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Management of *Meloidogyne incognita* in tomato using soil conditioner

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ABSTRACT

Soil conditioners have humic acid levels which can control nematodes and promote plant nutrition. The objective of this work was to evaluate different concentrations and doses of commercial soil conditioner Premium® on motility, mortality, hatching, infectivity and reproduction of *Meloidogyne incognita* in tomato. In order to evaluate motility, mortality, and hatching, second stage juveniles (J2) or eggs were subjected to incubation at different concentrations of soil conditioner. To evaluate infectivity and reproduction, infested soil with *M. incognita* were mixed to different concentrations of commercial soil conditioner. Thirty-day-old tomato seedlings (cv. Kada) were transplanted into the pots and after 30 days the numbers of galls, egg masses and eggs per gram of root were evaluated. The use of soil conditioner drastically reduced motility at a concentration of 66.67 g L^{-1} and caused mortality above 98% in *M. incognita* J2 at 133.33 g L^{-1}. Exposure of eggs to soil conditioner reduced J2 hatching by more than 50% at a concentration of 2.5 g L^{-1}. Highest concentrations of soil conditioner provided lower infectivity and reproduction of *M. incognita* in tomato. Soil conditioner at a dose of 5.0 g provided greater development of the root system, demonstrating the efficiency of this product.

Keywords: root-knot nematodes; humic acid; reproduction; control.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belongs to the family Solanaceae and is one of the most worldwide cultivated vegetables, ranked second, after potato (*S. tuberosum* L.). In 2022, Brazil was rated as the tenth largest tomato producer in the world, with 3.8 million tons produced in an area of approximately 54.5 thousand hectares.[1,2] Intensive cropping of tomato and susceptible solanaceous growing in the same areas has caused serious global problems, due to the attack of the root-knot nematodes, *Meloidogyne* spp., occurring mainly in tropical and subtropical regions.

In Brazil, *M. incognita* (Kofoid & White) Chitwood, *M. javanica* Treub (Chitwood), *M. arenaria* Neal (Chitwood), *M. hapla* Chitwood, *M. enterolobii* Yang & Eisenback and *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans are the most widely distributed species on solanaceous plants,[3-5] with the predominance of the first two on tomato.[6] The symptoms caused by *Meloidogyne* spp. are erroneously mistaken with mineral deficiency by farmers. *Meloidogyne* spp. causes galls on roots, interfering in plant physiological processes and leading to secondary symptoms such as yellowing, stunting and yield reduction, thus resulting in high losses of up to 100%, depending on the cultivar and the population density of the nematode and its distribution in the soil.

Managing *Meloidogyne* spp. with nematicides and crop rotation in vegetables is difficult and often economically unviable for farmers, so it is necessary to explore alternative control methods to reduce nematode populations. Soil conditioners are products containing higher levels of organic matter, humic and fulvic acids,[7] obtained from turf, mines or industrially synthetized. Soil conditioners are used to improve soil fertility and to promote physical, chemical and biological equilibrium,[8] improving the vegetative development and increasing the productivity in sugarcane, soybean and tomato crops.[9-11]

The substances constituting soil conditioners have been reported as potential products to manage *Meloidogyne* species,[12-18] in addition to stimulate plant growth and development.[19,20] Andaló *et al.*[21] evaluated the effects of exposing the nematode *Heterorhabditis amazonenses* MC01 to the soil conditioner Premium®, verifying its influence in the viability, infectivity, and nematode production in *Tenebrio molitor* L. larvae. However, studies defining concentrations and doses for the application of soil conditioners on populations of *Meloidogyne* spp. in tomato are still scarce. Thus, the objective of this work was to evaluate the effect of different concentrations and doses of the soil conditioner Premium® on motility, mortality, hatching, infectivity and reproduction of *M. incognita* in tomato.

MATERIAL AND METHODS

The experiments were performed in the Plant Pathology Laboratory and under greenhouse conditions at the Federal University of Minas Gerais (UFMG), *Campus* Montes Claros, Minas Gerais state, Brazil.

