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Attenuations of bacterial spot disease *Xanthomonas euvesicatoria* on tomato plants treated with biostimulants

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Abstract

Background: The bacterial-spot disease caused by different *Xanthomonas* species is one of the major tomato diseases that reduce crop production and quality. Pesticides indiscriminate usage has resulted in an increase in resistant bacterial strains as well as contamination of farmers, consumers and the environment. Plant growth-promoting bacteria and humic acids can act as elicitors of plant defence mechanism causing extensive transcriptional and metabolic reprogramming which, in turn, produce a range of plant chemical defences. The purpose of this study was to study how humic acids and plant growth-promoting bacteria, when applied to the substrate, affected the severity of bacterial spot symptoms in tomato leaves.

Materials and methods: One-month-old Micro-Tom tomato (*Solanum lycopersicum* L.) were transferred to 3 L pots filled with a sterile mixture of sand and vermiculite (2:1, v:v) and treated or not (control) with 250 mL of 4.5 mmol C. L^{-1} of humic acids, *Herbaspirillum seropedicae* (10^8 CFU. mL^{-1}) and the combination of humic acids plus *H. seropedicae*. One day after substrate treatment, the leaves were inoculated (or not) with *X. euvesicatoria* (*Xe*). The area below the disease progression curve based on severity scores and the number of symptomatic leaflets was used to assess phytopathogen virulence. The concentration of oxalic, citric and succinic acids in leaf extracts were determined using HPLC analysis.

Results: Sole or combined *H. seropedicae* (BAC) and humic acids (HA) application promoted shoot and root growth related to control when plants were challenged with *Xe* pathogen. For plants inoculated with *Xe*, more significant plant-growth promotion results were obtained for HA + BAC treatment. The first visible symptoms were observed 16 days after inoculation with 2×10^4 CFU. g^{-1} of *Xe* cells in leaves of control plants. HA and BAC applied alone or combined reduced disease severity. Only plants treated with HA were able to reduce disease incidence (number of the leaflets with symptoms). Organic acids, such as oxalic, citric and succinic acids, rose in Xe-inoculated leaves. The reduced amount of organic acids in diseased leaves treated with HA + BAC may be linked to a decrease in disease progression.

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Conclusion: Humic acids and *H. seropedicae* increased growth by modulating the content of organic acids in leaf tissue, attenuating the symptoms of the bacterial spot disease.

Keywords: Biocontrol, Systemic resistance, Plant-growth promotion, Humic substances, Endophytic bacteria

Introduction

Biofertilization with soluble humates and plant growth-promoting bacteria has attracted attention in recent years due to its beneficial effects on nutrient uptake and protection of plants against abiotic stress [1, 2]. This protection is generally due to increase of enzymatic and non-enzymatic antioxidant defenses, increase in compatible solutes production and changes in ion balance as well as boost in metabolism and plant growth. However, studies on the role of humates in mitigating plant diseases are very scarce [3]. Studies using stable organic matter applied to fungal diseases suppression have been widely reported [4–10]. Our group recently evaluated the concept of substrate biofertilization using humates and plant growth-promoting bacteria to produce vigorous tomato seedlings and, after field transplantation, showed higher crop yield [11]. The additional foliar application of humic acids combined with plant growth-promoting bacteria resulted in a lower incidence of *Phytophthora infestans* [11].

São José de Ubá is located in the northwest of the Rio de Janeiro state (Brazil), a region with one of the largest tomato producers in the Rio de Janeiro state. The diagnoses for pesticides use by tomato farmers showed that a mixture of different products is indiscriminately used without requirement, not respecting pre harvest interval (PHI) or waiting period, lacking technical assistance during all cultivation cycle and without using personal protective equipment [12, 13]. There are no products to control bacterial diseases, and farmers with fear of losing their production use many products prohibited or unlicensed in an attempt to preserve the harvest. The main bacterial disease in tomatoes in this region is caused by *Xanthomonas euvesicatoria* promoting loss in fruit productivity and quality due to the yellowing of the leaves, followed by defoliation, reducing the photosynthetic area and exposing the fruits to the sun, which can cause scalding in them. The control is difficult, and some chemical products are prohibited in other countries, because it is highly dangerous, and many microorganisms become resistant to chemical control [14].

Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two forms of induced resistance, wherein plant defences are preconditioned by prior infection or treatment that results in resistance against subsequent challenge by a pathogen [3]. The SAR is characterised by proteins related to pathogen (PR-proteins) and can change the plant morphology and anatomy and use salicylate as cell signalling. Differently, ISR did not show PR-protein accumulation and used other signalling pathways, such as ethylene and jasmonates with low plant morphology changes [15–17]. Plant growth-promoting bacteria can trigger the salicylic acid (SA)-dependent SAR and SA-independent ISR pathway [18]. Humic substances can increase the concentration of phenolic compounds [19] and peroxidase activity [20], as well as phenylalanine ammonia-lyase (PAL) activity [21] in leaves tissues. PAL is the key enzyme involved in synthesising phenolics and lignin compounds via the phenylpropanoid pathway.

Composts and water extracts could be used to induce plant resistance [22]. Dahmani et al. [23] investigated the efficacy of foliar sprays with compost water extracts in reducing the severity of bacterial spot of tomato caused by *Xanthomonas euvesicatoria* and observed decrease the bacterial spot disease damage in tomato, increasing fruit yield. These previous results indicate the possibility of natural products to reduce the plant diseases severity and, if not eliminate, at least reduce the toxic and lethal load of pesticides commonly applied to tomatoes. *Herbaspirillum seropedicae* is an endophytic diazotroph and forms nitrogen-fixing associations with several economically important crops in the family Poaceae, such as maize, rice, sorghum, and sugarcane, and can increase their growth and productivity by a number of mechanisms, besides nitrogen fixation, including the induction of systemic resistance to pathogens [24].

This work aimed to induce the systemic resistance of tomato against bacterial spot using humic acids isolated from vermicompost and *H. seropedicae* applied separately or in combination directly on the plant growth substrate.

Materials and methods

Plant assay

The sowing of the 'Micro-Tom' tomato (*Solanum lycopersicum* L.) seeds was carried out in 128-cell polystyrene trays with Vivatto® commercial substrate irrigated once a day. They were kept in a growth chamber at 28 °C and 80% relative humidity with a photoperiod of 16 h day and 8 h night. Thirty days after sowing, the seedlings were transplanted into 3 L pots in a greenhouse filled with sand: vermiculite mixture (2:1 ratio, v/v). This substrate

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was autoclaved three times at 121 °C, 1 atm for 1 h before pot filling.

Inoculum production

The inoculum of *Herbaspirillum seropedicae* strain HRC54, was obtained at the Laboratório de Biologia Celular e Tecidual (LBCT/UENF). The bacteria were grown in DYGS liquid medium (Dextrose Yeast Glucose Sucrose) under rotation of 120 rpm at 30 ºC for 24 h. The bacterial suspension was adjusted to 10^8 CFU mL^{-1} [25]. The inoculum of *Xanthomonas euvesicatoria* T1P3 [26] was prepared from the ENA 4135 isolate, provided by the Departamento de Fitopatologia da Universidade Federal de Lavras (UFLA), Brazil. This isolate was selected for its pathogenicity, which was previously tested [27] and resistance to copper-based pesticides. The isolate was grown in DYGS liquid medium for approximately 36 h, under agitation (100 rpm) at 28 °C. Then, the bacteria were cultured in Petri dishes containing the solid DYGS medium. After 36 h of growth, at 28 °C, the bacterial colonies were suspended in sterile water, and the cell concentration was adjusted to 10^8 CFU.mL^{-1} by visible spectrophotometry at 600 nm.

Humic acids extraction

Humic substances were extracted from vermicompost produced with sugarcane filter cake and *E. Andrei* with 0.1 mol L^{-1} NaOH 1:10 (v:v), under an atmosphere of N$_2$ for 4 h followed by centrifugation ($3000 \times g$). The separation of humic acids from the alkaline extract was obtained by acidification at pH 1 with 6 mol L^{-1} HCl. Dissolution and precipitation were repeated three times. After centrifugation, the HA fraction was washed with water until there was a negative test with AgNO$_3$ titrate until pH 7.00 with NaOH 0.01 mol L^{-1} and dialysed (molecular mass cutoff 1 kDa; Spectrapor, USA) and freeze-dried. The carbon content of the HA was measured using a CHN analyser (Perkin-Elmer 1483; Perkin-Elmer, Norwalk, CT, USA). The HA suspension was prepared dissolved 106 mg HA in 1 L of CaCl$_2$ 2 mmol L^{-1}. The pH was adjusted to $5.8 \pm 0,1$.

