Crop Protection 114 (2018) 97–105

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Efficacy of a *Magnolia officinalis* bark extract against grapevine downy mildew and apple scab under controlled and field conditions

Barbara Thuerig[a,*], Justine Ramseyer[b], Matthias Hamburger[b], Mathias Ludwig[a], Thomas Oberhänsli[a], Olivier Potterat[b], Hans-Jakob Schärer[a], Lucius Tamm[a]

[a] Research Institute of Organic Agriculture FiBL, Ackerstrasse 113, CH-5070 Frick, Switzerland
[b] Division of Pharmaceutical Biology, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstrasse 50, CH-4056 Basel, Switzerland

ARTICLE INFO

Keywords:
Plant extract
Fungicide
Botanical plant protection product
Honokiol
Magnolol
Magnolia officinalis

ABSTRACT

In organic agriculture, the control of several diseases is largely depending on copper fungicides. Yet, copper can accumulate in the soil if the annual input exceeds annual uptake by plants, which can have a negative impact on soil fertility. Its use should thus be avoided or reduced. The aim of the present study was to evaluate the efficacy of a bark extract of *Magnolia officinalis* Rehder and Wilson to control three pathogens including *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni (causing grapevine downy mildew), *Venturia inaequalis* (Cooke) G. Winter (causing apple scab), and *Phytophthora infestans* (Mont.) De Bary (causing potato and tomato late blight) under controlled and field conditions, and to identify compounds responsible for the antifungal activity of the extract. Under controlled conditions, *M. officinalis* bark extract showed a mean efficacy of 97% (*P. viticola*) and 93% (*V. inaequalis*) at 1 mg mL^{-1}, and EC_{50} between 0.14 and 0.20 mg mL^{-1}. Efficacy against *P. infestans* was comparatively low (52% at 1 mg mL^{-1}). Magnolol and honokiol were identified as the main active compounds, both with $EC_{50} \leq 0.08$ mg mL^{-1} against *P. viticola* and *V. inaequalis*. Under field conditions, preliminary formulations reached efficacies up to 71% at 1–2 mg plant extract mL^{-1} against grapevine downy mildew, whereas activity against apple scab could not be confirmed. *Magnolia officinalis* is a promising candidate for the development of a sustainable plant protection product against grapevine downy mildew due to a combination of good efficacy, high availability of the raw material at affordable prices, reasonable extraction efficiency, and expected low human toxicity due to its longstanding use in traditional Chinese medicine.

1. Introduction

Plant pathogens are a constant threat to crops and can seriously compromise yields. Besides indirect measures such as crop rotation, selection of resistant or tolerant varieties, and habitat management, direct plant protection by fungicides is often essential to avoid severe yield losses due to pathogen infections. However, there is growing demand to replace chemical fungicides by more sustainable alternatives due to concerns about their impact on human health and the environment (Bolognesi, 2003; Gilliom, 2007; Mullin et al., 2010; Schwarzenbach et al., 2010; Weisenburger, 1993). Copper-based fungicides have been widely used to control many devastating plant diseases, including fungal and bacterial leaf spots, blights, anthracnoses, downy mildews and cankers (Agrios, 2005). The use of copper-based fungicides has decreased in conventional agriculture with the introduction of synthetic pesticides, and in organic agriculture thanks to improved formulations and the implication of decision support systems. Yet, copper input often still exceeds its uptake by plants, resulting in accumulation in the soil, especially in the case of perennial high-value crops such as grapevine or apple trees, due to a combination of intensive spray programmes with no or limited crop rotation (Eijsackers et al., 2005). Natural products such as plant extracts might provide effective, sustainable, and environmentally-friendly alternatives (Isman, 2014; Seiber et al., 2014). The use of plant extracts as insecticides has quite a long tradition (Ntalli and Menkissoglu-Spiroudi, 2011). For example, extracts from *Tanacetum cinerariifolium* (containing pyrethrines) (Casida, 1980), *Azadirachta indica* (containing azadirachtine) (Schmutterer, 1990) or *Quassia amara* (containing quassin) (Mancebo et al., 2000) very efficiently control several insect pests. They are mainly used in high value crops, herbs and ornamentals due to their

