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Research article

Vermicompost in combination with *Trichoderma asperellum* isolate T13 as bioagent to control sclerotium rot disease on vegetable soybean seedlings

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Abstract

Importance of the work: Vermicompost is utilized as biofertilizer, especially in organic farming systems. It has potential for soilborne plant disease control but empirical studies are scarce.

Objectives: To test the effect of vermicompost from African nightcrawler in combination with *Trichoderma asperellum* isolate T13 on the mycelial growth of *Athelia rolfsii in vitro* and the incidence of sclerotium rot disease on vegetable soybean seedlings under greenhouse conditions.

Materials & Methods: Fungal confrontation assay using either vermicompost filtrate or in combinations with varied spore concentrations of *T. asperellum* isolate T13 was conducted to observe antagonistic activity against the mycelial growth of *A. rolfsii in vitro*. Vegetable soybean seeds were sown in the greenhouse in soil mixed with vermicompost and pathogen inoculum (mycelia or sclerotia) to observe the disease incidence on the seedlings.

Results: For the *in vitro* bioassay, adding half the proportion of *T. asperellum* isolate T13 spores (1×10^8 spores/mL) relative to the total volume resulted in 80.4% inhibition of *A. rolfsii* diameter growth, producing the best antagonistic activity. Under greenhouse conditions, the soil amended with mycelial inoculum and pure vermicompost led to significantly lower disease incidence (19.7%) than for the pathogen-inoculated control (40.7%) and the mixture of vermicompost and *T. asperellum* isolate T13 (34.7%). However, no disease incidence was observed with soil amendment using vermicompost, *T. asperellum* isolate T13 and sclerotia.

Main finding: Vermicompost can be used as a bioagent for soilborne disease control, especially when combined with *T. asperellum* isolate T13, suggesting a strategy to prevent sclerotium disease incidence rather than trying to control actively growing mycelia.

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Introduction

Sclerotium rot disease caused by the fungus *Athelia rolfsii* (Curzi) C.C. Tu. & Kimbr. (Anamorph: *Sclerotium rolfsii* Sacc.) has been a worldwide problem in plant production, leading to plant death and yield losses (Guclu et al., 2020). A major reason for this level of impact is the wide host range of approximately 500 plant species, including vegetables, woody– and ornamental plants (Billah et al., 2017). Additionally, its survival strategy via a modified structure, namely sclerotia, provides the advantage of allowing it to remain in soil for several years (Paul et al., 2017) and be resistant to extreme environments (Xu et al., 2008).

Several cultural practices (Punja, 1985), biological control (Shrestha et al., 2020) and chemical fungicides have been applied to control sclerotium rot disease (Huzar-Novakowiski et al., 2017). Indeed, the implementation of a combination of various methods to control sclerotium rot disease resulted in a significant reduction in disease incidence (Cilliers et al., 2003; Dwivedi and Prasad, 2016). Thus, integrated control strategies could have potential in disease prevention (Ganesan et al., 2007).

The global increase in demand for organic food has driven the growth of organic farming, especially organic plant production (Garg and Balodi, 2014; Nandwani and Nwosisi, 2016). While chemical pesticides are not allowed as a part of such cultivation processes, plant disease is one of major constraints in plant productivity. Thus, biological control is one alternative to overcome this limitation, especially regarding soilborne diseases, such as sclerotium rot disease.

Vermicompost, derived from organic matter digested by earthworms, together with the decomposition of associated microorganisms, has been utilized as biofertilizer (Wang et al., 2017) and also for soilborne plant disease control (Ersahin, 2011; Basco et al., 2017). Vermicompost-amended substrates revealed suppressive activity to soilborne plant diseases caused by several fungal pathogens, such as *Fusarium oxysporum*, *Phytophthora nicotianae*, *Pythium aphanidermatum* and *Rhizoctonia solani* (Ersahin, 2011). Regarding *A. rolfsii*, the growing substrate amended with vermicompost showed sclerotium rot disease suppression on chickpea in a greenhouse study (Sahni et al., 2008; Sahni and Prasad, 2020). However, there have been no published reports of experimental studies examining the antagonistic activity of vermicompost against *A. rolfsii in vitro*, rather only for the pathogenic fungus *Fusarium* (Szczech, 1999) and *Rhizoctonia solani* (Ersahin et al., 2009).

To increase the efficacy in controlling sclerotium rot disease, antagonistic microbes and vermicompost were added to the growing media before planting or at the seedling stage (Ersahin, 2011). For example, substrate amended with the combination of vermicompost and *Pseudomonas* sp. (PUR46) produced a significantly lower percentage of mortality caused by *A. rolfsii* than the pathogen-inoculated control in chickpea (Sahni and Prasad, 2020). Substrate amended with the combination of vermicompost and *T. asperellum* CB–Pin–01 showed a significant reduction in *Pythium aphanidermatum* colonization on lettuce roots under greenhouse conditions, compared with those having only vermicompost amendment (Charoenrak et al., 2019). Even though augmentation in controlling *Rhizoctonia solani* disease has been reported from the use of vermicompost and *T. harzianum* in the growing media (Ersahin et al., 2009), there has been no report on this effect on sclerotium rot disease. Since several *Trichoderma* spp., such as *T. asperellum* (Chamswarng and Intanoo, 2002; Charoenrak and Chamswarng, 2016; Pacheco et al., 2016), *T. harzianum* (Ozbay and Newman, 2004; Pacheco et al., 2016; Kamel et al., 2020; Junsopa et al., 2021) and *T. koningii* (Trutmann and Keane, 1990; Woo et al., 2014; Pacheco et al., 2016; Kamel et al., 2020) have been examined as effective biocontrol agents against soilborne plant pathogens, *T. asperellum* could be used for soil amendment together with vermicompost to control sclerotium rot disease.

The vegetable soybean (*Glycine max* (L.) Merr. 'Chiang Mai 84–2') was used as the tested plant variety as it is famous for its unique favor with seed fragrance (Somdee et al., 2017). It is widely cultivated in Thailand and Southeast Asia (Özcan and Al Juhaimi, 2014); however, sclerotium rot disease became a serious problem in crop production, specifically at the field crop station of Khon Kaen University, Khon Kaen province, Thailand, where *A. rolfsii* infected either seedlings or the mature stage of vegetable soybean plants. Diseased plants turn yellow and wilt and plants die within a few days of infection. During disease development, thick, white mycelia grow and extend from water-soaked lesions on the lower stems of the plants, called crown rot. Such fungal mycelia spread further on the soil surface around the collar region of the plant. The yellowish-to-brown sclerotia can be found on mycelia growing on both infected plant tissues and the adjacent soil surface (Fig. 1A). The crown rot caused by *A. rolfsii* observed on the vegetable soybean 'Chiang Mai 84–2' was similar to on infected soybean cultivars, as previously described by Chattopadhyay et al. (2015) and Zheng et al. (2020).

The current research aimed to test the effect of vermicompost from African nightcrawler (*Eudrilus eugeniae* Kinberg, 1867) on the mycelial growth of *A. rolfsii in vitro* and the sclerotium rot disease incidence on this vegetable soybean under greenhouse conditions. Furthermore, vermicompost added with *Trichoderma asperellum* isolate T13 was used to test for antagonistic activity against *A. rolfsii in vitro* and sclerotium rot disease control under greenhouse conditions.

