as test organisms in this study (Dr. Tatjana Popović, Collection of plant pathogenic bacteria, Institute for Plant Protection and Environment, Belgrade).

Table 1. Essential oils tested

Latin name	Common name	Manufacturer
<i>Abies alba</i>	Silver fir	Elmar
<i>Azadirachta indica</i>	Neem	Eterra
<i>Boswellia serrata</i>	Indian frankincense	Probotanic
<i>Cananga odorata</i>	Ylang ylang	Marigold
<i>Cedrus atlantica</i>	Atlas cedar	Oshadhi
<i>Cinnamomum camphora</i>	Camphor tree	Herba oils
<i>Cinnamomum verum</i> (bark)	Ceylon cinnamon - bark	Herba oils
<i>Cinnamomum verum</i> - leaf	Ceylon cinnamon - leaf	Oshadhi
<i>Citrus × aurantium</i>	Bitter orange	Herba oils
<i>Citrus × bergamia</i>	Bergamot orange	Marigold
<i>Citrus limon</i>	Lemon	Marigold
<i>Cymbopogon flexuosus</i>	Lemongrass	Oshadhi
<i>Cymbopogon martinii</i>	Palmarosa	Oshadhi
<i>Eucalyptus globulus</i>	Blue gum	Kirka Pharma
<i>Juniperus communis</i>	Common juniper	Elmar
<i>Juniperus virginiana</i>	Eastern red cedar	Razzmatazz
<i>Laurus nobilis</i>	Bay laurel	Elmar
<i>Melaleuca quinquenervia</i>	Niaouli	Aromatica
<i>Mentha × piperita</i>	Peppermint	Kirka Pharma
<i>Nigella sativa</i>	Black cumin	Granum
<i>Ocimum basilicum</i>	Basil	Marigold
<i>Origanum vulgare</i>	Oregano	Eterra
<i>Pimpinella anisum</i>	Aniseed	Herba oils
<i>Pinus mugo</i>	Dwarf mountain pine	Apothecary Benu
<i>Pinus sylvestris</i>	Scots pine	Elmar
<i>Pogostemon cablin</i>	Patchouli	Aromatica
<i>Rosmarinus officinalis</i>	Rosemary	Centrochem
<i>Santalum album</i>	Indian sandalwood	Marigold
<i>Syzygium aromaticum</i>	Clove bud	Probotanic
<i>Zingiber officinale</i>	Ginger	Oshadhi

Pure colonies of each strain were selected from nutrient agar plates in which they grew for 48 hours and were transferred into tubes containing 10 ml of sterile distilled water. McFarland standard was used as a reference to adjust the concentration of bacterial suspensions equivalent to 10^8 - 10^9 CFU/ml.

Assessment of antibacterial activity of EOs

The inhibitory effects of the EOs on bacterial growth was evaluated by agar-diffusion assay. Bacterial suspensions (5 ml) of each tested strain were mixed in nutrient agar (500 ml) to reach a pathogen concentration of approximately 10^6 cfu ml⁻¹ and then poured in sterilized Petri plates (90 mm in diameter). After the

media solidified, double layers of sterile filter paper discs (ø 5 mm) supplemented with c. 20 µl of each test EO were placed on media surface. There were four replicates (four filter paper discs treated with different EOs in each plate) for each of the tested EOs and each test bacterium. Plates inoculated with bacterial cultures and with paper discs supplemented with sterile distilled water served as the control. The plates were incubated at 26-27°C temperature for a period of three days. The experiment was performed in a completely randomized design.

After the incubation period of 72 hours, inhibition zones around paper discs were measured in millimetres (mm). According to the recorded diameter values, the sensitivity of individual bacteria to test EOs was ranked, using a modified scale given by Babu et al. (2011) as follows:

- *not sensitive* (no inhibition zone)
- *weakly sensitive* (total zone diameters ≤ 10 mm),
- *sensitive* (diameters between 11 and 24 mm);
- *very sensitive* (zone diameters between 25 and 34 mm);
- *highly sensitive* (zone diameter of 35 mm).

data. Mean values and standard errors (\pm SE) were determined. Significant differences among means were compared using Duncan's multiple range test at 5% probability level.

