Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 17(7): 2724-2734, December 2023

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Effect of two biocontrols on the accumulation of phenolic compounds and leaf pigments in sorghum [*Sorghum bicolor* (L.) Moench] leaves in northern Côte d'Ivoire

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Received: 12-09-2023 Accepted: 28-11-2023 Published: 31-12-2023

ABSTRACT

The aim of this study was to find an alternative to chemical control by improving sorghum's natural defense system. The effect of two biocontrols, Vacciplant and Calliet, on phenolic compound and leaf pigment levels was evaluated. Vacciplant was prepared at concentrations of 0.675; 0.9; 1.125; 1.35 and 1.575 mg/mL, while Calliet was prepared at concentrations of 4 ; 8 ; 12 ; 16 and 20 mg/mL. After preparation, the biocontrol solutions were sprayed onto sorghum leaves 60 days old. Incubation times of 24, 48, 72 and 96 hours were observed after spraying. The sorghum leaves were then harvested and freeze-dried for quantification of total phenols and leaf pigments. Results showed that Vacciplant at a concentration of 1.125 mg/mL after 72 hours of incubation allowed to have the highest contents of total phenols (76.63 mg/g MF), while Calliet at concentration of 4.00 mg/mL after 24 hours of incubation allowed to have the highest contents of total phenols (67.28 mg/g MF). With regard to photosynthetic activity, the results also showed an increase in leaf pigment content following the application of biocontrols. The use of biocontrols could therefore be an interesting alternative to chemical control in sorghum.

Keywords : Biocontrols, Vacciplant, Calliet, natural defense system, phenolic compounds, leaf pigments.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is an annual, gluten-free cereal belonging to the large Poaceae family. It is grown in tropical and semi-tropical regions. Today, this cereal plays a key role in human, animal and industrial nutrition (Djè et al., 2006). In Côte d'Ivoire, it is grown between latitude 4^{e} and 11^{e} degrees north, in the rainy season and on a traditional scale (Beninga, 2014). This plant ranks third among the country's most cultivated and consumed cereal,

DOI: https://dx.doi.org/10.4314/ijbcs.v17i7.10

due to national production estimated at 63,000 t/year for a sown area of 88,752 ha. For human consumption, the small, white, glassy grain is used to make local dishes (tô, couscous, porridge and fritters). On the other hand, the large, red, floury grain is used to make" tchapalo", a traditional commercial beer that is highly prized and dedicated to traditions of hospitality and conviviality just like corn (N'da, 2019). Unfortunately, sorghum is attacked by numerous pathogens, leading to a drop in production. Faced with this threat, growers resort to pesticides to ensure good productivity. However, while the use of pesticides meets agronomic imperatives, it is necessary to implement measures to reduce the risks to human health and the environment.

In this context, it seems necessary to seek more effective alternatives for the development of sustainable agriculture. One of these is to give plants the means to defend themselves, or to reinforce their own means of defense, rather than fighting the aggressor directly (Amari, 2012 ; Konan et al., 2014). In this category are Plant Natural Defense Stimulators (PNDS), which are compounds that act on the biosynthesis of secondary metabolites (Konan et al., 2014). Among the natural defense mechanisms developed by plants is the biosynthesis of compounds belonging to the polyphenol family (Belhadj et al., 2005; Pedras and Adio, 2008). These phenolic compounds, according to Ahuja et al. (2012) and Yin et al. (2013), accumulate in tissues adjacent to necrotic areas, suggesting that these compounds may be defensive. In addition, a healthy plant has good photosynthetic activity and therefore a good chlorophyll content. In general terms, this work is part of the implementation of sustainable agriculture. In this study it will specifically focus on:

- evaluate the impact of two biocontrols on the production of total phenols in sorghum leaves;
- and the second is to determine the effect of these biocontrols on the photosynthetic activity of sorghum leaves.

