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Non-aerated compost extracts: optimizing fermentation for enhanced antifungal effects against phytopathogenic fungi

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This study aimed to investigate the suppressive effect of non-aerated compost extracts on phytopathogenic fungi isolated from various diseased plants. Two commercial composts, C1 and C2, collected from two regions in Saudi Arabia and prepared in distinct ways, were used, and their physicochemical properties were characterized. To evaluate the individual and interactive effects of key parameters on their antifungal potential, compost extracts were prepared through a fermentation process, using a full factorial design based on the variation of four parameters at two levels: Temperature (25 and 44 ℃), compost concentration (1/5 and 1/8), glucose concentration (0 and 1 g/l), and fermentation time (3 and 7 days). The obtained extracts were characterized both physicochemically and microbiologically, then tested for their suppressive effect on the mycelial growth of four molecularly identified fungi (*Syncephalastrum racemosum* (F1), *Paramyrothecium roridum* (F2), *Fusarium oxysporum* (F3), and *Penicillium italicum* (F4)). The effect of the four variables on the inhibition percentage of these fungi showed that most compost extracts inhibited all studied fungi. Therefore, fermentation time significantly affects ($p<0.05$) the growth inhibition of *S. racemosum*. The concentration of compost and glucose, as well as fermentation time, significantly affected the growth inhibition of *P. italicum* ($p < 0.05$). However, not all studied variables significantly influenced *P. roridum* and *F. oxysporum*. The optimal conditions for the highest inhibition rate against *S. racemosum* were 25 °C, a compost concentration of 1/8, glucose supplementation at 1 g/l, and a fermentation time of 3 days. In contrast, the optimal conditions for *P. italicum* were 25 °C, a compost concentration of 1/8, without glucose addition, and fermentation times of 3 and 8 days. The Pearson test revealed that fermentation time was the key factor enhancing antifungal efficacy against *S. racemosum* and *P. italicum*. At the same time, nitrogen parameters (NTK, NH_4^+), microbial abundance, and phenolic content synergistically contributed to the suppression of pathogens. Autoclaving and filtration reduced fungal inhibition compared to non-sterile extracts, with autoclaving allowing greater fungal growth than filtration. The microbial composition and biochemical profile of the compost extracts, shaped by fermentation, likely contribute to their antagonistic effects against phytopathogenic fungi, driven by the microbial flora and organic/inorganic compounds.

Keywords Compost extracts, Biocontrol, Fermentation design, Suppressive composts, Sustainable agriculture

The increasing amount of organic waste produced by growing industrial and agricultural activities necessitates effective and sustainable management solutions. Composting has become a key method for treating biodegradable waste by converting it into stable, nutrient-rich organic matter through aerobic microbial processes. This not only reduces environmental pollution but also recycles organic materials back into the soil, improving its fertility and structure[1–3]. Compost, mainly decomposed plant residues with some animal or mineral matter, is rich in humic substances and nutrients, which boost soil organic matter, nutrient availability, and microbial activity to support

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sustainable crop growth and soil health[4–6]. Plant diseases caused by fungi, bacteria, and viruses pose a significant threat to global food security, with fungal pathogens accounting for 70–80% of crop losses[7,8]. Although chemical controls are standard, their environmental impact and increasing resistance among pathogens highlight the need for sustainable alternatives. Compost and products derived from it, such as extracts and teas, enrich soil with beneficial microbes and nutrients, helping to suppress pathogens. However, their effectiveness depends on their composition and preparation, stressing the importance of standardized production to ensure consistent quality[9,3].

Compost extracts, especially compost teas, are highly valued in sustainable agriculture for improving plant health and reducing phytopathogens. Made from composted organic materials, they are prepared as aerated compost teas (ACTs), which utilize oxygenation to promote beneficial aerobic microorganisms, or non-aerated compost teas (NCTs), which depend on passive fermentation[10]. These extracts act as environmentally friendly biocontrol agents, decreasing reliance on chemical pesticides. Their effectiveness is attributed to a range of microorganisms, including *Bacillus* spp., *Pseudomonas* spp., *Lactobacillus* spp., and *Streptomyces* spp., as well as fungi such as *Trichoderma* spp., *Aspergillus* spp., and *Penicillium* spp., along with bioactive substances like humic acids, phenolic compounds, and amino acids. These components help suppress pathogens, promote plant growth, enhance nutrient absorption, and improve stress tolerance [11–14]. Their mechanisms involve nutrient competition, activation of systemic acquired resistance (SAR), and antibiosis through compounds such as siderophores and lipopeptides (iturin, fengycin, surfactin), which damage pathogen membranes and lessen diseases caused by fungi like *Botrytis cinerea*, *F. oxysporum*, among others[15–19]. Liquid compost extracts can be integrated with fertigation systems to deliver nutrients and microbes via irrigation, foliar sprays, or soil drenches, supporting soil health and modern agriculture[20–22], Ros et al., 2020[23]. By reducing the use of synthetic agrochemicals, they promote soil biodiversity, decrease pollution, and support sustainable pest management[24]. However, the effectiveness of compost tea varies due to differences in compost quality, extraction techniques, and microbial community dynamics, which affect the stability of bioactive compounds and disease suppression potential[14,24,25]. Gaps remain regarding the best compost type, maturity, and compost-to-water ratios (ranging from 1:1 to 1:50), with inconsistent results on microbial growth and oxygen levels[23,26–29]. Fermentation times vary broadly (12 hours to 3 days for ACTs,7 to 14 days for NCTs), with unclear effects on nutrient release and overall effectiveness[30,31]. Aeration enhances microbial activity, but its implementation is limited by equipment costs and uncertain oxygen thresholds[32]. Additives such as glucose or molasses can enhance efficacy but often lack standardized formulations, which can lead to reproducibility and biosafety issues[22,14]. Further research is needed to develop standardized protocols for preparing compost extracts, ensuring they are consistent, effective, and safe.

In this context, the present study aimed to evaluate the antifungal potential of non-aerated compost extracts against phytopathogenic fungi isolated from infected plants. Specifically, it sought to determine how variations in fermentation parameters, assessed through a factorial experimental design, influence the suppressive activity of these extracts. Additionally, the study examined the impact of sterilization on their inhibitory efficiency.

Materials and methods

Composts

Two commercial composts, C1 and C2, were used and obtained, respectively, from agricultural product sale sites in Al Jouf (29° 29’ 59.99" N, 39° 29’ 59.99" E) and Al-Kharj (23° 55’ 0.01" N, 47° 30’ 0.00" E) cities in Saudi Arabia. Compost C1 was prepared with a blend of algae, pine bark, perlite, and vermiculite, while compost C2 was composed of proper plant peat and organic fertilizer.

Physico-chemical and microbial characterization of composts and their extracts

pH and electrical conductivity (EC) were measured directly in the compost extract, and after diluting the compost samples in distilled water (1:5 w/v), followed by agitation at 200 rpm for 2 hours. Then, they were settled and filtered through a Wattman paper (0.45 µm). The supernatant was analyzed for pH and electrical conductivity (EC) using a pH meter (Metrohm 744) and a conductivity meter (WTW LF 330), respectively, as described by Kebede[4]. The water content and total solids (TS) were determined before and after the samples were dried overnight at 105 °C. Volatile solids (VS) were determined gravimetrically by incinerating the dried compost at 550 °C for 4 hours. The ash content was calculated as the difference between total and volatile solid contents[33]. The chemical oxygen demand (COD) was determined according to the Knechtel method[79]. The total (TN) and mineral (NH_4^+) nitrogen concentrations were determined by the Kjeldahl method[33]. The total organic carbon (TOC) was determined by dichromate oxidation and measured using the Shimadzu TOC-500 analyzer after acidic hydrolysis of the sample with concentrated HCl[33]. Metals and heavy metals were determined by atomic absorption spectrophotometry[34]. The phenolic content was determined using the Folin-Ciocalteu reagent with catechol as a standard[35].

Microbial enumeration was performed according to ISO 7218[36], with results expressed as Colony Forming Units (CFU). Successive decimal dilutions of all samples were prepared in physiological water. For quantification, 0.1 ml aliquots of appropriate dilutions were surface-plated onto Plate Count Agar (PCA, Pronadisa, Cona, Spain) for total mesophilic bacteria (TMB) and Potato Dextrose Agar (PDA, Pronadisa, Cona, Spain) for yeasts and molds. PCA plates were incubated at 37 ± 1 °C for 24 ± 2 hours, while PDA plates were incubated at 25 °C for 5 days. The experiment was conducted in triplicate. Microbial concentration was calculated using the following formula[36]:

$$N = \frac{\sum Ci}{(N1 + 0.1N2)\, dxV}$$

ΣCi: Sum of characteristic colonies counted on all retained Petri dishes.

N_1: Number of Petri dishes retained at the first dilution.

N_2: Number of Petri dishes retained at the second dilution.

d: Dilution rate corresponding to the first dilution.

V: Inoculated volume (ml).

N: Number of microorganisms (CFU/ml).

For granulometric determination, filtration through different sieves was achieved to evaluate the fractions of composts in clay (< 2 µm), silt (2–5 µm), and sand (50–2000 µm) particles[34].

To assess the phytotoxic risk of the compost, a germination test was carried out on tomato (*Solanum lycopersicum* L.) and watercress (*Nasturtium officinale*) seeds. These seeds were obtained from Larosa Emanuele Sementi, Italy, a company certified under the CE Standard Category, License C.C.I.A.A. di Bari no. 160671, dated April 29, 1974. The studied compost was diluted in distilled water (1:5 w/v), stirred at 200 rpm for 2 hours, and then allowed to settle. It was subsequently filtered through Whatman paper (0.45µm). The obtained extract was used for the seeds' germination index. Therefore, ten seeds of watercress or tomato were placed on a 9 cm diameter Petri dish containing filter paper moistened with 10 ml of compost extract. The Petri dishes were incubated at 25 °C for 7 days in the dark. The control was conducted in the same manner using distilled water.

The germination index (GI) was calculated according to the following equation[34]:

$$\mathrm{GI}\ (\%) = \frac{\text{Number of germinated seeds (compost) x total radicle length of germinated seeds (compost) (cm)}}{\text{Number of germinated seeds (control) x total radicle length of germinated seeds (control) (cm)}} \times 100$$

All analyses were conducted in triplicate, and the results are reported as mean values with their corresponding standard deviations (± SD). The physicochemical and microbial characterization of the two composts studied, C1 and C2, is presented in Table 1 and Figure 1.

Isolation and identification of plant-pathogenic fungi

Pathogenic fungi were isolated from diseased plant tissues: *S. racemosum* (F1) from *Ficus benjamina* leaves, *P. roridum* (F2) from *Cucumis sativus* leaves, *F. oxysporum* (F3) from *Solanum lycopersicum* fruit, and *P. italicum* (F4) from *Citrus sinensis* fruit. These samples were collected from cultivated plants on a privately owned farm located in the Al Jouf region (29°54'16.3"N 40°09'54.3" E) with prior consent from the property landowner. Collections were conducted with minimal environmental impact to ensure ethical compliance. To eliminate saprophyte microorganisms, plant surfaces were disinfected with a 5% bleach solution and then rinsed with sterile distilled water. Under sterile conditions, plant fragments were cut with a sterile scalpel and then transferred to the surface of Potato Dextrose Agar (PDA, Sigma-Aldrich, Germany) supplemented with 0.05 g/l of rose Bengal. The Petri dishes were incubated at 25 °C for 7 days.

Isolated fungi were purified on PDA medium and then identified using microscopic observation followed by molecular identification. Fungal genera and species were identified based on their colonial and microscopic structures, using the taxonomic keys of Domsch et al.[37]. The microscopic examination of fungal colonies included the growth pattern, color, texture, basal and surface mycelium, the reverse of the colonies, and the rate of colony growth and diameter were determined.

