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# *Melia azedarach* fruit extracts for control of bacterial contaminants development in micropropagation

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## Abstract

The effect of *M. azedarach* fruit extracts was evaluated *in vitro vs* some bacterial isolates and contaminants (*Sphingomonas* spp., *Microbacterium* spp. and *Bacillus* spp.) of shoot cultures of calla lily (*Zantedeschia aethiopica*) and 'MRS 2/5' (*Prunus cerasifera* x *Prunus spinosa*), and on plant growth.

The aqueous extracts generally had a low antimicrobial activity. The ethanolic extract (PE) had a strong antimicrobial activity, but caused shoot death. PEE extract, that was obtained after ethanol evaporation from PE, was scarcely phytotoxic, bactericidal *vs* different *Bacillus* isolates and bacteriostatic/bactericidal against shoot contaminants.

KEY WORDS: NATURAL EXTRACTS, PERSIAN LILAC, SHOOT CULTURE

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## Introduction

Bacterial contamination can have variable effects on growth and metabolism of micropropagated shoots, even causing their death, with cost increases in commercial plant production.

Derivatives of plants of the *Meliaceae* family (e.g. 'neem' tree, *Azadiracta indica*) are known for their insecticidal (1) and antimicrobial activity. In particular, a leaf aqueous extract of *Melia azedarach* showed variable antimicrobial activities against phytopathogenic fungi and bacteria (2). If added to culture media, it was effective in eliminating some *Bacillus* spp, and *Sphingomonas* spp. contaminants from 'MRS 2/5' (*P. cerasifera* x *P. spinosa*) *in-vitro* grown shoots; however it caused up to 50% shoot death (3 and ref.).

PE fruit extract [40 g fruit pulp and skin/ 100 mL 50% ethanol (EtOH) solution] previously showed bacteriostatic/bactericidal activities *vs* different bacterial isolates, however it was phytotoxic to calla lily (*Zantedeschia aetiopica*) shoots; PEE extract, that was obtained after EtOH evaporation from PE, was bacteriostatic against some bacterial contaminants, if added at a 10% level in the medium, and not detrimental to the plant cultures (4).

Present research investigates: a) the antimicrobial activity of PE, PEE and of an aqueous fruit extract (4) on a wide range of bacterial species; b) the effectiveness of PEE for the control of bacterial contaminants development in calla lily and 'MRS 2/5' shoots when added to culture media at higher levels than those previously tested.

## Methods

'MRS 2/5' and calla lily shoots were grown on modified Murashige and Skoog (MS) (5) media enriched with 0.5 mg $L^{-1}$ and 3.0 mg $L^{-1}$ 6-benzyladenine (BA), respectively (3, 4). Rooting of calla lily occurred on the same medium enriched with BA 0.5 mg $L^{-1}$ that was used for plum shoot multiplication. Standard culture conditions were: 22±2 °C and a 16 h photoperiod at 30 µmoles $m^{-2}$ $s^{-1}$ photosynthetic active radiation (PAR).

The bacterial strains used in the present research were supplied by the Microbiology area of the Department of Agricultural Sciences of Bologna University, or were previously isolated from *in vitro* shoot cultures of fruit plants. Shoots were separately inoculated (3) with *Microbacterium oleivorans* (Mo), *Sphingomonas melonis* (Sm), *Bacillus circulans* (Bc) and *B. subtilis* (Bs), and subcultured three times on standard proliferation media to allow bacteria spread inside plant tissues.

The aqueous and ethanolic fruit extracts were prepared as previously reported (4). The activity of the undiluted extracts was tested by the 'spot agar test' (6) *vs* Mo, Sm, Bc, Bs and other *Bacillus* spp. isolates.

The effect of variable PEE doses [0 (control), 1, 5, 10 and 20 %] added to standard culture media was investigated on shoot and root growth, respectively in 'MRS 2/5' and calla lily shoots free of cultivable bacteria. This trial aimed to determine the highest extract dose that was not harmful to the shoots, to be used to counteract bacterial spread in contaminated cultures.

