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Original article

Antifungal activity of Moroccan plants against citrus fruit pathogens

N. Ameziane, H. Boubaker*, H. Boudyach, F. Msanda, A. Jilal, A. Ait Benaoumar

Laboratoire de Biotechnologie et de Valorisation des Ressources Naturelles, Faculté des Sciences, Université Ibn Zohr, Agadir, Morocco

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Abstract – The aim of this study was to find an alternative to the chemical fungicides currently used in the control of postharvest citrus fruit diseases. Here we screened twenty-one medicinal and aromatic plants used in southern Moroccan traditional medicine for their activity against *Penicillium digitatum, Penicillium italicum* and *Geotrichum candidum*. The antifungal efficacy of powders, essential oils and solvent extracts of these plants was tested in vitro by using the agar plates method. Our results show that among the 21 plants tested, the powders of *Thymus leptobotrys*, *Cistus villosus* and *Peganum harmala* plants totally inhibited the growth of all three pathogens. Furthermore, the powder of the *Eucalyptus globulus* plant totally inhibited the mycelial growth of both *G. candidum* and *P. digitatum*, whereas the powders of *Juglans regia* and *Myrtus communis* plants completely inhibited the mycelial growth of *G. candidum*, and the powder of the *Arenaria rubra* plant totally inhibited the growth of the *P. digitatum* fungus. The essential oils, as well as the methanolic and chloroformic extracts of plants with the highest antifungal activity, were tested against the mycelial growth of the three pathogens. The results indicate that only the essential oils and the chloroformic extract of the *T. leptobotrys* plant totally inhibited the three pathogens. These results demonstrate that plant-derived products have a high potential to control fungal diseases of citrus fruits. Such biopesticides therefore represent a sustainable alternative to the use of chemical pesticides.

medicinal and aromatic plants / Citrus fruit / antifungal activity / *Penicillium digitatum* / *Penicillium italicum* / *Geotrichum candidum* / *biopesticide*

1. INTRODUCTION

During storage, fruits and vegetables are often subject to varying levels of microbial decay, mainly due to pathogenic fungi which usually infect the host through wounds sustained during harvest, handling and/or processing (El Ghaouth et al., 2002). In the case of citrus fruits, losses are mainly caused by *Penicillium digitatum*, *Penicillium italicum* and *Geotrichum candidum* (Eckert and Ogawa, 1985; Laville, 1971).

Currently, control of postharvest citrus diseases relies mainly on the use of synthetic fungicides, principally thiabendazole and imazalil, sprayed on fruit during waxing operations at packing facilities (Brown and Miller, 1999). The emergence of strains of pathogens resistant to these fungicides (Spotts and Cervanntes, 1986; Suhr and Nielsen, 2003; Eckert, 1987), as well as the growing concern for human safety and the protection of the environment (Suhr and Nielsen, 2003; Wilson et al., 1997), compel us to search for alternatives to the use of synthetic fungicides in the control of postharvest diseases. Biological control using naturally-occurring substances has been recently explored for managing postharvest decay of fruits. Because of their non-phytotoxicity and systemicity (Fawcett and Spencer, 1970), as well as biodegradability, plant-derived products can be potent and valuable reagents in pest management (Mishra and Dubey, 1990; Shukla and Tripathi, 1987; Tripathi and Dubey, 2004; Xuan et al., 2006; Javaid et al., 2006). For example, essential oils and other medicinal and aromatic plant extracts have been suggested as possible means of controlling citrus postharvest rots (Wilson et al., 1997; Mari and Guizzardi, 1998; Chebli et al., 2003).

In this study, twenty-one medicinal and aromatic plants used in southern Moroccan traditional medicine were screened for their antifungal activity against the principal postharvest fungal pathogens of citrus fruits, i.e. *Penicillium digitatum*, *Penicillium italicum* and *Geotrichum candidum*.

2. MATERIALS AND METHODS

2.1. Plant material

Twenty-one fresh plant samples were collected from the Souss valley (Agadir, Morocco) during March and April of 2003 (Tab. I). Voucher specimens were identified, and were deposited in the herbarium of the laboratory of plant ecology, Faculty of Sciences, Ibn Zohr University, Agadir, Morocco. Different parts of the plants were used (stem, leaves, flowers and seeds). Tests were carried out with powders and essential oils, as well as methanolic and chloroformic extracts.

2.2. Fungal cultures

The fungi used in this study, *Penicillium digitatum*, *Penicillium italicum* and *Geotrichum candidum*, were isolated from decayed citrus fruits. The fungi were maintained on Potato

* Corresponding author: hassanboubaker@yahoo.fr

Table I. Antifungal activity of twenty-one plant powders against three postharvest pathogens of citrus fruit.

