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Inhibitory Potential of Lime Fruit (*Citrus aurantitolia*) Bark Extract on Mycelial Growth of *Colletotrichum falcatum,* Causal Organism of Sugercane Red Rot Disease

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Authors' contributions

This work was carried out in collaboration between both authors. Author OAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author GTS managed the analyses of the study. Authors OAA and GTS managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Biocontrol of plant pathogens involves the use and manipulation of living organisms or bioorganic compounds to reduce inoculum density and to maintain the pathogen population below the disease threshold level. This study investigated the inhibitory potential of lime fruit (*Citrus aurantitolia*) bark extract in the control of sugar cane Red Rot fungus, *Collectotrichum falcatum.* Three different concentrations of the extract (i.e 20, 40 and 60%) was prepared and incorporated into sterilized potato dextrose agar (PDA) plates. Each treatment concentration was prepared in four replicate. The test organism maintained in pure sub-culture was inoculated into the four replicate plates of each of the treatment concentration. The control treatment was also inoculated but the extract was not incorporated in to the medium. Activity of the extract against *C. falcatum* was determined by

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the measurement (mm) of percentage inhibition of mycelial growth extension of the organism over a 7-day period. Two way analysis of Variance (ANOVA) and the Least Significant Differences (LSD) at 5% level of probability was used to analyse data obtained from measurement of the treatment replicates. The fungal mycelial growth inhibition was very significant at 40% concentration level than at 20 and 60%.

Keywords: Sugarcane; red rot disease; Colletotrichium falcatum; biocontrol.

1. INTRODUCTION

The entry, growth and invasion of plant cells by different species of disease causing micro organisms constitutes a major threat to the global quest for food security. This is because the activities of these pathogens result in the infection of crops, which lead to reduction in yield, hunger and sometimes outright epiphytosis, famine, migration and eventual death of numerous members of the affected population. Different species of the heterotrophic fungi have long been implicated as one of the major group of micro organisms that causes the most devastating damage to plants, whether in the field or in post-harvest [1].

The fungus, *Colletorichum falcatum* causes red rot disease in sugar cane and was first reported by Went in 1893 from Java (Indonosia). It is a destructive disease of sugar cane found in many areas of the world [2]. In India for instance, it has caused extensive losses in Bihar and Uttar Pradesh and remains endemic in severe form in other parts of the country [3]. The first symptom of red rot in the field is discolouration of the young leaves. The margins and tips of the leaves whither and the leaves droop. The discoloration and withering continues from the tip to the leaf base until the whole crown withers and the plant dies within 4-8 days [3].

Sugar cane (*Saccharum officinarum)* belongs to the grass family (Poaceae), an economically important seed plant family that includes maize, wheat, rice and sorghum and many forage crops. Sugarcane is the world's largest crop. It was cultivated on about 23.8 million hectares, in more than 90 countries, with a world-wide harvest of 1.69 billion tones. Brazil was the larges producer of sugarcane in the world. The next five major producers, in decreasing amounts of production, were India, china, Thailand, Pakistan and Mexico [4].

The world demand for sugar is the primary driver of sugarcane agriculture. Cane accounts for 80% of sugar produced; most of the rest is made from sugar beets. The sugar in sugar cane is sucrose. A molecule of sucrose consists of one molecule of glucose and one of fructose. Other than sugar, products derived from sugarcane include falernum, molasses, rum, cachaca (a traditional spirit from Brazil), bagasses and ethanol. In some regions, people use sugarcane reeds to make pens, mats, screens and various thatch [5]. With all these, it becomes important to ensure that the sugar cane plant remains in good health by controlling the activities of the pathogens that affects it. This is so because the red rot disease fungus alone is responsible for about 10-15% yield reduction [6].

