Antifungal activity of banana rachis leachate on some fungi responsible for banana (*Musa acuminata* Colla) post-harvest diseases

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***Abstract*—** *Post-harvest diseases are a major problem for banana yield. Despite treatments with chemical fungicides, a persistence of diseases is noticed. This study aims at proposing a biological control method against banana post-harvest diseases by using banana rachis leachate. The effect of leachate has been tested in vitro on mycelial growth, conidial germination and in vivo on pathogenic fungi virulence. All leachate concentrations (5, 15 and 20%) tested showed antifungal activity on the tested fungi. However, the 20% concentration was more effective with complete inhibition of mycelial growth and conidial germination of all fungi. No symptoms of crown rot and anthracnose were observed after treatment of bananas with leachate. However, with azoxystrobin, the prevalence of crown rot and anthracnose was 60% and 30%, respectively. Banana rachis leachate recorded highly significant reduction of banana finger rot prevalence compared to azoxystrobin. Banana rachis leachate have strong antifungal properties that may be useful to control banana post-harvest disease as a safe alternative option to chemical fungicides*

***Keywords*— banana; post-harvest diseases; banana rachis leachate, antifungal activity.**

I. INTRODUCTION

Banana (*Musa acuminata* Colla.) plays a key role in the food security of more than 400 million people in developing countries (Arias *et al*., 2003). Banana is the fourth agricultural product worldwide after rice, wheat and maize (Lassoudière, 2007). Côte d'Ivoire is among the greatest African producer of banana dessert with 330 460 tons in 2016 (Faostat, 2016). The banana sector in Côte d'Ivoire provides employment to nearly 10 000 people (MINAGRI, 2015).

However, banana yield is facing many threats of biotic origin especially post-harvest diseases. These infections, such as crown rot, anthracnose, and finger rot, cause significant losses to producers (Dadzie and Orchard, 1997). These post-harvest diseases are caused by *Botryodiplodia theobromae*, *Colletotrichum musae*, *Fusarium* sp. and *Musicillium theobromae* (Lassois *et al*., 2010, Ewané *et al*., 2012, Abd-Alla *et al*., 2014). In Côte d'Ivoire, chemical fungicides with different active ingredients such as Azoxystrobin, Boscalid and Imazalil are used against banana post-harvest diseases. However, this control method has a high cost and the effectiveness of synthetic fungicides has been reduced by the frequent development of resistance by the pathogens. Currently, the search for natural products with novel uses, particularly related to pest management is very active. Recently, studies have focused on the use of composted organic matter as a biological control method (Oka and Yermiyahu 2002, Siddiqui 2004). Thus, the effectiveness of compost leachate in phytosanitary protection against several pathogenic fungi has been demonstrate (Weltzien 1992, Zhang *et al*., 1998). More recently, compost tea has being promoted as an effective tool to control rose powdery mildew (Ingham, 2005) as well as grape powdery mildew, leaf anthracnose and cherry brown rot (Rollins, 2004). The antifungal properties of leachate stemming from banana rachis composting were also demonstrated by Escobar *et al*. (2005) on *Mycosphaerella* spp, causal agent of Sigatoka. Moreover, DE Lapeyre *et al*. (2006) reported a significant control of *Mycosphaerella fijensis* by leachate stemming from plantain rachis composting.

This study aims at assessing the antifungal activity of banana rachis leachate on the fungi responsible for banana post-harvest diseases in Côte d'Ivoire.

II. MATERIAL AND METHOD

3.1 Fungal material

Pathogens fungi, *Botryodiplodia theobromae*, *Colletotrichum musae*, *Fusarium verticillioides*. and *Musicillium theobromae*, were obtained from the fungi collection of the plant pathology laboratory of the University Nangui Abrogoua, in Côte d'Ivoire and cultivated in potato dextrose agar (PDA) medium and incubated at 27 ± 1 °C.

