horticultura brasileira

Research

SCHONS, BC; BRITO, ODC; CECATTO JUNIOR, R; TSUTSUMI, CY; STANGARLIN, JR; KUHN, OJ. 2022. Rosemary extract formulated with hydrogel in the control of root-knot nematode and in the activation of defense mechanisms in tomato. *Horticultura Brasileira* 40:107-114. DOI: http://dx.doi.org/10.1590/s0102-0536-20220114

Rosemary extract formulated with hydrogel in the control of root-knot nematode and in the activation of defense mechanisms in tomato

Bruna Caroline Schons [1]; Olivia DC Brito [1]; Roberto Cecatto Junior[1]; Claudio Y Tsutsumi [1]; José Renato Stangarlin [1]; Odair José Kuhn [1]

[1]Universidade Estadual do Oeste do Paraná (UNIOESTE), Marechal Cândido Rondon-PR, Brasil; bru.schons@hotmail.com; odc.brito@gmail.com; junior_cecatto@hotmail.com; cytsutsu@gmail.com; jose.stangarlin@unioeste.br; ojkuhn@gmail.com

ABSTRACT

Root-knot nematodes (*Meloidogyne incognita)* are responsible for causing great damage to tomato crop, demanding various specific management strategies. In order to find an effective alternative to control this pathogen, the aim of this study was to test the rosemary (*Rosmarinus officinalis*) extract in formulation with hydrogel. The formulation was used at doses of 0.25; 0.50; 0.75; 1.00 and 1.25 g per pit, at seedling transplanting, and as controls, 1.25 g of hydrogel (without rosemary) and absolute control (only water). The nematological variables evaluated were number of egg masses, number of galls, total nematodes per root, and reproduction factor. To verify whether plant resistance induction occurred, root samples were collected at different time periods and the activity of the enzymes phenylalanine ammonia-lyase (FAL), peroxidase (POX) and polyphenoloxidase (PFO) were measured. For the nematological variables, we verified proportional dose-dependent reduction of nematode infection, indicating its effectiveness in disease control. For the enzymes, FAL showed no significant change in any treatment or time period tested, whereas POX and PFO showed peaks of activity in different treatments and times, mainly at the dose 1.25 g of the formulation. These results indicated that the control of *M. incognita* in tomato crop can be achieved by a direct effect of the rosemary extract on the nematode population as well as by plant resistance in response to the pathogen action.

Keywords: *Rosmarinus officinalis*, *Solanum lycopersicum*, *Meloidogyne incognita*, resistance induction.

RESUMO

Extrato de alecrim formulado com hidrogel no controle de nematoide de galhas e na ativação de mecanismos de defesa em tomateiro

Nematoides formadores de galha, como *Meloidogyne incognita*, representam grande problema na tomaticultura, demandando o uso de diversas medidas para o seu manejo. Buscando alternativa eficaz, o objetivo desse trabalho foi testar o extrato de alecrim (*Rosmarinus officinalis*) em formulação com hidrogel para controle de *M. incognita* em tomateiro. O formulado foi utilizado nas doses 0,25; 0,50; 0,75; 1,00 e 1,25 g por cova no momento do transplantio das mudas e, como testemunhas, 1,25 g de hidrogel (sem alecrim) e testemunha absoluta (apenas água). As variáveis nematológicas analisadas foram número de massa de ovos, de galhas, de nematoides por raiz e fator de reprodução. Para verificar se houve indução de resistência, amostras de raízes foram recolhidas em diferentes tempos e medidas as atividades das enzimas fenilalanina amônia-liase (FAL), peroxidase (POX) e polifenoloxidase (PFO). Nas variáveis nematológicas, houve redução proporcional ao aumento da dose do formulado, indicando controle efetivo do nematoide. Para as enzimas, a FAL não mostrou alteração significativa em nenhum tratamento ou tempo testado, enquanto que POX e PFO tiveram picos de atividade em diferentes tratamentos em diferentes momentos, principalmente para a dose 1,25 g do formulado. Isto evidenciou que o controle obtido de *M. incognita* pode ser resultado tanto pelo efeito direto do formulado de alecrim sobre a população do nematoide como pela indução de resistência da planta em reposta à ação do patógeno.

Palavras-chave: *Rosmarinus officinalis*, *Solanum lycopersicum*, *Meloidogyne incógnita*, indução de resistência.

Received on August 11, 2021; accepted on January 21, 2022

The tomato (*Solanum lycopersicum*) production chain is one of the most important for food industry. This production has been increasing in the last decades. The success of the crop is due to, mainly, the high productivity and its high market value, making this cultivation a profitable activity, contributing to its expansion (Minami & Mello, 2017).

According to Food and Agriculture Organization of the United Nations (FAO, 2020), the worldwide tomato production was approximately 138 million tons. About 93 million tons were destined for fresh consumption and 45 million tons for industry, which shows the importance of consumption and production of fresh tomato. According to CONAB (2020), about 52% of the tomato in Brazil is grown using tutoring method; however, pests and pathogens make the crop management difficult, increasing the costs of cultural practices

(Vilela *et al*., 2012).

Among the phytosanitary problems, nematodes have gained prominence, since the damages in production are quite impressive and the lack of efficient control methods has been a major obstacle (Mafessoni *et al*., 2019). This problem is even bigger in areas where tomatoes are grown in protected environment, using direct cultivation in soil, due to the difficulty in rotating crops / non-host plants, resulting in an increase of pathogen population in soil.

