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SALICYLIC ACID AND ACIBENZOLAR-S-METHYL INDUCED RESISTANCE AGAINST TOXIC EFFECT OF JUGLONE, A TOXIN OF MYCOSPHAERELLA FIJIENSIS CAUSAL AGENT OF BANANA BLACK LEAF STREAK DISEASE

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ABSTRACT

Salicylic acid and its analogues are considered the most important compounds which can be activated a systemic acquired resistance (SAR) in plants. The disadvantages and limits related to the usual methods in particular fungicide spray to the control of black leaf streak disease (BLSD) require research of approaches more respectful of the environment for this disease management such as the use of SAR inducers. The effects of Salicylic acid (SA) and Acibenzolar-S-methyl (ASM) on the interaction of two susceptible cultivars of banana (Orishele and Corne 1) with the hemibiotrophic fungal Mycosphaerella fijiensis and his toxin (juglone) were investigated. The results showed that SA and ASM at low concentrations (25 and 50 µg/ml) did not affect M. fijiensis development but have the capacity to induce protection into sensitive banana against juglone toxic effect. These SAR inducers reduced the intensity of the necrosis due to the juglone and lengthened the incubation period of M. fijiensis after inoculation of banana leaves. The expression of the resistance induced was related to the variety of banana. More significant effectiveness of protection was obtained with ASM in particular on Corne 1. A total protection against the induction of necrosis was kept up to 100 µg/ml of juglone 2 to 3 weeks after application of ASM on the soil and on the leaves of banana. ASM constitutes a viable and non-contaminant option in the fight against to BLSD because of his non-inhibiting action on M. fijiensis and excellent protection into banana when roots and leaves were treated.

Key words: Acibenzolar-S-methyl; Banana; Black leaf streak disease; juglone; Mycosphaerella fijiensi;, Salicylic acid; Systemic acquired resistance.

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INTRODUCTION

The black leaf streak disease (BLSD) caused by Mycosphaerella fijiensis, is the most destructive important disease of banana (Carlier et al., 2000; Ganry, 2010). M. fijiensis is a hemibiotrophic fungus of a high level of genetic diversity (Hayden and al., 2003).This airborne fungal leaf spot disease affects the photosynthetic area of the host plant, causing yield losses up to 50 % when the disease is not controlled (Jones, 2000; Castelan et al., 2012).

M. fijiensis produces a number of secondary metabolites (fijiensine, juglone…) which are toxic to banana plants (Churchill, 2011). These pathogen phytoxic compounds cause symptoms which appear like elongated necrotic lesions surrounded by chlorosis on bananas. Toxins such as juglone function like an aggressiveness factor as its production results in an increase in disease severity (Hoss et al., 2000). At sub-cellular level, juglone induces many biochemical effects such as disturbing the proton electrochemical gradient across the plasmalemma membrane and increasing electrolyte leakage (Etamé, 2003).

However, fungicide spray is not an appropriate method for small farmers to control BLSD because of many socio-economical and environmental reasons. In these last years, an increase in the frequency of M. fijiensis populations with decreased sensitivity to fungicides has been observed (De Lapeyre et al., 2010). The use of resistant cultivars of banana breeding programs is not always providing fruits with good organoleptic characteristics which have not showed interesting domestic consumption (N'Guessan et al., 2000). The resistance of hybrids of banana can also be lost with the time. Due to its destructive nature and lack of efficient control measures, the development of alternative or complementary approaches for management of this disease is highly desirable.

A control practice that has shown promise for plant disease management is the use of systemically induced plant resistance. Plant defenses can be activated to protect it from diseases. This defense response is a systemic acquired resistance (SAR) – induction of wide-spectrum protection that developed during the evolution of plants in response to attacks by natural enemies. It may be located at the site of application of an inducer and can also be transmitted systemically to other plant tissues (Kessmann et al., 1994). SAR inducers can be chemical compounds, metabolic substances of the host plant or microorganisms, which induce plant resistance through activation of plants' signaling pathways such as the salicylic acid pathway (Achuo et al., 2004; Adie et al., 2007). Salicylic acid (SA) and its analogues are considered the most important compounds which can be activated a SAR in plants. However, these compounds are toxic to most cultivated plants and therefore have no potential for commercial use as protective products. Recently, an analogue of SA, a S -methyl esther of the benzo-(1,2,3)-thiadiazole-7 carbotioic acid (acibenzolar-S-methyl, ASM), a compound in the benzothiadiazole group, was found to behave as a potent activator of SAR (Lyon and Newton, 1997).

The aim of this study was to investigate effects of SA and ASM on in vitro growth of M. fijiensis and the resistance of banana against development of BLSD due to juglone and the pathogen.

MATERIAL AND METHODS

Plants material

Two banana cultivars showing BLSD-susceptibility but those fruits having interesting quality and domestic consumption were used in this study. The cultivar "Orishele" (AAB) has a high sensitivity to M. fijiensis while cultivar "Corne 1" (AAB) is lowly sensitive. Both cultivars were produced from tissue culture and grown under controlled conditions in the greenhouse (16 h photoperiod and temperature of 25° C). Fertilizers were used when necessary but no pesticides.

SAR inducers

Salicylic acid (SA) purchased from Aldrich (Natick, MA, USA) and Acibenzolar-S-methyl (ASM) provided by Syngenta Crop protection (Greensboro, NC, USA) were used as SAR inducers in this study.

Fungal strains and toxin

Five monospore strains of M. fijiensis (δ66, δ104, δ106) from Côte d'Ivoire were provided by Plant Physiology Laboratory of Université Félix Houphouët-Boigny Abidjan, Côte d'Ivoire (Camara, 2011). M. fijiensis were grown on potato dextrose agar (PDA) at 25° C for 30 days. These strains were used to determine in vitro the effect of SAR inducers on M. fijiensis. The strain δ104 was used for inoculation to banana leaves after SAR inducers application.

A commercial juglone (Sigma-Aldrich), toxin of M. fijiensis, which is capable of causing the BLSD was used to induce necrosis on banana plant leaves. Plants were used for SAR inducers applications, juglone treatments or fungal inoculations at the stage of five leaves after potting.

Effect of SAR inducers on mycelia growth of M. fijiensis

The edge colony of each strain was crushed in a sterile mortar with 5 ml of sterile distilled water. The mixture was placed on PDA and incubated at 25 °C with 12-h photoperiod for 12 days. A disc of mycelia (2 mm in diameter) was taken from the colony and placed at the centre of PDA Petri dish amended with each SAR inducers at a final concentration of 0, 25, 50, 100, 250, 100, 500 and 1000 µg/ml. Triplicate plates were used for ach concentration and the dishes were incubated at 25 °C with 12 h of photoperiod. Two perpendicular colony diameters were measured per dish 7 days after incubation then, every week during 3 weeks. Diameter of colonies on SAR inducer amended medium was calculated for each concentration using the means of two perpendicular colony diameters. The relative rate (%) of reduction mycelia

growth by SAR inducers was determined as described by Keinath (2007). The experiment was conducted twice under the similar conductions.

