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Research Article

In vitro and *in vivo* Antibacterial Activity of Three Natural Substances, for the Control of *Xanthomonas albilineans* Responsible for Sugarcane Leaf Scald in Côte d'Ivoire

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Abstract

Background and Objective: Sugarcane cultivation in Côte d'Ivoire is subject to numerous biotic constraints, notably leaf scald caused by *Xanthomonas albilineans*. The present study was initiated with a view to controlling this disease using natural substances. **Materials and Methods:** The experiment was carried out in the laboratory. The *in vitro* efficacy of three essential oils was evaluated on three strains of *Xanthomonas albilineans* by deposition on YPGA medium. Their efficacy was tested on leaf slices of the R585 sugarcane variety, inoculated by soaking for 3 hrs in the laboratory. An analysis of variance was applied to the recorded data. **Results:** The essential oils of *Ocimum gratissimum* L. and the combination of *Zingiber officinale*+*Ocimum gratissimum* L. were the most effective *in vitro*, at concentrations of 4000 and 8000 ppm with diameters greater than 11 mm. Under *in vivo* conditions and at a concentration of 8000 ppm, the essential oil of *Ocimum gratissimum* L. (Oci) and the combination of *Zingiber officinale*+*Ocimum gratissimum* L. (ZiOci) proved the most effective. **Conclusion:** The essential oils tested were effective *in vitro* at concentrations of 4000 and 8000 ppm and *in vivo* at 6000 and 8000 ppm. These natural substances could provide an alternative to chemical control of sugarcane leaf scald.

Key words: *Xanthomonas albilineans*, scalding, natural substances, sugarcane, essential oils, Côte d'Ivoire

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a tropical Poaceae whose cultivation is widespread with around 25 billion hectares worldwide[1]. Within the West African Economic and Monetary Union (UEMOA), sugar production is estimated at nearly 400,000 ton/year. The Ivorian sugar sector is made up of two sub-sectors. The first is made up of irrigated industrial sugarcane cultivation, practiced by sugar companies, while the second is a sub-sector of rain-fed village sugarcane cultivation practiced by smallholders. It covers a total area of around 30,000 ha, 5,000 of which are under village cultivation[1]. These complexes specialize in the industrial production and processing of sugar cane. Peasant sugarcane farms are also found around the large plantations. National sugar production is estimated at over 214,000 ton/year, representing 3.3% of agricultural Gross Domestic Product (GDP), or 1% of national GDP and providing over 10,000 jobs[1]. In terms of sugar production, Côte d'Ivoire is ranked 53rd in the world, 16th in Africa and first in the UEMOA Region[1]. Sugar cane faces a number of abiotic and biotic constraints that reduce its yield[2]. Recently, the work of Sorho *et al.*[3] and Jacques-Edouard *et al.*[4], have shown that diseases remain a threat to the development of sugar industries. The main constraints are biotic, notably pests (borers) and diseases (viral, bacterial and fungal), including sugarcane scald[5]. Sugarcane leaf scald affects both cane yield at harvest and the quality of the extracted juice[6]. In Guadeloupe, losses have been estimated at 13 tonnes of cane per hectare, taking into account the difference in yield between healthy and diseased cane. On this island, field yield losses of between 15 and 20% have been noted for the susceptible variety B 69379[7]. In a bid to reduce crop loss, growers resort to chemical control, which is the most commonly used method[8]. However, its intensive use is harmful to the health of farmers, consumers and the environment[9]. What's more, these pesticides can encourage the emergence of resistant strains in pathogens[10]. In view of the economic consequences of this disease, effective and sustainable pathogen control strategies need to be proposed in order to guarantee sugarcane production, with a focus on the use of natural substances. In this context of adopting environmentally friendly approaches, plant extracts represent a promising avenue for combating pathogens responsible for fungal, bacterial and viral diseases[11]. Pesticidal plants are used to control crop pests and diseases. In Côte d'Ivoire, several research studies have proven the effectiveness of biopesticides based on pesticidal plants[12]. These bio-pesticides are known for their antimicrobial activity and some are classified as safe substances. They can be used to prevent the growth and multiplication of pathogenic microorganisms such as bacteria, viruses and fungi[13]. In addition, natural substances are effective at a low dose without risk of contamination for the user, the environment and production[14]. Thus, the present study aims to contribute to the sustainable production of sugarcane in Côte d'Ivoire, through the control of leaf scald disease using natural substances. Specifically, these are:

- *In vitro* evaluation of the antibacterial activity of natural substances against *Xanthomonas albilineans* strains
- Laboratory evaluation of the efficacy of natural substances *in vitro* on leaves of the R585 sugarcane variety

MATERIALS AND METHODS

Study area: The study was carried out between May and December, 2022. The work was carried out in the Laboratory of the Pedagogical and Research Unit of Plant Physiology and Pathology, of the Training and Research Unit Biosciences, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire.

Material

Plant material: The plant material consisted of 6 months old leaves of the R585 sugarcane variety. Cuttings of this variety were collected from the SUCRIVOIRE sugar complex in Zuénoula, then multiplied on an experimental site at the Félix Houphouët-Boigny Université in Abidjan, Côte d'Ivoire.

