Stimulation of Polyphenol Production by Three Biocontrols (Vacciplant®, Callel®, and Calliete®) in Plantain (*Musa* spp. Group AAB [Musaceae])

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Abstract

The heavy use of pesticides in banana cultivation is associated to environmental and human health degradation. Environmentally friendly alternatives for sustainable agriculture are needed to mitigate the negative impacts of chemicals. This study aims to evaluate the efficiency of biocontrols in the synthesis of bioactive secondary metabolites, mainly polyphenols. Thus, three biocontrols (Vacciplant®, Callel®, and Calliete®) were applied on three cultivars of banana (Orishele, Corne, and French) for 24 h, 48 h, and 72 h and the polyphenol contents were measured. Results showed that the polyphenol contents varied in function of the incubation time and the variety of banana. The highest polyphenol contents were 170.8 and 169.47 µg/g FM and were obtained with Corne-Calliete (Co-Ca) and Corne-Vacciplant (Co-V) respectively. Biocontrols Vacciplant® and Calliette® showed had the best action in stimulating polyphenol production in the three cultivars of banana.

Keywords: biocontrol, cultivars, plantain, polyphenols, secondary metabolites, sustainable agriculture

1. Introduction

Banana (*Musa* spp., Group AAB) is an herbaceous monocotyledonous plant belonging to the Musaceae family and the genus *Musa*. It is cultivated primarily for its fruit, which is consumed fresh for dessert banana or cooked for plantain (Lassoudière, 2007). Bananas are cultivated in more than 120 countries in the tropics and subtropics, where they play an important role in food as well as in social and economic terms (Bakry et al., 1997). Banana is the fourth largest agricultural product worldwide after rice, wheat, and corn. In Côte d'Ivoire, it ranks third after yam and cassava and its annual production varied between two and three million tons, which is far to meet the population's food needs (National Agronomic Research Center [CNRA], 2015). This insufficient production is partly the direct consequence of the increasingly harmful actions of bioaggressors, particularly nematodes and fungi (N'cho et al., 2017). Pesticides were used to control these biopests, and their action has made it possible to effectively control biopests and to increase banana production. However, studies have revealed that the massive use of pesticides causes human health problems (*e.g.*, cancer, sterility), pollution of groundwater, and even disruption of biodiversity (Faurie et al., 2009). Thus, pesticides are being increasingly abandoned because of environmental and health problems.

Faced with these constraints, the interest in seeking alternatives to chemical control for sustainable agriculture emerges. In fact, plants can most often resist bio-aggressors to prevent the development of the disease through secondary metabolites production as phenolic (Faurie et al., 2009; Yin et al., 2013; Konan et al., 2014). Indeed, several authors have reported the important role of phenolic compounds in plant resistance to pathogens (Grayer et al., 2001; Ahuja et al., 2012; Hildago et al., 2016). However, the accumulation of phenolic compounds in the tissues is favored by the application of elicitors or biocontrols. These are substances that activate the metabolism of defense compounds without the plant being attacked by pathogens (Belhadj, 2005; Ahuja et al., 2012).

Moreover, some plants remain sensitive to pathogens and disease establishment, not by an absence of defense compounds but because of a weak synthesis or a delay in the synthesis of these compounds (Grayer et al., 2001; Konan et al., 2014; N'goran et al., 2015).

Thus, it is necessary to research the impact of elicitors or biocontrols on the production of phenolic compounds in the protection of bananas. The objective of this work is to find an alternative to chemical control for sustainable agriculture. Thus, we hypothesized that there are an optimal application concentration of biocontrols and ideal post-application incubation timing of biocontrols to induce optimal accumulation of polyphenols in banana leaves in function of cultivars.

2. Materials and Methods

2.1 Plant Material

The plant material consisted of suckers of three plantains cultivars mainly cultivated in Côte d'Ivoire but also in West and Central Africa, Latin America, and the Caribbean (Bakry et al., 1997). These are triploid hybrids: French (AAB), Corne (AAB) and Orishele (AAB) (Lassois et al., 2009). The suckers were taken from mother banana plants in the experimental plot located at Nangui Abrogoua University (Abidjan-Côte d'Ivoire).

2.2 Methods

2.2.1 Experimental Site

Laboratory and greenhouse experiments were carried out at Nangui Abrogoua University (UNA) located in the district of Abidjan, southern Côte d'Ivoire. The UNA is situated between latitudes 5° 17' and 5° 31' N and between longitudes 3°45′ and 4°22′ W (Brou, 2005) (Figure 1). The climate is humid tropical type. Thus, temperatures and average precipitation based on data recorded on July 2019 were 27.9 °C and 1735.37 mm per year, respectively. The experimental site soil is ferruginous and loose type. The soil pH is more acidic on the surface (Coulibaly et al., 2019).
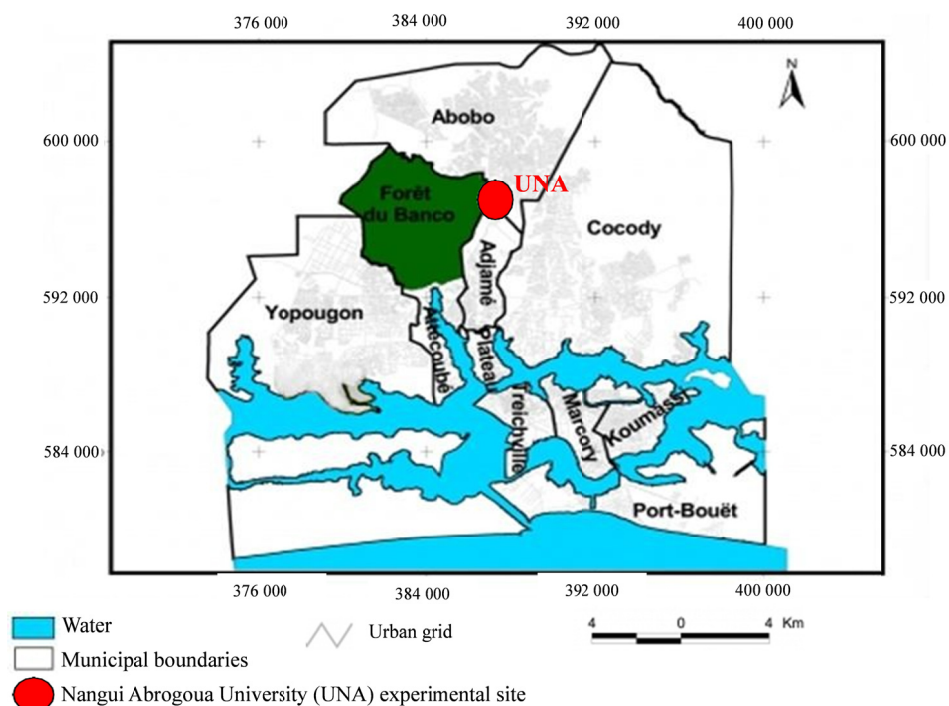