Materials and Methods

Fungal pathogen

Pathogen isolation and culture

Athelia rolfsii samples were isolated from diseased soybean plants [*Glycine max* (L.) Merr. 'Chiang Mai 84–2'] collected from the agricultural fields of Khon Kaen University, Khon Kaen province, Thailand. Infected plant stems and roots were further incubated in a moist chamber at 25±2 °C and 90% hygrometry for 7–10 d to induce sclerotium production (Pandey et al., 2005). Then, individual sclerotia were surface sterilized with 5% sodium hypochlorite (NaOCl) for 1 min followed by rinsing 2–3 times in distilled water. Then the sclerotia were placed on potato dextrose agar (PDA; HiMedia; Mumbai, India) medium, at 39.00 g/L of distilled water in a 90 mm diameter Petri dish and incubated at 25±2 °C for 5–7 d as described by Paparu et al. (2020). Mycelia grown from the sclerotia were transferred to new PDA medium to obtain pure culture (Fig. 1B) for further use *in vitro* and in the greenhouse experiment.

Pathogen inoculum for greenhouse experiment

A. rolfsii inoculum was prepared following the method described by Paparu et al. (2020). Fungal mycelia were grown on PDA and then inoculated on sterile sorghum. Mycelial inoculum was used as actively growing mycelia on sterile sorghum grains at 25±2 °C for 7 d. The sorghum grains covered with mycelia were well mixed daily for homogeneous growth of the fungus.

Antagonistic Trichoderma fungi

Three selected fungi (*Trichoderma asperellum* isolate T13, *T. hazianum* isolate T9 and *T. koningii* isolate 67) were used for testing the antagonistic activity against *A. rolfsii in vitro* because they exhibited antagonistic activity against *Rhizoctonia* spp. *in vitro* in other experiments (Vongphachanh et al., 2016). All fungi were provided by the Mycology Laboratory, Department of Entomology and Plant Pathology, Faculty of Agriculture, Khon Kaen University, Khon Kaen province, Thailand. They were maintained and multiplied on PDA at 25±2 °C. For the laboratory experiment, *Trichoderma* species aged 3 d were used in fungal confrontation assays (Junsopa et al., 2021). For the greenhouse experiment, this species was multiplied in steamed rice according to Charoenrak and Chamswarng (2016), while sclerotia were collected from the culture on PDA (Fig. 1C) at the same radial distance from the initial mycelial agar plug at the center (Nájera et al., 2018).

Vermicompost

Vermicompost was provided by the Research Developing and Learning Center on Earthworms for Agriculture and Environment, Integrated Water Resource Management Research and Development Center in Northeast Thailand, Khon Kaen University, Thailand. It was derived from African nightcrawler (*Eudrilus eugeniae* Kinberg, 1867) fed with various substrates including cow manure, cassava peel, rice husk ash and soil and was taken after 45 d of the composting process (Iwai et al., 2018). All vermicompost used in the current study came from the same batch.

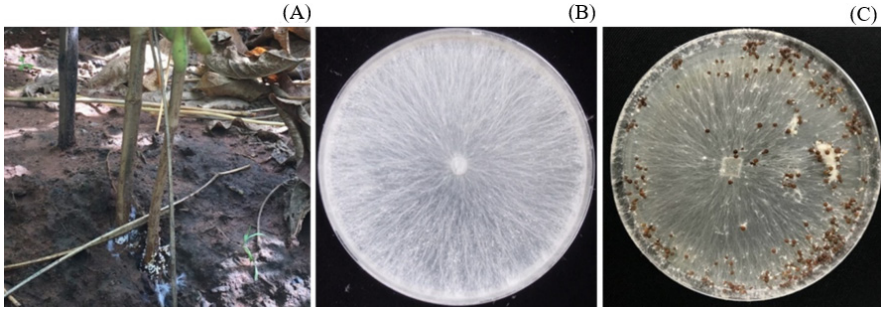

Fig. 1 Characteristics of *Athelia rolfsii*: (A) sclerotium rot disease on vegetable soybean shown with sclerotia on collar region of infected plants and white mycelia on soil surface; (B) mycelial growth on potato dextrose agar (PDA); (C) sclerotia on mycelia grown on PDA

Plant material

Seeds of Thai vegetable soybean, *Glycine max* 'Chiang Mai 84–2', were obtained from the Department of Agronomy, Faculty of Agriculture, Khon Kaen University, Thailand. This cultivar is commercially popular throughout Thailand and adjacent areas (Somdee et al., 2017).

Basic physico-chemical properties of soil, vermicompost and soil-vermicompost mixture

The soil used in the greenhouse experiment was collected (approximately 0–20 cm depth) from an agricultural field of the Faculty of Agriculture, Khon Kaen University, Khon Kaen province, Thailand. It was from the Yasothon soil series described as a bright, reddish-brown structureless sand (Soil Survey Division Staff, 1993). Basic physico-chemical properties were analyzed—pH, electrical conductivity (EC), organic carbon (OC), total nitrogen (N), exchangeable potassium (K), and available phosphorus (P) of non–amended soil and soil amended with 20% of vermicompost (weight per weight). The pH and EC were measured using pH and EC meters, respectively, after preparing the suspension with a ratio of 1:1 w/w and with a ratio of 1:5 soil (or soil amended with vermicompost)–to–water for the pH and EC, respectively. For pure vermicompost, a vermicompost suspension was prepared with a ratio of 1:10 vermicompost–to–water before the pH and EC measurement. The OC was estimated using the method of Walkley-Black (Walkley and Black, 1934). Total Kjeldahl nitrogen (TN) was measured using the method described by Jackson (1958), available P was measured according to Bray and Kurtz (1945), while extractable K was analyzed via ammonium acetate extraction and determined using the flame photometric method. The C:N ratio was calculated according to Allison (1965), total P and total K were determined based on the digestion of the samples with HNO_3:$HClO_4$ (2:1) and after that the total P was determined using the spectrophotometric molybdovanadophosphate method at 420 nm (Olsen and Sommers, 1982). Total K was determined using the flame photometric method.

Fungal confrontation assays

Experiment 1: appropriate technique for testing antagonistic activity of vermicompost

The vermicompost tested in this experiment was in the form of filtrate using Whatman® qualitative filter paper No.1 as described by Gopalakrishnan et al. (2011). A sample (10 g) of vermicompost was separately suspended in 90 mL of physiological saline (0.85% NaCl) in a flask and placed on a shaker (at 100 revolutions per minute) at 25±2 °C for 1 h. Five techniques for confrontation assay (Table 1) were used to observe the antagonistic activity of vermicompost against *A. rolfsii*. Each technique operated on three biological replicates with respective control of which single fungal pathogen was placed on PDA. A total of 100 µL vermicompost filtrate was applied on each PDA plate in all techniques, based on Ersahin et al. (2009). For the dual culture technique except for T5 (Table 1), fungal pathogen mycelia prepared as agar plugs (5 mm in diameter) and vermicompost filtrate were placed equidistantly on the border on opposite sides of a 9 mm diameter Petri dish. The swab plate technique involved spreading 100 µL of vermicompost filtrate on PDA in each Petri dish followed by placing an agar plug of fungal pathogen mycelia at the center of the Petri dish, according to Szczech (1999). Then, the plates were incubated at 25±2 °C and daily observed for radial growth (for T1–T4) or diameter growth (for T5) of the fungal pathogen. The percentage inhibition of diameter growth (PIDG) was calculated following Ebadzadsahrai et al. (2020) as PIDG = $[(D_1 - D_2)/ D1] \times 100$, where D_1 is the diameter growth of *A. rolfsii* on the control plate and D_2 is the diameter growth of *A. rolfsii* on the plate spread with vermicompost filtrate.