Control products: The natural substances used were supplied by the Industrial Research Unit (IRU) on biopesticides at the Félix Houphouët-Boigny University. These substances consisted of essential oils of *Ocimum gratissimum* L. (Oci), *Cymbopogon citratus* (cym) and *Zingiber officinale* (Zin). The action of the essential oils was compared with Callicuivre, a synthetic product based on copper oxychloride at a concentration of 50%. This product was supplied by the Callivoire company in Côte d'Ivoire (Table 1).

Bacterial strains: Bacterial strains of *Xanthomonas albilineans* were isolated from sugarcane leaves of different varieties showing symptoms of bacterial blight. These leaf samples were collected during a survey of the SUCRIVOIRE Integrated Agricultural Unit (UIA) sugar site in Zuénoula. Three strains coded S150, S164 and S229 were used for testing.

Table 1: Essential oils and combinations tested

Tested formulas	Product code
Ocimum gratissimum L.	Oci
Cymbopogon citratus	Cymbo
Zingiber officinale	Zin
Zingiber officinale + *Ocimum gratissimum* L.	ZiOci
Zingiber officinale + *Cymbopogon citratus*	ZiCym
Ocimum gratissimum L. + *Cymbopogon citratus*	OciCym
Callicuivre (Oxyde of cuivre)	Cal

Table 2: *Xanthomonas albilineans* strain susceptibility rating scale[20] (method modified)

Sensitivity of strain	Inhibition diameters
Non-sensitive strains (-)	≤8 mm
Sensitive strains (+)	9-14 mm
Very sensitive strains (++)	15-19 mm
Extremely sensitive strains (+++)	≥20 mm

Methods

In vitro evaluation of the antibacterial activity of natural substances and synthetic product

Culture of bacterial strains: Bacterial strains previously isolated and stored at -80°C were recultured on YPGA (yeast extract, peptone, glucose and agar) media. After 48 hrs of incubation, the Petri dishes containing the strains were purified on new culture media to obtain younger colonies.

Preparation of culture media: Two culture media, YPGA 100 and 75%, were prepared for this test. The 75% YPGA medium was obtained by a calculation based on the composition of the 100% YPGA medium. After autoclaving at 121°C for 30 min, the 100% medium was dispensed into 90 mm diameter Petri dishes at a supercooling temperature of 45°C. The YPGA 75% medium was allowed to supercool to 25°C, then transferred to 50 mL tubes at a rate of 45 mL per tube. At this temperature (25°C), the 75% medium containing a small amount of agar does not set and the incorporated bacteria are kept alive.

Inoculum preparation and seeding: An inoculum solution of each strain was prepared from a 48 hrs pure colony, in test tubes with sterile distilled water. The resulting suspension was calibrated at an optical density (OD) of 0.2, at a wavelength of 600 nm, corresponding to 10^8 CFU mL^{-1}. A volume of 150 µL of the bacterial suspension was mixed into a 45 mL volume of the liquid YPGA medium (75%) contained in the test tubes. This medium was distributed evenly over the surface of the solidified YPGA medium (100%) at a rate of 15 mL per box.

Obtaining product doses and seeding: The doses of the essential oils and the synthetic product were obtained according to the method described by Carine *et al.*[15]. The antibacterial activity of the natural substances and the synthetic product (calli-copper) was evaluated for four concentrations : 250, 1000, 4000 and 8000 ppm. Doses were prepared in 1 mL of sterile distilled water. A drop of Tween 20 was added to each concentrated solution to facilitate the miscibility of the oil with water. For the synthesis product, a 5 g L^{-1} callicuivre stock solution (5000 ppm) was prepared in an Erlenmeyer flask from the stock solution and the various solutions at different concentrations were obtained.

After solidification of the culture media, three deposits of 10 µL (per deposit) of each product concentration were made in each Petri dish. After diffusion of the deposits, the cultures were incubated at 28°C. Three replicates were performed per concentration.

Measuring inhibition diameters: After 24 hrs of incubation, the diameters of the inhibition zones were measured daily for 4 days. Measurements were made along two perpendicular lines drawn on the reverse side of each Petri dish, intersecting at the center of each inhibition point (Fig. 1).

Evaluation of strain sensitivity: The sensitivity of *Xanthomonas albilineans* strains to the products was assessed using the modified[16] method, based on the size of the inhibition zone diameters and the inhibition rate (Table 2).

Laboratory evaluation of the efficacy of essential oils on inoculated sugarcane leaves

Preparation of sugar cane leaf slices and inoculation: The efficacy of the essential oils and the synthetic product was evaluated using leaves infected with three strains of *Xanthomonas albilineans* S150, S164 and S229. The R585 cane leaves used for the evaluation tests were taken from 6 months old plants. The leaves were cut into 4.5 cm discs, washed thoroughly with tap water and then rinsed with sterile distilled water.

After wiping on sterile blotting paper, the discs were inoculated by soaking in the inoculum of each strain prepared as above for 3 hrs, the experiment was repeated three times (Fig. 2).

