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PATHOGENICITY OF *PUCCINIA KUEHNII* STRAINS AND *IN VITRO* ANTIFUNGAL ACTIVITY OF THREE ESSENTIAL OILS AGAINST ORANGE RUST OF SUGARCANE IN CÔTE D'IVOIRE

[a]N'GUESSAN Aya Carine*, [a]KONE Klinnanga Noël, [b]KOUAME KONAN Didier, [c]YAO Kouadio Jacques Edouard, [b]KASSI Koffi Fernand Jean-Martial, [d]KAMARA Adjata, [b]KONE Daouda

[a] Peleforo Gon Coulibaly University, Faculty of Biological Sciences, Department of Plant Biology, Korhogo, Côte d'Ivoire.
[b] Félix Houphouët Boigny University, Faculty of Biosciences, Department of Plant Physiology and Pathology, Abidjan 22, Côte d'Ivoire.
[c] Jean Lorougnon Guédé University, Faculty of Agroforestry, Department of Plant Physiology, Daloa, Côte d'Ivoire.
[d]Alassane OUATTARA University, Faculty of Science and Technology, Plant Physiology Laboratory, Bouaké 01, Côte d'Ivoire.

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ABSTRACT

Rationale: Sugarcane plays an important role in Côte d'Ivoire. It is subject to various biotic attacks, notably orange rust caused by *Puccinia kuehnii*. Existing methods of controlling this fungus have their limitations. **Objectives**: This study aimed to evaluate the infectivity of *P. kuehnii* strains and to test the antifungal activity of three essential oils against these strains in the laboratory. **Methods**: The pathogenicity of 19 strains was evaluated on Petri dishes using 5-cm leaf pieces from the SP711406 sugarcane variety. Inoculation of healthy leaves was carried out by contact with symptomatic leaves. The antifungal activity of the essential oils of *Ocimum gratissimum* L., *Zingiber officinale,* and *Cymbopogon citratus*, at concentrations of 500, 1000, and 2000 ppm, was tested by spraying the leaves in Petri dishes, which were then inoculated by contact. The parameters evaluated were incubation time, incidence, and disease severity index. **Results**: The optimal incubation period observed was three days. The highest severity indices ranged from 12.14% to 12.85%, while the highest incidence ranged from 50% to 50.89%. Essential oils exhibited variable inhibitory effects on disease progression at different concentrations. Furthermore, the concentration of 2000 ppm proved to be the most effective with all strains. **Conclusion**: These essential oils may be an alternative for controlling orange rust in sugar cane in Côte d'Ivoire.

Corresponding Author: N'GUESSAN Aya Carine
Email: knkone333@gmail.com ORCID: https://orcid.org/0009-0000-2612-4931
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INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a grass mainly cultivated in tropical and subtropical regions for its high sucrose content. From prehistoric times, sugar cane and honey were the only sources of sugar available to humankind. Sugar cane contains up to 16% sucrose in its stems, 96% of which can be extracted through an industrial process (Alfa, 2005).

Sugarcane cultivation has experienced production declines closely linked to numerous biotic constraints (Kouamé *et al*., 2018). Among these biotic constraints is orange rust disease caused by *Puccinia kuehnii*. Orange rust can affect yield components such as stem biomass and sucrose content, causing sugar losses per unit area exceeding 50% in some susceptible cultivars (Raid, 2010). The pathogen affects plant growth and yield by reducing leaf chlorophyll content, carbon sequestration efficiency, stomatal conductance, leaf transpiration rate, and net photosynthesis (Zhao *et al*., 2011). Orange rust has been reported to cause yield losses exceeding 40% in

Florida (Raid, 2010). Similar high-yield losses have also been documented in Australia following the emergence of orange rust on the Q124 variety in the late 1990s (Braithwaite *et al*., 2009). The emergence of orange rust on the Q124 variety reduced the area under cultivation of this variety from 85% in 2000 to approximately 55% in 2001 (Magarey *et al*., 2001b). In Côte d'Ivoire, orange rust was observed for the first time in 2009 in Zuénoula and in 2010 in Borotou-Koro, on the SP 71-6180 and Co 997 varieties (Saumtally *et al*., 2011).

To combat sugarcane diseases, producers rely on chemical control, the most commonly used method. However, its intensive use is harmful to the health of farmers, consumers, and the environment (Gouda *et al*., 2018).

In Côte d'Ivoire, little work has been done on orange rust. The productivity of sugar complexes could be improved by using sustainable, effective, and environmentally friendly methods to combat this pathogen. It is therefore necessary to propose and implement a biological control method based on natural substances. Indeed, natural substances are effective and pose no risk of contamination to users, the environment, or producers (Kassi *et al*., 2014). Some biopesticides are classified as safe substances. They can be used to prevent the growth and multiplication of pathogenic microorganisms, including bacteria, viruses, and fungi (Gachkar *et al*., 2007).

The overall objective of this work is therefore to contribute to improving sugarcane productivity by better understanding orange rust and developing biological control strategies based on natural substances. Specifically, the study aims to:

- Evaluate the infectivity of *P. kuehnii* on the leaves of the SP711406 variety of sugarcane;
- Test *in vitro* the antifungal activity of formulations based on natural substances against fungal strains.

MATERIAL AND METHODS

Plant Material

The plant material consisted of nineteen (19) leaves showing symptoms of orange rust, constituting the sugarcane samples (Table I). These leaves were collected from several varieties within the SUCRIVOIRE Integrated Agricultural Units, including three (03) taken from Zuénoula and sixteen (16) from Borotou-Koro. In addition, asymptomatic leaves of the SP711406 variety were used. These leaves were collected from a cutting propagation plot located at Felix HOUPHOUËT-BOIGNY University.

Table 1. Sample references tested.

Localities	Samples references	Varieties	Sample Codes
Borotou-Koro	BPI19/BCO997.R34	CO997	35B
	BP15DR570.R49	R570	51bB
	B15DR570.51	R570	53B
Zuénoula	ZPI22AM1400/86.R389	M1400/86	389Z
	ZPI30AR570.R114	R570	343Z
	ZPI1CSP711406.R135	SP711406	364Z
	ZPIC97R570.B53	R570	392Z
	ZPI30AR570.R122	R570	351Z
	ZPI1BR570.R152	R570	381Z
	ZPI1BR570.R154	R570	383Z
	ZPI1CSP711406.R130	SP711406	359Z
	ZPI1BR570.R140	R570	369Z
	ZPI30AR570.R116	R570	345Z
	ZPI2AR96/2569.R78	R96/2569	304Z
	ZPI1BR570.R148	R570	377Z
	ZPI30AR570.R129	R570	358Z
	ZPI22AM1400/86.R156	M1400/86	386Z
	ZPI17CSP711406.R107	SP711406	336Z
	ZPI1BR570.R144	R570	373Z

Natural Substances and Synthetic Products

The antifungal activity of essential oils from *Zingiber officinale*, *Cymbopogon citratus*, and *Ocium gratissimum* L. was evaluated. These natural substances were provided by the Industrial Research Unit (URI) on biopesticides at Félix HOUPHOUËT-BOIGNY University in Abidjan (Ivory Coast). The synthetic product callicuivre was used as a positive control. This product was provided by Callivoire (Côte d'Ivoire).

