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Synthetic Elicitor-Induced Defense Responses in Tomato (*Solanum lycopersicum*) Cultivated in Côte d'Ivoire against Bacterial Wilt Caused by *Ralstonia solanacearum*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors participated equally in the study idea, literature review, data collection and analyses, methodology, statistical analyses, tabulating the data, results validation, writing and revising the whole manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Bacterial wilt caused by *Ralstonia solanacearum* still constitutes tomato (*Solanum lycopersicum*) economical disease in the world. This disease is favored by very humid soils and high temperatures. Genetic control currently used is the most effective strategy. Disease control in crops has also become possible through the induction of plant defense reaction. This work aims at assessing the

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effect of both synthetic elicitors (BABA and ASM) supposed to induce tomato defense reaction against bacteria and particularly *Ralstonia solanacearum*. BABA and ASM solutions were each applied at concentrations of 5 to 100 ppm on *R. solanacearum in vitro*, and its growth was recorded. Then, the *in vivo* development of bacterial wilt was assessed following different elicitors application modes to both local tomato cultivars Tropimech and Caraïbo respectively sensitive and tolerant to this disease. Up to 100 ppm, BABA and ASM elicitors showed no antibacterial effect against *R. solanacearum*. However, these elicitors revealed a protective action against the development of bacterial wilt after inoculation of *R. solanacearum*. Compared to leaf treatment, the supply of elicitors to roots or successively to roots and leaves reduced the development of bacterial wilt by more than 50%. Both tomato varieties (Tropimech and Caraibo) expressed identical resistance levels facing *Ralstonia solanacearum* after BABA and ASM application. The elicitation of tomato plants could be an ecological approach for effective control of *R. solanacearum.*

Keywords: Elicitor; bacterial wilt; plant defense; Ralstonia solanacearum; Solanum lycopersicum; tomato.

1. INTRODUCTION

Tomato is the most consumed vegetable in Côte d'Ivoire because it is used to cook most sauces and all forms of salad. Tomatoes annual yield fluctuates between 22,000 and 35,000 tons. The grown varieties are mostly introduced from Europe or Israel [1]. However, the yield remains below demand. Several factors can explain these low yields. Among them, biotic factors are the most important. Among bacterial diseases impacting negatively tomato yield, bacterial wilt caused by *Ralstonia solanacearum* is a dangerous one that causes serious damage in the nursery as well as in plantation. It can cause up to 100 % yield loss depending on the variety [2].

In Côte d'Ivoire, bacterial wilt has frequently occurred [3]. The complexity of this bacteria makes its control difficult. Based on the use resistant varieties, this control is potentially the most effective. But it is confronted to the genotypic and phenotypic variability of strains, the high genomic plasticity, often the quantitative nature of host resistance associated with strong interactions with environmental conditions [4].

Unfortunately, pesticides of chemical origin are increasingly in the hot seat for questions of toxicity, environmental pollution, human health and biodiversity [5,6]. In this context, it seems necessary to look for more effective alternatives for sustainable agriculture development. One of them is to give means of plants to defend themselves, or to strengthen their own defenses, rather than directly to control the aggressor. In this category are the elicitors or stimulators of plant defense, which could contribute to the alternative management of bacterial wilt caused by *R. solanacearum* in more ecological production systems [7,8,9]. Thus, several elicitors have been tested to stimulate plants defense. Among them, ASM and BABA are well known activate the defense in many plants. Therefore, they play an important role in inducing resistance in plants against pathogens infection [10,11].

The aim of this study was to increase the resistance of tomato to bacterial wilt caused by *R. solanacearum* in order to decrease the use of pesticides in control of the disease. This objective is part of the establishment of sustainable agriculture in Côte d'Ivoire, i.e. research for more effective alternatives for sustainable agriculture development. In fact, it is a question of finding an alternative solution allowing to limit the chemical fight for a better protection of the environment and the human health. It is in this context that this has considered the evaluation of the effect of two synthetic elicitors such as ASM and BABA on bacterial wilt in two tomato varieties grown in Côte d'Ivoire.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material used in the research project was composed of two cultivars of tomato (*Solanum lycopersicum* L.) which are Tropimech and Caraibo, respectively sensitive and tolerant to bacterial wilt caused by *Ralstonia solanacearum* [12].

Tomato seeds were placed in well-divided seed bins and each containing sterile potting soil. After 14 days of seeding, seedlings were transplanted into 250 mL seed pots each containing 200 mL of potting soil. Plants were grown in a greenhouse and regularly watered until they were used at the five-leaf stage (i.e. 25 days after transplanting) for elicitation and inoculation tests.

2.2 Bacterial Material

Strains of *Ralstonia solanacearum* (RUN 1743; CIRAD collection, Island of reunion, France) belonging to phylotype I and pathoprofil F was used in this study. These bacterial strains are pathogenic for tomato grown in Côte d'Ivoire.

2.3 Elicitor Preparation

Two elicitors used in the elicitation experiment were ASM (Acibenzolar-S methyl, an analog of salicylic acid) and BABA (β-Amino-Butyric acid, a non-protein amino acid). ASM and BABA were dissolved in sterile distilled water (with 10% methanol) to obtain five concentrations such as 5, 10, 25, 50, 75 and 100 ppm. Water-treated leaves were used as control.

