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Evaluation of essential oils for preventive or curative management of melon gummy stem blight and plant toxicity

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Melon cultivation is frequently heavily reliant on synthetic fungicides, including products used to control gummy stem blight caused by Stagonosporopsis cucurbitacearum. The essential oils used in controlling plant pathogens may offer an alternative to chemical pesticides. This study evaluated the effectiveness of essential oils to control the gummy stem blight in melon plants. In vitro tests were carried out using essential oils obtained from ripe noni fruit (Morinda citrifolia) and dehydrated leaves of the following plants: lemon grass (Cymbopogon citratus), citronella grass (Cymbopogon nardus), basil (Ocimum basilicum) and Mexican tea (Chenopodium ambrosioides) at different concentrations. A synthetic fungicide was used as control treatment. Results showed that the essential oils from noni and lemongrass had the highest effect on mycelial growth inhibition in S. cucurbitacearum. When applied on melon plants as a preventive measure, the essential oils from noni and lemongrass controlled gummy stem blight at the following concentrations: 0.03, 0.05, 0.1 and 0.3%. These results highlight the potential of essential oils to manage melon fungal diseases, which may result in reduction of pesticide application.

Key words: Cucumis melo, Stagonosporopsis cucurbitacearum, Didymella bryoniae, alternative control, plant disease.

INTRODUCTION

Gummy stem blight (GSB) is a major disease that strikes several cucurbit species and is caused by Stagonosporopsis cucurbitacearum (Fr.) Aveskamp, Gruyter & Verkley, also known as Didymella bryoniae (Auersw.) Rehm (Steward et al., 2015). The fungus causes seedling damping-off, foliar lesions, as well as, stalk and stems cankers. It can be universally found in every continent and attacks at least 12 genera and 23

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species of cucurbit plants (Keinath, 2011), including some of the most popular crops grown in Brazil, such as watermelon, *Citrullus lanatus*, (Thunb.) Matsum. & Nakai and melon (*Cucumis melo* L.). Growers have considered synthetic fungicides to control cucurbit diseases. However, in addition to environmental pollution and contamination of consumable products, several cases of resistance to the main active fungicides on the market have been reported (Thomas et al., 2012; Keinath, 2013). Thus, there is a need for alternative control measures, such as dry season cultivation, thus avoiding environmental conditions favorable to the disease. According to Santos et al. (2011), leaf wetness, high relative humidity and temperatures between 20 and 30°C favor the development of the pathogen. Other methods of reducing the damage caused by GSB, include the use of partial genetic resistance (Santos et al., 2009) and biocontrol (Zhao et al., 2012).

Plant essential oils have been in use around the world mainly as insect repellents (Isman, 2000). More recently, their effect on the control of plant pathogens has aroused the interest of a number of researchers. For example, Guimarães et al. (2011) confirmed the inhibition of *Alternaria alternata in vitro* by the essential oil of lemon grass (*Cymbopogon citratus* (DC.) Stapf) and Aguiar et al. (2014) demonstrated the fungitoxic potential of eucalyptus essential oils (*Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson) and citronella grass (*Cymbopogon nardus* (L.) Rendle) in the *in vitro* control of *Pyricularia grisea*, *Aspergillus* spp. and *Colletotrichum musae*. In another study, Hussain et al. (2008) described how basil oil (*Ocimum basilicum* L.) showed fungitoxic activity on *Aspergillus niger*, *Mucor mucedo*, *Fusarium solani*, *Botryodiplodia theobromae* and *Rhizopus solani*.

This study describes the effectiveness of the application of essential oils of noni fruit (*Morinda citrifolia* L.), lemon grass, citronella, basil and mastruz (*Chenopodium ambrosioides* L.) in the control of *S. cucurbitacearum* and of the gummy stem blight disease in melon plants.

**MATERIALS AND METHODS**

The study was carried out at the Phytopathology Laboratory of the Federal University of Tocantins, Gurupi campus as follows: one isolate of *S. cucurbitacearum* was taken from naturally infected melon plants in the municipality of Gurupi, TO. The identity of the isolate was confirmed by using the primers D7S (Koch and Utkhede, 2002) and UNLO28S22 (Bakkeren et al., 2000), which amplified 535 bp fragments of *S. cucurbitacearum*, in accordance with Gasparotto et al. (2011).