Evaluation of bacterial strain resistance

The experiment to evaluate resistance to bacterial strain was conducted in a greenhouse. The experimental design was entirely random, in a 4×2 factorial scheme using four treatments in the substrate (CaCl$_2$ 2 mmol. L^{-1}—as a control; *H. seropedicae* 10^8 UFC. mL^{-1}—BAC; Humic acids at 4.5 mmol C. L^{-1}—HA; *H. seropedicae* + Humic acids—HA + BAC), and two treatments (without and with *X. euvesicatoria* inoculation) applied on leaves. Ten replicates and one plant per pot were used. One day after transplantation, treatments were applied in the substrate using 250 mL of control; HA; BAC; HA + BAC). HA and bacterial suspension were prepared as described above but placed in the proportion of 4 HA: 1 BAC (v:v). The plants were irrigated with water, but 2 days a week were irrigated with Clark's nutrient solution, in which in the first week it was with ¼ concentration, in the second week with ½ in the third ¾ and from the fourth week complete concentration. The inoculation of the bacterial suspension of *X. euvesicatoria* occurred 21 days after applying treatments (56 days after sowing). Applying in half of each treatment pots (5 pots) by spraying on the leaves and water was used in control. After *X. euvesicatoria* inoculation, a humid chamber > 90% relative humidity was performed using plastic bags. After 72 h in a humid chamber, the bags were removed, and observations of the disease's symptoms (severity and number of infected leaflets) began. The evaluations were carried out on alternate days in a total of seven evaluations of resistance to bacterial spot (*X. euvesicatoria*—Xe) based on a severity scale using notes adapted from Mello et al. [28] according to Fig. 1.
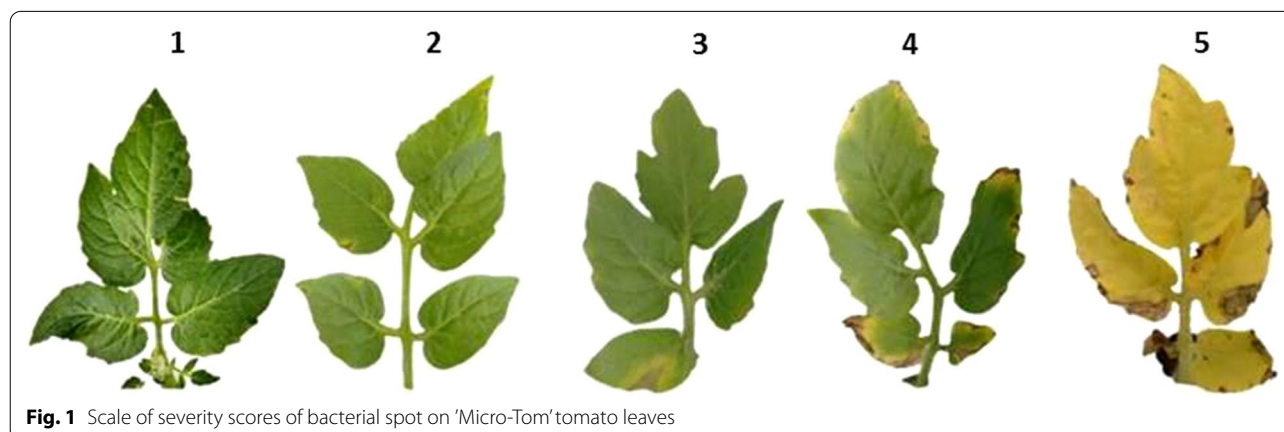
Fig. 1 Scale of severity scores of bacterial spot on 'Micro-Tom' tomato leaves

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Using the notes of severity and number of leaflets, the area under the disease progress curve (AUDPC) was calculated, in which the data obtained were integrated and calculated using the following equation: $AUDPC = \sum \left[(y1 + y2)/2 \right] * (t2 - t1)$, where $y1$ and $y2$ refer to two successive assessments of disease intensity performed at times $t1$ and $t2$, respectively [28].

