Research Article

Stimulation of phenolic compounds production in cotton (*Gossypium hirsutum* L.) by oligosaccharide filtrates of *Fusarium oxysporum* f. sp. *vasinfectum*

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Abstract

Disease control is largely based on the use of fungicides, bactericides, and insecticides-chemical compounds toxic to plant invaders, causative agents, or vectors of plant diseases. However, the hazardous effect of these chemicals or their degradation products on the environment and human health strongly requires the search for new, harmless means for disease control. There must be some natural phenomenon inducing resistance to protect plants from disease. Elicitors are such compounds which activate chemical defense in plants. Various biosynthetic pathways are involved in treated plants and mainly the phenolic compounds. Phenolic compounds are secondary metabolites that encompass several classes structurally diverse of natural products biogenetically arising from the phenylpropanoid pathways. Plants need phenolic compounds for resistance to pathogens and for many other functions. In this study, extract of *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) culture filtrate or extract of oligosaccharides from FOV (OSEF) was used as elicitor. it was sprayed onto the cotton leaves in order to investigate its effect on phenolic compound biosynthesis. The results showed that OSEF triggers the induction of phenolic compound biosynthesis. Indeed, in OSEF-treated plants, thirteen phenolic compounds were identified while in control plants, eight phenolic compounds were produced. The chlorogenic acid, epicatechin, astragalin, pterostilbene and piceid are the new synthesized phenolic compounds under the action of OSEF. Besides, content of phenolic compounds is higher in OSEF-treated plants than in control. OSEF positively affects both the quality and quantity of phenolic compounds in cotton. The phenolic compounds identified in this study can be classified into five groups which are the stilbenes (piceatannol, piceid, pterostilbene, and resveratrol), hydroxycinnamic acids (caffeyol-D-glucose, ferulic acid), chlorogenic acid (acid 3p-coumaroylquinic, chlorogenic acid), flavonoids (astragalin, catechin, epicatechin and gossypetin) and terpene (gossypol). These phenolic groups have a positive action in plants protection against pathogens. Oligosaccharides are also able to induce phenolic phytoalexins accumulation in cotton. These compounds would act as a chemical barrier against pathogens. Their presence in plant could strigger a resistance to fungal diseases. This approach can represent a valuable strategy to protect cotton against diseases and can also serve as an alternative or complementary method to pesticides use.

Keywords: fungal filtrate; *Fusarium oxysporum* f. sp. *vasinfectum*; oligosaccharide; *Gossypium hirsutum*; phenolic compounds.

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Introduction

Cotton (*Gossypium hirsutum* L.) is one of the most important fiber crops. It's the principal raw material for textile industries. In addition, cotton seeds are an important source of proteins which can be used in human and animal nutrition. Therefore, cotton represents an interesting source of currency, particularly for developing countries [1]. In West Africa, cotton is regarded as "the white gold" of the economy. Indeed, it is of considerable economic and social importance, because it doesn't only provide a livelihood to a substantial part of the population, but it is also a significant source of foreign exchange earnings. However, cotton plants are challenged by a variety of biotic stresses

like fungal, bacterial, or viral infections. This lead to a great loss to cotton yield. In West Africa, particularly in Côte d'Ivoire, cotton diseases are usually the main cause of production losses estimated between 15 and 25%. *Fusarium oxysporum* f. sp. *vasinfectum* (FOV), the causal agent of *Fusarium* wilt causes most damages. During unfavorable years, a non-riding or mishandled parasitism can cause production losses greater than 50% and sometimes up to almost total destruction of potential production [2]. Thus, to protect plants against diseases and get a good crop, various options are available. Some options include development of resistant cultivars, biological control, crop rotation, tillage, and chemical pesticides. Nearly all chemical pesticides or fungicides have a direct antibiotic principle. But their use at commercial level is uneconomical, application is cumbersome, and some are proved to be carcinogenic [3]. Therefore, considerable efforts have been accomplished to devise environmental-friendly strategies for the check of plant diseases and thus to save mankind from health hazard [4].