Effect of SAR inducers on spore germination

The edge colony of each strain was crushed in a sterile mortar with 5 ml of sterile distilled water. The mixture was placed on V8 juice agar (V8 juice, 100 ml; CaCO3, 0,2 g; agar, 20 g; distilled water, 900 ml) and incubated at 25 °C with 12-h photoperiod for 12 days. The dishes were flood with sterile distilled water and a suspension of spores was constituted. Five drops of spore suspensions were streaked on gelose (agar, 20g; distilled water, 1000 ml) amended with SAR inducers at concentrations of 0, 25, 50, 100, 250, 500 and 1000 µg/ml. The experiment was repeated twice with triplicate dishes used for each concentration in each experiment. The dishes were incubated at 25 °C for 24 h and germinated spores were counted for 100 spores on each dish under stereomicroscope at 100x magnification. The relative rate of germinated spores (%) on SAR induced amended medium was determined as described by Keinath (2007).

Application of SAR inducers in greenhouse

Banana plants grown in pot (1 l) at the stage of five leaves were submitted to different treatments with SAR inducers (SA and ASM). On the one hand, some plants for each cultivar (Orishele and Corne 1) were treated with the SAR inducers by applying 50 ml of solution at 25 or 50 µg/ml only on the soil into each pot (ST). On the other hand, soil into each pot of others plants for each cultivar was treated with the SAR inducers as previously. And for these last plants after 7 days, the leaves were treated with the compounds at the same concentrations by spraying 20 ml of solution (S+LT) using a hand held garden sprayer. In each case, plants were arranged in randomized complete blocks with four replicates under greenhouse and for each treatment three plants were used per cultivar. The experiment was repeated three times under the same conditions and plants non-treated with SAR inducers were used as controls.

Induction of necrosis with juglone

Plants on soils only treated and plants on soils and leaves treated with SAR inducers were used for this test 7 days after SAR inducers applications then every week during one month. Before use, plants in each case were kept under a saturated atmosphere for 48 h. Juglone solutions of 12.5; 25; 50; 100 and 500 µg/ml concentrations were prepared in 10 % methanol and used as well as the 10 % methanol solution (MeOH 10 %) and the distilled water as control (0 µl of juglone). Twenty microliter of juglone or MeOH 10 % or H_2O solutions were injected into the lower surface of the lower fully expended leaf using a syringe with a rubber stopper covering its needle. Four replicates per concentration and per leaf (2 per half-limb) were conducted on three plants of each cultivar and the whole experiment was repeated three times independently. Plants were incubated after the injections under greenhouse with a natural photoperiod during 48 h.

Evaluation of necrosis severity

For each cultivar, the minimum concentration of juglone (Cmin) inducing necrosis was determined 48 h after infiltration of solutions into banana leaves. Necrotic lesions are recognizable by brown or black spot observed at the site of injection.

Severity of necrosis observed at the injection site, 48 h after treatment, was evaluated by a visual scale adapted from standard values established by Stierle *et al.* (1991). According to the necrosis surface (Sn) as compared to the infiltrated surface (Si), using values ranging from 0 to 4, a necrosis index (i) was affected to each site injected with the toxin. In this scale: 0 = no necrosis; 1 = Sn lower than ¼ of Si; 2 = Sn between ¼ and ½ of Si; 3 = Sn between ½ and ¾ of Si and 4 = Sn higher than ¾ of Si. Using the formula of infection index defined by Townsend and Herberg (Perez *et al.*, 2002), necrosis intensity (IN) (%) was calculated for each cultivar per solution injected in the leave:

$$IN\% = (\Sigma\, in / 4N) \times 100$$

Where, i is the index of the necrosis observed for each infiltrated site per solution, n the number of the injected sites with the same necrosis index for each solution, N the total number of infiltrated sites for each solution N = 4.

For each treatment with SAR inducers, the efficacy of protection (% by for the control) of the banana leaves to necrosis induction by juglone was calculated using the formula:

$$EP\% = ((IN_{non\text{-}treated} - IN_{treated}) / IN_{non\text{-}treated}) \times 100$$

Where, IN is the necrosis intensity for each solution injected into the leaves of banana plants treated or non-treated with SAR inducers

For each treatment with SAR inducers and per cultivar, the incubation time (IT_{50}) was determined when leaves were infiltrated with juglone solution at 100 µg/ml. According to necrosis intensity, the incubation times were transformed into log of concentrations. The IT_{50}, inducing 50 % of necrosis on infiltrated surface for each banana cultivar was then calculated using linearization of the necrosis intensity evolution curves.

Application of M. fijiensis inoculum in greenhouse study

Colony of the M. fijiensis strain (δ104) grown on V8 medium, a suspension of spores adjusted from 2.10^4 to 2.10^5 spores/ml was constituted as described previously. Banana plants were inoculated 7 days after the applications of SAR inducers on soil and leaves. Before use, plants were kept under a saturated atmosphere for 48 h. For inoculation the lower surface of the first two fully expended leaves were sprayed with 2 ml of spore suspension for each leaf. Non-inoculated

plants were treated with the same amount of sterile distilled water. Plants were maintained in moisture conditions for 24 h after inoculation and kept in the greenhouse. The experiments were repeated three times under the same conditions.

Evaluation of disease

BLSD development was evaluated 10 days after M. fijiensis inoculation and then every two days. Disease was evaluated according to methods described by Meredith and Lawrence (1969) and Fouré (1987). The symptoms evolution time (SET), days between the appearance of first symptoms and the appearance of spots with dry centers, was calculated with the formula: **SET = DDT – IT**, which incubation time (IT) = days between inoculation and appearance of first symptoms of the disease (yellowish depigmentation on the lower leaf surface) and disease development time (DDT) = days between inoculation and the appearance of spots with dry centers.

Statistical Analysis

Experiments were performed using a completely randomized design. Data were subjected to analysis of variance (ANOVA) using Statistica software (release 7.0). Difference between means was compared using Newman-Keuls test. Differences at $p< 0.05$ were considered as significant.