Meloidogyne is the main nematode genus which can cause significant damage to tomato crop. In the world, we can still find other genera of nematodes, such as *Belonolaimus*, *Pratylenchus* and *Rotylenchulus* causing some kind of disease in crops, being less problematic, though. However, in Brazil, the problems are basically restricted to *Meloidogyne incognita* and *Meloidogyne javanica* (Pinheiro, 2017). The genre *Meloidogyne* tends to form root-knot galls, due to parasite-induced hypertrophy and hyperplasia of nourishing cells (Ferraz, 2018).

The conventional control of root-knot nematodes in tomatoes is carried out through chemical nematicides; however, these products have shown low control effectiveness, high cost, besides being prejudicial to the environment. Due to the lack of efficient control methods, many researches have been searching for alternative measures (Soares *et al*., 2017).

Rosemary (*Rosmarinus officinalis*), an easy-cultivated medicinal plant in Brazil, has been studied by phytopathology for controlling several diseases (Lorenzetti *et al*., 2018) and has shown nematicidal potential to be explored. Mattei *et al*. (2014) observed a reduction in *Meloidogyne javanica* population in soybean treated with rosemary essential oil, which was also effective against *M. incógnita* race 2 (Moreira *et al*., 2009).

The form the rosemary extract is applied needs to be viable for the producer's reality. It needs to be a ready-made formula, easy to prepare and apply, and available throughout the year, otherwise exploring this oil can be difficult (Coltro-Roncato *et al*., 2018). Thus, the use of hydrogel as a means of slow release of the extract is a good option, since it is easy to apply, being able to be easily applied at seedling transplanting.

When the use of plant extracts shows to be favorable for controlling pathogens, such as the use of rosemary extracts, at least two hypotheses can be elaborated. The first is regarded to the direct toxicity of its components to the pathogen, as shown by Müller *et al*. (2014), who tested *in vitro* the effect of the direct contact of the aqueous extract of rosemary, observing nematostatic and nematicidal effects. In this case, the authors attributed the nematicidal action to the rosmarinic acid present in abundance in the extract.

The other hypothesis would be the activation of plant defense mechanisms, through the induction of resistance (Stangarlin *et al*., 2011). The induction of resistance in plants involves the activation of some defense mechanisms, for example phytoalexins and enzymes such as peroxidase, polyphenoloxidase and phenylalanine ammonia lyase. Such activation can occur either by biotic factors, such as the presence of the pathogen, or by abiotic factors, such as the application of chemical inducers or plant extracts (Mazaro *et al*., 2008). Resistance induction may also have a broad-spectrum activity, since these enzymes, when activated, can result in plant defense against various pathogens (Pascholati & Dalio, 2018).

In the induction of resistance, phenylalanine ammonia-lyase plays an important role in the reactions of the metabolism of phenolic compounds, being responsible for the deamination of L-phenylalanine, transforming it into trans-cinnamic acid and ammonia (Schwan-Estrada *et al*., 2008).

Peroxidases are pathogenesis-related proteins (RP-proteins) belonging to RP-9 family. The enzymes of this group are studied in plant defense and resistance processes because they play an important role in lignification, suberization and cell wall metabolism, and may also be present in hypersensitivity responses and phytoalexin production (Stangarlin *et al*., 2011).

Polyphenoloxidases are enzymes responsible for catalyzing the oxidation reaction of polyphenols to transform them into highly toxic quinones. In general, they are released when the cell ruptures, either by mechanical damage or by the action of insects and pathogens (Hendges *et al*., 2021).

These three enzymes are strongly linked to lignin synthesis of phenolic compounds, phenylpropanoid pathway. When nematodes infect the plant, they activate the synthesis of these enzymes, which can harm them. This biochemical defense resource can be induced prior to the contact of nematodes with plants with the application of inducing products, resulting in a more efficient control (Sankar *et al*., 2017; Mattei, 2018).

The aim of this study was to evaluate the efficiency of rosemary oil-based hydrogel formulations to control *Meloidogyne incognita* in tomato crop and verify activation of plant defense mechanisms after applying this product.

MATERIAL AND METHODS

The experiments were carried out under climate controlled greenhouse conditions, located in the protected cultivation area at Núcleo de Estações Experimentais da Universidade Estadual do Oeste do Paraná, Campus Marechal Cândido Rondon-PR.

The local climate, according to Köppen is Cfa, being characterized as subtropical with an average temperature of the coldest quarter below 18°C (mesothermic), and in the hottest quarter, above 22°C, with hot summers, infrequent frost events and a tendency to concentrate rainfall in the summer months, no defined dry season, though. Normal average annual rainfall totals for the region range from 1,600 to 1,800 mm, with an average temperature between 22 and 23°C (Caviglione *et al*., 2000).

The experimental design used was randomized blocks, due to possible weather variations inside the greenhouse. The treatments consisted of doses of 0.25; 0.50; 0.75; 1.00 and 1.25 g of rosemary hydrogel formulation per pit. Two controls were

used, at dose 0, corresponded to 1.25 g of rosemary extract-free hydrogel (in order to verify any possible effect of hydrogel on plants), and the absolute control, corresponding to only distilled water. For nematological analysis five replicates were used, and for the biochemical analysis three replicates were used, for each of the five collect times: 0, 5, 10, 20 and 50 days after inoculation, being carried out three days after transplanting/treatment. Each plot consisted of 1-L pot containing a non-autoclaved 3:1 substrate mixture and commercial substrate Humusfértil.

The authors used Santa Clara seedlings, produced in a greenhouse in 128-cell expanded polystyrene trays, with commercial substrate and the transplanting was done 20 days after emergence.

