Chemical composition and antioxidant properties of beetroot parasitized by *Meloidogyne javanica* and treated with elicitors

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Abstract

Beetroot is rich in nutrients and bioactive compounds, such as, betalains and phenolics. The objective of this work was to analyze the chemical composition and antioxidant properties of beetroot parasitized by *Meloidogyne javanica* and treated with elicitors. Beetroot seedlings were inoculated with 1000 eggs and second-stage juveniles of *M. javanica* and treated with elicitors based on mannan oligosaccharides, citrus biomass, or acibenzolar-S-methyl (ASM). At 60 days after inoculation, plants were evaluated for nematode reproduction factor (RF), proximate composition, betalains, phenolic compounds, and antioxidant activity. Beetroot was found to be susceptible to M. javanica, with RF values ranging from 15.26 to 27.94. ASM and citrus biomass treatments increased ash content by 15–25% in nematode-inoculated plants. There was no treatment effect on moisture or protein content, but uninoculated plants had higher total phenolic content (~35%) than inoculated plants. Nematode infection was found to compromise betalain production but did not impact antioxidant activity. Nematode-inoculated plants showed reduced sucrose content and increased glucose and fructose levels in all treatments. Regardless of the elicitor used, beetroots inoculated *M. javanica* exhibit deformities that render them unmarketable in fresh form. However, the results indicate that nematode-infected beetroot may be suitable for industrial processing and compound extraction.

Keywords: Beta vulgaris, betalains, nematodes, phenolic compounds, sugars

Introduction

Beetroot (*Beta vulgaris* L.) contains several bioactive compounds associated with health benefits, such as betalains and phenolics (Clifford et al., 2015; Corleto et al., 2018). Silva et al. (2018) found 7.05 mg g⁻¹ of betalains and 65.62 mg g⁻¹ of total phenolic compounds in beetroot. Such compounds, synthesized as secondary metabolites in plants, have antioxidant activity and are associated with health maintenance and prevention of cardiovascular and carcinogenic diseases (Ravichandran et al., 2013; Clifford et al., 2015). Furthermore, they can decrease lipid and protein oxidation, preserving the nutritional quality of food products (Gengatharan et al., 2015).

Phenolics are compounds containing aromatic rings with one or more hydroxyl substituents, its production in plants is influenced by stress conditions such as injuries, infections, and UV radiation (Gobbo-Neto & Lopes, 2007). The major phenolic compounds in beetroot are epicatechin, rutin, and caffeic acid, all of which are easily absorbed by the human body (Clifford et al., 2015).

Betalains can be divided into two groups, betacyanins and betaxanthins, comprising red-violet and yellow-orange pigments, respectively (Ravichandran et al., 2013; Cliford et al., 2016). The precursor of betalain biosynthesis is betalamic acid, which reacts with derivatives of cyclo-3,4-dihydroxyphenylalanine (cyclo-DOPA) to form betacyanins or with amines or amino acids to produce betaxanthins (Nakatsuka et al., 2013). Betanin, a betalain pigment, is widely used as a natural dye (E162) in the food industry because of its high solubility in water and absence of toxicity (Ravichandran et al., 2013).

Plant-parasitic nematodes can negatively affect the health of beetroot plants, resulting in yield losses and alterations in nutritional quality (Débia et al., 2020a; 2020b; Castanheira et al., 2020; 2021). The nematodes

Débia et al. (2023)

Meloidogyne incognita Chitwood and M. javanica (Treub) Chitwood are the most harmful to vegetables (Jiang et al., 2018). These species infect the vascular cylinder and surrounding cells and induce the formation of feeding sites characterized by cell hypertrophy and hyperplasia, seen macroscopically as root galls. Such parasitism compromises water and nutrient absorption, interfering with the commercial quality of the final product (Hussain et al., 2016; Débia et al., 2019).

Controlling plant-parasitic nematodes is a complex task that requires the adoption of integrated management practices (Walters et al., 2005). In recent years, environmentally friendly management approaches have been stimulated to minimize the negative impacts of chemical nematicides on humans and ecosystems. One such practice is the use of elicitors of plant resistance, which act by activating natural defense responses through different biochemical routes, ultimately inducing systemic resistance in plants (Mostafa et al., 2014).

Mannan-oligosaccharides, citrus biomass, and acibenzolar-S-methyl have been investigated to control phytonematodes (Miamoto et al., 2017). The use of these elicitors is interesting due to the lack of any direct antimicrobial activity, unlike traditional pesticides, avoiding direct selective pressures on pathogen populations. The resistance induced by these agents has a broad spectrum and long lasting, however, it rarely provides complete control of infection (Molinari & Baser, 2010). Furthermore, it was not found studies that investigated the effect of elicitors application on the chemical composition and antioxidant properties of beetroot parasitized by M. javanica, which constitutes the aim of this work.