Plants can activate separate defense pathways depending on the type of pathogen encountered [5]. Jasmonic acid (JA) and ethylene dependent responses seem to be initiated by necrotrophs, whereas salicylic acid (SA) dependent response is activated by biotrophic pathogens. The mechanisms responsible for this differential recognition and response may involve crosstalk among these three different signal transduction pathways: JA, ethylene, and SA. The better understanding of plant signaling pathways has led to the discovery of natural and synthetic compounds called elicitors that induce similar defense responses in plants as induced by the pathogen infection [6–7]. Different types of elicitors have been characterized, including lipids, glycopeptides, glycoproteins and particularly carbohydrate polymers. Among the last compounds, the oligosaccharides derived from fungal and plant cell wall polysaccharides are well-defined elicitors that, in some cases, can induce defense responses at a very low concentration [8, 9–10]. Moreover, Fanizza *et al.* [11] showed that the elicitor activity may be due to the presence in the culture filtrate of extracellular polysaccharides. Polysaccharides or oligosaccharides are the most studied signal molecules in elicitation pathways and these compounds can substitute for fungal elicitors during a pathogen attack [12–14]. The elicitor's application has caused defensive reactions and increased resistance of many plants to pathogens [15]. Furthermore, several studies have already reported the effectiveness of elicitors in plant resistance to pathogens by stimulating the antifungal compounds synthesis like polyphenols [3, 16]. Moreover, polyphenols accumulate in adjacent tissues at the necrotic areas, suggesting that these compounds may be defensive [17]. Their role in plant resistance to fungi was reported by recent studies [18–19]. In plants, a complex array of defense response is induced after detection of microorganism via recognition of elicitor molecules released during plant-pathogen interaction. Following elicitor perception, the activation of signal transduction pathways generally lead to the production of phytoalexins biosynthesis, reinforcement of plant cell wall associated with phenylpropanoid compounds, active oxygen species, deposition of callose, synthesis of defense enzymes, and the accumulation of pathogenesis-related (PR) proteins. Some of these compounds, especially the phenolic phytoalexins (polyphenols) are effective against diverse pathogens, including fungi [19–22]. Oligosaccharides are the most studied signal molecules in elicitation pathways and these compounds can substitute for fungal elicitors during a pathogen attack [13].

The aim of the present study is the development of an alternative treatment of cotton to chemical fight through research for elicitors of natural origin able to induce defense responses that can protect it against FOV. The stimulating effects of fungal filtrate or oligosaccharide extract of FOV (OSEF) on the production of phenolic compounds of cotton was investigated.

Experimental

Plant Material

Seeds of cotton (*Gossypium hirsutum* L. cv. Y764AG3) were obtained from CNRA (Centre National de Recherche Agronomique, Côte d'Ivoire). This cotton cultivar is known to be susceptible to Fusarium wilt.

Seed germination

Seeds of cotton were delinted with sulphuric acid. Plump and mature seeds were chosen and surface sterilized by dipping in 70% (v/v) ethanol (1 min) prior to a 20 min exposure to 2.5 % sodium hypochlorite (v/v). Seeds were rinsed 3 times with sterile distilled water for 5 min and germination was initiated in the dark during 48 h. Seedlings

were cultivated in 500 mL pots containing substrate (soil) previously sterilized and incubated in a greenhouse during two months.

Fungal material

The virulent strain of fungi *Fusarium oxysporum* f. sp. *vasinfectum* (strain CBS-116626; Centraalbureau voor Schimmelcultures, Baarn, The Netherlands) was provided by the Phytopathology Laboratory of the Superior School of Agronomy of Félix Houphouët-Boigny National Polytechnic Institute, Yamoussoukro-Côte d'Ivoire.

Preparation of fungal extracts for inoculation.

The extraction of oligosaccharide from *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) filtrate used in this study was similar to that described previously by Ngoran *et al.* [23]. Briefly, the FOV spores suspensions were placed on an orbital shaker at 80 rpm during 10 days in darkness under a 12h photoperiod at 28 ± 2 °C. Then, cultures were maintained in darkness without any agitation for 4 weeks [11]. FOV filtrate was collected after mycelium removal by filtration on partial vacuum through a 30 µm nylon mesh and autoclaved 20 min at 121 °C. This filtrate was considered as crude fungal extract or crude oligosaccharide extract of FOV.

Elicitation of cotton by oligosaccharide extract of FOV

The crude oligosaccharide extract of FOV is dissolved in sterile distilled to obtain 10% of concentration. In fact, Ngoran *et al.* [23] reported that this elicitor concentration is appropriated to stimulate the production of phenolic compounds in cotton plants. At the diluted oligosaccharide extract of FOV (OSEF), 0.1% triton X-100 was added as a surfactant. OSEF solutions (10 mL) were applied on two month old cotton plants as a foliar spray. Inoculated plants were maintained in the greenhouse. Humidity was maintained at 90% through regular water spraying system in the enclosure. Water-treated leaves were used as controls. Watering of the seedlings was ensured according to the moisture of the substrate. Thereafter, ten plants were incubated during 72 h and experiment was triplicate.

