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Spent *Pleurotus ostreatus* Substrate Has Potential for Controlling the Plant-Parasitic Nematode, *Radopholus similis* in Bananas

Anthony Fredrick Tazuba ¹, Walter Ocimati ^{1,*} , Geoffrey Ogwal ¹ , Betty Nyangwire ², Francis Onyilo ³ and Guy Blomme ⁴ 

¹ Bioversity International, Kampala P.O. Box 24384, Uganda; tazubatony@gmail.com (A.F.T.); ogwalgeff1996@gmail.com (G.O.)

² Department of Crop Science, Muni University, Arua P.O. Box 725, Uganda; b.nyangwire@muni.ac.ug

³ Department of Sciences and Vocational Studies, Lira University, Lira P. O. Box 1035, Uganda; francisonyilo@lirauni.ac.ug

⁴ Bioversity International, c/o ILRI, Addis Ababa P.O. Box 5689, Ethiopia; g.blomme@cgiar.org

* Correspondence: w.ocimati@cgiar.org

Abstract: Spent mushroom substrate (SMS), a waste product from mushroom cultivation, in addition to being rich in essential nutrients for crop growth, contains actively growing mushroom mycelia and metabolites that suppress some plant pathogens and pests. SMS thus has potential for fostering the suppressiveness of soil-borne pathogens of farms. This study determined the potential of using the spent *Pleurotus ostreatus* substrate (SPoS) to suppress the plant-parasitic nematode *Radopholus similis* in bananas. *R. similis* is the most economically important nematode in bananas worldwide. The effect of SPoS on *R. similis* was assessed through two in vivo (potted plants) experiments between May 2023 and June 2024. Five-month-old East African highland banana (genome AAA) plantlets that are highly susceptible to *R. similis* were used. In the first experiment, the plantlets were established in 3 L pots containing (i) pre-sterilized soil, (ii) pre-sterilized soil inoculated with nematodes, (iii) pre-sterilized soil mixed with 30% (*v/v*) SPoS, (iv) pre-sterilized soil mixed with 30% (*v/v*) SPoS followed by nematode inoculation, (v) SPoS without soil, and (vi) SPoS without soil inoculated with nematodes. The SPoS was already decomposed; thus, it may or may not have contained active mycelia. The nematodes were introduced two weeks after the SPoS application. In the second experiment, SPoS was introduced two weeks after nematode inoculation. The SPoS treatments without soil were not evaluated in the second experiment. Both experiments were monitored over a three-month period. Each greenhouse treatment contained four plants and was replicated thrice. In the first experiment, data were collected on changes in soil nutrient content, below- and aboveground biomass, root deaths, root necrosis due to nematode damage, and *R. similis* population in root tissues and soil. In the second experiment, data were collected on root deaths and the number of nematodes in root tissues and the soil. The SPoS improved crop biomass yield, reduced root damage, and colonization by *R. similis*. The potential of SPoS to improve the management of *R. similis* and banana production under field conditions needs to be determined.

Keywords: circular farming; environment; organic production; plant pathogens; soil health; spent mushroom substrate



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1. Introduction

Edible mushrooms are an important source of food and income globally, with 85% of the production coming from five genera: *Agaricus bisporus* and *A. subrufescens* (30%), *Pleurotus* sp. (27%), *Lentinula edodes* (17%), *Auricularia* sp. (6%), and *Flammulina* sp. (5%) [1]. To

cultivate these mushrooms, a wide range of agricultural and agro-industrial products and waste, including corn cobs, straw, sawdust, sugarcane bagasse, cottonseed hulls, and coffee husks, are repurposed as substrates [2]. Generally, about 5–6 kg of spent mushroom waste (SMS) is produced for each kilogram of fresh mushroom harvested [3,4], thus generating significant amounts of waste. This poses a big challenge to waste management for producers. Agricultural use offers an economically and ecologically acceptable way of disposing of this waste [5]. Circularity on farms can reduce waste build-up, prevent pollution, and preserve essential nutrients by recycling organic waste, for instance, into organic fertilizers to support agricultural production [6–9].

Additionally, SMS extracts and composts have been reported to suppress plant pathogens and pests. For instance, different edible mushroom species and their wastes have been shown to suppress plant pathogens such as *Fusarium oxysporum* f. sp. *lycopersici* [10,11], *Ralstonia solanacearum* in tomatoes, and *Xanthomonas* spp. [12]; *F. oxysporum* f. sp. *cepae* [13]; and *F. oxysporum* f. sp. *cubense* (Foc) race 1 [14]. *Pleurotus ostreatus* and its wastes have also been reported to suppress various plant parasitic nematodes, including the root-knot nematode (*Meloidogyne incognita*) [15–17], *Caenorhabditis elegans* [18], and *Panagrellus* spp. [19,20]. The fungus captures and predaes on the nematodes as a source of nutrients [21]. This is facilitated through the release of chemical toxins by the actively growing hyphae of *P. ostreatus* to immobilize the nematodes. These toxins have been reported to cause shrinking of the head and impairment of the esophagus of the nematode [21,22].

