Original Research Article

The Pre-treatment of Cotton with Polysaccharide Biocontrols Inducing Tolerance to *Fusarium oxysporum* f. sp. vasinfectum Causal Agent of Fusarium Wilt in Cotton (*Gossypium hirsutum* L.)

ABSTRACT

Fusarium wilt caused by *Fusarium oxysporum* f. sp. vasinfectum (FOV) is a major damaging disease of cotton. Chemical control is currently ineffective and has led to resistance of the pathogen. In addition, pesticides are indexed for their negative impact on the environment and on human health. The objective of this study was to evaluate the impact of sugarcane stem residue filtrate (FCS) and cotton stem residue filtrate (FTC) residue filtrate on FOV causal agent of the disease. These two polysaccharide biocontrols allowed a good accumulation of gallic acid, caffeoyl D-glucose, catechin, gossypetin and 3-p-coumaroylquinic acid and the de novo synthesis of epicatechin, protocatechic acid, ferulic acid, trans-resveratrol, trans-piceide, pterosilbene, 5-caffeylquinic acid, rutin, astringin and isoquercetin. However, 5-caffeylquinic acid and rutin are specific to FCS while astringin and isoquercetin are specific only to FTC. This difference in polyphenol synthesis caused 1% mortality in FTC and 8% in FCS. This indicates that astringin and isoquercetin are phenolic markers of resistance to FOV followed by 5-caffeylquinic acid and rutin. FTC can be used as a preventive control of Fusarium wilt in cotton.

Keywords: biocontrol, cotton, *Fusarium oxysporum* f. sp. vasinfectum, polyphenol, tolerance

1. INTRODUCTION

Cotton in Côte d'Ivoire and around the world is of vital economic importance. Côte d'Ivoire is the second largest cotton producer in Africa behind Benin, with nearly 558,000 tons of cotton in production during the 2020-2021 season [1] . Cotton constitutes a very significant source of income as it supports more than 3.5 million people in Côte d'Ivoire [2,3]. With climate change, seed cotton production in West Africa, especially in Côte d'Ivoire, is greatly affected [4]. In fact, cotton production is increasingly threatened by several pathogens, including the most damaging, Fusarium oxysporum f. sp. vasinfectum (FOV), can cause almost total crop loss [5,6]. Furthermore, the disease caused by this pathogen (fusarium wilt) is difficult to control chemically [7]. This fungus can actively grow in soil and persist for many years even in the absence of the host plant. In this context, it appears important to find effective alternatives to chemical control for sustainable agriculture, respectful of the environment and human health. Thus, one of them is to give plants the ability to defend themselves by strengthening their defense instead of directly fighting the pathogen [8,9]. This category includes plant biocontrols. Plants can most often naturally resist pathogens with natural pathogen- or plant-derived molecules that activate de novo synthesis of defense compounds [10,11]. They are derived from cell walls and have a polysaccharide nature as reported by Korsangruang *et al.* [12]. These biocontrols are effective at very low doses and inactive on pathogens as acting on the plant [13], are completely biodegradable and have a generally interesting eco-toxicological profile. So they're very environmentally friendly molecules. Biocontrols are substances capable of inducing (or preparing for induction) resistance to bioaggressors in treated plants (Blanchard 2018). Also according to Mejri [14], molecules with the ability to induce a cascade of defense reactions in the plant are called biocontrols. They are also capable after application of stimulating the mechanisms of natural defense of plants in the absence of pathogens. The application of biocontrol to a plant activates defensive

reactions leading to increased resistance to pathogens [8]. Indeed, its binding to a receptor in the plant cell triggers a cascade of reactions that leads to the synthesis of defense compounds such as polyphenols. In addition, the oxidation of polyphenols leads to the production of compounds with bactericidal and fungicidal actions [15, 16]. Consequently, the aim of the present study was to investigate the efficacy of natural defense stimulators of polyssacharide origin in the protection of cotton against Fusarium wilt caused by FOV.

2. MATERIALS AND METHODS

2.1. Plant material

Cotton seeds (*Gossypium hirsutum* L. cv. 331B) originated from Côte d'Ivoire and were provided by the Centre National de Recherche Agronomique (CNRA). The cultivar used is highly susceptible to *Fusarium oxysporum* f. sp. vasinfectum (FOV), causative agent of Fusarium wilt [17].

2.2. Fungal Material

The fungal material consists of isolate of FOV. These isolates were supplied by the phytopathology laboratory of the 'Ecole Supérieure d'Agronomie' of the 'Institut Polytechnique Houphouët-Boigny of Yamoussoukro. They were packaged in Petri dishes on PDA medium.

2.3. Sowing of Plants

The cotton seeds were sown on 6 m long and 60 cm wide ridges. Three blocks were formed and separated by 3m from each other. Five seeds were sown per hole at 5 cm depth. At emergence, the plants were dismantled and only the most vigorous plant was left by potting. Each ridge contains three rows of 10 cotton plants, for a total of 30 plants per ridge. Watering was performed after two days interval. Two-month-old plants were used in this study.

2.4. Preparation of biocontrol solutions

The stem residues of sugarcane (after extraction of the juice) and cotton were dried in the oven at 60°C for 48 h and then ground to obtain a powder. Thereafter, 500 g of each powder were mixed in 1 L of water and kneaded by hand for 15 min. After settling for 24 hours, the supernatant was recovered and made the 100% stem filtrate stock solution. Moreover, of Triton X-100 at 0.1% was added to the solutions and then homogenized and the whole constituted the biocontrol solution.

