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Stimulatory Effects of Salicylic Acid and Benzothiadiazole on Phenolic Compounds Biosynthesis in Cotton Leaves [Gossypium Hirsutum L (Malvaceae)]

N'CHO Achi Laurent[1*], AMARI Ler-N'Ogn Dadé Georges Elisée[2], KOUAKOU Konan Yao Francois [3], KONE Dramane[1], KOUAKOU Tanoh Hilaire[1]

[1]Université Nangui Abrogoua, UFR des Sciences de la Nature, Laboratoire de Biologie et Amélioration des Productions Végétales, 02 BP 801 Abidjan 02, Côte d'Ivoire

[2]Université Félix Houphouët Boigny, UFR Biosciences, Laboratoire de Physiologie Végétale, 22 BP 582 Abidjan 22, Cote d'Ivoire

[3]Université Jean Lorougnon Guédé, UFR Agrophysiologie, BP 150 Daloa, Côte d'Ivoire

* Corresponding author: N'CHO Achi Laurent, Laboratoire de Biologie et Amélioration des Productions Végétales, UFR des Sciences de la Nature, Université Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire Tel: (+225) 20 30 42 00,

Abstract

Impact of benzothiadiazole (BTH) and salicylic acid (SA), as natural defense stimulators, was tested on phenolic compounds biosynthesis in cotton (Gossypium hirsutum L., cv. Y764AG). Cotton leaves were spread with BTH and SA at six concentrations (1.0, 2.5, 5.0, 8.0 and 10 mM) and incubated during 24, 48, 72 and 96 h. Plant-treated with 1.0 mM BTH and 2.5 mM SA, respectively 96 and 24 h of incubation time induced the highest levels of phenolic compounds for each type of stimulator. These both concentrations were then combined for the co-treatment of the plants, with the previous incubation times retained. Results showed that co-treatment with BTH and SA was not beneficial for the induction of phenolic compounds compared to elicitors used alone.

Keywords: Cotton; benzothiadiazole (BTH); elicitors; Gossypium hirsutum; phenolic compounds; salicylic acid (SA).

1. Introduction

Cotton is a tropical plant belonging to the genus Gossypium, of which four species are currently cultivated. G. hirsutum is the most cultivated species, accounting for almost 95 % of world cotton production [1]. African cotton-producing countries account for only 8% of world production [2]. Cotton plays important, economic and social role in Africa. Cotton represents a considerable economic and social importance in Africa. For example, it constitutes 7% of agricultural exports in Côte d'Ivoire and contributes to 1.7% of the annual gross domestic product. Thus, cotton is the fourth most important export product after cocoa, rubber and cashew and generates about 100 billion CFA franc currency in Côte d'Ivoire [3]. Cotton constitutes a substantial part of the population needs on one hand, but it is also an important source of foreign exchange earnings, one the overhand. However, to improve its production, cotton is threatened by various pests and diseases that reduces production and fiber and seeds quality [4]. So, uncontrolled parasitism can lead to more than 50 % losses of production and sometimes. It can also destroy the production potential. In tropical climates, the incidence of parasitism on cotton growing is so important that chemical control is the main control strategy. Intensive cultivation of cotton is particularly dependent on the application of very large quantities of pesticides. According to Liao et al. [5], about 25 % of the world's pesticides are devoted to cotton protection during growing. Excessive use of pesticides over time negatively affects farmers' incomes and causes an environmental problem. Moreover, the use of pesticides currently facing many oppositions ethically because they are indexed on toxicity, environmental pollution of environment, public

health and even destruction of biodiversity [6]. So, it becomes necessary to look for more effective alternatives for the development of sustainable agriculture. One of them consists in enabling plants to self defend, or to strengthen their own defenses, rather than direct fighting aggressor [7, 8]. In this defense category plant natural defense stimulators (NDS) constitute a scientifically and agronomically interesting future solution [9, 10]. Indeed, most often plants can naturally resist their attackers. However, some plants are more susceptible to pathogens and disease establishment than others by a slow defense response or a low level of synthesis compounds rather than an absence of defense mechanism [11, 12]. Among the natural defense mechanisms developed by plants compound biosynthesis, such as polyphenol has a prominent place [13, 14]. Belhadj [15] and Ahuja et al. [16], reported that phenolic compounds accumulate in the adjacent tissues of necrotic areas, revealing their defensive role in attacking plants. Cotton produces a large number of phenolic compounds which are determined in disease resistance [17-19]. The biosynthesis of these compounds can be stimulated by SDNs. These are usually analogues or derivatives of natural molecules, including salicylic acid and benzothiadiazole. However, little data is available on the use of both elicitors in cotton natural defenses stimulation.

