CROTALARIA AND MILLET AS ALTERNATIVE CONTROLS OF ROOT-KNOT NEMATODES INFECTING OKRA

CROTALÁRIA E MILHETO COMO ALTERNATIVAS NO CONTROLE DE NEMATOIDES DE GALHA INFECTANDO QUIABEIRO

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ABSTRACT: The relationship of crops grown in rotation or in succession has increased every day and the use of antagonistic plants and/or non-host plants is one of the most efficient practices of integrated management of nematodes. This study aimed to evaluate the efficiency of crotalaria (*Crotalaria spectabilis* Roth) and millet [*Pennisetum glaucum* (L.) Leeke] 'ADR 300' in reducing the population of *Meloidogyne incognita* and *M. javanica* and in increasing the productivity of okra [*Abelmoschus esculentus* (L.) Moench] when cultivated in succession. The experiment was conducted in an area cultivating okra (host culture) in rotation, with a history of severe infestation by phytonematodes. The experimental design involved randomized blocks with six treatments and four replicates, with the following treatments: T1, 15 kg.ha^{-1} of millet seeds; T2, 30 kg.ha^{-1} of crotalaria; T3, 10 kg.ha^{-1} of millet + 20 kg.ha^{-1} of crotalaria; T4, 20 kg.ha^{-1} of millet + 6 kg.ha^{-1} of crotalaria; T5, 6 kg.ha^{-1} of millet + 36 kg.ha^{-1} of crotalaria; and T6, control. The nematode populations in the soil and roots were evaluated about 60 d after planting okra, and the yield was evaluated at the end of the crop cycle. Simple treatment with millet or crotalaria reduced the nematode population by 61% and 72%, respectively. The millet-crotalaria intercropping treatments reduced the nematode population by up to 85% compared with the control. In terms of productivity, there was an increase of 787 kg.ha^{-1} in the millet treatment and 2,109 kg.ha^{-1} in the intercropping treatments. Both the single cultivation of crotalaria or millet and the consortia of crotalaria and millet were effective in controlling the root-knot nematodes, and increased the productivity of okra.

KEYWORDS: *Meloidogyne*. *Crotalaria spectabilis*. *Pennisetum glaucum.* Intercropping. Cover crop. Green manure.

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) are considered, among all plant-parasitic nematodes, to be the main agents that damage crops worldwide (SAUCET et al., 2016). In tropical climates, they are even more menacing, as the environmental conditions favor their development and reproduction (HUSSAIN et al., 2012).

Okra, *Abelmoschus esculentus*, typically cultivated in warmer regions, is highly affected by *Meloidogyne* spp. (HUSSAIN; MUKHTAR; KAYANI, 2011; MUKHTAR et al., 2014), with *Meloidogyne incognita*, *M. javanica*, *M. arenaria*, and *M. enterolobii* being the most frequently observed species on the cultivated crops (PINHEIRO et al., 2013).

Okra plants infested with *Meloidogyne* spp. exhibit reduced growth, yellowing of leaves and wilting, as the species damages the plant root system, thus reducing productivity, and can lead to the total loss of the crop (DARAMOLA et al., 2015). In addition, the roots infested and damaged by this pathogen become more susceptible to secondary infections by fungi and bacteria (MOTA et al., 2013).

According to Marin et al. (2017), because of the high risk associated with the root-knot nematodes during the cultivation of okra, many studies have sought to find resistant varieties. However, the varieties resistant to *Meloidogyne* spp. have not been identified within the genus *Abelmoschus* (MUKHTAR et al., 2014). Furthermore, the chemical control of these phytoparasites is often inefficient, mainly owing to their high population density (FERREIRA et al., 2010).

Among the measures employed to control *Meloidogyne* spp., the most prominent are crop rotation and the use of antagonistic plants. According to Ponte and Franco (1981), crop rotation with non-host plants is an efficient method, and is

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used in areas experiencing infestation to reduce nematode populations to levels at which they can no longer damage crops. However, the cultivation of antagonistic plants, both in rotation/succession or in consortium with the main crop, has been gaining interest in the integrated management of nematodes, as they allow to substantially reduce infestation. This is because these plants produce nematicidal substances, making the control more efficient than a simple crop rotation with a non-host plant (FERRAZ et al., 2010).

