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ScienceDirect
South African Journal of Botany 75 (2009) 375–379
SOUTH AFRICAN JOURNAL OF BOTANY
www.elsevier.com/locate/sajb

Short communication

The activity of extracts of seven common invasive plant species on fungal phytopathogens

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Received 30 May 2008; received in revised form 5 February 2009; accepted 5 February 2009

Abstract

Acetone extracts from different parts of seven common invasive plant species occurring in South Africa were studied as potential sources of antifungal agents for selected phytopathogenic fungi (*Penicillium janthinellum*, *Penicillium expansum*, *Aspergillus niger*, *Aspergillus parasiticus*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Trichoderma harzianum*, *Phytophthora nicotiana*, *Pythium ultimum* and *Rhizoctonia solani*. The invasive plant species were *Cestrum laevigatum* (flowers and leaves), *Nicotiana glauca* (flowers, leaves and seeds), *Solanum mauritianum* (fruits and leaves), *Lantana camara* (fruits, flowers and leaves), *Datura stramonium* (seeds), *Ricinus communis* (leaves) and *Campuloclinium macrocephalum* (leaves and flowers). All extracts exhibited moderate to good activities on all tested fungi with minimum inhibitory concentrations (MICs) ranging from 0.08 mg/ml to 2.5 mg/ml. In all cases leaf extracts were more active than seed or flower extracts. The growth of *A. niger*, *P. expansum* and *R. solani* was the most sensitive to all the extracts tested, with average MICs of 0.81, 0.83 and 0.84 mg/ml respectively. *C. macrocephalum* leaf extract was the most active against *C. gloeosporioides* with an MIC of 0.05 mg/ml. If extracts of these species do not have deleterious effects against plants infected by the fungi or the environment, it may be useful to protect organically grown crops.

Keywords: Antifungal activity; Fungal phytopathogens; Invasive plant species; Organic production

1. Introduction

More than 800 million people in developing countries do not have adequate food supplies and at least 10% of food is lost due to plant diseases (Strange and Scott, 2005). Plant diseases are caused by pathogens such as fungi, bacteria, nematodes and viruses. Compared to other plant parasites, fungi cause the greatest impact with regard to diseases and crop production losses. This includes considerable foliage and post harvest losses of fruits and vegetables which are brought about by decay due to fungal plant pathogens.

Common fungal diseases include powdery mildew, rust, leaf spot, blight, root and crown rots, damping-off, smut, anthracnose, and vascular wilts. Some notorious plant pathogenic fungi include *Pythium*, *Phytophthora*, *Fusarium* and *Rhizoctonia* spp, which cause root and crown rot, and seedling damping-off in many vegetables and ornamental plants. Apart from causing diseases in plants, many species of *Fusarium*, *Aspergillus*, *Penicillium* and *Alternaria* are also sources of important mycotoxins of concern in animal and human health (Robert and Richard, 1992; Eaton and Gallagher, 1994; Smith, 1997; Placinta et al., 1999). For example, aflatoxins produced by *Aspergillus flavus* and *A. parasiticus* may cause liver cancer.

The most important method of protecting plants against fungal attack is the use of fungicides. However, many fungicidal agents in the market are toxic and have undesirable effects on other organisms in the environment. Furthermore, halogenated hydrocarbons such as methyl bromide, widely used to control soil-borne pathogens, have ozone-depleting potential (Abritton and Watson, 1992). Some synthetic fungicides are non-biodegradable, and hence can accumulate in soil, plants and water, and consequently effect humans through the food chain.

The development of resistance of pathogenic fungi towards synthetic fungicides is of great concern. There is, therefore, a

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doi:10.1016/j.sajb.2009.02.003

motivation to find safe, efficacious and environmentally friendly fungicides.

Plants have, and continue to be, sources of antifungal agents (Hostettmann et al., 2000). Random screening of the leaves of 350 tree species by the Phytomedicine Programme of the University of Pretoria for antimicrobial activities revealed that large percent of plant species investigated have antifungal activities with MICs values lower than 0.1 mg/ml against animal fungal pathogens (unpublished data).

Many plant species therefore contain antifungal compounds. To develop commercial products, a large quantity of the species has to be cultivated, raising an additional level of complication. If invasive and weedy species contain good antifungal activity they may be a useful source of antifungal compounds or extracts because large quantities of material are available.

