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Aqueous extract of spent hops suppresses root-knot nematode and enhances soil biological activity

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ABSTRACT

Hops are known worldwide for their medicinal and antimicrobial properties, but their applications have been little studied in the field of agriculture. Furthermore, there are few studies about the nematicidal effect of the generated hops residue by the brewing industry. This study aimed to evaluate the potential of a 5% aqueous extract of hops residues in controlling *Meloidogyne javanica* in tomato plants and assess its impact on soil biological activity. Two experiments were conducted at different times. In both experiments, tomato seedlings grown in pots in a greenhouse received an application of 5% sent hops extract or water (control), using a spray volume of 50 L/ha. The treatments were applied in a hole made in the soil, close to the root system of the plant, approximately 2 cm deep. Plants were then inoculated with 1,000 eggs + eventual second-stage juveniles (J2) of *M. javanica*/plant, depositing the suspension containing the nematodes in the same hole made in the soil. In the laboratory, in the first experiment, basal respiration, soil microbial biomass, and nematode reproduction were determined at 30 days after inoculation (DAI), and in the second experiment, *M. javanica* reproduction was evaluated at 30 and 60 DAI, while basal respiration and soil microbial biomass were evaluated at 60 DAI. In the first experiment, the extract reduced total nematode number and number of nematodes per gram of root by up to 70% and 82%, respectively. In the second experiment, the respective reductions were 71% and 83% at 30 DAI and 80% and 73% at 60 DAI. The results showed that, in general, soils under plants treated with spent hop extract had higher basal respiration and microbial biomass carbon in both years, even when infected with nematodes. Thus, hops extract demonstrates potential for use in the management of *M. javanica*. However, further studies are necessary to elucidate the modes of action against these phytopathogens and their effects on soil.

Keywords: *Humulus lupulus*, *Meloidogyne javanica*, control, soil microbial biomass carbon, soil basal respiration, organic matter.

RESUMO

Extrato aquoso de resíduo de lúpulo suprime o nematoide das galhas e aumenta a atividade biológica do solo

O lúpulo é conhecido mundialmente por suas propriedades medicinais e antimicrobianas, mas é pouco estudado na agricultura. Também existem poucas informações na literatura sobre o efeito nematicida do resíduo da cultura gerado na indústria cervejeira. Diante desse cenário, os objetivos deste trabalho foram avaliar a capacidade do extrato de resíduo de lúpulo, na dosagem de 5%, no controle de *Meloidogyne javanica* em tomateiro, e verificar seu impacto na atividade biológica do solo. Foram conduzidos dois experimentos em épocas distintas. Em ambos os experimentos, plântulas de tomate, cultivadas em vaso, em casa de vegetação, receberam a aplicação de 5% de extrato de resíduo de lúpulo ou água (controle), utilizando-se volume de calda de 50 L/ha. Os tratamentos foram aplicados em um orifício aberto no solo, próximo ao sistema radicular da planta, com aproximadamente 2 cm de profundidade. Em seguida, estas foram inoculadas com 1.000 ovos + eventuais juvenis de segundo estádio (J2) de *M. javanica*/planta, depositando-se a suspensão contendo os nematoides no mesmo orifício aberto no solo. Em laboratório, no primeiro experimento, foram determinadas a respiração basal, a biomassa microbiana do solo e a reprodução do nematoide, aos 30 dias após a inoculação (DAI) e, no segundo experimento, a reprodução de *M. javanica* foi avaliada aos 30 e 60 DAI, enquanto a respiração basal e a biomassa microbiana do solo foi avaliada aos 60 DAI. No primeiro experimento, o extrato reduziu o número total de nematoides e nematoides por grama de raiz em até 70% e 82%, respectivamente, em relação à testemunha. No segundo experimento, as respectivas reduções aos 30 DAI foram de 71% e 83%, e aos 60 DAI, foram de 80% e 73%, respectivamente, em relação à testemunha. Para a atividade biológica do solo, em geral, as plantas que receberam a aplicação do extrato obtiveram maiores médias de respiração basal e biomassa microbiana do solo, mesmo quando inoculadas em ambos os experimentos. Dessa forma, o extrato de lúpulo estudado demonstra potencial de uso no manejo de *M. javanica*. Entretanto, estudos adicionais são necessários para elucidar os modos de ação contra esses fitopatógenos bem como seus efeitos no solo.

