Meloidogyne javanica parasitism on the vegetative growth and nutritional quality of carrots

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ABSTRACT: *Meloidogyne javanica is a plant-parasitic nematode that infects a wide range of vegetables. Its negative effects on crop yield and value are well documented. However, few studies have investigated the impact of the parasite on the nutritional value of vegetables. This study aimed to assess the effect of M. javanica parasitism on the vegetative characteristics, nematological parameters, chemistry composition and antioxidant activity of carrots. Seedlings were inoculated with 0 (control), 1000, 2500, or 5000 eggs and eventual second-stage juveniles (J2) of M. javanica. At 60 days after inoculation, plants were harvested and evaluated. Plants inoculated with 2500 eggs and J2 of M. javanica had higher root and tuber fresh weight than the control. Gall number increased with increasing inoculum density. The number of nematodes in the roots increased until 3000 specimens, decreasing thereafter. Proximate analysis revealed that plants inoculated with 1000 eggs and J2 of M. javanica or more had higher protein content in roots. In contrast, inoculation with 1775 nematodes or more resulted in a decrease in carotenoid content. There was no effect of inoculation on total phenolic content or antioxidant activity. Although, M. javanica infection did not have a marked impact on the nutritional quality of carrots, gall formation resulted in deformed roots of low commercial value.*
Key words: *Daucus carota, root-knot nematodes, phenolic compounds, carotenoids, proteins.*

Parasitismo de *Meloidogyne javanica* no crescimento vegetativo e na qualidade nutricional de cenouras

RESUMO: *Meloidogyne javanica é um nematoide parasita de plantas que infecta uma grande variedade de vegetais. Seus efeitos negativos sobre o rendimento e o valor das culturas estão bem documentados. No entanto, poucos estudos investigaram o impacto do parasita no valor nutricional dos vegetais. Este estudo teve como objetivo avaliar o efeito do parasitismo por M. javanica sobre as características vegetativas, parâmetros nematológicos, composição química e atividade antioxidante de cenouras. As plântulas foram inoculadas com 0 (controle), 1000, 2500 ou 5000 ovos e eventuais juvenis (J2) de M. javanica no segundo estádio. Aos 60 dias após a inoculação, as plantas foram colhidas e avaliadas. As plantas inoculadas com 2500 ovos e J2 de M. javanica apresentaram maior peso fresco das raízes e tubérculos que o controle. O número de galhas aumentou com o aumento da densidade do inóculo. Verificou-se aumento do número de nematoides nas raízes até 3000 espécimes, diminuindo posteriormente. A análise imediata revelou que plantas inoculadas com 1000 ovos e J2 de M. javanica ou mais tinham maior teor de proteína nas raízes. Por outro lado, a inoculação com 1775 nematoides ou mais resultou em diminuição no conteúdo de carotenoides. Não houve efeito da inoculação no conteúdo fenólico total ou na atividade antioxidante. Embora a infecção por M. javanica não tenha impactado significativamente a qualidade nutricional das cenouras, a formação de galhas resultou em raízes deformadas e de baixo valor comercial.*
Palavras-chave: *Daucus carota, nematoides das galhas, compostos fenólicos, carotenoides, proteínas.*

INTRODUCTION

Carrot (*Daucus carota* L.) is a root vegetable with great economic importance worldwide (BONTEMPO et al., 2017; GRABAU et al., 2017). In Brazil, it is one of the most produced and consumed vegetables (BONTEMPO et al., 2017). Carrots provide many health benefits because of their high content of carotenoids (0.51 g kg⁻¹ dry weight), which are important vitamin A precursors (ZHANG & HAMAUZU, 2004; RODRIGUEZ-CONCEPCION & STANGE, 2013; HIRANVARACHAT &

DEVAHASTIN, 2014), and phenolic compounds, which have high antioxidant activity. The tuberous root is also a source of proteins, carbohydrates, fibers, and minerals, particularly potassium, sodium, phosphorus, calcium, and magnesium (USDA, 2019). Carrots are typically consumed raw or cooked and can be processed into canned foods, juices, and baby foods.

The yield, nutritional quality, and commercial value of the vegetable are influenced by many factors, such as exposure to insects, pathogens, and ultraviolet radiation in the field (SELJASEN et al., 2013). Variation in composition can alter the color

and flavor characteristics of carrots (TALCOTT & HOWARD, 1999).

