

# ESSENTIAL OIL OF *Rosmarinus officinalis* IN THE CONTROL OF *Meloidogyne javanica* AND *Pratylenchus brachyurus* IN SOYBEAN

## ÓLEO ESSENCIAL DE *Rosmarinus officinalis* NO CONTROLE DE *Meloidogyne javanica* E *Pratylenchus brachyurus* EM SOJA

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**ABSTRACT:** The aim of this study was to assess the nematicidal activity of the essential oil of *Rosmarinus officinalis* applied directly to the soil to control *Meloidogyne javanica* and *Pratylenchus brachyurus* in soybean. The experiments were conducted in the greenhouse in a fully randomized design with a 4 x 2 factorial arrangement consisting of four concentrations of oil (0, 1, 2 and 3%) and two treatment frequencies (fortnightly and monthly), with four replications. Plantlets of soybean cv. CD 206 were transplanted to 1-liter pots and the first treatment applied one day after transplanting. One week after this treatment, the pots were inoculated with 2000 eggs of *M. javanica* or 1000 specimens of *P. brachyurus* per plant. The plants were collected 60 days after inoculation and plant growth variables assessed (height, aerial part fresh weight, root fresh weight and aerial part dry weight). The galls number and eggs per gram of root was assessed for *M. javanica* and the number of nematodes per gram of root and per 100 cm<sup>3</sup> soil for *P. brachyurus*. Was observed that the population of *M. javanica* was reduced by *R. officinalis* essential oil, but none of the concentrations studied affected the population of *P. brachyurus*.

**KEYWORDS:** Alternative control. Rosemary. Root-knot nematode. Root-lesion nematode.

## INTRODUCTION

Nematodes are among the major factors that impair soybean yields in Brazil. The main species that affect the soybean crop are root-lesion nematode *Pratylenchus brachyurus* (Godfrey) Filipjev and Schuurmans Stekhoven and root-knot nematodes *Meloidogyne javanica* (Treub) Chitwood and *M. incognita* (Kofoed and White) Chitwood (DIAS et al., 2010). Of these, *M. javanica* is cited as the most aggressive and widely disseminated in Brazilian soybean cropping areas (EMBRAPA, 1996), and *P. brachyurus* is increasingly worrying because it is occurring more and more frequently and causing significant damage to the crop (DIAS et al., 2010).

Controlling nematodes is complex, especially if alternative methods have to be used to satisfy the requirements of organic crops. In this context, a number of studies have been carried out in an attempt to discover new compounds in plant extracts and oils with nematicidal activity (MANI; CHITRA, 1989; RAO; REDDY, 1992; CHITWOOD, 2002).

The advantages of using plant extracts or oils to control nematodes are lower toxicity, rapid biodegradation, extensive activity and the fact that

they are produced from renewable resources (QUARLES, 1992). Furthermore, these products can be used in organic cropping, where conventional practices are ruled out.

Medicinal and aromatic plants contain many compounds with nematicidal potential, including essential oils (LEELA et al., 1992; OKA et al., 2000). One example of plant that produces essential oils with numerous biological properties is rosemary, *Rosmarinus officinalis* Lam., in the family Lamiaceae (SACCHETTI et al., 2005; SERPA et al., 2006), with proven effects as an acaricide (MIRESMILLI et al., 2006), bactericide (BURT, 2004), fungicide (VESALTALAB et al., 2012) and insecticide (KOSCHIER; SEDY, 2003). Studies conducted by Giordani et al. (2004) attribute the antimicrobial property of rosemary essential oil to the presence of  $\alpha$ -pinene, 1,8-cineole, camphene, limonene, camphor, verbenone and borneol. Of these, cineole is cited by Sangwan et al. (1985; 1990) as effective in the control of juveniles of *Anguina tritici* (Steinbuch) Chitwood, *M. javanica*, *Heterodera* spp. Schmidt and *Tylenchulus semipenetrans* Cobb.

Against this backdrop, the aim of this study was to evaluate the activity of rosemary essential oil

applied directly to the soil for controlling *M. javanica* and *P. brachyurus* in the soybean crop.

