

*Full Length Research Paper*

# **Spatiotemporal evolution and identification of oil palm phenolic compounds in response to vascular wilt caused by *Fusarium oxysporum* f.sp. *elaeidis* in Côte d'Ivoire**

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Received 23 April, 2022; Accepted 3 August, 2022

***Fusarium oxysporum* f.sp. *elaeidis* (Foe) is a fungus that attacks oil palm and affects its production. This study aimed at characterizing phenolic compounds and evaluating their spatiotemporal evolution in two cultivars (sensitive and tolerant to fusariosis). For this, total phenols were quantified during the latency period [0 to 28 Days After Inoculation (DAI)] of Foe. Thereafter, phenolic extracts from plant organs (roots and pseudobulbs) were used for *in vitro* inhibition tests of Foe spore germination. Lastly, Gas Chromatography coupled to Mass Spectrophotometry (GC-MS) was used to detect polyphenols. These results revealed that phenolic reaction in roots was earlier (1st DAI or T0) in the tolerant cultivar than in the sensitive (8th DAI). However, it occurred simultaneously in the pseudobulbs (3rd DAI) in both cultivars. Spore Germination Inhibition Rate (SGIR) of Foe reached 100% in the tolerant cultivar against the sensitive cultivar in which a maximum rate of 49, 64% was recorded. GC-MS revealed that in roots, para-hydroxybenzoic acid is a phytoanticipin while meta-hydroxybenzoic acid is a phytoalexin. However, in pseudobulbs, these two polyphenols are phytoalexins. These results will allow exploring the biosynthetic pathway of phenolic acids in order to improve the phenolic response of disease-sensitive but productive hybrids.**

**Key words:** *Fusarium oxysporum* f.sp. *elaeidis*, inhibition, polyphenols, GC-MS, meta-hydroxybenzoic acid, para-hydroxybenzoic acid.

## **INTRODUCTION**

The African oil palm (*Elaeis guineensis* Jacq.) is a tropical perennial plant cultivated for its fruits (Demol et al., 2002) that produce both palm oil and kernel oil. In Africa, Côte d'Ivoire is the second most productive

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country of palm oil after Nigeria (Agro, 2022). This oil holds 4th position in the Ivorian economy and represents 3.73% of the Gross Domestic Product (GDP) (Palmafrique, 2013). Ivorian market consumes only 45% of the country's oilseed production and the remaining 55% are destined towards the continent's other markets. Nonetheless, this production remains insufficient to cover needs estimated at around 500,000 tons in the West African Economic and Monetary Union (WAEMU) area and over 1,800,000 tons in the Economic Community of West African States (ECOWAS). Faced with this situation, Côte d'Ivoire aims at increasing its annual palm oil production to 800,000 tons in order to achieve self-sufficiency in oilseed products and satisfy subregional needs (Palmafrique, 2013).

Unfortunately, this target is difficult to achieve due to the ravages and of oil palm diseases, particularly fusariosis, which is the most damaging disease in tropical African palm groves. In Côte d'Ivoire, except for Iboké area, this disease occurs in all oil palm production areas (Gogbé et al., 2016). *Fusarium oxysporum* f. sp. *elaeidis* (Foe) (Ascomycetes), a soil-borne fungus, is this disease causal agent. It enters plants only through a mechanically or biologically wounded root. When it meets the root, Foe uses its hydrolytic enzymes to overcome the epidermal barriers (Renard, 1970; Trique, 1971). It then migrates upward along the xylem intra- and inter-cellularly to the pseudobulb. Destruction of plant cells by this fungus causes its reaction through accumulation of gums and thylle formation in the xylem vessels and polyphenol production. These gums obstruct vessel lumina and block sap drainage resulting in dehydration and more or less rapid dies back of affected palms (De Franqueville and Diabate, 1995; Tengoua and Bagkoumé, 2008). Symptoms include plants stunting, yellowing and leaves drying, leading to the death of the plant. Stipe and rachis cross section shows numerous vessels browned by these gums. This disease causes the plant's total wilting, which undermines its growth and production.

In order to combat this disease, the control method implemented is mainly genetic, which has significantly reduced its incidence. However, this approach is very time-consuming (8 months). Besides genetic control, several researchers (Taquet, 1985; Diabaté et al., 2010) explored physiological control using the natural defense reaction pathway through the synthesis of phenolic compounds. Indeed, they highlighted some families of polyphenols involved in an oil palm's natural defense against certain diseases. Secondary metabolites, particularly phenolic compounds, are one of the most common groups of substances usually found in plants and recognized for their toxicity and numerous roles in the defense mechanism against microbial pathogens. They are reported as phytoanticipins, phytoalexins, structural barriers, pathogenicity modulators, and plant defense gene activators (Arfaoui et al., 2007; Mazid et al., 2011). On the other hand, Asssohoun et al. (2016)

showed that after root invasion, Foe takes three weeks to reach the pseudobulbs in the sensitive cultivar. Then it becomes imperative to identify the polyphenols that prevent Foe spore proliferation and migration from roots to pseudobulbs during the lag time. Indeed, understanding the molecular basis of the pathogenesis and the resistance mechanism is necessary to develop an effective management strategy against this disease. Our objective is to evaluate the spatial and temporal variations of oil palm polyphenols and to determine them from infected organs of two cultivars (sensitive and tolerant).

## MATERIALS AND METHODS

### Preparation of plant and fungal materials

Plant material consisted of healthy germinated oil palm nuts of the tolerant C1001F and sensitive C1001 cultivars from La Mé Research Station of the National Center for Agronomic Research (CNRA). Whose head office is located at Adiopodoumé KM 17, Road to Dabou 01 BP 1740 Abidjan 01, Côte d'Ivoire (West Africa). After sowing, the three-month-old seedlings (Figure 1) were sorted into two blocks (sensitive and tolerant). The fungal material consisted of *F. oxysporum* f. sp. *elaeidis* strain L60 (Foe) spores (Figure 2). This strain was isolated from rachis collected from a fusaried oil palm and proved to be the most aggressive.

### Inoculation of oil palm seedlings with *F. oxysporum* strain L60

The inoculation method adopted in this experiment was that of Diabaté et al. (2010). It consisted in digging up each seedling from its pot in order to hurt its roots with a knife. After rinsing the roots with tap water, they were soaked in fungal inoculum for 1 min and replanted in their initial pots. Control seedlings were treated the same way as the trials except that the inoculum was replaced by sterile distilled water. For each cultivar, 10 plants were used for each sampling time (0, 3, 5, 8, 13, 18, 23 and 28 days after inoculation).

