RESEARCH/INVESTIGACIÓN

IRON NANOPARTICLE AND BIOLOGICAL NEMATICIDE AGENT FOR THE MANAGEMENT OF *MELOIDOGYNE JAVANICA* IN SOYBEAN

T. Benedetti[1,2]*, E. Sordi[2,3], Z. I. Antoniolli[4], J. R. P. Santos[4], and E. C. Bortoluzzi[3]

[1]Department of Horticulture, Oregon State University, Corvallis, OR, 97330, USA; [2]Graduate Program in Agronomy, University of Passo Fundo, Passo Fundo, Brazil; [3]Land Use and Natural Resources Laboratory, University of Passo Fundo, Passo Fundo, Brazil; [4]Federal University of Santa Maria, Santa Maria, Brazil; *Corresponding author: tatibenedetti@outlook.com

ABSTRACT

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The root-knot nematodes, *Meloidogyne* spp., have a wide geographical distribution and are among the most aggressive and economically important genera of nematodes worldwide. The phase out of some chemical nematicides makes it necessary to search for new tools to manage these plant-parasitic nematodes. The use of biological agents (BA) has received attention for nematode management, but some characteristics limit the efficacy of these agents. Nanoparticles (NP), due to their specific properties, represent a tool to mitigate the limitations of BA and have also shown potential in the management of plant diseases. Therefore, the objective of this study was to verify the efficiency of iron NP co-inoculation with a BA for the management of *Meloidogyne javanica* in soybean. The application of iron NP at 75 mg/kg + BA at 1 ml/kg resulted in an 88% reduction in the density of *M. javanica* females in roots. The mode of action responsible for the mortality of *M. javanica* appeared to be linked to the generation of reactive oxygen species. Co-inoculation of iron NP and BA has the potential as a *M. javanica* management strategy in soybean.

Key words: Biological control, nanotechnology, plant-parasitic nematodes, radical oxygen species, root-knot nematodes

RESUMO

Benedetti, T., E. Sordi, Z. I. Antoniolli, J. R. P. Santos, and E. C. Bortoluzzi. 2023. Nanopartículas de ferro e agente nematicida biológico no manejo da *Meloidogyne javanica* em soja. Nematropica 53:89-103.

O nematoide das galhas das raízes, *Meloidogyne* spp., apresenta ampla distribuição geográfica e está entre os nematoides mais agressivos e economicamente importantes do mundo. A proibição de uso de alguns nematicidas químicos importantes tornou necessário a busca por novas ferramentas de controle deste patógeno. A utilização de agentes biológicos (BA) tem recebido atenção no manejo do nematoide porém algumas características limitam a eficiência destes produtos. Devido a propriedades específicas as nanopartículas (NP) apresentam-se como uma ferramenta interessante para mitigar as limitações dos BA além de apresentarem potencial para o manejo de doenças de plantas. Portanto, o objetivo deste estudo foi avaliar a eficiência da co-inoculação de NP de ferro a um agente biológico nematicida no manejo de *Meloidogyne javanica* em soja. A aplicação de 75 mg/kg de NP de ferro + 1 ml/kg de BA resultou em uma

redução de 88% na densidade de fêmeas de *M. javanica* no sistema radicular da soja. O modo de ação que causa a mortalidade da *M. javanica* parece estar ligado à geração de espécies reativas de oxigênio. A co-inoculação do NP de ferro ao BA demonstra um bom potencial para uso na estratégia de manejo do *M. javanica* em soja.

Palavras-chave: Controle biológico, nanotecnologia, nematoides parasitas de plantas, radicais livres de oxigênio, nematoides das galhas das raízes

INTRODUCTION

Plant-parasitic nematodes (PPN) are among the most damaging plant pathogens and are difficult to manage worldwide (Trudgill and Blok, 2001; Wesemael *et al*., 2011). Within the PPN, the root-knot nematodes, *Meloidogyne* spp., are included in the most important genera with more than 100 species described worldwide (Jones *et al*., 2013). Among them, *M. incognita* and *M. javanica* stand out as the most cosmopolitan, polyphagous, and harmful to agriculture and are considered the most economically important in the world (Brito *et al*., 2008). In Brazil, *Meloidogyne* spp. are widely distributed due to the ideal climatic conditions for feeding and reproduction (Ferraz and Brown, 2016). Feeding by *Meloidogyne* spp. results in a reduction in the translocation of nutrients and water in a plant, generating plant physiological disturbances (Carneiro *et al*., 2002).

Different tools have been used for the control of *Meloidogyne* spp. Biological agents (BA), such as *Bacillus* spp., have gained attention in recent decades due to the need for molecules less harmful to the environment and humans (Alvarado-Herrejón *et al*., 2019; Mazzuchelli *et al*., 2020; Moazezikho *et al*., 2020). In recent years, Brazil has experienced an extraordinary expansion of the biological control market for nematode management. Biological nematicides have overtaken the market for chemical nematicides accounting for 44% of nematicide sales in the country (Bettiol and Medeiros, 2023). During the 2021-2022 season, biological nematicides comprised 94% of the market of nematicides sold for use in soybean. It is worth noting that in most field trials, BA based on bacteria, fungi, or a combination of both, outperformed chemical nematicide in reducing densities of most PPN (Bettiol and Medeiros, 2023). Currently, 96 products based on *Bacillus* spp. are registered as bionematicides in Brazil (EMBRAPA, 2023). Rhizobacteria in the genus *Bacillus* reduced galling caused by *Meloidogyne* spp. (Benedetti *et al*., 2021). *Bacillus* spp. are considered a good option for biocontrol (Engelbrecht *et al*., 2018) due to attributes such as the production of antibiotics, enzymes, exotoxins, and metabolites that contribute to their potential as bionematicides (Abbasi *et al*., 2014). Xiang *et al*. (2017) demonstrated that *Bacillus* was the bacterial genus that resulted in the most *M. incognita* second-stage juvenile (J2) mortality compared to other bacterial genera. *Bacillus subtillis* was responsible for the greatest reductions in *M. javanica* infection in eggplants (Abassi *et al*., 2014). However, limitations, specifically the slow speed of action and variability of results under varying environmental conditions, including geography and climate, create uncertainty about the effectiveness of BA (Engelbrecht *et al*., 2018). Narrow host specificity can also limit the potential of BA (Mnif and Ghribi, 2015), making them less attractive than broad-spectrum nematicides. Nanomaterials have been reported as a tool to reduce the limitations of pesticides, such as the short residual period (Ma *et al*., 2022) and the slow speed of action of BA (Engelbrecht *et al*., 2018).

