# EFFECT OF ELICITATION OF METHYL JASMONATE, SALICYLIC ACID AND CALLIETE IN LINKING WITH THEIR POST-TREATMENT CONTACT TIME ON ACCUMULATION OF PHENOLIC COMPOUNDS IN PINEAPPLE (ANANAS COMOSUS L.)

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## Abstract:

*The aim of this study was to evaluate the effect of three elicitors on the biosynthesis of phenolic compounds in two pineapple cultivars as a function of incubation time. The cultivars Cayenne smooth and MD2 were used and their leaves were sprayed with different concentrations of methyl jasmonate, salicylic acid and calliete. For each concentration, several incubation times were evaluated and then the concentration combined with the incubation time of each elicitor that caused the greatest accumulation of phenolic compounds in the leaves was selected. The best combination of elicitors was determined by coupling them two by two. Thus, the phenolic compounds induced in the leaves of the two cultivars sprayed with the best elicitor combination were identified by HPLC. The results showed that MeJA resulted in the best accumulation of phenolic compounds for a concentration of 10 mM after an incubation time of 72 h, followed by 5 mM SA after an incubation time of 24 h and 5 mM Ca after 48 h of post-treatment. The best combination of elicitors was MeJA and Ca after 72 h incubation. NWP from the smooth Cayenne cultivar induced six (6) phenolic compounds while PTCa, PTM and PTMCa synthesized eight (8), ten (10) and twelve (12) phenolic compounds, respectively. NWP from cultivar MD2 induced eight (8) phenolic compounds while TPCa caused de novo synthesis of ferulic acid. PTM induced de novo pterosilbene and kaempferol in addition to those of PTCa whereas PTMCa induced in addition to those of PTM, quercetin synthesis. The elicitors thus increased the level of accumulation of phenolic compounds in the treated plants..*

**Keywords**: *Elicitors, Phenolic compounds, pineapple, incubation time, Smooth Cayenne, MD2*

**Abbreviations**: *SA, Salicylic Acid; Ca, calliete; MeJA, methyl jasmonate, HPLC, High Performance Liquid Chromatography. PNT: untreated plants; PTCa: plants treated with Calliete; PTAS: plants treated with Salicylic Acid; PTM: plants treated with MeJA; PTMCa: plants treated with MeJA and Calliete*

## Introduction

Pineapple is a tropical plant, native to South America (Jacobs, 2010). The species *Ananas comosus* (L. Merril) is the most cultivated mainly for its edible fruit (Kanga, 2022). World production is estimated at nearly27.82 million tons (Shahbandeh, 2022) and represents the second largest tropical fruit after banana with the Philippines as the world's largest producer (FAO, 2020). In Africa, Nigeria is the leading producer followed by Ghana, Benin, Cameroon and Kenya (Kanga, 2022; Shahbandeh, 2022). In Côte d'Ivoire, pineapple represents 0.6% of GDP. However, numerous diseases including black spot, an emerging disease that is beginning to cause significant damage in Côte d'Ivoire, threaten pineapple production. Currently, there is no chemical control method to limit the disease, caused by *Fusarium moniliforme* (CNRA, 2005). The plant is attacked as soon as the flowers open and the black spots only appear or are visible when the fruit is ripe and split. Moreover, this disease makes the pineapple unfit for consumption. Indeed, inhalation or ingestion of the black powder present inside the diseased fruits causes health problems for humans (Boonpasart *et al*., 2002; Vismer *et al*., 2002). Therefore, pineapple black spot disease contributes to reduce the quality and commercial value of the fruits. In this context, it seems necessary to look for effective control methods against this pathology. One of them consists in stimulating the pineapple defense systems with elicitors in order to increase the natural resistance of the plant to this disease. Indeed, elicitors are generally molecules that are integrated in the signaling mechanisms leading to the synthesis of defense molecules directed against a wide range of pathogens. Moreover, plants can naturally resist pathogen attacks. However, some plants are more susceptible to pathogens and disease establishment than others by either a slow defense response or a low level of phenolic compound biosynthesis or rather by an absence of defense mechanism (Konan, 2014). Among, the defense mechanisms developed by the plant, phenolic compounds have an important place. Indeed, several studies have reported that phenolic compounds accumulate in adjacent tissues of necrotic plant tissues, indicating their defensive role during plant-pathogen interactions (N'cho, 2019; N'goran, 2019). In addition, pineapple produces a large number of phenolic compounds that are instrumental in resistance against diseases (Yapo, 2013). Elicitors can stimulate the biosynthesis of these compounds. These are usually analogues or derivatives of natural molecules such as salicylic acid, methyl jasmonate or ethylene (N'cho, 2017). However, few data are available on the use of elicitors in the stimulation of natural defenses in pineapple against pathogens. Therefore, an evaluation of the effect of elicitors on the biosynthesis of phenolic compounds in pineapple. Specifically, the impact of the concentration of salicylic acid, methyl jasmonate, calliete as well as the post-treatment contact time on the accumulation of phenolic compounds will be studied and finally, the phenolic compounds synthesized under the action of these elicitors will be identified according to the pineapple cultivar.

## 2. Materials and methods

### 2.1. Plant material

The plant material used is composed of suckers from the Smooth Cayenne and MD2 pineapple cultivar whose mass varies between 350 and 500 g were used. These different releases come from pineapple plantations in Bonoua (South), which is the largest pineapple growing area in Côte d'Ivoire.

### 2.2. Chemical products

The chemicals used were gallic acid, salicylic acid, methyl jasmonate, ethanol, methanol, sodium carbonate, triton X-100 and Folin-Ciocalteu's reagent were purchased from Sigma-Aldrich (Natick , MA, USA). Calliete was purchased from Callivoire-SA (Abidjan, Côte d'Ivoire). The fertilizers used for cultivation were represented mainly by urea, nitrogen, potassium sulfate, potassium oxide, sulfur, phosphorus, potassium, and magnesium oxide.