2.4 Evaluation of *In vitro* Activity of ASM and BABA on Bacterial Growth

A bacterial inoculum of 10^8 CFU mL^{-1} concentration (optical density 0.1 to 600 nm) was prepared from 24 hour-old colonies suspended in sterile distilled water. The CPG medium was prepared by autoclaving at 121°C under a pressure of 1 bar for 30 minutes and then distributed in Petri dishes after cooling at room temperature. Approximately, 1 mL of bacterial inoculum was added to CPG medium and the whole has been homogenized. After medium gelling, three wells were made using a sterilized punch. In a Petri dish, 40 µL of an elicitor concentration were placed at the rate of one elicitor per well. In the third well, the sterile distilled water that is considered the control has been deposited. Petri dishes were incubated in an oven at 37°C. After 24 h of incubation, the diameter of inhibition zones of bacterium growth caused by elicitors was evaluated. Each test has five Petri dishes per concentration and the experiment was triplicated.

2.5 Treatment of Tomato Plants with Elicitor

Elicitor solutions (5 mL) were applied to tomato plants according to three modalities: foliar spray, roots level and foliar and roots co-treatment. After three days the treatment was repeated as before.

2.6 Bacteria Inoculation of Tomato Plants

Tomato plants treated with elicitors (after the second application of elicitors) as well as control plants were inoculated with the addition of 5 mL of 108 CFU/mL bacterial suspensions. Bacterial inoculum was spread over the base of the stem of each plant. For each mode of application of the elicitors, five plants of each cultivar used. Treated-plants were transferred in the greenhouse according to a split-plot design taking into account factors of elicitors (ASM, BABA), elicitor application mode (foliar, root and root and leaf treatment) and cultivar type.

2.7 Assessment of Disease

Disease assessment was started three days after inoculation and stopped and this for 28 days i.e. 31 days after inoculation. Disease severity (mean wilt score) was evaluated using the disease symptom scoring scale [13]; score 0, no symptoms; score 1, one leaf wilted; score 2, two-three leaves wilted or half of the leaves wilted; 3, all leaves wilted and score 4, stem folded or plant death. Several parameters were calculated:

- The wilting index (WI) reflects the disease incidence following grades: 3 and 4, that is the proportion of plants showing end-stage symptoms: $\mathrm{WI}\ (\%) = \frac{N_3+N_4}{N_T}\ X\ 100$, with, N_3 was the number of plants rated 3, N_4 was the number of plants rated 4 and N_T was the total number of plants used for each treatment
- The disease index (DI) expresses the severity of the disease or the intensity of symptoms observed on tomato plants: $\mathrm{DI}\ (\%) = \frac{\Sigma(N_i . z_i)}{N_T . Z}\ X\ 100$, with, N_i was the number of plants that received the same grade, Z_i was grade (0; 1; 2; 3 or 4), N_T was the total number of plants used for each treatment and Z was highest grade (4).
- The reduction of disease rate (RDR) reflects the effectiveness of treatment against the bacterial wilt development. It was determined from the IM of inoculated plants and the non-elicited inoculated control: $\mathrm{RDR}\ (\%) = \frac{(IM_C - IM_T)}{IM_C .}\ X\ 100$, with IM_C was the disease index of non-elicited inoculated control and IM_T was the disease index of elicited and inoculated plants (trial).

2.8 Assessment of Latent Infections on Asymptomatic Plants

At the end of trial, latent infections were estimated on the plants showing no symptoms. Pathogen isolates were obtained from the stem fragments of tomato plants in order to verify the presence or the absence of *R. solanacearum*. For each plant, after stem disinfection, sections of 2 to 3 cm were cut at the collar and transferred into 5 mL of Tris buffer. Stem remained fragments were incubated for 2 h at room temperature for easing the release of bacterial colonies into the buffer. A volume of 50 µL of the extract was spread on CPG medium. The Petri dishes were then incubated at 28°C for three to four days. Asymptomatic plants were noted positive for latent infection when characteristic colonies of *R. solanacaerum* were observed. Data obtained from latent infections made it possible to calculate the colonization index (CI) for each treatment according to the formula:

CI = WI + (NS + RS), with WI was wilting index, NS was asymptomatic plants rate and RS was infected asymptomatic plants rate [14].

2.9 Statistical Analysis

Experiments were performed using a completely randomized design. Data were subjected to analysis of variance (ANOVA) using STATISTICA software (release 7.1) and differences between means were compared using Newman-Keuls test. Differences at $P < 0.05$ were considered as significant.

3. RESULTS

3.1 Effect of ASM and BABA on *in vitro* Growth of *Ralstonia solanacearum*

The result showed that no bacteria growth inhibition zone was observed on CPG medium at the wells which received elicitor solutions for the different concentrations tested. Bacteria growth was identical around the wells containing the sterile distilled water (control) to those receiving elicitor solutions (Fig. 1).

3.2 Incidence of ASM and BABA Application on *in vivo* Development of Bacterial Wilt

3.2.1 Effect of ASM and BABA on wilting index

Tomato plants showed different wilt indices depending on the mode of application of the elicitors, after 28 days of bacteria post-inoculation (Fig. 2). Significant differences were observed (P <0.0001). Thus, the wilting index was 100% for the control plants while it was less than 65% for the plants treated with the elicitors.

