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RESEARCH

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Control of *Meloidogyne javanica* with *Pleurotus djamor* spent mushroom substrate

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Abstract

Background The interest in the development of products that cause less damage to the environment associated with the loss of efficiency of chemical nematicides for the control management of nematodes is growing. Thus, the adoption of biological control or the use of biopesticides are excellent options for these products like those based on chemical compounds, such as commercial pesticides and anthelmintic (AH) drugs. Spent mushroom substrate (SMS), a product of the mushroom production industry, has great potential for biological control due to its high levels of mycelium, residual enzymes, high humidity and unique microbiota that may contain other nematode antagonists. For this reason, this study aimed to evaluate the potential of spent mushroom substrate (SMS) from *Pleurotus djamor* cultivation in the control of *Meloidogyne javanica* in lettuce and assess its effects on plant resistance enzymes and soil biological activity.

Results SMS reduced by 98.68% the nematode reproduction, and a plateau was reached at SMS concentrations above 15%. For the population density of nematode (nematode g-1 root), this reduction was 99,75%. Higher concentrations of SMS caused phytotoxicity in lettuce, with reduction of vegetative variables, chlorophyll content and nitrogen balance in the leaves; however, SMS increased the anthocyanin content. Guaiacol peroxidase activity was the highest in treatments containing 0% and 30% SMS and phenylalanine ammonia-lyase activity was the highest in the 60% SMS treatment, suggesting induction of resistance to *M. javanica*. The maximum soil basal respiration was estimated to be achieved with 25.75% SMS, whereas the maximum soil metabolic quotient was estimated to be achieved with 8.8% SMS. Soil biomass carbon increased with increasing SMS proportion.

Conclusions Spent substrate from *P. djamor* cultivation incorporated in soil at proportions of 15, 30, 45 and 60% is efficient in controlling *M. javanica* in lettuce.

Keywords Root-knot nematode, Nematophagous fungi, Biological control, Edible mushrooms, Agro-industrial waste

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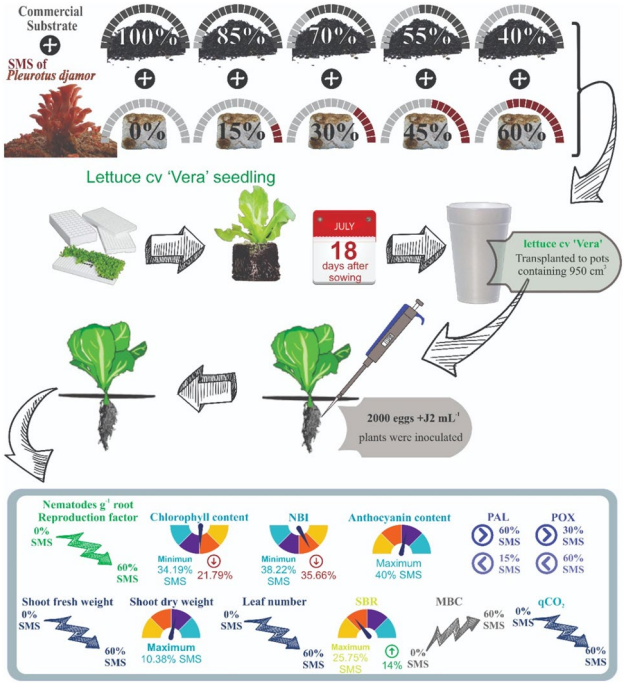
Graphical Abstract



Background

The global edible mushroom market is estimated at US$16.7 billion and projected to reach US$20.4 billion by 2025 [19]. In Brazil, predictions indicate a growth in mushroom production and consumption. This is due to the process of cultural interconnection, which includes changing culinary habits and focusing on healthier and more functional foods (Martínez-Ibarra 2019). In most Western countries, a large part of mushroom production is still carried out under rustic conditions by small rural producers, who combine fungiculture with other agricultural activities [36].

Mushrooms can be grown on different substrates, such as wood, fruit pulp and peel, banana leaves, coffee pulp [45, 55], grasses [41, 43], olive pruning residues [1], and industrial effluents [28], often combined with nitrogen or protein sources to maintain an adequate carbon/nitrogen (C/N) ratio [39]. Examples of nitrogen and protein sources include plant and oilseed meals (originating from soybean, cotton, sunflower, wheat, and maize, rice straw, sugarcane bagasse, juice waste, and wine waste [8, 9]. After a given number of cycles, which may vary according to fungal species and growing medium and commercial production, the substrate used for mushroom production has to be changed, generating a residue commonly known as spent mushroom substrate (SMS. The latter can be used as animal feed [60] as well as for bioremediation and organic fertilization [54], e.g., for tomato and cauliflower plants [29] [66], Sirić et al. 2022. An interesting characteristic is that some cultivated mushrooms have nematophagous activity, holding potential in the control of soil nematodes [5, 23, 41].