RESULTS

Effect of SAR inducer on mycelia growth and spore germination

The development of M. fijiensis strains was generally not inhibited by the SAR inducers in particular with ASM on mycelia growth and spore germination (Table 1 and Figure 1). The reduction rate of mycelia growth did not exceed 50 % after 3 weeks. Differences were observed in toxicity effect between SAR inducers treatments. AS was significantly not inhibited mycelia growth until 250 µg/ml. But at 250 µg/ml or higher concentrations, AS reduced spore germination of M. fijiensis strains (Figure 1). The inhibition of spores germination was correlated with increasing concentrations of AS.

Minimal concentration of juglone inducing necrosis on banana leaves

During 4 weeks after the application of SAR inducers, minimal concentrations of juglone inducing necrosis on infiltrated leaves were weaker with banana treated by ASM than those treated by SA in particular with the cultivar Corne 1 (Table 2). With controls, 12.5 µg/ml of juglone were sufficient to induce necrosis, but 25 µg/ml or higher concentrations raised 250 µg/ml of juglone were required at 2 or 3 weeks after the application of SAR inducers on soil or on soil and leaves. For both cultivar Orishele and Corne 1, juglone at 50 µg/ml or higher concentrations were necessary to induce necrosis after the treatment of soil and leaves by ASM. Two weeks after applications on soil and leaves with ASM at 25 and 50 µg/ml, juglone at 100 µg/ml was required to induce necrosis on the foliar limb of the cultivar Corne 1 (Table 2). Minimal concentrations of juglone inducing necrosis were generally weak at 1 or 4 after SAR inducers applications in particular when soil was only treated.

Intensity of necrosis induced by juglone on banana leaves

SAR inducers treatments at 25 or 50 µg/ml on soil only or on soil and leaves of Orishele and Corne 1 cultivars significantly reduced necrotic lesions induced by foliar injections of juglone (Tables 3 and 4). Differences were observed in sensitivity response to juglone toxic effect between banana plants treated by both SAR inducers. Necrosis intensities were generally weaker with banana treated by ASM at 50 µg/ml in particular 2 and 3 weeks after soil and foliar applications of cultivar Corne 1. Banana non-treated with SAR inducer were highly sensitive to juglone toxic effect. Necroses at 50 % or higher intensities were obtained at 25 µg/ml of juglone with control banana (Tables 3 and 4). Corne 1 was more resistant to juglone toxicity than Orishele after inducers applications. Necrosis intensities were at 50 % or higher at 50 µg/ml of juglone for Orishele and 100 µg/ml of juglone for Corne 1 particularly after soil and foliar treatments by SAR inducers (Table 4). Indeed, 2 and 3 weeks after ASM applications on soil and foliar, necrosis intensities were lower to 50 % at juglone concentrations lower than 250 µg/ml. For both cultivars Orishele and Corne 1, necrosis intensities were higher at 1 or 4 weeks after inducers applications on soil or on soil and leave (Tables 3 and 4).

Efficacy of protection against juglone toxicity after inducers applications on bananas

Compared to the controls, SA and ASM showed effectiveness against the toxic effect of juglone infiltrated in foliar limb (Tables 5 and 6). Differences were observed in sensitivity response to juglone toxic effect between banana plants after treatments by both SAR inducers. Treatments with ASM generally were a greater effectiveness against necrosis induction. Total efficacies of protection (100 %) were observed at 12.5 and 25 µg/ml of juglone respectively after SA and ASM applications only on soil (Table 5). But after inducers applications on soil and leaves, total efficacies of protection (100 %) were observed at 50 and 100 µg/ml of juglone in particular 2 and 3 weeks after ASM (at 50 µg/ml) treatment into cultivar Corne 1 (Table 6). SAR inducers generally showed a lower effectiveness between 1 and 4 weeks after applications (Tables 5 and 6). With cultivar Orishele, 1 and 4 weeks after applications of SA on soil, efficacy of protection against the effect of the toxin was already lower than 50 % at 12.5 µg/ml of juglone (Table 5).

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Effect of SAR inducers applications on incubation time inducing 50 % of necrosis (IT_{50}) on total foliar surface infiltrated with juglone at 100 µg/ml

During the 4 weeks after activators applications on soil and on leaves of banana plants, time requested to induce 50 % of foliar surface necrosis following the infiltration of juglone at 100 µg/ml, lies between 4 and 205 h for the whole of the treatments (Table 7). The highest values of IT_{50} (> 69 hours) were observed in cultivar Corne 1 treated with ASM in particular 2 and 3 weeks after applications of SAR inducers. For both cultivars of banana (Orishele and Corne 1) SA treatments showed values of IT_{50} relatively low (7-24 hours) in particular 4 weeks after application of SA at 25 µg/ml. For bananas non-treated by SAR inducers, the values of IT_{50} were low to 10 hours (Table 7).

Sensitivity of banana cultivars after soil and leaves SAR inducers-applications to BLSD

Significant differences were observed between the treatments after applications of defense activators for incubation time (IT), disease development time (DDT), symptoms evolution time (SET) under green house conditions (Table 8). Incubation time among all treatments with SA and ASM for both cultivars Orishele and Corne 1 was varied 15 to 20 days. The IT of banana treated by ASM particularly with ASM at 50 µg/ml was longer than other treatments and was higher than 19 days. The cultivar Corne 1, exhibited infestation periods (DDT, SET) longer than the cultivar Orishele (Table 8). For the DDT, with bananas treated by ASM, BLSD development was significantly slower as compared to bananas treated by AS and controls. The DDT of bananas treated with ASM was about 1 to 2 weeks longer than that of SA and controls (Table 8). Slower disease development also resulted in longer symptoms evolution time of treatment with ASM in particular in the cultivar Corne 1 where the SET was 1 or 2 weeks longer than that of SA and controls (Table 8).

DISCUSSION

The assessment of direct inhibitory effect of SAR inducers on the mycelia growth and spore germination exhibited that SA and ASM at low concentrations do not inhibit the development of M. fijiensis particularly at 25 and 50 µg/ml (Koné et al., 2009). However, salicylic acid was showed significant inhibitory effects on the mycelia growth and spore germination of M. fijiensis when the concentration was higher than 250 µg/ml with rates of spore germination lower than 50 %. Salicylic acid at high concentrations was reported to inhibit mycelia growth and zoospore germination of Pythium aphanidermatum (Chen et al., 1999). ASM exhibited good SAR inducer properties because it's almost inactive on M. fijiensis. Similar results were obtained by Koné and al. (2009) with Phytophthora capsici, causal agent of phytophthora blight on squash, except at 500 and 1000 µg/ml ASM reduces sporangium production of P. capsici. When used at lower concentrations (25 and 50 µg/ml) that were in the range generally recommended for SAR inducers applications, SA and ASM did not have a direct inhibitory effect on M. fijiensis.