2.5. Plants Inoculation

Agar fragments of 14-day-old FOV fragments were collected and crushed under sterile conditions in the presence of 5 mL of sterile distilled water. The crushed material was filtered on sterile gauze (retains mycelium fragments but allows the spores to pass). Approximately, 1 mL of filtrate was deposited per Petri dish containing PDA medium and then incubated for 7-days in a culture room at 25±2°C under a 12h photoperiod. In each Petri dish, the FOV colonies obtained were submerged with 5 mL of sterile distilled water containing a drop of Tween 20. Afterwards, the culture surface was gently scraped using a curved sterile Pasteur pipette to obtain a spore suspension. This method of obtaining the inoculum avoids possible morphological variations. The spore suspension was filtered through a sieve of mesh size 80 µm in order to remove the large fragments. FOV spores concentration was determined using a Malassez cell and the inoculum was calibrated to 10^4 spores/mL (FOV filtrate).

2.6. Inoculations of Plants

The 14-day cotton plants, at the 10-leaf stage, were sprayed with FTC (20%) or FCS (25%), because these concentrations were the best (data not shown). Each treated plant received approximately 50 mL of biocontrol and one batch of plants untreated with biocontrols was conducted. The fungal inoculation (5 µL spores suspension) was done from neck wounds with

a sterile needle 3 days (72 h) after treatment of the plants with or without biocontrols. According to Bouhot and Rouxel [18], inoculation of nursery plants is done by injecting the suspension of spores of *Fusarium oxysporum* directly into the part surrounding the root. The following treatments were performed:

- (i) Control plant (CP);
- (ii) Control plant infected with FOV (CPI);
- (iii) FCS-treated plant infected with FOV (FCS-PI);
- (iv) FTC-treated plant infected with FOV (FTC-PI);

For each treatment, thirty cotton plants were used and then exposed under cover covered with transparent plastic film in a completely randomized design. The experiment was repeated three times. The plants were watered according to the humidity of the substrate. The incidence of disease caused by FOV was assessed every other day until day 30 after inoculation. Leaves from each stage were collected for analysis.

2.7. Extraction and Purification of Polyphenols

The extraction and purification of the phenolic compounds were carried out according to the method described by Kouakou *et al.* (2009) [19]. Approximately 100 mg of leaf lyophilisate from each plant was placed in a hemolysis tube and 20 mL pure methanol was added. The mixture was placed at 4°C for 15 h. After, a 5min sonication in ultrasound (FAME, Emmi-12HC), the mixture was centrifuged at 5,000 rpm for 10 min. Thereafter, 4 mL of methanolic supernatant where the crude extract of phenolic compounds was evaporated using a mini-concentrator under vacuum, Speed Vac (Savant, USA). The dry residue obtained was dissolved in 1 mL of 30% methanol (v/v) and then placed on a mini-column of C18 grafted silica (Sep pack®; Macherey-Nagel, Düren, German) in the Supelco Visiprep™ charging system. The columns were previously equilibrated by successive washing with 100% methanol (2 mL), 50% methanol (2 mL) and purified water (6 mL). After deposition of the

methanol extract, a washing with 2 mL of distilled water was performed and the polyphenols were eluted with 4 mL of 90% methanol (v/v). The resulting eluate was evaporated using Speed Vac, then the dry residue was taken up in 1 mL of 50% methanol (v/v) and finally filtered through a Millipore membrane at 0.45µm. The filtrate obtained was diluted with ultra pure distilled water (50/50, v/v) and represents the purified extract of phenolic compounds to be analyzed.

2.8. Chromatographic Conditions

Ultra-high performance liquid chromatography (U-HPLC) analysis was performed according to Verdu (2013)'s method. U-HPLC analysis was carried out on two coupled Agilent chains (Agilent LC-1100 ; Agilent LC-1200). The chromatographic separation was performed analytical column Agilent Zorbax Eclipse XDB-C18 (150×4.6 mm, 5.0 µm) using solvent A, Water : Trifluoroacetic acid 1% (97.5:2.5) and solvent B, Acetonitrile : solvent A (80:20) at a flow rate 1.3 mL/min. The injection volume 10 µL and the run time was 20 min. using a elution gradient of 5% B (0-5 min), 10% B (5-10 min), 20% B (10-16 min), 80% B (16-17 min), 10% B (17-18 min) and 5% B (18-20 min). UV detection was carried out at 254 nm. Agilent system was coupled to a nuclear magnetic resonance spectrometer (Bruker Avance III) which a frequency of 600 MHz for a proton.

A reference library containing the retention times and NMR spectra of phenolic compounds likely to be present in cotton was previously carried out [8, 20, 21]. The retention time and/or the NMR spectra of the separated compounds were compared with those of the reference library for their identification. The content of each polyphenol, expressed as mg/g dry matter, was calculated using calibration curves made with pure polyphenols isolated from samples and/or those obtained commercially.

2.9. Evaluation of the Mortality Rate of Cotton

The protection of cotton against Fusarium wilt following treatment with biocontrols was assessed by determining the mortality rate of plants treated and FOV-inoculated plants in comparison with control (untreated and uninoculated and untreated and inoculated). The plants were grown on ridges. After the experiments, a black plastic film was used to cover the plot for 2-months to destroy pathogens in the soil. Mortality of inoculated plants started to be quantified as untreated and inoculated control plants were dried and died.

2.10. Statistical analyzes

Data were analyzed using Statistica 7.1 software. Analysis of variance was performed on all treatments applied. When this analysis shows a difference between the means, the Duncan test was carried out in order to determine the significant differences between the treatments at the 5% threshold. For percentages, the Kruskal-Wallis test was used to determine significant differences ($P < 0.05$) between treatments.