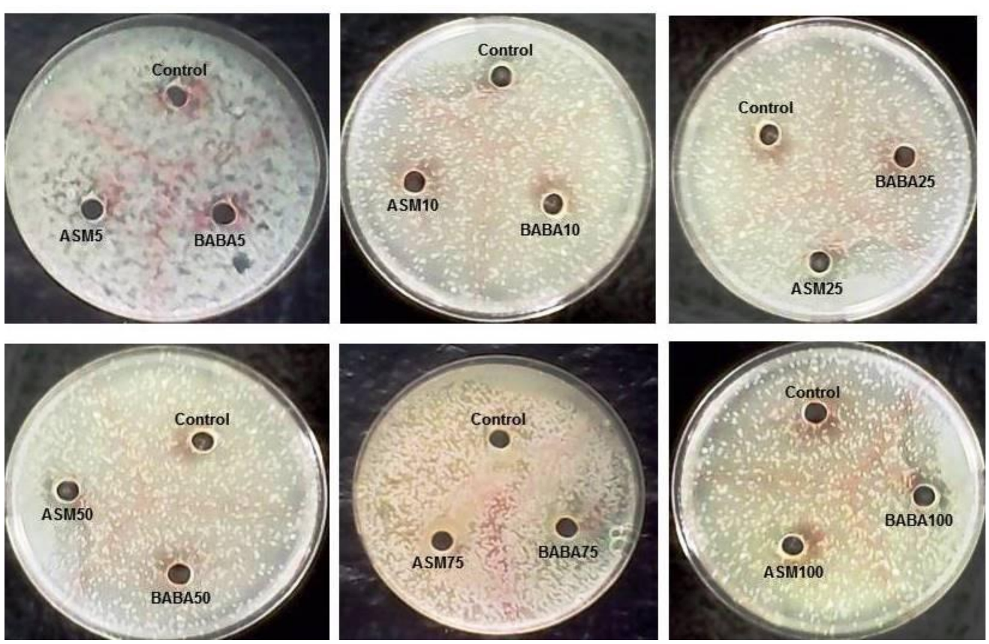

Fig. 1. Bacterial cultures of *Ralstonia solanacearum* strain (RUN 1743) on CPG medium with wells having received ASM and BABA solutions at 5; 10; 25; 50; 75 and 100 ppm

ASM, Acibenzolar-S methyl; BABA, β-Amino-Butyric acid; Control, distilled water

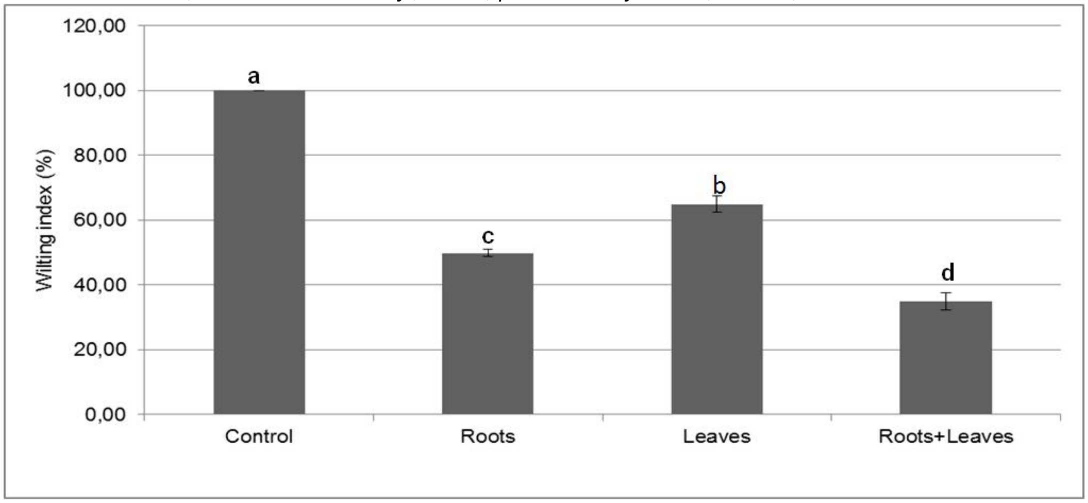

Fig. 2. Effect of elicitors application mode on tomato wilting index after 28 days of *Ralstonia solanacearum* strain (RUN 1743) inoculation

ASM, Acibenzolar-S methyl; BABA, β-Amino-Butyric acid; histograms topped with significantly different (Newman-Keuls test at 5 %).

In co-treated plants with ASM and BABA at the level of roots and leaves, the wilting index was significantly low (35%). On the other hand, alone elicitor application on roots (49.88%) and leaves (64.99%) were significantly significant. However, alone leaves treated had a higher wilting index than treated roots alone. Thus, the elicitor application influenced very significantly (P <0.0001) the plant wilting index (Fig. 3). Furthermore, results show that ASM with 61.34% plant wilt index was more effective compared to BABA (63.56%). The results reveal also that a varietal effect was observed. In fact, the tomato variety influenced the wilt index with highly significant differences ((P <0.0001). Caraibo variety showed the lowest wilt index (58.27%) compared to Tropimech variety which, with 66.66%, had the highest index (Fig. 4).

