ISSN 1819-1894

Asian Journal of Agricultural Research

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OPEN ACCESS Asian Journal of Agricultural Research

ISSN 1819-1894
DOI: 10.3923/ajar.2023.51.62

Research Article
Biological Control of *Puccinia kuehnii* Uredospores Germination, Responsible Agent for Orange Rust of Sugarcane (*Saccharum officinarum* L.)

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Abstract

Background and Objective: Sugarcane faces several biotic constraints, including the orange rust disease caused by *Puccinia kuehnii*, which can affect yield components. This study aimed to contribute to the improvement of sustainable sugarcane production in Côte d'Ivoire. **Materials and Methods:** The germinative capacity of *Puccinia kuehnii* urediniospores was assessed from 137 samples of sugarcane leaves symptomatic of orange rust collected from the Borotou-Koro and Zuénoula sugar complexes. Samples were cut into 10 cm sections, then incubated in test tubes containing 20 mL sterilized distilled water for 14 hrs in the dark. The efficacy of *Ocimum gratissimum* L., *Cymbopogon citratus* and *Zingiber officinale* essential oils was evaluated *in vitro*, on the germination of urediniospores from eight strains of *Puccinia kuehnii*. Germination rates ranged from 80 to 100%. Three concentrations, 500, 1000 and 2000 ppm, were tested. A 20 mL volume of each concentration was incorporated into the test tubes, then a drop of Tween 20 was added to facilitate miscibility. **Results:** The uredospores observed are characteristic of *Puccinia kuehnii*. The germination frequency of the strains was 78.83%, with a germination frequency of 86.90% (higher) for the Zuénoula strains. Essential oils had an inhibitory effect on uredospore germination at all concentrations. The best inhibitory concentration was 2000 ppm and *Zingiber officinale* oil was the most effective. In addition, the most effective IC_{50} and IC_{90} concentrations were recorded with *Cymbopogon citratus* and *Zingiber officinale* essential oils. **Conclusion:** Zuénoula strains had the highest germination capacity. The efficacy of essential oils was proven on the germination of *Puccinia kuehnii* urediniospores. At a concentration of 2000 ppm, these essential oils could be recommended for testing in the greenhouse and then in the field against orange rust disease.

Key words: Sugarcane, orange rust, *Puccinia kuehnii*, uredospores, germination

Citation: Kone, K.N., K.D. Kouame, A.C. N'Guessan, K.F.J-M. Kassi and K.J-E. Yao *et al*., 2023. Biological Control of *Puccinia kuehnii* Uredospores Germination, Responsible Agent for Orange Rust of Sugarcane (*Saccharum officinarum* L.). Asian J. Agric. Res., 17: 51-62.

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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 perennial plant of the Poaceae family (grasses) cultivated for its stalks, which contain a sweet juice[1]. It accounts for 85% of the sugar sold on the international market. Sugarcane occupies almost 20 million hectares worldwide, in 80 countries. Sugarcane production has fallen sharply as a result of numerous biotic and abiotic constraints[2]. Abiotic constraints are the effects of climate change and biotic constraints are the order of parasitic pressures causing disease. These include rust, brown rust or common rust caused by *Puccinia melanocephala* and orange rust caused by *Puccinia kuehnii*. The first observations of *Puccinia kuehnii* in La Réunion date back to July and August, 2018 on varieties R06/2006 and R06/2007, which were under selection at the time[3]. Orange rust of sugarcane, caused by the fungus *Puccinia kuehnii* (W. Krüger) E.J. Butler, is present in many parts of the world[4]. Indeed, the pathogen affects plant growth and yield by reducing leaf chlorophyll content, carbon sequestration efficiency, stomatal conductance, leaf transpiration rate and net photosynthesis[5]. From the 1990s onwards, the disease caused significant losses, while in Australia damage to the Q124 variety proved to be of the order of 24%. The most damaging effects of the disease occur in countries where production is concentrated around a few susceptible varieties[6]. To reduce crop losses, growers resort to chemical control, which is the most commonly used method of combating orange rust on sugarcane[7]. This method is based on the use of chemicals such as pyraclostrobin and azoxystrobin from the strobilurin group of fungicides, metconazole and propiconazole from the triazole group of fungicides and carboxamides. However, their intensive use is harmful to the health of farmers, consumers and the environment[8].

In Côte d'Ivoire, little work has been done on sugarcane orange rust since it first appeared in 2011[9]. In this context, there is a need for strategic studies aimed at proposing biological control methods based on natural substances. Furthermore, the work of Kassi *et al.*[10] has shown that natural substances are effective and risk-free in terms of contamination for the user, the environment and the producer. Some biopesticides are classified as safe substances and can be used to prevent the growth and multiplication of pathogenic microorganisms such as bacteria, viruses and fungi[11]. To contribute to the improvement of sustainable sugarcane production in Côte d'Ivoire, through *in vitro* control of the fungus responsible for this disease.

These were:

- To determine the germinative capacity of *Puccinia kuehnii* urediniospores *in vitro*
- Evaluate *in vitro* the antifungal activity of local plant essential oils on the germination of *Puccinia kuehnii* urediniospores

MATERIALS AND METHODS

Study area: The study was carried out between March and September, 2023. 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.

Materials

Plant material: The plant material consisted of sugarcane leaves infected with orange rust. These leaves were collected from the Zuénoula and Borotou-Koro sugar complexes. Healthy leaves were taken from a cutting propagation plot at the Félix Houphouët-Boigny University.

Fungal material: The fungal material consisted of uredospores of *Puccinia kuehnii*. These strains were obtained from samples of sugarcane leaves showing symptoms of orange rust. The samples were collected in the Integrated Agricultural Units (UAI) of Borotou-Koro and Zuénoula in Côte d'Ivoire.

Essential oils: The activity of four formulations based on natural substances from *Zingiber officinale*, *Cymbopogon citratus* and *Ocimum gratissimum* L. was evaluated. These hydro-distilled essential oils were supplied by the Industrial Research Unit (URI) of the Félix Houphouët-Boigny University.

