Control of postharvest diseases caused by Penicillium spp. with myrtle leaf phenolic extracts: in vitro and in vivo study on mandarin fruit during storage

Fadda Angela (Orcid ID: 0000-0002-1384-5956)

Control of postharvest diseases caused by *Penicillium* spp. with myrtle leaf phenolic extracts: *in vitro* and *in vivo* study on mandarin fruit during storage

Running title: antifungal activity of myrtle leaf extracts

Angela Fadda*[1], Giorgia Sarais[2], Carla Lai[2], Luana Sale[3], Maurizio Mulas[3,4]

[1] Institute of the Sciences of Food Production, National Research Council, Traversa La Crucca, 3 07100 Sassari, Italy

[2]Department of Life and Environmental Sciences, University of Cagliari, University Campus, I-09042 Monserrato (Cagliari), Italy

[3]Department of Agriculture, University of Sassari, Via E. De Nicola, 1, 07100 Sassari, Italy.

[4]Centre for Conservation and Evaluation of Plant Biodiversity, University of Sassari, Via De Nicola 9, 07100 Sassari, Italy.

Corresponding author: Angela Fadda

e-mail: angela.fadda@cnr.it; Institute of the Sciences of Food Production, National Research Council, Traversa La Crucca, 3 07100 Sassari, Italy

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Abstract

BACKGROUND: In postharvest handling of horticultural commodities, plant extracts with fungicidal activity are a valid alternative to synthetic fungicides. Fungicidal activity of myrtle leaf extracts from eight cultivars was studied *in vitro* against *Penicillium digitatum*, *Penicillium italicum* and *Penicillium expansum* and on artificially inoculated mandarins with green and blue moulds before storage for 12 d at 20 °C and 90% RH.

RESULTS: Hydroxybenzoic acids, hydrolysable tannins, and flavonols were identified by HPLC. Despite sharing the same phenolic profile, extracts of eight myrtle cultivars significantly differed for phenolics' concentrations. Hydrolysable tannins are the principal subclass representing nearly 44.9% of the total polyphenols, whereas myricitrin was the most abundant flavonol in all cultivars. Myrtle extracts strongly inhibited conidial germination of the pathogens tested, although the greatest efficacy was observed against *P. digitatum*. At the concentration of 20 g L^{-1}, all the extracts completely inhibited fungi growth; only 'Angela', 'Tonina' and 'Grazia' extracts were effective at lower concentrations (15 g L^{-1}). On inoculated fruit, myrtle extracts significantly controlled rot development. As preventive treatment 'Ilaria' and 'Maria Rita' extracts significantly reduced the rate of fruit with green mould decay lesions. When applied as a curative treatment, all the exacts decreased decay incidence. Against *P. italicum*, all the extracts applied as preventive treatment effectively controlled decay, while as curative treatment some of the extracts were not effective. All the extracts reduced the size of the infected areas.

CONCLUSION: The results propose myrtle extracts as a possible natural alternative to synthetic fungicides.

Keywords: myrtle, plant extracts, fungicidal activity, phenols, storage, *Penicillium*.

Introduction

Green and blue moulds caused by *P. digitatum*, *P. italicum* and *P. expansum* determine severe economic losses during storage, transport and marketing of fresh fruits. On a commercial scale,their management is based on pre- or postharvest application of thiabendazole, imazalil, pyrimethanil and fludioxonil.[1] However, the development of fungicide-resistant strains and the greater environmental and health awareness of consumers has determined a growing interest towards safe and eco-friendly control methods. Plant extracts are alternative methods effective for the management of several postharvest diseases.[2-8] Medicinal and aromatic plants can control, *in vitro* and *in vivo*, the development of many fungal infections. On *Citrus*, the use of plant extracts as stand-alone treatments or in combination with reduced doses of fungicides is a promising control measure used in sustainable agriculture for their low environmental toxicity.[1] Plant secondary metabolites such as flavonoids, tannins, alkaloids, terpenes, and saponins are the main responsible for the antifungal activity of plant extracts.[9] On navel oranges, the treatment with quercetin, scopoletin and scoparon delayed the onset of infection. The same treatment after 8 d of storage significantly reduced green mould incidence and severity.[10] Similarly, pomegranate peel extract inhibited conidial germination of *P. digitatum* and *P. italicum* and was effective in controlling, on lemons, rot development both in curative and in preventive treatments.[11] Extracts of *Sanguisorba minor* completely inhibited *in vitro* conidial germination of *Monilinia laxa*, *P.digitatum* and *Aspergillus niger*.[12] In *in vivo* trials *S. minor* completely inhibited brown rot in nectarines and apricots; while *Orobanche crenata* extracts reduced grey mould, brown rot and green mould on table grapes, apricots and oranges, respectively.[12] Ethanolic extracts of *Ficus hirta* displayed antifungal activity against *P. italicum* and *P. digitatum*. The flavonone pinocembroside, obtained from the fruit of *Ficus hirta*, significantly reduced the mycelial growth of *P. italicum* and inhibited in a dose-dependent manner the development of blue mould on 'Newhall' navel oranges.[13, 14] The mode of action of phenols is not clear yet but, according to the most recent studies, they can exert their effect on fungi cells or triggering the fruit defence mechanism.[1] Some studies suggested a membrane-targeted mechanism in the inhibition of fungi growth.[15, 16] On *P. italicum*, the treatment with phenolic extracts has changed the fungi cell ultrastructure and has modified the membrane fluidity causing the leakage of cytoplasmatic material and the

modification of cellular energy homeostasis. Polyphenols may stimulate the defence mechanism of the fruit. Pomegranate peel extracts, maybe trough a priming effect, determined an over-accumulation of ROS and activated the expression of genes related to plants defense mechanism.[17] Myrtle (*Myrtus communis* L.) is an aromatic plant typical of the Mediterranean area. In folk medicine, it was used as an anti-inflammatory and antiseptic agent, while, recently its antioxidant and antimicrobial properties have been studied.[18-21] Flavonoids, tannins, hydroxyl benzoic acids and ellagic acid are the responsible for the antioxidant, health-promoting and antimicrobial properties of myrtle extracts.[21-26]

On *P. italicum,* myrtle leaf extracts reduced *in vitro* the fungi growth by 50%. However, the myrtle antifungal activity against postharvest moulds was tested *in vitro* only, whereas no data is available, so far, on the effect of myrtle leaf extracts by *in vivo* trials.[2, 27]

Plant extracts should meet specific requirements for their use as antifungal agents in commercial formulations[1] and the standardisation is one of the most important. A problem often associated with the use of plant extracts is the high variability of the chemical composition within the same plant species. Previous studies on myrtle berries and leaves highlighted substantial differences in the metabolic profile of different genotypes.[22, 25, 26] This should be carefully considered when studying the biological activities of the plant extracts. Moreover, as highlighted by Chen et al. [28], the antifungal effect of plant extracts is mainly due to the action of some components or to the synergy of several components. In this contest, the study of the antifungal activities of plant extracts belonging to the same species, that share the same phenolic profile but are different in phenolic concentration, may add information on the role of phenolic compounds in antifungal activity.