Nanoparticles (NP) are molecular aggregates with at least one dimension between 1 and 100 nm (Ball, 2002; Roco, 2003). They exhibit physicochemical properties different from those of the raw material that gave rise to them. Nanoparticles, such as silver (Saad *et al*., 2021), gold (Thakur *et al*., 2018), magnesium (Tauseef *et al*., 2021), and zinc (Hrács *et al*., 2018) have been applied to control PPN. Nanoparticles have multi-site modes of action against nematodes and no environmental toxicity (Bhau *et al*., 2016) and are an alternative to synthetic nematicides with high environmental risk without toxicity to plants (Cromwell *et al*., 2014). Among the NP with nematicidal potential are iron NP (Ch *et al*., 2019; Khan et al., 2022; Kumar *et al*., 2022). Besides the benefits of iron in plant nutrition (Jalali *et al*., 2016; Bastani *et al*., 2018; Golshahi *et al*., 2018), iron is

an environmentally benign element, unlike elements such as copper and titanium. Iron NP have excellent physicochemical properties, such as high surface area (Jin *et al*., 2018), and are superparamagnetic (Lachowicz *et al*., 2017), making them one of the most explored nanomaterials as carrier molecules.

In this context, co-inoculation of iron NP with BA has the potential to be an efficient way to improve the action of BA. However, it is a challenge to test the combination of iron NP and BA since the following are not known: i) the effective dose of NP to control PPN; ii) the reaction of the plant and the BA to the NP; and iii) the possible mode-of-action of NP on *M. javanica*. It is known that iron can potentiate the formation of reactive oxygen species (ROS), through the Fenton reaction (Gill and Tuteja, 2010) and by being neurotoxic by concentrating mainly in the nervous system of organisms (Ayton *et al*., 2012; Davies *et al*., 2013; Visanji *et al*., 2013). As such, ROS formation is an essential toxicity biomarker and can be used in evaluating the efficiency of iron NP against PPN. Therefore, this study aimed to evaluate the potential of co-inoculation of iron NP and a BA on the control of *M. javanica* in soybean. Furthermore, the possible mechanism acting on *M. javanica* by the NP/BA combination was explored using ROS formation as an indicator of toxicity.

MATERIALS AND METHODS

Obtaining the biological agent

The BA used in the trials was Votivo®, a commercial biological nematicide produced by BASF, Brazil. This product is a formulation of *Bacillus firmus* (strain I-1582) at a concentration of 4.8 x 10 CFU/ml.

Obtaining the nematode inoculum

Meloidogyne javanica eggs used in this study were kindly provided by Bruno Barbosa Consulting Nematology (Jaboticabal, SP, Brazil) and the Department of Phytosanitary Defense, Federal University of Santa Maria (Camobi, Santa Maria, RS, Brazil). The eggs were extracted by the NaOCl technique (Hussey and Barker, 1973) from the roots of a pure culture of *M. javanica* maintained on tomato cv. Santa Cruz grown in earthen pots in the greenhouse (24 ± 2°C). To obtain J2, the eggs were placed in incubation chambers and J2 were collected after 24 hr. The J2 suspension was adjusted to 2,000 J2/ml.

Synthesis and characterization of the iron nanoparticle

Iron was precipitated from coal mining waste with a selective precipitation method (Wei *et al*., 2005; Menezes *et al*., 2009;). The pH of the residue was adjusted to 3.6 ± 0.1 with the addition of NaOH solution so that the precipitation of iron as ferric hydroxide occurred. The precipitate was filtered to remove excess water and calcined in a muffle furnace at 750°C for 30 min. After drying, the product was ground on an agatha crucible and sieved on a 25-µm (500 mesh) sieve. Particles below 500 mesh were exposed to 10 min of ultrasound and centrifuged for 10 min at 800 g to obtain the desired particle size. The nanoparticle was analyzed for particle size and zeta potential by dynamic light measurements (DLS) (Ashizawa, 2019). The morphological analyses were performed on a scanning electron microscope (SEM; Tescan, model Vega LM3, Tescan, Brno, Czech Republic). The crystallographic composition was analyzed for Ray-X diffraction (XRD, D8 diffractometer; Bruker, Billerica, MN) under the following operating conditions: Cu K radiation (300 Kv 10 MA^{-1}), goniometer speed of 0.020 2Θ per step with a counting time of 0.5 sec per step and collected from 3 to 700 2Θ. The nanoparticles were analyzed for the presence of functional chemical groups by Fourier transform infrared spectroscopy (FTIR; Agilent Technologies, Cary, NC) and the FTIR measurement was carried out at room temperature in the range of 650 to 4000/cm. The SEM images showed the spherical shape of the individual particle; however, it was possible to observe particle aggregation. The DLS analysis demonstrated that 95% of the particles were under 100 nm, and the electric charge of the particles was 0.66 mV. X-ray diffraction analyses showed that the iron nanoparticle obtained was hematite (Fe_2O_3). The synthesized IONP exhibited one well-defined peak at 657/cm due to the presence of iron–oxygen (Fe-O) which confirmed that the synthesized nanoparticles were iron oxide (Hwang *et al*., 2014). The observed FTIR and DRX results together confirmed that the synthesized nanoparticles were iron oxide (Benedetti, 2022).