Data analyses

Statistical analysis was performed using the software package Statistica 8.0 (StatSoft, Inc.). To evaluate the growth inhibitory effects of the essential oils against the test bacteria, we used an analysis of variance (ANOVA). The analysis was performed on log-transformed

RESULTS

Data on the sensitivity of bacterial strains of *E. amylovora*, *X. campestris* pv. *campestris* and *P. syringae* pv. *syringae* to 30 tested EOs, shown as absence or presence of inhibition zone (mm), are given in Tables 2-4 along with statistical analysis for each pathogen.

Table 2. *In vitro* growth inhibition (mm) of *Erwinia amylovora* subjected to 30 different essential oils

Essential oils		Inhibitory zone (mm)
Common name	Latin name	$\bar{X} \pm SE$
Ceylon cinnamon - bark	<i>Cinnamomum verum</i> (bark)	35.00 \pm 0 a
Oregano	<i>Origanum vulgare</i>	35.00 \pm 0 a
Clove bud	<i>Syzygium aromaticum</i>	35.00 \pm 0 a
Palmarosa	<i>Cymbopogon martinii</i>	35.00 \pm 0 a
Ceylon cinnamon - leaf	<i>Cinnamomum verum</i> - leaf	35.00 \pm 0 a
Rosemary	<i>Rosmarinus officinalis</i>	25.25 \pm 0.25 c
Basil	<i>Ocimum basilicum</i>	35.00 \pm 0 a
Blue gum	<i>Eucalyptus globulus</i>	22.00 \pm 0.41 d
Peppermint	<i>Mentha x piperita</i>	35.00 \pm 0 a
Camphor tree	<i>Cinnamomum camphora</i>	14.25 \pm 0.25 i
Lemongrass	<i>Cymbopogon flexuosus</i>	28.00 \pm 0.41 b
Aniseed	<i>Pimpinella anisum</i>	26.00 \pm 0.41 c
Ylang ylang	<i>Cananga odorata</i>	20.00 \pm 0.41 f
Silver fir	<i>Abies alba</i>	25.75 \pm 0.25 c
Lemon	<i>Citrus limon</i>	18.00 \pm 0.41 g
Dwarf mountain pine	<i>Pinus mugo</i>	10.25 \pm 0.25 k
Bay laurel	<i>Laurus nobilis</i>	20.75 \pm 0.25 e
Scots pine	<i>Pinus sylvestris</i>	15.25 \pm 0.25 h
Niaouli	<i>Melaleuca quinquenervia</i>	7.25 \pm 0.25 l
Atlas cedar	<i>Cedrus atlantica</i>	8.00 \pm 0.41 l
Black cumin	<i>Nigella sativa</i>	0 \pm 0 m
Indian frankincense	<i>Boswellia serrata</i>	0 \pm 0 m
Bergamot orange	<i>Citrus x bergamia</i>	0 \pm 0 m
Common juniper	<i>Juniperus communis</i>	12.25 \pm 0.25 j
Bitter orange	<i>Citrus x aurantium</i>	0 \pm 0 m
Neem	<i>Azadirachta indica</i>	0 \pm 0 m
Eastern red cedar	<i>Juniperus virginiana</i>	0 \pm 0 m
Patchouli	<i>Pogostemon cablin</i>	0 \pm 0 m
Indian sandalwood	<i>Santalum album</i>	0 \pm 0 m
Ginger	<i>Zingiber officinale</i>	0 \pm 0 m
Negative control		0 \pm 0 m
F		5896.6
P		0
df		30,93

Means marked by the same letter are significantly different

In the experiment with *E. amylovora* strain, the bacterium was: *highly sensitive* to the EOs of Ceylon cinnamon (leaf and bark), oregano, clove bud, palmarosa, basil and peppermint; *very sensitive* to lemongrass, aniseed, silver fir and rosemary; *sensitive* to blue gum, bay laurel, ylang ylang, lemon, scots pine, camphor tree and common juniper; *weakly sensitive* to dwarf mountain pine, Atlas cedar and niaouli; *not sensitive* to black cumin, Indian frankincense, bergamot orange, bitter orange, neem, Eastern red cedar, patchouli, Indian sandalwood and ginger.