The plants harvested occurred 21 days after the inoculation of the pathogen. The plants were divided into two parts, leaves and root. The collected roots were washed until the substrate was completely removed and washed once more in distilled water. Subsequently, the fresh mass was determined, and the dry mass was obtained after drying at 60 °C to be subsequently used for extraction of organic acids analysed in HPLC. The AUDPC data of the plants inoculated with *X. euvesicatoria* and the mass data were subjected to analysis of variance and means comparison test (Tukey's test) at a level of 5% probability using the SAEG statistical program.

Identification and quantification of organic acids on HPLC

The analysis of organic acids was performed by high-performance liquid chromatography (HPLC) for the leaves previously dried at ± 60 °C. 0.250 ± 0.001 g were mixed with 3.5 mL methanol: acetic acid 85:15 (v / v) and macerated in the porcelain mortar until it acquires liquid consistency, as homogeneous as possible. The extract obtained was transferred to a beaker and remained in the ultrasound for 30 min. The final volume was adjusted to 5 mL of solution with ultra-pure water. This diluted extract was centrifuged at 100 rpm at 25 °C for 5 min. Organic acids were identified and quantified by HPLC using a Younglin Instrument chromatograph, with a 210 nm wavelength UV detector, using a REZEX ROA—Organic acid H$^+$ column (300×7, 8 mm). The injected volume of the sample was 20 µL. 280 µL of sulfuric acid/L (2.4 mmol sulfuric acid/L) was used as the mobile phase, with a flow rate of 0.6 mL/min. It is carried out at room temperature. The peaks corresponding to each acid were identified by the retention time, using the standards' retention times as a comparison. The data obtained were subjected to analysis of variance (ANOVA), and the statistical differences were determined using the DMSt means comparison test (Tukey's test) at a significance level of 5%. The concentration obtained was expressed in mg L^{-1} of organic acid.

Results

We evaluated the population size of *H. seropedicae* cells established in the plant growth substrate using the most probable number (MPN) methodology at 5 and 15 days after suspension application. We found 1.3 and 1.5×10^5 CFU. mL^{-1} for BAC treatment and 2.0 and 2.5×10^4 CFU. mL^{-1} for HA + BAC treatment. As expected, *H. seropedicae* was not detected in the control and HA treatment (data not shown). When *X. euvesicatoria* was inoculated in leaves, *H. seropedicae* population number was increased in BAC and BAC + AH treated substrate.

The virulence of the *X. euvesicatoria* ENA 4135 isolate used to inoculate the tomato was attested by the characteristic symptoms of the bacterial spot disease observed in treated plants: necrotic spots with yellow halos, mainly from the border to the centre of the leaf, and the presence of anticipated senescence of older leaves with a greater symptomatic area (Fig. 2).

Fig. 2 Symptoms of bacterial spot disease on 'Micro-Tom' tomato leaves inoculated with *Xanthomonas euvesicatoria* T1P3: necrotic spots (left) with yellowish edges (middle) and generalised yellowing indicating pre-senescence (right)

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The fresh weight of leaves and root tissues treated or not (control) with humic acids (HA) and *H. seropedicae* (BAC) in the presence or not of *Xanthomonas euvesicatoria* are shown in Fig. 3. The presence of pathogen decreased both leaf and root fresh weight. However, the treatment with HA + BAC increased the leaf and root weight in the plants inoculated with *X. euvesicatoria* (*Xe*), compared to non-biostimulated plants without *Xe* (Fig. 3), probably due to a synergistic plant-growth stimulation by both HA and BAC. The HA-treated plants enhanced the roots fresh weight 1.5 fold compared to control in the absence of the pathogen. The lowest root fresh weight was found in the *H. seropedicae* treatment in the pathogen's presence on the leaves. However, the substrate treatment HA + BAC showed a significant increase of root fresh weight in plants inoculated with the pathogen (Fig. 3B).