Identification of fungal isolates was carried out by extracting and amplifying genomic DNA using PCR, followed by sequencing analysis using the ITS1 region of the 18S rRNA gene. By using a mortar and pestle, a small portion of the fungal culture was ground in liquid nitrogen. Then, 100 mg of mycelium was used for genomic DNA extraction using the i-genomic Series DNA Extraction Mini Kit (iNtRON), which is specifically designed for extracting genomic DNA from fungal species, following the manufacturer's instructions. ITS gene amplification was performed using the ITS universal primer (Sigma-Aldrich, Germany), ITS1 (TCCGTAGGT GAACCTTGCGG), and ITS4 (TCCTCCGCTTATTGATGC), producing ~600 bp of amplicon products. PCR Master Mix (Promega ™) was used to amplify the ~600-bp region of the ITS region. A negative control (PCR mix without template DNA) has also been performed in all PCR experiments. A DNA thermal cycler (BIO-RAD S1000, USA) was used for DNA amplification. The PCR reaction conditions were 95 °C for 2 min (1 cycle), followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, and extension at 72 °C for 2 min, before a final extension at 72 °C for 15 min (1 cycle). PCR products were analyzed for the presence of specific fragments of the expected length in a 1% agarose gel electrophoresis stained with Ethidium bromide. The purified PCR products were subjected to sequencing using an ABI 3730xl DNA sequencer, with sequencing performed using both forward and reverse primers. Each sequence was subjected to an individual BLAST search to be verified in the Gene Bank. Sequences have been edited using MEGA (Molecular Evolutionary Genetics Analysis) software[38]. Phylogenetic Analyses of sequences were newly obtained from our isolates and were aligned with highly similar sequences from the Gene Bank using the MUSCLE Multiple Sequence Alignment Program. Sequences were aligned using ClustalX version 2.0 software[39], and phylogenetic analyses were performed using MEGA 4.0[40] for neighbor-joining and bootstrap analyses. 1,000 replications generated bootstrap values. The BLASTn similarity search program was used to identify homologous sequences in the NCBI nucleotide database, confirming the species-level similarity with the query sequence of the isolates. The percentage of replicate trees was shown, in which the related taxa were grouped in the bootstrap test alongside the branches. The phylogenetic tree is plotted at scale, with branch lengths in the same units as the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method[41] and expressed in units of base substitutions per site.

Compost C2	Compost C1	Parameter
Basic		
7.96 ± 0.03	6.44 ± 0.02	pH
1.990 ± 0.02	0.72 ± 0.01	EC (mS/cm)
75.25 ± 1.42	81.78 ± 1.23	TS (%)
39.58 ± 0.82	52.52 ± 0.32	VS (%)
35.67 ± 0.55	29.26 ± 0.45	Ash (%)
32.44 ± 0.48	36.74 ± 0.62	TOC (%)
2.93 ± 0.10	2.72 ± 0.08	Total nitrogen (%)
11.07	13.50	C/N
3.17 ± 0.13	4.30 ± 0.50	Phenolic compounds (g/kg)
4.131 ± 0.034	3.478 ± 0.044	CEC (mq/100g)
Minerals (ppm)		
584.40 ± 1.24	662.3 ± 2.35	Total phosphorus
11980 ± 10	22100 ± 20	Calcium
779.5 ± 1.5	1129 ± 16	Iron
1791 ± 3	1503 ± 12	Magnesium
2300 ± 10	600 ± 5	Sodium
2550 ± 20	43500 ± 20	Potassium
29.32 ± 1.14	16.92 ± 1.24	Zinc
9.11 ± 0.99	16.38 ± 0.88	Copper
8.43 ± 0.52	7.57 ± 0.52	Boron
5.35 ± 0.10	1.78 ± 0.08	Cobalt
35.18 ± 0.12	33.38 ± 032	Chromium
60.97 ± 0.58	46 ± 1	Manganese
14.73 ± 0.17	17.05 ± 0.55	Nickel
<0.008	36.02 ± 0.80	Plomb
<0.01	<0.01	Vanadium
Microbial analysis **(ufc/g)**		
$(4.6 \pm 0.3) \times 10^8$	$(3.2 \pm 0.1) \times 10^6$	TMB
$(3.0 \pm 0.1) \times 10^6$	$(2.8 \pm 0.2) \times 10^5$	Yeasts
$(2.3 \pm 0.2) \times 10^5$	$(1.3 \pm 0.1) \times 10^6$	Molds
GI (%)		
81.35 ± 0.35	81.58 ± 0.42	Tomato
73.25 ± 0.15	77.57 ± 0.23	Watercress

Table 1. Physical-chemical and microbial characteristics of the two studied composts, C1 and C2. EC, electrical conductivity; TS, total solids; VS, volatile solids; TOC, total organic carbon; CEC, cation exchange capacity; C/N, ratio of carbon to nitrogen; GI, germination index.

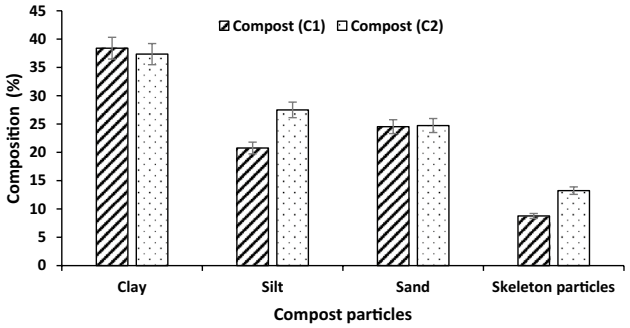

Fig. 1. Granulometric composition (%) of the studied composts C1 and C2 by particle size.

Phytopathogenicity test of isolated fungi

To evaluate the phytopathogenicity effects of the isolated fungi, the method described by Ewekeye et al.[42] was used with slight modifications. Ripe and healthy tomato fruits were sterilized with 5% sodium hypochlorite solution for 5 min and then washed with sterile distilled water. A sterile cone was carefully punctured on one side of the epidermal layer of tomato tissue. A spore solution containing a concentration of 10^6 spores/ml of the tested fungi was then applied to the fruit cavity. The fruit samples were incubated at 25 ℃ for 7 days in sterile plastic vials. Disease symptoms on the fruits were evaluated visually by comparison with a negative control (healthy, untreated fruit). The symptom appearance time was recorded as the point at which visible signs of disease first appeared. At the end of the incubation period, the diameter of the lesion was measured in centimeters. Additionally, the initial and final weights of the tomato fruits were recorded to assess changes over the incubation period. The experiment was conducted in triplicate, and the data were expressed as mean ± standard deviation.

Compost extract preparation

The compost extracts were prepared through a controlled compost fermentation process. The experimental design optimized fermentation conditions using four factors at two levels (Table 2): Temperature (25 °C and 44 °C), compost concentration (1/5 and 1/8 m/v), glucose concentration (0 and 1 g/l), and fermentation time (3 and 7 days). Fermentation occurred in 1 L sealed plastic containers without oxygenation, with daily manual homogenization. Extracts were obtained by decanting the samples, filtering them through gauze, and then storing them in 50 ml sterile tubes at 4 °C. To determine the optimal levels of the four studied variables, an experimental design was used to analyze the inhibition percentage of phytopathogenic fungi. All experiments were conducted in triplicate, and the data were analyzed statistically using SPSS software (Version 24). The assumptions for the statistical model, specifically normality and homogeneity of variances, were verified before analysis. A Pearson correlation coefficient was calculated to assess the linear relationship between the compost extract parameters and their antifungal effects. A p-value of less than 0.05 was considered statistically significant.

Antifungal activity of compost extracts

The study of the antifungal activity of compost extracts against isolated phytopathogenic fungi was conducted using the diffusion method on agar. Specifically, the compost extracts were mixed with PDA medium at dilutions of 1/2, 1/4, and 1/8. After the agar solidified, a 6 mm disc of the studied fungus was placed in the center of each Petri dish. PDA medium without compost extract served as a control medium. Then, the Petri dishes were incubated at 25 °C until the fungal mycelial filaments reached the edge of the control dish. The assessment of compost extract inhibition of phytopathogenic fungi was estimated by calculating the inhibition percentage of fungal mycelial growth in both control and dishes containing different dilutions of compost extracts, using the following equation[43]:

$$\text{Inhibition percentage } (\%) = \frac{(D - d)}{D} \text{ x } 100$$

Where D is the average diameter (cm) of the fungal mycelium growth on the control medium, and d is the average diameter (cm) of the fungal mycelium growth on PDA with diluted compost extract.

All the experiments were conducted in triplicate.

Test	Temperature (°C)	Compost concentration (m/v)	Glucose concentration (g/l)	Fermentation time (days)
1	25	1/8	0	3
2	44	1/8	0	7
3	25	1/5	0	7
4	44	1/5	0	3
5	25	1/8	1	7
6	44	1/8	1	3
7	25	1/5	1	3
8	44	1/5	1	7
9	25	1/8	1	3
10	44	1/8	1	7
11	25	1/8	0	7
12	44	1/8	0	3
13	25	1/5	1	7
14	44	1/5	1	3
15	25	1/5	0	3
16	44	1/5	0	7

Table 2. Full factorial design of 4 parameters at 2 levels.

Sterilization effect of compost extracts against phytopathogenic fungi

To determine the effect of sterilizing the compost extracts on phytopathogenic fungi, five extracts from each compost that showed a high inhibition percentage were sterilized using autoclaving at 121 °C for 20 minutes and then filtered through a 0.22 µm membrane.

Statistical analysis

Quantitative variables were analyzed by calculating the mean and standard deviation (mean ± SD), the 95% confidence interval (CI95%), the median, and the interquartile range (minimum–maximum). The Shapiro–Wilk test was used to evaluate the normality of data distribution. Parametric tests, such as the independent t-test and analysis of variance (ANOVA), were performed to identify significant associations among the physicochemical and microbial parameters of composts and their extracts across the selected studies. Pearson's correlation test examined relationships between the physicochemical and microbial characteristics of compost extracts and their antifungal activity against phytopathogenic fungi (n = 48). Only statistically significant results are reported. A factorial experimental design was employed to evaluate the impact of fermentation parameters on antifungal activity during the preparation of compost extracts. The level of statistical significance was set at 5%. All analyses were conducted using SPSS software (version 24).

Results and discussion

Physico-chemical characterization of the studied composts

The physicochemical characterization of composts, including pH, EC, organic matter, ammonium, C/N ratio, and mineral and heavy metal concentrations (Table 1), was used to assess compost stability and maturity[44].

The compost C1 exhibited a relatively acidic pH and low EC compared to C2, which showed a more alkaline pH and higher EC (Table 1). According to the British Standards PAS 100[45] and the Gulf Cooperation Council Standards[46], the pH range for composts used in agriculture typically ranges from 6 to 8.5. However, the maximum allowable EC is 4 dS/m according to PAS-100 and 10 dS/m per GCST[46]. Both composts studied met the standards of PAS-100 and GCST. Al-Turki et al.[47] noted that soil in Saudi Arabia tends to become arid with an alkaline pH and recommended against applying composts with a pH higher than 7.5. Additionally, Omar et al.[48] reported that high EC levels can increase soil salinity, which negatively impacts plant growth.

The two composts showed high levels of organic matter, including total and volatile solids, TOC, and nitrogen. Additionally, C1 had a higher concentration of phenolic compounds compared to C2. The minimum organic matter percentage recommended by PAS-100[45] is 25%, and by GCST[46], it is 40%. C1 and C2 needed high organic matter, with values of 52% and 40%, respectively. Brown and Cotton[49] stated that organic matter helps maintain soil structure, water retention capacity, and nutrient availability. Recently, Rossi et al.[50] noted that high organic and nitrogen content enhances mineral immobilization in soil and improves fertilizer nitrogen availability. The C/N ratios were 13.5 and 11.07 for C1 and C2, respectively, indicating that both composts are rich in nitrogen matter (especially algae, peat, and organic fertilizers). The C/N ratio is an important indicator of the decomposition rate of organic materials during compost production[51]. According to GCST[46] and PAS-100[45], the maximum acceptable C/N ratio for final compost products is 20.

Its richness in macro and micro-elements distinguished the two composts. Indeed, the C1 exhibited the highest levels of phosphorus, calcium, iron, potassium, and copper. However, C2 was relatively higher in magnesium, sodium, and zinc. The two composts showed a relatively high nitrogen content (2.72% for C1 and 2.93% for C2) (Table 1).

Potassium, phosphorus, and nitrogen are among the most essential elements for microbial growth during the composting process. Additionally, their positive effect on plant growth is mainly due to the increased availability of nutrients from compost application[25]. Dang et al.[52] noted that applying compost at a rate of 5 kg/ha improves the soil's cation exchange capacity (CEC), boosts the availability of calcium and magnesium, and promotes soil fertilization. Potassium (K) is vital for crop productivity and quality, playing a crucial role in metabolism, enzyme activation, nutrient transport, photosynthesis, and stress resistance[53]. Zinc is also essential for plant growth, aiding cell multiplication, biochemical processes, nutrient uptake, photosynthesis, and overall productivity[54,55].

Recent studies have shown that nitrogen buildup during composting is often linked to the breakdown of carbon-rich materials, resulting in carbon dioxide (CO_2) emissions and a subsequent decrease in carbon content. This process increases the relative concentration of nitrogen in the compost. Materials such as algae and peat moss, which are the primary components of compost types C1 and C2, respectively, naturally contain high nitrogen content, thereby increasing nitrogen levels in the final compost product. These dynamics are essential for optimizing compost quality and improving nutrient availability for plant growth[56,26].

