Scientia Agraria Paranaensis – Sci. Agrar. Parana.
ISSN: 1983-1471 – Online
DOI: https://doi.org/10.18188/sap.v21i3.29707

CONTROL OF *Meloidogyne incognita* IN SOYBEAN PLANTS WITH *Pycnoporus sanguineus* EXTRACT

Gisela Paula Tasca[1*], Olívia Diuelen Costa Brito[2], José Renato Stangarlin[3], Odair José Kuhn[4]

SAP 29707 Received: 15/05/2021 Accepted: 22/08/2022
Sci. Agrar. Parana., Marechal Cândido Rondon, v. 21, n. 3, jul./sep., p. 258-262, 2022

ABSTRACT - *Meloidogyne incognita* is an endoparasitic and sedentary nematode that forms galls on the roots and damage several agricultural crops, including soybean. Its control needs several management methods, including the use of microbial extracts. Thus, the objective of this work was to verify the influence of the fungus *Pycnoporus sanguineus* extract on the control of *M. incognita* in soybean. The treatments were concentrations of the crude extract of basidiocarps from *P. sanguineus* (0%; 2.5%; 5%; 7.5%; and 10%) which were applied to the aerial part of the plants, and after 72 h the nematodes (eggs + second stage juveniles - J2) were inoculated. After 60 days were evaluated fresh and dry masses of the aerial part and height of aerial part, and the nematological parameters number of egg masses, number of galls and total number of nematodes. It was concluded that there was a reduction in total number of nematodes in a dose-dependent manner for crude extract of *P. sanguineus*, where the calculated value of 17% was the concentration which more reduced de number of nematodes. Considering that the aqueous extract of *P. sanguineus* was applied to the aerial part of the soybean, it can be concluded that this fungus controls *M. incognita* in the roots, probably by inducing resistance.
Keywords: alternative control, resistance induction, root-knot nematode.

CONTROLE DE Meloidogyne incognita EM PLANTAS DE SOJA COM EXTRATO DE Pycnoporus sanguineus

RESUMO - *Meloidogyne incognita* é um fitonematoide endoparasita e sedentário que forma galhas nas raízes e danos significativos para diversas culturas agrícolas, inclusive para a soja. Para um controle efetivo é necessário aliar-se vários métodos de manejo, incluindo métodos considerados alternativos como, por exemplo, o uso de extratos microbianos. Dessa forma, o objetivo desse trabalho foi verificar a influência do extrato do fungo *Pycnoporus sanguineus* no manejo de *M. incognita* em soja. Os tratamentos foram concentrações do extrato bruto aquoso de basidiocarpos de *P. sanguineus* (0%; 2,5%; 5%; 7,5%; e 10%) que foram aplicadas por aspersão nas folhas de soja, e, após 72 h, as plantas foram inoculadas com ovos e juvenis de segundo estádio (J2) do fitonematoide. Após 60 dias da inoculação foram realizadas as avaliações vegetativas de massas fresca e seca da parte aérea e altura da parte aérea, e as análises nematológicas número de massa de ovos, número de galhas e número de ovos + J2. Houve redução do número de nematoides totais com o aumento das concentrações do extrato de *P. sanguineus*, representado por uma equação de segundo grau, sendo o valor calculado de 17% a concentração que proporcionaria a maior redução. Considerando-se que o extrato aquoso de *P. sanguineus* foi aplicado na parte aérea da soja, conclui-se que este fungo controla *M. incognita* nas raízes provavelmente por indução de resistência.
Palavras-chave: controle alternativo, indução de resistência, nematoide das galhas.

INTRODUCTION

In the current scenario, soybeans are the most important agricultural crop for the Brazilian economy. Moreover, it is a product that has been showing significant growth over the years (HIRAKURI; LAZZAROTTO, 2014). According to data from CONAB (2021), soybeans showed growth in area of 4.1% compared to the last crop, moreover, the productivity of the crop of 2020/21 is estimated at 133,817 million tons, surpassing the previous crop.