Several members of the mushroom genus *Pleurotus* have been reported to interact with nematodes, such as *P. cornucopiae* [23, 69], *P. cystidiosus*, *P. strigosus*, *P. subareolatus* [69], *P. florida* [23], *P. ostreatus* [23, 41, 69], *P. sajor-caju* [23], and *P. tuber-regium* [24, 41]. Some species can act as predators of plant–parasitic nematodes [69], extending their hyphae and paralyzing these phytoparasites through secretion of toxins, such as ostreatins, or production of toxin-containing structures known as toxocysts [6, 15, 24]. It is noteworthy that the information on interactions between nematodes and *Pleurotus djamor* is scarce. In addition to controlling nematodes, SMS can be used to improve soil microbiological quality and increase organic matter and mineral availability, stimulating plant development [20] and reducing leaching. Furthermore, studies suggested that SMS amendment may induce plant resistance against pathogens, owing to the presence of secondary metabolites and toxic fungal structures [4,

57]. It may be feasible to apply SMS as substrate/supplement for vegetable seedling production. An interesting advantage of such use is that, at the time of planting in the field, seedlings would have been pre-exposed to fungi and their metabolites.

Herein, it was hypothesized that spent substrate from *P. djamor* cultivation may be able to control nematodes and activate natural defense mechanisms in plants. This study aimed to assess the potential of *P. djamor* SMS in the control of *Meloidogyne javanica* in lettuce and investigate its effects on plant development, total chlorophyll content, nitrogen balance, flavonoid content, anthocyanin content, defense enzyme activities, and soil microbial activity.

Methods

Installation of the experiment and seedling production

The experiment was carried out in a greenhouse (23°47′34.5″S 53°15′22.1″W, 430 m above sea level) between July and September 2021. The design was completely randomized with five treatments and six replications. Treatments consisted of five proportions of SMS (0%, 15%, 30%, 45%, and 60%) added to a commercial substrate (Bioplant®) for seedling production. SMS, obtained after two cycles of *P. djamor* cultivation, was kindly donated by a local mushroom grower. The growing substrate was initially composed of 80% *Pine* spp. sawdust pellets, 18% wheat bran, 1% hydrated lime, and 1% calcitic limestone. Before start the process, the components of cultivation substrate are pasteurized. The producer mixes all the dry components in a concrete mixer, such as sawdust and wheat bran. When the mixture is quite homogeneous, water is added until reaching a moisture content of 62–65%. Afterward, the mixture is placed in heat-resistant plastic bags and the substrate is pasteurized for 6 h at 95°C.

Mixtures of SMS and commercial substrate at the proportions defined in the experimental design were placed in 128-cell polystyrene trays and sown with seeds of lettuce 'Vera'. At 18 days after sowing, seedlings were transplanted to pots containing 950 cm^3 of autoclaved (120 °C, 2 h) soil and sand at a ratio of 2:1, previously limed (0.7 g pot^{-1}) and fertilized with NPK fertilizer (15-09-12, 0.12 g pot^{-1}). After 10 days, plants were inoculated with a suspension containing 2000 eggs and eventual second-stage juveniles (J2) of *M. javanica*. Nematodes were extracted from a pure population maintained on soybean M6210 IPRO, according to the method proposed by Hussey and Barker and adapted by Boneti and Ferraz [11]. The suspension was quantified under an optical microscope (Motic® BA210E) using a Peters' chamber and calibrated to 2000 eggs + J2 mL^{-1}. Nitrogen top dressing (2% urea, 0.1 g pot^{-1}) was performed 30 days after transplanting.

Vegetative and physiological parameters

After 50 days of cultivation, plants were evaluated for total chlorophyll index, nitrogen balance index (NBI), flavonoid, content, and anthocyanin content using a portable chlorophyll meter (Force A, Dualex Scientific™, Orsay, France). Results are the mean of three readings from the middle third of each plant. Plants were then removed from the pots and divided into shoots and roots. Shoot fresh weight was determined using an analytical balance. Leaf number was determined by counting the number of leaves per plant, starting from the basal region to the last expanded leaf and excluding yellow and/or dry leaves, whenever present [10]. Head height was measured from the stem to the top of the head using a millimeter ruler. Shoot dry weight was determined by placing samples in paper bags and drying in a forced-air oven at 65 °C (Marconi MA35/1000) to constant weight. Root samples were thoroughly washed, placed on paper towels to remove excess water, and weighed to obtain the root fresh weight.

Nematode analysis

After fresh weight determination, roots were subjected to the above-mentioned method for nematode extraction and quantification. The number of nematodes per root system was divided by root fresh weight to obtain the number of nematodes per gram of root (population density). Reproduction factor was calculated as the ratio of final to initial population [42].