### 2.3. Study environment

The field experiments were carried out on the experimental site of Nangui Abrogoua University in the district of Abidjan (Côte d'Ivoire). The site is located in a sedimentary basin. These sedimentary formations have a sandy-clayey texture, which is favorable to the cultivation of pineapple. The site is made up of ferralitic soils and hydromorphic soils with a low acid pH and an organic matter content that varies from 2 to 3%. The climate is humid tropical. The average monthly temperatures during the cultivation period varied from 24.8°C to 29.2°C and the average monthly rainfall was from 725 mm to 10.67 mm (SODEXAM, 2021). The geographical coordinates of this site are 5°17' and 5°31' North latitude between 3°45' and 4°22' West longitude (N'cho, 2019).

**2.4. Study of the impact of elicitor concentration and incubation time on the accumulation of phenolic compounds**

**2.4.1. *In situ* production of pineapple plants**

The pineapple plants were produced from suckers of Smoth Cayenne cultivars. The suckers were first sorted according to size, followed by trimming. Surface disinfection was carried out by soaking in a solution containing a mixture of insecticide (Chlorpyriphos® 0.03%), fungicide (Mefenoxan® 0.02%), bactericide and nematicide (Carbosulfan® 0.03%) for 30 min. After three rinses with sterile distilled water, the suckers were immersed in 70% ethanol for 30 min, then in 2.5% active chlorine bleach for 30 min. After three rinsings with distilled water, the shoots were planted on the ridges of the experimental plot.

**2.4.2. Experimental design**

The Experimental design was made up of three blocks (block A, block B and block C) 5 m apart. The plants of the different blocks were treated respectively with salicylic acid, calliete then with methyl jasmonate. Each block was made up of six plots (A, B, C, D, E and F). These plots were separated from each other by 2 m. Each plot contained ridges 6 m long and 1 m wide, spaced 1 m apart. Each ridge was composed of two rows of 10 pineapple plants each. The plants were distant from each other by 50 cm. The plots were composed of three ridges (Figure 1).

**2.4.3. Preparation of elicitor solutions**

In order to obtain elicitor solutions, salicylic acid (SA), methyl jasmonate (MeJA) and calliete (Ca), were dissolved separately in 5 mL of 80% ethanol, in the presence of 0.5 mL of Triton 1% X-100, then the final volume of each elicitor was made up to 500 mL with distilled water. Five different concentrations that are 2.5; 5; 10; 15 and 20 mM were prepared for each elicitor. For the preparation, amounts of: 0.172; 0.345; 0.690; 1.035; 1.381 g salicylic acid, 0.280; 0.560; 1.121; 1.682; 2.243 mL of MeJA and 0.312; 0.624; 1.248; 1.872; 2.496 g of calliete corresponding respectively to the different concentrations were taken. These elicitors were prepared aseptically under the laminar flow hood. The control solution, with a final volume of 500 mL, consisted of 5 mL of 80% ethanol, distilled water and 0.5 mL of 1% Triton X-100. Triton X-100 acts as an adjuvant and allows a longer retention of the product on the leaves while conferring a penetrating power in them.

