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Modulation of Secondary Metabolites and Pigments Chlorophyll in Cotton *Gossypium hirsutum* L (Malvaceae) Sensible to *Fusarium oxysporum* by Treatments of Biocontrol Products

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A B S T R A C T

The objective of this work is to find an alternative to chemical control, by improving the natural defenses of the cotton plant using biocontrol products. To do this, the effect of vacciplant and calliet was tested at different concentrations (1, 2, 3, 4 and 5%) by observing three incubation times (24, 48 and 72 h) on cotton plants *in vivo* by evaluating the total phenol content. The results thus revealed that the concentration of 3% of vacciplant and calliet after 72 and 48 h of incubation respectively induced the highest total phenol contents which were respectively 69.87 and 38.70 mg/g MF in these treated plants. These optimal concentrations and incubation times were then used to treat open field cotton plants in order to determine again the total phenol contents and know their physiological state by evaluating the content of chlorophyll pigments (a, b and total). These results showed that the plants treated with vacciplant presented both the significant contents of total phenols (70.71 mg/g MF) as well as total chlorophyll (319.30 µg/g MF) and chlorophyll a (182.09 µg/g MF), but the lowest content of chlorophyll b (118.96 µg/g MF). However, the total phenol contents of the plants in culture *in vivo* and those in the open field were statistically identical. Thus, these high levels compared to the controls are indications of induction of cotton plant well-being by this product.

Introduction

In Ivory Coast, both in Africa and in the world, cotton is of indispensable economic and social importance, because it provides a livelihood for a part of the population and represents an important source of foreign currency income. In Ivory Coast, it occupies the 4th place after cocoa, coffee and cashew nuts. Moreover, in West

Africa, Ivory Coast is the 4th producing country behind Benin, Burkina Faso and Mali (Schwartz, 2012; Anonyme, 2013). It provides substantial income to the peasant masses of the Center and the North of the country which are the main cotton production regions. Unfortunately, climate change has a negative impact on Ivorian cotton production. Indeed, cotton cultivation is increasingly threatened by parasites and various diseases which reduce production, the quality of fibers and seeds (Vaissayre, 1994). The pathogens responsible for cotton diseases are bacteria, virus, mycoplasma and in particular fungi (Delhove *et al.,* 1992). Among all cotton diseases, fusarium wilt caused by *Fusarium oxysporum* f. vasinfectum (FOV) appears to be the most serious, causing yield losses of up to 50 % or almost all harvests. In West Africa, specifically in Ivory Coast, cotton diseases are the main cause of production loss. Unfortunately, methods for controlling these pests are costly for farmers and ineffective (emergence of resistance) given the extent of the damage caused in cotton fields (Demol and Drion, 1992; Ochou, 1998). Also, chemical control is increasingly being indexed for issues of toxicity, environmental pollution, public health and even destruction of biodiversity (Faurie *et al.,* 2009).

In this context, it appears necessary to seek more effective alternatives for the development of healthy and sustainable agriculture. One of these is to give plants the means to defend themselves, by strengthening their own defenses, rather than directly fighting the aggressor (Amari, 2012; Konan *et al.,* 2014). In this category are the stimulators of natural plant defenses (SDN).

Indeed, plant-pathogen recognition is achieved through certain compounds called elicitors or biocontrol products, derived from the pathogen or the plant itself. However, some plants remain susceptible to pathogens and the establishment of the disease. This is not due to a lack of defense mechanism, but rather due to a slow defense (Benhamou, 1996; Graver and Kokubon, 2001). Among the natural defense mechanisms developed by plants is the biosynthesis of compounds belonging to the phenol family (Pedras et Adro, 2008; Yin *et al.,* 2013). These polyphenols accumulate in tissues adjacent to necrotic areas, suggesting that they may be defensive, as their oxidation leads to the production of compounds with bactericidal and fungicidal action (Belhadj, 2005; Ahuja *et al.,* 2012). Cotton produces a large number of polyphenols that are instrumental in disease resistance (Kouakou, 2013; Konan *et al.,* 2014).

Also, a healthy plant has good photosynthetic activity, in other words a good content of chlorophyll pigments.

Thus, in the current vision of reducing agricultural inputs, biocontrol products aimed at helping plants express their full potential and better exploit the resources present in their environment in order to better resist pedoclimatic constraints are booming.