Effect of soil conditioner in the motility, mortality and hatching of Meloidogyne incognita

Meloidogyne incognita eggs were obtained from roots of Santa Cruz cv. Kada tomato plants grown in greenhouse. Infected roots were gently rinsed in a bucket with tap water, cut in pieces of approximately 2 cm and macerated in a blender containing sodium hypochlorite solution 0.5% for 20 s at low speed, according Hussey & Barker,[22] modified by Boneti & Ferraz.[23] Eggs retained on the 0.025 mm sieve were harvested using a wash-bottle and transferred into a beaker, for the subsequent use of eggs suspension in the experiments.

In the experiment to evaluate motility and mortality, the second-stage juveniles (J2) were obtained from a hatching chamber and quantified using a Peters' slide under light microscope. Only J2s hatched on the third day were used in the experiment. After quantification of the nematode suspension, an aliquot of 1 mL with 1,000 *M. incognita* J2 was transferred to a test tube containing aqueous solution of the soil conditioner Premium® (27% total carbon - organic and mineral, 12.85% organic carbon – C/N ratio of 10/1, Ca 4.85%, Mg 3.61%, P_2O_5 4.85%, K_2O 3.97%, N 1.66%, S 0.38%, Cu 74 mg kg^{-1}, Mn 616 mg kg^{-1}, Zn 875 mg kg^{-1}, Fe 12.359 mg Kg^{-1}, B 57 mg kg^{-1}, Na 1.740 mg kg^{-1} and pH 7.9) at concentrations of: 0.0; 66.67; 133.33; 266.67; 400.00 and 533.33 g L^{-1}. The soil conditioner solution was made by weighting the soil conditioner Premium® and then adding and diluting the product in 1 liter of water, which was sifted using filter paper for further use. Subsequently, the test tubes were sealed and incubated at 26 ºC for 24 hours. After the incubation period, the J2s were collected from each test tube with an automatic pipette of 1 mL and transferred to a Peters' slide for randomly quantification of 100 motile J2s under light microscope. Then, J2s were transferred to 11 µM sieve, rinsed in water and returned to test tubes using a wash-bottle with water. The J2 were incubated for

24 h as described above. After this exposition period, the number of J2 apparently inactive was assessed under a light microscope. The J2 were considered inactive when they did not move or when the body was straight. Juveniles which remained inactive for 24 h in water were considered dead. Next, the percentage of motility and mortality of the J2s was estimated. The experiment was performed in a completely randomized design, comprising six treatments and six replications. Based on these data the best concentration for experiments evaluating hatching, infectivity, and mortality was studied.

In order to evaluate hatching, hatching chambers made with thin screen and double-thickness paper tissue on Petri dishes (9.0 cm in diameter) were used. Soil conditioner solutions were deposited in the hatching chambers, at concentrations of 2.5; 5.0; 7.5; 10.0 and 12.5 g L^{-1}. Subsequently, 1 mL of the aqueous suspension with 10,000 *M. incognita* eggs was added on the hatching chamber. Eggs incubated in distilled water were used as control. The hatching chambers were incubated under laboratory conditions at 26 ± 2 ºC. The experiment was performed in a completely randomized design, with five treatments plus the control and five replications for each treatment. After this procedure, the number of hatched J2 was counted using Peters'slide under light microscope (40x), each 48 h until reaching 192 h.

Effect of soil conditioner on infectivity and reproduction of Meloidogyne incognita in tomato

Infested soil, collected in a field of central-pivot irrigation area grown with pumpkin (*Cucurbita pepo* L.) was used in the experiment. Roots with galls were also collected for nematode identification. Analyses of a composite soil sample showed the following physicochemical characteristics: 10% clay, 82% sand, 8% silt, pH in water 6.6; and 2.6% of organic matter. Nematode identification based on the morphology of perineal pattern and by the phenotype of α-esterase according to Taylor & Sasser,[24] Esbenshade & Triantaphyllou[25] and Hartman & Sasser,[26] confirmed only the *M. incognita* species. To determine initial nematode population in the infested soil, the J2s were extracted according Jenkins.[27] The average nematode density in the infested soil was of 954 *M. incognita* J2/100 cm^3 of soil, which was estimated from the counting under light microscope.