Root-knot nematodes, such as *Meloidogyne javanica* and *M. incognita*, are one of the major limiting factors to carrot crop yield, leading, in some cases, to total production loss (COLLANGE et al., 2011; VIGGIANO et al., 2014). During feeding, nematodes inject esophageal secretions into plant tissues, causing hypertrophy and hyperplasia of cortical cells of the host root. This process leads to the formation of root galls, which alter root shape, affect water and nutrient transport, and; consequently, reduce vegetative growth (HUSSAIN et al., 2016). Changes caused by root-knot nematode infection, even when at low levels, are responsible for reduced commercial value (GUGINO et al., 2006). Affected carrots; albeit being unsuitable for the consumer market, can be used in the food industry for compound extraction or processing. However, studies on tomato and bean have shown that stress caused by nematodes can alter the chemical and nutritional composition of vegetables (AHMED et al., 2009; ATKINSON et al., 2011).

Research on the effects of nematodes on carrot plants is limited to susceptibility analyses. There is little information about the influence of such parasitism on the proximate composition and nutritional quality of the vegetable. This study aimed to fill this research gap by assessing and quantifying the nutritional loss and physical damage caused by root-knot nematode infection in carrots and presenting alternative uses for affected roots.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse using a completely randomized design with four treatments, four replications for vegetative parameters and nematode population density, and three replications for proximate composition and antioxidant activity. Treatments consisted of the following inoculum levels: 0 (T1, control), 1000 (T2), 2500 (T3), and 5000 (T4) eggs and eventual second-stage juveniles (J2) of *M. javanica* plant^{-1}.

Carrot cv. Carandai (Horticeres®) was sown in pots filled with 2.8 L of a 2:1:1 mixture of sandy soil, commercial substrate (sphagnum peat, coconut fiber, rice husk, pine bark, vermiculite, and nutrients; Bioplant Agrícola Ltda., Nova Ponte, Brazil), and sand. The mixture was previously homogenized and sterilized in an autoclave for 2 h at 120 °C. The soil was fertilized with a granular fertilizer containing N (40 g), P_2O_5 (140 g), and K_2O (80 g) (Dimy, Cajamar, Brazil).

Thirty days after planting, carrot seedlings were thinned to one per pot and inoculated with 4 mL of a suspension containing different levels of *M. javanica* eggs and second-stage juveniles (J2). Eggs and J2 were extracted according to the method of HUSSEY & BARKER (1973) adapted by BONETI & FERRAZ (1981) and counted using a Peters chamber under a light microscope (BA210 Binocular, Motic, Hong Kong, China). Irrigation was performed daily, and foliar fertilization was conducted every 15 days using 5 g L^{-1} Nutrijá® (380 g of N, 380 g of P_2O_5, and 380 g of K_2O; Agrária, Jardinópolis, Brazil).

Plants were collected 60 days after inoculation, and the material was separated into shoots and roots (tuber + secondary roots). The following vegetative parameters were assessed: shoot fresh weight, shoot dry weight, shoot height, root fresh weight, tuber fresh weight, and tuber length. Shoot height was measured with a millimeter ruler and tuber length was measured using a Pantec® digital caliper. Shoot dry weight was determined after drying in a forced air-oven at 65 °C for 3 days.

Nematodes were extracted from roots and tubers following the method of CHARCHAR et al. (2006), with modifications. Roots and tubers were peeled to a depth of about 3 mm, cut into 1–2 cm pieces, and homogenized with 0.5% hypochlorite solution in a blender for 30 s at high speed. The suspension was sieved through 60- (0.250 mm) and 500-mesh (0.025 mm) sieves. The material retained in the bottom sieve was washed with water to obtain the nematode extract. Eggs and J2 were counted using a Peters counting chamber under a light microscope (BA210 Binocular, Motic, Hong Kong, China). The total number of nematodes was divided by the root fresh weight to obtain the nematode population density (nematodes g^{-1} root). The reproduction factor (RF) was calculated as the ratio of total population to inoculum level (OOSTENBRINK, 1966).