Treatment of inoculated sugarcane leaf slices: The effect of Oci and Zin essential oils and their combination of Oci+Zin

Table 3: Scald severity rating scale for sugar cane leaves[17]

Scores	Percentage	Characteristics
0	0	No symptom
1	1-20	Slight presence of white or yellow streaks or lines on leaf blades parallel to the veins
2	21-40	Average presence of striae on leaf blades
3	41-60	Slightly heavy streaking on leaf blades
4	61-80	Strong presence of striations on leaf blades
5	81-100	Average presence of streaks on leaf blades, leaves completely necrotic

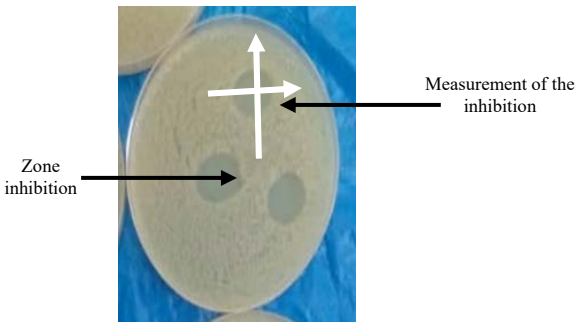

Fig. 1: Inhibition diameters of ZiOci on the growth of germs from strain S150, S164 and S229 (strains of *Xanthomonas albilineans)*

Fig. 2(a-c): Leaf discs inoculated by dipping with strains (a) S150, (b) S164 and (c) S229

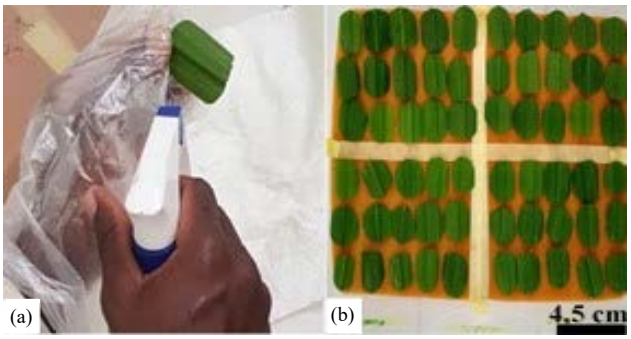

Fig. 3(a-b): Treatment of inoculated leaf discs with natural substances by (a) Sprinkling and (b) Experimental set-up

(at 50% of each oil) was evaluated for each strain. These essential oils were chosen on the basis of their antibacterial activity *in vitro*. Three concentrations were tested: 4000 ppm, 6000 ppm and 8000 ppm (CI1, CI2 and CI3, respectively). Callicuivre (Cal), the synthetic product, was tested as the reference product, at a concentration of 5 g L^{-1} (5000 ppm).

A 10 mL solution of each concentrate was prepared for treatment. After inoculation of the leaves with the bacterial strains, treatments were carried out by spraying the leaf discs according to product concentration. The leaves were placed in trays (Fig. 3a-b). Treatments were repeated three times. Control leaves were treated with the synthetic product.

Assessment of phytopathological parameters: The leaves were regularly monitored and disease severity and incidence were assessed every day for 5 days. For severity, the modified symptom rating scale of Champoiseau *et al.*[17] was used (Table 3).

The disease severity index was determined for each treatment using the following equation from Kranz[18]:

$$Is\ (\%) = \frac{\sum (x_i - n_i)}{N \times Z} \times 100$$

Where:

Is = Severity index
x_i = Rating or index on severity scale from 0 to 5
n_i = Number of sheets with note x_i
N = Total number of inoculated leaves
Z = Highest score on the scale

Disease incidence was calculated as the ratio of the number of attacked leaves to the total number of leaves. The aggressiveness of the strains was defined on the basis of the incidences noted. Once the pathogenicity of the various strains had been assessed, isolations were made in the laboratory from leaves showing symptoms of leaf scald disease.

Statistical analysis: An analysis of variance was applied to the recorded data and in the event of a significant effect of the factor studied, the Newman-Keuls Statistical Test, with a threshold of 5%, was used to separate the means into homogeneous groups.

RESULTS AND DISCUSSION

Results

In vitro antibacterial activity of the products tested

Effect of products on strain S150: The products evaluated showed effects that varied according to the concentrations studied (Fig. 4). With ZiOci, concentrations C4 and C3 induced the greatest inhibition diameters, with values of 14.04 and 11.41 mm, respectively. With ZiCym, inhibition diameters were statistically identical for concentrations C3 and C4. These doses had the largest inhibition diameters of 11.40 mm (C3) and 11.48 mm (C4). For OciCym and Cym, the highest inhibition diameters were recorded at concentration C4, with values of 13.25 and 12.87 mm, respectively. The Oci and Zin induced low inhibition diameters compared with the other products. Inhibition values obtained with these products ranged from 0 to 7 mm. Overall, all the essential oils showed greater antibacterial activity than the synthetic product, which had a maximum inhibition rate of 1.69 mm at C4 concentration.

Effect of products on strain S164: The highest inhibition diameters were obtained at concentrations C3 and C4 with products Oci, ZiOci and Cymbo. At these respective concentrations, inhibition diameters were greater than 11 mm for products Oci (14.18, 19.59 mm) and ZiOci (11.83, 13.13 mm). For Cymbo, inhibition diameters were 10.81 and 11.13 mm at concentrations C3 and C4, respectively (Fig. 5).

