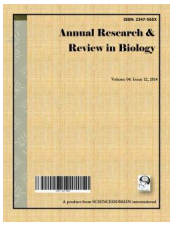

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Effects of Salicylic Acid and Acibenzolar-S-methyl on Phenols Content and Antioxidant Activity against Toxicity of Juglone in the Development of Black Leaf Streak Disease of Banana

Amari Ler-N'Ogn Dadé Georges Elisée[1*], N'guessan Aya Carine[2], Johnson Félicia[3], Chérif Mamadou[1] and Kone Daouda[1,4]

[1]Laboratoire de Physiologie Végétale, UFR Biosciences, Université Félix Houphouët Boigny, 22 BP 582, Abidjan 22, Côte d'Ivoire.
[2]Département de Biologie Végétale, UFR Sciences Biologiques, Université Péléforo Gon Coulibaly, BP 1328, Korhogo, Côte d'Ivoire.
[3]Laboratoire de Zoologie et Biologie Animale, UFR Biosciences, Université Félix Houphouët-Boigny, 22 BP 582, Abidjan 22, Côte d'Ivoire.
[4]Centre d'Excellence Africain sur les Changements Climatiques, la biodiversité et l'Agriculture Durable, Université Félix Houphouët-Boigny, 22 BP 582, Abidjan 22, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration between all authors. Authors ALNDGE, NAC and KD designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JF and CM managed the analyses of the study. Author ALNDGE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the effect of salicylic acid (SA) and acibenzolar-S-methyl (ASM) on the minimal concentration of juglone (C_{min}) inducing foliar necrosis- and to determine the total phenol content and antioxidant activity of extracts of banana leaves after SA and ASM applications and toxin injection.

**Corresponding author: E-mail: amariler3@gmail.com, amariler@yahoo.fr;*

Methodology: Banana cultivars Orishele and Corne 1 were subjected to root and foliar applications of elicitors, then leaves were injected with juglone a toxin of *M. fijiensis* to the determination of C_{min}. The determination of total phenols extracted from leaves was carried out using Folin Reagent. The antioxidant activity of phenolic crude extracts (PCE) was determined through the DPPH radical scavenging ability.
Results: In banana without elicitor applications, the minimum concentration inducing necrosis varied between 12.5 and 25 ppm of juglone but reached 250 ppm of juglone into banana treated with elicitors, particularly with ASM at 50 ppm. The phenol contents were highest 14 days after elicitors application. After this incubation time, with ASM at 50 ppm and AS at 25 ppm, the levels were 16.25 mg GAE/g DW in Orishele and 17.20 mg GAE/g DW in Corne 1, respectively. But the levels were lower 28 days after banana elicitation with 50 ppm of SA into Orishele and Corne 1 respectively 5.60 and 6.79 mg GAE/g DW. Treatments with 50 ppm of ASM showed the highest antioxidant activity between 7 and 28 days after elicitors application on banana leaves. With this treatment, the lowest concentration of phenolic crude extract scavenging 50% of DPPH was 13.09 µg/mL at 21 days after foliar elicitation.
Conclusion: The applications of elicitors SA and ASM affect phenol content and antioxidant activity for the detoxification of foliar tissues of banana cultivars Orishele and Corne 1 infiltrated with toxin.

Keywords: Banana; elicitors; phenolic compounds; juglone; oxidative stress; antioxidant activity.

1. INTRODUCTION

Black leaf streak disease (BLSD) caused by *Mycosphaerella fijiensis* is actually the most serious constraint on banana and plantain production in the world [1,2]. Juglone, one of the most aggressive toxins of *M. fijiensis*, contributes to the spread of necrotic foliar symptoms and increases the severity of banana BLSD [3]. At the subcellular level, juglone induces many biochemical effects such as disturbing the proton electrochemical gradient across the plasmalemma membrane [4]. Also, it is known that plants in contact with pathogens or their secondary toxic metabolites are inevitably exposed to different states of stress [5]. Oxidative bursts generate active oxygen species (AOS) such as superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH). These AOS can induce lipid peroxidation with membrane destruction, protein inactivation, or DNA mutation, resulting in tissue injury and the death of plants [5,6].

In banana production areas, chemical control is less effective against the damages caused by the infection of *M. fijiensis*. Consequently, the use of synthetic fungicides induces environmental negative impact, human health pollution and resistance of pathogen [7,8]. With these limitations of a conventional method to fight BLSD, induction of plant natural defence mechanisms against bio-aggressors constitutes a promising alternative strategy. Among the plant defence elicitors, chemical compounds including salicylic acid (SA) and its analogues, especially acibenzolar-S-methyl (ASM), would provide efficient protection against plant diseases [9,10].

Indeed, plants can develop multiple and complex defence strategies to limit pathogen invasions and to manage various environmental stress situations [11]. Plant protection mechanisms can reduce or completely eliminate AOS through enzymatic (superoxide dismutases, proxidases, catalases) and/or non-enzymatic (ascorbate, glutathione) antioxidant systems [6]. These defence responses can be artificially induced within plant through several metabolic pathways including salicylic acid pathway. Salicylic acid plays an especial role in resistance mechanisms against infections and others aggressions within plants. It is a plant immunity system (for `self - protection') [12]. The induced resistance can activate the production of defence proteins which can destroy aggressors and the synthesis of other proteins such as chitinases and glucanases, phytoalexins and secondary metabolites, especially phenolic compounds [13,14].

Most phenolic compounds are involved in defence reactions. These phenolic compounds are very sensitive to environmental variations and can be accumulated in the plant under the influence of various factors [15]. More recent studies have revealed the phenolic compounds involvement (hydroxycinnamic acid derivatives), in defence responses against different biotic and abiotic stresses [6,16]. However, there is limited information with regards to the role of the defence elicitors in the phenolic compounds

production against the development of BLSD symptoms in banana. This study aimed to evaluate the effect of SA and ASM against-juglone foliar necrosis induction and to determine the total phenol content and the antioxidant activity of leaf extracts after elicitor and toxin treatments of two BLSD-susceptible banana cultivar. Knowledge of the foliar necrosis induction and the variability in phenolic metabolism will help to select the most effective elicitor treatment against *M. fijiensis* infestation under natural conditions in BLSD-susceptible cv. Corne 1 and BLSD-highly susceptible cv. Orishele.

2. MATERIALS AND METHODS

2.1 Plant Material

Two banana cultivars showing differential behaviours against BLSD were used in this study. These are the BLSD-highly susceptible cv. Orishele and BLSD-susceptible cv. Corne 1 [17]. Both cultivars are from Plantain group and were produced from tissue culture (*in vitro*) on the solid medium of Murashige and Skoog [18] slightly modified and provided by Centre National de Recherche Agronomique (CNRA).

2.2 Synthesis Products

2.2.1 Toxin

Juglone (5-hydroxy-1,4-naphthoquinone), one of *M. fijiensis* toxins and marketed by Sigma-Aldrich, was used in this study. This toxin is strongly involved in the pathogenesis of *M. fijiensis* [19,20].

