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THE INHIBITORY EFFECT OF LAVENDER COMPOST EXTRACTS ON VARIOUS PATHOGENS

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Abstract

Different types of composts have gained attention for their potential applications in sustainable agriculture and for their inhibitory effects against plant pathogens. This study investigates the suppressive effects of lavender compost extracts on common plant and soil pathogens and evaluates the composts' microbial composition. Pathogenic fungi, including Sclerotinia sclerotiorum, Fusarium oxysporum, Botrytis cinerea, and Alternaria alternata, were subjected to in vitro assays with varying concentrations of the extracts. The results revealed inhibitory effects due to compost microbiota; several fungal genera (Aspergillus, Penicillium, Trichoderma) and bacteria were detected. Microbial analysis of the compost extracts indicated the presence of beneficial microorganisms, such as Trichoderma spp., which may contribute to plant pathogen suppression. These findings suggest that lavender compost extracts can be a natural, eco-friendly alternative for managing plant pathogens and promoting sustainable agricultural practices. Further studies regarding the mechanisms underlying their biocontrol properties are recommended.

Key words*: lavender compost, plant pathogen suppression.*

INTRODUCTION

Through the European Green Deal (EGD) elaborated by the European Commission (EC), the European Union (EU) sets the path to a green transition, aiming to reach climate neutrality by 2050. Some of the EGD's main goals refer to circular economy (CE), a healthier environment, and more sustainable farming. Strategies such as the Zero Pollution Action Plan, Farm to Fork and Biodiversity are the key deliverables of EGD. In 2018, the legislative framework on waste (Directive 2008/98/EU) was updated, setting ambitious recycling targets of 60% by 2030 and 65% by 2035 (Directive 2018/851/EU).

The EU is reshaping food production and consumption in Europe to minimize the environmental impact of food systems. Consequently, in 2022, EC proposed a new regulation on the sustainable use of plant protection products (EC, 2022). The goal is to reduce the use of chemical pesticides by 50% and decrease the use of more hazardous pesticides to 50% by 2030, in alignment with the EU's Farm to Fork and Biodiversity strategies.

In recent years, the application of compost has attracted significant attention due to its potential contributions to sustainable agriculture. The application of compost serves various purposes, including enriching soil quality, enhancing plant growth (Garcia-Gil et al., 2000; Albiach et al., 2001; Whalen et al., 2003; Bastida et al., 2007; Iovieno et al., 2009; Bellino et al., 2015), reducing the need for chemical fertilizers and pesticides, promoting soil microbial activity (Bellino et al., 2015; Emmerling et al., 2010; Ros et al., 2006; Reimer et al., 2023), and acting as a biocontrol agent (Veeken et al., 2005; Trillas et al., 2006; De Corato, 2020; Greff et al., 2023).

Among the various compost types tested by other authors, lavender compost has been less analyzed, as lavender waste is not easily compostable due to its physicochemical composition (Lesage-Meessen et al., 2018). However, it is important to consider whether the composted lavender waste has the capacity to suppress certain soil and plant pathogens, thereby serving as an effective component within plant protection strategies, considering that the essential oils of this plant exert some

antifungal activity (Widmer & Laurent, 2006; Erdoğan et al., 2016).

Notable pathogens, such as *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Botrytis cinerea*, and *Alternaria alternata*, are recognized for their capacity to induce substantial crop losses and compromise plant health.

Sclerotinia sclerotiorum is an ascomycetous fungal pathogen with a notably broad host range, infecting over 400 plant species across multiple families, including Brassicaceae, Fabaceae, Solanaceae, Asteraceae and Apiaceae (Boland & Hall, 1994; Bolton et al., 2006; Jiang et al., 2013). The fungus survives between growing seasons by forming sclerotia, which can germinate in two ways depending on the environmental conditions: myceliogenically and carpogenically (Steadman, 1979; Roper et al., 2010; Jiang et al., 2013). Infection typically causes soft rot symptoms and rapid plant death. The fungus produces abundant aerial hyphae on infected tissues, hence the name white mold. At later stages, new sclerotia form on lesions, contaminating soil, plant debris, or seeds (Jiang et al., 2013).