It was noticed that in some treatments, the symptoms of bacterial spot started at the edges, with small spots that did not progress but entered the count of symptomatic leaflets. Despite the absence of statistical difference for disease incidence, a significant reduction for AUCDP severity was noticed for all treatments related to control (Fig. 4A). The severity of the bacterial spot disease was reduced around 50% by the substrate treatment with HA and *H. seropedicae*.
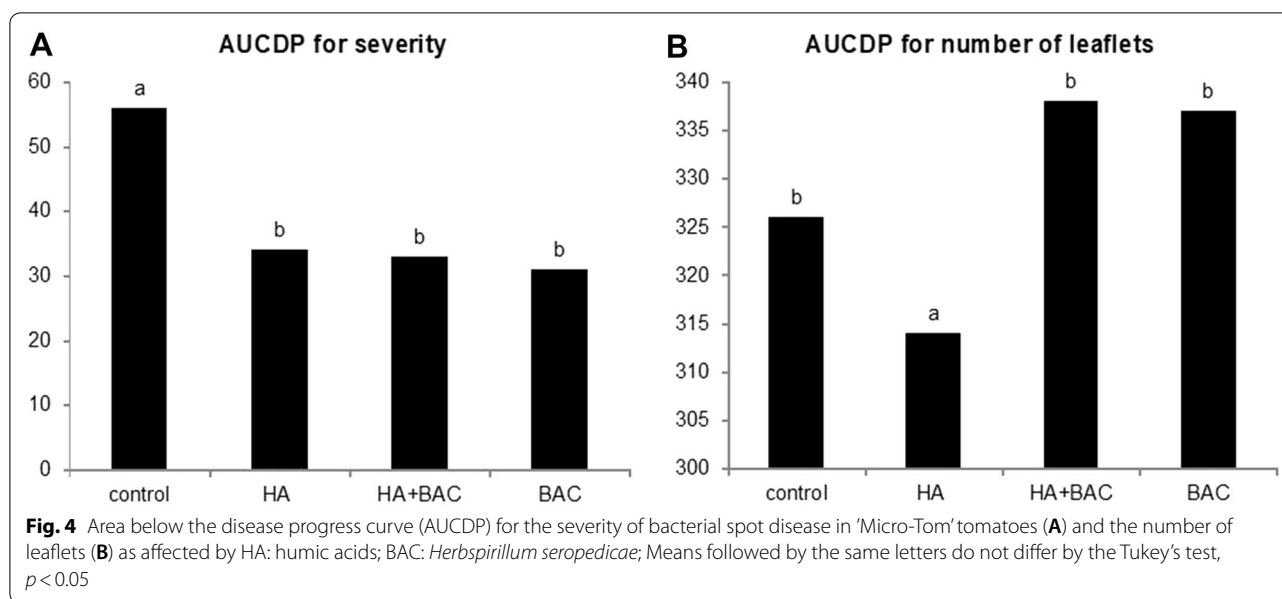
The control with pathogen inoculation received a higher severity score on the last day of the assessment. HA-treatment obtained the lowest AUCPD value for the number of leaflets on the last day of evaluation (Fig. 4B). The incubation period for *Xe* was 8 days for the control and HA, 10 and 11 days for BAC and AH + BAC, respectively. We noticed that the longer the time for first symptoms to appear, the less aggressive is the disease.