Seed treatment

Seeds of soybean 'Elite' (Brasmax, Londrina, Brazil), susceptible to *M. javanica*, were treated with BA at the recommended rate (1 ml/kg of seed) and with iron NP at the following doses: 0, 75, and 300 mg/kg of seed. In addition to NP and BA, the seeds received the dye CM Flo Rite® 1197 Green (BASF, Brazil). The seed treatment was performed using industrial seed treatment equipment to achieve a homogeneous treatment distribution. For the positive control, the seeds were treated with BA alone and for the negative control, the seeds did not receive the BA.

Obtaining the plants and nematode inoculation

Soybean seeds already treated with the different doses of iron NP, with and without BA, were sown in seedling production trays with sterilized sand (100%). Ten days after emergence, the plants were transferred to 500 ml-capacity cups. The substrate used was sand:soil composite in a 2:1 ratio sterilized in an autoclave (120°C, 2 hr). At transplanting, the plants were inoculated with 2,000 *M. javanica* J2. The J2 were placed near the root system in a 2-cm-deep hole. The plants received sufficient irrigation for their development and were fertilized once a week with the nutrient solution Kristalon Orange (Yara, Brazil). The experiment was conducted in a greenhouse that was maintained at 25 ± 2°C and a photoperiod of 12 hr. The experiment was arranged in a randomized design, with each treatment replicated 36 times to allow for destructive sampling over time (see below). The experiment was conducted twice, and the data from each experiment were combined.

Evaluating M. javanica *development in soybean roots*

To evaluate *M. javanica* development in soybean roots, six plants were destructively harvested 5, 10, 15, 20, 25, and 30 days after inoculation (DAI). The aerial part of the plant was removed, and the roots separated from the soil. The root system was washed in water to remove the soil adhering to the roots and cut into pieces of approximately 2 cm. Washed roots were stained according to Byrd *et al*. (1983). After staining, the roots were kept in glycerin under refrigeration. The root system was evaluated under an optical microscope (Nikon, USA). The entire root system was evaluated, and all nematode life stages were considered.

Measuring of Reactive Oxygen Species (ROS)

To measure the production of ROS, *M. javanica* J2 were exposed to two doses of the iron NP used in the seed treatment (75 and 300 mg/L). Water was used as a negative control, and AVICTA® Complete (Abamectin, Thiamethoxam, Mefenoxam, Fludioxonil; Syngenta, Brazil) (ABA) at the commercial dose (1 ml/kg) as the positive control. In this trial, the BA was not evaluated due to the mode-of-action. *Meloidogyne javanica* J2 were exposed to the treatments for 1 hr (Wang *et al*., 2008), washed three times in M9 buffer (3.0 g KH_2PO_4, 6.0 g Na_2HPO_4, 0.5 g NaCl, 1.0 g NH_4Cl, bring to 1 l with H_2O), transferred to a microcentrifuge tube, and frozen in liquid nitrogen three times. Samples were sonicated (Ultrasonic processor UP200Ht, Hielscher, Germany) five times for 15 s at 10-s intervals, on ice at 30% amplitude. Then the samples were centrifuged for 30 min at 15,000 rpm, and the supernatants were collected. A volume of 10 µl of the sample was added to 10 µl of 0.1 mM carboxy-2',7'-dichloro-dihydro-fluorescein diace-tate (DCFH-DA), and 130 µl of phosphate buffer (TFK) and incubated in the dark at 20°C for 5 min. Spectrophotometry measured the rate of change of absorbance at 570 nm (emission) and 545 nm (excitation). The results were expressed as DCF/mg protein. The treatments were replicated three times in each trial, and the experiment was repeated three times.

Statistical analysis

The data were analyzed for effects of the interaction between iron NP dose and the presence or absence of BA using ANOVA. The iron NP dose and the presence or absence of BA were treated as random factors. Regression analysis was performed for quantitative data (doses of iron NP) and a comparison of means by the Tukey test and Student test at a 5% significance level for qualitative data (presence or absence of BA). Data analysis was performed in the RStudio software RStudio Team, 2021). The ROS data did not meet the assumptions of ANOVA; therefore, data were

analyzed using Kruskal–Wallis and Duncan's multiple range tests to separate means ($P \leq 0.05$) in the GraphPad Prism 8.0.1 software (San Diego, CA).

RESULTS

M. javanica *development in soybean roots*

The BA decreased the number of *M. javanica* in soybean roots by 30% compared to the control without BA at 5 DAI (Table 1). At 15 DAI, the presence of BA resulted in a 34% reduction in the total number of *M. javanica*. This difference in *M. javanica* in roots at 30 DAI was 69% compared to the treatment without BA (Table 1). When iron NP were present at 75 mg/kg, the addition of BA up to 15 DAI had no significant effect on *M. javanica* densities. However, at 20 DAI, the addition of BA reduced the total number of *M. javanica* by 46% compared to the control without BA. At 30 DAI, the presence of BA with iron NP at 75 mg/kg resulted in a 67% reduction in the number of *M. javanica* compared to the control without BA (Table 1). When iron NP at 300 mg/kg was evaluated at 5 DAI, in the absence of BA, the density of *M. javanica* was 62% lower than with BA. At 10 DAI, the addition of BA increased the total number of *M. javanica* 4.8 times compared to the treatment without BA, and at 15 DAI, the addition of BA increased 3.8 times the number of *M. javanica*. No significant effect was observed with iron NP at 300 mg/kg at 30 DAI (Table 1). At 30 DAI, the BA and iron NP at 75 mg/kg, both alone, resulted in an equal reduction in the number of *M. javanica* in soybean roots by approximately 68%. However, the combination of iron NP at 75 mg/kg + BA was more effective in reducing *M. javanica* female densities, with 88% compared to the total females found in the iron NP at 0 mg/kg and 78% compared with iron NP at 0 mg/kg + BA. Iron NP at 0 mg/kg + BA resulted in a 44% reduction in females compared to the control of iron NP at 0 mg/kg without BA. This result was similar to that obtained in the iron NP at 300 mg/kg without BA, and much lower than the values obtained when iron NP at 300 mg/kg were applied with BA (Fig. 1).