Fusarium oxysporum is a widely represented anamorphic species in soils and the rhizosphere of plants worldwide (Burgess, 1981; Gordon & Martyn, 1997; Fravel et al., 2003). All strains are saprophytic, capable of surviving on organic matter, though they differ in pathogenicity: some induce vascular wilts or root rots, while others remain nonpathogenic (Garrett, 1970; Olivain & Alabouvette, 1997; Fravel et al., 2003). The pathogenic strains are classified into more than 120 formae speciales and races, based on host range and cultivar specificity (Fravel et al., 2003). These pathogens show high host specificity, and new races can emerge that overcome host resistance, making disease management challenging (Fravel et al., 2003).

Traditional management strategies rely on resistant cultivars (e.g., tomato varieties resistant to *F. oxysporum* f. sp. *lycopersici*). However, breeding is difficult for some crops, and new pathogen races often overcome resistance. Chemical soil fumigation, historically with broad-spectrum agents such as methyl bromide, is environmentally damaging. Research efforts have increasingly focused on biological control strategies, particularly the use of nonpathogenic *F. oxysporum* strains as sustainable alternatives (Fravel et al., 2003), along with the exploration of essential oils from aromatic and medicinal plants, which have shown disease-suppressive properties (Wogiatzi et al., 2009; Ahmad et al., 2020).

Botrytis cinerea is one of the most recognized plant pathogenic fungi, commonly causing gray mold on soft fruits such as strawberries (Elad et al., 2004; Williamson et al., 2007; Pearson & Bailey, 2013). Its symptoms include abundant aerial hyphae and massive sporulation, producing gray conidiospores. While often a postharvest issue, it is also an important pathogen of growing plants, with a host range exceeding 235 species as early as 1968 (Macfarlane, 1968; Pearson & Bailey, 2013). The fungus is widespread globally, thriving particularly in humid environments such as tropical regions, dense canopies, and protected cropping systems. It infects multiple plant tissues (petals, leaves, stems, tubers) by germinating conidia that penetrate directly or through wounds. Pathogenesis is aided by cell wall–degrading enzymes (Urbanek & Zalewska-Sobczak, 2003; Pearson & Bailey, 2013) and toxins (Choquer et al., 2007; Pearson & Bailey, 2013). After colonizing host tissues, the fungus sporulates and spreads to new hosts. Importantly, infection is not always immediately symptomatic: *B. cinerea* can enter a latent phase, remaining hidden until host physiology favours fungal growth (Jaspers et al., 2012; Pearson & Bailey, 2013).

Alternaria alternata is a fungus with a broad host range, acting as both a pathogen of economically important crops and an asymptomatic endophyte (Zamora et al., 2008; Woudenberg et al., 2013; Woudenberg et al., 2015; Armitage et al., 2015; Lawrence et al., 2016; El Gobashy et al., 2018; Armitage et al., 2020; DeMers, 2022). It causes leaf spots, rots, and blights on over 380 plant species, with yield losses up to 79% in tomato and significant postharvest damage due to latent infections (Abbo et al., 2014; Dube, 2014; Troncoso-Rojas & Tiznado-Hernández, 2014; Tozlu et al., 2018). Conventional control through fungicides is effective but raises concerns about cost, resistance, and environmental impact (Chaerani & Voorrips, 2006; Heydari & Pessarakli, 2010; Tozlu et al., 2018). As a result, research has

focused on sustainable alternatives such as fungal and bacterial biocontrol agents (Benhamou & Chet, 1993; Tozlu et al., 2017; Gao et al., 2017; Tozlu et al., 2018) and natural compounds, with cassia and thyme oils showing strong antifungal activity. These strategies highlight the potential of eco-friendly approaches for the management of *A. alternata* diseases (Feng & Zheng, 2007; Troncoso-Rojas & Tiznado-Hernández, 2014).

On the other hand, there are also beneficial fungi, such as *Trichoderma* spp., which was also included in our study.