***In vitro* Evaluation of the Pathogenicity of *Puccinia kuehnii* Strains**

Preparation of leaves

Young leaves from 6-month-old SP711406 sugarcane plants were harvested and cut into 5 cm long pieces. These leaves were rinsed with sterile distilled water and then placed in 90 mm diameter Petri dishes containing sterile blotting paper.

Sporulation test

The urediospore sporulation test consisted of placing the symptomatic samples on blotting paper in 90 mm diameter Petri dishes. The dishes were incubated overnight in the dark at 22°C with 97% relative humidity, conditions considered optimal for spore germination and infection (Infante *et al*., 2009; Minchio *et al*., 2011).

Leaf inoculation

The inoculation technique used was contact inoculation. After incubation, the infected samples were cut into pieces approximately 2 cm in size and placed on new 5 cm leaves in Petri dishes (Figure 1).

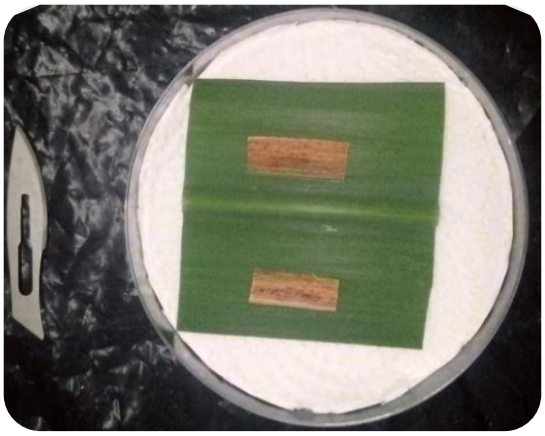


Figure 1. Incubation by contact with sugarcane leaves.

Four replicates per sample were performed. The control was inoculated with asymptomatic samples. The Petri dishes were incubated at 22°C with a relative humidity of 97%. Sporulation was confirmed by the appearance of orange rust symptoms on the leaves.

Evaluation of Phytopathological Parameters

The phytosanitary evaluations, conducted over seven (07) days, focused on the incubation period, severity index, and incidence of orange rust disease. The incubation period was defined as the time from inoculation to the appearance of the first symptoms. The severity index provided a quantitative assessment of the degree of disease attack (Table 2 and Figure 2). The latter expresses the intensity of the symptoms observed on the leaves. Scores were assigned to the symptoms observed on each leaf fragment according to the symptom rating scale as modified by Purdy *et al*. (1981). This severity index was calculated using the following formula:

$$\text{SI (\%)} = \frac{\sum \text{Xi. ni}}{\text{N. Z}} X100$$

SI: Severity index; Xi: score for disease i; ni: number of stems with score Xi;

N: total number of inoculated leaves; Z: highest score on the scale.

The incidence of disease is defined here as the proportion of diseased leaves in a given proportion of leaves. It was determined using the formula developed by Bansal *et al*. (1994):

$$\text{Incidence (\%)} = \frac{number\ of\ infected\ leaves}{\text{total number of leaves}} X100$$

The aggressiveness of the strains was determined by the observed incidence and severity. Following evaluation of the pathogenicity of the different strains, microscopic observations were conducted in the laboratory on leaves showing symptoms of orange rust in sugarcane.

***In vitro* Evaluation of the Antifungal Activity of Natural Substances on the Incidence and Severity of Orange Rust Disease**

Obtaining concentrations and leaf treatments

In this study, essential oils at concentrations of 500, 1000 and 2000 ppm from *Z. officinale*, *C. citratus,* and *O. gratissimum* L. were tested. The mixtures were prepared in test tubes containing 20 ml of sterile distilled water. A drop of Tween 20 was added to each test tube to facilitate the miscibility of the oil with water. The synthetic product Callicuivre (copper oxide) was used as a positive control at a single concentration of 5 g/l (5000 ppm), while the negative control consisted of sterilized distilled water (EDS). A curative control method was used, and the treatments were applied by foliar spraying (Figure 3).

Table 2. Rating scale for the severity of orange rust in sugarcane, modified from Tai *et al*. (1981).

Scores	Percentage (%)	Characteristics
0	0	No infection
1	1 to 20	Leaves showing chlorotic or necrotic spots, no sporulation
2	21 to 40	Small, thick pustules bearing spores, but easily identifiable
3	41 to 60	More abundant pustules distributed across the entire surface of most older leaves
4	61 to 80	More numerous pustules spread across the entire surface of the leaf
5	81 to 100	Large, coalescing pustules

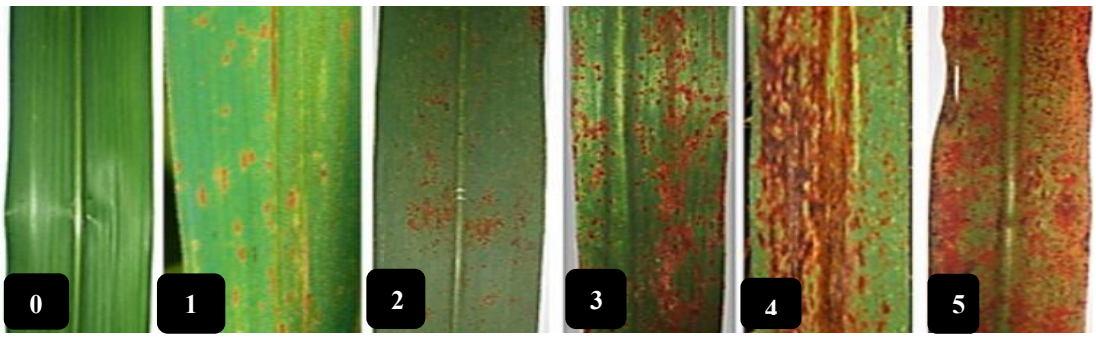


Figure 2. Rating scale for orange rust symptoms modified from Tai *et al*. (1981).

Inoculum production and leaf inoculation

Inoculum production was carried out as previously (pathogenicity test). As a result, the effects of the formulations were determined for six samples (51bB, 53B, 358Z, 373Z, 381Z, and 386Z). Incidence and severity index were the parameters evaluated during this activity.



Figure 3. Treatment of leaf fragments by spraying (A) and placement in Petri dishes (B and C).