Enzyme analysis

PAL and POX activities were measured in plant leaves and roots at the end of the experimental period. Fresh shoot and root samples (0.5 g) were collected from the median portion of each organ for enzymatic analysis, with three replications per treatment. Samples were ground in a mortar with liquid nitrogen, polyvinylpyrrolidone, and 50 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA. After centrifugation, the extract was transferred to 1.5 mL microtubes and stored in a freezer (− 8 °C) for subsequent protein quantification [12]. Protein concentration (mg mL^{-1}) was determined against a standard curve of bovine serum albumin.

Guaiacol peroxidase (POX; EC 1.11.1.7) activity was measured based on the conversion of guaiacol to tetraguaiacol in the presence of hydrogen peroxide, and the results were expressed in Δabs_{470} min^{-1} mg^{-1} protein [34]. Phenylalanine ammonia-lyase (PAL,EC 4.3.1.5)

activity was determined according to Umesha [71]. Absorbance readings were taken at 290 nm. Enzymatic activity was calculated from the difference between the absorbance of sample and blank based on a standard curve of *trans*-cinnamic acid. Results were expressed in mg *trans*-cinnamic acid h^{-1} mg^{-1} protein [71].

Soil biological analysis

At the end of the experiment, around 400 g of soil were collected from three replicates per treatment for biological analysis. Soil microbial biomass carbon was determined according to the fumigation–extraction method [64]. Soil basal respiration and metabolic quotient (qCO_2, representing the ratio of soil basal respiration to microbial biomass carbon) were estimated according to Silva et al. [65]. All analyses were performed in triplicate.

Statistical analysis

Data were subjected to analysis of variance at the 5% significance level. When necessary, to meet Shapiro–Wilk normality assumptions, data were transformed to $\sqrt{x+1}$ before regression analysis at the 5% significance level, with the exception of enzyme data, whose means were compared using Tukey's test at the 5% significance level. Statistical analyses were performed using Sisvar software [17]. Data for nematode population were subjected to linear-plateau segmented regression analysis using R statistical language (R Core Team 2021) [50]. The regression model consisted of two segments, the first describing an increasing or decreasing line up to a given p value (response plateau) and the second assuming a nearly constant value [59].

Results

Nematode population density and reproduction factor decreased with increasing SMS proportion, reaching the plateau at about 15% SMS (Fig. 1A, B) with reductions of 99.75% and 98.68%, respectively, compared to the control (0% SMS) (Additional file 1).

There were no statistical differences in root fresh weight or head height between treatments, whose means ranged from 4.41 to 6.22 g and from 1.97 to 2.42 cm, respectively (data not shown). On the other hand, increasing SMS proportions led to a reduction in shoot fresh weight (Fig. 2A), by 30.19% in seedlings grown in substrate supplemented with 60% SMS and by 2.69% and 4.67% in seedlings grown on 15% and 30% SMS, respectively, compared with the control. Leaf number followed the same pattern (Fig. 2C), with 60% SMS leading to a 33.33% reduction and 15% SMS causing a reduction of only 1.33%. There was a quadratic effect on shoot dry weight, which was estimated to reach the maximum value at 10.83% SMS (Fig. 2B).

Chlorophyll content decreased with SMS addition (Fig. 3A), being lowest in seedlings grown in substrate containing 34.19% SMS, corresponding to a 21.79% reduction. In this study, NBI had a similar behavior to that of chlorophyll content, with lower values observed in the treatment containing 38.22% SMS, which led to a reduction of 35.66% (Fig. 3B). Anthocyanin content increased with SMS treatment, reaching a maximum at 40% SMS (Fig. 3C). For flavonoid content, there were no significant differences between treatments, whose means ranged from 0.62 to 0.79 (data not shown).

Both enzymes were found to be activated only in leaves (Fig. 4A, B). PAL activity increased in seedlings grown on substrate containing 30% SMS (Fig. 4A). POX activity was the highest in treatments containing 0% and 30% SMS (Fig. 4B).

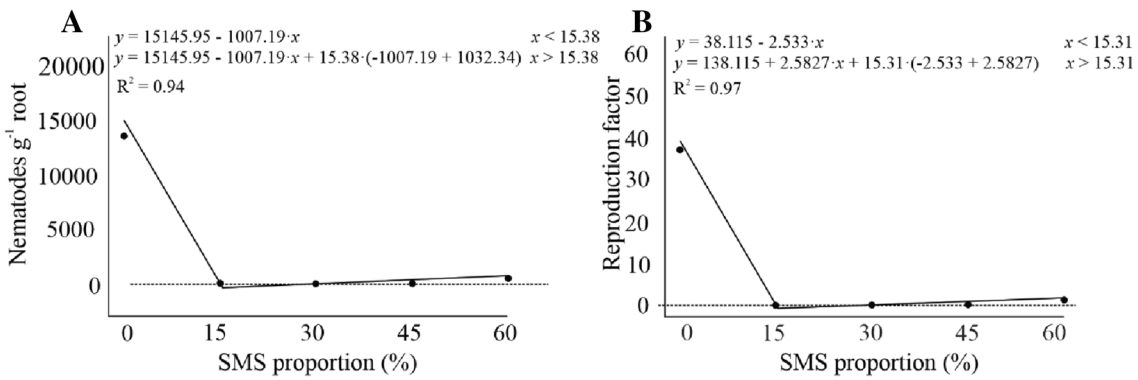


Fig. 1 **A** Number of nematodes per gram of root and **B** reproduction factor of *Meloidogyne javanica* on pre-grown seedlings of lettuce transplanted to substrate containing different proportions of spent mushroom substrate (SMS) from *Pleurotus djamor* cultivation. Coefficient of variation: A, 19.31%; B, 15.87%