Material and Methods

Greenhouse experiment

The experiment was conducted in a greenhouse $(23^{\circ}47'34.5''S 53^{\circ}15'22.1''W, 430 \text{ m a.s.l.})$. Analyses were performed at the Laboratory of Nematology and Laboratory of Physicochemical Analysis of Foods. The experimental design was completely randomized in a 4×2 factorial arrangement, with eight replications of nematode assays and three replications of the other analyses. Treatments consisted of three elicitors, in the presence or absence of *M. javanica*.

Seeds of 'Kestrel' hybrid beetroot (Sakata[™] Seed Sudamerica Ltd.) were sown in polypropylene trays containing commercial substrate (Bioplant®). After 15 days, seedlings received the first application of one of the following elicitors: mannan oligosaccharides (MOS) (Agro-Mos®, composed of phosphorylated mannan oligosaccharides from Saccharomyces cerevisiae cell walls complexed with 28.04 g L⁻¹ sulfur, 36.90 g L⁻¹ copper, and 24.60 g L⁻¹ zinc; Alltech Crop Science), citrus biomass (CB) (Ecolife®, citrus biomass containing 108.3 g L⁻¹ organic carbon, 1.71 g L⁻¹ boron, polyols, and carboxylic acids; Quinabra-Química Natural Brasileira Ltd.), and acibenzolar-S-methyl (ASM) (Bion®, 500 g kg⁻¹ acibenzolar-S-methyl; Syngenta Brazil). Elicitors were applied at the dosages recommended by the manufacturers for vegetable crops (MOS = 1.5 mL L⁻¹, CB = 2.0 mL L⁻¹, and ASM = 0.5 g L⁻¹). The fourth treatment consisted of water alone (control). Elicitor treatments were applied by spraying the aerial part of plants to the point of runoff.

After five days of treatment application, seedlings were transplanted to pots (one seedling per pot) containing 2.8 L of a mixture of sandy soil, potting substrate (Bioplant), and sand (2:1:1), previously sterilized in an autoclave for 2 h at 120 °C. Then, plants were inoculated or not with 1000 eggs and eventual secondstage juveniles (J2) of M. javanica. The inoculum was obtained from a pure population kept on tomato. Nematodes were extracted from plant roots by the method proposed by Hussey and Barker and adapted by Boneti and Ferraz (1981). The nematode suspension was calibrated to 1000 eggs + J2 mL⁻¹ by using a Peters chamber under an optical microscope. The inoculum was deposited in four 3 cm deep equidistant holes made in the soil around the base of each plant, which were covered with soil following application. At 20 days after nematode inoculation, the aerial part of beetroot plants received a second application of elicitor treatment (Débia et al., 2020b).

Reproduction factor of Meloidogyne javanica

At 60 after inoculation, plants were harvested and their roots (root system + taproot) collected. Roots were thoroughly washed under running water to remove excess soil. Then, nematodes were extracted and counted as described above. The total number of nematodes (final population) was divided by the initial population (1000 eggs + J2) to obtain the reproduction factor (RF), according to Oostenbrink (1966).

Chemical composition

The determination of moisture, ashes and proteins in beetroot were performed using methods 925.09, 923.03 and 920.87, respectively, of the Association of Official Analytical Collaboration (AOAC) International (Horwitz & Latimer, 2005). To determine sugars composition, an extraction was conducted using 80% (v v⁻¹) ethanol, in a proportion of 1:40 (m v⁻¹), by sonication (Schuster, L100, Shenzhen, China), at 42,000 Hz at 40 °C for 10 min. The samples were centrifugated (Metroterm, MTD III PULS, Porto Alegre, Brazil) at 1000 × g for 10 min and the supernatants were filtered through a 0.45 μ m hydrophobic Filtrilo PDVF membrane (Filtrilo, PDVF, Colombo, Brazil) (Castanheira et al., 2020).

Afterwards, 10 µL of each sample was applied in the high-performance liquid chromatography (HPLC), following the recommendation of Giombelli et al. (2020). The HPLC Prominence 20AD (Shimadzu, Chiyoda-ku, Tokyo, Japan) was used with a differential refractive index detector (RID-20A, Shimadzu), column oven (CTO-20A, Shimadzu), automatic injector (SIL-10A, Shimadzu) and the software Lab Solution Single Lite. The analytical column Shim-pack GIST NH2 (250 X 4.6 mm x 5 µm, Shimadzu) was maintained at 40 °C during the chromatograph run, with acetonitrile and ultrapure water (Milli-Q), 70:30 (v v-1) as mobile phase at a flow rate of 1 mL min⁻¹ (isocratic mode). Analytical curves ($\mathbb{R}^2 \ge 0.99$) were obtained with glucose, fructose and sucrose solutions (0.5 to 15 mg mL⁻¹) and the results were expressed as g 100 g⁻¹ (d.b.).