Experiment 2: selection of Trichoderma species with best antagonistic activity

Dual culture assay was conducted as described above in the confrontation assays using three *Trichoderma* species (*T. asperellum* isolate T13, *T. hazianum* isolate T9, and *T. koningii* isolate 67). The plates were incubated at 25±2 °C and then the radial growth of the pathogen was observed

Table 1 Five confrontation assay techniques used for testing antagonistic activity of vermicompost filtrate against mycelial growth of *Athelia rolfsii*

Confrontation technique	Type of vermicompost filtrate (100 µL per plate)	Reference
T1 = dual culture	Paper disc diffusion	Cheng et al. (2012)
T2 = dual culture	Well diffusion assay	Ersahin et al. (2009)
T3 = dual culture	Drop diffusion	Ersahin et al. (2009)
T4 = dual culture	Half plate spread	Kamal et al. (2015)
T5 = swab plate	Whole plate spread	Szczech (1999)

daily and measured for antagonistic activity. The percentage inhibition of radial growth (PIRG) was calculated following Muniroh et al. (2019) as PIRG = $[(R_1 - R_2)/ R_1] \times 100$, where R_1 is the radial growth of *A. rolfsii* on the control plate and R_2 is the radial growth of *A. rolfsii* on the dual culture plate. The assay used three biological replicates.

Experiment 3: confrontation assay for testing antagonistic activity of mixtures

The mixtures used in this experiment were prepared by mixing vermicompost filtrate with *Trichoderma* spore suspension, applying two proportions by volume of 75:25 and 50:50, respectively. In each proportion, the *Trichoderma* spore suspension varied in concentrations of 1×10^3 spores/mL, 1×10^5 spores/mL and 1×10^8 spores/mL. To prepare the mixtures, the selected *Trichoderma* from experiment 2 was grown on PDA for 7 d at 25±2 °C. Then, fungal spores were prepared in physiological saline solution (0.85% of NaCl) to obtain a spore suspension (Singh et al., 2016) that was adjusted to the desired concentration using a hemocytometer (Neubauer-ruled Bright Line counting chambers; Gizmo Supply Co.; Germany), according to Wolk et al. (2000). An appropriate confrontation assay that was applied in the swab plate technique mentioned in experiment 1 was used in this section.

Experiment 4: dual culture assay between A. rolfsii and isolated antagonists from vermicompost

Microorganisms producing an inhibition zone against the mycelial growth of *A. rolfsii* from experiment 3 were isolated and multiplied on synthetic media. Fungi were cultured on PDA at 25±2 °C for 7 d, whereas the bacteria were cultured on nutrient agar (NA) at 25 ± 2 °C for 48 h. Mycelial plug of *A. rolfsii* was prepared as described in experiment 1. Dual culture assay was conducted on PDA followed by the calculation of the PIRG (Muniroh et al., 2019) as shown in the experiment 2. Mycelial plug was used for antagonistic fungi while streak method was used for establishing the bacterial colony in dual culture assay as previously described by Archana et al. (2020) and Rivera–Méndez et al. (2020). The assay was conducted on three biological replicates.

Greenhouse experiment

Inoculation method

Inoculation of *A. rolfsii* on vegetable soybean was simultaneously conducted when the vegetable soybean seed was sown in each plastic pot (4 cm height and 5 cm diameter) containing 20 g of soil (Yasothon soil series) together with either a sorghum grain fully covered with mycelia or a single sclerotium of *A. rolfsii* in the respective treatment (Mahadevakumar et al., 2018; Paparu et al., 2020). Then, each pot was watered until reaching field capacity to trigger germination of sclerotia and growth of fungal mycelia. All pots were kept at 25±2 °C average temperature and 90% relative humidity (Sun et al., 2020), with a 12 h-12 h light-dark photoperiod via supplemental light (1,412.8 cd). Disease incidence was observed and recorded daily at 4:00 p.m. until 21 d after germination. Additionally, inoculated plants were grown until the plants died to observe additional disease incidence. Pathogen-inoculated controls of both inoculum types were used. There were four treatments: 1) seed + mycelia of *A. rolfsii* + vermicompost; 2) seed + mycelia of *A. rolfsii* + vermicompost + *T. asperellum* isolate T13; 3) seed + sclerotia of *A. rolfsii* + vermicompost; and 4) seed + sclerotia of *A. rolfsii* + vermicompost + *T. asperellum* isolate T13. The water-inoculated control was included for both inoculations of both mycelial and sclerotia. For the treatments inoculated with *T. asperellum* isolate T13, the pathogen inoculum was simultaneously inoculated with two grains of rice covered with *T. asperellum* isolate T13. The experimental design was arranged in a completely randomized design with five replicates, each containing seven experimental units.

Disease incidence

Disease incidence (DI) was calculated following Tarafdar et al. (2018), where DI (%) = (the number of plants with sclerotium rot disease in the replicate / the number of germinating seeds in the replicate) × 100. The percentage DI measured from all replicates was averaged and then compared among treatments and controls.

Data analysis

Data on PIDG, PIRG and DI were subjected to analysis of variance and subsequent pairwise comparisons were done using the least significant difference (LSD) at the 95% level of significance using the package 'agricolae' version 1.3-5 (de Mendiburu, 2021) and RStudio Desktop 1.4.1717 and the R software package (R Core Team, 2021).

Results and Discussion

Appropriate method to test antagonistic activity of vermicompost

Of the five tested fungal confrontation assay procedures,

all methods produced antagonistic activity against mycelial growth of *A. rolfsii* (Fig. 2). The observed diameter of *A. rolfsii* colonies grown on PDA spread thoroughly with vermicompost filtrate, as described by Szczech (1999), showed the highest antagonistic activity (53.3%) (Fig. 2). Thus, the whole plate spread method was used to confirm the results and used for testing the effect of the mixture of vermicompost and spore suspension of *T. asperellum* isolate T13 on *A. rolfsii* mycelial growth.

Selection of Trichoderma spp. producing best antagonistic activity

Among the three examined species, *T. asperellum* isolate T13 demonstrated PIRG at 40.7% that was significantly higher than for *T. harzianum* isolate T9 (31.2%) and *T. koningii* isolate 67 (24.9%), respectively (Fig. 3). Surprisingly, the antagonistic activities of all *Trichoderma* spp. were significantly greater than for the chemical fungicide (azoxystrobin + difenoconazole) for these experimental conditions (Fig. 3). Therefore, *T. asperellum* isolate T13 was selected to mix in the vermicompost filtrate for testing the antagonistic activity against mycelial growth *in vitro* and sclerotium rot disease suppression under greenhouse conditions. Previous empirical studies also indicated the *in vitro* antagonistic activity of *T. asperellum* against *A. rolfsii* (Pacheco et al., 2016) and *Sclerotium cepivorum* (Rivera-Méndez et al., 2020).