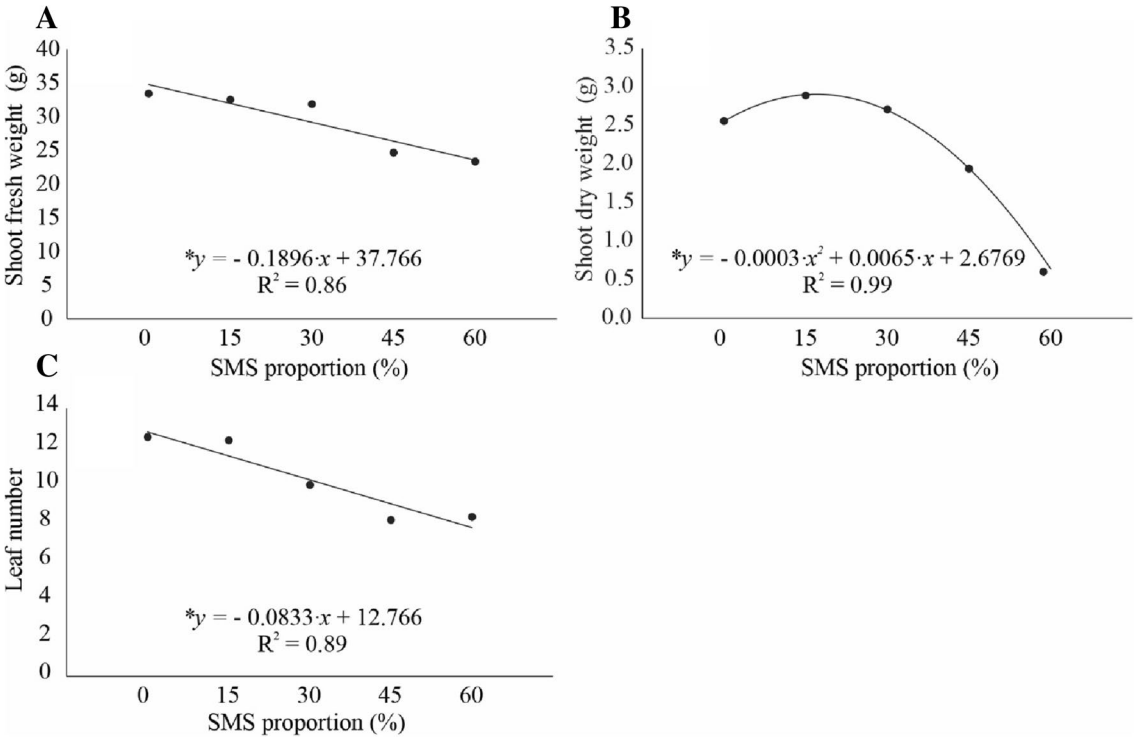


Fig. 2 **A** Shoot fresh weight, **B** shoot dry weight, and **C** leaf number of lettuce seedlings grown in substrate supplemented with different proportions of spent mushroom substrate (SMS) from *Pleurotus djamor* cultivation. Coefficient of variation: A, 12.83%; B, 3.81%; C, 11.64%. For analysis of leaf number, original data were transformed to $\sqrt{x+1}$

Regression models did not provide a good fit to soil basal respiration data. Experimental results indicated a peak of biological activity in substrate supplemented with 25.75% SMS, whose basal respiration was 14% higher than that of the control.

Microbial biomass carbon, which is the living portion of the soil [27], increased proportionally to SMS concentration (Fig. 5B). qCO_2, by contrast, had the opposite behavior, and decrease as a function of increasing SMS concentration (Fig. 5C).

Discussion

Spent substrate from *P. djamor* cultivation efficiently controlled *M. javanica* reproduction on lettuce, demonstrating the nematicidal potential of metabolites released during mushroom cultivation. Hyphae remaining from mushroom cultivation might have contributed to this effect by releasing hydrolytic enzymes, including proteases, collagenases, and chitinases, which penetrate and digest the cuticle of nematodes [2]. The results of the current study may also be explained by predation of *Pleurotus* spp. on nematodes. These fungi form traps to capture and predate or parasitize nematodes [32, 63]. *Pleurotus ostreatus* and other species of the genus contain specialized cells in hyphae that are capable of secreting tiny droplets of toxins, which paralyze nematodes within 30 s of contact, without killing the parasites [70]. Although alive, nematodes remain immobile, and the liquids that extravasate from their tissues stimulate hyphal growth via chemotaxis, hyphae may then penetrate and digest nematode tissues, absorbing the nutrients released during this process [6, 35, 69, 70].