In the absence of BA, there was a linear reduction in the number of *M. javanica* at all doses of iron NP. At 5 DAI, the reduction was in the order of 0.04 for each mg of iron NP (Fig. 2). At 10 DAI, this reduction was 0.19 for each mg of iron NP added (Fig. 3). At 15 DAI, each mg of added iron NP reduced 0.48 nematodes (Fig. 4). At 20 DAI, the reduction was 0.51 (Fig. 5). At 25 DAI, the addition of each milligram of NP reduced 0.2 nematodes (Fig. 6), and at 30 DAI, the reduction was 0.94 nematodes for each milligram of NP added (Fig. 7). The addition of iron NP at 300 mg/kg resulted in a 75% decrease in the total number of *M. javanica* compared to iron NP at 0 mg/kg. In the presence of BA, there was no significant effect of the concentration of iron NP on *M. javanica* densities.

Reactive Oxygen Species (ROS) formation

The enzymatic test applied to evaluate ROS formation by *M. javanica* exposed to different doses of iron NP showed an increase in ROS generation in *M. javanica* treated with BA and with iron NP at 75 mg/kg. Although the means did not differ statistically, these results suggest that the toxicological damage caused by the iron NP at a concentration of 75 mg/kg was similar to the

Table 1. *Meloidogyne javanica* densities in soybean roots at different concentrations of iron nanoparticles (NP) with and without biological agent (BA) *Bacillus firmus*, a formulation of *Bacillus firmus*.

	Iron NP doses (mg/kg)					
	0		75		300	
[-1]DAI	With BA	Without BA	With BA	Without BA	With BA	Without BA
5	14 b[z]	20 a	12 n/s	9 n/s	13 a	5 b
10	59 n/s	77 n/s	56 n/s	41 n/s	72 a	15 b
15	123 b	186 b	81 n/s	124 n/s	113 a	30 b
20	201 n/s	263 n/s	122 b	226 a	169 n/s	112 n/s
30	165 b	528 a	86 b	263 a	94 n/s	190 n/s

[z]Averages followed by the same letter in the same row do not differ statistically based on the Tukey test at 5% probability. n/s - non-significant interaction.

Table 2. Reactive Oxygen Species (ROS) identification in *Meloidogyne javanica* treated with different concentrations of iron nanoparticle (NP). Water was a negative control (-) and abamectin was a positive control (+).

	Treatments (DCF/mg protein)[z]			
	(-)	Aba	75	300
Mean	385278	616340	665468	317768
Std. Deviation	228954	446667	409508	171728

[z](-): negative control; Aba: abamectin, positive control; 75: iron NP 75 mg/kg; 300: iron NP 300 mg/kg.

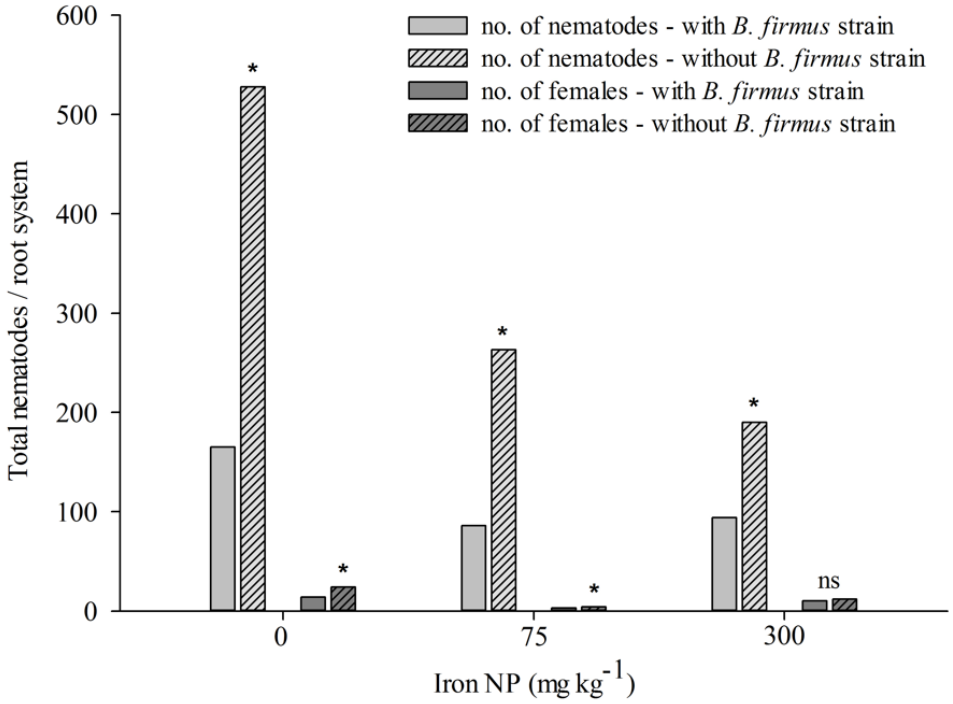

Figure 1. *Meloidogyne javanica* densities (second- and fourth-stage juveniles) and females in soybean roots 30 days after inoculation and treatment with iron nanoparticles (NP), with or without the biological agent *Bacillus firmus*. The number of nematodes and females were compared within each concentration of iron NP by Student's t-test ($P < 0.05$). * significant value, and (ns) non-significant value. Bars represent the mean of n = 16.

damage caused by ABA (Table 2). These results corroborate those found in the development test of *M. javanica* on soybean, where iron NP at 75 mg/kg, both with and without BA, had the lowest mean number of *M. javanica* females at 30 DAI (Fig. 1).

