

***In vitro* Antibacterial Activity of Four Essential Oils on *Xanthomonas* sp., Responsible of the Bacteriosis of the Cashew Tree**

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Abstract : Cashew (*Anacardium occidentale* L.), an important crop in Côte d'Ivoire is prone to many diseases, one of the most redoutable of which is bacteriosis caused by *Xanthomonas* sp. To limit the use of pesticides in the fight against this bacteriosis, an alternative solution based on the use of essential oils has been evaluated. Antibacterial tests were carried out *in vitro* with the essential oils of *Eucalyptus citriodora*, *Cymbopogon citratus*, *Citrus limon*, NECO (formulation based on the essential oil of *Ocimum gratissimum*) and a synthetic pesticide, the callicuivre. The smallest MICs (10 ppm and 60 ppm respectively) were obtained with NECO and the essential oil of *Cymbopogon citratus*. The highest antibacterial activities were obtained with the essential oil of *Cymbopogon citratus* and NECO biopesticide. at the dose of 4000 ppm (for inhibition diameters of 20.67mm and 27.33mm respectively). NECO and the essential oil of *Cymbopogon citratus* could be used as an alternative to the synthetic product in the fight against bacteriosis of cashew.

Keywords: Bacteriosis; *Xanthomonas* sp., *Anacardium occidentale* L., Essential Oil; Biological Fighting

Introduction

Cashew (*Anacardium occidentale* L.), a plant native to Northeast Brazil, is cultivated for its fruit. It is composed of two parts: the cashew apple or fake fruit and the cashew nut whose almond is marketed worldwide. The cashew apple, juicy and rich in vitamin C, is used in the form of fresh fruit, jam, juice, alcohol, vinegar or syrup (Djaha *et al.*, 2008). Cashew culture contributes significantly to the socio-economic development of the producing countries (Martin 2003, Marlos *et al.*, 2007). In Côte d'Ivoire, it is practiced in the northern and central regions and contributes significantly to improving the living conditions of producers by ensuring regular income. Since 2008, with 380,000 tonnes of raw nuts, Côte d'Ivoire has become Africa's leading

exporter of cashew nuts (Djaha *et al.*, 2010). Due to the high production of cashew nuts, the country is ranked as the world's largest producer of cashew nuts. The total quantity of cashew nuts marketed increased from 649,787 tonnes in 2016 to 673,236 tonnes in 2017, an increase of 3.72 tonnes (Anonymous, 2017). Despite this increase, its cultivation is subject to many biotic constraints that limit its productivity. Among these biotic constraints, bacteriosis caused by *Xanthomonas* sp causes significant damage to this crop. Indeed, a study carried out to make the sanitary map of the cashew nut has made it possible to highlight the incidence and the severity of this bacterial infection in certain production areas (Bouaké, Bondoukou, Korhogo, Odiénné and Séguéla). This study showed

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that bacterial disease is more severe in Ségoula (1.85%) and causes more damage to Odienné with an incidence of 57.24% (Soro *et al.*, 2015). Symptoms are oily and translucent angular spots on leaves with or without a chlorotic halo. The infection can spread to the whole plant leading to the death of it. It causes yield losses and depreciates the quality of cashews (Afouda *et al.*, 2013).

To control this bacteriosis, different methods are developed. However, circumvention of varietal resistance by some strains and the problem of availability of adequate plant material limits the effectiveness of these strategies. Biological control, an alternative solution to chemical control, could be the most environmentally friendly means of control because it uses natural antagonists and involves complex mechanisms that are difficult to circumvent by the parasite. In addition, the effectiveness of essential oils has been demonstrated in the fight against various pathogens of cultures.

Thus, this work aims to assess the sensitivity of 4 essential oils to the bacterial cashew caused by *Xanthomonas* sp.

1- Materials and Methods

1-1- Materials

1-1-1- Bacterial strains

The bacterial strain used was isolated from cashew leaves showing typical symptoms of *Xanthomonas* sp. These leaf samples were taken from a cashew tree orchard at Dikodougou (in the Korhogo area) in northern Côte d'Ivoire.

1-1-2- Essential oils and synthetic product

Four essential oils of vegetable origin were tested. These are essential oils extracted from the plant species of *Ocimum gratissimum*, *Cymbopogon citratus*, *Eucalyptus citrodora* and *Citrus limon*. The essential oil of *Citrus limon* has been extracted from the bark of the fruit (skin); the others come from the hydrodistillation of the leaves of the plant species concerned.