3. RESULTS AND DISCUSSION

3.1. U-HPLC Separation and identification of Polyphenols Extracted from Cotton Leaves Treaed with Polysaccharide Biocontrols

The samples analysis by U-HPLC allowed the separation and identification of the polyphenols present in the leaves treated by the biocontrols. Thus, by comparing the retention time of each chromatogram with those of the reference library of compounds, the various polyphenols revealed by U-HPLC were identified. Therefore, the chromatographic profile of the biocontrol-treated and infected cotton leaves shows that the sugarcane stem residue filtrate-treated and infected (FCS-PI) and cotton stem residue filtrate-treated and infected (FTC-PI) plants have peaks with greater amplitudes than untreated or control plants (CP) followed by untreated and infected or control infected plants (CPI), **Figure 1**. These results suggest that the application of biocontrols increases the content of phenolic compounds

synthesis. Indeed, a plant becomes diseased due to lack of synthesis level of pre-existing defense compounds or constituent compounds [9, 22]. This is suggested by Grayer *et al*. [23] a slow defense response therefore favors the disease establishment. Thus, increasing the accumulation of polyphenols by eliciting cotton plants through the filtrate of sugar cane and cotton stem residues allows plants to quantitatively increase their defense response, i.e., to rapidly mobilize defense molecules and induce acquired systemic resistance [24]. Moreover, the accumulation of phenolic compounds in plants has been reported as a potential activator of plant defense or phenolic phytoalexins as it has been shown to induce resistance against fungal attacks [25]. Indeed, during the cotton-Fusarium interaction, an increase in the level of phenolic compound synthesis was observed in cotton [26, 27]. The disappearance of (4) gentisic acid, (5) monoglucosyl-3 cyanidine and (6) ferulic acid immediately after fungal attack in plants not treated by biocontrols shows that they do not participate in the defense in cotton.

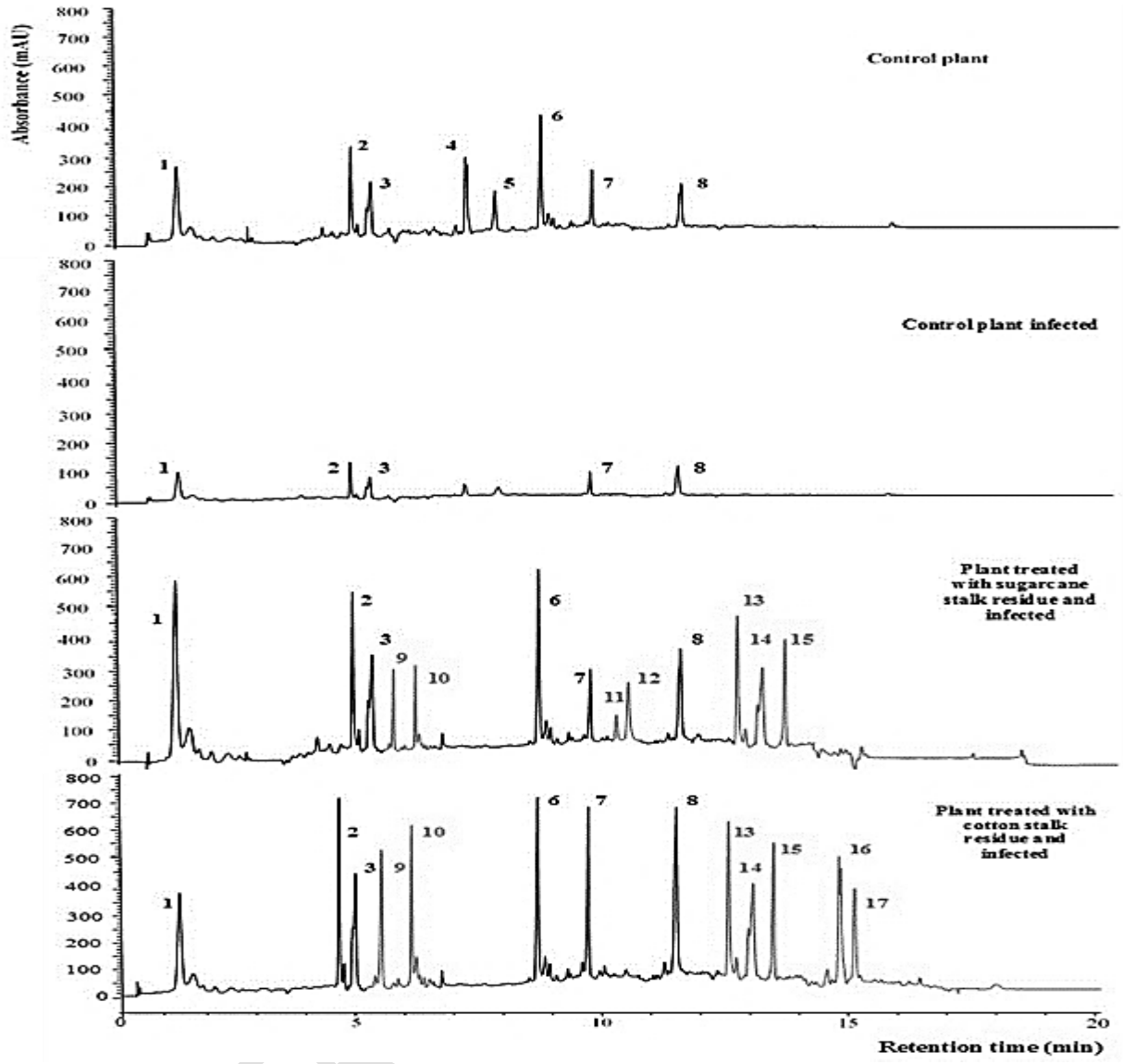

Figure 1. U-HPLC Chromatographic Profiles of Phenolic Compounds Extracted from Cotton Leaves Treated with Sugarcane and Cotton Residue Stem Filtrates and Infected by *Fusarium oxysporum* f. sp. vasinfectum.