2.4.4. Plant

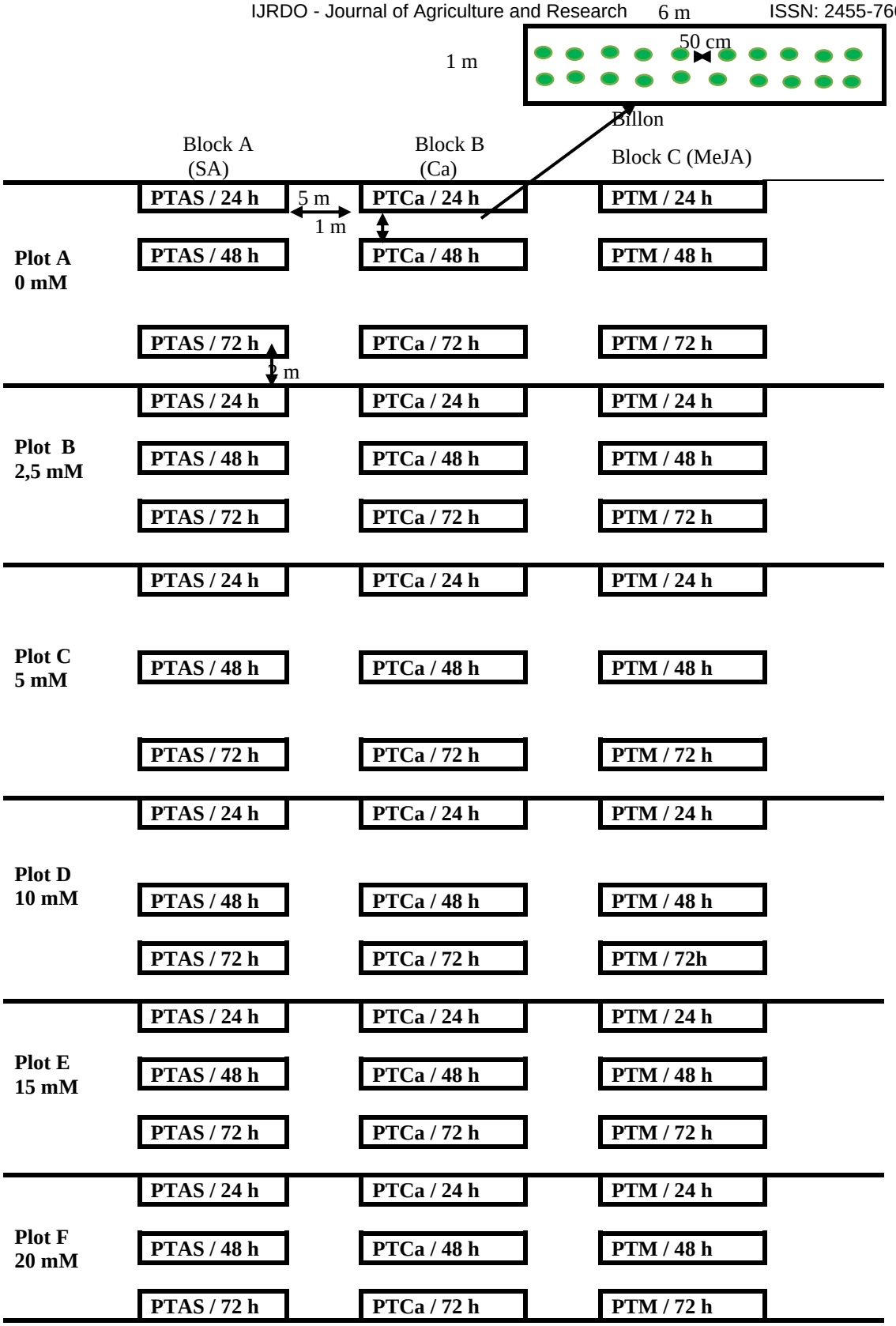


**Figure 1. Experimental design of pineapple treatment with elicitors**
PTAS: Plants treated with salicylic acid; PTM: Plants treated with Calliète; Plants treated with methyl jasmonate

## Treatment

Plants were treated with D-leaf sprays, which reflect the physiological condition of the plants. The general methods of maintenance and spraying of D-leaves were as described by N'cho (2019). After seven months of culture, the elicitor substances were sprayed on the lower and upper sides of the D leaves of the plants. Each D-leaf was sprayed with 50 mL of the elicitor solution, except for the control, which was sprayed, with 50 mL of the control solution. For each elicitor, five concentrations were made up, namely, 2.5; 5; 10; 15 and 20 mM with a variation in post treatment leaf incubation time of 24, 48 and 72 h for each concentration. Ten plants were treated per incubation time, i.e. thirty plants per concentration. The treatment was performed using a mini-sprayer previously washed with 3.6% sodium hypochlorite and

cleaned with 80% ethanol. During each treatment, plastic bags were used to separate the treated plants from the others, to prevent their contact with the solution.

**2.4.5. Leaf Harvest D**

After treatment and post-treatment incubation time, the D leaf of each plant was harvested after seven months of cultivation, i.e. ten leaves per treatment. The leaves were freeze-dried and stored at -18°C for analysis.

**2.4.6. Extraction of phenolic compounds**

The extraction of phenolic compounds was performed according to the method of Kouakou *et al.* (2008; 2009). Thus, a sample of 500 mg of lyophilized leaves from each treatment was immersed in 10 mL of pure methanol (96%) and placed in the dark for 18 h at 4°C corresponding to the time necessary for the extraction of phenolic compounds. The leaves were then removed and the methanolic extract obtained was centrifuged at 1000 turns/min during 10 min. The supernatant obtained constituted the extract of crude phenolic compounds. The total phenol content of crude extract was done according to the method of Singh *et al.* (2000). Thus, 0.5 mL of Folin-Ciocalteu reagent and 0.9 mL of distilled water were added to 0.1 mL of extract of phenolic compounds. After stirring at room temperature, 1.5 mL of 17% sodium carbonate solution and 6 mL of water were added. After 35 min of incubation, the color intensity (proportional to the concentration of phenolic compounds) was monitored using a spectrophotometer (Model MS-V 5100 spectrophotometer) at 765 nm. The level of phenolic compounds was determined using a standard curve ($y = 0.537\ x$; $R^2 = 0.999$ where y is the absorbance and x is the concentration of gallic acid) produced with different concentrations of a gallic acid stock solution (200 µg/mL) and expressed in milligrams per gram of dry matter (mg/g of DM). The best concentration and the best incubation time, which made it possible to have the highest levels of phenolic compounds induced by the treated D leaves, were retained for each elicitor. In order to investigate the effect of the best co-treatment on the phenolic compound content, the elicitors were associated two by two.

**2.4.7. Effect of plants co-treated by elicitors on phenolic compounds content**

Plants were made as before. The elicitor solutions of methyl Jasmonate, calliete and salicylic acid were prepared as before at the concentration at which each elicitor induced the highest content of phenolic compounds during the previous study. The elicitors were associated two by two at equal volume. For each association of elicitor, ten plants were formed per incubation time. The treatment was carried out as before. Thus, three types of association were established: salicylic acid and methyl jasmonate (AS + MeJA); salicylic acid and calliete (AS + Ca); methyl jasmonate and calliete (MeJA + Ca). After the treatment, the best post-treatment incubation time for each elicitor was respected for the harvest of the D leaves. The extraction and dosage of the phenolic compounds were performed as before.

**2.5. Identification and quantification of phenolic compounds by HPLC**

**2.5.1. Extraction and purification of phenolic compounds in pineapple leaves D**

The extraction of phenolic compounds was carried out as in the previous experiment. The crude phenolic extract obtained after centrifugation was subjected to sonication for 5 min in ultrasound (FAME, Emmi-12HC), then centrifuged at 10,000 rpm for 10 min. Four (4) mL of the supernatant was evaporated using a vacuum concentrator (Speed Vac). The dry residue obtained was dissolved in 1 mL of 30% methanol and then placed on a C18 grafted silica mini-column (Sep pack®; Macherey-Nagel, Düren, German) in the Supelco Visiprep™ system (Kouakou *et al.*, 2009). The columns were previously equilibrated by successive washing with 100% methanol (2 mL), then with 50% methanol (2 mL) and finally three times with distilled water (2 mL). The sample was then placed on the silica column and then washed with 2 mL of distilled water. Phenolic compounds were eluted with 4 mL of 90% methanol. The eluate obtained was evaporated on a Speed Vac then the dry residue was taken up in 1 mL of 50% methanol and finally filtered through a Millipore membrane (0.45 µm). The extract obtained constituted the purified crude extract of phenolic compounds to be analyzed by high performance liquid chromatography (HPLC).

**2.5.2. Analysis conditions**

High Performance Liquid Chromatography (HPLC) was carried out according to the method of Belhadj *et al.* (2006) and modified by Kouakou *et al.* (2009). The analysis of the samples was carried out on two HPLC chains; the first line (Agilent LC 1100 series) is equipped with a degasser, an automatic injector, a high pressure binary pump and a UV-visible detector. The second chain (Agilent LC 1200 series) includes a quaternary pump connected to an iodine bar detector, to a nuclear magnetic resonance spectrometer (Bruker Avance III) whose frequency is 600 MHz for a proton.