Moisture, ash, and protein contents were determined according to AOAC methods 925.09, 923.03, and 920.87, respectively (HORWITZ & LATIMER, 2005). Moisture content was determined by oven-drying (SL-102, Solab, Piracicaba, Brazil) samples at 105 °C to constant weight. Ash content was determined by burning samples at 550 °C in a muffle furnace (Q318M, Quimis, Diadema, Brazil). Proteins were quantified by the Kjeldahl method (HORWITZ & LATIMER, 2005).

Carotenoids were extracted by maceration of 3 g of freshly harvested carrots with 30 mL of acetone. The mixture was filtered, and the liquid fraction was transferred to a separatory funnel

containing 60 mL of petroleum ether. The organic phase was washed three times with 300 mL of distilled water to remove the acetone and used as carotenoid extract. Absorbance was read spectrophotometrically (700Plus, Femto, São Paulo, Brazil) at 453 nm. Carotenoid content (C, μg g^{-1}) was calculated as follows: $C = A \times V \times 10^4 \div E \times W$, where A is the absorbance, V is the total volume of carotenoid extract, E is the extinction coefficient of β-carotene in petroleum ether, and W is the weight of sample used to prepare the extract (RODRIGUEZ et al., 1976).

Antioxidant compounds were extracted according to RAVICHANDRAN et al. (2013), with minor modifications. Fresh samples were dissolved in 20 mL of 50% ethanol, stirred for 4 h on an orbital shaker, and centrifuged (MTD III plus, Metroterm, Porto Alegre, Brazil) at 3000 rpm for 10 min. The supernatant was collected, and the extraction process was repeated twice more with 5 mL of 50% ethanol. The ethanolic extract was used for measuring total phenolic content (TPC) and antioxidant activity.

The TPC was determined by the Folin–Ciocalteu method, according to CHEN et al. (2015). Absorbance was read spectrophotometrically at 765 nm (700Plus, Femto). A standard curve of gallic acid was constructed, and the results are expressed in mg gallic acid equivalents (GAE) g^{-1} sample.

Antioxidant activity was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and the ferric reducing antioxidant potential (FRAP) assays. Briefly, the DPPH assay was performed by mixing 3.80 mL of DPPH solution (0.011 g mL^{-1}) with 0.20 mL of ethanolic extract, incubating the solution for 30 min at room temperature, and measuring the absorbance at 515 nm (700Plus spectrophotometer, Femto) (RAVICHANDRAN et al., 2012). For the FRAP assay, an aliquot of ethanolic extract was mixed with water and a solution containing 300 mM acetate buffer (pH 3.6), 20 mM ferric chloride, 10 mM 2,4,6-tri(2-pyridyl)-*s*-triazine (TPZT), and 40 mM HCL. After 30 min of incubation at 37 °C, the absorbance was read at 593 nm (HIRANVARACHAT & DEVAHASTIN, 2014). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was used as standard. DPPH and FRAP results are expressed in μmol Trolox g^{-1} sample.
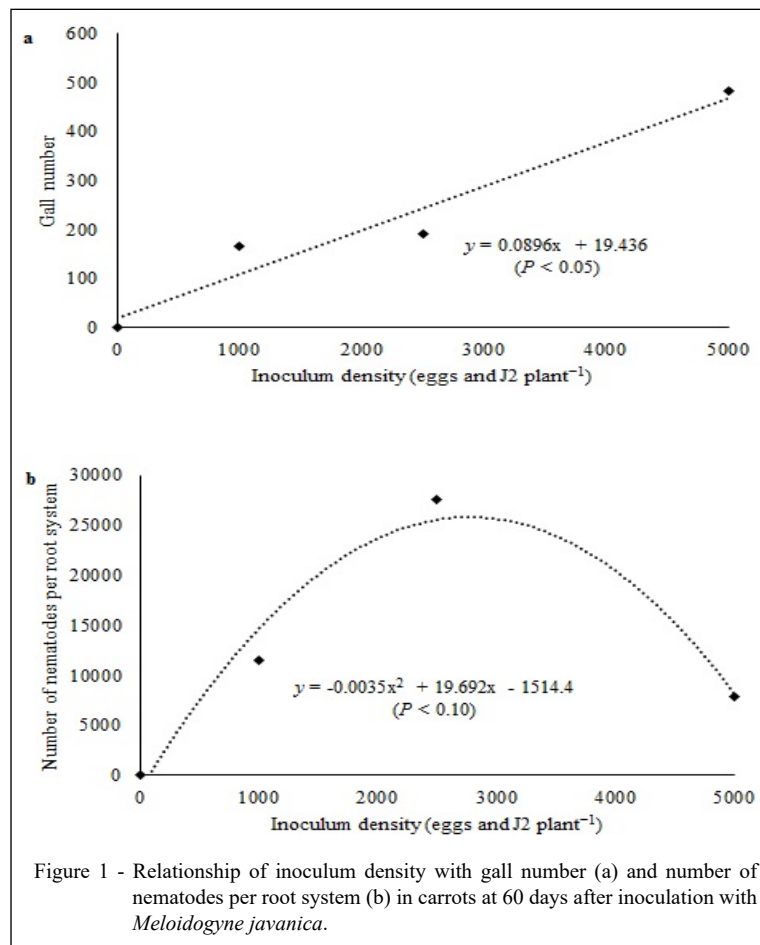
Data were subjected to analysis of variance and regression analysis at the 5% and 10% significance levels using the Sisvar software version 5.6. Nematode and vegetative growth data were transformed to $\sqrt{x + 1}$ to meet normality assumptions based on the Shapiro–Wilk test. Pearson correlation analysis was performed at the 5% significance level using Statistica version 8.