Effect of products on strain S229: The results of the products' antibacterial activity revealed that with product ZiOci, inhibition diameters were greatest at concentrations C3 (12.08 mm) and C4 (13.43 mm). In contrast, Cymbo and Oci recorded statistically identical inhibition diameters at C4 concentration, with values of 8.13 and 8.40 mm, respectively. The synthesis product, on the other hand, recorded the lowest inhibition diameters (Fig. 6).

Effect of natural substances and synthetic products on the leaves of sugarcane variety R585 in the laboratory

Severity and incidence of leaf scald induced by strain S150 on treated sugarcane leaf slices: Severity was low on leaves treated with products at CI1 and CI2 concentrations (Fig. 7). These results are statically identical at the 5% threshold according to the Newman-Keuls Test. However, severity was nil at CI3 for Zin and Oci. ZiOci was less effective at this concentration, with a severity of 1.60%. On the other hand, the severity observed with the synthetic product was higher, at 8.38%.

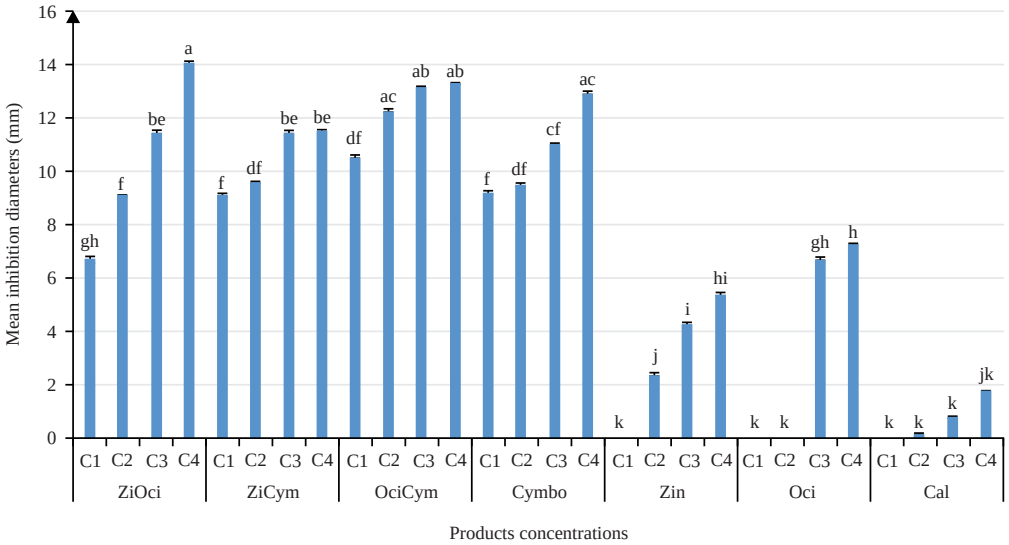

Fig. 4: Average inhibition diameters of strain S150 by-products at different concentrations

Error bars surmounted by the same letter are statistically identical according to the Newman-Keuls Test at threshold $\alpha = 0.05$

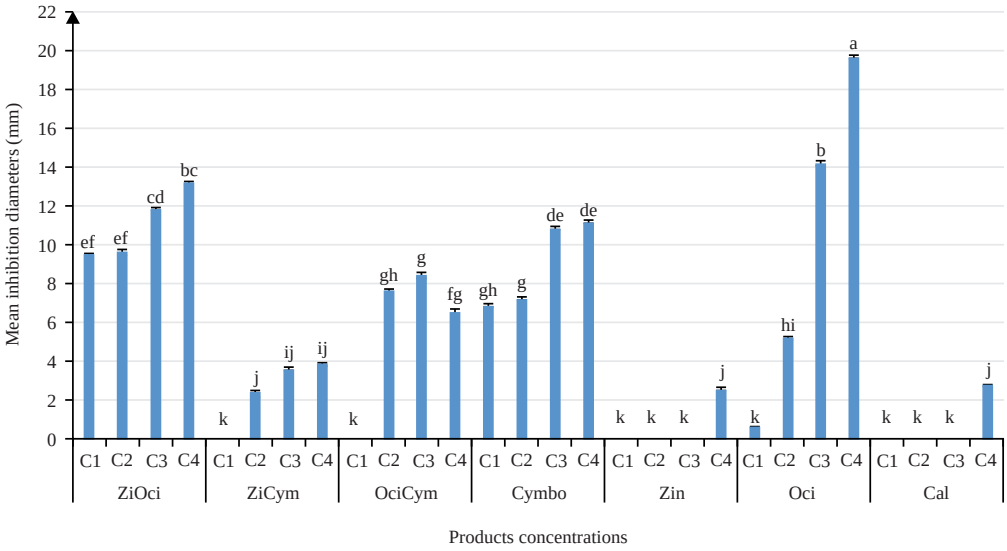

Fig. 5: Average inhibition diameters of strain S164 by products at different concentrations
Error bars surmounted by the same letter are statistically identical according to the Newman-Keuls Test at threshold α = 0.05