### Evaluation of the phenolic response of plants

#### Sample collection and handling

After oil palm seedling pre-nursery inoculation with fungal L60 strain, their roots and pseudobulbs were sampled at different times: 0, 3, 5, 8, 13, 18, 23, and 28 days after inoculation (DAI) recorded, respectively T0, T3, T5, T8, T13, T18, T23, and T28. This 28-days period corresponds to the pathogen's lag time (Kablan et al., 2016). The collected roots and pseudobulbs were then tagged and each sample was ground in liquid nitrogen and the resulting powder was used for the determination of total phenolic compounds.

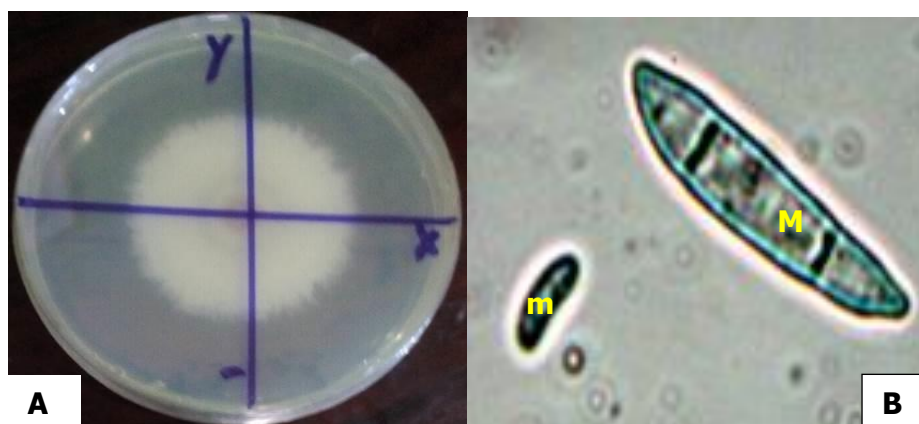
#### Phenolic compounds extraction

Extraction method adopted was that of Diabaté et al. (2009b). To each flask containing 20 ml of 70% ethanol and an aliquot of powder from each sample was added 1 ml of 2% Sodium Meta-Bisulfate (SMB), respectively. Each flask was boiled under vacuum for 30 min. After cooling, the resulting extract was filtered and dried at 60°C using a rotative evaporator. The residues were stripped



**Figure 1.** Three-month-old healthy oil palm seedling after germination.

Source: Author



**Figure 2.** Fungal colony of *Fusarium oxysporum* f. sp. *elaedis* strain L60 on Petri dish (A) and microscopic view (x 400) of its macroconidia (B: M) and microconidia (B: m).

Source: Author

with 70% ethanol and added with 0.1 ml of 2% SMB. The extracts were filtered using organic filter (0.3  $\mu\text{m}$ ) by syringe and adjusted to equal weight of the sample and stored in a refrigerator at 4°C.

#### **Total phenolic compounds determination**

Phenols were determined with the Folin-Ciocalteu reagent according to the modified method of Swain and Hillis (1959). So, to each 30  $\mu\text{l}$  sample of phenolic extract were added 0.25 ml of Folin-Ciocalteu reagent, 0.5 ml of sodium carbonate (20%, w/v) and final reaction volume was adjusted to 25 ml with distilled water. One tube containing no phenolic extract was the control for each incubation time (0, 3, 5, 8, 13, 18, 23, and 28 DA). The reaction blends were shaken then incubated in the dark for 1 h at 28°C. The absorbance was read with a spectrophotometer at a wavelength of 725 nm. The phenolic compounds content of the extract was determined by means of a calibration line performed with different concentrations of chlorogenic acid at 100 mg/L. For each extract

(roots and pseudobulbs), the mean content of total phenols, expressed as microgram of Chlorogenic Acid equivalent per gram of fresh tissue ( $\mu\text{g CA/g TF}$ ), was calculated from three replicates.

#### **Fungitoxicity test of phenolic extracts from roots and pseudobulbs**

The fungitoxic activity of each phenolic extract was evaluated in liquid medium based on Foe spore germination according to Taquet (1985). Consequently, 10 ml of the previously prepared inoculum was added to 90 ml of distilled water to reach 100 ml (1/100th) to obtain the necessary inoculum for the fungitoxicity tests. The germination control for this test was 30  $\mu\text{l}$  of 70% (v/v) ethanol added to 4 ml of the inoculum in an Erlenmeyer flask. For trials, the extract volume collected was previously calculated for a concentration of 300 mg/ $\mu\text{l}$ . The whole sample was incubated for 15 h at 28°C under magnetic stirring in an incubator. After incubation, an aliquot of the solution was spread on a Malassez cell and the

number of germinated and non-germinated spores was determined by counting under a photonic microscope at X400 zoom. This process was repeated three times for each phenolic extract. The

Spore Germination Inhibition Rate (SGIR) was calculated according to the formula of Diabaté et al. (2009a):

$$SGIR = \frac{\text{Germination \% of control spores} - \text{Germination \% of treatment spores}}{\text{Germination \% of control spores}}$$

### Polyphenols identification by GC-MS

In this study, samples' composition was analyzed on an Agilent GC-MS chain. The whole system, composed of a chromatograph with automatic injector is linked to a mass spectrometry and a computer which allows controlling it, thanks to the Masshunter software. Helium was used as the carrier gas and the HP5 MS column was used. For each extract from roots and pseudobulb, a volume of 1 µl was injected into the column for analysis. The chromatograph was defined for each extract, a chromatogram consisting of peaks, with its retention time and mass spectrum.

### Statistical analysis

All numerical data collected were statistically analyzed using Statistica 7.1 software. Total phenol content of organs and spore germination inhibition rate were compared based on the treatments using analysis of variance (ANOVA) with one or two ways at the 5% level of classification. When a significant difference was detected (P 0.05), the ANOVA was completed by Fisher's Least Significant Difference (LSD) test for the constitution of homogeneous groups. For total phenolic compounds identification, chromatographic data were visualized using Masshunter software (Agilent Technologies, 2010). Polyphenols occurring in oil palm roots and pseudobulbs were identified by comparing the mass spectra with those of the reference library of phenolic compounds of the computer. This library contains the retention time and mass spectra of the chromatographed phenolic compounds.