Trichoderma spp. are fungi commonly found in soils, rotting wood, and plant tissues, with some strains isolated from unusual habitats such as shellfish and termites (Samuels, 1996; Druzhinina et al., 2006; Harman et al., 2004; Blaszczyk et al., 2014). They are fast-growing, produce abundant conidia, and are widely known for their antagonism against plant pathogens through mycoparasitism, antibiosis, competition, and induction of systemic resistance (Howell, 2003; Benitez et al., 2004; Schuster & Schmoll, 2010; Blaszczyk et al., 2014). Beyond disease suppression, *Trichoderma* spp. enhances plant growth, nutrient uptake, and yield (Vinale et al., 2008; Carvalho et al., 2018; Zin & Badaluddin, 2020), while also improving composting processes and facilitating soil detoxification (Rai et al., 2016; Carvalho et al., 2018; Zin & Badaluddin, 2020). Their industrial importance lies in the production of antifungal enzymes, which are synergistic in pathogen control and useful in engineering resistant plants (Kubicek et al., 2001; Blaszczyk et al., 2014; Zin & Badaluddin, 2020). Field studies in crops like soybean, cucumber, and tomato confirm their value as sustainable biocontrol agents (Almeida et al., 2001; Ethur et al., 2008; Carvalho et al., 2018).

Aligned with the regulations and goals of the EU, this study investigates the inhibitory effects of lavender compost extracts on these prevalent plant and soil pathogens. Through the execution of *in vitro* assays utilizing varying concentrations of the extracts, we seek to assess the suppressive capabilities and microbial composition of the compost. Identifying beneficial microorganisms, such as *Trichoderma* spp., present in the compost extracts accentuates the potential of lavender compost as a natural and environmentally sustainable alternative for the management of plant pathogens.

This paper further explores the mechanisms underlying the bio-control properties of lavender compost extracts and their implications for sustainable agricultural practices.

MATERIALS AND METHODS

Composts used

This study analyzed four compost samples: one from compost that underwent fermentation in 2023 (C1) and three from compost produced in 2024 (C2, C3, and C4). The samples consisted of co-composted manure (sheep and goat), lavender waste (including undistilled lavender biomass and distilled lavender stalks), and wheat straw (C2). Details of the compost recipes are provided in Table 1.

Table 1. Recipes of the composts used

C1 (2023)	C2 (2024)	C3 (2024)	C4 (2024)
500 kg of sheep manure	±33% (kg/kg) of sheep manure	±33% (kg/kg) of sheep manure	±33% (kg/kg) of sheep manure
200 kg mix of undistilled and distilled lavender stalks	±33% (kg/kg) goat manure	±33% (kg/kg) goat manure	±33% (kg/kg) goat manure
6 kg compost activator	±33% (kg/kg) wheat straws	±33% (kg/kg) distilled lavender stalks	±33% (kg/kg) undistilled lavender biomass

Counting of microbial load in compost teas

To determine the cultivable microbial load, the samples were brought to the laboratory for analysis within 24 hours of collection. Serial decimal dilutions were prepared from the compost to quantify the microbial load and perform quantitative microbiological analyses. For the first dilutions (10^{-1}), 10 grams of compost were processed and infused in 90 ml of sterile distilled water for 1 hour at room temperature, under agitation at 150 rpm. Subsequently, serial decimal dilutions were made up to 10^{-5}.

For the quantitative microbiological tests, different agar media were used, such as: (i) Plate Count Agar (PCA) medium for determining cultivable bacterial load, (ii) Rose Bengal Chloramphenicol Agar (RBC) medium for isolating and counting fungal load, and (iii)

Eosin Methylene Blue (EMB) Agar according to Levine's recipe for isolating, counting, and differentiating bacteria from the Enterobacteriaceae family (Dușa et al., 2022, 2023).

For testing, the lawn inoculation method was used on the surface of the agar, with 100 µl suspension from the 10^{-2} to 10^{-5} dilutions, depending on the type of analysis and the culture medium used. After processing, the samples were inoculated on PCA medium to determine the total cultivable bacterial load and incubated at 28°C. In contrast, those inoculated on EMB medium for determining enterobacterial load were incubated at 36°C. The samples inoculated on RBC medium, processed to determine fungal load, were incubated at 26°C for 7 days to ensure the thermal conditions were suitable for the development of this category of microorganisms and sufficient time for fungal colony growth.

In vitro suppression assay

Fungi used in this assay were *S. sclerotiorum*, *Trichoderma* spp., *F. oxysporum*, *B. cinerea,* and *A. alternata* (Figure 1), which were maintained on potato dextrose agar (PDA) medium at 20°C until the incubation test.

Compost teas (CT) with a 20% concentration were made for the experiment. For the CTs, compost samples were mixed with distilled water (20 g of compost and 180 mL of distilled water). The mixture was homogenized for 10 minutes and left to rest for 24 hours. The aqueous extract was passed through a filter paper; the filtrate was centrifuged at 4000 rpm for 20 minutes.

