SUMMARY

Bacterial wilt of sweet pepper, caused by Ralstonia solanacearum (Rs), is an important disease in Brazil. The effectiveness of bergamot, blue gum, cinnamon, clary sage, copal, fennel, lemon, lemongrass, lemon-scented eucalyptus, mint, palmarosa and sweet orange essential oils was evaluated for disease control via biofumigation. Soil infested by Rs CGM-8 was biofumigated with all oils (0.14%, v:v) in the greenhouse, and with bergamot, sweet orange and palmarosa oils in the field for four days. Variables evaluated were: latency period (LP50), incidence, bacterial wilt index (BWI), area under the disease progress curve (AUDPC), plant development, Rs population in the soil, soil characteristics and the in vitro growth of Rs. Palmarosa, bergamot and sweet orange oils increased LP50 (15%), reduced BWI (60%) and AUDPC (64.4%), in the greenhouse. In the field, only the palmarosa oil increased LP50 (38%), reduced BWI (36%) and AUDPC (38%), and increased the number of fruits per plant. Only greenhouse soils biofumigated with bergamot and sweet orange oils presented significant high sodium levels. The in vitro growth of Rs was reduced in 66.9% by palmarosa oil. These results indicate that palmarosa essential oil is a potential alternative for the management of bacterial wilt of sweet peppers.

Key words: Ralstonia solanacearum, sweet pepper, bacterial wilt control, essential oils, palmarosa, Cymbopogon martini

In the state of Pernambuco (Brazil), Rs race 1 has caused major damages in sweet pepper-producing municipalities. Most of the isolates belong to phylotype I, biovar 3, bio-type 8, but some are from phylotype II, biovar 1, biotypes 3 and 6 (Garcia et al., 2013).

No adequate control measures exist for curing bacterial wilt. Public interest in environmental issues has prompted agricultural alternatives which, among other measures, advocate control of plant diseases with plant extracts and essential oils (Schwan-Estrada et al., 2003). Soil biofumigation with essential oils was shown to reduce the incidence of bacterial wilt, even up to 100%. In a greenhouse, tomatoes transplanted to soils biofumigated with essential oils of thyme, palmarosa, lemongrass, Greek oregano and tea tree exhibited no symptoms of the disease (Pradhanang et al., 2003), the same as tomato and geranium plants in soils treated with clove oil (Huang and Lakshman, 2010).

Since there are no apparent literature report on the use of essential oils for managing bacterial disease in sweet peppers, a study was carried out for evaluating the effects of these oils via soil biofumigation on the control of bacterial wilt and their direct effects on Rs growth.

Oils (100% pure and natural) of bergamot (Citrus aurantium var. bergamia L.), blue gum (Eucalyptus globulus Labill.), cinnamon (Cinnamomum zeylanicum Blume), clary sage (Salvia sclarea L.), copal ( Copaifera officinalis (Jacq.) L.), fennel or lemon balm (Foeniculum vulgare dulce Mill.), lemon (C. limon (L.) Burm.), lemongrass (Cymbopogon citratus Stapf), lemon-scented eucalyptus (Eucalyptus citriodora Hook), mint (Mentha x piperita L.), palmarosa (Cymbopogon martini (Roxb.) J.F. Watson) and sweet orange (Citrus sinensis L.), were obtained from Bioessência (Florananda Industry and Commerce of Cosmetics and Natural Products, Brazil). These oils were emulsified by hand shaking in Tween 20 (1:1 ratio) to enhance their solubility.

The Rs strain CGM-8, phylotype I, race 1, biovar 3, bio-type 8 (Garcia et al., 2013), was cultured on TZC (triphenyl tetrazolium chloride) medium (Kelman, 1954) for 48 h at 30±2°C and transferred to NYDA (nutrient yeast dextrose agar) (Pusey and Wilson, 1984).

For the initial soil infestation, 60 ml of the Rs CGM-8 suspension (5×10⁸ CFU ml⁻¹) was added to 2 kg plastic bags containing natural soil, with the following characteristics: pH 6.8; P: 25 mg dm⁻³; Na⁺: 1.13 cmolₑ dm⁻³;
soil pH was further analyzed as follows: (a) prior to biofumigation with the 12 essential oils at 0.14%, (b) 1 h after, (c) 4 days after and (d) 7 days after biofumigation.

The soil in the greenhouse was biofumigated according to Paret et al. (2010). Seven days after bacterial soil infestation, 2.1 ml of each of the 12 oils were emulsified in 80 ml of water, uniformly distributed and mixed with 2 kg of infested soil (0.14% v:v). The soil was incubated for four days in sealed clear plastic bags, which were then left open for three days to release volatile compounds. Controls were: (i) Tween control I: containing Rs, Tween 20 and covered with plastic, but without the essential oil, to test the Tween effect on Rs; (ii) Tween control II: containing only Tween 20 to test Tween effect on the plants; and (iii) Solarization control: containing Rs, plastic-covered but without the essential oil and without Tween 20.