The use of *Crotalaria spectabilis* and *Pennisetum glaucum* has been reported to be effective in controlling some nematodes in other cultures. However, there are no studies on the use of these plants to control *M. javanica* and *M. incognita* in okra culture, even these nematodes being the most important for the culture (GABRIEL et al., 2019; OSEI et al., 2010). Therefore, this study aimed to evaluate the efficiency of crotalaria, *C. spectabilis*, and millet, *P. glaucum* (L.) 'ADR 300', in reducing the population of *M. incognita* and *M. javanica* and in increasing the productivity of okra cultivated in succession.

MATERIAL AND METHODS

The experiment was conducted in the region of Barretos, São Paulo, Brazil (20º30'45"S, 48°38'08"W), in an area cultivating okra, with a history of infestation of *Meloidogyne* spp.. Soil analysis was performed to verify the presence of nematodes in the area by the Laboratory of Nematology (LabNema) in São Paulo State University, Jaboticabal campus. The results revealed high infestation of *M. incognita* and *M. javanica.* The soil was prepared in a conventional way, with plowing and two harrowing, in order to disrupt and eliminate invasive plants established in the field, thus allowing greater emergence of the seeds.

The treatments with millet ADR 300 and *C. spectabilis* included the following: T1, 15 kg.ha^{-1} (200,000 seeds.ha^{-1}) of millet; T2, 30 kg.ha^{-1} (1,600,000 seeds.ha^{-1}) of crotalaria; T3, 10 kg.ha^{-1} (133,000 seeds.ha^{-1}) of millet + 20 kg (1,060,000 seeds.ha^{-1}) of crotalaria; T4, 20 kg.ha^{-1} (266,000 seeds.ha^{-1}) of millet + 6 kg (320,000 seeds.ha^{-1}) of crotalaria; T5, 6 kg.ha^{-1} (78,000 seeds.ha^{-1}) of millet + 36 kg (1,920,000 seeds.ha^{-1}) of crotalaria; and T6 , control (fallow). A randomized complete block design was used, with four replications and an experimental plot of length and width 5 and 2 m, respectively.

The seeding of C. spectabilis and millet was performed by broadcasting, and after approximately four months, the plants were mowed and incorporated into the soil. Twenty-eight days after incorporated, okra seeds were sown in two planting lines spaced 1 m apart from each other and 0.5 m away from the edge of the plot. Forty-six days after okra sowing, the productivity was evaluated for a period of 14 d, according to the harvest point. At the end of this period, the values obtained per plot were converted to Kg.ha-1.

Approximately 60 d after sowing okra, soil and root samples were collected from each plot. Five plants per plot were collected for root samples and five points were selected for soil samples; 200 g of roots and 1 kg of soil were obtained per point. The samples from different points were conditioned in plastic bags and stored at a mean temperature of 6°C until extraction.

The nematodes were extracted in the soil laboratory of the University Center of the Educational Foundation of Barretos, UNIFEB. The nematodes were extracted from the soil samples (100 cm³) by the centrifugal flotation method in sucrose solution (JENKINS, 1964) and from the roots by the same method with addition of kaolin (COOLEN; D'HERDE, 1972). After extraction, the suspension was taken to the LabNema, where the population of nematodes present in the same was counted in a Peters chamber using an optical microscope (SOUTHEY, 1970).

The species of root-knot nematodes were identified based on the (1) perineal pattern, according to Taylor and Netscher (1974), (2) morphology of the labial region of the males (EISENBACK et al., 1981), and (3) esterase isoenzymatic phenotype obtained by the method of Esbenshade and Triantaphyllou (1990), using a traditional BIO-RAD Mini Protean II vertical electrophoresis system.

