

Meloidogyne javanica parasitism and its impacts on the vegetative parameters, physicochemical composition, and antioxidant potential of beet

Abstract – The objective of this work was to evaluate the effects of infestation by *Meloidogyne javanica* on the vegetative development, nutritional characteristics, and antioxidant potential of beet (*Beta vulgaris*). Inoculation in beet seedlings was performed with 0, 1,000, 2,500, 5,000, and 10,000 eggs and with possible second-stage juveniles (J2) of nematodes. Plants were collected 60 days after inoculation for the evaluation of the nematological and vegetative parameters, the physicochemical composition, and the content and activity of antioxidant compounds. The population levels of *M. javanica* reduced the mass and diameter of the tuber, comparing inoculum levels of 0 and 10,000 eggs + J2 of nematodes. There was a significant difference for the number of galls and nematodes per root system, with a proportional increase of population density according to the inoculation level. The reproduction factor in all infected treatments was above 1. Although the infection by *M. javanica* reduced the plant protein content, other compounds, such as betalains, sugars, and phenolics, were not affected. The inoculation by *M. javanica* negatively affects tuber development, but does not alter the antioxidant potential of beet.

Index terms: *Beta vulgaris*, betalains, gall nematodes, phenolic compounds, tuber.

Parasitismo por *Meloidogyne javanica* e seus impactos sobre os parâmetros vegetativos, a composição físico-química e o potencial antioxidante da beterraba

Resumo – O objetivo deste trabalho foi avaliar o efeito da infestação por *Meloidogyne javanica* sobre o desenvolvimento vegetativo, as características nutricionais e o potencial antioxidante da beterraba (*Beta vulgaris*). A inoculação foi feita em plântulas de beterraba com 0, 1.000, 2.500, 5.000 e 10.000 ovos e prováveis juvenis de nematoide em segundo estágio (J2). As plantas foram coletadas 60 dias após a inoculação, para a avaliação dos parâmetros nematológicos e vegetativos, da composição físico-química e do teor e da atividade dos compostos antioxidantes. Os níveis populacionais de *M. javanica* reduziram a massa e o diâmetro do tubérculo, comparando os níveis de inóculo 0 e 10.000 ovos + J2 de nematoide. Houve diferença significativa no número de galhas e nematoides por sistema radicular, com aumento proporcional da densidade populacional de acordo com o nível de inoculação. O fator de reprodução em todos os tratamentos com inoculação foi maior do que um. Embora a infecção por *M. javanica* tenha reduzido o teor de proteínas, outros compostos como betalainas, açúcares e fenólicos não foram alterados. A infestação por *M. javanica* afeta negativamente o desenvolvimento do tubérculo, mas não altera o potencial antioxidante da beterraba.

Termos para indexação: *Beta vulgaris*, betalainas, nematoides-das-galhas, compostos fenólicos, tubérculo.

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Introduction

Beet (*Beta vulgaris* L.) is commercially cultivated in Brazil due to its sensory characteristics and nutritional value (Stagnari et al., 2014; Carrillo et al., 2017). It contains approximately 1.9 g protein, 11.1 g carbohydrates, 3.4 g fiber, and 0.9 g ashes per 100 g of fresh weight (USDA, 2019). Its most abundant minerals are phosphorus (867 mg L⁻¹), potassium (2,825 mg L⁻¹), magnesium (259 mg L⁻¹), zinc (1,132 µg L⁻¹), and iron (2,206 µg L⁻¹) (Wruss et al., 2015). Besides, beet is a source of antioxidants such as phenolic compounds and betalains. Betalains are a class of pigments, formed by two structural groups, betacyanins and betaxanthins, which are responsible for the tuber's color; they can be used in the food industry as a source of natural color (Sawicki et al., 2016).

Beet production can be affected by a series of phytosanitary issues including nematodes belonging to the genera *Heterodera* and *Meloidogyne* (Held et al., 2000; Krall et al., 2000; Rosa et al., 2015). *Meloidogyne incognita* (Kofoid & White) Chitwood and *M. javanica* (Treub) Chitwood frequently parasitize horticultural plants, with gall formation in the tuberous root, resulting in yield and quality losses. Beet cultivar 'Oscarpoly', infected by *M. javanica*, showed a reduction of fresh root and shoot mass (Korayem, 2006). Hanse et al. (2011) and Melakeberhan et al. (2018) stated the importance of adequate nematode management to reduce losses in beet cultivation.