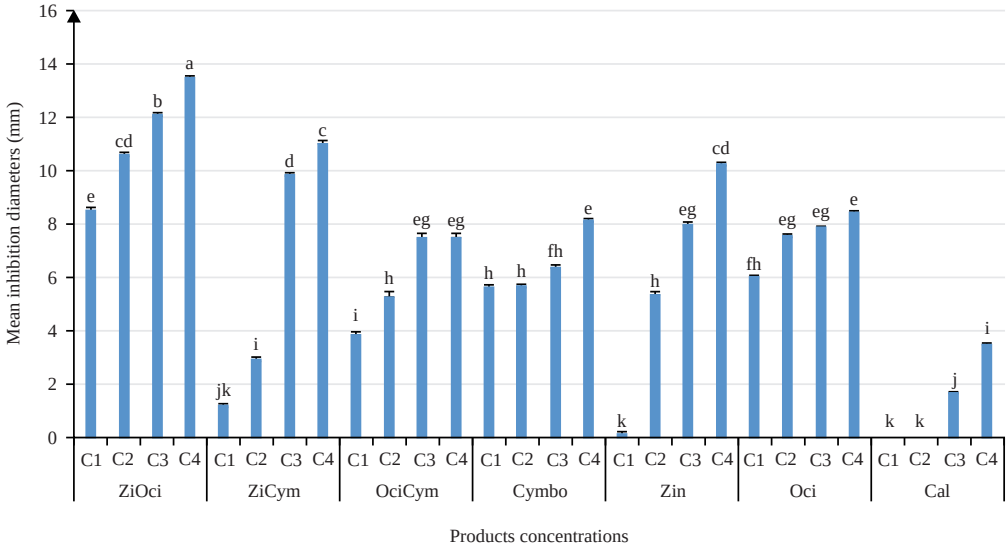

Fig. 6: Average inhibition diameters of strain S229 by products at different concentrations
Error bars surmounted by the same letter are statistically identical according to the Newman-Keuls Test at threshold α = 0.05

The incidence of disease (S150 strain) on leaves after treatment showed that the different products tested had different antibacterial effects. However, Zin and ZiOci recorded the highest incidences of 7.99 and 9.33%, respectively at CI1 concentration. On the other hand, no discs were infected at CI3 concentration with Zin and Oci products. These values differ from those of leaves treated with the synthetic product, which recorded a higher incidence of 29.32% (Fig. 8).

Severity and incidence of leaf scald induced by strain S229 on treated sugarcane leaf slices: Analysis of the results showed low disease severity on leaves treated with all products except Callicuivre. Oci was the most effective product, with total inhibition of disease development at CI3 concentration. At CI3, Zin and ZiOci were less effective, with infection rates of 0.26 and 0.79%, respectively. All the products tested were more effective than the synthetic product, which had a severity of 12.43% (Fig. 9).

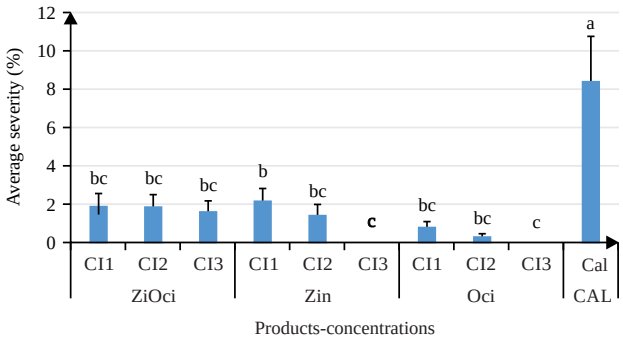

Fig. 7: Severity of S150 strain on treated leaf discs as a function of product and concentration

Error bars surmounted by the same letter are statistically identical according to the Newman-Keuls Test at threshold α = 0.05

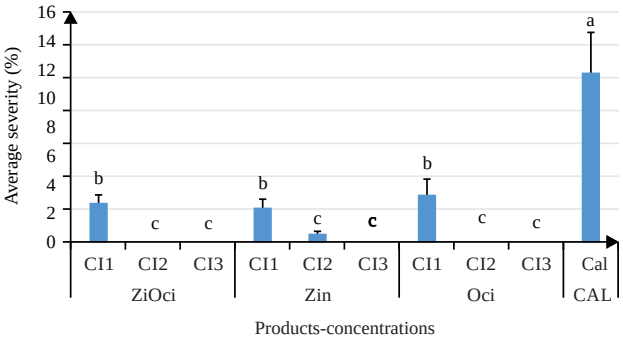

Fig. 8: Impact of strain S150 on treated leaf discs as a function of products tested and concentrations

Error bars surmounted by the same letter are statistically identical according to the Newman-Keuls Test at threshold α = 0.05

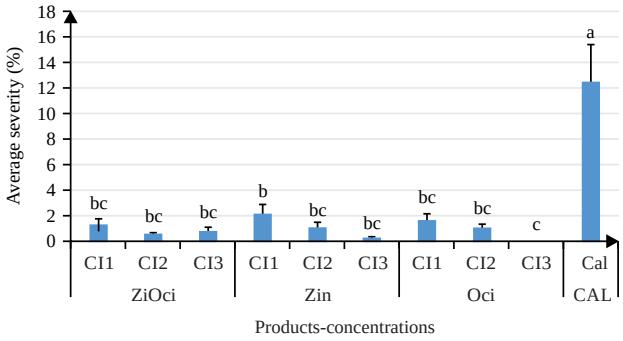

Fig. 9: Severity of strain S229 on treated leaf discs as a function of product and concentration