## MATERIAL AND METHODS

The experiments were conducted at the Umuarama Regional Campus of the State University of Maringá, with a fully randomized design and 4 x 2 factorial arrangement (four concentrations of rosemary oil and two treatment frequencies), with four replications of each treatment. The experiments were repeated over two different periods: October-December 2011 (2011) and December 2011-February 2012 (2012) for *M. javanica*, and January-March 2012 (2012-A) and February-April 2012 (2012-B) for *P. brachyurus*. In the first experiment, the pots were kept on stand in field conditions and, in the second, in greenhouse.

First, plantlets of soybean cv. CD 206 were grown on 128-cell polystyrene trays containing Plantmax<sup>®</sup> commercial substrate. When the first pair of true leaves was fully expanded (stage V1), the plantlets were transplanted to pots containing 2 L of an autoclaved (120°C for two hours) soil/sand mixture (2:1, v:v).

The treatments consisted of using a solution of rosemary essential oil (Lazlo, 100% pure) diluted in water, using 1% plant oil (Natur'l Óleo) as an adjuvant. We evaluated concentrations of 0, 1, 2 and 3% rosemary essential oil. The solution (9 mL) was applied directly to the soil one day after transplanting, covering a radius in which the root system was probably concentrated. Treatments were applied either fortnightly or monthly.

One week after the first treatment, the pots were inoculated with the nematode, each pot receiving 2000 eggs and any second-stage juveniles (J2) of *M. javanica* or 1000 specimens of *P. brachyurus*. The inocula of *M. javanica* and *P. brachyurus* were obtained from pure populations kept in the greenhouse in tomato (*M. javanica*) and maize (*P. brachyurus*) roots, and the nematodes were extracted using the methods proposed by Hussey; Barker (1973) and Coolen; D'Herde (1972). After extraction, the nematodes were counted on a counting slide under an optical microscope, calibrating the suspension to 500 eggs + juveniles of *M. javanica* mL<sup>-1</sup> and 250 specimens of *P. brachyurus* mL<sup>-1</sup>. Inoculation consisted of inserting 1 mL of the suspension into each of four holes approximately 4 cm deep, opened up in the soil around the plant.

The plants were collected for analysis one week after the last spraying (60 days after inoculation) and the following parameters assessed: plant height, aerial part fresh weight (APFW), root fresh weight (RFW) and aerial part dry weight (APDW). Aerial part dry weight was obtained after drying at 65 °C in a fan oven until the weight remained constant. To assess the nematological parameters, the galls number of *M. javanica* per root system and eggs/juveniles per gram of root were determined. For *P. brachyurus*, in addition to the number of specimens g<sup>-1</sup> root extracted and assessed as described, we also assessed the number of nematodes in 100 cm<sup>3</sup> soil, extracted using the method proposed by Jenkins (1964), which was added to the number of specimens g<sup>-1</sup> root, resulting in parameter number of total specimens.

The data obtained were evaluated by factor analysis, splitting where there was interaction between factors. The F-test at 5 % probability was used to compare treatment mean values. Where essential oil concentration was significant, linear and quadratic regression analysis were applied. For analysis, all the data were transformed using  $\sqrt{(x+0.5)}$ .

## RESULTS

### *Meloidogyne javanica*

The results show that in 2011, plant growth parameters were unaffected by the concentration of essential oil (Table 1), but the treatment frequency was significant for plant height, which fell statistically at more frequent applications. Similar results were obtained in 2012, where essential oil concentration did not affect aerial part development, but fortnightly treatment affected plant height and aerial part dry and fresh weights (Table 1).

The concentration x frequency interaction was significant for plant height in 2011 (Table 1). There were significant differences between plant heights for plants treated fortnightly with a 3% solution of essential oil (57.57cm) and those treated monthly (98.70cm). Aerial part fresh weight was also significant for the interaction, since in 2012, the plants treated with 0% essential oil were shorter and those treated with a 1% solution taller. Splitting also showed that the 0 and 2% solutions applied fortnightly resulted in a lower accumulation of fresh weight in the plants (4.55 and 13.18 g, respectively) by comparison with monthly treatment (6.02 and 13.26 g, respectively).