Figure 1. Location of the study site (UNA) in the district of Abidjan (Kouakou et al., 2010)

2.2.2 Production of Plantain *vitro* Plants for Elicitation

(1) Induction of Leafy Shoots

(a) Preparation of Culture Medium

MS base medium (Murashige & Skoog, 1962) was used in this study. This medium was supplemented with vitamin MS, 1238 mg/L NH_4NO_3, 80 mg/L adenine hemisulfate, 80 mg/L ascorbic acid, 5 mg/L BAP, 15 g/L sucrose and 15 g/L glucose. After homogenization, the medium pH was adjusted to 5.8 with 1 N NaOH (sodium hydroxide) or 1 N HCl (hydrochloric acid) solutions. The culture medium was solidified by adding 6 g/L Agar. The culture medium was dispensed into test tubes and the whole set was sterilized by autoclaving for 30 minutes at 121 °C, under a bar pressure.

(b) Plant Material Collection and Disinfection

For each cultivar (French, Corne and Orishele), 15 first-row suckers (the first suckers emitted by the mother plant and the most developed) were collected at the UNA experimental plot (Figure 2A). On the same day, the suckers were washed in the laboratory with tap water mixed with liquid soap for 10 min before being trimmed to reduce the pseudostem by two-thirds of its volume. Then, under a laminar flow hood, the trimmed suckers were soaked in 70% (v/v) ethanol for 30 seconds then, rinsed three times with sterile distilled water before being transferred to a bath 7% (m/v) calcium hypochlorite supplemented with 0.1% tween 20 for 30 minutes (Koné, 2013). After three rinses with sterile distilled water, the rejects were trimmed again with a sterile blade mounted on a scalpel until an apical meristem explant of dimensions 1 cm × 0.5 cm × 0.5 cm was obtained. Thus, the explant obtained was cultured on the shoot induction medium at the rate of one explant per jar or a test tube of dimensions 150 × 22 (L × ø mm). The test tubes containing the explants were closed then sealed with foil and placed in the culture chamber (Figure 2E).

(c) Rooting of Plantain Shoots

Induced buds, approximately 2 cm in length, were individualized and transferred to rooting medium whichwas composed of ½ MS medium (Murashige & Skoog, 1962) supplemented with vitamin MS, 80 mg/L ascorbic acid; 20 g/L sucrose; 1 mg/L BAP; 2 g/L activated carbon. The medium was solidified with 4 g/L Agar-agar after adjusting the pH to 5.8.

(d) Culture conditions

All cultures were placed in a 20 m^2 room at 29±2 °C with 70% of relative humidity. For leafy shoots induction, cultures were stored in continuous darkness for four weeks while incubation for rhizogenesis was performed in continuous darkness for two weeks followed by a 12 h photoperiod for 60 days (Koné, 2013).

(2) Acclimatization of Plantain *vitro* Plants

(a) Weaning of Plantain *vitro* Plants

The well-rooted *vitro* plants, about 15 cm tall, were gently removed from the agar medium with forceps. Then, they were rinsed with water to remove the agar at the base. Then, the oldest roots were cut and the *vitro* plants were placed in a plastic container with a transparent lid, containing forest soil (Nangui Abrogoua University forest topsoil) sterilized at 121 °C for 1 h under one bar pressure. The plastic container with seedlings inside, was placed in the culture chamber at 25 °C under a 12/12 h (12 light and 12 dark) photoperiod. The seedlings were watered regularly with distilled water. After four weeks of weaning, the seedlings were transferred to a mini greenhouse for 28 days, at a temperature ranging from 30 to 32 °C with a relative humidity oscillating between 88 and 91%. Regular watering during this step maintained saturated humidity conditions (Figure 2H).