Methods

***In vitro* evaluation of *Puccinia kuehnii* uredospore germination:** The germination capacity of *Puccinia kuehnii* spores was determined *in vitro* on symptomatic sugarcane leaves. The study involved a total of 137 leaf samples, of which fifty-three were collected from the Borotou-Koro sugar complex and eighty-four from Zuénoula. These samples, which had been collected and oven-dried at 28°C for 72 hrs to facilitate preservation, were cut into 10 cm long fragments (Fig. 1). The cut leaves were then placed in test tubes containing 20 mL of sterile distilled water (Fig. 1). Tubes were incubated in the dark at an optimum germination temperature of 22°C for 14 hrs, using the method of Minchio *et al.*[6], to facilitate uredospore germination. After incubation, pustules were removed from each sample using a scalpel blade and then spread in a drop of sterile distilled water between the slide and coverslip. Microscopic observations were then made at 40× magnification.

Fig. 1: Incubation of sugarcane leaves symptomatic of orange rust in test tubes

A total of three observations were made for each sample, as follows counting a set of fifty spores per observation. Urediniospores were considered to have germinated when the germ tubes were greater than or equal to their diameter[6].

Thus, the germination rate was determined by the ratio between the number of spores with germ tubes and the total number of spores observed. This rate was calculated using the following formula adapted from Dossa *et al.*[12].

Thus, the germination rate was determined by the ratio of the number of spores with germ tubes to the total number of spores observed. This rate was calculated using the following formula:

$$Tg\ (\%) = \left(\frac{n}{N}\right) \times 100$$

Where:

Tg = Germination rate
n = Number of urediniospores with germinated tubes
N = Total number of urediniospores observed

The germination rate of the strains was also determined as a function of the sugar-growing sites using.

The germination frequency of the strains was also determined according to the sugar-growing sites using the following formula:

$$\text{Frequency}\ (\%) = \left(\frac{\text{Number of stumps that have germinated}}{\text{Total number of souhe}}\right) \times 100$$

***In vitro* evaluation of the effect of essential oils on the germination of *Puccinia kuehnii* urediniospores**

Obtaining doses: Three formulations based on natural substances from three plants *Zingiber officinale*, *Cymbopogon citratus* and *Ocimum gratissimum* L. were evaluated *in vitro* against eight *Puccinia kuehnii* samples with high germination rates. For each formulation, three concentrations of 500, 1000 and 2000 ppm of each natural substance were tested.

Sample preparation: The effect of essential oils on spore germination was assessed on eight samples namely 381Z, 383Z, 389Z, 392Z, 35B, 51bB, 53B and 56B recording germination rates equal to or greater than 80%. Symptomatic leaf samples were cut into 10 cm long pieces and incorporated into test tubes containing 20 mL of sterile distilled water. A drop of Tween 20 was added to each test tube, homogenized by vortexing, to facilitate the miscibility of the natural substances with the water. The tubes were then incubated in the dark for 14 hrs at 22°C and 97% relative humidity. Three replicates were performed for each concentration.

Microscopic observation and spore counting: After incubation, spores were observed using an optical microscope at G×400 magnification. After incubation, the uredospores were observed at magnification 400 (G×400), using a ZEISS Axio Lab A1 optical microscope manufactured by the Carl Zeiss subsidiary in Jena, Germany. Uredospores were counted using the method described by Minchio *et al.*[6], which involved searching each test tube for 50 uredospores. Uredospores with germ tubes longer than their diameters were considered to have germinated. The germination rate was calculated as before.

IC_{50} and IC_{90} inhibitory concentrations of natural substances on uredospore germination: Inhibitory concentration values at 50% (IC_{50}) and 90% (IC_{90}) were determined using sigmoidal curves of probit values of uredospore germination inhibition rate versus base-10 logarithm according to the formula reviewed in Paranagama *et al.*[13]. The regression lines are established as follows:

$$Y = a \log x + b$$

Where:

a = Being the regression coefficient
b = Constant
x = Fungicide concentration
y = Probit
log = Decimal logarithm

The equations of these regression lines were used to determine the IC_{50} and IC_{90}, which are the concentrations that reduce fungal spore germination by 50 and 90%, respectively[14].

Statistical analysis: The data collected were recorded in Excel 2016 and analyzed using STATISTCA 7.1 software. To evaluate the different product doses on *Puccinia kuehnii* spore germination, an ANOVA (Analysis of Variance) was performed. In the event of a significant difference, the Newman-Keuls comparison test at the 5% threshold was used to separate the means into homogeneous groups.

RESULTS

Germinative capacity of *Puccinia kuehnii* urediniospores

Frequency of sprouted strains: Figure 2 illustrated the overall germination rate of strains according to sugar sites. The results show an overall germination rate of 78.83% for the two sites. Furthermore, the Zuénoula strains had the highest germination rate at 86.90%, compared with 66.03% for the Borotou-Koro strains. Analysis of results shows a significant difference between germination rates at the 5% threshold according to the Newman-Keuls text.

Microscopic characteristics of *Puccinia kuehnii* urediniospores observed: Figure 3a-b show urediniospores from strains 35B and 381Z, respectively, observed under the light microscope (Fig. 3). The *Puccinia kuehnii* uredospores observed are obovoid and elongated. They are orange in color, covered with a few spines and show an apical swelling of the wall.