**Securing the essential oils**

The essential oils obtained from ripe noni fruit and the dehydrated leaves of lemon grass, citronella grass, basil and mastruz were extracted by the hydro distillation method, which consists of depositing 0.02 kg of material for extraction in 500 ml of distilled water for a period of two hours in a *Clevenger apparatus* (1928). At the end of the extraction period, the essential oils were collected in the form of supernatant, stored in amber flasks, identified, and finally kept at 4°C until the bioassay was implanted (Seixas et al., 2012, adapted).

**Identification and quantification of the chemical constituents of essential oils**

Qualitative analyses of the essential oils were carried out by gas chromatography coupled to GC-MS mass spectrometry. The constituents were identified by comparing their spectral profiles with those in the databases of the 229 Nist and Wiley libraries. Compounds had their identities confirmed by comparing their calculated retention indices with those found in Nist (2015) and in the literature (Adams, 2007). The retention index of a component is a number, obtained by interpolation, which links the retention time of the component under study to the retention time of two standards (usually hydrocarbons) eluted before and after the peak of the compound examined (Inczedy et al., 1998).

The quantification of compound contents, expressed as a percentage based on the normalization of areas, was obtained by gas chromatography and Shimadzu GC-2010 flame ionization detector (DIC).

**In vitro mycelial growth inhibition tests**

*In vitro* bioassays were carried out on Petri dishes (70 mm diameter) by testing different concentrations of essential oils (0.0, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0%) and were compared with the synthetic fungicide Methyl Thiophanate + Chlorothalonil at 2000 ppm (Cerconil®), which served as a control treatment. A completely randomized experimental design was used in a factorial scheme, with four replications over five evaluation periods (2, 4, 6, 8 and 10 days of incubation). Each plate with an oil combination of X concentration was considered an experimental unit.

The different essential oil concentrations were diluted in a sterile water solution added to Tween 80 (1.0%). Subsequently, 100 µl of the different concentrations were scattered on the surface of the BDA culture medium with the aid of a Drigalsky loop. Next, in the center of each plate, a disk (4 mm) containing mycelium of *S. cucurbitacearum* was placed. The plates were sealed, identified and kept in an incubation chamber at 25°C for ten days and a 12 h photoperiod.

The evaluation consisted of measuring the mean diameter of the colonies in two diametrically opposed directions, at regular intervals of 48 h. From the values obtained of the mean diameter of the fungus, the mycelial growth rate index (MGVI) was calculated, using the formula described by Maia et al. (2011). Regression equations of mycelial growth in cm vs. time period in days were also calculated.

**Phytotoxicity tests in melon plants**

Phytotoxicity tests were performed applying the essential oils of noni fruit and lemon grass to melon plant at five concentration levels (0.1, 0.5, 1.0, 1.5 and 2.0% v/v). Two controls were used: one with the application of distilled water only and the other with a distilled water solution mixed with Tween 80.

Aliquots of essential oils were added to a stock solution of Tween 80 (1.0%), to obtain the different concentration levels, duly homogenized in a laboratory stirrer. Melon plants cv. Eldorado 300° were sprayed with 10 mL of essential oil solutions using a hand
spray. The plants were then kept in the laboratory at 25°C for 24 h. Phytotoxicity was estimated based on scales adapted from Dequech et al. (2008), Freitas et al. (2009) and Cogliatti et al. (2011); 0% = absence of phytotoxicity; 1 - 25% = mild leaf necrosis or mild chlorosis of the plant; 26 - 50% = moderate leaf necrosis or moderate chlorosis of the plant; 51 - 75% = high leaf necrosis or high chlorosis of the plant; 76 - 100% = wilt and dryness of the plant.

Preventive and curative control tests on melon plant

Following the results of the phytotoxicity tests, the concentrations of essential oils of noni fruit and lemongrass were suitably adjusted for testing the controllability of GSB in melon plants cv. Eldorado 300®, in preventive and curative applications. Six different concentrations at graduated levels (0.03, 0.05, 0.1, 0.3, 0.5 and 0.75% v/v) were prepared in the same manner as described above, in addition to treatment with the fungicide Methyl Thiophanate + Chlorothalonil at 2000 ppm (Cerconil®) and a control with distilled water only.