Betalains, phenolic compounds and antioxidant activity

For analysis of betalins, phenolic compounds and antioxidant activity, an extraction was realized following the recommendations of Ravichandran et al. (2013), with some modifications. Fresh samples (1 g) were dissolved in 20 mL of 50% (v v^{-1}) ethanol, shaken for 4 h on an orbital shaker (Marconi, MA-259) and centrifuged at 3000 rpm for 10 min. The supernatant was collected, and the extraction was repeated twice.

The betalain content was determined in a spectrophotometer (Kasuaki, IL-227), being the reading performed for betacyanin at 480 nm and for betaxanthin at 538 nm. The following equation was used to calculate the results: content (mg L⁻¹) = [(A×DF×MM×1000)/(e×I)]; where A is the absorbance, DF is the dilution factor, I is the bucket length, MW is the molecular weight and e is the molar extinction coefficient. The values used for MW

and were 550 g mol⁻¹ and 60,000 L mol⁻¹ for betacyanins and 308 g mol⁻¹ and 48,000 L mol⁻¹ cm for betaxanthines, respectively.

The analysis of total phenolic compounds (TPC) was carried out by the Folin-Ciocalteu method, according to the method of Chen et al. (2015). The ethanolic beetroot extract (0.5 mL), 2 mL of Folin-Ciocalteu reagent (10% v v⁻¹) and 2.5 mL of sodium carbonate (7.5 % v v⁻¹) were mixed and incubated in a water bath at 50 °C for 5 min. The absorbance was read in spectrophotometer at 760 nm. The analytical curve was prepared with gallic acid, and the results were expressed in mg of gallic acid equivalent (GAE) per 100 g of sample.

The antioxidant activity by the method of scavenging of DPPH (2,2-diphenyl-1-picryl-hydrazyl) was performed according to Ravichandran et al. (2013). Stock solution was prepared by dissolving 0.011 g of DPPH in 25 mL of ethanol. Subsequently, 6 mL of this solution was dissolved in 100 mL of ethanol. The reaction was carried out using 3.80 mL of this DPPH solution and 0.20 mL of ethanolic beetroot extract and, after incubation for 30 min at room temperature, the absorbance reading was performed at 515 nm. Trolox solutions were used to obtain the analytical curve and to express the results in µmol of Trolox g⁻¹ of sample.

The results obtained were submitted to analysis of variance (ANOVA) at 5% probability. When significant, the means of inducers were compared using the Tukey test and the presence of nematodes using the t test (LSD), both at 5% probability. Analyzes were performed using the Sisvar statistical program (Ferreira, 2011).

Results and Discussion

Reproduction factor of Meloidogyne javanica

The RF of *M. javanica* on beet plants inoculated and treated with elicitors ranged from 15.26 to 16.91. These values were lower than that of the control (RF =27.94) (**Figure 1**).

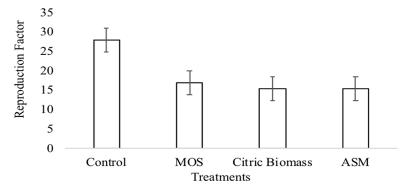


Figure 1. Reproduction factor of *Meloidogyne javanica* in beetroot after 60 days of inoculation with 1000 eggs and second-stage juveniles of *M. javanica* and treated with elicitors based on mannan oligosaccharides (MOS), citrus biomass, or acibenzolar-*S*-methyl (ASM).

Such high RF values are evidence of the susceptibility of the beetroot cultivar used in this study to *M. javanica*. In fact, literature data have shown that beetroot is susceptible to root-knot nematodes, with RF > 1 in some experiments (Rosa et al., 2013; Débia et al., 2019). The commercial value of beetroot is negatively affected by the presence of symptoms of root-knot nematode infection, often leading to the discarding of infected beetroots, as they are not well accepted for fresh consumption by consumers. Visual analysis of beetroots during the experiment confirmed the presence of galls and deformations.

Chemical composition

Elicitor treatment × Nematode inoculation did not exert significant interaction effects on beetroot moisture content (**Table 1**). However, the factor nematode inoculation was significant, and the mean moisture content was higher in inoculated plants.

Ashes contents were significantly influenced by the interaction Elicitor treatment × Nematode inoculation (Table 1). However, the ash content of uninoculated treated plants did not differ from that of the control. In plants inoculated with *M. javanica*, application of ASM and CB increased ash content by 15% and 25%, respectively, compared with the control (water).

A previous study on beetroot inoculated with M. javanica (0 to 10,000 nematodes) showed that parasitism did not influence beetroot moisture content, but ash content increased in plants inoculated with up to 2500 eggs + J2 (Débia et al., 2019). It is known that nematodes cause damage to plant roots, interfering with water and nutrient absorption (Hussain et al., 2016). Thus, it was expected that parasitism would negatively affect ash content, which is an indication of the amount of inorganic residues, that is, the content of minerals in the sample. Sodium, potassium, and iron salts are the most common minerals in beetroot (Tivelli et al., 2011). Nematode parasitism may, however, induce plants to produce new secondary roots as a form of compensation (Carneiro et al., 2002). In the case of beetroot, M. javanica inoculation was reported to increase root weight from 1.82 g (uninoculated) to 15.12 g (10,000 eggs + J2) (Débia et al., 2019), which could explain the results of the current study.