Error bars surmounted by the same letter are statistically identical according to the Newman-Keuls Test at threshold α = 0.05

The incidence of strain S229 on leaves after treatment showed that the ZiOci and Zin products recorded the highest incidence of 3.99 and 0.73% at CI3 concentration (Fig. 10). At CI3, however, leaves treated with Oci were not infected. Leaves treated with the synthetic product showed an infection rate of 36.88% (Fig. 10).

Severity and incidence of leaf scald induced by strain S164 on treated sugarcane leaf slices: Low severity was observed on leaves inoculated with strain 164 and treated with all products except callicopper. The CI2 and CI3 concentrations of the ZiOci and Oci products totally inhibited disease development. In the case of Zin, a severity of

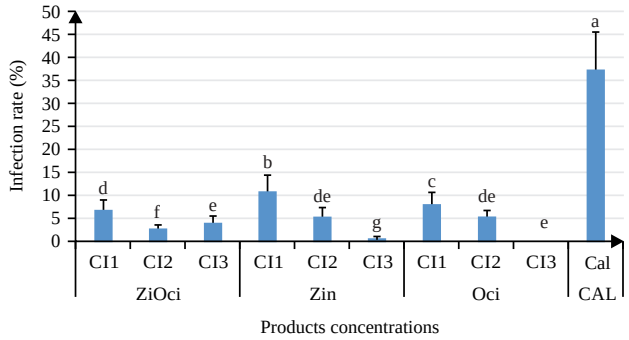

Fig. 10: Impact of strain S229 on treated leaf discs as a function of products tested and concentrations

Error bars surmounted by the same letter are statistically identical according to the Newman-Keuls Test at threshold α = 0.05

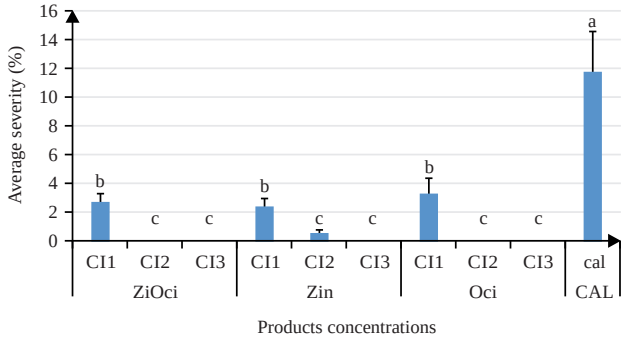

Fig. 11: Severity of strain S164 on treated leaf discs as a function of product and concentration

Error bars surmounted by the same letter are statistically identical according to the Newman-Keuls Test at threshold α = 0.05

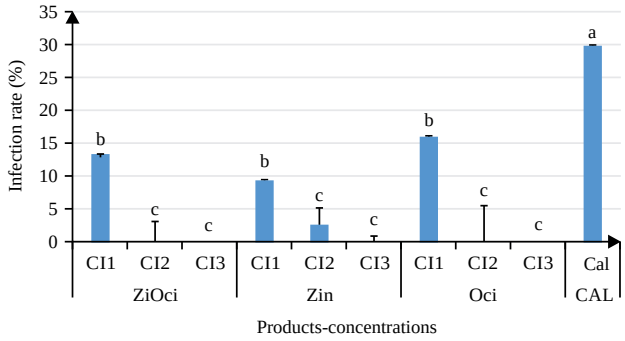

Fig. 12: Impact of strain S229 on treated leaf discs as a function of products tested and concentrations

Error bars surmounted by the same letter are statistically identical according to the Newman-Keuls Test at threshold α = 0.05

0.53% was observed at CI2 concentration. Leaves treated with the synthetic product showed a high severity of 11.73% (Fig. 11).

Zin recorded an incidence of 2.66% at CI2 and 0% at CI3. ZiOci and Oci were the most effective products, completely inhibiting infection at CI2 and CI3. On the other hand, the synthetic product recorded the highest infection rate at 29.77% (Fig. 12). The effect of natural substances on the development of leaf scald disease caused by strain S164 was shown in Fig. 13.