3.2 Leachate preparation

The leachate was obtained from banana rachis previously disinfected with sodium hypochlorite diluted at 1% and then rinsed with distilled water. Banana rachis were crushed and mixed with distilled water (rachis:water ratio of 1:5) placed in a plastic container and stirred twice during a 10 day incubation at (27 ± 1 °C) according to Elad *et al* (1994) method slightly modified. After incubation, the leachate was collected and filtered with a 250 µm mesh screen (Znaidi, 2012).

3.3 Evaluation of banana rachis leachate activity on mycelial growth

Assessment of the antifungal effect of the leachate was carried out on *B. theobromae, C. musae, F. verticillioides*. and *M. theobromae*. For the preparation of culture media, dilutions of 10; 15 and 20% leachate were carried out in supercooling PDA medium. The positive control consisted of PDA media amended with azoxystrobin at a concentration of 1200 ppm. The negative control consisted of PDA medium. The culture media thus prepared were run into sterile Petri plate. A fungal disc (5 mm) cut from the periphery of a 7-day-old culture was placed in the center of each Petri plate. Five repetitions per dilution were made for each pathogenic fungus. The cultures were incubated at 27 ± 1 °C temperature. After 7 days of incubation, diameter of fungal growth was measured in each case, by averaging two diameter of fungal colony at right angle to one another and the percent inhibition of mycelial growth was calculated by using the formula (1) given by Harlapur *et al*. (2007). The sensitivity of each fungus was determined using the Kumar *et al*. (2007) sensitivity scale, I > 90 %: Highly sensitive (S +); 75 % < I < 90 %: Sensitive (S); 60 % < I< 75 %: Moderately resistant (R -); 40 % < I < 60 %: Resistant (R); I < 40 %: Highly resistant (R+).

$$\mathrm{I}\,(\%) = \left(\frac{\mathrm{C} - \mathrm{T}}{C}\right) \times 100 \quad (1)$$

Where : I = inhibition rate ; C = diameter of the fungus colony on medium without fungicide ; T =diameter of the fungus colony in the presence of treatment

3.4 Evaluation of banana leachate activity on conidial germination

Agar plates amended with different concentrations (10; 15 and 25%) of leachate were inoculated with 0.2 ml of conidial suspension (10^6 conidia/ml) from pure culture (7 days old). Agar plate amended with azoxystrobin at the manufacturer's concentration (1200 ppm) served as positive control and negative control were agar plate without leachate. For each leachate concentration and controls, three Petri plate were prepared per fungus. All inoculating plates were incubated at 27 ± 1 °C temperature. Conidial germination was observed under a microscope 24 hours after incubation. Conidial germination was considered effective when the length of the germ tube was greater than the smallest conidia diameter according to Serghat *et al.* (2004) method. The count of germinated conidia was carried out on a total of 100 conidia. The inhibition rate of conidial germination was calculated according to formula (2).

$$. \mathrm{I}\,(\%) = \frac{Gt - Ge}{Gt} \times 100 \quad (2)$$

Where : *I* = inhibition rate; *Gt* = number of germinated conidia without fungicide (control); *Ge* = number of germinated conidia in the trial

3.5 Effect of leachate on post-harvest disease prevalence

Banana hands of the Cavendish subgroup free of visual defects with uniform shape and weight were selected for the experiment. Fruits were disinfected with sodium hypochlorite diluted at 1% for 5 min, rinsed twice with distilled water and then dried with sterile blotting paper under a hood. A conidial suspension concentrated at 10^6 conidia/ml of each fungus was sprayed on entire surface of banana hand. Based on the results of *in-vitro* susceptibility test, only the most efficience concentration of banana rachis leachate was used in the subsequent *in-vivo* susceptibility assay. For the positive control, the inoculated bananas were treated with azoxystrobin at the manufacturer's concentration (1200 ppm). The negative control consisted of bananas inoculated with the conidial suspension without treatment. The incubation of bananas was done in sterile plastic tubs under laboratory conditions (27 ± 1 °C) and arranged in completely randomized design. Ten bananas were used per trial. After 21 days of incubation, the prevalence of each post-harvest disease on bananas was assessed using formula (3).

$$\mathrm{P}(\%) = \frac{Ni}{\mathrm{Nt}} \times 100 \quad (3)$$

Where : P = disease prevalence ; Ni = number of infected bananas ; Nt = total number of bananas

3.6 Statistical analyses

All experiments were conducted in a completely randomized design with three repetitions, for each treatment. The statistical analysis of the results was conducted by one-way analysis of variance (ANOVA 1) with the Statistica 7.1 software. Differences between means were determined by the least significant difference (LSD) test at $P < 0.05$.