Rejmanek and Richardson (1996) stated that there is general pessimism on the prospect of predicting which organisms are likely to become successful invaders. They investigated pine trees and concluded that three parameters can be used to predict invasive potential: small seed size, short juvenile period and a short interval between large seed crops. They speculated that these parameters may also be useful for other woody species. Vila and Weiner (2004) considered whether invasive plant species are better competitors than native plant species and concluded that this may be the case. There is another possibility that have not been addressed as far as we could ascertain. If fungal pathogens play an important role in the growth or establishment of plant species, invasive species may have better resistance against plant pathogens. We have found that a weedy species *Melianthus comosus* has excellent antifungal activity (Eloff et al., 2006).

In this work we investigate weeds and invasive plant species as a potential source of antifungal agents. Most invasive species have been introduced into an environment in which they did not evolve and thus frequently have no natural enemies to limit their reproduction and spread. They compete with agricultural crops for water, light and nutrients, causing enormous losses in food production. Some are also toxic to livestock. In South Africa, more than 120 species have been declared unwanted invasive plants (Bromilow, 2001). An estimated 8%, or 10 million hectares, of South Africa has been invaded by different alien species (Versveld et al., 1998). By using weeds and invasive plant species as raw material for plant-derived fungicides, we may protect indigenous South African plants, and at the same time may create economic uses for these unwanted species.

2. Material and methods

2.1. Plant collection

Leaves, flowers and fruits were collected, in summer, from labelled plant species in the University of Pretoria, Faculty of Veterinary Science toxic plant garden in the Onderstepoort Campus, South Africa. Voucher specimens were verified and easily identified by Prof. C.J. Botha because they are so well known before being deposited in the Herbarium of the Department of Paraclinical Sciences, Faculty of Veterinary Science, Onderstepoort, University of Pretoria. Samples collected were from the following seven species: *Cestrum laevigatum* Schtdl [inkberry] (flowers and leaves), *Nicotiana glauca* Graham [wild tobacco tree] (flowers, leaves and seeds), *Solanum mauritianum* Scop. [bug weed] (fruits and leaves) (Solanaceae), *Lantana camara* L. [tick berry] (fruits, flowers and leaves) (Verbenaceae), *Datura stramonium* L. [thorn apple] (seeds) (Solanaceae) and *Ricinus communis* L. [castor-oil plant] (leaves) (Euphorbiaceae). *Campuloclinium macrocephalum* (Less.) DC. [pom pom weed] (leaves and flowers) (Asteraceae) were collected along the roadside in Pretoria. Voucher specimen numbers are PM010 to PM016 respectively.

2.2. Plant storage

Leaves, flowers and fruits were separated from the stems and dried at room temperature. Most scientists have tended to use dried material because there are fewer problems associated with the large-scale extraction of dried rather than fresh plant material (Eloff, 1998a). The dried plants were milled to a fine powder in a Macsalab mill (Model 200 LAB), Eriez®, Bramley and stored at room temperature in closed containers in the dark until used.

2.3. Extraction procedure

Plant samples from each species were individually extracted by weighing 1.0 g of finely ground plant material and extracting with 10 ml of acetone (technical grade — Merck) in polyester centrifuge tubes. Acetone was elected based on its ability to extract compounds with a wide range of polarities (Eloff, 1998b) Tubes were vigorously shaken for 15 min on a Labotec model 20.2 shaking machine at a high speed. After centrifuging at 3500 rpm for 10 min the supernatant was decanted into pre-weighed labelled containers. The process was repeated two times on the marc to exhaustively extract the plant material and extracts were combined. The solvent was removed under a stream of air in a fume cupboard at room temperature. To quantify the subsequent processes (Eloff, 2004) extract were made up to 10 mg/ml in acetone.

2.4. Phytochemical analysis

Chemical constituents of the extracts were analysed by thin layer chromatography (TLC) using aluminium-backed TLC plates (Merck, silica gel 60 F_{254}). The TLC plates were developed under saturated conditions with one of the three eluent systems developed in our laboratory i.e. ethyl acetate/methanol/water (40:5.4:5): [EMW] (polar/neutral); chloroform/ethyl acetate/formic acid (5:4:1): [CEF] (intermediately polar/acidic); benzene/ethanol/ammonium hydroxide (90:10:1): [BEA] (non-polar/basic) (Kotze and Eloff, 2002).

To detect the separated compounds, vanillin-sulphuric acid (0.1 g vanillin (Sigma®): 28 ml methanol: 1 ml sulphuric acid) was sprayed on the chromatograms and heated at 110 °C to optimal colour development (Stahl, 1969).