Journal of Plant Sciences
2021; 9(3): 77-83
http://www.sciencepublishinggroup.com/j/jps
doi: 10.11648/j.jps.20210903.12
ISSN: 2331-0723 (Print); ISSN: 2331-0731 (Online)

Sustainable Control of the Sugarcane Smut Disease Caused by *Sporisorium scitamineum* Piep. Using an Essential Oil of *Cymbopogon citratus*

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To cite this article:

Yao Kouadio Jacques-Edouard, Kassi Koffi Fernand-Jean Martial, Kouamé Konan Didier, Kouamé Koffi Gaston, N'guessan Aya Carine, Bolou Bi Bolou Antoine, N'guessan Kangah. Martial, Koné Daouda. Sustainable Control of the Sugarcane Smut Disease Caused by *Sporisorium scitamineum* Piep. Using an Essential Oil of *Cymbopogon citratus*. *Journal of Plant Sciences*. Vol. 9, No. 3, 2021, pp. 77-83. doi: 10.11648/j.jps.20210903.12

Received: February 1, 2021; **Accepted**: February 9, 2021; **Published**: May 14, 2021

Abstract: Sugarcane smut disease, caused by *Sporisorium scitamineum*, is one of the most damaging fungal diseases in the world. This study aims to evaluate the effect of treatment with a formulation based on *Cymbopogon citratus* essential oil on the incidence of *S. scitamineum* in sugarcane cultivation. The study was conducted under controlled conditions at the research site of the Bingerville scientific pole in Côte d'Ivoire. Some sugarcane cuttings of NCo376 variety were inoculated by dipping in a teliospores solution of 5.10^6 teliospores/ml. Before planting, the cuttings were cold treated in a *C. citratus* essential oil. Propiconazole-treated cuttings served as a reference control and untreated cuttings served as a negative control. Agronomic parameters of the cane and descriptors of smut disease were monitored for eight months to assess the health status of treated and untreated plants. Analysis of variance and comparison of means were performed using the Newman-Keuls test at 5% significance level using Statistica 7.1 software. The results showed that, the pre-treatment of cuttings with *C. citratus* oil had a benefic effect on the cutting's germination, height, tillering, stem diameter, internode length, number of internodes and biomass of sugarcane plants with the doses of 500-ppm and 1000-ppm. Moreover, the incidence of smut disease was greatly reduced from the 500-ppm of *C. citratus* essential oil. In addition, the essential oil-based treatment had similar effects to propiconazole and much better than the untreated controls. Therefore, this pre-treatment of cuttings with *C. citratus* essential oil could be an alternative to the use of chemicals in the fight against sugarcane smut disease.

Keywords: Smut Disease, *Sporisorium scitamineum,* Sustainable Control, *Cymbopogon citratus*

1. Introduction

Sugarcane (*Saccharum officinarum*) is a plant cultivated for the sugar contained in its stalks. It accounts for more than 60% of global sugar production [8] and is a major contributor to the global economy and agri-food. In recent years, sugarcane has been used to produce ethanol, biofuels and energy [19]. In Côte d'Ivoire, sugarcane is grown on four sugar complexes (Ferké 1, Ferké2, Zuénoula and Borotou-Koro). Cultivation is practiced on a total area more than 25,000 ha and the country's production is estimated at more than 214,000 tons, which generates 1% of GDP, 3.3% of the

agricultural sector and provides more than 7,000 jobs [9].

However, since the establishment of the sugar industry in the 1970s, average cane yields have remained at 80 tons/ha [23]. This situation is mainly due to pests that cause considerable yield losses. The sugarcane smut disease is one of the most serious constraints to global sugarcane production. *Sporisorium scitamineum* described as a basidiomycete fungus [21] is a biotrophic pathogen. This pathogen has been in Africa since 1877 from Natal and has spread to all sugarcane production areas since the 1970s [22]. The life cycle of *S. scitaminum* can be described in three distinct phases: haploid sporidia, dikaryotic hyphae and diploid téliospores. In contact with sugarcane, these teliospores germinate rapidly under favorable conditions of optimal humidity and temperature of 25-30°C [25].