Based on these considerations, this work aimed at evaluating the antifungal properties of leaf extracts of eight myrtle cultivars against *P. digitatum*, *P. italicum* and *P. expansum*. The extracts were tested *in vitro*, and *in vivo* on artificially inoculated fruit to evaluate extracts efficacy in preserving fruit from diseases development.

Materials and methods

Chemicals

Ethanol and acetonitrile were of HPLC grade and purchased from Sigma (Milano, Italy). Water was purified and filtered through a Milli-Q apparatus (Millipore, Milan, Italy). Orthophosphoric acid was purchased from Carlo Erba Reagents (ACS ISO, for analysis, 85%).

Standards of myricetin-3-*O*-galactoside, myricetin-3-*O*-rhamnoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-rhamnoside, quercetin-3-*O*-galactoside and vitexin were purchased from Extrasynthese (Lyon, France). Gallic acid was purchased from Sigma (Merck Life Science S.r.l., Milano, Italy).

Plant material

Leaves of eight myrtle cultivars (*Myrtus communis* L.): 'Grazia', 'Angela', 'Ika' 'Maria Rita', 'Maria Antonietta', 'Tonina', 'Ilaria' and 'Erika' were used to prepare the extracts. Leaves were collected from June to July at the experimental orchard located at the "Antonio Milella" station of the University of Sassari (Central Western Sardinia, Italy 39°54'20.95" N, 8°35'29.79" E, 5 m a.s.l.). About 500 g of leaves, randomly collected from fifteen plants per cultivar, were delivered to the postharvest laboratory under refrigerated conditions (5 °C). Leaves free from visible defects were washed with tap water, freeze in liquid nitrogen, freeze-dried (Edwards Ivophiliser, Bolton, UK), ground, and stored in the dark at room temperature and under low RH conditions until the preparation of the extracts.

Preparation of leaf extracts

Two grams of freeze-dried leaves were finely pulverized to obtain a homogeneous sample and extracted twice with 40 mL of an ethanol/water solution (70% EtOH). The mixtures were sonicated in an ultrasonic cleaner (VWR International, Leuven Belgium) for 1 hour at 25 °C then centrifuged at 3000 x g for 10 minutes. The organic extracts were filtered with Whatman 4 filter paper, evaporated to dryness under nitrogen flow to remove ethanol, then freeze-dried to remove water.

Chromatographic analysis: identification and quantification of phenolic compounds

Separation and quantification of phenolic compounds were performed by HPLC using an Agilent 1100 system (Agilent Technologies, Milan, Italy) equipped with a quaternary pump, a degasser and coupled with a DAD detector UV 6000 (ThermoFinnigan, Milan, Italy).[22] The dried residue was dissolved with 0.22 M phosphoric acid water solution (1 mL) and injected (10 µL) into the chromatographic system. A reversed-phase Luna column (3u, PFP (2), 100 A, 150x4.6mm, Phenomenex) was used for chromatographic separation.

Linear gradient elution of acetonitrile (mobile phase A) and 0.22 M phosphoric acid (mobile phase B) was performed, at 0.6 mL min^{-1} flow rate, with the following program: 0 min, 95% B; 35 min 85% B; 50 min 70% B. The equilibration of the column took 1 min and the isocratic conditions were maintained up to 15 min.

Phenolic compounds were identified based on standards' retention times and spectra or based on literature data when standards were not available.[24] Stock standard solutions (1000 mg L^{-1}) were appropriately diluted with 0.22 M phosphoric acid to get the final concentrations in the range between 0.02 – 20 mg L^{-1}. All standard solutions were stored in the dark at -20 °C until use. The levels of gallic acid derivatives (galloyl-glucosides and galloyl-quinic acid) were calculated using gallic acid as a reference. Results were expressed as mg of active ingredient per kg of dry weight. All analyses were replicated three times.

Fungal cultures and inoculum preparation

Fungal strains were purchased from the Spanish Type Culture Collection (CECT). Monosporic isolates of *P. digitatum* (CECT 20796), *P. italicum* (CECT 20332) and *P. expansum* (CECT 20906) were cultured on potato dextrose agar (PDA Merk and Co, Whitehouse Station, NY) amended with streptomycin sulphate and oxytetracycline hydrochloride (100+100 mg L^{-1}). Conidial suspensions (1 x 10^6 conidia mL^{-1}) of *P. digitatum, P. italicum* and *P. expansum* isolates were prepared by scraping the colony surface with a sterile scalpel and suspending in a sterile ringer (NaCl 0.9 % in sterile water). The conidial suspension was filtered through two layers of sterile cheesecloth and counted with a hemocytometer.

***In vitro* antifungal activity of myrtle leaves extracts**

The antifungal activity of myrtle leaf extracts was tested on spore germination and fungal growth. For *in vitro* antifungal assays, the dried extracts were dissolved with sterile water to get the final concentration of 90 g L^{-1}, filtered through 0.45 µm sterile pore size membrane filter (Millipore, Bedford, MA, USA) then stored at −20 °C until use. The spore germination test was performed according to Gatto et al.[12] with some modifications. Briefly, 50 µL spore suspension ($1x10^6$ spore mL^{-1}) were mixed with 50 µL of PDB (Potato Dextrose Broth, 24 g L^{-1} in distilled water) and 300 µL of the extract to get the final concentration of 65 g L^{-1}. In control, the extract was replaced by Ringer's solution (NaCl 0.09% in sterile water). The cultures were incubated at 25 °C for 24 hours then germinated and not germinated conidia were counted under a microscope (40 x magnification). The percentage of germinated conidia was calculated according to the following formula: g/G (*100), where g is the number of germinated conidia and G is the number of the total amount of conidia counted in the plate.

Fungi growth inhibition was evaluated on PDA (Potato Dextrose Agar) amended with myrtle extracts at the final concentrations of 10, 15, 20 and 30 g L^{-1}. In control plates, water replaced the extracts. Five µL of conidial suspensionsof *P. digitatum*, *P. italicum* or *P. expansum* at the concentration of $1x10^4$ conidia mL^{-1} were dropped onto the amended media. Three replicate plates were used for each experiment. Plates were incubated at 25 °C and radial growth was measured daily. The antifungal effect of the plant extract was evaluated when the control colonies had completely covered the plate surface. The results were expressed as a percentage of growth inhibition calculated as follows:

$$GI=100*(G \times g)/G$$

where GI is the growth inhibition, G is the growth (cm) of the control without extract and g is the growth of the colony in the media with extracts. The experiment was repeated twice.

Antifungal activity of myrtle leaf extracts on inoculated fruit

The effects of preventive and curative treatments with myrtle leaf extracts against *P. digitatum* or *P. italicum* strains were evaluated on artificially inoculated Clementine fruit cv. 'Freemont'. Commercially mature Clementine mandarins (*Citrus reticulata* Blanco; syn. *Citrus x Clementina*) were harvested in January 2019 at

the “Antonio Milella” station of the University of Sassari (Central Western Sardinia, Italy 39°54’20.95” N, 8°35’29.79” E, 5 m above sea level) and immediately delivered to the postharvest laboratory. Healthy fruit, free from visible defects, uniform in size (80-120 g) and ripening stage were washed with tap water, superficially disinfected by dipping for 30 s in a household bleach solution (0.1 mg L^{-1}, sodium hypochlorite), rinsed with tap water and left to dry overnight at 20 °C.