Tests of *X. campestris* pv. *campestris* susceptibility to different EOs revealed the following results: *highly sensitive* to the EOs of Ceylon cinnamon (leaf and bark),

oregano, clove bud, palmarosa, rosemary, blue gum and camphor tree; *very sensitive* to basil, peppermint, ylang ylang, silver fir, lemon and aniseed; *sensitive* to lemongrass, dwarf mountain pine, bay laurel, scots pine, black cumin, niaouli, Indian frankincense, Atlas cedar and bergamot orange; *weakly sensitive* to bitter orange; *not sensitive* to common juniper, neem, Eastern red cedar, patchouli, Indian sandalwood and ginger.

The bacterium *P. syringae* pv. *syringae* showed the following reactions to test EOs: *very sensitive* to Ceylon cinnamon (bark) and oregano; *sensitive* to rosemary, blue gum, basil, clove bud, scots pine, dwarf mountain pine, palmarosa, Ceylon cinnamon (leaf), aniseed, lemon, silver fir,

Table 3. *In vitro* growth inhibition (mm) of *Xanthomonas campestris* pv. *campestris* subjected to 30 different essential oils

Essential oils		Inhibitory zone (mm)
Common name	Latin name	$\bar{X} \pm SE$
Ceylon cinnamon - bark	<i>Cinnamomum verum</i> (bark)	35.00±0 a
Oregano	<i>Origanum vulgare</i>	35.00±0 a
Clove bud	<i>Syzygium aromaticum</i>	35.00±0 a
Palmarosa	<i>Cymbopogon martinii</i>	35.00±0 a
Ceylon cinnamon - leaf	<i>Cinnamomum verum</i> - leaf	35.00±0 a
Rosemary	<i>Rosmarinus officinalis</i>	35.00±0 a
Basil	<i>Ocimum basilicum</i>	30.00±0.41 b
Blue gum	<i>Eucalyptus globulus</i>	35.00±0 a
Peppermint	<i>Mentha x piperita</i>	30.00±0.41 b
Camphor tree	<i>Cinnamomum camphora</i>	35.00±0 a
Lemongrass	<i>Cymbopogon flexuosus</i>	24.75±0.48 e
Aniseed	<i>Pimpinella anisum</i>	26.00±0.41 d
Ylang ylang	<i>Cananga odorata</i>	29.75±0.25 b
Silver fir	<i>Abies alba</i>	28.25±0.25 c
Lemon	<i>Citrus limon</i>	28.00±0.41 c
Dwarf mountain pine	<i>Pinus mugo</i>	24.75±0.25 e
Bay laurel	<i>Laurus nobilis</i>	24.00±0.41 e
Scots pine	<i>Pinus sylvestris</i>	20.50±0.29 f
Niaouli	<i>Melaleuca quinquenervia</i>	17.25±0.25 h
Atlas cedar	<i>Cedrus atlantica</i>	13.25±0.25 j
Black cumin	<i>Nigella sativa</i>	18.00±0.41 g
Indian frankincense	<i>Boswellia serrata</i>	15.00±0.41 i
Bergamot orange	<i>Citrus x bergamia</i>	11.00±0.41
Common juniper	<i>Juniperus communis</i>	0±0 m
Bitter orange	<i>Citrus x aurantium</i>	8.00±0.41 l
Neem	<i>Azadirachta indica</i>	0±0 m
Eastern red cedar	<i>Juniperus virginiana</i>	0±0 m
Patchouli	<i>Pogostemon cablin</i>	0±0 m
Indian sandalwood	<i>Santalum album</i>	0±0 m
Ginger	<i>Zingiber officinale</i>	0±0 m
Negative control		0±0 m
F		4525.2
P		0
df		30,93

Means marked by the same letter are significantly different

bay laurel and camphor tree; *weakly sensitive* to bitter orange, lemongrass, niaouli, neem, Atlas cedar and ylang ylang; *not sensitive* to peppermint, black cumin, Indian frankincense, bergamot orange, common juniper, Eastern red cedar, patchouli, Indian sandalwood and ginger.