MATERIALS AND METHODS

Materials

Plant material

The plant material consisted of sorghum [*Sorghum bicolor* (L.) Moench] seeds of the cultivar SOUMALIMBA, an improved variety. This is an improved cultivar from the cereal station of the Centre National de Recherche Agronomique (CNRA) in Ferkessédougou (Côte d'Ivoire).

Chemical products

The chemicals used were gallic acid, ethanol, methanol, sodium carbonate, triton X-100 and Folin-Ciocalteu reagent, purchased from Sigma-Aldrich (Natick, MA, USA). The two natural biocontrols were purchased commercially from Callivoire (Abidjan, Côte d'Ivoire). They are Vacciplant and Calliet, with Laminarin (ß-1,3-glucans) and Fosetyl alumina as active ingredients respectively.

Methods

Study area

The trial was conducted at the Centre National de Recherche Agronomique (CNRA) experimental station in Ferkessédougou. The department of Ferkessédougou is located in northern Côte d'Ivoire in the Tchologo region, between longitudes 10°30" and 8°35" West and latitudes 5°55' and 3°30' North. Annual rainfall generally ranges from 1,000 mm to 1,400 mm, with average temperatures fluctuating between 28°C and 32°C. Soils are ferralitic and shallow on granite or gneiss (Lasm et al., 2012).

Experimental set-up and implementation

A complete block design with three (03) replicates was chosen. Each repetition consists of eleven elementary plots. The dimensions of the elementary plots were 5 m long and 4 m wide

(5 x 4 = 20 m^2). The distance between neighbouring elementary plots was 1 m and the

distance between replicates was 2 m. The total experimental area was 494 m^2. Sowing took place after a good rain, with three (3) seeds per pocket. Each elementary plot consisted of four (4) rows of seedlings, with ten (10) bunches in each row, spaced 0.5 m apart. One seedling was removed from each plot 15 days after germination, in order to homogenize the population of the elementary plots. The experimental field was set up in July.

Preparation of biocontrol solutions at different concentrations

Solutions of Vacciplant (45 mg/mL) and Calliet (800 mg/mL) were prepared at five different concentrations starting from the initial concentration. One volume (x) of biocontrol was taken and made up to 2000 mL with water; Tamol adjuvant (motor oil) was added at a rate of ten (10) drops per 2000 mL solution. Final concentrations were calculated according to the formula below and the results reported in Table 1.

$$Cf = \frac{Ci.Vi}{Vf}$$

Cf : Final biocontrol concentration ;
Ci : Initial biocontrol concentration ;
Vi : Volume of biocontrol taken ;
Vf : Final volume (biocontrol + distilled water)

Treatment of sorghum plants with different concentrations of biocontrol solutions

Two-month-old sorghum plants (60 days) were sprayed with biocontrol solutions (Figure 1). Each individual plot received 2000 mL of solution. Control plants were sprayed with water and Tamol adjuvant. During treatment, complete randomization of the different concentrations of the two biocontrols was observed. After treatment, incubation times of 24, 48, 72 and 96 hours were observed, the times required for induction of total phenols after application of biocontrols or Natural Defense stimulators. After each incubation time, 5 g of leaves from each concentration were collected and freeze-dried for total phenol assay.

Study of the effect of two biocontrols on phenolic compounds content

Extraction and determination of total phenolic compounds in sorghum leaves

Polyphenol extraction was carried out using the method of Kouakou et al. (2008 ; 2009). A 500 mg sample of leaves from each treatment was placed in 10 mL of pure methanol, then kept in the dark for 18 hours, the time required for polyphenol extraction. After centrifugation at 2,000 rpm for 10 min, the resulting supernatant was filtered through a Millipore membrane (0.45 µm) to form the crude phenolic extract. Total phenolic compounds were determined using the method of Singh et al. (2002). The principle of the method is based on the oxidation of phenolic compounds by the Folin-Ciocalteu reagent. Thus, 0.5 mL of Folin-Ciocalteu reagent and 0.9 mL of water were added to 0.1 mL of phenolic extract. After stirring at room temperature, 1.5 ml of 17 % sodium carbonate solution and 6 mL water were added. After 35 min incubation, the intensity of coloration proportional to phenolic compounds concentration was monitored with a spectrophotometer. Optical density (OD) was read at 765 nm. The phenolic extract was replaced by distilled water as a control. Phenolic compounds content was determined using a standard curve and expressed in milligrams per gram of fresh matter (mg/g FM).