Data were submitted to variance analysis and regression test using SAS University Edition software (SAS Institute Inc., 2014).

Obtaning hydrogel formulation with rosemary

Fresh rosemary leaves were crushed in a blender with distilled water to obtain aqueous extract at a concentration of 21% (mass/volume). This extract was filtered through qualitative filter (11 µm pore diameter), then transformed into soluble powder in a spray dryer. The soluble rosemary powder was diluted with distilled water until a concentration of 15% was obtained. For each 1 L of diluted extract, 12 g of hydrogel Hidroterragel were added (dose recommended by the manufacturer), obtaining a dough-like consistency mass, which was reserved for 24 hours, until the hydrogel completely absorbed the extract. Afterwards, the mixture was dried in an oven at 60°C, for 48 hours, until getting a solid consistency again. This material was then crushed in a blender to obtain the powder, which corresponded the rosemary-oil-based hydrogel formulation. The product was weighed with the aid of an analytical balance to obtain the doses, which were put into the pits made in the pots at the transplanting.

***M. incognita* inoculation**

The inoculum was obtained using the methodology described by Collen & D'Herde (1972), in which tomato roots containing pure populations of *M. incognita* race 3 were sectioned with scissors and ground in a 0.5% sodium hypochlorite solution in a blender for approximately 30 seconds. The suspension passed through a set of overlapping sieves, 60 and 500 mesh, and the content retained in the latter was collected in a beaker in order to be counted. As the sample was quite clean, the centrifugation step was discarded.

Counting was carried out in Peter's chamber under an optical microscope. The authors inoculated 2,000 eggs + second-stage juveniles (J2) of *M. incognita* per experimental plot for nematological experiment and 1,000 eggs + J2 per plot in the experiment of biochemical analyses. In both cases, inoculation took place three days after treatments were applied and seedlings were transplanted.

Nematological analysis

Forty five days after inoculation, we collected material to evaluate nematological variables to determine the effectiveness of the rosemary hydrogel formulation for *M. incognita* control.

To quantify the egg masses, we used the phloxin B, staining methodology (Taylor & Sasser, 1978). The roots were washed under running water, then they were put inside a container, fully immersed in the staining solution for 20 minutes. Afterwards, they were taken out and washed to remove the excess of the solution. The colorful galls represented the number of egg masses.

Afterwards, the eggs and J2 were extracted to be counted, following the methodology of Collen & D'Herde (1972), as previously mentioned.

In order to obtain reproduction factor, the initial population was used, videlicet, the amount of inoculated nematodes (eggs + J2), and the amount of eggs + J2 of *M. incognita* found in the root after 45 days of inoculation, which corresponded to the final population. Reproduction factor (FR) was calculated according to the methodology of Oostenbrink (1966), in which the final population is divided by the initial population (inoculum) (FR=Pf/Pi).

Biochemical analyses

The biochemical analyses were based on the collect of the roots in inoculated plants of the treatments in different times. Right after being taken from the plants, the roots were washed under running water and dried with paper towel. We collected, approximately, 0.5 g from the middle region of the root and immediately packed in an aluminum foil envelope for freezing at -20°C.

After all the collects, the samples were ground with 4 mL of 0.01 M sodium phosphate buffer (pH 6.0) in a porcelain mortar previously cooled with the aid of liquid nitrogen. The homogenate obtained was centrifuged at 6,000 g for 20 minutes at 4°C. The supernatant, considered as the fraction containing soluble proteins, was stored in microtubes at -20°C for further biochemical analysis (Lusso & Pascholati, 1999).

The phenylalanine ammonia-lyase activity was determined by colorimetric quantification of trans-cinnamic acid released from the substrate L-phenylalanine, according to the methodology described by Umesha (2006). The reading was performed in a spectrophotometer at 290 nm and the enzymatic activity was expressed in µg of trans-cinnamic acid min^{-1} mg^{-1} of protein.

In order to determine peroxidase activity, the authors used the methodology proposed by Hammerschmidt *et al*. (1982), using a spectrophotometer at 470 nm, with annotation of absorbance values every 15 seconds for 1 minute. The results were expressed as a variation of absorbance units min^{-1} mg^{-1} of protein.

To determine activity of polyphenoloxidases, the methodology of Duangmal & Apenten (1999) was used. The readings were carried out at 420 nm, each 15 seconds for 1 minute. The differential between the last and the first reading was used to determine the activity, whose results were expressed as absorbance min^{-1} mg^{-1} of protein.

Total proteins were determined according to the method proposed by Bradford (1976), in which 200 µL of each sample were mixed to 600 µL of

5 M phosphate buffer solution (pH 7.0) and, under vortex agitation, 200 µL of Bradford's solution was added. After 10 minutes, the absorbance readings were carried out in a spectrophotometer at 595 nm, in glass cuvettes. The protein concentration expressed in terms equivalent to µg of bovine serum albumin (ASB) in one mL of sample (µg protein mL^{-1}) was determined using the standard curve of ASB concentration.

