From Forest Nursery Notes Winter 2013

156. © Antimicrobial activity of chestnut extracts for potential use in managing soilborne plant pathogens. Hao, J. J., Liu, H., Donis-Gonzalez, I. R., and Lu, X. H. Plant Disease 96(3):354-360. 2012.

Antimicrobial Activity of Chestnut Extracts for Potential Use in Managing Soilborne Plant Pathogens

Jianjun J. Hao, Huawei Liu, Irwin Ronaldo Donis-Gonzalez, and **Xiao Hong Lu,** Department of Plant Pathology, **A. Daniel Jones,** Department of Biochemistry & Molecular Biology and Department of Chemistry, and **Dennis W. Fulbright,** Department of Plant Pathology, Michigan State University, East Lansing 48824

Abstract

Hao, J. J., Liu, H., Donis-Gonzalez, I. R., Lu, X. H., Jones, A. D., and Fulbright, D. W. 2012. Antimicrobial activity of chestnut extracts for potential use in managing soilborne plant pathogens. Plant Dis. 96:354-360.

Chestnut extracts were studied for antimicrobial activity against selected microorganisms, including plant pathogens. Chestnut extract on paper discs was applied to an agar medium to evaluate the inhibition to multiple microorganisms or the extract was added at various concentrations to a culture medium to evaluate the growth of target microorganisms. Chestnut type, tissue of plants (shell, pellicle, and leaf), extraction methods, and physical characteristics were studied to determine antimicrobial activity. Most test microorganisms were inhibited by the extracts at different effective concentrations for 50% growth inhibition (EC_{50}). *Pseudomonas fluorescens* was the most sensitive (EC_{50} = 4.4 µg/µl), *Phytophthora cambivora* was one of the least inhibited (EC_{50} = 185 µg/µl), and *Cryphonectria parasitica* was not inhibited. Extracts of the Japanese × European chestnut (*Castanea crenata* × *C. sativa*) 'Colossal' showed a greater inhibition than those of wild trees of the Chinese species (*C. mollissima*). High temperature did not affect the inhibitory effect. Extracts from chestnut pellicle had the highest concentration of antimicrobial compound, compared with leaf and shell. The active fraction contained several substances with molecular masses consistent with one flavonol glycoside and several terpenoid substances. Pellicle and shell tissue reduced radish scab disease caused by *Streptomyces scabies* in the greenhouse.

Species of *Castanea* are perennial plants varying from small shrubs and large trees in forests and orchards of Europe, Asia, and North America (6,9,11,22). Despite the worldwide dissemination of several devastating pathogens and pests, such as chestnut blight (*Cryphonectria parasitica* (Murrill) M.E. Barr) (2) and Asian gall wasp (*Dryocosmus kuriphilus*) (10,34), chestnut remains one of the most popular nut-bearing trees throughout Eurasia (5,6). Today, South Korea, China, Italy, and Turkey account for more than 70% of worldwide chestnut production (4,7). Chestnut farming and production are increasing in Australia, New Zealand (19), Chile (17), and the United States (15). A large research program to restore chestnut blight-resistant American chestnut (*Castanea dentata*) to the forest environment is currently being pursued in North America (12).

The popularity of many *Castanea* spp. is based on several attributes of various species, including the production of nutritious, low-fat nuts and decay-resistant, high-quality wood; the ability of roots to sprout after harvest, eliminating the need to replant; and the role of several species in forest ecosystems. The chestnut fruit is known for its flavor, nutritional value, and potential uses as nutritional supplements or for medical purposes (1,7,8,32).

In addition to these attributes, tissues of *Castanea* spp. have been found to contain compounds with inhibitory effects on microorganisms and plants (3). Tannins, flavonoids, and antioxidants in shell extracts of Chinese chestnut (*C. mollissima*) (27) appeared inhibitory to bacteria and fungi, including *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae,* and *Aspergillus niger* (23,24). Recently, Donis-Gonzalez (13) reported that the greatest antimicrobial activity was found in the pellicle, a thin fibrous tissue found between the shell and kernel. When chestnut pellicle, shell, and nut kernel were directly placed on agar media, the pellicle tissue was the only tissue found to inhibit fungi (including yeast) and bacteria. Acetone-extracted pellicle and shell had inhibitory activity against the test organisms, with the pellicle showing a higher level of inhibition. Chestnut kernel tissue extract showed no inhibitory activity (13).

The objectives of this work were to determine the level of the inhibitory effect on a range of microorganisms, the tissues producing the effect, and potential uses for chestnut shell material as a soil amendment in managing soilborne diseases.

Materials and Methods

Microorganisms and media. Strains of bacteria, fungi, and oomycetes were collected from various sources (Table 1). The microorganisms were grown in the laboratory on appropriate culture media, including potato dextrose agar (PDA; EMD Chemicals, Inc., Gibbstown, NJ) for fungi and some oomycetes, rye seed agar medium (29) for *Phytophthora infestans* Leonean, yeast malt extract agar (YME) (28) for *Streptomyces* spp., and Lysogeny broth (LB)-Miller agar medium (EMD Chemicals, Inc.) for other bacteria.

Chestnut extracts. Compounds were extracted from two different chestnut sources: wild, ungrafted Chinese chestnut trees (*C. mollissima*) growing in established Michigan orchards and commercially grafted 'Colossal' chestnut (*C. sativa* × *C. crenata*), also found on various farms in Michigan (15). The extract from shell and pellicle used in this study was obtained from hand-peeled chestnuts, unless otherwise stated. Hand-peeling was done by cutting the chestnut in half with a knife and then manually separating the pellicle from the shell, which were stored at –20°C until use.

Some shell and pellicle were obtained by processing chestnut through a commercial brulage peeling line (Boema S. P. A., Neive, Italy; (18) located at the Michigan State University Roger's Reserve, Jackson, MI. The peeling line uses propane burners coupled with hot water (70°C) to separate chestnut shell material from kernels. Shell material from the peeling line, which is often contaminated with pellicle tissue, was collected, placed in plastic bags, and stored at 4°C. This mixed material was used for greenhouse studies. For laboratory studies, the shell material was separated by removing as much attached pellicle tissue as possible. Pure pellicle

Corresponding author: J. J. Hao, E-mail: jjhao@msu.edu

Accepted for publication 30 September 2011.

http://dx.doi.org/10.1094/PDIS-03-11-0169