Study of the effect of the two biocontrols on leaf pigment content

To determine the effect of biocontrols on leaf pigment content, only the concentrations and incubation times that gave the highest total phenol content was used.

Extraction and determination of sorghum leaf pigments

Extraction and assay of sorghum leaf pigments were carried out according to the method described by Lichtenthaler (1987). 200 mg of leaves were cut into small fragments and

placed in a test tube containing 5 mL of acetone. They were then kept at 4 °C overnight. The new solution obtained was the crude leaf pigment extract. The mass of leaves used and the volume of crude extract were determined, and the absorbance was measured with a spectrophotometer. OD (Optical Density) readings were taken at 647 nm, 663 nm and 470 nm, against a control sample made with acetone. The chlorophyll a, b and total (Chl a, Chl b and Chl t) and carotenoid content of leaves was expressed in µg/mL using the following formulas (Lichtenthaler, 2001) :

Chl a (µg/mL) = [12.25 x DO_{663} – 2.79 x DO_{647}] x V / 1000m

Chl b (µg/mL) = [21.5 x DO_{647} – 5.10 x DO_{663}] x V / 1000m

Chl t (µg/mL) = [7.15 x DO_{663} + 18.71 x DO_{647}] x V / 1000m

Car (µg/mL) = [1000 x DO_{470} – 1.82 x Chla – 85.02 x Chlb] / 198 x V / 1000m

Where V is the volume of crude chlorophyll extract (mL) and m is the mass of leaves (FM) used (g).

Gain in chlorophyll production

The gain in foliar pigment production induced by the application of Vacciplant and Calliet was determined in relation to the control. It is expressed as a percentage and is calculated using the following formula (Lichtenthaler, 2001) :

GPF (%) = [(Trial P - Control P) / Control P] x 100

Where P is chlorophyll content (total chlorophylls) and G is chlorophyll gain.

Statistical analysis

Statistical analyses were performed using STATISTICA 7.1 software. For each study, an analysis of variance (ANOVA 1) was performed. When this analysis showed a difference between means, Duncan's test was performed to determine significant differences between treatments at the 5% threshold.

Table 1: Different final concentrations of the two biocontrol agents.

Biocontrols	Volume of biocontrol to be sampled (mL)	Volume of water (mL) to fill	Final concentration (mg/mL)
Vacciplant at 45 g / mL	30	1970	0.675
	40	1960	0.9
	50	1950	1.125
	60	1940	1.35
	70	1930	1.575
Calliet at 800 mg / mL	10	1990	4
	20	1980	8
	30	1970	12
	40	1960	16
	50	1950	20

Figure 1: 60-day-old sorghum plants.

RESULTS

Effect of the two biocontrols on phenolic compounds accumulation in sorghum leaves

Effect of Vacciplant concentration and incubation time on phenolic compounds accumulation in sorghum leaves

Table 2 shows the total phenol content of Vacciplant-treated leaves as a function of concentration and incubation time. The results show that the application of Vacciplant to sorghum leaves leds to an increase in phenol levels compared with the control after incubation times of 24.48 and 72 hours, while after 96 hours phenol levels feil. Phenol levels varied according to concentration and incubation time. However, the concentration of 1.125 mg/mL after 72 hours incubation has the highest total phenol content (76.63 mg/g FM).