A conidial solution of S. cucurbitacearum was prepared by adding 20 mL of distilled water to fungal colonies growing in PDA medium. Conidia were then removed using a soft-bristle brush. The solution was filtered through gauze and the conidia quantified in a Neubauer chamber, adjusting the concentration to 10⁶ conidia mL⁻¹ before inoculation to the melon plants.

The evaluation of the effect of preventive application of the different concentrations of essential oils was carried out by manually spraying plants at the growth stage of two well-defined fully expanded leaves (20 days after planting). Once the essential oil solution had dried (c. 2 h after the application), the pathogen was inoculated to the leaves and the plants were kept in a humid and dark chamber for 48 h. Subsequently, the melon plants were transferred to a natural environment with temperatures ranging from 26 to 34°C for four more days. The severity of the disease was assessed four days after inoculation, using the scale adopted by Santos et al. (2005) where 0 = healthy plant; 1 = less than 1% of diseased leaf area; 2 = 1 to 5% of diseased leaf area; 3 = 6 to 25% of diseased leaf area; 4 = 26 to 75% of diseased leaf area; 5 = 76 to 90% of diseased leaf area; 6 = more than 90% of diseased leaf area.

To verify the curative effect of the essential oils, plants of the same age described in the previous experiment were first inoculated with 10 mL of conidia solution at a concentration of 10⁵ conidia mL⁻¹ and kept in a dark, humid chamber for 48 h. After five days, the presence of lesions characteristic of GSB in the leaves was confirmed, after the application of different concentrations of essential oils. Disease severity assessments were made following the methodology prescribed for preventive control.

The in vitro data of mycelial growth as a function of essential oil dosage, phytotoxicity and in vivo effect to the melon plants were evaluated by analysis of variance, and divergence of means by Tukey test, as well as regression analysis. The quality of fit of the regression equations was evaluated to determine whether coefficients had 1 or 5% probability.

RESULTS

In vitro mycelial growth inhibition tests

The results of the mycelial growth tests of S. cucurbitacearum at different essential oils concentrations are shown separately for noni fruit and lemon grass (Table 1) and citronella grass, basil and mastruz (Table 2).

Starting at the 0.5% dosage level, there was inhibition of mycelial growth by noni fruit essential oil, rising to total inhibition of fungus growth at concentrations of 1% and above (Table 1). The reading of the mycelial growth index (MGI) was also significantly reduced at the 0.5% level (Table 1). There is little information on noni fruit essential oil in terms of antifungal activities.

Lemon grass presented an inhibitory effect on mycelial growth in S. cucurbitacearum starting at the 3.5% concentration level, where total inhibition of fungal development was observed (data not shown). From the 2.5% concentration level upwards, the inhibition measured by the MGI was equivalent to the inhibition produced by the synthetic fungicide (Table 1). On the tenth day of incubation, the fungus almost reached
maximum mycelial growth (73 mm) irrespective of concentration level, except for the concentration of 3.0% lemon grass with a diameter of 67.2 mm. Only the synthetic fungicide was able to significantly reduce the development of fungus, with mycelial growth of 45.2 mm by the tenth day.

The essential oils effects of citronella grass, basil and mastruz on the mycelial growth of *S. cucurbitacearum* are presented in Table 2. On the tenth day of the evaluation, Citronella grass oil caused the lowest mycelial growth (23.0 mm) and MGVI (3.1) at a concentration level of 5.0%. Basil and mastruz did not control fungus growth satisfactorily.

### Chemical constituents of essential oils

The qualitative and quantitative analyzes of the components present in noni fruit and lemongrass oils are presented in Table 3. The analyses showed the presence of octanoic acid, also called caprylic acid, as the major component of noni essential oil (82.24%), followed by hexanoic acid (caproic acid, 8.26%). Liu et al. (2008), reported high inhibitive capacity of mycelial growth for both caprylic and caproic acids on *Altermaria solani*, *Colletotrichum lagenarium*, *Fusarium oxysporum f. sp. cucumerinum* and *Fusarium oxysporum f. sp. lycopersici*, as well as the inhibition of spore germination.