Study of protective effect of SAR inducers against the toxicity of juglone highlights the potential of salicylic acid and ASM to reduce susceptibility to the toxic effect of juglone of BLSD-sensitive banana cultivars. These SAR inducers increased Orishele and Corne 1 resistance to the induction of foliar necrosis by the toxin. Best levels of resistance to the induction of necrosis were generally obtained 2 or 3 weeks after applications of the inducers. With ASM, the minimal concentration inducing necrosis reached 250 µg/ml of juglone. However, with BLSD-resistant hybrids of bananas (PITA 14 and FHIA 23) non-treated with SAR inducers, the range of minimal concentrations inducing necrosis was from 50 to 100 µg/ml of juglone (Amari et al., 2011). During 4 weeks of assessment, the intensities of necrosis were generally weaker, in particular with banana plants whose soil and leaves were treated with ASM at 50 µg/ml. The values of protective effectiveness were maintained up to 100 % until to 100 µg/ml of juglone and they were lower than 50 % at 250 ppm or higher concentrations of the toxin. The longest incubation times (higher than 48 H) inducing 50 % of necrosis (IT_{50}) on total foliar surface infiltrated with juglone at 100 µg/ml, were observed in particular with ASM treatments on soil and leaves of Corne 1 cultivar.

ASM confirms its reputation of excellent activator (Pajot et al., 2007) because of its greater protection conferred to bananas against the induction of necrosis by toxin. Salicylic acid although as inducing a sufficient resistance, ensured a protection less than the ASM. The difference in performance between both activators would be determined by the fact that in the way of regulation ASM intervenes after salicylic acid the (Ryals et al., 1996; Durner et al., 1997; Gullino et al., 2000). According to some authors, salicylic acid would have disadvantage for not being systemic (it induces a resistance only in the treated leaves), being quickly degraded on the surface of the leaves and toxic at weak concentrations (Percival, 2001). Sometimes, the protective effect of salicylic acid at 50 µg/ml was weaker than that observed at 25 µg/ml and would be explained by a possible toxicity of this molecule at 50 µg/ml or higher concentrations into bananas.

Although the resistance induced against toxin effect in banana leaves was weak after only soil-application of SA or ASM, this result indicates a systemic action of these SAR inducers. The optimal resistance to necrosis induction by juglone being observed at 2 and 3 weeks after activators applications, 1 month could constitute the maximal duration of the induced protection. The period of 3 weeks could be also used as the frequency of treatment with SA and ASM into BLSD-susceptible banana cultivars in field conditions.

The cultivar Corne 1 exhibited less sensitive to oxydative stress due to juglone than Orishele cultivar before and after SAR inducers application. The expression of the resistance induced seems to depend on the variety of banana.Compared to most resistant varieties to the MRN, the most significant varieties could have their systems of defense less activated by the SAR inducers. Corne 1 would then have a greater affinity with the action of the activators of resistance than Orishele. Similar results were reported at varieties of tomato sensitive and partially resistant to the

bacterial wilt caused by Ralstonia solanacearum (Pradhannugand al 2005). The studies in greenhouse showed that ASM significantly increases resistance at the varieties of tomato partially resistant (Neptune and BHN 466) to this bacterium that at those which are sensitive (Equinox). Nevertheless, from the contrary results were obtained on soya by Dann et al. (1998) which showed that the resistant one induced by activators (INA or ASM) against Sclerotinia sclerotiorum was more significant at the sensitive cultivars than resistant. In this part of our study, it would be difficult to justify this difference of behavior between Corne 1 and Orishele by the factors which are: the genotype and the environment in particular nutrition which would influence the expression of resistance induced to field (Walters et al., 2005). Indeed, these two cultivars have the same genotype (AAB) and the work was carried out in conditions controlled and standardized.

Bananas treated or non-treated with SAR inducers have been sensitive to M. fijiensis infection under greenhouse conditions. However, the assessment of incubation time (IT), disease development time (DDT) and symptoms evolution time (SET) is correlated with the sensitivity of the juglone toxic effect into bananas after applications of defense activators. The gradients of sensitivity to BLSD established with the various treatments are the same for both varieties tested (Orishele and Corne 1). Plants treated with SAR inducers presented long incubation and necrosis appearance periods compared to non-treated bananas. Our results showed differences between the treatments with SAR inducers. The behavior of the bananas treated with SAR inducers and particularly with ASM, is similar to BLSD-resistant hybrid (FHIA 21) of bananas breeding programs (Molina and Castano-Zapata, 2003; Traoré, 2008). ASM opposed a resistance to the progression of the pathogen. The SAR inducers (ASM) application could reduce the effect of phytotoxines in the extension of the lesions due to M. fijiensis. According to Madrigal et al. (1998), ASM is a very promising SAR activator which delays symptoms appearance on banana leaves, inducing a less severity of BLSD.

CONCLUSION

This study highlighted that SAR inducers activate sensitive banana cultivars resistance to toxins and M. fijiensis, which are causal agents of the development of the black leaf streak disease. ASM conferred to bananas a greater protection than SA and constituted an excellent activator because it does not inhibit the development of M. fijiensis particularly at 25 and 50 µg/ml which are the concentrations recommended for its application in the field. In greenhouse conditions, best levels of protection to the pathogen and its toxin, were obtained when banana plants roots (soil) and leaves were treated with the SAR inducers. These results indicate that ASM constitutes a viable and non-contaminant option in the fight against to BLSD caused by M. fijiensis.

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FIGURES AND TABLES

Table 1. Rate reduction of Mycosphaerella fijiensis mycelia growth on PDA medium amended with salicylic acid and acibenzolar-S-methyl

