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ACTION OF FORMULATED ROSEMARY AGAINST *Meloidogyne incognita* IN SOYBEAN

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ABSTRACT - The damage caused by nematodes has been severe in many crops in Brazil, causing a reduction in agricultural yield. The objective of this work was to evaluate the efficiency of a formulation, elaborated with extract of the rosemary (*Rosmarinus officinalis*) and cassava bark, on the control of nematode *Meloidogyne incognita* in soybean. The doses evaluated were 0.0; 25; 50; 100; 150 and 200 mL of the dried formulation per pot of 3 L containing soybean plants. The experiment was conducted in a greenhouse, in a randomized block design, with five replications. We evaluated dried and fresh masses of aerial part, fresh mass of root, and the number of egg mass, galls and second stage juveniles (J2) and eggs in root and soil. The soybean plants showed growth reduction resulting from the interaction of the formulation, maybe due a phytotoxic effect from cassava bark. There was allelopathic effect in soybean plants treated with formulated made with cassava bark + aqueous extract of rosemary, evidenced by inhibition in plant development according to the increase in doses of the formulated. Moreover, the formulated was not efficient in the control and or reduction of *M. incognita*.
Keywords: *Rosmarinus officinalis* L., alternative control, root-knot nematode, medicinal plant.

AÇÃO DE FORMULADO DE ALECRIM CONTRA Meloidogyne incognita NA SOJA

RESUMO - O dano causado por nematoides tem sido severo em diversas lavouras do Brasil, provocando redução na produção agrícola. O objetivo desse trabalho foi avaliar a eficiência de um formulado elaborado com extrato de alecrim (*Rosmarinus officinalis*) e casca de mandioca, para controle do nematoide *Meloidogyne incognita* na soja. As doses avaliadas foram de 0,0; 25; 50; 100; 150 e 200 mL do formulado seco por vaso de 3 L contendo plantas de soja. O experimento foi conduzido em casa de vegetação, em delineamento blocos casualizados, com cinco repetições. Foram avaliados parâmetros de crescimento vegetal como massas fresca e seca de parte aérea e massa fresca de raiz, e parâmetros nematológicos como número de massas de ovos, de galhas e de ovos e juvenis de segundo estádio (J2) no solo e raiz. As plantas de soja tiveram redução de crescimento resultante da interação com o formulado, reflexo de possível fitotoxidez da casca de mandioca. Houve efeito alelopático em plantas de soja tratadas com formulado feito com cascas de mandioca + extrato aquoso de alecrim, evidenciado pela inibição no desenvolvimento das plantas de acordo com o aumento das doses do formulado. Além disso, o formulado não se mostrou eficiente no controle e ou redução de *M. incognita*.
Palavras-chave: *Rosmarinus officinalis* L., controle alternativo, nematoide de galhas, planta medicinal.

INTRODUCTION

Brazil ranks second in the world ranking of soybean production, which is the main commodity cultivated in the country. For the 2018/19 season there was an increase in the area cultivated with oilseeds, of 1.9% compared to the previous harvest, corresponding to 35,818.8 thousand hectares (CONAB, 2019).

In Paraná, the estimated area for this crop is approximately 5.42 million hectares, however, it is expected an increase in productivity, and the yield can thus reach 16.2 million tonnes (CONAB, 2019). There are many pests and diseases that impair the development and production in soybean crop, especially nematodes, which can cause direct and indirect damage such as reduced productivity or the infeasibility of cultivation areas, respectively (CORTE et al., 2014).

The nematodes of the genus *Meloidogyne* are easily identified by the formation of galls in the root system. This stands out among the other genera of nematodes worldwide, due to the large number of host plant species and the ability to reduce productivity and quality. More than 90 species of the genus *Meloidogyne* have been identified in different crops worldwide (SILVA et al., 2016; LIMA et al., 2016).

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Production reductions in commercial crops can range from 30% to 50% in areas infested by these nematodes, however, the effect of the attack of this pathogen can intensify when under edaphoclimatic conditions unfavorable to the crop, such as drought and in sandy soils, where damage can reach 100% (FERRAZ, 2018).

Currently, conventional agriculture is dependent on the use of agrochemicals, which can generate imbalance in soil microbial activity. This imbalance ultimately favors the development of pathogens, especially nematodes, which cause losses equivalent to US$ 141 billion annually around the world (FLEMING et al., 2016). In addition, the use of chemicals presents high toxicity, which can cause damage to human health and contamination to the environment if overused or as the only form of control (PIMENTEL and BURGUESS, 2014).