* Corresponding author.
E-mail addresses: barbara.thuerig@fibl.org (B. Thuerig), justine.ramseyer@unibas.ch (J. Ramseyer), matthias.hamburger@unibas.ch (M. Hamburger), mathias.ludwig@fibl.org (M. Ludwig), thomas.oberhaensli@fibl.org (T. Oberhänsli), olivier.potterat@unibas.ch (O. Potterat), hans-jakob.schaerer@fibl.org (H.-J. Schärer), lucius.tamm@fibl.org (L. Tamm).

https://doi.org/10.1016/j.cropro.2018.08.011
Received 15 January 2018; Received in revised form 25 July 2018; Accepted 8 August 2018
0261-2194/ © 2018 Elsevier Ltd. All rights reserved.

relatively high prize (Isman, 2008). In contrast, only few plant extracts can be used in Europe against plant diseases, e.g. fennel oil (against powdery mildews and rust), lecithine (against powdery mildews), coconut potassium soap (against rainspot disease of apples), laminarine (an algae-derived stimulator of natural defense mechanisms in plants), and an extract of *Equisetum arvense* (against various diseases) (Expert Group for Technical Advice on Organic Production EGTOP, 2016; Yoon et al., 2013a).

To identify extracts with antimicrobial activity, we screened a library of more than 3000 extracts originating from approximately 800 plant and 100 fungal species for activity against important plant pathogens of high value crops, including *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni (causing grapevine downy mildew), *Venturia inaequalis* (Cooke) G. Winter (causing apple scab) and *Phytophthora infestans* (Mont.) De Bary (causing tomato and potato late blight) (Thuerig et al., 2016). These plant pathogens can cause up to 100% yield losses in non-treated plants, and plant protection in organic production is largely depending on copper fungicides (Finckh et al., 2015). Among the extracts tested, an ethyl acetate extract of *Magnolia officinalis* Rehder and Wilson bark showed promising activity. *Magnolia officinalis* is a deciduous tree distributed throughout subtropical China at elevations between 300 and 2000 m above sea level, and growing in natural broadleaf forests and plantations (Tong et al., 2002; Xiao et al., 2012). *Magnolia officinalis* is a well-known Asian medicinal plant, and its stem bark has been traditionally used in China, Korea and Japan to treat gastrointestinal disorders, anxiety and allergic diseases (Lee et al., 2011). It contains alkaloids and phenolic compounds, with the neolignans magnolol and honokiol contributing to 40–90% of total polyphenols (Poivre and Duez, 2017; Tong et al., 2002). The bark contains up to 7% of the two compounds, depending on factors including origin and age of the tree (Tong et al., 2002). Chinese and European Pharmacopoeia require a minimal content of 2% of neolignans in the herbal drug (Council of Europe (2013); CPC (Chinese Pharmacopoeia Commission), 2010). Numerous therapeutic properties have been described for magnolol and honokiol (Lee et al., 2011). Activity against human pathogens has been documented, including viruses (Lan et al., 2012), bacteria (e.g. acne causing bacteria (Park et al., 2004), periodontal pathogens (Ho et al., 2001), multi-drug resistant bacterial strains (Jacobo-Salcedo et al., 2011)), and fungi (Bang et al., 2000; Clark et al., 1981; Jacobo-Salcedo et al., 2011). *Magnolia* sp. extracts and isolated compounds (honokiol, 1-methoxyhonokiol, magnolol and eudesmone) reportedly showed *in vitro* activity against some wood-decay fungi and plant pathogens (Choi et al., 2009; Mori et al., 1997a, 1997b). An *in vivo* effect against some plant diseases (rice blast, tomato late blight, wheat leaf rust, barley powdery mildew, red pepper anthracnose) was shown under controlled conditions (Choi et al., 2009), and a formulated powder from *M. officinalis* stem bark reduced rust diseases of *Perilla* and *Zoysia* grass under field conditions (Yoon et al., 2013b).

The aim of the present study was to evaluate the potential of a *M. officinalis* extract to protect grapevine plants, apple trees and tomatoes against *Plasmopara viticola*, *Venturia inaequalis* or *Phytophthora infestans* under controlled and field conditions, and to identify the active constituents of the extract.

2. Material and methods

2.1. Phytochemistry

2.1.1. Chemicals

Solvents and formic acid were obtained from Scharlau (Barcelona, Spain). For extraction, technical grade solvents were used after re-distillation. For high-performance liquid chromatography (HPLC), HPLC-grade solvents were employed. HPLC grade water was obtained from a MilliQ water purification system (Merck Millipore, Darmstadt, Germany). Magnolol and honokiol references were purchased from Tokyo Chemical Industry (Tokyo, Japan).

