Influence of *Meloidogyne javanica* parasitism on soybean development and chemical composition

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Abstract

Background: Soybean is a major economic crop worldwide, but its yield and quality are greatly affected by root-knot nematode infection. This study aimed to assess the effects of *Meloidogyne javanica* parasitism on soybean growth, yield, and quality. Soybean plants were inoculated with 0, 1000, 2000, or 4000 eggs + second-stage juveniles (J2) of *M. javanica* and evaluated for vegetative and nematode parameters. Grains from each treatment were analyzed for yield, proximate composition, total phenolic content, total flavonoid content, isoflavone profile, and antioxidant capacity.

Results: Plants free of nematodes had lower vegetative growth and higher yield than nematode-infected plants. The maximum estimated reproduction factor was 34.85, achieved by inoculation of 2433 eggs + J2. Moisture, fiber, and protein contents decreased with increasing inoculum levels. Lipid content increased with inoculum level until reaching 22.59 g 100 g⁻¹ of sample. Total phenolic and flavonoid contents decreased with increasing inoculum level up to about 2000 eggs + J2 and then increased until reaching 219.20 mg gallic acid equivalents 100 g⁻¹ of sample and 121.67 mg quercetin equivalent 100 g⁻¹ of sample, respectively, at 4000 eggs + J2. A similar behavior was observed for antioxidant capacity determined by the 2,2-diphenyl-1-picrylhydrazyl radical scavenging, superoxide radical scavenging, and ferric reducing antioxidant power assays. The highest contents of malonylgenistin (539 mmol g⁻¹), acetyl-glycitin (106 mmol g⁻¹), and genistin (87 mmol g⁻¹) were found in grains from plants inoculated with 4000 eggs + J2.

Conclusions: *M. javanica* inoculum level affected soybean development, grain production, yield, composition, and antioxidant capacity.

Keywords: Root-knot nematode, Biotic stress, Oilseed, Antioxidant capacity, Isoflavones

Background

Soybean (*Glycine max* (L.) Merrill) is one of the most important oilseeds and food crops in the world because of its great production potential, chemical composition, and high nutritive value. This legume crop has a multiplicity of applications in human and animal nutrition, fulfilling a relevant socioeconomic role in many countries [39].

Soybean grains contain 40% high-quality proteins, 20% lipids (including a high proportion of polyunsaturated fatty acids), 35% carbohydrates, considerable levels of B vitamins, and 5% minerals (such as magnesium, phosphorus, iron, and zinc) [6]. Grains and derivatives are important sources of phenolic compounds—secondary metabolites synthesized by plants during normal development and in response to stress conditions, including pathogen infection. Phenolics are formed from

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phenylalanine and tyrosine and can be grouped into different classes, such as simple phenols, phenolic acids, flavonoids, coumarins, stilbenes, condensed and hydrolyzable tannins, and isoflavones [28]. Isoflavones have attracted great interest from researchers, health agencies, and the general population for their diverse biological properties, including antioxidant, radical scavenging, antifungal, estrogenic, and anticancer activities [43, 44].

Root-knot nematodes, such as Meloidogyne javanica (Treub) Chitwood and M. incognita (Kofoid & White) Chitwood, are major limiting factors to soybean yield [40]. During feeding, the parasites inject esophageal secretions into plant tissues, causing hypertrophy and hyperplasia of xylem or cortical cells in 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 [20]. Infected plants also show chlorotic spots on leaves and necrosis between leaf veins [10].

In the literature, there are several studies on the effects of nematode infection on plant physiology; however, little is known about the influence of parasitism on the composition and antioxidant potential of grains. Such information can contribute to our understanding of the physiological behavior of nematode-infected plants during reserve mobilization and grain filling. This study aimed to investigate the effect of different levels of M. javanica inoculation on vegetative growth, nematode parameters, yield, chemical composition, and antioxidant capacity in soybean.