The first nematicidal compound isolated and characterized from mushrooms of the genus *Pleurotus* (*P. ostreatus*) was *trans*-2-decenedioic acid, derived from linoleic acid. The compound was obtained from aqueous extract of *P. ostreatus* substrate and found to have nematotoxic action. The toxin affects not only nematodes but also insects and fungi, possibly by altering cell membrane permeability [30]. These authors observed that, at a concentration of 300 μg mL^{-1}, the nematicidal compound immobilized *Panagrellus redivivus* by 95% in 1 h. Other compounds, such as saturated fatty acids (palmitic acid, lauric acid, stearic acid), unsaturated fatty acids (oleic acid, linoleic acid), fatty acid methyl esters (oleic acid methyl esters), carbonyl compounds, and alcohols (*p*-anisyl alcohol) were found to have high nematicidal activity [67]. These findings show the potential of the direct use of mushroom residues for the control of plant–parasitic nematodes [46]. There are reports that *Pleurotus* releases

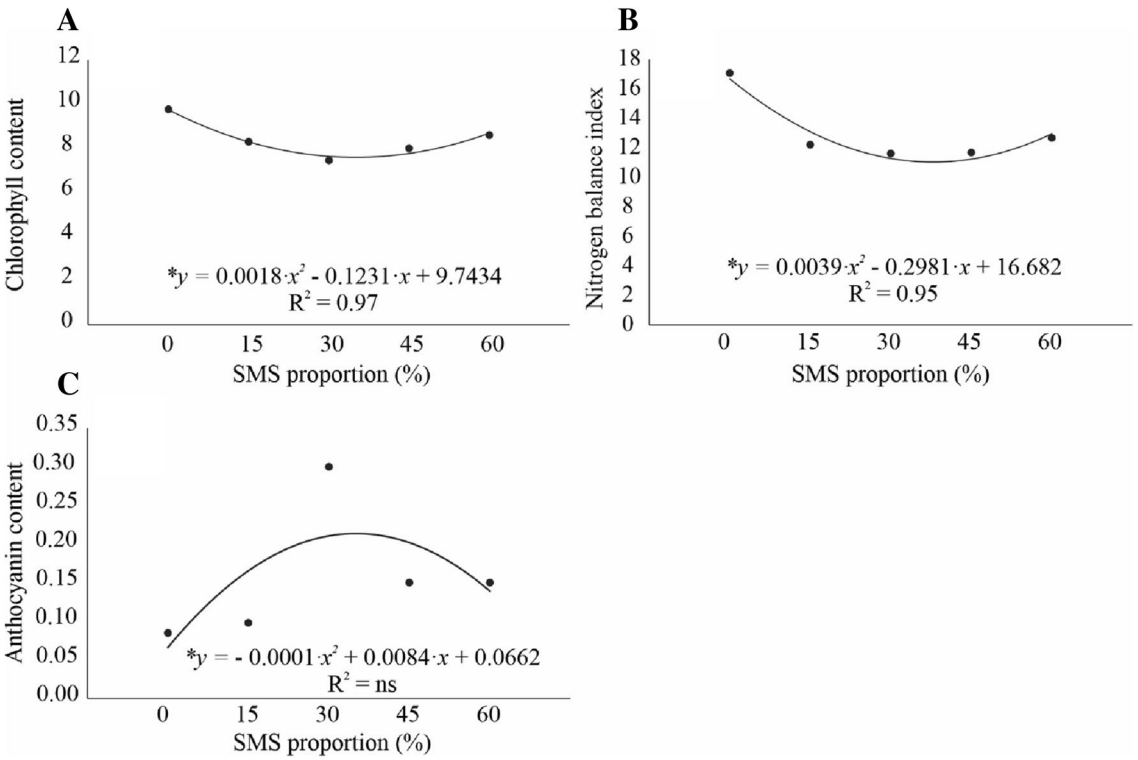


Fig. 3 **A** Chlorophyll content, **B** nitrogen balance index, and **C** anthocyanin content of lettuce seedlings grown in substrate supplemented with different proportions of spent mushroom substrate (SMS) from *Pleurotus djamor* cultivation. Evaluations were performed at 40 days after inoculation of *Meloidogyne javanica*. Coefficient of variation: A, 10.85%; B, 7.72%; C, 0.86%. For analysis of NBI and anthocyanin content, original data were transformed to $\sqrt{x+1}$