Plastic bags (1 L capacity) were filled with 800 cm^3 of soil infested with *M. incognita*. Subsequently, the doses of 2, 4, 6, 8 and 10 g of the soil conditioner were added and homogenized to obtain concentrations of 2.5; 5.0; 7.5; 10.0 and 12.5 mg/cm^3 of the product. The soil treated with different concentrations was placed in pots (1 L). Then, seedlings of tomato cv. Kada, previously grown in sterilized substrate Solomax®, for 30 days, were transplanted to the pots containing the treated soil with soil conditioner infested with *M. incognita*. Seedlings cultivated in infested soil were used as control. The pots were maintained under greenhouse conditions and irrigated when necessary. The experiment was performed in a completely randomized design with five treatments plus the control and five replications.

Thirty days after inoculation, tomato root systems were collected, weighted and the egg masses were stained according Rocha *et al*.[28] Subsequently, the number of egg masses, galls and eggs per gram of root were determined. Eggs were extracted according to the Hussey & Barker[22] method, modified by Boneti & Ferraz.[23]

Statistical analyses

Statistical analyses were performed using the statistical software R (R Development Core Team.[29] The normality of data was analyzed by Saphiro-Wilk test, and as the data did not show normal distribution, general linear models (GLM) were built. GLM models were built with binomial distribution error, being the overdispersion corrected with Quasi-binomial, followed by contrast analysis with *car* package. The influence of the treatments on the percentage of immobile J2s after 24 h and on the mortality after 48 h were assessed. To analyze the relationship between different concentrations of the soil conditioner and the infectivity and reproduction of nematodes a Poisson distribution error was used, being the overdispersion corrected with Quasi-poisson. Averages were compared by the analysis of contrast with car package. Values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The soil conditioner caused immobility and mortality in *M. incognita* J2s and significantly reduced hatching compared to control (Figures 1 A and B). Incubation of *M. incognita* J2s for 24 h, starting at a concentration of 133.33 g L^{-1} caused 100% reduction in motility (Figure 1A), while the mortality of 96.2 and 97.8% occurred at concentrations of 133.33 and 266.67 g L^{-1}, respectively. High reduction in J2 hatching occurred within the first 48 h

of incubation of *M. incognita* eggs. The hatching of the J2s was reduced to 63.59% at a concentration of 12.5 mg mL^{-1} of soil conditioner and 192 h of incubation, when compared to control (Figure 1B). As doses/concentrations of the soil conditioner applied to infested soil increased when compared to control, but a crescent reduction in infectivity and reproduction (Figure 2) was verified. Higher fresh weight of roots was observed in tomato plants with soil conditioner from the concentration of 5 mg/cm^3 (Figure 2D), when compared to control (Figure 3).

Humic substances are compounds originated from the decomposition of soil organic matter, with the most important compounds of the humic fractions being the humic and fulvic acids.[7] The rapid reduction observed in hatching, motility, and mortality of *M. incognita* J2s at low concentrations may be explained by the high levels of humic substances present in the soil conditioner tested. In fact, low hatching rate and high mortality stabilized after 48 hours of eggs exposition and 24 h of J2 exposition to a concentration of 133.33 g L^{-1} (Figures 1A and B). Ahmad *et al.*[15] reported that exposition of *M. javanica* eggs at 0.25-1% of humic acid inhibited J2 hatching to 16.8-59.8%. The authors also observed that the increasing concentrations of humic acid and exposure periods of *M. javanica* J2 resulted in highest percentage of mortality (89.5%) at 1% of humic acid and exposure for 10 days. Seenivasan & Senthilnathan[13] verified that humic acid, a by-product from lignite coals, inhibited *M. incognita* J2 hatching in 50-100% when incubated in humic acids at 0.08-2.0%, and increased J2 immobilization up to 100% with the increment of the humic acid concentration (higher than 0.08%) incubated for 48 h, being the lethal concentration (LC50) determined as 0.02% of humic acid. In a different study, Jothi & Poornima[14] demonstrated that the humic acid at all tested concentrations (0.2 to 1%) caused significant inhibition of *M. incognita* J2s hatching between 24 and 96 h of exposition (89% of inhibition after 24 h of exposure), and mortality of 93% J2s at 0.4% of humic acid and 48 h exposure. Santos *et al.*[12] evaluated the effect of fulvic acid fractions, humic acid and humic material on the mortality of *M. javanica* and verified that humic acid fractions caused higher mortality in relation to fulvic acid and humic material, providing higher than 90% J2s mortality after 48 h exposure.