**Methods**

**Soybean cultivation**

The experiment was carried out in a greenhouse with the coordinates 23°45′59″ S 53°19′30″ W, 442 m elevation from November 2017 to March 2018 at 19 to 32 °C. It was used a completely randomized design with four treatments (inoculum levels of 0, 1000, 2000, and 4000 M. javanica eggs + eventual second-stage juveniles, J2). A total of 20 replications per treatment were cultivated (10 plants for evaluation of nematode and vegetative parameters and 10 plants for assessment of grain yield, chemical composition, and antioxidant capacity).

Soybean 'Pintado' seeds were sown in plastic pots containing 600 mL of a 2:1 mixture of soil and sand previously autoclaved at 120 °C for 2 h. Three days before sowing, the soil was fertilized with 1 g of granular 16-16-16 NPK (nitrogen/phosphorus/potassium) per pot. Sowing was carried out in the greenhouse by placing the seeds (two per pot) in holes in the soil. Plants were thinned to one per pot soon after emergence. Each plant was treated as an experimental unit.

Plants were inoculated with the respective inoculum levels at 7 days after sowing by addition of 4 mL of nematode suspension into four equidistant holes (about 3 cm deep) in the soil around the plant. Nematodes were obtained from a single-species population maintained on tomato plants. For extraction, tomato roots were collected with a trowel, ground in 0.05% (v/v) sodium hypochlorite solution, and sieved through a 500 mesh sieve [8, 21]. Inoculum levels were determined by counting nematodes in a Peters chamber under an optical microscope. During the experimental period, plants were irrigated two to three times a day, as required.

**Evaluation of vegetative and nematological parameters**

At 60 days after inoculation, half of the plants (10 replications per treatment) were evaluated for vegetative and nematode parameters. Plants were harvested, and shoots were separated from roots. Shoot height was determined using a millimeter ruler. Shoot fresh and dry weights were assessed by gravimetry using a semi-analytical scale. For dry weight determination, shoots were dried in a forced air oven at 65 °C for 3 days.

Roots were carefully washed to remove excess soil, and the root fresh weight was determined using a semi-analytical balance. Subsequently, roots were subjected to nematode extraction using a blender and sodium hypochlorite solution, as proposed by Hussey and Barker (1973) and adapted by Boneti and Ferraz (1981). Total nematode number was determined by counting in a Peters chamber under an optical microscope. The number of nematodes per gram of root was calculated as the ratio between total nematode number and root fresh weight. The reproduction factor (RF) was determined as the quotient of final and initial nematode populations [31].

**Determination of grain yield parameters and proximate composition**

Ten plants per treatment were grown under greenhouse conditions until the end of the crop cycle (120 days). Then, grains were harvested and analyzed for number of pods per plant and thousand grain weight.

Harvested grains were ground in a rotary mill, homogenized, and analyzed in triplicate for moisture, ash, protein, lipid, and fiber contents. Results are expressed as g 100 g⁻¹ sample (dry basis—d.b.). Moisture content was assessed by oven drying at 105 °C to constant weight, and ash content was determined by calcination at 550 °C in a muffle oven. Protein content was determined by digestion with sulfuric acid (98% v/v), followed by distillation with sodium hydroxide (50% v/v) and titration with HCl (0.1 M), according to the Kjeldahl method. Lipid contents
were evaluated by Soxhlet extraction using petroleum ether. Fiber content was determined by the acid detergent digestion method [2].

**Analysis of total phenolic content, total flavonoids, and antioxidant capacity**

Soybean grains were harvested at the end of the crop cycle (120 days), and extractions were obtained by mixing 1 g of ground sample with 10 mL of 80% (v/v) ethanol. The mixture was incubated on a shaker at 25 °C and 150 rpm for 6 h, centrifuged at 3000 rpm for 10 min, and the supernatant was collected. The residue was washed with 10 mL of 80% ethanol and centrifuged. The supernatants were used as crude extract for analysis.