Peak detection was performed at 254 nm; UHPLC (Ultra High Performance Liquid Chromatography); (1) Gallic acid (1.323 min); (2) Caffeoyl D-glucose (4.492 min) ; (3) Catechin (4.855 min); (4) Gentisic acid (7.246 min); (5) Monoglucosyl-3 cyanidine (7.898 min); (6) Ferulic acid (8.695 min); (7) Gossypetin (9.782 min) ; (8) 3-p-coumaroylquinic acid (11,666 min); (9) Epicatechin (5,434 min); (10) Protocatechic acid (6,086 min); (11) caffeyl 5-quinic acid (10,289 min); (12) Rutin (10,507 min) ; (13) *trans*-Resveratrol (12.753 min); (14) *trans*-Piceid (13.260 min); (15) Pterosilbene (13.695 min); (16) Astringin (15.070 min); (17) Isoquercetin (15.289 min).

These compounds appear to be related to cultivars as studies on other cotton cultivars does not indicate its presence in the identified induced or constituent phenolic compounds [28, 29]. In addition, the compounds (1) gallic acid, (2) caffeoyl D-glucose, (3) catechin, (7) gossypetin and (8) 3-p-coumaroylquinic acid whose contents increase after treatment suggests that it is not the quality of these polyphenols that activates the defense reactions but rather their quantity as already reported by N'cho *et al*. [28]. These compounds would be phytoanticipins capable to protect cotton pgainst pathogen aggression.

Results show also that compounds 1, 2, 3, 7 and 8 respectively gallic acid, caffeoyl D-glucose, catechin, gossypetine and 3-p-coumaroylquinic, were synthesized concomitantly by FTC-PI, FCS-PI and CPI. The compounds (4) gentisic acid, (5) monoglucosyl-3 cyanidine and (6) ferulic acid disappeared in the infected control plants (CPI), while the compounds (4) gentisic acid, (5) monoglucosyl-3 cyanidine disappeared in the pretreatment and infected plants. In addition, in biocontrol elicited and infected plant (FTC-PI, FCS-PI), *de novo* induction of seven phenolic compounds was observed. However, among these induced compounds, (6) Ferulic acid, (9) epicatechin, (10) protocatechic acid, (13) *trans*-resveratrol, (14) *trans*-piceide and (15) pterosilbene are present in plants treated with both types of biocontrols. In contrast, compounds (11) 5-caffeylquinic acid, and (12) rutin are induced only in plants treated with sugarcane stem filtrates (FCS-PI), while compounds (16) astringin (15.070 min) and (17) isoquercetin are present only in plants treated with stem filtrates cotton and infected (FTC-PI). Thus, both biocontrols are able, after application, to stimulate the natural defense mechanisms of the plants in the absence of pathogen. They activate defense reactions, leading to increased resistance to pathogens such as FOV [8, 30]. Their binding to a receptor in the plant cell triggers a cascade of reactions that results in de novo induction of compounds such as polyphenols for their defense [8, 31, 32]. Furthermore, the difference between their ability to induce tolerance in cotton against fusarium wilt is linked to the nature

of the induced polyphenols. Ferulic acid, epicatechin, protocatechic acid, trans-resveratrol, trans-piceide and pterosilbene, which are common to both biocontrols, would allow rapid reinforcement of defense reactions. Indeed, Kouakou [33] reported the presence of trans-resveratrol, a stilbene already identified as vine phytoalexin (an antifungal molecule produced during a pathogen attack), in cotton cells. Phenolic compounds, derived from the phenylpropanoid pathway, are known for their antifungal properties against pathogenic fungi, and their involvement in the resistance of certain cultivars to these same parasites has already been reported [30, 34]. Their role in plant resistance to fungi has been demonstrated [25, 31] and their possible involvement in disease tolerance is supported by recent studies |29, 32, 35]. In particular, the influence of some compounds on the growth of fungi involved in wood diseases has been demonstrated [8, 29, 36]. In addition, cotton stem residue filtrate stimulates the synthesis of isoquercetin and astringin which appear to have greater antifungal potency than 5-caffeylquinic and rutin induced by the application of sugarcane residue filtrate [37]. Thus, the effectiveness of these compounds in protecting cotton against fusarium wilt is due to the activation of different pathways for the biosynthesis of phenolic compounds

3.2. Polyphenol Contents in Elicited Cotton Leaves and Mortality Rate

Table 1 shows that at the level of untreated and infected plants (CPI), the absence of protocatechic acid, ferulic acid, epicatechin, rutin, isoquercetin, trans-resveratrol, *trans*-piceide, pterosilbene and astringin. However, the phenolic compounds identified in CPI are low and statistically identical, i.e. 3-p-coumaroylquinic acid (4.13 mg/g DM), caffeoyl D-glucose (2.91 mg/g DM), gossypetine (2.65 mg/g DM), gallic acid (2.51 mg/g DM) and catechin (1.29 mg/g DM). Regarding the plants treated with the sugarcane stem residue filtrate (FCS-PI), there is still a lack of isoquercetin and astringin.

