

***Thuja occidentalis* Essential Oil: *in vitro* and *in situ* Antibacterial Potential against Phytopathogenic Bacteria and Insecticidal Activity**

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Abstract

Thuja occidentalis essential oil (TOEO) was evaluated for antibacterial activity against phytopathogenic bacteria *in vitro* (disc diffusion) and *in situ* (fruit/vegetable models), and for insecticidal activity against *Megabruchidius dorsalis*. Tested bacteria were *Xanthomonas arboricola*, *Pectobacterium carotovorum*, *Pseudomonas syringae* and *Agrobacterium radiobacter*. *In vitro*, the largest inhibition zone was observed for *X. arboricola* (11.67 mm), while the smallest was for *A. radiobacter* (7.67 mm). *In situ*, the strongest effect occurred at 62.5 µg/mL on strawberry against *carotovorum* (78.96% inhibition) and on beetroot against *A. radiobacter* (79.56% inhibition). Insecticidal tests showed increasing mortality of *M. dorsalis* with rising TOEO concentration, reaching 100% at 100%. Within the tested ranges and models, TOEO exhibited measurable antibacterial and insecticidal effects. Findings are limited to the employed matrices, concentrations and incubation conditions, and do not imply broader applicability without additional sensory/toxicity and shelf-life testing.

Keywords: *Thuja occidentalis* essential oil, phytopathogenic bacteria, antibacterial and insecticidal effect

1. Introduction

One of the most productive coniferous trees, *Thuja occidentalis* (*T. occidentalis*), is used to treat a wide range of ailments with its oil and leaves. It is native to parts of Europe and the south of the United Kingdom [1]. Western Asia is home to a large number of species of species of this plant, including those that are found in various regions of the Kingdom of Saudi Arabia [2]. *T. occidentalis* has been shown to have anti-inflammatory [3], anti-tumour [4-6], antioxidant [7], antibacterial and antifungal [8,9], antidiabetic [10], hypolipidaemic and atheroprotective [11],

gastroprotective [12], antiviral and immunostimulating [13] activity. Its immunostimulant and antiviral properties have been demonstrated [14,15]. Torres et al. [3] investigated the effects of thujone on glioblastoma using both *in vitro* and *in vivo* models. *T. occidentalis* has been shown to be antimicrobial against a significant number of species including *Salmonella* spp., *Enterobacter cloacae*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Shigella flexneri*, *Candida albicans*, *Proteus vulgaris*, and *Enterococcus faecalis* [3]. They observed that the two components, α -thujone and β -thujone, were protective against Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. They also observed a weak effect against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* [16]. *Thuja occidentalis*

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also showed significant antibacterial activity against bacteria and fungi [17]. The antimicrobial profile of the essential oil of *Thuja occidentalis* has also been demonstrated by Tsiri et al. [18]. *T. occidentalis* has antifungal properties against *Saccharomyces cerevisiae*, *Aspergillus parasiticus*, *Aspergillus niger*, *Aspergillus flavus*, *Trichophyton rubrum*, *Macrophomina*, and *Fusarium solani* [19,20]. In the recent study by Bellili et al. [21], essential oil extracted from leaves and cones of *Thuja occidentalis* exhibited antimicrobial activity against Gram-negative bacteria (*E. coli*, *S. typhimurium*, *Aeromonas hydrophila*, etc.), Gram-positive bacteria (*S. aureus*, *Listeria monocytogenes*, and *Bacillus cereus*), fungi (*Aspergillus flavus* and *Aspergillus niger*) and yeasts (*Candida albicans*) [21]. Our aim was to study the antimicrobial activity of *Thuja occidentalis* essential oil *in vitro* and *in situ*, and its insecticidal activity against *Megabruchidius dorsalis*.

2. Materials and methods

2.1. Essential oil

Thuja occidentalis essential oil was purchased from Inevita SK (Bratislava, Slovakia). The essential oil was stored at 4 °C before use. The country of origin was India.