2.2.2 Plant defence elicitors

Plant defence elicitors used were 2-hydroxybenzoic acid called salicylic acid (SA) and its analogue S-methyl benzo (1,2,3) - thiadiazole-7-carbothiotate called acibenzolar-S-methyl (ASM). These elicitors especially ASM is an elicitor marketed in USA and France under the names of Actigard 50WG and Bion 50WG respectively [12].

2.3 Elicitor Applications on Banana

Plants of the two banana cultivars (Orishele and Corne 1) were grown in pots (1.5 L) containing soil and kept under greenhouse (12 h photoperiod and temperature of 25°C). Plants were used for SA and ASM treatments at the stage of 4-5 fully expanded leaves (age of 3-4 months).

Before elicitor applications, plants have not received water during two days then the first application of 50 mL of elicitor at 25 or 50 ppm was done on roots.

Seven days after the first elicitation, a second application was done with 20 mL of elicitor at the same concentration (25 or 50 ppm) sprayed on the leaves. Twelve (12) plants of each banana cultivar were used per treatment (concentration x elicitor).

Plants were incubated after the elicitation in a greenhouse under natural temperature and photoperiod conditions. Plants without elicitor treatment were used as controls. The plants without or with elicitor treatment were used at different periods for juglone injection. The whole experience was repeated three times.

2.4 Banana Plants Acclimatisation and Juglone Injection into the Leaf

One week (7 days) after banana leaves elicitation for one month, a toxin injection was performed according to the protocol described by El Hadrami et al. [6]. Juglone solutions at different concentrations (0; 12.5; 25; 50; 100; 250; 500 ppm) were injected into the leaves of banana plants previously incubated in a humid atmosphere (90% relative humidity). Distilled water and 10% methanol (0 ppm of juglone) were used to prepare juglone solutions and were constituted control solutions. The injection was weekly performed on the lower surface of the fully-expanded oldest leaf with a microsyringe. Four replicates (20 µL per injection site) per concentration and per leaf were conducted on three plants of each cultivar. The whole experiment was repeated independently three times. After the injections, banana plants were incubated in the greenhouse under ambient conditions. Banana plants reactions to elicitor and/or juglone applications were evaluated at different periods.

2.5 Determination of Minimum Concentrations of Juglone Inducing Foliar Necrosis

The effect of elicitation on foliar necrosis development was determined by the minimum concentration of juglone (C_{min}) inducing necrotic lesions 48 h after the injection of toxin. The C_{min}

was the lowest concentration of juglone causing the formation of necrotic lesions on the leaves. C_{min} reflects the effectiveness of elicitor treatment against the phytotoxicity of juglone. Necrosis was recognisable by brown or black spots at the injection sites.

2.6 Foliar Sample Collection and Preparation

During one month, foliar samples were weekly harvested after the elicitor application on the leaves of banana cultivars Orishele and Corne 1 no-treated with juglone.

However, each week during one month, a harvest of leaves was carried out at different periods following the injection of the juglone at 100 ppm into another batch of banana of the cv. Corne 1, after the application of elicitor on the leaves. These successive samples of leaves were made at 0; 6; 12; 24 and 48 hours after the injection of the toxin solution at 100 ppm. The 100 ppm concentration of juglone corresponds to the minimum concentration (C_{min}) of necrosis-inducing toxin for all treatment modalities of banana plants with or without elicitor.

After harvest, leaf samples were placed in clean plastic bags under shade and air dried. Samples from salicylic acid treatments at 25 and 50 ppm were identified with the codes AS 25 and AS 50 respectively, while those from acibenzolar-S-methyl treatments were assigned with the codes ASM 25 and ASM 50 respectively.

2.7 Extraction of Total Phenolic Compounds

The leaf samples were dried for 5 days in an oven at 35°C. After drying, leaf samples were ground into powder using a grinder (Moulinex type). Five (5) g of powder were suspended in 25 mL of methanol (80%) and 1 mL of sodium metabisulfite (0.5%). The suspension was then evaporated in an oven at 60°C until a dry residue is obtained. Five (5) mg of this residue were dissolved in 1 mL of methanol (80%) to prepare a solution of phenolic crude extract (PCE) at 5 mg/mL. For each sample, the phenolic crude extract was stored at 4°C in the dark until used for phenolic and antioxidant analysis.

2.8 Determination of Total Phenol Content

The total phenol content was determined using the Folin-Ciocalteu colourimetric method according to Aleman et al. [21] with a few modifications. The reaction mixture was composed of 250 µL of PCE (5%) and 250 µL of Folin reagent. After homogenisation and incubating at room temperature for 5 min, 500 µL of sodium carbonate (17%) were added to the reaction mixture and then adjusted with distilled water to final volume of 3 mL. The absorbance was measured at 750 nm with a spectrophotometer (Milton Roy Spectronic 1001 Plus) after homogenisation and incubation of the reaction mixture for 30 min at 60°C in the dark.

The total phenol content was determined following a set of standard solution of gallic acid (10 to 100 µg/mL). The results were expressed as mg Gallic Acid Equivalent (GAE) / g Dry Weight (DW) of the plant.

2.9 Evaluation of Phenols Antioxidant Activity by DPPH Test

Measurement of the antioxidant activity of the total phenols was performed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test according to the method of *Rawat* et al. [22] with a few modifications. In the presence of free radical scavengers (phenolic extract), DPPH (purple colour) is reduced to 2,2-diphenyl-1-acrylrylhydrazine (yellow colour).

The DPPH solution at 0.1 mM concentration was prepared in methanol (80% v/v) and stored at 4°C in darkness. A volume of 2 mL of DPPH solution was added to 2 mL of phenol crude extract (PCE) at different concentrations (25 - 250 µg/mL) previously prepared in 80% methanol. The reaction mixture was incubated at room temperature in the dark for 30 min. The DPPH reduction in absorbance was recorded at 517 nm with the spectrophotometer. The mixture of methanol (80%) and DPPH (0.1 mM) constituted the control (0 µg / ml of EPB) and the methanol at 80% was used as a blank. All measurements were done in triplicates.

The percent decolourisation (PD) of the DPPH solution by the phenolic crude extract (PCE), was calculated from control following the formula:

$$\mathrm{PD}\ (\%) = \frac{\mathrm{Ac} - \mathrm{As}}{\mathrm{Ac}} x\ 100$$

(where Ac represents the absorbance of the mixture without phenolic extract and As the absorbance of the mixture with phenolic extract)

The antioxidant activity of the phenolic crude extracts was expressed by the concentration (CD_{50}) decolorising 50% of the DPPH solution. Following percent decolourisation (PD), the PCE concentrations were converted to log concentration. The CD_{50} was calculated using the equations from the linearisation of the curves of PCE antioxidant activities (percent decolourisation of DPPH).

2.10 Statistical Analysis

The total phenol contents and antioxidant activities data were analysed using one-way analysis of variance (ANOVA 1) with the STATISTICA 7.0 software. The Newman-Keuls test at $P<0.05$ was used to compare the mean values in case of significant differences. The means were calculated from data collected on three replicates for each experiment.

