**Efficacy of biofumigation with essential oils in the control of postharvest rots on nectarines**

# Efficacy of biofumigation with essential oils in the control of postharvest rots on nectarines

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**Abstact**

**The most common postharvest pathogens on nectarines are *Monilinia fructicola* (G. Winter) Honey and *M. laxa* (Aderh. & Ruhland) Honey, the agents of brown rot. The efficacy of five essential oils (EOs) at 0.1%, 0.5% and 1% concentration was evaluated in vitro against *M. fructicola* strain CVG1539 after 14 days at 25°C. Subsequently, biofumigation was realized with the three most effective EOs (fennel, basil, and lemon), this time at 2% concentration. Disease incidence was evaluated after a longer storage (28 days) at 1°C and after further 5 days shelf-life at 20°C. At the end of storage, nectarines treated with EOs showed a significant reduction in rots, which were caused by *Monilinia* spp. At the end of shelf-life, *Monilinia* spp., *Penicillium* spp., *Botrytis* spp., *Alternaria* spp., and *Rhizopus* spp. were isolated from the rots. Fruit quality and microbiome composition were analysed at harvest, after 28 days of storage, and after 5 days of shelf-life. The firmness in the treated fruits with EOs was higher compared to the untreated ones. Treatments with biofumigation with EOs are promising tools for the control of postharvest rots.**

**Keywords:** biofumigation, essential oils, *Monilinia* spp., nectarine, postharvest disease.

## INTRODUCTION

Despite a steady decline over the last three decades, peaches and nectarines still represent the third most important fruit in Italy, with over one million tonnes in 2020 (FAOSTAT, 2020). Several fungal pathogens are associated to post-harvest production loss, such as *Monilinia* spp. (causal agent of brown rot), *Botrytis cinerea* (causal agent of grey mould decay), *Penicillium* spp. (causal agent of blue mould decay), *Rhizophus nigricans* (causal agent of *Rhizopus* decay), but also *Alternaria* spp., *Aspergillus* spp. and *Mucor* spp. (Mari *et al.*, 2019). Among all mentioned pathogens, *Monilinia* spp. is the most common and economically important, with production loss up to 100% in favourable environmental conditions (Papavasileiou *et al.*, 2020). In recent years, several control strategies against post-harvest disease have been proposed as alternatives to fungicide treatments with fludioxonil, such as hot water dipping (Spadoni *et al.*, 2013) or the use of biocontrol yeasts (Mari *et al.*, 2012). Another line of research

has focused on using essential oils (EOs), which are oily substances extracted from several plant species belonging to order Lamiales, Asterales, Rutales and Apiales. The antimicrobial efficacy of EOs is associated with several molecular mechanisms, which include alteration of cell wall and membrane permeability, impairment of quorum sensing mechanisms and changes in gene expression patterns, including those of mycotoxin associated genes (Maurya *et al*., 2021). Moreover, EOs have been shown to act indirectly by priming host defence mechanisms (Banani *et al*., 2018). Despite this, practical application of these substances is hampered by phytotoxicity phenomena even at moderate EO concentration (Lopez-Reyes *et al*., 2013). One of the possible solutions for this problem is biofumigation, where EOs are slowly released in the atmosphere from dispensers. In this study, we first analyzed the in vitro efficacy of five essential oils against *M. fructicola*, previously considered a quarantine pathogen in Europe (Pellegrino *et al*., 2009) and now the main postharvest pathogen on nectarines in Italy (Franco Ortega *et al*., 2019). We then chose the three most effective ones and run an in vivo biofumigation test on naturally inoculated nectarines. Our aim was to find suitable candidates for the treatment of nectarines against post-harvest rots, to validate our biofumigation protocol and to quantify the impact of the treatment on fruit quality parameters.

## MATERIALS AND METHODS

### Preparation of EOs plates

Essential oils plates for the in vitro inhibition test were prepared by pouring a slightly modified PDA medium added with essential oils in petri dishes. In particular, 18.5 g of standard PDA formulate were added to 499 ml of deionized water and 1 ml of Tween-20, which were then autoclaved. Afterwards, media with 0.1%, 0.5% and 1% EO content were created by adding 0.5 ml, 2.5 ml and 5 ml of essential oils to 499.5 ml, 497.5 ml and 495 ml of PDA medium, respectively. Selected oil for this step were thyme, basil, oregano, fennel and lemon (Flora, Lorenzana, Italy).