The effect of the combinations of application patterns of the elicitors on the index of wilting of tomato plants after 28 days of post-bacterial inoculation (Table 1), is characterized by the weakest wilting indexes of the plants for the

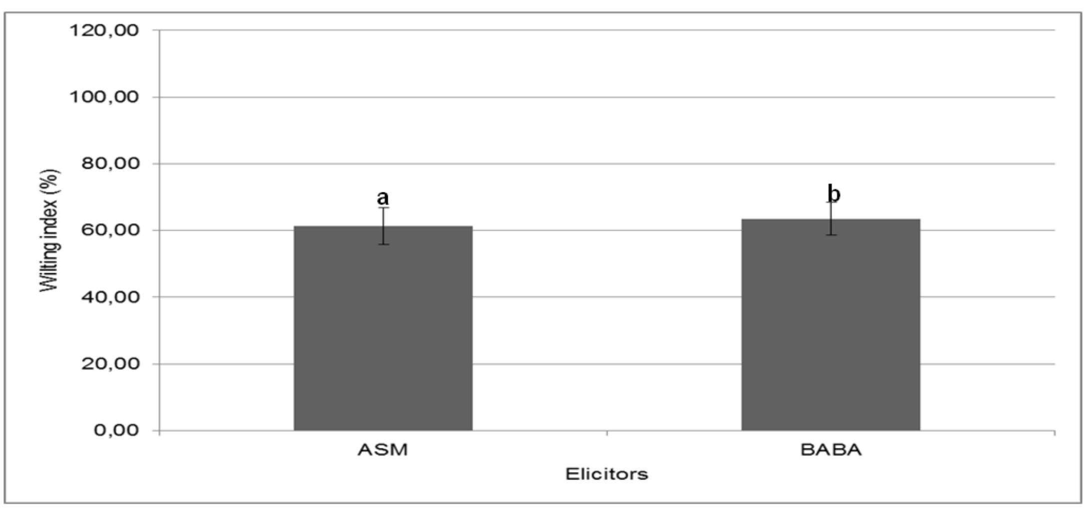

Fig. 3. Effect of elicitors (ASM and BABA) on tomato wilting index after 28 days of *Ralstonia solanacearum* strain (RUN 1743) inoculation

ASM, Acibenzolar-S methyl; BABA, β-Amino-Butyric acid; histograms topped with significantly different (Newman-Keuls test at 5 %)

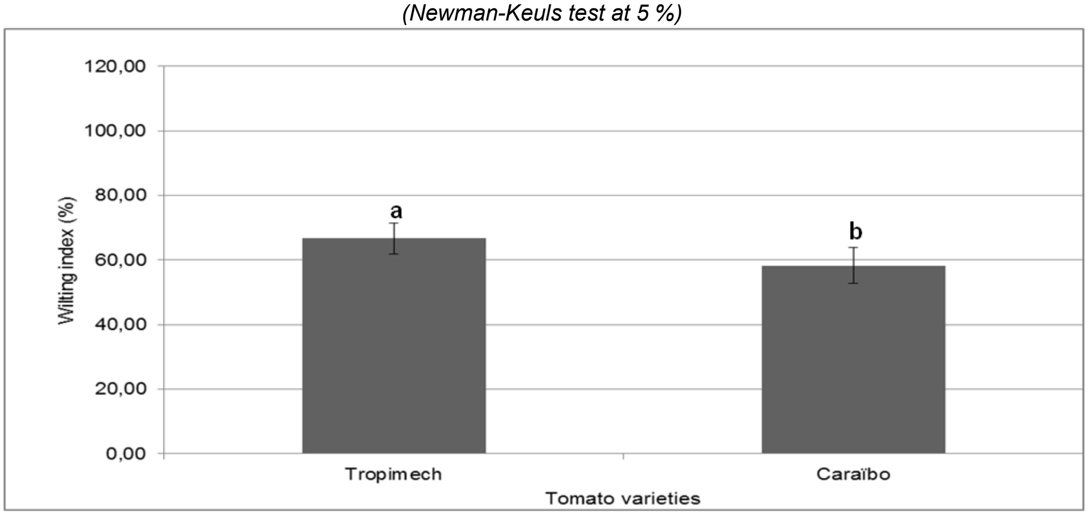

Fig. 4. Effect of variety on tomato wilting index after 28 days of *Ralstonia solanacearum* strain (RUN 1743) inoculation

ASM, Acibenzolar-S methyl; BABA, β-Amino-Butyric acid; histograms topped with significantly different (Newman-Keuls test at 5%)

Table 1. Effect of elicitor application mode on tomato wilting index after 28 days of *Ralstonia solanacearum* strain (RUN 1743) inoculation

Modes of application	Elictors	Wilting index (%) of tomato	
		Tropimech	**Caraïbo**
Control	water	100.0 ± 0.00 a	100.0 ± 0.00 a
Root	ASM	53.33 ± 0.00 c	44.33 ± 2.17 e
	BABA	53.33 ± 0.00 c	48.55 ± 0.95 d
Leaf	ASM	73.33 ± 0.00 b	60.00 ± 0.00 b
	BABA	73.33 ± 0.00 b	53.33 ± 0.00 c
Root/Leaf	ASM	40.00 ± 0.00 d	20.00 ± 0.00 g
	BABA	40.00 ± 0.00 d	40.00 ± 0.00 f
Means (± SD)		61.90 ± 0.00 b	52.32 ± 0.44 d
P		< 0.0001	< 0.0001

ASM, Acibenzolar-S methyl; BABA, β-Amino-Butyric acid; on a line and in a column, means followed by a different letter are significantly different (Newman-Keuls test at 5 %); data are expressed as the mean of triplicate; ±SD: standard deviation

simultaneous treatments on roots and leaves with ASM and BABA in the Tropimech variety and only with ASM at Caraibo. Overall, plants from combinations including root and leaf application mode were less favorable for wilt symptoms development at the stage 3 and 4. For all combinations, Caraibo variety had the weakest wilting index, unlike those of the Tropimech variety. These differences observed were significant (P <0.0001).