Fruits were wounded (2 wounds, diameter 3 mm, depth 3 mm) at two opposite points in the equatorial zone and allowed to dry for 3 hours. Twenty µL of plant extracts (concentration 85 g L^{-1}) were pipetted slowly into each wound, allowing the fruit to absorb the droplets. Each injury was inoculated with 10 µL of a conidial suspension 10^4 cells mL^{-1}. In control, sterile water was pipetted instead of the extract. In preventive treatment, pathogens were inoculated 36 h after the application of the extract, whereas in curative treatment the extracts were applied 24 h after pathogens inoculation.

After treatments or inoculation, fruits were arranged in plastic boxes (3 replications of 15 fruit each) and incubated at 20 °C and 90-95% RH.

After 12 d, decay incidence and decay severity were evaluated. The first was calculated as the percentage of fruit with lesions, of any size, of the total, whereas the severity of the decay was determined by calculating the lesion surface area (πr^2 where r is the radius of the lesion) on each fruit per replicate, the results were expressed in cm^2.

Statistical analysis

The statistical analysis was performed with GraphPad Prism7 for windows software (GraphPad software. Inc. La Jolla. CA92037, USA). Results of phenolic concentration, decay incidence, lesion surface, conidial germination and inhibition of mycelial growth were analyzed with a one-way ANOVA and mean separation was calculated by Tukey’s test at $P \leq 0.05$. Before statistical analysis, results expressed as a percentage were transformed into arcsin square root values to normalize distributions, but in tables, untransformed data were reported. The shapiro-Wilk test was used to check data normality. A Pearson’s correlation coefficient was calculated to identify the relationships among the concentration of the main phenolic compounds and in vitro and in vivo tests.

Results

Composition and concentration of phenolic compounds

Hydroxybenzoic acids, hydrolysable tannins and flavonols are the main classes of phenolic compounds identified in *Myrtus communis* leaves (Table 1). Hydrolysable tannins, based on hexa-hydroxyl diphenoyl-glucose (HHDP-glucose) group, represent about 44.9% of the total phenols. In myrtle leaves exist seven isomeric forms of HHDP-glucose with different concentrations based on the cultivar. In almost all samples, isomers 4 and 5 were the most concentrated (from 9435,3 ± 5,8 mg kg^{-1} to 3388,4 ± 1,1 mg kg^{-1} in 'Grazia' and 'Maria Rita' cultivars; from 14084,8 ± 5,3 mg kg^{-1} to 719,4 ± 16,6 mg kg^{-1} in 'Grazia' and 'Tonina' cultivars, respectively). In 'Tonina' leaves only, the most concentrated isomers were 6 and 3 (3946,9 ± 2,3 and 3419,7 ± 2,8 mg kg^{-1}, respectively).

Myrtle leaf extracts held galloyl and digalloyl quinic acids also. Their concentration ranged from 176,3 ± 1,4 to 496,4 ± 4,8 mg kg^{-1} and from 81,3 ± 5,4 to 5493,7 ± 8,3 mg kg^{-1}, respectively. Gallic acid was the only hydroxybenzoic acid identified in myrtle leaf extracts; its concentration ranged from 337,7 ± 5,9 to 2561,8 ± 0,7 mg kg^{-1}d.w.

The profile and the concentration of flavonoids were similar among the eight myrtle extracts. Myricitrin represents the most abundant compound in all samples ranging from 2401,5 ± 1,7 mg $kg^{-1}$to 5258,1 ± 4,7 mg kg^{-1} in 'Angela' and 'Grazia' respectively, followed by myricetin 3-galactoside ranging from 916,1 ± 3,0 mg kg^{-1} in 'Ilaria' to 4800,6 ± 2,4 mg kg^{-1} in 'Maria Antonietta'.

Vitexin, quercetin-3-galactoside, quercetin-3-glucoside and quercitrin were present but in low concentrations (<1% of total phenols).

In vitro antifungal effect of myrtle leaf extracts

Table 2 reports the effect of myrtle leaf extracts on conidial germination of *P. digitatum*, *P. italicum* and *P. expansum*. The extracts strongly inhibited spore germination, causing leakage of cytoplasmic material. Even when germinated, the *hyphae* showed visible malformations (Data not shown). In control, the germinated

spores produced a branched mycelium while, when treated with the extracts, conidia gave rise to a small germ tube.

Myrtle extracts significantly reduced the percentage of germinated conidia of all pathogen tested. The results indicated a different sensitiveness of the pathogens to the treatment with the extracts. In general, *P. expansum* was the least sensitive with percentages of germinated conidia ranging from 4.93 ±0.48 ('Ika') to 35.61 ± 0.56 ('Maria Antonietta'). By contrast, *P. digitatum* had percentages of germination ranging from 0 ('Maria Antonietta') to 6.52 ± 0.74 ('Tonina') thus showing a greater efficacy. 'Maria Antonietta' extracts, for example, completely inhibited *P. digitatum* germination but not that of *P. italicum* and *P. expansum*. The eight extracts had a different effect on spore germination of each pathogen. On *P. digitatum*, 'Maria Rita', 'Maria Antonietta' and 'Ika' leaf extracts showed a higher efficacy as indicated by the low percentage of germinated conidia. On the contrary, the extracts of 'Tonina' had the smallest effect, although germination was significantly lower than control. 'Ika' leaf extracts have shown the highest effectiveness against all the pathogens tested.

Table 3 reports the effect of myrtle leaf extracts on *P. digitatum*, *P. italicum* and *P. expansum* colony growth. On *P. digitatum*, all the extracts caused complete inhibition of the growth at the concentration of 20 g L^{-1} except for the cultivar 'Ilaria'; only 'Angela' and 'Tonina' brought about a full growth stop at 15 g L^{-1}. Even the lowest extract concentration employed strongly inhibited the development of the colonies, with percentages above 70%, except for 'Maria Antonietta'. On *P. italicum,* a remarkable dose effect was observed. All the extracts, except 'Grazia' and 'Erika', determined the inhibition of fungal development at 30 g L^{-1}. *P. expansum* was the less sensitive pathogen tested to the presence of the extracts since no or low inhibition occurred in plates amended with myrtle leaf extracts. Figure 1 describes the growth of *P. digitatum* and *P. Italicum* colonies as a function of time on PDA medium amended with some of the most representative extracts. The extracts caused a decrease in the pathogens growth rate proportional to their concentration. On *P. digitatum* the extracts from 'Ika' and 'Angela' myrtle cultivars determined a noticeable delay of the growth, which started after the third day from the inoculum. By contrast, on *P. italicum* the colonies in amended media began to grow together with the control, even if the growth rate was considerably lower.

***In vivo* fungicide effect of myrtle leaf extracts**

Tables 4 and 5 report the effect of myrtle leaf extracts on disease development on inoculated mandarins. *P. expansum* was not tested in *in vivo* trials due to the low efficacy of the extracts demonstrated under *in vitro* conditions.

After 12 d of storage at 20 °C and 95% RH, fruit inoculated with *P. digitatum* (control) displayed, in curative and in preventive treatments, a percentage of infected wounds respectively above 85% and 70%, whereas on fruits inoculated with *P. italicum* the percentage of infections was above 70%. Myrtle leaf extracts, applied at the concentration of 85 g L^{-1}, controlled the development of green or blue moulds.