Nematode infestation can also affect the nutritional value of the products. Potatoes infected with *M. arenaria* showed reduced carbohydrates and sugars (Korayem et al., 2012). Besides, there is a research that points to an increase of the production of sugars and phenolic compounds in tomatoes infected by *M. incognita* (Atkinson et al., 2011), showing that plants respond differently to stress.

Despite the proven interaction between root-knot nematodes and beet, there are no known studies addressing its effects on the nutritional value of tubers, nor the antioxidant potential of beet. It is worth mentioning that tubers infected by nematodes are discarded due to changes in their physical characteristics, such as deformation and diameter reduction, and they do not meet the demands of in natura commercialization (Rosa et al., 2015). However, other alternatives can be proposed to minimize these losses, such as extraction of pigments, antioxidants,

and other substances (Clifford et al., 2015). Thus, it is hypothesized that, although nematodes affect the production and commercial value of beets, the nutritional characteristics of the tubers may remain unchanged.

The objective of this work was to evaluate the effect of *M. javanica* parasitism on the vegetative development, nutritional quality, and antioxidant potential of beet.

Materials and Methods

The experiment was carried out between December 2014 and February 2015, in the greenhouse of the Nematology and Food Physicochemical Analysis Laboratory at Universidade Estadual de Maringá. Thirty-five plots were evaluated, each one composed of one plant, in a complete randomized design with four replicates, to analyze the vegetative and nematological parameters. Three replicates were conducted during the physicochemical composition and antioxidant analyses.

Beet 'Early Wonder Tall Top' (Top Seed) was sown in polystyrene trays containing vegetal substrat (Bioplant, Nova Ponte, MG, Brazil). After 20 days, seedlings were transplanted to pots (one seedling per pot) containing the following material: 2.8 L of sandy soil; Bioplant – composed of sphagnum peat, coconut fiber, rice husk, pine bark, vermiculite, and nutrients; and sand mixture (at 2:1:1). Prior to use, the mixture was autoclaved for 2 hours at 120°C. Before planting, the soil was fertilized with granulated fertilizer containing N (40 g), P₂O₅ (140 g), and K₂O (80 g).

The seedlings were infected 7 days after transplantation at different *M. javanica* inoculum levels, consisting of 0, 1,000, 2,500, 5,000, and 10,000 eggs, and possible second-stage juveniles (J2) per plant. The inoculum was obtained from a pure population kept in okra plants, according to the methodology proposed by Hussey & Barker (1973). The inoculum levels were evaluated by using Peters' slides under an optical microscope (BA210 Binocular, Motic, Hong Kong, China). Four (4) equidistant holes, approximately 3 cm deep, were opened in the soil around the plant for inoculation. The inoculum, held in 4 mL water, was deposited in the holes, which were subsequently closed. The plants were irrigated on a daily basis, depending on water requirements. Leaf fertilization was conducted every 15 days, using the leaf fertilizer Nutrijá at 380 g

N, 380 g P₂O₅, and 380 g K₂O (Agrária, Jardinópolis, Brazil) diluted in water at a 5 g L⁻¹.

After 60 days of inoculation, the plants were collected and carefully rinsed under running water to remove excess soil. Subsequently, shoots were separated from roots, and the following vegetative parameters were assessed: shoot fresh mass and height, secondary root and tuber mass, and tuber diameter. Shoot dry matter mass was also obtained after the samples were dried in a forced-air circulation oven for 3 days at 65°C.