The results reveal that *P. syringae* pv. *syringae* was less sensitive than the other two test bacteria (*E. amylovora* and *X. campestris* pv. *campestris*) because none of the test EOs was highly active against that bacterium.

In general, the EOs of Ceylon cinnamon (bark) and oregano were found to have the widest spectrum of activity, then clove bud, palmarosa, Ceylon cinnamon (leaf), rosemary, basil, blue gum and camphor tree.

The EOs of lemongrass, aniseed, ylang ylang, silver fir, lemon, dwarf mountain pine, bay laurel, scots pine, niaouli and Atlas cedar partially inhibited the growth of all tested bacteria. Some EOs gave variable reactions (less sensitive or no reaction) depending on test bacteria; peppermint oil showed a high inhibitory activity against *E. amylovora* and *X. campestris* pv. *campestris* but no activity against *P. syringae* pv. *syringae*; black cumin, Indian frankincense and bergamot orange acted against *X. campestris* pv. *campestris*, but no reaction was found against *E. amylovora* and *P. syringae* pv. *syringae*; common juniper evinced sensitive reaction of *E. amylovora* but no inhibition against *X. campestris*

Table 4. *In vitro* growth inhibition (mm) of *Pseudomonas syringae* pv. *syringae* subjected to 30 different essential oils

Essential oils		Inhibitory zone (mm)
Common name	Latin name	$\bar{X} \pm SE$
Ceylon cinnamon - bark	<i>Cinnamomum verum</i> (bark)	26.25±0.25 a
Oregano	<i>Origanum vulgare</i>	25.25±0.25 a
Clove bud	<i>Syzygium aromaticum</i>	20.25±0.75 d
Palmarosa	<i>Cymbopogon martinii</i>	18.75±0.25 f
Ceylon cinnamon - leaf	<i>Cinnamomum verum</i> - leaf	18.25±0.25 f
Rosemary	<i>Rosmarinus officinalis</i>	24.00±0.41 b
Basil	<i>Ocimum basilicum</i>	20.50±0.29 d
Blue gum	<i>Eucalyptus globulus</i>	22.50±0.29 c
Peppermint	<i>Mentha x piperita</i>	0±0 n
Camphor tree	<i>Cinnamomum camphora</i>	11.50±0.29 j
Lemongrass	<i>Cymbopogon flexuosus</i>	8.00±0.41 l
Aniseed	<i>Pimpinella anisum</i>	16.75±0.48 g
Ylang ylang	<i>Cananga odorata</i>	7.00±0.41 m
Silver fir	<i>Abies alba</i>	14.50±0.29 i
Lemon	<i>Citrus limon</i>	15.75±0.25 h
Dwarf mountain pine	<i>Pinus mugo</i>	19.25±0.25 ef
Bay laurel	<i>Laurus nobilis</i>	11.75±0.25 j
Scots pine	<i>Pinus sylvestris</i>	20.25±0.25 de
Niaouli	<i>Melaleuca quinquenervia</i>	8.00±0.41 l
Atlas cedar	<i>Cedrus atlantica</i>	7.50±0.29 lm
Black cumin	<i>Nigella sativa</i>	0±0 n
Indian frankincense	<i>Boswellia serrata</i>	0±0 n
Bergamot orange	<i>Citrus x bergamia</i>	0±0 n
Common juniper	<i>Juniperus communis</i>	0±0 n
Bitter orange	<i>Citrus x aurantium</i>	10.25±0.25 k
Neem	<i>Azadirachta indica</i>	8.00±0.41 l
Eastern red cedar	<i>Juniperus virginiana</i>	0±0 n
Patchouli	<i>Pogostemon cablin</i>	0±0 n
Indian sandalwood	<i>Santalum album</i>	0±0 n
Ginger	<i>Zingiber officinale</i>	0±0 n
Negative control		0±0 m
F		1905.6
P		0
df		30,93

Means marked by the same letter are significantly different

pv. *campestris* and *P. syringae* pv. *syringae*; bitter orange evinced sensitive reaction of *P. syringae* pv. *syringae* and *X. campestris* pv. *campestris* but no reaction against *E. amylovora*; neem oil showed weakly activity against *P. syringae* pv. *syringae* and no activity against *E. amylovora* and *X. campestris* pv. *campestris*.