Table 1. Content of Polyphenols Identified in Cotton Leaves Treated with Sugarcane and Cotton Residue Stem Filtrates and Infected with *Fusarium oxysporum* f. sp. vasinfectum

Phenolic group	Phenolic compounds	Content of phenolic compounds (mg/g MS)		
		CPI	FCS-PI	FTC-PI
Hydroxy benzoic acids	Gallic acid	2.51±0.11^{b}	24.66±0.43^{g}	11.21± 0.54^{d}
	Protocatechic acid	-	23.62±0.54^{g}	49.08±0.76^{k}
Hydroxy cinnamic acids	Caffeoyl D-glucose	2.91 ± 0.14^{b}	6.21± 0.76^{cd}	8.19 ± 0.79^{d}
	Ferulic acid	-	9.16±0.83^{d}	10.81 ± 0.34^{d}
chlorogenic acids	3-*p*-coumaroylquinic acid	4.13 ± 0.47^{c}	18.03 ± 0.87^{d}	35.96 ± 1.01^{c}
	Caffeyl-5 quinic acid	-	10.60 ± 0.42^{d}	-
Flavonoids	Catechin	1.29 ± 0,32^{a}	5.94 ± 0.47^{c}	8.61 ± 0.97^{d}
	Gossypetin	2.65 ± 0.23^{b}	14.72 ± 0.95^{e}	30.18 ± 1.05^{h}
	Epicatechin	-	15.75 ± 0.67^{e}	29.94 ± 0.77^{h}
	Rutin	-	14.81 ± 1.04^{e}	-
	Isoquercetin	-	-	13.12 ± 1.41^{e}
Stilbenes	*trans*-Resveratrol	-	21.94 ± 2.01^{f}	30.17 ± 1.70^{h}
	trans-Piceid	-	13.41 ± 0.90^{e}	18.12 ± 0.43^{d}
	Pterosilbene	-	29.62 ± 1.85^{h}	42.42 ± 2.01^{j}
	Astringin	-	-	13.71 ± 1.11^{e}

(-) absent; U-HPLC, ultra high performance liquid chromatography; DM, dry matter ; CPI, untreated and infected plant; FCS-PI, treated plant with sugarcane residue filtrate and infected; FTC-PI, treated plant with cotton stem residue filtrate and infected.

However, the pterosilbene content (29.62 mg/g DM) is higher, followed by gallic acid (24.66 mg/g DM), protocatechic (23.52 mg/g DM) and *trans*-resveratrol (21.94 mg/g DM). In contrast, *3-p*-coumaroylquinic acid (18.03 mg/g DM) followed by epicatechin (15.75 mg/g DM), gossypetine (14.72 mg/g DM), rutin (14.81 mg/g DM) and *trans*-piceide (13.41 mg/g DM) had average contents. However, ferulic acid contents (9.16 mg/g DM), caffeoyl-5-quinic acid (9.60 mg/g DM), caffeoyl D-glucose (6.21 mg/g DM) and catechin (5.94 mg/g DM) were the lowest. For cotton stem residue filtrate-treated and infected plants (FTC-PI), analysis revealed the absence of 5-caffeyl quinic acid and rutin. Protocatechic acid has the highest content (49.08 mg/g DM) followed by pterostilbene (42.42 mg/g DM), *3-p*-coumaroylquinic acid (35.96 mg/g DM), *trans*-resveratrol (30.18 mg/g DM), epicatechin (29.94 mg/g DM) and gossypetine (30.17 mg/g DM). The content of *trans*-piceide (18.12 mg/g DM) followed by isoquercetin (13.71 mg/g DM), astringin (13.12 mg/g DM), gallic acid (11.21 mg/g DM) and ferulic acid (10.81 mg/g DM) are intermediate. In contrast, caffeoyl D-glucose and catechin were the lowest contents, 8.19 mg/g DM and 8.61 mg/g DM, respectively. In addition, the contents of protocatechic acid (49.08 mg/g DM) and pterostilbene (42.42 mg/g DM) are higher in FTC-PI. In general, polyphenol contents are greater in FTC-PI than in FCS-PI. Concerning CPI, polyphenol content were lowest.

Konan [8] and N'Cho [38] showed that after biocontrol and inoculation of a pathogen, plants produce a large amount of polyphenols. Quantitative analysis of polyphenols indicates that flavonoids and stilbenes together constitute the major part of the phenolic pool with antifungal activities and are therefore thought to be heavily involved in plant defense [30, 39, 40]. In fact, the results of this study show that most of the induced phenolic compounds are flavonoids and stilbenes. According to Faurie *et al*. [34], they have an inhibitory action on the mycelial growth of several pathogens. Isoquercetin (flavonoid) and astringin (stilbene) inhibited growth of FOV mycelium by 20% and 11%, respectively, while rutin (flavonoid)

inhibited growth by only 2% and caffeyl 5-quinic acid (chlorogenic acid) stimulated mycelial growth by 1% (data not shown). This can be seen in the mortality rate (**Figure 2**), which is almost zero for FTC-PI (1%) and low for FCS-PI (8%) and CP (10%).