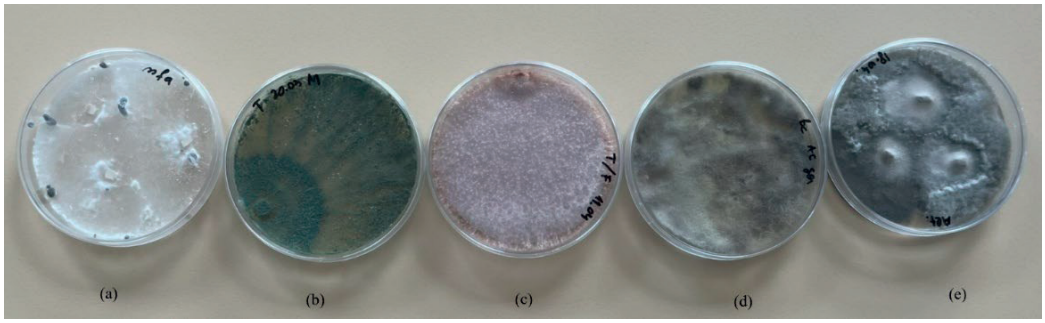

Figure 1. Fungi used for the incubation (a) *S. sclerotiorum*, (b) *Trichoderma* spp., (c) *F. oxysporum*, (c) *B. cinerea,* (d) *A. alternata*

The CTs were incorporated into the PDA medium to provide final concentrations of 5%, 2.5%, 1.25%, and 0.625%, resulting in 16 variants. The control consisted of only the PDA without CT. On each petri plate, 5 mL of medium with combined CTs was poured. One disc (0.5 cm) of mycelium of each fungus was placed centrally on the PDA medium. All plates were incubated at 25°C for 6 days.

The same test was repeated 2 weeks later with autoclaved, sterilized CTs.

The assay was performed as a completely randomized design with three repetitions.

RESULTS AND DISCUSSIONS

Counting of microbial load in compost teas

Microbiological tests revealed the cultivable microbial load present in the compost by microbial categories: bacteria and fungi. Table 2 shows that the microbial load in C1 is high, and there are no notable differences in microbial load between C2, C3, and C4. Composts C2, C3, and C4 each have a cultivable bacterial load of 108 CFU/g and a fungal load of 106 CFU/g. However, a slight decrease can be observed from C2 to C4 in both microbial categories analyzed (bacteria and fungi).

Substantial differences can be observed between C1 and C2, C3, and C4. The compost from 2023 has a microbial load 10 times lower than the composts from 2024, both in terms of bacteria and fungi (Figure 2). The analysis of enterobacteria presence in compost samples by plating dilutions (10^{-1} - 10^{-4}) on a semi-selective EMB medium suggests the absence of these bacteria categories in the compost or a load below the detection limit of 102 CFU/g.

Table 2. Microbial abundance and composition of microbial communities in composts

Substrate	Counting	Incubation period (days)	Composts			
			2023	2024		
			C1	C2	C3	C4
			(CFU/g)			
PCA	Cultivable bacteria	3	2.14×10^7	5.84×10^8	2.16×10^8	1.2×10^8
RBC	Fungi	7	2.6×10^5	4.9×10^6	3.3×10^6	2.1×10^6
EMB	Enterobacteriaceae	3-5	B.D.L.	B.D.L.	B.D.L.	B.D.L.

Legend: PCA = Plate Count Agar medium, RBC = Rose-Bengal Chloramphenicol agar medium, EMB = Eosin Methylene Blue agar medium, CFU = colony forming units, g = gram, B.D.L. = below the detection limit (102 CFU/ml)