When applied as a preventive treatment, only the leaf extracts of 'Ika', 'Ilaria', and 'Maria Rita' significantly reduced the rate of fruit with decay lesions. Conversely, in curative treatments, all the extracts significantly reduced the decay incidence. On *P. italicum*, the preventive treatment caused a significant reduction of the decay incidence in all cultivars with the exclusion of 'Maria Antonietta', while as curative treatment some of the extracts ('Tonina', 'Ilaria', 'Angela', and 'Maria Rita') were not effective.

The results of the effect of treatments with myrtle leaf extracts on the lesion surface area demonstrated that all the extracts significantly reduced the size of the infected area (Table 4 and 5). Even those extracts with a low or no efficacy on the incidence of the decay significantly reduced the lesion surface. This demonstrates that myrtle leaf extracts were able to slow down rot progression.

In general, the effectiveness against green mould infections was greater than that against blue mould. Similarly, curative treatments (24 h after inoculation) were more effective than preventive ones (36 hours before inoculation).

Correlation analysis

The results of the correlation analysis indicate a significant relationship among some phenolic compounds and the inhibition of conidia germination and fungi growth (Table 6). On *P. digitatum* a negative correlation was calculated among the percentage of germinated conidia and galloyl quinic acid, galloyl-HHDP glucose isomer 4, and isomer 5, whereas galloyl quinic acid only affected the fungi growth. On *P. italicum* a negative correlation was detected between galloyl-HHDP glucose isomer 5 and conidia germination while the inhibition of fungi growth was positively correlated with galloyl quinic acid and myricetin 3-galactoside.

According to the results of correlation analysis, the phenolic compounds did not affect the decay incidence of curative treatments of both *P. digitatum* and *P. italicum*. By contrast, in preventive treatments, decay incidence was positively correlated with galloyl-HHDP glucose isomer 1 and 3 for *P. digitatum* and myricetin 3-galactoside for *P.italicum*. Myricetin 3-galactoside and myricetin derivative were positively correlated to the lesion surface in decayed fruit.

Discussion

The use of plant extracts as food preservatives has been suggested as an alternative to conventional fungicides in postharvest treatments of horticultural commodities. [1, 6, 28, 29] The organic or the aqueous extracts of several plants were able to inhibit, *in vitro*, the fungi growth and to reduce the decay on harvested horticultural products.[6, 12]

The antifungal properties of plant extracts have been studied against a wide range of pathogens including *Penicillium* spp.[2, 12, 18] and several plant species were tested by *in vitro* and in *in vivo* trials against the main postharvest diseases. [12, 15, 30, 31] In this paper, the antifungal properties of myrtle leaf hydroalcoholic extracts were tested *in vitro* against *P. italicum*, *P. digitatum*, and *P. expansum* strains; and *in vivo* against *P. digitatum* and *P. italicum* on clementine mandarins. The results presented showed that myrtle extracts significantly inhibited, *in vitro*, the growth of *P. digitatum* and *P. italicum* but were not effective against *P.expansum*. A different sensitiveness of *P. expansum* to the treatments with plant extracts have been observed in other contexts as well. Gatto et al.[12] reported for *P. expansum* a lower inhibition of the germination than that of *P. digitatum* and *P. italicum* strain treated with the same plant extracts. This is not surprising considering that

some phenolics, with known antifungal activity, on *P. expansum* are not involved in the inhibition of the pathogen growth but in the production of patulin. Quercetin, for example, was effective in reducing patulin accumulation without affecting mycelial growth.[32]

The greatest antifungal activity was observed against *P. digitatum* where a complete inhibition of the colony growth was observed at 20 g L^{-1}, whereas on *P. italicum* it was achieved at the concentration of 30 g L^{-1}. These results are in agreement with Ameziane et al.[2] who reported a growth inhibition of 80% and 50% respectively for *P. digitatum* and *P. italicum,* with a myrtle leaf extracts concentration of 10% (w/v).

If the concentration of 30 g L^{-1} completely inhibited the growth of both pathogens, lower concentrations of the extracts caused a delay of the mycelial growth. The trials in PDA medium showed a delay of 3 d in *P. digitatum* development as compared to control, thus demonstrating a dose-dependent effect: fungistatic at low concentration of extract, and fungitoxic at higher concentrations. On *P. italicum* the growth delay was not as clear as in *P. digitatum* even if the growth rate was lower than control. These results, in agreement with Kanan and l Al-Najar [33] further highlight the different sensitiveness of the pathogens to the treatment with plant extracts.

The myrtle leaf extracts were good inhibitors of spore germination as all the extracts analyzed significantly inhibited the germination of the conidia of the tested fungi. Even in this case, however, myrtle leaf extracts, applied at the concentration of 65 g L^{-1}, controlled more effectively *P. digitatum* rather than *P. italicum* or *P. expansum*.

The extracts of the eight myrtle cultivars showed different effectiveness on spore germination: 'Maria Antonietta' extract, completely inhibiting the germination of *P. digitatum*, showed the best results followed by 'Maria Rita' and 'Ika'. The same extracts showed, on *P. Italicum* and on *P. expansum*, a lower ability to control germination being the percentage of germinated conidia sensibly higher.

Besides the ability to inhibit the germination of conidia, myrtle extracts determined malformations in the newly formed germinal tubes, causing the leakage of the cytoplasmic material. A similar effect was also observed by Gatto et al.[12]. Many of the plants extracts they tested inhibited the germ tube elongation rather than the spore germination. Similarly, Chen et al.[15] demonstrated by SEM and TEM analysis that mycelia treated with pinocembroside exhibited notable shrinking, thinned cell walls, insufficient cytoplasmic matrix

and leakage of cytoplasm, resulting in cell death. The influence of the *Penicillium* spp. developmental stage on the effectiveness of treatments with plant extracts has been also discussed by Askarne et al.[3] Conidia germination was less sensitive than mycelia growth to the treatments with the extracts. The results presented in this paper further confirm this aspect, as the concentration of extracts used for spore germination tests was sensibly higher than that used to inhibit mycelial growth, and 'Maria Antonietta' extracts only completely inhibited the germination of conidia.