## RESULTS

Results of total polyphenols content, spore germination inhibition test and identification of phenolic compounds by GC-MS are reported in Figures 3 to 8.

### Variation of oil palm total phenolic compounds content as a function of Foe lag time

#### *In tolerant cultivar C1001F*

In the tolerant cultivar, statistical analysis of Figure 3 indicated that, inoculated plants responded to infection by the synthesis of a higher content of phenolic compounds in roots as early as the first DAI or T0 compared to the control plants. However, at the same time, no phenolic compounds were synthesized nor accumulated in the pseudobulbs of these infected plants compared to control (untreated) plants of this cultivar. This roots phenolic content synthesis of inoculated plants steadily increased until the fifth DAI and remained stable until the 13th DAI. At the same time in pseudobulbs, the phenolic response occurring from the third DAI remained stable until the fifth

DAI before decreasing from eighth to 13th DAI. On 18th DAI, phenolic synthesis by inoculated plants reached its maximum value in roots and pseudobulbs of this tolerant cultivar. From 23rd to 28th DAI, phenols synthesis dropped in the roots and pseudobulbs. However, its content remained higher than that of the control in the different organs.

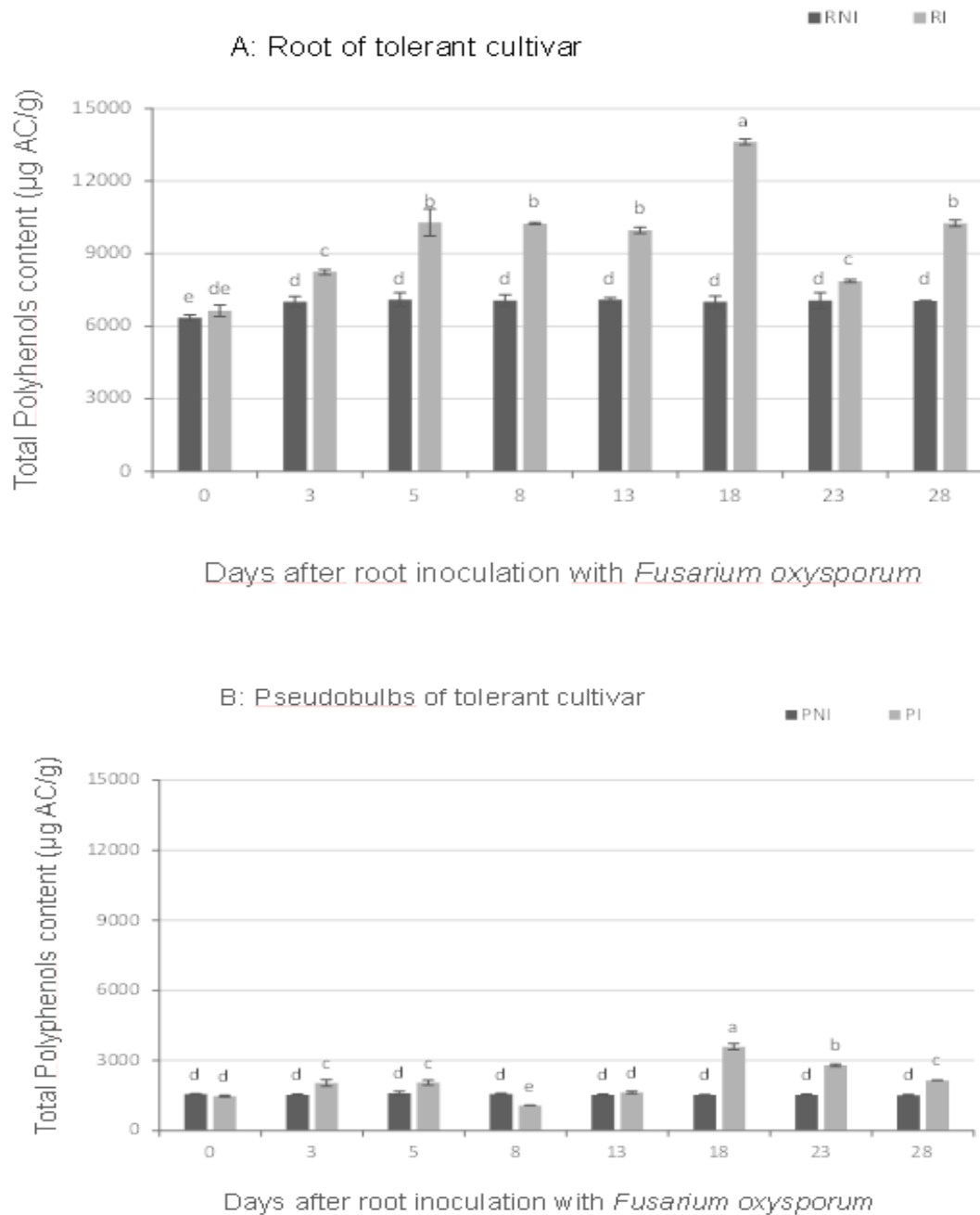
#### *In sensitive cultivar C1001*

In the sensitive cultivar C1001 (Figure 4), root total phenol content was higher in the control than in the inoculated plants on first DAI. However, in the pseudobulbs of the same plants, both the control and the inoculated plants synthesized the same total phenol content. As early as the third DAI, these phenols were strongly synthesized in the pseudobulbs of inoculated plants and starting from the eighth DAI in their roots. Then, this root synthesized phenols amount increased up to the 13th DAI as compared to the controls before decreasing to their level. However, in pseudobulbs, after this level of total phenol content increased on the 3rd DAI, it dropped in comparison to the control on the 5 to 18th DAI before increasing again from the 23rd to 28th DAI.

### Fungitoxicity of phenolic compounds extracted from roots and pseudobulbs of the both oil palm cultivars during the Foe latency period

In the tolerant cultivar C1001F, statistical analysis of Spore Germination Inhibition Rate (SGIR) of inoculated (IT) and non-inoculated (NI) plants indicated that phenolic reaction occurred as soon as inoculation (1st DAI or T0), in roots partially inhibited (35%) Foe spore germination (Figure 5). From the third to the fifth DAI, phenolic compounds synthesized in roots have been able to totally inhibit (100%) the germination of Foe spores. From the eighth to the 13th DAI, about 20% of the spores were inhibited from growing in the roots. However, from the 18th DAI, SGIR increased to ~80% before gradually decreasing to ~55% until the 28th DAI. Besides, in pseudobulbs, roughly 95% of the spores were also inhibited on the third DAI. Then dropping to less than 20% from the 5th to the 8th DAI, this SGIR rose to 100% at the 13th DAI before progressively dropping until the end of the experiment (18 to 28th DAI) in pseudobulbs.