The number of galls per root system was first assessed to measure the nematological parameters through direct naked-eye counting. Subsequently, eggs were extracted from roots and tubers, according to the method proposed by Charchar et al. (2006). For this, beet tubers were peeled, and a thin peel slice of approximately 3 mm was removed from them. Peel slices and the secondary roots were cut in pieces (1 and 2 cm) and ground in a blender (for 30 s, at maximum speed), using hypochlorite solution at 0.5% to cover the plant material. The suspension was sieved through 60 and 500 mesh and washed in water; the content on the last sieve was collected in a beaker. Subsequently, eggs and J2 were counted on Peters' slide under an optical microscope. The total number of nematodes was divided by the root mass and expressed as nematodes per gram of root. The reproduction factor (RF) was also calculated based on the ratio between the final population (eggs + J2) and the initial one (eggs + J2) (Oostenbrink, 1966).

The treatments were analyzed to determine moisture, ash, and protein contents by the methods 925.09, 923.03, and 920.87, respectively, described by Horwitz & Latimer (2005). The methodology proposed and adapted by Benkeblia & Lopez (2015) was used for sugar extraction. Briefly, fresh beet samples (5 g) were homogenized in 50 mL ethanol at 80%, and centrifuged at 1,650 g (Metroterm, MTD III PLUS, Porto Alegre, RS, Brazil) for 10 min. The supernatant was removed, and supernatant aliquots (0.5 mL) were placed in a forced-air circulation oven SL-102 (Solab, Piracicaba, SP, Brazil) at 50°C, until complete ethanol evaporation. Subsequently, the samples were resuspended in 20 mL of Milli-Q water (Millipore, Burlington, MA, EUA); glucose, fructose, and sucrose were determined via high-efficiency ion exchange chromatography (Dionex ICS 5000, Thermo Fischer Scientific, Waltham, MA, USA). The samples were filtered through a PVDF hydrophilic membrane of

0.22 µm Millex-GV (Millipore, Burlington, MA, USA), and a total volume of 10 µL was manually injected; separation was conducted in an analytical column CarboPac PA1 (Dionex Corporation, Sunnyvale, CA, USA). The run was performed at a solvent flow at 1.0 mL min⁻¹ at 30°C, using NaOH at 0.25 mol L⁻¹ for 35 min. Chromatogram analysis was performed using the software package Chromeleon version 6.8 (Dionex Corporation, Sunnyvale, CA, USA). Calibration curves were generated using different glucose, fructose, and sucrose concentrations, obtained from Sigma-Aldrich (New Orleans, MO, USA) (Bolanho et al., 2015), and the results were expressed in mg g⁻¹ of sample on a wet basis.

Phenolic compounds and betalains were extracted according to Ravichandran et al. (2013), with modifications. Fresh samples (1 g) were dissolved in 20 mL ethanol at 50%, stirred for 4 hours in an orbital stirrer MA-259 (Marconi, Piracicaba, SP, Brazil), and centrifuged in a MTD III plus (Metroterm, Porto Alegre, RS, Brazil) at 1,200 g for 10 min. The supernatant was removed, and the extraction was repeated twice by using 5 mL ethanol at 50%. Betalain content was determined in a spectrophotometer 700Plus (Femto, São Paulo, SP, Brazil), and the readings were conducted for betacyanins at 538 nm, and for betaxanthins at 480 nm (Ravichandran et al., 2013). The concentrations were calculated using the following formula: betalain content (mg L⁻¹) = [(A × DF × MM × 1000) / (e × l)], in which: A is absorbance; DF is the dilution factor; and l is the bucket length. Molecular mass (MM) and the molar extinction coefficients (e) were used for betacyanin and betaxanthin quantification, respectively, as follows: MM = 550 g mol⁻¹; e = 60,000 L mol⁻¹ cm⁻¹ of H₂O; MM = 308 g mol⁻¹; e = 48,000 L mol⁻¹ cm⁻¹ of H₂O.

The same extract was used to determine the contents of total phenolic compounds through the method of Folin-Ciocalteu, according to the protocol described by Chen et al. (2015), with modifications. Ethanolic beet extract (0.5 mL), 10% Folin-Ciocalteu (2 mL), and 7.5% sodium carbonate (2.5 mL) were stirred and heated in a water bath at 50°C for 5 min; absorbance was read at 760 nm in a spectrophotometer 700Plus (Femto, São Paulo, SP, Brazil). The calibration curve was prepared using gallic acid, and the results were expressed as gallic acid equivalent (GAE) in mg 100 g⁻¹ of sample.