The aim of this study was to investigate the effect of BTH and SA on phenolic compound biosynthesis. Indeed, many of these compounds are phytoalexins which are essential in the plant defense establishment.. Specifically, the effect of elicitor concentration and incubation time on total phenol content was evaluated, as well as impact of elicitor co-treatment.

2. Material and methods

2.1. Plant material

The plant material consists of cottonseed (Gossypium hirsutum L.) from cultivar Y764G3, originating in Côte d'Ivoire (West Africa). It is an improved cultivar, resulting from the cross between local lines and introduced lines (Hau, 1988). The seeds come from Korhogo (Côte d'Ivoire) and were provided by the Ivorian Textile Development Company.

2.2. Chemicals

All chemicals used were at least analytical grade. gallic acid, salicylic acid, ethanol, methanol, sodium carbonate, triton X-100 and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (Natick, MA, USA). BTH was obtained from Syngenta Crop Protection (Greensboro, NC, USA).

2.3. Site study

This experiment was carried out in the field on the experimental plot of the Nangui Abrogoua University (UNA) in Abidjan (southern Côte d'Ivoire). The geographical coordinates of this site are: 5 ° 17 and 5 ° 31 'North latitude between 3 ° 45' and 4 ° 22 'West longitude [20]. The forest relic of this university contains numerous plant species such as Chrysophyllum albidum G. Don (Sapotaceae), Synsepalum afzelii (Engl.) T.D. Penn. (Sapotaceae), Palisota hirsuta (Thunb.) K. Schum. (Commelinaceae). The soil is derived from sedimentary formations of the ferralitic type (Perraud, 1971). These sedimentary formations have a clay-sandy texture that is favorable to cotton growing. The mean annual rainfall and temperature are 1642 mm and 27.16 °C [21].

2.4. Implementation of experimental design

On a plot of one hectare, 48 blocks were laid. Blocks were spaced apart by 50 m to avoid the risk of interference by the different elicitors and their concentrations by wind during spray treatments. In each block, three ridges with a length of 3 m and a width of 1.0 m were set up. The plants were obtained from seeds. The seeds are shown on the ridges. On each ridge, two rows of seedling were formed. The both rows were separated by 60 cm and in each row seedlings were spaced with 20 cm. Thus, 30 plants were placed on each ridge, i.e. a total of 90 plants per block. Plant growth was monitored for two months.

2.5. Preparation and application of elicitors.

Benzothiadiazole (BTH) and salicylic acid (SA) solutions with concentrations of 1.0, 2.5, 5.0, 8.0 and 10 mM were made up in 1% ethanol containing 1% Triton X-100 as a surfactant.

Plants were treated by a foliar spray until runoff using two phases. First, 33 blocks were used. In each block, 2-month old plants were each sprayed with 10 mL of BTH and SA at five different concentrations (1.0, 2.5, and 5.0, 8.0 and 10 mM). Distilled water was used for control plants. Plants were incubated during 24, 48, 72 and 96 h after elicitor treatment. Then, plant leaves were removed and lyophilized. This experiment was replicated three times.

Secondly, 15 blocks were used. A co-treatment of both elicitors was applied to cotton plants. In this experiment, only the concentration of elicitor which gave the highest total phenol content after the previous experiment was used. Each elicitor was prepared in the same conditions as previously. Then different solutions consisting of an equal volume mixture of BTH and SA, at different combinations of concentrations were obtained. For each concentration, 10 mL of mixed

solution were sprayed on plants. Control plants were sprayed with 10 mL distilled water. Thereafter, plants were incubated during 24, 48, 72 and 96 h. Each treatment was performed in triplicates.

2.6. Total phenols extraction and quantification

Phenolic compounds were extracted following the method of [19]. A sample of 100 mg of freeze-dried leave derived from elicited plants was placed in 20 mL of pure methanol and then placed at 4 °C for 12 h. After centrifugation of the mixture at 2000 rpm for 10 min, the supernatant was filtered through a Millipore membrane (0.45 µm) and represented crude phenolic extract.