 Table 1. Moisture, ash nd protein contents of beetroots parasitized or not by Meloidogyne javanica and treated with elicitors.

Elicitors	Mois	sture (g 100) g-1)	As	hes (g 100 g	-1)	Prot	tein (g 100	g-1)
Elicitors	0	1000	Mean*	0	1000	Mean*	0	1000	Mean*
Water	83.90	84.56	84.23	1.22 aA	1.22 cA	1.22	1.40	1.25	1.33
MOS	82.90	85.00	83.95	1.27 aA	1.28 bcA	1.28	1.31	1.29	1.31
Citric Biomass	82.79	82.81	82.81	1.24 aB	1.52 aA	1.39	1.22	1.26	1.24
ASM	82.73	85.12	83.92	1.29 aB	1.41 abA	1.35	1.12	1.39	1.19
Mean	83.01 B	84.37 A		1.25 A	1.36 A		1.27 A	1.27 A	
CV (%)		1.11			6.48			8.02	

Within each evaluated parameter, means followed by the same lowercase letter in the column or the same uppercase letter in the row did not differ significantly at 5% significance. MOS - mannan oligosaccharides, ASM - acibenzolar-S-methyl, CV=Coefficient of variation, Mean*= mean values were calculated from the data of inoculated nematode population

Application of CB and ASM might have further contributed to the increase in ash content, given that inoculated treated plants exhibited higher media than uninoculated treated plants. It is possible that elicitors promoted changes in plant metabolism, reducing the damage caused by nematodes (Walters et al., 2013) and consequently contributing to the absorption of mineral salts from soil.

There were no significant interaction or main effects on total proteins (Table 1). These results indicate that there was no lack of proteins for the synthesis of plant defense metabolites (Lorencetti et al., 2015). We highlight that protein synthesis in plants is a complex process comprising several stages and occurring in distinct locations (Naoe et al., 2017). The mean values of moisture, ash, and protein contents obtained here were similar to or higher than those reported by TBCA (2021), in which raw beetroot was found to contain 86 g 100 g⁻¹ moisture, 0.90 g 100 g⁻¹ ash, and 1.90% g 100 g⁻¹ protein. Elicitor treatment × Nematode inoculation exerted significant effects on glucose, fructose, and sucrose levels, as can be seen in **Table 2**.

In all treatments applied in this study, sucrose levels were negatively influenced by nematode inoculation, whereas glucose and fructose levels increased, indicating sucrose hydrolysis. The increase in reducing sugar content with nematode inoculation might be a result of the energy demand generated by the activation of secondary metabolism processes, including synthesis and activation of pathogenesis-related proteins (Pansera-Espíndola, 2017). Atkinson et al. (2011) have reported that the sugar levels were maintained, when tomato plants were subjected to nematode stress. This fact can be associated to the high-metabolic activity in sick tissues, leading to a sugar-production compensation mechanism that can maintain sugar levels in plants

Javanica ana nea	ied with elicito	15.				
Elicitors	Sucrose (g 100g-1)	Glucose	(g 100g-1)	Fructose	(g 100g ⁻¹)
EIICHOIS	0	1000	0	1000	0	1000
Water	9.74 bA	6.77 bB	0.37 bB	0.56 cA	0.60 cB	0.95 cA
MOS	8.20 cA	7.73 aB	0.21 dB	1.28 bA	0.52 dB	1.14 bA
Citric Biomass	8.04 cA	7.52 aB	0.27 cB	0.56 cA	0.89 aB	1.14 bA
ASM	10.30 aA	5.52 cB	0.44 aB	1.63 aA	0.69 bB	1.34 aA
CV (%)	1.9	95	2.	54	2.	.86

 Table 2.
 Sucrose, glucose and fructose proteins contents of beetroots parasitized or not by Meloidogyne javanica and treated with elicitors.

Within each evaluated parameter, means followed by the same lowercase letter in the column or the same uppercase letter in the row did not differ significantly at 5% significance. MOS - mannan oligosaccharides, ASM - acibenzolar-S-methyl, CV=Coefficient of variation.

infected by sedentary endoparasite nematodes (Nayak & Mohanty, 2010).

In uninoculated plants, ASM promoted an increase in sucrose content, whereas MOS and CB caused a decrease. However, in inoculated plants, the opposite was observed, with ASM treatment decreasing sucrose content and MOS and CB increasing this variable.