RESULTS AND DISCUSSION

Gall number increased with increasing inoculum density (Figure 1a), as also observed in parsley and spring onion exposed to 0, 760, and 5700 *M. incognita* eggs + J2 (WALKER, 2002) and cucumber inoculated with *M. incognita* (KAYANI et al., 2017). The number of nematodes in the roots increased with inoculum density up to 2500 nematodes plant^{-1} but decreased thereafter (Figure 1b). Carrot plants were susceptible to all inoculum densities (RF > 1), but multiplication was affected by high initial population densities. At an inoculum density of 1000 nematodes plant^{-1}, the RF was 11.47, whereas at 5000 nematodes plant^{-1}, the RF was reduced to 1.57 (Figure 1b). This result is likely due to competition between nematodes, as previously observed in other plant pathosystems (HUSSAIN et al., 2016). No differences ($P > 0.10$) were observed between treatments in nematode density, ranging from 1133 to 1726 nematodes g^{-1} root.

Shoot dry weight (2.21–2.85 g) and tuber length (10.97–12.15 cm) were not affected by *M. javanica* inoculum levels. Conversely, shoot height, shoot fresh weight, root fresh weight, and tuber fresh weight increased ($P < 0.05$) up to inoculum densities of 4000, 2700, 2125, and 2250 nematodes plant^{-1}, respectively (Figure 2). Although, nematodes stunt the growth of the root system by inducing necrotic lesions and galls (AFSHAR et al., 2014; HUNT & HANDOO, 2009), root and tuber weight can increase with gall formation (KAYANI et al., 2017). Galls form from the hypertrophy and hyperplasia of vascular parenchyma in root cells affected by secretions of the dorsal esophageal glands of nematodes. Root galls hamper the formation of secondary roots, reduce water and nutrient absorption, and impair plant growth (ESCOBAR et al., 2015). It is possible that the 60-day experimental period was not long enough for the effects of *M. javanica* parasitism on shoot development to become evident. Moreover, plants may still develop when exposed to small nematode populations, as new roots can emerge (OLTHOF & POTTER, 1977). This could explain the high root fresh weight and tuber fresh weight of plants inoculated with up to 2125 and 2250 nematodes, respectively, compared with plants inoculated with higher nematode densities (Figure 2c and 2d).

Moisture and ash contents did not differ significantly between treatments, ranging from 852.36 to 853.86 g kg^{-1} and from 11.40 to 11.86 g kg^{-1} in T1 and T4, respectively. Protein levels increased with inoculum densities greater than 1000 nematodes plant^{-1} (Figure 3a). This result can be attributed to the plant's response to stress caused by nematode