RESULTS AND DISCUSSION

Nematological analyses

Considering all the evaluated variables, the controls (hydrogel without rosemary oil, which corresponded to the dose 0, and water as an absolute control) differed statistically from the doses of the formulation. For number of egg mass and galls, both in control and in dose 0, we observed values statistically higher than the others, it means, applying the

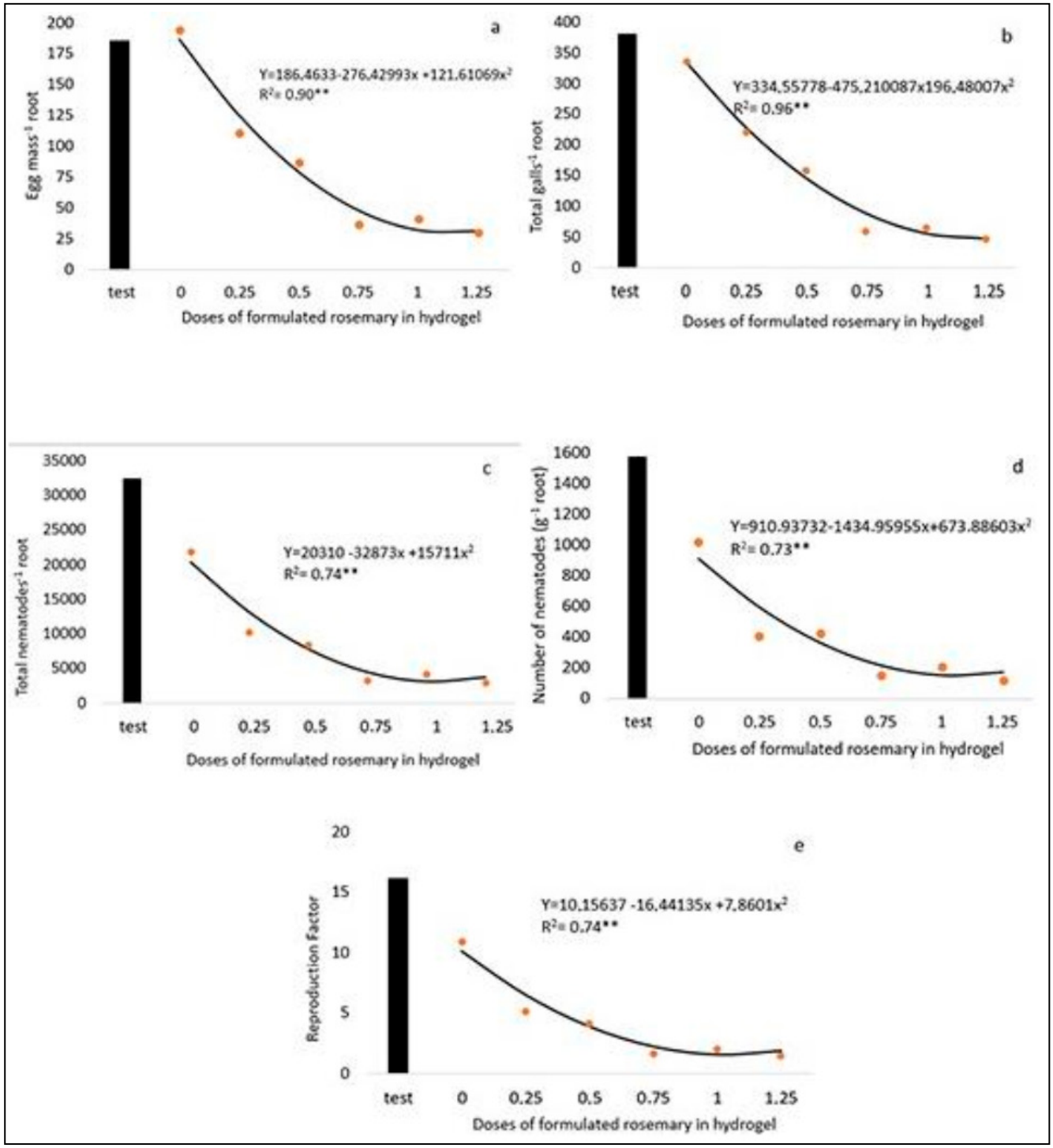

Figure 1. Effect of doses of formulated rosemary in hydrogel (g pit^{-1}) on nematological variables: number of egg masses (a), total galls (b), total nematodes per root (c), nematodes per gram of root (d) and reproduction factor (e) in tomato inoculated with *Meloidogyne incognita*, regression analysis at 5% probability. UNIOESTE, Marechal Cândido Rondon, 2019.

rosemary hydrogel formulation, at any dose, resulted in a reduction of these variables, showing that the formulation is able to control the nematode (Figure 1).

For total number of nematodes, nematodes per gram of root and reproduction factor, comparing the control with all the doses, we observed statistical difference. We verified a reduction in these variables by simply applying the hydrogel, even with no rosemary extract, which could be due to the physical impediment that the hydrogel caused. The barrier formed around the root may have made it difficult for nematodes to enter.

Another possible explanation is that the chemical hydrogel composition has caused alteration in root exudate gradients and, consequently, in the biochemical composition of the plant rhizosphere; through this root exudate gradient, the second stage juvenile of *Meloidogyne,* infective phase, moves in the soil until the root elongation part (Ferraz, 2018). The altered composition of the exudate can result in disorientation of these nematodes and reduce the amount of them reaching the roots.

It is possible to consider that the hydrogel had retained a lot of water, this is one of the main characteristics of this polymer, resulting in a decrease of free water around the roots. The nematodes in soil need free water to move up to the roots and start their infectious process (Pinheiro, 2017; Ferraz, 2018). A reduction in this amount of water would be harmful to displacement, consequently, it would decrease the nematode infection rate.

Quintela *et al*. (2015) observed in their study, with sugarcane x *M. incognita* pathosystem, the lowest number of eggs g^{-1} root and nematode reproduction factor in less-water-treated plants. The authors considered this reduction to three factors: the first is the difficulty in locomotion and colonization, as mentioned before. The second factor is the dehydration of the gelatinous matrix which covers the eggs of the nematode, interrupting its life cycle. Finally, the third factor refers to the low water supply which makes roots develop thicker layers and, depending on the plant species, the penetration of the parasite can become more difficult.