Plant-parasitic nematodes (PPNs) are a major biotic constraint to crop production globally. Annual yield losses of up to 12.3–12.6% globally due to PPN infestation have been reported [23–25]. These yield losses translate into an estimated economic loss of between USD 80 and USD 216 billion [24,25]. The most devastating PPNs worldwide are *Meloidogyne* spp., cyst nematodes (*Heterodera* and *Globodera* spp.), root-lesion nematodes (*Pratylenchus* spp.), and burrowing nematode (*Radopholus similis*) [26].

In bananas and plantains (*Musa* spp., hereafter bananas), PPNs are also a major problem worldwide [27]. Bananas are affected by diverse PPNs, often occurring in mixed populations [28]. These include *Meloidogyne* spp., *Pratylenchus* spp., the spiral nematode (*Helicotylenchus multicinctus*), and *R. similis* [28]. *R. similis*, an obligate migratory endoparasite, is the most economically important nematode in bananas worldwide [29]. *R. similis* is naturally polyphagous and infests numerous (sub)tropical plants like citrus, avocado, sugarcane, coffee, tea, weeds, vegetables, grasses, black pepper, coconut, and forest trees besides bananas [29,30].

Radopholus similis is harmful to crops at all development stages and is hard to eradicate once established [31]. In the process of feeding, it damages roots, reducing their ability to take up and translocate water and nutrients necessary for plant growth and development. This weakens root anchorage, making the plant more susceptible to toppling [31,32]. The damage caused in banana roots also slows sucker formation; delays fruiting; and reduces fruit size, weight, and plantation life span [32]. PPNs also indirectly affect crops through their complex interactions with plant pathogens by wounding plants, being vectors, modifying plant biochemistry and physiology, or changing the rhizosphere microbiome [33–35]. For example, the activity of nematodes might make banana plants more susceptible to soil-mediated Foc infections [36,37].

Chemical control, though easy to apply and an effective strategy against PPNs, poses environmental and human health risks. Additionally, nematodes also remain a major threat to organic production systems where the use of chemical pesticides is limited or prohibited. As an alternative to synthetic pesticides and for organic production systems, the use of mushroom waste (e.g., composts and teas) from mushroom production could offer a sustainable and environmentally and economically sound strategy for nematode

control. Though shown to work in other crop species [15,16], no similar evidence has been generated with respect to the management of nematodes in bananas.

This study assessed the potential of spent mushroom substrate (SMS) from *P. ostreatus*, a commonly cultivated mushroom species, to suppress the plant-parasitic nematode *R. similis* (burrowing nematode) in bananas. This study builds on our previous study, in which we showed that *P. ostreatus* and its filtrate cause mortality in root-knot nematode *Meloidogyne* spp. from egg plants in vitro and reduce root damage in potted egg plants [17]. If effective, spent *P. ostreatus* waste could be integrated into circular farming systems, where crop residues, including banana residues, are used for mushroom production, and the SPoS is repurposed as organic manure to enhance *R. similis* management in banana production.

2. Materials and Methods

2.1. In Vitro Multiplication and Preparation of *Radopholus similis* Inoculum

A pure culture of *R. similis* at different ages was obtained from the nematology lab of the National Agricultural Research Laboratories (NARL)—Kawanda, Uganda, and was used for this study. The *R. similis* culture was previously extracted using the modified Baermann plate method [38], multiplied, and maintained on sterile carrot discs as described by Coyne et al. [39]. To retrieve the nematodes for use in the experiments, the nematode-infested carrot discs were soaked in distilled water in a beaker for one hour to allow the nematodes to migrate from the discs into the water. The resultant suspension was transferred to a new beaker. For maximum extraction, the carrot discs were further sliced into smaller pieces and soaked in water for another hour. The resultant suspension was transferred into the original suspension. To determine the number of nematodes in the resultant suspension, nematodes in ten 1 mL aliquots were counted using a counting chamber on a Primostar microscope (Carl-Zeiss Microscopy Deutschland GmbH, Carl-Zeiss-Straße 22, 73447 Oberkochen, Germany; 400×; ×10 eyepiece and ×40 objective). The mean nematode count was used to obtain the total number of nematodes in the suspension as per the formula below.

$$\text{Number of nematodes} = \text{Mean nematode count} \times \text{Total volume of nematode suspension}$$

Only females and mixed stages of juveniles were counted. Features described in Roy et al. [40] were used to distinguish the female and juvenile *R. similis*. The stock suspension was adjusted to a working suspension of 125 nematodes per mL.

