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The effect of plant essential oils, *Solanum chilense* and *Aster sedifolius* raw saponins, plant-derived colourants and *Brassica carinata* glucosinolate-containing tissues, for the control of seedborne fungi *Alternaria dauci*, *Alternaria radicina*, *Colletotrichum lindemuthianum* and *Ascochyta rabiei*, was evaluated. *In vitro* assays indicated that oregano essential oil, bark chestnut-derived colourant and *B. carinata* meals, showed a large spectrum of activity against all pathogens. *S. chilense* raw saponins were effective against *A. dauci* and *A. radicina*, while those from *A. sedifolius* also inhibited significantly *C. lindemuthianum*. Screening showed the antifungal potential of natural compounds.

Keywords: dyeing plants; essential oils; glucosinolates; saponins; smart agriculture

Introduction

Seedborne pathogens cause diseases where the seeds are the main source of inoculum (Nome et al. 2002). The control of pathogens associated with the seeds is very problematic, because the infections departing from coat and embryo are difficult to control using only agronomic practices. Therefore, prevention of Seedborne diseases is based on the use of certified plant material free from pathogens and/or using resistant cultivars. However, because plant resistance against these pathogens is very erratic, the control of these microbes was currently entrusted to chemical fungicides. Nevertheless, the preference of the consumers for safe and organic productions has stimulated the research of sustainable alternative pesticides (Saba and Messina 2003). For this reason, plant-derived compounds with antifungal activity, such as essential oils (Isman 2000), saponins (Barile et al. 2006) and glucosinolates (Brown and Morra 1997) are promising tools to totally replace or integrate chemicals for disease management. Recently, plants used for dye extraction have been shown to possess antimicrobial activity own to the presence of a large amount of tannins, anthraquinones or naphthoquinones (Singh et al. 2005; Chengaiah et al. 2010). Seed application of plant-derived molecules may be considerate an integrated pest management strategy able to improve pathogen control and to reduce unsustainable use of traditional fungicides (Ntalli and Menkissoglu-Spiroudi 2011).

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This research was focused on environmentally compatible control of four Seedborne pathogens: *Alternaria dauci* (J.G. Kühn) J.W. Groves & Skolko, *Alternaria radicina* Meier, Drechsler & E.D. Eddy, *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib. and *Ascochyta rabiei* (Pass.) Labr. *A. dauci* and *A. radicina* are related pathogens causing severe blights on leaves, petioles and stems of carrot (*Daucus carota* Blotter). *A. radicina* can cause a further distinctive black rot symptom, consisting in damping-off and rotting of roots, crowns and seedlings of carrots that become unmarketable. *C. lindemuthianum* and *A. rabiei* are the causal agents of anthracnose of common bean (*Phaseolus vulgaris* L.) and leaf blight of chickpea (*Cicer arietinum* L.), respectively. The research of alternative to chemicals for the control of these diseases already proposed the use of seed treatment with physical methods, resistance inducers and antagonistic microorganisms (Tinivella et al. 2009; Koch et al. 2010). Aim of this study was to show a further screening of other phytochemicals, such as essential oils, natural dyeing, raw saponins and *Brassica carinata* L. meals containing glucosinolates for their *in vitro* antifungal activity against the described Seedborne pathogens.

Results and discussion

This study provided new data about *in vitro* inhibition activity of some plant-derived substances against Seedborne pathogens *A. radicina*, *A. dauci*, *A. rabiei* and *C. linde-muthianum*. *In vitro* assays indicate that plant essential oils showed variable effects on mycelial growth of the tested fungi. Oregano oil showed the largest spectrum of activity, because inhibited all tested fungi. On the contrary, rosemary oil does not affect any pathogen used in this work. *A. radicina* was more sensible to essential oils than *A. dauci*. A number of essential oils, including hyssop, oregano, basil, caraway, sage, marjoram and balm, were able to suppress black rot agent, whereas *A. dauci* was inhibited only by oregano and thyme oil (Table 1). About *C. lindemuthianum* and *A. rabiei*, results indicate that hyssop and oregano-derived essential oils inhibited both pathogens. Moreover, oils distilled from basil, caraway, sage and thyme blocked mycelial growth of *C. lindemuthianum*, while essential oils from lemon balm and verbena blocked *A. rabiei* growth. Essential oils generally were reported as very efficacious in order to reduce *in vitro* development of several fungi belonging to genus *Alternaria* (Tian et al. 2011), *Colletotrichum* (Chang et al. 2008; Arrebola et al. 2010; Maqbool et al. 2011) and *Ascochyta* (Zerroug et al. 2011). In agreement with our results, oregano essential oil revealed, by *in vitro* assays, a broad-spectrum antifungal activity vs. a plethora of fungi (Portillo-Ruiz et al. 2005).