(b) Breeding of Plantain *vitro* Plants

Weaned seedlings were transplanted into polyethylene bags of 25 cm × 30 cm, containing forest soil. The bagged plants were placed under shade for four months. Maintenance of plants at this stage of the study consisted of regular watering and weeding (Figure 2I).
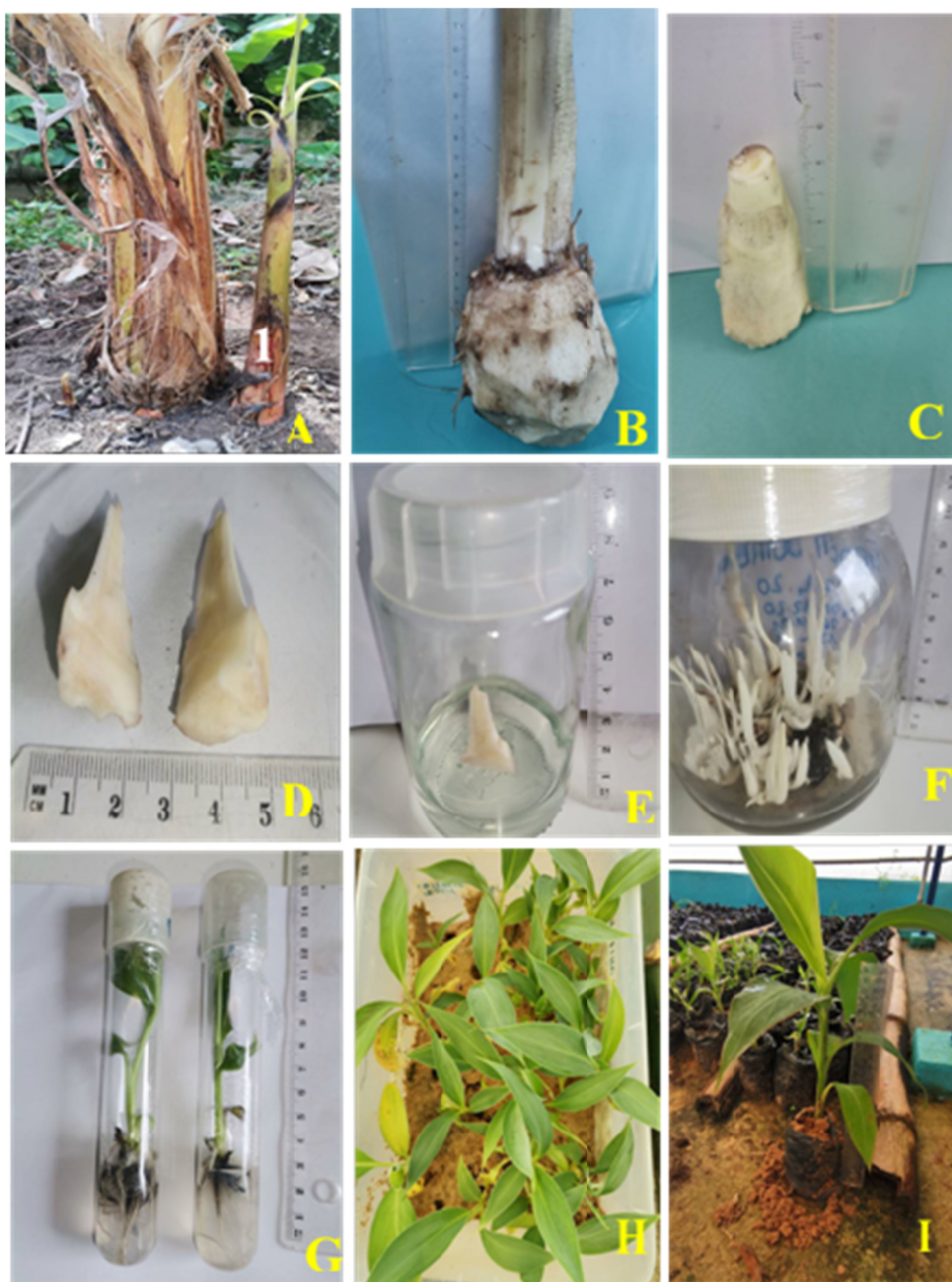
Figure 2. Stages in the production of plantain *vitro* plants

Note. A: Plantain mother plant with bayonet sucker 1, B: trimmed suckers, C: bulb explant, D: bulb explant split longitudinally in half, E: bulb seeded on initiation medium, F: induced shoots, G: *vitro* plants, H: weaning of plantain *vitro* plants in greenhouse, I: plantain plant acclimatized in a tunnel.

2.2.3 Preparation of the Biocontrols Concentrations

Different concentrations of biocontrols were prepared from two biocontrols of natural origin (Vacciplant® at 45 mg/mL and Callel® at 50 mg/mL) and another synthetic biocontrol (Calliete® at 800 mg/mL) (Table 1). Thus, from the initial concentrations of each biocontrol, five concentrations were prepared. A volume (Vi) of biocontrol was taken and diluted in 50 mL of distilled water. Then, a drop of motor oil (Tamol®) was added as an adjuvant to allow longer retention of the product on the leaves. The mixture was then well homogenized before use. The final concentrations were calculated according to the formula below and recorded in Table 2.

$$Cf = (Ci \times Vi)/Vf \tag{1}$$

Where, Cf: Final concentration of biocontrol; Ci: Initial concentration of biocontrol; Vi: Volume of biocontrol collected; Vf: Final volume (biocontrol + distilled water).

Table 1. Caracteristics of different biocontrols used in this study

Biocontrol	Manufacturer in CI	Manufacturer's URL address	Active ingredient	Price/kg (US $)
Vacciplant® (45 mg/mL)	Callivoire/UPL	https://www.upl-ltd.com/ci/product-details/Vacciplant-45-sl	laminarin	22
Callel® (500 mg/mL)	Callivoire/UPL	https://www.k-phyto.ci/stimulants/456-Callel-5-pa.html	Ethephon	10
Calliete® (800 mg/mL)	Callivoire/UPL	https://www.upl-ltd.com/ci/product-details/Calliete-80-wp	Fosetyl alumin	8

Note. CI: Côte d'Ivoire.

Table 2. Different concentrations of the prepared biocontrol solutions

Initial concentration of biocontrol	Volume of biocontrol collected (mL)	Water (mL)	Final concentration (mg/mL)
Vacciplant® (45 mg/mL)	1.4	50	C1 = 1.23
	1.6	50	C2 = 1.4
	1.8	50	C3 = 1.56
	2.0	50	C4 = 1.73
	2.2	50	C5 = 1.90
Callel® (500 mg/mL)	0.50	50	C1 = 0.495
	0.75	50	C2 = 0.739
	1.0	50	C3 = 0.98
	1.25	50	C4 = 1.22
	1.5	50	C5 = 1.456
Calliete® (800 mg/mL)	1.0	50	C1 = 15.69
	2.0	50	C2 = 30.77
	3.0	50	C3 = 45.28
	4.0	50	C4 = 59.26
	5.0	50	C5 = 72.73

Note. C: concentration.

2.2.4 Application of Biocontrols on Plantain Leaves

Biocontrols were applicated according to the method described by Belhadj (2005). Thus, 15 leaves (leaves number 2) were collected with their petiole from the 15 banana plants of each cultivar (Figure 3A). Then, in a greenhouse, these leaves were sprayed directly with biocontrols using a hand sprayer (Figure 3B). About 15 mL of biocontrol were sprayed on each side of the leaf, which corresponds to a slight runoff. A control was made of distilled water. The sprayed leaves were immersed in vases containing distilled water to prevent dehydration (Figure 3C).