The evaluated nematological variables were submitted to regression analysis and the authors verified quite similar results: a reduction in values, with an increase of the dose of rosemary hydrogel formulation (Figures 1a, 1b, 1c, 1d and 1e).

The evaluated variables can be separated into two groups. The first group refers to colonization and reproduction of the nematode, including number of galls, egg mass and reproduction factor. The second group refers to the presence of eggs and juveniles of *M. incognita* in roots, which is represented by nematodes per roots and nematodes per gram of root.

Although both groups showed similar results in this study, when testing doses of rosemary essential oil in *Meloidogyne javanica* x soybean pathosystem, Mattei *et al*. (2014) observed a reduction in the values of the reproductive variables as the dose increased; however, considering the presence of nematodes, no difference was noticed when the essential oil was applied, not even for the control. The authors believe that these results are due to the low doses, and that increasing the doses, the nematicidal effect observed in reproduction would also be observed in the amount of nematodes present in the roots.

Coltro-Roncato *et al*. (2016) evaluated the same nematological variables to control *M. incognita* on tomatoes treated with crambe (*Crambe abyssinica*) extract applying in different forms. The values of the evaluated variables were reduced and the best form to apply this extract, according to the study, was via soil. The authors attributed the control obtained to the direct contact, mainly, because of the damage that some plant extracts can cause to the nematode's external cuticle. Moreover, the inhibition action of the enzyme V-ATPase, which has a nematostatic and nematicide effect, may have contributed to the advanced control, which corroborates the results of the *in vitro* experiment carried out in this study (data not shown).

The active principles of essential oils and plant extracts can act directly on the nematode cuticle, changing its permeability. Under the action of these active principles, the activation of defense mechanisms can also occur, which can result in enzymatic modifications and physiological changes in the plant, which will impair the life cycle of the nematode in the host species

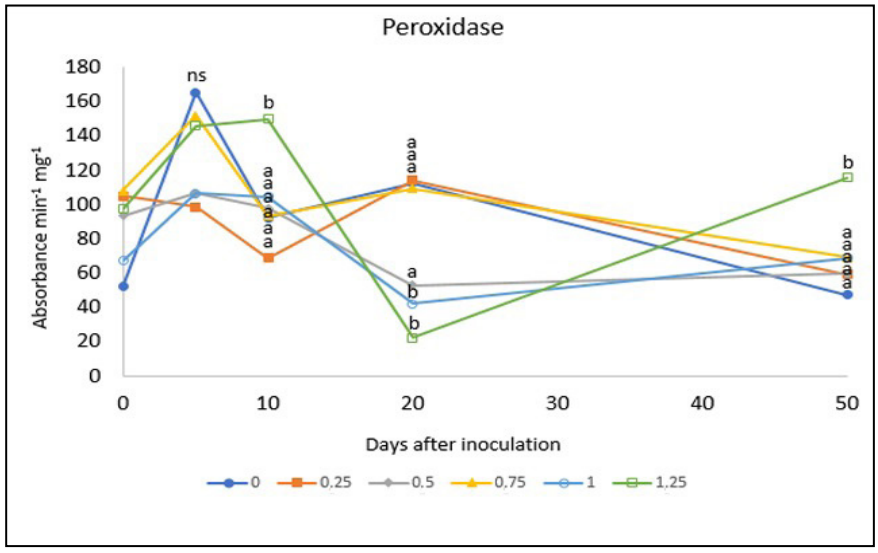

Figure 2. Peroxidase activity in relation to time after inoculation of tomato plants with *Meloidogyne incognita*. The treatment of plants with doses (g pit^{-1}) of the formulated rosemary in hydrogel occurred three days before inoculation. Means followed by the same letter within each time do not differ by Tukey's test at 5% probability. ns: not significant. UNIOESTE, Marechal Cândido Rondon, 2019.

(Lopes *et al*., 2005).

Biochemical analyses

The activity of the three enzymes, FAL, POX and PFO was measured to evaluate the activation of plant defense mechanisms after applying the rosemary hydrogel formulation. The authors highlight that the day zero is the inoculation time, however, the inoculation, actually, took place three days after the treatment been applied in soil, when the seedlings were transplanted, which explains the high values of some enzyme's activity on day zero.

In relation to FAL, no statistical difference was observed considering the treatments, so data were not shown. The same was verified in Kuhn & Pascholati (2010), who have not verified any alteration in FAL activity after the application of acibenzolar-S-metil and *Bacillus cereus* on beans against *Xanthomonas axonopodis* pv. *phaseoli*, even the first being a well-known resistance inducer.

For POX (Figure 2), the authors noticed activity at dose 1.25, 10 days after inoculation. At 20 days after inoculation (DAI), the treatments 0, 0.25 and 0.75 stood out statistically, due to its behavior, whose results can be observed in Figure 2. At 50 DAI, the treatment of dose 1.25 stood out comparing to the other ones. Despite the data observed at 20 DAI, the data at 10 and 50 days are quite consistent, as it was possible to observe the activity of this enzyme at the highest dose of the formulation.

The results shown by Formentini (2012) are similar to this study. The author observed the peroxidase enzyme activity after the application of the treatments *Bacillus cereus*, rosemary and turmeric extracts, when compared to the positive control (acibenzolar-S-metil) and to the absolute control (water). Besides, the treatments also promoted significant results for nematological variables when related to the *M. incognita* control, as well as in this study.