3. RESULTS

3.1 Juglone Toxicity on Banana Leaf Tissues after Application of the Elicitors

During the 4 weeks after application of the elicitors on banana leaves, minimum concentrations of juglone (C_{min}) inducing necrosis were ranged from 12.5 to 250 ppm. C_{min} was relatively the highest at 14 and 21 days after elicitor treatments into Orishele and Corne 1. But, 7 and 28 days after the foliar application of elicitors, the C_{min} inducing necrosis formation were generally the least (Fig. 1, Table 1).

The minimum concentration of juglone inducing necrosis on leaves of cv. Orishele were the least (12.5 - 25 ppm) with control plants. With salicylic acid treatments, the C_{min} initiating necrosis was between 12.5 and 50 ppm. In particular, 14 days after application of SA at 25 and 50 ppm on banana leaves, the minimum concentration varied between 25 and 50 ppm of juglone (Table 1). ASM applications increased the minimum concentrations inducing necrosis ($C_{min} \geq 50$ ppm) compared to those of control and SA treatments. The highest C_{min} (250 ppm of juglone) was obtained 14 and 21 days after elicitation of banana leaves with ASM at 50 ppm (Table 1).

With the cv. Corne 1, the minimum concentrations of juglone inducing necrosis were the least (12.5 - 25 ppm) on the leaves of control plants (Table 1). Cmin for SA 25-treatment, were between 12.5 and 50 ppm of juglone, however, 25 ppm of juglone was required to induce foliar necrosis at 14 days after leaf elicitation. With SA at 50 ppm, Cmin was between 25 and 100 ppm of juglone from 7 to 21 days after foliar application of the elicitors, while necrosis appeared on leaves with 12.5 ppm of juglone 21 days after the foliar elicitation (Table 1). With ASM applications on roots then leaves of cv. Corne 1, it took a minimum of 50 ppm of juglone to induce foliar necrosis. A minimum of 100 ppm of juglone was required for necrosis appearance at 14 and 21 days after treatment with ASM 25 and at 7 and 14 days after treatment with ASM 50. The highest C_{min} (250 ppm of juglone) was recorded especially with ASM 50 from 7 to 21 days after leaf elicitation (Table 1).

3.2 Content of the Total Phenols after the Application of the Elicitors on the Banana Trees

Total phenol levels in banana leave varied generally with treatments (concentration and type of elicitor), time after elicitation, and banana cultivar (Figs. 2 and 3). Significant differences were observed between the effects of elicitor treatments on banana roots and leaves. A general decrease of phenol content from 17.20 to 5.60 mg GAE/g DW was recorded between 7 and 28 days after application of SA and ASM on the leaves of both banana cultivars. The contents were the highest 16.25 mg GAE/g DW into cv. Orishele and 17.20 mg GAE/g DW into cv. Corne 1 at 14 days after foliar elicitation with ASM 50 and SA 25 respectively (Figs. 2 and 3). However, phenol contents were the least 5.60 and 6.79 mg GAE/g DW at 28 days after foliar elicitation with AS 50 into cv. Orishele and cv. Corne 1 respectively. At 7 and 21 days after application of the elicitors, the total phenol contents were relatively the same into Orishele or Corne 1 leaves (Figs. 2 and 3).

3.3 Total Phenol Content of cv. Corne 1 Leaves after Elicitation and Juglone Injection

The total phenol content of cv. Corne 1 leaves were investigated during one month in banana plants submitted to the toxic effect of juglone at 100 ppm after roots and leaf applications of the elicitors. The variation of the phenol content following the time after the injection of juglone

was different with the period after the elicitation. High significant differences (P < 0,001) were recorded between the phenol contents of banana leaves after elicitor applications and toxin infiltration.

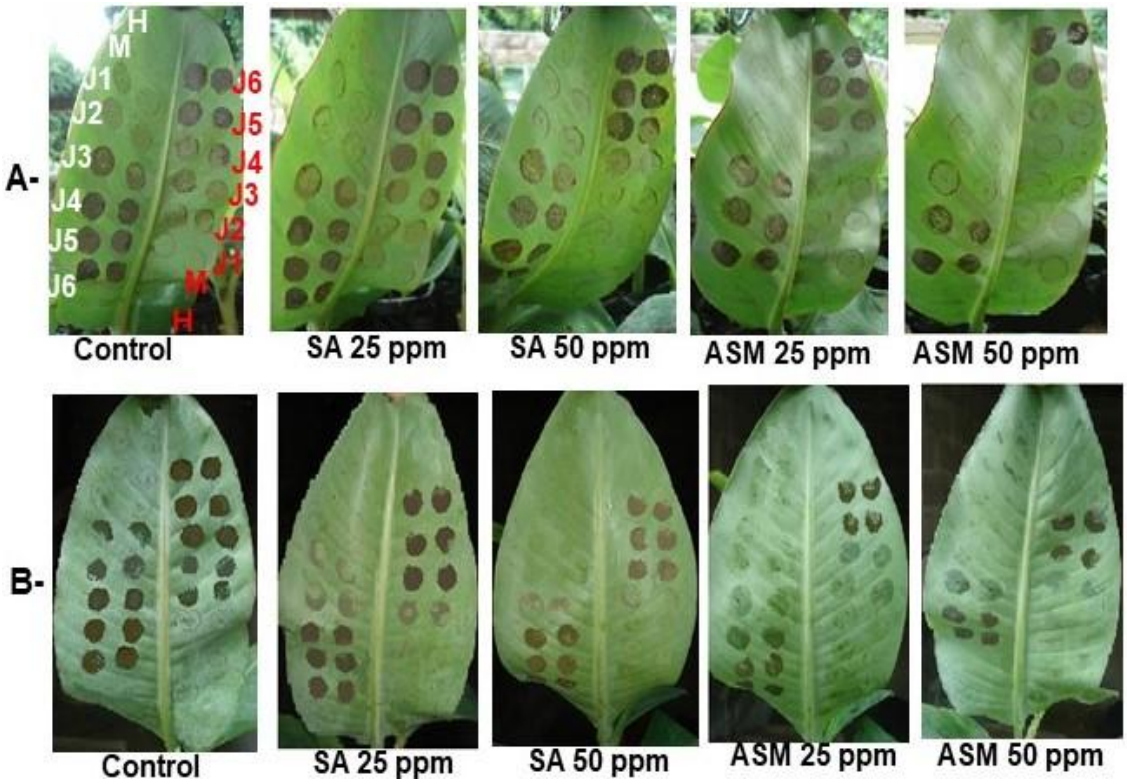

Fig. 1. Foliar necrotic lesions induced 48 h after injection with distilled water (H) or 10% methanol (M) or 12.5 ppm (J1) or 25 ppm (J2) or 50 ppm (J3) or 100 ppm (J4) or 250 (J5) or 500 ppm (J6) of juglone in banana cultivars Orishele (A) and Corne 1 (B) treated with salicylic acid (SA) and acibenzolar-S-methyl (ASM) or without elicitor treatment (Control).