However, the overzealous and indiscriminate use of most of these synthetic fungicides has created different types of environmental and toxicological problems [7]. Plants have ability to synthesize aromatic secondary metablitees like phenols, phenolic acids, quinines, flavones, flarovoids, flavonols, tannins and coumarins [8]. These groups of compounds show antimicrobial effect and serve as plant defense mechanisms against pathogenic microorganisms [9]. The lime fruit (*Citrus aurantifolia*) has been used for ages for the treatment of various ailments. The objective of this study is to investigate the inhibitory effect of lime fruit bark extract on *Collectorichum falcatum*, the fungus that causes sugarcane red rot diseases.

2. MATERIALS AND METHODS

2.1 Source of Inoculum

An infected sugarcane *(Saccharrum officinarum)* showing symptoms of red rod disease caused by *Colletotrichuni falcatum* was collected from Isi Road, Iwofe, Rumuoluineni Obio/Akpor Local Government Area of Rivers State and was brought to the laboratory for isolation.

2.2 Preparation of Culture Medium

The culture medium used for isolation of the causal organism was potato dextrose agar

(PDA). It was prepared by weighing 15.6 g of the PDA into 400 m1 of water in a 500nil conical flask. The potato dextrose agar was sterilized by autoclaving at the temperature of 121°C and 1.1 kg/cm^3 for 15 minutes. After autoclaving, the potato dextrose agar (PDA) was allowed to cool; 2.5 milligram of tetracycline antibiotics was poured into it to inhibit bacterial growth and swirled carefully. Thereafter, the medium was dispensed into sterilized Petri dishes, covered immediately and left to solidify.

2.3 Isolation of Pathogen from Infected Plant Material

The infected sugarcane was cut open, and then cut into small cubes, sterilized with tliymol in spirit for one minute, rinsed twice in distilled water. Using sterile forceps, the cut sugarcane pieces were placed in the Petri dishes plates containing potato dextrose agar (PDA) and kept in sterile incubator for 3 days. The fungal growth on the medium was sub cultured into freshly prepared Saboraud Dextrose Agar (SDA) in Petri dish plates to obtain and maintain pure culture of the pathogen.

2.4 Plant Materials

Lime fruits (*Citrus aurantifolia*) were purchased from mile 3 market, Diobu, Port Harcourt Local Government Area of Rivers State and brought to the laboratory for extraction. Lime fruits were washed and the bark was peeled from healthy lime fruit and washed thoroughly with water and 2% sodium hydrochloride (Chiorox). The bark was then grinded with sterile blender (MoerGold) to obtain 200 g of the sample.

The bark extract was gotten by adding 100 ml of warm distilled water (40°C) into 20, 40, and 60 g of the paste into separate 250 ml beakers. It was left under an ultraviolet light for 12 hours and the bark extract was obtained by filtration through sterile Whatman filter paper of 5.5 cm into test tubes with the various concentrations. This preparation gave 20, 40 and 60% warm aqueous extract. The extract was then sterilized in an autoclave at a temperature of 110°C.

2.5 *In vitro* Test

The *in vitro* test was carried out in sterile Petri dish plates of 9 mm diameter containing potato dextrose agar (PDA). The effect of *Citrus aurantfolia* extract on the radial growth of *Colletotrichum falcatum* in culture was determined by growing the fungus on a PDA medium containing 20, 40 and 60% of the plant extract in Petri dishes. The extract — PDA medium was prepared by adding 3 m1 of the extract into 17 m1 of the molten PDA in plates that were previously marked with two perpendicular lines at the bottom to indicate the centre of the plates, covered and left to solidify. Control plates also contain 3 m1 of distilled water in 17 m1 of molten (PDA). Using cock borer of 4 mm in diameter, the fungus was taken from already prepared extracts/agar plates and incubated at ambient temperature for 7 days and examined daily. Four replicates per treatments were set up in a completely randomized design (CRD). Radial growth of the pathogen was measured on each treated plates and the control.

Fungitoxicity was recorded in terms of percentage colony inhibition and calculated according to the formular of Pandey, et al [10].