Incubation time (day)	Concentration of inducer (µg/ml)	SA			ASM		
		δ66	δ104	δ106	δ66	δ104	δ106
7	25	- 0.64 bc	6.02 ab	- 0.99 b	3.33 a	15.22 a	- 2.54 b
	50	- 5.23 c	- 4.37 b	- 2.54 b	4.53 a	13.52 a	7.24 ab
	100	- 0.46 bc	7.99 ab	- 2.24 b	10.97 a	19.12 a	15.80 ab
	250	25.97 ab	30,36 a	23.28 ab	12.68 a	25.87 a	15.80 ab
	500	34.90 a	38.66 a	19.27 ab	10.60 a	22.30 a	19.11 ab
	1000	26.99 ab	31.34 a	36.77 a	13.10 a	25.48 a	28.20 a
14	25	- 6.73 b	1.50 c	2.85 c	8.83 a	8.34 a	5.43 b
	50	- 6.38 b	1.22 c	2.11 c	12.80 a	11.03 a	7.51 b
	100	- 2.37 b	4.33 c	2..22 c	9.29 a	8.61 a	7.87 b
	250	15.86 ab	19.62 bc	15.76 bc	15.89 a	13.05 a	16.58 ab
	500	36.26 a	40.16 a	35.13 ab	14.44 a	15.49 a	17.29 ab
	1000	34.55 a	31.4 ab	44.87 a	13.04 a	20.01 a	31.63 a
21	25	- 4.11 b	2.22 c	1.03 b	6.13 b	3.70 b	5.26 c
	50	- 5.21 b	4.73 bc	4.36 b	9.85 ab	5.97 b	7.04 c
	100	- 1.59 b	5.24 bc	5.04 b	14.46 ab	5.31 b	11.38 ab
	250	15.29 ab	20.73 ab	26.55 a	14.36 ab	16.29 ab	20.81 ab
	500	26.64 a	29.13 a	35.86 a	15.00 ab	12.06 ab	20.71 ab
	1000	17.92 ab	23,41 a	40.37 a	17.52 a	20.55 a	25.94 a

SA: salicylic acid; ASM: acibenzolar-S-methyl; δ66, δ104 and δ106: M. fijiensis strains; in the same column, means followed by different letters are significantly different (Newman-Keuls test at 5 %). Reduction of rate was compared to the control.

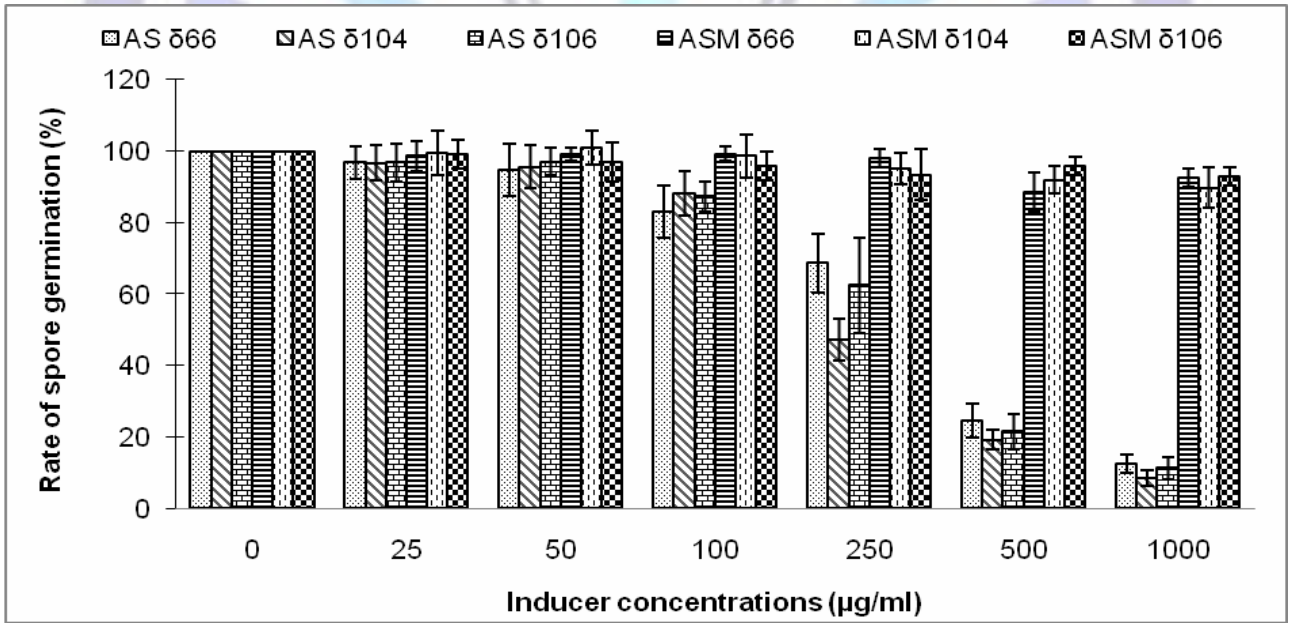

Figure 1. Effect of different concentrations of salicylic acid and Acibenzolar-S-methyl on spore germination of Mycosphaerella fijiensis

SA: salicylic acid; ASM: acibenzolar-S-methyl; δ66, δ104 and δ106: M. fijiensis strains.

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Table 2. Range of minimal concentrations of juglone inducing necrosis on leaves of two banana cultivars treated with salicylic acid and acibenzolar-S-methyl

Banana cultivar	Treatment of inducer (µg/ml)	Week after inducer treatment							
		1		2		3		4	
		ST	S+LT	ST	S+LT	ST	S+LT	ST	S+LT
Orishele	Control	12.5	12.5 – 25	12.5 – 25	12.5 – 25	12.5 – 25	12.5 – 25	12.5 – 25	12.5 – 25
	SA 25	12.5 – 25	12.5 – 50	25 – 50	25 – 50	25 – 50	12.5 – 50	12.5 – 25	12.5 – 50
	SA 50	12.5 - 25	12.5 - 25	12.5 – 50	25 – 50	12.5 – 50	12.5 – 50	12.5 – 25	12.5 – 50
	ASM 25	25 – 50	50 – 100	25 -50	50 – 100	25 – 50	50 – 100	12.5 – 25	25 – 50
	ASM 50	25 - 50	50 - 100	25 - 50	100 - 250	25 - 50	50 - 250	12.5 - 50	50 - 100
Corne 1	Control	12.5 – 25	12.5 – 25	12.5 – 25	25	12.5 – 25	12.5 – 25	12.5 – 25	12.5 – 25
	SA 25	25	12.5 – 50	12.5 – 50	25 – 50	25 – 50	25 – 50	12.5 – 25	25 – 50
	SA 50	25 – 50	25 – 50	25 – 50	25 – 100	12.5 – 25	25 – 50	12.5 – 25	12.5 – 50
	ASM 25	50	50 – 250	25 – 50	100 - 250	25 – 50	100 - 250	12.5 – 25	50 – 100
	ASM 50	50	100 - 250	25 - 100	100 - 250	25 - 100	250	25 - 50	50 - 250

SA: salicylic acid; ASM: acibenzolar-S-methyl; ST: soil-treated; S+LT: soil and leaves-treated

Table 3. Intensities of necrosis induced by different concentrations of juglone on leaves of two banana cultivars after soil-treatment with salicylic acid and acibenzolar-S-methyl