Data Analysis

The data collected were recorded using Excel 2016 and then analyzed using STATISTCA 7.1. An ANOVA was performed to evaluate the effects of different product doses on sugarcane leaf infection. In the event of a significant difference, the Newman-Keuls comparison test at the 5% significance level was used to separate the means into homogeneous groups.

RESULTS

Pathogenicity of *P. kuehnii* Strains on Sugarcane Leaf Fragments

Symptoms observed

The pathogenicity test on sugarcane leaf fragments recorded symptoms characteristic of orange rust. These symptoms are characterized by small lesions similar to elongated chlorotic spots surrounded by a yellow halo (Figure 4).

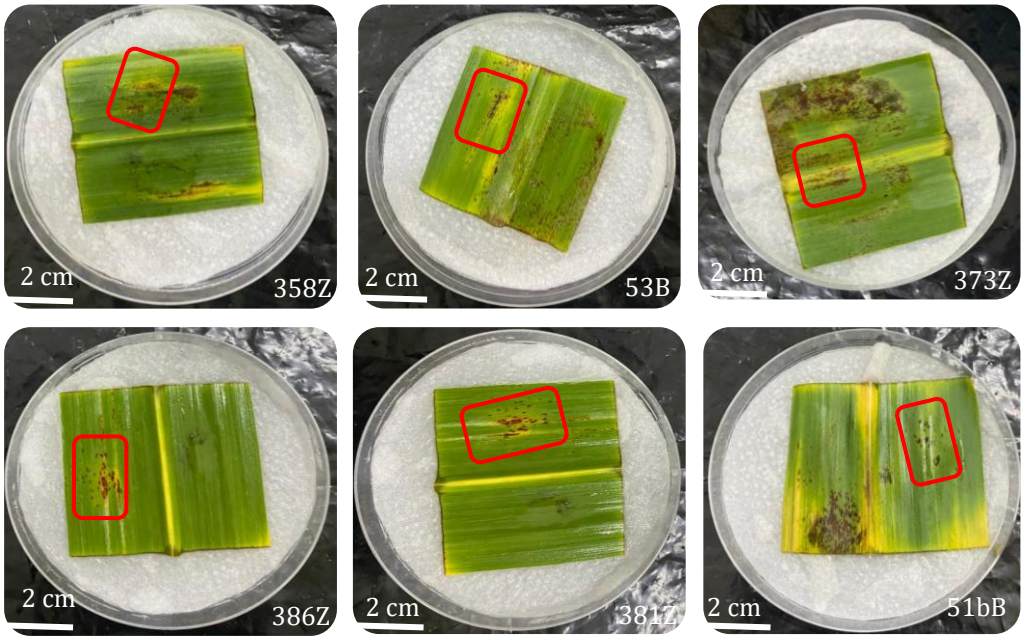


Figure 4. Presence of orange rust on detached sugarcane leaves 7 days after inoculation.

Incubation Time and Incidence of Orange Rust on Leaf Fragments

The first symptoms appeared three days after inoculation with samples 35B, 51bB, 53B, 343Z, 381Z, 377Z, 358Z, and 386Z. Samples 389Z, 345Z, 336Z, and 373Z had a four-day incubation period after inoculation. The longest incubation period was five days, observed with sample 383Z. The results on the average incidence of *Puccinia kuehnii* samples on sugarcane leaves as a function of time reveal four levels of infection rate: 00 ; 25 ; 50 and 75% on the seventh day after inoculation. An incidence of 68.75% was observed with samples 358Z and 373Z three days (D3) and four days after inoculation (D4), respectively. These incidences remained constant until the sixth day (D6), then peaked on the seventh day (D7) at 75%. Sample 381Z induced a gradual increase in incidence, from 50% on the third day to 75% on the fourth day, then remained constant until D7. The incidence curve was low with samples 304Z and 336Z, varying from 0 to 25% (Table 3).

Table 3.ss Evolution in the average incidence of orange rust by sample and time period.

Samples	Number of days after inoculation						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
S35	0.00±0,00	0.00±0.00	25±10.2	50±10.2	50±10.20	50±20.41	50±27.00
S51b	0.00±0.00	0.00±0.00	18.75±11.96	25±10.2	25±10.2	25±10.2	25±10.2
S53	0.00±0.00	0.00±0.00	31.25±15.72	50±20.41	75±30.61	75±30.61	75±30.61
S389	0.00±0.00	0.00±0.00	0.00±0.00	25±17.67	25±10.20	25±10.2	25±10.2
S343	0.00±0.00	0.00±0.00	25±10.20	50±20.41	50±20.41	50±20.41	50±20.41

Continue…

S364	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
S392	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
S351	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
S381	0.00±0.00	0.00±0.00	50±30.61	75±0.00	75±30.61	75±20.41	75±0.00
S383	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	25±10.20	27.5±13.14	50±20.41
S359	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
S369	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	25±10.20	25±10.20
S345	0.00±0.00	0.00±0.00	0.00±0.00	25±17.67	25±17.67	25±10.20	50±20.41
S304	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
S377	0.00±0.00	0.00±0.00	25±10.20	25±10.20	25±10.20	25±10.20	25±10.20
S358	0.00±0.00	0.00±0.00	68.75±25.76	68.75±25.76	68.75±25.76	75±0.00	75±0.00
S386	0.00±0.00	0.00±0.00	25±0.00	50±20.41	68.75±25.76	68.75±25.76	75±53.03
S336	0.00±0.00	0.00±0.00	0.00±0.00	25±10.20	25±10.20	25±10.20	25±10.20
S373	0.00±0.00	0.00±0.00	0.00±0.00	68.75±25.76	68.75±25.76	68.75±25.76	75±53.03

The highest average incidences were 50% and 50.89%, obtained with samples 381Z and 358Z, respectively. Intermediate average incidence values (between 40% and 43%) were observed for samples 53B, Z386, and 373Z. Samples 389Z, 383Z, 345Z, and 336Z showed statistically identical low infection rates, ranging from 2.85 to 3.57%. Leaves inoculated with samples 364Z, 392Z, 351Z, 359Z, and 304Z showed no symptoms of the disease (Figure 5).

