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# Alternative control of root-knot nematodes (*Meloidogyne javanica* and *M. enterolobii*) using antagonists

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**Key words:** Nematodes, Sustainable management, *Meloidogyne* sp, Antagonistic plants.

## Abstract

The use of alternative ways to control pests and diseases in agriculture is becoming increasingly popular, as they are methods that do not cause a negative impact on the environment, unlike chemical pesticides. This research aimed to evaluate antagonistic plants (sorghum, crotalaria, mucuna, guandu bean and neem) in reducing the infestation of *Meloidogyne javanica* and *M. enterolobi*. The experiment was conducted in a greenhouse belonging to the Federal Institute of Ceará - *Campus* Sobral, from May 17 to July 2, 2013. Five plant species, sorghum, crotalaria, mucuna, guandu bean and neem were used. 142.94 g of each fresh plant was mixed with soil infested with nematodes in plastic pots of 1 liter capacity, and the experimental design was randomized, EDR. The most promising results were observed in the neem and sorghum plants, in which the most significant variables for both species of nematode reductions were observed, highlighting the number of galls per root system and per gram of root. It is concluded that the control of these plant-parasitic nematodes through the use of neem and sorghum can be effective.

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## Introduction

The genus *Moloidogyne* comprises nematodes which cause galls on the root system of many plants, and these are the most important because they occur in several areas and cause considerable damage. Over the years, new species have been described and the genus *Meloidogyne* has become of greater economic interest in the world (Ferraz, 2001).

The use of nematicides, like other pesticides used in conventional agriculture, might cause adverse damage to the agricultural ecosystems in which they are used soon after their application. Although nematicides are being used in biological control to mitigate the damage caused by these populations' plant-parasitic nematodes, they are used in other microorganisms as entomopathogenic fungi, crop rotation, antagonistic plants among other ways.

Plants considered to be antagonistic are those that negatively affect the population of nematodes, like trap plants (when the nematode penetrates, but does not complete its development) unfavorable host (when there is no penetration, but a few nematodes develop) and those containing nematicide/nematostatic compounds in their tissues, which can either be released into the external environment or act only within the plant (Ferraz and Valle, 1997).

The use of plants with an antagonistic effect on nematodes, through interspersed use in planting, intercropping or rotation, is one of the most promising methods to control these organisms. Tells us that the use of antagonists plants in land cover in the form of fresh or dried leaves, leaf extracts applied to the soil, root exudates, saw-dust, seed coverage with extracts or oil, powder seed for soil application and treatment of plant roots by diving in plant extracts, provide significant amounts of organic matter and increase toxic activity, thus improving the general characteristics of the soil (Ferraz and Freitas, 2008).

These plants not only serve to control the infestation of these parasitic organisms, but can also be used as green cover, organic matter, thereby improving the general quality of the soil. The most commonly used species are found mainly in three families, namely Asteraceae mentioned in Zavaleta-Mejia and Gomez (1995) Moreira and Ferreira (2015); Poaceae (Grass) in Dias-Arieira *et al.*, (2003) and Fabaceae in Rosa *et al.*, (2004). Charchar *et al.*, (2009) cites different species of *Crotalaria*, *Mucuna*, *Brachiaria* and *Stylosanthes,* with antagonistic plants.

Since alternate control is an important tool to alleviate the growing populations of plant-parasitic nematodes, the aim of this trial is to evaluate the effect of antagonistic plants in reducing the infestation of two species of nematode galls, *Meloidogyne javanica* and *Meloidogyne enterolobii.*

## Material and methods

*Place and time of conducting the test*

The experiment was conducted in the agricultural greenhouse and Health Plant Laboratory at the Federal Institute of Education, Science and Technology of Ceará - *Campus* Sobral, in the city of Sobral - CE, in the period May to July 2013.