These increases in productivity are linked to various factors, such as advances in technology and proper management of culture, among others. However, phytosanitary problems are responsible for great damage, which compromises productivity, and hence its final quality. Among these problems are root-knot nematodes of the genus *Meloidogyne* Goeldi, such as *M. incognita* (Kofoid and White) Chitwood and *M. javanica* (Treub) Chitwood, which cause greater damage to soybean culture in Brazil (JUHÁSZ et al., 2013).

Symptoms caused by *Meloidogyne* spp. appear in bare spots, with decrease of the booth of plants, yellowish leaves and decrease of the growth, moreover, occurs the decrease in the number of roots and the appearance of branches (FERRAZ, 2018). These nematodes are considered to be one of the most important because they

[1]Universidade Estadual do Oeste do Paraná (Unioeste), Marechal Cândido Rondon *Campus*, PR, Brazil. E-mail: gi_paula_tasca@hotmail.com.
*Corresponding author.

have a wide range of hosts and have a high reproductive capacity, which may render the production of soybeans unviable (GRIGOLLI, 2012). As these pathogens are difficult to handle, integrated measures must be taken to make effective the control methods, which can be mechanical, cultural, chemical, biological, and also the induction of resistance (MÜLLER et al., 2016).

Plants have natural resistance against pathogens, through barriers and defense mechanisms that do not depend on the pathogen arrival at the site of infection, that is, they are pre-formed in relation to infection. However, the most efficient mechanism that the plant presents is when it is exposed to induction agents or to the very arrival of the pathogen, and, when this occurs, the resistance is called induced or postformed resistance. This can be classified into systemic acquired resistance (SAR) and induced systemic resistance (ISR), and both have phenotypic similarities, however, SAR is usually induced by abiotic factors or extracts of biotic origin, whereas in ISR the inductor is usually a non-pathogenic microorganism (PASCHOLATI; DALIO, 2018).

Various forms of resistance induction have been studied to reduce the environmental impact caused by the methods of disease control, mainly chemical. As an alternative for resistance induction, extracts obtained from plants and microorganisms such as the fungus *Pycnoporus sanguineus* (Fries) Murrill are used (SCHWAN-ESTRADA et al., 2012). This basidiomycete fungus, is a slow-growing saprophyte that usually develops over fallen tree trunks, where it forms red-orange sessile, coriaceous and shelf shaped basidiocarps (TÉLLEZ-TÉLLES et al., 2016). The potential of resistance induction of *P. sanguineus* has already been verified in the pathosystem *Pseudocercospora griseola* (VIECELLI et al., 2010) and *Xanthomonas axonopodis* pv. *phaseoli* (TOILLIER et al., 2010) in bean, and *Xanthomonas vesicatoria* (ASSI et al., 2017) and *Alternaria solani* (ALENCAR et al., 2020) in tomato. For phytonematodes, the efficiency of *P. sanguineus* has been verified only for control of *M. javanica* in tomato (BARBOSA et al., 2021). In view of the above, the present study aimed to evaluate the control of the nematode *M. incognita* in soybean, with concentrations of aqueous extract of *P. sanguineus*.

MATERIAL AND METHODS

The experiment was implemented in the Area of Protected Cultivation and Biological Control "Dr. Mário Cesar Lopes," of the State University of Western Paraná, *Campus* Marechal Cândido Rondon, in air-conditioned greenhouse, maintaining temperature of 28°C ±2°C. Experimental design of casualized blocks was used, with six repetitions and five treatments, where each 500 mL pot represented an experimental unit. Five concentrations of aqueous extract from *P. sanguineus* basidiocarps (0; 2.5; 5; 7.5 and 10%) were obtained and the method of application was by sprinkling in the aerial part of soybean plants.

The extract of *P. sanguineus* was obtained from basidiocarps of this fungus produced according to the technique of Martinazzo-Portz et al. (2022), which were dried in greenhouse air forced at 40°C for 12 h and ground in knife mill (PEITER-BENINCA et al., 2008). Dry powder of *P. sanguineus* basidiocarps was hydrated for 24 h to 4°C in sterile distilled water in the proportion of 14 mL for each gram of powder (DI PIERO et al., 2006) to obtain the aqueous extract. After this period, the mixture was filtered into Whatman filter paper n.1 and then into membrane 0.22 μm pore diameter. The extracted solution was called aqueous extract (AE) and stored at 4°C until use, not exceeding seven days of storage (BALDO et al., 2011).