III. RESULTS

3.1 Effect of banana rachis leachate on mycelial growth

Banana rachis leachate inhibited the mycelial growth of each fungus responsible for banana post-harvest diseases (Fig 1). This antifungal activity varied significantly ($P < 0.05$) depending on leachate concentrations in the culture medium. *C. musae* and *M. theobromae* were more sensitive to banana rachis leachate with total inhibition of mycelial growth at all concentrations. *B. theobromae* was sensitive to leachate at 15 and 20% concentrations with respective inhibition rates of 80 and 100%. However, a resistance of *B. theobromae* to the effect of azoxystrobin was noticed with an inhibition rate of 10% (Table 1). *F. verticillioides* strain also showed sensitivity to leachate at concentrations of 15 and 20% with successive inhibition rates of 75 and 100%. At 20% leachate concentration, the mycelial growth of all fungi was totally inhibited.

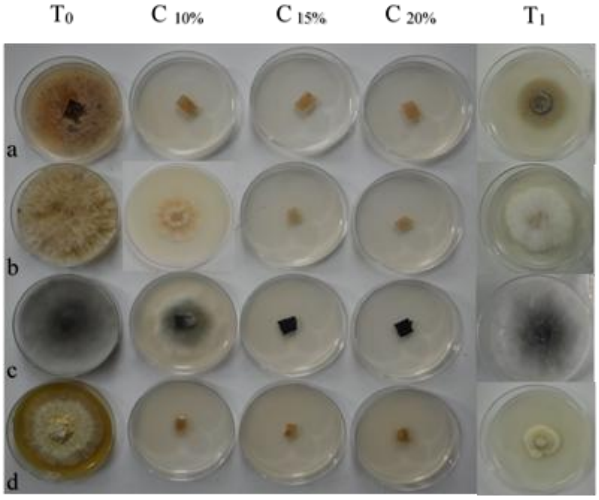

FIG 1: EFFECT OF LEACHATE AND AZOXYSTROBIN ON THE MYCELIAL GROWTH OF FOUR FUNGI RESPONSIBLE FOR BANANA POST-HARVEST DISEASES

a: Colletotrichum musae; b: Fusarium verticillioides; c: Botryodiplodia theobromae; d: Musicillium theobromae

T_0: Negative control (agar); T_1: Positive control (agar + azoxystrobin); C 10%; C 15%; C 20%: leachate concentrations

TABLE 1

INHIBITION RATE OF FUNGI MYCELIAL GROWTH

	Botryodiplodia. theobromae	*Colletotrichum musae*	*Fusarium verticillioides.*	*Musicillium. theobromae*
Inhibition rates (%)				
Leachate (10 %)	30^{c}	100^{a}	20^{d}	100^{a}
Leachate (15 %)	80^{b}	100^{a}	75^{b}	100^{a}
Leachate (20 %)	100^{a}	100^{a}	100^{a}	100^{a}
azoxystrobin (1200 ppm)	10^{d}	50^{b}	60^{c}	70^{b}

The values bearing the same letters in the same column are identical according to the LSD test at 5% threshold.

3.2 Effect of banana rachis leachate on the conidial germination of fungal strains

The antifungal activity of banana rachis leachate on conidial germination varied significantly ($P < 0.05$) depending on fungal strains (Fig 2). Germination of *C. musae* and *M. theobromae* conidia was completely inhibited by leachate at all concentrations. In contrast, with azoxystrobin, conidial germination of *C. musae* and *M. theobromae* was inhibited at 40 and 60% respectively. *B. theobromae* conidia were sensitive to banana rachis leachate at all concentrations with conidial germination inhibition rates greater than 80% (Table 2). However, *B. theobromae* was moderately sensitive to azoxystrobin with an inhibition rate of 50%. *F. verticillioides*. was sensitive to all leachate concentrations with inhibition rates ranging

between 90 and 100%, however with azoxystrobin the conidial inhibition rate of this fungus was 25%. At 20% concentration, the leachate totally inhibited conidial germination of all fungal strains (Table 2).