Effect of Calliet concentration and incubation time on polyphenol accumulation in sorghum leaves

Table 3 shows the total phenol content of leaves treated with Calliet as a function of concentration and incubation time. The results show that the application of Calliet to sorghum leaves leds to an increase in phenol levels compared with the control. These levels varied according to concentration and incubation time. However, the concentration of 4 mg/mL after 24 hours incubation has the highest total phenol content (67.28 mg/g MF).

Comparative effects of the two biocontrols on total phenol content in treated sorghum leaves

Figure 2 shows the comparative effect of Vacciplant and Calliet on total phenol content in sorghum leaves. In this comparison, only the highest total phenol levels from the previous study were used. Analysis of this figure shows that sorghum leaves treated with Vacciplant at a concentration of 1.125 mg/mL after an incubation time of 72 hours had a total phenol content of 76.63 mg/g MF, significantly higher than those treated with Calliet at a concentration of 4 mg/mL after an incubation time of 24 hours (67.28 mg/g MF). All plants treated with biocontrols had a higher total phenol content than the control (34.08 mg/g MF). Results for total phenol levels in sorghum leaves treated with biocontrols compared with the control (Figure 3) show that Vacciplant causes a 2.24-fold increase, while Calliet causes a 1.97-fold increase compared with the control.

Effect of the two biocontrols on leaf pigment content in sorghum leaves

The results presented in Tables 4 and 5 relate to the effect of biocontrols on leaf pigment content in sorghum leaves. Analysis of these results shows that under the action of biocontrols, chlorophyll pigment levels

(chlorophyll a, chlorophyll b and total chlorophyll) increase significantly in leaves, while carotenoid levels decrease compared with the control. In particular, after treating the plants with biocontrols, the highest chlorophyll a and total chlorophyll values came from plants treated with Vacciplant, which were 190.34 and 318.65

µg/mL respectively, compared with those treated with Calliet, which were 156.43 and 287.51 µg/mL respectively. With regard to the gain in chlorophyll production by sorghum plants, the results are shown in Figure 4. Plants treated with Vacciplant showed a greater gain in total chlorophyll production (34.57%) than those treated with Calliet (21.41%). These results correlate with those of Table 6 on indicators of functional leaf pigment equipment and leaf greenness**.** The Chl a/Chl b ratio giving an indication of functional leaf pigment equipment was 1.51 in leaves treated with Vacciplant and 1.17 in those treated with Calliet. Similarly, for leaf greenness (Chl t/Car), the results show that in plants treated with Vacciplant, the indicator of greenness is 15.81, and 11.39 in those treated with Calliet. All these ratios show significant differences and are higher than the control ratios.

Table 2: Total phenol content of Vacciplant-treated leaves as a function of concentration and incubation time.

Concentrations (mg/mL)	Total phenol content (mg/g FM)			
	Incubation time (hours)			
	24 h	48 h	72 h	96 h
C0 (0)	34.08 ± 0.11 e	34.28 ± 0.11 d	33.02 ± 0.01 e	35.12 ± 0.11 a
C1 (0.65)	38.28 ± 0.11 d	46.52 ± 0.23 c	52.12 ± 0.21 c	24.54 ± 0.11 b
C2 (0.9)	41.40 ± 0.12 c	59.58 ± 0.12 a	69.77 ± 0.11 b	22.32 ± 0.11 c
C3 (1.125)	43.88 ± 0.05 b	58.94 ± 0.01 a	76.63 ± 0.15 a	20.09 ± 0.11 d
C4(1.350)	44.53 ± 0.16 a	54.66 ± 0.02 b	52.38 ± 0.12 c	19.67 ± 0.11 d
C5 (1.575)	44.28 ± 0.04 a	53.47 ± 0.10 b	41.92 ± 0.06 d	20.11 ± 0.11 d

± S: standard error; in the same column, values followed by the same letter are not significantly different (Duncan test at 5%); the values represent the average of the three repetitions.

Table 3: Total phenol content of leaves treated with Calliet as a function of concentration and incubation time.