For quantification of resistance to bacterial wilt, 21-day-old sweet pepper seedlings of cv. Impact grown in polystyrene trays were transplanted to plastic pots containing 500 ml of soil previously infested and biofumigated with one of the 12 oils, and to the control pots. Plants were fertilized once a week (Souza, 2009), irrigated by wetting as needed and evaluated daily for 15 days for the presence of symptoms. The severity of the disease (SEV) was recorded using a scale from 0 to 4 (Nielsen and Haynes, 1960). Based on the SEV data the following components of disease resistance were determined: latency period (LP50), incidence (INC) (Silveira et al., 1999), bacterial wilt index (BWI) at 15 days (Empig et al., 1962), and area under the disease progress curve (AUDPC) (Shaner and Finney, 1977). The experimental design was randomized with five replicates. Each replicate consisted of four pots containing one plant each.

Based on the results obtained in the greenhouse, the bergamot, sweet orange and palmarosa oils (0.14%) were tested in the field. Biofumigation was conducted in plots of the experimental garden (UFRPE, Recife, Brazil) using the previously described methodology. Plastic bags containing 2 kg of natural soil infested with 60 ml of the pathogen suspension and biofumigated were arranged in the plots so that only 2 cm remained above the soil surface. Sweet pepper seedlings were transplanted at 21 days of age and sprinkler watered. Controls consisted of plants grown either in infested soil or in non-infested soil. LP50 and SEV were evaluated daily for 45 days, and SEV was used to calculate BWI and AUDPC. Surviving plants were brought to the laboratory to evaluate the fresh and dry biomass of roots and shoots (measured after 72 h at 65°C) and the sweet pepper yield (number and weight of fruits).

After the experiments, the Rs CGM-8 populations were analyzed by collecting soil samples (10 g) from the rhizosphere of each plant that were placed in Erlenmeyer flasks to which 90 ml of sterile distilled water were added. Serially diluted samples were plated on modified SMSA medium (semi-selective medium from South Africa) (Elphinstone et al., 1996) in triplicate. After incubation for 72 h at 30°C the bacterial population was determined as log CFU g⁻¹ soil. The experimental design was completely randomized with five replicates. Each replicate consisted of four plants per plot.

To analyze the in vitro effect on Rs, bergamot, sweet orange and palmarosa oils were added at 0.14% to NYDA medium and poured into dishes where aliquots (0.1 ml) of the pathogen suspension (5 × 10³ CFU ml⁻¹) were plated. The control consisted of NYDA medium without oil. After incubation for 72 h at 30°C the bacterial population was determined as log CFU g⁻¹ soil. The experimental design was completely randomized with three replicates. Each replicate consisted of one Petri dish.

The data were subjected to analysis of variance (ANOVA), and their means were compared by Tukey, least significant difference (LSD) or nonparametric Kruskal-Wallis tests (P ≤ 0.05) using Statistix 9 software. All experiments were duplicated at two different times. Because the data from each pair of experiments exhibited no significant differences, they were analyzed together.

Palmarosa oil significantly increased (P ≤ 0.05) the LP50 (6.6 days), while palmarosa, bergamot and sweet orange oils reduced BWI (18.5, 22.9, 26.4, respectively) and AUDPC (3.1, 3.5, 4.7, respectively) compared with the control (LP50 = 5.6; BWI = 46.0 and AUDPC = 8.7) (Table 1). Tween and solarization did not affect Rs CGM-8 and Tween did not affect the plants.

Under field conditions, only palmarosa oil maintained efficacy for disease control, significantly increasing (P ≤ 0.05) the LP50 (15.7) and reducing BWI (32.3) and AUDPC (1.6) compared with the control (11.4, 49.9 and 2.6, respectively) (Table 2).

Biofumigation is the most common method for applying essential oils to control diseases caused by soil-inhabiting pathogens, such as the agents of bacterial wilt of tomato (Huang and Lakshman, 2010; Ji et al., 2005; Pradhanang et al., 2003), geranium (Huang and Lakshman, 2010) and edible ginger (Paret et al., 2010). Palmarosa and thyme have provided complete control (100%) of bacterial wilt via biofumigation in greenhouse-grown tomatoes, although only palmarosa oil maintained a significantly lower (20%) incidence of wilt in a second experiment (Pradhanang et al., 2003). However Ji et al. (2005) demonstrated that tomato wilt incidence in the field was lower when the soil was biofumigated with thyme (33.1%) than with palmarosa (48.1%) oils.