2.1.2. Plant material

The bark of *Magnolia officinalis* var. *biloba* Rehder and Wilson was purchased from Peter Weinfurth, Bochum, Germany. The plant material was imported from China. A voucher specimen (Nr 216) is kept at the Division of Pharmaceutical Biology, University of Basel, Switzerland.

2.1.3. Extraction

The extract used for all controlled conditions bioassays and for field trials in 2014 was obtained as follows. The bark of *M. officinalis* was milled with a grinder SM 100 (Retsch, Haan, Germany). The powder (14 kg) was divided into three portions and mixed with sea sand (1:1). Each portion was then packed into a column, and percolated for 3 days with approx. 32 L of ethyl acetate. After evaporation under reduced pressure, a total amount of 1053 g of extract was obtained (yield 7.5%) (Extract 1).

For field trials in 2015, 20 kg of *M. officinalis* finely sliced bark was macerated with 120 L of ethyl acetate for 24 h. After filtration over a 50 µm polyester fleece the extract was concentrated under reduced pressure to a liquid extract (3.0 kg) containing 32.6% dry matter (yield 4.9%) (Extract 2).

2.1.4. HPLC microfractionation

Microfractionation was performed by semi-preparative HPLC on an Agilent 1100 Series with a PDA detector (Santa Clara, CA, USA) connected to a FC204 fraction collector (Gilson, Middleton, WI, USA). Separations were carried out at 25 °C on a SunFire™ Prep C_{18} column (5 µm, 150 × 10 mm i.d., Waters, Milford, MA, USA) equipped with a guard column (10 × 10 mm i.d.). The mobile phase consisted of water with 0.1% formic acid (Solvent A) and acetonitrile with 0.1% formic acid (Solvent B). A gradient of 5–100% B in 30 min was used, followed by 100% B for 5 min. The flow rate was 4 mL min^{-1}. The extract was dissolved in DMSO at a concentration of 50 mg mL^{-1}, centrifuged, and filtered. Two injections of 200 µL each were performed (20 mg of extract in total). Microfractions were collected every 90 s from 1 to 34 min (22 fractions for each run). After removal of the solvent in a Genevac EZ-2 evaporator (Stone Ridge, NY, USA), the fractions were redissolved in 300 µL of methanol. The corresponding fractions obtained from the two separations were combined and dried.

2.1.5. Quantification of the active constituents

Analyses were performed in triplicate on an HPLC Agilent 1100 Series with a PDA detector (Santa Clara, CA, USA). Separation was carried out at 25 °C on a SunFire™ C_{18} (3.5 µm, 150 × 3.0 mm i.d.) column equipped with a guard column (10 mm × 3.0 mm i.d.). The mobile phase consisted of water with 0.1% formic acid (Solvent A) and acetonitrile with 0.1% formic acid (Solvent B). The flow rate was 0.4 mL min^{-1}. The following gradient was used: 50–100% B in 20 min, followed by 100% B for 5 min. Samples were dissolved in DMSO at a concentration of 1 mg mL^{-1} for the extract, and 32–250 µg mL^{-1} for magnolol and honokiol. The injection volume was 10 µL. Detection was at 290 nm. Calibration curves were used to determine the concentration of the compounds in the extract: magnolol: 38.708x + 29.247 (r^2 = 0.9998); honokiol: 38.141x + 25.292 (r^2 = 0.9999).

2.2. Pathogens

Phytophthora infestans (Mont.) de Bary was cultivated on V8 agar (200 mL L^{-1} Campbell's V8 or "Biotta® Gemüsecocktail" (vegetable juice) (Biotta AG, Tägerwilen, Switzerland), 3 g L^{-1} $CaCO_3$, 1.5% Agar, pH 6.3) at 20 °C in the dark. *Venturia inaequalis* Cooke (Wint.) was maintained on apple (*Malus domestica* Borkh.) seedlings cv. 'Jonagold' as described below. Leaves with sporulating lesions were dried at room temperature before storing them in glass vessels at 4 °C in the dark. *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni was maintained on grapevine (*Vitis vinifera* L.) seedlings cv. 'Chasselas' by weekly re-inoculation (described below). Sporangia suspensions of *P. viticola*

Download English Version:

https://daneshyari.com/en/article/8877847

Download Persian Version:

https://daneshyari.com/article/8877847

Daneshyari.com