Antagonistic activity of vermicompost-T. asperellum isolate T13 mixture against A. rolfsii

The PIDGs observed from the confrontation assays on media spread with all types of mixture of vermicompost filtrate and *T. asperellum* isolate T13 spore suspension were significantly greater than for pure vermicompost (47.6%), as shown in Fig. 4. Of all the mixtures, adding a half proportion of 1×10^8 spores/mL spore suspension of *T. asperellum* isolate T13 relative to the total volume produced 80.4% PIDG, implying the best antagonistic activity (Fig. 4). Hence, adding spores of antagonistic fungi to the mixtures either with a higher spore concentration or a larger proportion tended to increase the antagonistic activity against *A. rolfsii*.

Of the 35 sown seeds in each treatment, the total of germinated seeds in each treatment was greater than 50% (Fig. 5). Within each treatment, there were more than three germinated seeds in every replicate (data not shown). After inoculation, the water-inoculated plants either with mycelia or sclerotia did not show any disease symptoms (% DI = 0). For the pathogen-inoculated control, sclerotium rot disease incidence in the treatment inoculated with mycelia was significantly greater than for sclerotium inoculation (40.7% and 10.9%, respectively), as shown in Fig. 5. This suggested that active mycelia of *A. rolfsii* caused a larger percentage of disease incidence, as reported by Mishra et al. (2021). Sclerotia, dormant structures of the fungal pathogen, appeared to delay the infection process due to late germination in unusual growth conditions (Xu et al., 2008).

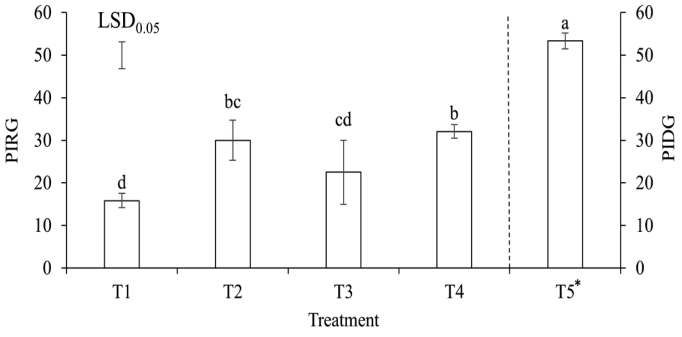

Fig. 2 Mean percentage inhibition of radial growth (PIRG) and diameter growth (PIDG) measured from tested methods of confrontation assay between mycelial plug of *Athelia rolfsii* and vermicompost filtrate at day 3 after culture (error bars = ± SD), different lowercase letters above bars indicate significantly ($p < 0.05$) different; dual culture assay was applied in T1–T4 while mycelial growth on media spread with vermicompost filtrate was used in T5*. Vermicompost filtrate used in T1 = paper disc diffusion, T2 = well diffusion assay, T3 = drop diffusion, T4 = half plate spread, T5 = whole plate spread

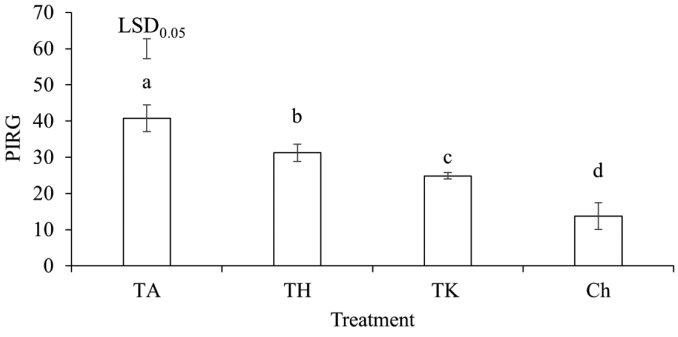

Fig. 3 Mean percentage inhibition of radial growth (PIRG) measured from dual culture assay between mycelial plug of *Athelia rolfsii* and individual *Trichoderma* species compared with chemical fungicide at day 5 after culture (error bars = ± SD), where different lowercase letters above bars indicate significantly ($p < 0.05$) different; TA = *T. asperellum* isolate T13, TH = *T. harzianum* isolate T9, TK = *T. koningii* isolate 67 and Ch = azoxystrobin + difenoconazole

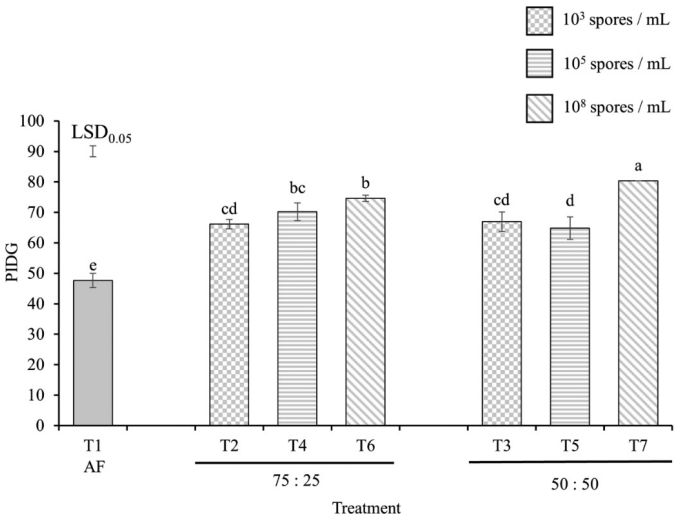

Fig. 4 Mean percentage inhibition of diameter growth (PIDG) observed from fungal confrontation assay via mycelial growth on media spread with various mixtures of vermicompost filtrate and spore suspension of *Trichoderma asperellum* isolate T13 at day 3 after culture (error bars = ± SD), where different lowercase letters above bars indicate significantly ($p < 0.05$) different; 75:25 and 50:50 = proportion between vermicompost volume and spore suspension volume in the mixture; T1 = pure vermicompost, T2, T4, T6 = mixture of 75% by volume of vermicompost + 25% of *T. asperellum* isolate T13 spore suspension volume with varied spore concentrations for each treatment (T2 = 1×10^3 spores/mL, T4 = 1×10^5 spores/mL, and T6 = 1×10^8 spores/mL); T3, T5, T7 = mixtures of 50% by volume of vermicompost + 50% of *T. asperellum* isolate T13 spore suspension volume (T3 = 1×10^3 spores/mL, T5 = 1×10^5 spores/mL and T7 = 1×10^8 spores/mL; AF = vermicompost

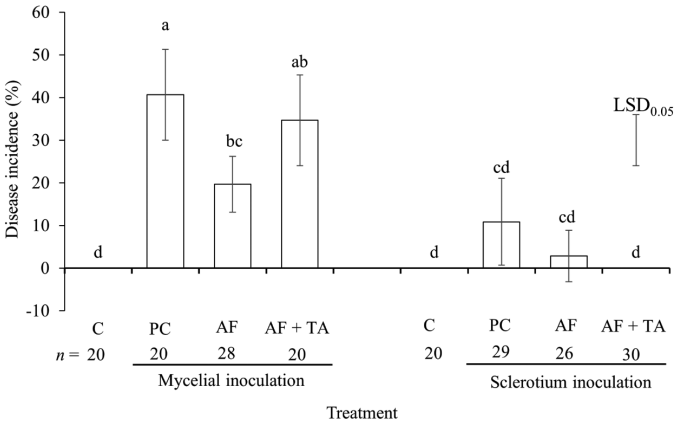

Fig. 5 Mean percentage sclerotium rot disease incidence observed on soybean seedlings at day 21 after inoculation with different pathogen inoculum types, where n = total number of germinated seeds of each treatment (error bars = ± SD); Different lowercase letters above bars indicate significantly ($p < 0.05$) different; C = water-inoculated control, PC = pathogen-inoculated control, AF = vermicompost, TA = *Trichoderma asperellum* isolate T13 and AF + TA = simultaneous inoculation of pathogen inoculum and *T. asperellum* isolate T13

For mycelial inoculation, the pathogen-inoculated control had 40.7% DI that was higher than the treatment with the combination of vermicompost and *T. asperellum* isolate T13. Interestingly, adding pure vermicompost produced the lowest DI (19.7%), as shown in Fig. 4, contrasting to the result observed from the *in vitro* confrontation assays (Fig. 3). Specific environmental conditions either on synthetic culture media or in the soil might have affected the growth of both the fungal pathogen and the antagonistic organism (Punja, 1985). Therefore, varied antagonistic activity levels *in vitro* and percent disease incidences in the greenhouse were observed in the current study.