Table 1. Minimum concentrations of juglone (C_{min}) inducing foliar necrosis at different times after application of the elicitors (SA and ASM) on the roots and leaves of banana cultivars (Orishele and Corne 1)

Cultivar of banana	Treatment	The range of minimum concentrations of juglone (ppm) inducing necrosis following the number of days after elicitor application on banana leaves			
		7	14	21	28
Orishele	**Control**	12,5 - 25	12,5 - 25	12,5 - 25	12,5 - 25
	AS 25	12,5 - 50	25 - 50	12,5 - 50	12,5 - 50
	AS 50	12,5 - 25	25 - 50	12,5 - 50	12,5 - 50
	ASM 25	50 - 100	50 - 100	50 - 100	25 - 50
	ASM 50	50 - 100	100 - 250	50 - 250	50 - 100
Corne 1	**Control**	12,5 - 25	25	12,5 - 25	12,5 - 25
	AS 25	12,5 - 50	25 - 50	25 - 50	25 - 50
	AS 50	25 - 50	25 - 100	25 - 50	12,5 - 50
	ASM 25	50 - 250	100 - 250	100 - 250	50 - 100
	ASM 50	100 - 250	100 - 250	250	50 - 250

SA and ASM refer to salicylic acid and Actigard 50WG, respectively, while 25 and 50 represent concentrations (ppm) of these products.

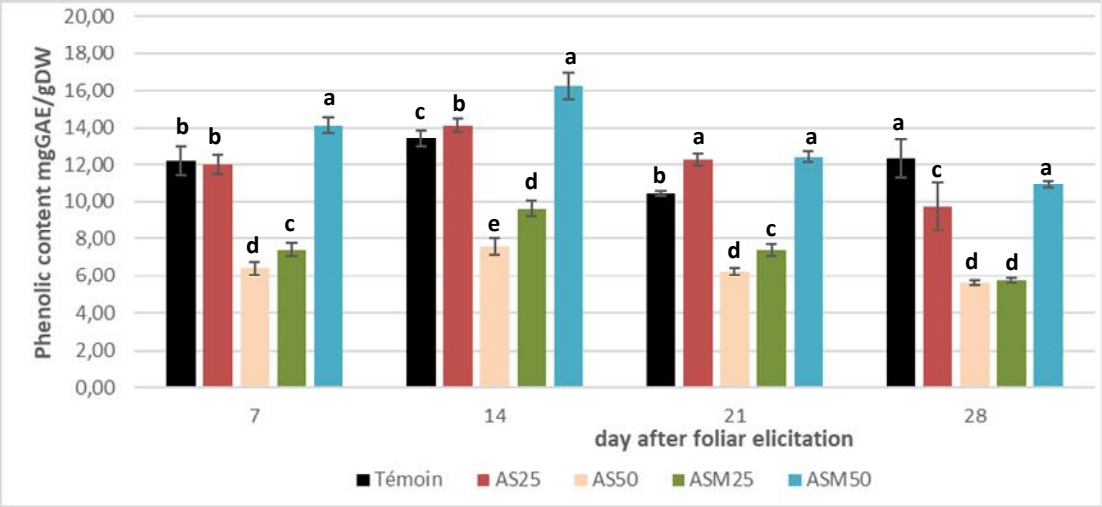

Fig. 2. Total phenol content in cv. Orishele leaves following different periods after application of the elicitors (SA and ASM) on the roots and leaves of banana plants

At 7 and 14 days after leaf applications of elicitors, the variation of the phenol contents during the time after juglone injection in the leaves was the same between the elicitor-treatments (Figs. 4 and 5). Between 0 and 6 h after the infiltration of the toxin, a high decrease of the phenol contents was observed in particular at 14 days after elicitation with ASM 50 (from 15.37 to 5.12 mg GAE/g DW). But, a weak increase of phenol contents was recorded after 6 h with all elicitor-treatments. However, between 24 and 48 h another decrease of phenol contents was observed in particular with plants of untreated control and those treated with SA 25 (Fig. 5). With ASM at 25 and 50 ppm treatments, between 24 and 48 h after juglone injection, the phenol contents were stable in banana leaves (Figs. 4 and 5).

At 21 and 28 days after banana elicitation, the variation of the phenol contents in injected leaves of juglone at 100 ppm was generally different of those observed at 7 and 14 days after leaf elicitation (Figs. 6 and 7). The decrease of the phenol contents during the first hours (between 0 and 6 hours after juglone infiltration) was weak particularly at 21 days after banana treatments with SA at 50 ppm and ASM at 25 and 50 ppm. However, during the same period, with SA 25 treatment, an increase of the phenol contents was observed, whereas the decrease of phenol content occurred at 6 h after juglone injection (Fig. 6). At 28 days after leaf application of the elicitors, treated banana with ASM at 50 ppm was showed again a high decrease of phenol contents in the first hours (between 0 and 6 h) after juglone injection. With the other elicitor-treatments, the decrease of phenol contents occurred later; at 6 and 12 h after juglone-injection in the bananas leaves for SA 25 treatment and SA 50 and ASM 25 treatments respectively (Fig. 7). Between 24 and 48 h, the effects of elicitor-treatments on phenol content were similar at 21 and 28 days after the elicitation on the contrary of untreated-control (Fig. 7).

3.4 Antioxidant Activity of Phenolic Crude Extracts on DPPH after Elicitation of Banana Roots and Leaves

The antioxidant activity of the phenolic crude extracts (PCE) of the leaves of cv. Corne 1 treated with or without elicitors, was varied according to PCE-concentration, type and concentration of elicitor and period after elicitation (Figs. 8, 9, 10 and 11). The variations of percent decolourisation of DPPH were significant differences ($P < 0.001$) between elicitor-treatments. The antioxidant activity of PCE was increased with increase in PCE concentration. At 25 µg/mL of PCE, low antioxidant activities were recorded with the percentages of DPPH-decolourisation less than 50% during 4 weeks after the elicitor applications except for the treatment with ASM at 50 ppm (Figs. 8, 9, 10 and 11). Indeed, for this elicitor treatment after 14 and 21 days, 25 µg/mL of phenol extract was discoloured more than 50%

of the DPPH (Figs. 9 and 10). On the contrary, for the other treatments with elicitors, the percent decolourisation of the DPPH was more than or equal to 50% with the PCE concentration at 50 µg/mL and more than (Figs. 8, 9, 10 and 11). However, for the control without elicitor treatment, a concentration of phenolic extract more than 50 µg/mL, was necessary to obtain a percent decolourisation of DPPH more than or equal to 50% (Figs. 8, 9, 10 and 11).

With the lowest CD_{50} and between 13.09 and 33.18 µg/mL for 50% decolourisation of DPPH, the treatments with ASM at 50 ppm were shown the highest antioxidant activity at 14 and 21 days after elicitor applications on the banana leaves (Fig. 12). ASM-treatments were exhibited the highest CD_{50} at 7 and 28 days after foliar elicitation. The lowest and the most active CD_{50} (13.09 and 26.46 µg/mL) were observed with ASM 50 and ASM 25 treatments at 21 days after foliar elicitation. With salicylic acid treatments, the most active CD_{50} (39.55 and 36.55 µg/mL) was noticed at 7 and 14 days after elicitation for AS 25 and AS 50 respectively. For the control (without elicitor-application), the lowest CD_{50} (72.98 µg/mL) was obtained at 14 days after elicitor-applications. CD_{50} is higher and less active with PCE of control leaves (Fig. 12).