The data obtained from the nematological and productivity evaluations were subjected to the analysis of variance by the F test and compared by Tukey's test at 1% probability using SAS version 9.4 (SAS INSTITUTE INC, 2011).

RESULTS AND DISCUSSION

The mean data and grouping of the number of second-stage juveniles (J2) in the soil, second-stage juveniles (J2) in the roots, and eggs in the roots is shown in Figure 1. The control differed significantly at 1% probability from almost all treatments, presenting the highest average for all nematological variables evaluated.

A significant difference in the number of second-stage juveniles (J2) in the soil was observed at 1% probability between the treatments and the control, which presented the highest mean, with 694 individuals. The treatment 6 kg.ha^{-1} of millet + 36 kg of crotalaria exhibited the lowest average (151) for J2 in the soil; however, it did not differ significantly from that of the other treatments.

Table 1. Summary of the analysis of variance for variables of second-stage juveniles number of *Meloidogyne* spp. (J2) in soil, number of J2 in roots, egg number in roots and productivity (kg.ha-1) evaluated in okra after cultivating different combinations of *Crotalaria spectabilis* and *Pennisetum glaucum*.

Sources of variation	*Degrees of freedom*	*Juveniles in soil*	*Juveniles in roots*	*Eggs in roots*	*Protuctivity (Kg.ha^{-1})*
Blocks	3	1.82 ^{ns}	0.8 ^{ns}	2.71 ^{ns}	5.09 ^{ns}
Treatments	5	11.99 **	5.77 **	7.38 **	165.4 **
Error	15	14 025.49	414 112.4	60 949.18	15 251.33
MSD	---	272.08	1 478.4	567.17	283.72
CV%	---	38.85	74.71	89.05	9.94

^{ns} = not significant; ** = significant at 1% probability by the F test. MSD, Minimum Significant Difference. CV%, coefficient of variation.

Although *C. spectabilis* is one of the most studied species in Brazil in relation to nematode control, its use still has some limitations, such as slow initial growth and poor adaptation to certain regions, often resulting in early flowering that, consequently, stunts its growth (FERRAZ; FREITAS, 2008). These factors favor the establishment of invasive plants, which are often hosts of nematodes, resulting in the multiplication of these parasites in the area and in the ineffectiveness of the management system.

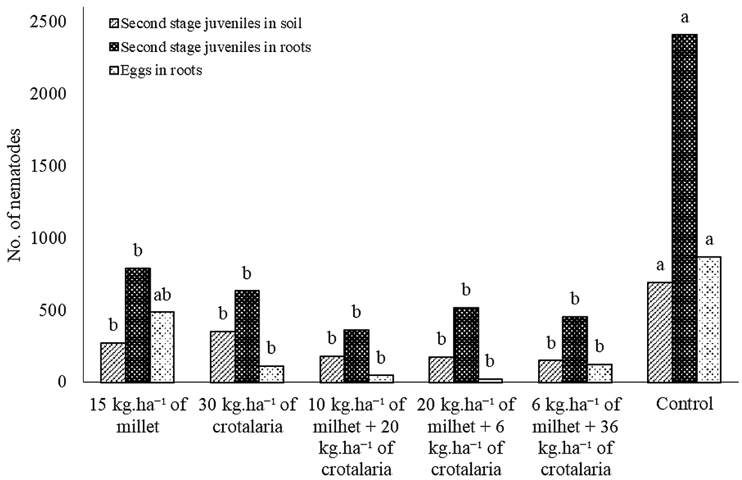

Figure 1. Comparison between the populations of *Meloidogyne incognita* and *M. javanica* found in the soil (100 cm^3) and the roots (10 g) of okra grown in succession with different treatments of *Crotalaria spectabilis* and millet ADR 300, in Barretos, São Paulo, Brazil.
The averages represented by the same letter did not differ statistically by Tukey's test at 1% probability.

