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The Biopotential of Bacterial Bioagents Isolated from Compost in Suppressing *Botrytis cinerea* **and** *Sclerotinia sclerotiorum*

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Introduction

The increasing global population is contributing to the rise in environmental pollution issues. Rising living standards have altered consumption habits, leading to changes in waste types. A large part of both household and industrial waste comes from organic material sourced from plants and animals. Plant waste includes various unprocessed parts, such as: cut grass, chopped stems, straw, leaves, branches, stalks, seeds, fruit peels. Additionally, it includes waste produced during the processing of plant products.

The recycling of plant (solid) waste helps protect both the environment and ecosystems, benefits urban ecology, reduces landfill waste, and prevents resource wastage.

Solid waste management, recycling, and the efficient use of resources are fundamental aspects of sustainability. Effective utilization of resources, which is the cornerstone of sustainability, is achieved through these practices.

Among the methods for ensuring sustainability and recycling plant waste, composting is considered the most effective (Akdoğan & Güleç, 2007). Composting is the process by which organic waste in solid waste is converted into simpler substances, or nutrients necessary for plants, through biochemical reactions by organisms. The primary reason composting has gained importance in solid waste management is that it is not only an environmentally friendly recycling method but also yields a product that is beneficial for agricultural purposes (Diaz et al., 2003). Compost is primarily used as a soil conditioner rather than a fertilizer. It has a high organic content, ranging from 90- 95%. However, compost generally contains lower concentrations of macro and micronutrients, including phosphorus, potassium, and nitrogen, compared to commercial fertilizers. In terms of its characteristics, it is similar to peat moss (Knight, 1997). Composting is a widely used method for the biological treatment of municipal and agricultural solid waste. Composting generally involves several stages: the processing of solid waste, the biological decomposition of its organic components, and the preparation and marketing of the final compost product. Utilizing waste in compost production is of significant importance both for contributing to the economy and for preventing environmental pollution (Tchobanoglous et al., 1993; Pekşen & Günay, 2009). Compost has commercial value and can be sold under names as organic fertilizer, soil conditioner, and growing medium. However, all these benefits can only be achieved through a controlled and successful composting process. For successful composting, the nutrient content of the waste used in the process is crucial. Materials must be combined in the correct proportions to start the decomposition and breakdown processes. The two fundamental elements required are nitrogen (N) and carbon (C). Materials such as kitchen scraps, egg shells, freshly cut grass, animal manure, and animal carcasses have high nitrogen content. In contrast, materials like dried leaves, branches, straw, sawdust, paper, and cardboard have high carbon content. In the composting process, materials high in nitrogen (N) are referred to as "green" materials, while those high in carbon (C) are referred to as "brown" materials. Typically, nitrogen-rich materials are green and include fresh grass, vegetables, and fruits, whereas carbonrich materials include straw, shredded branches, and sawdust. The ideal carbon-to-nitrogen (C/N) ratio for organic matter in a compost pile is generally considered to be around 25:1 or 30:1. This means that materials with 25 or 30 units of carbon should be mixed with materials containing 1 unit of nitrogen to achieve a balanced compost. Materials high in carbon content, such as straw and sawdust, are generally dry and lightweight. In contrast, materials high in nitrogen content, such as animal and poultry manure, fish scraps, and freshly cut grass, are wet, heavy, and dense. Manure from ruminants is preferred in composting because it is rich in microorganisms. Cow manure is particularly ideal, but manure from sheep, goats, and horses can also be used. Additionally, it is better to use manure in composting before it becomes excessively aged (Anonymous, 2021).

The concept of sustainable agriculture promotes the use of compost in farming. Compost not only provides nutrients to plants but also helps renew the organic matter content, contributing to more sustainable agricultural ecosystems (Marmo, 2008; Palanivell et al., 2013). Research conducted worldwide has shown that the use of compost can reduce dependency on chemical pesticides and fertilizers in agriculture (Hargreaves et al., 2008; Wang et al., 2011; Gonzalez-Gonzalez et al., 2024). Pesticides are

commonly used, especially in the management against fungal-origin diseases. However, the rapid development of resistance by pathogens to pesticides, along with the threat to natural balance, has led to the exploration of alternative methods. One of the increasingly important alternatives today is biological control, which has been extensively studied against various plant disease agents and has started to be used successfully as biopreparations (McDonald & Linde, 2002; Martin, 2003). In biological control, isolating microorganisms with antagonistic potential from nature and studying their effects on pathogen development are crucial (Soylu et al., 2021). The use of organic materials (such as animal manure, green manure, compost, and peat) is recommended in both traditional and biological systems, particularly for reducing disease rates caused by soil pathogens and improving soil structure (Gamliel & Stapleton, 2012; Kefalogianni et al., 2017). The application of compost alters microbial communities because it is a source of numerous and diverse beneficial microorganisms (Dai et al., 2018; Huang et al., 2019; Wang et al., 2020). A significant diversity of bacterial species has been isolated from compost to date, including microorganisms known as Plant Growth-Promoting Rhizobacteria (PGPR), which are believed to promote plant growth (Blaya et al., 2016). Additionally, compost bacteria can also act as biocontrol agents against phytopathogens, and their significant impact on the prevention of various plant diseases has been reported (Angelopoulou et al., 2014; Calderón et al., 2014; Baliah et al., 2016; Kefalogianni et al., 2017; Le et al., 2018; Özkaya & Soylu, 2022). Among these pathogens are *Sclerotinia sclerotiorum* (deBary), which causes white mold and can remain viable in soil for years as sclerotia, and *Botrytis cinerea* (deBary), known for gray mold, both of which cause significant economic losses in our country (Soylu et al., 2020).