The total phenol content of crude extract was determined using Folin-Ciocalteu's reagent according to the method of Siriwoharn et al. [22]. Briefly, an aliquot of crude extract (0.1 mL) was mixed with 0.9 mL of distilled water and 0.5 mL of Folin-Ciocalteu's reagent. The mixture added to 1.5 mL of sodium carbonate 17% was incubated at 25 °C for 35 min in the dark. The intensity of coloration which is proportional to phenolic compound concentration was monitored with a spectrophotometer at 765 nm. a standard curve was prepared using gallic acid (0-100 μg/mL). Total phenol content was calculated from the calibration plot and expressed as mg gallic acid equivalents (mg GAE) of phenol/g of freeze-dried extract (g FDE). The calibration equation for gallic acid was $y = 0.586x$, $R^2 = 0.998$, where y is absorbance and x is the concentration of gallic acid in mg/mL. All measures were performed in triplicate.

The increase in total phenol production induced by the application of BTH and SA was determined relative to control. It is expressed as a percentage (%) and calculated using the following formula: ITPP (%) = [(P test - P control)/P control] x 100. Where P is the highest total phenol content for each of the elicitors and ITPP, the production gain in total phenols. In addition, Ratio total phenol content induced by elicitors and control (BTH/Control; SA/control) was determined; the highest values in each case were the only ones to be taken into account.

2.7. Statistical analysis

Experiments were performed using a completely randomized design. Data were subjected to analysis of variance (ANOVA) were carried out for the experiment using Statistica software (release 7.5). Means of data were compared by Duncan's Multiple Range Test. Differences at $P \leq 0.05$ were considered as significant. For percentages which are nonparametric values, Kruskal-Wallis test was used at $P \leq 0.05$.

3. Results

Total phenol content varies according to the elicitor, their concentration and the incubation time of the leaves after treatment (Table 1). Applying the elicitor on cotton leaves caused an increase in the total phenol rate relative to control at 1.0 and 2.5 mM concentrations. At 1.0 mM concentration, BTH induced the best total phenol production (65.15 mg/g FDE) after 96 h incubation time against 41.36 mg/g FDE for SA. In addition, plants treated with 2.5 mM BTH had the highest total phenol levels compared to SA, regardless of incubation time after elicitation. However, these levels remain significantly lower compared to those induced with 1.0 mM. Moreover, at a concentration of 5.0 mM, the both elicitors have statistically identical total phenol contents. These contents are also identical to those induced by control. The concentrations 8.0 and 10 mM allowed to accumulate lowest total phenols for both elicitors, regardless of incubation time. Indeed, everything seems to happen as if these concentrations have no effect on phenolic compounds production are even statistically weak compared to those induced by control. In addition, with regard to BTH, the most important total phenol content was obtained with 1.0 mM after 96 h incubation (61.15 mg/g FDE). On the other hand, with SA, the highest total phenol content was observed at 2.5 mM after 24 h of incubation time (45.89 mg/g FDE).

Table 1. Effect of elicitor concentration and incubation time on total phenol content in cotton leaves

Elicitor (mM)	Incubation time (h)	Total phenols content (mg/g FDE)	
		SA	BTH
0.0	24	34.65 ± 1.15e	34.65 ± 1.15e
	48	36.60 ± 0.56e	36.60 ± 0.56e
	72	37.12 ± 0.32e	37.12 ± 0.32e
	96	37.46 ± 0.94e	37.46 ± 0.94e
1.0	24	42.68 ± 0.54d	44.56 ± 0.22c
	48	42.16 ± 0.80d	45.23 ± 0.44c
	72	42.56 ± 0.01d	48.67 ± 0.41b

	96	42.36 ± 0.06d	61.15 ± 0.34a
2.5	24	45.89 ± 1.11c	48.82 ± 0.84b
	48	42.97 ± 0.27d	47.13 ± 0.33b
	72	40.42 ± 0.92d	47.13 ± 0.33b
	96	38.61 ± 0.32de	47.08 ± 0.63b
5.0	24	37.89 ± 0.28e	36.29 ± 0.65e
	48	38.42 ± 0.10e	35.51 ± 1.53e
	72	37.91 ± 0.59e	35.97 ± 0.26e
	96	37.81 ± 0.88e	35.04 ± 0.80e
8.0	24	27.29 ± 0.15f	27.97 ± 1.01f
	48	27.84 ± 0.42f	27.29 ± 0.65f
	72	27.15 ± 0.45f	26.65 ± 0.14f
	96	27.29 ± 0.55f	26.29 ± 0.48f
10.0	24	17.29 ± 0.15g	19.97 ± 1.01g
	48	16.84 ± 0.42g	17.29 ± 0.65g
	72	18.15 ± 0.45g	18.65 ± 0.14g
	96	17.29 ± 0.55g	18.29 ± 0.48g