2.2. Bacterial strains

The antibacterial activity of *Thuja occidentalis* essential oil (TOEO) was evaluated against a range of Gram-negative (G⁻) bacterial strains including *Agrobacterium radiobacter* CCM 2926, *Pectobacterium carotovorum* CCM 1008^T, and *Pseudomonas syringae* CCM 2868 and *Xanthomonas arboricola* CCM 1441. All bacterial strains were obtained from the Czech Collection of Microorganisms (Brno, Czech Republic). Bacterial inocula were cultured in Mueller-Hinton broth (MHB, Oxoid, Basingstoke, UK) for 24 h at 37 °C before analysis. The optical density of the inocula was adjusted to the 0.5 McFarland standard on the day of the experiment. Strain maintenance/storage followed CCM guidance; only the *Pseudomonas* strain was kept at a lower storage temperature, while assay incubations were standardized at 37 °C for cross-strain comparability. The use of Mueller–Hinton media

(MHB/MHA) follows widely adopted antimicrobial screening methodology.

2.3. Disc diffusion method

In order to evaluate antimicrobial activity, the disc diffusion method was employed, as previously described. Small discs (6 mm in diameter) saturated with TOEO were placed on Mueller-Hinton agar (MHA) plates incubated with the bacterial strains. The bacterial strains were incubated at 37°C for 24 hours. The measurement of the inhibition zones was conducted in mm. All assays were performed in three independent repetitions, with results reported as mean ± SD. The blank discs served as negative controls, while the antibiotic discs (gentamicin, from Oxoid, Basingstoke, UK) served as positive controls [22].

2.4. *In situ* antimicrobial activity

In order to assess the *in situ* antimicrobial activity of TOEO, a range of substrates were tested, including commercial strawberry and beetroot, as well as the specific Gram-negative bacteria. The substrates were sliced into pieces measuring 0.5 mm, cleaned, and placed in 60 mm Petri dishes that had been inoculated with bacteria. TOEO was dispersed in ethyl acetate at concentrations of 500, 250, 125, and 62.5 µg/mL. Ethyl acetate filter sheets served as controls. Each experiment was conducted in three independent repetitions, with technical replications within each run. Graphs present mean ± SD values; the number of repetitions is specified in the text, not in the figure legends. The plates were sealed and incubated at 37 °C for 7 days. Microbial colony growth was assessed using ImageJ to calculate bacterial volume densities, along with standard methods for measuring *in situ* colony development [22].

2.5. Insecticidal activity

The insecticidal activity of TOEO was assessed using *Megabruchidius dorsalis* Fahreus, 1839 as the model organism. Groups of hundred *M. dorsalis* insects were placed in Petri dishes, each lined with sterile filter paper. Various concentrations of TOEO (100 %, 50 %, 25 %, 12.5 %, 6.25 %, and 3.125 %) were prepared by diluting the TOEO with a 0.1% polysorbate solution. After saturating sterile filter paper discs

with 100 μL of each TOEO concentration, the plates were sealed with parafilm and left at room temperature for 24 hours. The control group received 100 μL of the 0.1 % polysorbate solution. After 24 h, the number of living and dead insects was counted. This experimental procedure was successfully replicated in three independent studies.

3. Results and discussion

More recently, the prevalence of antimicrobial drug resistance has driven to the discovery of novel antimicrobial lead molecules for the treatment of various human pathogens [23]. Some of the synthetic drugs currently available are not able to inhibit a large number of pathogenic microbes. In addition, the use of synthetic chemicals to control pathogenic microorganisms is limited by their carcinogenic effects, acute toxicity and environmental hazards. In this regard, the use of essential oils for the control of epidemic multidrug resistant pathogenic microorganisms may be useful in the control of various infectious diseases [24]. The greatest antibacterial activity of TOEO was found against *Xanthomonas arboricola* (11.67 mm). The lowest antibacterial activity was found against *Agrobacterium radiobacter* (7.67 mm) (Figure 1). *In situ* analyses showed the best antibacterial activity in lowest concentration 62.5 $\mu\text{g/mL}$ on both models against