The essential oil of *Ocimum gratissimum* was used under the formulation of the NECO biopesticide. These essential oils were provided by the Industrial Research Unit (URI) on the essential oils of the Scientific and Innovation Cluster of the Felix Houphouët-Boigny University of Abidjan (UFHB).

The product of synthesis was the callicuivre.

1-2- Methods

1-2-1- Preparation of the culture media and obtaining doses of essential oils and synthetic products

Three media were prepared: YPGA 100% (yeast extract, 7 g peptone, 7 g glucose and 18 g agar),

YPGA 75%, and agar at 0.1%. The 75% YPGA medium was obtained by the calculation ratio as a function of the composition of the 100% YPGA medium. The 100% YPGA medium was distributed in Petri dishes. Cooled to a temperature of about 25 °C after autoclaving, the 75% YPGA medium was dispensed into Falcon tubes at 45 mL per tube. The 0.1% agar was used for the preparation of the different concentrations of the products. Different volumes of essential oils depending on the concentrations to be evaluated were added to test tubes containing 1 ml of 0.1% prepared agar medium. Having 1 ml of 0.1% agar medium, the volume of essential oil (V_i) to be sampled, in order to obtain the different concentrations, is given by the following formula:

$$\frac{C_f \times V_f}{C_i}$$

C_f is the final concentration; V_f the final volume and C_i the initial concentration of the essential oil.

For the synthetic product, a 1000 ppm stock solution was prepared from the manufacturer's recommended dose by taking 0.75g of Callicuivre in 10 mL of sterile distilled water.

Different concentrations were tested to determine the Minimum Inhibitory Concentration (MIC) and their antibacterial activity. Concentration ranges between 10 to 50 ppm, 60 to 90 ppm, and 100 to 5000 ppm were tested.

1-2-2- Inoculation of the amended Petri dishes of the different doses of essential oils and Callicuivre

A volume of 1 mL of inoculum was prepared from a pure 48-h old colony of *Xanthomonas* sp. The bacterial suspension obtained was calibrated at the optical density (OD) of 0.02, corresponding to 108 bacteria / mL. This suspension was then mixed with 75% YPGA medium at the rate of 15 µL of bacterial suspension for 45 mL of 75% YPGA. Using a pipette, 15 mL of the mixture were removed and uniformly distributed on the surface of 100% solidified YPGA medium previously cast in the Petri dishes. After solidification of the mixture, 1 to 3 deposits of 10 µL of each concentration of essential oils and Callicuivre were made per petri dish. After diffusion of the deposits, the cultures were incubated at 28 °C. Three repetitions were performed by concentration. After 96 hours, the diameters of the inhibition zones were measured using a graduated rule.

1-2-3- Statistical analyzes

The collected data were recorded with the Excel 2013 spreadsheet and then analyzed with the Statistica version 7.1 software. To evaluate the

effect of different doses of essential oils and Callicuivre on *Xanthomonas* sp., a one-way analysis of variance (ANOVA) was used. The Newman-Keuls average comparison test at the 5% threshold was performed.

2- Results and Discussion

2-1- Results

2-1-1- Effect of essential oils and synthetic product on the growth of *Xanthomonas* sp.

The different essential oils *Eucalyptus citriodora*, *Cymbopogon citratus*, *Ocimum gratissimum* and *Citrus limon* as well as the product of synthesis (Callicuivre) had variable activities according to the nature of the product and the concentrations tested (Figure 1). Thus, depending on the dose and the product, different antibacterial activities were observed.

2-1-2- Minimum inhibitory concentration (MIC) of essential oils and synthetic product

The results in Table I indicate that the NECO biopesticide has the lowest MIC (10 ppm) and an

average inhibition diameter of 2.17 mm. The highest MIC (500 ppm) was obtained with the essential oil of *Eucalyptus citriodora* for an average diameter of inhibition of 4.17 mm. However, the MICs of the oils tested were lower than that of the synthetic product (Callicuivre) which was 300 ppm, with the exception of *Eucalyptus citriodora* and varied between 10 ppm and 80 ppm.