**2.5.3. Separation of phenolic compounds by HPLC**

Ten (10) µL of the hydromethanolic extract are directly injected into the chromatograph and the detection of the chromatograms is carried out using a strip detector. The separation was performed on a reverse phase C18 column (Prontosil, 250 x 4.0 mm, 5 µm, Bischoff). The elution was carried out with a binary gradient composed of solvent A consisting of purified water acidified with 0.025% trifluoroacetic acid (TFA) and solvent B consisting of a methanol/acetonitrile mixture, acidified with 0.025% TFA. The profile of the elution gradient is presented in Table I. The detection of the chromatograms was carried out at 284 nm with a flow rate of 1 mL/min. A reference library consisting of phenolic compounds was previously produced with compounds purified by nuclear magnetic resonance (NMR) or purchased commercially (HPLC grade) depending on their possible presence in pineapple (Yapo, 2013; Coulibaly, 2019). This library gives the NMR spectra and the retention times of the compounds.

**Table 1. Elution gradient of phenolic compounds**

| Time (min) | Solvent A (%) | Solvent B (%) |
|---|---|---|
| 0-10 | 90 | 10 |
| 10-15 | 80 | 20 |
| 15-25 | 70 | 30 |
| 25-30 | 60 | 40 |
| 30-35 | 50 | 50 |
| 35-40 | 0 | 100 |
| 45-50 | 90 | 10 |

HPLC: High Performance Liquid Chromatography; solvent A (1% TFA in filtered distilled water); solvent B (1% TFA in acetonitrile); TFA = trifluoroacetic acid

**2.6. Statistical analyzes**

Statistical analyzes were performed using Statistica 7.1 software. An analysis of variance (ANOVA) was performed on all the treatments applied. When this analysis shows a difference between the means, the Newman Keuls test is carried out in order to determine the significant differences between the treatments at the 5% threshold.

**3. Results**

**3.1. Effect of concentration and incubation time of salicylic acid, calliete and methyl jasmonate on of phenolic compounds content**

Table 2 presents the contents of phenolic compounds in the D leaves of the Smooth Cayenne pineapple cultivar elicited by methyl jasmonate (MeJA), salicylic acid (SA) and calliete (Ca) as a function of concentration and dwell times. post-treatment incubation. The analysis shows that the contents of phenolic compounds varied according to the elicitor, the concentration and the post-treatment incubation time. The results showed no significant difference between the controls.

**Table 2. Contents of phenolic compounds in D leaves of the Cayenne Lisse pineapple cultivar according to the concentration and incubation time of the elicitors applied**

| Concentration of the elicitor (mM) | Incubation time (h) | Contents of phenolic compounds (mg /g DM) | | |
|---|---|---|---|---|
| | | Salicylic Acid | Calliete | Methyl jasmonate |
| 0 | 24 | 32,57 ± 0,12$^{r}$ | 32,57 ± 0,11$^{r}$ | 32,28 ± 0,18$^{r}$ |
| | 48 | 32,62 ± 0,03$^{r}$ | 32,29 ± 0,27$^{r}$ | 32,25 ± 0,19$^{r}$ |
| | 72 | 32,58 ± 0,12$^{r}$ | 32,44 ± 0,10$^{r}$ | 32,28 ± 0,21$^{r}$ |
| 2,5 | 24 | 45,58 ± 0,11$^{m}$ | 37,36 ± 0,22$^{p}$ | 49,54 ± 0,24$^{l}$ |
| | 48 | 40,21 ± 0,13$^{o}$ | 37,34 ± 0,25$^{p}$ | 55,71 ± 0,19$^{k}$ |
| | 72 | 40,07 ± 0,05$^{o}$ | 36,31 ± 0,14$^{p}$ | 55,71 ± 0,03$^{k}$ |
| 5 | 24 | **76,29 ± 0,15$^{f}$** | 60,25 ± 0,11$^{j}$ | 63,60 ± 0,19$^{i}$ |
| | 48 | 69,17 ± 0,15$^{g}$ | **66,39 ± 0,16$^{h}$** | 63,61 ± 0,11$^{i}$ |
| | 72 | 60,24 ± 0,08$^{j}$ | 59,20 ± 0,23$^{j}$ | 88,46 ± 0,14$^{d}$ |
| 10 | 24 | 59,14 ± 0,01$^{j}$ | 56,32 ± 0,06$^{k}$ | 93,32 ± 0,14$^{c}$ |
| | 48 | 55,66 ± 0,17$^{k}$ | 56,30 ± 0,18$^{k}$ | 100,84 ± 0,04$^{b}$ |
| | 72 | 50,57 ± 0,14$^{l}$ | 56,25 ± 0,32$^{k}$ | **132,21 ± 0,09$^{a}$** |
| 15 | 24 | 45,65 ± 0,20$^{m}$ | 43,38 ± 0,50$^{n}$ | 80,19 ± 0,09$^{e}$ |
| | 48 | 34,24 ± 0,12$^{q}$ | 45,08 ± 0,10$^{m}$ | 80,23 ± 0,02$^{e}$ |
| | 72 | 34,11 ± 0,61$^{q}$ | 43,24 ± 0,11$^{n}$ | 80,22 ± 0,06$^{e}$ |
| 20 | 24 | 22,58 ± 0,08$^{t}$ | 22,36 ± 0,27$^{t}$ | 70,36 ± 0,05$^{g}$ |
| | 72 | 27,15 ± 0,08$^{s}$ | 22,26 ± 0,14$^{t}$ | 70,26 ± 0,07$^{g}$ |
| | 48 | 27,13 ± 0,014$^{s}$ | 21,26 ± 0,11$^{t}$ | 65,54 ± 0,03$^{h}$ |

Values represent the average of three replicates; ± S, standard deviation; in a column and on a row, the values followed by the same letter are not significantly different (Newman Keuls test at 5%). DM: Dry Matter.