3.2.2 Effect of elicitors on bacterial wilt reduction rate

The elicitor application mode on tomato wilt reduction rate, 28 days after bacterium inoculation (DAI), showed very significant differences (P <0.0001) as shown in Fig. 5. The highest rate of disease reduction for eliciting applications was observed on roots and leaves (64.58%) simultaneously treated followed by root alone treatment (58.09%). Finally, leave elicitor application mode (50.60%) gave the lowest rate of plant wilt reduction. In addition, tomato wilt reduction rates were ranged between 56.19 and 59.32%

depending on elicitors (Fig. 6) and a significant difference was observed (P <0.001) between values.

The tomato variety was influenced significantly (P <0.0001) the rate of bacterial wilt reduction as shown in Fig. 7. Thus, the highest rates were reported with the Caraibo variety (60.95%).) while Tropimech variety (54.57%) showed the lowest value. Tomate plant wilt reduction rates was ranged from 48.56 to 60.00 for Tropimech and between 51.66 and 71.66 for Caraibo, and that according to the combinations of application mode and elicitors (Table 2). However, the differences observed were not significant (P = 0.087) at 5% threshold.

3.2.3 Effect of elicitors on bacterium colonization index in tomato

The tomato plants colonization index by bacterium recorded with the application mode of elicitors on leaves was higher than 80 % (Fig. 8). However, bacterium colonization index were lower on plants treated on roots and leaves (58.33 %) and those from treatment on roots (60.13 %) alone. This index was range from 60.64 to 74.4% depending on elicitors, but no

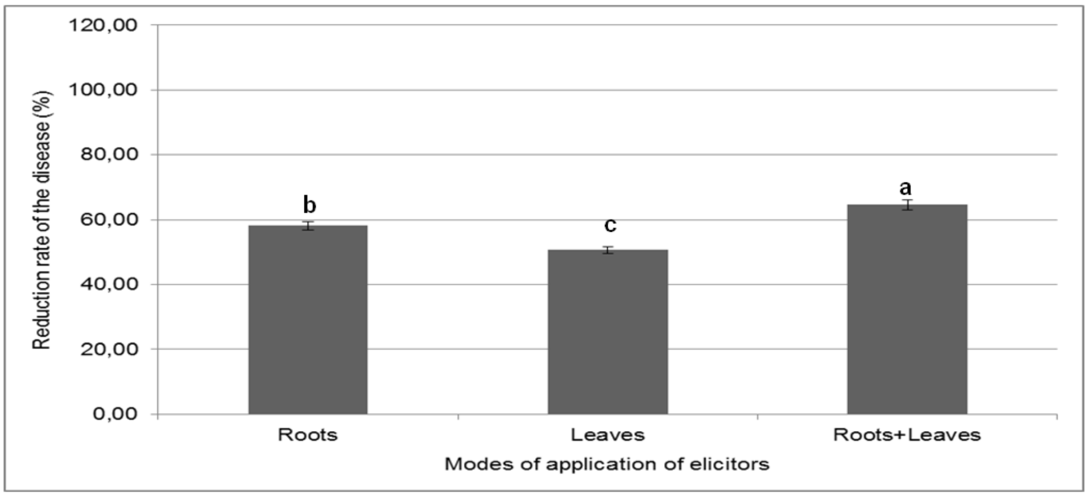

Fig. 5. Effect of elicitor application mode on tomato bacterial wilt reduction rate after 28 days of *Ralstonia solanacearum* strain (RUN 1743) inoculation

ASM, Acibenzolar-S methyl; BABA, β-Amino-Butyric acid; histograms topped with significantly different (Newman-Keuls test at 5 %)

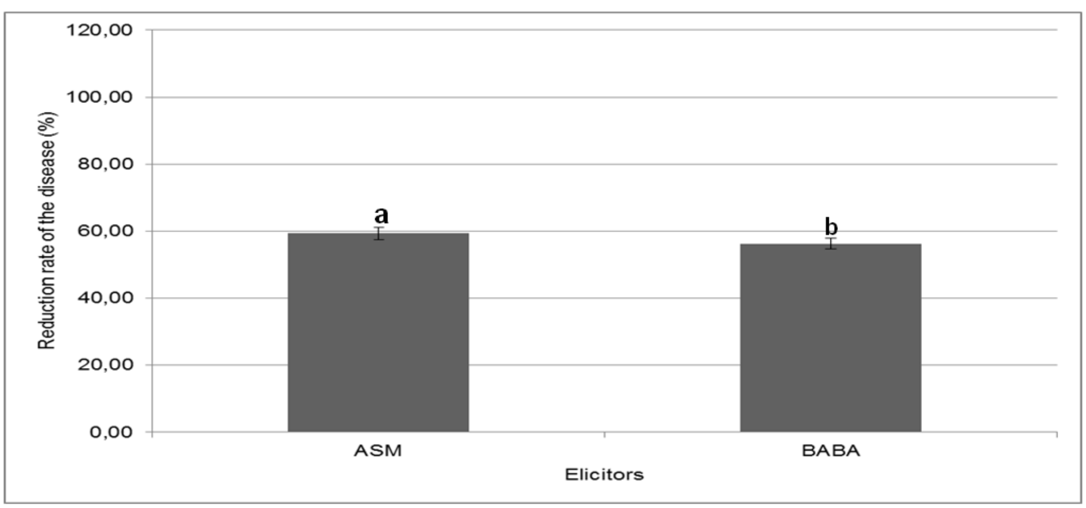

Fig. 6. Effect of elicitors on tomato bacterial wilt reduction rate after 28 days of *Ralstonia solanacearum* strain (RUN 1743) inoculation

ASM, Acibenzolar-S methyl; BABA, β-Amino-Butyric acid; histograms topped with significantly different (Newman-Keuls test at 5%).