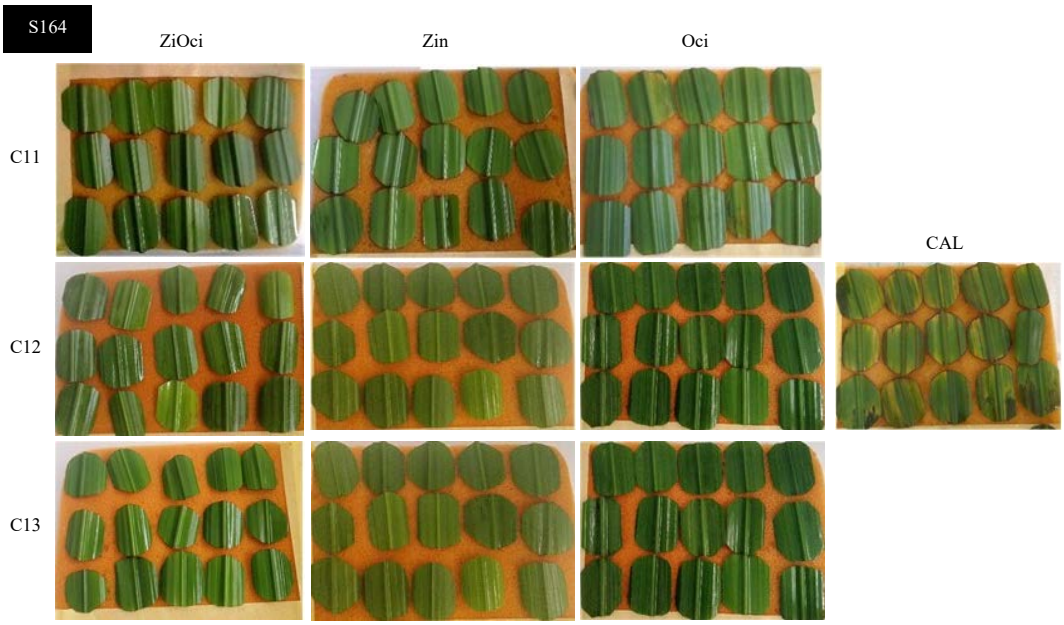

Fig. 13: Experimental set-up for studying the effect of natural substances and the synthetic product on the growth of strain S164 after 5 days
CI1: 4000 ppm, CI2: 6000 ppm and CI3: 8000 ppm

DISCUSSION

This study demonstrated the antibacterial activity of natural substances on the growth of *Xanthomonas albilineans* strains.

In vitro evaluation of the antibacterial activity of natural substances and the synthetic product (Callicuivre) on *Xanthomonas albilineans* strains showed different effects depending on the products and their concentrations. The natural substances *Zingiber officinale*, *Ocimum gratissimum* L., *Cymbopogon citratus* and the mixtures (*Zingiber officinale*+*Ocimum gratissimum* L., *Zingiber officinale*+*Cymbopogon citratus*, *Ocimum gratissimum*+*Cymbopogon citratus*) were more effective overall than the synthetic product (Callicuivre). The results of this study showed that, at the highest concentrations, the natural substances effectively inhibited the growth of bacterial strains. The antibacterial activity of these substances could be due to their chemical compositions. Indeed, the work of Kobenan *et al.*[19] showed that the essential oil of *Ocimum gratissimum* L. was predominantly composed of oxygenated monoterpenes including Thymol (24.57%), Camphor (5.53%) and hydrocarbon monoterpenes. According to Christian *et al.*[20], *Cymbopogon citratus* essential oil is composed of geranial (41.3%), neral (33%), myrcene (10.4%) and geranyl acetate (2.4%). The composition of this biopesticide could justify its effectiveness against bacteria. Wannissorn *et al.*[21] revealed that, *Cymbopogon citratus* essential oil inhibits bacterial growth. Furthermore, according to Tofiño-Rivera *et al.*[22], this essential oil is capable of destroying complex structures, such as the biofilm formed by Streptococcus.

The combination of *Zingiber officinale*+*Ocimum gratissimum* L. (ZiOci) was more effective at almost all concentrations. Most of the strains used were sensitive to the combination of products tested (ZiOci, ZiCym and OciCym) at different concentrations, with diameters ranging from 9 to 14 mm. In addition, the strains were sensitive to essential oils of *Zingiber officinale*, *Ocimum gratissimum* L. and *Cymbopogon citratus* at concentrations C3 (4000 ppm) and C4 (8000 ppm). Strain S164 was highly sensitive to Oci at 8000 ppm, with an inhibition diameter of 19.59 mm. Ponce *et al.*[16] showed that, a strain is said to be sensitive to a product when its inhibition diameter is between 09-14 mm and highly sensitive if the inhibition diameter is in the 15-19 mm range. According to them, a strain is extremely sensitive when the inhibition diameter is greater than 20 mm. Their study also showed that a strain can be non-susceptible (inhibition diameter less than or equal to 8 mm). Strain sensitivity is explained by the fact that the mixture of chemical compounds from two products acts effectively on the different growth stages of the bacteria. Lahlou[23] corroborated that, the biological activity of an essential oil is related to its chemical concentration and the synergistic effects between its components and that its efficacy takes account of all its constituents.

Compared with the other products, *Zingiber officinale* essential oil was less effective overall. The low efficacy of this product is probably due to the presence of molecules in the membrane structure of *Xanthomonas albilineans* strains, which prevent its inhibitory activity. Carine *et al.*[24] have shown in their work that the essential oil of *Zingiber officinale* has an effective antibacterial activity on the growth of *Xanthomonas albilineans* and could constitute a promising production activity in the fight against this bacterium in the Ivorian Agricultural Sector.