The concentrations of oxalic, citric and succinic acids in the leaves extracts with and without *X. euvesicatoria* are shown in Table 1. The concentration of oxalic acids in control inoculated with *X. euvesicatoria* was 67% higher than the plant treated with HA and inoculated with *X. euvesicatoria*. However, we noticed that HA treatment had different characteristics, compared to control plants inoculated with *X. euvesicatoria*, showing a rapid infection, but this severity found in the initial evaluation days did not increase. On the last day of evaluation, HA treatment presented a smaller area under the disease progress curve (AUDPC) among treatments (Fig. 4B). Oxalic acid has previously been linked to senescence and programmed cell death [29]. The rapid senescence was observed in control with *X. euvesicatoria* and the leaves yellowing, indicating greater severity of the disease due to the greater infected leaf area (greater necrotic area). The concentration of the applied pathogen was 10^8 CFU/mL, which is considered very high. Plants no infected with *X. euvesicatoria* showed similar content of citric and succinic acids in leaves among treatments (Table 1). The HA + BAC significantly increase the content of citric acid in plants infected with *X. euvesicatoria*, while the succinic acid was found in larger content in control plants.
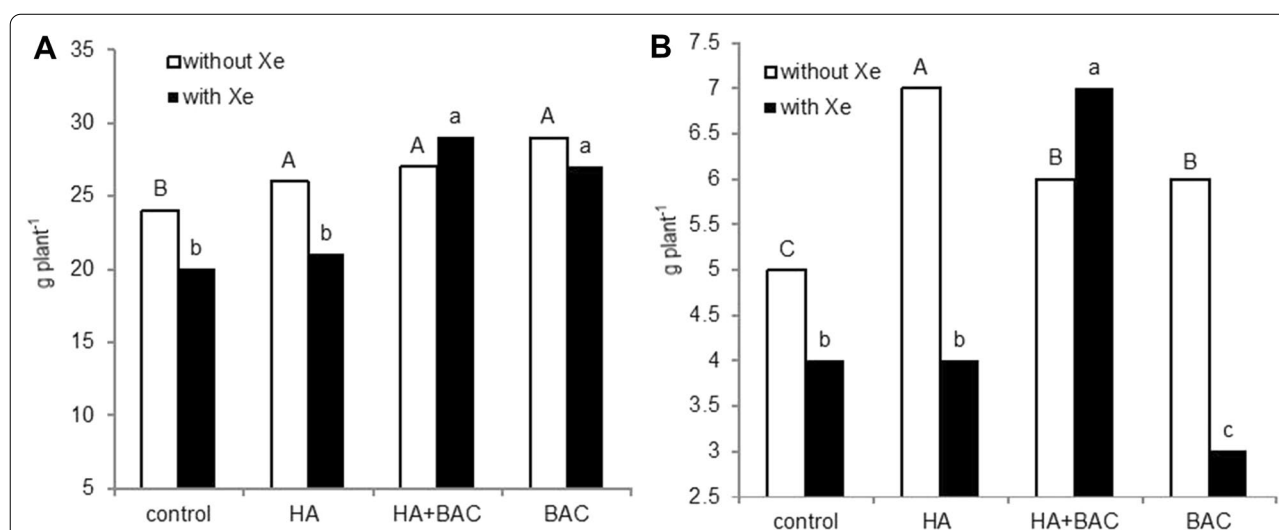
Fig. 3 Leaves (**A**) and roots (**B**) fresh weight of tomato plants grown on substrate treated or not (control) with humic acids (HA), *H. seropedicae* (BAC) and the combination of both (HA + BAC). White and black bars represent treatments without and with *Xanthomomas euvesicatoria* (Xe), respectively. Means followed by the same capital letters (without *Xe*) or followed by the same small letters (with *Xe*) do not differ by the Tukey's test, $p < 0.05$

Fig. 4 Area below the disease progress curve (AUCDP) for the severity of bacterial spot disease in 'Micro-Tom' tomatoes (**A**) and the number of leaflets (**B**) as affected by HA: humic acids; BAC: *Herbspirillum seropedicae*; Means followed by the same letters do not differ by the Tukey's test, $p < 0.05$

Table. 1 Concentration of oxalic, citric and succinic acids (mg kg^{-1}) in leaves extracts from MT Tomato growth in the substrate treated with humic acids (HA), *Herbaspirillum seropedicae* (BAC) and in combination (HA + BAC) after inoculation or not of *Xanthomonas euvesicatoria* on the leaves

	Without *X. euvesicatoria*	With *X. euvesicatoria*
	Oxalic acid	
Control	776 (± 103) Aa	1426 (± 1382)A a
HA	nd	944 (± 208) A a
BAC	458 (± 648) A a	563 (± 538) A a
HA + BAC	nd	700 (± 990) A a
	Citric acid	
Control	456 (± 188) A a	1169 (± 319) BC a
HA	748 (± 258) A a	1593 (± 1375) AB a
BAC	547 (± 92) A a	720 (± 398) AB a
HA + BAC	612 (± 327) A b	2244 (± 1115) C a
	Succinic acid	
Control	74 (± 0.1) A b	220 (± 129) A a
HA	79 (± 11) A a	131 (± 2) B a
BAC	84 (± 24) A a	75 (± 49) B a
HA + BAC	93 (± 2) A a	120 (± 50) B a