Chemicals

All solvents and chemicals for the HPLC analysis such as acetonitrile (Sigma-Aldrich), methanol (Sigma-Aldrich) and standard phenolic compounds (Sigma-Aldrich) were of HPLC grade. Ultrapure water was used to prepare some solutions for the HPLC method.

Phenol extraction

Phenol extraction was performed using the method of [24–25]. Approximately, 50 mg of freeze-dried leaf were dissolved overnight with 10 mL of methanol at 4 °C in a blender. Sample was centrifuged at 2000 g for 10 min. Supernatant was collected and represents the extract of phenol compounds. Approximately 1 mL aliquot was filtered through Millipore with 0.45 µm porosity using syringe into HPLC sample vials before injection into the HPLC system.

HPLC analysis

Analyses were performed on an Agilent HPLC unit (model-LC 1100 series). The samples were evaporated with speed vac and the dried extracts were dissolved in 1 mL of H_2O/MeOH (50/50, v/v). HPLC analysis was conducted using the method described by Belhadj [17]. A C18 reverse phase column (Prontosil, 250 x 4.0 mm, 5 µm, Bischoff) was used. The flow of the mobile phase was 0.8 mL/min with a binary gradient eluent composed of (A) H_2O/TFA 1% (97.5/2.5, v/v) and (B) acetonitrile/solvent A (80/20, v/v). The elution program was 10% B (0-40min), 10-50% B (40-41min), 50-100% B (41-50 min), 100-10% B (50-51min), and 10% B (51-70min). The chromatogram was monitored at 284 nm wavelength using a UV detector (Kontron 430, Germany). NMR spectra were recorded on LC-NMR (Agilent 1200 series PLC/Bruker Avance III spectrometer operating at 600 MHz for proton). A reference library of compounds was performed previously with purified compounds and identified by NMR in laboratory and also with commercially available compounds such as caffeic acid, catechin, chlorogenic acid, cinnamic acid, epicatechin,

ferulic acid, gallic acid, genistein, gossypin, naringenin, *p*-coumaric acid, piceatannol, pterostilbene, quercetin, quercitrin, resveratrol, rutin and salicylic acid. This database contains the retention time of these compounds and can be compared with those obtained from unknown samples and thus proceeds to their identification. NMR spectra were recorded on LC-NMR (Agilent 1200 series PLC/Bruker Avance III spectrometer operating at 600 MHz for proton). A reference library of compounds was performed previously with purified compounds and identified by NMR in laboratory and also with commercially available compounds or standard phenolic compounds such as caffeic acid, catechin, chlorogenic acid, cinnamic acid, epicatechin, ferulic acid, gallic acid, genistein, gossypin, naringenin, *p*-coumaric acid, piceatannol, pterostilbene, quercetin, quercitrin, resveratrol, rutin and salicylic acid. This database contains the retention time of these compounds and can be compared with those obtained from unknown samples and thus proceeds to their identification. Standard curves were obtained by plotting the peak areas of standard concentrations of phenolic compounds (0-25 µg/mL). The linear regression equation ($R^2 > 0.98$) was obtained. Quantification of phenolic compounds was based on peak area in comparison with the standard curves.

Statistical Analysis

All experiments were of complete randomized design and treatments consisted of five replications. Experiments were performed 3 times. Treatments were compared to controls by one-way ANOVA using the Duncan test ($P < 0.05$). The statistical analyses were performed with SAS (version 6.0).