Plant species	Part used	*P. digitatum*	*P. italicum*	*G. candidum*
		Growth inhibition[a](%)		
Arenaria rubra L.	Whole	100 ± 0.0	65 ± 0.2	79 ± 0.4
Artemesia reptans C.Sm.	Leaves + stem	47 ± 0.5	37 ± 0.3	26 ± 0.7
Cistus villosus L.	Leaves + stem	100 ± 0.0	100 ± 0.0	100 ± 0.0
Echium horridum Batt.	Leaves + stem	79 ± 1.1	52 ± 0.5	30 ± 0.3
Eucalyptus globulus Labill.	Leaves + stem	100 ± 0.0	67 ± 0.2	100 ± 0.0
Juglans regia L.	Bark	79 ± 0.2	72 ± 0.1	100 ± 0.0
Lavandula dentata L.	Leaves + stem + flowers	34 ± 0.3	26 ± 0.3	0.5 ± 0.7
Lavandula multifida L.	Leaves + stem + flowers	17 ± 0.3	–3 ± 0.1	–16 ± 0.6
Lippia citriodora H.B.et K.	Leaves	29 ± 0.1	27 ± 0.1	–2 ± 0.3
Marrubium vulgare L.	Leaves + stem + flowers	47 ± 0.1	42 ± 0.3	18 ± 0.5
Mentha pulegium L.	Leaves + stem	59 ± 0.0	21 ± 0.4	–15 ± 0.7
Mentha rotundifolia L.	Leaves + stem	59 ± 0.9	22 ± 0.7	–12 ± 0.2
Myrtus communis L.	Leaves + stem	80 ± 0.4	55 ± 0.4	100 ± 0.0
Nigella sativa L.	Seeds	81 ± 0.1	27 ± 0.1	74 ± 0.0
Ononis natrix L.	Leaves + stem	38 ± 0.1	41 ± 0.4	22 ± 0.1
Peganum harmala L.	Seeds	100 ± 0.0	100 ± 0.0	100 ± 0.0
Rosmarinus officinalis L.	Leaves + stem	56 ± 0.3	22 ± 0.4	8 ± 0.2
Ruta montana Mill.	Leaves + stem	28 ± 0.6	31 ± 0.5	34 ± 0.2
Schinus molle L.	Leaves + stem + fruit	47 ± 0.3	24 ± 0.3	44 ± 0.3
Thymus leptobotrys Murb.	Leaves + stem	100 ± 0.0	100 ± 0.0	100 ± 0.0
Thymus pallidus Coss.	Leaves + stem	38 ± 0.1	58 ± 0.6	45 ± 0.3

[a] Values are means of three replicates ± standard deviation. The negative values indicate a profungal activity against some fungi species.

Dextrose Agar plates at 5 °C, with periodic transfers through citrus fruit to maintain the aggressiveness of the pathogen. A one-week-old culture of each fungus was used to inoculate the agar plates.

2.3. Preparation of plant extracts and testing for antifungal activity

The plant samples were first air-dried and ground, and 10 grams of powders of each sample were added to 100 mL of Potato Dextrose Agar medium. The resulting suspensions were stirred for 10 min, autoclaved for 15 min and subsequently filtered through four layers of sterile cheesecloth before being dispensed into 9-cm diameter Petri dishes.

The essential oils of selected plants were obtained by 3 h of steam distillation of 50 g of fresh samples using a steam distillation apparatus. Due to concern that some of their components could be sensitive to high temperature, these oils were sterilized using two methods: autoclaving and ultra-filtration through 0.2-μm pore diameter Millipore Swinex filters, before being added to Potato Dextrose Agar medium.

The methanolic and chloroformic extracts were obtained from ground dry samples. Fifty grams of each plant were methanol- and chloroform-extracted (375 mL each) for 24 h using a Soxhlet apparatus. The extracts were filtered and the solvent completely removed using a rotary evaporator.