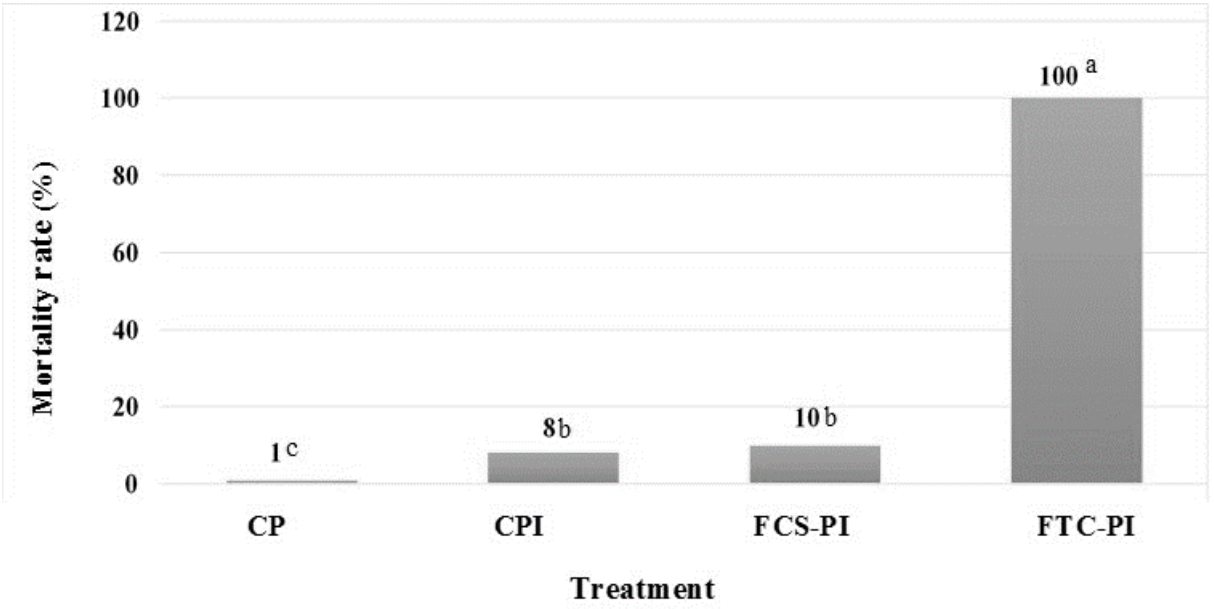

Figure 2. Mortality Rate of Cotton Plants Elicited or not by Biocontrols and Infected by *Fusarium oxysporum* f. sp. vasinfectum

CP: Control plant; CPI, control infected plant; FTC-PI, cotton stem residue filtrate-treated, infected plant; FCS-PI, sugarcane residue filtrate-treated, infected plant; values followed by the same letter are not significantly different (Kruskal wallis test at 5%).

In contrast, for PNT-I, the mortality rate was 100%, indicating the effectiveness of biocontrols on the protection of cotton against FOV (**Figure 3**). These results are consistent with those of several authors who reported the effective role of elicitors such as methyl jasmonate (MeJA), ethephon, and fungal filtrates on Fusarium wilt tolerance in cotton [28, 29]. As the mortality rate of plants treated with FTC was the lowest after infection, FTC could be recommended in the preventive control of FOV [41,42]. It could therefore be an interesting alternative to chemical control, as this would reduce the use of pesticides in cotton crops.

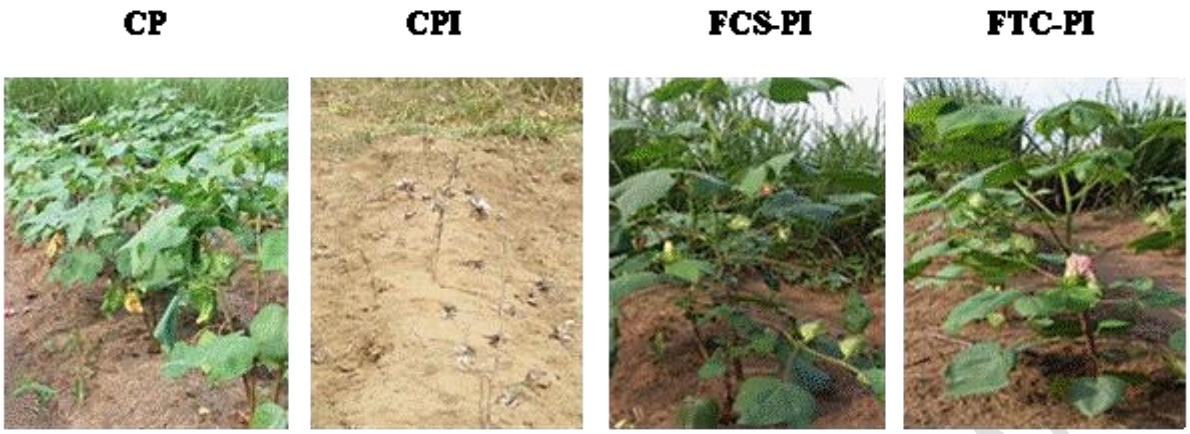

Figure 3. Appearance of Some Cotton Plants Treated with Biocontrols and Inoculated with *Fusarium oxysporum* f.sp. vasinfectum after 120 Days of Incubation

CP: Control plant; CPI, control infected plant; FTC-PI, cotton stem residue filtrate-treated, infected plant; FCS-PI, sugarcane stem residue filtrate-treated, infected plant.

4. CONCLUSION

The two biocontrols, Cotton Stem (FTC) and Sugar Cane Stem (FCS) residue filtrates, caused the elicited plants to increase the content of constituent polyphenols. In addition, they caused *de novo* synthesis or induction of specific polyphenols that are involved in cotton defenses against Fusarium wilt. However, FTC has been shown to be more tolerant than control plants due to the presence of isoquercetin and astringin, which have a greater inhibitory effect on FOV than those induced by FCS (5-caffeylquinic acid and rutin). Indeed, the mortality rate of plants treated with FTC is very low (1%) after infection, unlike FCS (8%) and the control (10%).