Concentrations (mg/mL)	Total phénol content (mg/g FM)			
	Incubation time (hours)			
	24 h	48 h	72 h	96 h
C0 (0)	34.78 ± 0.11 e	34.08 ± 0.11 e	34.02 ± 0.21 d	35.73 ± 0.55 e
C1 (4)	67.28 ± 0.11 a	40.22 ± 0.03 d	43.32 ± 0.21 c	42.03 ± 0.11 b
C2 (8)	54.40 ± 0.12 b	42.52 ± 0.19 c	45.47 ± 0.11 b	45.45 ± 0.11 a
C3 (12)	46.17 ± 0.05 c	48.95 ± 0.02 a	46.53 ± 0.15 a	43.11 ± 0.11 c
C4(16)	44.13 ± 0.16 d	48.06 ± 0.04 a	45.09 ± 0.12 b	44.67 ± 0.11 b
C5 (20)	44.09 ± 0.04 d	47.17 ± 0.11 b	42.82 ± 0.06 c	43.23 ± 0.11 c

± S: standard error; in the same column, values followed by the same letter are not significantly different (Duncan test at 5%); the values represent the average of the three repetitions.

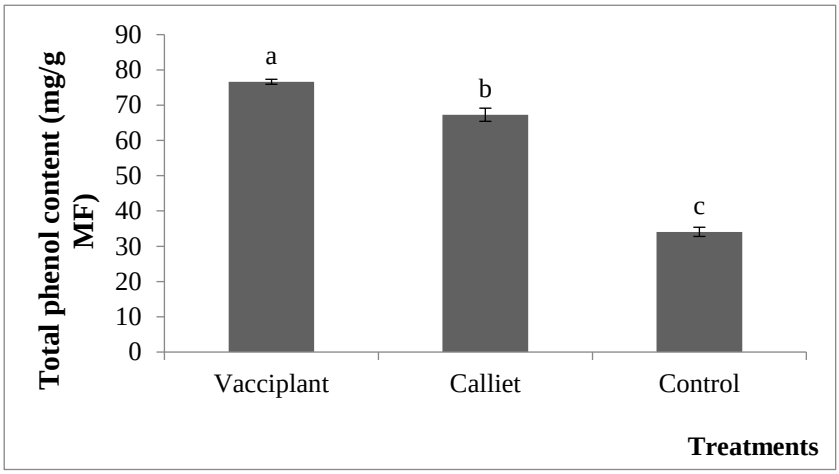

Figure 2: Total phenol content of sorghum leaves as a function of s biocontrols.
Bars topped by the same letter are significantly identical. Duncan test at 5%.

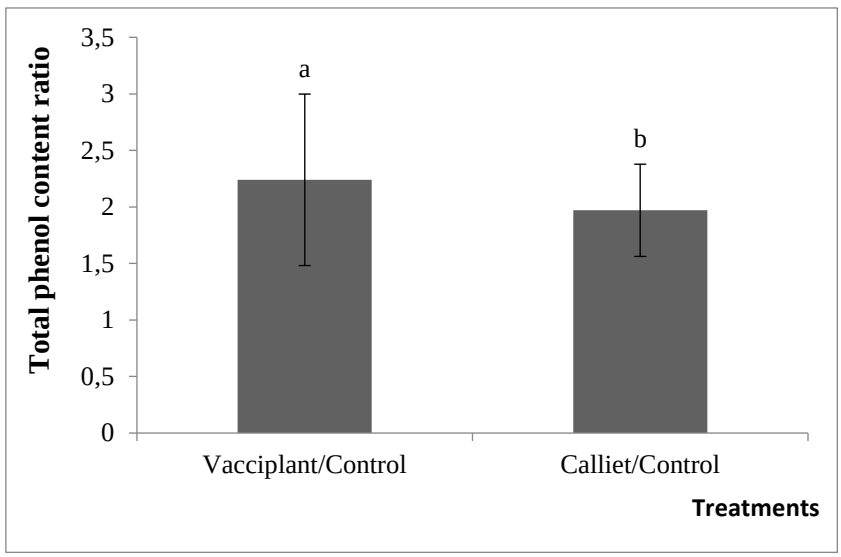

Figure 3: Ratio of total phenol content in treated sorghum leaves compared with the control.
Bars topped by the same letter are significantly identical. Duncan test at 5%.