2.2. Spent *P. ostreatus* Substrate Preparation

The SPoS was obtained from a single commercial farm in Wakiso district, in central region of Uganda. The mushroom gardens/raring units were made of cottonseed hull, and no additional nutrients were added to them. The SPoS was stored in Kawanda for a period of four months before use in the experiments. The SPoS was therefore already highly decomposed at the onset of trials and may or may not have contained active *P. ostreatus* mycelia (Supplementary Figure S1). The SPoS was not analyzed for the presence of naturally occurring nematodes and other soil organisms prior to use in the experiments. Different spent mushroom rearing units were crushed and thoroughly mixed to minimize potential variations that would arise from differences between the rearing units and to ease their application.

2.3. Screening for Nematicidal Effects of Spent *P. ostreatus* Substrate in Banana Plantlets Inoculated with *R. similis*

Two greenhouse experiments were conducted using five-month-old tissue culture-derived nematode-susceptible East African highland banana (genome AAA) plantlets

of the cultivar ‘Mbwazirume’ [41,42]. The plantlets were obtained from Agro-Genetic Technologies (AGT), a private tissue culture company, and transferred into pre-sterilized loam soil in 3 L potting buckets at trial initiation.

In the first greenhouse experiment conducted between May 2023 and August 2023, banana plantlets were subjected to six treatments that included (i) pre-sterilized soil only (0% SPoS), (ii) pre-sterilized soil (0% SPoS) inoculated with nematodes, (iii) pre-sterilized soil mixed with 30% SPoS, (iv) pre-sterilized soil mixed with 30% (*v/v*) SPoS and inoculated with nematodes, (v) SPoS without soil (100% SPoS) (Supplementary Figure S1), and (vi) SPoS without soil (100% SPoS) but inoculated with nematodes. The pre-sterilized soil-only bucket contained approximately 240 g of soil. The pre-sterilized soil was a mixture of top forest soil, lake sand, and chicken manure at a ratio of 3:1:1. Each treatment comprised three replicates of four plants each, with each plant acting as an experimental unit. The SPoS amendments were introduced first on day one, and the plants were allowed to establish themselves over a two-week period before nematodes were introduced for treatments with nematodes. For treatments inoculated with nematodes, 16 mL of nematode suspension, resulting in approximately 2000 *R. similis*, was aliquoted and drenched into three 5 cm deep and 2 cm wide equidistant holes drilled around the stem of each banana plantlet. During inoculation, the working suspension was mixed thoroughly to ensure the uniform distribution of the nematodes before sucking the 16 mL of suspension with a pipette. Inoculation was carried out using females and mixed stages of juveniles. The pot experiment was then observed over a 3-month period before trial termination.

Data were collected using the field book app, version 5.3.0 [43], an open-source Android-based application. Soil chemical parameters were measured at 8 weeks and 10 weeks (i.e., trial termination) after nematode infestation, using a portable hand-held soil integrated 5-in-1 sensor (JXCT Electronic Technology Co., Ltd., Weihai, Shandong, China). The assessed chemical parameters included nitrogen (N), phosphorus (P), and potassium (K). At trial termination, the plants were destructively sampled for assessments of root and shoot biomass, root damage, and the number of nematodes in living/functional roots and the surrounding soils following the procedure described in [38]. To assess root biomass, functional roots were sorted out and dried to a constant weight in an oven at 85 °C for 24 h. For the shoot weight, the plant portion above the soil was chopped into smaller portions and dried as for the roots.

Root damage was assessed by counting the number of both dead and functional primary roots on each rhizome/corm and scoring for root necrosis (%). Five functional roots were randomly selected from each plant, reduced to about 10 cm in length and split longitudinally into two halves and scored for root cortex necrosis [38]. To determine the nematode population in roots and soils, the modified Baermann plate technique was used [38]. The roots were washed with running tap water to remove the adhering soil and then rinsed with double-distilled water. Five sub-root samples were randomly selected, chopped into 1–2 cm pieces with a knife and thoroughly mixed. Only a 5 g sub-sample per plant was weighed for the subsequent steps in the Baermann plate method. For the soil extraction, the soil samples were thoroughly mixed, and a sub-sample of 100 g per plant was weighed for extraction. *R. similis* in the extracts were differentiated from saprophytic nematodes and other species using known prominent morphological characteristics [38,40]. For example, *R. similis* have stylets and pharyngeal glands that dorsally overlap the intestines, while the saprophytes lack stylets and have a continuous line separating the pharyngeal gland from the intestines. The Primostar microscope (Carl-Zeiss Microscopy Deutschland GmbH, Carl-Zeiss-Straße 22, 73447 Oberkochen, Germany; 400×; ×10 eyepiece and ×40 objective) was used for enumerating the nematodes and to morphologically differentiate them.