ASM treatment enhanced glucose and fructose levels in inoculated and uninoculated plants. Glucose level was also enhanced by MOS treatment in inoculated plants compared with the control. CB increased fructose content in uninoculated plants compared with the control, and all elicitors enhanced fructose levels in inoculated plants. The findings show that elicitors exerted different effects on the major sugars of beetroot. It can be inferred that ASM treatment afforded the highest conversion rate of sucrose to glucose and fructose in plants inoculated with M. javanica. Resistance induction is a complex process, involving allocation of chemical compounds, especially simple compounds such as sugars, for growth or defense; the balance between allocation and synthesis is determined by competition for common substrates and energy (Barros et al., 2010).

Betalains, phenolic compounds and antioxidant activity

Only nematode inoculation significantly influenced total phenolic content, betaxanthin content, and DPPH scavenging activity. Betacyanin content was significantly influenced by interaction effects (Table 3). Beetroot samples had total phenolic contents greater than 100 mg GAE 100 g⁻¹, similar to the value reported by Débia et al. (2019) in beetroot 'Early Wonder Tall Top' (Top Seed®) inoculated with M. javanica (0-10,000 nematodes). The highest total phenolic content observed by the authors was 80.88 mg GAE 100 g⁻¹. In the study of Stagnari et al. (2014), beetroot (B. vulgaris L. var. conditiva Alef. 'Piatta d'Egitto') subjected to water stress showed total phenolic contents of 50 to 100 mg GAE 100 g⁻¹.

In considering the mean values of treatments, it was observed that nematode inoculation caused a decrease in total phenolic content. Phenolic compounds might have been consumed during lignin synthesis, which

~~t:⊂: _	TPC (TPC (mg GAE100g	0g ⁻¹)	Betac	Betacyanins (mg L ⁻¹)	L ⁻¹)	Beta	Betaxanthins (mg L ⁻¹)	lg L ⁻¹)	DPPH (DPPH (µmol of Trolox g ⁻¹)	lox g ⁻¹)
	0	1000	Mean*	0	1000	Mean*	0	1000	Mean*	0	1000	Mean*
Water	130.07	101.87	115.97	54.75abA	40.12aA	47.44	42.73	36.04	39.39	124.65	117.61	121.13
MOS	154.94	101.97	128.46	62.42aA	47.80aB	51.30	47.20	37.02	42.11	137.36	150.42	143.90
CB	152.66	129.98	141.32	44.79bA	45.49aA	45.15	50.32	42.26	46.29	142.60	150.90	146.75
ASM	194.25	112.63	154.44	59.37abA	42.68aB	51.03	53.33	36.92	45.13	187.05	134.74	160.89
Mean	157.98A	112.11B		55.33A	40.37B		48.90A	38.06B		147.92A	138.42B	
CV(%)		32.56			22.01			23.63			22.01	

is part of plant defense mechanisms against pathogens. Lignification of plant cells increases wall rigidity, conferring greater resistance to penetration by parasites (Stangarlin et al., 2011). In a study on the resistance of *Lathyrus* spp. infected with Ascochyta lathyris, cell wall metabolism was found to be altered, as demonstrated by overexpression of genes involved in cellulose and lignin biosynthesis (Almeida, 2015). Bean plants treated with ASM showed a reduction in total phenolic content but an increase in lignin synthesis (Kuhn & Pascholati, 2010), possibly explaining why elicitors did not increase total phenolic content in the current study.

Currently, the sole betalain-based commercial source is from red beetroot. Betalain is composed by betaxanthins (yellow color) and red-violet betacyanins (Rodriguez-Amaya, 2016). The presence of nematodes decreased betaxanthin and betacyanin contents by about 25%, as shown by the mean values of treatments. In agreement with the results of Débia et al. (2019), here, betacyanin content was not influenced by nematode inoculation in control plants or plants treated with CB. On the other hand, in plants treated with MOS or ASM, nematode inoculation led to a decrease in betacyanin content, indicating that betacyanins might have been consumed to protect plants against pathogen-induced stress. By contrast, Kleinowski et al. (2014) reported an increase in betalain levels in Alternanthera philoxeroides (Mart.) Griseb., a medicinal plant commonly known as alligator weed, subjected to elicitation by the amino acid tyrosine.

In the present study, application of elicitors did not result in the activation of any mechanism associated with increased betalain production in beetroot. Previous studies on the use of elicitors to control pathogens demonstrated that compounds were able to suppress and/or damage nematodes, as evidenced by the decrease in infection area size, disease severity, or pathogen proliferation (Gomes et al., 2016; Neumann Silva et al., 2017; Antonio et al., 2018). Here such effects resulted in a decrease in RF. The induction of plant resistance to pathogens is triggered when an external signal (elicitor) binds to a receptor on the surface of plant cells; in this way, the primary signal is transmitted to the inside of cells, activating secondary messengers, which amplify the signal and regulate the expression of specific genes, determining the development of compatible (susceptibility) or incompatible (resistance) interactions (Barros et al., 2010). Resistance induction can be local or systemic, being classified as systemic acquired resistance or induced systemic resistance (Conrath et al., 2015).