Once in contact with the cuttings, the teliospores germinate when the conditions of optimal humidity and temperature are favourable to give Sporidia [6]. They are easily propagated as yeast-like haploid cells in the stems [17]. The infectious mycelium develops systemically in the plant by preferentially settling in each of the lateral meristems formed, thus disrupting stem elongation which then produces short internodes, tillering and death of the plants after the appearance of the smut whip [18].

Sugarcane tissue colonization also differs between susceptible and resistant genotypes [5]; [20]. In addition, NCo376 is highly susceptible to the disease whose damage is perceptible both on cane yield (30-50% losses) and on the loss of juice quality [10]. The control of this disease is mainly based on the use of synthetic fungicides, including propiconazole, benomyl, carbendazim, mancozeb, chlorothalonil and captan [3]. These fungicides can also be combined with thermotherapy treatments for the sanitation of cuttings [2]. These products are expensive, carcinogenic and present negative impacts on biodiversity [12]; [26].

Therefore, the search for alternative solutions that are effective, sustainable, without any risk for users is indispensable. However, in Côte d'Ivoire, to our knowledge, very few studies have been carried out in this field. With the exception of in vitro tests performed by [15]. No scientific information has been reported on phytosanitary treatments in Côte d'Ivoire. In view of this situation, it seemed appropriate to initiate research on this disease, which is a cause for concern in the production areas. Essential oils extracted from the leaves of *C. citratus* are suspected to have antifungal properties under *in vitro* conditions [29]. The objective of this study is to evaluate the effect of pre-treatment of cuttings with a *C. citratus* essential oil formulation on smut disease in Côte d'Ivoire.

2. Material and Methods

2.1. Production of Cuttings

Asymptomatic sugar cane stems of NCo376 variety were collected from the plots of Borotou-Koro industrial plantation. They were washed with water and then cut into two-eyed cuttings (30 cm). The cuttings were transferred to the experimental propagation site of the Félix HOUPHOUËT-BOIGNY University's scientific and innovation pole. The seedlings that emerged were carefully monitored for six months. The harvested stems were cut into two-eyed cuttings that were used for this study.

2.2. Preparation of Inoculum

The collected smutted whips were kept at room temperature in the laboratory for 5 days. Fragments (5 cm) of whips were then collected and placed in test tubes containing 10 ml of sterilized and distilled water. These tubes were shaken for 15 seconds to detach teliospores attached to the whip fragments from the smutted whips. The number of teliospores suspended in each tube was determined using the Malassez hematimeter. The result of the teliospores count was used in the following formula to determine its density in aqueous suspensions:

$$C = \left(\frac{N}{\text{Number of cells}}\right) X\ k$$

C: teliospores concentration, N: number of teliospores counted, k: constant.

This initial concentration was then adjusted to 5.10^6 teliospores/ml by dilution. Then a téliospore viability test was performed on PDA culture medium in the laboratory [1].

2.3. Infection of the Cuttings

During the greenhouse trials, tests were carried out on asymptomatic sugar cane stems of the variety NCo376, taken at the age of 6 months. The cuttings were washed and then disinfected by a short thermotherapy in hot water at 52°C for 30 min before inoculation [30]. The inoculum had been prepared from fragments of charcoal whips dissolved in sterilized water. The suspension was adjusted to a concentration of 5.10^6 teliospores/ml by dilution. The cuttings were inoculated by total immersion in the solution contained in a cooler for 1 hour 30 min. Beforehand, a germination test was used to evaluate the viability of the teliospores. Infected cuttings were then stored in plastic bags for 72 hours to promote teliospore germination [28]. Treatment of infected cuttings after 72 hours of incubation, the cuttings were subjected to phytosanitary treatments.