The population of *M. incognita* was obtained from Santa Clara tomato roots kept in air-conditioned greenhouse, and identified based on the configuration of the perineal region (HARTMAN; SASSER, 1985). To get nematodes and eggs from infected roots was used the methodology described in Freitas et al. (2007), based on the methodology of Hussey and Barker (1973), modified by Boneti and Ferraz (1981). The roots were cut and shredded into a stirrer for 20 sec, poured over a sieve of 0.427 mm opening (48 Mesh), coupled in the sieve of 0.037 mm opening (500 Mesh). The eggs and the second stage juveniles (J2), retained in the 400 Mesh sieve were transferred to Peters chamber and quantified with optical microscope.

Soybean seeds were placed in trays of 128 cells containing substrate for plants class A Humusfértil® 1.6 kg. After 15 days (V1 phenological stage) the plants were transplanted to 500 mL glasses containing mixture of substrate and humus (3:1, v/v). At the time of transplantation, the plants were sprayed into the leaves with concentrations of the extract of *P. sanguineus*, taking care not to slip into the soil. After three days of transplantation, the plants were inoculated with a suspension containing 1,000 eggs + J2 per plant, in three holes made around the seedling, 1.5 cm away from each other and 2.0 cm deep. These plants were irrigated daily and care was taken not to wash the inoculum deposited in the substrate, as described by Tihohod (1989) and the test conducted for 60 days in the greenhouse, with an average temperature of 28°C.

In these tests, vegetative assessments of fresh and dry air masses (g), aerial part height (cm) and fresh root mass (g) were carried out, and for nematological assessments: number of egg mass, number of galls and total number of nematodes at the root (g/root).

For evaluation of egg mass in the roots, these were coloured with Floxin B (15 mg L^{-1}) and after 20 min, washed to remove excess dye (TAYLOR; SASSER, 1978). The number of galls with and without egg mass was counted. The methodologies of Freitas et al. (2007) and Coolen and D'Herde (1972) were used to extract eggs and J2 from roots. First a solution of water and sodium hypochlorite (5 mL L^{-1}) was prepared to release the mass of eggs; then the roots were crushed into a stirrer for 20 sec. minimum speed. After, they were poured into 500 Mesh sieve and the crushed was washed with running water and collected for quantification, in Peters chamber, under optical microscope.

The results obtained were submitted to the normality test and analysis of variance and regression, at

5% probability of error, using the Sisvar statistical program (FERREIRA, 2011).

RESULTS AND DISCUSSION

For vegetative variables (Table 1), there was no significant difference among treatments. For the nematological variables number of egg masses, number of galls and number of nematodes per gram of root (Table 2) there was also no significance.

TABLE 1 - Fresh mass of aerial part (FMAP), dry mass f aerial part (DMAP), fresh mass f roots (FMR) and plant height (PH) of soybean treated in the aerial part with concentrations of *P. sanguineus* extract (PSE) and inoculated with *M. incognita*.

Concentrations (%) of PSE	FMAP (g)	DMAP (g)	FMR (g)	PH (cm)
0.0	33.84 ^{ns}	9.33 ^{ns}	18.36 ^{ns}	54.60 ^{ns}
2.5	33.26 ^{ns}	9.46 ^{ns}	19.98 ^{ns}	51.40 ^{ns}
5.0	34.48 ^{ns}	9.66 ^{ns}	19.66 ^{ns}	58.20 ^{ns}
7.5	35.28 ^{ns}	9.30 ^{ns}	18.60 ^{ns}	60.0 ^{ns}
10.0	35.80 ^{ns}	10.93 ^{ns}	18.86 ^{ns}	55.60 ^{ns}
CV(%)	5.97	25.81	10.72	8.52
Average CV (%): 12.76				

^{ns} = no significance.

TABLE 2 - Number of egg mass (NEM), number of galls (NG) and number of nematodes (NN) in soybean treated in the aerial part with concentrations of *P. sanguineus* extract (PSE) and inoculated with *M. incognita*.