The total phenolic content of the extract was quantified by the Folin–Ciocalteu method [41] using a standard curve of gallic acid. Absorbance was measured at 760 nm on a spectrophotometer (Femto 700 Plus). Results were expressed as mg gallic acid equivalents (GAE) 100 g⁻¹ sample.

Total flavonoids were quantified using 0.5 mL of extract and 150 µL of 50 g L⁻¹ NaNO₂. The mixture was incubated in a water bath at 50 °C for 5 min and received the addition of 15 µL of AlCl₃. After 5 min, 1.5 mL of 1 M NaOH was added together with 1.5 mL of deionized water. Absorbance was read at 415 nm using a spectrophotometer. Total flavonoid content was calculated on the basis of a standard quercetin curve and expressed as mg quercetin equivalents (QE) 100 g⁻¹ sample [7].

Antioxidant activity was evaluated by three methods. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined using an aliquot (0.1 mL) of each extract and 3.9 mL of DPPH ethanol solution. The absorbance was read at 517 nm using a spectrophotometer [9]. Ferric reducing antioxidant power (FRAP) assay was carried out using 90 µL of extract, 270 µL of distilled water, and 2.7 mL of FRAP reagent. After incubation at 37 °C for 30 min, the absorbance was read at 595 nm [5]. Calibration curves for DPPH and FRAP assays were prepared with Trolox solution, and the results were expressed as µmol Trolox equivalents (TE) g⁻¹ sample.

Superoxide radical (O₂⁻) scavenging (SRS) activity was assessed by reacting each extract (0.5 mL) with 4 mL of 50 mM Tris–HCl (pH 8.2) and 0.5 mL of 25 mM pyrogallol for 5 min at 25 °C. Then, 1 mL of 8 mM HCl was added, and the absorbance was read at 420 nm. The scavenging percentage was calculated as follows: scavenging percentage = [A₀ (A₁ − A₀)]/A₀ × 100, where A₀ is the absorbance of the control (prepared by adding Tris–HCl instead of sample), A₁ is the absorbance of the sample, and A₀ is the absorbance of the sample with pyrogallol [24].

**Isoflavone profile determination**

Isoflavone extraction was carried out in triplicate using crushed and defatted grains and a 1:1:1 (v/v/v) mixture of ultra-pure water, ethanol, and acetone as solvent [18]. Separation and quantification of isoflavones were performed by ultra-performance liquid chromatography (UPLC). The UPLC apparatus (Waters Corporation, Milford, MA, USA) was composed of an automatic injector (Acquity UPLC®System, Waters Corporation), a BEH reverse-phase C18 column (2.1 mm × 50 mm, 1.7 µm, Waters Corporation), and a diode array detector (Waters Corporation). The injection volume was 1.4 µL, the flow rate 0.3 mL min⁻¹, and the column temperature 27 °C. The binary mobile phase consisted of 0.4% formic acid (A) and acetonitrile (B), and the elution gradient was as follows: 0 min, 95% A; 8.5 min, 20% A. Compounds were detected at 260 nm. Retention times and UV spectra were compared with those of standards. Calibration curves were prepared for quantification purposes. Results were expressed as mmol g⁻¹ sample [15].

**Statistical analysis**

Data were subjected to analysis of variance (ANOVA). To meet normality assumptions, as assessed by the Shapiro–Wilk test, we transformed nematode data using √x + 1. Significant parameters were subjected to regression analysis at p < 0.05, except protein, lipid, and fiber contents, which were analyzed at p < 0.10. Analyses were performed using Sisvar [16].

**Results**

**Vegetative and nematological parameters**

Uninoculated plants had lower shoot height (95 cm) than plants inoculated with *M. javanica* (p < 0.05) (Fig. 1). In inoculated plants, shoot height increased with increasing inoculum levels up to 2450 eggs + J₂ and then decreased slightly (Fig. 1a). Shoot fresh and dry weights showed similar behavior, with peaks of 51.83 g for fresh weight (Fig. 1b) and 13.91 g for dry weight (Fig. 1c) at 2583 and 2666 eggs + J₂, respectively (p < 0.05).