2-1-3- *In Vitro* Effect of Essential Oils and Callicuivre at Concentrations of 10 to 50 ppm

The results showed that the essential oils of *Eucalyptus citriodora*, *Cymbopogon citratus*, *Citrus limon* and Callicuivre have no effect on the growth of the bacterial strain at the doses of 10 to 50 ppm (Figure 2). On the other hand, the NECO biopesticide showed a greater antibacterial activity. The inhibition diameters of the bacterium by NECO were the highest, however, these diameters are not statistically different from 30 ppm (Newman-Keuls test).

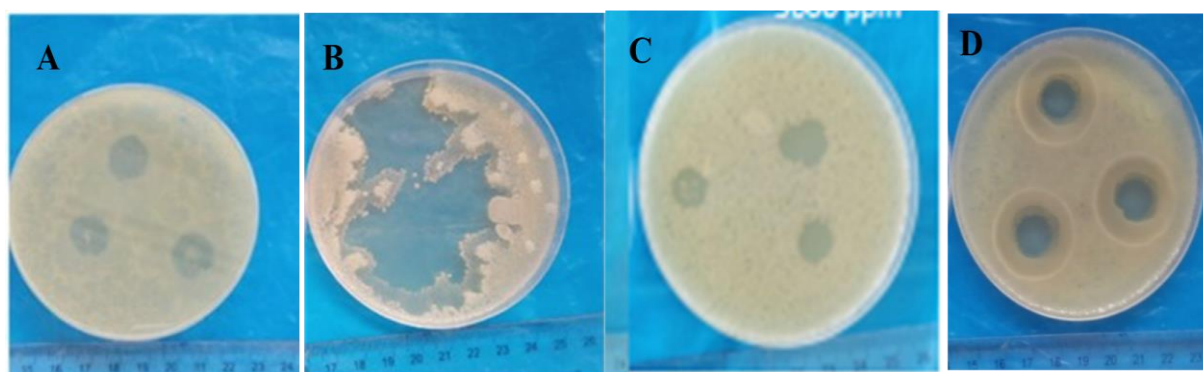


Figure 1: *In vitro* inhibition of *Xanthomonas* sp. grown on YPGA amended medium of essential oils and synthetic product at a concentration of 5000 ppm.

A : *Cymbopogon citratus*; **B:** *Ocimum gratissimum* (biopesticide NECO); **C :** *Eucalyptus citriodora*, **D :** Callicuivre.

Table I: Minimum inhibitory concentrations of different essential oils and Callicuivre.

Products	Minimal Inhibitory Concentrations (ppm)	Mean inhibition diameters (mm)
<i>Eucalyptus citriodora</i>	500	4,17
<i>Cymbopogon citratus</i>	60	2,33
<i>Citrus limon</i>	80	1,50
NECO	10	2,17
Callicuivre	300	3