Therefore, this work generally falls within the framework of the establishment of sustainable agriculture, improving the plant's natural defense system through a natural induction of its defenses.

Materials and Methods

Plant material

The plant material consisted of the cotton cultivar Y764 G3 (*Gossypium hirsutum* L.), originating from Ivory Coast. It is an improved cultivar, resulting from the cross between local lines and introduced lines (Hau, 1988). The seeds come from the Korhogo region and were supplied by the Ivorian Textile Development Company (CIDT). The cultivar Y764 G3 has a very high level of sensitivity to *Fusarium oxysporum* f. sp. vasinfectum, causative agent of fusarium wilt.

Biocontrol products

Calliet 80 WP

Calliet, with the active ingredient fosetyl-aluminum, is a systemic fungicide with protective and curative action, launched on the market by Rhône-Poulenc (now Bayer CropScience). Fosetil-aluminum is produced by the chemical reaction between phosphorous acid and an aluminum salt. Its initial concentration can vary between 200 g/L and 400 g/L, depending on the product marketed.

Vacciplant

Vacciplant is a biocontrol product used in agriculture to stimulate plants' natural defenses in order to prepare them for future attacks. Thus, vacciplant has preventative effectiveness. Its active ingredient is laminarin, a natural extract of brown algae (especially of the genre *Laminaria*), classified as an elicitor. It is a β-1,3-glucan, a complex sugar. Laminarin is not toxic to pathogens.

Study methods

Evaluation of the effect of concentration and post-treatment incubation time on cotton plant leaves *in vivo* aged 14 days on the biosynthesis of total phenols

Production of cotton plants *in vivo*

Cotton seeds were sown in 200 ml pots containing soil previously sterilized in an autoclave at 121 °C for 30 minutes and watered every other day with 100 ml of water per pot (Figure 1). Thus, 180 plants were obtained in the pots.

Determination of the optimal concentration coupled with the incubation time of the two biocontrol products

Since the two biocontrol products (calliet and vacciplant) had never been used on cotton, it was necessary to find the concentration and incubation time that best elicited it. Therefore, five concentrations of each biocontrol product (1%, 2%, 3%, 4% and 5%) were defined and each of them was subjected to three incubation times (24, 48 and 72 h).

Preparation of biocontrol product solutions

For each concentration, the corresponding quantities of calliet (CAL) and vacciplant (VAC) were taken and dissolved in distilled water. The preparation of the different concentrations was carried out volume by volume (V/V).

Finally, to all these solutions, 1 mL of a 5 % tamol oil solution dissolved in ethanol was added in order to allow long-term adhesion of the latter to the leaves and give them very good penetrating power.

Treatment of cotton plants *in vivo* by biocontrol products

This study was carried out on 180 cotton plants *in vivo*. Thus, using a mini sprayer, batches of 15 young cotton plants aged 14 days were treated with a single concentration of a very specific biocontrol product at a rate of 51 mL and subjected to different incubation times (24, 48 and 72 h). For this purpose, a total of 75 plants were treated with a biocontrol product. Thirty (30) control plants were treated with 51 mL of distilled water only. The plants were watered according to the humidity of the culture medium.

Extraction and determination of total phenols

The optimal concentrations coupled with the incubation time of vacciplant and calliet were determined from the total phenol content in the treated leaves. For this, the leaves of the treated or untreated plants were collected using a leafhopper according to the incubation times for molecular analyses.

Extractions of total phenols

The extraction of total phenols was carried out according to the method of Singleton *et al.,* (1999). Approximately 0.5 g of leaves taken from each batch were put in 80 % methanol overnight. After removing the 0.5 g of leaf using a strainer. Thus, the solution obtained constituted the crude extract with which the determination of total phenols was carried out.

Determination of total phenols

The determination of total phenols was carried out according to the method of Singh *et al.,* (2002), modified and adapted to our plant material. The reaction mixture is composed mainly of phosphotungstic acid and phosphomolibdic acid which will be reduced in an alkaline medium, in parallel with the oxidation of the phenols. The presence of phenols is revealed by the addition of 0.5 ml of crude extract of 0.5 ml of Folin Ciocalteu 1 N reagent and 1.5 ml of 17% sodium carbonate. The intensity of the blue color of the reaction mixture which is proportional to the concentration of total phenols is monitored with a spectrophotometer at 765 nm. During the assay, a control is carried out where the crude (phenolic) extract is replaced by distilled water. The total phenol content is determined using a standard curve produced with different concentrations of a stock solution of gallic acid (200 µg/ml) and is expressed in milligrams per gram of fresh matter (mg/g MF).