Root fresh weight showed no statistical difference between treatments (p < 0.05); mean values ranged from 32.39 g in control plants to 33.21 g in plants inoculated with 4000 eggs + J₂ (data not shown).

Nematode quantification (Fig. 2) revealed that the highest total number of nematodes (73,765) is obtained with an initial population of about 3500 nematodes (Fig. 2a) (p < 0.05). The maximum number of nematodes per gram of root (2830) was estimated to be achieved with an initial population of 3642 eggs + J₂ (Fig. 2b).
content was higher than that in the uninoculated control (p < 0.05).

The lowest DPPH (123 µmol TE g⁻¹, Fig. 5c) and FRAP (7.72 µmol TE g⁻¹, Fig. 5d) activities were detected in soybean grains from plants inoculated with 2000 eggs + J2 (p < 0.05). By the SRS method, the minimum value was 25.47% in grains of plants inoculated with 900 eggs + J2 (Fig. 5e). Similar to the behavior observed for total flavonoids (Fig. 5b), DPPH and SRS activities increased after reaching the minimum value; the highest antioxidant activity (140.15 µmol TE g⁻¹ for DPPH and 32.76% for SRS) was obtained by inoculation of 4000 eggs + J2.

**Isoflavone profile**

Of the 12 most well-known soybean isoflavones, 11 were identified in this study (Table 1). There was a significant difference in malonylgenistin (p < 0.05), acetyl-glycitin (p < 0.05), and genistin (p < 0.05) levels between treatments. The highest content of these isoflavones was observed at an initial population of 4000 eggs + J2. Total isoflavone content did not differ between treatments (2.43 to 2.72 mmol g⁻¹). The following isoflavone profile was observed: 58% malonyl-β-glucosides, 20% β-glucosides, 13% aglycones, and 9% acetyl-β-glucosides. The only undetected form was acetyl-β-genistin.

**Discussion**

Vegetative growth was highest in plants inoculated with low levels of *M. javanica*. This compensatory response of plants to nematode infection can be seen as a form of acquired defense. Plants activate defense responses when attacked by herbivores or pathogens, altering gene expression and metabolism [42]. However, at very high nematode levels, roots become more damaged and are unable to supply sufficient amounts of water and nutrients, affecting plant growth [14].

The results of root fresh weight can be explained by nematode-induced formation of root galls. It should be noted that root development in infected soybean plants varies according to nematode aggressiveness and the efficiency of plant responses to such infections [1].

Nematode population growth as a function of initial population followed the expected patterns: small inoculum levels resulted in exponential growth, whereas large inoculum levels affected nematode multiplication, probably because of nutrient competition and other environmental limitations [36].

The reduction in yield, a reflection of the reduction in pod number, was also expected, as pod number depends on the number of flowers produced during the reproductive period. One of the symptoms shown by
nematode-infested plants is an intense abortion of flowers and pods [27]. A similar greenhouse study assessing the effects of *Pratylenchus brachyurus* inoculum level (up to 2000 individuals) on soybean found that yield and pod number decreased by about 24% in inoculated plants [11].

The mean thousand grain weight of uninoculated plants (about 133 g) was similar to that of conventional soybean cultivated in different regions and at different sowing times (137–180 g) [12, 37]. Reduction in grain weight in nematode-infested plants can be attributed to photosynthesis impairment caused by root damage [30].

The protein content of soybean grains from inoculated and uninoculated plants agrees with values (31.70–51.90 g 100 g$^{-1}$) reported by the Germplasm Bank of the Brazilian Agricultural Research Corporation [35]. However, we observed that protein content decreased with increasing inoculum levels. It is known that protein synthesis in plants is a complex process that occurs at several stages and locations. Variations in environmental conditions throughout plant development can decrease protein levels by interrupting protein synthesis and favoring that of other compounds [29]. Protein synthesis can be reduced by up to 20% in plants subjected to water stress at the beginning of the reproductive phase [25].