Furthermore, the heavy metals varied between the two composts, C1 and C2. The permissible limits set by GCST[46] and PAS-100[45] are 350 ppm for Zn, 150 ppm for Cu, 25 ppm for Ni, 120 ppm for Pb and Cr, and three ppm for Cd. The heavy metal levels, shown in Table 1 for C1 and C2, stay within these limits, confirming that both composts are non-toxic. Some heavy metals, such as zinc (Zn), iron (Fe), copper (Cu), nickel (Ni), and manganese (Mn), are essential micronutrients for microorganisms and plants, crucial for supporting microbial biomass and activity, and aiding in nutrient cycling[57]. However, when their concentrations go above safe levels, they can become toxic to soil organisms and plants. Additionally, the microbial profiles of composts C1 and C2 were examined to measure mesophilic bacteria, yeasts, and molds (Table 1). Compost C2 showed a higher presence of bacteria and more yeast activity than C1, while molds were more prevalent in C1 than in C2. These differences highlight distinct microbial communities, likely influenced by variations in environmental conditions or processing methods.

Both composts exhibited a high seed germination index (almost 81% and 75% of tomato and watercress seeds, respectively), indicating their maturity and confirming their suitability for seed germination without any signs of phytotoxicity[58].

The results for the two studied composts, as shown in Figure 1, indicate the percentage of each fraction in the clay (< 2 µm), silt (2–50 µm), sand (50–2000 µm), and skeleton particle (> 2000 µm) size ranges.

The fine particles (clay) were the most abundant fraction in both composts, indicating their high-quality texture and suitability for soil amendment[59]. Compost C2 showed a slight increase in silt and skeleton particles compared to C1, while both composts exhibited similar sand fractions. The tiniest particle size fraction was found in the skeleton particles. No significant correlation was observed between the physicochemical and microbial parameters of the two studied composts ($p > 0.05$).

The beneficial effects of compost on soil properties and crop productivity are closely linked to its chemical, physical, and biological characteristics. Recent studies have shown that fine particle fractions generally have lower electrical conductivity (EC) and carbon-to-nitrogen (C/N) ratios but are enriched with essential nutrients, thereby improving soil fertility[2]. The silt fraction tends to accumulate heavy metals but also contributes to better compost stability and structure[60]. Although larger particle sizes increase soil aeration and reduce water retention, the fine particles in organic amendments help boost soil organic carbon and enhance soil aggregation, leading to improved soil structure and function[49].

Isolation and identification of phytopathogenic fungi

Four fungi (F1, F2, F3, and F4) were isolated from various diseased plants and fruit organs, then identified both morphologically and molecularly.

On PDA medium, the colonies of F1 grew rapidly (within 2 days), displayed a cottony appearance, and ranged in color from white to grey, turning dark grey as sporangia developed (Figure 2). Under the microscope, F1 was characterized by forming a terminal sporangiophore and exhibited the same morphological structure as *Syncephalastrum* sp. In contrast, the fungal F2 grew slowly on PDA medium, taking up to 7 days at 25 °C. Its colony initially appeared as white, fluffy mycelia and later developed concentric, olivaceous to grey-black sporodochia. These structures are composed of numerous phialides that produce cylindrical, hyaline to slightly olive-green conidia at their tips (Figure 2). All characteristics aligned with the description of *Paramyrotheciumsp*[61].. On PDA, the fungal F3 exhibited thin, aerial, and relatively dense colonies that were pinkish-purple. Microscopic examination of the isolate revealed septate hyphae that were branched and hyaline, along with microconidia and ellipsoid macroconidia. These characteristics were consistent with the morphology of *Fusarium* sp. Fungal colonies of F4 developed rapidly on PDA media, exhibiting a velvety texture, and had white, young mycelia that turned green-blue with maturation, along with an uncolored reverse. Under the microscope, the fungus appears similar to Penicillium spp. It presented septate hyaline hyphae with branched conidiophores. Cylindrical phialiads produced long chains of small oval conidia. (Figure 2)

Molecular identification of the isolated fungi was assessed using sequencing of the ITS rRNA genes, after submitting the nucleotide sequence to the European Nucleotide Archive, the isolated fungi were assigned accession numbers MZ057792 for *Syncephalastrum racemosum* (F1), MZ057761 for *Paramyrothecium roridum* (F2), OL913805 for *Fusarium oxysporum* (F3), and MZ057729 for *Penicillium italicum* (F4).

Phylogenetic analyses of sequences from our isolates were aligned with highly similar sequences from GenBank. The distance matrix results showed that *S. racemosum* isolates are monophyletic, with an average distance of 0.411 and 99.47% identity. Similarly, *P. roridum* isolates demonstrated a monophyletic relationship with an average distance of 0.560 and 99.83% identity. *P. italicum* isolates also formed a monophyletic group, with an average distance of 0.695 and 99.60% identity. *F. oxysporum* isolates had the closest relationship, showing an average distance of 0.231 and 100% identity, further supporting their monophyly.

The neighbor-joining phylogenetic tree (Figure 3), constructed using evolutionary distances, confirmed these results. The ITS rRNA gene sequence alignment showed that isolates within each species are closely related (monophyletic), while relationships between different species tend to be polyphyletic. Notably, *S. racemosum*, *P. italicum*, and *F. oxysporum* isolates cluster tightly within their species. In contrast, some *P. roridum* isolates exhibit polyphyletic relationships within their species but are monophyletic with *S. racemosum* (Figure 3).

Phytopathogenicity test of the studied fungi

The phytopathogenicity test on tomato fruits, detailed in Table 3, showed distinct virulence patterns among four fungal isolates: *S. racemosum*, *P. roridum*, *F. oxysporum*, and *P. italicum*. All isolates could cause post-harvest decay under controlled conditions. *S. racemosum* was the most virulent, causing rapid symptoms within 1 day, with extensive soft rot, tissue breakdown, yellowish discoloration, and liquid exudate. It also resulted in the highest weight loss (30.11%) and the largest lesion diameter (4.6 cm). *F. oxysporum* demonstrated aggressive growth, with white fluffy mycelium covering much of the fruit, along with a 22.05% weight loss, 3.5 cm lesions, and symptoms appearing within 1 day, likely due to Fusarium toxin-induced necrosis[62]. *P. italicum* caused moderate decay, with white mold and puncture symptoms around inoculation sites, leading to 15.33% weight loss, 1.2 cm lesions, and a 2-day onset. *P. roridum* was the least virulent, with symptoms appearing after 3 days, minimal 11.64% weight loss, and small 1.5 cm rot spots with sparse mycelial growth. The susceptibility of these fruits to fungal attack can be linked to the water present in their deeper layers. According to Estrada-Bahena et al.[63], the fruit's organic matrix undergoes dynamic physicochemical changes that are heavily influenced by water availability, its distribution in the tissue, and its mobility. Water activity (aw) is vital for microbial activity and enzyme reactions,fungal growth typically occurs when aw exceeds 0.90. Enzymes like amylases, phenoloxidases, and peroxidases become active at aw levels above 0.85, while lipases can still function even at aw below 0.3.

Effect of non-sterile compost extracts on phytopathogenic fungi

The compost extracts were prepared using a fermentation process with variations in parameters: Temperature (25 and 44 ℃), glucose concentration (0 and 1 g/l), compost concentration (1/5 and 1/8 w/v), and fermentation time (3 and 7 days). Sixteen extracts were obtained for each compost (Table 2). All the extracts were tested for

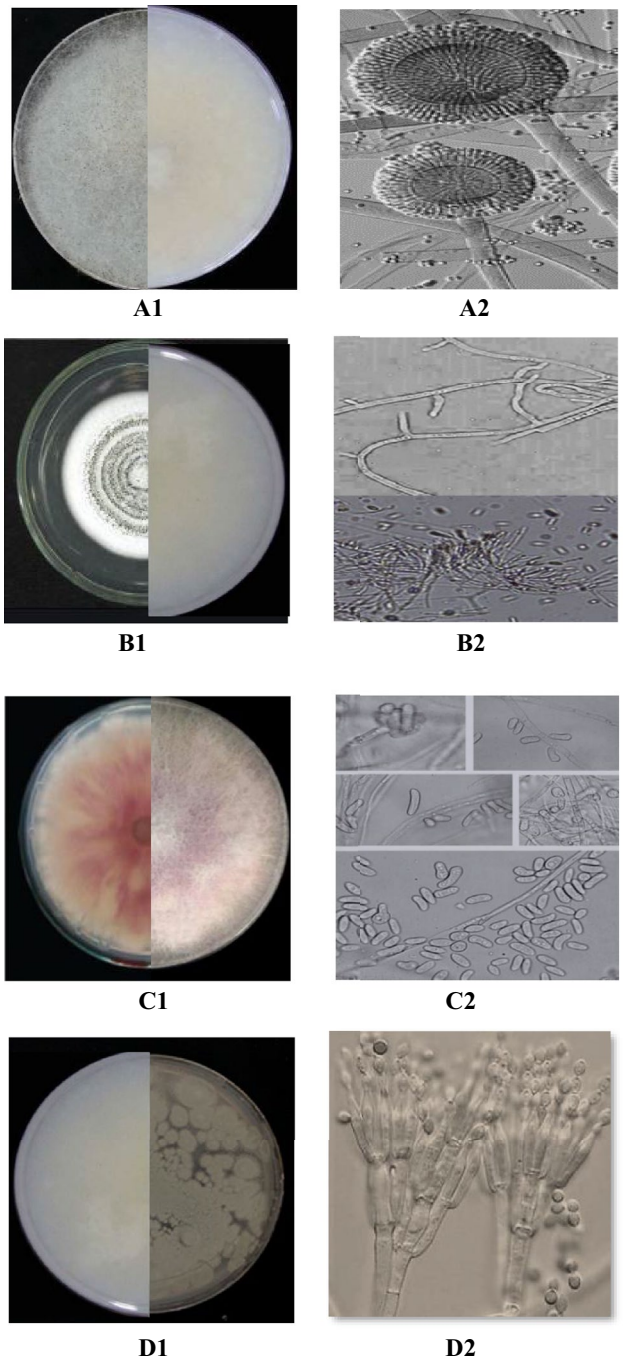

Fig. 2. Mycelial growth (surface and reverse) of F1 (A1), F2 (B1), F3 (C1), and F4 (D1) on PDA at 25 °C for 5 days and its microscopic observation (A2, B2, C2, and D2), respectively.

their suppressive effect against the four identified fungi: *S. racemosum* (F1); *P. roridum* (F2); *F. oxysporum* (F3), and *P. italicum* (F4).

The results were presented as the inhibition percentage (%) of mycelial growth of tested fungi by different fermented compost extracts, which were diluted at 1/2, 1/4, and 1/8 in Table 4 (C1) and Table 5 (C2). Additionally, the results showed that most of the extracts exhibited notable antifungal activity against the four isolated strains, with some fluctuations observed between the different tests. The extracts obtained by fermentation of C2 (Table 5) have a greater inhibitory activity against fungi than those obtained by fermentation of C1 (Table 4). This could be related to the raw material used for compost preparation, the organic compounds, and the diversity of microbial flora of the two composts[25]. Therefore, the most inhibited fungi were *P. roridum* > *F. oxysporum* > *P. italicum* > *S. racemosum*. *S. racemosum* exhibited high resistance to the majority of the extracts. At the same time, it showed complete inhibition (100%) with the extract prepared in test n°6, which was diluted at 1/2 and

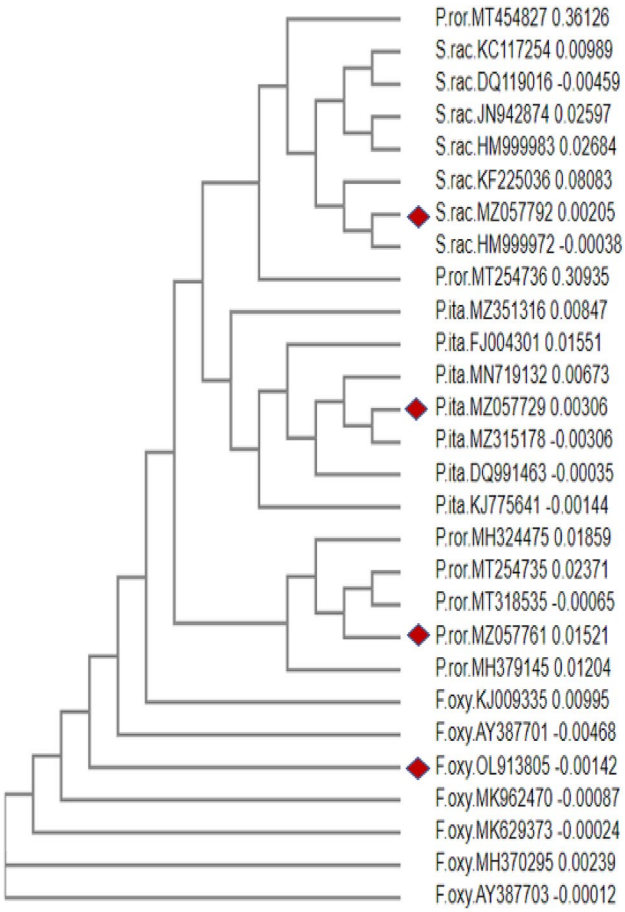

Fig. 3. Phylogenetic analysis of the ITS region derived from the Neighbour-Joining method using MEGA 4.0 software. (Diamond shapes represent our isolates, and the remaining are reference sequences.).