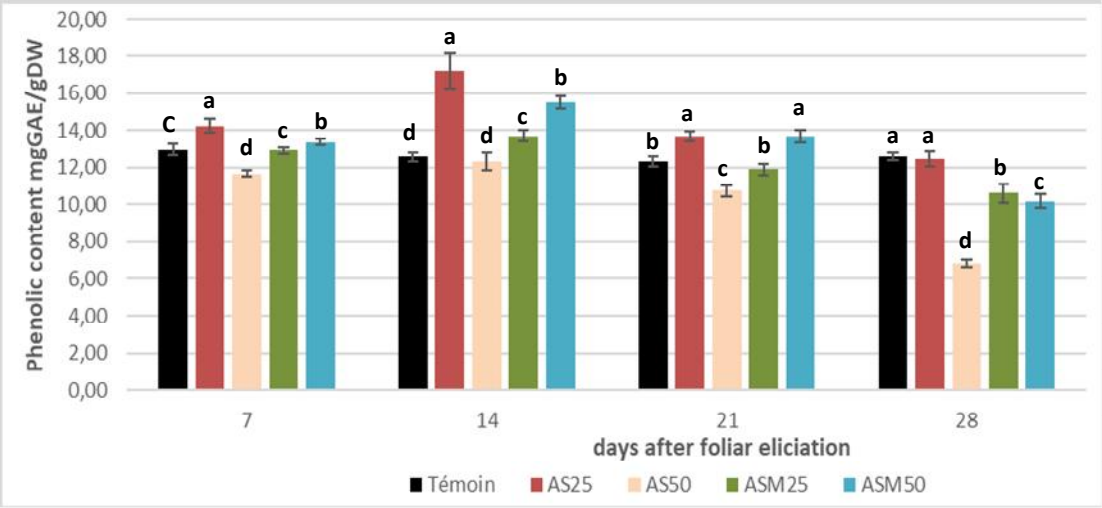

Fig. 3. Total phenol content in cv. Corne 1 leaves following different periods after application of the elicitors (SA and ASM) on the roots and leaves of banana plants

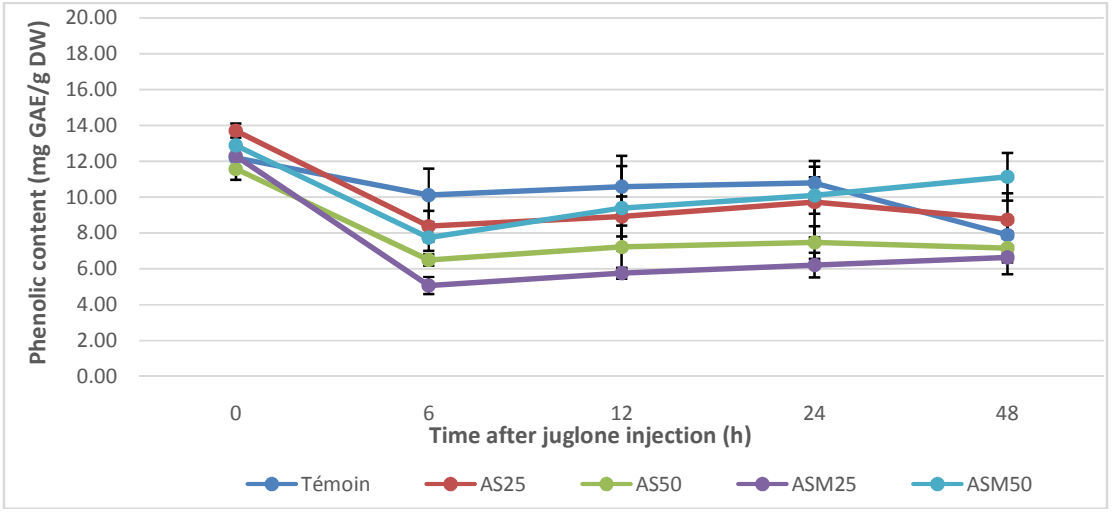

Fig. 4. Total phenol content of leaves at different times after juglone injection into cv. Corne 1 at 7 days after applications of the elicitors (SA and ASM) on the roots and leaves

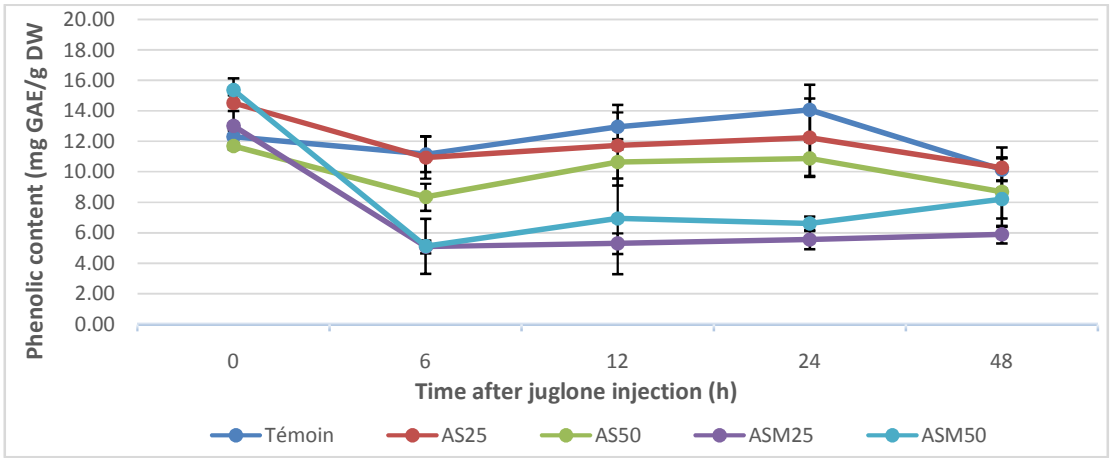

Fig. 5. Total phenol content of leaves at different times after juglone injection into cv. Corne 1 at 14 days after applications of the elicitors (SA and ASM) on the roots and leaves