*Obtaining the inoculum and installation of test*

The nematodes were obtained from soil collected in fruit growing areas in Irrigation Perimeter Baixo Acaraú, in Marco-CE. After the collection of materials, part of the samples were sent to the Nematology Laboratory, Department of Plant Science, Science Center Agricultural, Federal University of Ceará, *Campus* do Pici, in Fortaleza - CE, between February 20, 2013 and April 16 2013 for subsequent identification of the species contained in the soil. For the extraction of plant-parasitic nematodes from the soil the technique described by Barker & Hussey (1973) was performed. In order to identify them, the technique according to the pattern of esterase was used, and the technique developed by Carneiro and Almeida (2001), was employed confirming accurate and secure identification of plant pathogens. Subsequently they were multiplied in agricultural greenhouse in 'Santa Clara' tomato plants.

The antagonistic plants were grown and then picked in the Agricultural IFCE greenhouse - *Campus* Sobral, except the neem which was taken from adult plants that make up the forestation of said *Campus*; these were then collected and taken to the Plant Health Laboratory and cut into small pieces with pruning shears and weighed.

*Variables analyzed*

To conduct the test, ‘Santa Clara’ tomatoes were cultivated in pots with 2.5 liter capacity for 60 days in soil infested with the two nematodes, and then proceeded to the uprooting them and later incorporation of 142.94 g of biomass of each species. After a week the new seedlings of 'Santa Clara' tomato were transplanted. 50 days after transplanting, we performed the final evaluation of the test, measuring them according to: **a**. plant height: measuring with graduate centimeter ruler; **b**. number of sheets: counting the number of leaves per plant; **c**. dry weight of the aerial parts: After the measurements, the material was cut with the aid of pruning shears, was then packed in paper bags and placed in a forced circulation air oven for 24 hours at 85 °C; after this period the cases were removed from the oven and heavy material 0.01 g precision balance; **d**. number of galls: the number of galls present on each root system were counted with the aid of a stereomicroscope; **e**. number of eggs/juveniles per root system: After counting the number of galls the extraction of the nematodes took place, and this technique was performed as described by Barker & Hussey (1973) and measured with the aid of an optical microscope.

*Experimental design statistical analysis*

A completely randomized design, CRD in a factorial scheme 5 x 2 + 2 was used, with five species of antagonistic plants sorghum (*Sorghum bicolor*), crotalaria (*Crotalaria juncea*), mucuna (*Mucuna Pruriens*), guandu bean (*Cajanus cajan*) and neem (*Azadirachta indica*), two species of nematodes (*Meloidogyne javanica* and *M. enterolobii*), with 12 repetitions each. Two controls, one positive (tomato seedlings transplanted into infested soil, to demonstrate the quality of the inoculum) and one negative (tomato seedlings transplanted into sterile soil).

Data were tabulated with the help of the spreadsheet in Microsoft Excel®, then the analysis of variance was carried out, which was held at the statistical program Assistat®, 7.4 *beta*, and the variances compared by test F at level of 1.0% probability, and the statistical analyses, when significant, were performed by Tukey test at 1.0% probability.

**Results and discussion**

Table 1 shows the results of the statistical analysis. In the values obtained in the search for the nematodes factor, it was observed that there were no significant results for the variables analyzed. With regard to the antagonistic plants factor, most values showed up as significant with the exception of the Stem diameter (DC), and the same result was observed for the interaction between the factors A and B.

The botanical extracts have great advantages when compared to chemicals; they are rapidly biodegraded and do not have a high toxicity like pesticides, and they may be selective in terms of the organism. In addition, these characteristics are consistent with their use in organic agriculture, and can bring great benefits to production and the environment and these compounds can be studied so they can be handled properly.

Several alternative control methods have been studied because of the difficulties and disadvantages to cultivation, environment and man of using chemicals (Ferraz *et al.* 2010). The use of antagonist plants and/or traps in rotation schemes or consortium planting has proven a very attractive alternative for the control of nematodes (Moreira, 2007; Moreira and Santos, 2015).