Concentrations (%) of PSE	NEM	NG	NN (g/root)
0.0	208.2 ^{ns}	374.8 ^{ns}	1480.7 ^{ns}
2.5	146 ^{ns}	262.4 ^{ns}	1281.8
5.0	96 ^{ns}	248.8 ^{ns}	1313.5
7.5	102.2 ^{ns}	283.6 ^{ns}	1216.2
10.0	116.2 ^{ns}	274.2 ^{ns}	959.8
CV (%)	51.37	46.93	20.75
Average CV (%): 39.83			

^{ns} = no significance.

For the nematological variable total number of nematodes, however, there was significant effect of concentrations of *P. sanguineus* (Figure 1), represented by a second-degree equation, with the calculated value of 17% being the concentration that would provide the greatest reduction. In the literature there are many reports of *in vitro* nematicide activity of compounds extracted from basidiomycetes, as well as by biological control by direct parasitism (ELKHATEEB et al., 2021).

However, there is little work for *in vivo* nematode control when only the use of compounds produced by basidiomycetes is considered. In this context there is the work of Wille et al. (2019), in which extracts of *Pleurotus ostreatus*, *P. citrinopileatus*, *P. pulmonarius*, and *Boletus* sp., applied to soil infested with *M. incognita*, reduced on average 70% the reproduction of nematode in lettuce.

For *P. sanguineus*, reports of the use of extracts from this basidiomycete for nematode control are scarce. In tomatoes, the aqueous extract of *P. sanguineus* basidiocarps was sprayed weekly in the leaves three days before inoculation with *M. javanica*, resulting in up to 90% the disease, clearly indicating the potential resistance inducer of this fungus (BARBOSA et al., 2021).

In the present study it was found that, as a gradual increase in *P. sanguineus* extract concentrations occurred, it was possible to observe a decline in the number of total nematodes per root indicating control potential. This dose-dependent control potential has already been verified in other pathosystems. Viecelli et al. (2010) found that the severity of the angular leaf spot in bean was reduced by induction of systemic resistance by 71.6% and 69.4%, with the extracts of *P. sanguineus* at concentrations of 10% and 20%, respectively. Assi et al. (2017) also observed induction of systemic resistance by the extract of *P. sanguineus* against *Alternaria solani* and *Xanthomonas vesicatoria* in tomatoes, which corroborates with this work although it is another pathosystem.

It can be found that the extract of *P. sanguineus* presents potential in the control of diseases in plants, probably by induction of resistance, however, when evaluating this extract for the control of *M. incognita* in soybeans, the same was not significant for any of the vegetative variables, but only for the nematological variable total number of nematodes in the roots. This may indicate that although there was control of this radicular pathogen by the spraying of the extract in the aerial part of soybeans, this resistance induction did not generate metabolic cost for the plant.

Thus, future work is needed to investigate which metabolic pathways act in the process of resistance induction, since in this work there was no metabolic cost in inducing plant resistance.

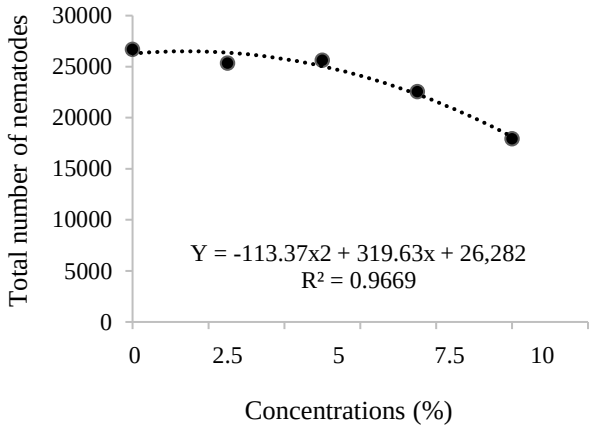

FIGURE 1 - Total number (eggs + second stage juveniles) of *Meloidogyne incognita* in soybean treated in aerial part with concentrations of *P. sanguineus* extracts.

CONCLUSIONS

The aqueous extract of *P. sanguineus* presents potential for controlling *M. incognita* in soybean, probably by induction of resistance not involving metabolic cost for the plant.

REFERENCES

ALENCAR, M.S.R.; SOLINO, A.J.D.S.; OLIVEIRA, J.S.B.; PASCHOLATI, S.F.; SCHWAN-ESTRADA, K.R.F. Induction of defense mechanisms in tomato plants by saprobic fungi filtrates against early blight disease. **Revista Caatinga**, v.33, n.3, p.671-678, 2020.