Pathogenic parameter	*S. racemosum*	*P. roridum*	*F. oxysporum*	*P. italicum*
Initial weight (g)	89.40 ± 2.35	81.50 ± 1.35	78.56 ± 2.22	88.05 ± 3.12
Final weight (g)	62.48 ± 2.41	72.34 ± 3.21	61.24 ± 2.50	74.55 ± 2.36
Weight loss (%)	30.11%	11.64%	22.05%	15.33%
Symptom appearance time (days)	1	3	1	2
Lesion diameter (cm)	4.62 ± 0.30	1.53 ± 0.21	3.56 ± 0.34	1.24 ± 0.12
Visible symptoms	significant soft rot, leading to tissue breakdown	localized rot with mycelial growth	fruit rot and aggressive colonization	puncture-associated decay

Table 3. Phytopathogenicity test of studied fungi: *S. racemosum*, *P. roridum*, *F. oxysporum*, and *P. italicum* on tomato fruit incubated for 7 days at 25 ℃.

1/4. This was also observed in test n°1, where the extract was diluted to 1/2 for compost C1, and in test n°13 for compost C2.

The extracts of compost C1 completely inhibited the fungus *P. roridum* at a 1/2 dilution for tests 1 and 5 and at a 1/8 dilution for test 2. However, the extracts of compost C2, at a 1/2 dilution, completely inhibited the mycelial growth of *P. roridum* in tests 1, 2, 4, 6, 9, 10, 11, 13, and 15; *F. oxysporum* in tests 6 and 9; and *P. italicum* in tests 1, 4, 6, 7, and 11. At a 1/4 dilution, the C2 extract achieved 100% inhibition of *P. roridum* in tests 6 and 9, *F. oxysporum* in test 9, and *P. italicum* in tests 6 and 9. At a 1/8 dilution, only the C2 extract from test 9 completely inhibited the growth of *P. roridum* and *F. oxysporum*.

The results also showed a concentration-dependent antifungal effect of compost extracts C1 and C2. Typically, the highest antifungal activity is seen at the least diluted concentration (1/2), with inhibition percentages decreasing as the extracts become more diluted (1/4 and 1/8). For example, non-sterile C1 extract at 1/2 dilution often achieves 100% inhibition against *S. racemosum* (F1) and *P. roridum* (F2), but this drops significantly at 1/4 and even more at 1/8. Similar patterns are observed for extract C2, where a 1/2 dilution generally results in

Compost extract preparation	Extract dilutions											
	1/2				1/4				1/8			
	Fungal inhibition percentage (%)											
Test	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
1	100.00	100.00	68.08±0.12	56.12±0.08	79.59±0.02	74.28±0.12	62.76±0.04	66.32±0.04	79.59±0.04	74.28±0.02	73.40±0.05	65.30±0.04
2	26.53±0.05	72.09±0.05	65.30±0.04	2.04±0.01	6.38±0.01	100.00	45.91±0.02	2.04±0.01	0.00	100.00	48.97±0.04	32.65±0.05
3	22.44±0.02	62.79±0.04	62.24±0.11	13.26±0.01	9.57±0.01	62.5±0.08	36.73±0.03	11.22±0.02	2.127±0.01	57.14±0.04	18.36±0.05	44.89±0.02
4	85.71±0.06	72.85±0.04	62.76±0.12	48.97±0.05	95.91±0.02	77.14±0.07	74.46±0.05	66.32±0.05	69.38±0.05	68.57±0.08	79.59±0.02	54.08±0.04
5	7.142±0.02	100.00	38.77±0.08	25.51±0.05	25.53±0.02	18.25±0.02	46.93±0.02	19.38±0.03	21.27±0.02	100.00	50.00±0.03	55.10±0.03
6	100.00	97.19±0.05	80.85±0.07	95.19±0.06	100.00	74.28±0.03	69.14±0.04	55.10±0.06	85.71±0.03	97.14±0.04	68.08±0.04	95.91±0.04
7	65.00±0.04	53.00±0.03	62.10±0.06	30.23±0.01	55.16±0.02	43.442±0.04	70.74±0.05	74.48±0.08	69.95±0.04	33.14±0.03	43.48±0.04	69.95±0.04
8	19.38±0.03	97.67±0.04	47.95±0.05	20.40±0.04	19.14±0.02	56.25±0.05	12.24±0.01	14.28±0.04	18.08±0.02	55.10±0.02	8.163±0.01	25.51±0.02
9	38.77±0.04	70.00±0.04	65.30±0.06	82.65±0.05	0.00	68.33±0.02	61.22±0.02	65.30±0.06	0.00	22.22±0.02	24.48±0.01	25.55±0.02
10	0.00	27.27±0.03	62.85±0.07	91.11±0.07	0.00	51.51±0.02	38.57±0.04	11.11±0.01	0.00	0.00	0.00	0.00
11	0.00	23.25±0.02	14.65±0.02	11.54±0.02	0.00	0.00	0.00	0.00	12.35±0.02	44.25±0.04	30.25±0.02	24.02±0.02
12	48.97±0.02	66.67±0.04	54.08±0.03	79.59±0.04	0.00	40.00±0.05	36.26±0.04	44.89±0.04	8.00±0.01	21.11±0.01	31.63±0.02	16.66±0.01
13	0.00	54.23±0.04	24.03±0.01	13.25±0.02	0.00	0.00	0.00	0.00	0.00	36.88±0.02	27.25±0.03	4.25±0.01
14	69.3±0.10	90.00±0.05	43.08±0.02	82.65±0.12	0.00	51.67±0.04	59.18±0.02	0.00	10.55±0.02	20.00±0.03	23.46±0.02	27.78±0.02
15	4.08±0.02	0.00	48.97±0.08	38.77±0.08	0.00	35.00±0.02	38.77±0.04	0.00	0.00	21.11±0.04	24.48±0.02	1.67±0.01
16	0.00	26.55±0.04	14.25±0.05	0.00	0.00	0.00	0.00	0.00	32.14±0.03	40.25±0.02	15.80±0.01	10.84±0.02

Table 4. The Suppressive effect of compost extracts (C1) used at different dilutions (1/2, 1/4, and 1/8) on phytopathogenic fungi is expressed as the inhibition percentage (%) of fungal mycelium growth. F1: *S. racemosum*; F2: *P. roridum*; F3: *F. oxysporum,* and F4: *P. italicum.*

Compost extract	Compost extract dilutions											
	1/2				1/4				1/8			
	Fungal inhibition percentage (%)											
Test	F1	F2	F3	F4	F1	F2	F3	F4	F1	F2	F3	F4
1	54.08±0.06	100.00	82.97±0.04	100.00	0.00	85.71±0.04	80.85±0.06	64.28±0.06	0.00	85.71±0.04	80.85±0.06	74.48±0.04
2	0.00	100.00	93.87±0.05	81.63±0.04	0.00	50.76±0.05	2.04±0.01	2.04±0.01	14.28±0.01	81.53±0.04	61.22±0.04	75.51±0.04
3	0.00	0.00	93.87±0.06	30.61±0.06	8.16±0.01	38.46±0.02	0.00	18.36±0.01	10.20±0.02	78.46±0.06	71.42±0.04	82.65±0.06
4	54.08±0.06	100.00	97.87±0.06	100.00	0.00	0.00	79.78±0.03	0.00	0.00	0.00	68.08±0.02	54.08±0.04
5	0.00	59.55±0.02	69.38±0.06	76.53±0.04	0.00	24.61±0.02	2.04±0.01	2.04±0.01	8.16±0.02	84.61±0.06	69.38±0.02	85.71±0.04
6	81.63±0.05	100.00	100.00	100.00	59.18±0.04	100.00	78.72±0.06	100.00	28.57±0.03	0.00	62.67±0.04	79.59±0.05
7	69.38±0.04	45.71±0.02	89.36±0.04	100.00	0.00	91.42±0.04	86.17±0.06	79.59±0.04	38.77±0.01	94.28±0.06	78.72±0.04	95.91±0.05
8	0.00	86.51±0.04	93.87±0.04	87.75±0.04	2.040±0.01	66.15±0.02	2.04±0.01	2.04±0.01	59.18±0.05	60.00±0.02	91.83±0.06	93.87±0.06
9	69.38±0.06	100.00	100.00	91.83±0.06	65.30±0.04	100.00	100.00	100.00	0.00	100.00	100.00	95.91±0.06
10	0.00	100.00	78.72±0.04	18.36±0.03	0.00	0.00	43.33±0.02	43.87±0.02	0.00	21.67±0.01	25.00±0.05	33.67±0.04
11	75.51±0.04	100.00	91.48±0.04	100.00	38.77±0.04	33.33±0.02	0.00	70.40±0.04	0.00	41.67±0.01	86.67±0.05	51.02±0.02
12	71.42±0.04	84.00±0.03	71.42±0.06	93.87±0.04	18.36±0.02	52.00±0.04	42.85±0.04	48.97±0.03	0.00	40.00±0.01	28.57±0.02	28.57±0.02
13	100.00	100.00	78.72±0.04	59.18±0.02	91.83±0.06	83.33±0.04	83.33±0.06	89.97±0.05	0.00	38.33±0.01	83.33±0.04	89.79±0.04
14	36.73±0.04	60.00±0.04	61.22±0.04	89.79±0.06	44.27±0.04	24.00±0.01	85.71±0.06	84.69±0.06	0.00	20.00±0.01	42.85±0.02	0.00
15	79.59±0.05	100.00	79.59±0.04	97.95±0.04	0.00	0.00	0.00	0.00	0.00	50.00±0.03	27.14±0.04	39.79±0.02
16	0	42.22±0.02	33.26±0.03	40.88±0.02	0.00	0.00	0.00	0.00	0.00	35.77±0.02	36.24±0.02	12.44±0.01

Table 5. Suppressive effect of compost extracts (C2) used at different dilutions (1/2, 1/4, and 1/8) on phytopathogenic fungi expressed as inhibition percentage (%) of fungal mycelium growth. F1: *S. racemosum*; F2: *P. roridum*; F3: *F. oxysporum,* and F4: *P. italicum*.

Parameter	Compost extract (C1)					Compost extract (C2)				
	1	6	9	12	14	9	10	11	13	15
pH	7.36±0.02	6.93±0.03	7.05±0.01	7.21±0.02	7.65±0.04	8.25±0.03	7.39±0.02	8.03±0.01	7.36±0.03	8.06±0.02
EC (mS/cm)	0.10± 0.03	0.22 ± 0.01	0.16 ± 0.01	0.12± 0.02	0.26±0.02	0.45±	0.36±0.04	0.25±0.01	0.62±0.02	0.57±0.01
COD (mg/l)	2568±2	9688±3	5507±3	3538±2	10235±4	2759±1	3064±2	1786±4	4526±1	3544±2
TOC (mg/l)	352±2	299±1	305±2	352±1	492±3	298±2	324±1	341±2	528±2	458±1
TNK (mg/l)	3.68±0.10	5.69 ±0.20	2.51±0.12	2.80 ±0.14	2.7 ±0.10	4.85±0.20	2.55±0.30	5.02±0.24	4.26±0.12	4.28±0.14
NH_4^+ (g/l)	3.05±0.03	4.25±0.05	2.45±0.04	2.11±0.05	2.35±0.04	4.12±0.03	1.75±0.02	4.19 ±0.04	3.56±0.02	3.85±0.05
Phenolic compounds (g/l)	1.45±0.03	2.56±0.04	0.89±0.05	0.57±0.02	1.35±0.01	1.52±0.02	0.85±0.03	1.78±0.01	1.56±0.02	1.47±0.04
TMB (log_{10} ufc/ml)	7.40±0.01	10.86±0.01	7.28±0.01	6.51±0.01	7.32±0.02	9.72±0.03	4.86±0.02	10.38±0.01	7.60±0.01	9.80±0.03
Yeast (log_{10} ufc/ml)	5.48±0.02	7.40±0.02	5.04±0.01	5.6±0.02	5.33±0.03	6.63±0.01	5.30±0.02	7.50±0.02	5.66±0.02	5.91±0.02
Molds (log_{10} ufc/ml)	4.36±0.01	6.40±0.02	4.15±0.02	4.30±0.01	4.31±0.01	6.31±0.02	4.00±0.01	6.30±0.01	4.48±0.01	5.78±0.02
GI (%)	120.32±2.21	89.25±1.14	95.47±2.24	109.11±1.01	87.58±1.02	112.22±3.52	103.24±1.03	135.25±2.02	92.47±1.01	96.58±2.00

Table 6. Physicochemical and microbial characterization of the main compost extracts C1 and C2 diluted to 1/2. EC: electrical conductivity; COD: chemical oxygen demand; TNK: total nitrogen Kjelgahl; TMB: total mesophilic bacteria; GI: germination index determined on tomato seeds.

better inhibition than a 1/4 or 1/8 dilution across different fungal strains. This suggests that the active antifungal compounds are most effective when less diluted, requiring higher concentrations to maximize fungicidal or fungistatic effects. Notably, some fungi, such as *P. italicum* (F4), can still be substantially inhibited even at higher dilutions, indicating different sensitivities among the fungi to these active components.