Previous papers dealing with the effects of plant extracts on the development of fungal diseases showed that extracts of plants belonging to different species may have different potential antifungal activities. Askarne et al.[3] in a screening evaluation of several plant extracts showed that 21 plants out of 50 reduced *in vitro* growth of *P. italicum* by more than 50%. The results presented in this paper demonstrated, for the first time, that within the same plant species, different cultivars may have different effects on fungi growth. This aspect should be carefully taken into consideration when working with natural extracts. The same problem has been frequently faced by pharmaceutical and cosmetic industries working with plants. A problem often associated with the use of phytocomplexes, as food preservatives or food additives, is the strong variability of the chemical composition of the extracts within the same species. The myrtle cultivars used in this study are the result of a selection program based on the identification, collection and reproduction of plants with excellent chemical and agronomical features. Some of the cultivars tested had white fruit whereas others had red-purple berries. This classification concerns the fruits only and does not involve the qualitative and quantitative phenolic profile of the leaves. All myrtle leaf extracts analyzed showed a homogeneous qualitative phenolic profile consisting of three classes of compounds: hydroxybenzoic acids, hydrolysable tannins and flavonols. Eighteen individual phenolic compounds were detected in all leaves extracts. Among these, hydrolysable tannins represent the main group followed by flavonols consisting mainly of myricetin and its derivatives. Gallic acid was the only hydroxybenzoic acid detected in the extracts. By contrast, the quantitative analysis highlighted a high variability among cultivars demonstrating that although cultivated in the same orchard, they retained an imprint linked to genetic factors and the different geographical origin. Hydrolysable tannins are characterized by the presence of seven isomeric forms whose concentration is extremely variable among cultivars. Galloyl-HHDP glucose isomer 5 was the principal tannin in the cultivars

'Grazia', 'Maria Antonietta', 'Ilaria', 'Ika' and 'Maria Rita' cultivars whereas the isomer 4 is the most abundant tannin in 'Erika' and 'Angela'. By contrast, the 'Tonina' showed a profile completely different from the other cultivars. These compounds display a strong antioxidant, anti-inflammatory and antimicrobial activity.[34, 35]The concentration of gallic acid was also extremely variable among myrtle cultivars.

The phenolic pool of plant extracts has been considered by several authors as the principal responsible for the antifungal activity.[36, 37] On fungi cells, phenols predominantly exert their fungicide activity by disrupting the cell membrane or depolarizing the mitochondrial membrane potential interfering with energy homeostasis.[16, 36-39] According to Jiang et al.[9], the antifungal properties of tea polyphenols might be linked to the induction of ROS. Indeed, it is known that, under particular conditions, some plant derived polyphenols, like ellagitannins, may act both as antioxidant or pro-oxidant or may have metal binding properties. In fungi cells where redox-active metals, like Cu (II), are present, ellagitannins could behave as pro-oxidants able to induce the production of ROS, via Fenton-like reactions, and increase lipid peroxidation. The significant correlations calculated among conidia germination, fungi growth and galloylquinic acid or some galloyl HHDP glucose isomers seems to support this hypothesis. However, further studies will be undertaken to analyse the role of molecules containing catechol groups on fungicidal activity.

Besides acting against fungi cells, plant polyphenols may act by enhancing fruit defence mechanism via a priming effect. On lemons, Pangallo et al.[17], following treatment with pomegranate peel extracts observed an increase of the expression of genes involved in the activation of defence response in plants thus demonstrating the role of induction of resistance mechanism in inhibition of *P. digitatum* and *P. italicum* disease development.

According to Gatto et al.[12], the phenolic profile rather than the phenolic concentration is involved in moulds inhibition. The results presented in this paper seem to demonstrate that, despite the similar phenolic profile, the eight myrtle cultivars analyzed displayed a different antifungal activity thus highlighting even the role and the importance of the concentration of the phenolic compounds on disease control. A concentration-dependent mechanism was demonstrated both by *in vitro* and in *in vivo* trials. Yang et al.[16] found that the respiration rate of *P. italicum* declined as the dose of poplar buds active fraction increased. Similarly, De Corato et al.[40] showed a remarkable reduction of lesion diameter on the fruit surface with increasing

concentrations of brown and red seaweed extracts. The results on *P. digitatum* and *P. italicum* mycelial growth, presented in this paper, further confirm the dose-effect mechanism of the treatments with myrtle leaf extracts.

Unfortunately, the control ability of many antimicrobials like plant extracts or natural antifungal compounds cannot be predicted by their activity *in vitro*. Even those antimicrobials with good or excellent properties *in vitro* once applied as postharvest treatments might not achieve the control level hypotized in *in vitro* tests. For this reason, the ability of myrtle leaf extracts to inhibit or delay the spoilage caused by green and blue mould has been studied by *in vivo* tests on citrus fruit. In postharvest treatments, the effectiveness of plant extracts has been demonstrated on several commodities including citrus, peaches, grapes and strawberries.[3, 5, 7, 12, 41]

In this paper, myrtle leaf crude extracts were applied as curative and preventive treatments to provide information about their effectiveness. The results on inoculated fruits demonstrated that on green mould disease, myrtle leaf extracts were more effective when applied after 24 h from inoculation, in agreement with *in vitro* assays, which demonstrated the efficacy of the extracts in the early stages of germ tube elongation. Inversely, the blue mould has been controlled more effectively with a preventive treatment applied 36 h before inoculation. As already observed in *in vitro* assays, even on mandarins, blue and green mould disease reacted differently to the treatment with the extracts. The fungicidal activity of myrtle extracts observed in wounded fruits may be associated to the metal chelating properties of ellagitannins.[42] Ballester et al.[43], highlighted the importance of the availability of Iron (Fe(II)) in the host in *P. digitatum* development and suggested the restriction of micronutrients trough metal chelation as a new possible target in fungal control. The role of metal chelation in the control of rot development was also highlighted by Lafuente et al. [44] These authors suggested that the activation of ferrochelatase induced by a treatment with LED blue light may be involved in the elicitation of resistance against *P. digitatum*.

The efficacy of preventive treatments indicates that the extract persists inside the wound until inoculation and is capable of damaging the newly formed germ tube. The persistence is related to the stability of the main compounds present in the extracts. The stability of ellagitannins, for example, is strongly influenced by the pH of the environment and by storage temperature; at 20 °C and pH 5-6, the natural pH within 2-mm-

deep citrus wounds, they are stable for 25 h.[35, 45] However, it is possible that during storage the active ingredients undergo hydrolysis or polymerization processes that alter their antimicrobial properties. The control of postharvest diseases caused by wound pathogens like *Penicillium* spp. depends on the ability of the antimicrobial to leave residues that remain unaltered during storage and that interact with the fungus and with the constituents of the rind. This kind of interactions may be the cause of the different effect on different fungi or to the failure of the antimicrobial activity predicted in vitro.

Moreover, as previously discussed in this paper, myrtle leaf extracts may induce the fruit defense mechanism that acts in synergy with the antifungal effects of the extracts.

The protection provided by myrtle extracts may be important to reduce, in addition to reduced fungicides doses or other alternative methods, the infections that occur during harvest and transport or that may arise during storage.

All myrtle extracts significantly reduced the surface lesion area, indicating a high ability to reduce the progression of the decay and to protect citrus fruit during storage and shelf life.

The results of the *in vivo* experiments further confirm the cultivar dependent effectiveness of the extracts. This aspect must be necessarily considered when studying the extracts' biological properties.

In conclusion, this paper provides evidence of the antifungal activity of myrtle leaf extracts *in vitro* and, for the first time, on inoculated fruit. The different ability of plant extracts obtained from different genotypes of the same specie to control postharvest pathogens was never taken into consideration. However, it is an aspect that should be carefully considered in the evaluation of the biological properties of plant extracts.

The results obtained so far are encouraging but further studies are needed to gain more information into the mode of action of the extracts and to select the myrtle extracts with the best antifungal properties and to evaluate if the extracts can trigger a fruit defense mechanism.