Among over 10 dyeing plants extracts assayed in this study, *Castanea sativa* L. bark extract partially inhibited *in vitro* growth of all the four pathogens tested in this study, probably due to the high content of tannins. *Rubia tinctorum* L. inhibited completely the radial growth of *A. dauci* and *A. rabiei* (Table 1) and these results are in accordance with the literature data inherent the antimicrobial activity of root extract of this plant (Manojlovic et al. 2005; Kalyoncu et al. 2006). A similar case was showed by the extracts from *in vitro* grown shoots of *Quillaja saponaria* that proved effectiveness against *Botrytis cinerea* (Ribera et al. 2008). The absence of inhibition of the other tested extracts can be attributed to the low dose adopted as reported from Prusty et al. (2010). So the natural dyes require more in-depth investigation into the effect of dose and dye structure on antimicrobial property.

Raw saponin-containing tissues showed encouraging specie-specific antifungal properties. In plate assays, raw saponins contained in tissue-meals of *Solanum chilense*

Table 1. Effect of different plant-derived products (essential oils and dyeing extracts) on mycelial growth of *A. dauci*, *A. radicina*, *A. rabiei* and *C. lindemuthianum*. Signs – and + were used to indicate inhibition rank.

Plant-derived products	Concentration used	Mycelial growth inhibition[a]			
		A. dauci	*A. radicina*	*A. rabiei*	*C. lindemuthianum*
Basil oil	1 vol.%	–	+++	–	+++
Caraway oil	1 vol.%.	–	+++	–	+++
Hyssop oil	1 vol.%.	–	+++	+++	+++
Majoram oil	1 vol.%.	–	+++	–	–
Melissa oil	1 vol.%.	–	+++	+++	–
Oregano oil	1 vol.%.	+++	+++	+++	+++
Rosmary oil	1 vol.%.	–	–	–	–
Sage oil	1 vol.%.	–	+++	–	+++
Thyme oil	1 vol.%.	+++	–	–	+++
Verbena oil	1 vol.%.	–	–	+++	–
Bark chestnut dye	1 vol.%.	+	+	+	+
Clover dye	1 vol.%.	–	–	–	–
Chrysanthemum dye	1 vol.%.	–	–	–	–
Heather dye	1 vol.%.	–	–	–	–
Wild fennel dye	1 vol.%.	–	–	–	–
Inula dye	1 vol.%.	–	–	–	–
Pomegranate dye	1 vol.%.	–	–	–	–
Mint dye	1 vol.%.	–	–	–	–
Madder dye	1 vol.%.	–	–	–	–
Elder dye	1 vol.%.	–	–	–	–

Note: [a]Inhibition rank: 100% (+++), >50% (++), <50% (+) significant growth reduction compared with control. Not inhibited (–).

reduced significantly radial growth rate of *A. dauci* and *A. radicina*, while those derived from *Aster sedifolius* inhibited significantly *A. radicina*, *C. lindemuthianum* and *A. rabiei*. Saponins are a class of natural compounds isolated from plants that can exhibit antimicrobial properties independently from their purity degree, as in the case of saponin-rich fraction of *Camellia oleifera* cake (Hu et al. 2012). In our work, finally, *B. carinata* glucosinolate-derived isothiocyanates were also significantly effective in *in vitro* control of all the four assayed pathogens (Figure 1) confirming the high antimicrobial activity showed by these molecules (Tierens et al. 2001).

Findings of the present work suggest that plant-derived products with antifungal properties can have a large potential for practical application in disease management, since they are composed of natural molecules compatible with environmental sustainable cropping systems. Results of the present screening gave encouraging indications regarding the potential utilisation of the assayed natural products in the protection against pathogens transmissible through the seeds. Here, we observed that the broad-spectrum efficacy of *B. carinata* seed meals, as-well-as that of some essential oils is in accordance with a number of literature data (Sridhar et al. 2003; Galletti et al. 2008; Lalitha et al. 2011). Similarly, raw saponins exhibited a great potential to implement the control of Seedborne diseases (Stuardo and San Martín 2008). All these substances with antifungal properties can be suitable biofungicides for direct seed applications, by coating or tanning, to improve plant protection against pathogens (Wulff et al. 2011). As matter of the fact, essential oils are reported to be promising for the protection of the seeds against