The decrease in total phenolic, betacyanin, and betaxanthin contents in beetroot inoculated with 1000 eggs + J2 of *M. javanica* led to a reduction in antioxidant activity. Similar effects were observed in soybean inoculated with up to 2000 eggs + J2 of *M. javanica* (Castanheira et al., 2021). On the other hand, in beetroot inoculated with up to 10,000 eggs + J2 of *M. javanica*, there was no change in antioxidant activity (Débia et al., 2019).

It is noteworthy that the betalain contents observed here are higher than those described by Ravichandran et al. (2013) for processed beetroot (subjected to irradiation, cooking, roasting, and vacuum treatment): 7.00–8.50 mg L^{-1} betacyanin and 5.20–5.80 mg L⁻¹ betaxanthin. Beetroot is well known for its high betalain content and antioxidant capacity compared with other vegetables, such as potato, carrot, cassava, and onion (Sreeramulu & Raghunath, 2010). Thus, it can be inferred that, despite the decrease in betalain content with nematode infection, nematode-infected beetroot may still be considered an important source of these components. Betalains inhibit oxidative reactions because of their high free radical scavenging activity, being associated with health benefits and prevention of some types of cancer (Picolli et al., 2010; Tivelli et al., 2010).

In line with the fact that elicitors are part of integrated management strategies and that the results of nematode control were partial, all inoculated plants, regardless of the type of elicitor used, exhibited galls. This was expected, given that elicitors contribute to the control of nematode reproduction but do not prevent symptom emergence (Chinnasri et al., 2003; Molinari & Baser, 2010; Débia et al., 2019). Beetroots with visible symptoms have reduced commercial value and are frequently discarded. Thus, the findings of the current study are relevant because they show that, even when infected, beetroots remain an important source of betalains, phenolic compounds, and sugars for extraction. Another alternative use for beetroots with visual defects is in the food industry for the enrichment of breads, cakes, biscuits, baby foods, and athlete supplements or as coloring agents in yogurts, ice cream, and sweets.

Conclusions

The hybrid beetroot 'Kestrel' is host to M. javanica, and an initial population of 1000 eggs + J2 is sufficient to negatively affect total phenolic, betalain, and betaxanthin contents as well as antioxidant activity. Nematode inoculation decreases sucrose levels but increases those of the monosaccharides glucose and fructose. CB and ASM treatments increase ash content in beetroot infected with *M. javanica*. ASM increases sucrose levels in healthy plants and glucose and fructose in inoculated or uninoculated plants. Galls and physical deformations may occur regardless of the type of elicitor used, possibly affecting the commercial value of beetroot; infected beetroot may be used for the preparation of processed foods or compound extraction.

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References

Almeida, N.F., Nicolas, K., Bjorn, R., Winter P., Rubiales, D., Vaz Patto, M.C. 2015. Lathyrus sativus transcriptome resistance response to Ascochyta lathyri investigated by deepSuperSAGE analysis. *Frontiers in Plant Science* 6: 1-11.

Antonio, G.L., Donato, R.L., Iossi, M.R., Firmino, A.C. 2019. Ação de indutores de resistência em cacaueiro infectados com *Ceratocystis cacaofunesta*. *Summa Phytopathologica* 45: 104-106.

Atkinson, N.J., Dew, T.P., Orfila, C., Urwin, P.E. Influence of combined biotic and abiotic stress on nutritional quality parameters in tomato (*Solanum lycopersicum*). 2011. Journal of Agricultural and Food Chemistry 59: 9673-9682.

Barros, F.C., Sagata, E., Ferreira, L.C.C., Juliatti, F.C. 2010. Indução de resistência em plantas contra fitopatógenos. *Bioscience Journal* 26: 231- 239.

Boneti, J.I.S., Ferraz, S. 1981. Modificação do método de Hussey & Barker para extração de ovos de Meloidogyne exigua em raízes de cafeeiro. *Fitopatologia Brasileira* 6: 553.

Bucur, L., Țarălungă, G., Schroder, V. 2016. The betalains content and antioxidant capacity of red beet (Beta vulgaris L. subsp. vulgaris) root. Farmacia 64:198-201.

Carneiro, R.G., Mazzafera, P., Ferraz, L.C.C.B., Muraoka, T., Trevelin, P.C.O. 2002. Uptake and translocation of nitrogen, phosphorus and calcium in soybean infected with *Meloidogyne incognita* and *M. javanica*. *Fitopatologia Brasileira* 27: 141-150.

Castanheira, C.M., Falcão, H.G., Ida. E.I., Dias-Arieira. C.R., Barros, B.C.B. 2020. *Pratylenchus brachyurus* parasitism on soybean: effects on productivity, vegetative and nematological parameters and chemical properties. *European Journal of Plant Pathology* 157: 651–661.