Palavras-chave: *Humulus lupulus*, *Meloidogyne javanica*, controle, carbono da biomassa microbiana, respiração basal do solo, matéria orgânica.

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Nematodes are among the most devastating pathogens for agriculture, particularly members of the genus *Meloidogyne*, whose management is complicated by their broad host range and wide geographic distribution (Jones *et al*., 2013). Strategies for nematode control in vegetable crops include antagonistic plants, crop rotation, elimination of crop residues, resistant cultivars, chemical agents, biological agents, and organic matter amendment (Pinheiro *et al*., 2019). Organic matter incorporation has the potential to control nematodes. Different materials can be used for this purpose, such as crop waste, organic fertilizers, chitinous waste, oil press cake, and any other organic matrix that releases toxic compounds upon decomposition (Lopes, 2019).

Plant extracts, essential oils, and agroindustrial wastes have been investigated as alternative methods for nematode control (Reiner *et al.*, 2016; Brito *et al.*, 2020, Tarini *et al.*, 2020). It is important to note that organic matter amendment may alter soil properties, affecting mineral nitrogen, pH, acetic acids, and soil basal respiration through the release of toxic compounds that act on root pathogens (Pathma & Sakthivel, 2012). Phenolic acids, a prominent example of such toxic compounds, play an important role in plant tolerance to abiotic or biotic stresses (Rehman *et al.*, 2012; Tuominen, 2013). The monitoring of soil biological activity can provide interesting information on the effects of organic amendments.

Hops, the female inflorescences of *Humulus lupulus* (L.), are widely used worldwide as raw material in the brewing industry. Hops are added during beer production for preservation purposes and to impart characteristic flavors resulting from the presence of different classes of compounds, including terpenoids, bitter acids, phenolic acids, and other phenolic compounds (Bocquet *et al*., 2018a). A study investigating the use of hops for nematode control found that a methanolic extract of hop inflorescences at a concentration of 2.5% (v/v) inhibited the hatching of *Meloidogyne incognita* juveniles by 76.3% and, at 10%, caused 100% mortality after 24 h of *in vitro* exposure (Akyazi, 2014). A whole plant extract of pre-dried *H. lupulus* obtained by cold water extraction inhibited *Meloidogyne arenaria* hatching by 95% after five days of *in vitro* exposure, whereas the extract obtained by hot water extraction reduced gall number by about 83% in tomato crops grown under controlled conditions (Aydinli & Mennan, 2014). However, little is known about the effect of spent hops on phytopathogens or soil biological activity.

Given the above, this study aimed to evaluate the potential of spent hop extract in the suppression of *M. javanica* reproduction on tomato and assess its impact on soil biological activity.

MATERIAL AND METHODS

Extract preparation

The spent hops residue was provided by the Tamuía brewing company, located in Umuarama, Paraná, Brazil. A crude extract was prepared by mixing spent hops and distilled water at a 1:1 (w/v) ratio. The mixture was kept at rest for 24 h and then filtered through a funnel lined with gauze. The resulting extract was diluted to 5% with distilled water for use in the experiments.

Greenhouse experiments

The first experiment was conducted in a greenhouse at the State University of Maringá, Umuarama, Paraná State, Brazil (23°47′29″S 53°15′24″W, 430 m elevation). The mean daily minimum and maximum temperatures during this period were 20°C and 32°C. The experiment followed a completely randomized design with seven replications of each of the following treatments: spent hop extract + nematode inoculation, spent hop extract only, nematode-inoculated control, and uninoculated control. The experiment lasted from November 2020 to January 2021.

First, seedlings of tomato 'Santa Clara' were grown in commercial substrate (Bioplant®) in polystyrene trays. Twenty days after emergence, seedlings were transplanted into plastic pots containing 1.2 kg of a 1:1 mixture of soil and sand, previously autoclaved (120°C, 2 h).