The soil conditioner used in this study has on its composition, organic compounds and macro and micronutrients, as early cited, in concentrations which interfere in *M. incognita* mortality. Then, the high efficiency of the soil conditioner may be also explained by the quantity and/or quality of some toxic components in the chemical composition, besides the C/N (10/1) ratio, which are factors that can inhibit J2s movement and cause death after 24 h exposure. In fact, nitrogen fertilizers used in the agriculture as $Ca(NO_3)_2$ and NH_4NO_3 at 1.0 M completely inhibit *M. arenaria* J2s hatching.[30] These authors evaluated five concentrations (0.1, 0.2, 0.3, 0.4 and 1.0 M) of NaCl, $CaCl_2$ and KCl, verifying higher inhibition of hatching with the increasing concentrations, up to completely inhibit hatching at the higher concentration. Osei *et al.*[31] evaluated various organic waste extracts on hatching of *M. incognita* eggs and observed hatching reduction of 93% when using citrus waste, which showed one of the highest organic carbon contents (36.4%), organic matter (72.8%), N (3.05%)

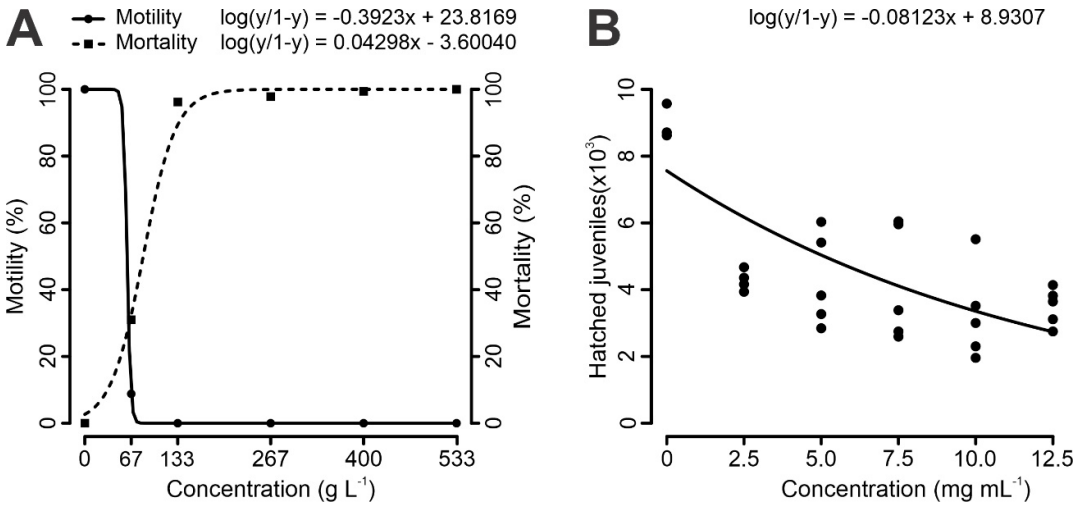

Figure 1: Percentages of motility and mortality (A) and hatching (B) of *Meloidogyne incognita* juveniles after exposure to different concentrations of soil conditioner.

and highest concentrations of P (0.46%) and K (0.8%) at pH 7.8. In the present study, the pH of the soil conditioner was of 7.9; this fact, added to the period of exposition and organic carbon (27%) and macro P (4.85%), K (3.97%), N (1.66%), Ca (4.85%), Mg (3.61%) and micro-nutrients concentrations on its composition, as well as the concentrations used in the experiments, may partially explain the control in hatching and mortality of *M. incognita* J2s.