In this study, the effects of beneficial bacteria obtained from compost mixtures produced as the end product of the aerobic decomposition of organic material on *S. sclerotiorum*, a pathogen causing root rot, and *B. cinerea* (deBary), which causes gray mold, were investigated *in vitro*.

Materials and Methods

Compost Production

Compost produced in 2021 and 2022 at the Selçuk University Çumra School of Applied Sciences experimental garden was used in the trial. The compost mixture, made from a combination of domestic and agricultural solid wastes, including fruit and vegetable peels, used tea leaves, eggshells, nut shells, freshly cut grass, tree leaves, pruned plant residues, and straw, is listed in Table 1.

Table 1. The materials used to create the compost from which the bacteria were obtained are as follows:

Compost Materials	Amount
Straw	25 kg
Dry, carbon-rich materials include dry leaves, shredded branches, either used alone or in combination	20 kg
Animal manure (Cow manure)	25 kg
Kitchen scraps (fruit and vegetable waste, nut shells, tea grounds)	30 kg
Freshly cut grass (left to sit for 1-2 days after mowing)	35 kg
Accelerators (to speed up the composting process), such as urea or any high-nitrogen material	1 kg urea
Enrichers: Egg shells (mineral-rich material)	Ap 10% *
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Approximately 10% of the total compost materials

Parameter	Method	Result
Water Saturation $(\%)$	Saturation	59.4
Salinity (EC) $(dS \, m^{-1})$	Saturation	2.84
pH	Saturation	7.11
$CaCO3(\%)$	Scheiber	25.01
Organic Matter (%)	Walkley-Black	1.82
K (mg kg)	Olsen	8.10
P(mg kg)	A.Asetat-ICP	252.23

Table 2. Basic Chemical Properties of the Compost

Preparation of Compost

A pit measuring 1.5 meters by 2.0 meters was dug in an unused area. At the bottom of the pit, layers of straw, dry branches, leaves, and shredded branches were placed. Manure, freshly cut grass, kitchen scraps, and soil were layered on top to form the compost pile. Urea was added to the second layer as an accelerator. The same process was repeated with four layers, stacking and lightly watering each layer until the pile reached a height of 1.5 meters. The pile was then covered with plastic. The pile was turned every eight days, completing the composting process in about a year. The basic chemical properties of the compost after fermentation are listed in Table 2.

Phytopathogenic Fungi

We obtained the fungal pathogens used in this study from the Mycology Laboratory of Selçuk University. These pathogens were *Botrytis cinerea*, isolated from tomatoes, and *Sclerotinia sclerotiorum*, isolated from sunflowers. These fungi were cultured on Potato Dextrose Agar (PDA) and maintained as pure cultures on slant agar at +4°C during the experiment.

Obtaining Bacterial Isolates from Compost

A compost sample was collected from three locations within the experimental garden of Selçuk University Çumra School of Applied Sciences, mixed, and used as a single sample. We isolated bacteria from the compost samples using the serial dilution plating technique (Aneja, 2003). The sample was serially diluted with sterile distilled water (serial dilutions ranging from 10^{-1} to 10^{-7}), 200 μ l from each dilution was spread onto Nutrient Agar (NA) plates. After incubating at 28°C for 48 hours, colonies were selected from these plates, and pure cultures were obtained and preserved long-term in 30% glycerol.

Identification of Bacterial Isolates

The identification of bacterial isolates used for biocontrol was performed using Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS), which accurately classified the bacterial isolates into species (Aktan & Soylu, 2020). The MALDI-TOF system is a cutting-edge diagnostic technology. It is based on the principle of comparing protein profiles obtained directly from bacteria by laser with those of known isolates in the device's database. Samples were sent to the Scientific Industrial and Technological Application Research Center at Bolu Abant İzzet Baysal University.