Banana cultivar	weeks after inducer treatment	Treatment of inducer (µg/ml)	Concentrations of juglone (µg/ml)						
			0	12.5	25	50	100	250	500
Orishel	1	Control	0 a	30.55 a	52.77 a	72.22 a	87.50 a	100 a	100 a
		SA 25	0 a	15.27 c	43.05 b	62.50 b	84.72 a	100 a	100 a
		SA 50	0 a	18.75 b	43.75 b	62.50 b	75.00 b	87.50 b	100 a
		ASM 25	0 a	0 d	6.25 c	38.19 d	63.88 c	81.90 c	90.97 c
		ASM 50	0 a	0 d	18.75 d	43.75 c	62.50 c	87.50 b	93.75 b
	2	Control	0 a	15.27 a	40.27 a	50 a	79.16 a	91.66 a	100 a
		SA 25	0 a	0 b	18.75 b	43.75 b	77.77 a	90.97 a	100 a
		SA 50	0 a	1.38 b	8.33 c	25.00 c	49.30 c	79.86 b	93.75 b
		ASM 25	0 a	0 b	9.02 c	36.11 c	62.50 b	81.25 b	93.75 b
		ASM 50	0 a	0 b	9.02 c	30.55 d	52.77 c	77.77 b	89.58 c
	3	Control	0 a	18.75 a	43.75 a	56.25 a	78.47 a	90.97 a	100 a
		SA 25	0 a	0 c	15.20 bc	40.27 bc	68.75 b	87.50 a	97.20 ab
		SA 50	0 a	2.77 b	21.52 b	46.52 b	68.75 b	84.72 a	93.70 bc
		ASM 25	0 a	0 c	18.75 c	43.75 c	61.80 c	77.77 b	95.10 bc
		ASM 50	0 a	0 c	15.27 c	40.27 c	55.55 d	75.00 b	90.97 c

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	4	Control	0 a	22.91 a	47.22 a	66.66 a	81.94 a	94.44 a	100 a
		SA 25	0 a	12.50 b	37.50 b	60.40 b	78.47 b	90.27 a	97.20 ab
		SA 50	0 a	9.72 bc	34.70 bc	56.25 b	72.22 b	85.41 b	96.5 ab
		ASM 25	0 a	6.25 cd	31.25 c	47.22 c	59.02 d	81.25 b	93.75 b
		ASM 50	0 a	2.77 d	20.13 d	46.52 c	64.58 c	75.00 c	90.27 c
Corne 1	1	Control	0 a	6.25 a	31.25 a	50.00 a	84.72 a	96.52 a	100 a
		SA 25	0 a	0 b	21.52 b	37.50 b	63.19 b	77.77 b	100 a
		SA 50	0 a	0 b	16.66 c	31.25 c	58.30 bc	75.00 b	100 a
		ASM 25	0 a	0 b	0 d	25.00 d	47.84 d	69.44 c	87.50 b
		ASM 50	0 a	0 b	0 d	25.00 d	50.70 cd	63.19 d	81.25 b
	2	Control	0 a	6.25 a	31.25 a	50.00 a	69.44 a	87.50 a	100 a
		SA 25	0 a	2.77 b	18.75 b	43.75 b	67.36 a	84.02 a	100 a
		SA 50	0 a	0 c	13.19 c	32.63 c	50.69 b	85.41 a	100 a
		ASM 25	0 a	0 c	6.25 d	31.25 c	56.25 b	83.33 a	100 a
		ASM 50	0 a	0 c	2.77 e	15.27 d	40.27 c	78.47 b	93.75 b
	3	Control	0 a	10.41 a	31.94 a	53.47 a	69.44 a	84.72 a	91.66 a
		SA 25	0 a	0 c	9.72 c	31.20 cd	40.97 e	72.22 b	88.19 a
		SA 50	0 a	3.47 b	15.27 b	40.97 b	61.80 b	80.55 a	88.88 a
		ASM 25	0 a	0 c	9.72 c	34.72 c	52.77 c	71.52 b	89.58 a
		ASM 50	0 a	0 c	9.02 c	28.47 d	47.22 d	66.66 b	84.72 a
	4	Control	0 a	15.97 a	40.97 a	65.97 a	78.47 a	87.50 a	100 a
		SA 25	0 a	12.50 b	37.50 b	59.72 b	78.47 a	87.50 a	100 a
		SA 50	0 a	6.25 c	31.25 c	53.47 c	62.50 b	76.38 c	92.36 b
		ASM 25	0 a	3.47 d	28.47 d	50.00 d	63.19 b	81.25 b	93.75 b
		ASM 50	0 a	0 e	12.50 e	36.80 e	56.25 c	75.00 c	90.97 b

SA: salicylic acid; ASM: acibenzolar-S-methyl; in the same column, means followed by different letters are significantly different (Newman-Keuls test at 5 %).

Table 4. Intensities of necrosis induced by different concentrations of juglone on leaves of two banana cultivars after soil and leaves-treatment with salicylic acid and acibenzolar-S-methyl

Banana cultivar	Time after inducer treatment (week)	Treatment of inducer (µg/ml)	Intensity of necrosis (%)						
			Concentrations of juglone (µg/ml)						
			0	12.5	25	50	100	250	500
Orishele	1	Control	0 a	13.80 a	36.80 a	64.58 a	85.41 a	90.97 a	96.52 a
		SA 25	0 a	4.10 c	27.77 b	54.86 b	74.30 b	82.63 b	93.00 ab
		SA 50	0 a	9.00 b	28.47 b	57.6 ab	75.00 b	80.55 b	95.13 a
		ASM 25	0 a	0 d	0 c	23.61 c	54.86 c	77.77 b	92.3 ab
		ASM 50	0 a	0 d	0 c	25.69 c	43.75 d	71.52 c	88.88 b
	2	Control	0 a	7.63 a	35.41 a	57.63 a	78.47 a	87.50 a	95.83 a
		SA 25	0 a	0 b	8.33 b	37.50 b	52.77 c	77.77 b	87.50 b