**Table 1.** Analysis of the variables of plant height and aerial part dry and fresh weight within the factors of rosemary essential oil concentration, treatment frequency and interaction of these two factors for plants inoculated with 2000 eggs and any juveniles of *Meloidogyne javanica* in two agricultural years (2011 and 2012)

Treatment	Height (cm)		Fresh weight (g)		Dry weight (g)	
Concentration (%) / Year	2011	2012	2011	2012	2011	2012
0	76.69 <sup>ns</sup>	26.46 <sup>ns</sup>	14.36 <sup>ns</sup>	8.87 <sup>ns</sup>	4.89 <sup>ns</sup>	2.95 <sup>ns</sup>
1	69.89	75.50	11.95	11.58	3.90	3.14
2	83.13	71.28	14.75	9.64	4.89	3.35
3	78.14	77.31	12.98	12.10	4.30	3.54
Frequency						
Fortnightly	69.17*	57.71*	14.14 <sup>ns</sup>	8.57*	4.67 <sup>ns</sup>	2.55*
Monthly	84.75	87.06	12.87	12.52	4.31	3.94
F-test						
Concentration (C)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Frequency (F)	*	*	n.s.	*	n.s.	*
Interaction (CxF)	*	n.s.	n.s.	*	n.s.	n.s.
R.L.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
R.Q.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
C.V. (%)	9.74	11.98	11.94	18.32	10.73	17.13

\*: significant at 5% probability; n.s.: not significant. At the same treatment frequency, \* indicates a significant difference in the F-test. R.L. = Regression Linear; R.Q. = Regression Quadratic.

Root fresh weight was not affected by isolated factors or interaction, irrespective of the year in which the experiment was conducted (Table 2). In terms of nematological parameters (Table 2), there were no changes in the galls number or eggs per g<sup>-1</sup> root in the 2011 experiment. In contrast, in the 2012 experiment, the higher treatment frequency (fortnightly) resulted in fewer galls, but the number

of eggs/g root was lower for monthly treatment compared to fortnightly treatment. In terms of the number of galls, splitting the interaction showed that only the oil concentration of 0% was significant, with higher values observed for monthly treatment (Table 2). We observed a large difference in the number of eggs/g root, possibly due the conditions under which the experiments were conducted.

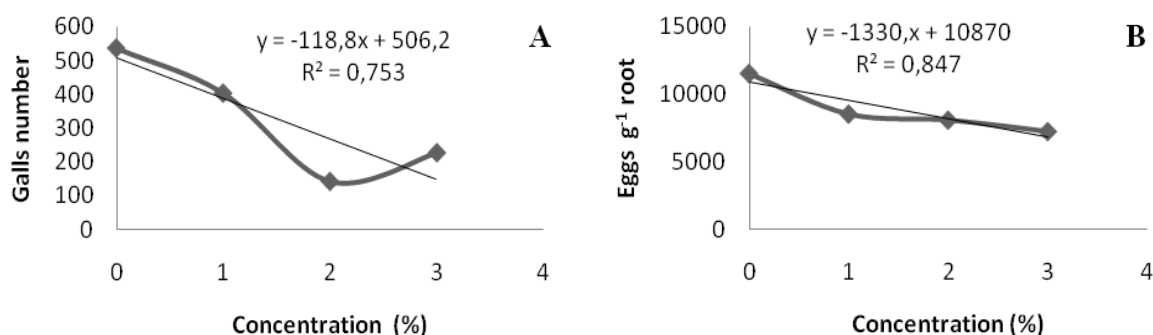
**Table 2.** Analysis of the variables of root fresh weight, galls number of *Meloidogyne javanica* and eggs per gram of root in soybean treated with different concentrations (%) of *Rosmarinus officinalis* essential oil at two frequencies and in two agricultural years (2011 and 2012)