The application of the elicitors on the D leaves of the pineapple causes an increase in the rate of phenolic compounds compared to the control for the concentrations of 2.5; 5; 10 and 15 mM. Thus, the MeJA allowed a better accumulation content of phenolic compounds for a concentration of 10 mM after an incubation time of 72 h (132.21 mg/g of DM), followed by SA 5 mM after a incubation time of 24 h (76.29 mg/g of DM) and the Ca 5 mM after a post treatment of 48 h (66.39 mg/g of DM). MeJA increased production of phenolics 4.09-fold in 72 h at 10 mM, Ca 2.05-fold in 48 h at 5 mM, and AS 2.34-fold in 24 h at 5 mM. 5 mM relative to their respective control (32.28 mg/g MS, 32.29 mg/g MS and 32.57 mg/g MS). On the other hand, the concentration of 20 mM induced statistically lower levels of phenolic compounds (SA: 27.13 mg/g of DM; Ca: 21.26 mg/g of DM) than those obtained for the controls (SA: 32 .58 mg/g DM; Ca: 32.44 mg/g DM and MeJA: 32.28 mg/g DM) with the exception of MeJA (65.54 mg/g DM). For each elicitor, the best concentration and the best incubation time that induced the greatest production of phenolic compounds were retained for the rest of the experiments. These are SA (5 mM; 24 h), Ca (5 mM; 48 h) and MeJA (10 mM; 72 h).

### 3.2. Effect of elicitor co-treatment on phenolic compounds content

Table 3 presents the comparison of the levels of phenolic compounds in the D leaves of pineapple co-treated two by two by the elicitors and treated by the elicitors alone as a function of the incubation time. The analysis of the table indicated that the association of the elicitors two by two, whatever the incubation period, positively stimulated the production of the phenolic compounds compared to the control. However, the greatest value of the phenolic compounds produced was obtained with the combination MeJA 10 mM and Ca 5 mM after an incubation time of 72 h (195.51 mg/g). It is an increase of 6.02 times compared to the control (32.45 mg/g of DM) or 4.76 times after 48 h of treatment whereas MeJA applied

alone increased production by 4.07 times in 72 h and Ca by 1.95 times in 48 h compared to the control (33.28 mg/g of DM for 48 h and 32.45 mg/g of DM for 72 h).

**Table 3. Contents of phenolic compounds extracted from leaves D of the cultivar Smooth Cayenne treated and co-treated by the elicitors according to the incubation time**

| Treatement | Time of incubation (hours) | Content of the phenolic compounds (mg/g of DM) |
|---|---|---|
| **Control** | **24** | 32,42 ± 0,31$^{j}$ |
| | **48** | 33,28 ± 0,30$^{j}$ |
| | **72** | 32,45 ± 0,44$^{j}$ |
| **SA (5 mM)** | **24** | 76,34 ± 0,01$^{f}$ |
| **Ca (5 mM)** | **48** | 66,41 ± 0,02$^{g}$ |
| **MeJA (5 mM)** | **72** | 132,25 ± 0,01$^{c}$ |
| **SA + Ca (5 mM + 5 mM)** | **24** | 90,57 ± 0,1$^{d}$ |
| | **48** | 81,50 ± 0,20$^{e}$ |
| **MeJA + Ca (10 mM + 5 mM)** | **48** | 158,30 ± 0,06$^{b}$ |
| | **72** | **195,51 ± 0,09$^{a}$** |
| **MeJA + SA (10 mM + 5 mM)** | **24** | 47,37 ± 0,08$^{h}$ |
| | **72** | 45,21 ± 0,04$^{i}$ |

Values represent the average of three replicates; ± S, standard deviation; in the same column, the values followed by the same letter are not significantly different (Newman Keuls test at 5%). DM: Dry Matter; SA: salicylic acid; Ca: calliete; MeJA: Methyl Jasmonate

D leaves co-treated with AS and Ca, then incubated for 24 h, showed a significant evolution (90.57 mg/g of DM) compared to the control (32.42 mg/g). Thus, a production of phenolic compounds 2.79 times higher than the control. That is 2.44 times after 48 hours of treatment. AS applied alone increased the production of phenolic compounds by 2.35 times in 24 h and the Ca by 1.99 times in 48 h compared to the control (32.42 mg/g of DM for 24 h and 33, 28 mg/g DM for 48 h). Regarding the co-treatment of D leaves with MeJA and SA, a slight increase was observed in the levels of phenolic compounds after an incubation time of 24 h (47.37 mg/g) then 72 h (45.21 mg / g) compared to the control (32.42 mg/g of DM in 24 h and 32.45 mg/g of DM in 72 h). The levels of phenolic compounds in the leaves treated with MeJA and SA were 1.39 times greater than in those of the control after an incubation time of 72 h. That is 1.46 times higher for a 24-hour post-treatment. In the controls, the levels of phenolic compounds were statically identical regardless of the time after treatment.

### 3.3. Chromatographic profile and identification of phenolic compounds in D leaves of pineapple cultivars treated with elicitors

**Figure 2** shows that the chromatographic profile of D leaves of the cultivar Smooth Cayenne treated with methyl jasmonate (PTM), calliete (PTCa) and the association of methyl jasmonate and calliete (PTMCa) as well as the untreated leaves have showed similarities and differences. Indeed, untreated leaves (PNT) induced six (6) phenolic compounds while those treated with Ca, MeJA, and MeJA + Ca synthesized eight (8), ten (10) and twelve (12) respectively. phenolic compounds. The results showed that the constituent compounds in the control were: 1, 2, 3, 4, 5, 6. Thus, the application of calliete on the D leaves, in addition to those synthesized in the control, caused de *novo* synthesis of compounds 7 and 8. Application of MeJA to D leaves caused de *novo* synthesis of compounds 9 and 12 in addition to those produced by Ca. However, the combination of MeJA + Ca induced *novo* compounds 10 and 11 in addition to the compounds synthesized by MeJA. The joint treatment MeJA+Ca therefore allowed an increase in the number of compounds compared to MeJA and Ca. Furthermore, the figure 3 shows the chromatographic profile of the D leaves of the MD2 cultivar. Thus, PNT induced compounds 1, 2, 3, 4, 5, 6, 7 and 8 while PTCa caused de *novo* synthesis of compound 9. PTM induced de *novo* compounds 10 and 12 in addition of those of the PTCa whereas the PTMCa provoked in addition to those of the PTM the compound 11. The results also showed that all the compounds identified after treatment with the elicitors present phenolic peaks of large amplitudes compared to the control. Thus, the amplification was greater in PTMCa followed by PTM and PTCa in both cultivars. The chromatographic peaks detected were all identified as phenolic compounds thanks