Evaluation of the effect of concentration and post-treatment incubation time of 14-day-old field cotton plant leaves on the biosynthesis of total phenols

In this study, only optimal concentrations and incubation times inducing high posttreatment total phenolic content

of each biocontrol product were used for the treatment of field cotton plants (in nature).

Production of cotton plants in the open field

Experimental device

The experimental design adopted was a Fisher randomized complete block design with three replicates for the treated plants and for the control (T). The experiment was carried out on a plot of 231 m^2 (21 m long and 11 m wide) located in the Korhogo region. The device is subdivided into three blocks spaced 1.5 m from each other. Each block is made up of three sub-plots called elementary plots with an area of 18 m^2 (6 m long and 3 m wide). The elementary plots each contain four ridges 5.5 m long and 0.4 m wide for an area of 2.2 m^2. These ridges are separated from each other by 1 m. Each ridge contains ten pockets 4 cm deep and each separated by 50 cm.

Sowing seeds and obtaining cotton plants in the open field

The seeds were sown on the ridges at a rate of three seeds per pocket at a depth of 4 cm. At emergence, the plants were thinned. Each ridge contains 10 cotton plants. Forty (40) plants per elementary plot were obtained. A total of 360 plants were used for our field experiments in which 120 plants were used for each biocontrol product (vacciplant and calliet) and for the control. At the level of the 120 plants in each block, 60 were used for the evaluation of the total phenolic content and then the other 60 for the chlorophyll a, b and total content. The water nutrition of the plants was carried out by watering every two days according to the needs, where each plant received 100 mL of water.

Preparation of solutions

Here, only the best concentration coupled with the incubation time of the two biocontrol products which induced the highest level of total phenols in the previous study was used for the continuation of our work. Therefore, the 3 % concentrations for each biocontrol product were prepared as previously (*Ref. 3.1.2.1*).

Treatment of plants in the open field

The treatment of the cotton plants was done as previously (*Ref 3.1.2.2*)

Extraction and determination of total phenols

Extraction of total phenols

The extraction of total phenols was done as in the previous study (*Ref 3.1.3.1*).

Determination of total phenols in the leaves of 14-day-old cotton plants

The dosage of total phenols was done as previously (*Ref 3.1.3.2*).

Evaluation of the chlorophyll pigment content in the leaves of cotton plants treated with biocontrol products

Extraction and dosage of chlorophyll pigments

The extraction and determination of chlorophyll pigments were carried out according to the method described by Lichtenthaler, (1987). Thus, 200 mg of leaves cut into small fragments were placed in a test tube containing 5 ml of 80 % methanol and then stored at 4 °C over night. The resulting liquid only solution constituted the crude chlorophyll extract. The mass of the crushed leaves and the volume of the crude extract were determined and the absorbance was measured using a spectrophotometer at 647 and 663 nm, against a control sample made with methanol. The chlorophyll a, b and total (Chl a, Chl b and Chl t) content of the leaves, expressed in mg/g of fresh leaf, was then calculated using the following formula:

Chl a (µg/ml) = (12.25 x DO_{663}- 2.79 x DO_{647}) x V/1000 m

Chl b (µg/mL) = (21.5 x DO_{647}- 5.10 xDO_{663})x V/1000 m

Chl t (µg/ml) = (7.15 x OD_{663}+ 18.71xDO_{647}) x V/1000 m

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

Gain in chlorophyll production

The gain in chlorophyll production induced by the application of calliet and vaciplant was determined compared to the control. It is expressed as a percentage and was calculated from the following formula:

GPF (%) = ((P test – P control) / P control) x 100

Where P is the chlorophyll content (chlorophylls a or b or total) and G the chlorophyll gain.

Statistical analyses

Statistical analyses were performed using SAS 6.0 software. Analysis of variance (ANOVA) was performed on all treatments applied. When this analysis showed a difference between means, Duncan's test was performed to determine significant differences between treatments at the 5 % level. For percentages, the Kruskal-Wallis test was used to determine significant differences ($P < 0.05$) between treatments.