Physicochemical and microbial analyses of compost extracts

Therefore, physicochemical and microbial analyses were conducted on five samples from each compost extract, C1 (1, 6, 9, 12, and 14) and C2 (9, 10, 11, 13, and 15), both diluted to 1/2. These samples showed the highest antifungal effect (Table 6). The initial composition of the compost serves as a foundational blueprint that significantly influences the final characteristics of its extract. This is evident when comparing composts C1 and C2, and their corresponding extracts. Compost C2, which started with a higher pH, greater mineral content, and larger microbial populations, consistently produced an extract that maintained these elevated characteristics throughout the fermentation process.

Indeed, the C2 extract generally showed a higher pH, greater electrical conductivity (EC) ranging from 0.25 to 0.62 mS/cm, and more microbial counts (TMB, yeast, and molds) compared to the C1 extract, whose EC remained lower (0.10–0.26 mS/cm). However, contrary to expectations, the C1 extract had higher levels

of organic matter, indicated by its generally higher COD values. In contrast, the C2 extracts contained greater amounts of TNK and NH_4^+, suggesting more advanced nitrogen mineralization. The microbial analysis revealed distinct population dynamics: Total mesophilic bacteria were the most abundant and variable group (ranging from 4.86 to 10.38 log ufc/ml), followed by yeasts (5.02 to 7.50 log ufc/ml), while molds were the least numerous but consistently present (4.00 to 6.40 log ufc/ml). These differences highlight how the starting material influences the unique chemical and biological trajectory of the extract. Moreover, fermentation appears to be a dynamic process, causing fluctuations in parameters like pH, EC, and microbial populations over time for both extracts, indicating that the fermentation duration is a key factor in determining the final chemical and microbial properties of the compost extract. The germination index (GI) of the compost extracts varies across samples, ranging from 87.58±1.02% to 135.25±2.02%, reflecting their diverse effectiveness in supporting seed germination, while also demonstrating their dual role in suppressing phytopathogenic fungi and significantly improving agricultural productivity through enhanced soil health, promoted plant growth, and sustainable farming practices.

The bivariate correlation between physicochemical and microbial compost extract parameters and their antifungal activity against phytopathogenic fungi was assessed using the Pearson test and is presented in Table 7. The correlation analysis of compost extracts revealed significant relationships between physicochemical parameters and microbial factors with antifungal activity against the studied fungi: *S. racemosum*, *P. roridum*, *F. oxysporum*, and *P. italicum*. This suggests that the antifungal potential arises from the combined effects of nutrient availability and diverse microbial communities, rather than solely from organic matter content. Notably, Nitrogen-related parameters (NTK and NH4+) and microbial abundance (TMB, yeasts, and molds) showed strong positive correlations with each other and with activity against all studied fungi (Table 7). This highlights the role of nitrogen availability in promoting antagonistic microbial populations, as supported by research showing that nitrogen dynamics sustain bacterial growth in manure composting systems, enabling them to outcompete fungi[64]. Phenolic compounds, as bioactive elements, showed a strongly positive and significant correlation with the antifungal effects of *S. racemosum* (r = 0.868*)* and *P. italicum* (r = 0.660), as well as a moderate positive correlation with the antifungal effect of *P. roridum* (r = 0.416), highlighting their direct inhibitory mechanisms. Findings also suggest that phenolic compounds, such as coumaric, caffeic, and ferulic acids, in compost extracts play a key role in controlling pathogenic fungi through antimicrobial actions[65]. pH and EC showed moderate positive correlations with antifungal efficacy against *P. roridum* (r = 0.468 and r = 0.540, respectively) and *F. oxysporum* (r = 0.472 and r = 0.467), suggesting that slightly alkaline conditions may enhance pathogen suppression by promoting bacterial growth over fungi[66]. Meanwhile, COD and TOC displayed mixed but often significantly negative correlations with *F. oxysporum*.

In the same context, several studies have shown that the antifungal efficacy of compost extracts is due to their diverse bioactive compounds, including phenolics, organic acids, enzymes, humic and fulvic acids, volatile fatty acids, and plant growth regulators, along with active microbial communities[1,67,68]. These components operate through multiple mechanisms, including nutrient and space competition, antibiosis, parasitism, and the induction of systemic resistance in host plants[1,69]. Key microbial genera, including *Bacillus*, *Pseudomonas*, *Trichoderma*, *Penicillium*, and *Streptomyces*, produce a wide spectrum of antifungal metabolites such as siderophores, lipopeptides (iturin, fengycin, surfactin), chitinases, proteases, and antibiotics[16–18,70–72]. Both aerated (ACTs) and non-aerated compost teas (NCTs) have been shown to suppress a broad range of pathogens, including *Fusarium oxysporum*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Alternaria alternata*, *Botrytis cinerea*, *Aspergillus niger, Rhizoctonia solani, Rhizoctonia bataticola, Pythium* spp., and *Verticillium dahliae* [73,28,51,74].

Optimal fermentation conditions of compost extracts

To determine the optimal levels of four variables (temperature, compost concentration, glucose concentration, and fermentation time) that exhibited high suppressive activity of compost extract against the studied fungi, an experimental design analysis was employed to enhance the inhibition percentage of phytopathogenic fungi. The data in Table 8 revealed a significant difference in antifungal efficacy between the two compost extracts. The normality test p-values were consistently low (≤ 0.04 for C1 and ≤ 0.002 for C2) across all fungal species, indicating that the inhibition data do not follow a normal distribution. This suggests that the inhibitory effects are not random but are likely influenced by specific, potent compounds within the extracts. The most important statistical insight comes from comparing the mean inhibition values and their 95% confidence intervals (CI95%). For each fungal pathogen tested, the confidence intervals for C1 and C2 do not overlap, with values higher in C2 extracts compared to C1. The complete separation between these ranges provides strong statistical evidence that the higher mean inhibition observed in C2 is not due to random chance but reflects a genuinely superior and more potent antifungal capability inherent to the C2 extract.

The ANOVA analysis assessing the effects of different preparation variables (temperature, compost concentration, glucose concentration, and fermentation time) on antifungal activity, measured as the growth inhibition percentage of compost extracts C1 and C2 against four fungal pathogens, is summarized in Table 9. For extract C1, fermentation time significantly influenced the growth inhibition of *S. racemosum* (F = 17.524, p = 0.002), while no variables significantly influenced *P. roridum* (all p > 0.05) and *F. oxysporum* (all p > 0.05). However, *P. italicum* inhibition was significantly dependent on compost concentration (F = 6.872, p = 0.024), glucose concentration (F = 6.491, p = 0.027), and fermentation time (F = 20.278, p = 0.001). For extract C2, fermentation time significantly impacted the growth inhibition of *S. racemosum* (F = 7.384, p = 0.020) and *P. italicum* (F = 9.152, p = 0.012), while no variables affected *P. roridum* and *F. oxysporum* (all p > 0.05).

The fermentation process is crucial in enhancing the antifungal effectiveness of both C1 and C2 extracts, as demonstrated by its consistent and significant impact on inhibiting the growth of *S. racemosum* and *P. italicum* across both extracts. The notable p-values for C1 and C2 against these fungi suggest that the length of fermentation likely contributes to the production or activation of bioactive compounds through microbial

Parameter		pH	EC	COD	TOC	NTK	NH4+	Phenols	TMB	Yeasts	Molds	GI	*S. racemosum*	*P. roridum*	*F. oxysporum*	*P. italicum*
pH	Pearson Correlation	1	0.486**	−0.438*	0.160	0.290	0.416*	0.086	0.375*	0.293	0.489**	0.428*	0.069	0.468**	0.472**	0.329
	Sig. (2-tailed)		0.006	0.015	0.398	0.120	0.022	0.650	0.041	0.116	0.006	0.018	0.715	0.009	0.009	0.076
EC	Pearson Correlation	0.486**	1	−0.146	0.558**	0.299	0.369*	0.100	0.181	0.053	0.240	−0.288	0.145	0.540**	0.467**	−0.028
	Sig. (2-tailed)	0.006		0.441	0.001	0.108	0.045	0.597	0.339	0.780	0.202	0.123	0.445	0.002	0.009	0.882
COD	Pearson Correlation	−0.438*	−0.146	1	0.219	−0.012	−0.066	0.367*	0.112	−0.007	−0.034	−0.760**	0.195	−0.153	−0.501**	0.219
	Sig. (2-tailed)	0.015	0.441		0.246	0.951	0.728	0.046	0.556	0.970	0.859	<0.001	0.302	0.419	0.005	0.245
TOC	Pearson Correlation	0.160	0.558**	0.219	1	−0.118	−0.031	0.068	−0.114	−0.325	−0.269	−0.406*	0.343	0.188	−0.354	−0.034
	Sig. (2-tailed)	0.398	0.001	0.246		0.534	0.869	0.719	0.549	0.080	0.151	0.026	0.063	0.320	0.055	0.859
NTK	Pearson Correlation	0.290	0.299	−0.012	−0.118	1	0.961**	0.859**	0.894**	0.900**	0.906**	0.206	0.689**	0.590**	0.717**	0.532**
	Sig. (2-tailed)	0.120	0.108	0.951	0.534		<0.001	<0.001	<0.001	<0.001	<0.001	0.275	<0.001	<0.001	<0.001	0.002
NH4+	Pearson Correlation	0.416*	0.369*	−0.066	−0.031	0.961**	1	0.863**	0.939**	0.823**	0.900**	0.214	0.725**	0.555**	0.713**	0.642**
	Sig. (2-tailed)	0.022	0.045	0.728	0.869	<0.001		<0.001	<0.001	<0.001	<0.001	0.257	<0.001	0.001	<0.001	<0.001
Phenols	Pearson Correlation	0.086	0.100	0.367*	0.068	0.859**	0.863**	1	0.881**	0.729**	0.733**	−0.037	0.868**	0.416*	0.329	0.660**
	Sig. (2-tailed)	0.650	0.597	0.046	0.719	<0.001	<0.001		<0.001	<0.001	<0.001	0.847	<0.001	0.022	0.076	<0.001
TMB	Pearson Correlation	0.375*	0.181	0.112	−0.114	0.894**	0.939**	0.881**	1	0.846**	0.935**	0.151	0.657**	0.366*	0.546**	0.828**
	Sig. (2-tailed)	0.041	0.339	0.556	0.549	<0.001	<0.001	<0.001		<0.001	<0.001	0.425	<0.001	0.047	0.002	<0.001
Yeasts	Pearson Correlation	0.293	0.053	−0.007	−0.325	0.900**	0.823**	0.729**	0.846**	1	0.921**	0.372*	0.440*	0.428*	0.651**	0.570**
	Sig. (2-tailed)	0.116	0.780	0.970	0.080	<0.001	<0.001	<0.001	<0.001		<0.001	0.043	0.015	0.018	<0.001	0.001
Molds	Pearson Correlation	0.489**	0.240	−0.034	−0.269	0.906**	0.900**	0.733**	0.935**	0.921**	1	0.260	0.452*	0.461*	0.714**	0.693**
	Sig. (2-tailed)	0.006	0.202	0.859	0.151	<0.001	<0.001	<0.001	<0.001	<0.001		0.165	0.012	0.010	<0.001	<0.001
GI	Pearson Correlation	0.428*	−0.288	−0.760**	−0.406*	0.206	0.214	−0.037	0.151	0.372*	0.260	1	0.005	0.169	0.408*	0.057
	Sig. (2-tailed)	0.018	0.123	<0.001	0.026	0.275	0.257	0.847	0.425	0.043	0.165		0.977	0.372	0.025	0.763
S. racemosum	Pearson Correlation	0.069	0.145	0.195	0.343	0.689**	0.725**	0.868**	0.657**	0.440*	0.452*	0.005	1	0.379*	0.142	0.491**
	Sig. (2-tailed)	0.715	0.445	0.302	0.063	<0.001	<0.001	<0.001	<0.001	0.015	0.012	0.977		0.039	0.455	0.006
P. roridum	Pearson Correlation	0.68**	0.540**	−0.153	0.188	0.590**	0.555**	0.416*	0.366*	0.428*	0.461*	0.169	,379*	1	0.611**	−0.138
	Sig. (2-tailed)	0.009	0.002	0.419	0.320	<0.001	0.001	0.022	0.047	0.018	0.010	0.372	,039		<.001	0.466
F. oxysporum	Pearson Correlation	0.472**	0.467**	−0.501**	−0.354	0.717**	0.713**	0.329	0.546**	0.651**	0.714**	0.408*	,142	0.611**	1	0.134
	Sig. (2-tailed)	0.009	0.009	0.005	0.055	<0.001	<0.001	0.076	0.002	<0.001	<0.001	0.025	,455	<0.001		0.480
P. italicum	Pearson Correlation	0.329	−0.028	0.219	−0.034	0.532**	0.642**	0.660**	0.828**	0.570**	0.693**	0.057	,491**	−0.138	,134	1
	Sig. (2-tailed)	0.076	,882	0.245	0.859	0.002	<0.001	<0.001	<0.001	0.001	<0.001	0.763	,006	0.466	,480	