In the second pot experiment conducted from March to June 2024, nematodes were introduced first, and SPoS was introduced two weeks later. This potentially allowed the nematodes to migrate and colonize the roots before the introduction of the SPoS. This experiment aimed at mimicking a scenario for SPoS application in banana fields that are already infested with nematodes. The use of SPoS without soil is not anticipated for the banana crop under field conditions and was omitted as such in the second experiment. This omission, as such, does not negatively impact the strength of the study findings. At trial termination, data were collected on belowground fresh root weight, the number of living and dead roots, root necrosis (damage) score, and *R. similis* counts in 5 g of root tissue and 100 g of soil sample per plant as described for experiment 1.

2.4. Statistical Analysis

All statistical analyses were performed in RStudio version 2024.4.2.764 [44], using R version 4.4.1 [45]. The treatments were considered as the independent variable and fixed factor, with the replications as random variables. All data were tested for normality using a normal quantile–quantile plot, Studentized histogram plot, and Shapiro–Wilk test (stats R package). Data were also checked for homogeneity of variance using Levene’s test (car R package) and skewness and kurtosis using the agricolae package. The data did not violate assumptions of ANOVA (they were not normally distributed, did not have equal variance, and were skewed), and hence were not transformed. A general analysis of variance (ANOVA) (agricolae R package) on untransformed data was run. Significant variables were entered in post hoc tests and mean separation based on Fisher’s protected least significant difference test at 5% significance.

3. Results

3.1. Soil Chemical Parameters for Treated Pots with Banana Plants

Except for the variation in soil nitrogen, the treatments, the two sampling points (8 weeks post-nematode infestation and trial termination at the 10th week), and their interaction significantly ($p < 0.05$) influenced the soil parameters (nutrients) (Figure 1). The SPoS treatments had a significantly lower ($p < 0.001$) soil N in the 8th week, later rising significantly higher than other treatments at the close of the experiment (10th week). In contrast, at eight weeks, soil N was higher for the sterilized soil with and without nematodes, declining at the close of the trial (Figure 1). At eight weeks, soil phosphorus was significantly lower ($p < 0.01$) in the treatments with SPoS, significantly increasing ($p < 0.001$) at the close of the trial across all the treatments, with higher increments in SPoS treatments and lower values in the sterilized soil with and without nematodes (Figure 1). For potassium, increments only occurred for SPoS and SPoS with nematode treatments. Declines in K were observed for the rest of the treatments, with higher declines in soils without SPoS (Figure 1).

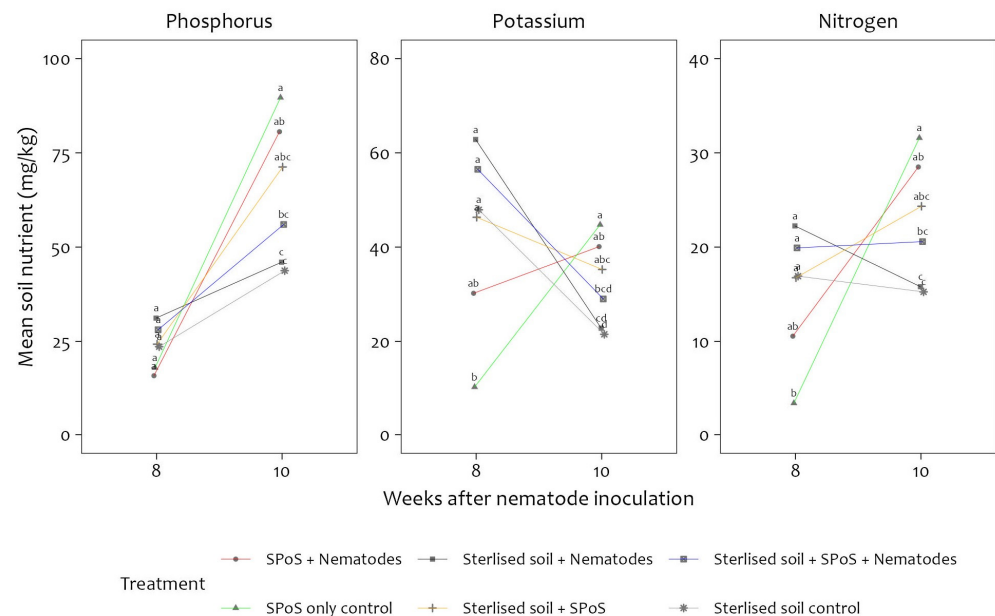


Figure 1. Changes in the amount of key soil nutrients (phosphorus, potassium, and nitrogen) from the 8th week post-nematode inoculation (pni) to the 10th week pni in pots subjected to different treatments. Nematodes were inoculated two weeks after application of spent *P. ostreatus* substrate. Significant differences ($p < 0.05$) in soil nutrient levels were observed between the treatments at the 8th and 10th weeks pni. For the same number of weeks, points/values followed by the same letter are not significantly different at 5% least significant difference.