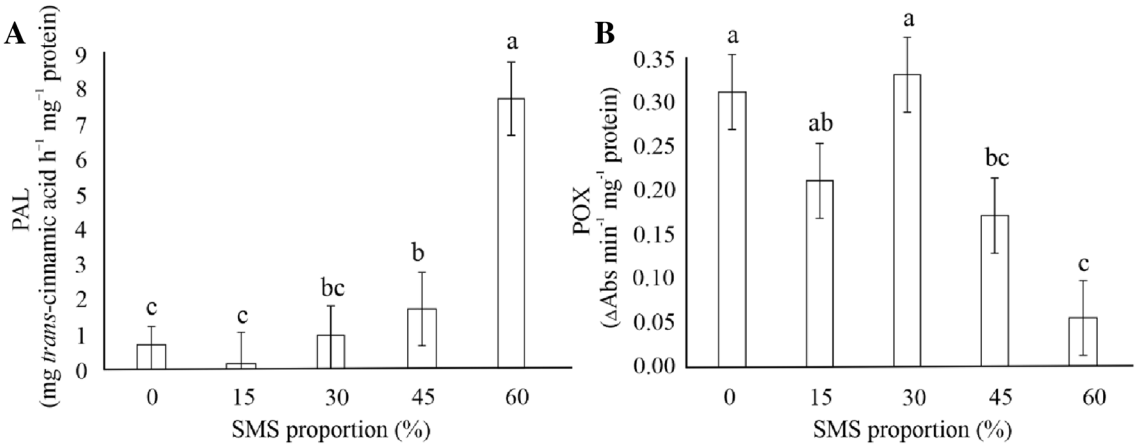


Fig. 4 **A** Phenylalanine ammonia-lyase (PAL) and **B** peroxidase (POX) activities in the leaves of lettuce crops grown in substrate supplemented with different proportions of spent mushroom substrate (SMS) from *Pleurotus djamor* cultivation. Coefficient of variation: A, 13.82%; B, 22.57%

fatty acids, such as linoleic acid, which are converted into highly reactive peroxides, instantly halting nematode activity [31, 58].

Previous studies demonstrated the nematicidal potential of toxins found in mushroom medium. The filtrates of liquid *Pleurotus* spp. medium afforded complete (100%) immobilization of *M. javanica* juveniles after 24 h of application; however, immobilization efficiency differed according to species [23]. Other researchers obtained positive results with the use of *Pleurotus* spp. for the control of root-knot nematodes [5, 41]. Aqueous extracts of 10 basidiomycetes were

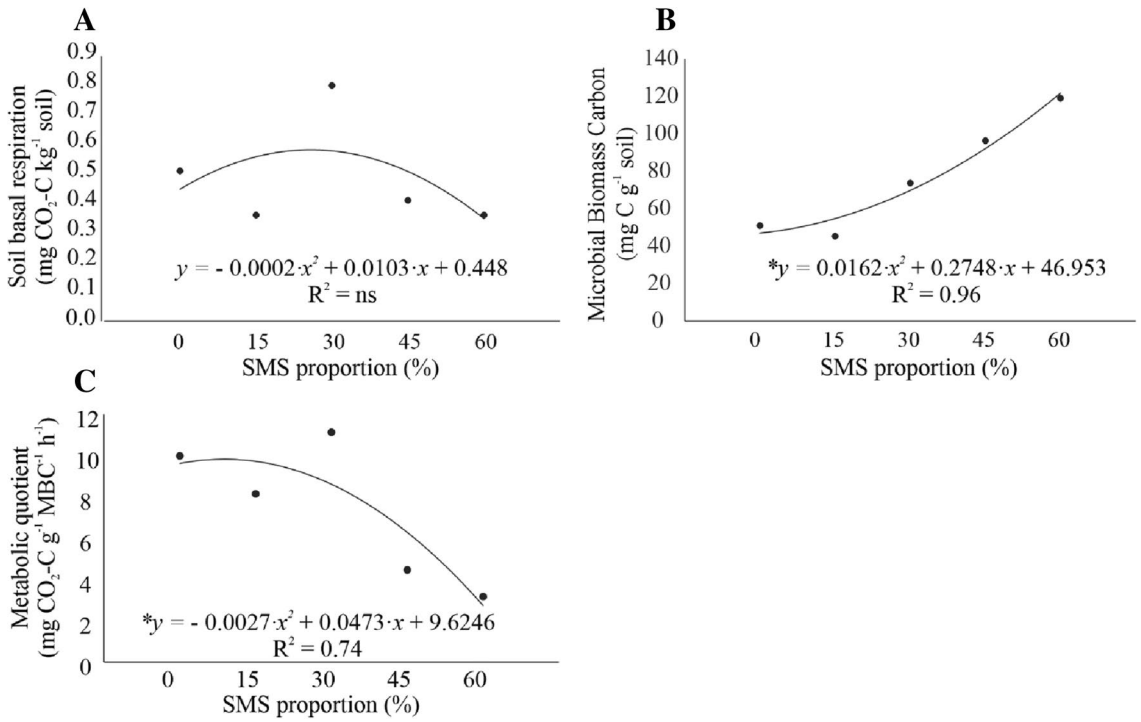


Fig. 5 **A** Basal respiration, **B** microbial biomass carbon (MBC), and **C** metabolic quotient of soil under lettuce seedlings grown in substrate supplemented with different proportions of spent mushroom substrate (SMS) from *Pleurotus djamor* cultivation. Coefficient of variation: A, 3.07%; B, 15.02%; C, 23.23%. For basal respiration and microbial biomass carbon, data were transformed by $\sqrt{x+1}$