This study on major cotton pathogen, FOV, was very interesting and the management of plant disease by polysaccharides were highly important to minimise the role and overuse of fungicides. Inducing the plant natural defense response can be the ultimate aim to manage plant diseases such as cotton. Therefore, FTC may be recommended as a preventive treatment of cotton against Fusarium wilt caused by the FOV pathogen.

DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

5. REFERENCES

[1] Olopo E. Côte d'Ivoire : la production cotonnière devrait désormais atteindre 600 000 tonnes en 2021. Accessed 20 March 2022. Available https://www.agenceecofin.com/coton/0511-92923-cote-d-ivoire-la-production-cotonniere-devrait-desormais-atteindre-600-000-tonnes-en-2021/2022.

[2] FIRCA. La filière coton. Filière du Progès. 2012; 9:1-48.

[3] FIRCA. Résultats du Programme de développement de la Filière Coton. In : Rapport annuel 2019, pp 44-50. Accessed 20 March 2022. Available https://firca.ci/wp-content/uploads/2020/11/RA-FIRCA-2019-FINAL.pdf.

[4] Traoré B, Pohé J. Risque de pullulation des ravageurs et d'émergence des maladies sur le cotonnier en condition irriguée dans le nord de la Côte d'Ivoire. Rev Ivoir Sci Technol. 2020;36:284-305.

[5] Sayegh M. La résistance du cotonnier à la bactériose causée par *Xanthomonas camprestris pathovar malvacearum* rôle du gène GhLOX1dans la réaction hypersensible. Thèse de l'Institut National Polytechnique de Lorraine-Nancy. 2009; (France). 155p.

[6] Renou A, Brévault T. Les ravageurs et maladies du cotonnier, gestion intégré des ravageurs. In : Le cotonnier. Éditions Quæ, CTA, Presses agronomiques de Gembloux. 2015; pp.109-154.

[7] Abo K. Contribution à l'étude de la fusariose vasculaire du cotonnier, en Côte d'Ivoire: caractérisation de populations de l'agent pathogène *Fusarium oxysporum* f. sp. vasinfectum (Atk.) Sn. & H. ; analyse des relations entre les facteurs de l'environnement des sols et l'expression de la maladie. Thèse de l'Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire. 2006;180p.

[8] Konan YKF. Effect of Methyl jasmonate on phytoalexins biosynthesis and induced disease resistance to *Fusarium oxysporum* f. sp. vasinfectum in Cotton (*Gossypium hirsutum* L.). Int J Agron. 2014;2014:1-11.

[9] N'goran ARB. Stimulation des défenses naturelles du cotonnier [*Gossypium hirsutum* L. (Malvaceae)] par des éliciteurs oligosaccharidiques extraits des suspensions de *Fusarium oxysporum* f. sp. vasinfectum, intérêt phytopathologique des composés phénoliques. Thèse de l'Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire. 2015;168p.

[10] Li MY, Lan WZ, Chen C, Yu LJ. The effects of oligosaccharides and spores from *Aspergillus niger*on the defense responses of *Taxus chinensis* leaves *in vitro*. J Phytopathol. 2003;151:540-545.

[11] Amari LDGE. 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* (Mycosphaerellaceae) en Côte d'Ivoire. Thèse de l'Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire. 2012;237p.

[12] Korsangruang S, Soonthornchareonnon N, Chintapakorn Y, Saralamp P, Prathanturarug S. Effect of abiotic and biotic elicitors on growth and isoflavonoid

accumulation in *Pueraria candollei* var *candollei* Benth. And P. *candollei* var *mirifica* benth. Cell suspension cultures. Plant Cell Tiss Orgc Cult. 2010;3:333-342.

[13] Lyon GD, Reglinski T, Newton AC. Novel disease control compounds : the potential to immunize plants against infection. Plant Patholol. 1995;44:407-427.

[14] Mejri S. Efficacités et modes d'action de nouveaux stimulateurs de défenses des plantes sur le pathosystème blé - *Zymoseptoria tritici.* Thèse de l'Université du Littoral Côte d'Opale, Calis, France. 2018;195p.

[15] Vessere RJ. Role of Peroxydase in cotton resistance to bacterial blight. Plant Sci Lett. 1980; 20:47-56.

[16] Nicholson RL, Hammershmidt R. Phenolic compounds and their role in desease resistance. Ann Rev Phytopathol. 1992;30:369-389.

[17] Assigbetse KB. Pouvoir pathogène et diversité génétique chez *Fusarium oxysporum* f. sp. vasinfectum: agent de la fusariose du cotonnier. Thèse de l'Université de Monptellier II, France. 193;205p.

[18] Bouhot D, Rouxel F. Deux techniques de determination du pouvoir pathogene des *Fusarium oxysporum.* Ann phytopathol. 1970;2:251-254.

[19] Kouakou TH, Kouadio YJ, Téguo PW, Valls J, Badoc A, Mérillon JM, Décendit A. Polyphenol levels in two cotton (*Gossypium hirsutum* L.) callus cultures. Acta Bot Gall. 2009;156(2):223-231.

[20] Kouakou TH, Téguo PW, Kouadio YJ, Valls J, Tristan R, Decendit A, Mérillon JM. Phenolic compounds and somatic embryogenesis in cotton (*Gossypium hirsutum* L.). Plant Cell Tiss Org Cult. 2007;90:25-29.

[21] N'cho AL, Yapo SES, Amari L-ND, Kouakou TH. Impact of elicitation by methyl jasmonate and ethephon on cotton (*gossypium hirsutum* L.) protection against *Fusarium oxysporum* f. sp. vasinfectum. *J Adv Agric.* 2018;9:1558-1575.