SA : acid salicylic ; BTH : benzothiadiazole; FED, freeze-dried extract; ± S : standard error; data are expressed as mean of three replicates; within row and column, values followed by a different letter are significantly different according to Duncan's multiple range test at α = 5%.

The increase of total phenol production in cotton was shown in Figure 1. BTH-treated plants had larger total phenol production profit than the SA-treated plants, at optimal concentration and best incubation time. Thus, plants treated with 1.0 mM BTH and incubated for 96 hours had a total phenol production gain of 63.24% compared with 32.43% for plants treated with 2.5 mM SA at 24 h post-treatment incubation compared to control. The ratio (Figure 2) revealed that the total phenol content of BTH-treated plants was 1.63 times higher than that of control whereas SA has made it possible to increase phenol content. than 1.32 times. The comparison between the elicited plants shows that the BTH makes it possible to increase 1.33 times the total phenol content that SA.

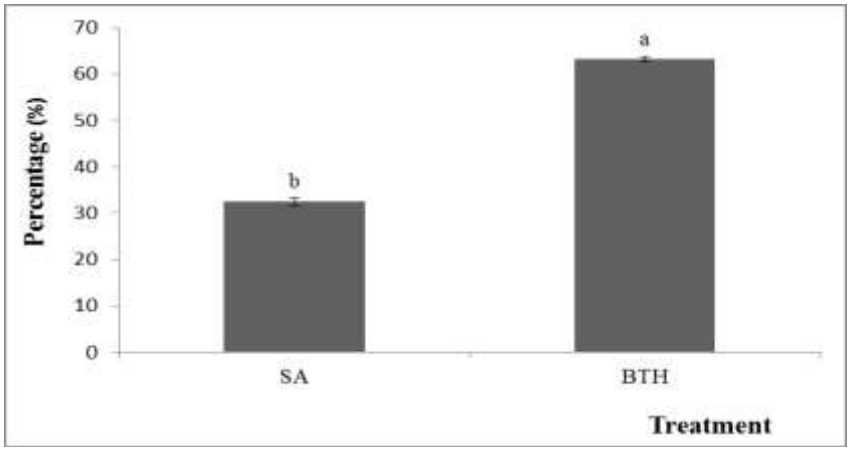

Figure 1. Profit of production on induced total phenols in cotton leaves compared with control elicitor treatment

BTH, Benzothiadiazole; SA, salicylic acid; data are the means of three replicates; vertical bars represent standard deviation.

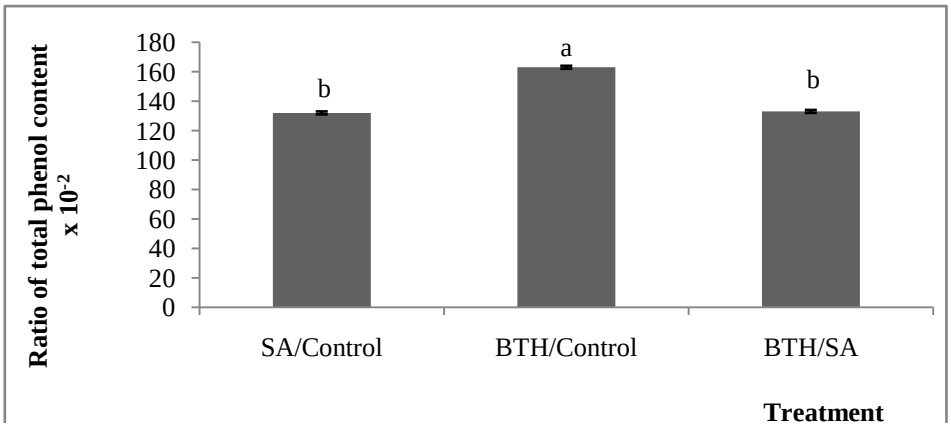

Figure 2. Ratio of total phenols in cotton treated-leaves with elicitor

BTH, Benzothiadiazole; SA, salicylic acid; data are the means of three replicates;

Vertical bars represent standard deviation.