2.4. Treatment of Infected Cuttings

2.4.1. Treatment with Formulations Based on Essential Oils

Essential oil of *C. citratus* was obtained from the fresh leaves, by training with saturated water steam carried out with the Clevenger device for 2 hours. This method consists of a conventional distillation in which leaves are not in direct contact with water. The leaves on a grid and a stream of water vapor flows through them. During the passage of steam, the cells of the leaves burst and release the essential oil which is carried to the condenser and then, the tank. The separation is done by decantation. The essential oil obtained is the active ingredient in the natural formulation. To do this,

a fixing element (mineral oil) and an emulsifier (1%) were added to the essential oil. With formulation based on essential oils. Three doses of formulation (C1=500-ppm, C2=1000-ppm and C3=1500-ppm) were evaluated. After the time of incubation, the cuttings were subjected to phytosanitary treatments. The solution of each dose were prepared for the three essential oils in 2 l jars. Then, eight inoculated cuttings were treated by dipping on each dose for 20 min. After treatment of the cuttings, they were planted in 3 l jars containing sterilized soil in the greenhouse.

2.4.2. Treatment with Synthetic Fungicide (Reference Control)

For treatment with propiconazole, the method of [2] has been adopted with a slight modification. This treatment was used as a reference control since this method is very paretic by producers. Three doses of propiconazole (C1=500-ppm, C2=1000-ppm and C3=1500-ppm) were swallowed. The cuttings were first given a short thermotherapy in water heated to 52°C for 30 min, before being soaked in the propiconazole slurry for 20 min [30]. Subsequently, eight inoculated cuttings were used in each dose for 20 min.

Control

Inoculated cuttings not treated with the natural formulation and propiconazole were used as controls.

2.5. Experimental Design

The experimental design adopted was a completely randomized block design with four replicates, i.e. 13 pots per block. The blocks were 30 cm apart and contained 13 treatments (pots). Each pot was filled with sterile soil and then two cuttings were planted per pot. All maintenance factors such as irrigation, fertilizer application, weeding were kept uniform to show the effect and dosage.

2.6. Data Collection

2.6.1. Assessment of Growth Parameters

(i). Germination Rate of Cuttings

The germination rate (Tg) of the cuttings used for the test was determined 45 days after planting. This rate was obtained by calculating the ratio of the number of germinated plants to the total number of eyes planted according to the following relationship [4]:

$$Tg\ (\%) = (n/N)*100$$

With n: number of germinated plants; N: total number of eyes planted

(ii). Tillering and Height of the Plants

The tillering consisted of counting the primary and secondary stems obtained in each pot. The evaluation was done monthly from the 2nd month to the 8th month after planting. In each pot, three seedlings were randomly selected and then labelled as 12 seedlings/treatment (pots). The height of the plants was measured with a tape measure. The size of the plants was taken monthly from the collar to the last ochrea visible on the leaves. Of the three marked plants, the number of nodes, the number of leaves and internodes were also assessed. These parameters were evaluated by counting every week [4].

(iii). Evaluation of Fresh and Dry Mass of Plants

After eight months of experimentation, the plants were harvested. The fresh mass was obtained by weighing the biomass after harvest using a scale. The seedlings were cut up and put in an oven at 70°C for 72 hours; the mass was determined using a balance regularly in order to obtain a constant dry mass [4].

2.6.2. Evaluation of Phytopathological Descriptors of Sugarcane Smut Disease

Phytosanitary evaluations have focused on phytopathological descriptors of smut disease. These were incubation period (IT), Disease Development Time (DDT) and disease incidence. The evaluation started after 1 month after planting. The number of affected plants was determined by counting the number of stems affected by anthrax each month. The progression of smut was monitored after 3.5 months for each dose and for each product. The number of stems showing symptoms characteristic of smut disease and the total number of stems were also recorded [7]. The incidence was calculated using the following formula proposed by [14]:

$$I\ (\%) = \frac{\text{number of stems attacked} * 100}{\text{total number of stems}}$$

2.7. Data Analysis

An analysis of variance was applied to the collected data and the comparison of the means was performed using the Newman-Keuls test (post-hoc ANOVA) at the 5% significance level using STATISTICA 7.1 software**.**

3. Results

3.1. Germination of Cuttings

The different treatments showed a significant difference in the germination of cuttings (Table 1). Germination was total (100%) in cuttings treated with the natural formulation, the synthetic fungicide at 500 and 1000-ppm and the control. However, at the maximum rate (1500-ppm), 50%. germination was achieved in cuttings treated with the natural formulation.