POX activity is related to lignification, since it catalyzes the oxidation of phenolic alcohols in this process. Lignification results in changes in the cell wall and provides greater resistance against pathogenic toxins (Carvalho, 2012) and also against nematode penetration.

Similar results were found by Lorenzetti *et al*. (2018). An increase in enzyme activity occurred after treatment with rosemary extract in soybean, and inoculation with *Macrophomina phaseolina*. As in the present study, the authors found two activity peaks for the same dose, both after the beginning of the infectious process.

Portz *et al*. (2011) used the example of ethylene and explained the behavior of POX with two activation peaks. In general, the second peak is lower than the first and it is due to the process of colonization of the host plant tissues by the pathogen, at the moment in which several defense mechanisms are expressed in a more pronounced way. In this study, at 50 DAI *M. incognita* probably ended its first life cycle and started the second, that is to say, it was when the J2 was returning to the roots for a new colonization.

As it is shown in Figure 3, PFO activity was also noticed. However, this activity was concentrated in the first 20 DAI, with emphasis on the treatment 0.25 g pit^{-1} at time 0 and 1.25 g pit^{-1} at 10 DAI. At 20 DAI, despite being visually close, the results of treatments 0 and 0.25 g pit^{-1} differed from the others.

According to Bonaldo *et al*. (2005), when the plant recognizes an elicitor molecule, protection against the pathogen occurs more quickly and efficiently. Nevertheless, in this study, we could notice a peak of activity in the treatment 0.25 g of the formulation which occurred at inoculation time. This can be harmful to the plant in terms of metabolic energy expenditure. Although the plant is able to delay or prevent the infectious process, in some cases the metabolic expense involved in the process makes the induction disadvantageous, as it can result in production losses, for example.

As for POX, in Formentini (2012), a response for PFO was also verified after applying the rosemary extract, when tomato cultivars, susceptible and resistant to *M. incognita,* were tested. The response of the resistant cultivar was more pronounced, which was attributed to the constitutive levels of the enzyme found in resistant plants. This is contrary to what was reported in Kuhn & Pascholati's (2010) study. In this case, the inducers, both abiotic (acibenzolar-S-methyl) and biotic (*Bacillus cereus*), did not change the action of PFO in common bean plants, it means, this route was not altered by the action of the inducers.

Viecelli *et al*. (2009) observed that

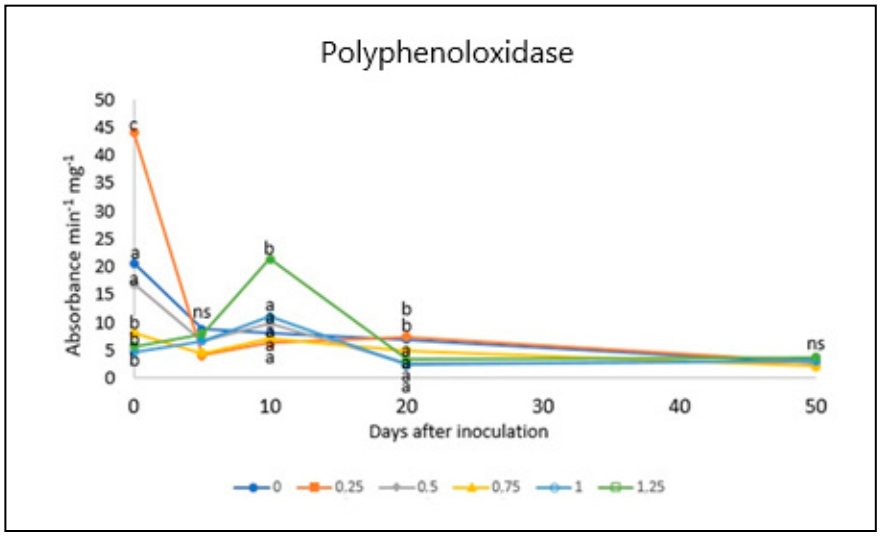

Figure 3. Activity of the polyphenoloxidase enzyme in relation to time after inoculation of tomato plants with *Meloidogyne incognita*. The treatment of the plants with doses (g pit^{-1}) of the formulated rosemary in hydrogel occurred three days before the inoculation. Means followed by the same letter within each time do not differ by Tukey's test at 5% probability. ns: not significant. UNIOESTE, Marechal Cândido Rondon, 2019.

the concentration of PFO altered after the treatment with Mycelium extracts from *Pycnoporus sanguineus* for the induction of resistance to common bean angular leaf spot. Enzyme activity was also concentrated in the first days after induction treatment has been applied. The higher initial concentration is explained by the action of this enzyme against phytopathogens, which occurs soon after penetration. When the enzyme is released from the thylakoids, after the cell ruptures by the pathogen, the oxidation of phenolic compounds occurs, which are also released from the vacuoles. Oxidation leads to the formation of quinones, which have antimicrobial action (Taiz & Zeiger, 2004; Liu *et al*., 2005).

According to Portz *et al*. (2011), the enzyme activity, sometimes high, sometimes low, is related to the plant response to the infectious process. According to the authors, when it is necessary, the plant increases the levels of these proteins; in order to save energy, in other moments, the level of the protein is reduced, though. This corroborates the idea that the induction of resistance by the application of the hydrogel formulation with rosemary in the studied pathosystem really occurred.

Given the above, we concluded that the rosemary hydrogel formulation was efficient to control *M. incognita* in tomato crop, showing a reduction proportional to the dose increase in all tested variables. This control may also have occurred by inducing resistance involving the activation of the enzymes peroxidase and polyphenoloxidase, as well as by the action of the hydrogel resulting in a possible interference in the migration and penetration into the roots by infective forms. Using this information, further studies shall be carried out in order to verify the feasibility of using this rosemary hydrogel formulation in commercial tomato cultivation.