For sclerotium inoculation, the DI percentages were not significantly different among inoculated treatments (Fig. 5). However, plants treated with the combination of vermicompost and *T. asperellum* had no sclerotium rot disease symptoms (0% DI; Fig. 5). This result suggested effective biological control of soilborne pathogens, especially *A. rolfsii* should be at the initial stage of pathogen growth, as reported by Qadri et al. (2020). In contrast, controlling *A. rolfsii* in the mycelial stage was partially successful when using only vermicompost (Fig. 4), which agreed with the empirical studies of Szczech (1999) and Ersahin et al. (2009).

Effect of vermicompost amendment on physico-chemical properties and disease incidence

The basic physico-chemical properties of non-amended soil, pure vermicompost and soil amended with 20% of vermicompost by volume are shown in Table 2. The soil amended with vermicompost showed significant differences in the physico-chemical properties from the non-amended soil (Table 2). The soil amended with vermicompost had lower values for pH and EC but higher levels of total N, available P and exchangeable K than the non-amended soil (Table 2). These results indicated that adding vermicompost to the soil led to short-term changes in some physico-chemical properties, as was also reported by Zhao et al. (2017). For example, the addition of vermicompost to the soil resulted in a change in the pH (Vo and Wang, 2014; Mahmud et al., 2018) and the OM (Tejada and Benítez, 2011; Wang et al., 2017).

In addition to fungal inoculum in the soil, the physico-chemical properties of the soil significantly correlated with soilborne disease incidence (Huber et al., 2012). For example, sclerotium disease incidence was positively correlated with an increasing level of N and OM but negatively correlated with the pH and K contents (Garain et al., 2020). Furthermore, the

decrease in pH with the increase in K revealed effective control of fusarium wilt (Wang et al., 2021). In the current study, the soil amended with vermicompost produced a decrease in the pH, whereas the exchangeable K increased (Table 2). This might have resulted in the significantly lower percentage of sclerotium rot disease incidence than in the non-amended soil (Fig. 5). The changes in the physico-chemical properties of the soil after amendment with vermicompost, such as the decrease in pH and the increase in K, could adversely affect soilborne pathogen growth and thus have the advantage of soilborne disease suppression (Sahni et al., 2008; Zhao et al., 2017).

Antagonistic activity of microbes isolated from vermicompost against A. rolfsii

Vermicompost added to growth media for potted plants showed effective soilborne disease suppression (Pathma and Sakthivel, 2012). For example, disease incidence in chickpea grown in substrate mixed with vermicompost was lower than in pure substrate (Sahni and Prasad, 2020), as well as in the current study for sclerotium rot disease on vegetable soybean (Fig. 5). The competition-based mechanisms among microorganisms for resources, including direct- and interference competition, have been used to explain how vermicompost encouraged soilborne disease suppression (Pathma and Sakthivel, 2012) because vermicompost amendment leads to an increase in OM in the substrate resulting in some microorganisms, from both the microflora of the substrate and the added vermicompost, readily multiplying and rapidly exploiting the organic substances at the expense of the soilborne plant pathogen. This direct resource competition provides unfavorable conditions for the growth of the infective stage of the pathogen (Zhao et al., 2019; Wang et al., 2021) and prevents germination (Amooaghaie and Korrani, 2018). Consequently, disease incidence is decreased in such circumstances compared to the pure substrate condition, as reported by Wang et al. (2021).

Furthermore, some microbial species from vermicompost can indirectly compete for resources via interference competition by secreting secondary metabolites to suppress the growth of soil borne pathogens (Gopalakrishnan et al., 2011; Jayakumar and Natarajan, 2013); thus, soilborne disease incidence decreases. In the current study, two antagonistic bacteria and one antagonistic fungus isolated from vermicompost showed the inhibition activity *in vitro* against *A. rolfsii* (Table 3). They produced distinct inhibition zones, implying an inhibitory mechanism for disease suppression.

The three examined *Trichoderma* spp. (*T. harzianum* isolate T9, *T. koningii* isolate 67 and *T. asperellum* isolate T13) had higher antagonistic activity against the mycelial growth of *A. rolfsii in vitro* than did the chemical fungicide (azoxystrobin + difenoconazole), as shown in Fig. 3. The *T. asperellum* isolate T13 had the best *in vitro* antagonistic activity among the three *Trichoderma* species (Fig. 3). The vermicompost filtrate clearly had antagonistic activity against the mycelial growth of

Table 3 Percentage inhibition (mean ± SD) of radial growth (PIRG) measured from dual culture assay between mycelial plug of *Athelia rolfsii* and three antagonistic microbes isolated from vermicompost filtrate at day 5 after culture on potato dextrose agar

Antagonistic microbe	PIRG (%)
Bacteria	
Bacillus sp. isolate B1	23.0±1.3[b]
Bacillus sp. isolate B2	51.5±5.8[a]
Fungus	
Trichoderma sp. isolate V1	55.0±3.9[a]

Mean values superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Table 2 Basic physico-chemical properties of soil, soil amended with vermicompost and vermicompost (mean ± SD)

Parameter	Soil	Vermicompost	Soil + Vermicompost
pH	6.90±0.01	6.61±0.01	6.63±0.01
EC (dS/m)	0.080±0.00	4.05±0.02	0.64±0.03
Total N (g/kg)	0.42±0.00	13.29±0.00	0.70±0.00
Total P(g/kg)	-	4.70±0.14	-
Total K (g/kg)	-	4.10±0.01	-
Available P (mg/kg)	312.50±0.00	-	512.50±0.00
Exchangeable K (mg/kg)	308.96±3.22	-	543.90±17.32
OC (%)	0.54±0.00	8.56±0.09	0.56±0.00
C/N	12.85±1.52	12.33±0.49	8.10±0.75

AF = vermicompost from African nightcrawler; EC = electrical conductivity; OC = organic carbon; C = carbon; N= nitrogen; K = exchangeable potassium; P= available phosphorus. Exchangeable potassium and available phosphorus were measured for non-amended soil and soil amended with vermicompost, in contrast to total potassium and total phosphorus for pure vermicompost.