Furthermore BTH/SA co-treatment effect on total phenols biosynthesis (Figure 3) shows that 1.0 mM BTH and 2.5 mM SA were used to prepare the four co-treatment solutions. Thus, the result reveals that BTH/SA (1.0 mM) induced the highest total phenol content (40.96 mg/g FDE). However, the best post-treatment incubation time was 48 h. This content still remains significantly lower than those induced by 2.5 mM SA alone after 24 h of incubation time (45.89 mg/g FDE) and by 1.0 mM BTH alone at 96 h of incubation time (61.15 mg/g FDE).

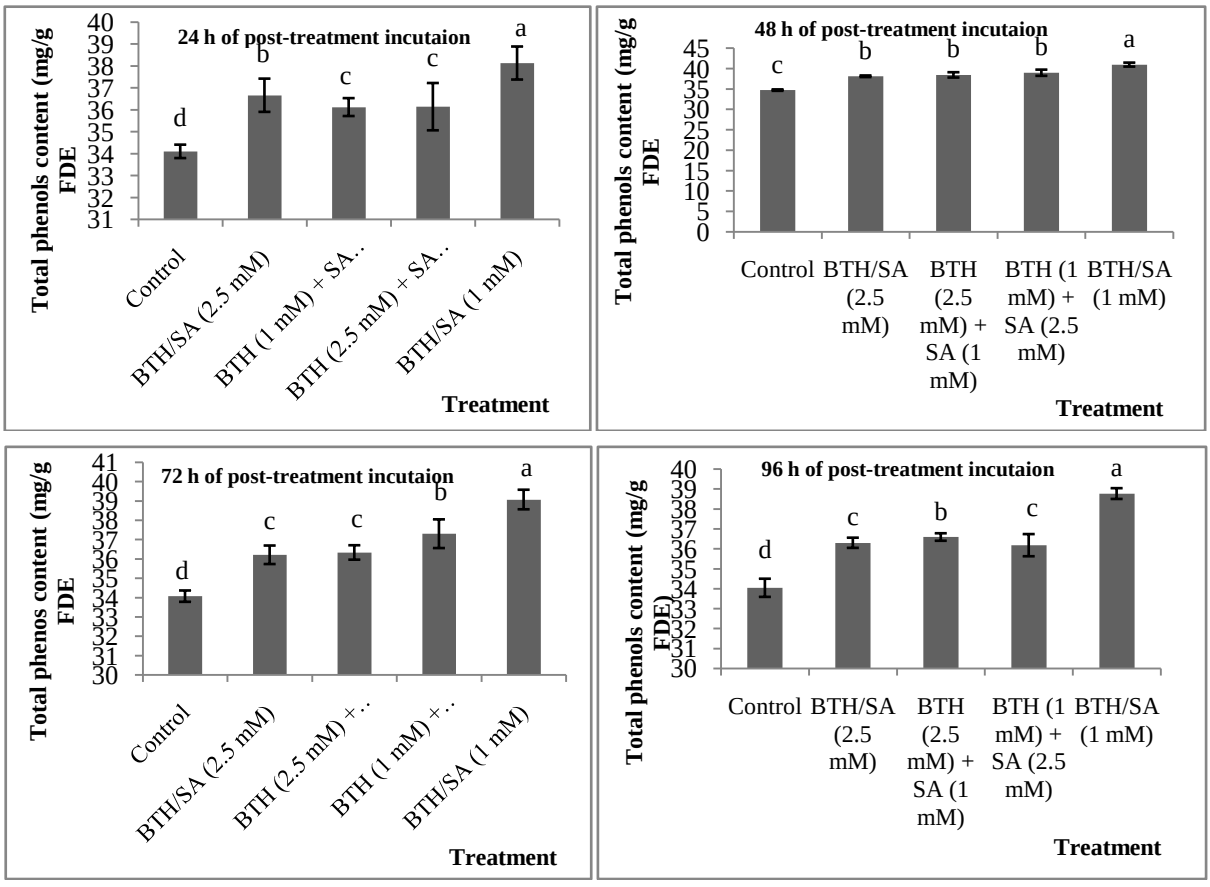

Figure 3: Total phenols content in cotton leaves after BTH and SA co-treated in function to incubation time

BTH, Benzothiadiazole; SA, salicylic acid;SA : acid salicylic ; BTH : benzothiadiazole; FED, freeze-dried extract; data are the means of three replicates; vertical bars represent standard deviation.