DISCUSSION

In this study we evaluated the potential of iron NP for *M. javanica* control and for the capacity to improve the action of a BA for nematode control. Our results suggest that iron NP are a viable alternative for managing *M. javanica* in soybean since it decreased the number of *M. javanica* females present in the root system. We did not evaluate soybean growth in this work, but there is evidence in the literature that higher female densities were related to lower productivity (Lü *et al*., 2023). The presence of iron NP improved the

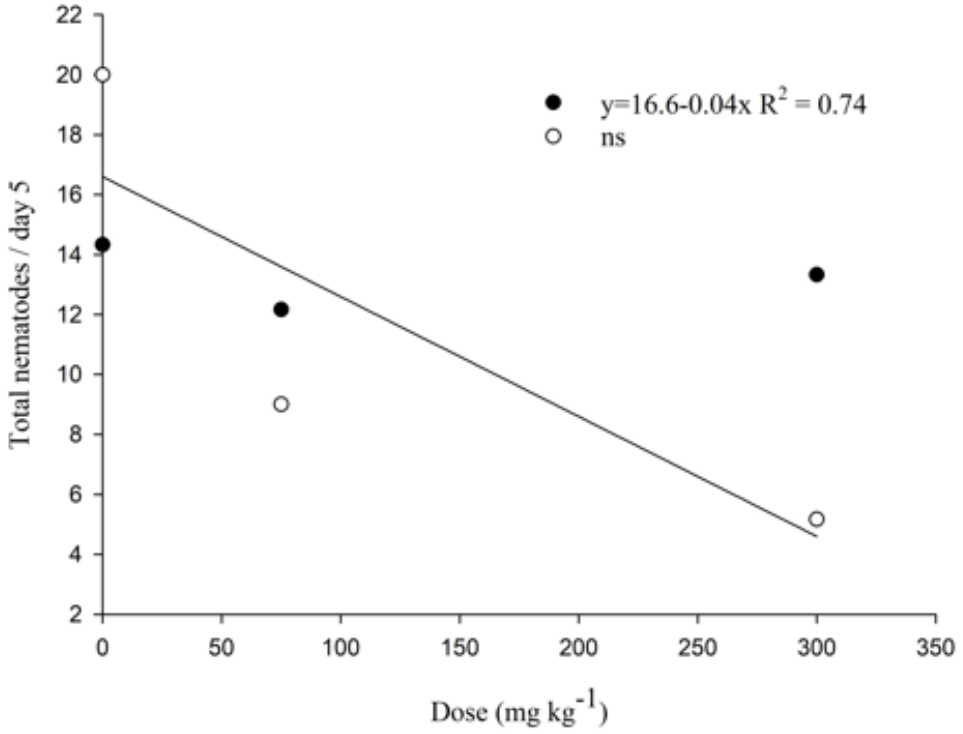

Figure 2. *Meloidogyne javanica* densities in soybean roots as a function of iron nanoparticle concentration at five days after inoculation.

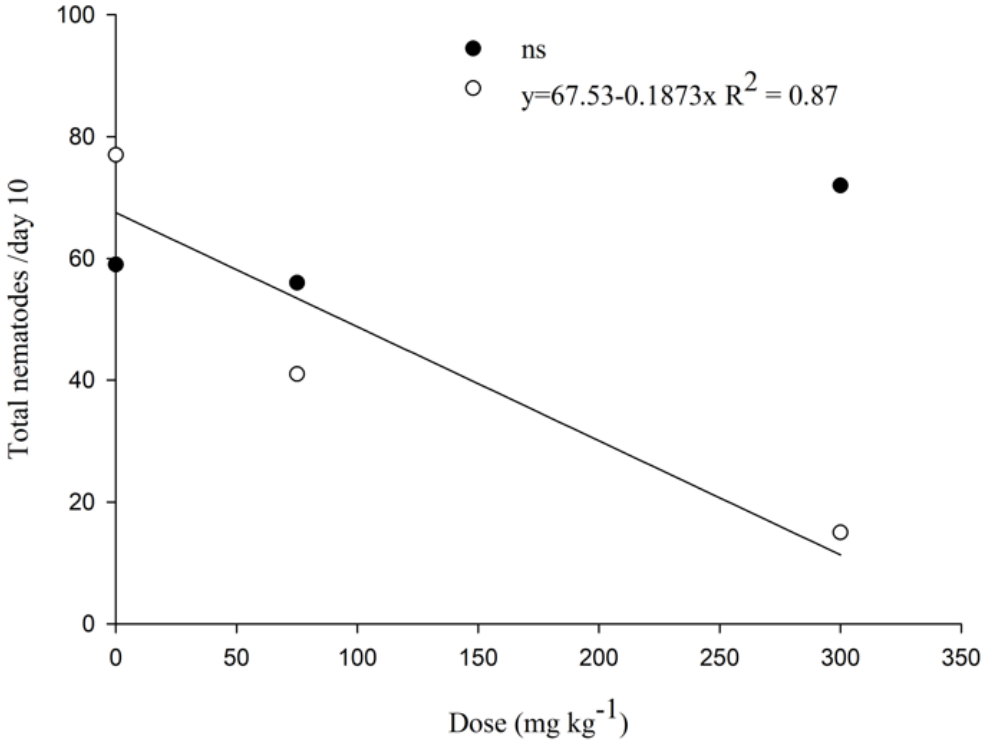

Figure 3. *Meloidogyne javanica* densities in soybean roots as a function of iron nanoparticle concentration at 10 days after inoculation.