Figure 1 - Relationship of inoculum density with gall number (a) and number of nematodes per root system (b) in carrots at 60 days after inoculation with *Meloidogyne javanica*.
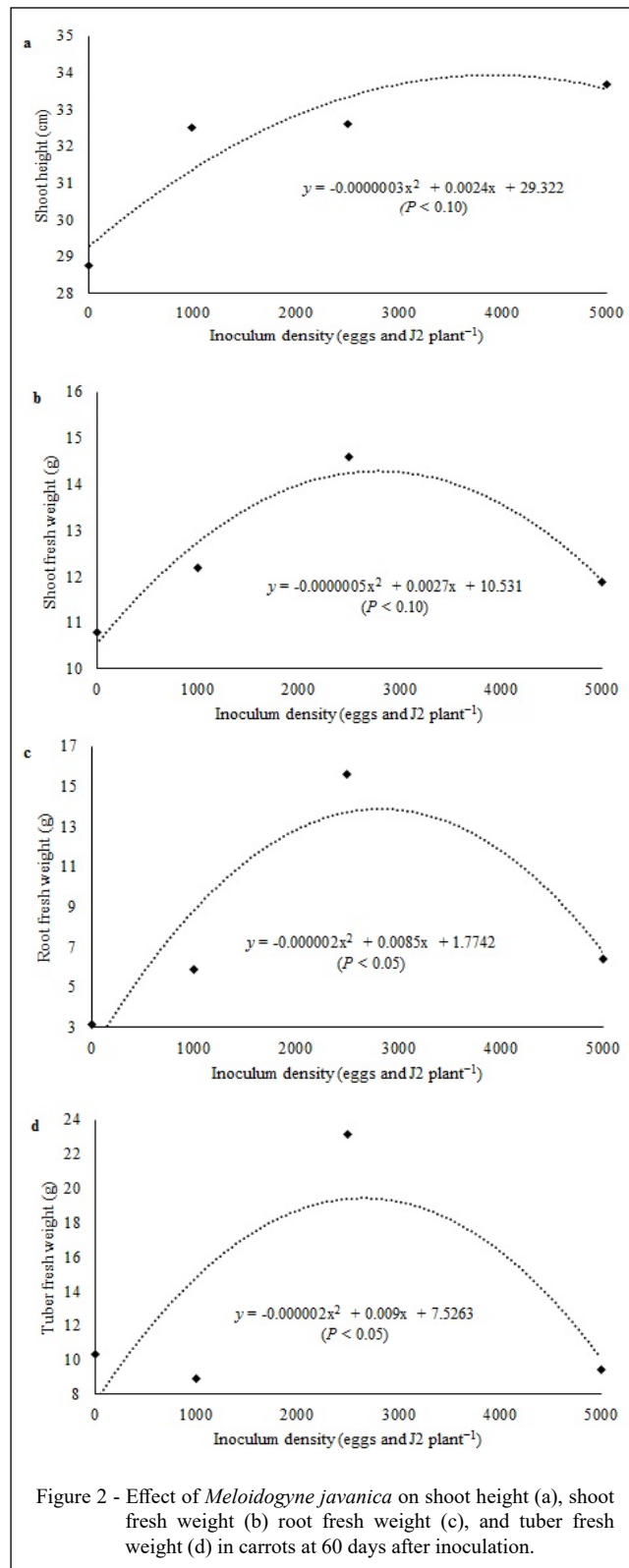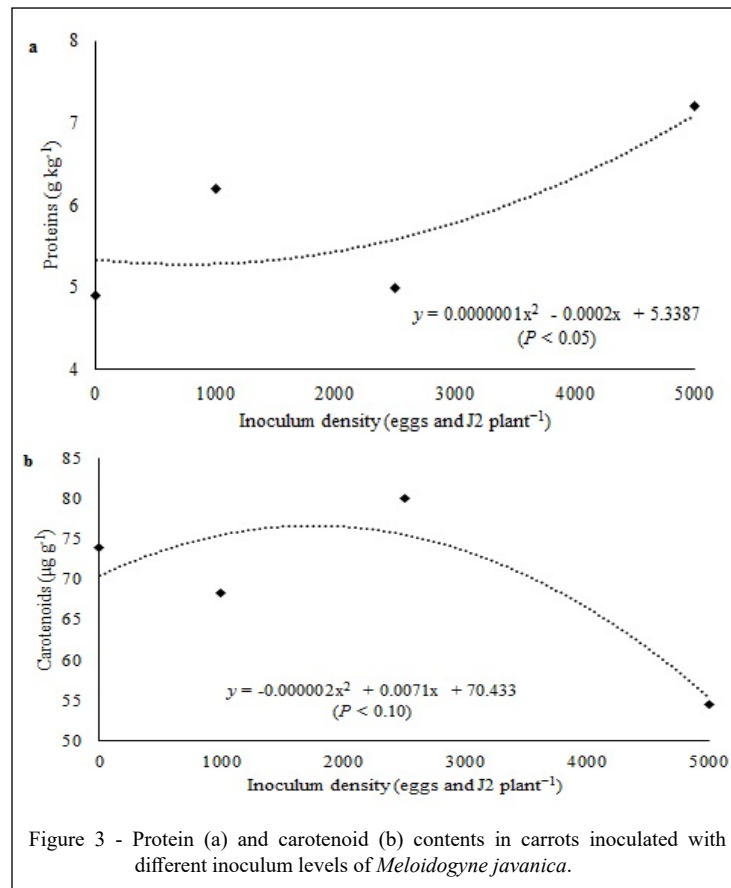
infection (ROCHA et al., 2015). Plants have a wide array of mechanisms to respond to biotic and abiotic stresses (ATKINSON et al., 2011), including metabolic activation, phytoalexin biosynthesis, phenolic compound accumulation, and increase in peroxidase, catalase, phenylalanine ammonia-lyase, and β-1,3-glucanase activities (DIAS-ARIEIRA et al., 2013). Thus; although the current study assessed the level of total proteins rather than that of pathogenesis-related proteins, it is likely that protein-related defense mechanisms were activated by high inoculum densities. A previous study showed that nematodes parasitism can alter the protein profile of plants (GHEYSEN & FENOLL, 2002). These effects should be further studied.