Results and Discussion

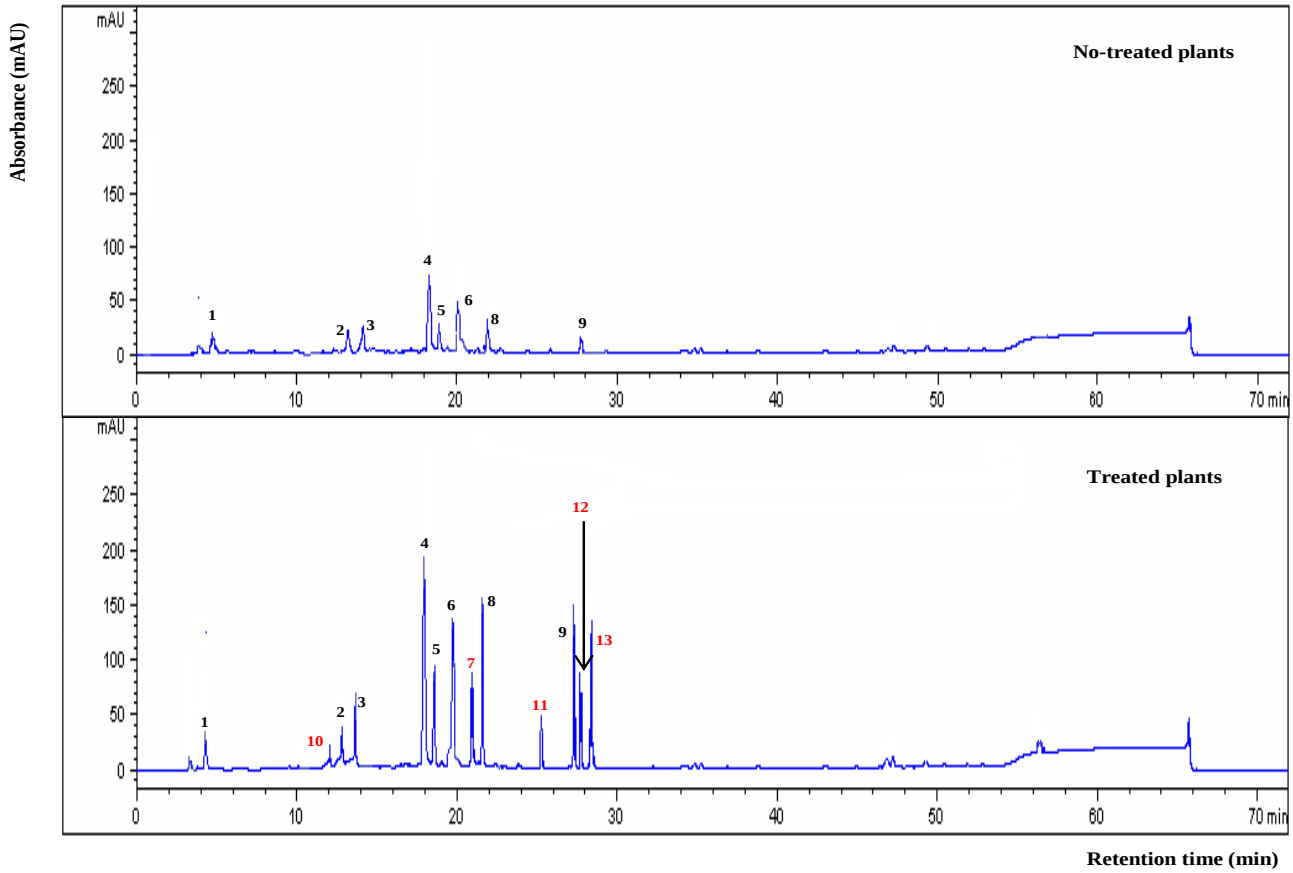

Figure 1 HPLC profile of phenolic compounds from leaves of cotton plants treated with oligosaccharide extract of FOV

To investigate the effect oligosaccharide extract of FOV (OSEF) on phenolic compound production in the cotton, the methanolic extract of leaves was analyzed by using reversed-phase HPLC (**Figure 1**).

Detection is shown at 284 nm. Peaks were identified by comparison with reference standards when available or by NMR data (retention time). (1) Gossypol(4.301 min); (2) caffeoyl-D-glucose (12.810 min); (3) catechin (13.720 min); (4) 3-*p*-Coumaroylquinic acid (18.015 min); (5) ferulic acid(25.603 min); (6) gossypetin (19.910 min); (7) chlorogenic acid (21.170 min); (8) piceatannol (21.725 min); (9) resveratrol (27.201 min); (10) Epicatechin (12.010 min); (11) Astragalin (25.603 min); (12) piceid (27.992 min); (13) Pterostilbene (28.215 min); HPLC: high performance liquid chromatography; FOV: *Fusarium oxysporum* f. sp. *vasinfectum.*