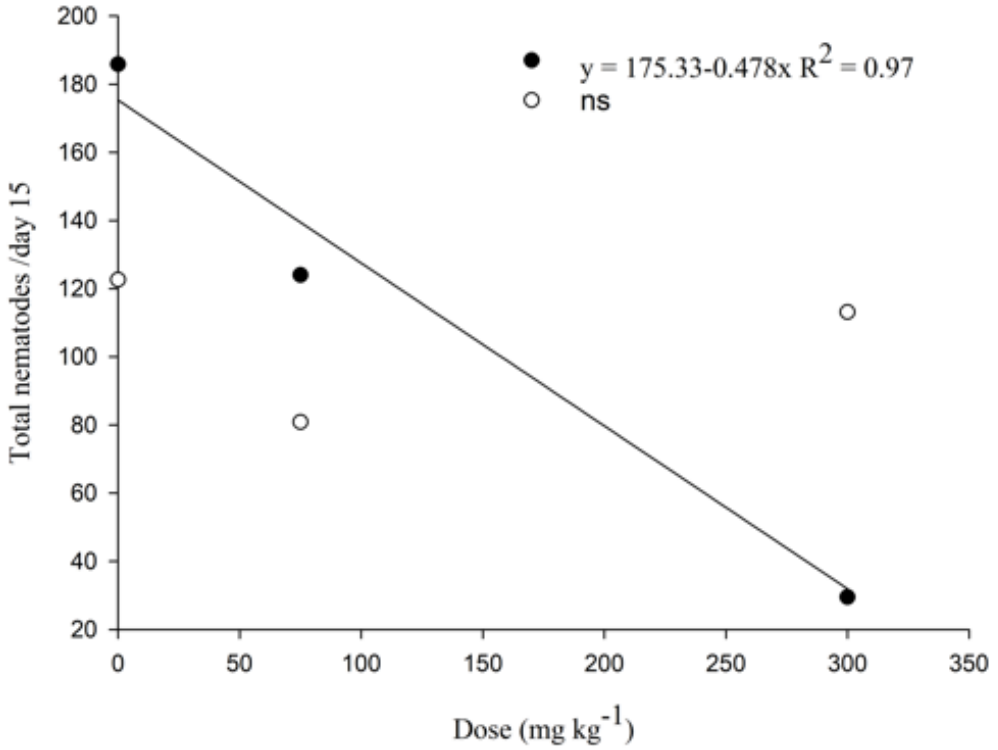

Figure 4. *Meloidogyne javanica* densities in soybean roots as a function of iron nanoparticle concentration at 15 days after inoculation.

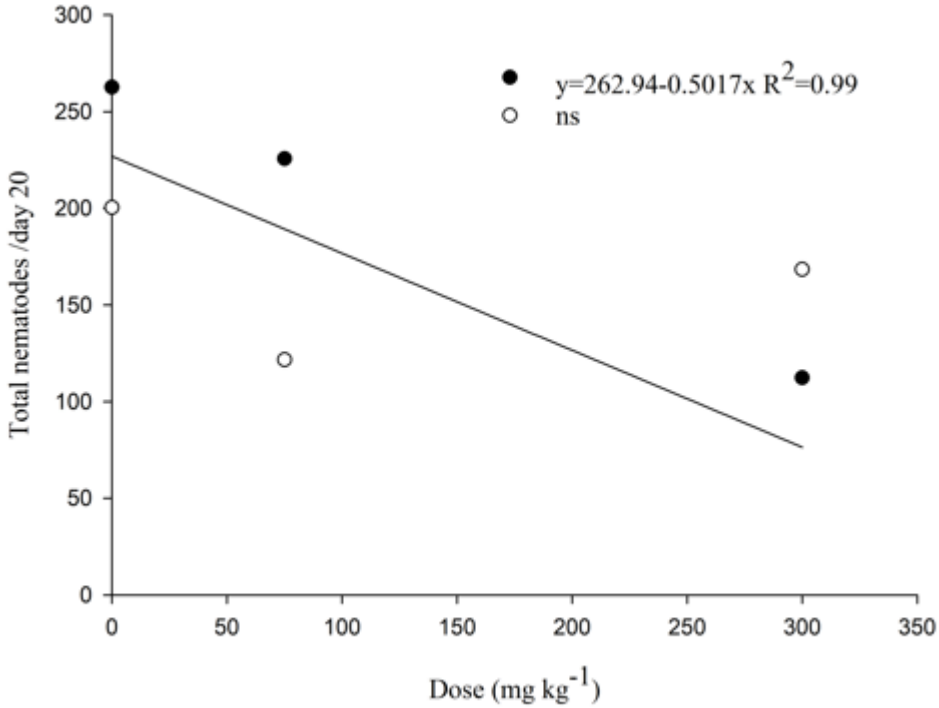

Figure 5. *Meloidogyne javanica* densities in soybean roots as a function of iron nanoparticle concentration at 20 days after inoculation.