[22] Belhadj *A*, Saigne C, Telef N, Cluzet S, Bouscaut J, Corio-costet MF. Methyl jasmonate induces defenses in grapevine and triggers protection against Erysiphe necator. J *Agric Food Chem.* 2006;54(24):9110-9125.

[23] Grayer RJ, Felix G, Boller T. Plant-fungal interactions : the search for phytoalexins and other antifungal compounds from higher plants. Phytochemistry. 2001;56:253-263.

[24] Zhao J, Zheng SH, Fujita K, Sakai K. Jasmonate and ethylene signaling and their interaction are integral parts of the elicitor-signalling pathway leading to betathujaplicin biosynthesis in *Cupressus lusitanica* cell cultures. J Exp Bot. 2004;55 (399):1003-1015.

[25] Zeneli GP, Krokene E, Christiansen, Krekling T, Gershenzon J. Methyl jasmonate treatment of mature Nor-way spruce (*Picea abies*) trees increases the accumulation of terp-enoid resin components and protects against infection by *Ceratocystis polonica*, a bark beetle-associated fungus. Tree Physiol. 2006;26:977–988.

[26] Zhu L, Tu L, Liu L, Yuan D, Jin L, Long L, Xianlong Zhang X. Lignin metabolism has a central role in the resistance of cotton to the wilt fungus *Verticillium dahliae* as revealed by RNA-Seq-dependent transcriptional analysis and histochemistry. J Exp Bot. 2011;62(15):5607-5621.

[27] N'goran AR, Kouakou TH, Konan FKH, Camara B, Kouassi NK, Kone D. Effet de la fraction oligosaccharidique de *Fusarium oxysporum* f. sp. vasinfectum, sur la protection du cotonnier (*Gossypium hirsutum* L.) contre la fusariose. Agron Afr. 2016; 28(3):1-10.

[28] N'cho AL, Yapo SES, N'goran AREB, Kouakou TH. Effect of the exogenous application of methyl and ethephon jasmonate on the accumulation of phenolic

compounds and efficacy against fusarium wilt in cotton [*Gossypium hirsutum* L. (Malvaceae)]. Chem Sci Int J. 2018;25(2):1-16.

[29] N'goran KD. Régulation de la biosynthèse des polyphénols par des éliciteurs oligosaccharidiques et mycéliens d'origine fongique; effet sur la tolérance du cotonnier [*Gossypium hirsutum* L. (Malvaceae)] à la fusariose. Thèse de l'Université Nangui Abrogoua, Abidjan, Côte d'Ivoire. 2019;197p.

[30] Ahuja I, Kissen R, Bones AM. Phytoalexins in defense against pathogens. Trends Plant Sci. 2012;17(2):73-90.

[31] Pedras MS, Adio AM. Phytoalexins and phytoanticipins from the wild crucifers *Thellungiella halophila* and *Arabidopsis thaliana*: rapalexin A, wasalexins and camalexin. Phytochemistry. 2008;69(4):889-893.

[32] Yin Z, Sadok A, Sailem H, McCarthy A, Xia X, Li F, Garcia MA, Evans L, Barr AR, Perrimon N, Marshall CJ, Wong ST, Bakal C. A screen for morphological complexity identifies regulators of switch-like transitions between discrete cell shapes. Nat Cell Biol. 2013;15(7):860-871.

[33] Kouakou TH. Embryogenèse somatique chez le cotonnier (*Gossypium hirsutum* L.) : variation des composés phénoliques au cours de la callogenèse et de la culture des suspensions cellulaires. Thèse d'état de l'Université Abobo-Adjamé Abidjan Côte d'Ivoire. 2009;137p.

[34] Faurie B, Cluzet S, Corio-costet M, Merillon J. Methyl jasmonate/etephon cotreatment synergically induces stilbène production in *Vitis vinifera* cell suspension but fails to trigger resistabce to *Erysiphe necator*. J Int Sci Vig Vin. 2009;43(2):99-110.

[35] Dufour MC, Lambert C, Bouscaut J, Merillon JM, Corio-Costet MF. Benzothiazole-primed defence responses and enhanced differential expression of defence genes in

Vitis vinifera infected with biotrophic pathogens *Erysiphe necator* and *Plasmopara viticol*a. Plant Pathol. 2013;62:370-382.

[36] Lambert C. Étude du rôle des stilbènes dans les défenses de la vigne malade contre les maladies du bois. Thèse de l'Université de Bordeaux 2, France. 2011;179p.

[37] N'cho AL. Diversité des systèmes de défenses induites du cotonnier [*Gossypium hirsutum* L. (Malvaceae)] *in natura* et efficacité sur la fusariose. Thèse de l'Université Nangui Abrogoua, Abidjan, Côte d'Ivoire. (2019);143p.

[38] N'cho XE. 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. Thèse de l'Université Nangui Abrogoua, Abidjan, Côte d'Ivoire. 2017;198p.

[39] Leiss KA, Maltese F, Choi YH, Verpoorte R, Klinkhamer PG. Identification of Chlorogenic Acid as a Resistance Factor for Thrips in *Chrysanthemum*. Plant Physiol. 2009;150:1567-1575.

[40] Shimoda K, Hamada M, Takemoto M, Hamada H. Synthesis of resveratrol glycosides by cultured plant cells. Nat Prod Comm. 2013;8(7):907-909.

[41] De Lamo FJ, Takken FL. Biocontrol by *Fusarium oxysporum* using endophyte-mediated resistance. Front Plant Sci. 2020;11(37):1-15.

[42] Martyn RD. Fusarium wilt of watermelon: 120 years of research. Hortic. Rev. 2014; 16(42):349-442.