ASSI, L.; MEINERZ, C.C.; STANGARLIN, J.R.; KUHN, O.J.; VIECELLI, C.A.; SCHWAN-ESTRADA, K.R.F. Controle de *Alternaria solani* e de *Xanthomonas vesicatoria* em tomateiro por extrato formulado de *Pycnoporus sanguineus*. **Scientia Agraria Paranaensis**, v.16, n.3, p.314-320, 2017.

BALDO, M.; STANGARLIN, J.R.; FRANZENER, G.; ASSI, L.; KUHN, O.J.; SCHWAN-ESTRADA, K.R.F. Detecção *in situ* de espécies reativas de oxigênio em feijoeiro tratado com extratos de *Pycnoporus sanguineus* e inoculado com *Colletotrichum lindemuthianum*. **Summa Phytopathologica**, v.37, n.4, p.174-179, 2011.

BARBOSA, J.A.; RAMOS, D.D.; RINALDI, L.K.; STANGARLIN, J.R.; FIORENTIN, F. *Pycnoporus sanguineus* extract in the control of *Meloidogyne javanica* in tomato. **Ensaios e Ciência**, v.25, n.5, p.783-787, 2021.

BONETI, J.I.S.; FERRAZ, S. Modificações do método de Hussey e Barker para extração de ovos de Meloidogyne exigua em raízes de cafeeiro. **Fitopatologia Brasileira**, v.6, n.3, p.553, 1981.

CONAB. COMPANHIA NACIONAL DE ABASTECIMENTO. **Acompanhamento da safra brasileira de grãos.** 2021. Brasília, v.8, safra 2020/21, sexto levantamento, 2021.

COOLEN, W.A.; D'HERDE, C.J. **A method for the quantitative extraction of nematodes from plant tissue.** Ghent: State Agriculture Research Centre, 1972. 77p.

DI PIERO, R.M.; WULFF, N.A.; PASCHOLATI, S.F. Partial purification of elicitors from *Lentinula edodes* basidiocarps protecting cucumber seedlings against *Colletotrichum lagenarium*. **Brazilian Journal of Microbiology**, v.37, n.2, p.175-180, 2006.

ELKHATEEB, W.A.; DABA, G.M.; GAZIEA, S.M. The anti-nemic potential of mushroom against plant-parasitic nematodes. **Journal of Microbiology and Biotechnology**, v.6, n.1, e-000186, 2021.

FERRAZ, L.C.B. **Nematoides.** In: AMORIM, L.; RESENDE, J.A.M.; BERGAMIN FILHO (Eds.). Manual de fitopatologia: princípios e conceitos. 5ª ed. Ouro Fino: Ceres, cap. 13, p.195-211, 2018.

FERREIRA, D.F. Sisvar: a computer statistical analysis system. **Ciência e Agrotecnologia**, v.35, n.6, p.1039-1042, 2011.

FREITAS, L.G.; NEVES, W.S.; OLIVEIRA, R.D.L. **Métodos em nematologia vegetal.** In: ALFENAS, A.C.; MAFIA, R.G. (Eds.). Métodos em Fitopatologia. 1ª ed. Viçosa: UFV, 2007. p.253-291.

GRIGOLLI, J.F.J. **Manejo de doenças na cultura da soja.** In: REIS, E.M.; CASA, R.T. (Eds.). Doenças da soja. Editora: Berthier, p.206-23, 2012.

HARTMAN, K.M.; SASSER, J.N. **Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology.** In: BARKER, K.R.; CARTER, C.C.; SASSER, J.N. (Eds.). An advanced treatise on *Meloidogyne*. Methodology, North Carolina: University Graphics, 1985. p.69-77.

HIRAKURI, M.H.; LAZZAROTTO, J.J. **O agronegócio da soja nos contextos mundial e brasileiro.** Documentos 349. Londrina: Embrapa Soja, 70p. 2014.

HUSSEY, R.S.; BARKER, K.R.A comparison of methods for collecting inocula of *Meloidogyne* spp. including a new technique. **Plant Disease Reporter**, v.57, n.12, p.1025-1028, 1973.