TABLE 2
INHIBITION RATES OF CONIDIAL GERMINATION

	Botryodiplodia. theobromae	***Colletotrichum musae***	***Fusarium verticillioides.***	***Musicillium. theobromae***
Inhibition rates (%)				
Leachate (10 %)	80^{c}	100^{a}	90^{c}	100^{a}
Leachate (15 %)	90^{b}	100^{a}	95^{b}	100^{a}
Leachate (20 %)	100^{a}	100^{a}	100^{a}	100^{a}
azoxystrobin (1200 ppm)	50^{d}	40^{b}	25^{d}	60^{b}

The values bearing the same letters in the same column are identical according to the LSD test at 5% threshold.

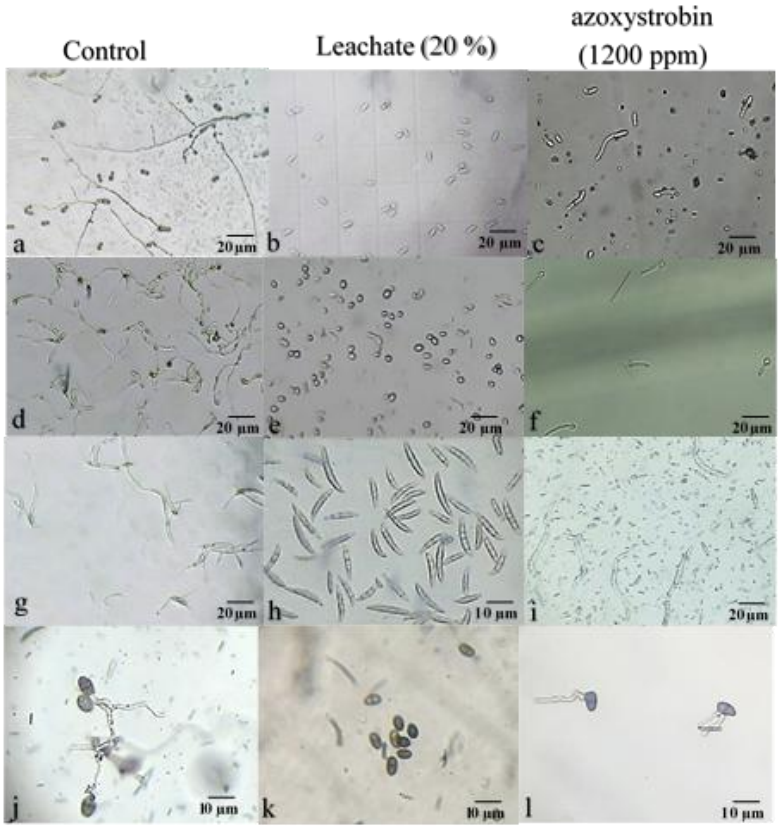

FIG 2: EFFECT OF LEACHATE AND AZOXYSTROBIN ON CONIDIAL GERMINATION OF THE FUNGI RESPONSIBLE FOR BANANA POST-HARVEST DISEASES
a, b, c: conidia of *Colletotrichum musae*; d, e, f: conidia of *Musicillium theobromae*; g, h, i: conidia of *Fusarium verticillioides*; j, k, l: conidia of *Botryodiplodia theobromae*

3.3 Effect of banana rachis leachate on post-harvest diseases

The treatment of bananas with banana rachis leachate showed a significant reduction ($P < 0.05$) in the prevalence of post-harvest diseases. The prevalence of crown rot was 10% for bananas treated with leachate, while for those treated with azoxystrobin, a prevalence of 60% was recorded. As for anthracnose, no symptom was observed on bananas treated with leachate, however a prevalence of 30% anthracnose was obtained with bananas treated with azoxystrobin (Fig 3). finger rot prevalence of bananas treated with leachate was significantly lower than that of bananas treated with azoxystrobin. Indeed bananas treated with leachate showed a prevalence of 5% to distal end rot while for those treated with azoxystrobin a prevalence of 50% was recorded.