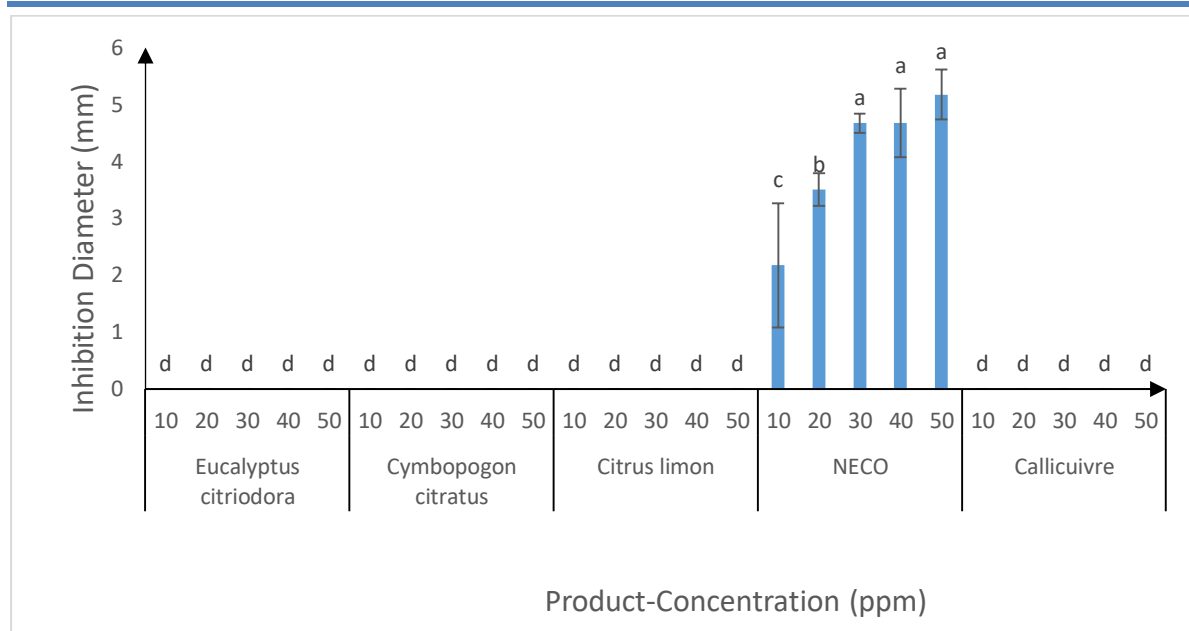


Figure 2: Mean growth inhibition diameters of *Xanthomonas* sp. depending on the essential oils and synthetic product at concentrations of 10 to 50 ppm.

The bars surmounted by identical letters indicate that there are no significant differences between the inhibition diameters.

2-1-4- *In vitro* effect of essential oils and callicuivre at doses of 60 to 90 ppm

All essential oils and Callicuivre were tested at doses of 60 to 90 ppm except for NECO whose MIC was determined at previously tested concentrations. The results showed that at concentrations of 60 to 90 ppm, the essential oil of *Eucalyptus citriodora* and the synthetic product (callicuivre) had no inhibitory effect on the bacterium (**Figure 3**). On the other hand, the oil of *Cymbopogon citratus* inhibited the growth of the bacteria at these doses. Inhibition diameters ranged from 2.33 mm to 6.67 mm at concentrations of 60 to 90 ppm.

As for the essential oil of *Citrus limon*, inhibition began only from the concentration of 80 ppm with a diameter of inhibition of 1.50 mm.

2-1-5- *In vitro* effect of essential oils and Callicuivre at doses of 100 to 5000 ppm

The Figure 4 shows that the diameters of inhibition are high when the concentration of the essential oil or the product of synthesis increases. However, for *Citrus limon* oils, the inhibition diameter decreases or even vanishes for concentrations greater than 300 ppm.

The highest inhibition diameters (27.33 mm) were recorded with the NECO biopesticide at a concentration of 4000 ppm. However, the essential oil of *Citrus limon* showed the smallest diameters of inhibition (1.50 mm) at the dose of 500 ppm. As for the synthetic product, the smallest and largest inhibition diameters were 3 mm and 15.67 mm respectively at concentrations of 300 and 5000 ppm (**Figure 4**).

2-2- Discussion

The antibacterial activity of the essential oils of *Ocimum gratissimum*, *Eucalyptus citriodora*, *Cymbopogon citratus*, *Citrus limon* and the synthetic product (callicuivre) on bacteriosis caused by *Xanthomonas* sp. showed different effects depending on the products and concentrations tested.

The essential oils of *Ocimum gratissimum*, *Cymbopogon citratus*, and *Citrus limon* had lower MICs than callicuivre with the exception of the essential oil of *Eucalyptus citriodora*. The difference observed between the MICs of the essential oils and the synthetic product could be related to a different chemical composition between the essential oils and the callicuivre. This difference in compounds could have an effect on the antibacterial activity of these products which would allow them to inhibit the growth of the bacterial strain differently.