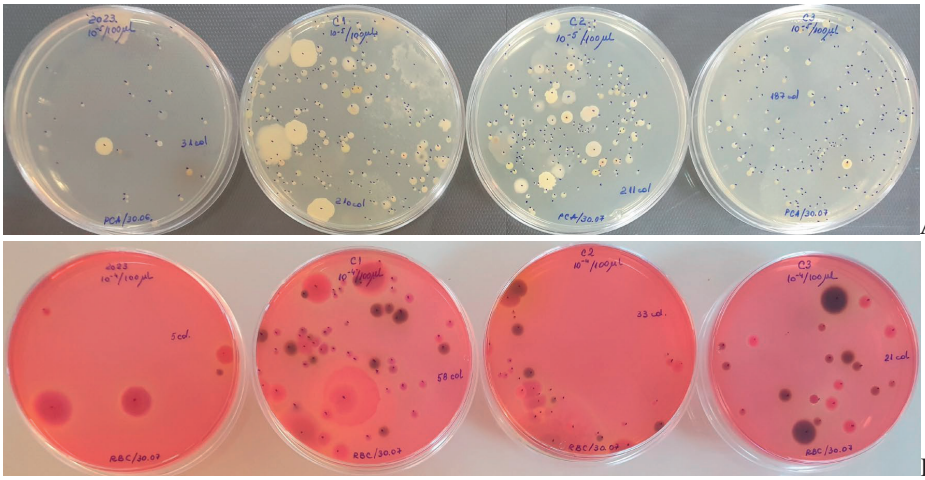

Figure 2. Bacterial (A) and fungal (B) load in the analyzed compost samples

CTs fungal suppressiveness

Experiments were conducted to evaluate the potential of different CTs in inhibiting the growth of various fungal pathogens. The results from Petri plate assays (Figures 3, 4, 5, and 6) indicated that the degree of mycelial growth reduction varied according to both the type of CT used and the concentration applied.

The results revealed considerable variability in the degree of fungal growth suppression, which depended on both the specific compost tea tested and its applied concentration. While some CTs exhibited notable inhibitory effects at certain concentrations, others demonstrated more limited activity. These observations highlighted the absence of a clear or consistent pattern of fungal inhibition across the tested samples and concentrations, making it difficult to draw definitive conclusions regarding the efficacy of any of the CTs tested.

Although the findings were not precise enough to establish firm trends, the experiments underscored the complex interactions between CT composition and fungal suppression. It is worth noting that current research is increasingly interested in the use of aromatic and medicinal plant extracts and composts for their potential to suppress or inhibit the growth of specific plant and soil pathogens, suggesting a direction for further investigations in this field (De Corato et al., 2019; De Corato, 2020; Paraschiv (Jafri) et al., 2023).

In contrast, when the compost teas were sterilised, they failed to exhibit any inhibitory effects on fungal growth. This outcome highlights that sterilised CTs were ineffective in suppressing fungal development under the conditions evaluated (data not shown).

Sclerotinia sclerotiorum

Experiments involving plates inoculated with *S. sclerotiorum* demonstrated that compost samples C3 and C4 were effective in inhibiting the growth of this pathogen. Notably, C4 exhibited the strongest suppressive effect among the tested samples. In the case of C3, the presence of *Trichoderma* species played a competitive role, actively preventing the development of *S. sclerotiorum* on the plates. Conversely, the results for compost samples C1 and C2 were inconclusive, as a clear pattern of inhibition could not be established based on the observations (Figure 3).

Previous studies have demonstrated that *Origanum* spp. and *Mentha* spp. are effective in suppressing the growth of *S. sclerotiorum* (Soylu et al., 2007; Boligłowa et al., 2009; Wogiatzi et al., 2009). Since all three plants are classified within the same botanical family (*Lamiaceae*), it is reasonable to infer that lavender may also demonstrate a comparable suppressive effect against this pathogen (Greff et al., 2023).

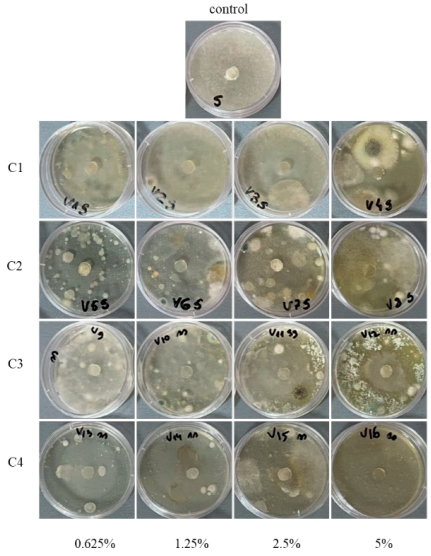

Figure 3. *Sclerotinia sclerotiorum*

Fusarium oxysporum

Figure 4 illustrates the suppressive potential of different CTs against *F. oxysporum*. At the lowest concentration tested, compost samples C1 and C2 demonstrated a modest inhibitory effect on fungal growth. However, as the concentration of these CTs increased, the composition of the microbial community within the compost also changed, with additional microorganisms emerging. This shift made it difficult to accurately assess the suppressive action of C1 and C2 at higher concentrations, as the presence of other bacteria and fungi may have interfered with the results.