Fig. 2: Overall germination rate of strains
Bars surmounted by the same alphabets are not significantly different at the 5% threshold according to the Newman-Keuls test

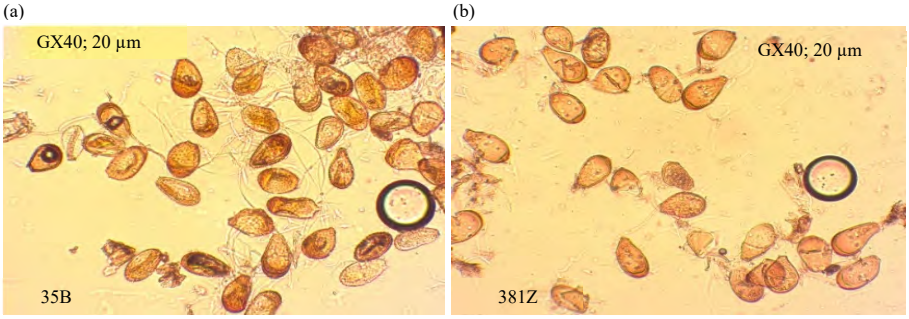

Fig. 3(a-b): Microscopic appearance of *Puccinia kuehnii* urediniospores from strains (a) 35B and (b) 381Z

Table 1: Germination rate and uredospore abundance of Borotou-Koro strains

Strains	Germination rate (%)	Abundance of urediniospores	Strains	Germination rate (%)	Abundance of urediniospores
38B	20.00^{gi}	16.67^{cf}	31B	16.67^{hi}	3.33^{df}
50B	26.67^{ei}	16.67^{cf}	51Ba	38.89^{bi}	8.33^{df}
8B	22.22^{fi}	5.00^{df}	28B	22.22^{fi}	25.00^{df}
17B	0.00^{i}	0.00^{f}	15B	0.00^{i}	0.00^{f}
2B	0.00^{i}	0.00^{f}	39B	0.00^{i}	0.00^{f}
56B	83.33^{ab}	50.00^{a}	13B	0.00^{i}	0.00^{f}
24B	30.00^{di}	16.67^{cf}	35B	81.90^{ab}	45.00^{ab}
7B	33.33^{ci}	3.3^{df}	29B	0.00^{i}	0.00^{f}
18B	0.00^{i}	0.00^{f}	10B	0.00^{i}	0.00^{f}
37B	73.33^{ad}	50.00^{a}	26B	0.00^{i}	0.00^{f}
11B	76.67^{ac}	50.00^{a}	51bB	96.67^{a}	43.33^{be}
19B	0.00^{i}	0.00^{f}	41B	66.67^{af}	50.00^{a}
5B	0.00^{i}	0.00^{f}	21B	53.33^{ah}	33.33^{bf}
1B	0.00^{i}	0.00^{f}	36B	33.33^{ci}	6.66^{df}
55B	0.00^{i}	0.00^{f}	12B	78.33^{ac}	28.33^{bf}
42B	26.67^{ei}	16.67^{cf}	53B	83.33^{ab}	18.33^{cf}
57B	26.67^{ei}	16.67^{cf}	44B	57.14^{ah}	28.33^{bf}
20B	0.00^{i}	0.00^{f}	27B	33.33^{ci}	36.66^{bf}
25B	63.33^{ag}	50.00^{a}	50B	33.33^{ci}	10.00^{cf}
30B	43.33^{bi}	33.33^{bf}	23B	60.00^{ah}	31.66^{bf}
34B	70.00^{ae}	50.00^{a}	48B	43.33^{bi}	20.00^{bf}
32B	0.00^{i}	0.00^{f}	14B	0.00^{i}	0.00^{f}
52B	0.00^{i}	0.00^{f}	4B	0.00^{i}	0.00^{f}
54B	0.00^{i}	0.00^{f}	3B	0.00^{i}	0.00^{f}
6B	0.00^{i}	0.00^{f}	40B	0.00^{i}	0.00^{f}
43B	64.17^{ag}	46.66^{ab}	49B	26.67^{ei}	16.67^{cf}
22B	76.67^{ac}	50.00^{a}			

Means bearing the same letter in the same column are statistically identical at the 5% threshold according to the Newman-Keuls test

Germination rate of Borotou-Koro strains: Strains 51bB, 53B and 56B recorded the highest germination rates, estimated at 83.33%. However, strains 2B, 18B, 5B, 55B, 20B, 32B, 52B, 54B, 6B, 14B, 4B, 3B, 40B, 29B, 10B and 26B had germination rates null. The other strains had intermediate germination rates, with values ranging from 20 to 76%. In terms of uredospore abundance, strains 56B, 37B, 11B, 25B, 34B, 22B and 41B had the highest abundance with an average of 50 spores, while low abundance ranging from 2.5 to 6.67% was observed in strains 362Z and 391Z. Intermediate abundances ranging from 16.67 to 46.67% were noted for the other strains (Table 1).

Germination rate of Zuénoula strains: Table 2 shows the germination rate and abundance of *Puccinia kuehnii* urediniospores observed on strains from the Zuénoula Integrated Agricultural Unit. Results showed that strains 381Z, 389Z, 304Z and 345Z achieved high germination rates with values of 96.67%, while germination was 100% for strain 386Z. On the other hand, strains 317Z, 319Z, 320Z, 327Z, 363Z, 393Z, 316Z and 318Z achieved germination rates of 0%. As for the other strains, germination rates ranging from 20 to 86% were recorded. With regard to uredospore abundance, the highest (50 uredospores) were obtained in thirty-six strains. However, an abundance of 0% was observed with strains 319Z, 363Z, 327Z, 320Z, 317Z, 316Z, 322Z, 318Z, 393Z and 353Z.

In vitro effect of antifungal activities of natural substances on orange rust spore germination

Effect of natural substances on uredospore germination in Zuénoula strains: Figure 4 shows the average spore germination rate according to Zuénoula strains. Analysis of the results revealed significant differences between strains according to the Newman-Keuls test at the 5% threshold. All four strains had variable germination rates depending on the products and concentrations applied.

With the *Ocimum gratissimum* L. based formulation, at a dose of 500 ppm, strain 392Z and 389Z had the lowest germination rates, with values of 42.22 and 37.78%, respectively. Strain 381Z had the highest germination rate, with an estimated value of 63.33%. At 1000 ppm, strains 381Z and 383Z recorded the highest germination rates of 46.67 and 51.67% respectively, which are statistically identical according to the Newman-Keuls test at the 5% threshold. Strains 389Z and 392Z achieved statistically identical germination rates of 36.67 and 34.44%, respectively. On the other hand, at a concentration of 2000 ppm, statistical germination rates were recorded for all four strains, ranging from 33.33 to 36.67%.