Antioxidant activity, based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenge method, was determined according to Ravichandran et al. (2012), with some modifications. To prepare the solution, 0.011 g of DPPH (Sigma-Aldrich, New Orleans, MO, USA) was weighed and dissolved in 25 mL ethanol. Subsequently, 6 mL of this solution were dissolved in 100 mL of absolute ethanol. The reaction was carried out by using 3.80 mL of this DPPH solution, and 0.20 mL of ethanolic beet extract. Absorbance was read in a spectrophotometer 700Plus (Femto, São Paulo, SP, Brazil) at 515 nm, after 30 min of incubation at room temperature. The antioxidant activity, based on the ferric-reducing/antioxidant power (FRAP) method, was determined by using a mixture containing 25 mL acetate buffer solution at 300 mmol L⁻¹ (pH 3.6), 2.5 mL of ferric chloride solution at 20 mmol L⁻¹, and 2.5 mL of 10 mmol L⁻¹ TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) dissolved in 40 mmol L⁻¹ HCl (Wootton-Beard et al., 2011). A test tube containing 0.27 mL water and 90 µL ethanolic extract was spiked with 2.7 mL of this mixture (FRAP reagent) for antioxidant capacity analysis. The tube was stirred and heated in a water bath at 37°C for 30 min; absorption was read in a spectrophotometer 700Plus (Femto, São Paulo, SP, Brazil) at 593 nm. Different Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) concentrations were used to generate the calibration curve through both antioxidant activity determination methodologies, and the results were expressed in µmol trolox g⁻¹ of sample.

Data were subjected to the variance analysis through regression, to assess the parameters presenting significant differences, at 5% probability. To meet the assumptions of normality, vegetative parameters and nematological data were transformed to (x+1)^{0.5}. The analyses were performed using the statistical program Sisvar (Ferreira, 2011).

Results and Discussion

The beet cultivar used in the present work was susceptible to *M. javanica*; the reproduction factors at all treatments with inoculation were higher than 1 (Table 1). The number of galls found in roots of beet increased linearly with increasing inoculum application levels. However, nematodes per root system and per gram of roots, as well as the reproduction factor, increased up to 2,500 eggs + J2, and decreased in

treatments with 5,000 and 10,000 eggs + J2. Based on previous studies, at higher inoculum levels, nematodes compete with each other for food and space, leading to a reduction of the population density. However, galls are formed early in the infestation, and therefore, gall number was not reduced (Asmus & Ferraz, 2002; Di Vito et al., 2004; Vovlas et al., 2005).

The increased nematode population did not change the fresh shoot mass and the height of beet plants (Table 2). However, the increases of the dry shoot mass and the fresh root mass were directly proportional to the inoculum level used in the experiment. Most likely, this is because nematodes cause several physiological disturbances in the plants, mainly in the roots, leading

Table 1. Nematological parameters of *Meloidogyne javanica* 60 days after the inoculation of beet (*Beta vulgaris*) with different nematode population levels.

Initial population	NG	NRS	NRG	RF
0	-	-	-	-
1,000	89*	3,732*	595*	3.73 ^{ns}
2,500	195	13,460	1,794	5.38
5,000	226	10,180	1,720	2.03
10,000	477	9,980	978	1.00
R ²	91.19 ⁽¹⁾	74.50 ⁽²⁾	89.50 ⁽³⁾	-
CV (%)	30.00	41.20	32.24	-

NG, number of galls; NRS, nematodes in the root system; NRG, nematodes per gram of root; RF, reproduction factor. ^{ns}Nonsignificant and *Significant at 5% probability; CV, coefficient of variation. ⁽¹⁾y = 0.0431x + 38.085. ⁽²⁾y = -0.0003x² + 4.018x + 975.88. ⁽³⁾y = -0.00006x² + 0.6758x + 76.831.

Table 2. Effect of *Meloidogyne javanica* on the vegetative parameters of beet (*Beta vulgaris*), 60 days after inoculation at different nematode population level.