### Inoculum preparation and in vitro inhibition test

*M. fructicola* strain CVG1539 was grown on MEA medium for 21 days. Mycelia plugs were removed and transferred in new MEA plates. Each MEA plate was then joined face-to-face with an essential oil plate in a sandwich configuration and closed with parafilm. Plates were incubated at 25°C for 14 days and subsequently the growth diameter of the pathogen was measured for the different treatments. Results were then compared to the untreated controls prepared in the same way using PDA. For each treatment, 5 biological replicates were measured.

### In vivo inhibition test of nectarines

For the in vivo test, fennel, basil and lemon essential oils were selected. EO dispenser plates were prepared as the in vitro inhibition test plates, but with a 2% EO. For each treatment, 3 replicates consisting of 30 uninoculated nectarines ‘Big Top’, were set up in plastic boxes inside a cabinet. Treatment was applied by placing 6 EO dispensers in each cabinet. In addition to these treatments, an untreated control and a chemical control treated with Switch (Syngenta Italia S.p.A., cyprodinil, a.i.: 37.5%;

fludioxonil, a.i.: 25%) formed by 3 boxes of 30 fruit each, were also set up. All the treatments were kept inside the cabinets at 1 ± 1 °C for 28 days and then transitioned to shelf-life conditions (20 ± 5 °C) for 5 days. Rot incidence was measured at the end of storage and the end of shelf-life.

**Quality analyses**

Quality analyses were performed at harvest ($t_0$), at the end of storage ($t_{0+28}$) and at the end of shelf-life ($t_{0+33}$). Selected quality parameters were fruit firmness, total soluble solids and titratable acidity. Fruit firmness was measured using a Fruit Texture Analyser penetrometer (FTA, Turoni, Italy) with a 8 mm tip. For each treatment, 5 fruit were measured twice on opposite sides of their equatorial region and the results mediated. Total soluble solids, in particular sugars, were measured using a NR151 refractometer (Rose Scientific Ltd, Canada). For each treatment, the juice of 3 groups of 3 fruit (one per replication) was extracted and a 1 ml aliquot was transferred on the instrument for the measurement. Finally, titratable acidity was measured using 6 g of each replication juice, which were added to 50 ml of water. Titration was carried out adding a 0.1 N NaOH solution up to a 8.2 pH endpoint, as measured by a FP20-Std-Kit FiveEasy Plus pHmeter (Mettler Toledo, Italy). Based on consumed NaOH volume, starting acidity was calculated using the formula:

$$\frac{VNaOH * 0.0067 * 100}{6}$$

where VNaOH are the millilitres of NaOH, 0.0067 is the acidity factor of malic acid and 6 is the grams of juice.

**RESULTS AND DISCUSSION**

Results for the in vitro inhibition test (**table 1**) show that, with the exception of 0.1% lemon, all considered EOs caused a significant reduction in *M. fructicola* growth compared to the untreated control, with the highest reduction associated to thyme, basil, fennel, and oregano. These results are in accordance with previous studies on essential oil carried out both in vitro and in vivo (Carovic-Stanko *et al.*, 2012; Lopez-Reyes *et al.*, 2013; Santoro *et al.*, 2018; Fontana *et al.*, 2021).

Table 1: percentage reduction in colony diameter for different treatments, compared to the untreated control. Significance groups were determined by one-way ANOVA followed by a Duncan post-hoc test, both with alpha < 0.05 .

| Treatment | Diameter reduction compared to control (%) | Significance group (Duncan test) |
|---|---|---|
| Thyme 1% | - 100 | a |
| Thyme 0.5% | - 100 | a |
| Thyme 0.1% | - 100 | a |
| Basil 1% | - 100 | a |
| Basil 0.5% | - 96 | a |
| Basil 0.1% | - 56 | b |

| | | |
|---|---|---|
| Oregano 1% | - 100 | a |
| Oregano 0.5% | - 100 | a |
| Oregano 0.1% | - 100 | a |
| Fennel 1% | - 100 | a |
| Fennel 0.5% | - 100 | a |
| Fennel 0.1% | - 28 | c |
| Lemon 1% | - 34 | c |
| Lemon 0.5% | - 16 | d |
| Lemon 0.1% | -9 | de |
| Control | / | e |

In the in vivo experiment on nectarines (**table 2**), all essential oil treatments showed an efficacy comparable with the chemical treatment in reducing rot incidence during storage. By the end of shelf-life, this efficacy was lost, with rot incidence values comparable to the untreated control. A possible explanation for this phenomenon is that the shelf-life was spent outside the cabinets, without atmosphere rich in essential oils. For this reason, we could hypothesize a fungistatic more than fungicidal activity.