Percentage growth inhibition

$$\frac{Dc—Dt}{Dc} \quad X \quad \frac{100}{1}$$

Where

Dc = Average diameter of fungal colony with control

Dt = Average diameter of fungal colony with treatment

Table 1. Measurement of mycelia growth inhibition on PDA Medium

Concentration of extract (%)	Total measurement	Mean measurement	Percentage inhibition
0	121	30.25	27%
20	130	32.50	29.01%
40	79	19.75	17.53%
60	118	29.50	26.33%

2.6 Statistical Analysis

Data obtained were analyzed by analysis of variance (ANOVA) using Statistical Analysis System (SAS) version 91, Institute 2002 USA and the least significant difference (LSD) between means established at 5% probability level ($P<0.05$) was used to separate the means.

$$LSD = \sqrt{\frac{t\ 2(MSE)}{r}}$$

3. RESULT PRESENTATION AND ANALYSIS

Mean separation using least significant difference (LSD).

$$LSD = +0.052 \frac{(MSE)}{r}$$

$$= 2.179 \frac{2 \times 45.76}{4}$$

$$= 2.179 \frac{91.52}{4}$$

$$= 2.179\ 22.88$$

$$= 2.179 \times 4.78$$

$$LSD = 10.41$$

4. DISCUSSION

The ultimate aim of recent researches into the use of bioorganic compounds as remedies for plant diseases has been the development of alternative control strategies to reduce dependency on chemical or synthetic fungicides. Plants have ability to synthesis aromatic secondary metabolites which contain thousand of constituents that are valuable sources of biologically active molecules' possessing antimicrobial properties. (8). Plant extracts have been demonstrated successfully to control diseases in plant (11, 12), yet little has been done in the control of *C. falcatum*. This spurred the investigation of the potential of *C. aurantifolia* bark extract in the control of *C. falcatum*, which causes sugarcane red rot disease.

Table 1 shows measurement of fungal mycelial growth inhibition on the 7th day by the extract on the PDA medium. The highest inhibition is seen in 40% concentration of the extract (i.e 17.63%), followed by the 60% concentration (i.e 26.33%). This is so because the total and average mycelia growth measurement (i.e 79 and 19.75) respectively was highest at 40% concentration of the extract.

The effectiveness of lime fruit (*Citrus aurantfolia)* bark extract in the control of *C. falcatum*, the fungus causing sugarcane red rot-disease was analyzed statistically.

Analysis of variance of the data from all the treatment as shown in table 2 indicates that the observed f-value (28.76) is higher than the tabulated f-value (3.49) at 5% level of probability. This significant difference points to the effectiveness of the extract against the pathogen.

Amienyo and Ataga (13) and Akinbode (14) reported similar effectiveness of the use of indigeneous plants extracts against plant pathogenic organism. Table 3 shows comparative effect of the different concentrations of the extract (20, 40, and 60%) on the pathogen *C. falcatum*. Using the least Significant Difference (LSD) to separate the mean, it shows a significance difference at 40% concentration because the mean value at 40% concentration (36.28) is greater than the LSD value (10.41).

Table 2. Analysis of variance (ANOVA)

Source of variance	Degree of freedom	Sum of square	Mean square	Observed f-value	Tabulated f-value	Required
Total	15	2298.56			5%	1%
Treatment	3	3949.36	131.45	28.76	3.49	5.95
Error	12	549.2	45.76			

Table 3. Comparative effect of different concentrations of Extract of *Citrus aurantifolia* on *C. falcatum*

Concentration	Mean value	LSD value	% Inhibition
40	-44.83ns		-46.39
40	36.28**	10.41	348.51
60	4.83ns		46.39

5. CONCLUSION

The findings of this research work confirms that the use of lime fruit bark extract *(Citrus aurantjfolia)* at 40% concentration has strong inhibitory activity against the fungi *C. falcatum* the cause of sugar cane red rot disease.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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