Table 4: Effect of Vacciplant concentration on variation in leaf pigment content in sorghum leaves.

Biocontrol concentrations (mg/mL)	**Leaf pigment content (µg/mL)**			
	Vacciplant			
	Incubation time (72 h)			
	Chla	Chlb	Chlt	Car
C (0)	123.58 ± 1.51b	113.21± 1.01b	236.79± 0.41b	75.48± 1.21a
C (1.125)	190.34± 0.21a	125.64± 1.22a	318.65± 0.13a	20.15± 0.33b

± S: standard error; in the same column, values followed by the same letter are not significantly different (Duncan's test at 5%); values represent the mean of the three repetitions; Chl a: Chlorophyll a; Chl b: Chlorophyll b; Because: Carotenoids.

Table 5: Effect of Calliet concentration on variation in leaf pigment content in sorghum leaves.

Biocontrol concentrations (mg/mL)	Leaf pigment content (µg/mL)			
	Calliet			
	Incubation time (24 h)			
	Chla	Chlb	Chlt	Car
C (0)	123.58± 0.03b	113.21± 0.22b	236.79± 2.18b	57.48± 0.27a
C (4)	156.43± 1.41a	133.65± 1.01a	287.51± 0.43a	25.23± 1.12b

± S: standard error; in the same column, values followed by the same letter are not significantly different (Duncan's test at 5%); values represent the mean of the three repetitions; Chl a: Chlorophyll a; Chl b: Chlorophyll b; Because: Carotenoids.

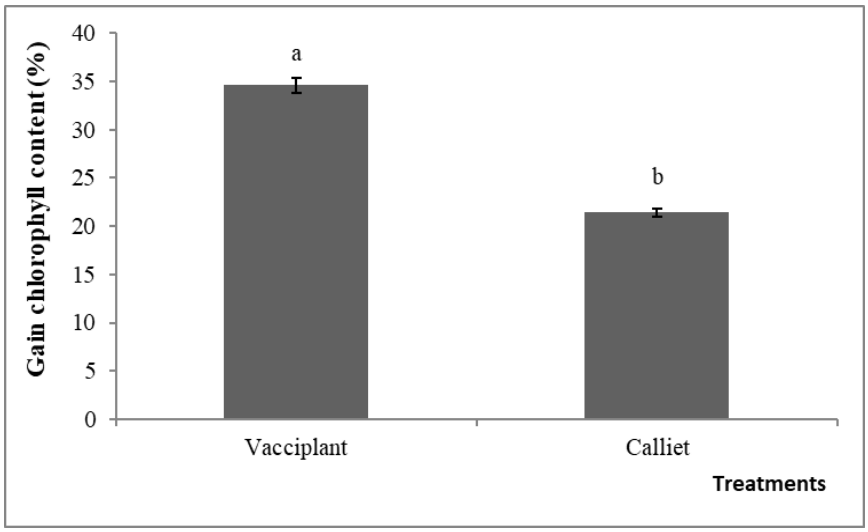

Figure 4: Gain in chlorophyll content in sorghum leaves treated with biocontrols compared to the control. Bars topped by the same letter are significantly identical. Duncan test at 5%.