Table 2: rot incidence values measured at the end of storage and at the end of shelf-life for nectarines treated with essential oils, as well as an untreated control and a chemical control. Significance groups were determined by one-way ANOVA followed by a Duncan post-hoc test, both with alpha < 0.05

| **Treatment** | **Rot incidence at the end of storage (%)** | **Rot incidence at the end of shelf-life (%)** |
|---|---|---|
| Untreated control | 9% b | 11% a |
| Chemical control | 1% a | 10% a |
| Lemon EO (2%) | 3% a | 11% a |
| Fennel EO (2%) | 2% a | 12% a |
| Basil EO (2%) | 4% a | 6% a |

The most frequently isolated genus from rotten fruit was *Monilinia* (84%), followed by *Penicillium* (7%), *Botrytis* (4%), *Alternaria* (2%) and *Rhizopus* (2%).

Fruit quality parameters (**figure 1**, **figure 2** and **figure 3**) were almost unaffected by EO treatments. No statistically significant difference was found in any time point for either titratable acidity or total soluble solids, whereas a statistically significant difference was found at the end of shelf-life, where treatments with all essential oils resulted in increased fruit firmness compared to the untreated control. Previous studies showed that application of essential oils through biofumigation in peaches and nectarines not only preserves fruit quality, but can also result in increased nutritional qualities, such as an higher title of vitamin C (Santoro *et al.,* 2018).

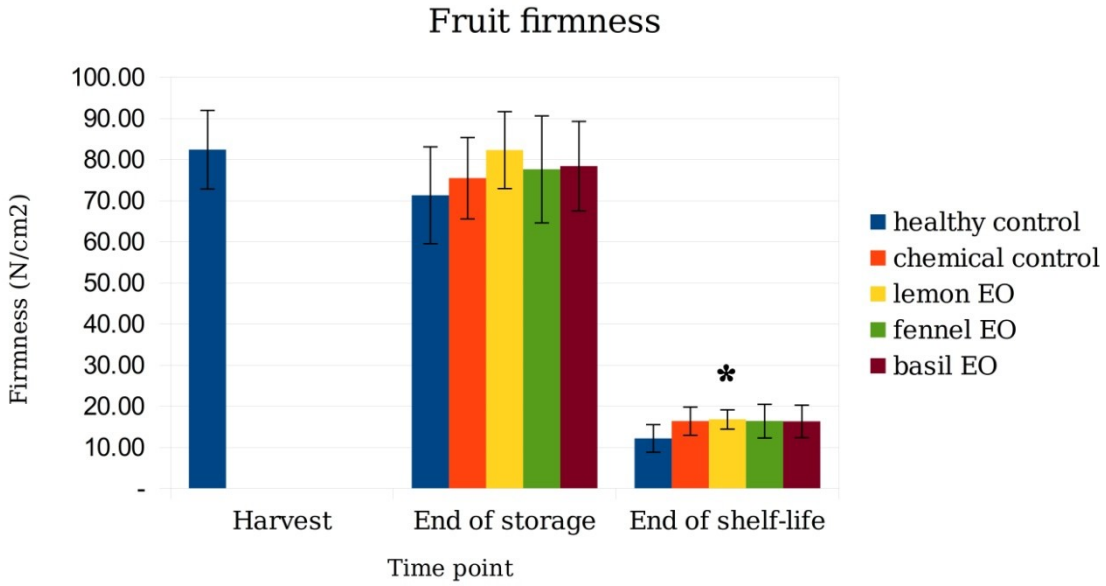


Figure 1: fruit firmness values measured for the three essential oil treatments, as well as for the untreated control and chemical control, at harvest, after 28 days of storage and after 5 days of shelf-life. The asterisk indicates the presence of a statistically significant difference, as measured by a one-way ANOVA followed by a Duncan posthoc test, both with alpha<0.05 .