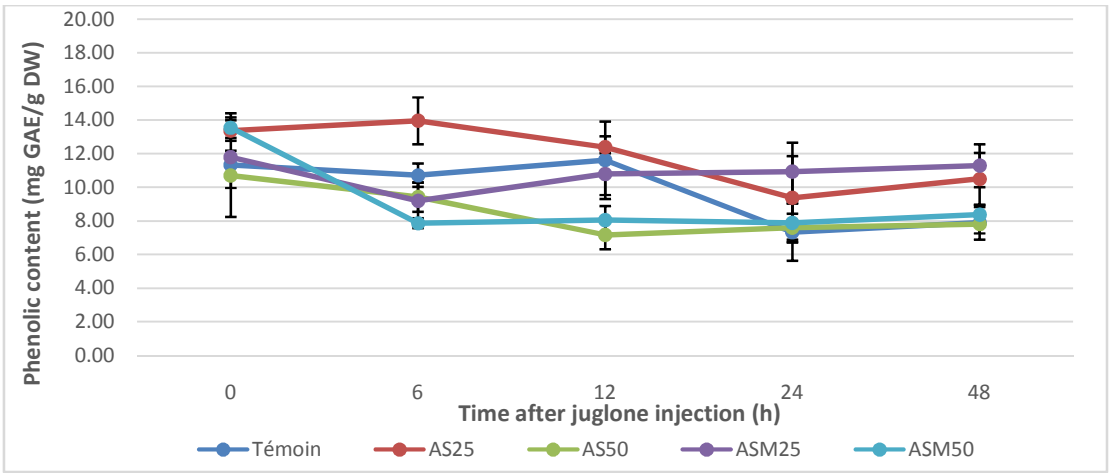

Fig. 6. Total phenol content of leaves at different times after juglone injection into cv. Corne 1 at 21 days after applications of the elicitors (SA and ASM) on the roots and leaves

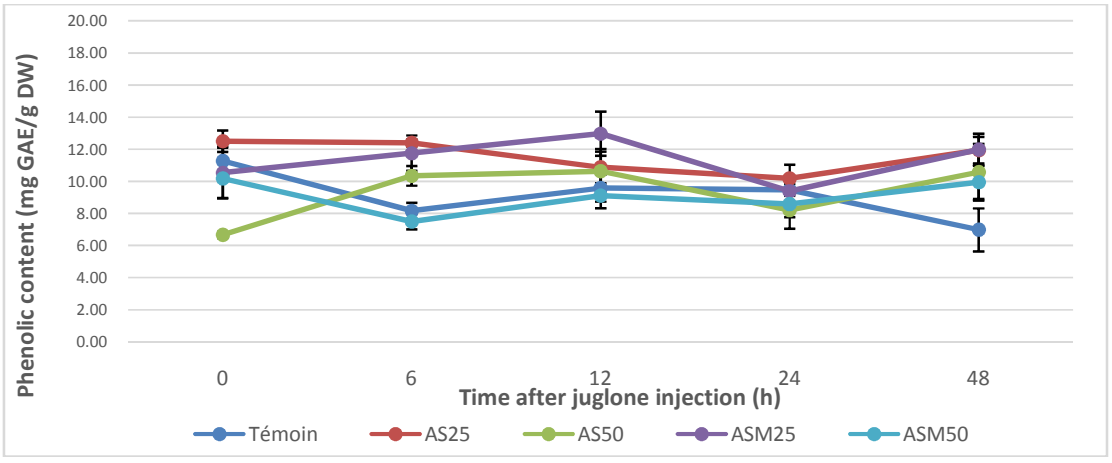

Fig. 7. Total phenol content of leaves at different times after juglone injection into cv. Corne 1 at 28 days after applications of the elicitors (SA and ASM) on the roots and leaves

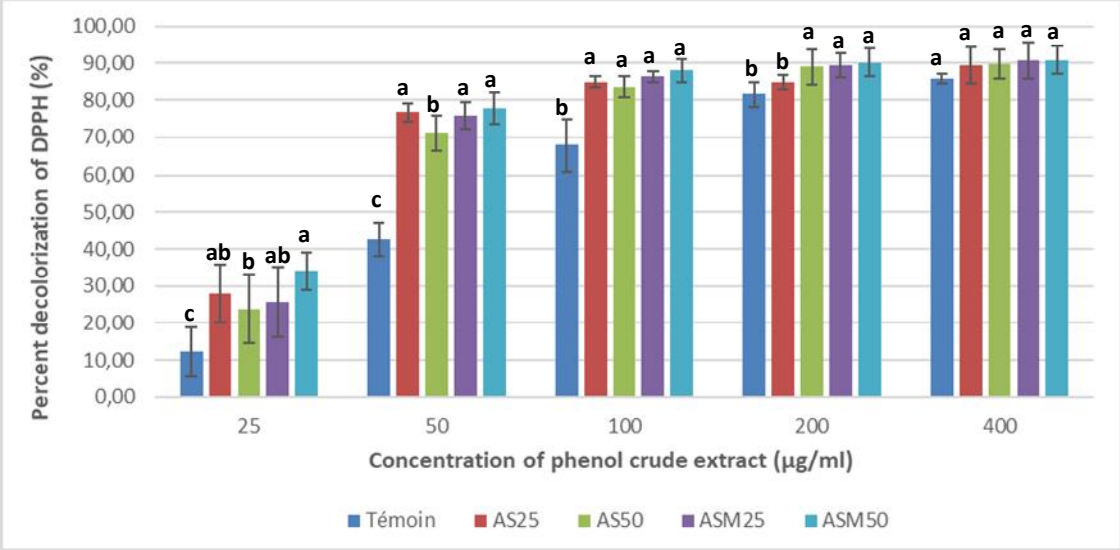

Fig. 8. Percent decolourisation of DPPH by phenol crude extracts of Corne 1 at 7 days after application of the elicitors (SA and ASM) on the roots and leaves

4. DISCUSSION

4.1 Protective Effect of Plant Defence Elicitors against the Toxicity of Juglone

Performance of plant defence elicitors such as salicylic acid (SA) and acibenzolar-S-methyl (ASM) was tested against the toxic effect of juglone injected in leaves of cv. Orishele and Corne 1 (susceptible to BLSD caused by *M. fijiensis*). SA and ASM increased banana resistance against foliar necrosis induction after juglone injection. Following elicitor applications on roots and leaves, the foliar necrotic symptoms due to juglone injection have been related to toxin concentration, banana cultivar, concentration and nature of elicitor and time after elicitor-treatment [9]. After banana elicitation, the minimum concentrations of juglone inducing necrosis formation were higher than those of untreated-control. Minimum concentrations reached 250 ppm of juglone for cv. Corne 1 treated with ASM against 12.5 ppm of juglone for control. The highest levels of resistance to necrosis induction were generally obtained 2 or 3 weeks after application of the elicitors. Similar results were obtained in tomato after treatment with BABA (β-aminobutyric acid) and ASM elicitors against bacterial wilt caused by *R. solanacerum* [10]. The present findings show that the high protection of banana plants was obtained between 2 and 3 weeks after ASM applications.

ASM has confirmed its reputation as an excellent elicitor for plant protection against pathogens or their toxic metabolites than in opposite of salicylic acid [12]. The difference in performance between ASM and salicylic acid would be related to the involvement level in the metabolic pathway. In fact, in the regulation of the metabolic pathway the role of ASM occurs after that of SA [23,24]. Since the protective effect of SA at 50 ppm observed in the present study was lower than these of SA at 25 ppm, it may suggest that there is a possible toxicity of the salicylic acid at concentrations of 50 ppm and more, as reported by Blanchard and Limache [12].