The EOs of Eastern red cedar, patchouli, Indian sandalwood and ginger did not show any inhibitory effect on bacterial growth in the experiment.

Bacterial growth was observed on all positive control treatments treated with sterilized water.

DISCUSSION

Essential oils have recently been found to provide fungicidal, bactericidal, nematocidal and insecticidal biological activity applicable in agriculture (Koul et al., 2008; Pavela & Benelli, 2016). Thus, when pests and plant pathogens are present, EOs act as agricultural chemicals to reduce damage, while posing a minimum risk to humans and environment.

In vitro and *in vivo* studies of various EOs have so far shown that they have varying degrees of antibacterial activity against different plant pathogenic bacteria (Hevesi et al., 2006; Vasinauskiene et al., 2006). According to Bajpai et al. (2011), EOs could become alternative industrial products to synthetic bactericides and be applied in agricultural industry to control severe bacterial diseases caused by *Xanthomonas* species. The results obtained in this study showed that EOs of the aromatic plants Ceylon cinnamon and oregano produced the highest *in vitro* antibacterial activity against *E. amylovora*, *X. campestris* pv. *campestris* and *P. syringae* pv. *syringae*. Dadasoglu et al. (2011) showed the EOs of *Origanum acutidens*, *O. rotundifolium* and *O. vulgare* to have a wide spectrum of antibacterial activity against 25 phytopathogenic bacteria, which is probably due to their phenolic components, such as carvacrol and thymol, resulting with inhibition zone diameters from 8 to 48 mm. We found the EO of *O. vulgare* to cause diameter zones from 25 (*P. syringae* pv. *syringae*, very sensitive reaction) to 35 mm (*E. amylovora*, *X. campestris* pv. *campestris*, highly sensitive reaction). In addition to our results, a study conducted by Vasinauskiene et al. (2006) also identified oregano oil as having the strongest inhibitory effect against several phytopathogenic bacteria (*Erwinia carotovora* subsp. *carotovora*, *Xanthomonas vesicatoria*, *Pseudomonas marginalis* pv. *marginalis*, *P. syringae* pv. *syringae*, *P. syringae* pv. *tomato* and *Bacillus* sp). Similarly, Kokoskova et al. (2011) found

Origanum compactum, *O. vulgare*, *Thymus vulgaris*, *Mellisa officinalis*, *Mentha arvensis* and *Nepeta cataria* to be effective against *E. amylovora* and *P. syringae* pv. *syringae*, highlighting the first three as significantly more effective. The authors suggested that the tested oils exhibited a higher level of antibacterial activity than streptomycin used as a standard. In our study, *Mentha x piperita* (peppermint) caused a highly sensitive reaction of *E. amylovora* and very sensitive reaction of *X. campestris* pv. *campestris*, but no reaction of *P. syringae* pv. *syringae* was noticed. Todorović et al. (2016) reported the strongest and broadest antibacterial activity of wintergreen, oregano and lemongrass oils against *X. campestris* pv. *phaseoli*, *Clavibacter michiganensis* subsp. *michiganensis* and *Pseudomonas tolaasii*, indicating the former bacterium as the most sensitive to plant EOs. In our study, lemongrass caused very sensitive or sensitive reaction of *E. amylovora* and *X. campestris* pv. *campestris*, respectively, but *P. syringae* pv. *syringae* had a weakly sensitive reaction.