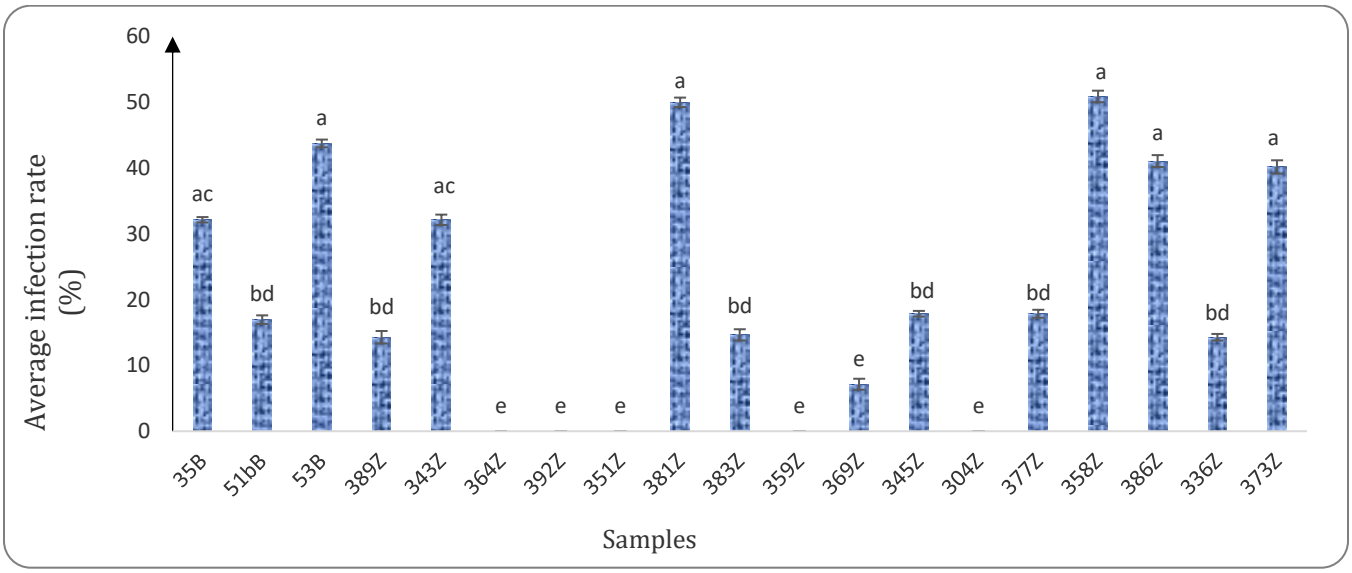


Figure 5. Infection rate of orange rust on inoculated leaves according to the samples.
Bars with the same letters are not significantly different at the 5% level according to the Newman-Keuls test.

Average Severity Index of Orange Rust on Sugarcane Leaf Fragments

The average severity index of samples on sugarcane leaves over time is shown in Table IV. Sample 386Z recorded a severity index of 15% on day 3, then remained constant until day 6. This severity index reached a maximum of 25% on day 7. From the third to the fourth day after inoculation, a severity index of 15% was recorded with sample 358Z. In addition, this severity index rose from 20% on day 5 to 25% on day 6 and then remained constant until day 7. The lowest severity indices were observed with sample 336Z (5%), 377Z (5%), and 304Z (0.00%). Samples 383Z. 343Z and 345Z showed intermediate developments with an identical severity index of 10%, while sample 51bB recorded an average value of 15%.

Average severity indices of 12.14%; 12.14% and 12.85% were obtained with samples 381Z, 358Z, and 386Z, respectively. However, with samples 364Z, 392Z, 351Z, 359Z and 304Z. Zero severity indices were observed. In addition, the others showed intermediate average severity indices ranging from 1.42 to 10% (Figure 6).

Table 4. Evolution of the orange rust severity index according to samples and time.

Samples	Number of days after inoculation						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
S35	0.00±0.00	0.00±0.00	5±2.04	10±2.04	10±2.04	10±4.08	10±5.40
S51b	0.00±0.00	0.00±0.00	3.75±2.39	10±4.08	15±6.12	15±6.12	15±6.12
S53	0.00±0.00	0.00±0.00	6.25±3.14	10±4.08	20±8.16	15±6.12	15±6.12
S389	0.00±0.00	0.00±0.00	0.00±0.00	5±3.53	5±2.04	5±2.04	5±2.04
S343	0.00±0.00	0.00±0.00	5±2.04	10±4.08	10±4.08	10±4.08	10±4.08
S364	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
S392	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
S351	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
S381	0.00±0.00	0.00±0.00	15±0.00	15±0.00	15±4.08	20±8.16	20±4.08
S383	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	5±2.04	10±4.08	10±4.08
S359	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
S369	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	5±2.04	5±2.04
S345	0.00±0.00	0.00±0.00	0.00±0.00	5±3.53	5±3.53	5±2.04	10±4.08
S304	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
S377	0.00±0.00	0.00±0.00	5±2.04	5±2.04	5±2.04	5±2.04	5±2.04
S358	0.00±0.00	0.00±0.00	15±0.00	15±0.00	15±6.12	15±6.12	25±10.20
S386	0.00±0.00	0.00±0.00	5±2.04	15±6.12	25±10.20	25±10.20	25±17.67
S336	0.00±0.00	0.00±0.00	0.00±0.00	5±2.04	5±2.04	5±2.04	5±2.04
S373	0.00±0.00	0.00±0.00	0.00±0.00	15±6.12	15±6.12	20±8.16	20±8.16

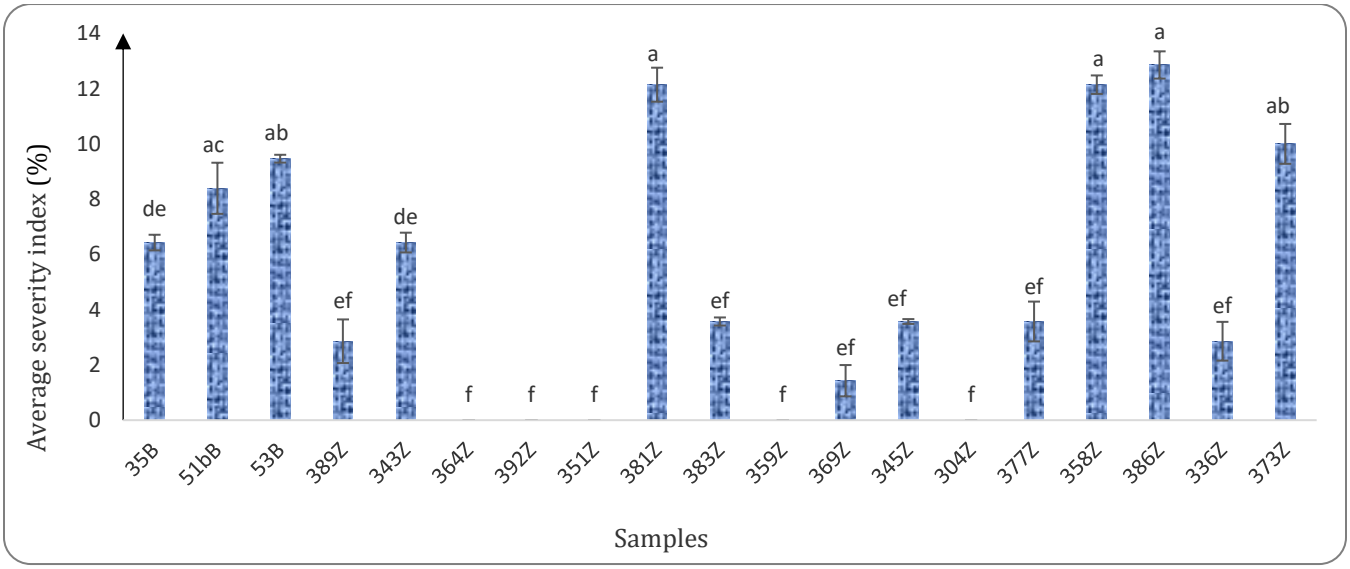


Figure 6. Severity index of orange rust on inoculated leaves according to samples.
Bars with the same letters are not significantly different at the 5% level according to the Newman-Keuls test.