to the comparison of their retention time and their NMR with those found in the reference library. Thus, the compounds are: 1. Gallic acid (2.667min); 2. Gentisic acid (5.164 min); 3. Protocatechuic acid (6.720 min); 4. Syringic acid (6.915 min); 5. caffeic acid (8.175 min); 6. Sinapic acid (9.599 min); 7. caffeoylquinic acid (11.167 min); 8. p-coumaric acid (12.508 min); 9. Ferulic acid (13.838 min); 10. pterosilbene (15.456 min); 11. Quercetin (17.530 min); 12. Kaempferol (17.701min).

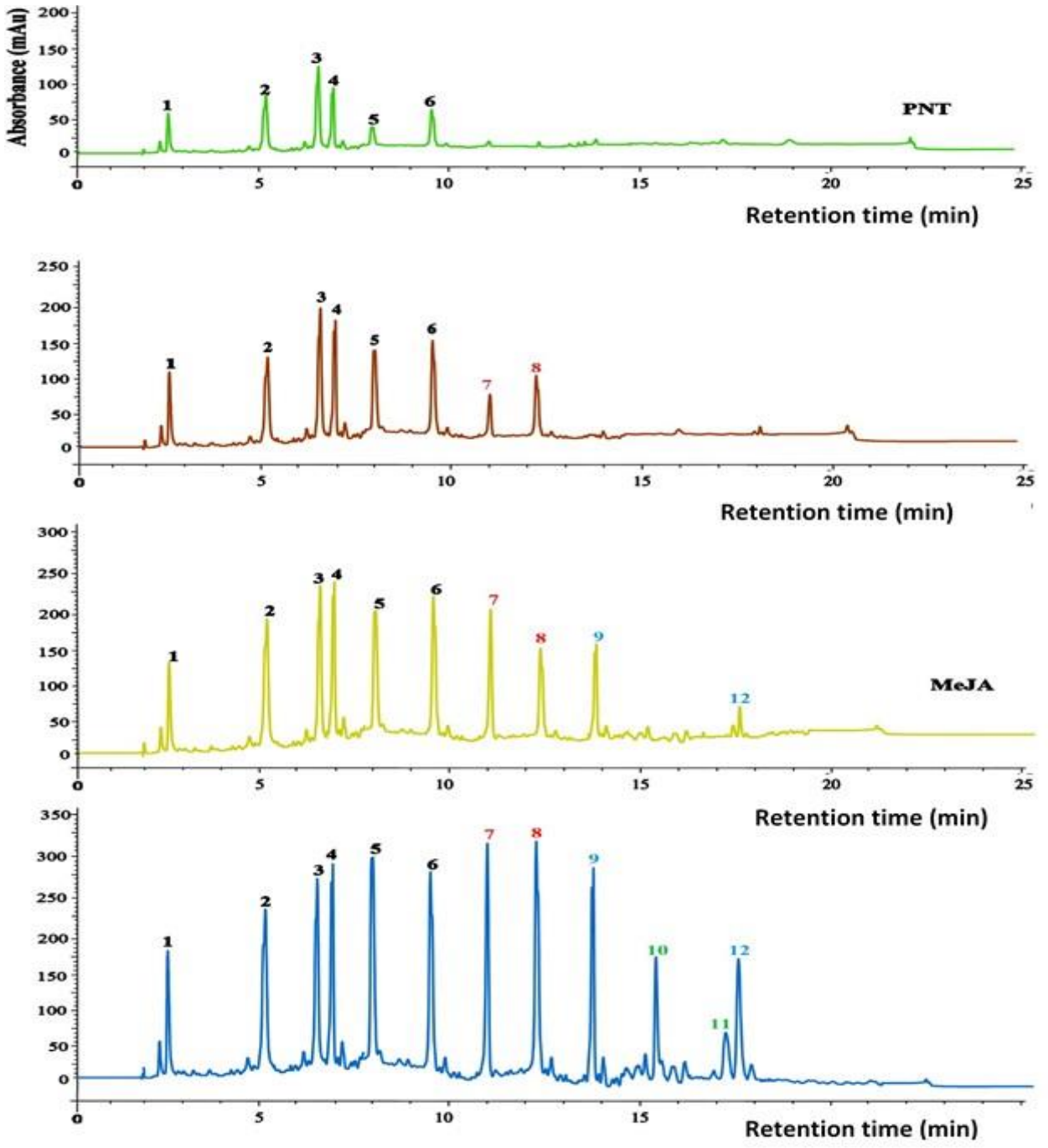


**Figure 2. Chromatographic profile of extracts from D leaves of the Smooth Cayenne cultivar treated with elicitors at 284 nm**

1. Gallic acid (2.667min); 2. Gentisic acid (5.164 min); 3. Protocatechuic acid (6.720 min); 4. Syringic acid (6.915 min); 5. Caffeic acid (8.175 min); 6. Sinapic acid (9.599 min); 7. Caffeoylquinic acid (11.167 min); 8. p-coumaric acid (12.508 min); 9. Ferulic acid (13.838 min); 10. Pterosilbene (15.456 min); 11. Quercetin (17.530 min); 12. Kaempferol (17.701 min). PNT: untreated plants; PTM: plants treated with MeJA; PTCa: plants treated with Calliete; PTMCa: plants treated with MeJA and Calliete