The major components of lemongrass essential oil were geranial (41.46%) and neral (32.43%) which were also detected in the essential oil of *Lippia rehmannii* by Linde et al. (2010), which, in similar concentrations, showed inhibition of the growth of hyphae of *Fusarium oxysporum* and *Rhizoctonia solani*, important plant pathogens. Thus, the origin of the geranial and neral compounds from botanical species of different families did not alter their fungistatic effect.

### Phytotoxicity of essential oils to the melon plant

Phytotoxicity tests were performed with the essential oils of noni and lemon grass, since they presented the best results in the *in vitro* tests (Figure 1). Noni fruit essential oil caused phytotoxicity right from the initial concentration level of 0.1%. However, at this concentration level, the damage was minimal, with 1 to 25% of the leaves presenting mild chlorosis and slight necrosis. Lemon grass essential oil caused phytotoxicity at a concentration level of 0.5% to the melon plant. However, at concentration levels in excess of 1.0%, there was increasing phytotoxic symptoms (chlorosis and necrosis).

### Evaluation of preventive and curative controls in melon plant

Both essential oils of noni fruit and lemongrass were efficient in preventive applications only, giving rise to major reductions in the severity of GSB (Table 4), and the concentration levels of 0.03, 0.05, 0.1 and 0.3% for the two essential oils were efficient in controlling the disease.
Table 3. Relative percentages (% area), obtained by Gas Chromatography, coupled to a Mass Spectrometry Detector, of the constituents of essential oils. Gurupi-TO, 2015.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Retention Index**</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Methyl-3-butenyl-1-acetate</td>
<td>888</td>
<td>-*</td>
</tr>
<tr>
<td>2-Heptanone</td>
<td>897</td>
<td>-</td>
</tr>
<tr>
<td>Methyl Hexanoate</td>
<td>922</td>
<td>-</td>
</tr>
<tr>
<td>Hexanoic Acid</td>
<td>987</td>
<td>8.26</td>
</tr>
<tr>
<td>Ethyl Hexanoate</td>
<td>999</td>
<td>2.48</td>
</tr>
<tr>
<td>Methyl octanoate</td>
<td>1123</td>
<td>-</td>
</tr>
<tr>
<td>Octanoic Acid</td>
<td>1177</td>
<td>82.24</td>
</tr>
<tr>
<td>Ethyl octanoate</td>
<td>1196</td>
<td>-</td>
</tr>
<tr>
<td>Isopentyl Hexanoate</td>
<td>1259</td>
<td>1.60</td>
</tr>
<tr>
<td>3-Methyl-2-butenyl Hexanoate</td>
<td>1292</td>
<td>-</td>
</tr>
<tr>
<td>3-Methylbutyl octanoate</td>
<td>1457</td>
<td>4.25</td>
</tr>
<tr>
<td>3-Methylbutyl-2-enyl octanoate</td>
<td>1489</td>
<td>-</td>
</tr>
</tbody>
</table>

**Cymbopogon citratus**

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Retention Index**</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mircene</td>
<td>986</td>
<td>9.73</td>
</tr>
<tr>
<td>(Z)-β-Oxime</td>
<td>1020</td>
<td>0.32</td>
</tr>
<tr>
<td>(E)-β-Oxygen</td>
<td>1029</td>
<td>0.16</td>
</tr>
<tr>
<td>Linalool</td>
<td>1074</td>
<td>1.64</td>
</tr>
<tr>
<td>Neral</td>
<td>1209</td>
<td>32.43</td>
</tr>
<tr>
<td>Geraniol</td>
<td>1220</td>
<td>4.52</td>
</tr>
<tr>
<td>Geranial</td>
<td>1239</td>
<td>41.46</td>
</tr>
<tr>
<td>2-Undecanone</td>
<td>1359</td>
<td>0.35</td>
</tr>
<tr>
<td>Geranyl acetate</td>
<td>1443</td>
<td>0.42</td>
</tr>
<tr>
<td>E-Caryofylene</td>
<td>1641</td>
<td>0.17</td>
</tr>
<tr>
<td>Others</td>
<td>-</td>
<td>8.8</td>
</tr>
</tbody>
</table>

*Not quantified (values < 0.05). **The retention index is a component of a number, obtained by interpolation, relating the retention time of a component under study to the retention time of two patterns (generally hydrocarbons) eluted before and after the peak in interest (Inczedy et al., 1998).

