



TITLE: DEVELOPMENT OF AN *IN VITRO* METHOD, AND BACTERICIDES DERIVED FROM NATURAL PRODUCTS IN ORDER TO CONTROL *XYLELLA FASTIDIOSA* IN CITRUS

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ABSTRACT

Purpose of study: Citrus is target of several diseases. Citrus Variegated Chlorosis (CVC) is one of the main diseases that cause large losses in Citrus production. This disease is caused by bacterium called *Xylella fastidiosa*. Unfortunately, we do not have specific bactericides in the market, or processes, in order to combat this disease, so far. The purpose of this study is to develop and validate biological methods, *in vitro* and evaluate new molecules derived from natural products for the control of *Xylella fastidiosa* in citrus,

Methods: The natural products and derived compounds used *in vitro* tests, in order to evaluate their bactericidal effects against *X. fastidiosa*, were: azadiracthin, hesperidin, naringenin, hesperidin and naringenin complexed with magnesium ($[\text{Mg}(\text{hesp})_2(\text{phen})]$ and $[\text{Mg}(\text{ngnina})_2(\text{phen})(\text{H}_2\text{O})_2]$, respectively), and naringenin complexed with ruthenium $[\text{Ru}(\text{ngnina})(\text{phen})_2]$. A method by microdilution in microplate was developed and applied for determining the Minimum Inhibitory Concentration (MIC) of the compounds able to inhibit the growth of bacteria. The MIC technique was performed according to the methodology of the "National Committee for Clinical Laboratory Standards". The MIC was determined visually through the addition of resazurin, which becomes pink and fluorescent (resorufin) due to the chemical reduction of oxidoreductases excreted by viable cells.

Results: The molecules that showed the best results in MIC assays are: 0.19 μM , naringenin complexed ruthenium; 0.34 μM , narigenin complexed magnesium; 1.40 μM , hesperidin complexed magnesium; 2.10 μM , azadiracthin, 3.27 μM , hesperidin; and 7.30 μM , naringenin. Phenanthroline, which was used in the synthesis of the complexes, did not present bacterial inhibition. This method presenting a low cost, fast and reliable results, and high sensibility. This is the first *in vitro* developed method to study the *X. fastidiosa* control in Brazil.

Conclusions: The complexed natural products showed better results because these composts not only increased the bacterial activity, but also their solubility in water, and stability in different pH's. Natural products and derived compounds are an important source of biodegradable pesticides. Moreover, they are not harmful to human and environment.