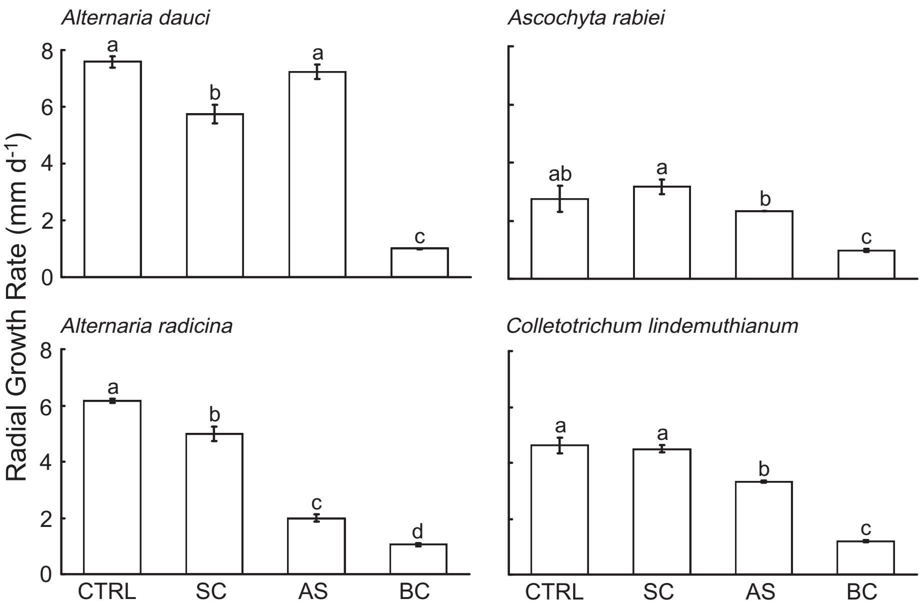

Figure 1. Effect of *S. chilense* (SC), *A. sedifolius* (AS) and *B. carinata* (BC) tissue meals on radial growth rate of *A. dauci*, *A. radicina*, *A. rabiei* and *C. lindemuthianum*, compared to the control (CTRL). Different letters indicate differences statistically significant. Data averages (± 1 SE) of three replicates.

infestation by specialised fungi, because they have penetration action (Lalitha et al. 2011). It is the case, for example, of the various oil seed treatments that have successfully controlled the cumin rot root disease caused by *Fusarium* spp. (Hashem et al. 2010). Among other non-chemical seed treatments, thyme oil has been proposed to reduce Seedborne diseases caused by *C. lindemuthianum* on bean and *Ascochyta* spp. on pea (Tinivella et al. 2009), *A. dauci* and *A. radicina* on carrot (Koch et al. 2010), *Phoma valerianellae* on lamb's lettuce (Schmitt et al. 2009) and *Alternaria brassicola* on cabbage (Amein et al. 2011). Likewise, seed treatments with *Brassica* spp. meals have also reported to control *Rhizoctonia solani* in cabbage (Chung et al. 2002). Previously, *B. carinata* seed meals have been proposed as geodisinfectant in a greenhouse lettuce system (Pane et al. 2012). While this product containing glucosinolates was also proposed, together some essential oils as foliar spray to improve processing tomato plant conditions in open field (Pane et al. 2013). The current research showed oregano essential oil, *B. carinata* seed meal and bark chestnut dye the most promising non-chemical fungicides that can be proposed against the examined Seedborne pathogens. However, as a perspective of this research, the practical applications of these natural products in disease control will have to be tested to evaluate the actual potential of the treatments.

Experimental

Fungal pathogens

A. radicina (strain ER1462), *A. dauci* (strain ER1530), *A. rabiei* (strain 1405AR) and *C. lindemuthianum* (strain ISCI9) were originally isolated from their specific hosts. The fungal isolates were stored at 4 °C in tubes containing potato–dextrose–agar (PDA, Difco).

In vitro plate assays

To evaluate the ability of plant-derived substances to inhibit growth of fungal plant pathogens *A. radicina*, *A. dauci*, *A. rabiei* and *C. lindemuthianum*, *in vitro* plate tests were performed. 10 essential oils of aromatic plants and 10 water extracts of dyeing plants were separately tested by submerging a plug (5 mm in diameter) removed from the edge of the growing mycelia in water suspensions. Essential oil emulsions were prepared at 1% concentration (vol./vol.). While to produce the dyeing plant extract solution 5 g of dried material in 800 ml of water for 60 min at 70 °C at neutral pH, and concentrated by rotavapor, until 1:4 in volume. After overnight incubation at 25 °C, the plug was transferred in the centre of a PDA Petri plates (diameter 90 mm). Assay with glucosinolates was carried out including, in inverted hermetically closed PDA platesand under the plug, a capsule containing 100 mg of *B. carinata* seed meals wetted with 500 μl of sterile distilled water. Raw saponins, instead, were tested amending PDA with *A. sedifolius* or *S. chilense* tissue meals at a rate of 0.5 $g\,ml^{-1}$. In all cases, control plates were prepared without the addition of the tested substances. For each treatment, plates were inoculated in triplicate and incubated at 25 °C. The diameter of the mycelia was measured daily until fungi reached the edge of control plates; data were expressed as growth rate ($mm\,d^{-1}$). Instead, in the case of essential oils and dyeing plant extracts, only *in vitro* inhibition levels were indicated as 100% (+++), >50% (++), <50% (+) significant growth reduction compared to control, and not inhibited (−).

Statistical analysis

Data from the plate inhibition assays of the fungi with raw saponins and glucosinolates were processed with the analysis of variance (ANOVA). When the ANOVA was significant ($P \leqslant 0.05$), means were separated with Duncan's test.

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