2.2.5 Extraction and Determination of Total Phenolic Compounds

The extraction of phenolic compounds was carried out according to the method of Kouakou et al. (2008, 2009). This method was adapted ton our plant material. Thus, 50 mg of fresh material (fresh leaf) were weight and placed in 10 mL of methanol (96%) contained in a hemolysis tube. The tube was placed overnight at 4 °C. After centrifugation at 2000 rpm for 10 min, the methanolic supernatant obtained was filtered through a Millipore membrane (0.45 μm) and constituted the crude phenolic extract.

The determination of total phenols was carried out according to the method described by Siriwoharn et al. (2004). 0.5 mL Folin-Ciocalteu reagent (0.5 N) and 0.9 mL water were added to 0.1 mL of phenolic extract. After stirring at room temperature, 1.5 ml of 17% (w/v) sodium carbonate solution was added to the mixture. After 20 min of incubation at 25 °C in the dark, the intensity of the blue coloration of the reaction mixture which is proportional to the phenolic compound's concentration was monitored with a spectrophotometer at 765 nm. The total phenol content, expressed in milligrams of gallic acid equivalents per gram of extract, was determined

using a calibration line made with gallic acid ($y = 0.021x + 0.053$; $R^2 = 0.999$; where y is absorbance and x is the concentration of gallic acid). Each measurement is repeated three times.
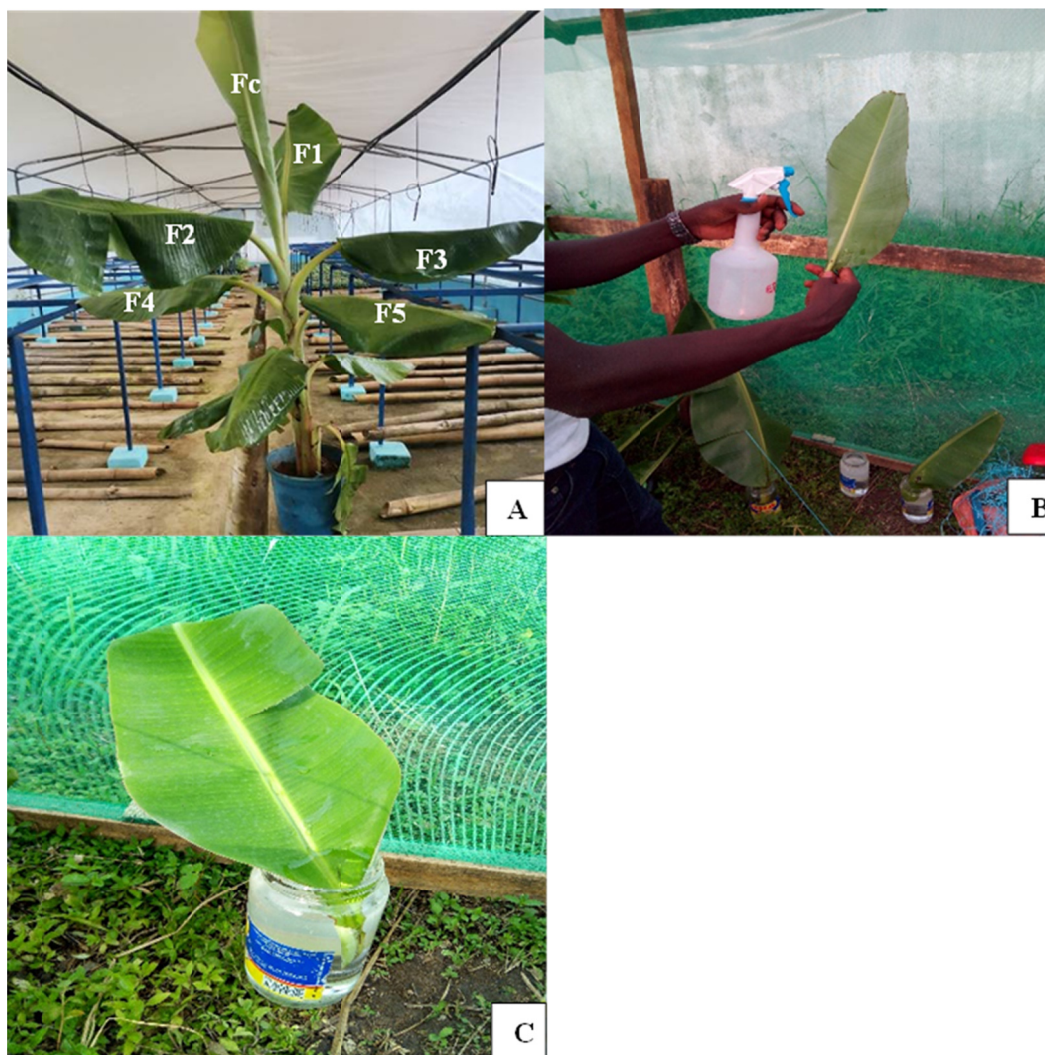

Figure 3. Treatment of plantain leaves using biocontrol solutions.

Note. A: banana tree showing the different leaves (Fc: cigar leaf; F1-F4: succession of leaves appearing before the cigar leaf); B: spraying the leaf; C: leaf put in a pot containing water.

2.2.6 Statistical Analysis

Statistical analysis of the experimental data was carried out using Statistica 7.1 software. An analysis of variance (ANOVA 2) was performed to determine significant differences between total phenols concentration means. In case of significant differences, the means were classified into homogeneous groups using Turkey's HSD test at the 5% threshold. All experiments were repeated three times.

3. Results

3.1 Effect of Different Biocontrol Concentrations and Incubation Time on Polyphenol Accumulation

3.1.1 Effect of Different Concentrations and Incubation Time of Vacciplant® on Total Polyphenols Accumulation

The values for phenol content under various concentrations of Vacciplant® and incubation time are presented in Table 3. According to the results, 24 h and 48 h after incubation, the values were not significantly different regardless of the biocontrol concentration applied to the cultivars concerned. They were statistically identical to each other and to the control values. However, at 72 h, C2 and C5 concentrations induced high phenol levels in

all three cultivars. Thus, the Corne cultivar to which C2 (1.40 mg/mL) was applied synthesized 169.47 µg/g fresh matter (FM). The Orishele and French cultivars synthesized 117.6 and 136 µg/g of FM, respectively, under the C5 concentration (1.9 mg/mL).