In the sensitive cultivar, less than 50% of Foe spores

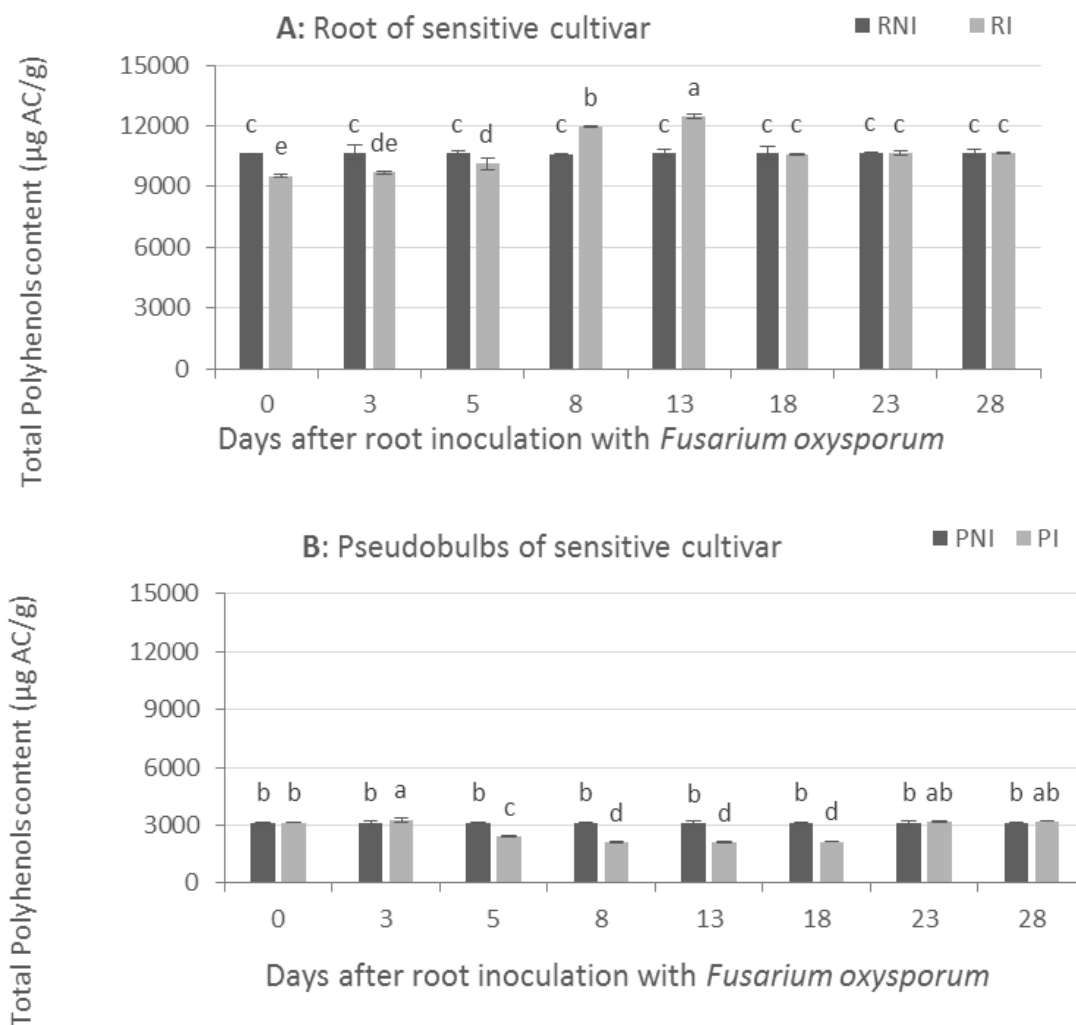


**Figure 3.** Evolution of total polyphenols content in roots (A) and pseudobulbs (B) of tolerant oil palm cultivar inoculated (I) or non-inoculated (NI) with *Fusarium oxysporum* (Foe). Histograms carrying the same letter are statistically equal (5% LSD test) and bars correspond to the standard deviations.  
Source: Author

were inhibited in both roots and pseudobulbs. The greatest number of fungal spores in roots (about 35%) was inhibited at the eighth DAI and in pseudobulbs (about 49.64%) at the 18th DAI. An example of the microscopic evolution of Foe spore germination in the presence of phenolic extracts of the tolerant cultivar is as shown in Figure 6.

#### Variation of phenolic compounds identified in root and pseudobulbs of both oil palm cultivars inoculated with Foe

The results from qualitative analysis of root and pseudobulbs extract chromatograms of both oil palm cultivars (sensitive and tolerant) obtained by GC-MS are