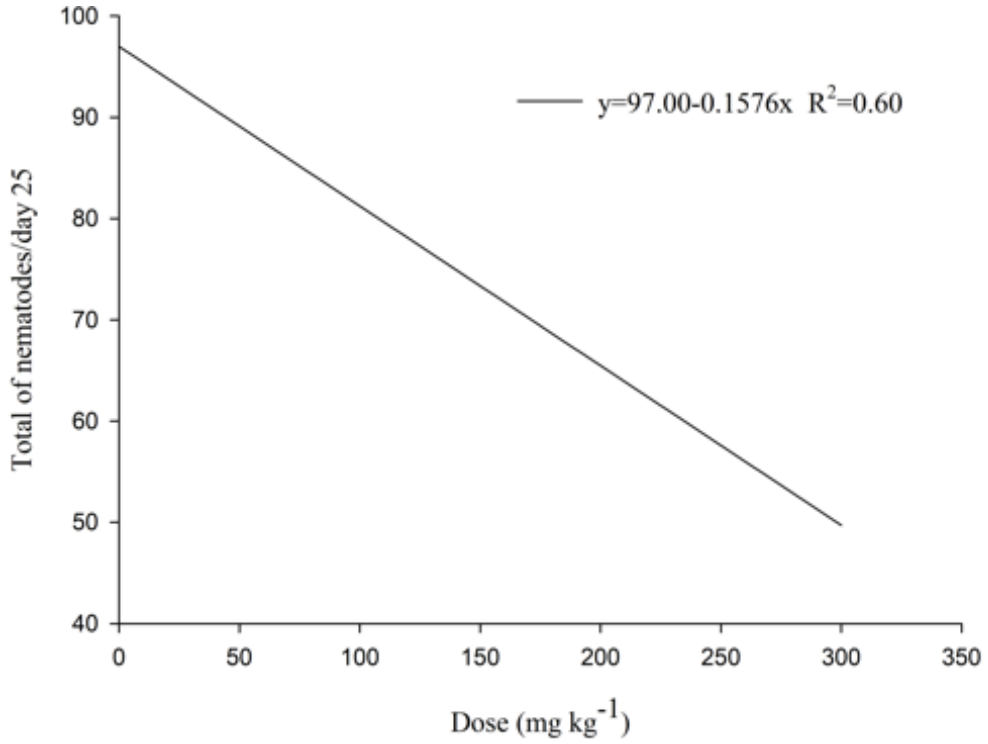

Figure 6. *Meloidogyne javanica* densities in soybean roots as a function of iron nanoparticle concentration at 25 days after inoculation.

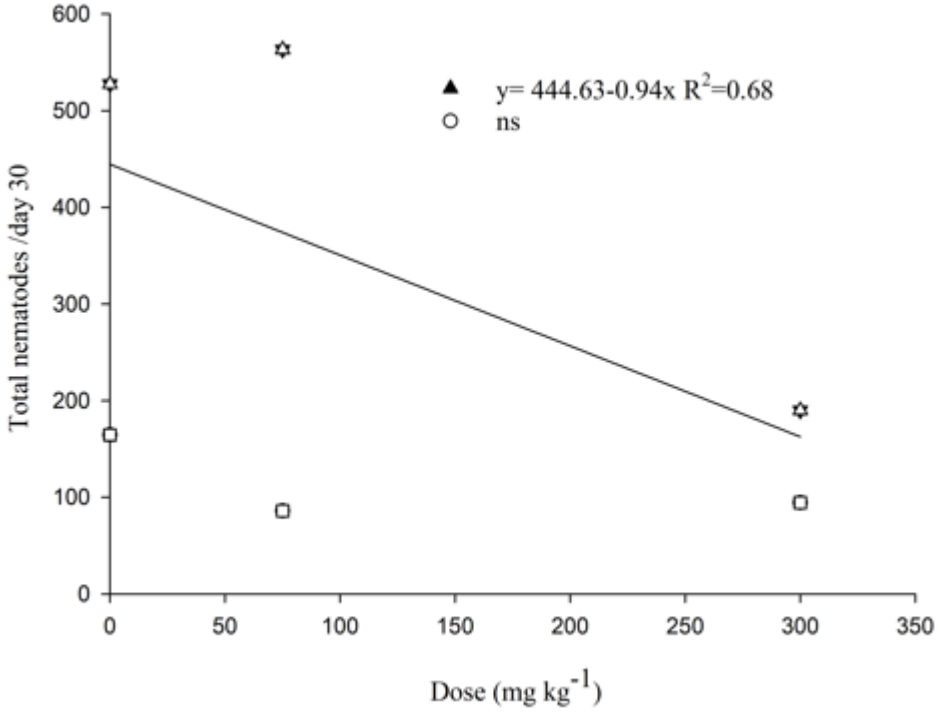

Figure 7. *Meloidogyne javanica* densities in soybean roots as a function of iron nanoparticle concentration at 30 days after inoculation.

levels of *M. javanica* suppression for the BA at specific doses when compared to the standard treatment exclusively with the BA. The addition of BA as a seed treatment resulted in a reduction of 69% in *M. javanica* population densities in the soybean roots compared to the untreated control at 30 DAI. These results corroborate the control levels of BA in field situations in Brazil (Miamoto *et al*., 2017; Dias-Arieira *et al*., 2018; Oliveira *et al*., 2019). Other studies have demonstrated that *Bacillus* was the genus that resulted in the greatest nematode mortality (Xiang *et al*., 2017) and reduced gall formation (Hussain *et al*., 2020; Benedetti *et al*., 2021). In the absence of BA, the addition of iron NP reduced densities and development of *M. javanica*, demonstrating that the iron NP has the potential for nematode control. Previous research demonstrated the benefit of iron for nematode control (Khan *et al*., 2022; Kumar *et al*., 2022). However, in the presence of BA, the addition of iron NP at 300 mg/kg did not reduce *M. javanica* densities, suggesting an antagonistic effect between iron NP and BA at the higher iron NP concentration. This may have occurred due to the action of iron on the cholinergic system, promoting disorganization in the signaling process of the BA (Dhakshinamoorthy *et al*., 2017) or due to a competition of the BA for the source of iron, since iron is a key element in bacteria (Borcherding *et al*., 2014).