3.2. Effect of Spent *P. ostreatus* Substrate Treatment on Banana Root and Shoot Biomass

In the first experiment, the nematode and SPoS treatments significantly ($p < 0.001$) affected and/or influenced the below- and aboveground biomass of banana plantlets (Figures 2 and 3). The soil inoculated with *R. similis* and without SPoS had the lowest root biomass (Figures 2A and 3; Supplementary Table S1). In contrast, root biomass yields were significantly higher (5% LSD) in the SPoS-treated plants. Yield declines were also observed in the 100% SPoS–nematode treatment with no significant difference (5% LSD) from the 100% SPoS control. The sterilized soil–SPoS–nematode treatment had a lower root biomass compared to the 100% SPoS–nematode treatment, despite not being statistically different at 5% LSD. The sterilized soil–SPoS–nematode treatment was also not statistically different (5% LSD) from the soil–SPoS control, the soil control, and the soil with *R. similis*. A similar trend was visible for the aboveground biomass, though the treatments with SPoS had significantly higher (5% LSD) biomass yields compared to the *R. similis* inoculated soil and nematode-free soil treatment (Figures 2B and 3; Supplementary Table S1).

In experiment 2, in which the nematodes were introduced two weeks before the application of SPoS, the treatments did not significantly ($p > 0.05$) differ in their influence on the belowground biomass (fresh root weight) of banana plantlets. Nevertheless, a similar trend to that in experiment 1 was observed with the lowest root biomass in the sterilized soil with nematodes compared to the soil without nematodes and the SPoS-inoculated soils (Figure 2).

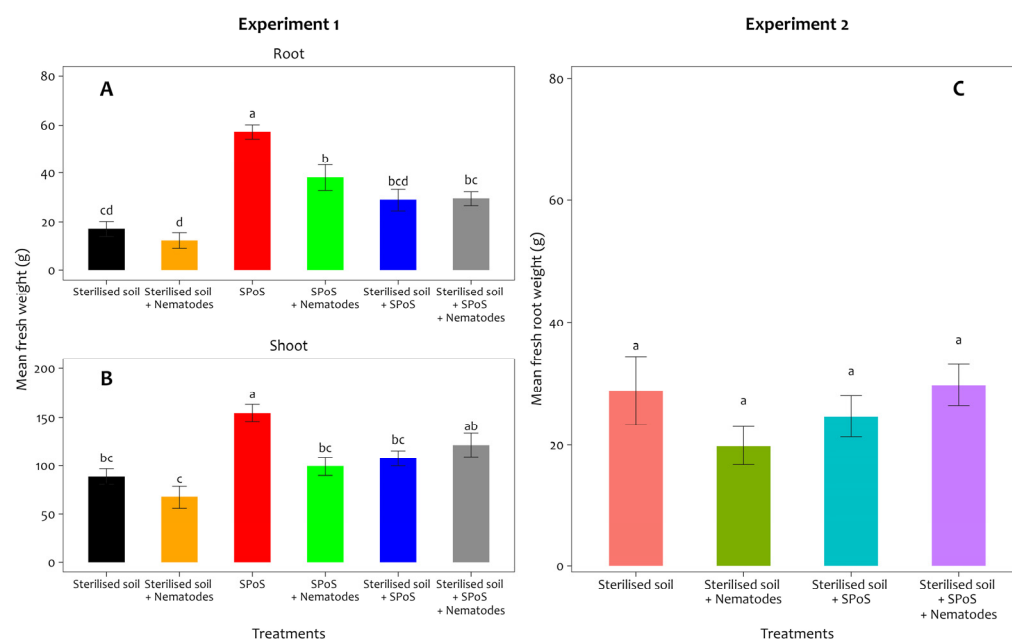


Figure 2. The effect of spent *P. ostreatus* substrate (SPoS) application and *R. similis* inoculation on root and shoot biomass of potted banana plants. The data were measured at trial termination, 10 weeks after inoculating nematodes into soils with SPoS. The differences are significant ($p < 0.001$) in (A,B) (experiment 1) and non-significant ($p > 0.05$) in (C) (experiment 2). Within A, B, and C, values followed by the same letter are not significantly different at 5% least significant difference.



Figure 3. Visual appearance and size of the aboveground plant (pseudostem and leaves) and roots of plants subjected to different treatments, including application of *P. ostreatus* substrate (SPoS) and *R. similis* (RS). Sterile soil (SS) without SPoS and *R. similis* (RS) served as a control. Stunting was observed in SS + RS, while leaf yellowing was observed in SS + RS, sterile soil control, and SS + SPoS + RS. A lower root mass with more dead roots was observed in treatments with *R. similis*.