Table 7. Assessment of bivariate correlations between physicochemical and microbial parameters of compost extracts and their antifungal activity against the studied fungi by using Pearson's Test. EC: electrical conductivity; TOC: total organic carbon; TMB: total mesophilic bacteria; GI: germination index. N = 30. **. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

	Fungal inhibition	Estimators	Values
C1	*S. racemosum* n=48	*Mean ± STD* $IC_{95\%}$ *q [Min – Max]* *Normality test (p-value)*	41,8246 ± 5,39910 [30.967, 52.682] 100.00 [0.00–100.00] 0.04
	P. roridum n=48	*Mean ± STD* $IC_{95\%}$ *q [Min – Max]* *Normality test (p-value)*	63,3687 ± 4,37651 [54.571, 72.167] 100[0–100] 0.034
	F. oxysporum n=48	*Mean ± STD* $IC_{95\%}$ *q [Min – Max]* *Normality test (p-value)*	50,9490 ± 2,76711 [45.383, 56.515] 66.80[14.20–81.20] 0.001
	P. italicum n=48	*Mean ± STD* $IC_{95\%}$ *q [Min – Max]* *Normality test (p-value)*	43,1817 ± 4,75190 95.25[33.628, 52.736] [0–95.25.25] 0.001
C2	*S. racemosum* n=48	*Mean ± STD* $IC_{95\%}$ *q [Min – Max]* *Normality test (p-value)*	43,8060 ± 5,33750 [33.069, 54.543] 100[0–100] 0.001
	P. roridum n=48	*Mean ± STD* $IC_{95\%}$ *q [Min – Max]* *Normality test (p-value)*	79,9362 ± 4,23544 [71.416, 88.456] 100[0–100] 0.001
	F. oxysporum n=48	*Mean ± STD* $IC_{95\%}$ *q [Min – Max]* *Normality test (p-value)*	82,2252 ± 2,46602 [77.266, 87.184] 66.80[33.20–100.20] 0.002
	P. italicum n=48	*Mean ± STD* $IC_{95\%}$ *q [Min – Max]* *Normality test (p-value)*	78,8565 ± 4,02171 [70.770, 86.942] 96.41[3.59–100.59] 0.001

Table 8. Statistical parameters of fungal inhibition by compost extracts C1 and C2.

	Dependent Variable: Growth inhibition percentage (%)															
	C1								C2							
	S. racemosum		*P. roridum*		*F. oxysporum*		*P. italicum*		*S. racemosum*		*P. roridum*		*F. oxysporum*		*P. italicum*	
Source	F	Sig.*	F	Sig*.	F	Sig*.	F	Sig.*	F	Sig.	F	Sig.	F	Sig.	F	Sig.
Temperature	1.166	0.303	0.467	0.509	0.423	0.529	3.942	0.073	2.648	0.132	0.320	0.583	0.528	0.483	0.227	0.643
Compost concentration	0.282	0.606	0.609	0.452	1.367	0.267	**6.872**	**0.024**	0.010	0.924	3.074	0.107	0.627	0.445	0.371	0.555
Glucose	0.013	0.913	1.681	0.221	0.229	0.641	**6.491**	**0.027**	0.032	0.861	0.046	0.834	0.126	0.729	0.055	0.820
Fermentation time	**17.524**	**0.002**	0.454	0.514	4.611	0.055	**20.278**	**0.001**	**7.384**	**0.020**	0.723	0.413	0.421	0.530	**9.152**	**0.012**

Table 9. Tests of between-subjects effects of compost extracts C1 and C2. *Significance at $p<0.05$.

processes, such as enzymatic breakdown or secondary metabolite formation[75]. This method appears to be especially effective for these pathogens, likely because specific inhibitory substances accumulate over time. The absence of significant effects on *P. roridum* and *F. oxysporum*, despite fermentation affecting other fungi, indicates that the compounds formed through fermentation may be selectively effective, possibly needing adjustments in fermentation conditions or additional strategies to expand their activity spectrum. These results highlight the importance of optimizing fermentation duration as a key factor in preparing compost extracts for targeted antifungal use in agriculture.

Table 10 shows the descriptive statistics, including the mean and standard deviation of growth inhibition for *S. racemosum* and *P. italicum* under different conditions (Temperature, Concentration, Glucose, and Time). The results showed that for *S. racemosum*, the greatest growth inhibition (Mean = 100.00%) occurred with a 1/5 concentration, positive glucose, and a long fermentation time. This suggests that higher concentrations of compost extract, combined with a glucose source and extended fermentation, are highly effective against this fungus. For *P. italicum*, the highest inhibition (Mean = 100.00%) was observed under two different conditions: both with a 1/8 concentration, negative glucose, and long fermentation time. This suggests that for *P. italicum*, a lower compost concentration without added glucose is very effective, provided the fermentation time is long. Clearly, long fermentation time is often part of the conditions leading to the highest mean inhibition for both fungi, reinforcing the conclusion from Table 10 that fermentation time is a key factor.

Table 11 presents the regression equations that describe the percentage of fungal inhibition in relation to the experimental parameters. A high R^2 value indicates that the model accurately fits the data. The models for *P. italicum* ($R^2 = 0.774$ for C1) and *S. racemosum* ($R^2 = 0.633$ for C1) have the strongest fit, meaning the equations

S. racemosum							*P. italicum*						
Temperature	**concentration**	**Glucose**	**Time**	**Mean**	**Std.Deviation**	**N**	**Temperature**	**concentration**	**Glucose**	**Time**	**Mean**	**Std.Deviation**	**N**
Low	1/5	Negative	Short	79.5900		1	Low	1/5	Negative	Short	97.9500		1
			Long	.0000		1				Long	30.6100		1
			total	39.7950	56.27863	2				total	64.2800	47.61657	2
		Positive	Short	69.3800		1			Positive	Short	99.0000		1
			Long	**100.0000**		1				Long	59.18000		1
			total	84.6900	21.65161	2				total	79.5900	28.86410	2
		Total	Short	74.4850	7.21956	2			Total	Short	98.9750	1.44957	2
			Long	50.0000	70.71068	2				Long	44.8950	20.20204	2
			total	62.2425	43.40368	4				total	71.9350	33.34102	4
	1/8	Negative	Short	54.0800		1		1/8	Negative	Short	**100.0000**		1
			Long	75.5100		1				Long	**100.0000**		1
			total	64.7950	15.15330	2				total	**100.0000**	.00000	2
		Positive	Short	69.3800		1			Positive	Short	91.8300		1
			Long	.0000		1				Long	76.5300		1
			total	34.6900	49.05907	2				total	84.1800	10.81873	2
		Total	Short	61.7300	10.81873	2			Total	Short	95.91500	5.77706	2
			Long	37.7550	53.39363	2				Long	88.2650	16.59580	2
			total	49.7425	34.36435	4				total	92.0900	11.06522	4

Table 10. Descriptive statistics of dependent variable *S. racemosum* (F1) and *P. italicum (*F4).

Compost extract (d= 1/2)	Strain	Modeling formula	R^2	F
C1	***S. racemosum***	I (%) = 0.741T + 6.92 CC +1.46 G + 13.64 FT + 68.22	0.633	4.74
	P. roridum	I (%) = 0.573 T+ 12.423 CC + 20.645 G - 2.683 FT + 28.056	0.226	0.803
	F. oxysporum	I (%) = 0.309 T + 10.563 CC + 4.325 G - 4.849 FT + 46.531	0.376	1.658
	P. italicum	I (%) = 0.978 T + 24.528 CC + 23.838 G - 10.533 FT + 13.428	0.774	9.396
C2	***S. racemosum***	I (%) = −1.343 T + 1.530 CC + 2.805 G - 10.649 FT + 139.108	0.478	2.518
	P. roridum	I (%) = 0.444 T + 26.139 CC + 3.194 G - 3.170 FT + 39.604	0.275	1.041
	F. oxysporum	I (%) = −0.363 T + 7.510 CC + 3.367 G - 1.539 FT + 89.488	0.181	0.425
	P. italicum	I (%) = −0.288 T + 7.007 CC - 2.688 G - 8.703 D + 123.568	0.471	2.451

Table 11. Modeling equations of fungal inhibition percentage obtained by experimental design analysis. I (%): Inhibition percentage; T: Temperature; CC: Compost concentration; G: Glucose concentration; FT: Fermentation time; R^2: R-squared; F: F-value.

are fairly reliable predictors of inhibition for these fungi under the C1 compost extract conditions. The other models have lower R^2 values, which suggests they are less effective as predictors.

Among the models presented, those for *P. italicum* and *S. racemosum* under compost extract C1 serve as the most reliable predictors. Analyzing their respective equations reveals which factors most significantly impact fungal inhibition. For *P. italicum*, the equation I (%) = 0.978 T + 24.528 CC + 23.838 G - 10.533 FT + 13.428 indicates that compost concentration (CC) and glucose (G) have the most significant positive coefficients, making them the most influential factors for increasing inhibition. In contrast, for *S. racemosum*, the equation I (%) = 0.741 T + 6.92 CC + 1.46 G + 13.64 FT + 68.22 identifies fermentation time (FT) as the most critical factor due to its significant positive coefficient. Overall, the data consistently show that fermentation time is the most influential parameter in enhancing the antifungal efficacy of compost extracts C1 and C2, especially against *S. racemosum* and *P. italicum*. While temperature appears to have no significant effect, certain combinations of compost concentration and glucose also play a role, particularly for *P. italicum* with extract C1. The descriptive statistics further emphasize that longer fermentation, combined with optimized concentrations and glucose levels, results in the highest inhibition percentages. Regression models support these conclusions, offering predictive equations that highlight the critical role of fermentation time, compost concentration, and glucose in inhibiting fungal growth.

Effect of sterile compost extracts on phytopathogenic fungi

To assess the sterilization effect of compost extracts on phytopathogenic fungi, selected extracts from C1 and C2, known for their strong antifungal activity, were sterilized using both autoclaving (121 ℃ for 20 minutes) and filtration methods.