Results and Discussion

Effect of concentration and incubation time of vacciplant and calliet on the accumulation of total phenols in cotton leaves *in vivo*

Effect of the vacciplant

Table I presents the total phenol content of the leaves treated with vacciplant as a function of concentration and incubation time. The results show that the application of the product on cotton leaves causes an increase in the phenol level compared to the control. These levels vary depending on the concentration and incubation time. However, the 3 % concentration after 72 hours of incubation has the highest total phenol content (69.87 mg/g of MF).

Effect of calliet

Table II shows the total phenol content of leaves treated with calliet as a function concentration and incubation time. The results show that the application of calliet on cotton leaves causes an increase in the level of total phenols compared to the control. These levels vary depending on the concentration and incubation time. However, the 3 % concentration after 48 h of incubation presents the highest total phenol content (38.70 mg/g of MF).

Comparative effect of biocontrol products on the total phenol content of cotton leaves elicited in the open field

For this comparison, only the concentration coupled with the incubation time which gave the highest total phenol content in each treatment following the previous study was retained.

Figure 2 presents the comparative effect of the total phenol content of the leaves of field cotton plants treated with vacciplant and calliet.

Statistical analysis shows that cotton leaves treated with vacciplant had a total phenol content of 70.71 mg/g MF significantly higher than that obtained with leaves treated with calliet (39.09 mg/g MF) followed by the control (36.01 mg/g MF).

Also, in order to determine an influence of the culture medium on the biosynthesis of polyphenols, a comparison of the total phenols of the results of the controlled culture in pot (in vivo) and that not controlled in open fields(in nature) was carried out (Table III).

These results revealed that the values taken two by two at the level of the controls, of the plants treated with vacciplant and those treated with calliet are statistically identical. However, the content of total phenols is higher at the level of the plants treated with vacciplant.

Results of the ratio of total phenolic contents of cotton leaves elicited in the open field compared to the control (figure 3) show that the vacciplant causes an increase 2.06 times more than that of the control while the calliet increases it only 1.14 times compared to the control.

Likewise, an analysis of the production gain in polyphenols (Table IV) revealed that the application of vacciplant on the leaves results in a production gain of 106.10% while that of calliet is only 13.93% compared to the control.

Effect of vacciplant and calliet on the chlorophyll pigment content of cotton leaves in the open field

The results of the Table V show that under the action of biocontrol products, chlorophyll b degrades, going from 142.10 µg/g MF in the leaves of control plants to 130.74 µg/g DM in those of plants treated with calliet (CAL) followed by 118.96 µg/g MF in the leaves of plants treated with vacciplant (VAC). However, the opposite was observed in the variation of chlorophyll a and total chlorophyll. Thus, the application of biocontrol products causes a significant increase in the contents of chlorophyll a and total chlorophyll. As a result, the chlorophyll a content increased from 151.20 µg/g DM

leaves (control) to 175.63 µg/g DM in leaves treated with calliet followed by 189.09 µg/g DM in leaves treated with vacciplant. Similarly, the total chlorophyll content increased from 293.30 µg/g DM in the leaves of untreated plants (control) to 316.24 µg/g DM and 334.18 µg/g DM in the leaves of plants treated with calliet and vacciplant, respectively. Although this increase was significant in plants treated with both biocontrol products, it was even more interesting and significant in those treated with vacciplant.

The gain in chlorophyll production by cotton plants is revealed by Figure 4. Plants treated with vacciplant showed gains in total chlorophyll production (13.94 %), greater than those treated with calliet (7.82 %).

These results are consistent with those of the Table VI which have the ratio chl a /chl b. Indeed, Note that all these ratios are greater than 1 (chl a / chl b- 1). Thus, this ratio increases progressively from 1.06 in the control plants (untreated) to 1.34 in the plants treated with calliet followed by 1.58 in those treated with vacciplant. However, statistical analysis reveals that this ratio is the most important in the plants treated with vacciplant.

Analysis of the results obtained showed that the total phenol content varies depending on the biocontrol products, concentrations and incubation time. All the biocontrol products used stimulate the production of polyphenols involved in plant defense mechanisms (Yamaji and Ichihara, 2012; Konan, 2015).

Indeed, many studies have shown that the application of biocontrol products to a plant activates defense reactions and also leads to an increase in its resistance to pathogens (Ahuja *et al.,* 2012; N'Goran *et al.,* 2015). Thus, vacciplant and calliet therefore stimulate the biosynthetic pathways of these polyphenols. They are then stimulators of natural plant defense (SDN) which, with their indirect mode of action, seem difficult to induce resistance as with pesticides (Giuliano *et al.,* 2000).