Castanheira, C.M., Dias-Arieira, C.R., Ida. E.I., Falcão, H.G., Barros, B.C.B. 2021. Influence of *Meloidogyne javanica* parasitism on soybean development and chemical composition. *Chemical and Biological Technologies in Agriculture* 8: 10-15. Chen, M., Zhao, Y., Yu, S. 2015. Optimization of ultrasonicassisted extraction of phenolic compounds, antioxidants, and anthocyanins from sugar beet molasses. *Food Chemistry* 172: 543–550.

Chinnasri, B., Sipes, B.S., Schmitt, D.P. 2003. Effects of acibenzolar-S-methyl application to Rotylenchulus reniformis and Meloidogyne javanica. Journal of Nematology 35: 110-114.

Clifford, T., Howatson, G., West, D.J., Stevenson, E.J. 2015. The Potential Benefits of Red Beetroot Supplementation in Health and Disease. *Nutrients* 7: 2801-2822.

Conrath, U., Beckers, G.J.M., Langenbach, C.J.G., Jaskiewicz, M.R. 2015. Priming for enhanced defense. *Annual Review of Phytopathology* 53: 97–119.

Corleto, K.A., Singh, J., Jayaprakasha, G.K., Patil, B.S. 2018. Storage Stability of Dietary Nitrate and Phenolic Compounds in Beetroot (*Beta vulgaris*) and Arugula (*Eruca sativa*) Juices. Journal of Food Science 83: 1237-1248.

Débia, P.J.G., Bolanho, B.C., Puerari, H.H., Dias-Arieira, C.R. 2019. *Meloidogyne javanica* parasitism and its impacts on the vegetative parameters, physicochemical composition, and antioxidant potential of beet. *Pesquisa Agropecuária Brasileira* 54: e00695.

Débia, P.J.G., Bolanho, B.C., Puerari, H.H., Dias-Arieira, C.R. 2020a. *Meloidogyne javanica* parasitism on the vegetative growth and nutritional quality of carrots. *Ciência Rural* 50: e20190585.

Débia, P.J.G., Bolanho, B.C., Dias-Arieira, C.R. 2020b. Mineral composition of beetroot treated with potential elicitors and inoculated with *Meloidogyne javanica*. *Chemical and Biological Technologies in Agriculture* 7:30.

Ferreira, D.F. 2011. Sisvar: a computer statistical analysis system. *Ciência e Agrotecnologia* 35: 1039-1042.

Gengatharan, A., Dykes, G.A., Choo, W.S. 2015. Betalains: Natural plant pigments with potential application in functional foods. *LWT—Food Science and Technology*, 64: 645–649.

Giombelli, C., Iwassa, I.J., Silva, C., Barros, B.C.B. 2020. Valorization of peach palm by-product through subcritical water extraction of soluble sugars and phenolic compounds. *The Journal of Supercritical Fluids* 165: 104985.

Gobbo-Neto, L.B., Lopes, N.P. 2007. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. *Química Nova* 30: 374-381.

Gomes, R.S.S., Demartelaere, A.C.F., Nascimento, L.C., Maciel, W.O., Wanderley, D.B.N.S. 2016. Bioatividade de indutores de resistência no manejo da antracnose da goiabeira (*Psidium guajava* L.). *Summa Phytopathologica* 42: 149-154.

Horwitz, W., Latimer, G. 2005. Official methods of analysis of AOAC International. AOAC International, Gaithersburg, USA.

Hussain, M., Kamran, M., Singh, K., Zouhar, M., Rysánek, P., Anwar, S.A. 2016. Response of selected okra cultivars to Meloidogyne incognita. Crop Protection 82: 1-6.

Jiang, C.H., Xiea, P., Li, K., Xiea, Y.S., Chenc, L.J., Wangd, J.S., Xua, Q., Guoa, J.H. 2018. Evaluation of root-knot nematode disease control and plant growth promotion potential of biofertilizer Ning shield on *Trichosanthes kirilowii* in the field. *Brazilian Journal of Microbiology* 49: 232-239.

Kleinowski, A.M., Brandão, I.R., Einhardt, A.M., Ribeiro, M.V., Peters, J.A., Braga, E.J.B. 2014. Pigment Production and Growth of Alternanthera Plants Cultured in vitro in the Presence of Tyrosine. *Brazilian Archives of Biology and Technology* 57: 253-260.

Kuhn, O.J., Pascholati, S.F. 2010. Custo adaptativo da indução de resistência em feijoeiro mediada pela rizobactéria *Bacillus cereus* ou acibenzolar-S-metil: atividade de enzimas, síntese de fenóis e lignina e biomassa. *Summa Phytopathologica* 36: 107-114.