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Figure captions

Fig. 1. Growth of the colonies of *Penicillium digitatum* (graphs A, C, E) and *Penicillium Italicum* (graphs B, D, F) on plates with PDA amended with increasing concentrations of 'Maria Rita' (A and B), 'Angela' (C and D), and 'Ika' (E and F) leaf extracts. The following extracts concentration were tested: 0 g L^{-1} (control) (●), 10 g L^{-1} (▼); 15 g L^{-1} (□); 20g L^{-1} (○).

Table 1. Concentration of phenolic compounds in *Myrtus communis* L. leaf extracts.

Compound	Maria Rita	Maria Antonietta	Angela	Ika	Tonina	Ilaria	Grazia	Erika
Hydroxybenzoic acid								
Gallic acid[$]	963,1 ± 7,1 **b**	1215,3 ± 9,4 **b**	2561,8 ± 0,7 **a**	2039,0 ± 4,0 **ab**	337,7 ± 5,9 **b**	657,1 ± 7,0 **b**	804,2 ± 8,7 **b**	882,0 ± 7,0 **b**
Hydrolysable tannins								
Galloylquinic acid[$]	355,3 ± 9,8 **a**	496,4 ± 4,8 **a**	214,0 ± 9,0 **a**	390,9 ± 7,1 **a**	176,3 ± 1,4 **a**	223,7 ± 6,8 **a**	253,8 ± 2,3 **a**	229,2 ± 1,5 **a**
Digalloylquinic acid[$]	3221,7 ± 6,9 **bc**	4883,0 ± 1,1 **a**	81,3 ± 5,4 **e**	1712,5 ± 5,3 **d**	5493,7 ± 8,3 **a**	2712,9 ± 4,8 **bc**	3795,3 ± 5,0 **b**	3528,3 ± 6,0 **b**
Galloyl HHDP glucose isomer 1[$]	581,6 ± 6,9 **b**	1329,5 ± 3,5 **a**	1841,2 ± 10,2 **a**	1359,1 ± 7,0 **a**	785,4 ± 9,4 **b**	442,7 ± 3,9 **b**	1061,4 ± 2,2 **a**	849,5 ± 9,0 **a**
Galloyl-HHDP glucose isomer 2[$]	216,6 ± 10,9 **b**	231,6 ± 0,8 **b**	1643,3 ± 7,4 **a**	1869,4 ± 8,3 **a**	1478,8 ± 5,6 **a**	216,0 ± 0,8 **b**	318,4 ± 5,4 **b**	170,3 ± 7,0 **b**
Galloyl-HHDP glucose isomer 3[$]	1458,7 ± 4,2 **c**	4069,0 ± 0,4 **a**	2988,2 ± 3,5 **b**	2946,2 ± 0,3 **b**	3419,7 ± 2,8 **ab**	1314,8 ± 2,3 **c**	3121,6 ± 4,1 **b**	4537,0 ± 6,3 **a**
Galloyl-HHDP glucose isomer 4[$]	3388,4 ± 1,1 **d**	7859,6 ± 6,0 **b**	4010,0 ± 2,9 **d**	3731,2 ± 2,7 **d**	938,0 ± 21,5 **e**	4224,8 ± 5,3 **c**	9435,3 ± 5,8 **a**	7317,8 ± 6,8 **b**
Galloyl-HHDP glucose isomer 5[$]	3639,4 ± 19,2 **e**	11841,0 ± 5,0 **b**	2847,3 ± 0,5 **f**	4829,8 ± 8,4 **d**	719,4 ± 16,6 **g**	6663,2 ± 4,6 **c**	14084,8 ± 5,3 **a**	6492,4 ± 8,2 **c**
Galloyl-HHDP glucose isomer 6[$]	2409,7 ± 20,8 **d**	5456,9 ± 2,3 **b**	2185,3 ± 4,1 **d**	3866,0 ± 4,9 **c**	3946,9 ± 2,3 **c**	4017,0 ± 4,4 **c**	8945,1 ± 5,8 **a**	5124,8 ± 6,8 **b**
Galloyl-HHDP glucose isomer 7[$]	842,5 ± 10,4 **b**	892,2 ± 2,9 **b**	1038,0 ± 9,6 **b**	2313,9 ± 6,3 **a**	879,9 ± 5,2 **b**	708,1 ± 0,8 **b**	1140,3 ± 4,4 **b**	583,0 ± 3,8 **b**
Flavonols								
Myricetin derivative1[£]	659,5 ± 9,9 **b**	2051,1 ± 1,1 **a**	513,9 ± 6,5 **b**	655,2 ± 6,3 **b**	1293,2 ± 4,8 **b**	452,6 ± 1,8 **b**	693,5 ± 4,4 **b**	857,0 ±7,1 **b**
Myricetin 3-galactoside	1587,5 ± 9,5 **c**	4800,6 ± 2,4 **a**	996,6 ± 8,6 **d**	1468,8 ± 5,3 **c**	1934,4 ± 4,8 **bc**	916,1 ± 3,0 **d**	1516,7 ± 4,3 **c**	2154,8 ± 11,0 **b**
Vitexin	174,8 ± 3,3 **a**	523,1 ± 2,8 **a**	133,3 ± 10,0 **a**	263,3 ± 10,5 **a**	259,8 ± 0,6 **a**	163,3 ± 2,4 **a**	248,5 ± 4,0 **a**	460,7 ± 6,3 **a**
Myricetin derivative2[£]	88,2 ± 5,2 **a**	90,7 ± 2,4 **a**	113,1 ± 4,0 **a**	114,2 ± 0,1 **a**	85,0 ± 6,1 **a**	83,2 ± 5,3 **a**	126,8 ± 7,1 **a**	95,6 ± 7,2 **a**
Myricitrin	2726,4 ± 6,3 **c**	5128,6 ± 1,8 **a**	2401,5 ± 1,7 **c**	2772,9 ± 6,0 **c**	4901,9 ± 6,6 **a**	3359,3 ± 1,8 **b**	5258,1 ± 4,7 **a**	4952,9 ± 5,8 **a**
Quercetin 3-galactoside	47,3 ± 6,9 **a**	135,7 ± 1,9 **a**	68,2 ± 1,3 **a**	78,7 ± 4,6 **a**	108,8 ± 3,9 **a**	53,1 ± 2,5 **a**	81,2 ± 5,0 **a**	122,8 ± 6,0 **a**
Quercetin 3-glucoside	46,5 ± 4,9 **a**	87,6 ± 1,2 **a**	71,3 ± 0,6 **a**	111,1 ± 5,7 **a**	112,2 ± 4,6 **a**	81,0 ± 3,0 **a**	116,2 ± 4,0 **a**	208,4 ± 5,4 **a**
Quercitrin	216,3 ± 5,6 **a**	348,5 ± 7,3 **a**	78,9 ± 2,4 **a**	227,4 ± 2,6 **a**	252,8 ± 7,0 **a**	133,0 ± 9,2 **a**	193,6 ± 3,9 **a**	158,7 ± 8,8 **a**

[$] Expressed as gallic acid equivalent; [£] Expressed as myricitrin equivalent. Results are expressed as mg kg^{-1} of d.w. and reported as mean (n = 3) ± standard deviation. In each row grouping, means separation was performed by Tukey's test (P≤0.05).

Table 2. Effect of myrtle leaf extracts at the concentration of 65 g L^{-1} on conidia germination of *P. digitatum*, *P. italicum* and *P. expansum*.