P. carotovorum on strawberry and *X. arboricola* on beetroot. Inhibition of *Pectobacterium carotovorum* on strawberry model was 78.96 % and *Xanthomonas arboricola* on beetroot was 79.56 % (Figure 1,2). The effect of the antibacterial activity of essential oils may be to inhibit the growth of bacteria (bacteriostatic activity) or to destroy bacterial cells (bactericidal activity) [25]. Rapid antibacterial screening of essential oils is usually performed using the agar diffusion technique. Essential oils are added to filter paper discs or wells, which are placed in agar that has been uniformly inoculated with a bacterial strain. After incubation, the inhibited zone represents the antimicrobial effect [26]. The efficacy of essential oils varies from one type to another, as well as against different target bacteria, depending on their structure (Gram-positive and Gram-negative bacteria). For example, sandalwood and vetiver oils have a higher inhibitory activity against gram-positive bacteria; however, they do not inhibit gram-negative strains of bacteria [83, 27]. Cinnamon, clove, pimento, thyme, oregano and rosemary essential oils were shown to be potent against *Salmonella typhi*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [28]. Some pathogenic bacteria (*Salmonella choleraesuis*, *Salmonella enteritidis*, *S. typhimurium* and *E. coli*) were inhibited by thyme and oregano essential oils [29].