The lipid contents observed in this study (21.13–22.59 g 100 g$^{-1}$) were similar to those found in conventional and transgenic soybean grains (8.00–25.40 g 100 g$^{-1}$) [22] and in grains from plants infected by *P. brachyurus* (20.91–22.53 g 100 g$^{-1}$) [11]. Lipids not only contribute to cell structure and energy reserves, but also serve as precursors to intracellular or long-distance signaling compounds, which are crucial for the activation of plant defense systems against adverse abiotic conditions [38]. An important factor to be considered is that certain conditions may favor lipid synthesis in detriment to that of proteins, as these biosynthetic processes compete for carbon chains [34]. Soil liming was shown to increase lipid but decrease protein content in soybean grains proportionally to the increase in soil K$_2$O [46].

**Fig. 1** Vegetative parameters of soybean plants relative to initial population of *Meloidogyne javanica* after 60 days inoculation. **a** Shoot height (cm); **b** fresh weight of shoot (g); **c** dry weight of shoot (g). Significant at 5% probability.
Fig. 2  Nematological parameters of *Meloidogyne javanica* in soybean relative to initial population after 60 days inoculation—

**a** total number of nematodes;  
**b** nematodes per gram of root;  
**c** reproduction factor. Significant at 5% probability

Fig. 3  Productivity parameters to initial population of *Meloidogyne javanica* after 120 days of inoculation—

**a** number of pods plant$^{-1}$;  
**b** thousand grain weight (g). Significant at 5% probability
Soybean grain fiber content decreased with increasing *M. javanica* inoculum levels, probably because of the reduction in grain size. Soybean fibers are concentrated mainly in grain hulls and are synthesized during grain filling. Insufficient nutrient accumulation resulting from nematode infection can reduce grain size and, consequently, fiber content. Dietary fibers are of great nutritional importance and have received much attention in the last three decades [26].

The reduction in total phenolics and flavonoids with increasing population levels up to 2000 eggs + J2 is suggestive of phytochemical translocation to other plant organs, such as leaves, stems, and roots, where the demand for antioxidants is likely to be greater. This conclusion is based on the hypothesis that active compounds are concentrated where most needed during plant defense responses to maximize adaptation to the environment [3]. However, the stress caused by abrupt or intense biotic or abiotic stimuli can change the natural functioning of organisms, leading to diverse responses, such as acclimatization and genetic modifications [32]. High inoculum levels might have subjected plants to intense stress, altering biosynthesis and translocation of phenolic compounds to grains, as observed in plants inoculated with more than 3000 eggs + J2.

There was a direct relationship of antioxidant capacity, particularly FRAP activity, with total phenolics and flavonoids (Fig. 5). This relationship is commonly observed in fruits and vegetables [23]. Higher levels of total phenolics and flavonoids indicate a higher capacity to scavenge free radicals (DPPH), reduce metal ions (FRAP), and sequester superoxide anions (O$_2^-$).

The isoflavone profile of soybean grains (Table 1) showed that synthesis of certain types of isoflavones may vary according to nematode inoculum level. Nevertheless, total isoflavone content was not affected. Total isoflavone levels ranged from 0.50 to 1.20 mg g$^{-1}$, in agreement with literature data (0.10–5 mg g$^{-1}$) [45]. Isoflavone distribution seemed to be related to the biosynthesis order of compounds. Malonyl-CoA is an
intermediate in the formation of chalcones, which are isomerized to originate flavanones (β-glucoside structures). These structures are converted into aglycones by the action of β-glucosidase [47]. A predominance of malonyl forms and β-glucosides has been observed in previous studies, with malonyl-β-glucosides accounting for 70 to 80% of soybean isoflavones and β-glucosides for 25% [4, 13].