Myrtle cultivars	Spore germination (%)		
	P. digitatum	*P. italicum*	*P. expansum*
Control	88.59 ± 4.36 **a**	86.09 ± 0.12 **a**	99.49 ± 0.71 **a**
'Maria Rita'	1.35 ± 0.20 **b**	7.55 ± 0.20 **b**	9.21 ± 0.17 **c**
'Maria Antonietta'	0.00 ± 0.00 **d**	1.21 ± 0.04 **c**	35.61 ± 0.56 **b**
'Ika'	1.75 ± 0.14 **b**	1.20 ± 0.05 **c**	4.93 ± 0.48 **c**
'Tonina'	6.52 ± 0.74 **c**	9.76 ± 2.40 **b**	26.94 ± 4.33 **b**
'Erika'	3.20 ± 0.27 **b**	8.05 ± 0.91 **b**	34.23 ± 2.49 **b**
'Ilaria'	3.02 ± 0.24 **bc**	5.42 ± 0.02 **b**	28.90 ± 2.65 **b**
'Grazia'	2.14 ± 0.60 **b**	1.20 ± 1.08 **c**	23.66 ± 0.82 **b**
'Angela'	2.77 ± 0.24 **b**	5.68 ± 0.01 **b**	24.57 ± 0.61 **b**

Each value is the mean of 3 replicates ± standard deviation. Percentages of germinated conidia were transformed in arcsin √x before statistical analysis. In each column grouping, means separation was performed by Tukey's test ($P \leq 0.05$).

Table 3. Effect of the extracts of the eight myrtle cultivars at the concentrations of 10, 15, 20 and 30 g L^{-1} on the growth of *Penicillium digitatum, Penicillium italicum* and *Penicillium expansum*. Results are expressed as inhibition of growth (%).

Extract concentration (g L^{-1})	Growth inhibition (%)							
	'Maria Rita'	'Ilaria'	'Tonina'	'Erika'	'Ika'	'Grazia'	'Angela'	'Maria Antonietta'
	Penicillium digitatum							
10	74,85 ± 1,0 **Cc**	70,86 ± 1,9 **Dd**	78,72 ± 1,9 **Bb**	79,37 ± 2,2 **Cb**	83,56 ± 1,0 **Ca**	78,72 ± 1,9 **Cb**	86,46 ± 1,9 **Ba**	49,06 ± 3,0 **Ce**
15	78,72 ± 1,9 **Bd**	80,66 ± 1,9 **Cd**	100,00 ± 0,0 **Aa**	89,68 ± 1,1 **Bc**	87,75 ± 1,1 **Bd**	92,26 ± 1,9 **Bb**	100,00 ± 0,0 **Aa**	67,12 ± 1,9 **Be**
20	100,00 ± 0,0 **Aa**	92,91 ± 1,1 **Bb**	100,00 ± 0,0 **Aa**	100,00 ± 0,0 **Aa**	100,00 ± 0,0 **Aa**	100,00 ± 0,0 **Aa**	100,00 ± 0,0 **Aa**	100,00 ± 0,0 **Aa**
30	100,00 ± 0,0 **Aa**	100,00 ± 0,0 **Aa**	100,00 ± 0,0 **Aa**	100,00 ± 0,0 **Aa**	100,00 ± 0,0 **Aa**	100,00 ± 0,0 **Aa**	100,00 ± 0,0 **Aa**	100,00 ± 0,0 **Aa**
	Penicillium italicum							
10	38,79 ± 1,2 **Db**	42,26 ± 2,3 **Dab**	45,73 ± 1,2 **Ca**	39,18 ± 3,5 **Db**	33,02 ± 2,3 **Db**	29,18 ± 1,3 **Dc**	40,72 ± 2,7 **Da**	43,03 ± 1,3 **Ca**
15	51,50 ± 2,3 **Cb**	50,73 ± 3,5 **Cb**	49,96 ± 3,5 **Cb**	50,73 ± 3,5 **Cb**	55,35 ± 2,7 **Cb**	53,81 ± 2,3 **Cb**	51,50 ± 2,3 **Cb**	69,21 ± 1,3 **Ba**
20	69,98 ± 2,3 **Bbc**	67,67 ± 2,3 **Bc**	74,60 ± 2,3 **Bb**	66,13 ± 1,3 **Bc**	63,05 ± 2,3 **Bc**	64,59 ± 1,3 **Bc**	74,60 ± 2,3 **Bb**	100,00 ± 0,0 **Aa**
30	100,00 ± 0,0 **Aa**	100,00 ± 0,0 **Aa**	100,00 ± 0,0 **Aa**	73,83 ± 3,5 **Ab**	100,00 ± 0,0 **Aa**	75,37 ± 2,7 **Ab**	100,00 ± 0,0 **Aa**	100,00 ± 0,0 **Aa**
	Penicillium expansum							
10	0,00 ± 0,0 **Ba**	4,76 ± 3,1 **Ca**	2,00 ± 1,7 **Aa**	7,70 ± 4,4 **Aa**	0,00 ± 0,0 **Aa**	0,00 ± 0,0 **Aa**	2,76 ± 4,8 **Aa**	2,00 ± 1,7 **Aa**
15	0,00 ± 0,0 **Ba**	4,76 ± 3,1 **Ca**	3,76 ± 4,2 **Aa**	8,64 ± 15,0 **Aa**	1,00 ± 1,7 **Aa**	0,00 ± 0,0 **Aa**	3,94 ± 6,8 **Aa**	4,94 ± 6,1 **Aa**
20	2,94 ± 0,1 **Ba**	8,29 ± 0,0 **Ba**	2,76 ± 4,8 **A**	0,00 ± 0,0 **Ba**	0,00 ± 0,0 **Aa**	0,00 ± 0,0 **Aa**	8,88 ± 5,1 **Aa**	5,94 ± 5,1 **Aa**
[illegible]	10,00 ± 1,7 **Ab**	27,10 ± 2,0 **Aa**	2,76 ± 4,8 **Ab**	3,76 ± 4,2 **Ab**	0,00 ± 0,0 **Ab**	0,00 ± 0,0 **Ab**	2,00 ± 1,7 **Ab**	4,94 ± 6,1 **Ab**

Each value is the mean of 3 replicates ± standard deviation. Capital letters relate to comparisons of the effects of extract concentration within each cultivar. Lower case letters refer to comparisons of the effects of cultivars within each extract concentration. In each row or column grouping, means separation was performed by Tukey's test ($P \leq 0.05$).

Table 4. Effect of myrtle leaf extracts on decay incidence (%) and lesion surface (cm^2) of green and blue moulds caused by *P.digitatum* and *P. italicum* on mandarin fruit stored for 12 days at 20 °C and 90% RH . Treatment with the leaf extracts was performed 36 h before inoculation.