In order to try to solve this problem, different methods of controls have been employed, such as chemical control (CORTE et al., 2014), the crop rotation with green manure (PEREIRA et al., 2017), and the use of resistant varieties, although there are still few resistant or tolerant varieties of high efficiency against nematodes (HUSSAIN et al., 2016).

It is known that plants generally produce secondary metabolites (CAMPOS et al., 2016) and that they have various functions, such as protecting against the attack of pathogens and pests (MAUCH-MANI et al., 2017). Extracts of some plant species such as *Azadirachta indica*, commonly known as neem (JAVED et al., 2008), and rosmarinic acid, a substance found in rosemary extracts (*Rosmarinus officinalis)* (RUPPELT et al., 2015) has shown promise in the control of nematodes (MATTEI et al., 2014; MÜLLER et al., 2016). In addition to these, other plant species have also been studied to control these pathogens, such as Santa-Maria grass (*Chenopodium ambrosioides*) (MELLO et al., 2006), garlic (*Allium sativum*), thyme (*Thymus vulgaris*) (CETINTAS and YARBA, 2010), and fern (*Pteridium aquilinum* (L.) Kuhn) (NEVES et al., 2010). It is also worth mentioning that the "manipueira" – residue of industrial cassava processing – which has nematicide effect (FONSECA et al., 2018) remains little studied.

Among other plant extracts, some agro-industrial residues, such as manipueira, has been used to control nematodes of the genus *Meloidogyne*, presenting significant results (FONSECA et al., 2018). However, there are few studies related to the use of these residues in the control of nematodes of the genus *Meloidogyne* in soybean, especially when referring to other types of cassava processing waste, such as the bark.

In order to add value to an agro-industrial residue of wide availability, both in Brazil and in other countries, cassava bark can be used as a component to make available plant extracts, since direct application of the extract in the soil may be ineffective or economically unfeasible when it comes to large areas of cultivation.

Thus, the objective of this work was to evaluate the efficiency for controlling the nematode *M. incognita* in soybean with aqueous extract of rosemary formulated in cassava bark.

MATERIAL AND METHODS

The experiment was conducted in greenhouse. The experimental design was randomized blocks (DBC), with six treatments (0.0 mL; 25 mL; 50 mL; 100 mL; 150 mL and 200 mL per pot 3 L of rosemary formulated) and five replicates.

Cassava bark were obtained in cassava industry and then washed in a sieve in running tap water in order to eliminate possible impurities. Subsequently, they were dried at 60ºC until constant mass, and ground in a knife mill and then were sieved in order to maintain a particle size of 2 mm. A sample of this formula was used to perform chemical analysis.

Fresh rosemary leaves, harvested in spring and during the morning (between 10:00 h and 12:00 h), were crushed with distilled water in the proportion of 12% (mass/volume) in a blender. This suspension was filtered in cheesecloth for removing of solid waste and, subsequently, the mixture was made in a ratio of 1:1, being one part of cassava bark and one part of rosemary extract. This mixture was dried at 60ºC for 48 h. After this procedure, the mass was deboroated and re-sieved to maintain a 2 mm granulometry, thus obtaining the formulated used in the assays.

The substrate for the pots was prepared in the proportion of 1:1:2 (v:v:v) of sand, vermiculite and claysoil. This substrate was autoclaved (120ºC, 1 atm por 1 h) and packed in 3 L pots, being irrigated for 15 days before soybean sowing.

To perform the treatments, the formulated in the indicated dosages was incorporated into the 0.05 m surface of the substrate placed in the pots, in order to simulate the distribution of the formulated next to the seed in the sowing furrow. After incorporation, five seeds of the cultivar BRS 284 (conventional soybean) was sowed in every vessel of 3 L. When the plants were in phenological stage V1, thinning was performed resulting in only one plant per pot.

The inoculum of *M. incognita* used was obtained from Santa Cruz tomato plants kept in a greenhouse. When most soybean plants were in phenological stage V2, nematodes were inoculated at the concentration of 900 eggs + second stage juveniles (J2) mL^{-1} aqueous suspension per pot. After inoculation the plants were cultivated for 50 days, and in this period there was daily replacement of soil moisture by manual irrigation in order to reach the field capacity.

At the end of this period, the plants were removed from the pots and there was separation between shoots and roots. The aerial parts were packed in paper bags for drying in a forced air circulation oven at 60ºC for 48 h. The roots were washed in running water and packed in plastic bags for nematological assessments.