It can also be used as a way to improve the chemical and physical qualities of soil, as its decomposition becomes a source of nutrients, which in turn helps in the mineral nutrition of the plants by means of transformation into organic matter. These are all benefits in the fight to control plant parasitic nematodes.

Table 2 presents the results regarding the number of galls caused by *M. javanica* observed in the root systems of the positive control. It was higher than the number observed in the positive control in *M. Enterolobii*. Although they were smaller than the galls caused by *M. enterolobii* in treatments the averages obtained were satisfactory as with regard to the galls on *M. javanica* better control was observed in all treatments relative to *M. enterolobii*. This is because, despite showing a higher number of galls on positive control in treatments it showed a smaller amount compared to the amount of *M. enterolobii*, so observing the difficulty in controlling the infestation of *M. enterolobii*.

**Table 1**. Summary of the analysis of variance with mean squares of the variables plant height (PH), number of leaves (NL), stalk diameter (SD), fresh weight of roots (FWR), weight dry aerial part (WDAP ), number of galls (NG) and number of galls per root system (NGRS) in tomato plants depending on biomass incorporation of the five species of antagonistic plants. IFCE - *Campus* Sobral. Sobral-CE, 2015.

| Sources of variation | GL | Average square | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | PH | NL | SD | FWR | WDAP | NG | NGRS |
| Nematodes (A) | 1 | 18.10714$^{ns}$ | 25.19048$^{ns}$ | 0.05101$^{ns}$ | 272.66423$^{**}$ | 0.07088$^{ns}$ | 22737.19048$^{**}$ | 602.67857$^{**}$ |
| Antagonist Pls. (B) | 6 | 1754.38095$^{**}$ | 39.20635$^{**}$ | 2.78918$^{ns}$ | 175.62762$^{**}$ | 23.97987$^{**}$ | 114062.79762$^{**}$ | 981.68651$^{**}$ |
| Interaction (A x B) | 6 | 1675.96825$^{**}$ | 22.13492$^{**}$ | 2.24990$^{ns}$ | 391.11745$^{**}$ | 21.35166$^{**}$ | 173110.05159$^{**}$ | 1685.28968$^{**}$ |
| Treatments | 13 | 1584.63095$^{**}$ | 30.24908$^{**}$ | 2.32965$^{ns}$ | 282.54882$^{**}$ | 20.92770$^{**}$ | 134290.32967$^{**}$ | 1277.27198$^{**}$ |
| Residue | 70 | 198.89286 | 6.47619 | 1.27287 | 17.10202 | 2.60270 | 470.65238 | 24.56429 |
| DMS (A) | - | 6.13980 | 1.107 | 0.49118 | 1.80040 | 0.70236 | 9.44484 | 2.1577 |
| DMS (B) | - | 17.4653 | 3.151 | 1.39720 | 5.12142 | 1.99792 | 26.86685 | 6.1378 |
| CV (%) | - | 15.09 | 16.34 | 18.40 | 28.41 | 10.95 | 11.81 | 33.28 |

** Significant at 1.0% probability by F test

ns – Not significant by F test.

As noted in other variables, the treatments that worked best were Neem (N) and sorghum (S) in the control of *M. javanica* and *M. enterolobii*, noting the lowest average for these treatments, there was no statistical difference between the values obtained for these two plants. Neem has been used in different ways in the research into the control of Plant-parasitic nematodes: soil cover with fresh or dried leaves, leaf extracts applied to the soil, root exudates, powder-saw, seed coverage with extracts or oil, powder seed applied to the soil or as a seed coverage of interest in agriculture, treatment of roots of plants per dip in plant extracts, etc. (Chitwood, 2000).

According to Chitwood (2002) this is because many plant species produce nematicide substances such as alkaloids, fatty acids, *iso*-thiocyanates, glycosides, terpenoids, phenolic compounds, among others.