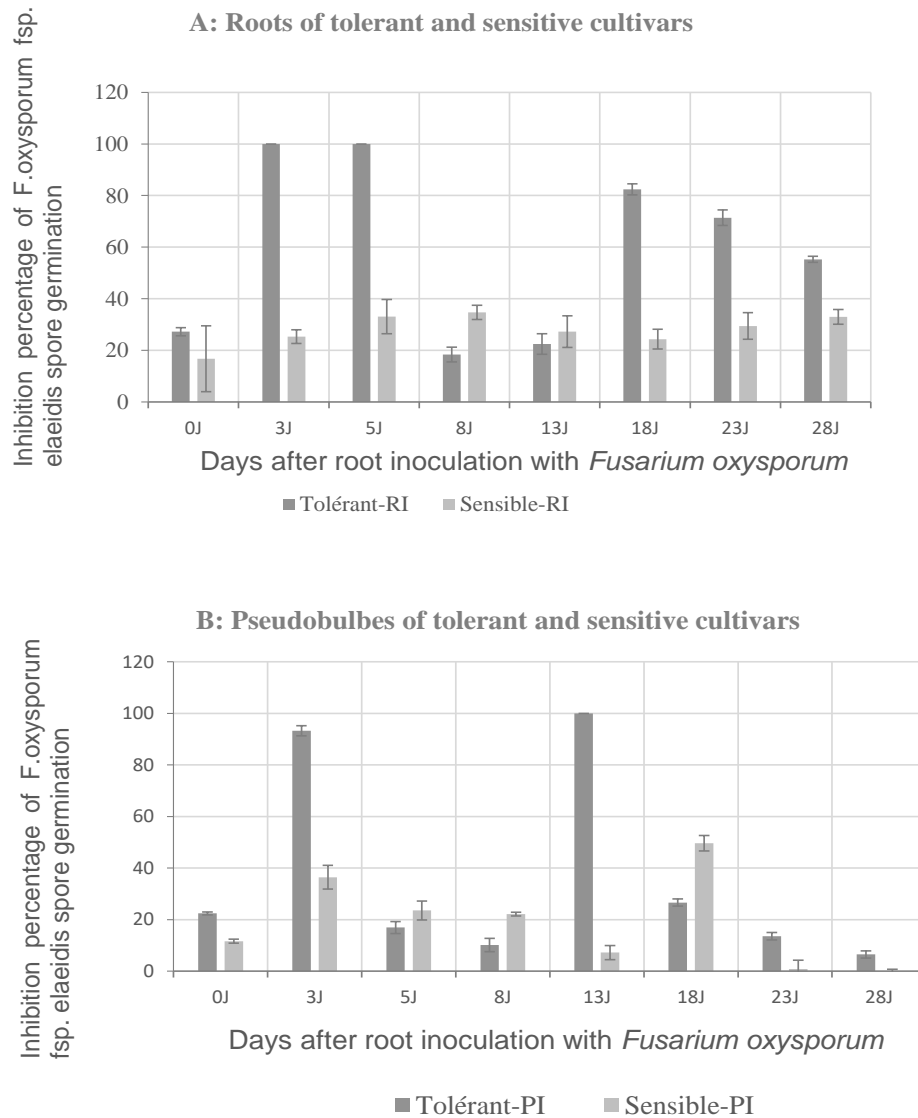
**Figure 4.** Evolution of total polyphenols content in roots (A) and pseudobulbs (B) of sensitive oil palm cultivar inoculated (I) or not inoculated (NI) with *Fusarium oxysporum* (Foe). Histograms carrying the same letter are statistically equal (5% LSD test) and bars correspond to the standard deviations.  
Source: Author

summarized in Table 1. For brevity, however, only few chromatograms necessary for a good explanation of these results were presented (Figures 7, 8 and 9). Both roots and pseudobulbs of inoculated and not inoculated plants of both cultivars expressed two phenolic compounds: 2,5-Di-tert-butylphenol (at 9.05 min) and orciprenaline (at 9.93 min) (Figures 7, 8 and 9). In addition, para-hydroxybenzoic acid (at 13.5 min) and meta-hydroxybenzoic acid (at 13.49 min) were also detected in the roots of control and inoculated plants of both cultivars, respectively (Table 1). In contrast, both compounds appeared only in the pseudobulbs of inoculated plants (Figure 8).

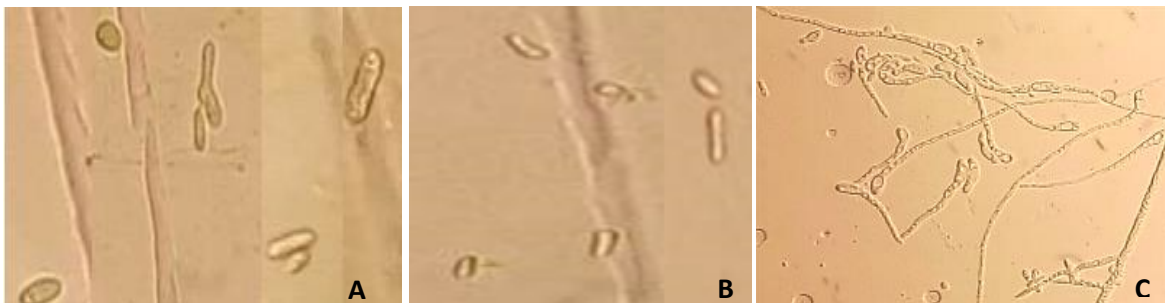
Analysis of Table 1 reveals that, with the exception of pseudobulbs from control plants of the tolerant cultivar, para-hydroxybenzoic acid (PHBA) was present in root

extracts from control (non-inoculated) plants of both cultivars (tolerant and susceptible). However, the presence of this compound (PHBA) only inhibited the germination of 30% of the fungal spores at T0. In addition, other phenolic compounds present in the pseudobulbs of the control and inoculated plants of the susceptible cultivar could not be detected.

Para-hydroxybenzoic acid (PHBA) persisted until the third day after inoculation (DAI) in the tolerant cultivar plants roots where it appeared in their pseudobulbs and completely inhibited *Foe* spores germination of both organs. At the fifth DAI, PHBA disappeared in these organs in favor of meta-hydroxybenzoic acid (MHBA) leading to the total inhibition (100%) of *Foe* spore germination in the roots (SGIR). From the eighth DAI, both antifungal compounds (PHBA and MHBA)



**Figure 5.** Evolution of *Foe* spore germination incubated with phenolic extracts from roots and pseudobulbes of tolerant and sensitive oil palm cultivars  
Source: Author



**Figure 6.** Microconidia and macroconidia of *Fusarium oxysporum* f.sp. *elaedis* showing complete inhibition of germination in the presence of phenolic extracts of roots (A) and pseudobulb (B) of the tolerant oil palm cultivar and its fungal filaments developing after complete germination without these extracts (C).  
Source: Author

**Table 1.** Phenolic compounds with fungitoxic activity identified by GC-MS in root and pseudobulb extracts of both (tolerant and sensitive) oil palm cultivars inoculated with *Fusarium oxysporum* f.sp. *elaeidis*.