Just after the transplanting, seedlings were inoculated with 1,000 eggs + eventual second-stage (J2) of *M. javanica*. The nematode inoculum was deposited into the planting hole, onto the roots, after application of 5% hop extract (at 0.48 mL/plant) or distilled water (control) at a spray volume of 50 L/ha. The inoculum was obtained from a pure nematode population multiplied on okra for two months in a greenhouse. Nematodes were extracted according to the method proposed by Hussey & Barker (1973) and adapted by Boneti & Ferraz (1981).

At 30 days after nematode inoculation (DAI), plants were harvested, the aerial parts discarded, and the roots washed and evaluated for fresh weight on a semi-analytical balance. Subsequently, nematodes were extracted from roots according to the above-mentioned method and total nematode number was determined in a Peter's chamber under a light microscope. Total nematode number was divided by the root fresh weight to obtain the nematode population density (number of nematodes per gram of

root). The soil in each pot was collected and stored in a refrigerator at about 5°C until analysis of microbial biomass carbon (MBC) and soil basal respiration (SBR).

The experiment was repeated from March to June 2021 using the same procedures, except that nematode reproduction was evaluated at 30 and 60 DAI and soil biological activity at 60 DAI. The mean daily minimum and maximum temperatures were 19°C and 29°C, respectively.

MBC determination

MBC was determined by adapting the method originally proposed by Vance *et al.* (1987). Soil samples were collected from experimental units and sieved through 2 mm mesh sieves to remove plant fragments. For this analysis, the soil of each experimental unit was separated into two 20 g aliquots, one of which was fumigated. Samples were stored in 100 mL amber glass flasks. Fumigation was performed by adding 1 mL of ethanol-free chloroform using a pipette and incubating samples in the dark for 24 h. On the following day, flasks were opened under a fume hood and allowed to rest until complete evaporation of the solvent.

For MBC determination, samples received the addition of 50 mL of potassium sulfate (K_2SO_4) solution and were stirred for 30 min. Then, the supernatant was filtered through a paper filter attached to a funnel over a 100 mL beaker. At the end of filtration, 8 mL of each sample was transferred to 250 mL Erlenmeyer flasks and added with 2 mL of 0.066 M potassium dichromate, 5 mL of orthophosphoric acid P.A., and 10 mL of sulfuric acid P.A. After cooling, 70 mL of distilled water and four drops of diphenylamine were added for titration with 0.033 M ferrous ammonium sulfate solution. Titration was completed when the color changed from purple to green. The volume (mL) of ferrous ammonium sulfate used in this step was recorded.

The carbon content of extracts was determined by using the formula $C = [(V_b - V_a)\ M \times 0.003 \times V_1 \times 10^6]/(S_w \times V_2)$, where C is the carbon content extracted from soil (mg C/kg soil), V_b is the volume of ferrous ammonium sulfate used for titration of the blank (mL), V_a is the volume of ferrous ammonium sulfate used for titration of the sample (mL), M is the exact molarity of ferrous ammonium sulfate, V_1 is the volume of extractor (K_2SO_4), V_2 is the aliquot of the extract used for titration, and S_w is the soil dry weight (g). MBC was then calculated using the formula $MBC = F_c \times k_c^{-1}$, where MBC is the microbial biomass carbon extracted from soil (mg C/kg soil), F_c is the flux obtained by the difference between the C content estimated from the previous equation, and k_c is a correction factor (0.33).

SBR determination

SBR was quantified by the method of Jenkinson & Powlson (1976), with modifications. Briefly, 50 g of soil sample from each experimental unit was deposited in 100 mL flasks. For each flask containing soil, an identical flask received the addition of 10 mL of 1 M sodium hydroxide (NaOH) using an automatic pipette. Both flasks were transferred to a larger glass bottle, which was covered with film paper to prevent the entrance or exit of CO_2. Flasks containing NaOH solution only were used as blank. Samples remained in the dark for seven days.