The agar plates method was used to screen for antifungal activity. As described above, powders were tested at a concentration of 10% (w/v). Methanolic and chloroformic extracts were prepared as described above, added to the Potato Dextrose Agar medium and then autoclaved for 15 min. Plates with Potato Dextrose Agar with and without solvents were used as control. Agar plates were inoculated with one of the three fungal pathogens (*P. digitatum*, *P. italicum* or *G. candidum*), using a 5-mm diameter agar disk taken from one-week-old cultures, mycelial surface facing down. The agar plates were then incubated at 25 °C for six days. Radial growth was determined by measuring colony size along two perpendicular axes. The antifungal activity was expressed in terms of percentage of mycelial growth inhibition and calculated according to the following formula:

$$\% \text{ mycelial growth inhibition} = \frac{\text{Control diameter} - \text{Plant extract diameter}}{\text{Control diameter}} \times 100$$

3. RESULTS AND DISCUSSION

3.1. Effect of powders on mycelial growth

The screening of the 21 plant powders for antifungal activity against the three fungal species *Penicillium digitatum*, *Penicillium italicum* and *Geotrichum candidum* uncovered significant antifungal activity of *Thymus leptobotrys*, *Cistus villosus* and *Peganum harmala* (Tab. I). The powders of these plants completely inhibited the mycelial growth of the three pathogens. Some other plant powders from *Juglans regia*, *Eucalyptus globulus*, *Myrtus communis* and *Arenaria rubra* inhibited mycelial growth by more than 50%. The remainder of

the plant powders tested exhibited much lower antifungal activities, and some even enhanced the mycelial growth of *G. candidum.* Of the three fungal species tested, *Geotrichum candidum* was clearly the most susceptible to inhibition, with six plant powders completely inhibiting its growth, whereas only five and three powders were efficient against *P. digitatum* and *P. italicum*, respectively (Tab. I). In a previous study, Bautista-Banos et al. (2003) found that the powder of the *Pithecellobium dulce* plant at 20 g/L inhibited not more than 58% of the mycelial growth of *P. digitatum*.

3.2. Effect of essential oils

The results shown in Figure 1 indicate that of the plant species tested, only the essential oil of *T. leptobotrys* at 1.2 g/L had the highest fungistatic effect (100%), compared with the essential oils of *E. globulus*, *C. villosus* and *P. harmala*, where the growth inhibition was less than 40% on the three fungal pathogens. The essential oil of *T. leptobotrys* inhibited all three pathogens to the same degree as the corresponding powder. Interestingly, the antifungal activity of *T. leptobotrys* essential oil was not heat-labile, as it was unaffected by autoclaving.

In a previous study, the antifungal active ingredient of essential oil derived from *T. capitatus*, a species closely related to *T. leptobotrys*, was characterized as carvacrol (Arras and Picci, 1986). Unlu et al. (2003) reported that thymol, carvacrol and borneol were the main components of *Thymus pectinatus* plant essential oil. An antimicrobial activity test carried out with fractions of the essential oil showed that the activity was mainly observed in those fractions containing thymol and carvacrol. Soylu et al. (2005) found that essential oils from *Origanum syriacum* and *Foeniculum vulgare* plants possessed strong antimicrobial activity against conidial germination and germ tube elongation of *P. digitatum*. Other studies demonstrated the antifungal effect of plant essential oils and encouraged the use of these natural products as an alternative to chemical fungicides for citrus fruit treatments (Aligiannis et al., 2001; Cosentino et al., 1999; Elgayyar et al., 2001; Mishra and Dubey, 1994; Shin and Kim, 2005).

3.3. Effects of chloroformic and methanolic extracts

The effects of chloroformic and methanolic extracts on the mycelial growth of the three pathogens are listed in Table II. As in the case of powder and essential oils, *T. leptobotrys* chloroformic and methanolic extracts exhibited a significant fungistatic activity, 100% inhibition of fungal growth by the chloroformic extract at a concentration of 0.3% (w/v), and a 71–76% inhibition by the methanolic extract at a concentration of 1.5% (w/v). Similarly, chloroformic and methanolic extracts of *P. harmala* tested at a concentration of 1% and 2% (w/v), respectively, exhibited a pronounced activity against the three pathogens. Indeed, these extracts completely inhibited the mycelial growth of the *P. digitatum* and *P. italicum* pathogens, whereas the chloroformic extract allowed some residual *G. candidum* mycelial growth (88% inhibition). In contrast, *C. villosus* and *E. globulus* chloroformic

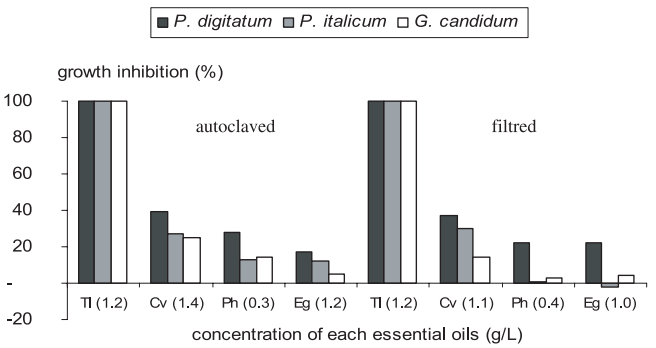

Figure 1. Effect of essential oils from *Thymus leptobotrys* (Tl), *Cistus villosus* (Cv), *Peganum harmala* (Ph) and *Eucalayptus globulus* (Eg) on mycelial growth of three pathogens using two methods of sterilization.