In vitro Effect of Natural Substances on the Severity Index and Incidence of Orange Rust

Effect of _O. gratissimum_ L. Essential Oil on the Severity Index and Incidence of Orange Rust

Leaves inoculated with sample 381Z recorded a severity index of 28.57%, which was the highest, while the lowest was obtained with sample 358Z (13.33±3.41%) at 500 ppm. At a concentration of 1000 ppm, samples 358Z, 373Z, and 386Z had the lowest severity indices of 7.94±2.55%, 6.67±2.16%, and 5.71±1.61%, respectively. Furthermore, an intermediate severity index of 16.19±4.84% was observed with samples 51bB and 53B. In contrast, the highest severity index, estimated at 19.05±5.02%, was observed in sample 381Z. At a concentration of 2000 ppm, a severity index of zero was obtained with samples 358Z, 373Z, and 386Z. As for the synthetic product, the severity index was low in leaves inoculated with samples 358Z, 373Z, 386Z, 51bB, and 53B, with respective values of 0.00±0.00, 10.48±3.30, 3.81±1.1, 7.62±2.35, and 7.62±3.79%. However, a higher severity index of 23.81±6.48% was observed with sample 381Z. With sterile distilled water, high severity indices of 31.43±9.19% and 30.48±8.48% were obtained for samples 381Z and 386Z, respectively. The results indicate a significant difference at the 5% level according to the Newman-Keuls test with an estimated p-value of 0.0001 (Table 5).

Figure 7 reveals that at a concentration of 500 ppm, samples 381Z and 51bB had a higher incidence of 71.42%. Samples 358Z, 373Z, and 386Z recorded lower incidences at a concentration of 1000 ppm, with values of 25.39%, 19.04%, and 23.80%, respectively.

Table 5. Severity index of orange rust on leaf fragments according to concentrations of _O. gratissimum_ L. essential oil.

Products	Concentrations (ppm)	Samples					
		358Z	373Z	381Z	386Z	51bB	53B
Ocimum gratissimum L.	500	13.33±3.41ac	16.83±4.38ac	28.57±7.93a	17.14±5.26ac	23.81±6.40ab	17.14±4.73ac
	1000	7.94±2.55bc	6.67±2.16bc	19.05±5.02ac	5.71±1.61bc	16.19±4.84ac	12.38±3.45ac
	2000	0.00±0.00c	0.00±0.00c	13.33±3.65ac	0.00±0.00c	8.57±2.53bc	0.00±0.00c
Callicuivre	5000	0.00±0.00c	10.48±3.30ac	23.81±6.48ab	3.81±1.1c	7.62±2.35bc	7.62±3.79bc
Control	0.0	18.73±5.13ac	17.14±5.55ac	31.43±9.19a	30.48±8.48a	26.67±7.86a	26.67±5.34a
p-value		0.0001					
C.V		1.69					

Means followed by the same letters in the same column are not significantly different at the 5% level according to the Newman-Keuls test.

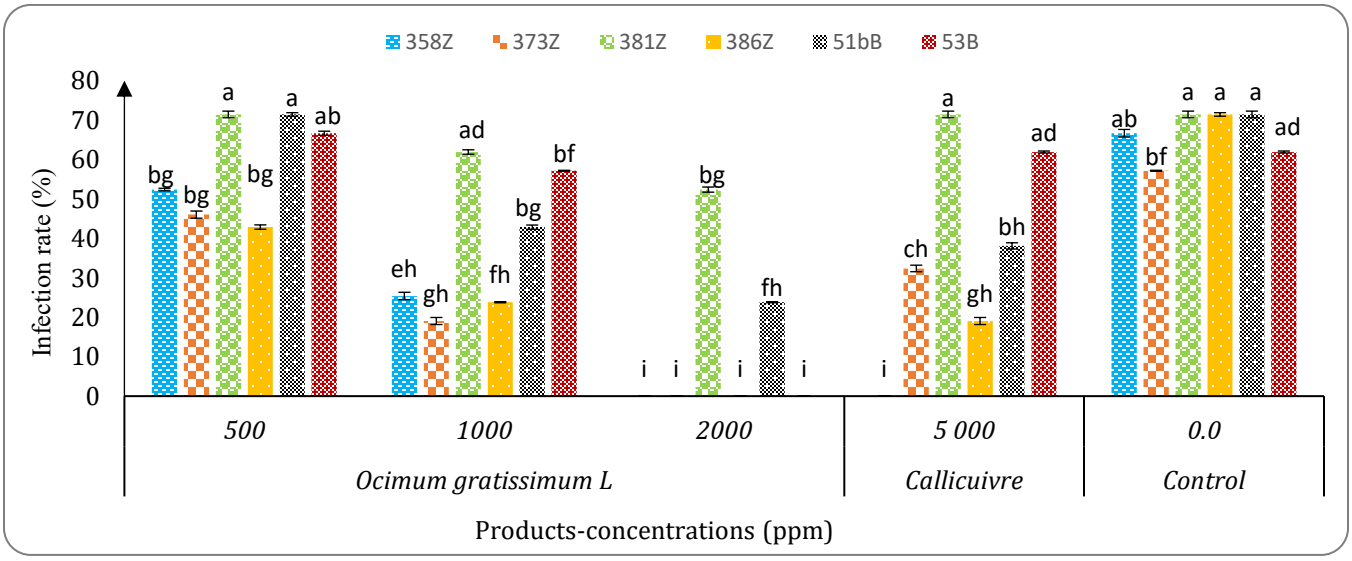


Figure 7. Incidence of orange rust on leaf fragments according to concentrations of _Ocimum gratissimum_ L. essential oil. Bars with the same letters are not significantly different at the 5% level according to the Newman-Keuls test.