[1] Means ± standard deviation followed by same capitals letters in the column and lowercase in the lines are not different by Tukey test. *nd* not detected in the four repetitions

Discussion

Humic acids and plant growth-promoting bacteria can reduce the use of pesticides and fertilisers, significantly cutting down on the costs of production and promoting farmers and consumers' health. The potentiality of vermicompost extracts as bio-control agents has been recognised [30]. The foliar application of aqueous vermicompost extracts is suggested to be utilised by the farmers as an easy, cheap, and efficient system of crop protection with high-yielding capacity [31].

Plant growth-promoting bacteria can elicit plant defence mechanisms consisting of an eco-friendly strategy to reduce pesticides and it is widely used in some crops [3]. Beneficial associations of *H. seropedicae* with sorghum, sugar cane, rice, and maize have been reported but some isolates can causes red stripe disease on some sorghum varieties and can cause mottled stripe disease on sugarcane and induced disease symptoms in rice [32]. The response of the host plant to *H. seropedicaes* includes both the recognition of the bacteria as non-pathogenic and the induction of systemic resistance to pathogens [24, 32].

The fact that organic matter and its fractions also elicit plant defence responses is less recognised, despite previous evidence [21, 23, 30]. Here we reported the results obtained with direct application of HA and BAC on the substrate aiming to attenuate the symptoms of bacterial spot on tomatoes leaves. The substrate treated with biostimulants and further inoculation of leaves with *Xanthomonas euvesicatoria* resulted in fewer symptoms of bacterial spot. Previous reports showed that humic substances and aqueous extracts of composts and vermicomposts can suppress fungal and bacterial diseases, such as Pythium, Rhizoctonia, and *Plectosporium, Fusarium* and *Verticillium* [4–10]. Studies considering plant-bacterial disease control by humic substances are scarce. Al-Damahy et al. [23] showed the possibility of suppressing bacterial spot of tomato with

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foliar sprays of compost extracts. They reported a moderate but statistically significant reduction in the severity of bacterial spot in the greenhouse bioassays and ineffective in the field against the disease's foliar phase. The mechanisms in which aqueous extracts of compost suppress the plant bacterial disease are still speculative. The use of HA on the substrate promoted the reduction of bacterial spot severity (Fig. 4), indirectly indicating the occurrence of ISR mechanism. In line with this, compost amendments into soil decreased the disease severity of some aboveground diseases in tomatoes [21].

Several biochemical and physiological changes have been associated with pathogen infection, including cell death and oxidative burst, deposition of callose and lignin, and the synthesis of phytoalexins and novel proteins [33]. The main characteristics of SAR mechanism are the production of PR proteins mediated by the salicylic acid (SA) pathway. We found no reports showing direct evidence that humic substances could promote differential gene expression encoding PR; however, the conversion of phenylalanine to *trans*-cinnamic acid catalysed by PAL is part of the SA biosynthetic pathway. The conversion of *trans*-cinnamic acid into SA has been proposed to proceed via chain shortening to produce benzoic acid, followed by hydroxylation at the C-2 position to derive SA [33]. The last step is likely catalysed by a cytochrome P450 monooxygenase, called benzoic acid 2-hydroxylase. Both PAL and P450 monooxygenase were found in a high transcription level in plants treated with HA [19, 33]. The larger concentration of SA, benzoic and cinnamic acids were found in leaves tissues in plants treated with HA and HA + BAC [35, 36]. The presence of phenolics compounds and their oxidation products in the plant tissue is toxic to pathogen growth. Different types of plant biostimulants, including humic substance, enhanced phenolics content in plants [37].

Moreover, Nunes et al. [38] showed that HA could increase several antioxidant enzymes, among them 2-Cys peroxiredoxin BAS1, a putative heat shock protein 90 family protein, glutathione peroxidase and PAL. Heat shock proteins are found ubiquitously in plant and animal cells and are involved in heat shock and a wide variety of stresses, including biotic stresses [39]. In addition, HA triggers expressions of heat shock proteins both in the absence and presence of heat stress [40].