Days after inoculation	Phenolic compounds found in roots extracts		Phenolic compounds found in pseudobulbs extracts	
	Tolerant Cultivar	Sensitive Cultivar	Tolerant Cultivar	Sensitive Cultivar
0	2,5-Di-tert-butylphenol			ud
	Orciprenaline			ud
	Para-hydroxybenzoic acid (PHBA)		Absent	ud
3	Absent	Meta-hydroxybenzoic acid (MHBA)	Absent	ud
	Para-hydroxybenzoic acid (PHBA)			ud
5	Absent	PHBA	Absent	ud
	Meta-hydroxybenzoic acid (MHBA)		Absent	ud
8	Para-hydroxybenzoic acid (PHBA)			ud
	Meta-hydroxybenzoic acid (MHBA)			ud
13	PHBA		Absent	ud
	Absent	MHBA		ud
18	Absent	PHBA		ud
	Meta-hydroxybenzoic acid	Absent	Absent	ud
23	Para-hydroxybenzoic acid		Absent	ud
	Absent	Meta-hydroxybenzoic acid	Absent	
28	Para-hydroxybenzoic acid		Absent	ud
	Meta-hydroxybenzoic acid	Absent	Absent	ud

ud: Undetermined by GC-MS.

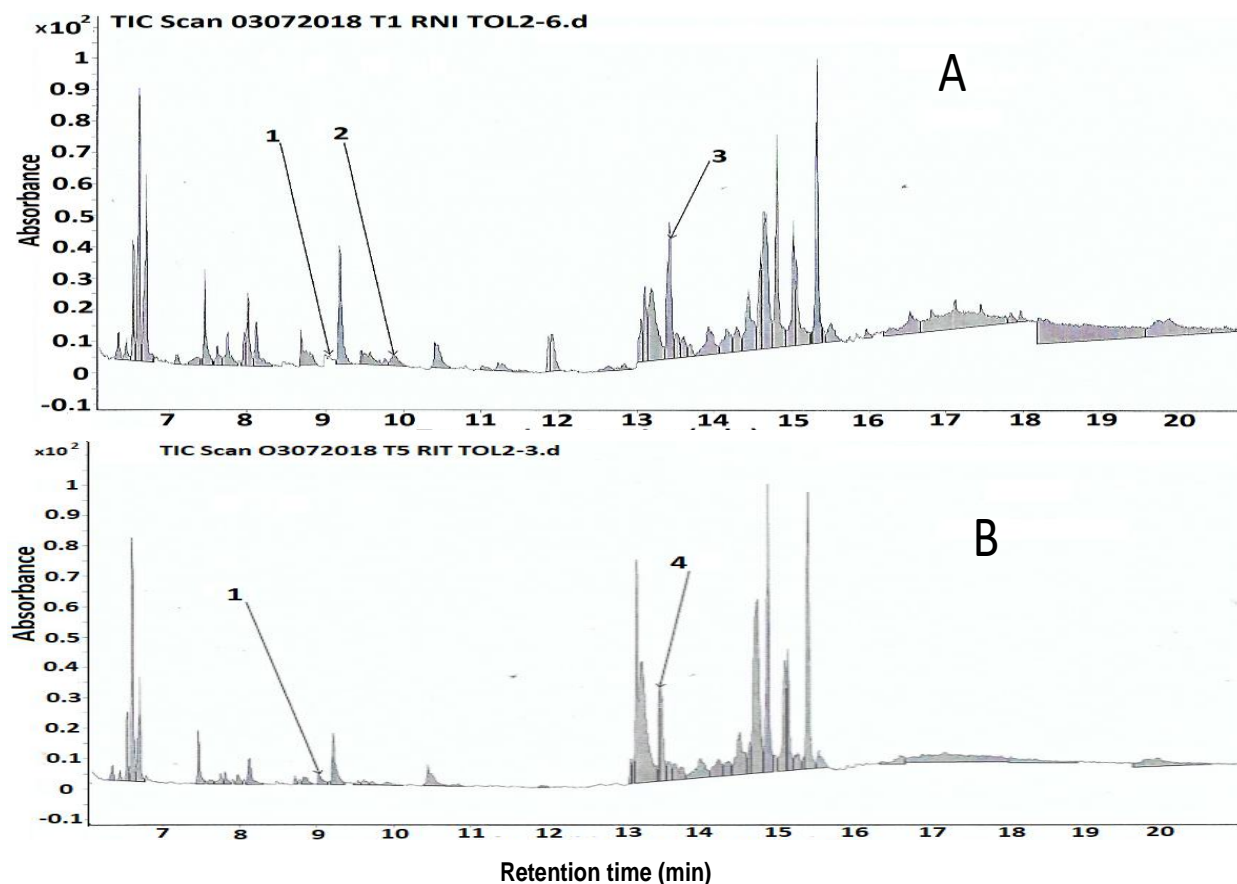
Source: Author

simultaneously persisted in the phenolic extracts of the roots and pseudobulbs, leading to a drastic decrease of the SGIR. This SGIR stilled decreasing in the roots at 13th DAI in the presence of PHBA while it increased in the pseudobulbs in the presence of MBHA alone. So, the presence of AMHB alone is sufficient to

promote the fungal spores germination inhibition through the SGIR. At 18th DAI, there was synthesis of meta hydroxybenzoic acid favoring the increase of SGIR in the roots. However, in the pseudobulbs, the para form of this acid was synthesized and this favored the fall of the SGIR in this cultivar. From the 23<sup>rd</sup> to 28th DAI, the

synthesis of both antifungal compounds (PHBA and MHBA) in the pseudobulbs of this tolerant cultivar plants was interrupted at the same time as the inhibition of the Foe spores germination. However, plants continued synthesizing both forms (para and meta) of the acid in the roots which did not block spore germination.





**Figure 7.** GC-MS chromatogram of phenolic compounds extracted from the oil palm tolerant cultivar roots not inoculated (A) and inoculated (B) with *Fusarium oxysporum* f. sp. *elaeidis*. The obtained compounds are 1: 2,5-Di-tert-Butylphenol (9.05 min). 2: Orciprenaline (9.93 min). 3: Para-hydroxybenzoic acid (13.5 min). 4: Meta-hydroxybenzoic acid (13.49 min). RNI: Roots of uninoculated plants. RIT: Roots from inoculated plants. Source: Author

In the sensitive cultivar, from the third to the 13th DAI, plant roots synthesized both fungitoxic compounds (PHBA and MHBA). However, their presence in small quantities did not prevent the germination of Foe spores. At the 180th DAI, only the para form of the acid was synthesized in the roots, while higher (about 50%) SGIR was expressed in the pseudobulbs of this cultivar. Then, from the 23rd to the 28th DAI, the SGIR was almost null in the pseudobulbs but increased in the roots without reaching 50% in the presence of both forms of the acid at the 23rd DAI and the para form alone at the 28th DAI. In this cultivar, meta-hydroxybenzoic acid content during the different organ sampling times is almost zero according to the amplitude of the peaks observed on the chromatograms.