Table 4. Severity of gummy stem blight in melon plant as a function of preventive and curative application of different concentrations of the essential oils of noni fruit (*Morinda citrifolia*) and lemon grass (*Cymbopogon citratus*). Gurupi-TO, 2015.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration/Severity (%age Diseased Foliar Area)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>Noni preventive</td>
<td>75.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Noni curative</td>
<td>75.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lemon grass preventive</td>
<td>75.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lemon grass curative</td>
<td>75.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Means followed by the same letter in the column do not differ from each other according to Tukey test at 5% probability.

Concentrations of 0.5 and 0.75% were also tested; however, these levels were found to provoke a high degree of phytotoxicity in plants. The curative application was not efficient in disease control and, therefore, is not recommended for GSB management.
FIGURE 1. Percentage of foliar area damaged (phytotoxicity) as a function of different concentration levels of two essential oils of noni fruit (*Morinda citrifolia*) and lemon grass (*Cymbopogon citratus*) in melon plant.

**DISCUSSION**

**In vitro essays**

There are reports relative to the use of juice from noni fruits to treat diabetes, diarrhea, pain, hypertension, arthritis, stress and cancer. More than 160 chemical constituents present in noni fruit juice have been identified, including asperuloside, scopolamine and antraquinones, as well as vitamins and several amino acids (Franchi et al., 2008). However, so far, the effect of noni extract on the causative agent of GSB is yet to be investigated. Sarmento-Brum et al. (2014) found mycelial growth of *D. bryoniae* (= *S. cucurbitacearum*) of 22.4 mm at ten days of incubation at a concentration of 0.15% of citronella grass essential oil. Seixas et al. (2012) also reported that citronella grass essential oil showed higher inhibition "in vitro" of the causative agent of GSB than the other essential oils. However, these results were not confirmed by the present study. The essential oils from basil and mastruz had no fungitoxic potential at ten days of incubation, though there was some delay on the growth rate of hyphae, as measured by MGVI (mainly basil essential oil).

**Phytotoxicity**

The phytotoxicity test determines the dosage that is effective for controlling the disease, whilst sparing the plant host of damage. Thus, an essential oil efficient in controlling such a disease may be inadequate for use, due to the intolerance of plants to the chemical constituents present in the oil, even in low concentrations. Silva et al. (2012) did not observed lemon grass phytotoxicity to lettuce (*Lactuca sativa*) after application at concentration <1.0%. The evaluation of phytotoxicity is an important both at field level as well as in post-harvest too. Oliveira Junior et al. (2013) used essential oil of redfish, *Schinus terebinthifolius*, at a concentration level of 0.5% (v/v) in papaya fruit, to test protection against *Colletotrichum gloeosporioides*. Despite the promising "in vitro" response, the oil cannot be recommended due to the high levels of phytotoxicity to the fruit which make it unfit for marketing.

**In vivo essays**

Perini et al. (2011) have also observed similar results with those obtained in the present study, evidencing the superiority of preventive over curative control in rice blast control. Another result that proves the efficiency of the essential oils effect against pathogenic fungi was developed by Tomazoni et al. (2017), which obtained control of *Alternaria* in tomato plants, both in preventive and curative treatment. These results demonstrate that the efficiency of the application type varies according to
the essential oil content and fungus species.

Conclusions
Noni fruit essential oil reduced GSB disease levels between 60 and 70.6%, depending on the concentration used. As for the lemongrass essential oil, the decrease in disease severity at the same concentration was even higher, between 70 and 73.8%, similar to that observed with the application of fungicide that showed a reduction in 75% of the affected leaf area. In all, the essential oils of lemongrass and noni fruit showed that control is effective when applied preventively against gummy growth in melon plant. This result demonstrates the potential of essential oils as an additional tool in the management of plant diseases, which can, thus, contribute to reducing the use of pesticides in the control of GSB in the melon crop.

CONFLICT OF INTERESTS
The authors have not declared any conflict of interest.

REFERENCES