The difference in phenolic profile was observed between OSEF-treated plants and control. Thirteen phenolic compounds were detected in OSEF-treated plants leaves while only eight were identified in control. Moreover, amplitudes of the peaks are definitely more significant in OSEF-treated plants than in control. Identification and peak assignment of phenolic compounds were based on comparison of their retention time with that of standard phenolic compounds such as caffeic acid, catechin, chlorogenic acid, cinnamic acid, epicatechin, ferulic acid, gallic acid, genistein, gossypin, naringenin, *p*-coumaric acid, piceatannol, pterostilbene, quercetin, quercitrin, resveratrol, rutin, salicylic acid (**Table 1**), and polyphenols previously purified and identified by NMR. The retention time of these compounds was stored in a reference library of our database. Comparing their retention times with standards, peaks 1 to 6, 8 and 9 were synthesized by OSEF-treated plant and untreated plant i.e. control. These compounds were identified as (1) gossypol (4.301 min), (2) caffeoyl-D-glucose (12.810 min), (3) catechin (13.720 min), (4) 3-*p*-coumaroylquinic acid (18.015 min), (5) ferulic acid (25.603 min); (6) gossypetin (19.910 min), (8) piceatannol (21.725 min) and (9) resveratrol (27.201 min). In addition, after plant treated with OSEF, the appearance of peaks 7 and 10 to 13 were observed and identified as (7) chlorogenic acid (21.170 min), (10) epicatechin (12.010 min), (11) astragalin (25.603 min), (12) piceid (27.992 min) and (13) pterostilbene (28.215 min).

Table 1 HPLC retention time of standard phenolic at 284 nm

Phenolic compounds	Retention time (min)
Gossypol	04.301
Gallic acid	05.496
Gossypin	07.113
Genistein	11.544
Epicatechin	12.341
Catechin	13.595
Quercetrin	15.963
p-Coumaric acid	17.616
Ferulic acid	18.525
Piceid	18.816
Rutin	19.301
Salicylic acid	19.617
Cafeic acid	20.816
Chlorogenic acid	20.993
Piceatannol	21.546
Naringenin	21.905
Astringin	22,496
Cinnamic acid	24.730
Quercetin	24.855
Astragalin	25.603
Resveratrol	26.992
Pterostilbene	28.345

HPLC (chromatographie Liquide Haute Performance); min (minute)

All identified compounds were quantified and **Table 2** shows the comparison of phenolic contents in OSEF-treated plants and control leaves. However, it is wise to report that the content of the phenolic compounds in OSEF-treated plants are significantly higher than those of control. Gossypetin (9.12 µg/g FED), piceatannol (8.26 µg/g FDE), 3-*p*-Coumaroylquinic acid (11.23 µg/g FED), resveratrol (2.20 µg/g FDE), ferulic acid (0.85 µg/g FED) and gossypol (0.026 µg/g FDE) which had a low content in control leaves were increased after application of OSEF. The amount of their compounds was 4, 7, 9, 10, 30 and 937-fold higher than that of control. The 3-*p*-Coumaroylquinic

acid (99.84 µg/g FDE) followed gossypetin (89.60 µg/g FDE) and piceatannol (56.70 µg/g FDE) are the major compounds. Similarly, considering phenolic group, we notified that stilbenes (148.02 µg/g FDE) followed flavonoids (133.09 µg/g FDE) and chlorogenic acids (118.24 µg/g FDE) are majority. In addition, all of these phenolic compounds are substances which possess biological such as antifungal and antimicrobial activities [15, 26–28]. It was reported that these compounds were derived of phenylpropanoid pathway [25, 29–30]. Many phenolic compounds produced through this pathway can be induced by elicitors such as oligosaccharides [8, 31]. In this study, the relation between quality and the quantity of phenolic compounds and OSEF treatment was established. The OSEF stimulated the biosynthesis of *novo* phenolic compounds.

Table 2 Phenolic composition of cotton leaves treated with oligosaccharide extract of *Fusarium oxysporum* f. sp. *vasinfectum*