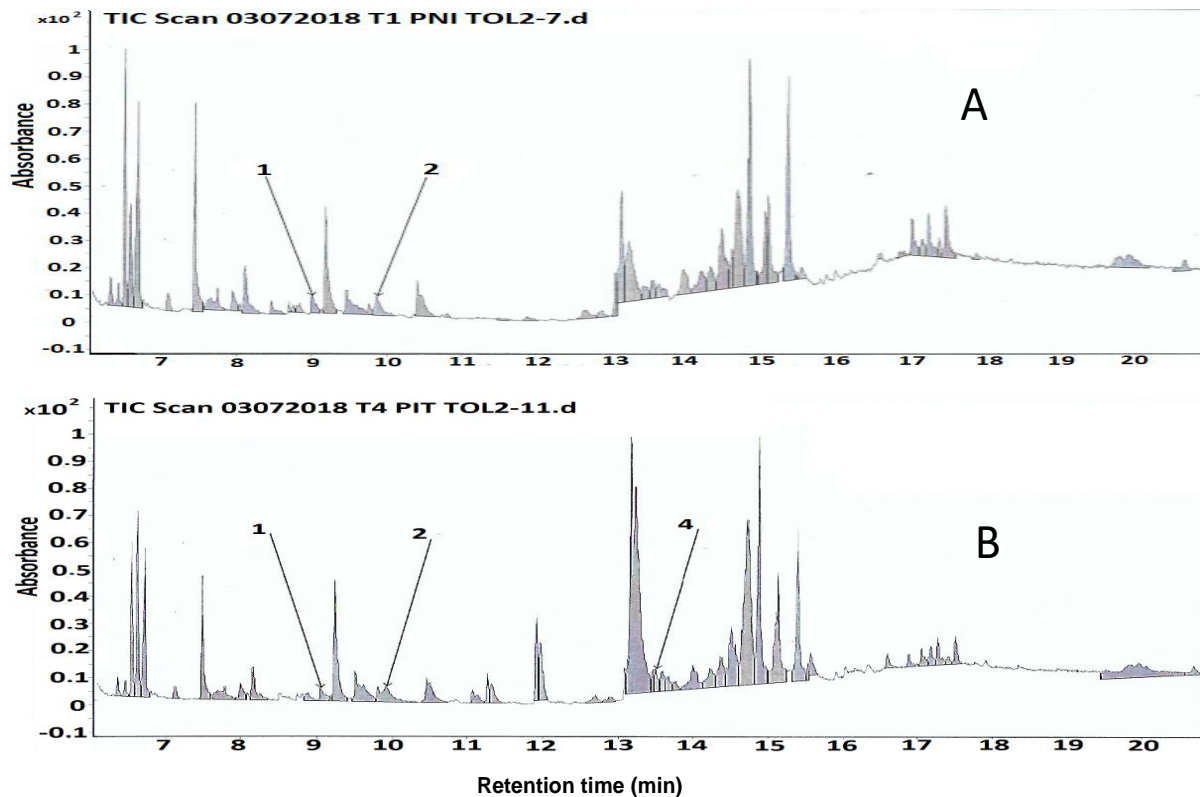
## DISCUSSION

This study aimed at investigating the spatiotemporal

distribution and identification of total phenolic compounds in roots and pseudobulbs of oil palm both tolerant and sensitive cultivars during the latency period after inoculation with the Foe pathogen.

Total phenolic compounds determination showed that oil palm naturally synthesizes and accumulates five times more phenolic compounds in roots than in pseudobulbs. Roots being the first organs exposed to this earthborn pathogen attack could explain this; plant would naturally synthesize constitutively phenolic compounds as pre-formed inhibitors.

Oil palm plants reaction to Foe infection, through the synthesis of phenolic compounds after infection, was early (1st DAI or T0) in the tolerant cultivar roots but late (8th DAI) in the sensitive one. Compared to the control, this increase in phenolic compounds content could be explained by their role in plant defense. Indeed, in response to infection, the plant synthesizes new phenolic compounds that are considered part of an active defense response (N'goran et al., 2019). These compounds are



**Figure 8.** GC-MS chromatogram of phenolic compounds extracted from the oil palm tolerant cultivar pseudobulb not inoculated (A) and inoculated (B) with *Fusarium oxysporum* f. sp. *elaeidis*. The obtained compounds are 1: 2,5-Di-tert-Butylphenol (9.05 min). 2: Orciprenaline (9.93 min). 3: Para-hydroxybenzoic acid (13.5 min). 4: Meta-hydroxybenzoic acid (13.49 min). PNI: Pseudobulb of uninoculated plants. PIT: Pseudobulb from inoculated plants  
 Source: Author

classified as phytoalexins and possess antimicrobial properties (Hammerschmidt, 1999). They accumulate in adjacent areas to infection sites and participate in plant wall reinforcement to slow pathogen penetration and confine it to these infection sites (Massala et al., 1980; Bompeix et al., 1981; Gogbeu et al., 2015a). Therefore, the tolerant cultivar's early response to infection could be explained by its rapid ability to recognize the pathogen.

This would have elicited defense mechanisms including new phenolic compounds synthesis as phytoalexins or the increase in phytoanticipin quantity (El Modafar et al., 1996). Furthermore, defense compounds were synthesized so late in the sensitive cultivar (up to the eighth day after infection) that they could not prevent the pathogen from establishing and developing, resulting in the disease. These results confirm the work carried out by Mondolot-Cosson et al. (1997) who showed that in the susceptible grapevine species (*Plasmopara viticola*), the lack of an early reaction is favorable to its important and rapid development because of the late and weak appearance of flavonoids. Therefore, the delayed synthesis of these compounds could be an indicator of plant resistance. Plant tolerance to infection depends

both on how quickly the plant detects the pathogen (Jones and Dangl, 2006) and on how quickly the defense mechanisms are set up to synthesize and accumulate certain compounds, including polyphenols (Mondolot-Cosson et al., 1997; Hammerschmidt, 1999). Other studies have established a positive correlation between the resistance of *Carmellia oleifera* and *Lupinus angustifolius* cultivars and the rapid and intense production of flavonoids (Calatayud et al., 1994; Chen et al., 2007).