The roots were stained with Floxin B for 20 min to facilitate the identification of nematode egg masses and subsequent counting. After counting the number of egg masses and galls in the roots, they were submitted to nematode extraction procedures, using the methodology described in Freitas et al. (2007), based on the methodology of Hussey and Barker (1973) modified by Boneti and Ferraz (1981), and the method of Coolen and D'Herde (1972) with centrifugal flotation in sucrose solution. Later, with the use of an optical microscope and Peters' camera, eggs and J2 were quantified. The data were tabulated and submitted to regression analysis by the Sigma Plot 12.0 program.

RESULT AND DISCUSSION

According to the results obtained, it can be inferred that the treatments had an influence on the biometric parameters evaluated in soybean plants. From germination and the first phenological stage it was possible to notice the difference in plant development according to the increase of formulated doses compared to the control (Figure 1).

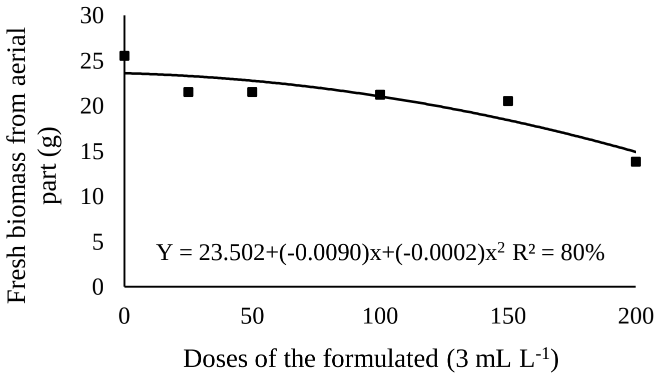

FIGURE 1 - Fresh biomass from aerial part (g) of soybean plants inoculated with *Meloidogyne incognita* and grown on a substrate containing doses of formulated cassava bark + aqueous rosemary extract.

Figure 2 shows a similar behavior to that found in Figure 1, in which it is clear the inhibitory effect of the development of soybean plants caused by the use of the formulated made of cassava bark + rosemary, indicating allelopathic effect. Studies carried out by Barbosa et al. (2012) demonstrated a reduction in the length of lettuce roots according to the increase in doses of rosemary extract, thus reinforcing the presence of allelopathic compounds in this medicinal plant.

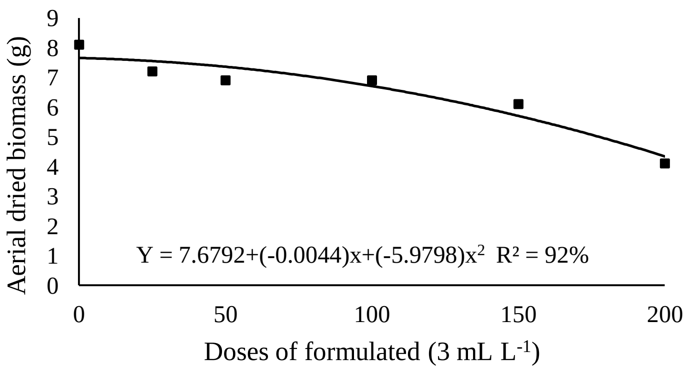

FIGURE 2 - Aerial dried biomass (g) of soybean plants inoculated with *Meloidogyne incognita* and grown on a substrate containing doses of formulated cassava bark + aqueous rosemary extract.

Cassava bark, as well as manipueira, are by-products of cassava processing, so there are similarities between these materials, like the presence of cyanides and the change in pH over time, and the greater volatilization of cyanide is linked to the lowering of the pH. These data corroborate studies conducted by Leonel and Cerada (1995), which evaluated the pH of the manipueira in different times and concluded that the pH decreased from 6.2 of the first 24 h to 4.0 and stabilized at 3.6 with 72 h. The incorporation of this residue with low pH in a low pot volume may have reflected in a higher acidification of the substrate and a nutritional restriction on soybean plants in this experiment.

Figure 3 shows a decreasing and linear behavior in the root masses in relation to the increase in the doses of the formulated. This reduction in the number of roots carries serious risks to soybean plant, since it restricts the volume of soil exploited by them and the absorption of water and nutrients, in addition, the effects of this inhibition on root masses can be aggravated in situations of water deficit.

Chemical characterization studies of industrial cassava residues have shown that this material is rich in nitrogen and potassium and can be used as a source of organic fertilizer for various crops. According Fioretto (1994) has a potassium content higher than 2.000 mg L^{-1}, while for nitrogen, has mean values of 5.51 kg m^{-3} urea (45% N). The influence of organic compounds on pH changes in soils occurs mainly due to the nitrification process (YAN et al., 1996). This was evidenced in works performed with brachiaria as rotation crop and subsequent incorporation, and they showed decreased pH over time (SANTOS et al., 2012).