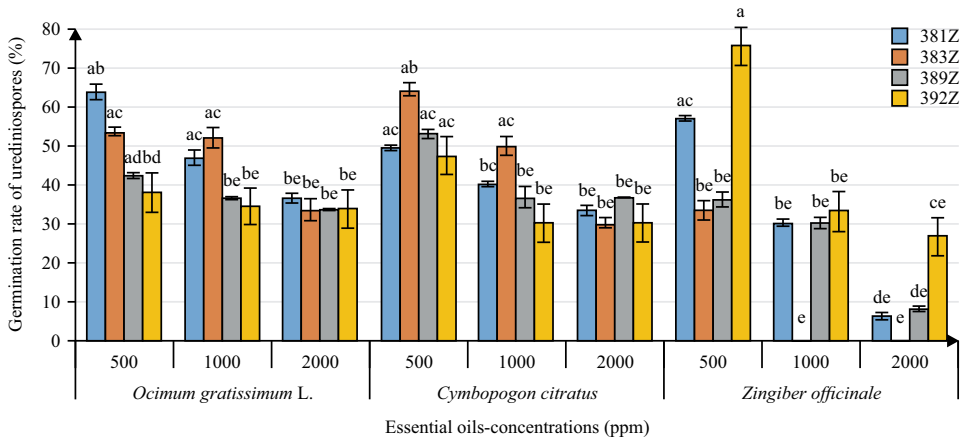

Fig. 4: Effect of products and their concentrations on urediniospore germination in Zuénoula strains of *Puccinia kuehnii*
Bars surmounted by the same letters are not significantly different at the 5% threshold according to the Newman-Keuls test

Table 2: Germination rate and uredospore abundance of Zuénoula strains

Strains	Germination rate (%)	Abundance of urediniospores	Strains	Germination rate (%)	Abundance of urediniospores
307Z	56.67^{cf}	50.00^{a}	304Z	96.67^{a}	50.00^{a}
388Z	76.67^{af}	50.00^{a}	361Z	66.67^{bf}	40.00^{ad}
381Z	96.67^{a}	50.00^{a}	397Z	66.67^{bf}	40.00^{ad}
341Z	66.67^{bf}	50.00^{a}	362Z	66.67^{bf}	5.00^{g}
330Z	60.00^{bg}	50.00^{a}	384Z	70.00^{af}	50.00^{a}
366Z	22.22^{eg}	50.00^{a}	333Z	60.00^{bg}	50.00^{a}
336Z	93.33^{ab}	50.00^{a}	369Z	84.44^{ac}	43.33^{ad}
351Z	83.33^{ac}	50.00^{a}	353Z	0.00^{g}	0.00^{g}
377Z	80.00^{ae}	50.00^{a}	390Z	60.00^{bg}	40.00^{ad}
344Z	73.33^{af}	50.00^{a}	308Z	66.67^{bf}	50.00^{a}
354Z	20.00^{eg}	20.00^{ag}	321Z	11.11^{fg}	5.00^{g}
349Z	73.33^{af}	50.00^{a}	393Z	0.00^{g}	0.00^{g}
360Z	76.67^{af}	50.00^{a}	301Z	46.67^{cf}	23.33^{ag}
398Z	33.33^{cg}	6.66^{fg}	318Z	0.00^{g}	0.00^{g}
375Z	70.00^{af}	50.00^{a}	334Z	73.33^{af}	50.00^{a}
370Z	56.67^{cf}	50.00^{a}	364Z	80.00^{ae}	41.66^{ad}
379Z	72.22^{af}	38.33^{ad}	365Z	56.67^{cf}	50.00^{a}
347Z	66.67^{bf}	50.00^{a}	322Z	0.00^{g}	0.00^{g}
323Z	73.33^{af}	41.66^{ad}	312Z	70.00^{af}	41.66^{ad}
376Z	71.52^{af}	41.5^{ad}	392Z	86.67^{ac}	50.00^{a}
325Z	63.33^{bg}	41.66^{ad}	358Z	86.67^{ac}	36.66^{ae}
339Z	66.67^{bf}	50.00^{a}	316Z	0.00^{g}	0.00^{g}
385Z	55.55^{cf}	50.00^{eg}	367Z	66.67^{bf}	50.00^{a}
319Z	0.00^{g}	0.00^{g}	395Z	59.26^{bg}	28.33^{ag}
363Z	0.00^{g}	0.00^{g}	329Z	60.00^{bg}	50.00^{a}
327Z	0.00^{g}	0.00^{g}	317Z	0.00^{g}	0.00^{g}
368Z	63.33^{bg}	50.00^{a}	318Z	72.38^{af}	43.33^{ad}
382Z	66.67^{bf}	16.66^{dg}	371Z	46.67^{cf}	33.33^{af}
389Z	96.67^{a}	38.33^{ad}	345Z	96.67^{a}	23.33^{ag}
340Z	76.67^{af}	50.00^{a}	355Z	76.67^{af}	50.00^{a}
374Z	45.00^{cf}	15.00^{dg}	396Z	76.67^{af}	50.00^{a}
348Z	33.33^{cg}	3.33^{g}	320Z	0.00^{g}	0.00^{g}
213Z	29.44^{eg}	3.67^{dg}	337Z	60.00^{bg}	50.00^{a}
349Z	73.33^{af}	50.00^{a}	357Z	73.33^{af}	50.00^{a}
373Z	83.33^{ac}	6.66^{fg}	310Z	16.67^{fg}	6.66^{fg}
383Z	88.89^{ac}	8.33^{fg}	343Z	80.00^{ae}	50.00^{a}
359Z	86.67^{ac}	50.00^{a}	309Z	63.33^{bg}	23.33^{ag}
387Z	70.00^{af}	38.33^{ad}	378Z	65.71^{bf}	45.00^{ad}
306Z	33.33^{cg}	16.66^{g}	401Z	70.00^{af}	50.00^{a}
386Z	100.00^{a}	50.00^{a}	326Z	70.00^{af}	50.00^{a}
352Z	76.67^{af}	50.00^{a}	300Z	6.67^{fg}	21.66^{ag}
356Z	33.33^{cg}	3.33^{g}	391Z	16.67^{fg}	2.5^{g}