Table 3. Effect of Vacciplant® concentration and incubation time on total phenols synthesis in the three plantain cultivars

Total phenols (µg/g FM)				
Vacciplant® concentration (mg/mL)	Incubation time (h)	Cultivars		
		Orishele	Corne	French
Control (0)	24	28±0.5[a]	30±0.6[a]	34±0.7[a]
	48	27.5±0.5[a]	30.5±0.7[a]	33±0.7[a]
	72	26.8±0.5[a]	29±0.5[a]	33±0.7[a]
C1 (1.23)	24	26.47±2[a]	58±9[a]	41.4±5[a]
	48	33.7±0.1[a]	69.07±1[b]	49.2±3[a]
	72	90.27±8[b]	22.4±2[a]	69.47±9[b]
C2 (1.4)	24	26.80±7[a]	115±3[bc]	35.8±7[a]
	48	56.27±1[b]	107.9±1[bc]	17.2±1[a]
	72	90.40±8[b]	169.47±7[c]	82±5[b]
C3 (1.56)	24	37.86±14[a]	84.93±8[b]	34.06±10[a]
	48	54.80±3[b]	108±2[bc]	56.27±14[a]
	72	102±7[bc]	128±7[bc]	102.73±6[bc]
C4 (1.73)	24	37.33±3[a]	152±4[bc]	17.93±0.1[a]
	48	34±4[a]	88.93±2[b]	135.2±6[b]
	72	80.27±6[b]	87.27±6[b]	105.6±6[bc]
C5 (1.90)	24	16.80±8[a]	86.27±10[b]	33.53±9[a]
	48	38.27±3[a]	123±1[bc]	62±3[a]
	72	117.6±6[c]	93.47±6[b]	136±4[c]

Note. In the same row and in the same column, numbers followed by the same letter are not significantly different at the 5% level (Turkey at 5% level); FM: fresh matter.

3.1.2 Effect of Different Callel® Concentrations and Incubation Time on Total Phenols Synthesis

Table 4 shows the effect of different concentrations of Callel® over time on total polyphenol synthesis. After 24 h of incubation, the applied concentrations had no significant effects on phenol synthesis in all cultivars. After 48 h, following the treatments, only the Corne cultivar recorded a high phenol content (100.6 µg/g FM) in the presence of C2 (0.74 mg/mL). As for the French and Orishele cultivars, they induced highly significant phenol levels (83.2 and 98.27 µg/g FM, respectively) after 72 h, in the presence of C4 (1.22 mg/mL) for one and C1 (0.50 mg/mL) for the other.

Table 4. Effect of Callel® concentration and time on total phenol synthesis in the three plantain cultivars

| Callel® concentration (mg/mL) | Incubation time (h) | Total phenols (µg/g FM) Cultivars | | |
		Orishele	Corne	French
Control (0)	24	28 ± 0.5^a	30 ± 0.6^a	34 ± 0.7^a
	48	27.5 ± 0.5^a	30.5 ± 0.7^a	33 ± 0.7^a
	72	26.8 ± 0.5^a	29 ± 0.5^a	33 ± 0.7^a
C1 (1.50)	24	22 ± 9^a	30 ± 7^a	18.94 ± 3^a
	48	55.87 ± 15^b	98.6 ± 7^{bc}	52.2 ± 2^b
	72	98.27 ± 10^c	22.4 ± 0.1^a	37.87 ± 0.1^a
C2 (0.74)	24	25 ± 7^a	29.73 ± 1^a	68 ± 0.1^b
	48	43.6 ± 10^a	100.6 ± 6^c	44 ± 7^a
	72	69.2 ± 1.1^b	59.73 ± 0.5^b	13.60 ± 1.4^a
C3 (0.98)	24	21.94 ± 2^a	46.34 ± 0.6^a	17.74 ± 1.4^a
	48	70.53 ± 5^b	36.50 ± 3^a	61.87 ± 0.8^b
	72	43.87 ± 1.7^a	91.87 ± 3^{bc}	35.47 ± 0.1^a
C4 (1.22)	24	27.14 ± 8^a	41.60 ± 3^a	18.54 ± 1.3^a
	48	68.8 ± 9^b	46.40 ± 13^a	70 ± 12^b
	72	54.27 ± 8^b	83.2 ± 6^{bc}	83.2 ± 6^{bc}
C5 (1.46)	24	22.14 ± 8^a	11.13 ± 6^a	26.14 ± 5^a
	48	80.27 ± 5^b	52.2 ± 1^b	66.93 ± 3^b
	72	36.4 ± 0.6^a	22.27 ± 4^a	52.27 ± 7^b

Note. In the same row and in the same column, numbers followed by the same letter are not significantly different at the 5% level (Turkey at 5% level); FM: fresh matter.

3.1.3 Effect of Different Calliete® Concentrations and Incubation Time on Total Phenols Synthesis

The determination of phenol levels in the three plantain cultivars after analysis revealed a variation depending on the treatments and incubation time (Table 5). Indeed, for the first day (24 h), the values of total phenol contents are not significantly different from each other and from the control values. But from 48 h, a peak of 170.8 µg/g FM was noted for the C2 concentration (30.77 mg/mL) with the Corne cultivar. Also, with the French cultivar, the peak of 126 µg/g FM is reached for a C5 concentration (72.73 mg/mL). In Orishele, the 72 h delay allowed the production of the highest value of phenols (102 µg/g FM) in the presence of C3 concentration (45.28 mg/mL).