However, the infection rate of 61.90% was high with sample 381Z. In addition, at a concentration of 2000 ppm, samples 358Z, 373Z, and 386Z recorded zero incidences. As for the synthetic product Callicuivre, it recorded higher incidences of 71.42% and 61.90% with strains 381Z and 53 B, respectively. These results are statistically identical to the control according to the Newman-Keuls test at the 5% threshold. In contrast, samples 358Z and 386Z recorded incidence rates of 0% and 19.04%, respectively. The average incidences obtained with samples 386Z and 51bB were higher in leaves inoculated and treated with sterilized distilled water than those treated with natural substances (control).

Effect of *Z. officinale* Essential Oil on Rust Severity Index and Incidence

The severity indices obtained for leaves inoculated with samples 381Z, 386Z, 51bB, and 53B, and treated with sterilized distilled water, were higher than those treated with natural substances and copper hydroxide. In this regard, the highest severity index observed with sample 381Z was 31.43±9.19% (Control). At a concentration of 500 ppm, sample 381Z recorded the highest severity index of 28.57±9.55%, while the lowest was observed in sample 358Z (7.62±2.12%). At 1000 ppm, samples 386Z, 373Z and 358Z had the lowest severity indices, with values of 6.67±2.16. 6.67±2.16, and 7.62±2.12%. Furthermore, zero severity indices were observed for samples 373Z and 386Z at a concentration of 2000 ppm. Additionally, the highest severity index, at 23.80%, was observed in sample 381Z, which was treated with a copper-based fungicide. However, with the synthetic product, sample 358Z showed no severity index for orange rust (Table 6).

Table 6. Severity index of orange rust on leaf fragments as a function of *Z. officinale* essential oil concentrations.

Products	Concentrations (ppm)	Samples					
		358Z	373Z	381Z	386Z	51bB	53B
Zingiber officinale	500	7.62±2.12bc	11.11±3.57ac	28.57±9.55a	20.95±5.75a	21.90±5.89ac	21.90±5.76ac
	1000	7.62±2.12bc	6.67±2.16bc	16.19±4.38ac	6.67±2.16bc	17.14±4.73ac	17.14±5.16ac
	2000	7.62±2.12bc	0.00±0.00c	16.19±4.38ac	0.00±0.00c	13.33±6.52ac	12.38±3.60ac
Callicuivre	5000	0.00±0.00c	10.48±3.30ac	23.81±6.48ab	3.81±1.1bc	7.62±2.35bc	7.62±3.79bc
Control	0.0	18.73±5.13ab	17.14±5.55ac	31.43±9.19a	30.48±8.48a	26.67±7.86a	26.67±5.34a
p-value		0.0001					
C.V		1.68					

Means followed by the same letters in the same column are not significantly different at the 5% level according to the Newman-Keuls test.

The results show a significant difference at the 5% threshold according to the Newman-Keuls test.

Leaves inoculated with samples 381Z, 386Z, and 51bB and treated with sterile distilled water showed statistically identical high incidence values of 71.42%. Samples 381Z, 386Z, 51bB and 53B induced statistically identical incidences ranging from 69.85% to 71.42% with *Z. officinale* oil at 500 ppm. At a concentration of 1000 ppm, samples 358Z. 373Z and 386Z recorded severities of 23.80 ; 19.04 and 19.04%, respectively, which were the lowest. At a concentration of 2000 ppm, the lowest incidence was observed with samples 358Z (23.8%), 373Z (0%), 386Z (0%), and 51bB (19.04%). The lowest incidences obtained with the synthetic product at 5000 ppm were 0% and 19.0%, recorded with samples 358Z and 386Z, respectively (Figure 8).

Effect of *C. citratus* Essential Oil on the Severity Index and Incidence of Orange Rust

Table 7 presents severity indices that differ significantly according to the Newman-Keuls test at the 5% significance level (p=0.0001). Samples 381Z and 51bB recorded higher severity indices of 24.76±6.67% and 22.86±6.28%, respectively, while the lowest severity indices were obtained with samples 358Z (10.48±3.30%) and 373Z (13.02±3.33%). As for sample 386Z, it recorded a severity index of zero (0.00±0.00c%) at a concentration of 500 ppm. At 1000 ppm, all samples obtained severity indices ranging from 0 to 13.65±3.61%. The results reveal zero severity indices for samples 358Z, 386Z, 51bB, and 53B at a concentration of 2000 ppm. In contrast, the highest severity index, at 23.81±6.48%, was observed in sample

381Z with copper-copper. Samples 358Z, 373Z, 386Z, 51Bb, and 53B yielded low severity indices ranging from 0.00±0.00 to 10.48±3.30%. Compared with natural substances and the synthetic compound (copper), the average severity indices for samples 381Z (31.43±9.19%) and 386Z (30.48±8.48%) were higher in leaves treated with sterilized distilled water.

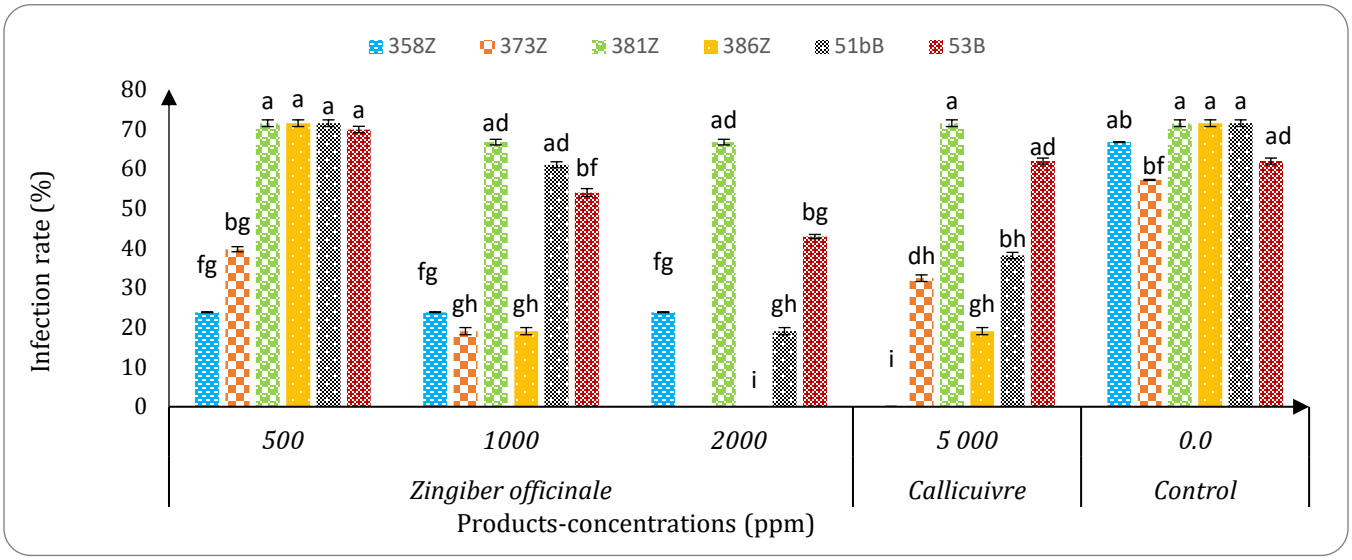


Figure 8. Incidence of orange rust on leaf fragments according to concentrations of *Z. officinale* essential oil. Bars with the same letters are not significantly different at the 5% level according to the Newman-Keuls test.