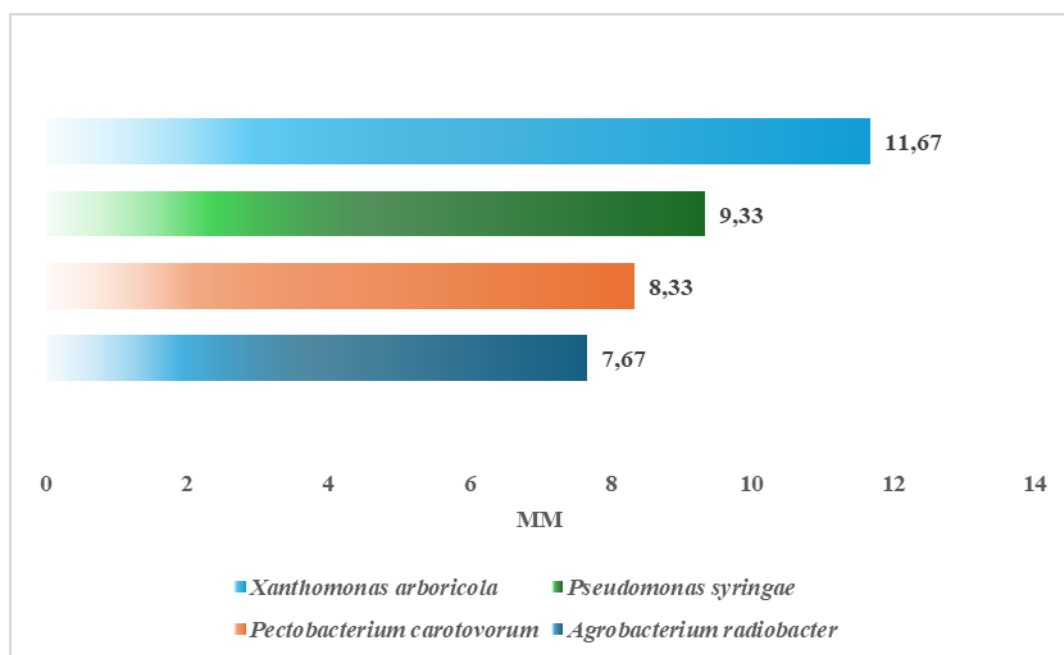


Figure 1. Antibacteria activity of TOEO by disc diffusion method in mm

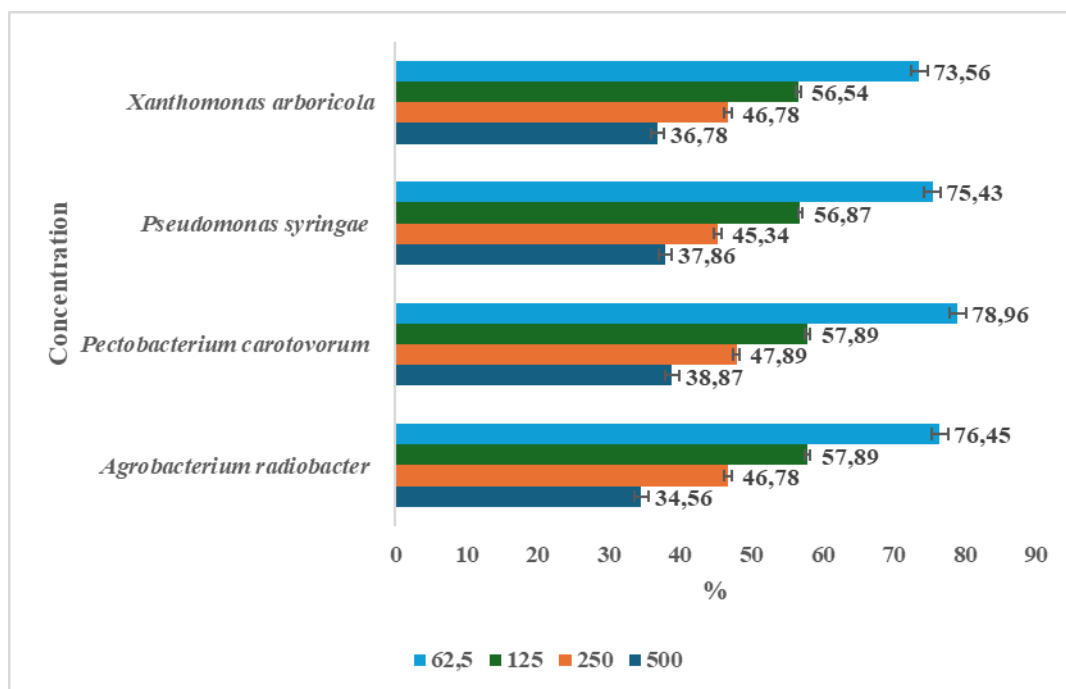


Figure 2. Antibacterial activity of TOEO *in situ* on strawberry model (% inhibition)