tested for *M. incognita* control; all extracts, particularly that of *Pleurotus*, inhibited hatching and increased J2 mortality [73]. Furthermore, these authors found that treatment of soil with fungal extracts reduced nematode reproduction by about 70%.

In addition to controlling root-knot nematodes, *P. djamor* spent substrate may efficiently promote plant development compared to commercial substrate and can, therefore, be used as an ingredient of plant substrates [36]. The positive effect of SMS on root development was attributed to reduction in soil compaction, clod and surface crust formation, and diurnal temperature changes, as well as an increase in aggregate stability (organic matter) and water infiltration rate [68]. Aeration and moisture retention are two of the many benefits provided by SMS application in plant substrate, which may positively influence germination in a variety of plant species [36]. However, it is worth mentioning that SMS may have phytotoxic effects depending on its concentration, as observed in the current study; thus, care should be taken so as not to compromise plant development.

Plants treated with 60% SMS showed reduced development throughout the production cycle. Thus, at the time of transplanting, seedlings subjected to high SMS concentrations showed a noticeable difference in size, explaining the lower shoot fresh weight, shoot dry weight, and leaf number of these plants. Phytotoxic effects were also observed in plants treated with 75% SMS. The results of this treatment are not reported, because such effects culminated in plant death. Spent *Agaricus bisporus* substrate had a similar effect on tomato seedlings, causing a 2-day delay in germination [16].

Because SMS contains lignocellulosic compounds (e.g., wheat or rice straw, sugarcane bagasse, sawdust), which act as a source of carbon, and additional protein nutrients (organic bran or mineral elements) [18], it is possible that seedling development and plant growth were affected by nitrogen imbalance. It is also possible that the salinity of the culture medium increased after the production cycle [48], resulting from addition of inputs, such as limestone, gypsum, and chemical fertilizers. Furthermore, the substrate used here was not subjected to composting, a practice that tends to promote stability and reduce salinity (Colella et al. 2019). It should be noted that the negative effects of SMS on vegetative development were negligible at low concentrations, thus not precluding the use of the material. The application of SMS in legume production can contribute to integrating production chains. Producers may optimize space, minimize costs, and maximize product diversity [36].

There was a decrease in chlorophyll content, which might be due to nitrogen imbalance. Nitrogen is indispensable for the synthesis of chlorophyll, a compound that is essential for plant growth and adaptation to the most varied environments. Chlorophyll index is an indicator of the physiological status of plants [53], and the quality of the molecule is related to photosynthetic activity [75]. Some saprophytic fungi, such as basidiomycetes, can only survive on substrates with high C/N ratios and, therefore, depend on nitrogen supplementation. These fungi, to meet their nitrogen needs, developed nematode predation strategies [33, 69]. Thus, the higher demand for nitrogen in the growth medium might have compromised nitrogen absorption by plants, but remaining fungal populations might have been favored.

NBI had a similar behavior to chlorophyll. The index is an estimate of nitrogen in plants, obtained from the relationship between chlorophyll index and flavonoid content [51]. Around 70% of nitrogen contained in the leaves is found in chloroplasts, participating in the synthesis and structure of chlorophyll molecules [74]. These findings further support the interaction between nematodes and microorganisms from SMS.

Anthocyanins act as photoprotectors and antioxidants [13]. Higher anthocyanin levels in plant tissues tend to confer greater resistance to abiotic stresses, particularly drought stress [52]. However, there are few reports in the literature on anthocyanin accumulation in plants exposed to biotic stress, such as that caused by phytonematodes.

Peak PAL activity was observed in the treatment with the highest SMS concentration (60%). It is possible that microorganisms from SMS or their byproducts served as elicitors of plant resistance. In general, when plants are exposed to nematodes, resistance induction occurs within 8 days of inoculation [44, 49, 56]. In the current study, enzyme activity was evaluated only at the end of the experiment, which may explain the non-activation of POX and PAL routes in the root system and the reduction in POX activity as a function of increasing SMS levels.

PAL contributes to plant resistance to pathogens, as it is involved in the first step of phenylpropanoid synthesis, whereby phenylalanine is converted into *trans*-cinnamic acid, resulting in compounds, such as phytoalexins and, mainly, lignin [14, 49]. Lignin, in turn, confers resistance to plant cell walls, restricting nematode activity at feeding sites [49]. PAL also acts as a precursor of several compounds, such as benzoic acid derivatives, coumarins, lignin precursors, flavones, isoflavones, flavonols, anthocyanins, condensed tannins, caffeic acid, ammonia, and other simple phenylpropanoids, all of which are important for plant defense against pathogens [61, 62].