Table 7. Severity index of orange rust on leaf fragments as a function of *C. citratus* essential oil concentrations.

Products	Concentrations (ppm)	Samples					
		358Z	373Z	381Z	386Z	51bB	53B
Cymbopogon citratus	500	10.48±3.30ab	13.02±3.33ab	24.76±6.67a	0.00±0.00c	22.86±6.28ab	11.42±3.23ac
	1000	6.67±2.16ac	9.52±2.46ac	13.65±3.61ac	0.00±0.00c	6.67±2.16ac	3.80±1.18bc
	2000	0.00±0.00c	7.62±2.12ac	8.57±2.53ac	0.00±0.00c	0.00±0.00c	0.00±0.00c
Callicuivre	5000	0.00±0.00c	10.48±3.30ac	23.81±6.48ab	3.81±1.1bc	7.62±2.35ac	7.62±3.79ac
Control	0.0	18.73±5.13ac	17.14±5.55ac	31.43±9.19a	30.48±8.48a	26.67±7.86a	26.67±5.34a
p-value		0.0001					
C.V		1.87					

Means followed by the same letters in the same column are not significantly different at the 5% level according to the Newman-Keuls test.

Figure 9 shows that at a concentration of 500 ppm, samples 381Z and 51bB had the highest incidence of 71.42%. In contrast, the lowest incidences, estimated at 38.09%; 19.04% and 44.44% were obtained with samples 358Z, 373Z, and 386Z. At concentrations of 1000 and 2000 ppm. *C. citratus* essential oil recorded low incidences with all strains compared to the infection rate of plants treated with sterilized distilled water and copper sulphate. The synthetic product recorded the highest incidence rates, at 71.42% and 61.90%, with samples 381Z and 53 B, respectively. respectively. However, samples 358Z and 386Z recorded low incidence rates of 0% and 19.04 %.

Effect of Natural Substances on the Severity Index and Incidence of Orange Russt

The lowest severity indices were recorded at a concentration of 2000 ppm for the essential oils of *O. gratissimum* L. and *C. citratus*, with respective values of 7.78 and 5.56% (Table 8). In contrast, the highest severity indices were 34.44% and 39.64%, obtained, respectively, with essential oils of *O. gratissimum* L. and *Z. officinale* at 500 ppm. As for the synthetic product and the untreated

inoculated plants, the severity indices were 17.78% and 53.33%, respectively.

Regarding the incidence of the disease, the results indicate infection rates that are statistically identical to the 5% threshold according to the Newman-Keuls test, with estimated values of 100% in inoculated and untreated plants and 83% in plants treated with *O. gratissimum* L. essential oil at 500 ppm. At a concentration of 2000 ppm, the essential oils of *O. gratissimum* and *C. citratus* had the lowest incidences, 22.22% and 16.67%, respectively. The synthetic product (Callicuivre at 5000 ppm), and the essential oils of *C. citratus* (1000 ppm). *Z. officinale* (500 and 1000 ppm) and *O. gratissimum* L. (1000 ppm) essential oils showed intermediate infection rates, statistically identical to the 5% threshold according to the Newman-Keuls test, ranging from 61.11 to 79.62%.

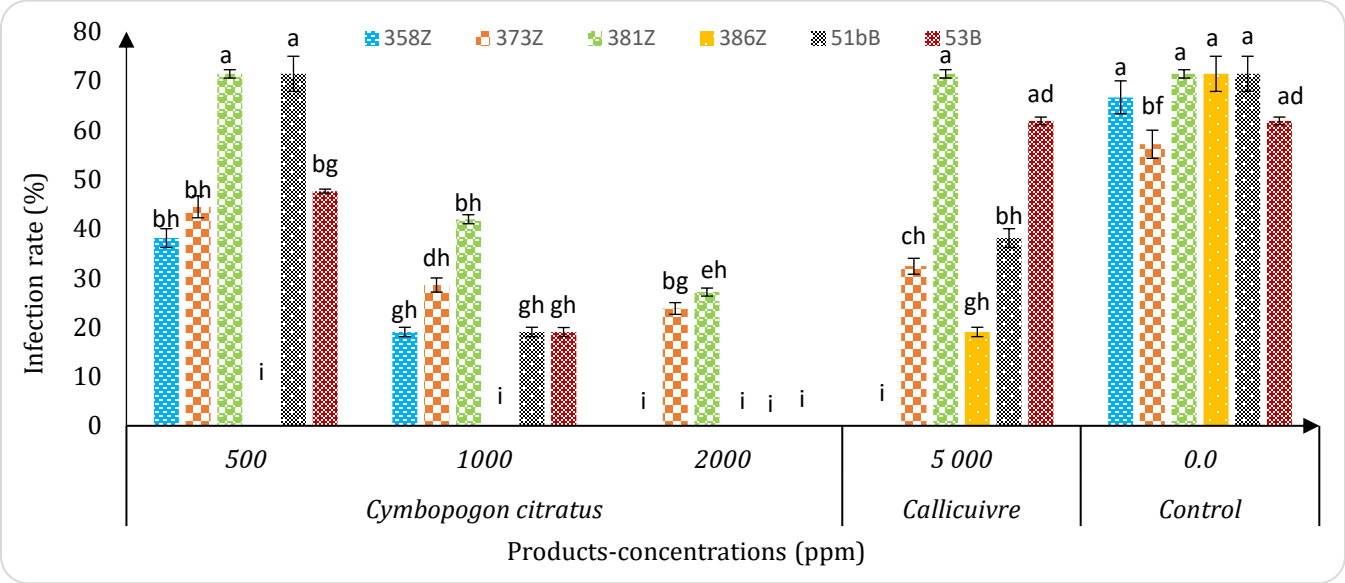


Figure 9. Incidence of orange rust on leaf fragments according to concentrations of *C. citratus* essential oil.
Bars with the same letters are not significantly different at the 5% level according to the Newman-Keuls test.

Table 8. Effect of essential oils at different concentrations on the severity index and incidence of orange rust.