REFERENCES

BONALDO, SM; PASCHOLATI, SF; ROMEIRO, RS. 2005. Indução de resistência: noções básicas e perspectivas. In: LARA, FM (ed). *Indução de resistência em plantas a patógenos e insetos.* Piracicaba, BR: FEALQ. cap. 1, p.11-28.

BRADFORD, MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254.

CARVALHO, NL. 2012. Resistência genética induzida em plantas cultivadas. *Revista eletrônica em Gestão, Educação e Tecnologia Ambiental* 7, 1379-1390.

CAVIGLIONE, JH. 2000. Cartas climáticas do Paraná. Versão 1.0. Londrina: *Instituto Agronômico do Paraná*: (CD-ROM).

CAVIGLIONE, JH.; CARAMORI, PH; KIIHL, LRB; OLIVEIRA, DD. 2000. *Cartas climáticas do Paraná. Versao 1.0* (No. CD 551.6 C322). Instituto Agronómico do Paraná, Brasilia, PA (Brasil).

COLLEN, WA; D'HERDE, CJ. 1972. *A method for the quantitative extraction of nematodes from plant tissue.* Ohio, EUA: State Nematology and Entomology Research Station. 77p.

COLTRO-RONCATO, S; STANGARLIN, JR; KUHN, OJ; GONÇALVES, AC; DILDEY, ODF; GONÇALVES, EDV; RISSATO, BB; BROETTO, L; FARIA, VDO. 2018. Controle de *Meloidogyne incognita* em tomateiro pelo extrato de crambe em diferentes formas de aplicação. *Summa Phytopathologica* 44: 261-266.

COLTRO-RONCATO, S; STANGARLIN, JR; KUHN, OJ; GONÇALVES, EDV; DILDEY, ODF; RISSATO, BB; BROETTO, L; HENKEMEIER, NP; MIORANZA, TM; HELING, AL; FARIA, VO; STEFANELLO, VV; WEBLER, TFB. 2016. Nematicidal effect of *Crambe abyssinica* leaf extracts to *Meloidogyne javanica* on tomato. *African Journal of Agricultural Research* 32: 3004-3011.

CONAB, Companhia Nacional de Abastecimento. 2020. Tomate: análise dos indicadores da produção e comercialização no mercado mundial, brasileiro e catarinense. *Compêndio de estudos Conab* 21: 11-12.

DUANGMAL, K; APENTEN, RKO. 1999. A comparative study of polyphenoloxidases from taro (*Colocasia esculenta*) and potato (*Solanum tuberosum var. romano*). *Chemistry* 64: 351-359.

FAO, food and agricultural organization. 2020. *FAO Statistical Yearbook.* Nova York, USA. Available www.fao.com. Accessed December 10, 2021.

FERRAZ, LCCB. 2018. Nematoides. In: AMORIM, L; REZENDE, JAM; BERGAMIN-FILHO, A (eds). *Manual de Fitopatologia, v. 1. Princípios e conceitos.* Piracicaba, SP: Ouro Fino: Agronômica Ceres. cap. 13, p.195-211.

FORMENTINI, HM. 2012. *Avaliação de indutores de resistência bióticos, abióticos e extratos vegetais no controle de Meloidogyne incognita em tomateiro*. Marechal Cândido Rondon, BR: 93p. (Ph.D. thesis).

HAMMERSCHMIDT, R; NUCKLES, EM; KUĆ, J. 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiological Plant Pathology* 20: 73-82.

HENDGES, C; STANGARLIN, JR; ZAMBAN, VC; MASCARO, MDHN; CARMELO, DB. 2021. Antifungal activity and control of early blight in tomato through tea tree essential oil. *Crop Protection* 148: 1-8.

KUHN, OJ; PASCHOLATI, SF. 2010. Custo adaptativo da indução de resistência em feijoeiro mediada pela rizobactéria *Bacillus cereus* ou acibenzolar-S-metil: atividade de enzimas, síntese de fenóis e lignina e biomassa. *Summa Phytopathologica* 36: 107-114.

LIU, H; JIANG, W; BI, Y; LUO, Y. 2005. Postharvest BTH treatment induces resistance of peach (*Prunus persica* L. cv. Jiubao) fruit to infection by *Penicillium expansum* and enhances activity of fruit defense mechanisms. *Postharvest Biology and Technology* 35: 263-269.

LOPES, AE; FERRAZ, S; FREITAS, LG; AFONSO FERREIRA, P; XAVIER AMORA, D. 2005. Efeito dos extratos aquosos de mucuna preta e de manjericão sobre *Meloidogyne incognita* e *M. javanica*. *Nematologia Brasileira* 29: 67-74.

LORENZETTI, E; STANGARLIN, JR; KUHN, OJ; PORTZ, RL. 2018. Indução de resistência à *Macrophomina phaseolina* em soja tratada com extrato de alecrim. *Summa Phytopathologica* 44: 45-50.

LUSSO, MFG; PASCHOLATI, SF. 1999. Activity and isoenzymatic pattern of soluble peroxidases in maise tissues after mechanical injury or fungal inoculation. *Summa Phytopathological* 25: 244-249.

MAFESSONI, AB; BAHIA, BL; SOUZA, IVB; SILVA, RF; REBOUÇAS, TNH; PORTO, JS. 2019. Fungos antagonistas e suas combinações contra *Meloidogyne* spp. em solo de cultivo de tomate sem a presença de hospedeiro. *Acta Biológica Catarinense* 6: 54-60.