Initial population	FSM (g)	DSM (g)	FRM (g)	SH (cm)	TM (g)	TD (mm)
0	7.48 ^{ns}	0.55*	1.82*	17.77 ^{ns}	25.06*	34.31*
1,000	6.51	0.90	9.16	18.37	18.15	32.38
2,500	7.01	0.83	9.18	16.47	12.91	23.95
5,000	9.45	1.09	6.83	18.42	11.93	23.44
10,000	9.41	1.32	15.12	17.35	13.38	20.96
R ²	-	87.15 ⁽¹⁾	96.10 ⁽²⁾	-	99.98 ⁽³⁾	94.12 ⁽⁴⁾
CV (%)	21.28	28.65	32.35	11.38	20.14	10.95

FSM, fresh shoot mass; DSM, dry shoot mass; FRM, fresh root mass; SH, shoot height; TM, tuber mass; TD, tuber diameter. ^{ns}Nonsignificant and *Significant at 5% probability. CV, coefficient of variation. Original data transformed by (x+1)^{0.5} for statistical analysis. ⁽¹⁾y = -0.00000002x² + 0.000242x + 0.5596. ⁽²⁾y = 0.00000001x² + 0.000862x + 4.9662. ⁽³⁾y = 0.0000003x² - 0.004356x + 23.4654. ⁽⁴⁾y = 0.0000002x² - 0.003650x + 34.3398.

to the formation of new secondary roots (Carneiro et al., 2002). In addition, the growth of the aerial part tends to be proportional to the growth of the root system (Leskovar, 1998).

Tubers were negatively affected by nematode infestation, with mass and diameter reductions (Table 2), which can be attributed to the fact that nematodes act as a photoassimilate drain in the plant (Premachandra & Gowen, 2015), and may reduce the contents of important nutrients such as nitrogen, phosphorus, and potassium in beet cultivars (El-Sherif et al., 2013). These results lead us to infer that photoassimilates are used in the formation of tubers for root and shoot maintenance. This hypothesis is supported by the fact that ash contents were reduced at a population level of 2,500 eggs + J2 of *M. javanica* (Table 3), as ashes represent mineral content present in the sample (Tivelli et al., 2011).

The different inoculum levels did not affect beet moisture (Table 3), which ranged from 814.5 to 855.4 g kg⁻¹ in the control and in the treatment with 10,000 eggs + J2. This result was similar to that reported by the United States Department of Agriculture in studies using fresh beet (860 g kg⁻¹) (USDA, 2019). The control treatment showed the highest-protein content, 12.6 g kg⁻¹ (Table 3); this value is similar to that detected in the beet 'Bikor' (13 to 14 g kg⁻¹) (Petek et al., 2012). However, protein levels decreased linearly with increasing nematode populations, which may be related to the increase of the gall number – that was directly proportional to the inoculum levels; such gall are the same ones formed as a response to feeding of the nematodes (Hoth et al., 2005), and for which the nutrients, including proteins, are drained and directed to feed these pathogens.

No significant differences in the sugar contents were recorded among the treatments; sucrose was the most abundant sugar found in the tubers (Table 3). Beet is rich in sugars, with sucrose contents ranging from 28.4 to 29.3 g kg⁻¹, depending on the cultivar and on the cultivation system (Bavec et al., 2010). Similarly, 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 infected by sedentary endoparasite nematodes (Nayak & Mohanty, 2010).

There was no significant difference in the production of betalains and phenolic compounds as a result of the application levels of nematode inoculum (Table 4). Betacyanin and betaxanthin contents ranged from 5.22 to 10.26 mg L⁻¹ and from 4.55 to 10.01 mg L⁻¹, respectively; these values are within the range reported in the literature, namely of 7.00 to 8.50 mg L⁻¹ for betacyanins, and 5.20 to 5.80 mg L⁻¹ for betaxanthins

Table 3. Moisture, ash, protein, glucose, fructose, and sucrose contents, on a wet basis, of beet (*Beta vulgaris*) subjected to different *Meloidogyne javanica* population levels.