Means bearing the same letter in the same column are statistically identical at the 5% threshold according to the Newman-Keuls test

Table 3 : Average urediniospore abundance of Zuénoula strains of *Puccinia kuehnii*

		Urediniospores abundance by concentration (ppm)		
Strains	Essential oils	500	1000	2000
381Z	*Ocimum gratissimum* L.	50.00^{a}	50.00^{a}	50.00^{a}
	Cymbopogon citratus	50.00^{a}	50.00^{a}	41.67^{ac}
	Zingiber officinale	50.00^{a}	50.00^{a}	50.00^{a}
383Z	*Ocimum gratissimum* L.	33.33^{ag}	26.66^{ah}	18.33^{bh}
	Cymbopogon citratus	36.67^{af}	25.00^{ah}	13.33^{dh}
	Zingiber officinale	10.00^{fg}	5.00^{hg}	0.00^{h}
389Z	*Ocimum gratissimum* L.	50.00^{a}	50.00^{a}	38.33^{af}
	Cymbopogon citratus	50.00^{a}	41.67^{ac}	13.33^{dh}
	Zingiber officinale	50.00^{a}	46.67^{ab}	13.33^{dh}
392Z	*Ocimum gratissimum* L.	45.00^{ac}	35.00^{af}	23.33^{ah}
	Cymbopogon citratus	50.00^{a}	45.00^{ac}	16.67^{ch}
	Zingiber officinale	50.00^{a}	45.00^{ac}	13.67^{dh}

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

The results observed with *Cymbopogon citratus* essential oil show that, at a concentration of 500 ppm, the highest germination rate, estimated at 63.89%, was recorded with strain 383Z. Statistically identical germination rates, ranging from 47.14 to 52.78%, were recorded with the other three strains. Then, at the 1000 ppm concentration, strains 381Z, 389Z and 392Z showed the lowest germination rates, with values ranging from 30 to 40%. Strain 383Z achieved a germination rate of 49.73%. Statistically identical germination rates ranging from 30 to 36.67% were observed at the 2000 ppm concentration in all strains.

At a concentration of 500 ppm of *Zingiber officinale* essential oil, the lowest germination rates were 33.33 and 35.83% (statistically identical), recorded with strains 383Z and 389Z, respectively. Strain 381Z showed an intermediate germination rate of 56.67%, while the highest germination rate of 75% was recorded with strain 392Z.

The results revealed that at 1000 ppm, a zero germination rate was observed with strain 383Z. The other three strains had statistically identical germination rates ranging from 30 to 32.85%. At the 2000 ppm concentration level, the highest germination rate was 26.67%, recorded with strain 392Z. On the other hand, a zero germination rate was obtained with strain 383Z. On the other hand, strains 381Z and 392Z recorded the lowest germination rates, with intermediate values of 6.67 and 8.33%, respectively.

Effect of natural substances on uredospore abundance of Zuénoula strains: At concentrations of 500 and 1000 ppm, an abundance of 50 urediniospores of strain 381Z was observed with all essential oils. On the other hand, at the 2000 ppm concentration, *Ocimum gratissimum* L. and *Zingiber officinale* essential oils recorded an abundance of 50 urediniospores, while *Cymbopogon citratus* essential oil obtained an average abundance of 41.67 urediniospores (Table 3).

Uredospore abundance of strain 383Z, observed with *Ocimum gratissimum* L. essential oil, was 33.33 and 18.33, respectively at concentrations of 500 and 2000 ppm. An intermediate abundance of 26.66 urediniospores was obtained at the 1000 ppm concentration. With *Cymbopogon citratus* essential oil, abundances of 36.67, 25 and 13.33 urediniospores were noted at 500, 1000 and 2000 ppm, respectively.

As for *Zingiber officinale* essential oil, it recorded the lowest uredospore abundances at all concentrations, with values of 10, 5 and 0 respectively at concentrations of 500, 1000 and 2000 ppm.

An abundance of 50 uredospores of strain 389Z was observed with all essential oils at the 500 ppm concentration. On the other hand, at the 1000 ppm concentration, the abundances obtained were not statistically identical according to the Newman-Keuls test and showed values of 50, 41.67 and 46.67, respectively with *Ocimum gratissimum* L., *Cymbopogon citratus* and *Zingiber officinale* essential oils. *Cymbopogon citratus* and *Zingiber officinale* essential oils recorded a low average abundance of 13.33 urediniospores at 2000 ppm, while *Ocimum gratissimum* L. essential oil had a higher abundance of 38.33 urediniospores.

With strain 392Z, *Cymbopogon citratus* and *Zingiber officinale* essential oils had the highest uredospore abundance (50) at the 500 ppm concentration. However, these natural substances recorded the lowest average abundances at the 2000 ppm concentration, with values of 16.67 and 13.67 urediniospores, respectively. In the case of *Ocimum gratissimum* L., essential oil, mean abundances of 45 and 23 urediniospores, respectively were recorded at the 500 and 2000 ppm concentrations. An intermediate abundance of 35 urediniospores was obtained at 1000 ppm.