3.3. The Effect of *R. similis* and Spent *P. ostreatus* Substrate on Survival of Banana Roots

In experiment 1, no significant difference ($p > 0.05$) was observed in the number of living banana roots (Figures 3 and 4A; Supplementary Table S1). However, banana plants without SPoS amendment generally had a significantly lower number of living roots at 5% LSD. In contrast, a significantly higher ($p < 0.05$) number of dead roots was observed in treatment infested with *R. similis*, with five times more root death in the *R. similis*-inoculated soils compared to the control soil without nematodes and SPoS (Figures 3 and 4B; Supplementary Table S1). The application of SPoS significantly (5% LSD) reduced root death, with a higher reduction in the SPoS-only treatment.

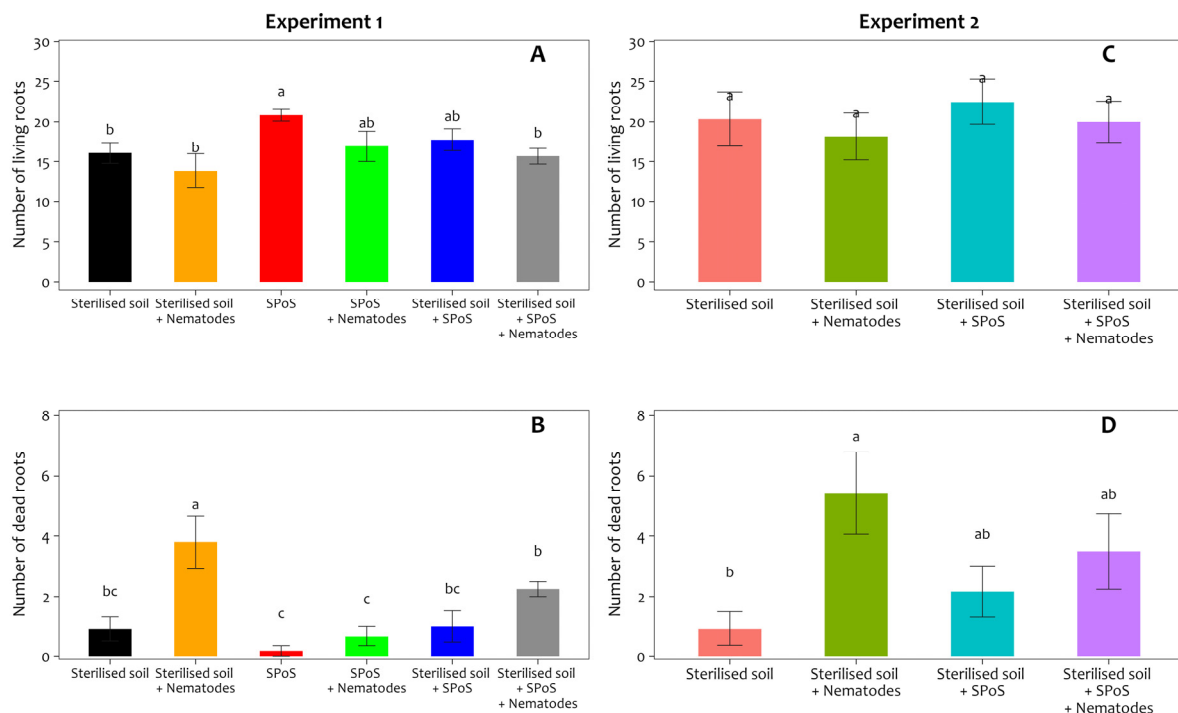


Figure 4. The effect of *R. similis* and spent *P. ostreatus* substrate (SPoS) on roots of potted banana plantlets in experiment 1 (A,B) and experiment 2 (C,D). No significant ($p > 0.05$) difference was observed in (A,C), while a significant ($p < 0.05$) difference was observed in (B,D). Within A, B, C, and D, values followed by the same letter are not significantly different at 5% least significant difference.

No significant difference ($p > 0.05$) was observed in the number of living banana roots between the treatments in the second experiment in which the plants were first exposed to nematodes prior to the introduction of SPoS (Figure 4C; Supplementary Table S1). However, the plants in the soil inoculated with nematodes had the lowest number of living roots. In contrast, significant differences ($p < 0.05$) were observed in the number of dead roots. A similar trend to Figure 4A,B was observed in the number of dead roots when nematodes were introduced two weeks after SPoS application, with a higher number of dead roots in the sterilized soils inoculated with nematodes but without SPoS (Figure 4C,D; Supplementary Table S1).