	SA 50	0 a	0 b	10.41 b	39.58 b	58.33 b	79.16 b	90.27 b
	ASM 25	0 a	0 b	0 c	6.94 c	40.27 d	67.36 c	86.80 b
	ASM 50	0 a	0 b	0 c	0 d	13.19 e	48.61 d	65.97 c
	Control	0 a	11.80 a	45.13 a	69.44 a	84.02 a	93.75 a	97.91 a
	SA 25	0 a	4.86 b	28.47 b	47.22 b	65.27 b	82.63 b	93.75 a
3	**SA 50**	0 a	2.08 bc	19.44 c	46.52 b	68.75 b	82.63 b	95.83 a
	ASM 25	0 a	0 c	0 d	9.02 c	34.02 c	64.58 c	85.41 b
	ASM 50	0 a	0 c	0 d	1.38 c	20.83 d	54.16 d	79.16 c
	Control	0 a	29.86 a	51.38 a	68.05 a	80.55 a	87.50 a	96.52 a
	SA 25	0 a	11.11 b	36.80 b	55.55 b	72.91 b	84.72 a	90.97 a
4	**SA 50**	0 a	2.08 c	30.55 c	56.94 b	74.30 b	83.33 a	93.05 a
	ASM 25	0 a	0 c	16.66 d	36.11 c	49.30 c	78.47 b	90.27 a
	ASM 50	0 a	0 c	0 e	18.05 d	50.00 c	73.61 c	84.02 b
	Control	0 a	12.50 a	45.13 a	59.02 a	79.16 a	89.58 a	97.91 a
	SA 25	0 a	9.02 b	29.86 b	45.83 b	69.44 b	84.72 a	90.97 b
1	**SA 50**	0 a	0. c	14.58 c	40.97 c	72.22 b	84.72 a	95.83 a
	ASM 25	0 a	0 c	0 d	6.94 d	31.94 c	72.22 b	86.10 bc
	ASM 50	0 a	0 c	0 d	0 e	29.16 c	68.75 b	88.19 c
	Control	0 a	6.25 a	34.72 a	48.61 a	71.52 a	86.11 a	96.52 a
	SA 25	0 a	0 b	20.83 b	43.05 b	63.88 b	80.55 b	93.05 a
2	**SA 50**	0 a	0 b	4.16 c	31.25 c	50.00 c	72.91 c	93.05 a
	ASM 25	0 a	0 b	0 c	0 d	23.60 d	56.94 e	80.55 b
	ASM 50	0 a	0 b	0 c	0 d	8.33 e	66.66 d	81.25 b
Corne 1	**Control**	0 a	6.25 a	23.61 a	45.13 a	70.13 a	89.58 a	95.83 a
	SA 25	0 a	0 b	9.72 b	34.02 b	59.72 b	79.86 b	91.66 a
3	**SA 50**	0 a	0 b	4.86 c	29.86 c	49.30 c	71.52 c	91.66 a
	ASM 25	0 a	0 b	0 d	0 d	10.41 d	41.79 e	81.25 b
	ASM 50	0 a	0 b	0 d	0 d	0 e	62.50 d	81.25 b
	Control	0 a	19.44 a	39.58 a	60.41 a	74.30 a	88.19 a	97.91 a
	SA 25	0 a	0 c	10.41 c	39.58 c	59.72 b	72.22 c	92.30 ab
4	**SA 50**	0 a	6.9 b	22.22 b	45.83 b	61.80 b	79.86 b	95.80 ab
	ASM 25	0 a	0 c	0 d	10.41 d	46.52 c	70.13 c	90.27 b
	ASM 50	0 a	0 c	0 d	6.94 d	26.38 d	60.41 d	89.58 b

SA: salicylic acid; ASM: acibenzolar-S-methyl; in the same column, means followed by different letters are significantly different (Newman-Keuls test at 5 %).

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Table 5. Efficacy (% compared to control) of protection to necrosis induction by different concentrations of juglone on leaves of two banana cultivars after soil-treatment with salicylic acid and acibenzolar-S-methyl

Banana cultivar	Time after inducer treatment (week)	Treatment of inducer (µg/ml)	Concentrations of juglone (µg/ml)					
			12.5	25	50	100	250	500
Orishele	1	SA 25	47.59 b	18.05 c	13.29 c	3.12 c	0 c	0 c
		SA 50	37.22 c	16.82 c	13.46 c	14.30 b	12.50 b	0 c
		ASM 25	100 a	88.42 a	47.13 a	26.90 a	18.05 a	9.02 a
		ASM 50	100 a	64.66 b	39.47 b	28.49 a	12.50 b	6.25 b
	2	SA 25	100 a	51.04 b	12.50 d	1.70 c	0.74 b	0 c
		SA 50	94.44 a	77.75 a	50.00 a	37.53 a	12.63 a	6.25 b
		ASM 25	100 a	76.16 a	27.77 c	21.08 b	11.09 a	6.25 b
		ASM 50	100 a	75.33 a	38.88 b	33.19 a	14.80 a	10.41 a
	3	SA 25	100 a	65.07 a	27.99 a	12.25 b	3.61 b	2.77 b
		SA 50	85.18 b	50.79 b	16.54 a	12.25 b	6.69 b	6.25 ab
		ASM 25	100 a	57.14 ab	22.06 a	20.94 ab	14.39 a	4.86 ab
		ASM 50	100 a	65.07 a	27.71 a	28.84 a	17.10 a	9.02 a
	4	SA 25	43.70 b	19.57 b	9.41 b	4.09 d	4.35 c	2.77 b
		SA 50	53.33 b	24.77 b	15.30 b	11.74 c	9.35 b	3.47 b
		ASM 25	70.00 ab	33.04 b	29.15 a	27.57 a	13.86 b	6.25 ab
		ASM 50	85.18 a	55.53 a	29.49 a	21.04 b	20.49 a	9.72 a
Corne 1	1	SA 25	100 a	31.11 c	24.73 c	25.28 b	19.39 c	0 c
		SA 50	100 a	46.66 b	37.27 b	31.15 b	22.22 bc	0 c
		ASM 25	100 a	100 a	49.82 a	37.84 a	27.82 ab	12.50 b
		ASM 50	100 a	100 a	49.82 a	40.73 a	34.25 a	18.75 a
	2	SA 25	55.55 b	40.00 c	12.50 c	2.86 d	3.96 b	0 b
		SA 50	100 a	57.77 b	34.72 b	27.67 b	2.38 b	0 b
		ASM 25	100 a	80.00 a	37.50 b	18.63 c	4.76 b	0 b
		ASM 50	100 a	91.11 a	69.44 a	41.96 a	10.31 a	6.25 a
	3	SA 25	100 a	70.18 a	41.51 a	40.75 a	14.71 a	3.61 a
		SA 50	74.07 b	52.22 b	23.45 c	10.99 d	4.94 b	2.93 a
		ASM 25	100 a	69.25 a	35.03 b	23.56 c	15.50 a	2.27 a
		ASM 50	100 a	72.03 a	46.913 a	31.70 b	21.48 a	7.18 a
	4	SA 25	18.51 d	7.93 c	9.29 c	0 c	0 d	0 b
		SA 50	59.25 c	23.28 b	18.88 b	20.22 b	12.69 b	7.63 a
		ASM 25	79.62 b	30.42 b	24.04 b	19.44 b	7.14 c	6.25 a
		ASM 50	100 a	69.84 a	44.34 a	28.34 a	14.28 a	9.02 a

SA: salicylic acid; ASM: acibenzolar-S-methyl; in the same column, means followed by different letters are significantly different (Newman-Keuls test at 5 %).