Products	Concentrations (ppm)	Severity index mean (%)	Incidence (%)
Ocimum gratissimum L.	500	34.44ac	83.33a
	1000	20bc	61.11ab
	2000	7.78c	22.22c
Zingiber officinale	500	39.64ab	79.62ab
	1000	23.33bc	62.96ab
	2000	14.44bc	38.89b
Cymbopogon citratus	500	24.44bc	66.67ab
	1000	12.22bc	33.33b
	2000	5.56c	16.67c
Callicuivre	5000	17.78bc	61.11ab
Control	C0	53.33a	100a
	P-Value	0.000007	0.002363
	CV (%)	135.01	121.31

DISCUSSION

Pathogenicity of *Puccinia kuehnii* Strains

The pathogenicity of *P. kuehnii* strains was evaluated in the laboratory on detached leaves of the sugarcane variety SP711406 by contact.

The inoculated leaves showed symptoms of orange rust of sugarcane. Environmental conditions such as relative humidity (97%) and temperature (22°C) in Petri dishes could be factors favouring the onset of the disease. These conditions would be conducive to the development of orange rust of sugarcane. Our results corroborate those of Martins *et al*. (2010), who showed that sugarcane orange rust symptoms could develop under growth chamber conditions at temperatures between 20 and 25 °C and with more than 8 hours of leaf wetting.

The symptoms appeared as clusters of spots, in small successive dashes with a yellow halo. These results are consistent with those of Magarey *et al*. (2000), who showed that orange rust symptoms appear as lesions and small, elongated, chlorotic spots surrounded by a pale yellow-green halo, and quickly develop into orange-to-orange-brown pustules.

The study revealed varying incidence and severity indices of orange rust among strains on sugarcane leaf fragments. To this end, the highest incidences ranged from 40 to 50%, while the highest severity was 12.85%. This pathogenic diversity among strains could be related to their physiological mechanisms for overcoming the host's resistance mechanisms. Our results confirm those of Yohannes *et al*. (2010), who showed that physiological functioning varies among strains. In addition. Sanjel's (2016) study, conducted using inoculation tests on detached leaves to assess the pathogenic diversity of four isolates in Florida, also revealed at least two virulence groups among the strains.

In vitro Antifungal Effect of Natural Substances

The results of this study recorded a severity index and incidence of 00% with *O. gratissimum* L. oil at a concentration of 2000 ppm in samples 358Z, 373Z, and 386Z. As for the synthetic product (Callicuivre), a severity index and incidence of 00% were observed only with sample 358Z. The biopesticide containing the essential oil of *Ocimum gratissimum* L. was more effective. The effectiveness of this essential oil could be due to the presence of oxygenated compounds (thymol and terpinene) and hydrocarbons that act against orange rust. Studies by Camara *et al*. (2007) and Kassi *et al.* (2014) have shown the antifungal effect of *O. gratissimum* L. oil on black cercosporiosis of banana trees at a concentration of 1000 ppm. In addition, studies conducted by Janine d'Aquino *et al*. (2005) on the chemical composition of essential oils revealed that the essential oil of *O. gratissimum* L. consists primarily of eugenol, thymol, citral, ethyl cinnamate, and linalool.

Furthermore, the strong antifungal activity observed may be due solely to thymol. Indeed, according to Dorman and Deans (2000), thymol inhibits the synthesis of trehalase, an enzyme involved in glucose metabolism in fungi. Cowan (1999) and Lopez-Malo *et al*. (2005**)** confirm this assertion, noting that phenolic terpenes act on fungi through various mechanisms based, on the one hand, inactivating fungal enzymes that contain the SH group in their active site and, on the other hand, by binding to the amine and hydroxylamine groups of microbial membrane proteins, thereby causing an alteration in permeability and leakage of intracellular components. This oil has been effective against soil-borne fungi in vegetable crops (Doumbouya *et al*., 2012).

Z. officinale essential oil has an infection rate of 00% at a concentration of 2000 ppm with samples 373Z and 386Z. These results could be explained by the presence of antifungal properties in this essential oil. According to Khellah (2017). *Z. officinale* essential oil has antioxidant, antimicrobial, antifungal, and antibacterial properties. In addition, the work of Mvuemba *et al*. (2009) on ginger showed that *Z. officinale* oil at a concentration of 5000 ppm caused 100% inhibition of the fungi *Aspergillus niger* and *Fusarium sambusium*.

C. citratus oil, at a concentration of 2000 ppm, was more effective than the synthetic product. This could be explained by the active compounds inhibiting fungal enzymes. Our results are consistent with those of Danielli *et al*. (2018), who showed that the terpene components of essential oils inhibit membrane enzymes (ATPases, ergosterol synthase), leading to a loss of membrane integrity and inhibition of fungal growth. Guici and Boucetta (2017) noted in their study that the essential oil of *C. citratus* possesses antifungal properties that can effectively treat certain fungal infections. Furthermore, Tiendrebeogo *et al*. (2017) demonstrated that this essential oil inhibits the mycelial growth rate of *Pyricularia oryzae* v. Our results also confirm those of Yao *et al*. (2021), who revealed that the essential oil of *C. citratus* exhibits very high fungicidal efficacy against *S.*

scitamineum, the causative agent of sugarcane charcoal disease. The biotrophic nature of *Puccinia kuehnii* strains may confer genetic diversity upon them. This could lead to a different response than that of necrotrophic fungi when exposed to the natural substances tested.

CONCLUSION AND OUTLOOK

The objective of this study was to determine in the laboratory the infectivity of *Puccinia kuehnii* strains on leaves of the SP711406 variety of sugarcane, and to test the antifungal activity of three essential oils on the development of orange rust disease.

The results of the pathogenicity test showed that the optimal incubation period for orange rust disease in the laboratory is three days. Incidence and severity were higher with samples 381Z, 358Z, and 386Z.

All essential oils prevented the spread of orange rust on sugarcane. Furthermore, a concentration of 2,000 ppm proved to be the most effective for each product tested, with low severity and incidence.

These biopesticides could be an alternative to chemical control. To this end, these studies should be further investigated to better understand the effectiveness of fungicides at different stages of *P. kuehnii* growth.

It would therefore be advisable to continue this study with a view to:

- Evaluating the antifungal activity of essential oils on the severity and incidence of *P. kuehnii* strains in a greenhouse setting;
- Determining the effectiveness of these natural substances on orange rust disease in the field;
- Evaluate the economic value of phytosanitary treatments based on natural substances in the field.

AUTHOR CONTRIBUTIONS

N'G.A.C.: Writing - original draft, Investigation, Methodology. K.K.N.: Data curation, Formal analysis. K.K.D.: Resources, Funding acquisition. Y.K.J.E.: Review and editing. K.K.F.J.: Review and editing. K.A.: Writing - original draft, Writing - review and editing. K.D.: Supervision, Writing - original draft, Writing – review and editing. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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