Božik et al. (2017) indicated that cinnamon, thyme, oregano and clove EOs have the potential to be used as antimicrobial agents against *Pseudomonas* spp. (*fluorescens*, *putida*, *syringae*) and *Pectobacterium* spp. (*carotovorum*, *antroseptica*), emphasising cinnamon as the most effective among the tested oils. The EOs of clove bud, palmarosa, rosemary, basil and blue gum also showed strong (high) efficacy in our study, but the reactions of test bacteria varied from very sensitive to highly sensitive.

It is important to point out that none of the tested EOs caused a highly sensitive reaction of *P. syringae* pv. *syringae* in this study and the bacterium was therefore classified as less sensitive (more resistant) than the other two test bacteria, *E. amylovora* and *X. campestris* pv. *campestris*. Different levels of sensitivity of plant pathogenic bacteria to EOs had already been reported before. Vasinauskiene et al. (2006) reported *X. vesicatoria* as the most sensitive organism to oregano, caraway, peppermint, fern-leaf and willow-leaved yarrow, while a weak antibacterial activity was found in some *Pseudomonas* spp. and *E. carotovora* subsp. *carotovora*. According to Huang & Lakshman (2010), clove oil has antibacterial activity on *Agrobacterium tumefaciens*, *E. carotovora*, *P. syringae* pv. *syringae*, *Ralstonia solanacearum*, *X. campestris* pv. *pelargonii*, *Rhodococcus fascians* and *Streptomyces* spp., with *R. solanacearum* being the most sensitive one. EOs obtained from four *Thymus* species (*vulgaris*, *serpyllum*, *citriodorus*, *citriodorus* “Archer’s Gold”) held a controlling effect against Gram-negative plant pathogenic bacteria, with *X. campestris* pv. *vesicatoria* and *P. syringae* pv. *phaseolicola*

as the two most sensitive bacterial pathogens (Horváth et al., 2004). Similarly, *Tagetes minuta* oils were found effective against halo and common blight pathogens of bean, and *X. axanopodis* pv. *manihotis* and *P. syringae* pv. *phaseolicola* (halo blight) were the most susceptible pathogens (Gakuubi et al., 2016).

There are various other reports on strong antibacterial activity of other EOs against plant pathogenic bacteria. According to Kotan et al. (2010), *Satureja spicigera* and *Thymus fallax* oils have the potential for controlling certain important agricultural plant pathogenic bacteria, and for being seed disinfectants. They demonstrated a potent antibacterial activity against a broad spectrum of 25 phytopathogenic bacteria (such as *C. michiganensis* subsp. *michiganensis*, *E. carotovora* subsp. *atroseptica*, *E. chrysanthemi*, *E. rhapontici*, *Pseudomonas cichorii*, *P. syringae* pv. *tomato*, *X. hortorum* pv. *pelargonii*, *X. axanopodis* pv. *malvacearum*, *X. axanopodis* pv. *vesicatoria*, *X. axanopodis* pv. *vitians*, *X. campestris* pv. *raphani*, *X. campestris* pv. *zinnia*), including three test bacteria used in our present study: *E. amylovora*, *X. campestris* pv. *campestris* and *P. syringae* pv. *syringae*. Bajpai et al. (2010a, 2010b) reported antibacterial activity of EOs derived from cones of *Metasequoia glyptostroboides* and *Cleistocalyx operculatus* buds which were quantitatively assessed against the plant pathogenic bacteria *X. campestris* pv. *campestris*, *X. campestris* pv. *vesicatoria*, and *X. oryzae* pv. *oryzae* in *in vitro* experiments. *In vivo* tests conducted on greenhouse-grown oriental melon plants, using the oil of *M. Glyptostroboides*, exhibited potent antibacterial effect against *X. campestris* pv. *vesicatoria* with 100% disease suppression efficacy (Bajpai et al., 2010b). Popović et al. (2017) highlighted the EOs of *Thymus vulgaris*, *Cinnamomum cassia*, *Origanum vulgare*, *Boswellia serrata*, *Eucalyptus globulus* and *Satureja montana* as having antibacterial potential against the soft rot pathogen *Pectobacterium carotovorum*.