	Test	Dilution 1/2				Dilution 1/8			
		S. racemosum	*P. roridum*	*F. oxysporum*	*P. italicum*	*S. racemosum*	*P. roridum*	*F. oxysporum*	*P. italicum*
C1									
Non-sterile	1	100.00	100.00	68.08±0.12	56.12±0.08	79.59±0.04	74.28±0.02	73.40±0.05	65.30±0.04
	6	100.00	97.19±0.05	80.85±0.07	95.19±0.06	85.71±0.03	97.14±0.04	68.08±0.04	95.91±0.04
	9	38.77±0.04	70.00±0.04	65.30±0.06	82.65±0.05	0.00	22.22±0.02	24.48±0.01	25.55±0.02
	12	48.97±0.02	66.67±0.04	54.08±0.03	79.59±0.04	8.00±0.01	21.11±0.01	31.63±0.02	16.66±0.01
	14	69.3±0.10	90.00±0.05	43.08±0.02	82.65±0.12	10.55±0.02	20.00±0.03	23.46±0.02	27.78±0.02
Autoclave	1	0.00	21.42±0.02	57.44±0.04	0.00	0.00	24.28±0.03	15.95±0.03	0.00
	6	0.00	28.57±0.04	11.70±0.01	0.00	0.00	31.42±0.02	15.95±0.03	0.00
	9	0.00	35.71±0.02	22.34±0.02	0.00	0.00	31.42±0.03	20.21±0.02	0.00
	12	0.00	25.71±0.02	9.57±0.01	0.00	0.00	22.85±0.02	25.53±0.02	0.00
	14	0.00	8.57±0.01	25.53±0.02	0.00	0.00	22.85±0.02	21.53±0.03	0.00
Filtration	1	0.00	41.42±0.03	46.80±0.02	0.00	0.00	45.71±0.04	27.44±0.04	21.22±0.02
	6	14.28±0.03	38.57±0.04	36.82±0.03	12.22±0.02	0.00	38.57±0.04	21.70±0.02	47.55±0.04
	9	23.46±0.02	28.57±0.02	56.44±0.02	77.55±0.03	0.00	0.00	11.06±0.01	21.63±0.02
	12	28.57±0.02	22.85±0.02	37.44±0.02	71.42±0.04	0.00	17.14±0.02	27.06±0.02	9.59±0.02
	14	0.00	35.71±0.02	36.17±0.03	74.48±0.04	0.00	7.12±0.01	20.12±0.02	21.42±0.03
C2									
Non-sterile	9	69.38±0.06	100.00	100.00	91.83±0.06	0.00	100.00	100.00	95.91±0.06
	10	0.00	100.00	78.72±0.06	18.36±0.03	0.00	21.67±0.02	25.00±0.02	33.67±0.04
	11	75.51±0.04	100.00	91.48±0.04	100.00	0.00	41.67±0.01	86.67±0.05	51.02±0.02
	13	100.00	100.00	78.72±0.04	59.18±0.02	0.00	38.33±0.01	83.33±0.04	89.79±0.04
	15	79.59±0.05	100.00	79.59±0.04	97.95±0.04	0.00	50.00±0.03	27.14±0.04	39.79±0.02
Autoclave	9	0.00	34.28±0.03	0.00	2.75±0.01	0.00	50.00±0.06	36.17±0.03	25.53±0.02
	10	0.00	21.42±0.02	27.65±0.03	4.00±0.01	0.00	10.00±0.01	25.53±0.02	28.75±0.02
	11	33.67±0.03	57.14±0.04	0.00	0.00	0.00	20.00±0.02	16.80±0.02	38.08±0.04
	13	13.26±0.02	37.14±0.03	30.85±0.02	3.70±0.02	0.00	30.00±0.02	25.53±0.02	22.95±0.03
	15	0.00	35.71±0.03	30.85±0.02	3.00±0.02	0.00	34.28±0.02	25.00±0.02	14.89±0.01
Filtration	9	28.57±0.02	54.28±0.04	52.12±0.04	68.36±0.04	0.00	51.42±0.03	53.19±0.04	35.40±0.02
	10	18.36±0.02	85.71±0.04	51.06±0.04	14.28±0.03	0.00	20.00±0.02	21.06±0.03	29.30±0.01
	11	14.26±0.02	39.80±0.03	35.44±0.03	41.50±0.04	0.00	35.22±0.02	46.54±0.06	41.25±0.03
	13	18.36±0.04	64.28±0.02	46.80±0.02	69.38±0.06	0.00	37.00±0.02	27.57±0.02	25.10±0.03
	15	8.22±0.01	45.54±0.03	51.02±0.04	52.00±0.04	0.00	44.20±0.04	25.35±0.03	25.82±0.01

Table 12. Sterilization effect (by autoclave and filtration) of compost extracts (C1 and C2) against fungal growth.

Table 12 carefully describes how sterilization methods affect the antifungal activity of compost extracts C1 and C2, providing essential insights into their active compounds and the influence of microbial diversity. The non-sterile extracts consistently show the highest inhibition, such as 100% against *S. racemosum* (C1, dilution 1/2, Test 1, 6) and *P. roridum* (C1, dilution 1/2, Test 1, 6), and 95.19% against *P. italicum* (C1, dilution 1/2, Test 6). When autoclaved, which kills microbes and destroys heat-sensitive compounds, the antifungal activity drops significantly, often to 0.00% inhibition for *S. racemosum* and *P. italicum,* suggesting that many of the active components are heat-sensitive and likely microbially produced. Conversely, filtration, which removes microbes but retains heat-stable compounds, still yields some antifungal activity, generally lower than in non-sterile extracts. For example, C1 extract at dilution 1/2, after filtration, still inhibited *P. roridum* by 41.42% (Test 1) and *P. italicum* by 74.48% (Test 14), indicating the presence of heat-stable, soluble antifungal compounds that do not rely on live microbes. This variation in response to sterilization highlights the complexity of the antifungal mechanisms in compost extracts, which seem to depend on interactions between the live microbial community and its stable secondary metabolites, with the microbial portion playing a key role in achieving the most potent inhibition. Past studies support this, showing that sterilization methods such as autoclaving and microfiltration significantly disrupt the active microbial populations in compost extracts, thereby considerably reducing their disease-suppressing abilities[9,76]. Importantly, non-sterilized extracts retain vigorous biocontrol activity, whereas sterilized ones lose their capacity to induce systemic resistance[77,78].

Conclusion

Effective control of fungal plant diseases remains a major challenge, requiring sustainable and environmentally friendly alternatives to harmful agrochemicals. Among these strategies, the use of compost extracts has emerged as a promising method for maintaining plant health and productivity. In this study, compost extracts produced

through a fermentation process involving four variables at two levels showed strong antifungal activity against four phytopathogenic fungi, identified morphologically and molecularly as *S. racemosum* (F1), *P. roridum* (F2), *F. oxysporum* (F3), and *P. italicum* (F4). The inhibitory effects of the extracts varied depending on the compost source, fermentation conditions, and dilution levels. Compost C2 consistently outperformed Compost C1, likely due to its more favorable physicochemical profile, higher pH, electrical conductivity, and nitrogen content, as well as a more diverse microbial community. Statistical analysis confirmed that fermentation time was the most crucial factor for increasing antifungal effectiveness, especially against *S. racemosum* and *P. italicum*, while temperature had no significant impact. Compost concentration and glucose addition also played a role, particularly in enhancing inhibition of *P. italicum*. Pearson correlation results identified nitrogen-related parameters (NTK, NH_4^+), microbial abundance, and phenolic content as key factors in pathogen suppression, highlighting the combined importance of nutrient availability and microbial activity. Sterilization tests further showed that disease suppression results from the combined effects of active microbial communities and their heat-stable metabolites. Non-sterilized extracts consistently exhibited the most potent inhibition, while autoclaving removed activity, and filtration only partially preserved efficacy. These results highlight the crucial role of microbial activity in achieving maximum suppression, with secondary metabolites exerting residual effects. Future research focusing on microbial species and their bioactive compounds could offer deeper insights into the mechanisms behind antifungal activity, aiding in the identification of effective microbial consortia or metabolites for sustainable disease control.

Data availability

The datasets analyzed during the current study are available in the [Supplemented Materials] repository, [[Supplemented Materials](https:/joufuniversity-my.sharepoint.com/:b:/g/personal/rjazouzi_ju_edu_sa/EUarulbvFZxIuM_4eJIXM7kBXeC6E4itSxYIU3yUL-HYCA?e=0lfdpx)].

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References

1. Bouchtaoui, E. M. et al. Exploring mechanisms of compost-mediated suppression of plant pathogens: A critical review. *Appl. Soil Ecol.* **203**, 105644. https://doi.org/10.1016/j.apsoil.2024.105644 (2024).
2. Domene, M. Á. et al. Comparative analysis of horticultural and animal waste compost: Physicochemical properties and impact on plant growth. *Agronomy* **15**, 516. https://doi.org/10.3390/agronomy15030516 (2025).
3. Waqas, M. et al. Composting processes for agricultural waste management: A comprehensive review. *Processes* **11**, 731. https://doi.org/10.3390/pr11030731 (2023).
4. Kebede, T., Diriba, D. & Boki, A. The effect of organic solid waste compost on soil properties, growth, and yield of Swiss chard crop (Beta vulgaris L.). *Sci. World J.* https://doi.org/10.1155/2023/6175746 (2023).
5. Mosa, W. F. A. et al. Effect of the combined application of compost with the spraying of some nano fertilizers on the performance of mango. *Sustainability* **16**, 10239. https://doi.org/10.3390/su162310239 (2024).
6. Oueld Lhaj, M. et al. Sustainable agriculture through agricultural waste management: A comprehensive review of composting's impact on soil health in Moroccan agricultural ecosystems. *Agriculture* **14**, 2356. https://doi.org/10.3390/agriculture14122356 (2024).
7. Rehman, S. U., De Castro, F., Aprile, A., Benedetti, M. & Fanizzi, F. P. Vermicompost: Enhancing plant growth and combating abiotic and biotic stress. *Agronomy* **13**, 1134. https://doi.org/10.3390/agronomy13041134 (2023).
8. Zhou, J. et al. Progress in research on prevention and control of crop fungal diseases in the context of climate change. *Agriculture* **14**, 1108. https://doi.org/10.3390/agriculture14071108 (2024).
9. Luo, Y. et al. Effects of sterilization and maturity of compost on soil bacterial and fungal communities and wheat growth. *Geoderma* **409**, 115598. https://doi.org/10.1016/j.geoderma.2021.115598 (2022).
10. Ingram, D. T. & Millner, P. D. Factors affecting compost tea as a potential source of Escherichia coli and Salmonella on produce. *J. Food Prot.* **70**, 828–834. https://doi.org/10.4315/0362-028X-70.4.828 (2007).
11. Aguilar-Paredes, A. et al. Microbial community in the composting process and its positive impact on the soil biota in sustainable agriculture. *Agronomy* **13**, 542 (2023).
12. Naidu, Y., Meon, S. & Siddiqui, Y. In vitro and in vivo evaluation of microbial-enriched compost tea on the development of powdery mildew on melon. *BioControl* **57**, 827–836 (2012).
13. Naidu, Y., Meon, S., Kadir, J. & Siddiqui, Y. Microbial starter for the enhancement of biological activity of compost tea. *ISSN Online* **12**, 1560–8530 (2010).
14. Yin, J. et al. Compost tea: Preparation, utilization mechanisms, and agricultural applications potential – A comprehensive review. *Environ. Technol. Innov.* **38**, 104137. https://doi.org/10.1016/j.eti.2025.104137 (2025).
15. Dahmani, H., Abbasi, P., Miller, S. & Hoitink, H. Suppression of bacterial spot of tomato with foliar sprays of compost extracts under greenhouse and field conditions. *Plant Dis.* **87**, 913–919 (2003).
16. Horn, J. N., Cravens, A. & Grossfield, A. Interactions between fengycin and model bilayers quantified by coarse-grained molecular dynamics. *Biophys. J.* **105**, 1612–1623 (2013).
17. Hua, M. et al. Iturin A strongly inhibits the growth and T-2 toxin synthesis of Fusarium oxysporum: A morphological, cellular, and transcriptomics study. *Food* **12**, 1278 (2023).
18. Pandur, Z. et al. Surfactin molecules with a cone-like structure promote the formation of membrane domains with negative spontaneous curvature and induce membrane invaginations. *J. Colloid. Interfac. Sci.* **650**, 1193–1200 (2023).
19. Santos, M., Diánez, F. & Carretero, F. Suppressive effects of compost tea on phytopathogens. In Natural products in plant pest management, Chapter 12, (ed Dubey, N. K.) (CABI, 2011).
20. Litterick, A. M., Harrier, L., Wallace, P., Watson, C. A. & Wood, M. The role of uncomposted materials, composts, manures, and compost extracts in reducing pest and disease incidence and severity in sustainable temperate agricultural and horticultural crop production—A review. *Crit. Rev. Plant. Sci.* **23**, 453–479. https://doi.org/10.1080/07352680490886815 (2004).
21. Morales-Corts, M. R., Sánchez, R. P. & Gomez-Sanchez, M. A. Efficiency of garden waste compost teas on tomato growth and its suppressiveness against soilborne pathogens. *Sci. Agric.* **75**, 400–409 (2018).
22. Shaban, H., Fazeli-Nasab, B., Alahyari, H., Alizadeh, G. & Shahpesandi, S. An overview of the benefits of compost tea on plant and soil structure. *Adv. Bio. Res.* **6**, 154–158 (2015).
23. Wu, F. et al. Factor analysis of compost tea production and utilization in agriculture. *Chin. J. Biol. Control.* **36**, 972–980 (2020).