3.4. Effect of Spent *P. ostreatus* Spent Substrate on the Number of *R. similis* in Plant Tissues and Associated Root Necrosis

In experiment 1, the number of *R. similis* in the banana root tissues and root necrosis due to *R. similis* was significantly reduced ($p < 0.001$) in nematode treatments to which *P. ostreatus* was added (Figure 5A,B). The amount of *R. similis* was highest in the *R. similis*-inoculated soil without SPoS and the least in SPoS only inoculated with nematodes. The

amount of *R. similis* was, however, not significantly different (5% LSD) between the *R. similis*-inoculated soils and the soils inoculated with both *R. similis* and SPoS (Figure 5A; Supplementary Table S1). No nematodes were isolated from the control tissues to which *R. similis* was not inoculated. A similar trend was observed for root necrosis. Over 40% of root necrosis was observed in soils inoculated with *R. similis* compared to about 13% and 1% in treatments that had soil inoculated with SPoS and *R. similis*, and 100% SPoS and *R. similis*, respectively (Figure 5B; Supplementary Table S1). No damage was observed in the roots of banana plants in the nematode-free control treatments.

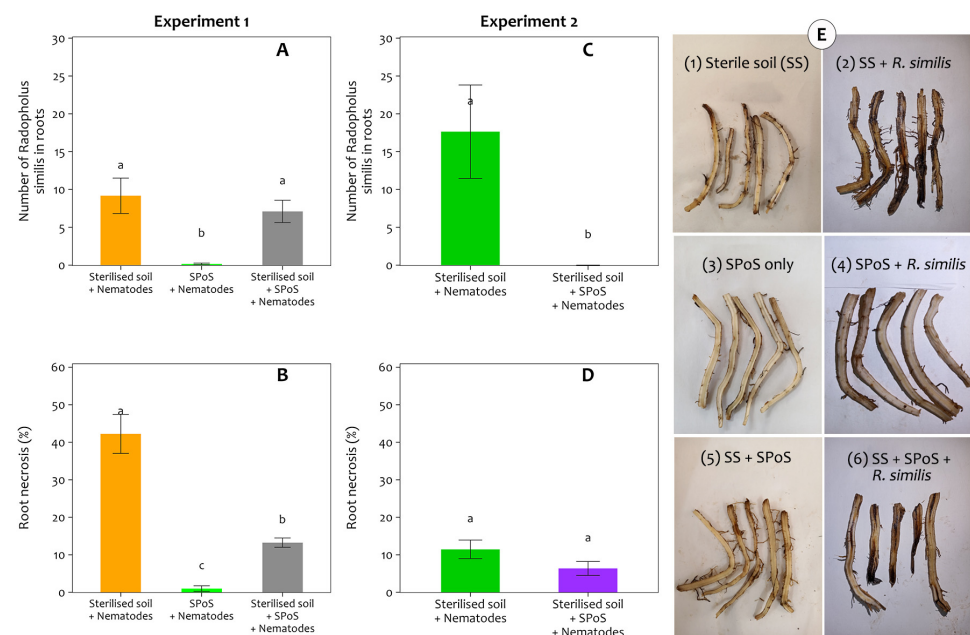


Figure 5. Effect of spent *P. ostreatus* substrate (SPoS) on the amount of *R. similis* (A,C) and banana root necrosis (B,D,E) in plants subjected to different treatments. SS denotes sterile soil. Significant differences ($p < 0.001$) were observed in (A,B,C) and non-significant differences ($p > 0.05$) in (D). Within A, B, C and D, values followed by the same letter are not significantly different at 5% least significant difference.

Similar trends to experiment 1 were observed in the number of nematodes and root necrosis in experiment 2, where *R. similis* was introduced two weeks before the application of SPoS (Figure 5C,D; Supplementary Table S1). The amount of *R. similis* isolated from banana roots was significantly reduced ($p < 0.001$) in the treatment with SPoS compared to the sterilized soil with nematodes. In contrast, no significant difference ($p > 0.05$) was observed in root necrosis due to nematodes between the soil inoculated with nematodes and SPoS and the control that had soils only inoculated with nematodes. In both experiments 1 and 2, there were no *R. similis* isolated from the soil samples or SPoS substrate samples across the various treatments.

4. Discussion

Environmentally sustainable strategies for the effective management of nematodes require an integrated approach. This study provides evidence that spent *P. ostreatus* substrate (SPoS) can potentially be used as an integral part of the integrated pest management strategy for *R. similis* in bananas. SPoS improved soil health as shown by the increase in soil nitrogen and potassium over time. Higher increments also occurred for phosphorus in the SPoS-inoculated pots. Prabu et al. [46] similarly reported a higher content of soil organic matter and nutrients, including N, P, and K, in compost prepared using SMS. The increase in the amount of available soil nutrients with time can be attributed to multiple factors,

including the high C:N ratio in the SPoS, which initially immobilizes the nutrients, coupled with a slow mineralization process and lower leaching of nutrients [46,47]. The use of SPoS as a biocontrol agent requires the presence of actively growing *P. ostreatus* mycelia. Thus, the use of SPoS to suppress nematodes will lead to trade-offs with essential nutrients that are fixed in the early stages of SPoS decomposition.