DISCUSSION

Total phenol production varies with biocontrol concentrations (Vacciplant and Calliet) and incubation time. Both biocontrols used stimulate the production of phenolic compounds (Yamaji and Ichihara, 2012 ; Konan, 2015). At a concentration of 1.125 mg/mL, treatment of sorghum leaves with Vacciplant induced the highest content of total phenols (76.63 mg/mL), while with Calliet the highest content was obtained at a concentration of 4 mg/mL (67.28 mg/mL). These results show that laminarin and fosetyl-alumina, the active ingredients in Vacciplant and Calliet respectively, are more effective in inducing phenolic compound synthesis in sorghum at these different concentrations. In fact, natural defense stimulators are effective in inducing phenolic compounds at variable concentrations depending on the plant. In cotton, for example, concentrations of 1 mM BTH and 5 mM MeJA are effective in inducing phenolic compound synthesis (N'cho et al., 2017). However, Vacciplant appears to be more active than Calliet. This translates into a higher phenolic compound content. The exogenous application of Vacciplant and Calliet to sorghum plants seems to induce defense responses through the

production of phenolic metabolites (Larronde et al., 2003 ; Lattanzio et al., 2006 ; Bellow, 2012). Moreover, several studies have reported that phenolic compounds are involved in pathogen control (Lambert, 2011; Konan et al., 2014 ; N'goran, 2016). The binding of Laminarin (the active ingredient in Vacciplant) and Fosetyl-alumina (the active ingredient in Calliet) to a plant cell receptor triggers a cascade of actions and chemical reactions leading to the synthesis of defense compounds such as phenolics (Konan et al., 2014). The increase in phenolic compound content in treated plants compared to the control seems to show a biosynthesis of phenolic compounds which, according to Belhaj et al. (2005), enables a pathogen-susceptible variety to resist attack. This mobilization of phenolic compounds would argue in favor of an increase in sorghum defenses under the action of Vacciplant and Calliet. The results also showed that Calliet had the shortest induction time (24 h) compared with Vacciplant (72 h). This difference in induction times shows that Calliet acts on the synthesis of phenolic compounds in constitutive defense, whereas Vacciplant provokes the synthesis of phenolic compounds involved in induced defense. In fact, Calliet acts rapidly to increase the level of synthesis of pre-existing phenolic compounds, whereas Vacciplant acts both to increase phenolic content and to stimulate de *novo* synthesis of these compounds. These results were also reported by Belhaj et al. (2005) in grapevine and N'cho et al. (2017) in cotton. As far as the physiological state of plants is concerned, leaf pigments provide an assessment of the plant's sanitary state, i.e. its vigor. Results show that under the action of biocontrols, total chlorophyll content rose from 236.79 µg/mL leaves (control) to 318.65 µg/g leaves in leaves treated with Vacciplant and to 287.51 µg/mL in leaves treated with Calliet, i.e. a gain of 34.57 % with Vacciplant and 21.41% with Calliet. In this study, the Chl a/Chl b ratio was greater than 1 (chla/chlb> 1) and significantly higher in plants treated with Vacciplant (1.51) and Calliet (1.17). Untreated plants (controls) had the lowest Chl a/Chl b ratio (1.09). These results would indicate good photosynthetic activity and therefore a good physiological state of the plants after treatment with biocontrols (Hoffman, 2003). The same cannot be said for the indicator of leaf greenness (chlt/car). The ratio of chlorophyll pigments (total chlorophylls) and carotenoids (chlt/car) in treated plants is greater than 3.5, but higher in treated sorghum plants. According to Lichtenthaler (2001), a plant is green if the ratio (Chl t/Car) is greater than 3.5. The exogenous application of biocontrols to sorghum plants would therefore have had a positive effect on leaf greenness.

Conclusion

Treatment of sorghum plants with the biocontrols resulted in an increase in total phenols and leaf pigments. The two molecules (Laminarin and Fosetyl-alumina) contained in the two biocontrols thus demonstrated their ability to induce an increase in the level of synthesis of these compounds, enabling the plant to equip itself with natural defense equipment against disease pathogens.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

ALN : This author is the designer and director of this study. HAN : This author is the breeder of the sorghum variety that was used in this study. He evaluated the growth parameters of the plants in the field. GY : This author followed the phytopathological aspect of the plants in the field. LF : This author contributed to the statistical analyzes and corrections of the manuscript.

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