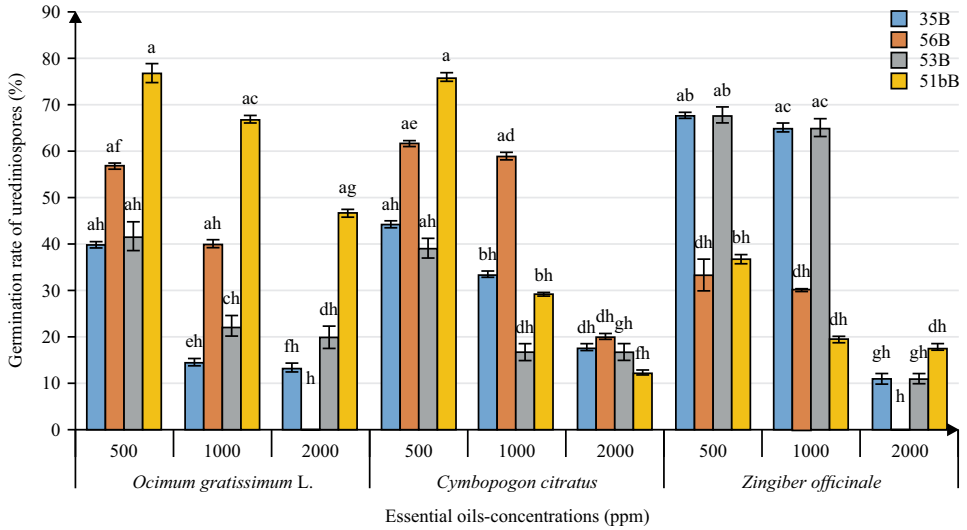

Fig. 5: Effect of products and their concentrations on uredospore germination in Borotou-Koro strains of *Puccinia kuehnii*
Bars surmounted by the same letters are not significantly different at the 5% threshold according to the Newman-Keuls test

Effect of natural substances on the germination of Borotou-Koro orange rust spores: Figure 5 show that the germination rate of *Puccinia kuehnii* urediniospores decreases for each strain as essential oil concentrations increase.

Strain 51bB recorded the highest germination rate of 76.67%, at a concentration of 500 of the essential oil of *Ocimum gratissimum* L. An intermediate germination rate of 56.67% was recorded with strain 56B, while strains 35B and 53B showed intermediate germination rates of 40 and 41.67%, respectively. The lowest germination rate of 14.44% was recorded with strain 35B at 1000 ppm. At this concentration, a higher germination rate of 66.67% was obtained with strain 51bB. The lowest germination rate was recorded with strain 66.67%. At a concentration of 2000 ppm, zero germination was achieved with strain 56B, while strain 51bB had a higher germination rate of 46.67%.

The essential oil of *Cymbopogon citratus* recorded statistically identical germination rates in strains 35B and 53B at a concentration of 500 ppm, with values of 44.04 and 38.89%, respectively. Strains 56B and 51bB achieved the highest germination rates at 61.50 and 75.66%, respectively. At 1000 ppm, strains 56B and 35B recorded the lowest and highest germination rates, at 33.33 and 58.73%, respectively. At 2000 ppm, the germination rates of strains 35B and 56B were statistically identical at 17.78 and 20%, respectively.

Analysis of the results shows that with *Zingiber officinale* essential oil, at a concentration of 500 ppm, strains 35B and 53B had a higher germination rate of 67.62%. However, strains 56B and 51bB had germination rates of 33.33 and 36.67%, respectively. At 1000 ppm, the lowest germination rates were 30% (56B) and 19.44% (51bB). With strains 35B and 53B, an estimated germination rate of 64.81% was obtained. A germination rate of 0% was obtained with strain 56B at the concentration. Strain 51bB recorded a higher germination rate of 17.78%. Strains 35B and 53B had statistically identical germination rates, with a value of 11.11%.

Urediniospores abundance as a function of essential oil concentration is shown in Table 4. Statistical analysis of the data indicates that there is a significant difference between the uredospore abundance of the products and concentrations, according to the Newman-Keuls test at the 5% threshold.

Thus, an average abundance of 50 *Puccinia kuehnii* urediniospores was observed with strain 35B, at a concentration of 500 ppm of *Ocimum gratissimum* L. and *Cymbopogon citratus* essential oils. For *Zingiber officinale* essential oil, uredospore abundance was 28.33% at 500 ppm. At 1000 ppm and 2000 ppm, *Ocimum gratissimum* L., essential oil recorded the highest abundances, with values of 41.67 and 40 urediniospores, respectively. The lowest uredospore abundances, 21.67 (1000 ppm) and 11.67 (2000 ppm), were obtained with *Zingiber officinale* essential oil.

The results show that with strain 56B, the essential oil of *Cymbopogon citratus*, recorded the highest abundance of urediniospores at 500, 1000 and 2000 ppm, with values of 50, 35 and 21.67 urediniospores, respectively. However, the lowest abundances of 1.67 and 00% (statistically identical) were observed at the 2000 ppm concentration, with *Ocimum gratissimum* L. and *Zingiber officinale* essential oils, respectively.

Table 4: Average urediniospore abundance of *Puccinia kuehnii* (Borotou-Koro strains)

		Urediniospores abundance by concentration (ppm)		
Strains	Essential oils	500	1000	2000
35B	*Ocimum gratissimum* L.	50.00^{a}	41.67^{ad}	40.00^{ad}
	Cymbopogon citratus	50.00^{a}	36.67^{af}	28.33^{ah}
	Zingiber officinale	28.33^{ah}	21.67^{aj}	11.67^{gj}
56B	*Ocimum gratissimum* L.	33.33^{ag}	25.00^{ai}	1.67^{j}
	Cymbopogon citratus	50.00^{a}	35.00^{af}	21.67^{aj}
	Zingiber officinale	35.00^{af}	3.33^{ij}	0.00^{j}
53B	*Ocimum gratissimum* L.	21.67^{aj}	16.67^{fj}	5.00^{ij}
	Cymbopogon citratus	20.00^{hj}	8.33^{dj}	3.33^{ij}
	Zingiber officinale	28.33^{ah}	21.67^{aj}	11.67^{gj}
51bB	*Ocimum gratissimum* L.	50.00^{a}	46.67^{ab}	15.00^{fj}
	Cymbopogon citratus	43.33^{ac}	35.00^{af}	28.33^{ah}
	Zingiber officinale	50.00^{a}	20.00^{dj}	18.33^{ej}

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

Table 5: Concentration of natural substances reducing by 50% and 90% the germination of *Puccinia kuehnii* spores from Zuénoula strains

	381Z		383Z		389Z		392Z	
Essential oils	CI_{50} (ppm)	CI_{90} (ppm)	CI_{50} (ppm)	CI_{90} (ppm)	CI_{50} (ppm)	CI_{90} (ppm)	CI_{50} (ppm)	CI_{90} (ppm)
Ocimum gratissimum L.	1100.00	1297.42	889.83	2750.00	1091.25	8234.10	4722.40	20722.40
Cymbopogon citratus	239.72	3799.89	731.67	1070.86	301.72	4045.86	291.53	4373.16
Zingiber officinale	574.62	679.21	877.21	2982.47	177.28	2304.94	985.68	4177.19

Uredospore abundance observed with strain 53B, was relatively low at all concentrations of each essential oil.