SPoS increased both the shoot and root weight of bananas. This could be partially explained by the high nutrient content of SPoS-treated pots as reported above. Similar observations have been reported in different crop species, including bananas, due to SMS compost application [14,17,46,48]. In addition to the abundance of microorganisms, the SMS of *P. ostreatus* is also known to contain enzymes like cellulases, peroxidases, and proteases [49] that degrade plant tissue components (e.g., cellulose) to release nutrients for the improved shoot and root growth. SMS has also been reported to improve soil physical properties, including increasing soil moisture, decreasing soil bulk density, and aggregation [46,50]. Improvement in these soil properties favours root development and ultimately crop growth.

The improved crop growth can also be attributed to the protective effect of the SPoS against the root-damaging nematode *R. similis*. This is supported by the fact that the SPoS-treated plants had a higher number of living roots compared to those without SPoS. Similar observations have been reported for the root-knot nematode *Meloidogyne* spp. in eggplants [17] and *Foc* [14]. Nyangwire et al. [17] reported that *P. ostreatus* supports a higher build-up of free-living nematodes that could potentially outcompete PPNs, reducing damage to the banana plants.

According to Khalil et al. [51], the increased number of live roots in treatments having SMS could also be due to its bioremediation potential and the fact that it acts as a biofertilizer. In contrast, the sterile soils inoculated with nematodes had a higher number of dead roots. *R. similis* causes root necrosis and openings that provide entry points for fungal and bacterial invasions. This results in the rotting of the roots, therefore reducing the number of living roots [52].

SPoS reduced root necrosis and the population of *R. similis* in plant root tissues. This could partly be attributed to active predation and/or immobilization through secondary metabolites released by *P. ostreatus* hyphae. The hyphae of *P. ostreatus* have been shown to directly penetrate through the cuticle of some nematode species and colonize the nematode body [17,53]. In addition, secondary metabolites, especially proteases released by *P. ostreatus*, have been reported to cause the disintegration of the nematode cuticle, which facilitates the penetration of the fungal hyphae into the nematode body [53]. The secondary metabolites of *P. ostreatus* also contain hydrolytic enzymes capable of hydrolyzing the juveniles and the eggs of nematodes, including *R. similis*, thus reducing their overall population [54,55]. The lack of *R. similis* in the soil samples could be attributed to their obligate nature, requiring a living plant host to survive [32].

The application of SPoS either before or after the introduction of *R. similis* resulted in improved crop vigour and crop protection from the nematodes. This suggests that the SPoS can be beneficial in either protecting the banana crop from infection or in reducing existing inoculum in banana plant roots.

This study thus shows that SPoS has the potential to boost soil health and crop growth and vigour while at the same time directly suppressing *R. similis* infestation in bananas. The study relied on spent mushroom waste that could have a lower potency. The substrate was heavily decomposed and potentially lacked or had a reduced presence of active *P. ostreatus* metabolites or mycelia. The observed effects could be attributed entirely or partly to the residual nutrients from SPoS observed above and the effect of other SPoS-associated microbes. Diverse microbial profiles, including bacteria and fungi capable of

offering different services, including biodegradation, bioremediation, the promotion of plant growth, the boosting of plant defences, and suppressing plant pests and pathogens, have been reported in spent mushroom composts and substrates [49,51]. For example, Yang et al. [56] reported that spent mushroom vermicompost increases the diversity of nematode-trapping fungi, thus suppressing *M. incognita* in tobacco plants. Exploring the efficacy of the actively growing mycelia of *P. ostreatus* on other growth/carrier media as an alternative delivery channel is thus recommended. The current study is also based on potted trials that do not depict the complexity of field conditions that often affect the efficacy of biological agents. On-farm studies under varying soil conditions and infestation levels are thus recommended for the validation of *P. ostreatus* effects on *R. similis*. If validated under field conditions, the integration of *P. ostreatus* waste in a circular farming setting will potentially improve yields, incomes, and the overall farm sustainability of banana-based systems.

5. Conclusions

The current study shows SPoS has a high potential of reducing the impact of *R. similis* on banana plants. In a circular farming system, the SPoS could be repurposed as an organic manure for managing *R. similis* in banana production systems, while crop residues are used for mushroom production. Further studies are, however, needed for decoding the mechanisms of the SPoS suppression of *R. similis*. These could include assessing the suppressiveness of SPoS at different stages of decomposition and identifying *R. similis* suppressing secondary metabolites and microbes in SPoS. Studies to understand the efficacy of SPoS against *R. similis* under field conditions are needed.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agronomy15051040/s1>: Figure S1: Banana plantlets transplanted into a partially composted spent *P. ostreatus* substrate; Table S1: Dry and/or fresh shoot and root weight, number of living and dead roots, number of *R. similis* per 5 g of root tissues and root necrosis score for plants subjected to different treatments.

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