In the greenhouse, the pH of soils biofumigated with the 12 essential oils did not differ significantly (P ≤ 0.05) from that of controls for each sampling period. However,
regardless of the treatment, soil pH before and 1 h after biofumigation differed significantly from that at four and seven days post biofumigation for the pH originally ranged from 7.51 to 7.81 and after seven days, it ranged from 6.68 to 7.24 (Fig. 1). Thus, pH-dependent reactions in the soil are not influenced by biofumigation. 

The final levels of P, K+, Ca^{2+}+Mg^{2+}, Ca^{2+}, Al^{3+} and H+Al did not differ significantly (P ≤ 0.05) between controls and soils treated with bergamot, sweet orange and palmarosa oils in the greenhouse experiments. However, soils treated with bergamot (0.98 cmol, dm^{-3}) and sweet orange (0.63 cmol, dm^{-3}) oils exhibited higher Na levels compared with the control (0.08 cmol, dm^{-3}) and with palmarosa oil treated soils (0.05 cmol, dm^{-3}). There were no differences between these two last treatments. Rs supports NaCl levels in liquid culture medium up to 2% (Álvarez et al., 2010) and can enter the viable but nonculturable stage (VBNBC) in response to a saline solution, among other factors (Grey and Steck, 2001).

In the field, no significant differences (P ≤ 0.05) were observed among controls and soils treated with bergamot, sweet orange and palmarosa oils at 0.14% for levels of P, K+, Ca^{2+}+Mg^{2+}, Ca^{2+} and Na+, root and shoot fresh and dry biomass (g plant^{-1}), or average yield (g fruit plant^{-1}).

Palmarosa oil significantly increased the number of fruits per plant (10.2) grown in non-infested soil over that of the infested control (6.0). Thus, in addition to reducing the incidence and severity of the disease, palmarosa oil allowed the plant to finish its cycle and produce the same as a healthy crop.

The average fruit weight of cv. Impact (30.5 g), including the control, was less than the standard weight of a commercial green sweet pepper fruit, probably due the occurrence of temperatures above 35°C during cultivation (Henz et al., 2007). In contrast, the growth and yield of ginger plants were unaffected in soils treated with essential oils of lemongrass, blue gum and palmarosa (Paret et al., 2010).

The level of the Rs CGM-8 population in the rhizosphere soil at the end of the experiments did not vary significantly (P ≤ 0.05) between that registered in the control (4.89 log CFU g soil^{-1}) and in soils treated with palmarosa (4.64 log CFU g soil^{-1}), sweet orange (4.68 log CFU g soil^{-1}) and bergamot oils (4.75 log CFU g soil^{-1}) at 0.14%. Pradhananag et al. (2003) reported a decline in the Rs race 1 populations to undetectable levels in soils treated with thyme, palmarosa and lemongrass oils in pots planted with tomatoes. In addition, neither immunostrips nor plating on SMSSA medium were able to detect Rs race 4 populations in soils biofumigated with palmarosa and lemongrass oils after the removal of ginger plants (Paret et al., 2010). In the present study, chemotaxis exerted by several amino acids, organic acids and specific plant exudates from host plants may explain Rs survival in the rhizosphere and surrounding soil (Álvarez et al., 2010).

### Table 1. Reduction of bacterial wilt in greenhouse-grown sweet peppers via soil biofumigation with essential oils evaluated according to the latency period (LP_{50}), the bacterial wilt index (BWI) and the area under the disease progress curve (AUDPC).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LP_{50}^{b}</th>
<th>BWF</th>
<th>AUDPC^{d}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmarosa</td>
<td>6.6^{a}</td>
<td>18.5</td>
<td>3.1</td>
</tr>
<tr>
<td>Copal</td>
<td>6.4 ab</td>
<td>32.4</td>
<td>abcd 5.9</td>
</tr>
<tr>
<td>Lemon-scented eucalyptus</td>
<td>6.1 ab</td>
<td>28.6</td>
<td>abcd 4.7</td>
</tr>
<tr>
<td>Bergamot</td>
<td>6.0 ab</td>
<td>22.9</td>
<td>cd</td>
</tr>
<tr>
<td>Lemongrass</td>
<td>5.9 ab</td>
<td>34.8</td>
<td>abcd 6.0</td>
</tr>
<tr>
<td>Sweet orange</td>
<td>5.9 ab</td>
<td>26.4</td>
<td>bc</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>5.9 ab</td>
<td>37.1</td>
<td>abc</td>
</tr>
<tr>
<td>Blue gum</td>
<td>5.9 ab</td>
<td>37.5</td>
<td>abc</td>
</tr>
<tr>
<td>Clary sage</td>
<td>5.7 ab</td>
<td>38.9</td>
<td>abc</td>
</tr>
<tr>
<td>Fennel</td>
<td>5.6 b</td>
<td>37.9</td>
<td>abc</td>
</tr>
<tr>
<td>Lemon</td>
<td>5.6 b</td>
<td>45.5</td>
<td>a</td>
</tr>
<tr>
<td>Mint</td>
<td>5.5 b</td>
<td>43.1</td>
<td>ab</td>
</tr>
<tr>
<td>Control</td>
<td>5.6 b</td>
<td>46.0</td>
<td>a</td>
</tr>
</tbody>
</table>