In contrast, CTs derived from C3 and C4 displayed the highest suppressive effects at the highest concentrations applied. Notably, compost sample C3 exhibited an early presence of *Trichoderma* spp., which originated from the compost itself and may have contributed to its enhanced inhibitory activity against *F. oxysporum*.

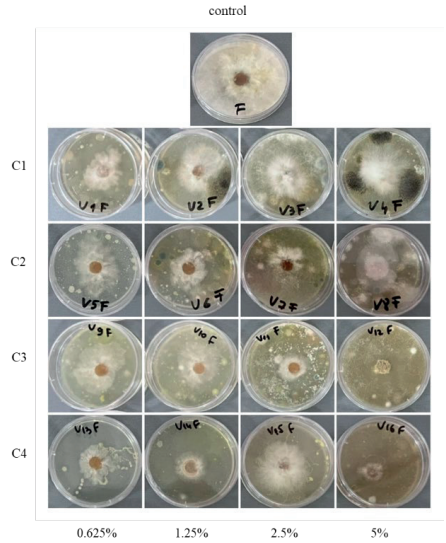

Figure 4. *Fusarium oxysporum*

Similar to the results obtained for *S. sclerotiorum*, additional members of the *Lamiaceae* family have also demonstrated suppressive effects against *F. oxysporum*. Extracts from *Thymus pallescens* (Moutassem et al., 2019) and *Salvia officinalis* (Ahmad & Matsubara, 2020) were tested and found to be effective in inhibiting the growth of *F. oxysporum* (Wogiatzi et al., 2009; Moutassem et al., 2019; Ahmad & Matsubara, 2020). These findings reinforce the potential of the plants within the *Lamiaceae* family to serve as valuable resources for the biocontrol of plant pathogens.

Botrytis cinerea

Composts C1 and C2 were found to contain a variety of microorganisms, including bacteria and fungi such as *Aspergillus* and *Penicillium*. The presence of these microorganisms interfered with the ability to accurately assess the suppressive effects of these compost teas against *B. cinerea*. As a result, the evaluation of their antifungal activity was complicated by microbial interactions within the samples.

In contrast, CTs derived from C3 and C4 consistently exhibited suppressive effects against *B. cinerea*, regardless of the concentration applied. The CT from C3 contained its own strains of *Trichoderma* spp., which were present across all tested concentrations, further contributing to its suppressive properties. Notably, C4 displayed the most substantial antifungal activity at the highest tested concentration of 5%, demonstrating its effectiveness in inhibiting fungal growth (Figure 5).

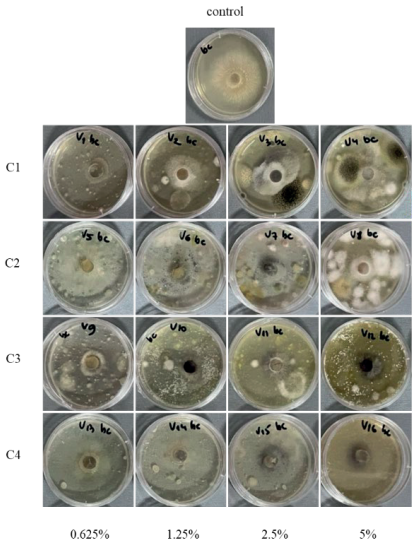

Figure 5. *Botrytis cinerea*

Alternaria alternata

At the lowest concentration tested (0.625%), CTs derived from samples C1 and C4 demonstrated the highest suppressive effects on the growth of *A. alternata*. As the concentrations of C1 and C2 CTs increased, there was a noticeable emergence of additional bacterial and fungal species within these samples, indicating shifts in microbial community composition that accompanied the higher application rates. Notably, *Trichoderma* spp. appeared in C3 at higher concentrations, suggesting a dynamic change in the microbial profile influenced by the strength of the CT.

Among all the CTs evaluated, C4 consistently exhibited the strongest antifungal activity against *A. alternata*, regardless of the concentration applied. This finding underscores the superior inhibitory potential of C4 in controlling fungal pathogens, as illustrated in Figure 6.