It is interesting to note that aglycone forms are the most suitable for human consumption because of their high...
bioavailability and activity. Isoflavones can be converted by heat or enzymatic hydrolysis. Heat conversion, which normally occurs during product processing, increases the bioactivity of isoflavones by enhancing liposolubility and reducing molecular weight, thereby contributing to their absorption through the intestinal wall [17, 33]. As thermal treatment was not used in this study, isoflavone conversion may have occurred in the plant via enzymatic hydrolysis. β-Glucosidases convert glucoside forms into aglycones and hydrolyze preformed isoflavones (malonyl-glucosyl-daizzein and malonyl-glucosyl-genistein), releasing precursor molecules, such as glyceollin and coumestrol, in response to physical, chemical, and biological stresses [48]. Although no significant differences were found between treatments, we observed a relationship between aglycone structures and nematode population levels, suggesting the occurrence of isoflavone conversion in response to biotic stress. Analysis of glyceollin levels in soybean extracts could contribute to testing this hypothesis.

Flavonoids, such as isoflavones, are secondary metabolites with the ability to scavenge free radicals; they are produced in situations of metabolic imbalance or stress [19]. Given that aglycone isoflavones are highly bioavailable, grains of soybean plants inoculated with nematodes may serve as sources of aglycones for human consumption.

**Conclusions**

Soybean plants were susceptible to *M. javanica* at the evaluated inoculum levels. Vegetative parameters were negatively affected by the highest population levels, whereas yield decreased at all inoculation levels. Grain moisture, fiber, and protein contents decreased and lipid content increased with increasing inoculum level. Total phenolic and flavonoid contents were lower in grains from plants inoculated with low nematode levels (up to about 2000 eggs + J2) and higher in grains from plants inoculated with high nematode levels. A positive correlation was observed between antioxidant capacity and total phenolic and flavonoid contents. Nematode infection altered the isoflavone profile of soybean grains. Further studies are needed to understand the influence of nematodes on isoflavone accumulation in soybean.

**Abbreviations**

J2: Second-stage juvenile; GAE: Gallic acid equivalents; QE: Quercetin equivalents; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant power; TE: Trolox equivalents; ANOVA: Analysis of variance; SRS: Superoxide radical scavenging.

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**Authors’ contributions**

All authors contributed equally to the research and agree with the submission of the manuscript to the journal. All authors read and approved the final manuscript.

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**Availability of data and materials**

Additional data are available on request from the corresponding author.

**Ethics approval and consent to participate**

Ethical approval and consent to participate were not required for this research, as it did not involve human or non-human animals.

**Consent for publication**

Not applicable.

### Table 1 Isoflavone profile (mmol g⁻¹) from soybean infected with *M. javanica*

| Isoflavone | Initial population (eggs + J2) | CV (%) | R² |
|------------|-------------------------------|--------|----|
|            | 0    | 1000 | 2000 | 4000 |
| Mal-Daidzin | 664  | 689  | 651  | 701  | 14.29 | ns |
| Mal-Glicitin | 339  | 353  | 353  | 354  | 13.57 | ns |
| Mal-Genistin | 485b | 521b | 396b | 539a | 6.85  | 0.36 |
| Acet-Daidzin | 131  | 147  | 118  | 151  | 9.79  | ns |
| Acet-Glicitin | 94b  | 99b  | 82b  | 106a | 9.16  | 0.55 |
| Daidzin     | 197  | 206  | 176  | 207  | 16.07 | ns |
| Genistin    | 65b  | 76b  | 66b  | 87a  | 8.29  | 0.26 |
| Glicitin    | 217  | 215  | 242  | 218  | 8.70  | ns |
| Daidzein    | 171  | 163  | 183  | 185  | 14.81 | ns |
| Genistein   | 71   | 79   | 79   | 84   | 12.18 | ns |
| Glicitein   | 86   | 82   | 82   | 87   | 18.78 | ns |
| Total       | 2520 | 2640 | 2428 | 2719 | 10.29 | ns |

*ns not significant at 5% probability. Different letters on the same line indicate statistical difference at 5% probability. Mal malonyl-β-glycoside, Acet acetyl-β-glycoside*
Competing interests
The authors have no conflict of interest to declare.

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