Table 1. *Germination rate (p.c.) of cuttings after different treatments.*

	Germination rate (%)	
Doses (ppm)	**Synthetic Fungicide (Propiconazole)**	**Natural Formulation (*Cymbopogon citratus*)**
500	100,0 ± 0,0a	100,0±0,0a
1000	100,0 ± 0,0a	100,0 ± 0,0a
1500	100,0 ± 0,0a	50,0 ± 8,86b
Control	100,0 ± 0,0a	

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

3.2. Effects of Treatments on Plant Elongation

Chemical (Propiconazole) and natural formulation (*C. citratus*) treatments at all rates tested induced higher plant height growth than the control (Table 2). Values ranged from 75-105 cm for the chemical fungicide and 69-101 cm for the natural formulation. The highest rate (1500-ppm) resulted in the highest height growth for both chemical and natural formulation treatments.

Table 2. *Average height of seedlings according to treatments applied.*

Doses (ppm)	Average plant height (cm)	
	Synthetic Fungicide (Propiconazole)	**Natural Formulation (*C. citratus*)**
500	75,0 ± 12,13bc	69,5 ± 4,941b
1000	90,0 ± 10,60bc	99,0 ± 13,91a
1500	105,25 ± 5,25a	101,25 ± 9,012a
Control	62,75 ± 3,30b	

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

Table 3. *Average number of stems (tillering) of plants as a function of treatments.*

Doses (ppm)	Average number of rodss	
	Synthetic Fungicide (Propiconazole)	**Natural Formulation (*C.citratus*)**
500	7,0±1,03ab	6,25±0,478ab
1000	5,25±0,25b	5,25±0,25b
1500	4,0±0,408b	3,0±0,408b
Control	12,25±0,25a	

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

3.3. Effect of Treatments on Tillering

A significant effect of fungicide treatments was observed on the number of stems carried by the cuttings planted (Table 3). Untreated control shoots were distinguished by the greatest number of stems produced. The average number of stems per shoot was 12.25. For the fungicide treatments, the rate of the product had a significant effect on stem production. Based on these effects, the fungicide treatments were classified into two homogeneous groups (Table 3). The first group is represented by the two products at the 500-ppm rate that produced the highest number of stalks produced. The second group with mean numbers of stalks produced that ranged from 3 to 5 is formed by the two fungicides at 1000 and 1500-ppm.

3.4. Effect of Treatments on Plant Growth

Growth parameters included stalk diameter, number of leaves, number of internodes and internodes length (Table 4). Average stem diameters were statistically different depending on the treatment applied to the cuttings. The natural formulation (*C. citratus*) at the 500, 1000 and 1500-ppm rates promoted similar diameter to the synthetic fungicide (Propiconazole) at the higher rates (1000 and 1500-ppm). More leaves were produced (9.5 leaves) in cuttings treated with Propiconazole at 1500-ppm (Table 4). This value is not statistically different from that obtained with the natural fungicide treatment at the rate of 1000-ppm in *C. citratus* essential oil. The control treatment and those containing the lowest rate (500-ppm) of propiconazole and *C. citratus* essential oil induced the lowest number of leaves. Regarding the number of internodes and nodes, the results showed a similar effect of the treatments. Based on these effects, the fungicide treatments were classified into two homogeneous groups (Table 4). The first group is represented by the two products at the rates of 500-ppm and 1000-ppm for the natural fungicide and 1000-ppm and 1500-ppm for the chemical fungicide that resulted in the highest number of nodes and internodes. The second group with mean numbers of nodes and internodes generated by the 1500-ppm natural formulation, the 500-ppm chemical fungicide and the control treatment. With respect to internode length, treatments with natural formulation at 1000-ppm and synthetic fungicide at 1000 and 1500-ppm differed from the others with the best values being 8.28, 9 and 8.53 cm, respectively.