After incubation, for quantification of CO_2 respiration, 2 mL of 10% (w/v) barium chloride was added to flasks containing the NaOH solution for CO_2 precipitation, and the large jars were closed. Sample flasks were opened only before titration with 0.5 M hydrochloric acid (HCl). The end of titration was determined by a change in color from pink to colorless. The volume of HCl (mL) used to reach the color change was recorded.

Basal respiration was estimated by the following equation: $SBR = (V_b - V_a) \times M \times 6 \times 1000/(S_w \times T)$, where V_b is the volume of hydrochloric acid used for titration of the blank (mL), V_a is the volume of hydrochloric acid used for titration of the sample (mL), M is the exact molarity of HCl (mol/L), S_w is the soil dry weight (g), and T is the incubation time (h). The soil metabolic quotient (qCO_2) was calculated as qCO_2 = SBR/MBC × 1000.

Statistical analysis

Nematode reproduction and soil biological activity data were subjected to analysis of variance at the 5% significance level. When significant, data were assessed by Tukey's test at the 5% level using Sisvar version 5.3 (Ferreira 2014).

RESULTS AND DISCUSSION

Application of spent hop extract reduced nematode reproduction at 30 DAI in the first and the second experiments (Table 1). The total nematode number was reduced by 70% and nematode population density (number of *Meloidogyne javanica* per gram of root) by 82%, compared to the control. In the second experiment, at 30 DAI, the total nematode number was reduced by 71% and nematode population density by 83% (Table 1). At 60 DAI, extract treatment provided a reduction of about 80% and 73% in total nematode number and population density, respectively, compared to the control.

Although hops are little investigated for their efficiency in nematode control, previous *in vitro* experiments showed that acetone hop extract (2.5% w/v) inhibited *M. incognita* hatching and an aqueous extract inhibited *M. arenaria* hatching (Akyazi, 2014, Aydinli & Mennan, 2014). We highlight that various agroindustrial wastes have exhibited activity against nematodes under *in vitro* and greenhouse conditions (Brito *et al.*, 2020; Tarini *et al.*, 2020), including wine industry waste, which deformed nematode eggs and/or juveniles (Reiner *et al.*, 2016).

Table 1. Total number (TNMj) and number of *Meloidogyne javanica* per gram of root (NGRMj/g) in tomato plants treated with a 5% concentration of aqueous extract of spent hops. Evaluations were performed at 30 days after nematode inoculation (DAI) in Experiment 1 and at 30 and 60 DAI in Experiment 2. Umuarama, UEM, 2021.

Treatment	Exp. 1 (30 DAI)		Exp. 2 (30 DAI)		Exp. 2 (60 DAI)	
	TNMj	NGRMj/g	TNMj	NGRMj/g	TNMj	NGRMj/g
Inoculated control	2874 a	398 a	2529 a	360 a	17340 a	537 a
Extract + inoculation	884 b	71 b	729 b	62 b	3481 b	146 b
CV (%)	32.30	26.17	14.49	18.69	12.68	20.40

Means within columns followed by the same letter are not significantly different from each other by Tukey's test (p<0.05). CV (%)= coefficient of variation.

The significant reduction in nematode population observed here may be attributed to a series of factors, including the action of compounds present in or released by spent hop extract. Hop plants synthesize prenylated chalcones, such as xanthohumol and acylphloroglucinol derivatives (α- and β-acids), also known as bitter acids, which have antimicrobial action (Bocquet *et al.*, 2019).

Bitter acids present in hops were found to exhibit activity against Gram-positive (Bocquet *et al.*, 2019) and Gram-negative bacteria, including *Brucella* (Shapouri & Rahnema, 2011). The compounds also exerted antifungal effects on *Fusarium* (Rój *et al.*, 2015). Compounds synthesized by hop plants act on the cell membrane of these microorganisms (Rój *et al.*, 2015) and are hypothesized to have a similar action on *M. javanica*. In another study, hop methanolic extract inhibited enzymes that are crucial for the survival of several microorganisms, such as α-amylase (EC 3.2.1.1), urease (EC 3.5.1.5), and acetylcholinesterase (EC 3.1.1.7) (Keskin *et al.*, 2019).