Carotenoid content increased up to inoculum densities of 1775 nematodes plant[-1], decreasing thereafter (Figure 3b). A similar effect was observed in mung bean (*Vigna radiata*) inoculated with 2000 *M. javanica* eggs + J2. The reduction in

carotenoid content was attributed to a reduction in leaf area (AHMED et al., 2009). Oxidation is the main cause of carotenoid degradation. The compound is easily oxidized because of its large number of double bonds. Carotenoids are protected from oxidation in intact tissues; however, tissue damage increases susceptibility to oxidation (SAINI et al., 2015). Activation of plant defense responses to nematode infection probably increased the activity of enzymes such as peroxidase (DIAS-ARIEIRA et al., 2013), promoting carotenoid degradation (UENOJO et al., 2007). There was a negative correlation (-0.60, $P < 0.05$) between total carotenoid and protein contents, suggesting that the increase in protein content affected carotenoid accumulation in roots.

The TPC (24.77–27.97 mg GAE g[-1]) and antioxidant activity were not influenced by *M. javanica* inoculum density ($P > 0.10$). DPPH activity was 0.01 μmol Trolox g[-1], regardless of inoculum density. According to the FRAP assay, all samples had

Figure 2 - Effect of *Meloidogyne javanica* on shoot height (a), shoot fresh weight (b) root fresh weight (c), and tuber fresh weight (d) in carrots at 60 days after inoculation.

Figure 3 - Protein (a) and carotenoid (b) contents in carrots inoculated with different inoculum levels of *Meloidogyne javanica*.

an antioxidant activity of 0.12 μmol Trolox g^{-1}, except for T2, which had an activity of 0.14 μmol Trolox g^{-1}. Similar results were observed in tomato plants subjected to water and nematode stress (ATKINSON et al., 2011). Plants accumulate phenolic compounds as protection against nematode infection (NACZK & SHAHIDI, 2004). These compounds are toxic to the parasite and limit the penetration of nematodes and other pathogenic microorganisms (TALCOTT & HOWARD, 1999).

Although, carrot tubers affected by *M. javanica* had presented visible damage and reduction of carotenoids, the TPC and antioxidant activity were not affected, indicating that they could still be used in the food industry. This strategy can reduce food waste. In a study by GULL et al. (2015), for instance, carrot pomace was used in pasta production as a partial, highly nutritional substitute for wheat.

CONCLUSION

Although, carrot tuber length was not negatively influenced by inoculum density, tuber weight significantly decreased with inoculum densities greater than 2250 nematodes $plant^{-1}$. Antioxidant activity, TPC, moisture content, and ash content were not influenced by inoculum density. The decrease in carotenoid content was associated with an increase in protein levels. Root galls caused visible changes to tuber morphology, which negatively affects the vegetable's commercial value. Nevertheless, affected tubers may find application in the food processing industry, as they still have high nutritional quality.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the

collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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