4.2 Effect of Elicitation on the Quantity and the Antioxidant Activity of Phenolic Compounds

The applications of the Salicylic acid and acibenzolar-S-methyl induced a modification of the metabolism of the phenolic compounds and the antioxidant activity able to detoxify foliar tissues of the banana cv. Orishele and cv. Corne 1, after injection of juglone at 100 ppm. These responses were related to the banana cultivar, the concentration and the nature of elicitors and the period after their applications and the time after toxin injection.

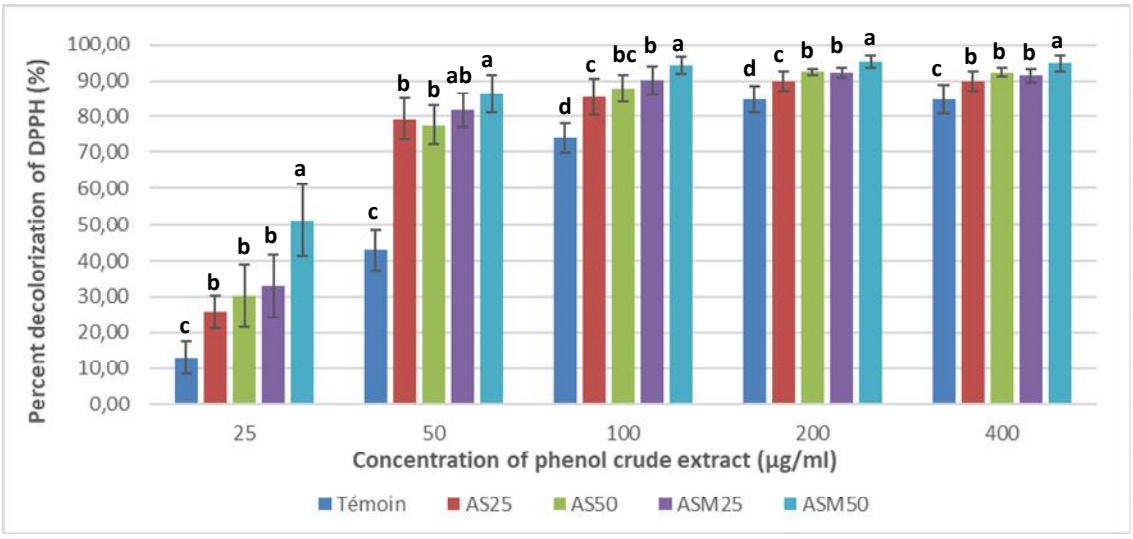

Fig. 9. Percent decolourisation of DPPH by phenol crude extracts of Corne 1 at 14 days after application of the elicitors (SA and ASM) on the roots and leaves

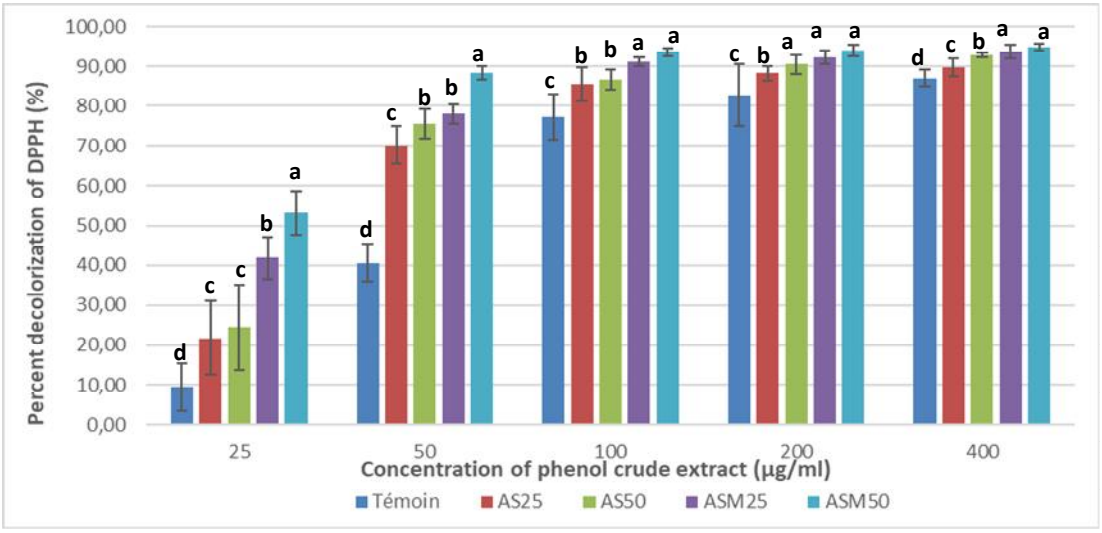

Fig. 10. Percent decolourisation of DPPH by phenol crude extracts of Corne 1 at 21 days after application of the elicitors (SA and ASM) on the roots and leaves

Fluctuations were observed on the synthesis of total phenols between 7 and 28 days in the plants of both banana cultivars treated with the elicitors. Cultivar Corne 1 was shown relatively higher levels of phenols than cv. Orishele, it could be explained by the difference of behaviour to BLSD between these two tested cultivars. The findings are similar to those of El Hadrami et al. [6] who found that there is a difference in the content of performed polyphenols observed in BLSD-susceptible cv. Grande Naine as compared to BLSD-tolerant cv. Fougamou. After application of the elicitors, phenol concentrations were higher at 14 days after elicitation, particularly with salicylic acid treatments at 25 ppm and ASM at 50 ppm. With AS 50 ppm and ASM 25 ppm, phenol levels were lower, especially in cv. Orishele. This might explain the low protective effect to juglone necrosis induction observed for banana plants treated with SA at 50 ppm. However, this low accumulation of phenolic compounds in banana plants, especially with applications of ASM at 25 ppm, which also induces in the plants a high level of resistance to

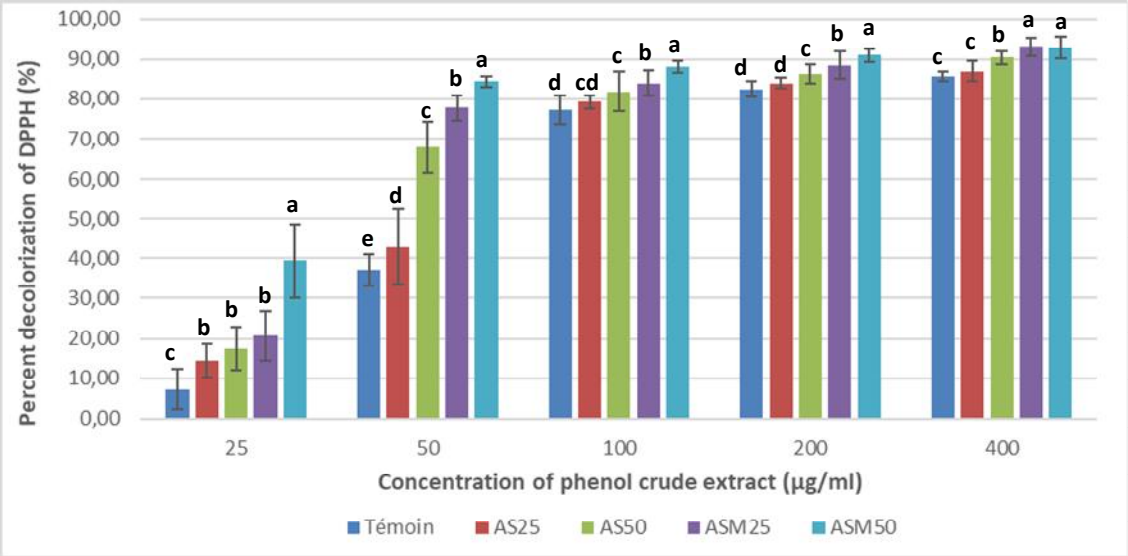

Fig. 11. Percent decolourisation of DPPH by phenol crude extracts of Corne 1 at 28 days after application of the elicitors (SA and ASM) on the roots and leaves