Hops showed positive results in the control or suppression of other pathogens of agricultural importance, including *Zymoseptoria tritici* (Desm.), *Colletotrichum lindemuthianum* (Sacc. & Magnus) (Briosi & Cavara, 1889), *Penicillium* spp., *Aspergillus parasiticus* (Speare), and *Aspergillus niger* (van Tieghem), under both greenhouse and *in vitro* conditions (Truylio, 2015, Bocquet *et al.*, 2018b). The antimicrobial activity of plant extracts is generally attributed to the presence of biologically active compounds, particularly that of polyphenols.

Regarding soil biological activity, in the first experiment (30 DAI), treatment with spent hop extract combined with nematode inoculation led to significant differences in SBR and MBC (Table 2). SBR increased by more than 100% with extract + inoculation compared with the inoculated or uninoculated control, and extract only (Table 2). The extract + inoculation treatment differed in MBC from the other treatments, promoting an increase of 39.7%, 93.3%, and 34.0% compared with extract only, the inoculated and uninoculated control, respectively (Table 2).

In the second experiment (60 DAI), the SBR of extract-treated plants was three times higher than that of the controls, regardless of inoculation (Table 2). The MBC of extract-treated plants (inoculated or uninoculated plant) differed significantly from that of the controls; the means of inoculated and uninoculated controls were 83.6% and 52.0% lower. There were no significant differences in qCO_2 between treatments in either experiment.

These results may be related to the indirect effects of extract application. It is known that application of organic compounds improves soil characteristics and increases organic matter contents, positively affecting soil physical parameters, nutrient cycling, and the levels of nutrients necessary for the maintenance of microorganisms in the soil (Sharma & Garg 2018). In general, it is observed that the use of biofertilizers increases the availability of readily metabolizable carbon, contributing to the increase in microbial biomass (Yu *et al.*, 2013).

SBR represents the respiratory activity of soil microorganisms, which results in CO_2 production. The parameter can be used to identify changes that influence soil microbial activity (Polli & Pimentel, 2005). Recent studies investigated the respiratory activity of soil and nematodes by adding biological agents to soil samples (Oliveira *et al.*, 2019, Miamoto *et al.*, 2021). Here, we did not add biological agents and the soil was autoclaved; however, given the increase in MBC and SBR, we hypothesize that spent hop extract contained microorganisms that altered the levels of these variables. Such microorganisms might have acted directly or indirectly on pathogens. To confirm this hypothesis, it is necessary to conduct studies to identify microorganisms or enzymes in soil.

Table 2. Soil basal respiration (SBR), microbial biomass carbon (MBC), and metabolic quotient (qCO_2) of soils infested or not with *Meloidogyne javanica* at 30 and 60 days after inoculation (DAI) with or without application of a 5% aqueous extract of spent hops. Umuarama, UEM, 2021.

Treatment	SBR (mg CO_2-C/kg soil/h)	MBC (mg C/kg soil)	qCO_2 (mg CO_2-C/g C/h)
	Experiment 1 (30 DAI)		
Extract + inoculation	2.92 a	773.27 a	3.77^{ns}
Extract only	1.55 bc	553. 29 bc	2.80
Inoculated control	1.27 c	399.97 c	3.17
Uninoculated control	1.60 b	576.62 b	2.77
CV (%)	11.35	19.29	55.52
	Experiment 2 (60 DAI)		
Extract + inoculation	3.60 a	6045.79 a	0.59^{ns}
Extract only	3.31 a	5395.33 a	0.61
Inoculated control	0.59 b	1823.61 b	0.32
Uninoculated control	1.73 b	3641.41 b	0.48
CV (%)	38.06	24.32	61.03

Means within columns followed by the same letter are not significantly different from each other by Tukey's test (p<0.05). CV (%)= coefficient of variation; ns= not significant.

It was possible to conclude that the 5% spent hop extract is efficient in the control of *M. javanica* in tomato, providing reductions of about 70% in nematode populations. The extract is also efficient in improving SBR and MBC. However, despite these benefits, including the potential for using the extract in nematode management and the opportunity for repurposing this industrial byproduct, further research is needed to clarify its mechanisms of action on nematodes and their impact on soil health.

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