The inhibition rate of the natural substances was determined according to each concentration and *Xanthomonas albilineans* strain. The ZiOci formulation showed the highest inhibition rates with strains S150 and S229, while the essential oil of *Ocimum gratissimum* L. (Oci), showed the highest inhibition rate with strain S164. OciCym, ZiCym, Cymbo and Zin induced similarly high inhibition rates, which differed according to strain. On the other hand, compared with natural substances, the antibacterial activity of the synthetic product was lower overall. The efficacy of the ZiOci and Oci products could be explained by their ability to infiltrate the membrane and wall of bacterial strains. Also, the high antibacterial activity of these products could probably be attributed to the flexibility of bacteria's outer membranes, which surround the cell walls and also limit the diffusion of hydrophobic compounds by covering lipopolysaccharides. The low antibacterial activity of some products is due to their inability to cross the membrane of strains. The results obtained are consistent with those of Nguefack *et al.*[25], Paret *et al.*[26] and Amari *et al.*[27], who assert that essential oils have both antibacterial and fungal properties. In addition, the effectiveness of essential oil combinations stems from the existence of several active ingredients with very strong antibacterial properties that inhibit the growth of strains. In fact, the number of compounds present in each oil and their ability to react could be at the root of their efficacy. According to Kpoviessi *et al.*[28], the essential oil of *Cymbopogon citratus*, contains 29 characterized compounds whose main constituents are geranial, neral and β-pinene and cis-geraniol.

Control tests carried out on leaf discs in the laboratory assessed the antibacterial activity of natural substances on leaf discs inoculated with three strains of *Xanthomonas albilineans*, S150, S164 and S229. The *Ocimum gratissimum* L. product was the most effective, recording a total inhibition rate of 0% at CI3 concentration, thus preventing disease development compared with the other biopesticides and the synthetic product. These rates were constant up to day five. The S164 strain was sensitive to the antibacterial activity of natural substances, while the S150 strain was less sensitive to the antibacterial activity of natural substances. Strain S229 was resistant to the antibacterial activity of natural substances. The work of Gabaze *et al.*[12] has also shown that *Phytophthora palmivora* is sensitive to biological products (NECO, ASTOUN, FERCA and DECO). Our results corroborate those of Balakissa *et al.*[29], who showed that the biofungicides NECO and ASTOUN recorded a very significant inhibition rate against Phytophthora palmivora, the agent responsible for brown pod rot, compared with the synthetic fungicide CALLOMIL SUPER. These results provide further evidence of the fungicidal or fungistatic properties of biological products, already mentioned by a number of researchers. Kassi *et al.*[14] demonstrated that banana plants in plots treated with the NECO biofungicide proved less prone to infection by *Mycosphaerella fijiensis*, the agent responsible for black cercosporiosis, than those in control plots. The application of biopesticides to the leaf discs inoculated with the bacterial strains reduced the susceptibility and severity of the strains on the leaves. The reduction in disease severity could be explained by the chemical composition of these natural substances. The results of the current work give great hope to the sugar industry and sugarcane growers in Côte d'Ivoire. These biopesticides could play an important role in the search for sustainable control strategies and healthy production.

These studies should be pursued with a view to improving sugarcane production and quality for export and also in the interests of the sugar industries in Côte d'Ivoire. It would therefore be interesting to continue this study with a view to:

- Determine the pathogenicity of *Xanthomonas albilineans* strains under semi-controlled conditions
- Evaluate the effect of natural substances on strains under semi-controlled conditions

CONCLUSION

The present study demonstrated the antibacterial activity of the natural substances of the synthetic product (Callicuivre) against *Xanthomonas albilineans* strains *in vitro*. The efficacy of these products was determined on inoculated R585 sugarcane leaves. The inhibitory effect of the products was assessed. In fact, all the products tested had antibacterial effects, depending on the concentrations and strains of *Xanthomonas albilineans.* Moreover, on the whole, the natural substances were more active than the synthetic product. Specifically, under *in vitro* conditions, the essential oils of *Ocimum gratissimum* L., *Cymbopogon citratus* and the mixture of *Zingiber officinale*+*Ocimum gratissimum* L. had

the best growth inhibition diameters for bacterial strains at concentrations of 4000 and 8000 ppm. At a concentration of 8000 ppm (Cl3), the essential oil of *Ocimum gratissimum* L. (Oci) and the combination of *Zingiber officinale*+*Ocimum gratissimum* L. (ZiOci) effectively reduced the incidence and severity of the disease on sugarcane leaves. These natural substances could provide an alternative to chemical control, since, unlike synthetic pesticides, their use would be safe for the environment, have no effect on beneficial fauna and be non-toxic to humans.

SIGNIFICANCE STATEMENT

The aim of this project was to contribute to the sustainable production of sugarcane in Côte d'Ivoire, by controlling leaf scald disease using natural substances. This work is in line with the adoption of environmentally friendly approaches to plant extracts, which represent a promising avenue for controlling the pathogens responsible for fungal and bacterial diseases. This study revealed that all essential oils inhibit the growth of bacterial strains. In addition, *Ocimum gratissimum* L. oils and the combination of *Zingiber officinale*+*Ocimum gratissimum* L. were the most effective at concentrations C3 (4000 ppm) and C4 (8000 ppm) with diameters greater than 11 mm.

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