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Table 6. Efficacy of protection to necrosis induction by different concentrations of juglone on leaves of two banana cultivars after soil and leaves- treatment with salicylic acid and acibenzolar-S-methyl

Banana cultivar	Time after inducer treatment (week)	Concentration of inducer (µg/ml)	Concentrations of juglone (µg/ml)					
			12.5	25	50	100	250	500
Orishele	1	SA 25	61.11 b	22.96 b	14.81 b	12.94 b	9.15 b	3.56 ab
		SA 50	29.62 c	22.11 b	10.49 b	12.08 b	11.42 b	1.38 b
		ASM 25	100 a	100 a	62.96 a	35.71 a	14.44 b	4.16 ab
		ASM 50	100 a	100 a	59.56 a	48.65 a	21.37 a	7.82 a
	2	SA 25	100 a	76.29 b	34.81 c	32.76 c	11.08 c	8.65 b
		SA 50	100 a	70.74 b	31.35 c	25.56 d	9.37 c	5.74 b
		ASM 25	100 a	100 a	88.02 b	48.71 b	22.78 b	9.30 b
		ASM 50	100 a	100 a	100 a	83.11 a	44.37 a	31.11 a
	3	SA 25	50.00 c	35.07 c	31.46 c	21.93 c	11.77 c	4.25 c
		SA 50	72.22 b	54.34 b	32.47 c	17.58 c	11.57 c	2.08 c
		ASM 25	100 a	100 a	87.00 b	59.24 b	31.11 b	12.68 b
		ASM 50	100 a	100 a	98.06 a	75.12 a	42.19 a	19.12 a
	4	SA 25	62.40 b	28.54 d	18.57 b	9.51 b	3.07 c	5.60 ab
		SA 50	93.14 a	40.58 c	16.94 b	7.66 b	4.66 bc	3.51 b
		ASM 25	100 a	67.59 b	46.66 a	38.38 a	9.94 b	6.25 ab
		ASM 50	100 a	100 a	73.28 a	37.64 a	15.64 a	12.59 a
Corne 1	1	SA 25	22.22 b	33.33 c	22.09 d	11.90 b	5.18 b	7.03 b
		SA 50	100 a	67.52 b	30.49 c	8.59 b	5.23 b	2.08 c
		ASM 25	100 a	100 a	88.02 b	59.25 a	19.11 a	11.99 a
		ASM 50	100 a	100 a	100 a	62.83 a	22.98 a	9.90 ab
	2	SA 25	100 a	39.81 c	11.30 c	10.52 d	6.41 d	3.51 b
		SA 50	100 a	87.96 b	35.71 b	29.96 c	15.20 c	3.61 b
		ASM 25	100 a	100 a	100 a	66.91 b	33.88 b	16.57 a
		ASM 50	100 a	100 a	100 a	88.38 a	22.58 a	15.74 a
	3	SA 25	100 a	58.70 c	24.00 a	15.05 d	10.79 d	4.40 b
		SA 50	100 a	78.88 b	33.39 b	30.10 c	20.05 c	4.16 b
		ASM 25	100 a	100 a	100 a	85.13 b	41.79 b	15.09 a
		ASM 50	100 a	100 a	100 a	100 a	30.15 a	15.09 a
	4	SA 25	100 a	73.54 b	34.32 b	18.97 c	17.94 b	5.60 a
		SA 50	63.88 b	44.44 c	23.95 c	16.33 c	9.31 c	2.08 a
		ASM 25	100 a	100 a	82.83 a	37.10 b	20.27 b	7.87 a
		ASM 50	100 a	100 a	88.51 a	64.99 a	31.33 a	8.42 a

SA: salicylic acid; ASM: acibenzolar-S-methyl; in the same column, means followed by different letters are significantly different (Newman-Keuls test at 5 %); % compared to control.

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Table 7. Incubation time inducing 50 % of necrosis on total foliar surface infiltrated with juglone at 100 µg/ml after salicylic acid and acibenzolar-S-methyl application on soil and leaves of two cultivars banana

Banana cultivar	Concentration of inducer (µg/ml)	Week after inducer treatment							
		1		2		3		4	
		IT_{50} (h)	R^2	IT_{50} (h)	R^2	IT_{50} (h)	R^2	IT_{50} (h)	R^2
Orishele	0	3.84	0.86	7.94	0.87	6.52	0.96	8.03	0.91
	SA 25	12.86	0.97	15.5	0.99	12.57	0.96	9.61	0.97
	SA 50	13.82	0.98	15.47	0.98	13.09	0.98	12.28	0.97
	ASM 25	26.31	0.99	41.50	0.94	38.56	0.94	22.67	0.99
	ASM 50	54.94	0.98	101.29	0.89	49.96	0.98	31.33	0.96
Corne 1	0	9.13	0.93	10.19	0.94	80.60	0.99	8.00	0.96
	SA 25	23.79	0.98	15.32	0.97	9.80	0.97	7.57	0.97
	SA 50	19.46	0.98	18.10	0.99	13.82	0.96	10.75	0.99
	ASM 25	89.17	0.94	110.89	0.87	69.20	0.96	40.52	0.99
	ASM 50	163.78	0.98	204.51	0.85	179.34	0.99	68.86	0.98

AS: salicylic acid; ASM: acibenzolar-S-methyl; R^2 = Coefficient of correlation, IT_{50} = Incubation time inducing 50 % of necrosis on total foliar surface infiltrated with juglone at 100 µg/ml.

Table 8. Sensitivity to BLSD of two banana cultivars after treatments of soil and leaves with salicylic acid and acibenzolar-S-methyl

Banana cultivar	Treatment of inducer	Incubation time (d)	Disease development time (d)	Symptoms evolution time (d)
Orishele	**Control**	15.13 b	32.13 c	17.00 c
	SA 25	15,66 b	38.13 b	22.46 b
	SA 50	15.93 b	37.26 b	21.33 b
	ASM 25	17.33 b	44.46 a	27.13 a
	ASM 50	19.46 a	44.66 a	25.20 a
Corne 1	**Control**	15.00 b	33.20 c	18.20 c
	SA 25	17.20 ab	37.93 c	20.73 c
	SA 50	17.66 ab	35.33 c	17.66 c
	ASM 25	17.53 ab	45.66 b	28.13 b
	ASM 50	19.40 a	53.26 a	33.86 a

SA: salicylic acid; ASM: acibenzolar-S-methyl; BLSD: black leaf streak disease; in the same column, means followed by different letters are significantly different (Newman-Keuls test at 5 %).