Thus, with the essential oils of *Zingiber officinale*, *Cymbopogon citratus* and *Ocimum gratissimum* L., respective (lower) abundances of 11.67, 3.33 and 5 urediniospores were noted at the 2000 ppm concentration. On the other hand, at 500 ppm, higher abundances of 28.33, 21.67 and 20 urediniospores were obtained with *Zingiber officinale*, *Cymbopogon citratus* and *Ocimum gratissimum* L., essential oils, respectively.

All three essential oils recorded high abundances of *Puccinia kuehnii* urediniospores of strain 51bB, ranging from 45 to 50 at the 500 ppm concentration. Abundances of 23.33, 16.67 and 13.67 were observed at the 2000 ppm concentration, respectively with *Ocimum gratissimum* L., *Cymbopogon citratus* and *Zingiber officinale* essential oils.

Inhibitory concentrations of natural substances reduce *Puccinia kuehnii* spore germination by 50 and 90%, respectively

50 and 90% inhibitory concentration of natural substances on the germination of *Puccinia kuehnii* spores from Zuénoula: Table 5 shows the concentrations of natural substances that reduce germination of *Puccinia kuehnii* spores by 50 and 90% in the Zuénoula strains. Inhibitory concentrations reducing uredospore germination by 50% (IC_{50}) induced by *Ocimum gratissimum* L., essential oil were lower with strain 383Z, but higher with strain 392Z, at 889.83 and 4722.40 ppm, respectively. The IC_{90} values were 1297.42 ppm (lower) and 20722.40 (higher) with strains 381Z and 392Z, respectively.

Concentrations of *Cymbopogon citratus* essential oils from the different strains ranged from 239.72 to 731.67 ppm for IC_{50} and from 1070.86 to 4373.16 ppm for IC_{90}.

With *Zingiber officinale* essential oil, the highest IC_{50}, estimated at 985.68 ppm, was obtained with strain 392Z. On the other hand, strain 389Z had the lowest IC_{50} of 177.28 ppm. The 90% inhibitory concentrations ranged from 679.21 to 4177.19 ppm.

50 and 90% inhibitory concentration of natural substances on the germination of *Puccinia kuehnii* spores from Borotou-Koro: Table 6 shows the inhibitory concentrations reducing germination of *Puccinia kuehnii* urediniospores by 50 and 90% from Borotou-Koro strains. *Ocimum gratissimum* L., essential oil recorded the lowest IC_{50} at 167 and 350.13 ppm with strains 51bB and 56B, respectively. While the highest IC_{90} were revealed with strains 35B and 53B, for values of 3210 and 3683.09 ppm, respectively.

The 50% inhibitory concentrations observed with *Cymbopogon citratus* essential oil ranged from 105.92 ppm (35B) to 874.88 ppm (53B). The IC_{90} concentrations ranged from 880 ppm (51bB) to 4024.48 ppm (53B).

Table 6: Concentration of natural substances reducing by 50 and 90% the germination of *Puccinia kuehnii* urediniospores from Borotou-Koro

	35B		56B		53B		51bB	
Essential oils	CI_{50} (ppm)	CI_{90} (ppm)	CI_{50} (ppm)	CI_{90} (ppm)	CI_{50} (ppm)	CI_{90} (ppm)	CI_{50} (ppm)	CI_{90} (ppm)
Ocimum gratissimum L.	685.45	3210	350.13	700.00	558.04	3683.09	167.00	1833.33
Cymbopogon citratus	105.92	2219.47	311.50	1055.42	874.88	4024.48	154.55	880.73
Zingiber officinale	111.87	1112.82	71.67	1788.41	111.87	1112.82	154.37	4758.99

The lowest IC_{50} and IC_{90} were recorded with *Zingiber officinale* essential oil, with concentrations of 71.67 ppm (56B) and 1112.82 (35B and 53B), respectively. The highest IC_{50} and IC_{90} concentrations were 111.82 ppm (35B and 53B) and 154.55 ppm (51bB), respectively.

DISCUSSION

A total of 137 samples of sugarcane leaves symptomatic of orange rust were observed for the identification of *Puccinia kuehnii* urediniospores. The results revealed the presence of an apical bulge in the urediniospores observed. The urediniospores were obovoid in shape, sparsely covered with spines and orange in color. This description was in line with that of Virtudazo *et al.*[15] and Pérez-Vicente *et al.*[16], who showed that *Puccinia kuehnii* urediniospores are obovoid to pyriform in shape, variable in size and indented, with spines evenly distributed along the contour.

This study determined the germination rate of urediniospores from samples from each production zone. The results showed that 78.83% of the strains showed urediniospores characteristic of *Puccinia kuehnii*. On the other hand, strains 304Z, 386Z, 389Z and 381Z from Zuénoula and strains 35B, 56B, 53B and 51bB recorded the highest germination rates, ranging from 81.90 to 100%. These results could be explained by the fact that the temperature of 22°C used in the *in vitro* tests was conducive to spore germination. Current study results corroborated those of Minchio *et al.*[6] and Sanjel *et al.*[17], who revealed that in *in vitro* tests, the germination of *Puccinia kuehnii* spores was favourable at temperatures between 20 and 25°C. In addition, an incubation time of 14 hrs for samples in tubes also facilitated uredospore germination. Current study results were similar to those of Minchio *et al.*[6], who revealed that the optimum temperature for *Puccinia kuehnii* germination was 21°C with an incubation period of 14 hrs.