A. rolfsii in vitro, with augmented efficacy when combined with spores of *T. asperellum* isolate T13 (Fig. 4). While this indicated the antagonistic activity of the vermicompost against *A. rolfsii in vitro*, the sole amendment of vermicompost to the growing media led to a lower incidence of sclerotium rot disease caused by mycelial inoculation on vegetable soybean than either the pathogen-inoculated control or vermicompost combined with *T. asperellum* isolate T13 under greenhouse conditions (Fig. 5). It certainly appeared that sole vermicompost could be utilized for controlling sclerotium rot disease on vegetable soybean seedlings. This was contradictory to the results of previous experiments which indicated that soil amended with the combination of vermicompost and *T. asperellum* CB–Pin–01 had greater suppression of *P. aphanidermatum* colonization on lettuce seedling roots than vermicompost alone (Charoenrak et al., 2019), as well as the amendment of combination of vermicompost and *Pseudomonas syringae* (PUR46) that reduced chickpea seedling mortality caused by *A. rolfsii* (Sahni et al., 2008). However, the combination of vermicompost and *T. asperellum* isolate T13 amended to the soil completely suppressed sclerotium rot disease following the inoculation with sclerotia of *A. rolfsii* (Fig. 5). This suggested that the combined vermicompost and *T. asperellum* isolate T13 should be used for sclerotium rot disease prevention rather than for the control of *A. rolfsii* mycelial colonization.

The results of the current study indicated that sole vermicompost and its combination with *T. asperellum* isolate T13 had the potential to be used as a bioagent to control sclerotium rot disease, especially on potted plants. The effective control of this disease would prevent disease incidence by amending the soil with combined vermicompost with *T. asperellum* isolate T13 in the growing substrate before seed sowing. The protocol of vermicompost and its combination with *T. asperellum* isolate T13 application in this research would alternatively provide a means of controlling sclerotium rot disease on various plants. It would be necessary to further investigate the mechanisms of sclerotium disease suppression via either sole vermicompost or the combination of vermicompost and *T. asperellum* isolate T13. Intensive analysis of the microbial community in the vermicompost and identification of isolated antagonistic microbes should be conducted to identify antagonistic microbial species and their antagonistic activities against *A. rolfsii*. In addition, the antagonistic activity of additional species of *Trichoderma* against *A. rolfsii* should be examined and compared with the current results. The antagonistic activity of vermicompost should be examined against various soilborne pathogens.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

Allison, L.E. 1965. Organic carbon. In: Norman, A.G. (Ed.). Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties. American Society of Agronomy Inc. Madison, Wisconsin, WI, USA, pp. 1367–1378.

Amooaghaie, R., Korrani, F.M. 2018. *Bacillus subtilis* and vermicompost suppress damping-off disease in *Psyllium* through nitric oxide-dependent signaling system. Russ. J. Plant Physiol. 65: 435–445. doi.org/10.1134/S1021443718030093

Archana, T., Rajendran, L., Manoranjitham, S.K., Krishnan, V.P.S., Paramasivan, M., Karthikeyan, G. 2020. Culture-dependent analysis of seed bacterial endophyte, *Pseudomonas* spp. EGN 1 against the stem rot disease (*Sclerotium rolfsii* Sacc.) in groundnut. Egypt. J. Biol. Pest Control 30: 119. doi.org/10.1186/s41938-020-00317-x

Basco, M.J., Bisen, K., Keswani, C., Singh, H.B. 2017. Biological management of Fusarium wilt of tomato using biofortified vermicompost. Mycosphere 8: 467–483. doi.org/10.5943/mycosphere/8/3/8

Billah, K.M.M., Hossain, M.B., Prince, M.H., Sumon, M.M.P. 2017. Pathogenicity of *Sclerotium rolfsii* on different host, and its over wintering survival: A mini review. Int. J. Adv. Agric. Sci. 2: 1–6.

Bray, R.H., Kurtz, L.T. 1945. Determination of total organic and available forms of phosphorus in soils. Soil Sci. 59: 39–45. dx.doi.org/10.1097/00010694-194501000-00006

Chamswarng, C., Intanoo, W. 2002. Production of *Trichoderma* fresh culture by a simple technique for controlling damping-off of yard long bean caused by *Sclerotium rolfsii*. In: Proceedings of 40th Kasetsart University Annual Conference (Subject: Plants). Bangkok, Thailand, pp. 114–122.

Charoenrak, P., Chamswarng, C. 2016. Efficacies of wettable pellet and fresh culture of *Trichoderma asperellum* biocontrol products in growth promoting and reducing dirty panicles of rice. Agr. Nat. Resour. 50: 243–249. doi.org/10.1016/j.anres.2016.04.001

Charoenrak, P., Chamswarng, C., Intanoo, W., Keawprasert, N. 2019. The effects of vermicompost mixed with *Trichoderma asperellum* on the growth and Pythium root rot of lettuces. Int. J. Geomate 17: 215–221. doi.org/10.21660/2019.61.4728

Chattopadhyay, C., Kolte, S.J., Waliyar, F. 2015. Diseases of Edible Oilseed Crops. CRC Press. Boca Raton, FL, USA.

Cheng, C. H., Yang, C.A., Peng, K.C. 2012. Antagonism of *Trichoderma harzianum* ETS 323 on *Botrytis cinerea* mycelium in culture conditions. Phytopathology 102: 1054–1063. doi.org/10.1094/PHYTO-11-11-0315

Cilliers, A.J., Pretorius, Z.A., Van Wyk, P.S. 2003. Integrated control of *Selerotium rolfsii* on groundnut in South Africa. J. Phytopathol. 151: 249–258. doi.org/10.1046/j.1439-0434.2003.00715.x

de Mendiburu, F. 2021. Agricolae: Statistical procedures for agricultural research. R package version 1.3-5. https://CRAN.R-project.org/package=agricolae, 24 May 2021.

Nájera, J.F.D., Castellanos, J.S., Hernández, M.V., Serna, S.A., Gómez, O.G.A., Verduzco, C.V., Ramos, M.A. 2018. Diagnosis and integrated management of fruit rot in *Cucurbita argyrosperma*, caused by *Sclerotium rolfsii*. Plant Pathol. J. 34: 171–181. doi.org/10.5423/PPJ.OA.08.2017.0185

Dwivedi, S., Prasad, G. 2016. Integrated management of *Sclerotium rolfsii*: An overview. Eur. J. Med. Pharm. Sci. 3: 137–146.

Ebadzadsahrai, G., Higgins Keppler, E.A., Soby, S.D., Bean, H.D. 2020. Inhibition of fungal growth and induction of a novel volatilome in response to *Chromobacterium vaccinii* volatile organic compounds. Front. Microbiol. 11: 1035. doi.org/10.3389/fmicb.2020.01035

Ersahin, Y.S., Haktanir, K., Yanar, Y. 2009. Vermicompost suppresses *Rhizoctonia solani* Kühn in cucumber seedlings. J. Plant Dis. Prot. 116: 182–188. doi.org/10.1007/bf03356308

Ersahin, Y.S. 2011. The use of vermicompost products to control plant diseases and pests. In: Karaca, A. (Ed.). Biology of Earthworms, Vol. 24: Soil Biology. Springer. Berlin, Germany, pp.191–213.

Ganesan, S., Kuppusamy, R.G., Sekar, R. 2007. Integrated management of stem rot disease (*Sclerotium rolfsii*) of groundnut (*Arachis hypogaea* L.) using *Rhizobium* and *Trichoderma harzianum* (ITCC - 4572). Turk. J. Agric. For. 31: 103–108.

Garain, P.K., Mondal, B., Dutta, S. 2020. Influence of edaphological factors on *Sclerotium rolfsii* Sacc., causing collar rot of betel vine (*Piper betle* L.) under coastal saline zone of West Bengal. Plant Arch. 20: 1943–1948.