The effect of neem against nematodes is probably due to the presence of various chemical substances such as azadirachtin, nimbin, salanin, among other (Chitwood, 2002). According to Schumettere (1997) the products do not affect the neem based spiders or the adults of various species of beneficial insects. He believes that due to their relative selectivity, these products can be recommended in many integrated management programs as they will probably not pollute the environment.

Many pesticide formulations made from neem have been developed in the United States, India and other countries, mainly for use as insecticides (Margosan-O®, Nimbecidine®, Neemgold®, Neemazal®, Neemax®, Fortune Aza®, Neemix®, Achook®, Neemrich®, Neemark®, Econeem®, Rakshak®, Repeln®, Welgrow®, Azatin®, Turplex®, Align®, Bioneem®, Benefit® and others). Some of these products have shown good nematicide activity (Akhtar, 2000).

According to the results shown in Table 2, the treatment showed better efficacy in reducing root galls per gram than was used Neem (N) *M. javanica*, along with sorghum (S) that can be regarded as the same effect of these plants, because their results did not differ among themselves. To control damage caused by *M. enterolobii* it was found that treatment with leaves of neem (N) showed a greater reduction in the number of galls per root grass, followed by sorghum (C), which also showed a great reduction where the median values of these two treatments differed statistically from each other and from other treatments. Andrade & Ponte (1999) and Ribas *et al.*, (2002) evaluated the use of *C. juncea* and *C. spectabilis* Roth. intercropped with okra (*Abelmoschus esculentus* Moench.), and observed a reduction in the number of galls formed by Plant-parasitic nematodes, in okra roots and a consequent increase in productivity. This was, however, in conventional cultivation.

**Table 2.** Average of negative control (NC), positive control (PC) concentrations of antagonist plants: Crotalaria, Mucuna, Sorghum, Neem and Guandu bean incorporated in soil with tomato plants in the alternate control *Meloidogyne javanica* and *M. enterolobii*. IFCE – *Campus* Sobral, Sobral-CE, 2015.

| Nematodes species | Antagonistic plants | | | | | | |
|---|---|---|---|---|---|---|---|
| | NC | PC | Crotalaria | Mucuna | Sorghum | Neem | Guandu bean |
| | ……… Number of galls……… | | | | | | |
| *M. javanica* | 0.00Aa | 580.16Ab | 181.16Ac | 123.16Ad | 87.66Ae | 73.66Ae | 140.83Adf |
| *M. enterolobii* | 0.00Aa | 348.33Bb | 226.16Bc | 275.66Bd | 162.00Be | 158.16Be | 214.66Bf |
| | ……… Number of galls per root gram……… | | | | | | |
| *M. javanica* | 0.00Aa | 56.33Ab | 15.50Ac | 14.00Ac | 8.16Acd | 7.33Ad | 14.16Af |
| *M. enterolobii* | 0.00Aa | 34.50Bb | 12.33Bc | 10.83Bc | 1.66Bc | 5.33Ad | 16.33Afc |
| | ……… Plant height……… | | | | | | |
| *M. javanica* | 110.6Aa | 68.00Ab | 87.66Ac | 96.50Aac | 106.16Aac | 88.33Adc | 83.16Ad |
| *M. enterolobii* | 122.3Ba | 74.33Ab | 71.00Bcb | 90.66Ade | 111.00Aae | 93.33Ae | 105.00Bae |

Capital letters mean differences between columns.
Lowercase letters mean differences between lines.

The results in Table 2, which also shows the height of the plant data, show that the plants infested by *M. enterolobii* Sorghum and treated with (S) had the best development, showing the effectiveness in controlling plant parasitic nematode, which can be compared with the results observed in treatments with Mucuna (M), Neem (N) and Guandu bean (G), which the same effect, and were not therefore statistically different (between these three). The treatment with Crotalaria (C) with an average of 71.0 cm, was not statistically different compared to positive control.