Phenolic group	Polyphenol compounds	Polyphenol content in cotton leave (µg/FDE)			
		Control	total content	Treated plants	total content
Chlorogenic acids	Chlorogenic acid	nd	11.23 ± 0.06^{a}	18.40 ± 0.30^{l}	118.24 ± 0.80^{j}
	3-*p*-CQA	11.23± 0.06^{c}		99.84 ± 1.50^{a}	
Hydroxycinnamic acids	Ferulic acid	0.85 ± 0.02^{d}	05.45 ± 1.50^{f}	25.60 ± 0.33^{b}	33.18 ± 0.50^{d}
	Caffeyol-D-glucose	04.60± 0.05^{b}		07,58 ± 0.07^{g}	
Flavonoids	Catechin	05.15 ± 0.04^{b}	14.27 ±1.50^{a}	20.32 ± 0.10^{j}	133.09 ± 1.10^{k}
	Gossypetin	09.12 ± 0.03^{d}		89.60 ± 0.95^{i}	
	Epicatechin	nd		08.25 ± 0.04^{d}	
	Astragalin	nd		14.92 ± 0.05^{c}	
	Pterostilbene	nd	10.46 ±1.50^{a}	40.03 ± 0.30^{n}	148.02 ± 0.90^{m}
Stilbenes	Resveratrol	02.20 ± 0.01^{e}		15.89 ± 0.05^{h}	
	Piceatannol	08.26± 0.05^{d}		56.70 ± 0.70^{m}	
	Piceid	nd		35.40 ± 0.20^{p}	
Terpenes	Gossypol	0.026 ± 0.001^{a}	0.026 ± 0.001^{p}	24.37 ± 0.25^{f}	24.37 ± 0.15^{c}

FDE: freeze-dried extract; nd: not detected; data are expressed as mean of three replicates; ±SD: standard deviation; within row means, numbers followed by a different letter are significantly different according to Duncan's multiple range test at $P = 0.05$ level; OSEF: oligosaccharide extract of FOV; FOV: *Fusarium oxysporum* f. sp. *vasinfectum*; 3-p-CQA: 3-*p*-coumaroylquinic acid.

For example, height phenolic compounds (3-*p*-coumaroylquinic acid, ferulic acid, caffeyol-D-glucose, catechin, gossypetin, resveratrol, piceatannol and gossypol) were common to OSEF-treated and control plants. This result suggests that these compounds have no direct relation with OSEF application. However, the content of these compounds which were exponentially increased in OSEF-treated plants indicates the presence of an intense biosynthetic activity after applying OSEF. Certainly it is the high amount of these compounds which would have a fungitoxic effect.

Oligosaccharide seems to play an important role in the accumulation of the phenolic compound in cotton. Moreover, similar results were obtained by Konan *et al.* [19] after application of methyl jasmonate on cotton leaves. They also reported the existence of a close relationship between quantity and antifungal activity of phenolic compounds. Moreover, other authors revealed the bioactive property of these phenolic compounds [3, 32]. As far as caffeyol-D-glucose, catechin, ferulic acid and resveratrol, the content was increased slightly after application of OSEF. However, some studies showed the contribution of these phenolic compounds in plant preformed defense more than plant induced defense [27–33]. In our case the stimulation of defense is induced. Regarding gossypol, their content has dramatically increased from 0.026 µg/g FDE in control to 24.37 µg/g FDE in OSEF-treated plant, i.e. an increase of 937 times (0.026 µg/g FDE). This result showed the effect of OSEF on accumulation of gossypol with the cotton. In fact the gossypol is a sesquiterpene which was considered as phytoalexin able to induce plant resistance against pathogens [34]. Although this rate is high, the amount produced is still low compared to other phenolic groups. However, Tao *et al.* [35] reported that a slight quantity of gossypol is efficient against pathogens. Thus the quantities of gossypol induced by application of OSEF were sufficient to defend cotton against *Fusarium oxysporum* f. sp. *vasinfectum* (FOV). So everything seems to happen as OSEF was only on the phenolic content. However, HPLC analysis revealed the biosynthesis of new phenolic compounds in cotton leaves after treatment with OSEF. These

compounds such as chlorogenic acid, epicatechin and astragalin (flavonoids), pterostilbene and piceid (stilbene) have a close link with natural defense stimulation in cotton. Indeed, chlorogenic acid is an antifungal activity and according some authors it implicated in plants defense [36–37]. As to pterostilbene, it is a very active biological compound and present in slight quantities in plants [38]. Its beneficial effect in plants protection against the mildiou, *Botrytis* and others pathogens was demonstrated [15, 39–40]. The functions of flavonoids in plants range from physiological development to obviously plant responses to abiotic and biotic (pathogen infection) stresses [40-17-3]. Flavonoid such as kaempferol and its glycosylated form astragalin are reported to enhance growth inhibition of *Spodoptera lituracaterpillars* on groundnut [41–42]. These studies indicate that flavonoids also contribute to plant defense against diseases. Moreover, little is known about the defense-related function of flavonoids against fusarium wilt. However, [43] reported bioactive action of epicatechin and their role in plants defense. In addition some authors reported the presence of pterostilbene and chlorogenic acid in plants under methyl jasmonate treatment [16–19, 40]. Piceid is one of major resveratrol derivates in plants [44–45]. Resveratrol might induce the piceid by glycosylation, the pterostilbene by methylation or the piceatannol by oxidation presumably under the action of peroxidases [46–47].