The reduction of infectivity and reproduction, represented by the number of galls, egg masses and number of eggs per gram of roots was already observed at the lowest concentration, with a decline of more than 50% at the second concentration, with decrease verified also for other concentrations (Figures 2A, B and C). In addition, the application of soil conditioner provides visible and significant increase in the development and growth of tomato root

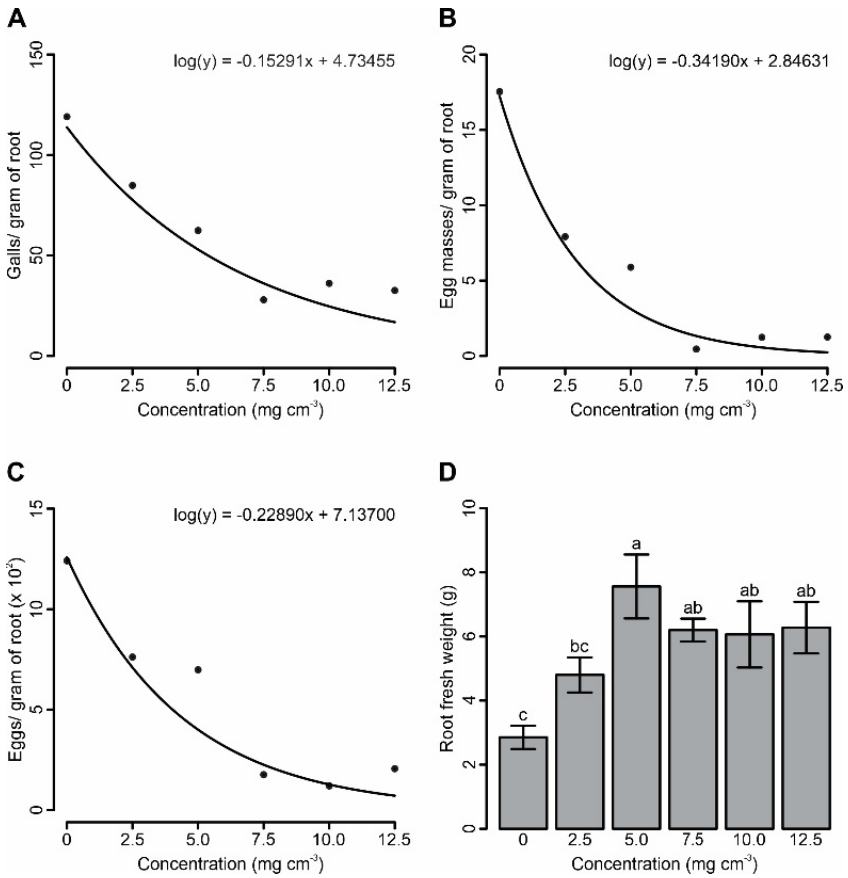

Figure 2: Nematicidal effect of different concentrations of soil conditioner on the number of galls/g of roots (A), number of eggs masses/g of roots (B), number of eggs/g of roots (C) and fresh weight of roots (D) in tomato 30 days after inoculation with *Meloidogyne incognita*.

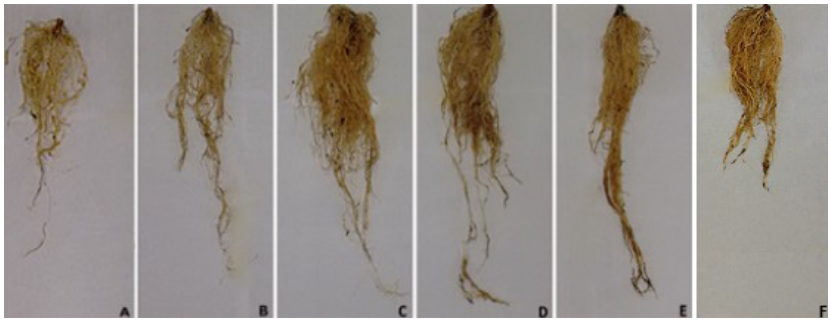

Figure 3: Tomato root system after 30 days on soil infested with *Meloidogyne incognita* or treated with soil conditioner at different concentrations. A: Control; B: 2.5 mg/cm³ of soil conditioner; C: 5.0 mg/cm³ of soil conditioner; D: 7.5 mg/cm³ of soil conditioner; E: 10.0 mg/cm³ of soil conditioner; F: 12.5 mg/cm³ of soil conditioner.