Garg, A., Balodi, R. 2014. Recent trends in agriculture: Vertical farming and organic farming. Adv. Plants Agric. Res. 1: 142–144. https://doi.org/10.15406/apar.2014.01.00023

Gopalakrishnan, S., Pande, S., Sharma, M., et al. 2011. Evaluation of actinomycete isolates obtained from herbal vermicompost for the biological control of *Fusarium* wilt of chickpea. Crop Prot. 30: 1070–1078. doi.org/10.1016/j.cropro.2011.03.006

Guclu, V., Aydogdu, M., Basak, M., Kizil, S., Uzun, B., Yol, E. 2020. Characterization of a groundnut collection to stem rot disease caused by *Sclerotium rolfsii*. Australas. Plant Pathol. 49: 691–700. https://doi.org/10.1007/s13313-020-00748-y

Huber, D., Römheld, V., Weinmann, M. 2012. Relationship between nutrition, plant diseases and pest. In: Marschner, P. (Ed.). Plant Diseases and Pests. Marschner's Mineral Nutrition of Higher Plants, 3rd ed. Academic Press. San Diego, CA, USA, pp. 283–298.

Huzar-Novakowiski, J., Paul, P.A., Dorrance, A.E. 2017. Host resistance and chemical control for management of Sclerotinia stem rot of soybean in Ohio. Phytopathology. 107: 937–949. doi.org/10.1094/PHYTO-01-17-0030-R

Iwai, C.B., Oo, A.N., Kruapukdee, A., Chuasavatee, T. 2018. Vermicompost as soil amendment for sustainable land and environment in Thailand. In: Rakshit, A., Sarkar, B., Abhilash, P. (Eds.). Soil Amendments for Sustainability: Challenges and Perspectives. CRC Press. Boca Raton, FL, USA, pp. 321–348.

Jackson, M.L. 1958. Soil Chemical Analysis. Prentice Hall Inc., Englewood Cliffs, New Jersey, NJ, USA.

Jayakumar, P., Natarajan, S. 2013. Molecular and functional characterization of bacteria isolated from straw and goat manure based vermicompost. Appl. Soil Ecol. 70: 33–47. doi.org/10.1016/j.apsoil.2013.03.011

Junsopa, C., Saksirirat, W., Saepaisan, S., Songsri, P., Kesmala, T., Shew, B.B., Jogloy, S. 2021. Bio-control of stem rot in Jerusalem artichoke (*Helianthus tuberosus* L.) in field conditions. Plant Pathol. J. 37: 428–436. doi.org/10.5423/PPJ.OA.04.2021.0067

Kamal, M.M., Lindbeck, K.D., Savocchia, S., Ash, G.J. 2015. Biological control of Sclerotinia stem rot of canola using antagonistic bacteria. Plant Pathol. 64: 1375–1384. doi.org/10.1111/ppa.12369

Kamel, S.M., Farag, F.M., Arafa, R.A., Essa, T.A. 2020. Bio-control potentials of *Trichoderma* spp. against *Sclerotium rolfsii* the causative of root and crown rot in tomato, common bean and cabbage. Egypt J Phytopathol. 48: 122–136. doi.org/10.21608/ejp.2020.54217.1018

Mahadevakumar, S., Chandana, C., Deepika, Y.S., Sumashri, K.S., Yadav, V., Janardhana, G.R. 2018. Pathological studies on the southern blight of China aster (*Callistephus chinensis*) caused by *Sclerotium rolfsii*. Eur. J. Plant Pathol. 151: 1081–1087. doi.org/10.1007/s10658-017-1415-2

Mahmud, M., Abdullah, R., Yaacob, J.S. 2018. Effect of vermicompost amendment on nutritional status of sandy loam soil, growth performance, and yield of pineapple (*Ananas comosus* var. MD2) under field conditions. Agronomy 8: 183. doi.org/10.3390/agronomy8090183

Mishra, S., Mishra, P., Mishra, A.K., Singh, J. 2021. Evaluation of different inoculation techniques on pathogenicity of *Sclerotium rolfsii*. Pharma Innov. 10: 758–759. doi.org/10.22271/tpi.2021.v10.i3k.5876

Muniroh, M.S., Nusaibah, S.A., Vadamalai, G., Siddique, Y. 2019. Proficiency of biocontrol agents as plant growth promoters and hydrolytic enzyme producers in *Ganoderma boninense* infected oil palm seedlings. Curr. Plant Biol. 20: 100116. doi.org/10.1016/j.cpb.2019.100116

Nandwani, D., Nwosisi, S. 2016. Global trends in organic agriculture. In: Nandwani, D. (Ed.). Sustainable Development and Biodiversity, Vol. 9: Organic Farming for Sustainable Agriculture. Springer. Cham, Switzerland. pp. 1–35.

Olsen, S.R., Sommers, L.E. 1982. Phosphorus. In: Page, A.L. (Ed.). Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties. American Society of Agronomy Inc. Madison, Wisconsin, WI, USA, pp. 403–430.

Ozbay, N., Newman, S.E. 2004. Biological control with *Trichoderma* spp. with emphasis on *T. harzianum*. Pak. J. Biol. Sci. 7: 478–484. doi.org/*10.3923/pjbs*.2004.478.484

Özcan, M.M., Al Juhaimi, F. 2014. Effect of sprouting and roasting processes on some physico-chemical properties and mineral contents of soybean seed and oils. Food Chem. 154: 337–342. doi.org/10.1016/j.foodchem.2013.12.077

Pacheco, K.R., Viscardi, B.S.M., de Vasconcelos, T.M.M., Moreira, G.A.M., do Vale, H.M. M., Blum, L.E.B. 2016. Efficacy of *Trichoderma asperellum*, *T. harzianum*, *T. longibrachiatum* and *T. reesei* against *Sclerotium rolfsii*. Biosci. J. 32: 412–421. doi.org/10.14393/BJ-v32n2a2016-32732

Pandey, M.K., Sarma, B.K., Singh, U.P. 2005. Induction of sexual stage and colony morphology of some isolates of *Sclerotium rolfsii* causing spotted leaf rot in plants. Mycobiology 33: 7–11.

Paparu, P., Acur, A., Kato, F., Acam, C., Nakibuule, J., Nkuboye, A., Musoke, S., Mukankusi, C. 2020. Morphological and pathogenic characterization of *Sclerotium rolfsii*, the causal agent of Southern blight disease on common bean in Uganda. Plant Dis. 104: 2130–2137. doi.org/10.1094/PDIS-10-19-2144-RE

Pathma, J., Sakthivel, N. 2012. Microbial diversity of vermicompost bacteria that exhibit useful agricultural traits and waste management potential. SpringerPlus 1: 26. doi.org/10.1186/2193-1801-1-26

Paul, N.C., Hwang, E.J., Nam, S.S., et al. 2017. Phylogenetic placement and morphological characterization of *Sclerotium rolfsii* (Teleomorph: *Athelia rolfsii*) associated with blight disease of *Ipomoea batatas* in Korea. Mycobiology 45: 129–138. https://doi.org/10.5941/MYCO.2017.45.3.129

Punja, Z. 1985. The Biology, ecology, and control of *Sclerotium rolfsii*. Ann. Rev. Phytopathol. 23: 97–127. https://doi.org/10.1146/annurev.phyto.23.1.97

Qadri, R., Azam, M., Khan, I., Yang, Y., Ejaz, S., Akram, M.T., Khan, M.A. 2020. Conventional and modern technologies for the management of post-harvest diseases. In: Ul Haq, I., Ijaz, S. (Eds.). Sustainability in Plant and Crop Protection, Vol. 13: Plant Disease Management Strategies for Sustainable Agriculture through Traditional and Modern Approaches. Springer. Cham, Switzerland. pp. 137–172.