In this study, the EOs of Eastern red cedar, patchouli, Indian sandalwood and ginger showed no reaction against any of the three tested bacteria. Some EOs have been shown not to inhibit many plant pathogenic bacteria, such as common yarrow and sweet-flag (Vasinauskiene et al., 2006).

CONCLUSION

Development of natural products to be used as antimicrobial agents in agricultural production is a major step towards a reduction in negative effects

associated with synthetic chemical pesticides, and enormously contributes to the implementation of the REACH regulation in the Republic of Serbia. This study confirmed the antibacterial activity of 30 different EOs against three plant pathogenic bacteria, *E. amylovora*, *X. campestris* pv. *campestris* and *P. syringae* pv. *syringae*. *In vitro* tests revealed that the EOs of Ceylon cinnamon and oregano had remarkable antibacterial activity against all three test bacteria. Based on inhibitory zone means, the tested EOs were more effective against *E. amylovora* and *X. campestris* pv. *campestris* than to *P. syringae* pv. *syringae*. However, to confirm the potential use of EOs in control of plant pathogenic bacteria, they should be tested for minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC), and validated in field *in vivo* tests. Some important risks in conventional and organic agriculture will thus be definitely reduced, food safety would improve, and one important step on the path to sustainable development will be made.

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Preliminarna ispitivanja antibaktericidnog delovanja etarskih ulja na ekonomski značajne fitopatogene bakterije

REZIME

Brojna naučna istraživanja širom sveta potvrđuju da je poljoprivreda u 21. veku posebno osetljiva na klimatske promene koje su uzrok širenja fitopatogenih bakterija. Stoga je jasna hitna potreba za ublažavanjem ovog rizika u poljoprivrednoj proizvodnji (u konvencionalnoj i organskoj poljoprivredi). Cilj ovog rada je određivanje antibakterijske aktivnosti 30 etarskih ulja prema tri ekonomski značajne fitopatogene bakterije, *Erwinia amylovora*, *Xanthomonas campestris* pv. *campestris* i *Pseudomonas syringae* pv. *syringae*. Istraživanja su vršena u *in vitro* uslovima, korišćenjem agar-difuzne metode. Etarska ulja pravog cimeta (od lista i kore), origana, zatim karanfilića i palmaroze, su pokazala antibakterijsku aktivnost prema testiranim sojevima bakterija, ostvarujući zone inhibicije maksimum prečnika 35 mm dobijene u slučaju *E. amylovora* i *X. campestris* pv. *campestris* (visoko osetljiva reakcija), a u slučaju *P. syringae* pv. *syringae* manju, od 18.25-26.25 mm (osetljiva do vrlo osetljiva reakcija). Maksimalni prečnik inhibicije zone (35 mm) je takođe dobijen primenom ulja bosiljka i pitome nane prema *E. amylovora* i ruzmarina, eukaliptusa i ravsare prema *X. campestris* pv. *campestris*. Kod *P. syringae* pv. *syringae* ni u jednom slučaju primene ulja nije postignut maksimalan prečnik inhibicije od 35 mm, na osnovu čega je ova bakterija svrstana kao slabije osetljiva.

Etarska ulja limun trave, anisa, ilang-ilanga, evropske jele, limuna, planinskog bora, lovora i belog bora su rezultirala osetljivom reakcijom testiranih sojeva bakterija. Pitoma nana, čurukot, tamjan, begramot, kleka, gorka pomorandža i nim su izazvali varijabilnu reakciju, od potpune inhibicije, do slabe ili čak i bez inhibicije. Slaba aktivnost je ostvarena kod niaoulja i atlaskog kedra. Sve tri testirane bakterije nisu pokazale reakciju prema virdžinijskoj kleki, pačuliju, sandalovini i đumbiru. Rezultati dobijeni u ovom radu daju osnovu za dalja istraživanja *in vivo*, sa svrhom razvoja "prirodnih pesticida" koji se mogu primeniti za suzbijanje fitopatogenih bakterija, čime se daje značajan doprinos u smanjenju gubitaka prinosa u poljoprivredi i održivom razvoju.

Ključne reči: Etarska ulja; Fitopatogene bakterije; Baktericidi