Table 5. Effect of Calliete® concentration and incubation time on total phenol synthesis in the three plantain cultivars

Calliete® concentration (mg/mL)	Incubation time (h)	Total phenols (µg/g FM)		
		Cultivars		
		Orishele	Corne	French
Control (0)	24	28±0.5[a]	30±0.6[a]	34±0.7[a]
	48	27.5±0.5[a]	30.5±0.7[a]	33±0.7[a]
	72	26.8±0.5[a]	29±0.5[a]	33±0.7[a]
C1 (15.69)	24	39.47±0.7[a]	26.20±10[a]	25±11[a]
	48	99.64±14[bc]	44±4[a]	80.27±0.1[b]
	72	100.6±1.7[bc]	79.8±1.3[b]	102±18[bc]
C2 (30.77)	24	35.33±3.6[a]	23.93±7.5[a]	27.87±5[a]
	48	68±3[b]	170.8±3[c]	85.47±14[b]
	72	70.4±8[b]	143±8[c]	90.4±6[b]
C3 (45.28)	24	32.13±10[a]	34.93±0.5[a]	32.33±11[a]
	48	36.8±3[a]	62±2.7[b]	90±7[bc]
	72	102±5[c]	52.93±13[a]	117.63±3[c]
C4 (59.26)	24	33.93±12[a]	48.13±0.5[a]	35.8±11[a]
	48	37.73±3[a]	124.8±0.2[bc]	100.8±5[bc]
	72	36.40±5[a]	116.8±2[bc]	53.86±20[a]
C5 (72.73)	24	13.53±3[a]	40.2±8[a]	34±6[a]
	48	79.2±5[b]	65.87±2[a]	126±9[c]
	72	74.47±8[b]	30.5±6[a]	37.47±2[a]

Note. In the same row and in the same column, numbers followed by the same letter are not significantly different at the 5% level (Turkey at 5% level); FM: fresh matter.

3.2 Comparative Effect of Biocontrols on Polyphenol Production in the Three Plantain Cultivars

Table 6 reports the effects of cultivar-treatment combinations on total phenol content. After the application of biocontrols, total phenol production varied according to the banana cultivar-biocontrol pairs. Indeed, only the Corne-Calliete (Co-Ca) and Corne-Vacciplant (Co-V) pairs produced very high levels of phenols (170.8 and 169.47 µg/g FM, respectively). These values are followed by the French-Vacciplant (Fr-V) pair with 136.27 µg/g FM. As for the French-Calliete (Fr-Ca) and Orishele-Vacciplant (Or-V) pairs, they produced 126 and 117.6 µg/g FM, respectively. On the other hand, in the Orishele-Callel (Or-C), Corne-Callel (Co-C), French-Callel (Fr-C) and Orishele-Calliete (Or-Ca) pairs, the phenol levels were the lowest and statistically identical.

Table 6. Comparative table of the effect of biocontrols on the synthesis of total phenols according to plantain cultivars

Biocontrols	Total phenols content (µg/g FM)		
	Plantain cultivars		
	Orishele	Corne	French
Vacciplant®	117.6±1.3[ab]	169.47±1.1[c]	136±0.5[b]
Callel®	98.27±1.7[a]	100.6±0.9[b]	83.2±1.2[a]
Calliette®	102±0.4[a]	170.8±0.6[c]	126±1.4[b]

Note. In the same row and in the same column, numbers followed by the same letter are not significantly different at the 5% level (Turkey at 5% level), FM: fresh matter.

4. Discussion

The search for new control strategies against pathogens to overcome recurrent problems of environmental pollution, the effectiveness of plant protection products, observed resistance and possible risks to human health

(De Lapeyre de Bellaire et al., 2010) related to the abusive use of pesticides in banana cultivation, justify this study, in which the effect of three biocontrols (Vacciplant®, Callel®, and Calliete®) on polyphenols production was evaluated. This study was carried out on plant material produced *in vitro*. The selection of banana *vitro* plants for elicitation aims to eliminate the hypothesis that the polyphenol content of treated banana leaves could be partly related to an immune response of bananas in the presence of pathogens. Indeed, *vitro* culture allows to obtain plants free of any contamination. In this case, such plant material allows us to evaluate the exclusive effect of the different elicitors tested on polyphenol production. In other words, the variation in polyphenol content observed is exclusively related to elicitation.

The analysis of the different results obtained after the application of the biocontrol showed that the levels of phenols vary depending on the cultivar, the post-treatement incubation time, the type, and the concentration of the biocontrol used. After 48 h, the Corne cultivar was the first to produce a high level of total phenols with the Calliete®, the active ingredient of which is fosetyl alumina, followed by the Vacciplant® (laminarin) after 72 h of incubation. This content observed in Corne with fosetyl alumin and laminarin could be explained by the fact that the different biosynthetic pathways of polyphenols were activated by these elicitors (fosetyl alumin and laminarin). This argument agrees with that of Martin and Andriantsitohaina (2002) that the structural diversity of polyphenolic compounds is due to a dual biosynthetic origin and is further enhanced by the possibility of simultaneous participation of both pathways (shikimate and acetate) in the development of compounds of mixed origin, such as flavonoids. Also, this result would be justified by the fact that in the Corne cultivar the photosynthetic activity is very intense; hence the accumulation of large quantities of carbohydrates, the first precursors of polyphenols. This could also be justified by a high stomatal conductance in this cultivar.

The difference time frame observed suggests that fosetyl-aluminum acts spontaneously while laminarin acts gradually. In the French and Orishele cultivars, which received Calliete® and Vacciplant® respectively, an intermediate level of phenols was observed after 48 h for one and 72 h for the other. This result would be due either to a low amount of carbohydrates accumulated in the thylakoid matrix of the plant due to lack of minimal photosynthetic activity or to an inability of these substances (fosetyl alumin and laminarin) to activate both biosynthetic pathways of polyphenols in banana has its delays (Amari, 2012).

The results also showed a cultivar effect on the accumulation of phenolic compounds. The Corne cultivar, which has a high level of accumulation of phenolic compounds after application of elicitors, would be less sensitive to pests than the French and Orishelé cultivars. This result suggests the possibility for susceptible bananas to acquire disease tolerance after treatment with elicitors (N'cho, 2017).

