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The potency of lemon (*Citrus limon* L.) essential oil to control some fungal diseases of grapevine wood

*Les huiles essentielles de citron (*Citrus limon *L.) pour lutter contre certaines maladies fongiques du bois de la vigne*

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ABSTRACT

This study aimed to evaluate the in vitro antifungal activity (AA) of the essential oil (EO) of lemon (*Citrus limon* L.) against three pathogenic fungi attacking grapevine wood. The composition of the EO was also studied. Ten volatile components were identified by gas chromatography–mass spectrometry. The results showed that the EO consists of volatile components where monoterpene hydrocarbons are the most abundant ones. Four major components were identified, which represent 99.9% of the total EO (limonene, neral, β-pinene, and γ-terpinene). The AA of the EO was evaluated against three pathogenic fungi attacking grapevine wood (*Eutypa* sp., *Botryosphaeria dothidea*, and *Fomitiporia mediterranea*). The results showed that the EO exerts AA against all tested fungi and significantly inhibits their growth. *Eutypa* sp. is the most sensitive fungus. These results show, for the first time, a new use for the EO of lemon (*C. limon* L.) to control fungal diseases of grapevine wood.

Mots clés :
Activité antifongique
Citrus limon L.
Maladies du bois
Huile essentielle
Vigne

RÉSUMÉ

Cette étude examine l'activité antifongique in vitro (AA) de l'huile essentielle (EO) de citron (*Citrus limon* L.) contre trois champignons pathogènes du bois de la vigne. La composition de l'EO a également été étudiée. Dix composants volatiles ont été identifiés par chromatographie en phase gazeuse–spectrométrie de masse. Les résultats ont montré que l'EO est constituée de composants volatils où les hydrocarbures de monoterpènes sont majoritaires. Quatre composants principaux ont été identifiés, ce qui représente 99,9 % de l'EO totale (limonène, néral, β-pinène et γ-terpinène). L'AA de l'EO a été évaluée contre

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trois champignons pathogènes du bois de vigne (*Eutypa* sp., *Botryosphaeria dothidea* et *Fomitiporia mediterranea*). Les résultats ont montré que l'EO exerce une AA contre tous les champignons testés et inhibe de manière significative leur croissance. *Eutypa* sp. a été le plus sensible. Ces résultats montrent, pour la première fois, une nouvelle utilisation pour l'EO du citron pour lutter contre les maladies fongiques du bois de vigne.

1. Introduction

Fungi are the main infectious agents in plants, causing alterations during developmental stages, and are the main microorganisms responsible for losses in agriculture. The decline of the vine is due to fatal diseases such as esca, eutypiosis, and BDA (black dead arm) developed in vineyards [1,2]. These diseases are the most destructive ones of the woody tissues of grapevine and have been found on some hosts, causing decline and loss of productivity [3]. This kind of fungus colonizes wood tissue and causes dieback; the first disease, called esca, is a complex disease, including vascular symptoms and internal white rot in the trunk, which gradually changes the hard wood into a soft one [1]. *Eutypa* dieback, caused by the fungus Eutypa lata, and BDA, caused by the Botryosphaeria family, threatens the sustainability of vineyards, especially young ones. It is becoming a serious problem in most vine growing regions. These fungi produce toxic metabolites that are transported through vascular tissue to the foliage, causing necrosis of leaves, vascular symptoms, and internal brown rot in the trunk [4]. Since the ban of sodium arsenite, the only known way to fight the esca and the Black Dead Arm (BDA) in 2001, there is a worrying progression of these diseases in the vineyards around the world. The removal of sodium arsenite due to its toxicity on both environment and human health is causing concern to growers in that no satisfactory replacement control methods have existed up till today. The control methods available are prophylactic, and they involve the removal of cut wood and dead vineyard. Painting wounds with a drying product, following the ban, since 2007, of Escudo, fungicide to brush. Biocontrol, by treating size wounds with a preparation with *Trichoderma harzianum*, significantly reduces the development of the pathogenic fungi Botryosphaeria, Phaeomoniella, and Phaeoacremonium. However, this last preparation showed its prophylactic efficacy in greenhouse only [5,6].

Fungi are generally controlled by synthetic fungicides; however, the use of these is increasingly restricted due to the harmful effects of pesticides on human health and the environment [7]. Wood diseases of the vine and many other trees are very complex, involving several fungi, and no effective control exists for the time being against this kind of pathogen. Natural products having components with various modes of action might provide an effective solution to some sanitary plant problems [8]. These products are now an endless source of interesting molecules for scientists and the industry. They have similar active ingredients that have specific properties giving them an intrinsic behavior [9]. Phyto-compounds are expected to be far more advantageous than synthetic pesticides due to the sheer magnitude of their complexity, diversity and novelty of chemicals, reactions, and phenomena [10], as they are bio-degradable in nature, non-pollutant and possess no residual or phytotoxic properties [11].

Citrus essential oil (EO) has been identified in different parts of fruits as well as in leaves (particularly present in fruit flavedo), showing that limonene, β-myrcene, α-pinene, *p*-cymene, β-pinene, terpinolene, and other elements are the major aromatic compounds of many citrus species [12–15]. *Citrus limon* EO is used for many applications such as food, medicines, cosmetics and perfumes, detergents, aromatherapy, pathogen inhibition, and insect control [16], but, to our knowledge, no previous studies have been carried out on the use of *C. limon* EO against phytopathogenic fungi attacking grapevine wood trees or to control plant diseases.

This study aims to investigate, for the first time, the in vitro antifungal activity (AA) of *C. limon* EO against three phytopathogenic fungi (*Eutypa* sp., *Fomitiporia mediterranea* and *Botryosphaeria dothidea*) that attack grapevine wood trees. Also, this study determined the EO composition when extracted by steam distillation from the epicarp of *C. limon* fruits. The results showed that EO exerts a significant AA against the studied fungi attacking the wood of the grapevine and could be of use to control fungal diseases in agriculture.

2. Materials and methods

The present study also confirmed the AA of citrus oils already described in the literature, for example the antifungal efficacies of citrus oils against *Penicillium digitatum* and *P. italicum*, and found that *P. digitatum* was more sensitive to the inhibitory action.

2.1. Isolation of the EO

Fresh epicarps of lemon were collected from Blida (Northern Algeria) in April 2013. The EO was extracted by steam distillation of fresh plant material collected (100 g of epicarps). The bottles of oil were covered with aluminum paper to protect them from any negative effects of light and were stored in a refrigerator at a temperature of 4 °C.

2.2. Gas chromatography–mass spectrometry (GC–MS) analysis

The oils obtained were analyzed according the method previously used for lemon [17–19]. GC–MS was performed using a PerkinElmer Clarus 600 mass spectrometer with a

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