The proteomic analysis showing the promotion of several antioxidant enzymatic activities [39] can be associated with SAR mechanisms triggered by humic substances. Superoxide dismutase (SOD) and catalase are antioxidants involved in oxygen radical scavenging that play a critical role in determining the consequences of plant–pathogen interactions [41]. The promotion of SOD

and CAT activities in leaf extracts of sugarcane treated with HA were previously observed [42].

Induced systemic resistance (ISR) does not produce PR and is mediated by a jasmonate/ethylene sensitive pathway for cell signalling and emerged as an important mechanism by which selected plant growth-promoting bacteria and other agents prime the whole plant body for defence against a broad range of pathogens and insect herbivore [3]. Some previous evidences that humic substances can act as a prime chemical agent and that compost and vermicompost-amended soils might improve plant defence were reported [21, 23, 34]. In addition, it was previously reported that HA could enhance the content of jasmonic acid (JA) and its derivatives [43–45], including JA conjugate with isoleucine (JA-Ile), the most bioactive form. De Hita et al. [44] observed a large SA concentration in roots when HA was applied on leaves. Unfortunately, the authors did not measure or present the leaves' JA concentration values when HA was applied only to the roots [42]. Moreover, it was previously indicated that JA and methyl-JA could regulate the ascorbate–glutathione cycle, two crucial nonenzymatic compounds involved in defence against oxidative stress [46]. The larger concentration of ascorbate in plants treated with HA and *H. seropedicae* and their combination was previously reported in a plant metabolome study [33], and the high level of proteins involved in the ascorbate–glutathione cycle was also reported [38].

Finally, it is well known that the pathogen's presence increases the concentration of organic acids [29], which is also evidenced here by the larger organic acids concentration in the tomatoes inoculated with *X. euvesicatoria* (Table 1). All the treatments (HA, BAC and HA + BAC) decreased the concentration of the organic acids. The oxidative burst, defined as controlled release of O_2 – and H_2O_2, is one of the earliest and universal responses observed in plants following pathogen challenge [29]. The oxidative burst is also known to be suppressed at low cell pH [46]. Humic acids, such as short-chain organic acids, decrease the intracellular pH showed by the increased fluorescence of BCECF probe ((2,7-bis(2-carboxyethyl)-5(and 6)-carboxyfluorescein, acetoxymethyl ester) and can act directly avoiding the oxidative burst [47].

Conclusion

Bacterial spot of tomato, caused by *X. euvesicatoria*, is a serious problem of tomatoes in North of Rio de Janeiro, Brazil. Once the disease is established in a crop, currently available management strategies are only partially effective and farmers indiscriminately use a cocktail of pesticides that are harmful to health and the environment. Plants are able to mount a number of defense responses upon pathogen attack. Induced resistance is a state of

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enhanced defensive capacity developed by a plant when appropriately stimulated. Humic acids and *H. seropedicae* when applied to growth medium acting as abiotic and biotic elicitors triggering defense mechanisms prior to infection by *X. euvesicatoria* reducing the symptoms in the leaves*.* The biostimulant based on humic acids and *H. seropedicae* reduced foliar disease severity and the incidence of bacterial spot.

Abbreviations
HA: Humic acids isolated from vermicompost; BAC: Herbaspirillum seropedicae; HA + BAC: Suspension with HA plus BAC.

Authors' contributions
APSS carried out the experiments; CPS was responsible by *X. euvesicatoria* inoculum and supervised the greenhouse experiment; RMS and VC reviewed the scientific literature and supervised the data analysis; NAC developed the HPLC method and supervised the analysis. LPC, CPS, FLO and LEPP conceived the experimental idea, designed the experiment and were responsible for writing the manuscript. All authors read and approved the final manuscript.

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
This manuscript is an original paper and has not been published in other journals. The authors agreed to keep the copyright rule.

Consent for publication
The authors agreed to the publication of the manuscript in this journal.

Competing interests
The authors declare that they have no competing interests.

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