The benefit of co-inoculation with BA and iron NP was seen after 20 DAI and specifically at an iron NP concentration of 75 mg/kg. The presence of BA co-inoculated with the iron NP at 75 mg/kg decreased the total number of nematodes by 54% compared to iron NP at 75 mg/kg without BA and 60.7% compared to iron NP at 0 mg/kg with BA characterizing a synergistic effect. This treatment also had the lowest number of females in roots at 30 DAI. Our data corroborates the efficiency of the BA used commercially for the management of *M. javanica* in soybean crops in Brazil. However, in accordance with our hypothesis, the addition of iron NP improved the levels of *M. javanica* suppression for the BA at this specific dose when compared to the standard treatment exclusively of BA. This behavior was expected since the bacteria takes time to colonize the roots to start its beneficial effects. The slower action of BA in controlling pathogens is well documented in the literature (Engelbrecht *et al*., 2018). This synergism occurred at this specific dose probably because at this dose there was a higher bioavailability of iron. Rohner *et al*. (2007) demonstrated that the absorption, and hence the bioavailability *in vivo* was greatly increased at the nanoscale (Rohner *et al*., 2007). Consequently, there was more bioavailable iron at this dose and higher absorption by the nematodes, strongly pronouncing its toxic effect. Lower doses can even increase the toxicity of a given substance due to decreasing the spontaneous particle clumping that can occur at higher concentrations (Bell *et al*., 2014; Patiño-Ruiz *et al*., 2020). We observed in the iron NP characterization data that there was agglomeration in the NP system. These agglomerations generate nanoaggregates that decrease the particle's bioavailability and therefore their toxicity (Mudunkotuwa and Grassian, 2011). The agglomeration process also explains the reduced bioavailability of iron in the system at iron NP 300 mg/kg generating an antagonism between the iron NP and BA at this concentration. In this case, the BA could be using the iron NP as an iron source for growth and nutrition. Therefore, the system lost bioavailable iron, part for agglomeration process and part for bacterial biosynthesis, making the available iron insufficient to be toxic to nematodes.

Our data showed that the addition of iron NP reduced nematode parasitism since the number of nematodes seen within the roots at 5 DAI was reduced by 0.04 individuals for each milligram of iron NP added, while at 30 DAI this reduction was 0.9 individuals for each mg of iron NP added. *Meloidogyne javanica* also did not develop normally. When conditions are favorable for parasitism, *M. javanica* can undergo ecdysis through juvenile stages in approximately 14 days and finally to the adult stage (Moens *et al*., 2009). It was possible to see the third and fourth-stage juveniles (J3 and J4) on soybean roots starting at 15 DAI. At a dose of 75 mg/kg iron NP, with and without BA, the presence of females was not observed before 25 DAI. The number of females in these treatments at 30 DAI was reduced 84% and 88%, respectively, compared to the control. The action of iron NP caused changes in the locomotion of the nematodes, reducing the motility of the nematode and thereby the precision in locomotion (Charlotte *et al*., 1997). The ability of nematodes to find a host depends on a complex and sensitive chemosensory system (Curtis, 2008). Tests of learning and memory behavior in nematodes have

been established using the *C. elegans* (Rankin *et al*., 1990), which has a highly developed chemosensory system (Bargmann, 2006) and exhibits a high ability to respond to changes in the environment and adjust its behavior (McDiarmid *et al*., 2015). The chemo-orientation of nematodes to locate a host is dependent on the presence of various phytochemicals released by the plant through root exudates. The possible disorientation and lethargy in nematode locomotion can drastically affect their ability to find a host, decreasing the chances of infection.

When we evaluated ROS generation, by *M. javanica* J2 in the presence of iron NP, we observed that a higher level of ROS was produced by J2 exposed to iron NP at 75 mg/kg. The ROS level of *M. javanica* J2 at this iron NP concentration was similar to the level of ROS in J2 exposed to abamectin, the standard chemical control of nematodes in Brazil. The high levels of ROS in the system should lead to *M. javanica* death. The more pronounced effect at the lower dose may be due to the high bioavailability of iron and, with it, the higher absorption by organisms (Rohner *et al*., 2007), causing the generation of ROS. Iron is an essential metal for animal and plant life since it is fundamental in biochemical processes such as the formation of the heme group of hemoglobin in animals and the photosynthetic process in plants. However, it can also promote cell toxicity through the Haber-Weiss mechanism or the Fenton reaction, generating the OH* radical, the ROS with the greatest potential for cell damage (Gill and Tuteja, 2010). Although it was impossible to statistically differentiate the results, we observed consistent behavior with the results obtained in the *in vivo* test with *M. javanica* as well as in the nematode penetration and development test. In these two tests, we verified the greatest toxicity of iron NP at 75 mg/kg. It is well documented that various abiotic stresses lead to the overproduction of highly reactive and toxic ROS that can result in oxidative stress (Gill and Tuteja, 2010). Reactive oxygen species are considered vital molecules in regulating cellular processes. However, when produced in excess they can exert toxic effects on cells causing oxidative damage to nucleic acids, lipids, and proteins that can render them non-functional. Thus, the evaluation of the expression of ROS enzymes and determination of oxidative damage (FOX) can serve as a biomarker of the toxicity of a molecule. Additional studies are needed to determine the reproduction factor of the nematode, as well as other evaluations including the number of galls and eggs. Thus, the addition of iron NP to BA is a management strategy for *M. javanica* in soybean, because it decreased the number of *M. javanica* females present in the root system. This study is important proof-of-concept that iron NP can be a potential and ecologically friendly alternative for managing *M. javanica* in soybeans.

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