CV (%) 8.8 30.6 32.0

* For biofumigation, the essential oils were emulsified in Tween 20 (1.1, v:v) and mixed with water. This emulsion was added and mixed to the soil (0.14%) infested with *Ralstonia solanacearum* CGM-8. The soil was incubated for four days in sealed transparent plastic bags. The 21-day-old sweet pepper plants were transplanted to pots with infested soil seven days after soil treatment.

b LP_{50} = Latency period, number of days required for the appearance of wilt in 50% of the plants.

BWI = Bacterial wilt index, calculated according to Empig et al. (1962).

AudPCI = Area under the disease progress curve, calculated according to Shaner and Finney (1977).

Mean of eight replicates. Each value represents the mean of two experiments.

Mean values followed by the same lowercase letter in the column do not differ significantly according to the Tukey test (P ≥ 0.05).

### Table 2. Reduction of bacterial wilt in field-grown sweet peppers via soil biofumigation with essential oils evaluated according to the latency period (LP_{50}), the bacterial wilt index (BWI) and the area under the disease progress curve (AUDPC).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LP_{50}^{b}</th>
<th>BWF</th>
<th>AUDPC^{d}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmarosa</td>
<td>15.7^{a}</td>
<td>32.3</td>
<td>b</td>
</tr>
<tr>
<td>Bergamot</td>
<td>13.6 ab</td>
<td>36.4</td>
<td>ab</td>
</tr>
<tr>
<td>Sweet Orange</td>
<td>13.0 b</td>
<td>38.5</td>
<td>ab</td>
</tr>
<tr>
<td>Control</td>
<td>11.4 b</td>
<td>49.9</td>
<td>a</td>
</tr>
</tbody>
</table>

CV (%) 19.1 41.2 43.9

* For biofumigation, the essential oils were emulsified in Tween 20 (1.1, v:v) and mixed with water. This emulsion was added and mixed to the soil (0.14%) infested with *Ralstonia solanacearum* CGM-8. The soil was incubated for four days in sealed transparent plastic bags. The 21-day-old sweet pepper plants were transplanted to pots with infested soil seven days after soil treatment.

b LP_{50} = Latency period, number of days required for the appearance of wilt in 50% of the plants.

BWI = Bacterial wilt index, calculated according to Empig et al. (1962).

AUDPC = Area under the disease progress curve, calculated according to Shaner and Finney (1977).

Mean of eight replicates. Each value represents the mean of two experiments.

Mean values followed by the same lowercase letter in the column do not differ significantly according to the Tukey test (P ≥ 0.05).
In vitro growth of *R.* CGM-8 was significantly reduced by palmarosa essential oil (4.91 log CFU ml⁻¹) (Fig. 2) compared with the control (7.34 log CFU ml⁻¹), in accordance with the notion that the growth of *R.* races 1 and 4 was inhibited by various essential oils, such as palmarosa, lemon-grass (Paret et al., 2010), citronella, rosemary, lemon balms (Amorim et al., 2011; Martins et al., 2009, 2011), cloves and ginger (Amorim et al., 2011). These oils contain volatile antimicrobial substances that participate in the biofumigation process. The monoterpenes, primary component of palmarosa oil (73-80%) (Jirovetz et al., 2006) exhibit lipophilic characteristics which cause the disruption of cell wall, alteration of molecular transport, inhibition of the enzymatic activity, and coagulation of cytoplasmic content (Sikkema et al., 1995).

In conclusion, soil biofumigation with palmarosa essential oil reduced the severity of bacterial wilt of sweet peppers in both the greenhouse and field, it was not detrimental to plant growth and increased the number of fruits per plant. Furthermore, it did not affect the pH nor the characteristics of biofumigated soil, and caused a clear-cut...

---

**Fig. 1.** Effects of biofumigation with the essential oils on the soil pH, evaluated prior to application of the oil and 1 h, 4 days and 7 days after application.

**Fig. 2.** Effects of the essential oils on the growth of *Ralstonia solanacearum* CGM-8 in the culture medium after 72 h incubation, evaluated by counting the colonies on the plates. The data were transformed using log (CFU ml⁻¹). Mean values followed by the same lowercase letter do not differ significantly according to the non-parametric Kruskal-Wallis test (P ≤ 0.05).
decrease of Rs growth, thus representing a potential alternative for controlling bacterial wilt of sweet peppers.

ACKNOWLEDGEMENTS

The authors thank the CNPq and FACEPE for financial aid (Proc. 309.697/2011-5 and APQ 1574-5.01/12 respectively), and for the scholarships provided.

REFERENCES