Then the activity of PEE at a 20% level was evaluated against shoot contaminants by plate counts (3). Living cell count was taken by tenfold serial dilutions of shoot homogenates in physiological solutions, and plating 1 mL on Merck Plate Count agar (pH 7). In each experiment, the number of bacteria 'colony forming units' (cfu) $g^{-1}$ fresh tissue was recorded on three samples per culture type after 24-48 h at 30 °C.

## Results and Discussion

The aqueous extracts showed a low antimicrobial activity against bacterial isolates in repeated experiments in two different years.

PE had a strong antimicrobial activity, but caused death of many shoots if used at a 20% level.

The undiluted PEE was bactericidal *vs* the isolates

of Mo, Bc, Bs (Figure 1), *B. mycoides*, *B. thuringensis* and *B. laterosporus*.

Moreover, PEE 10% and 20% only reduced shoot and root growth of bacteria-free shoots (Figure 2), and did not cause their death.

Therefore, the antimicrobial activity of PEE 20% was also tested against Mo, Sm, Bc, Bs contaminants of the shoots. PEE at a 20% level had a bacteriostatic activity on Mo and Bc contaminants of calla lily (the cfu $g^{-1}$ number was reduced by about one order of magnitude compared to the untreated shoot cultures) and was even effective in getting heavily-contaminated 'MRS 2/5' shoots (cfu $g^{-1}$, mean ± standard deviation, 2.23 ± 0.83 x $10^5$) rid of Mo bacterial contaminants, without negatively affecting shoot growth after transfer to standard media. Instead, visible Sm and Bs contamination was found at the shoot base of both plant genotypes already at the first transplant after PEE treatment.

## Conclusion

Antibiotic treatments are the most widely used method to eliminate bacterial contamination from plant *in vitro* cultures. However, they are sometimes phytotoxic, and can lead to the selection of resistant bacterial strains after long-lasting exposure. The results reported above suggest that *M. azedarach* extracts could be an alternative to antibiotics for bacteria control in micropropagation, although further studies are needed to determine the optimal extract doses to be used in the different *in vitro* culture phases, and with different bacterial strains and plant species.

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Figure 1. Inhibition effect of undiluted PEE on *Bacillus subtilis* isolates detected by the 'spot agar test'

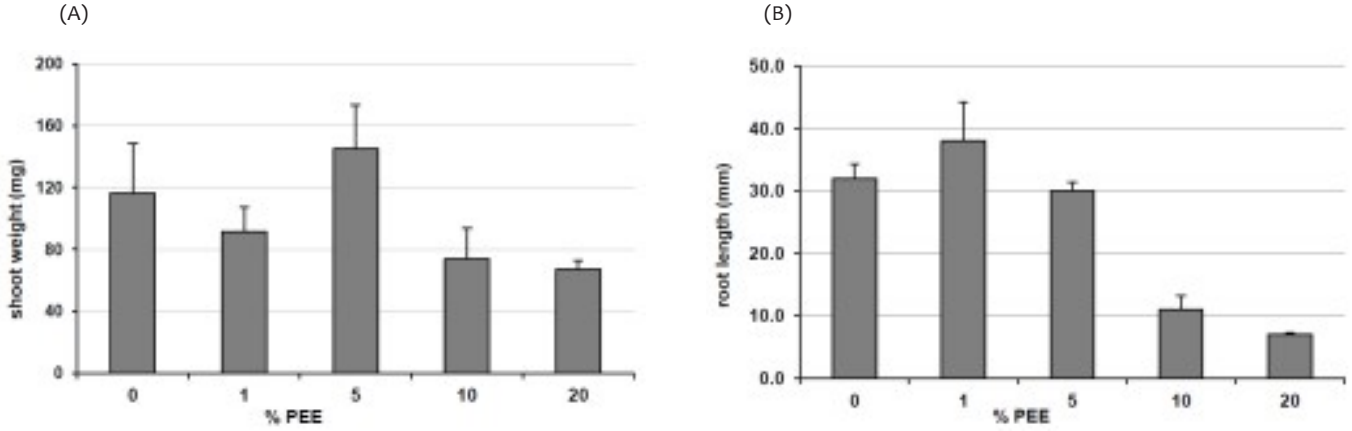


Figure 2. Effect of PEE on: (A) shoot weight of 'MRS 2/5'; (B) root length of calla lily (mean values are based on six shoots per treatment; bars represent standard errors)