ADR 300, in turn, presents a low reproduction factor to *Meloidogyne* spp. (RIBEIRO et al., 2002). It is a good alternative in the management system, because of its characteristics of accelerated initial growth, competitive advantage over invasive plants, and the potential to cover the possible failures in the germination of crotalaria. In addition, millet is an important cover crop, owing to its high potential for straw production and nutrient cycling (NASCENTE; LI; CRUSCIOL, 2013).

There was a difference in the number of J2 in the roots between the treatments and control, which presented the highest average of 2,410 individuals. Among the treatments, the highest average of 789 individuals was observed in the treatment 15 kg.ha^{-1} of millet and the lowest average (363 individuals) was observed in the treatment 10 kg.ha^{-1} of millet + 20 kg.ha^{-1} of crotalaria.

The number of J2 in the soil and in the roots showed that *C. spectabilis* and millet ADR 300, individually or together, regardless of seed density, reduced the populations of *Meloidogyne* spp. In their study on the consortium of okra with *C. spectabilis* to control *M. incognita*, Andrade and

Ponte (1999) obtained similar results, with > 50% reductions in root galls, reflecting an increase in the growth of okra, and consequently, an increase in its productivity.

The highest number of eggs in the roots was observed in the control (869.00 eggs), which did not differ significantly only from the millet treatment 15 $kg.ha^{-1}$, with a mean of 486.80 eggs. With respect to other treatments, the means < 486.80 eggs did not differ from each other. In general, the main cultivated Poaceae members are good hosts of *Meloidogyne* spp.; however, millet is among those with the lowest reproduction factor. This is evident by the reduction in the number of J2, but it was not efficient in reducing the number of nematode eggs when used alone. Carneiro et al. (2007) reported that millet ADR 300 is resistant to race 1 of *M. incognita*. However, it is susceptible to race 3 of *M. incognita* and *M. javanica*, and even with a reproduction factor of 1.4, which is considered low for a susceptible plant, still hosts these species.

Another factor that might favor the maintenance of eggs/J2 in the soil is the non-destruction of the cultural remains of host plants, allowing the females to remain within the roots, and consequently preserve their eggs for a long time in the soil. It is important to observe the interval between the cultivation of millet and okra, in order to reduce the number of roots with galls from the previous crop (DUTRA et al., 2006).

A significant difference in productivity between the treatments was observed, with the highest productivity in the treatments with a consortium of millet and crotalaria. However, among the consortia, the treatment 20 $kg.ha^{-1}$ of millet + 6 kg of crotalaria was the least productive. The treatments 15 kg.ha-1 of millet and 30 kg.ha-1 of crotalaria exhibited the least increase in the productivity of okra, yet they were higher than the control (Figure 2).

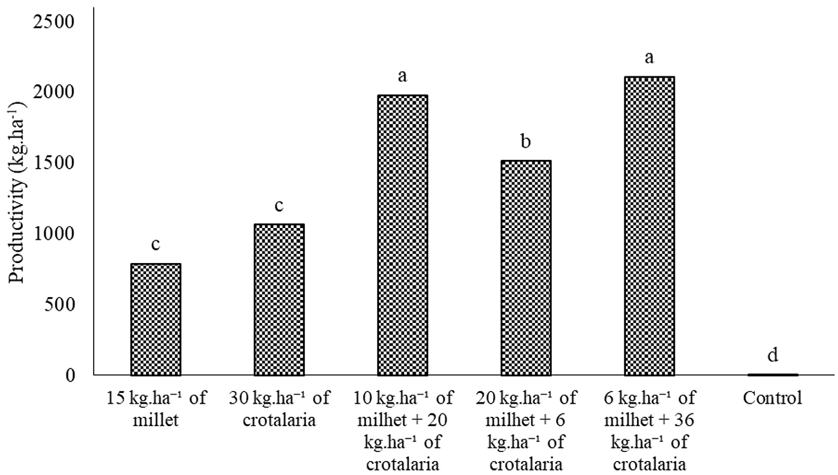

Figure 2. Comparison between the productivity ($Kg.ha^{-1}$) of okra cultivated in succession with different treatments of *Crotalaria spectabilis* and millet ADR 300, in Barretos, São Paulo, Brazil.
The averages represented by the same letter did not differ significantly by Tukey's test at 1% probability.