Table 4. *Growth parameters values as a function of treatments applied.*

Treatments	Doses (ppm)	Stem diameters (cm)	Number of leaves	Number of internodes	Number of nodes	Length of internodes (cm)
Natural formulation (*C. citratus)*	500	18,74±1,15a	6,0±0,81b	10,25±2,75a	11,25±2,75a	6,875±1,93b
	1000	19,14±1,55a	8,75±1,89a	11,50±2,64a	12,50±2,64a	8,28±0,88a
	1500	17,75±1,86a	6,50±1,29b	7,00±1,82b	8,00±1,82b	6,875±0,75b
Synthetic fungicide (Propiconazole)	500	15,39±0,72b	6,25±0,95b	8,75±0,50b	9,25±0,50b	6,25±0,86b
	1000	18,95±3,24a	7,00±1,41ab	13,50±1,73a	14,50±1,73a	9,00±01,35a
	1500	17,69±1,24a	9,5±1,29a	11,50±2,64a	12,50±2,64a	8,53±0,41a
Control	T0	15,76 ±0,48b	6,0±0,81b	8,50±1,29b	8,25±1,89b	4,0±01,47c

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

3.5. Effect of Treatments on Matter Production

The production parameters concerned the mass of fresh matter and the mass of dry matter of the plants (Table 5). The mass of fresh matter and the mass of dry matter produced were statistically different depending on the fungicide applied. The weight of fresh material was higher (393.75 g) with the chemical treatment (Propiconazole) at the 1000-ppm rate. This fresh mass was statistically identical in plants

treated with the natural fungicide at the 500-ppm rate; 1000-ppm and the synthetic fungicide at the 1500-ppm rate. Concerning the dry matter mass, the chemical and natural treatments at the 1000-ppm rate favored 130 g and 120 g respectively. The control plants were in the group of the lowest value both for the of fresh and dry matter.

Table 5. *Values of cane production parameters according to the treatments applied.*

Treatments	Doses (ppm)	Fresh masses(g)	Dry masses (g)
Natural	500	330,75 ± 50,00ab	105,5 ± 40,99ab
Formulation	1000	343,75 ± 75,57ab	120,0 ± 69,28a
(*C. citratus*)	1500	288,75 ± 04,57b	95,0 ± 10,15b
Synthetic	500	245,5 ± 56,00b	99,75 ± 37,95b
fungicide	1000	393,75 ± 28,18a	130,25 ± 38,30a
(Propiconazole)	1500	349,25 ± 69,77ab	103,25 ± 13,37ab
Control	T0	284,50 ± 23,68b	94,5 ± 25,98b

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

3.6. Effects of Treatment on the Incidence of Smut Disease

Symptoms of smut disease were only observed on control plants (Table 6). 51.28 p.c. of plants from untreated cuttings showed symptoms characteristic of smut disease. Sanitation treatment of cuttings with synthetic fungicide (Propiconazole) and natural formulation (*C. citratus*) at 500; 1000 and 1500-ppm prevented expression of the smut disease in emerged plant.

Table 6. *Incidence of the smut disease.*

Doses (ppm)	Incidence of disease (p.c)	
	Fongicide de synthèse (Propiconazole)	Naturel formulation (*C. citratus)*
500	0,0b	0,0b
1000	0,0b	0,0b
1500	0,0b	0,0b
Control	51,28 ± 4,70a	

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

4. Discussion

Smut disease in sugarcane is caused by *S. scitamineum*, a basidiomycete that develops from diploid teliospores. Infection of cuttings with the teliospores promotes the production of infectious promycelium. This mycelium evolves physiologically and cytologically by systematically contaminating the plant preferably in each of the lateral meristems formed. This disrupts the growth pattern of the stems, which then produce short internodes, strong tillering and plant death [7]. Taking into account the harmful consequences of chemicals on the health of producers and consumers and seeking alternative methods to the use of these chemical inputs, a new strategy to control smut disease has been considered that is easily applicable in the farming environment.