MATTEI, D. 2018. Alterações bioquímica e física da soja tratada com silicato de potássio visando o controle de *Meloidogyne incognita*. Marechal Cândido Rondon: UNIOESTE. 116p (Ph.D. thesis).

MATTEI, D; DIAS-ARIEIRA, CR; BIELA, F; ROLDI, M; SILVA, TRB; RAMPIM, L; TAVAREZ-SILVA, CA. 2014. Essential oil of *Rosmarinus officinalis* in the control of *Meloidogyne javanica* and *Pratylenchus brachyurus* in soybean. *Bioscience Journal* 30: 469-476.

MAZARO, SM; CITADIN, I; GOUVÊA, A; LUCKMANN, D; GUIMARÃES, SS. 2008. Indução de fitoalexinas em cotilédones de soja em resposta a derivados de folhas de pitangueira. *Ciência Rural* 38: 1824-1829.

MINAMI, K; MELLO, SC. 2017. *Fisiologia e nutrição do tomateiro*. Curitiba, PR: Senar. 1185p.

MOREIRA, FJC; SANTOS, CDG; INNECCO, R; SILVA, GSD. 2015. Controle alternativo de nematoide das galhas (*Meloidogyne incognita*) raça 2, com óleos essenciais em solo. *Summa Phytopathologica*. Botucatu, v. 41, n. 3, p. 207-213.

MÜLLER, MA. MIORANZA, TM; FUCHS, F; BATISTTUS, AG; STANGARLIN, JR; KUHN, OJ. 2014. Mortalidade e motilidade de *Meloidogyne incognita* em extrato aquoso de alecrim. *Scientia Agraria Paranaensis* 13: 343-346.

OOSTENBRINK, M. 1966. Major characteristics of the relation between nematode and plants. *Mededelingen Landbouhogeschool* 66: 3-46.

PASCHOLATI, SF; DALIO, RJD. 2018. Fisiologia do parasitismo: como as plantas se defendem dos patógenos. In: AMORIM, L; REZENDE JAM; BERGAMIN FILHO, A (eds). *Manual de Fitopatologia, volume 2: Princípios e Conceito*. 5.ed. Ouro Fino: Agronômica Ceres Ltda, cap. 35, p.424-450.

PINHEIRO, JB. 2017. *Nematoides em hortaliças*. Brasília, BR: Embrapa. 421 p.

PORTZ, RL. FLEISCHMANN, F; KOEHL, J; FROMM, J; ERNST, D; PASCHOLATI, SF; OSSWALD, WF. 2011. Histological, physiological and molecular investigations of *Fagus sylvatica* seedlings infected with *Phytophthora citricola*. *Forest Pathology* 41: 202-211.

QUINTELA, MP. PEDROSA, EM; WILLADINO, L; ROLIM, MM; SILVA, ÊFDF; DAVID, MFDL. 2015. Intensity and duration of water deficit on the pathosystem sugarcane x *Meloidogyne incognita*. *Revista Brasileira de Engenharia Agrícola e Ambiental* 19: 22-30.

SANKAR, C; SOORIANATHASUNDARAM, K; KUMAR, N; KARUNAKARAN, G; SIVAKUMAR, M. 2017. Induction of resistant to *Radopholus similis* and defence related mechanism in susceptible and resistance banana hybrids infected with *Radopholus similis*. *International Journal of Current Microbiology and Applied Sciences*. 6: 1668-1684.

SAS Institute Inc. 2014. *SAS University Edition: Installation guide*. Cary, NC: SAS Institute Inc.

SCHWAN-ESTRADA, KRF; STANGARLIN, JR; PASCHOLATI, SF. 2008. Mecanismos bioquímicos de defesa vegetal. In: PASCHOLATI, SF (eds). *Interação planta patógeno – fisiologia, bioquímica e biologia molecular.* Piracicaba, p.227-248.

SOARES, PLM; NASCIMENTO, DD; VIDAL, RL; VIZENTINI, LR. 2017. Controle biológico de nematoides. In: BALDIN, ELL; KRONKA, AZ; SILVA IF (eds). *Inovações em manejo fitossanitário.* Botucatu: EPAF, cap. 11, p.167-232.

STANGARLIN, JR. KUHN, OJ; TOLEDO, MV; PORTZ, RL; PASCHOLATI, SF. 2011. A defesa vegetal contra fitopatógenos. *Scientia Agraria Paranaensis* 10: 18-46.

TAIZ, L; ZEIGER, E. 2004. *Fisiologia Vegetal*. 3 ed. Porto Alegre, RS: Artmed. 719 p.

TAYLOR, AL; SASSER, JN. 1978. *Biology, identification and control of root-knot nematode (Meloidogyne species).* Raleigh, NC: Cooperative Publication of the Departament of Plant Pathology. 111p.

UMESHA, S. 2006. Phenylalanine ammonia lyase activity in tomato seedlings and its relationship to bacterial canker disease resistance. *Phytoparasitica* 34: 68-71.

VIECELLI, CA; STANGARLIN, JR; KUHN, OJ; SCHWAN-ESTRADA, KRF. 2009. Indução de resistência em feijoeiro por filtrado de cultura de *Pycnoporus sanguineus* contra *Pseudocercospora griseola*. *Tropical Plant Pathology* 24: 87-96.

VILELA, NJ; GUEDES, IM; PINHEIRO, JB; SUINAGA, FA. 2012. *Produção de tomate para processamento industrial.* Brasília-DF: Embrapa hortaliças. 18p.