Several studies have shown an increase in productivity in the subsequent cultivation following the use of crotalaria as a green manure (SANTOS et al., 2010; QUEIROZ et al., 2010). This is mainly because of the contribution of nutrients, such as K, Mg, Ca, and P (PERIN et al., 2010), and also owing to the accumulation of N, the latter being more efficient when there is an association of crotalaria + millet (PERIN et al., 2004). It is noteworthy that, even in small proportions, the single cultivation of crotalaria and millet increased the productivity of okra, indicating that the damage caused by nematodes to the control plants was so severe that it was impossible for them to produce.

The intercropped use between different species of cover plants can bring great benefits to the management system. Menezes et al. (2009) observed greater phytomass production in intercropped cultivation of crotalaria and millet than when they were cultivated alone. In addition, AMORIM et al. (2019) obtained greater control of Pratylenchus brachyurus and greater soybean production in succession when using the intercrop system millet and crotalaria. Both crotalaria and millet are excellent accumulators of phytomass, and their combined use can not only increase the content of organic matter, but also provides a greater diversity of compounds and nutrients in the soil.

Such characteristics provide greater quantity and microbial diversity in the system, enabling soils more suppressive to the phytonematodes and to other root pathogens.

CONCLUSIONS

The use of millet ADR300 and *Crotalaria spectabilis* is efficient, in both single and intercropping cultivation, to reduce the population of *M. incognita* and *M. javanica* infesting okra cultivated in succession.

The use of the consortium, crotalaria + millet, significantly increased the productivity of okra.

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RESUMO: A relação de culturas cultivadas em rotação ou em sucessão tem aumentado a cada dia, e a utilização de plantas antagônicas e/ou plantas não hospedeiras é uma das práticas mais eficientes de manejo integrado de nematoides. O trabalho teve como objetivo avaliar a eficiência do cultivo de crotalária (*Crotalaria spectabilis* Roth) e milheto [*Pennisetum glaucum* (L.) Leeke] 'ADR 300' no incremento de produtividade e redução populacional de *Meloidogyne incognita* e *M. javanica* em quiabeiro [*Abelmoschus esculentus* (L.) Moench] cultivado em sucessão. O experimento foi conduzido em área de rotação com quiabeiro (cultura hospedeira), que tem histórico de grande infestação dos referidos fitonematoides. O delineamento experimental adotado foi o de blocos casualizados com seis tratamentos e quatro repetições, sendo T1 = 15 kg.ha^{-1} de sementes de milheto, T2 = 30 kg.ha^{-1} de crotalária, T3 = 10 kg.ha^{-1} de milheto + 20 kg.ha^{-1} de crotalária, T4 = 20 kg.ha^{-1} de milheto + 6 kg.ha^{-1} de crotalária, T5 = 6 kg.ha^{-1} de milheto + 36 kg.ha^{-1} de crotalária e T6 = Testemunha. Foram avaliadas as populações de nematoides no solo e nas raízes cerca de 60 dias após o plantio do quiabeiro e a produtividade ao final do cultivo. Os tratamentos milheto e crotalária solteiros reduziram a população de nematoides em 61 e 72%, respectivamente. Nos cultivos consorciados, obteve-se a redução de 85%, comparado com a testemunha. Quanto a produtividade, houve acréscimo de 787 kg.ha^{-1} no tratamento com milheto solteiro e de 2.109 kg.ha^{-1} nos tratamentos consorciados. Tanto o cultivo solteiro de crotalária e milheto, quanto seu uso em consórcio, efetivaram o controle dos nematoides de ganha e elevaram a produtividade do quiabeiro.

PALAVRAS-CHAVE: *Meloidogyne*. *Crotalaria spectabilis*. *Pennisetum glaucum.* Consorciação. Plantas de cobertura. Adubação verde.

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