The essential oils of *Ocimum gratissimum* L., *Cymbopogon citratus* and *Zingiber officinale* were subjected to *in vitro* efficacy tests on the germination of *Puccinia kuehnii* urediniospores. Results showed that, in general, the germination rate and uredospore abundance decreased with increasing concentration for all essential oils. These results demonstrated that all three essential oils have inhibitory effects on *Puccinia kuehnii* uredospore germination at all concentrations between 500 and 2000 ppm. Present study results concurred with those of Didier *et al.*[2], who showed *in vitro* the effect of these essential oils on the growth of strains of *Sporisorium scitamineum*, the agent responsible for sugarcane smut disease.

Essential oils reduced uredospore germination more than all strains at the 2000 ppm concentration. In contrast, *Ocimum gratissimum* L., essential oil was effective on urediniospores germination at this concentration (2000 ppm), with an inhibition rate of 33.33% obtained with strain 383Z.

As for *Cymbopogon citratus* essential oil, the lowest inhibition rate was 11.11%, observed with strain 35B. At 2000 ppm, *Zingiber officinale* essential oil was more effective. The nature of the chemical composition of the essential oils tested may be responsible for the variability of these results. Indeed, the work of Kobenan *et al.*[18] showed that the essential oil of *O. gratissimum* L., is made up of eighteen compounds representing 100% of the components identified. This essential oil is composed of sesquiterpenes such as β-cis-caryophyllene (4.42%), selinene (1.55%), copaene (1.14%) and α-caryophyllene (0.63%). In the hydrocarbon monoterpene group, p-cymene with a content of 37.79% and sabinene (6.60%) were the most represented terpene elements. Thymol (24.57%) and camphor (5.53%) dominated the oxygenated monoterpenes present in this essence. Oussou *et al.*[19], in a study carried out in Côte d'Ivoire, also showed that the essential oil of *Ocimum gratissimum* L., is predominantly rich in para-cymene, γ-terpinene and thymol. Work by Pauli[20] has shown that thymol is a phenolic compound with powerful antimicrobial activity.

The antifungal activity of *Ocimum gratissimum* L., essential oil in this study would be justified by the presence of this compound. However, the activity of this essential oil would not be due solely to the presence of thymol, as the presence of compounds such as alcohols, aldehydes, monoterpene ketones, phenylpropanes and monoterpenes could act through synergy or addition of effect. These various compounds are known for their antimicrobial properties[21-23]. Previous studies have reported on the efficacy of formulations based on *Ocimum gratissimum* L., essential oil. Mohamed *et al.*[24] focused on telluric fungi of vegetable crops, while Kassi *et al.*[10] and Paranagama *et al.*[13] demonstrated

the antifungal effect of *Ocimum gratissimum* L., oil on black cercosporiasis of banana. The antifungal effect of *Cymbopogon citratus* oil has already been demonstrated by Ohno *et al.*[25] and Dutta *et al.*[26]. The synergistic action of its compounds is thought to contribute to its efficacy. The main constituents of *Cymbopogon citratus* are citral, which ranges from 65 to 86%, with equal proportions of neral and gerial. Other major compounds are myrcene (20%), camphene (10%) and geraniol (2-10%). Sallé[27] and Akhila[28] have shown that these compounds are accompanied by geranyl acetate, linalool, nerol, citronellal and 2-methylhept-5-en-one. In addition, according to de Billerbeck[29], *Cymbopogon citratus* essential oil has antifungal properties that can radically treat certain mycoses.

As for *Zingiber officinale* oil, its fungicidal effect was demonstrated by the work of Gaston *et al.*[30] on the pathogen responsible for anthracnose in mango fruit. Studies by Jeena *et al.*[31] showed that *Zingiber officinale* oil has a high proportion of arcurcumene (59%), -myrcene (14%), 1,8-cineole (8%), citral (7.5%) and zingiberene (7.5%).

The essential oils of *Cymbopogon citratus* and *Zingiber officinale* showed the most effective IC_{50} and IC_{90} on urediniospores germinability, compared with the essential oil of *Ocimum gratissimum* L. The antifungal activity of essential oils against *Puccinia kuehnii* strains may be linked to the sensitivity of the cytoplasmic membrane of urediniospores.

CONCLUSION

The overall aim of this work was to contribute to the improvement of sustainable sugarcane production by gaining a better understanding of orange rust and developing biological control strategies based on essential oils. This study demonstrated the *in vitro* germinative capacity of *Puccinia kuehnii* urediniospores from 137 samples. It also demonstrated the antifungal effect of *Ocimum gratissimum* L., *Zingiber officinale* and *Cymbopogon citratus* essential oils on uredospore germination. The results showed that 78.83% of the strains had urediniospores characteristic of *Puccinia kuehnii*. Furthermore, Zuénoula strains had the highest germination rate of 86.90%, against a germination rate of 66.03%.

All the essential oils tested (*Ocimum gratissimum* L., *Zingiber officinale* and *Cymbopogon citratus*) had an inhibitory effect on uredospore germination. The higher the concentration of essential oils, the greater the inhibition of urediniospores germination. The concentration of 2000 ppm was the best for each essential oil. *Zingiber officinale* essential oil proved the most effective overall.

These essential oils could provide an alternative to chemical control. These studies should be pursued to improve sugarcane production and quality for the benefit of the Côte d'Ivoire industry. In addition, a better understanding of the efficacy of fungicides on the different fungal growth stages of *Puccinia kuehnii* is essential to optimize control of the orange rust disease and maximize yields. It would therefore make sense to continue this study with a view to:

- Greenhouse evaluation of the antifungal activity of essential oils on the severity and incidence of *Puccinia kuehnii* strains
- Determine the efficacy of natural substances on orange rust disease in the field

SIGNIFICANCE STATEMENT

The objective of this study was to contribute to the improvement of sustainable sugarcane production in Côte d'Ivoire, through *in vitro* control of *Puccinia kuehnii*, the agent responsible for orange rust disease in Côte d'Ivoire. 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 diseases of sugarcane. This study revealed that all Zuénoula strains had the highest germination capacity. In addition, the efficacy of essential oils was demonstrated in the germination of *Puccinia kuehnii* urediniospores. Essential oils were most effective at a concentration of 2000 ppm.

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