system (Figures 2D and 3). Therefore, besides its direct activity on *M. incognita* and according to the *in vitro* results, the soil conditioner also stimulates the development of tomato root system, allowing a better distribution of roots and absorption of water and nutrients, thus reducing the stress caused by the parasitism of *M. incognita*. In addition to enhancing root growth, humic and fulvic acids improve the biomass of the aerial parts of plants.[7] This is due to the activation of the proton pumping ATPases present in the cell membrane, which lead to higher exchange of ions and higher nutrients absorption, as nitrates, which favor the vegetative growth in cucumber and maize.[32-36]

Many studies have reported the nematicide activity and/or nematostatic effect of humic acids and organic compounds on *Meloidogyne* species infectivity and reproduction in tomato, peppers and banana. [12,15,18,37] The application of 2.5 mL of humic acid in the soil around previously inoculated with *Meloidogyne* spp. tomato roots significantly reduced the infectivity, expressed by the galls index, and increases the fresh and dry weight of roots.[16] Hammad *et al.*[17] evaluated the influence of humic acid in the development of olive plants (*Olea europaea* L.) and the control of *M. incognita* verifying the application of 20 mL of humic acid per pot (3.5 kg soil) reduced the reproduction factor (71.5%) and increased the fresh (403.8%) and dry (400%) weight of the aerial part and fruit weight (145.87%). In another experiment, Podestá *et al.*[38] demonstrated the application of 10 g of soil conditioner Ribumin® per pot reduced the number of galls and eggs of *M. javanica* in tomato in 24 and 3%, respectively.

Seenivasan & Senthilnathan[13] reported the use of 2 liters of humic acid (pH 8.5) per 20 kg of soil, at concentrations of 0.04, 0.08, 0.2 and 0.4%, significantly reduced: *M. incognita* population density (53.5-56.7%), root infection (61.9-63.8%), number of eggs (61.9-63.8%) and reproduction rate (55.7-56.6%) in banana. In a different study, Kesba & Al-Shalaby[39] verified the application via drenches (200 mL per pot) of product-based on humic acid (Actosol®) + NPK (10:10:10) + micronutrients (Fe - 2.9%, Mn - 0.7%, Cu - 1.4%) significantly reduced the infectivity (galls), penetration, and the reproduction factor of *M. incognita* in tomato. These authors also observed that humic acid + NPK or micronutrients inhibited hatching (2.3-15.7%) and increased mortality (15.3-25.6%). They confirmed, still, that two applications of humic acid + NPK proportioned the best plant growth results (fresh and dry weight) in sandy loam soil. Hua *et al.*[40] evaluated the attraction of *M. incognita* and *M. hapla* in response to the effect of pH and to a gradient of inorganic salts ($MgCl_2$, KCl, KNO_3, $MgSO_4$, Na_2SO_4), and verified weak attraction to base and salt at low concentrations of J2s (300 J2 mL^{-1}), but increasing the attraction at higher concentrations of both nematode species. These authors also reported that basic pHs (8.53 to 8.97 and 9.5 to 10.1) promoted higher attraction of J2s, while in low pHs (4.84 and 4.56) no significant increase in mortality between 2 and 24 hours was verified, indicating *Meloidogyne* species may support lower pH levels; on the other hand, 50 mM of NaOH (pH 12.81) is lethal. In the present work, soil pH was of 6.6 and soil conditioner pH was of 7.9, indicating that the effect of pH favored the attraction of *M. incognita* J2s at a concentration of 954 J2 of *M. incognita*/100 cm^3 of soil (control), but the application of soil conditioner negatively affected the attraction and/or the penetration of J2s in tomato roots.

CONCLUSIONS

Soil conditioner Premium® reduced hatching and motility, caused mortality of *M. incognita* J2s, and offered a possibility for managing root-knot nematodes in tomato and improving plant development.

FULL DISCLOSURE

The authors inform that there is no conflict of interest in carrying the research and publishing this manuscript.

AUTHOR CONTRIBUTION

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Methodology: Fernando da Silva Rocha.

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