In our study, resveratrol content was slightly increased more than 72%. The content which was 2.20 µg/g FDE in control increased to 15.89 µg/g FDE in OSEF-treated plants. Pterostilbene and piceid are missed in control, so it is plausible that the increase rate of resveratrol in OSEF-treated plant is due to the biotransformation of piceatannol. Nevertheless, other biosynthesis pathways should not be excluded. Several studies have reported that resveratrol and its derivatives are involved in plant resistance to various pathogens [48–49]. They also significantly inhibit conidial germination and mycelial growth of several fungi [38–3]. The ability of oligosaccharides to induce stilbenes could therefore give him role of biotic elicitor of natural defense in cotton like other plants in which their application induces the production of stilbene against pathogens [16, 40].

All phenolic compounds identified in this study can be classified into five groups which are the phenolic stilbenes (piceatannol, piceid, pterostilbene, and resveratrol), hydroxycinnamic acids (caffeyol-D-glucose, ferulic acid), chlorogenic acid (acid 3p-coumaroylquinic, chlorogenic acid), flavonoids (astragalin, catechin, epicatechin, gossypetin) and terpene (gossypol) (**Figure 2**). These phenolic groups have beneficial action on plant protection against pathogens [15, 26]. These results led us to the hypothesis that, in plants such as cotton, oligosaccharides from fungal origin would regulate the gene expression known to be inducible elicitors, and this could activate the biosynthesis of defensive compounds such as stilbenes, phenolic acids (chlorogenic and hydroxycinnamic acids), flavonoids and terpene which are regulated by pathogen attack. These compounds accumulated in OSEF-treated plants behave as antibodies that will protect the plants against potential pests. Thus, cotton seems to be equipped with compounds (phytoanticipins) able to act against any pathogen attacks. These phytoanticipins would act as a chemical barrier against a wide range of bioaggressors and would be appropriate in resistance of cotton to fungal diseases [50–51].Oligosaccharides represent true essential auxiliary to the proper functioning of plants. The interest on their application is growing because they are considered as biological activators. Oligosaccharides are biological modulators involved in many signaling events. Thus, oligosaccharides from degradation of plant polysaccharides (xyloglucan and pectin), or fungi (β-glucan and chitin) are broadly described as biological regulators active on plant defense reactions [52]. During the assault of a plant by pathogen, different elicitors' signals are output. In the early stages, oligogalacturonates from degradation pectocellulose wall will trigger a systemic acquired plant resistance [53]. In relation to elicitation activities of plant defense responses *via* oligosaccharides, their uses as biopesticides and plant protection agents have been considered in agribusiness. Thus, the production of penicillin from filamentous fungi as *Penicillium chrysogenum* is largely powered by oligomannan trace and oligoalginates from *Laminaria hyperborean* [54].

Conclusion

Commonly tested chemical elicitors are salicylic acid, methyl jasmonate, ethylene and so forth which affect production of phenolic compounds in plants. In this study, oligosaccharides from fungal polysaccharides (*Fusarium oxysporum* f. sp. *vasinfectum* or FOV) degradation were used as elicitors. The exogenous application of the oligosaccharides extract from FOV (OSEF) allowed the synthesis of thirteen phenolic compounds while in control plants only eight compounds were synthesized. Eight compounds were common to treated plants and non-treated

plants with the OSEF. The difference between both types of plant was *de novo* synthesis of astragalin, chlorogenic acid, epicatechin, piceid and pterostilbene. Besides, content of phenolic compounds identified was amplified after OSEF application on plants. OSEF allowed the stimulation of natural mechanism defense through the synthesis of phenolic compound as phytoalexins with cotton. OSEF was also able to accumulate the phytoalexins with cotton. Their use in agricultural practice could reduce the scope of chemical control, thus contributing to the development of sustainable agriculture. This approach could represent a valuable strategy to protect cotton against FOV, causal agent of Fusarium wilt.