Compared to the two previous results, the phenols contents obtained with Callel®, whose active ingredient is ethephon, are very low. Ethephon would act partially on cinnamic acids and flavonoids accumulation as well as the total phenolic pool. This hypothesis supports the arguments of Konan (2015) for whom, in the form of ethephon, ethylene exerts a weak activation of the natural defenses of the cotton plant. The best concentrations observed with biocontrols (having produced high levels of phenols) are those that would have triggered a cascade of action leading to the synthesis of defense compounds such as phenolic compounds (Konan et al., 2014).

These results agree with the work of other authors who reported that phenolic compounds levels increase after treatment of banana leaves by optimal concentrations of elicitors or biocontrols (De Ascensao & Dubery, 2003; Amari, 2012). Indeed, elicitors are able, under certain conditions, to stimulate natural plant defense mechanisms in the absence of pathogens. Thus, the binding of an elicitor to a plant cell receptor triggers a cascade of actions that result in the synthesis of defense compounds such as phenolic compounds (Konan et al., 2014; N'cho, 2017). The use of biocontrols could reduce the quantity of pesticides needed to protect a crop. This pest control technique is called natural defense stimulation (NDS). The use of natural defense stimulators appears to be a new approach in the field of plant protection and, by inducing the plant's defense reactions, allow it to mobilize its own resources. It is a systemic acquired resistance (SAR) effective against a wide spectrum of aggressors and in many cultures (Kauffmann et al., 2001). Biocontrols are effective at very low doses, inactive on pathogens because they act on the plant. A small amount of biocontrol is therefore sufficient to immunize plants, unlike chemical control, which is very expensive. Moreover, these biocontrols are completely biodegradable and have a generally favorable eco-toxicological profile (Lyon et al., 1995). They are therefore very environmentally friendly molecules. Moreover, with their indirect mode of action, it seems difficult for biocontrols to induce resistance like pesticides (Gullino et al., 2000). Furthermore, the use of biocontrols alternating with "classic" phytosanitary products would allow to reduce the number of fungicide treatments thanks to the gain in efficiency, to avoid or delay the appearance of resistance to these products and therefore increase their resistance. sustainability (Jakab et al., 2001). Thus, the use of biocontrols is part of the new integrated pest management

approach, which is a response to growing expectations relating to respect for the environment and human health (N'cho, 2017).

5. Conclusion

The production of total polyphenols under the action of biocontrols is a function of the concentration of the product applied, the incubation time and the cultivar.

Thus, the cultivar that reacted best with biocontrols was Corne. The highest levels of polyphenols were obtained at 72 h of incubation with Vacciplant® and at 48 h with Callel® and Calliete®. The short incubation time observed with Callel® and Calliete®, shows that they induce plant defense more quickly than Vacciplant®. However, the Corne-Calliete (Co-Ca) and Corne-Vacciplant (Co-V) couples produced the highest phenol levels (170.8 and 169.47 µg/g FM, respectively). The use of biocontrols therefore constitutes an interesting alternative to chemical control. It can be introduced into agricultural practices and thus contribute to the development of agriculture that is more respectful of the environment and human health. Its low-dose efficiency and reasonable cost make it an essential alternative for the development of sustainable agriculture, especially for middle-income farmers.

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References

Ahuja, I., Kissen, R., & Bones, A. M. (2012). Phytoalexins in defense against pathogens. *Trends Plant in Science, 17*(2), 73-90. https://doi.org/10.1016/j.tplants.2011.11.002

Amari, E. G. D. L. (2012). *Stratégies d'évaluation et de gestion par stimulation des défenses naturelles des bananiers à l'infection de la maladie des raies noires causée par Mycosphaerella fijiensis morelet (Mycosphaerellaceae) en Côte d'Ivoire* (p. 237, Thèse de doctorat, Université Félix Houphouët Boïgny, Abidjan, Côte d'Ivoire).

Bakry, F., Charrier, A., Jacquot, M., Hamon, S., & Nicolas, D. (1997). L'amélioration des plantes tropicales. *Les bananiers* (pp. 109-140). CIRAD and ORSTOM, Paris and Montpellier, France.

Belhadj, A. (2005). *Stimulation des défenses naturelles de la vigne par le méthyl jasmonate: Impact sur la biosynthèse des polyphénols et sur la résistance aux champignons* (p. 188, Thèse de doctorat, Université de Bordeaux 2).

Brou, Y. T. (2005). *Climat, mutations socio-économiques et paysages en Côte d'Ivoire* (p. 212). Mémoire de synthèse des activités scientifiques présenté en vue de l'obtention de l'habilitation à diriger des recherches: Université des Sciences et Technologies de Lille.

CNRA. (2015). *Le CNRA en 2015* (p. 40).

Coulibaly, S., Yapo, S. E. S., N'cho, A. L., Kouadio, O. K. S., & Kouakou, T. H. (2019). Effects of Potassium Fertilization for Pineapple on Internal Browning of Fruit in Post-Harvest Conservation. *Journal of Agriculture and Crops, 5*(6), 100-108. https://doi.org/10.32861/jac.56.100.108

De Ascensao, A. R. F. D. C., & Dubery, I. A. (2003). Soluble and wall-bound polyphenols and phenolic polymers in *Musa acuminata* roots exposed to elicitors from *Fusarium oxysporum f.sp. cubense*. *Phytochemistry, 63*, 679-686. https://doi.org/10.1016/S0031-9422(03)00286-3

De Lapeyre de Bellaire, L., Fouré, E., Abadie, C., & Carlier, J. (2010). Black Leaf Streak Disease is challenging the banana industry. *Fruits, 65*, 327-342. https://doi.org/10.1051/fruits/2010034

Faurie, B., Cluzet, S., & Merillon, J. M. (2009). Multiple signaling pathways mediate methyl jasmonate-induced defense responses in *Vitis vinifera* cell cultures. *Journal of Plant Physiology, 166*, 1863-1877. https://doi.org/10.1016/j.jplph.2009.05.015

Grayer, R. J., & Kokubun, T. (2001). Plant fungal interactions: the search for phytoalexins and other antifungal compounds from higher plants. *Phytochemistry, 56*(3), 253-263. https://doi.org/10.1016/S0031-9422(00)00450-7