4. Discussion

The production of total phenols varies following elicitors (BTH and SA) concentrations and the cotton plants incubation time. At 1.0 mM concentration, BTH-treatment of cotton leaves induced the best total phenol content. These results are similar to those obtained by Inbar et al. [23] who reported that BTH application of leaves increases the enzyme activity of phenolic compound biosynthesis, hence the phenolic compound accumulation. For salicylic acid (SA), the highest total phenol content was obtained with 2.5 mM. This result is in agreement with that of Soler et al. [24] who reported an accumulation of phenolic compounds under the action of SA in plants. In addition, Dogbo et al. [25] showed that SA significantly induced phenolic compounds synthesis in cassava [26, 27]. In addition, some authors reported that SA induces a defense reaction which affects growth and cell death. Sood et al. [28] also showed that an increase in phenol content after application of BTH and SA on rice. Thus, result shows that BTH and SA are able to induce phenolic compounds biosynthesis in cotton. Otherwise, 1.0 mM BTH allowed to induce the most important phenol level irrespective of incubation time after leaves treatment. The concentrations 2.5 to 10 mM revealed no significant differences between BTH and SA as well as between incubation times. Therefore, BTH and SA seem to stimulate phenolic compound production identically except at 1.0 mM. BTH is SA analogue, which has no direct effect on pathogen and stimulates plant defenses.

SA is known for its involvement in setting up systemic resistance in plants. The molecule causes the plant to believe that a pathogen has attacked [29, 30]. The plant is therefore preparing by putting in place preventive defense mechanisms, including the synthesis of defense molecules such as phenolic compounds. However, the significantly important action of BTH on the biosynthesis of phenolic compounds at 1.0 mM compared to SA suggests that this elicitor has a greater impact on defense mechanisms. Moreover, the increase in phenolic compounds production is greater with BTH. Similar results have already been reported by several authors in many plants [31-33]. So, having been dependent for a long time on pesticides, global agriculture today is being made with more sustainable and more environmentally friendly practices. In this context, the development of biological molecules capable of stimulating the natural defenses of plants (SND) is a strategy that attracts much attention. A SND molecule is an elicitor likely to trigger a series of biochemical events that can lead to the expression of disease resistance in plant. Signal perception and its transduction by various signaling pathways lead to the synthesis and accumulation of defensive molecules, among which phytoalexin-type phenol having a high antimicrobial potential [16, 34]. Thus, elicitors that increase the phenolic compounds production such as BTH would be able to stimulate cotton "immune system" by mimicking the effect of pathogens more than SA. These promising results suggest that elicitors or stimulators of plant defenses will have their place in the future among cotton protection solutions, thereby reducing the use of pesticides. Moreover, cotton culture consumes a little more than 25% of pesticides manufactured in the world [5, 19]. However, the need to apply very high doses of SA because plants metabolize it promptly has eliminated any potential marketing potential for agronomic purposes. On the other hand, the possibility that other compounds mimicking SA effect may be used has aroused interest since the discovery of BTH which is capable to induce systemic acquired resistance (SAR) as SA [30, 33, 35]. Thus, use BTH in combination with SA did not allow to observe additive or synergistic effects. The reduction in phenolic compound level observed after co-treatment of both elicitors shows an infra-additive effect and therefore a lower efficiency and therefore the acquisition of a lower level of resistance than when the elicitors are used alone. BTH and SA would be antagonistic because they are analogous compounds. Their co-treatment would increase salicylate content that would become the phototoxic effect for plants [32, 36]. Moreover, the results have shown that BTH concentrations greater than 1.0 mM cause a decrease in the stimulation of phenolic compounds production. Thus, for better cotton protection against pathogens, treatment with elicitor alone would be desirable.

5. Conclusion

This study showed that BTH used at 1.0 mM and after 96 h of incubation time was the best elicitor to induce phenolic compounds biosynthesis compared to SA. Its application on the leaves would allow the cotton plant to equip itself with compound capable of anticipating a possible attack of pests. The joint application of BTH and SA showed a non-beneficial effect on phenolic compound biosynthesis in cotton.

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