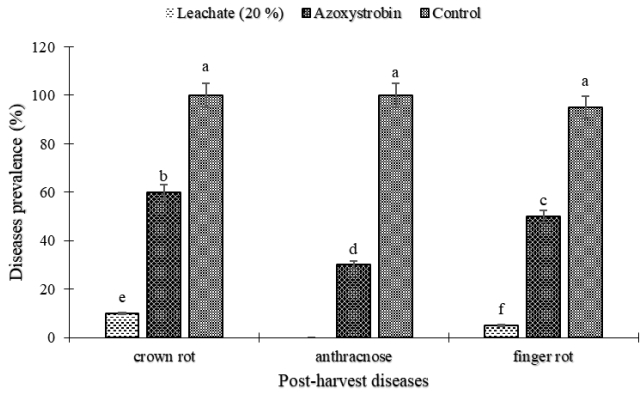

FIG 3: EFFECT OF LEACHATE AND AZOXYSTROBIN ON POST-HARVEST DISEASE PREVALENCE
Histograms bearing the same letters represent statistically identical prevalence according to the LSD test at 5% threshold

IV. DISCUSSION

Mycelium and conidia of the fungi responsible for post-harvest diseases were significantly affected by leachate, which demonstrate the ability of the extract to act on the different development stages of pathogenic fungi. Sikirou *et al* (2010) observed similar results on *Sclerotium rolfsi*, the agent responsible for tomato rust. Indeed banana rachis extract inhibited mycelial growth and germination of *S. rolfsi* sclerotia. The inhibitory activity of banana rachis leachate on conidial germination would help prevent the development of post-harvest diseases. Arauz (2000) and Ploetz (2003) have shown that post-harvest diseases occur as a result of fruit infections by fungal conidia. The application of leachate would stop penetration of the fungus into the host by inhibition of conidial germ tube emission.

Disease prevalence reduction after banana treatment with leachate at 20% concentration suggests that banana rachis leachate has the ability to control post-harvest diseases. Muñoz *et al* (2005) have indicated in their works a better activity of banana rachis leachate at a concentration of 25%. Other works conducted by Toribio (1989) cited by Messiaen *et al* (1991) mentioned that banana rachis extract induced antifungal activity on *S. rolfsi*. According to the same author, a one-tenth dilution of rachis extract showed excellent antifungal properties.

The antifungal activity of banana rachis leachate might be related to its fulvic acid composition. Fulvic acids contain a high concentration of potassium which tends to induce resistance to many diseases (Álvarez *et al*., 2002). Studies conducted by Weltzein (1992) and Yohalem *et al*. (1994) report that leachate has been used for many years in leaf sprinkling for the control of plant fungal diseases. Moreover, the study of Álvarez *et al*. (2002) showed that applications at 5% of fulvic acids stemming from banana leachate reduced the severity of powdery mildew in rose. Furthermore, Escobar *et al* (2005) reported that fulvic acids at 0.5% reduce the incidence of Black Sigatoka in banana tree.

The antifungal properties of compost leachate would also be justified by its composition in several active ingredients other than fulvic acid. These compounds might be responsible for the effectiveness of compost leachate in plant protection against fungal diseases. The results obtained by Al zaemey *et al* 1993 describe the inhibitory effect of a variety of organic acids stemming from compost leachate on the growth of *C. musae in vitro*. These authors indicated that potassium, sodium benzoate and propionic acid are active ingredients present in these organic acids. The effectiveness of compost leachate has also been demonstrated by Welke *et al* (2004). Their works showed a significant reduction of gray mold of strawberry caused by *Botrytis cinerea* by leachate of different types of compost.

V. CONCLUSION

This study has showed the sensitivity of fungal agents responsible for post-harvest diseases to leachate stemming from the composting of banana rachis. Banana rachis leachate has antifungal compounds capable of controlling banana post-harvest diseases.

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