This argument agrees with that of Martin and Andriantsitohaina (2002) according to which the structural diversity of polyphenolic compounds is due to a double biosynthetic origin and would increase by the simultaneous participation of the two pathways (shikimate and acetate). However, vacciplant seems to be the most active. This is further reflected in a very significant rate of increase and gain in plants treated with biocontrol product. Thus, vacciplant stimulates the biosynthesis of polyphenols better than calliet. To this end, the exogenous application of vacciplant on plants therefore induces defense responses such as the accumulation of phytoalexins which are phenolic in nature (Larronde *et al.,* 2003; Lattanzio *et al.,* 2006; Bellow *et al.,* (2012). Moreover, several studies have reported that their use would allow the control of pathogens for the defense of cotton (Belhadj *et al.,* 2006; Lambert, 2011; Konan *et al.,* 2014). Thus, the fixation of a biocontrol product such as the latter (vacciplant) on a receptor of the plant cell would trigger a cascade of actions which results in the synthesis of defense compounds such as polyphenols (Konan *et al.,* 2014) added to this, it leads to the production of reactive oxygen species (ROS), the flow of intracellular calcium and the activation of hormonal pathways (salicylic acid, jasmonate, ethylene).

Laminarin (vacciplant) is thus recognized as an attack signal through its specific receptors on the plant even in the absence of pathogens. This therefore triggers an immune-type reaction. This is a systemic acquired resistance (SAR) effective against a broad spectrum of aggressors (Kaufmann *et al.,* 2001; Belhadj *et al.,* 2006; Konan, 2015).

Therefore, the use of biocontrol products could help reduce the quantity of pesticides in cotton crops. Also, due to the composition of its active ingredient which is also a polysaccharide, vacciplant applied to plants would contribute to the strengthening of cell walls (deposition of callose, suberin and lignin), to ionic modifications leading to calcium entry, to the activation of defense genes and to the production of pathogenesis-related proteins (PR proteins) such as chitinases and glucanases, which are antifungal enzymes. The plant enters a state of "preparation" called "alert state," or "wakeful state," where it becomes faster and more efficient at defending itself if a real attack occurs.

The observed difference in timescales shows that calliet acts more on the synthesis of preformed phenolic compounds in the constitutive defense than on the induced defense, whereas vacciplant causes the synthesis of phenolic compounds that intervene in the induced defense. Indeed, at the level of calliet, fosetyl-alumina is very quickly degraded in the soil and quickly absorbed by the leaves or roots of the plant with translocation towards the bottom of the plant and vice versa.

Table.1 Total phenol content of leaves treated with vacciplant as a function of concentration and incubation time

	Total phenols content (mg/g de MF)		
Concentration (%)	Incubation time		
	24 h	48 h	72 h
0	35,08 ± 0,11^{a}	34,28 ± 0,11^{d}	34,22 ± 0,11^{e}
1	34,28 ± 0,11^{a}	45,52 ± 0,23^{b}	54,02 ± 0,01^{c}
2	28,88 ± 0,05^{c}	37,94 ± 0,01^{e}	56,33 ± 0,13^{b}
3	31,40 ± 0,12^{b}	57,58 ± 0,12^{a}	69,87 ± 0,12^{a}
4	26,53 ± 0,16^{d}	30,66 ± 0,02^{f}	56,33 ± 0,02^{b}
5	24,28 ± 0,04^{e}	34,47 ± 0,10^{d}	43,90 ± 0,07^{d}
P (%)	0,001	0,001	0,002

± S: standard error; in the same column, values followed by the same letter are not significantly different (5% Newman-Keuls test); MF: fresh matter; values represent the average of three repetitions; P (%): percentage probability.

Table.2 Total phenol content of leaves treated with calliet as a function of concentration and incubation time

	Total phenol content (mg/g de MF)		
Concentration (%)	Incubation time		
	24 h	48 h	72 h
0	35,08 ± 0,11 a	34,28 ± 0,01 b	34,22 ± 0,11 a
1	29,99± 0,10 d	33,09± 0,12 b	28,83± 0,06 d
2	34,24± 0,13 b	30,46± 0,13 d	31,08± 0,03 c
3	30,93± 0,20 d	**38,70± 0,11 a**	30,70± 0,15 c
4	19,38± 0,15 f	32,91± 0,04 c	33,42± 0,15 b
5	26,10± 0,05 e	34,91± 0,05 b	30,78± 0,12 c
P (%)	0,002	0,001	0,001

± S: standard error; in the same column, values followed by the same letter are not significantly different (5 % Newman-Keuls test); MF: fresh matter; P (%): probability in percentage.