and methanolic extracts showed relatively lower inhibitory effects in comparison with their corresponding powders. The methanolic extracts allowed a 73% to 82% inhibition of *P. digitatum* and *G. candidum* mycelial growth, while the chloroformic extracts exhibited an inhibitory effect lower than 35%.

Also of interest, we found that the methanolic extract of *P. harmala* was more effective than the chloroformic one, and at a low concentration (1% w/v). Shahverdi et al. (2005) have reported that the seeds of *P. harmala* were rich in alkaloid substances with antimicrobial activity. Therefore, the antimicrobial activity of these compounds against postharvest decay microorganisms of citrus fruits needs to be investigated.

The *E. globulus* and *C. villosus* methanolic extracts exhibited a potent antifungal activity against *P. digitatum* and *G. candidum*: mycelial growth was inhibited above 70%. In contrast, the chloroformic extracts were less effective. The fungal growth inhibition was less than 35%. Bouamama et al. (1999) reported the antibacterial and antifungal activities of leaf extracts obtained from two Moroccan *Cistus* species plants (*C. villosus* and *C. monspeliensis*) against five strains of bacteria and fungi. According to these authors, *C. villosus* extracts exhibited more activity than *C. monspeliensis* extracts.

In our study the methanolic extracts of *E. globulus* and *C. villosus* were more active against *P. digitatum* and *G. candidum* than the chloroformic extracts. Thus, it can be suggested that methanol is a better solvent for extraction of antifungal compounds from these two plant species. In the case of *P. italicum*, more studies are needed to explain the variation observed and to determine the nature of the active ingredients of these plants.

4. CONCLUSION

In the present study, we demonstrate that several Moroccan medicinal and aromatic plants possess potent antifungal activities with potential practical applications in the treatment of postharvest fungal diseases of citrus fruits. Among the 21 plants tested, *T. leptobotrys*, *C. villosus*, *E. globulus* and *P. harmala* showed high antifungal activities against the tested

Table II. Antifungal activity of chloroformic and methanolic extracts of four medicinal plants against three postharvest pathogens.

Plants	Type of extract	Concentration	*P. digitatum*	*P. italicum*	*G. candidum*
		(% w/v)	Growth inhibition[a](%)		
Thymus leptobotrys	Chloroformic	0.1	68 ± 0.1	70 ± 0.1	26 ± 0.0
		0.3	100 ± 0.0	100 ± 0.0	100 ± 0.0
	Methanolic	0.1	8 ± 0.0	9 ± 0.1	–5 ± 0.2
		1.5	73 ± 0.1	77 ± 0.1	71 ± 0.0
Eucalyptus globulus	Chloroformic	0.1	20 ± 0.1	6 ± 0.1	6 ± 0.1
		2.0	23 ± 0.2	16 ± 0.1	31 ± 0.1
	Methanolic	0.1	18 ± 0.2	1 ± 0.1	18 ± 0.0
		0.7	73 ± 0.1	35 ± 0.1	82 ± 0.1
Cistus villosus	Chloroformic	0.1	12 ± 0.2	3 ± 0.1	12 ± 0.2
		0.7	27 ± 0.0	27 ± 0.1	21 ± 0.0
	Methanolic	0.1	15 ± 0.1	2 ± 0.1	12 ± 0.1
		0.8	77 ± 0.0	17 ± 0.1	79 ± 0.0
Peganum harmala	Chloroformic	0.1	9 ± 0.1	6 ± 0.1	–1 ± 0.2
		2.0	100 ± 0.0	100 ± 0.0	88 ± 0.0
	Methanolic	0.1	29 ± 0.1	16 ± 0.1	11 ± 0.1
		1.0	100 ± 0.0	100 ± 0.0	100 ± 0.0

[a] All the values are means of three replicates ± standard deviation.

pathogens. This antifungal activity was found in the plant powders, as well as in essential oils and solvent extracts. Plant powders showed a stronger antimicrobial activity than plant fractions. Further phytochemical research is needed to identify the active principles responsible for the antifungal effects of each plant and to make this a practical option for farmers' use.

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