Evaluation of the antifungal activity of five aromatic plants essential oils against *Botrytis cinerea* and their efficiency for keeping quality of fresh raspberries and strawberries

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Abstract

Five essential oils (EOs) were hydrodistilled in a Clevenger-type apparatus from: *Artemisia absinthium*, *Artemisia herba-alba*, *Lavandula dentata*, *Thymus broussonnetii* and *Thymus leptobotrys* plants growing in Morocco. The EOs were tested for their antifungal activity first in vitro against *Botrytis cinerea* using agar dilution and disk diffusion methods. And then three EOs were tested for their ability to extend the shelf life of fresh raspberries and strawberries conserved at 4°C. In vitro, the difference between the concentrations of both EOs was significant. The essential oils of *Thymus leptobotrys* and *Thymus broussonnetii* inhibited the growth of fungus at 250 µl/L and 500 µl/L respectively. The both EOs were found more efficient preservatives for raspberries and strawberries during 12 days of cold storage. The results suggest that essential oils vapour may improve fruit quality of fresh raspberries and strawberries during storage and transit.

*Keywords*: essential oils, antifungal activity, *Botrytis cinerea*, shelf life, raspberries, strawberries.

1. Introduction

Raspberries and strawberries are highly perishable and susceptible to mechanical injury, physiological deterioration, water loss, and postharvest decay. These detriments have been identified as a major factor causing significant economic loss, especially in the fruit marketing chain [1]. *Botrytis cinerea* is one of the commonly present fungi causing severe post-harvest losses to fruits and vegetables. In recent years and according to its scientific and economic extent, this fungus was placed in the second rank in the world’s top ten pathogens listed [2].

With the aim of finding safe and eco-friendly alternatives to synthetic pesticides, which on one hand control the postharvest diseases and on the other hand conserve the quality of fresh fruits, essential oils (EOs) are catching the attention of scientists worldwide [3]. Due to their favourable characteristics
(being natural, volatile, environmentally safe, and biodegradable) they were considered as hopeful biopesticides to produce safe food [4].

The aim of our study was to evaluate first the in vitro antifungal potential of five aromatic plant essential oils against B. cinerea, and secondly the efficiency of three EOs to delay the postharvest decay of fresh raspberries and strawberries stored at 4°C.

2. Material and Methods

2.1. Essential oils

Aerial parts of five wild plants were collected from different regions of Morocco: *Artemisia absinthium*, *Artemisia herba-alba*, *Lavendula denta*, *Thymus broussonnetii*, and *Thymus leptobotrys*. A specimen was deposited in the herbarium of the laboratory of biotechnology, at the National School of applied sciences, Ibn Zohr University, Agadir, Morocco. Essential oils were obtained by hydrodistillation of the air-dried plants for 3h using a Clevenger type apparatus and preserved in the dark at 4°C until use.

2.2. Fungal culture of Botrytis cinerea

*Botrytis cinerea* was isolated from naturally decayed strawberries, using potato dextrose agar medium (PDA) amended with gentamycin (10 mg/L). A pure culture of this fungus was maintained on PDA and stored at 4°C.

2.3. In vitro antifungal assays

Two different antifungal assays were carried out with 6 concentrations of EOs: 125, 250, 500, 1000, 2000, and 4000 µL/L.

An agar dilution method was realized according to [5]. To obtain the desired concentrations, it consisted of dissolving pure essential oils in water containing 0.2 % Tween 80 (v/v) and adding appropriate amounts of this basic solution into autoclaved potato dextrose agar medium (PDA) maintained at 45°C just before pouring into Petri dishes. Then, a mycelial plug, 6 mm in diameter, was excised from a pure culture of the fungus and placed at the center of each Petri dish. Control consisted of un-amended PDA. The dishes were sealed with parafilm and incubated at 25°C.

A volatile effect was tested using a disk diffusion method according to [6]. Different amounts of pure essential oils were deposited on sterile filter paper disks, which were then placed on the inner surface of the Petri dish lids. The dishes were sealed with parafilm and incubated upside-down at 25°C. Control consisted of sterile distilled water-soaked filter paper in the lid of Petri dish. For both methods, the test was stopped when control Petri dishes were covered by mycelial culture.

All the experiments were done in three replicates, and for each replicate three plates were used. The percentage inhibition of fungal growth was determined on the growth in test plate compared to the control plate according to the equation: Inhibition %=100 (C-T)/C, where C was the diameter of fungal growth on the control and T was the diameter on the test plate.

2.4. Evaluation of essential oils on the shelf life of raspberries and strawberries

Three concentrations (200, 400, and 600 ppm) of *A. herba-alba*, *T. broussonnetii*, and *T. leptobotrys* EOs were prepared as aqueous emulsions using 0.4% Tween 80 and distilled water under vigorous
stirring. The aluminium and filter paper sheets used in this treatment were autoclaved at 120° during 30 minutes.

Batches of ten raspberries and six strawberries were tested separately per replicate. The berries were placed inside the containers under the aluminium foil. The EOs emulsions were sprayed onto fresh fruits at room temperature. A sterile filter paper was deposited in the container in the middle of the fruits to absorb the excess of the EOs solution. After spraying, fresh fruits were allowed to dry for 15 min on sterile aluminium sheets. Then, they were covered using a second sterile aluminium sheet and were stored at 4°C. Visual decay of raspberries and strawberries was evaluated for 12 days and the contamination level (percentage of berries showing decay signs) was calculated during storage. For treatments and controls (untreated), three replicates were made.

2.5. Statistical analysis
All data were subjected to analysis of variance (ANOVA) using SPSS 16.0 software. The means comparisons were made using Newman & Keuls tests. The results are presented as mean values ± standard deviation with different letters which are statistically significant at 5% level probability.