Moreover 3rd DAI, all pseudobulbs of inoculated plants showed a phenolic reaction in both oil palm cultivars (tolerant and sensitive). This result shows that roots being the first organs exposed to the attack of this soilborne pathogen, when infected, they synthesized some signaling compounds such as salicylic acid (Dogbo et al., 2008; Gogbeu et al., 2012; Ojha and Chatterjee, 2012). These compounds migrate into the plant's higher organs (bulb or stem and leaves) and induce the synthesis of defense compounds for systemic resistance.

GC-MS analysis coupled with fungitoxicity tests of organs phenolic extracts reveals that para-hydroxybenzoic acid (PHBA) is naturally present in oil

palm roots and not in pseudobulbs. Previously, Manuja et al. (2013) made similar observations. PHBA is believed to be involved in the plant's defense system against pathogens. According to Sawada et al. (2006) under stress conditions, the induction and control of para hydroxybenzoic acid is important for antioxidant system because salicylic acid biosynthesis is catalyzed by 2-hydroxylase of benzoic acid and bound to para hydroxybenzoic acid.

On the 1st DAI, the possible increase of PHBA (phytoanticipin) content would have favored the inhibition of spore germination through the SGIR followed in the tolerant cultivar roots. Subsequently (at 3rd DAI), the rise in this PHBA content continued in the roots so that it appeared in the pseudobulbs to completely inhibit spore germination in both organs of the tolerant cultivar. Specifically, Smith et al. (1998) showed that PHBA is synthesized *de novo* in stems and petioles in response to a motile signal by *Pseudomonas syringae* pv. *Syringae*. Besides, the synthesis and accumulation of meta hydroxybenzoic acid (MBHA) alone was sufficient to keep Foe spore germination completely inhibited (100%) in roots at 5 DAI and subsequently in pseudobulbs at 13 DAI. To our knowledge, this is the first time that the fungitoxicity of this phytoalexin has been demonstrated in oil palm. It was found to be the most toxic form of phenolic acid to Foe on root and pseudobulb. According to Wu et al. (2009) and Manuja et al. (2013), para- and meta-hydroxybenzoic acids possess antifungal and bactericidal properties, whose effectiveness depends on their concentration. These authors also showed that at high concentration (1600 mg/L) on culture medium, para-hydroxybenzoic acid totally (100%) inhibits the germination of *F. oxysporum* f. sp. *niveum* spores. It demonstrates that their protective actions are indeed dependent on their concentration in plant. The presence of para- and meta-hydroxybenzoic acids in sufficient quantities in palm roots or pseudobulbs inhibits germination of Foe spores by close to 80 to 100%. Therefore, the fungitoxicity of these molecules is due to their optimal concentration reached after infection of the oil palms. In the sensitive cultivar, despite the individual or simultaneous presence of these same compounds, they could not totally inhibit (100%) the germination of spores in the studied roots and pseudobulbs. This could be explained by the fact that in this susceptible cultivar's roots, meta hydroxybenzoic acid is synthesized and accumulated in very low quantities as revealed by the chromatograms. Thus, the SGIR was 49.64% at 18th DAI in pseudobulbs in presence of para hydroxybenzoic acid and 35% in roots on 8th DAI in presence of both compounds. This result demonstrates that the sensitive cultivar is unable to quickly respond to infection by increasing the content of the pre-existing compound which only becomes toxic at high concentrations. Despite its earlier synthesis, meta-hydroxybenzoic acid (as early as the 3rd YD) is more than the tolerant cultivar. This is

because the low content of this compound would not allow a consistent antifungal activity to contain the pathogen; therefore, the disease settled. Clériver et al. (1996) and El Modafar et al. (1996) showed that plant resistance to infection and antimicrobial activity would be positively correlated to the content of pre-existing compounds called phytoanticipins. At 13th DAI, due to meta hydroxybenzoic acid, the tolerant cultivar would contain the roots infection and prevent its evolution to pseudobulbs at 18th DAI with a high content of this compound that converted to the para form until the end of the experiment. However, both compounds would not be the only ones contributing to the complete inhibition of Foe spores during infection. In fact, the work of Chong et al. (2011) highlighted the presence of a series of phenolic acids (syringic, caffeic and para-hydroxybenzoic acids) in oil palm root extracts as well as their *in-vitro* antimicrobial role against the fungus *Ganoderma boninense*.

Thus, the oil palm's defense reaction to Foe during the latency period is expressed by adjustments in the kinetics of the synthesis of phenolic compounds toxic to the pathogen. Consequently, this resistance type set up by the plant is not specific because it is not expressed in an all-or-nothing manner (resistance/sensitivity). Nevertheless, it could be of partial or quantitative sort. It therefore requires complex and often polygenic mechanisms as shown by Meunier et al. (1979) in oil palm. This resistance is less effective than monogenic resistance but is more appreciated by breeders. These results confirm that there are rather tolerant and non-resistant oil palm cultivars to fusarium.

This study could provide an opportunity to exploit the phenolic acid biosynthetic pathway in order to confer immunity to cultivars/hybrids sensitive to the disease but with good production.

## Conclusion

Oil palm synthesizes and accumulates phenolic compounds, whose nature and content vary with Foe lag time. Plant phenolic response was early (on 1st DAI or T0) in tolerant cultivar roots and late (up to 8th DAI) in sensitive ones. It occurred in the pseudobulbs by the 3rd DAI in both studied cultivars (sensitive and tolerant). The major phenolic compounds are para-hydroxybenzoic acid naturally synthesized in oil palm roots and meta-hydroxybenzoic acid which was found only in Foe infected cultivar roots as revealed by GC-MS. Both compounds were found in pseudobulbs only after infection.

The study showed that following infection with Foe, three mechanisms can take place: either the para-hydroxybenzoic acid content increases in roots, or plant synthesizes it *de novo* in pseudobulbs, or meta-hydroxybenzoic acid is induced in both organs as a function of time. On the contrary, Foe spore germination

inhibition rate depends on the para- or meta-hydroxybenzoic acid content synthesized in plant's organs. The simultaneous presence of the two fungitoxic compounds in the roots of plants of the susceptible cultivar did not completely inhibit the germination of *Foe* spores due to the small amount accumulated in them. This highlights the importance of both the dose of any substance for its effectiveness in any biological process.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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