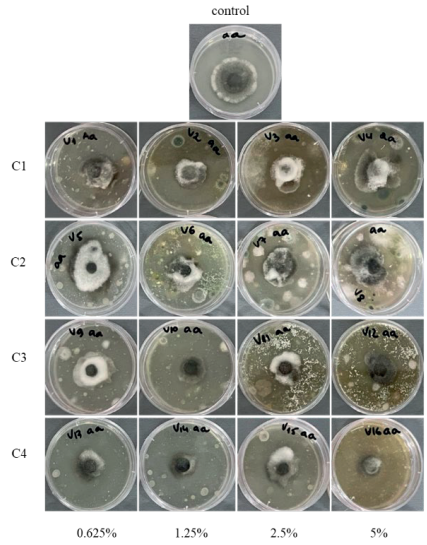

Figure 6. *Alternaria alternata*

***Trichoderma* spp.**

The evaluation of CTs against *Trichoderma* spp. revealed diverse differences in suppressive capabilities among the compost samples. Specifically, composts C1 and C2, which previously exhibited limited suppressive effects on other fungal species, were also found to have no inhibitory impact on *Trichoderma* spp. This observation is clearly illustrated in Figure 7, where the absence of suppression by C1 and C2 is noticed.

The compost C3 presented a unique challenge for assessment, as it naturally contained its own *Trichoderma* strains. The presence of these endogenous strains complicated the evaluation of any potential inhibitory effects exerted by the CT, making it difficult to draw definitive conclusions regarding its suppressive properties against *Trichoderma* spp.

In contrast, the compost C4 demonstrated a consistent and effective ability to inhibit fungal growth, including *Trichoderma* spp. The suppressive effect of C4 was observed across all tested fungal species, highlighting its superior antifungal activity and its potential role in biocontrol strategies.

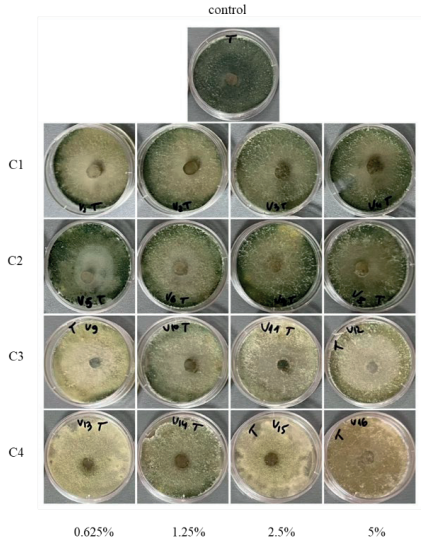

Figure 7. *Trichoderma* spp.

CONCLUSIONS

The microbiological analysis of compost samples from 2024 demonstrated a markedly higher microbial load compared to those from 2023, with bacterial and fungal counts of 108 CFU/g and 106 CFU/g, respectively, indicating enhanced microbial activity in the more recent compost. In contrast, in the 2023 composts, the microbial load was 10 times lower, with 107 bacterial CFU/g and 105 fungal CFU/g detected, respectively. Importantly, enterobacteria were undetected in all samples, with counts below the threshold of 102 CFU/g, suggesting the composts are microbiologically safe regarding this group of bacteria.

The suppressive effects of the CTs varied notably among the samples and target organisms. C4 emerged as the most effective CT, consistently displaying high inhibitory activity against *A. alternata* and *Trichoderma* spp, across all concentrations tested. This highlights the potential of C4 as a promising candidate for biocontrol strategies in managing fungal pathogens. The appearance of additional microbial species at higher CT concentrations, particularly in C1 and C2, indicates that increasing the strength of CTs can alter microbial community composition, possibly enhancing or diminishing suppressive effects.

Notably, C1 and C2 showed limited to no suppression of *Trichoderma* spp. At the same time, the assessment of C3 was complicated by the natural presence of endogenous *Trichoderma* strains, making it challenging to isolate the effects of CT application. Overall, C4's broad-spectrum antifungal activity suggests that specific compost formulations can be optimised to maximise disease suppression. The absence of enterobacteria further supports the microbiological safety of these composts for agricultural use.

Collectively, these findings highlight the significance of compost composition and microbial dynamics in developing effective and safe biocontrol agents. Optimising compost and CT formulations could play a significant role in sustainable disease management and soil health improvement strategies. However, further studies regarding the mechanisms underlying compost biocontrol properties should be continued.

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