The development of the tomato with the treatment of Sorghum (S), also proved to be more effective in controlling as it grew higher than the other tomatoes infected with *M. javanica* and treated with other antagonists plants. Statistically there was a difference compared to positive control, in which the results with Mucuna, Crotalaria and Neem, were not statistically different between such treatments. In pea and bean crops irrigated by conventional sprinkler systems, pivot rotation with the prior crotalarias (*Crotalaria juncea* or *C. spectabilis*), corn (*Zea mays*) and sorghum (*Sorghum vulgare*) is used by farmers to practice population reduction in nematodes of the genus *Meloidogyne* (Charchar and Aragão 2005;

Charchar *et al.* 2009).

Species sorghum (*Sorghum sudanense* and *S. bicolor*) containing durrin cyanogenic-glycoside with nematicidal activity proved able to reduce by 55% the penetration of juveniles of *M. hapla* in lettuce roots (Widmer and Abawi, 2000).

In the USA, grain sorghum is used for rotation with soybeans in areas infested with nematodes, because the available genotypes are considered poor hosts from *M. arenaria*, *M. incognita* and *M. javanica* (Rich and Kinloch, 2012). In Brazil there is no information on the use of sorghum for the management of *M. javanica* (Brida, 2012).

**Table 3**. Average of negative control (NC), positive control (PC) concentrations of antagonist plants: Crotalaria, Mucuna, Sorghum, Neem and Guandu bean incorporated in soil with tomato plants in the alternate control of *Meloidogyne javanica* and *M. enterolobii*. IFCE – *Campus* Sobral, Sobral-CE, 2015.

| Nematodes species | Antagonistic plants | | | | | | |
|---|---|---|---|---|---|---|---|
| | NC | PC | Crotalaria | Mucuna | Sorghum | Neem | Guandu bean |
| | ........................................... Number of leaves........................................... | | | | | | |
| *M. javanica* | 14.33Aad | 11.16Abd | 17.33Aac | 17.00Ac | 16.00Bcd | 15.50Acd | 12.66Bd |
| *M. enterolobii* | 17.66Bab | 13.83Bbc | 14.83Bcd | 15.66Bde | 20.33Aa | 15.66Ade | 16.00Ae |
| | .......................................... Fresh root weight.......................................... | | | | | | |
| *M. javanica* | 11.01Aa | 10.41Aa | 13.11Ba | 8.96Ba | 10.65Aa | 11.52Ba | 10.86Ba |
| *M. enterolobii* | 10.70Abf | 10.09Acf | 23.57Ad | 26.56Ad | 12.52Aef | 30.23Aa | 13.53Af |
| | .......................................... Diameter of stalk........................................... | | | | | | |
| *M. javanica* | 5.79Aa | 6.18Aa | 5.53Aa | 5.49Ba | 5.63Aa | 7.06Aa | 5.99Aa |
| *M. enterolobii* | 5.77Aa | 5.64Aa | 5.50Aa | 7.14Aa | 6.35Aa | 7.09Aa | 6.64Aa |
| | ....................................... Dry weight of shoot.................................... | | | | | | |
| *M. javanica* | 16.36Ba | 12.96Abc | 14.31Ac | 13.42Adc | 15.20Aa | 14.60Aac | 14.18Bc |
| *M. enterolobii* | 19.43Aa | 12.03Ab | 12.60Bcb | 14.11Adb | 15.98Ae | 15.65Ae | 15.39Ae |

Capital letters mean differences between columns.
Lowercase letters mean differences between lines.

According to the results shown in Table 3, the roots infested by *Meloidogyne enterolobii* showed a greater weight than the weight of roots infested by *M. javanica*, and compared to treatments with antagonistic plants for the control of *M. javanica*, treatment with Crotalaria (C) had the highest average. It can be noted that these results were not statistically different, proving that treatments with antagonist plants did not influence the results of the fresh weight of the roots. To control *M. enterolobi*, the treatment that stood out with good results was the Neem (N) with a fresh weight of the root system superior to other treatments, in which this result was statistically different compared to the result obtained for the treatment with same plant. For the control of *M. javanica*, neem also differed from the other results with different plants used in research, thus showing its influence in improving the fresh weight of the roots.