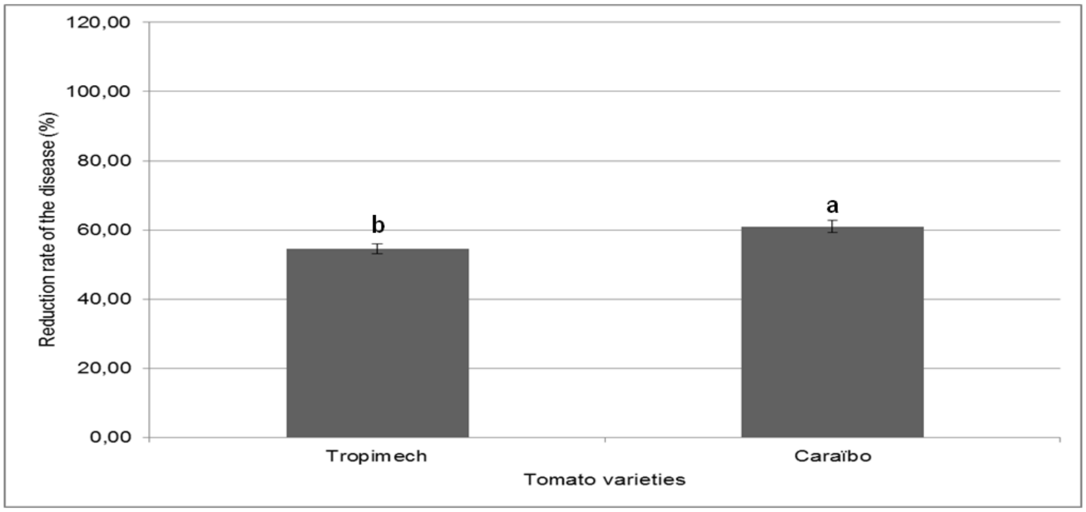

Fig. 7. Effect of tomato variety on bacterial wilt reduction rate after 28 days of *Ralstonia solanacearum* strain (RUN 1743) inoculation

ASM, Acibenzolar-S methyl; BABA, β-Amino-Butyric acid; histograms topped with significantly different (Newman-Keuls test at 5%)

Table 2. Effects of elicitor application mode on tomato bacterial wilt reduction rate after 28 days of *Ralstonia solanacearum* strain (RUN 1743) inoculation

Modes of application	**Elictors**	**Bacterial wilt reduction rate (%)**	
		Tropimech	**Caraibo**
Root	ASM	58.33 ± 0.00 a	63.75 ± 0.00 a
	BABA	51.67 ± 0.00 a	58.64 ± 0.02 a
Leaf	ASM	48.89 ± 2.22 a	53.33 ± 0.00 a
	BABA	48.56 ± 3.97 a	51.66 ± 0.00 a
Root/Leaf	ASM	60.00 ± 0.00 a	71.66 ± 0.00 a
	BABA	60.00 ± 0.00 a	66.66 ± 0.00 a
Means (± SD)		54.57 ± 1.03 a	60.95 ± 0.00 a
P		0.3033	0.2304

ASM, Acibenzolar-S methyl; BABA, β-Amino-Butyric acid; on a line and in a column, means followed by a different letter are significantly different (Newman-Keuls test at 5 %); data are expressed as mean of triplicate; ±SD: standard deviation.

significant difference (P = 0.56) was observed at 5 % threshold between the applications of ASM and BABA (Fig. 9).

The tomato plants colonization index oscillated between 60.68% with the Caraïbo variety and 74.44% with the Tropimech variety (Fig. 10). The observed differences were not significant (P = 0.058) at 5% threshold. For all combinations of application modes and elicitors, the tomato plants colonization index varied from 43.33 to 100.00% (Table 3). However, there was no significant difference between these combinations in Tropimech (P = 0.98) and Caraïbo (P = 0.88) varieties.

4. DISCUSSION

The direct antibacterial effect of ASM and BABA was studied *in vitro* from diffusion trials of these elicitors in culture media of the Ivorian strain of *Ralstonia solanacearum* (RUN 1743). No inhibition growth zones of the bacterium were recorded around the wells containing the five concentrations (5, 10, 25, 50 and 100 ppm) of the elicitors tested. This lack of bacterial growth inhibition confirms that the ASM and BABA elicitors have no direct inhibitory action on the *Ralstonia solanacearum* bacterium. This result is in accordance with that obtained by Ben Hassena [15] on the aerial fungus *Botrytis cinerea* with the Dalgin Active elicitor, supposed to have an inductive action of defense reactions in plants attacked by pathogens.

In fact, elicitors have characteristics of protecting crops against pathogens by increasing the defense and resistance potential of plants [16,17]. Elicitors are plant defense stimulators (PDS) showing inactive characteristic on pathogen since they act on the plant. However, Amari et al. [9] reported that using ASM at high concentrations (> 100 ppm) weakly inhibits mycelial growth and conidial germination.