24. Lerma-Moliz, R. et al. Antioxidant and biofertilizing effect of compost extracts on horticultural crops to minimize the use of agrochemicals. *Environ. Technol. Innov.* **36**, 103776. https://doi.org/10.1016/j.eti.2024.103776 (2024).
25. Zhao, B. et al. Improving suppressive activity of compost on phytopathogenic microbes by inoculation of antagonistic microorganisms for secondary fermentation. *Bioresour. Technol.* **367**, 128288. https://doi.org/10.1016/j.biortech.2022.128288 (2023).
26. Liu, Y. et al. A global meta-analysis of greenhouse gas emissions and carbon and nitrogen losses during livestock manure composting: Influencing factors and mitigation strategies. *Sci. Total Environ.* **885**, 163900. https://doi.org/10.1016/j.scitotenv.2023.163900 (2023).
27. Raza, A. Standardization of protocol for the formulation of compost tea and its efficacy study on potato. *Pure Appl. Biol.* **12**, 1044–1055 (2023).
28. Seddigh, S. & Kiani, L. Evaluation of different types of compost tea to control rose powdery mildew (Sphaerotheca pannosavar. rosae). *Int. J. Pest. Manag.* **64**, 178–184 (2017).
29. Zaccardelli, M., Pane, C., Scotti, R., Palese, A. M. & Celano, G. Use of compost-teas as biopesticides and biostimulants in horticulture. *Italus. Hortus.* **19**, 17–28 (2012).
30. Saba, Z., Magwaza, L. S., Sithole, N. J., Mditshwa, A. & Odindo, A. Physico-chemical analysis of vermicompost mixtures. *Agronomy* **13**, 1056 (2023).
31. Scheuerell, S. Understanding how compost tea can control disease. *BioCycle* **44**, 20 (2003).
32. Wang, X. et al. Short-chain fatty acid producers in compost tea as affected by brewing time and aeration condition. *J. Soil. Sediment.* **23**, 3096–3107 (2023).
33. JIS. JIS handbook: Wastewater treatment. (Japanese Standards Association,Tokyo, 1995).
34. Jarboui, R., Dhouib, B. & Ammar, E. Effect of food waste compost (FWC) and its non-aerated fermented extract (NFCE) on seeds germination and plant growth. *Open J. Soil Sci.* **11**, 122–138. https://doi.org/10.4236/ojss.2021.11200 (2021).
35. Chtourou, M., Ammar, E., Nasri, M. & Medhioub, K. Isolation of a yeast: *Trichosporum cutaneum* able to use low molecular weight phenolic compounds: Application to olive mill waste water treatment. *J. Chem. Technol. Biotechnol.* **79**, 869–878 (2004).
36. ISO. Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations. ISO 7218, International Standards Organisation, Geneva (2007).
37. Domsch, K. H., Gams, W. & Anderson, T. H. Compendium of soil fungi. *Eur. J. Soil Sci.* **59**, 1007–1011. https://doi.org/10.1111/j.1365-2389.2008.01052.x (2008).
38. Umashankar, V., Arunkumar, V. & Dorairaj, S. ACUA: A software tool for automated codon usage analysis. *Bioinformation* **2**, 62–63. https://doi.org/10.6026/97320630002062 (2007).
39. Larkin, M. A. et al. Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947–2948 (2007).
40. Tamura, K. et al. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**, 2731–2739. https://doi.org/10.1093/molbev/msr121 (2011).
41. Meyer, I. M. & Miklos, I. SimulFold: Simultaneously inferring RNA structures including pseudoknots, alignments, and trees using a Bayesian MCMC framework. *PLoS Comput. Biol.* **3**, 149. https://doi.org/10.1371/journal.pcbi.0030149 (2007).
42. Ewekeye, T. S. et al. Studies on post-harvest deterioration of some fruits and vegetables in selected markets in Lagos State Nigeria. *Am. J. Res. Commun.* **1**, 209–223 (2013).
43. Kilani-Feki, O., Zouari, I., Culioli, G., Ortalo-Magné, A. & Jaoua, S. Correlation between synthesis variation of 2-alkylquinolones and the antifungal activity of a *Burkholderia cepacia* strain collection. *World J. Microbiol. Biotechnol.* **28**, 275–281. https://doi.org/10.1007/s11274-011-0817-0 (2012).
44. Redon, R. et al. Rapid on-site maturity and stability assessment of sewage sludge compost by mathematical treatment of UV and fluorescence spectroscopic data. *Waste Manag. Bull.* **3**, 1–9. https://doi.org/10.1016/j.wmb.2025.02.007 (2025).
45. BSI. Specification for composted materials. British Standards Institution, (London, 2005).
46. GCST. Standards of fertilizers and soil conditioners in Gulf Countries. Gulf Countries Standards, Riyadh (2006).
47. Al-Turki, A., El-Hadidy, Y. & Al-Romian, F. Assessment of chemical properties of locally produced composts in Saudi Arabia. *Int. J. Curr. Res.* **5**, 3571–3578 (2013).
48. Omar, M. M. et al. Modeling the electrical conductivity relationship between saturated paste extract and 1:2.5 dilution in different soil textural classes. *Front. Soil Sci.* https://doi.org/10.3389/fsoil.2024.1421661 (2024).
49. Brown, S. & Cotton, M. Changes in soil properties and carbon content following compost application: Results of on-farm sampling. *Compost. Sci. Util.* **19**, 88–97. https://doi.org/10.1080/1065657X.2011.10736983 (2011).
50. Rossi, G., Beni, C. & Neri, U. Organic mulching: A sustainable technique to improve soil quality. *Sustainability* **16**, 10261. https://doi.org/10.3390/su162310261 (2024).
51. Kong, Y. et al. Applicability and limitation of compost maturity evaluation indicators: A review. *Chem. Eng. J.* **489**, 151386. https://doi.org/10.1016/j.cej.2024.151386 (2024).
52. Dang, L. V., Ngoc, N. P. & Hung, N. N. Effects of biochar, lime, and compost applications on soil physicochemical properties and yield of pomelo (Citrus grandis Osbeck) in alluvial soil of the Mekong Delta. *Appl. Environ. Soil Sci.* **2022**, 5747699. https://doi.org/10.1155/2022/5747699 (2022).
53. Johnson, R. et al. Potassium in plants: Growth regulation, signaling, and environmental stress tolerance. *Plant Physiol. Biochem.* **172**, 56–69. https://doi.org/10.1016/j.plaphy.2022.01.001 (2022).
54. Faizan, M., Faraz, A., Yusuf, M., Khan, S. T. & Hayat, S. Zinc oxide nanoparticle-mediated changes in photosynthetic efficiency and antioxidant system of tomato plants. *Photosynthetica* **56**, 678–686. https://doi.org/10.1007/s11099-017-0717-0 (2018).
55. Khanm, H., Vaishnavi, B. & Shankar, A. Raise of nanofertilizer era: Effect of nano scale zinc oxide particles on the germination, growth and yield of tomato (*Solanum lycopersicum*). *Int. J. Curr. Microbiol. Appl. Sci.* **7**, 1861–1871 (2018).
56. Huang, D. et al. Carbon and N conservation during composting: A review. *Sci. Total Environ.* **840**, 156355. https://doi.org/10.1016/j.scitotenv.2022.156355 (2022).
57. Lazcano, C., Decock, C. & Wilson, S. G. Defining and managing for healthy vineyard soils: Intersections with the concept of terroir. *Front. Environ. Sci.* **8**, 68. https://doi.org/10.3389/fenvs.2020.00068 (2020).
58. Wang, G. et al. Key factors affecting seed germination in phytotoxicity tests during sheep manure composting with carbon additives. *J. Hazard Mater.* **421**, 126809. https://doi.org/10.1016/j.jhazmat.2021.126809 (2022).
59. Matus, F. J. Fine silt and clay content is the main factor defining maximal C and N accumulations in soils: A meta-analysis. *Sci. Rep.* **11**, 6438. https://doi.org/10.1038/s41598-021-84821-6 (2021).
60. Ejileugha, C., Onyegbule, U. O. & Osuoha, J. O. Use of additives in composting promotes passivation and reduction in bioavailability of heavy metals (HMs) in compost. *Rev. Environ. Contam. Toxicol.* **262**, 1–31. https://doi.org/10.1007/s44169-023-00055-9 (2024).
61. Armand, A., Bundhun, D., Bhunjun, C. S., Punyaboon, W. & Jayawardena, R. S. *Paramyrothecium amorphophalli* sp. Nov., a causal agent of leaf blight on elephant foot yam in northern Thailand. *Curr. Res. Environ. Appl. Mycol.* https://doi.org/10.5943/cream/13/1/4 (2023).
62. Qu, Z. et al. Fusarium mycotoxins: The major food contaminants. *mLife* https://doi.org/10.1002/mlf2.12112 (2024).
63. Estrada-Bahena, E. B. et al. Influence of water activity on physical properties, fungal growth, and ochratoxin-A production in dry cherries and green-coffee beans. *J. Food Process. Preserv.* **46**, e16226. https://doi.org/10.1111/jfpp.16226 (2022).
64. Cao, L. et al. Assessment of Chinese medicinal herbal wastes compost inoculated with antagonistic fungi: Nitrogen retention and microbial community in phytopathogenic soil. *Water Air. Soil. Pollut.* **235**, 747. https://doi.org/10.1007/s11270-024-07534-2 (2024).

65. Jarboui, R., Azab, S. M., Bilel, H. & Moustafa, M. N. S. Antifungal effect of fresh and stored olive mill wastewater and its ethyl acetate extract against plant pathogenic fungi. *Plant Protect. Sci.* https://doi.org/10.17221/91/2023-PPS (2024).
66. Priyadarshini, C. et al. Plant disease suppressiveness enhancement via soil health management. *Biology* **14**, 924. https://doi.org/10.3390/biology14080924 (2025).
67. El-Saadony, M. T. et al. Medicinal plants: Bioactive compounds, biological activities, and applications in human and animal health. *Sci. Rep.* **15**, 1–26 (2025).
68. Parra-Pacheco, B. et al. Bioactive compounds from organic waste: Extraction, characterization, and applications. *Molecules* **29**, 2243. https://doi.org/10.3390/molecules29102243 (2024).
69. Chen, Q., Song, Y., An, Y., Lu, Y. & Zhong, G. Soil microorganisms: Their role in enhancing crop nutrition and health. *Diversity* **16**, 734. https://doi.org/10.3390/d16120734 (2024).
70. Kredics, L. et al. Recent advances in the use of Trichoderma-containing multicomponent microbiological inoculants for sustainable agriculture. *World J. Microbiol. Biotechnol.* **40**, 1–22. https://doi.org/10.1007/s11274-024-03965-5 (2024).
71. Thepbandit, W., Pongdet, P., Pumas, C. & Pekkoh, J. Rhizosphere microorganisms supply availability of soil nutrients and induce plant defenses: A review. *Front. Plant Sci.* **15**, 1–18 (2024).
72. Xie, W. et al. Physicochemical properties and microbial community structure of rhizosphere soil of *Panax notoginseng* under different cultivation years. *3 Biotech* **15**(1), 15 (2025).
73. Islam, R., Sultana, S., Islam, M. R. & Mondal, C. Effect of aerated and non-aerated compost tea against some fungal phytopathogens. *J. Bangladesh Agric. Univ.* **17**, 142–147. https://doi.org/10.3329/jbau.v17i2.41936 (2019).
74. Jiménez, R. et al. Phytoprotective behaviour of compost extracts against aerial pathogenic fungi after rhizosphere application. *Environ. Technol. Innov.* **37**, 104062. https://doi.org/10.1016/j.eti.2025.104062 (2025).
75. Peres Fabbri, L., Cavallero, A., Vidotto, F. & Gabriele, M. Bioactive peptides from fermented foods: Production approaches, sources, and potential health benefits. *Food* **13**, 3369. https://doi.org/10.3390/foods13213369 (2024).
76. Lutz, S. et al. Harnessing the microbiomes of suppressive composts for plant protection: From metagenomes to beneficial microorganisms and reliable diagnostics. *Front. Microbiol.* **11**, 1810. https://doi.org/10.3389/fmicb.2020.01810 (2020).
77. Xing, Z. et al. Isolation and identification of induced systemic resistance determinants from *Bacillus simplex* Sneb545 against *Heterodera glycines*. *Sci. Rep.* **10**, 11586. https://doi.org/10.1038/s41598-020-68548-4 (2020).
78. Meena, M. et al. PGPR-mediated induction of systemic resistance and physiochemical alterations in plants against the pathogens: Current perspectives. *J. Basic Microbiol.* **60**, 828–861. https://doi.org/10.1002/jobm.202000370 (2020).
79. Knechtel, R. J. A more economical method for the determination of chemical oxygen demand. *J. Water Pollut. Control. Fed.* **116**, 25–29 (1978).

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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