Table.3 Influence of culture conditions on total phenol content post-treatment

Total phenol contents (mg/g MF)		
	Growing conditions	
Treatments	*In vivo*	*In natura*
Vacciplant	69,87^{a}	70,71^{a}
Calliet	38,7^{b}	39,09^{b}
Witness	35,08^{c}	34,31^{c}
P (%)	0,001	0,001

In the same column and on the same line, the values followed by the same letter are not significantly different (Newman-Keuls test at 5%); MF: fresh matter; P (%): probability in percentage.

Table.4 Total phenol gains in treated cotton leaves compared to the control

Treatments	Polyphenol gains (%)
Calliete	13.93b
Vacciplant	106.10a
P	0.0001

In the same column, values followed by the same letter are not significantly different (NewmanKeuls test at 5%); MF: fresh matter; %: percentage; P (%): probability in percentage.

Table.5 Chlorophyll content in cotton leaves as a function of biocontrol products

	Chlorophyll pigment content (µg/g MF)		
Treatments	Chl a	Chl b	Chl t
Witness	151.20 ± 0.02c	142.10 ± 0.06a	293.30 ± 0.05c
Calliet	175.63 ± 0.01b	130.74 ± 0.22b	316.24 ± 0.22b
Vacciplant	189.09 ± 0.11a	118.96 ± 0.32c	334.18 ± 0.11a
P (%)	0.001	0.001	0.001

Values followed by the same letter in the same column are statistically identical at 5% (Newman-Keuls test); ± S: standard error; MF: fresh matter; Chl a: chlorophyll a; Chl b: chlorophyll b; Chl t: total chlorophyll; P (%): probability in percentage.

Table.6 Ratio of chlorophyll a content to chlorophyll b content in leaves of cotton plants treated with biocontrol products

Treatments	Chl a / Chl b ratio
Witness	1.06 ± 0.02^{c}
Calliete	1.26 ± 0.07^{b}
Vacciplant	1.45 ± 0.00^{a}
P (%)	0.0001

Values followed by the same letter are statistically identical (5% Newman-Keuls test); Chl a: chlorophyll a; Chl b: chlorophyll b; P (%): probability in percentage.

Figure.1 Two-week-old cotton seedlings in cultivation *in vivo*

Figure.2 Total phenol content of field-grown cotton leaves as a function of biocontrol products

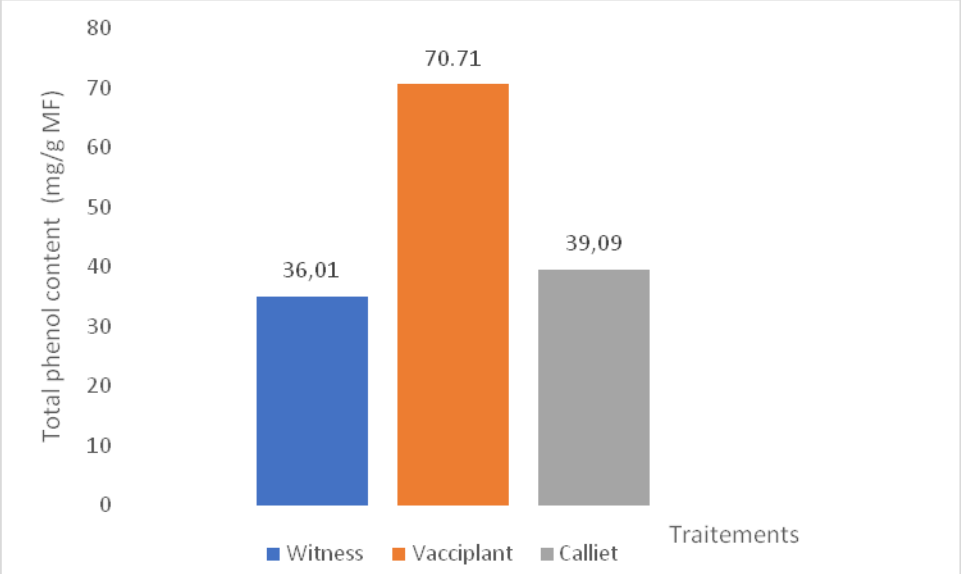


Figure.3 Increase in total phenolic contents of elicited cotton leaves compared to the control