Initial population	Moisture (g kg ⁻¹)	Ash (g kg ⁻¹)	Proteins (g kg ⁻¹)	Glucose (g kg ⁻¹)	Fructose (g kg ⁻¹)	Sucrose (g kg ⁻¹)
0	814.5 ^{ns}	12.1*	12.6*	2.80 ^{ns}	1.70 ^{ns}	17.1 ^{ns}
1,000	823.5	12.6	11.9	2.90	1.80	16.5
2,500	841.4	15.5	11.3	2.70	1.70	16.4
5,000	814.5	11.9	10.0	2.60	1.70	16.8
10,000	855.4	11.9	7.80	2.50	1.70	16.4
R ²	-	77.55 ⁽¹⁾	99.70 ⁽²⁾	-	-	-
CV (%)	2.90	8.97	13.34	5.99	3.90	3.76

^{ns}Nonsignificant and *Significant at 5% probability; CV, coefficient of variation. ⁽¹⁾y = 0.00000005 + 0.0004 + 12.63. ⁽²⁾y = -0.00047x + 1.25.

Table 4. Contents of betacyanins, betaxanthins, phenolic compounds, and antioxidant activity via DPPH and FRAP methods, in beet (*Beta vulgaris*) subjected to inoculation at different *Meloidogyne javanica* levels.

Initial population	Betacyanins (mg L ⁻¹)	Betaxanthins (mg L ⁻¹)	Phenolic compounds (mg GAE 100 g ⁻¹)	DPPH (μmol trolox g ⁻¹)	FRAP (μmol trolox g ⁻¹)
0	6.17 ^{ns}	7.23 ^{ns}	62.03 ^{ns}	42.86 ^{ns}	312.51 ^{ns}
1,000	5.22	4.55	73.81	52.19	350.65
2,500	10.26	10.01	80.88	66.35	413.19
5,000	8.43	6.24	68.72	39.60	284.93
10,000	7.04	7.97	64.26	43.78	298.55
R ²	-	-	-	-	-
CV (%)	26.72	39.56	23.80	35.61	21.38

^{ns}Nonsignificant and *Significant at 5% probability. CV, coefficient of variation.

(Ravichandran et al., 2013). The contents of phenolic compounds (62.03 to 80.88 mg of GAE 100 g⁻¹) were also within the range reported in previous studies (50 to 100 mg of GAE 100 g⁻¹) (Stagnari et al., 2014). These compounds are secondary metabolism products and related to defense mechanisms against pathogens, injuries, or other stress types (Naczki & Shahidi, 2004). Against this background, the contents of these substances are expected to be relatively high in infected plants. However, it can be inferred that these compounds were used to maintain the development of the plants through the translocation of phytochemicals to other organs, such as leaves and stems, where the demand for antioxidants should be greater to maximize plant suitability in the environment (Taiz et al., 2017).

There were no significant differences for the antioxidant activity in the treatments evaluated, and values ranged from 39.60 to 66.35 µmol trolox g⁻¹ via the DPPH method, and from 284.93 to 413.19 µmol trolox g⁻¹ via the FRAP methodology (Table 4). Most likely, this can be explained by the fact that the values of total phenolic contents (TPC) and the betalains were similar in the studied samples (p>0.05). According to Silva et al. (2018), in red beet extracts, there is a positive correlation between TPC and antioxidant activity via the DPPH method, and between betalains content and antioxidant activity via the FRAP method.

The levels of nematode infestation studied did neither alter betalain and TPC contents nor the antioxidant potential, but there was a significant impact on the physical characteristics of the tubers, such as a reduction of mass and diameter. The extraction of betalain, for instance as a dye in the food industry, or the isolation of other antioxidant compounds, may be alternatives to minimize the losses of beets infected by *M. javanica*.

Conclusions

1. The population levels of *Meloidogyne javanica* reduce the main vegetative characteristics of beets (*Beta vulgaris*), namely the mass and diameter of the tuberous root.
2. The protein content decreases irrespectively of the applied inoculums levels.
3. Sugar levels, total phenolic compounds, betalains, and antioxidant activity are not reduced in the presence of *M. javanica*.

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