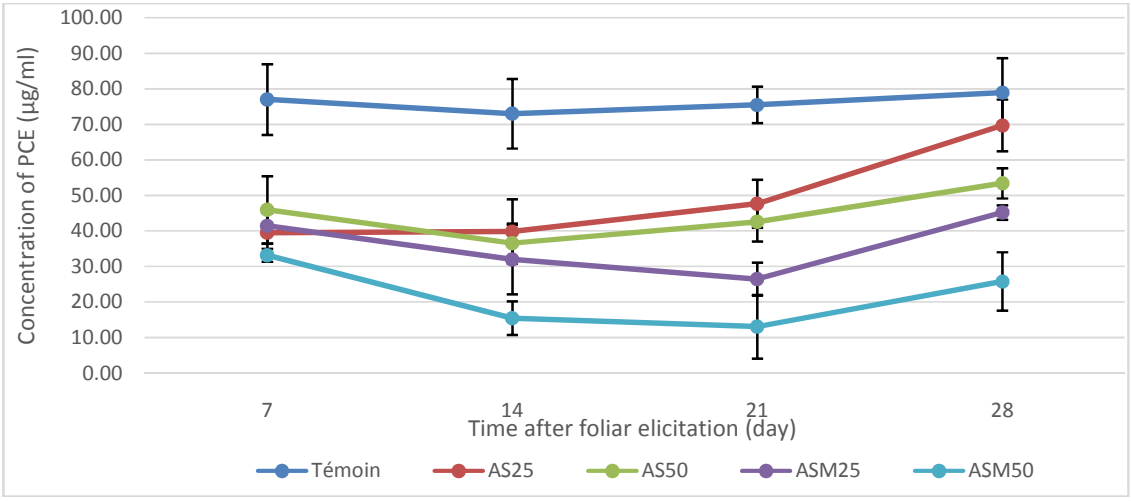

Fig. 12. Total phenol content of crude extract (PCE) for 50% decolourisation of DPPH (CD_{50}) after different periods of application of the elicitors (SA and ASM) on the roots and leaves

juglone necrosis induction, reveals an apparent contradiction between the phenol content and the resistance to the toxin. Such as contradiction can be attributed to the fact that the phenolic compounds are not only the antioxidant system which determines the resistance to juglone and therefore to BLSD. Other compounds might be involved in the mechanisms of protection against oxidative stress. Indeed, it has been reported the involvement of PR proteins, enzymes such as peroxidases and glucanases, lipopeptides (lipoxygenase, lipid hydroperoxidase) in plant defence mechanisms [25,26]. An increase in the phenolic compounds content was also observed in cassava leaves after injury and application of salicylic acid at 5 mM with the study of Dogbo et al. [27] which showed. N'Cho et al. [28] recorded high levels of phenols at 24 h, and 96 h in cotton leaves (*Gossypium hirsutum* L.) treated with 1 mM BTH and 2.5 mM SA, respectively.

Also, the low phenol content in banana leaves after elicitation especially with SA at 50 ppm might confirm the phytotoxicity associated with

an excessively increase concentration of this elicitor [12]. But with ASM at 25 ppm, the concentration of this elicitor would still be low to induce an abundant production of certain phenolic compounds. The resistance to toxicity of juglone might depend more on the species and the quantities of phenolic compounds. This is the case with applications of SA at 25 ppm which resulted in relatively high levels of phenols but with less active phenolic crude extracts (PCE) to reduce the DPPH radicals and consequently with high CD_{50} (phenol crude extract concentration to reduce 50% of DPPH). This elicitor-treatment resulted in low C_{min} (low concentration of juglone for necrosis induction).

Similar variations of phenol content in the leaves injected with juglone at 100 ppm were observed at 7 and 14 days after elicitor applications. This was different at 3 and 4 weeks after foliar elicitation, where the variations of the phenol content were different between elicitor-treatments. The time after elicitor-application might determine phenolic compounds biosynthesis scavenge of oxidative stress due to the injection of the toxin into the leaves. The decrease in phenol content quickly after juglone injection is an important indicator of banana susceptibility to the physiological and biochemical disturbance due to the toxin effects. Such disturbances have been reported by Traore et al. [17] after inoculation of banana plants with nematodes. However, with the highest decreases in phenol content, plants treated with acibenzolar-S-methyl, showed the highest scavenging ability to oxidative stress generated in plant tissues by juglone. This excellent antioxidant activity was also demonstrated with the DPPH test of phenolic extracts from banana leaves treated with ASM. Indeed, the total phenol concentrations (CD_{50}) for 50% decolourisation of DPPH, the lowest and consequently the most active, were recorded at 2 and 3 weeks after treatments with ASM at 25 and 50 ppm. The results of this study seem to highlight the persistence of the protection induced by salicylic acid and acibenzolar-S-methyl in the banana plant, i.e. 2-3 and 3-4 weeks respectively after application of these elicitors [9].

5. CONCLUSION

This study has shown that plant defence elicitors induce resistance to *M. fijiensis* toxins involved in the BLSD development. Banana plants treated with elicitors have relatively high levels and high antioxidant capacities, particularly with ASM at 50 ppm. ASM compared to SA is an excellent elicitor of defence mechanisms of the banana plant against black leaf streak disease. In opposite of cv. Orishele, cv. Corne 1 was less susceptible to the toxicity of juglone with or without applications of defence elicitor. After infiltration of juglone in banana leaves, the variation in phenolic metabolism occurs differently with cvs Orishele and Corne 1 and with periods after elicitor-applications. Following the period after elicitation, banana plants have developed various protective mechanisms to reduce or scavenge the active oxygen species induced by the toxin. A period of 3 weeks would be required for the frequency of elicitors application (salicylic acid and acibenzolar-S-methyl) in banana in natural conditions. However, resistance to oxidative stress generated by juglone could more depend on the qualitative biosynthesis of phenolic compounds and other compounds. Investigations should be conducted into other biochemical and molecular analysis on the determinism of induced resistance into the banana plant by the elicitor AS and ASM.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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