R Core Team. 2021. R: A language and environment for statistical computing R foundation for statistical computing. Vienna, Austria. http://www.R-project.org/, 24 May 2021.

Rivera-Méndez, W., Obregón, M., Morán-Diez, M.E., Hermosa, R., Monte, E. 2020. *Trichoderma asperellum* biocontrol activity and induction of systemic defenses against *Sclerotium cepivorum* in onion plants under tropical climate conditions. Biol. Control 141: 104145. doi.org/10.1016/j.biocontrol.2019.104145

Sahni, S., Prasad, B.D. 2020. Management of collar rot disease using vermicompost and a PGPR strain *Pseudomonas* sp. and their effect on defense-related enzymes in chickpea. Indian Phytopathology 73: 301–311. doi.org/10.1007/s42360-020-00203-4

Sahni, S., Sarma, B.K., Singh, D.P., Singh, H.B., Singh, K.P. 2008. Vermicompost enhances performance of plant growth-promoting rhizobacteria in *Cicer arietinum* rhizosphere against *Sclerotium rolfsii*. Crop Prot. 27: 369–376. doi.org/10.1016/j.cropro.2007.07.001

Shrestha, U., Dee, M.E., Piya, S., Ownley, B.H., Butler, D.M. 2020. Soil inoculation with *Trichoderma asperellum*, *T. harzianum* or *Streptomyces griseoviridis* prior to anaerobic soil disinfestation (ASD) does not increase ASD efficacy against *Sclerotium rolfsii* germination. Appl. Soil Ecol. 147: 103383. doi.org/10.1016/j.apsoil.2019.103383

Singh, V., Upadhyay, R.S., Sarma, B.K., Singh, H.B. 2016. *Trichoderma asperellum* spore dose depended modulation of plant growth in vegetable crops. Microbiol. Res. 193: 74–86. doi.org/10.1016/j.micres.2016.09.002

Soil Survey Division Staff. 1993. Soil Survey Manual. U.S. Department of Agriculture Handbook No. 18. U.S. Department of Agriculture, U.S. Government Printing Office. Washington, DC, USA.

Somdee, T., Mahaweerawat, U., Wibulutai, J., Dungkokruad, N., Yungyuen, S. 2017. Polyphenol contents, antioxidant and anticancer activity (MCF-7) of soybean products in Thailand. Chiang Mai J. Sci. 44: 176–183.

Sun, S., Sun, F., Deng, D., Zhu, X., Duan, C., Zhu, Z. 2020. First report of southern blight of mung bean caused by *Sclerotium rolfsii* in China. Crop Prot. 130: 105055. doi.org/10.1016/j.cropro.2019.105055

Szczech, M.M. 1999. Suppressiveness of vermicompost against Fusarium wilt of tomato. J. Phytopathol. 147: 155–161.

Tarafdar, A., Rani, T.S., Chandran, U.S.S., Ghosh, R., Chobe, D.R., Sharma, M. 2018. Exploring combined effect of abiotic (soil moisture) and biotic (*Sclerotium rolfsii* Sacc.) stress on collar rot development in chickpea. Front. Plant Sci. 9: 1154. doi.org/10.3389/fpls.2018.01154

Tejada, M., Benítez, C. 2011. Organic amendment based on vermicompost and compost: Differences on soil properties and maize yield. Waste Manag. Res. 29: 1185–1196. doi.org/10.1177/0734242X10383622

Trutmann, P., Keane, P.J. 1990. *Trichoderma koningii* as a biological control agent for *Sclerotinia sclerotiorum* in Southern Australia. Soil Biol. Biochem. 22: 43–50. doi.org/10.1016/0038-0717(90)90058-8

Vo, H.M., Wang, C.H. 2014. Physicochemical properties of vermicompost-based substrate mixtures and their effects on the nutrient uptake and growth of muskmelon (*Cucumis melo* L.) seedlings. Biol. Agric. Hortic. 30: 153–163. doi.org/10.1080/01448765.2014.885851

Vongphachanh, P., Saksirirat, W., Seapaisan, S. 2016. Biological control of sheath diseases of rice caused by *Rhizoctonia oryzae* and *Rhizoctonia solani* by *Trichoderma* spp. *J. Pure Appl. Microbiol.* 10: 1735–1744.

Wang, L., Liu, J., Nie, Y., Wang, D., Wang, H. 2021. Effects of vermicompost on tomato Fusarium wilt and soil microbial community structure. Acta Agr. Scand. B-S P. 71: 835–851. doi.org/10.1080/09064710.2021.1952301

Wang, X.X., Zhao, F., Zhang, G., Zhang, Y., Yang, L. 2017. Vermicompost improves tomato yield and quality and the biochemical properties of soils with different tomato planting history in a greenhouse study. Front. Plant Sci. 8: 1978. doi.org/10.3389/fpls.2017.01978

Walkley, A.J., Black, I.A. 1934. Estimation of soil organic carbon by the chromic acid titration method. Soil Sci. 37: 29–38.

Wolk, D.M., Johnson, C.H., Rice, E.W., Marshall, M.M., Grahn, K.F., Plummer, C.B., Sterling, C.R. 2000. A spore counting method and cell culture model for chlorine disinfection studies of *Encephalitozoon* syn. *Septata intestinalis*. Appl. Environ. Microbiol. 66: 1266–1273. doi.org/10.1128/AEM.66.4.1266-1273.2000

Woo, S.L., Ruocco, M., Vinale, F., et al. 2014. *Trichoderma*-based products and their widespread use in agriculture. Open Mycol. J. 8: 71–126. doi.org/10.2174/1874437001408010071

Xu, Z., Gleason, M.L., Mueller, D.S., et al. 2008. Overwintering of *Sclerotium rolfsii* and *S. rolfsii* var. *delphinii* in different latitudes of the United States. Plant Dis. 92: 719–724. https://doi.org/10.1094/PDIS-92-5-0719

Zhao, F., Zhang, Y., Dong, W., Zhang, Y., Zhang, G., Sun, Z., Yang, L. 2019. Vermicompost can suppress *Fusarium oxysporum* f. sp. *lycopersici* via generation of beneficial bacteria in a long-term tomato monoculture soil. Plant Soil. 440: 491–505. doi.org/10.1007/s11104-019-04104-y

Zhao, H.T., Li, T.P., Zhang, Y., Hu, J., Bai, Y.C., Shan, Y.H., Ke, F. 2017. Effects of vermicompost amendment as a basal fertilizer on soil properties and cucumber yield and quality under continuous cropping conditions in a greenhouse. J. Soils Sediments 17: 2718–2730. doi.org/10.1007/s11368-017-1744-y

Zheng, B., He, D., Liu, P., Wang, R., Li, B., Chen, Q. 2020. Occurrence of collar rot caused by *Athelia rolfsii* on soybean in China. Can. J. Plant Pathol. 43: 1–5. doi.org/10.1080/07060661.2019.1703819