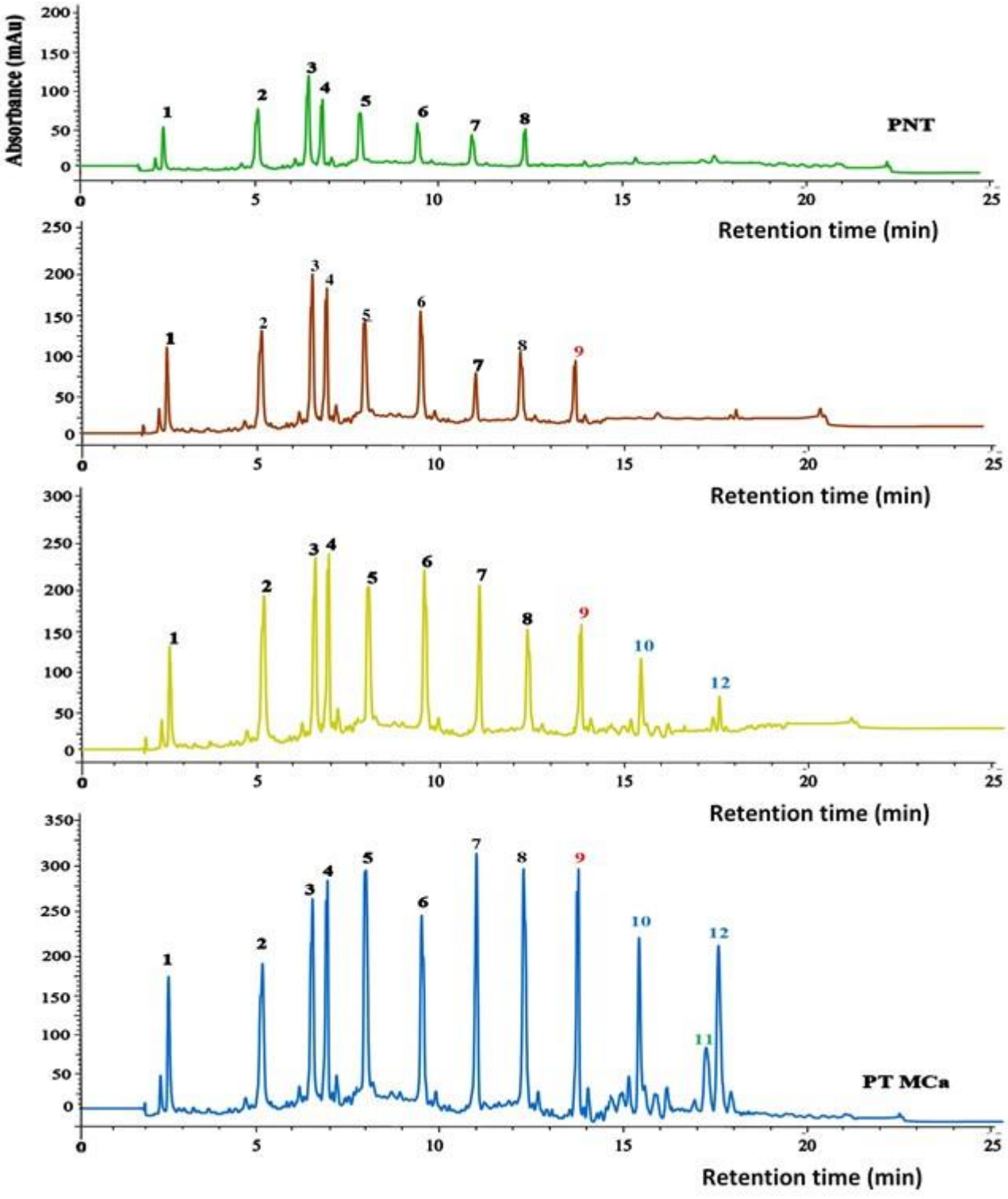


**Figure 3. Chromatographic profile of extracts from D leaves of cultivar MD2 treated with elicitors at 284 nm**

1. Gallic acid (2.667min); 2. Gentisic acid (5.164 min); 3. Protocatechuic acid (6.720 min); 4. Syringic acid (6.915 min); 5. Caffeic acid (8.175 min); 6. Sinapic acid (9.599 min); 7. Caffeoylquinic acid (11.167 min); 8. p-coumaric acid (12.508 min); 9. Ferulic acid (13.838 min); 10. Pterosilbene (15.456 min); 11. Quercetin (17.530 min); 12. Kaempferol (17.701 min). PNT: untreated plants; PTM: plants treated with MeJA; PTCa: plants treated with Calliete; PTMCa: plants treated with MeJA and Calliete.

## 4. Discussion

The results relating to the effect of the concentration and the incubation time of the elicitors on the biosynthesis of phenolic compounds in the D leaves of pineapple indicate that the elicitors significantly influence the content of the phenolic compounds. Thus, the content of phenolic compounds varies according to the type of elicitor, the concentration and the post-treatment incubation time. The application of the elicitors increased the phenolic quantity in the treated leaves compared to the controls. The levels of phenolic compounds increased to reach an optimum and then fell with the incubation time of the treated leaves. The application of MeJA at the concentration of 10 mM after 72 h of incubation made it possible to obtain the highest content of phenolic compounds, while salicylic acid and calliete were at the concentration of 5 mM after a respective incubation time of 24 h and 48 h. MeJA accumulates more phenolic compounds, followed by AS and then Calliete. MeJA appears as the elicitor that induces the greatest production of phenolic compounds in pineapple. This result could be explained by the greater stimulation of phenolic compound synthesis pathways by MeJA in contrast to other elicitors. Indeed, similar observations were observed by Opitz *et al.* (2008) after exogenous application of jasmonic acid to plants. Moreover, a significant accumulation of phenolic compounds after spraying plants with MeJA has also been observed in cotton and grapevine (Belhadj *et al.*, 2006; Konan et *al*., 2014).. In addition, studies have reported that the use of MeJA 10 mM would have been necessary to increase the accumulation of phenolic compounds in banana leaves in the fight against Sigatoka. These results are in contradiction with other works showing that a better accumulation of phenolic compounds was obtained after elicitation of vine and cotton plants with 5 mM (Belhadj *et al.*, 2006; Konan *et al.*, 2014). Thus, the concentrations of MeJA, calliete and AS capable of inducing the best responses depend on the plant species. Furthermore, pineapple plants treated with MeJA 15 and 20 mM showed symptoms of stress or toxicity in the form of chlorosis; which led to a decrease in the content of the leaves in phenolic compounds. This result seems to suggest that in pineapple, MeJA induces the accumulation of phenolic compounds up to an optimum beyond which it becomes toxic. Indeed, the phytotoxicity of MeJA at high concentrations has already been reported in pine (Heijari *et al.*, 2008; Moreira *et al.*, 2009). Results would have indicated that SA acts early to mobilize phenolic compounds in elicited leaves. It would therefore have set up a hypersensitivity reaction (HR) as reported by Beckers and Spoel (2006). Furthermore, SA was effective at 5 mM after 24 h as also shown by N'cho (2017) in cotton. In fact, SA would relay the distress signal caused by the presence of a pathogen to the nucleus of the attacked cell to induce specific defense responses. Furthermore, 5 mM calliete would also enhance natural plant defenses (Agriclean, 2008; Konan, 2014; N'cho, 2019).