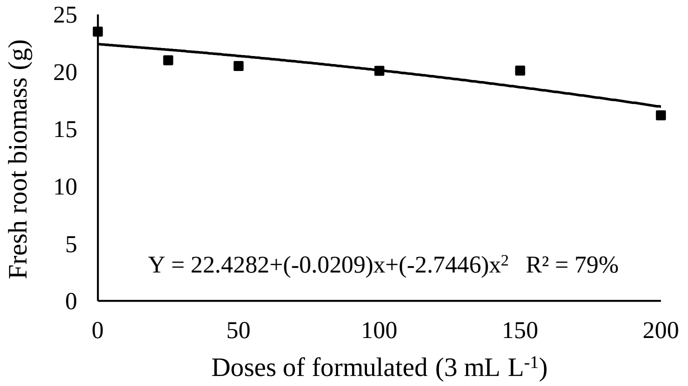

FIGURE 3 - Fresh root biomass (g) from soybean plants inoculated with *Meloidogyne incognita* and grown on a substrate containing doses of formulated cassava bark + aqueous rosemary extract.

This event may have occurred with the decomposition of the formulated cassava bark + rosemary used here, emphasizing the reduction of the pH of the substrate solution in the pot. Thus, acidification of the substrate could have occurred through nitrification. In addition, toxicity may have occurred due to excess of potassium, since cassava bark have high levels of this element. This hypothesis corroborates with Salton et al. (2002), where there was a marked reduction of shoots, root system, plant height and root system length of soybean plants, depending on the increase in potassium doses. This behavior was very similar to that obtained in this work due to the increase in doses of the formulated.

The results obtained from the chemical analysis of the formulated for pH in water and potassium content showed an average of 4.98 and 1.047 mg dm^{-3}, respectively. The potassium level found in the formulation corroborate those found in the literature, however, would not be sufficient to cause excess phytotoxicity, since it is an organic compound and its release and dynamics in the soil are linked to the decomposition of the formulated.

According Lopes (1998), at pH above 6.0 there is the highest availability of potassium in the soil solution, values lower than 6.0 start to make the same unavailable, and at pH 5.0 there is the lowest release of this element in the soil for the plants. The effects of toxicity and potassium deficiency are very similar in soybean plants, in addition, allelopathic effects are still little known, more studies are needed.

Concerning the number of eggs and J2, it can be inferred that there was no significant regression adjustment for such data, because in the doses of 25 and 150 mL a greater presence of individuals was noted than in the other doses or even in relation to the control without the formulated (Figure 4).

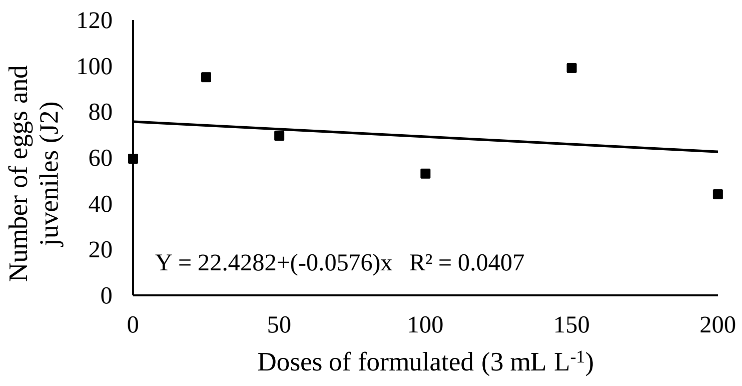

FIGURE 4 - Number of eggs and juveniles of second stage (J2) *Meloidogyne incognita* in soybean plants, grown on substrate containing doses of formulated cassava bark + aqueous rosemary extract.

This behavior may be related to the specific characteristics of interaction between plant extracts, since they release secondary metabolites, which may be toxic to nematodes. In addition, it is important to mention the weakness of soybean plants in relation to the allelopathic effects resulting from the formulated, which, considering that *M. incognita* is a biotrophic pathogen, would greatly limit its nutrition and development.

These results show the need for further studies on the interaction of plant extracts in plants and its ability to control nematodes.

CONCLUSIONS

From this work it becomes clear the alelopathic effect in soybean plants treated with formulated made with cassava bark + aqueous rosemary extract, evidenced by the inhibition in the development of the plants, according to the increase of the doses of the formulated.

In addition, the formulation has not been effective in controlling and/or reducing *M. incognita*.

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