Treatment	Root fresh weight (g)		Galls Number		Eggs g <sup>-1</sup> root	
Concentration/Year	2011	2012	2011	2012	2011	2012
0	25.69 <sup>ns</sup>	16.43 <sup>ns</sup>	661 <sup>ns</sup>	537a	885 <sup>ns</sup>	11556a
1	24.88	21.64	667	404ab	831	8559ab
2	31.09	18.26	741	143c	823	8111ab
3	26.90	22.20	700	228bc	1082	7269b
Frequency						
Fortnightly	29.06 <sup>ns</sup>	15.52 <sup>ns</sup>	703 <sup>ns</sup>	241*	925 <sup>ns</sup>	11356*
Monthly	29.06	21.76	681	414	885	6391
F-test						
Concentration (C)	n.s.	n.s.	n.s.	*	n.s.	*
Frequency (F)	n.s.	n.s.	n.s.	*	n.s.	*
Interaction (CxF)	n.s.	n.s.	n.s.	*	n.s.	n.s.
L.R.	n.s.	n.s.	n.s.	*	n.s.	*
Q.R.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
C.V. (%)	12.35	19.42	18.19	28.08	24.75	14.02

\*: significant at 5% probability; n.s.: not significant. At the same treatment frequency, \* indicates a significant difference in the F-test. R.L. = Regression Linear; R.Q. = Regression Quadratic.

In regression analysis, the linear model gave the best fit to the galls number (Figure 1-A) and especially to the eggs  $g^{-1}$  root (Figure 1-B). In both

cases, a concentration of 3% essential oil produced the best results for controlling the nematode.



**Figure 1.** Regression analysis on the galls number (A) of *M. javanica* and eggs/  $g^{-1}$  root (B) in soybean treated with different concentrations of *Rosmariuns officinalis* essential oil.

### *Pratylenchus brachyurus*

In the experiment on *P. brachyurus*, essential oil concentration was significant for plant development in terms of height in experiment 2012-A and aerial part fresh and dry weight in both experiments (2012-A and 2012-B, see Table 3).

Treatment frequency was significant for aerial part fresh weight in experiment 2012-A, with lower values for monthly application. There was no significant interaction between factors for any of the plant development parameters associated with the aerial part (Table 3).

**Table 3.** Analysis of the variables of plant height and aerial part dry weight within the factors of rosemary oil concentration, treatment frequency and interaction between the two factors for plants inoculated with 1000 specimens of *Pratylenchus brachyurus* in two experiments (2012-A and 2012-B)

Treatment	Plant height (cm)		Aerial part fresh weight (g)		Aerial part dry weight (g)	
Concentration (%) / Year	2012-A	2012-B	2012-A	2012-B	2012-A	2012-B
0	30.01a	30.59 <sup>ns</sup>	2.47a	1.65a	0.96a	0.58a
1	31.34a	26.95	2.25a	1.23a	0.82ab	0.45a
2	25.59ab	25.33	0.85b	1.16a	0.37bc	0.42a
3	20.43b	26.10	1.24ab	0.49b	0.32c	0.26b
Frequency						
Fortnightly	27.33 <sup>ns</sup>	144.09 <sup>ns</sup>	2.09*	1.23 <sup>ns</sup>	0.72 <sup>ns</sup>	0.46 <sup>ns</sup>
Monthly	26.35	25.39	1.31	1.03	0.53	0.38
F-test						
Concentration (C)	*	n.s.	*	*	*	*
Frequency (F)	n.s.	n.s.	*	n.s.	n.s.	n.s.
Interaction (CxF)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
L.R.	*	n.s.	*	*	*	*
Q.R.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
C.V. (%)	12.32	19.57	20.02	12.74	14.37	7.01

\*: significant at 5% probability; n.s.: not significant. At the same treatment frequency, \* indicates a significant difference in the F-test. R.L. = Regression Linear; R.Q. = Regression Quadratic.