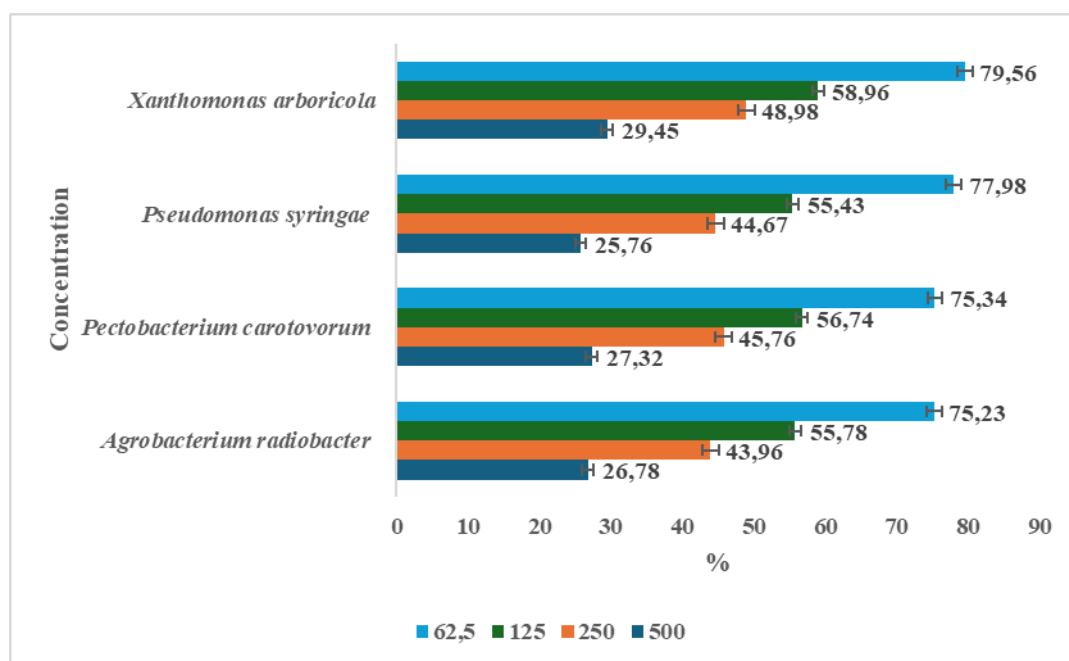


Figure 3. Antibacterial activity of TOEO *in situ* on beetroot model (% inhibition)

The essential oils had an MIC of 0.25 % to $\geq 2\%$ v/v. In another study, the essential oils of *Salvia* spp. (*S. officinalis*, *S. sclarea* and *S. lavandulifolia*) and *Thuja* spp. (*T. plicata* and *T. occidentalis*) showed potent antibacterial properties against human pathogens [16]. The main components (α -thujone and β -thujone) of these sage species showed high inhibitory activity

against *P. aeruginosa* and *K. pneumoniae*, whereas *S. aureus* and *E. coli* were moderately inhibited. The essential oils of *Thuja* spp. (*T. plicata* and *T. occidentalis*) effectively inhibited *P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *E. coli* [16].

The present study examined the insecticidal efficacy of TOEO against *M. dorsalis*, as

illustrated in Table 1. The results demonstrated that the highest levels of insecticidal activity were observed in trials where 25 %, 50 % and 100 % of the TOEO concentrations were applied. Nevertheless, concentrations of 6.25 of the TOEO did not demonstrate repellent effect against *M. dorsalis*.

The results showed a variation in the activity of the EOs against the larvae of *A. diaperinus*: it was closely related to the composition of the EOs and to the age of the larvae. *Thuja* oil and α -thujone were more active compared to tansy oil and β -thujone, but there were no significant differences in the activity of the oil versus its major component. In many cases, the major components of EOs determine their activity. However, the role

of the remaining compounds and antagonistic/synergistic interactions between EO components cannot be ignored [30]. Sometimes an EO is more insecticidal than its major component and vice versa. For example, the oil of *Majorana hortensis* L. showed stronger activity against *Spodoptera littoralis* Boisduval larvae and *Aphis fabae* Scopoli adults than its main components, γ -terpinene and terpinen-4-ol, suggesting a synergistic effect of the other components [31]. Conversely, *A. diaperinus* larvae were less sensitive to thyme oil than its major components, thymol and carvacrol. These monoterpene phenols had a strong effect on the growth and development of the larvae, while thyme oil stimulated the growth of the larvae [32].

Table 1. Insecticidal activity of TOEO against *Megabruchidius dorsalis* (n=50)

Concentration (%)	Number of Living Individuals	Number of Dead Individuals	Insecticidal Activity (%)
100	0	100	100.00 $\pm$ 0.00
50	10	90	90.00 $\pm$ 0.00
25	20	80	80.00 $\pm$ 0.00
12.5	50	50	50.00 $\pm$ 0.00
6.25	90	10	10.00 $\pm$ 0.00
3.125	100	0	0.00 $\pm$ 0.00
Control group	100	0	0.00 $\pm$ 0.00

4. Conclusions

Our study demonstrates that TOEO exhibits antimicrobial activity against plant-pathogenic bacteria and shows insecticidal activity under the tested conditions. Specifically, TOEO inhibited the growth of *Xanthomonas arboricola*, *Pectobacterium carotovorum*, *Pseudomonas syringae*, and *Agrobacterium radiobacter* in disc diffusion assays and reduced surface growth in plant-tissue matrix models (strawberry, beetroot) under vapor-phase exposure. Insecticidal tests showed concentration-dependent mortality of *Megabruchidius dorsalis*. Results are reported as mean $\pm$ SD from three independent repetitions and pertain to the tested strains, matrices, concentration range (62.5–500 μ g/mL), and incubation settings.

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