	Decay incidence (%)								Lesion surface (cm^2)							
Treatment	*P. digitatum*				*P. italicum*				*P. digitatum*				*P. italicum*			
Control	86.67	±	6.6	**a**	71.67	±	1.6	**a**	20.5	±	1.3	**a**	4.99	±	0.1	**a**
Ika	58.33	±	1.6	**b**	29.17	±	0.8	**d**	3.01	±	0.1	**c**	1.39	±	0.1	**c**
Tonina	75.00	±	1.7	**ab**	35.00	±	3.3	**cd**	4.15	±	1.5	**b**	1.72	±	0.4	**c**
Ilaria	19.80	±	1.6	**c**	28.33	±	0.0	**d**	0.26	±	0.06	**d**	0.92	±	0.06	**cd**
Grazia	87.87	±	2.1	**a**	18.33	±	1.6	**e**	6.07	±	1.1	**b**	0.59	±	0.07	**d**
Erika	73.80	±	2.8	**ab**	42.22	±	3.8	**c**	5.23	±	0.8	**bc**	1.39	±	0.2	**c**
Angela	85.57	±	7.6	**a**	17.50	±	0.8	**e**	5.82	±	0.8	**b**	1.42	±	0.2	**c**
Maria Antonietta	72.80	±	10	**ab**	66.67	±	5.0	**ab**	3.66	±	0.3	**b**	2.54	±	0.08	**b**
Maria Rita	57.00	±	3.7	**b**	61.67	±	5.0	**b**	3.84	±	1.1	**b**	2.75	±	0.07	**b**

Each value is the mean of 3 replicates of 15 fruit each ± standard deviation. Percentages of decay incidence were transformed in arcsin √x before statistical analysis. In each column grouping, means separation was performed by Tukey's test ($P \leq 0.05$).

Table 5. Effect of myrtle leaf extracts on decay incidence (%) and lesion surface (cm^2) of green and blue moulds caused by *P. digitatum* and *P. italicum* on mandarin fruit stored for 12 days at 20 °C and 90% RH. Treatment with the leaf extracts was performed 24 h after inoculation.

	Decay incidence (%)								Lesion surface (cm^2)							
Treatment	*P.digitatum*				*P.italicum*				*P. digitatum*				*P. italicum*			
Control	99.44	±	5.1	**a**	75.00	±	7.1	**a**	26.25	±	2.4	**a**	6.59	±	1.2	**a**
Ika	64.44	±	6.9	**bc**	53.94	±	0.6	**b**	7.36	±	0.7	**b**	0.95	±	0.2	**c**
Tonina	57.77	±	6.9	**bc**	64.84	±	0.5	**a**	9.22	±	0.7	**b**	4.76	±	0.1	**b**
Ilaria	52.22	±	1.9	**c**	72.62	±	5.4	**a**	3.65	±	0.8	**c**	4.32	±	0.3	**b**
Grazia	52.22	±	1.9	**c**	57.69	±	7.6	**b**	4.14	±	0.2	**c**	4.64	±	0.1	**b**
Erika	61.11	±	6.9	**b**	57.24	±	9.3	**b**	9.00	±	1.9	**b**	1.49	±	0.2	**c**
Angela	53.33	±	3.3	**bc**	60.71	±	7.1	**a**	5.01	±	0.1	**c**	1.90	±	0.5	**c**
Maria Antonietta	58.33	±	5.0	**bc**	55.36	±	1.7	**b**	9.86	±	0.6	**b**	2.11	±	0.2	**c**
Maria Rita	68.33	±	8.3	**b**	60.71	±	0	**a**	9.59	±	1.3	**b**	2.19	±	0.1	**c**

Each value is the mean of 3 replicates of 15 fruit each ± standard deviation. Percentages of decay incidence were transformed in arcsin √x before statistical analysis. In each column grouping, means separation was performed by Tukey's test ($P \leq 0.05$).

Table 6. Pearson's correlation coefficients (r) calculated among the results of in vitro and in vivo assays on *P. digitatum* and *P. italicum* and the concentration of the main phenolic compounds identified in myrtle leaves.

Phenolic compounds	*Penicillium digitatum*						*Penicillium italicum*					
	In vitro assays		*In vivo* assays				*In vitro* assays		*In vivo* assays			
			Curative treatment		Preventive treatment				Curative treatment		Preventive treatment	
	Conidia germination	Fungi growth	Decay	Lesion surface	Decay	Lesion surface	Conidia germination	Fungi growth	Decay	Lesion surface	Decay	Lesion surface
Gallic acid	-0.62	-0.07	0.11	-0,15	0,20	0,21	-0.51	0.27	-0.48	**-0.77***	-0,11	0.06
Galloyl quinic acid	**-0.87****	**-0.80***	0.49	0,39	-0,07	-0,15	-0.65	**0.79***	-0.47	-0.54	0,63	0.56
Digalloyl quinic acid	0.04	-0.44	0.27	0,42	-0,24	-0,28	0.01	0.21	0.04	0.35	0,49	0.16
Galloyl HHDP glucose isomer 1	-0.26	0.24	-0.12	0,01	**0,72***	0,61	-0.47	0.41	-0.48	-0.46	-0,22	-0.01
Galloyl-HHDP glucose isomer 2	0.40	0.64	-0.05	-0,09	0,28	0,16	-0.01	-0.18	-0.11	-0.09	-0,51	-0.14
Galloyl-HHDP glucose isomer 3	0.08	0.22	-0.06	0,35	**0,74***	0,63	-0.14	0.36	-0.37	-0.25	0,001	-0.03
Galloyl-HHDP glucose isomer 4	**-0.74***	-0.44	-0.18	-0,25	0,14	0,21	-0.61	0.43	-0.28	-0.28	0,06	-0.19
Galloyl-HHDP glucose isomer 5	**-0.78***	-0.58	-0.18	-0,28	-0,03	-0,01	**-0.73***	0.50	-0.17	-0.16	0,10	-0.21
Galloyl-HHDP glucose isomer 6	-0.08	-0.14	-0.34	-0,13	0,21	0,14	-0.47	0.32	-0.02	0.38	-0,08	-0.44
Galloyl-HHDP glucose isomer 7	-0.22	0.14	0.17	-0,14	0,14	0,03	-0.61	0.17	-0.46	-0.28	-0,31	-0.13
Myricetin derivative1	-0.05	-0.35	0.16	**0,72***	0,35	0,16	-0.06	0.69	-0.11	-0.01	0,62	0.59
Myricetin 3-galactoside	-0.31	-0.53	0.29	**0,74***	0,31	0,18	-0.19	**0.77***	-0.27	-0.21	**0.73***	0.57
Vitexin	-0.20	-0.39	0.21	0,59	0,24	0,15	-0.21	0.60	-0.26	-0.23	0.51	0.22
Myricetin derivative2	-0.25	0.39	-0.22	-0,44	0,59	0,61	-0.59	0.01	-0.55	-0.18	0.59	-0.50
Myricitrin	0.17	-0.11	-0.24	0,23	0,29	0,21	-0.04	0.31	0.11	0.43	0.18	-0.12
Quercetin 3-galactoside	0.10	-0.01	-0.06	0,47	0,50	0,35	-0.09	0.49	-0.13	-0.11	0.21	0.07
Quercetin 3-glucoside	0.32	0.29	-0.15	0,05	0,26	0,25	-0.04	-0.06	-0.09	-0.02	-0.25	-0.49
Quercitrin	-0.19	-0.53	0.44	0,65	0,01	-0,13	-0.24	0.59	-0.23	0.05	0.65	0.48

* Significant at $p \leq 0.05$; **significant at $p \leq 0.01$.