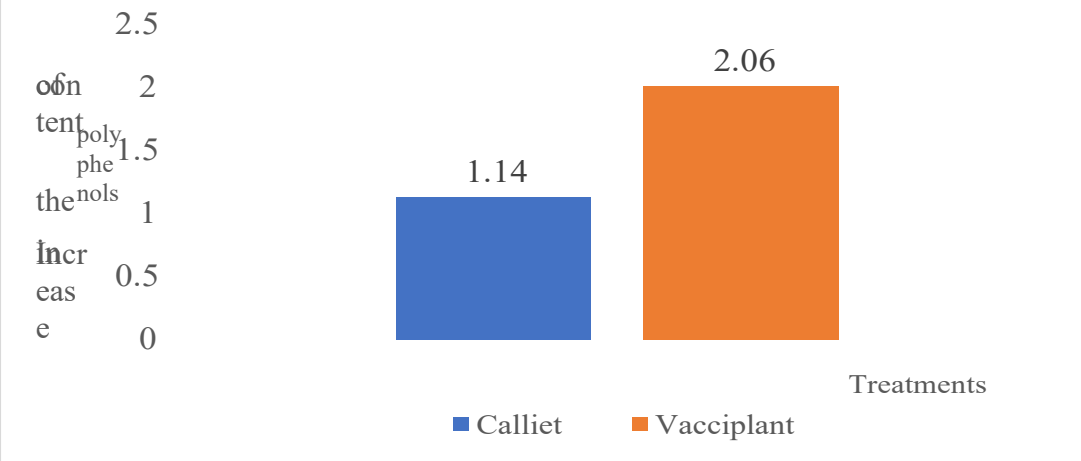
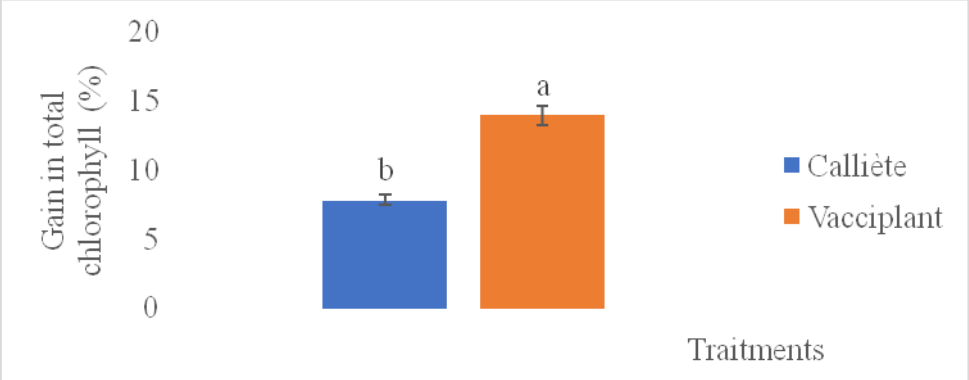


Figure.4 Gain in total chlorophyll content of cotton plant leaves elicited compared to the control



Values followed by the same letter are statistically identical (5% Newman-Keuls test); %: percentage.

This would intervene quickly to increase the level of synthesis of preexisting polyphenols in the plant, whereas vacciplant would intervene both on the increase in phenolic contents and on the synthesis ofnewof these compounds. These results were also reported by Belhadj *et al.,* (2006) in the vine and N'cho (2017) in the banana tree.