Gullino, M. L., Leroux, P., & Smith, C. M. (2000). Uses and challenges of novel compounds for plant disease control. *Crop Protection, 19*, 1-11. https://doi.org/10.1016/S0261-2194(99)00095-2

Hidalgo, W., Chandran, N. J., Menezes, R. C., Otalvaro, F., & Schneider, B. (2016). Phenylphenalenones protect banana plants from infection by *Mycosphaerella fijiensis* and are deactivated by metabolic conversion. *Plant Cell and Environment, 39*, 492-513. https://doi.org/10.1111/pce.12630

Jakab, G., Cottier, V., Toquin, V., Rigoli, G., Zimmerli, L., Métraux, J. P., & Mauch-Mani, B. (2001). Beta-aminobutyric acid-induced resistance in plants. *European Journal of Plant Pathology, 107*, 29-37. https://doi.org/10.1023/A:1008730721037

Kauffmann, S., Dorey, S., & Fritig, B. (2001). Les stratégies de défense. *Pour la Science* (pp. 16-121).

Konan, Y. K. F. (2015). *Stimulation des défenses naturelles du cotonnier (Gossypium hirsutum L., Malvaceae) par le méthyl jasmonate et l'éthéphon: Effet sur la biosynthèse des composés phénoliques et sur la résistance à Fusarium oxysporum f. sp. Vasinfectum, agent causal de la fusariose* (p. 207, Thèse de doctorat, Université Nangui Abrogoua, Abidjan, Côte d'Ivoire).

Konan, Y. K. F., Kouassi, K. M., Kouakou, K. L., Koffi, E., Kouassi, K. N., Sékou, D., ... Kouakou, T. H. (2014). Effect of methyl jasmonate on phytoalexins biosynthesis and induced disease resistance to *Fusarium oxysporum f.* sp. *Vasinfectum* in Cotton (*Gossypium hirsutum* L.). *International Journal of Agronomy, 2014*, Article ID 806439. https://doi.org/10.1155/2014/806439

Koné, T. (2013). *Optimisation de la production de matériel végétal sous forme de semences chez trois cultivars (Orishele, Corne 1 et French 2) de bananiers plantain [Musa spp., AAB (Musaceae)] cultivés en Côte d'Ivoire* (p. 161, Thèse de doctorat unique, Université Nagui Abrogoua, Abidjan, Côte d'Ivoire).

Kouakou, T. H. (2009). *Embryogenèse somatique chez le cotonnier [Gossypium hirsutum L. (Malvaceae)]: variation des composés phénoliques au cours de la callogenèse et de la culture des suspensions cellulaires* (p. 137, Thèse de doctorat d'état, Université d'Abobo-Adjamé, Abidjan, Côte d'Ivoire). https://doi.org/10.4314/afsci.v5i1.61718

Kouakou, T. H., Koné, M., Koné, D., Kouadio, Y. J., Amani, N. G., Teguo, W. P., ... Decendit, A. (2008). Trans-resvératrol as phenolic indicator of somatic embryogenesis induction in cotton (*Gossypium hirsutum* L.) cell suspensions. *African Journal of Biochemistry Research, 2*(1), 015-023.

Kouakou, Y. E., Koné, B., Bonfoh, B., Kientga, S. M., N'Go, Y. A., Savane, I., & Cissé, G. (2010). L'étalement urbain au péril des activités agro-pastorales à Abidjan. *VertigO—La Revue Électronique en Sciences de l'Environnement, 10*(2), 47-53.

Lassois, L., Busogoro, J.-P., & Jijakli, H. (2009). La banane: De son origine à sa commercialisation. *Biotechnology, Agronomy, Society and Environment, 13*, 575-586.

Lassoudière, A. (2007). *Le bananier et sa culture*. Versailles, France: Editions Quæ.

Lyon, G. D., Reglinski, T., & Newton, A. C. (1995). Novel disease control compounds: The potential to immunize plants against infection. *Plant Pathology, 44*, 407-427. https://doi.org/10.1111/j.1365-3059.1995.tb01664.x

Martin, S., & Andriantsitohaina, R. (2002). Mécanismes de la protection cardiaque et vasculaire des polyphénols au niveau de l'endothélium. *Annales de Cardiologie et d'Angéiologie, 51*, 304-315. https://doi.org/10.1016/S0003-3928(02)00138-5

N'goran, A., Régine, É. B., Yapo, S. E., Modeste, K. K., Kouassi, K. N., Sékou, D., … Tanoh, H. K. (2015). Stimulation of polyphenols production in cell suspensions of cotton (*Gossypium hirsutum* L.) by oligo-saccharide fraction of *Fusarium oxysporum* sp. *Vasinfectum*, causal agent of fusarium wilt. *International Journal Agri Crop Sciences, 7*(15), 1570-1576.

Ncho, X. (2017). *Élicitation du bananier par le méthyle jasmonate et l'acide salicylique: Impact sur les composés phénoliques, efficacité sur Mycosphaerella fijiensis responsable de la maladie des raies noires* (p. 189, Thèse de doctorat unique, Université Nagui Abrogoua, Abidjan, Côte d'Ivoire).

Ncho, X. E., Konan, Y. K. F., Mohamadou, D. L., Traore, S., & Kouakou, T. H. (2017). Évaluation de la protection des bananiers (*Musa* spp.) élicites par le methyle jasmonate contre la maladie des raies noires causée par Mycosphaerella Fijiensis Morelet en Côte d'Ivoire. *European Journal of Scientific Research, 147*(1), 6-21.

Siriwoharn, T., Wrolstad, R. E., Finn, C. E., & Pereira, C. B. (2004). Influence of cultivar, maturity, and sampling on blackberry (*Rubus* L. hybrids) anthocyanins, polyphenolics, and antioxidant properties. *Journal of Agricultural and Food Chemistry, 52*(26), 8021-8030. https://doi.org/10.1021/jf048619y

Yin, Z., Sadok, A., Sailem, H., McCarthy, A., Xia, X., Li, F., … Bakal, C. (2013). A screen for morphological complexity identifies regulators of switch-like transitions between discrete cell shapes. *Nature Cell Biology, 15*(7), 860-871. https://doi.org/10.1038/ncb2764