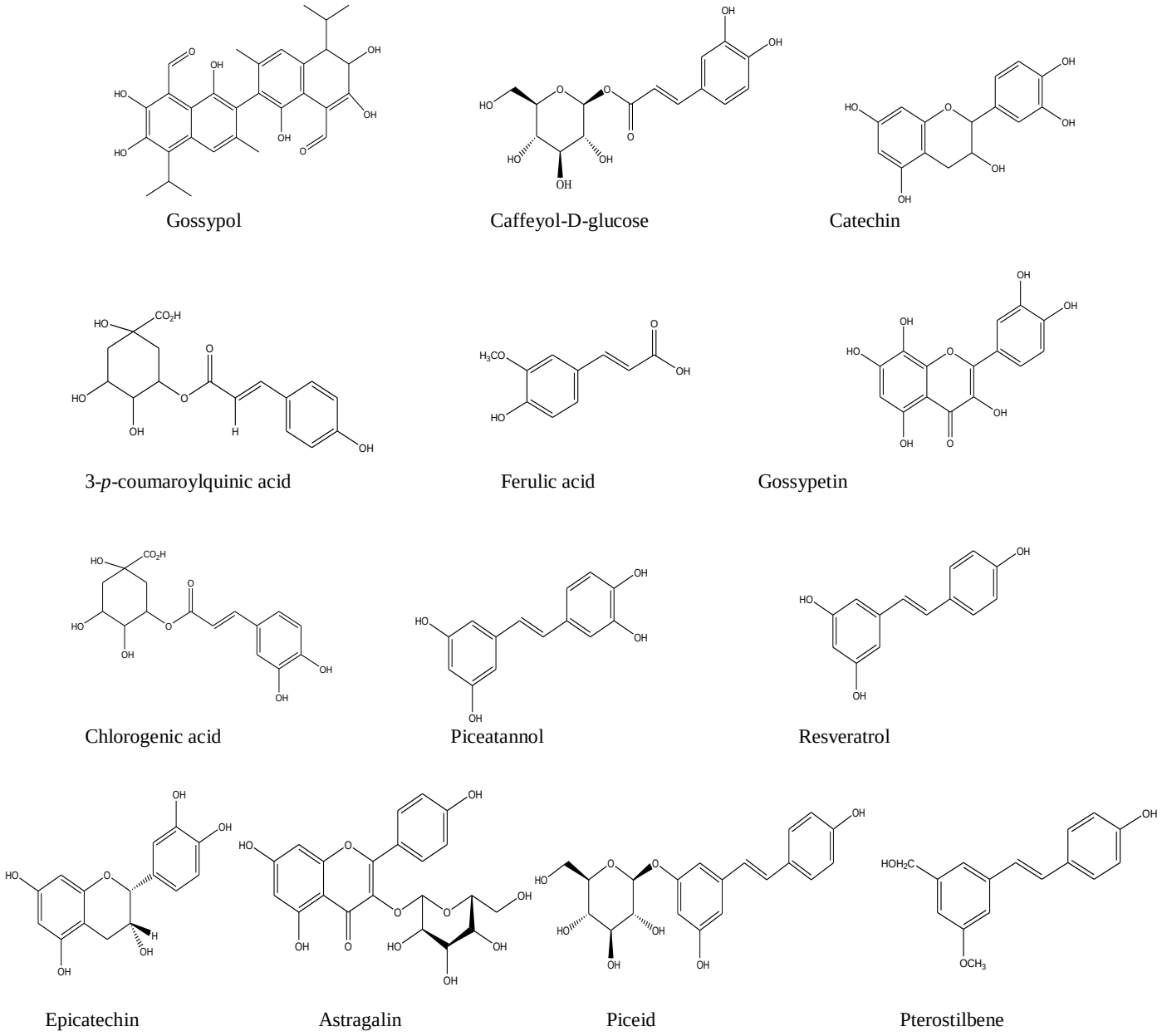

Figure 2 Chemical structure of phenolic compounds isolated from leaves of cotton plants treated with oligosaccharide extract of FOV

Phenolic compounds can be classified into five phenolic groups as follows: (1) stilbenes: piceatannol, piceid, pterostilbene and resveratrol; (2) hydroxycinnamic acids: caffeoyl-D-glucose and ferulic acid; (3) chlorogenic acids: 3-*p*-coumaroylquinic acid and chlorogenic acid; (4) flavonoids: astragalin, catechin, epicatechin, and gossypetin; (5) terpene aldehyde: gossypol; FOV: *Fusarium oxysporum* f. sp. *vasinfectum*.

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