In regression analysis, irrespective of the parameter or year in which the experiment was conducted, the linear model produced the best fit (Table 4). In experiment 2012-A, plants were taller than the control only at an essential oil concentration of 1%. Essential oil concentration

significantly affected root fresh weight in experiment 2012-A, and a linear model produced the best fit to explain the results (Table 4). Treatment frequency was also significant for this parameter in experiment 2012-A, indicating that monthly treatment resulted in poorer root system

development. In regard to nematological parameters, only treatment frequency was significant for specimens  $g^{-1}$  root in experiment

2012-B, with a significant drop in numbers following monthly treatment (Table 5).

**Table 4.** Equations and fits for regression analysis on the effects of rosemary essential oil concentration on plant growth parameters in soybean inoculated with 1000 specimens of *Pratylenchus brachyurus* in two experiments: 2012-A and 2012-B

Year	Parameter evaluated	Equation	Fit*
2012-A	Height	$y=3.449x+32.01$	0.815
	Aerial part fresh weight	$y=0.509x+2.466$	0.708
	Aerial part dry weight	$y=0.237x+0.973$	0.911
	Root fresh weight	$y=0.728x+3.997$	0.919
2012-B	Aerial part fresh weight	$y=0.355x+1.665$	0.912
	Aerial part dry weight	$y=0.099x+0.576$	0.944

\*Significant at 5% probability.

**Table 5.** Analysis of the variables of root fresh weight, number of specimens of *Pratylenchus brachyurus* per gram of root and number of total specimens (root + 100  $cm^3$  soil), in soybean treated with different concentrations (%) of *Rosmarinus officinalis* essential oil and at different frequencies in two experiments (2012-A and 2012-B)

Treatment	Root fresh weight (g)		Specimens $g^{-1}$ root		Total specimens	
Concentration (%)/Year	2012-A	2012-B	2012-A	2012-B	2012-A	2012-B
0	3.92a	2.63 <sup>ns</sup>	117 <sup>ns</sup>	360 <sup>ns</sup>	411 <sup>ns</sup>	857 <sup>ns</sup>
1	3.56a	2.25	124	353	458	793
2	2.19ab	2.77	93	240	173	634
3	1.95b	1.81	195	335	303	428
Frequency						
Fortnightly	3.46*	2.64 <sup>ns</sup>	106 <sup>ns</sup>	416*	346 <sup>ns</sup>	880*
Monthly	2.34	2.09	159	228	327	476
F-test						
Concentration (C)	*	n.s.	n.s.	n.s.	n.s.	n.s.
Frequency (F)	*	n.s.	n.s.	*	n.s.	*
Interaction (CxF)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
L.R.	*	n.s.	n.s.	n.s.	n.s.	n.s.
Q.R.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
C.V. (%)	17.76	17.05	25.51	29.43	26.05	31.26

\*: significant at 5% probability; n.s.: not significant. At the same treatment frequency, \* indicates a significant difference in the F-test.

## DISCUSSION

Essential oil concentration did not influence plant development (height, aerial part fresh and dry weight and root fresh weight) in the experiment aimed at controlling *M. javanica*, in line with the results reported by Gardiano et al. (2011), who treated cotton plants with an aqueous extract of rosemary for controlling *Rotylenchulus reniformis* Linford; Oliveira and also found no difference in plant height, aerial part fresh and dry weight and stem diameter compared to the control. In tomato plants, the use of rosemary essential oil did not exhibit any phytotoxic effect (CETINTAS; YARBA, 2010). However, in the experiments to control *P. brachyurus*, plant growth parameters

were significantly affected at higher concentrations. One of the factors that could explain this difference is that the oil was not effective in controlling *P. brachyurus* and nematode parasitism may have made the plant more susceptible to abiotic stresses.

In the experiment to control *M. javanica*, fortnightly treatment with the essential oil resulted in poorer development of the aerial part but did not affect root system development. However, in the experiment to control *P. brachyurus*, fortnightly treatment in experiment 2012-A increased aerial part and root fresh weight. As basic parameters for measuring plant growth, Peixoto (2010) cites accumulated dry matter and leaf area, since he considered the leaf to be a “factory” for producing dry matter. Thus, if there is more fresh weight in the

aerial part, it is expected that the root fresh weight will also be higher.