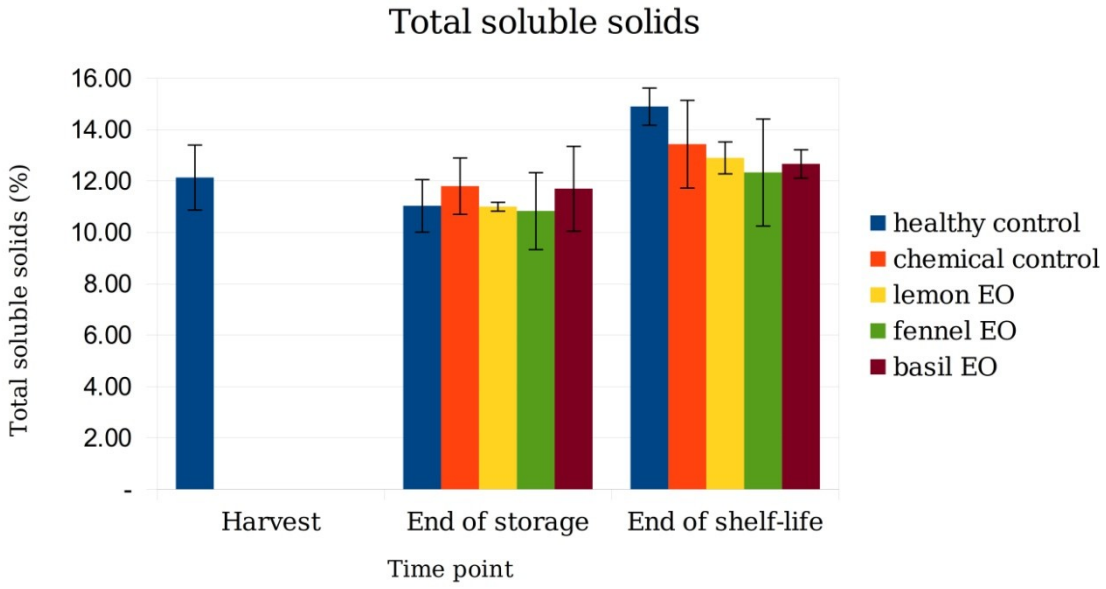


Figure 2: total soluble solids values measured for the three essential oil treatments, as well as for the untreated control and chemical control, at harvest, after 28 days of storage and after 5 days of shelf-life. No statistically significant difference was found, based on a one-way ANOVA with alpha<0.05 .

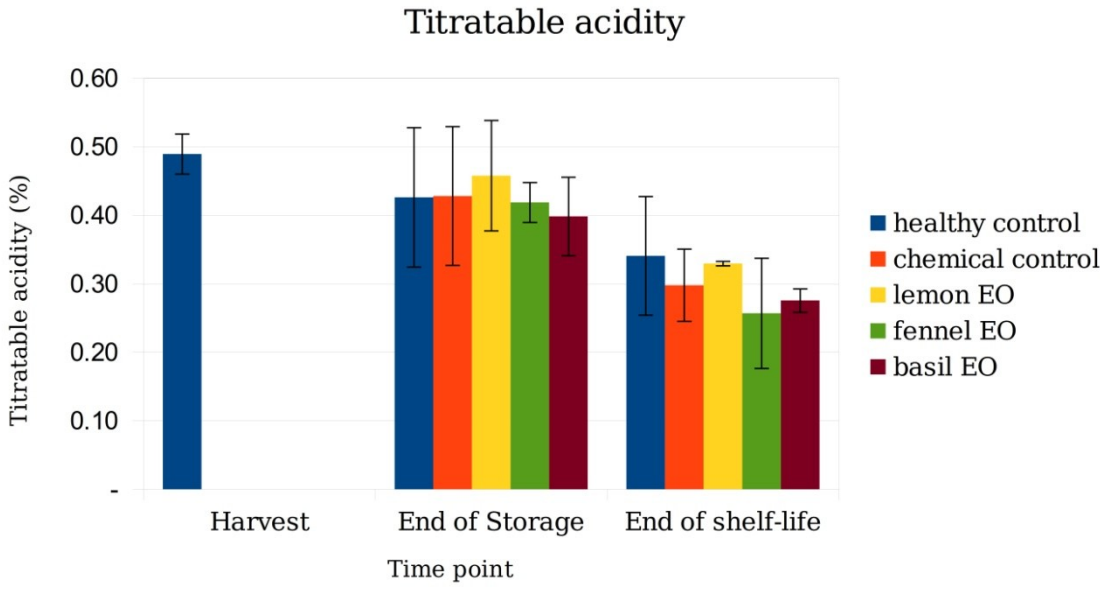


Figure 3: titratable acidity values measured for the three essential oil treatments, as well as for the untreated control and chemical control, at harvest, after 28 days of storage and after 5 days of shelf-life. No statistically significant difference was found, based on a one-way ANOVA with alpha<0.05 .

## CONCLUSIONS

Five essential oils (thyme, basil, oregano, fennel and lemon) were selected to test in vitro efficacy against *M. fructicola* growth. Three of them (lemon, fennel and basil) were then chosen to test biofumigation efficacy in

preventing natural *M. fructicola* inoculum development in nectarines. Results demonstrate that post-harvest control of *M. fructicola* can be achieved by biofumigation with essential oils. Additional studies will be necessary to improve the efficacy of this strategy and simplify the process, in particular regarding the necessity of a controlled atmosphere.

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