In relation to the number of leaves, plants that showed the largest number of these leaves were treated with Sorghum (S), with an average of 20.3 leaves, the development of these plants in terms of the number of leaves shows a good result, by reducing the damage caused by *M. enterolobii*, statistically differing from all other treatments by antagonists, and compared to the results observed for the control of *M. javanica*, there was also a statistical difference. For the control *M. javanica* the most effective treatment in relation to the number of leaves was found to be Crotalaria (1C), with an average of

17.3 leaves. The literature depicts the effectiveness of this legume plant parasitic nematodes in control, besides having an efficacy of green manure and fixing N to the soil.

Many *Crotalaria* species may be non-host or host, may increase the population of antagonistic microorganisms and even produce nematotoxic compounds (Wang *et al.*, 2002). Several species are used in green manure, improving crop yields in succession. *Crotalaria juncea*, for example, produces about 30 tons of green mass by hectare can may fix up to 450 kg N.ha-1.year-1 (Wutke, 1993).

Villar and Zavaleta-Mejia (1990) observed in two experiments in the greenhouse, that the incorporation of *C. longirostrata* waste into the ground was enough to reduce galls on tomato roots caused by *M. incognita* and *M. arenaria*.

Among the promising Fabaceaes for the practice of green manure in organic farming, are: the velvet bean (*Stilozobium aterrimum* Piper and Tracy), the (*Crotalaria juncea* L.) and the jack bean (*Canavalia ensiformis* D.C.), because they are rustic and efficient vegetative growth plants, adapted to conditions of low fertility and high temperatures (Pereira *et al.* 1992) conditions which are found throughout the year in the Northeast. These species also possess the ability to reduce the density of plant parasitic nematodes. The effectiveness of these plants in controlling the development of plant-parasitic nematodes is of great interest and socio-economic importance.

For the results relating to the diameter of stems of tomato plants, the best results were observed in velvet bean treatments (M) and Neem (N) *M. enterolobii*, and treatments Neem (N) and Guandu bean (G) to *M. javanica*, showing they have more vigorous stems, noting that these results showed no statistical difference among themselves.

With the dry matter of the shoot, the most promising results were obtained in treatments with sorghum (S) and Neem (N). *M. enterolobii*, has the highest values of dry matter in relation to the other treatments which did not differ statistically between their values, but statistical differences compared with the control. With regard to the *M. javanica* treatments, the best treatments were found to be Sorghum (S) and Neem (N). It is important to note that sorghum and Neem performed well, showing vigorous plants compared to treatments studied for both plant parasitic nematode species in the study. According to Inomoto *et al.* (2006) some Poaceaes and Fabaceaes already known to have effects on other species of nematode galls.

As observed in the results presented in this research, treatments with antagonist plants can be used as an alternative control of plant-parasitic nematodes. Highlighted in this experiment are the extracts of Neem and sorghum plants, which have an excellent response in reducing the negative effects caused by these plant parasites, and thus can be used in the cultivation of plants susceptible to such plant parasitic attack and may also improve the physical characteristics of the soil to be incorporated.

Since one can always devise several pieces of research to look at the beneficial effects of these plants, it is proposed to study the substances present in these antagonist plants, which cause inhibition of plant-parasitic nematode proliferation of this kind, in the plant roots.

**Conclusions**

- Efficacy was found in two antagonistic plants in decreased proliferation of these pathogens, and these Neem and sorghum plants for the control of *M. javanica* and *M. enterolobii*;
- The species *M. enterolobii* was more aggressive compared to *M. javanica*, causing major damage;
- The species *M. javanica* is more likely to be controlled with antagonistic plants.

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