Research on resistance induction stimulated by *Pleurotus* is still scarce. A previous study showed that these fungi induce resistance to *M. incognita* in tomato when used alone or in combination with rabbit manure and wheat straw [57, 73] observed resistance induction in lettuce with the use of aqueous extracts of fruiting bodies of five *Pleurotus* species. The extracts showed nematostatic activity in vitro, reduced nematode reproduction factor, and increased nematode control. Hahn et al. [22] obtained good results with the use of *Pleurotus* extracts, including *P. djamor* extract, for *M. javanica* immobilization and mortality.

POX activity in lettuce leaves was the highest in plants treated with 0% and 30% SMS (Fig. 4B). POX is an antioxidant enzyme that accumulates in tissues subjected to some type of oxidative stress, in this case, nematodes. The enzyme is involved in the release of reactive oxygen species to inhibit parasitic activity at feeding sites [40]. The high POX activity in the untreated sample can be explained by the high nematode population density, which likely promoted oxidative stress in plant tissues. Besides, the presence of fungal residues in SMS may activate enzymes involved in resistance induction (POX and PAL) and reduce the efficiency of microorganisms in utilizing available carbon for growth.

The application of SMS at a concentration of 25.75% increased soil basal respiration by 14% compared to the control. This parameter represents the amount of carbon in the form of CO_2 resulting from the respiration of decomposing organisms present in the soil [37]. Species of the genus *Pleurotus* decompose wood and plant residues [21] and are known for their ability to produce ligninolytic enzymes, such as laccase and manganese peroxidase [26]. Laccase is an oxidoreductase capable of catalyzing the oxidation of various aromatic compounds (especially phenol) while concomitantly reducing oxygen to water [72]. This information explains the high basal respiration in the 25.75% SMS treatment.

Although high respiration values generally indicate favorable soil conditions, it should be considered that, in the short term, high respiration rates imply the release of nutrients to plants, but, in the long term, they may imply loss of organic carbon from the soil to the atmosphere [47]. Treatments with low SMS concentrations were likely influenced by abiotic factors, such as substrate porosity and, consequently, aeration. SMS has high water retention capacity, allowing the creation of a high-humidity microclimate, which might have compromised microbial respiration [65]. In the experimental units, microbial populations were composed of nematodes and any remaining fungi in SMS, given that the soil used was previously autoclaved, eliminating other organisms [25].

Therefore, the results agree with the hypothesis that treatments with a high concentration of spent *P. djamor* substrate promoted soil microbial biomass.

Anderson and Domsch [3] argued that qCO_2 is a relevant factor for assessing environmental and anthropogenic effects on soil microbial activity. In this study, the highest qCO_2 was observed in treatments containing 8.8% SMS (Fig. 4C). qCO_2 is related to the efficiency of microorganisms in using available carbon for growth [7]. High values of qCO_2 indicate a correlation with low biomass indices and low C and N contents [38]. A similar correlation was found in this study, where the lowest qCO_2 values occurred in treatments with the highest microbial biomass carbon.

Conclusions

Spent substrate from *P. djamor* cultivation is efficient in controlling *M. javanica* in lettuce cultivation. However, high SMS concentrations may negatively influence shoot development, with no negative effects on roots.

The interaction of SMS microorganisms with nematodes may, on one hand, affect chlorophyll, NBI, and anthocyanin contents but, on the other hand, protect the photosynthetic apparatus. The presence of fungal residues in SMS may activate enzymes involved in resistance induction (POX and PAL) and reduce the efficiency of microorganisms in utilizing available carbon for growth.

Overall, the present results demonstrated the potential of *P. djamor* spent substrate in the control of *M. javanica*. Further studies are needed to assess SMS efficiency and possible interactions of remaining hyphae with nematodes. SMS concentrations of 15–30% are effective for nematode control and do not exert phytotoxic effects on plant development.

Abbreviations

SMS	Spent mushroom substrate
C/N	Carbon/nitrogen ratio
NPK	Nitrogen:phosphorus:potassium
NBI	Nitrogen balance index
EDTA	Ethylenediaminetetraacetic acid
POX	Guaiacol peroxidase
PAL	Phenylalanine ammonia-lyase
qCO_2	Metabolic quotient
MBC	Microbial biomass

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00380-0.

Additional file 1 . Graphical abstract

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Author contributions

AL, SG and CDA designed experiments; AL, SG, RS and MC carried out experiments; AL, SG, and CDA analyzed experimental results; AL and CRA wrote and edited the manuscript. All the authors read and approved the final manuscript.

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Availability of data and materials

All data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors have no competing of interests to declare.

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