When assessing the protective effect of ASM and BABA at 50 ppm on tomato against *R. solanacearum*, two applications of these elicitors within three days were made only on roots or leaves and finally, successively on roots and then on leaves. The results obtained showed that the disease development was less intense in the plants treated with elicitors than in the control plants not elicited but inoculated. These results related to the elicitors application mode showed that root and leaf supplies were more effective against bacterial wilt than mere applications ones. Root and then leaf treatment might enable

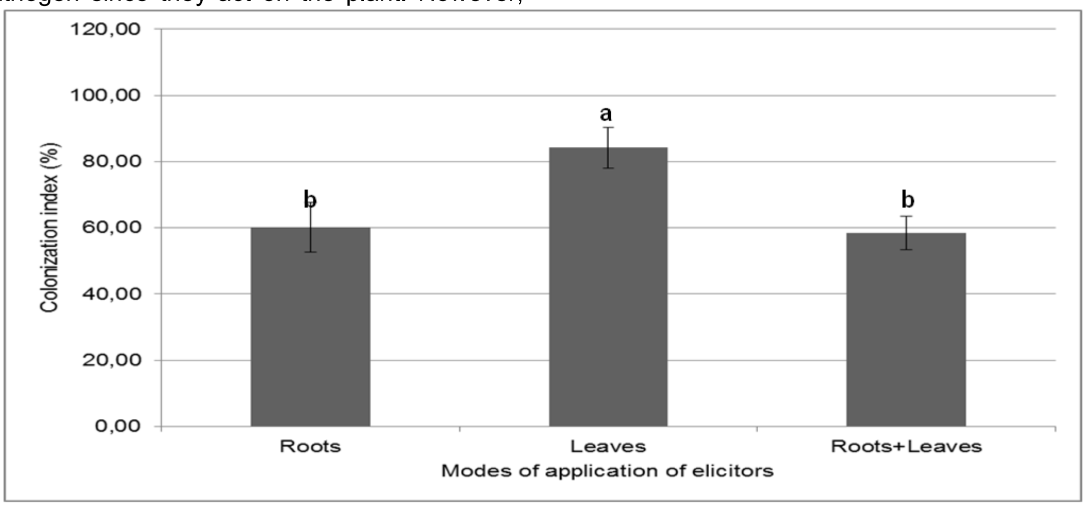

Fig. 8. Effect of elicitors application mode on tomato colonization index after 28 days of *Ralstonia solanacearum* strain (RUN 1743) inoculation

ASM, Acibenzolar-S methyl; BABA, β-Amino-Butyric acid; histograms topped with significantly different (Newman-Keuls test at 5%).

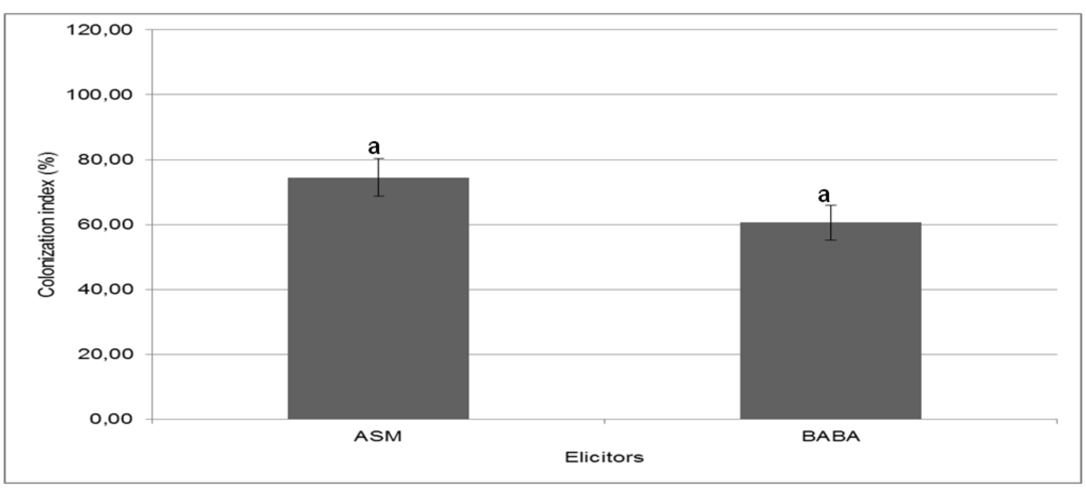

Fig. 9. Effect of elicitor on tomato colonization index after 28 days of *Ralstonia solanacearum* strain (RUN 1743) inoculation

ASM, Acibenzolar-S methyl; BABA, β-Amino-Butyric acid; histograms topped with significantly different (Newman-Keuls test at 5%)