Lorencetti, G.A.T., Mazaro, S.M., Potrich, M., Lozano, E.R., Barbosa, L.R., Luckmann, D., Dalacortti, S. 2015. Produtos alternativos para controle de *Thaumastocoris peregrinus* e indução de resistência em plantas. *Floresta e Ambiente* 22: 541-548.

Molinari, S., Baser, N. 2010. Induction of resistance to root-knot nematodes by SAR elicitors in tomato. *Crop Protection* 29: 1354-1362.

Mostafa, F.A.M., Khalil, A.E., Nour El Deen, A.H., Ibrahim, D.S. 2014. Induction of systemic resistance in sugar-beet against root-knot nematode with commercial products. *Journal of Plant Pathology and Microbiology* 5: 1-7.

Nakatsuka, T., Yamada, E., Takahashi, H., Imamura, T., Suzuki, M., Ozeki, Y., Tsujimura, I., Saito, M., Sakamoto, Y., Sasaki, N., Nishihara, M. 2013. Genetic engineering of yellow betalain pigments beyond the species barrier. *Scientific Reports* 3: 1-7.

Naoe, A.M.L., Peluzio, J.M., Sousa, J.P. 2017. Estresse ambiental na cultura da soja. *Revista Integralização Universitária* 11: 71-80.

Nayak, D.K., Mohanty, K.C. 2010. Biochemical changes in brinjal induced by root-knot nematode, *Meloidogyne incognita*. *Indian Journal of Nematology* 40: 43-47.

Neumann Silva, V., Do Amaral, J.C., Martinelli, V., Cigel, C., Wordell Filho, J.A. 2017. Resultados preliminares do efeito de indutores de resistência no controle da cercosporiose em beterraba. *Horticultura Argentina* 36: 91.

Miamoto, A., Silva, M.T.R., Dias-Arieira, C.R., Puerari, H.H. 2017. Alternative products for *Pratylenchus brachyurus* and *Meloidogyne javanica* management in soya bean plants. *Journal of Phytopathology* 65: 635-640.

Oostenbrink, R. 1966. Major characteristics of the relation between nematodes and plants. Mededeelingen der Landbouw-Hoogeschool 66: 1-46. Pansera-Espíndola, B. 2017. Indução de resistência e vaporização de óleos essenciais no controle de podridões pós-colheita em pêssegos 'chimarrita'. 84p. (Tese de Doutorado) - Universidade do Estado de Santa Catarina, Lages, Brasil.

Picolli, A.A., Faria, D.B., Jomori, L.L., Kluge, R.A. 2010. Avaliação de biorreguladores no metabolismo secundário de beterrabas inteiras e minimamente processadas. *Bragantia* 69: 983-988.

Ravichandran, K., Saw, N.M.M.T., Mohdaly, A.A.A., Gabr, A.M.M., Kastell, A., Riedel, H., Cai, Z., Knorr, D., Smetanska, I. 2013. Impact of processing of red beet on betalain content and antioxidant activity. *Food Research International* 50: 670-675.

Rodriguez-Amaya, D.B. 2016. Natural food pigments and colorants. *Current Opinion in Food Science*.7:20-26.

Rosa, J.M.O., Westerich, J.N., Wilcken, S.R. 2013. Reprodução de *Meloidogyne javanica* em olerícolas e em plantas utilizadas na adubação verde. *Tropical Plant Pathology* 38: 133-141.

Silva, H.R.P., Silva, C., Bolanho, B.C. 2018. Ultrasonicassisted extraction of betalains from red beet (Beta vulgaris L.). Journal of Food Process Engineering 46: 1-6.

Sreeramulu, D., Raghunath, M. 2010. Antioxidant activity and phenolic content of roots, tubers and vegetables commonly consumed in India. *Food Research International* 43: 1017–1020.

Stagnari, F., Galieni, A., Speca, S., Pisante, M. 2014. Water stress effects on growth, yield and quality traits of red beet. *Scientia Horticulturae* 165: 13–22.

Stangarlin, J.R, Schulz, D.G, Franzener, G., Assi, L., Schwan-Estrada, K.R.F., Kuhn, O.J. 2010. Indução de Fitoalexinas em Soja e Sorgo Por Preparações de Saccharomyces boulardii. Arquivos do Instituto Biológico 77: 91-98.

TBCA. Tabela Brasileira de Composição de Alimentos. 2021. http://www.fcf.usp.br/tbca <Acessed on: Jul. 2021>

Tivelli, S., Factor, T.L., Teramoto, J.R.S., Fabri, E.G., Moraes, A.R.A., Trani, P.E., May, A. 2011. *Beterraba: do plantio* à comercialização. Boletim Técnico IAC 210. Instituto Agronômico, Campinas, Brazil. 45p.

Walters, D.R., Ratsep, J., Havis, N.D. 2013. Controlling crop diseases using induced resistance: challenges for the future. *Journal of Experimental Botany* 64; 1263-1280.

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