Thus, culture conditions did not influence polyphenol biosynthesis. This could be due to comparable environmental conditions. Indeed, the abiotic conditions (light, temperature, humidity, substrate, nutrition) could be sufficiently close between the two cultivation systems. Also, this relatively stable level would indicate that the plants are not subjected to major stresses differentiated between the two environments. Regarding the physiological state of the plants, the chlorophyll pigments give an assessment of the photosynthetic activity, that is to say their vigor. It is therefore the chlorophylls which give the plants the power of photosynthesis, that is to say the biosynthesis of carbohydrates from the light energy captured by these chlorophylls. Thus, the results show that under the action of biocontrol products, the contents of chlorophyll a and total chlorophylls increase in cotton leaves compared to the control. Indeed, the products of photosynthesis are the starting point of the biosynthesis of secondary metabolites (Hoffmann, 2003). In addition, the significant increase in chlorophyll a after treatment with biocontrol products would indicate a good production of photosynthetates, because chlorophyll a is the main photosynthetic pigment. Thus, the role of biocontrol products would be to protect chlorophyll a in order to maintain photosynthetic activity and hence the biosynthesis of secondary metabolites such as phenolic phytoalexins important for the induction of defense mechanisms. In addition, they would stimulate the biosynthesis of chlorophylls, therefore photosynthesis. Moreover, the work of Couderchet *et al.,* (2003) reported a close link between Pathogenesis-Related (PR) proteins, induced phytoalexins and protoporphyrogen oxidase, an enzyme involved in chlorophyll biosynthesis. Vacciplant and calliet appear to have a beneficial effect on the chlorophyll a and total chlorophyll content. However, that of vacciplant is immediately more important with a gain and Chlorophyll a/Chlorophyll b ratio higher in the plants treated with this biocontrol product. These results indicate good photosynthetic activity and therefore a good physiological state of the plants after treatment with the latter. In the same sense, the increase in the total chlorophyll content would be due to a progressive increase in the magnesium ion (Jacquot *et al.,* 2011). Indeed, chlorophyll is a chlorine chelating a magnesium cation (Mg_{2+}) at the center of the macrocycle and esterifying a terpenoid alcohol at C_{20}, phytol, which is hydrophobic and serves as an anchor for proteins in thylakoid membranes (Raven *et al.,* 2007). The increase in chlorophyll content is caused according to Lourtie (2008) by an activation of magnesium chelatase and chlorophyllase involved in the biosynthesis of chlorophylls. These two enzymes would be located on chlorophyll a. Vacciplant seems to be the biocontrol which strongly stimulates the activity of magnesium chelatase and chlorophyllase, compared to calliet.

This biocontrol product would better protect the active site of the enzymes and would attach itself to the active site of magnesium chelatase, thus causing its inactivation (Langmeier *et al.,* 1993). This results in an increase in the biosynthesis of chlorophylls by this product. Also,

Folly and Engel, (1999) explain these results by the fact that there would be a bioconversion of chlorophyll b into chlorophyll a. This suggests that photosynthetic activity is mainly linked to chlorophyll a (Folly, 2000; Zarco-Tejada *et al.,* 2001).

The weight ratio of Chl a and Chl b (Chl a/b ratio) is an indicator of the functional pigment equipment and light adaptation (acclimatization) of the photosynthetic apparatus.

Chl b is found exclusively in the pigment antenna system, whereas Chl a is present both in the pigment antenna and in the reaction centers of photosystems I and II.

Furthermore, the increase in the chl a/chl b ratio (> 1) shows good photosynthetic activity and therefore a good physiological state of the plant; which translates according to Morot-Gaudry and Farineau, (2011), into good availability of carbohydrate assimilation and activation of the secondary metabolism responsible for the formation of defense molecules.

The results obtained indicate good photosynthetic activity, therefore resulting in a good physiological state and good vigor of the plants after treatment with biocontrol products, particularly with vacciplant.

Ultimately, biocontrol products (preferably vacciplant) would have increased the chlorophyll levels of the leaves either by protecting the chlorophylls against degradation or aging, or by inserting themselves into a cascade of signals which would promote their biosynthesis, in particular chlorophyll a.

At the end of this study, we can conclude that the elicitation of cotton plants with vacciplant and calliet led to an increase in the total phenol and chlorophyll pigment content.

However, vacciplant is the biocontrol product that best induces polyphenol biosynthesis and chlorophyll pigment content. It would therefore be the most suitable for the natural defense of cotton plants. (*Gossypium hirsutum* L., Malvaceae, cv. Y764G3). It should also be noted that this phytosanitary control technique called stimulation of natural defenses (SDN) appears to be a new avenue opened in the field of plant protection by inducing defense reactions. This would allow it to mobilize its own defense mechanisms naturally.

Author Contributions

Idrissa Coulibaly: Investigation, formal analysis, writing—original draft. Brahima Ouattara: Validation, methodology, writing—reviewing. Désiré Kouakou N'goran:—Formal analysis, writing—review and editing. Lucien Gbongué Dan Gogbeu: Investigation, writing—reviewing. Hilaire Tanoh Kouakou: Resources, investigation writing—reviewing. Tchoa Koné: Validation, formal analysis, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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