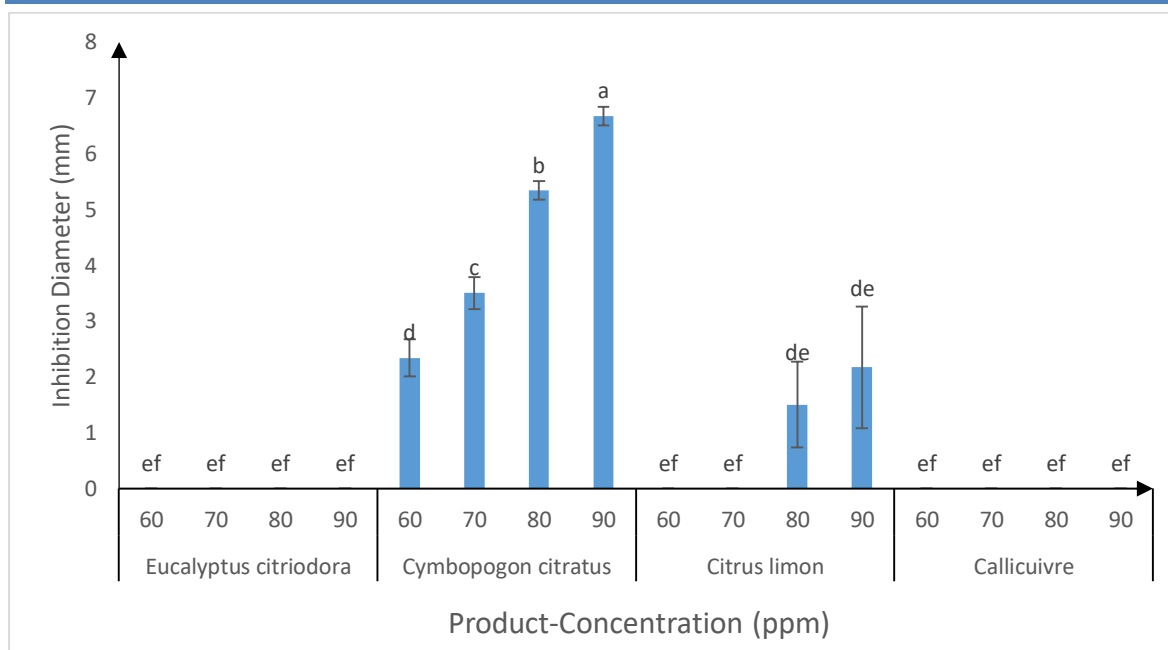


Figure 3: Mean growth inhibition diameters of *Xanthomonas* sp. depending on the essential oils and synthetic product at concentrations of 60 to 90 ppm.