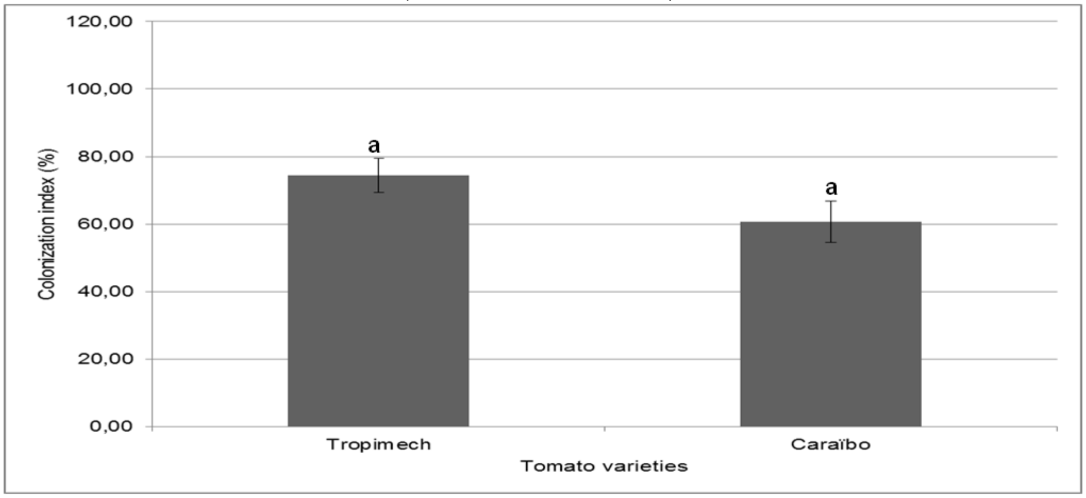

Fig. 10. Effect of tomato variety on colonization index after 28 days of *Ralstonia solanacearum* strain (RUN 1743) inoculation

Histograms topped with significantly different (Newman-Keuls test at 5 %)

Table 3. Effects of elicitor application modes and their interactions on the tomato varieties (Tropimech and Caraïbo) plants colonization index 28 days after inoculation of the *Ralstonia solanacearum* strain (RUN 1743)

Modes of application	Elictors	Colonization index (%) of tomato varieties	
		Tropimech	**Caraïbo**
Root	ASM	66.66 ± 13.33 a	66.93±23.77 a
	BABA	63.33 ± 12.01 a	43.60±11.75 a
Leaf	ASM	100.00 ± 0.00 a	86.66±13.33 a
	BABA	93.33 ± 6.66 a	56.93±8.56 a
Root+Leaf	ASM	60.00 ± 11.54 a	66.66±6.66 a
	BABA	63.33 ± 3.33 a	43.33±14.52 a
Means (± SD)		74.44 ± 7.81 a	60.68±13.10 a
P		0.980799	0.888734

ASM, Acibenzolar-S methyl; BABA, β-Amino-Butyric acid; on a line and in a column, means followed by a different letter are significantly different (Newman-Keuls test at 5 %); data are expressed as the mean of triplicate; ±SD: standard deviation.

the elicitor to act more effectively on the root and leaf systems of the plant at the same time. However, the efficacy of sole elicitation of roots than that of leaves could be explained by the fact that these bacteria are telluric.

Plant infection with the bacterium starting from the roots, lead to a rapid and stronger accumulation of defense compounds in these organs after elicitation. This might then promote a more effective control of disease unlike to elicitors application on leaves. The root and then leaf application mode could be an important criterion for effective control of *R. solanacearum* bacterium. Moreover, Lang et al. [18] showed that the preventive effect of ASM (26.3 g active ingredient/ha applied twice 7-days apart) resulted in a 48% reduction in bacterial wilt caused by *Xanthomonas axonopodis* in the onion, can be improved by varying the mode of treatment.

The effect of ASM and BABA on the disease expression symptoms showed that theses elicitors act differently. Contrarily to BABA, ASM with a lower wilting index and a higher disease reduction rate has reduced more effectively the development of initial and terminal stage symptoms. However with a lower plant colonization index than ASM, BABA favored the development of longer bacterial wilt symptoms latency. This difference between both elicitors action might be based on the fact, contrarily to BABA, ASM once applied in the presence of a pathogen or not, causes defense reactions in plant tissues [19].

BABA only triggers the first steps of resistance induced after application but it will allow a faster and more effective response from the plant during an infection. BABA is therefore qualified as a potentiator because it has a priming effect that ensures a long period for the initiation of defense compounds biosynthesis, resulting to a delay in the expression of symptoms following plant infection.

Considering the development of bacterial wilt symptoms of tomato plants, the Tropimech variety was more sensitive than the Caraibo one even after elicitation. These results are similar to those of Soro et al. [20] on the behavioral study of tomato varieties facing pathogens. These authors describe the Tropimech tomato variety as more sensitive one to fungal agents. There is, therefore, a varietal effect related to elicitor treatment as reported also by Amari et al. [9] in banana. Indeed, they reported that the application of elicitors such as ASM and salicylic acid on banana induces a higher resistance to black Sigatoka in the less sensitive cultivar (corn I) than in the highly susceptible cultivar (Orishele). Thus, in tomato varieties (Tropimech and Caraibo) whose susceptibility to bacterial wilt is variable, this same induction kinetics and defense compounds seem to be observed [21].

5. CONCLUSION

The objective of this study was to control bacterial wilt caused by *R. solanacearum* using ASM and BABA as elicitors of tomato defense. Two tomato cultivars that are Tropimech and Caraïbo respectively sensitive and tolerant to bacterial wilt were used. The results show that ASM and BABA haven't any direct action on the development of *R. solanacearum* colonies following pathogen growth inhibition tests. However, this study showed a 50% of disease severity reduction after ASM and BABA application on roots and leaves or on roots alone compared to control. Tropimech which is the sensitive cultivar expressed identical resistance levels facing *Ralstonia solanacearum* after BABA or ASM application as Caraibo, the tolerant cultivar. Thus, ASM and BABA can be used in control to bacterial wilt of tomato. This technique can be considered as an ecological approach and an alternative to chemical control of bacterial wilt caused by *R. solanacearum* in tomato.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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