3. Results and discussion
3.1. In vitro antifungal assays
In both antifungal assays, the tested EOs reduced the growth of fungus at all concentrations, but to different degrees (Table 1). EOs from *T. broussonnetii* and *T. leptobotrys* completely inhibited the fungus in both antifungal methods at 500 and 250μL/L, respectively. These two EOs showed better effect in disc diffusion than in agar dilution method. All other EOs inhibited *B. cinerea* by 100% at 2000μL/L. The greatest antifungal potential for these three EOs was recurrent between the two assays. Our EOs have shown varying antifungal potential to inhibit mycelial growth of *B. cinerea*. This difference in the antifungal activity may be due to their chemical composition, structural configuration of these constituents, the activity of their functional groups and possible synergistic interactions between these constituents [7]. In general, the active antimicrobial compounds of essential oils are terpenes which have the potential to strongly inhibit microbial pathogens [8]. Other studies of antifungal effect of *Melissa officinalis* and *Pulicaria mauritanica* EOs against *B. cinerea* revealed that they were both better in diffusion than in dilution method [6; 9]. In our work, the antifungal effect of EOs in the two assays were generally no equal, it may be related to the difference volatility of major compounds in each EOs. To our knowledge, this is the first report on the antifungal activity of the tested EOs against *B. cinerea* isolated from strawberry fruit. Previous study was proved greatest antifungal effect of Thymus genus against *B. cinerea* [5]. In contrast to the present study, there was no efficiency of *A. absinthium, A. herba-alba* and *L. dentata* against *B. cinerea* isolated from tomato plant [5, 10]. This may be due to the concentrations tested or to the difference of fungal strains.
Table 1. Antifungal assays of essential oils from five aromatic plants against *Botrytis cinerea*

<table>
<thead>
<tr>
<th>Antifungal assays</th>
<th>Essential oils</th>
<th>Concentrations (µL/L)</th>
<th>Percent inhibition of mycelia growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>125</td>
<td>250</td>
</tr>
<tr>
<td><strong>Agar dilution</strong></td>
<td><em>A. absinthium</em></td>
<td>42.5±2.50 d</td>
<td>44.17±1.44 d</td>
</tr>
<tr>
<td></td>
<td><em>A. herba-alba</em></td>
<td>6.67±6.29 f</td>
<td>23±5.20 de</td>
</tr>
<tr>
<td></td>
<td><em>L. dentata</em></td>
<td>17.50±6.61 e</td>
<td>25.83±1.44 de</td>
</tr>
<tr>
<td></td>
<td><em>T. broussonnetii</em></td>
<td>40±4.33 d</td>
<td>55±6.61 c</td>
</tr>
<tr>
<td></td>
<td><em>T. leptobotrys</em></td>
<td>45.83±5.77 b</td>
<td>100±0.00 a</td>
</tr>
<tr>
<td><strong>Disc diffusion</strong></td>
<td><em>A. absinthium</em></td>
<td>30.83±12.33 e</td>
<td>40±6.61 de</td>
</tr>
<tr>
<td></td>
<td><em>A. herba-alba</em></td>
<td>15±2.50 ef</td>
<td>20±2.50 de</td>
</tr>
<tr>
<td></td>
<td><em>L. dentata</em></td>
<td>16.67±1.44 e</td>
<td>45±2.50 c</td>
</tr>
<tr>
<td></td>
<td><em>T. broussonnetii</em></td>
<td>70.83±5.77 b</td>
<td>98.33±1.44 a</td>
</tr>
<tr>
<td></td>
<td><em>T. leptobotrys</em></td>
<td>98.33±1.44 a</td>
<td>100±0.00 a</td>
</tr>
</tbody>
</table>

3.2. Evaluation of essential oils on the shelf life of raspberries and strawberries

After 12 days the non-treated controls of raspberries and strawberries were completely decayed. For raspberries, the lowest postharvest contamination (16.67±15.27%) was observed for fruits treated with 600ppm of *T. broussonnetii* EOs (Figure 1). Treatments with 400ppm and 600ppm *A. herba-alba* provided the higher postharvest decay (70±30%).

For strawberries, the lowest postharvest decay (44.33±9.81%) was observed for fruits treated with 400ppm *T. broussonnetii* EOs (Figure 2). Treatment with 400ppm *A. herba-alba* provided no protection.

Overall, *T. broussonnetii* EOs seemed to have the best efficiency to extend shelf life of raspberries and strawberries. *T. leptobotrys* EOs, which showed the highest level of in vitro inhibited of *B. cinerea* (Table 1), provided less protection than *T. broussonnetii* on fruits (Figure 1). Similar results were reported by [11] on *Origanum compactum*, which showed strong antimicrobial effect in in vitro tests but had little bioactivity in in vivo conditions. This may be due to interactions of phenolic compounds with the food matrix [12]. *Thymus vulgaris* and *Mentha piperita* EOs were also tested on strawberries;
and they were found efficient preservatives during 14 days [11]. Other natural volatile compounds were also found efficient to retard postharvest decay on raspberries [13].

**Figure 1.** Level of postharvest decay in raspberries treated with three EOs after 12 days at cold storage.

**Figure 2.** Level of postharvest decay in strawberries treated with three EOs after 12 days at cold storage.

### 4. Conclusion

Our results suggest that vapours from essential oils may preserve fruit quality during fresh raspberries and strawberries storage and transit. As essential oils are composed of many volatile compounds, the residual levels would be low after storage. However more work on other quality properties of raspberries and strawberries after storage is required.
References