With the aim of controlling *M. javanica*, in studying the interactions between oil concentration and treatment frequency, we observed that some parameters were affected by a higher treatment frequency, such as plant height at a concentration of 3% and aerial part fresh weight at concentrations of 0 and 2%. One hypothesis that could explain these results is the phytotoxic effect of the essential oil. However, since this effect was also observed for the control (0%), we assume that the adjuvant could have had a phytotoxic effect on the soybean. This is more likely, since rosemary essential oil has not been found to be phytotoxic in other studies on various plants (CETINTAS; YARBA, 2010; GARDIANO et al., 2011).

The galls number of *M. javanica* and the quantity of eggs g<sup>-1</sup> root dropped as the rosemary oil concentration was increased, i.e. a concentration of 3% was the most effective in controlling the nematode. An *in vitro* study on the nematicidal activity of rosemary essential oil on a population of *M. graminicola* showed potential for controlling 78.33% of J2 juveniles after exposure for 48 hours (STEFFEN et al., 2008). Ibrahim et al. (2007) observed a drop of 90.9% in the number of galls and 91.2% in the egg masses of *M. incognita* in sunflower grown in soil incorporating dry rosemary. Rosemary essential oil was also effective in reducing the hatching and mobility of juveniles of

*M. javanica* by comparison with the control, but better results were obtained with other oils evaluated in the same study (OKA et al., 2000).

Although rosemary essential oil at a concentration of 3% was the most effective treatment in the control of *M. javanica*, the nematode population found per gram of root was still higher than the original figure on inoculation, which means that further studies will be required using higher concentrations, since many studies have shown the potential of natural products, such as essential oils, with nematicidal or nematostatic activity (BAUSKE et al., 1994; NEVES et al., 2008; SALGADO; CAMPOS, 2003).

The numbers of *P. brachyurus* per gram of root and per 100cm<sup>3</sup> soil were not affected by essential oil concentration. The use of an aqueous extract of rosemary did not alter the final population of *R. reniformes* in cotton compared to the control, cutting nematode multiplication by only 4% (GARDIANO et al., 2011). Rosemary essential oil also failed to kill juveniles of *Ditylenchus dipsaci* (Kühn) Filipjev, even after exposure for three or six hours (ZOUHAR et al., 2009).

For these reasons, we can conclude that rosemary essential oil was effective in cutting the population of *M. javanica* by comparison with the control, but had no effect on the reproduction of *P. brachyurus*. Plants infected with *P. brachyurus* exhibited impaired development when treated with rosemary essential oil.

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**RESUMO:** Objetivou-se avaliar a atividade nematicida do óleo essencial de *Rosmarinus officinalis*, aplicado diretamente ao solo, visando o controle de *Meloidogyne javanica* e *Pratylenchus brachyurus* em soja. Os ensaios foram conduzidos em casa de vegetação, em delineamento inteiramente casualizado, em esquema fatorial 4 x 2, sendo quatro concentrações do óleo (0, 1, 2 e 3%) e duas frequências de tratamento (quinzenal e mensal), com quatro repetições. Plântulas de soja cv. CD 206 foram transplantadas para vasos de 1 L, recebendo o primeiro tratamento um dia após o *transplantio* e inoculadas uma semana após esse tratamento com 2000 ovos de *M. javanica* ou 1000 espécimes de *P. brachyurus* por planta. As plantas foram coletadas 60 dias após a inoculação, avaliando-se variáveis vegetativas da cultura (altura, massa fresca de parte aérea e de raiz e massa seca de parte aérea); número de galhas e ovos por grama de raiz para *M. javanica* e nematoides por grama de raiz e por 100 cm<sup>3</sup> de solo para *P. brachyurus*. Observou-se que a população de *M. javanica* foi reduzida pelo óleo essencial de *R. officinalis*, porém nenhuma das concentrações estudadas afetou a população de *P. brachyurus*.

**PALAVRAS-CHAVE:** Controle alternativo. Alecrim. Nematóide das galhas. Nematóide das lesões radiculares.

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