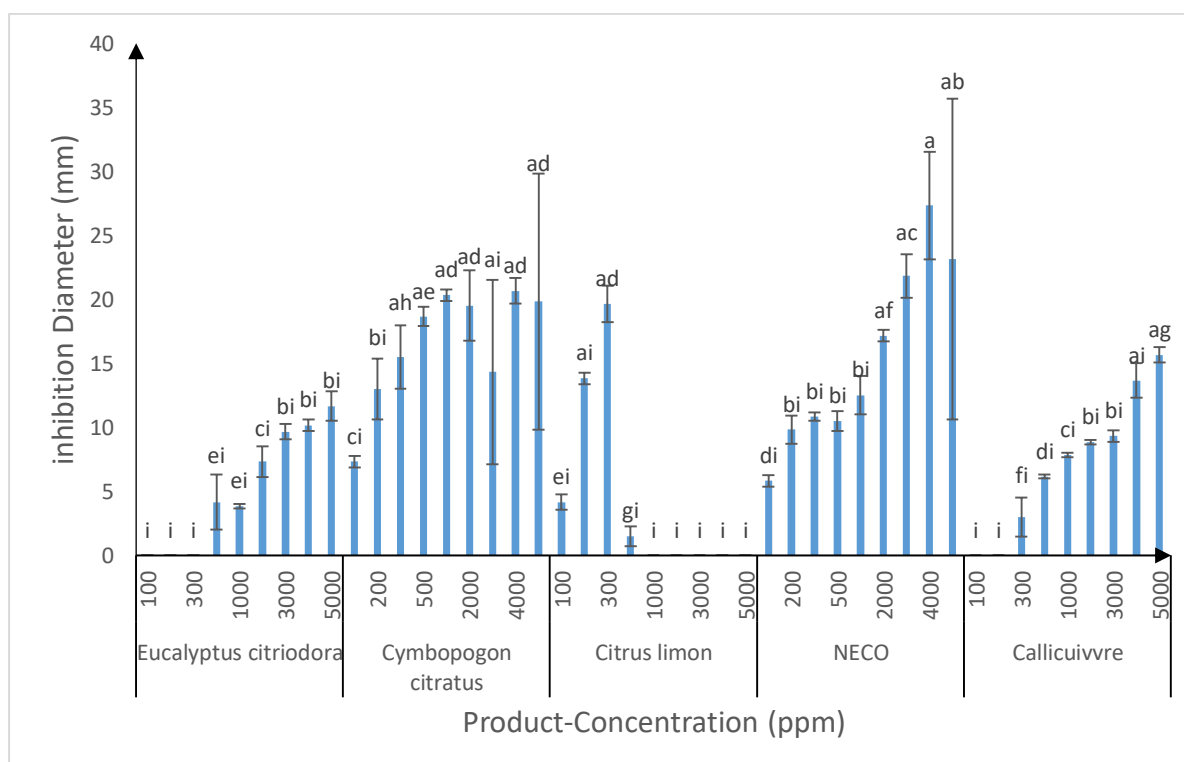


Figure 3: Mean growth inhibition diameters of *Xanthomonas* sp. depending on the essential oils and synthetic product at concentrations of 100 to 5000 ppm.

Indeed, each essential oil is composed of various chemical substances that are able to slow the growth of a pathogen according to their effectiveness and concentration (Lahlou, 2004). NECO biopesticide

and *Cymbopogon citratus* oil had greater inhibitory activity than callicuivre, and were also more effective than other tested oils. Indeed, these two oils had the greatest inhibitory activity at the

concentration of 4000 ppm. But NECO has been shown to be the best inhibitor of *Xanthomonas* sp. than *Cymbopogon citratus* oil in all doses tested. The fact that the biopesticide NECO composed of essential oil of *Ocimum gratissimum* inhibits the bacterium and is more active could be explained by the effect of its constituents which would cause different inhibition reactions compared to other products. The existence of an active ingredient with a very strong antibacterial property in NECO would inhibit the growth of the bacterial strain of *Xanthomonas* sp. Indeed, Oussou (2009), Kanko (2010) and Kassi (2015) showed that the NECO biopesticide is mainly composed of 46.1% of thymol and 17.6% of γ -terpene, and these compounds would allow it to act differently on the same pathogen. These results are consistent with those of Doumbouya *et al.* (2012), who showed that the essential oil of *Ocimum gratissimum* is highly effective on the different life stages of *Fusarium oxysporum* compared to callicuivre.

Essential oils showed varying inhibitory effects on the strain of *Xanthomonas* sp. Indeed, the inhibitory activity of the oils varies according to the concentrations. In addition, the bacterium does not have the same sensitivity towards oils and callicuivre. According to Rossi *et al.* (2007), oils are considered active if they produce inhibition diameters greater than or equal to 15 mm. For this purpose, the essential oils of *Ocimum gratissimum*, *Cymbopogon citratus*, and *Citrus limon* were active in the presence of *Xanthomonas* sp. The number of compounds present in each oil and their capacity of reaction could be at the origin of their effectiveness. Kpoviessi *et al.* (2014) showed that the essential oil of *Cymbopogon citratus* contains 29 characterized compounds whose main constituents are geranial, neral, β -pinene and cis-geraniol. These different compounds would be less active than those of NECO. Moreover, the chemical composition of the essential oil of *Ocimum gratissimum* used under the formulation of the NECO biopesticide is variable. Five chemotypes (eugenol, thymol, citral, ethyl cinnamate and linalool) have been characterized and described as major constituents of the oil (Kishore *et al.*, 2000). These different compounds could therefore be at the origin of its effectiveness. In addition, studies by Kassi *et al.* (2014) showed that the NECO biopesticide had an antifungal effect on *Deightonella torulosa* (SYD.) ELLIS, a banana leaf parasite. The very low activity of the essential oil of *Eucalyptus citriodora* is probably due to the presence of molecules in the membrane structure of the bacterium that would prevent its inhibitory activity or a minority presence of alcohol or phenol compounds in its structure. These results corroborate those of Skocibusic *et al.*, (2006);

Shariffar *et al.* (2007), which revealed that the antibacterial activity of essential oils is due to the presence of long-chain alcohols and phenol compounds that inhibit the growth of bacteria.

CONCLUSION AND PERSPECTIVES

The antibacterial activity of essential oils has shown that they have been very active as the callicuivre with the exception of *Eucalyptus citriodora* oil. The biopesticide NECO and the essential oil of *Cymbopogon citratus* had the best inhibition rates. These two oils could be used in the fight against this bacteriosis. In order to better control bacterial wilt and thus limit chemical pesticides, NECO biopesticide and *Cymbopogon citratus* essential oil should be evaluated under field conditions.

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