Application of biosynthetic silver nanoparticles as inhibitor of citrus canker

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Abstract
The citrus canker affects all kind of citrus plants contaminated by the microorganism Xanthomonas axonopodis pv. citri (Xac). The disease has its symptoms evident on the leaves, and it causes plant death, and also affects flowers and fruits, with wounds, thus unable oranges fruit commercialization. Citrus canker is responsible for millions of dollars loss on world citrus industry. The silver nanoparticles (AgNP) synthetized from an extracellular extract using the fungus Fusarium oxysporum had shown remarkable stability (with zeta potential of -37.1 mV) and have been successfully used as agrochemicals against Xac, in vitro. The application in vivo shown that AgNP helped to prevent the development of citrus canker (when compared to placebo) and improved the performance of the commercially used inhibitor based on copper (II) ions.

Key words: canker, citrus, nanoparticles.

Introduction
The experiment's main objective is to assess an optimal conditions for applying a solution of AgNP on orange trees as agrochemical. The study is based on the analysis of the progression of citrus canker based on a scale of severity of the wounds on their leaves. The AgNP, which were synthetized from the extracellular extract of F. oxysporum, were produced during Daniela Pott's research project and they are characterized as follows: zeta potential of -37.1 mV, 28 nm diameter and 91.11 mg.mL\(^{-1}\). AgNP was diluted 1:100. The experiment was performed on 4 different groups of trees with 10 trees in each group: control, AgNP, Cu, Cu+AgNP. All groups were contaminated with Xac. And all of them, except control, were treated with the corresponding agrochemical.

Results and Discussion
In order to assess the wounds, the techniques described on the reference 1 were applied. The relative treatment efficiency was determined in each case and expressed as severity of the disease. The last encreased along the time. And fot the joint action of AgNP and Cu (II) ions, the minor average severity was observed. The AgNP concentration was 9.11 mg.mL\(^{-1}\) and the copper's was 100 μg.mL\(^{-1}\).

<table>
<thead>
<tr>
<th>Group</th>
<th>Average Severity /a.u.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
</tr>
<tr>
<td>AgNP</td>
<td>10</td>
</tr>
<tr>
<td>AgNP + Cu</td>
<td>7</td>
</tr>
<tr>
<td>Cu</td>
<td>9</td>
</tr>
</tbody>
</table>

Chart 1: Average wounds severity at the end of the experiment, 30 days from the beginning.

Conclusions
The AgNP were effective when applied without copper (II) ions. When AgNP were combined with the traditional agrochemical - copper (II) ions, an improved AgNP performance has been observed. This probably occurred because AgNP facilitated copper (II) ions action by binding onto bacterial membrane proteins. It is expected to improve even more the AgNP performance by adjusting this agrochemical solution stability and composition.

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