JUHÁSZ, A.C.P.; PÁDUA, G.P.; WRUCK, D.S.M.; FAVORETO, L.; RIBEIRO, N.R. Desafios fitossanitários para a produção de soja. Defesa vegetal e sustentabilidade do agronegócio. **Informe Agropecuário**, v.34, n.276, p.66-75, 2013.

MARTINAZZO-PORTZ, T.; MIORANZA, T.M.; STANGARLIN, J.R.; KUHN, O.J. Substrate with lignocellulosic residues for *Pycnoporus sanguineus* cultivation. **Revista Caatinga**, v.35, n.2, p.243-253, 2022.

MÜLLER, M.A.; MIORANZA, T.M.; STANGARLIN, J.R.; KUHN, O.J.; BATTISTUS, A.G.; ISTCHUK, A.N.; FUCHS, F. *In vitro* toxicity and control of *Meloidogyne incognita* in soybean by rosemary extract. **Semina.** Ciências Agrárias, v.37, p.103-110, 2016.

PASCHOLATI, S.F.; DALIO, R.J.D. Fisiologia do parasitismo: como as plantas se defendem dos patógenos. In: AMORIN, L.; REZENDE, J.A.M.; BERGAMIN FILHO, A. (Eds.). **Manual de Fitopatologia:** princípios e conceitos. 5ª edição. Ouro Fino: Agronômica Ceres, 2018. p.424-450.

PEITER-BENINCA, C.; FRANZENER, G.; ASSI, L. IURKIV, L.; ECKSTEIN, B.; COSTA, V.C.; NOGUEIRA, M.A., STANGARLIN, J.R.; SCHWAN-ESTRADA, K.R.F. Indução de fitoalexinas e atividade de peroxidases em sorgo e soja tratados com extratos de basidiocarpos de *Pycnoporus sanguineus*. **Arquivos do Instituto Biológico**, v.75, n.3, p.285-292, 2008.

SCHWAN-ESTRADA, K.R.F.; VIECELLI, C.A.; DI PIERO, R.M.; SILVA, C.M.; MESQUINI, R.M.; SANTOS NETO, J.; STANGARLIN, J.R. Extratos de cogumelos no controle de doenças de plantas. **Revisão Anual de Patologia de Plantas**, v.20, p.177-205, 2012.

TAYLOR, A.L.; SASSER, J.N. **Biology, identification and control of root-knot nematodes (*Meloidogyne* sp.).** Raleigh: North Carolina State University, 1978. 111p.

TÉLLEZ-TÉLLEZ, M.; VILLEGAS, E.; RODRÍGUEZ, A.; ACOSTA-URDAPILLETA, M.L.; DÍAZ-GODÍNEZ, G. Mycosphere Essay 11: Fungi of *Pycnoporus* genus: morphological and molecular identification, worldwide distribution and biotechnological potential. **Mycosphere**, v.7, n.10, p.1500-1525, 2016.

TIHOHOD, D. **Nematologia Agrícola.** Jaboticabal: FCAV, 1989. 80p.

TOILLIER, S.L.; IURKIV, L.; MEINERZ, C.C.; BALDO, M.; VIECELLI, C.A.; KUHN, O.J.; SCHWAN-ESTRADA, K.R.F.; STANGARLIN, J.R. Control of bacterial blight (*Xanthomonas axonopodis* pv. *phaseoli*) and biochemical analyses of bean resistance treated with *Pycnoporus sanguineus* extracts. **Arquivos do Instituto Biológico**, v.77, n.1, p.99-110, 2010.

VIECELLI, C.A.; STANGARLIN, J.R.; KUHN, O.J.; SCHWAN-ESTRADA, K.R.F. Indução de resistência em feijoeiro a mancha angular por extratos de micélio de *Pycnoporus sanguineus*. **Summa Phytopathologica**, v.36, n.1, p.73-80, 2010.

WILLE, C.N.; GOMES, C.B.; MINOTTO, E.; NASCIMENTO, J.S. Potential of aqueous extracts of basidiomycetes to control root-knot nematodes on lettuce. **Horticultura Brasileira**, v.37, n.1, p.54-59, 2019.