The leaves treated jointly with the elicitors associated two by two showed a decrease in the content of phenolic compounds for the treatments with MeJA+SA compared to each of them used individually. These results suggest that treatment of plants with these different combinations of elicitors inhibits the synthesis of phenolic compounds, although they are good elicitors when applied separately. There would therefore be an antagonistic interaction between these elicitors. The activation of phenolic compound biosynthetic pathways in pineapple seems to be reduced by these treatments. Indeed, methyl jasmonate induces the expression of genes such as PR3 and PR4 while salicylic acid induces the expression of the PR2 and PR12 genes. Thus, a treatment combining these different elicitors leads to the demonstration of a negative interaction between the different genes, resulting in an antagonistic effect (Pennicks *et al.*, 1998; N'cho, 2017). In short, the MeJA and SA co-treatment has an infra-additive effect on the biosynthesis of phenolic compounds. This type of treatment is therefore not profitable for pineapple on the elicitation of phenolic compounds. This similar result had been observed by several authors (El Oirdi, 2009). The concomitant application of MeJA + Ca and SA + Ca on the leaves therefore seems to have a supra-additive or potentiating effect on the accumulation of phenolic compounds in pineapple. Thus, MeJA + Ca (175.51 mg/g) stimulates the phenolic content more than SA + Ca (90.57 mg/g). This synergistic or cooperative effect of MeJA and Calliete on the accumulation of phenolic compounds in pineapple is similar to that observed in several plant species (arabet, vine, cotton) co-treated with MeJA and ethephon (Larronde , 2003; Zhang et al., 2013; Konan, 2014). This joint MeJA and Ca treatment is part of a signaling cascade resulting in the mobilization of defense molecules. The combination of these two stimulators seems to be the best for triggering more heightened defense mechanisms. Thus, a joint treatment of pineapple plants with these two molecules could increase resistance gains and protect the plant against pathogens.

High-performance chromatographic (HPLC) analysis made it possible to identify the phenolic compounds present in the D leaves of pineapple elicited by MeJA and calliete revealed that the nature of the phenolic compounds varies according to the type of treatment. However 12 phenolic compounds have been identified. They are: 1. Gallic acid; 2. Gentisic acid; 3. Protocatechuic acid; 4. Syringic acid; 5. caffeic acid; 6. Sinapic acid; 7. caffeoylquinic acid; 8. p-coumaric acid; 9. Ferulic acid; 10. Pterosilbene; 11. Quercetin; 12. Kaempferol. The D leaves of the cultivar Smooth Cayenne treated with MeJA revealed the presence of 10 compounds while those of MD2 revealed the presence of 11 compounds. The D leaves of the cultivar Smooth Cayenne treated with Ca revealed 8 phenolic compounds while those of MD2 indicated 9 phenolic compounds. On the other hand, the profiles of the compounds present in the PNT leaves serving as a control, reveal the presence of six constitutive compounds within the D leaves of the Smooth Cayenne cultivar and eight for MD2. These constituent phenolics are common to MeJA-treated leaves, Ca-treated leaves, and untreated leaves (PNT). However, their number increased sharply following treatment with MeJA or Calliete, which shows the important role that these molecules play in the accumulation of phenolic compounds (Faurie, 2009; Konan, 2014). Meja and Ca co-treatment of the two cultivars induced more phenolic compounds compared to each treatment separately. This treatment induced respectively in Smooth Cayenne and MD2 two phenolic compounds and one compound more than in the leaves treated with MeJA alone. This diversity of biosynthesis of phenolic metabolites has already been reported by Konan (2015) then N'goran (2016) in cotton. These results showed that MeJA is the elicitor that allows good biosynthesis of phenolic compounds compared to Ca. It is therefore a key molecule in the induction and accumulation of PR proteins and phytoalexins. Thus,

MeJA and calliete would be essential elicitors in the signaling cascade leading to the effective defense artillery of the plant, particularly in pineapple. The induction of molecule 11 would be proof of the synergistic effect between MeJA and Ca. This result is similar to that observed in several plants co-treated with MeJA and ethephon (Konan, 2014; N'cho, 2019).

## 5. Conclusion

In this study, we tested the effect of known compounds (methyl jasmonate, salicylic acid, Calliete) as alarm signals in plants on the establishment of defense reactions in pineapple. Thus, this study showed that in the Smooth Cayenne cultivar, the SA used at 5 mM after an incubation time of 24 h, the Ca at 5 mM after an incubation time of 48 h and the MeJA at 10 mM after an incubation time of 72 h were better at inducing high levels of phenolic compounds. The best concentrations and their previously selected incubation times were used for the joint treatment. The MeJA and SA co-treatment induced an infra-additive effect while the MeJA and Calliète co-treatment induced a supra-additive effect on the accumulation of phenolic compounds. The co-treatment (MeJA+ Ca) selected allowed the stimulation of the production of strong contents of phenolic compounds at pineapple. This large variety of phenolic compounds induced was identified by HPLC.

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