Thus, efficacy tests of an organic product based on *C. citratus* oil were carried out in greenhouses. Sanitation of cold sugarcane cuttings with the natural fungicide *C. citratus* showed a favorable effect on the development of sugarcane growth parameters with 500-ppm and 1000-ppm. This showed that at these doses, no toxicity effects were manifested. Regarding the plants treated at the 1500-ppm dose, toxicity responses acting on cuttings germination and development were the most noticeable effects. This result suggests that the toxicity threshold is below 1500-ppm.

By regularly observing the health status of the plants, the three doses of the natural formulation tested inhibited the development of smut by completely reducing the incidence of the disease from 500-ppm upwards. Unlike untreated plants, the disease manifested itself with an infection rate of more than 50%. Indeed, diseased plants showed a stunted appearance, short internodes and a low number of leaves. This is consistent with the symptoms described by the work of [2]. This proves that the formulations totally inhibited the development of the pathogen. This formulation based on *C. citratus* oil had a very good fungicidal effect against *S. scitamineum*. This result also suggests the existence of at least one active ingredient with very strong antifungal properties that would inhibit growth. These results corroborate those obtained by [15], who reported the antifungal effects of this oil under *in vitro* conditions on *S. scitamineum*. Indeed, the antifungal activity of this essential oil is closely related to its chemical composition. As for *C citratus* oil, according to the work of [27] and [16], the essential oil extracted from the leaves is characterized by the presence of five main constituents which are E-citral, Z-citral, beta-myrcene, selina-6-en-4-ol and cis-ocimene. These compounds are believed to act synergistically by altering membrane permeability and denaturing the proteins of the pathogen [13]. The absence of symptoms in the treated seedlings shows that they were protected from infection by the pathogen from 500-ppm upwards.

This treatment will therefore be very beneficial for the sustainable productivity of sugarcane in Côte d'Ivoire. Indeed, *C citratus* is available in quantity for the production of natural plant substances. Moreover, the method is easily applicable in a farming environment. In addition, seedlings treated with propiconazole did not manifest the disease. Indeed, propiconazole is one of the most effective and widely used active ingredients against *S scitamineum* [3].

The results showed that the *C citratus* formulation had similar effects to the synthetic fungicide. This demonstrated that the essential oil of *C citratus* has comparable efficacy to that of propiconazole [11]. However, propiconazole could cause skin allergy and is suspected to be carcinogenic [26]. This oil could be used as an alternative for sanitizing sugar cane cuttings. This method could be effective without any risk to users and protect the environment [24]. This method could be developed and tested on a large scale in plantations in Côte d'Ivoire to control sugarcane smut.

5. Conclusion

This study evaluated the effect of a natural formulation

based on *C. citratus* essential oil on agronomic parameters and against sugarcane smut disease. The treatments induced a favorable effect on the growth and development parameters of sugarcane. In addition, they controlled smut disease at the low dose of 500-ppm. In addition, the formulations had similar effects to those of the synthetic propiconazole-based fungicide, and could be used as an alternative for the disinfection of sugarcane cuttings. The formulation based on *C citratus* appears to be effective against anthrax and could reduce the use of synthetic products and thus reduce the serious risks associated with their use. This would promote the return of many varieties said to be highly susceptible to smut disease and set aside for this purpose. However, it would be interesting to evaluate the efficacy of these formulations in the field in virgin crops and in ratoon crops in other production areas and on other sugarcane varieties.

Acknowledgements

Our thanks go to the World Bank for funding this research through the African Centre of Excellence on Climate Change, Biodiversity and Sustainable Agriculture (ACE-CCBAD). We are also grateful to the sugar companies, SUCAF-CI and SURCRIVOIRE, for authorizing the prospecting in their sugar cane plantations. I express my deep gratitude to all the teachers of the Laboratory of Plant Physiology at UFR Biosciences.

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