Determination of In Vitro Antagonistic Activities of Bacteria Isolated from Compost

Bacterial isolates obtained from compost were evaluated for antifungal activities against *Botrytis cinerea* and *Sclerotinia sclerotiorum* using dual culture method. The fungal pathogens used in this study were first inoculated onto Potato Dextrose Agar (PDA) plates containing Streptomycin sulfate and incubated at 25°C for 4-5 days to obtain fresh cultures. The bacterial isolates used in the experiment were spread onto Nutrient Agar (NA) plates and incubated at 25°C for 24 hours before pathogen inoculation. Dual culture assays were performed on antibiotic-free PDA plates. A disc of agar with fresh pathogen culture was placed in the center of a Petri dish (90 mm diameter), and bacterial isolates were streaked in a circle with a radius of 1.5 cm around the pathogen (Salman et al., 2022). The control group was not inoculated with bacteria. The Petri dishes were incubated at 27°C for a duration of 5 days. After incubation, the mycelial growth of the pathogen towards the bacterial isolates (measured in mm) was recorded, and the percentage of colony growth inhibition was calculated using the formula: %Inhibition = (1- (Treatment Growth / Control Growth)) \times 100 (Tariq et al., 2010). *In vitro* biocontrol tests were conducted with three replicates for each treatment and repeated at two different times.

Statistical Analysis

Data were analyzed using one-way ANOVA with SPSS statistical software (SPSS Inc., version 17.0), and differences between isolates were compared using Tukey's test ($p \leq 0.05$).

Results and Discussion

A total of 8 bacterial isolates were obtained from compost samples collected from the experimental garden at Selçuk University, Çumra Applied Sciences School (Table 1). In dual culture assays, 6 of the isolates showed antagonistic effects against the fungal pathogens. Among these isolates, only 3 effectively inhibited the mycelial growth of *Botrytis cinerea* and *Sclerotinia sclerotiorum*, and these isolates were identified at the species level using MALDI-TOF MS analysis. According to the analyses, all the isolates were determined to belong to the genus *Bacillus* spp. The SÖ 1-2/SÖ 1-7 isolates, which have high antagonistic activity, were identified at the species level as *Bacillus cereus*, while the SÖ 1-16 isolate was identified as *Bacillus megaterium*.

Subsequently, the *in vitro* antagonistic potentials of the 8 candidate bacteria were determined. The bacterial isolates significantly inhibited the growth of phytopathogenic fungi in dual culture tests. The *in vitro* antagonistic potentials of the 8 antagonist bacterial isolates were evaluated against the gray mold pathogen *B. cinerea*, and it was found that 6 isolates of *Bacillus spp.* inhibited mycelial growth by 23.3% to 63.3%. Among the tested antagonist bacterial isolates, the one that most effectively suppressed mycelial growth was SÖ 1-16 (*Bacillus megaterium*), with up to 63.3% inhibition. This isolate was followed by SÖ 1-2 (*Bacillus cereus*) with a 48.8% inhibition rate and SÖ 1-7 (*Bacillus cereus*) with a 47.7% inhibition rate. Other isolates (SÖ 1-14, SÖ 1-4, and SÖ 1-5) inhibited mycelial growth of *B. cinerea* by 23.3%, 26.6%, and 31.1%, respectively. Among the 8 tested isolates, SÖ 1-1 and SÖ 1-3 did not inhibit the mycelial growth of the fungal pathogen (Table 3, Figure 1).

Among the tested antagonist candidates, *Bacillus cereus* (SÖ 1-7) was the most effective in inhibiting the

mycelial growth of the root rot pathogen *S. sclerotiorum*, achieving 83.3% inhibition and a mycelial growth of 1.5 mm.This isolate was followed by *Bacillus cereus*(SÖ 1-2), which showed 80% inhibition with a mycelial growth of 1.8 mm. Other antagonist bacteria were unable to inhibit the mycelial growth of *S. sclerotiorum* (Table 3, Figure 1).

The increasing interest in compost application in horticultural practices is a result of the integrated approach to managing plant diseases (Scharenbroch et al., 2011). The pH (neutral) and EC (high) values of composts, which depend on organic and mineral components, are important not only for plant growth and potential toxicity but also for the formation of microbial populations necessary to inhibit fungal pathogens. To prevent phytotoxic reactions, compost should be diluted with water before application to plants. However, more research is needed to evaluate the individual biotic factors of each component in determining their disease-suppressive effects (Pane et al., 2012).

Table 3. The potential of antagonist bacterial isolates obtained from compost to inhibit the mycelial growth of fungal pathogens under in vitro conditions^a.

	r ----- <i>- -</i> -----	B. cinerea		S. sclerotiorum	
Isolates	Genus/Species Names	Mycelial growth $(cm)^b$	%Inhibition	Mycelial growth (cm)	%Inhibition
$SO1-1$	Bacillus spp.	9.00a	$_{0}$	9.00a	
$S\ddot{\mathrm{O}}$ 1-2	Bacillus cereus	4.6c	48.8	1.8 _b	80
$S\ddot{\mathrm{O}}$ 1-3	Bacillus spp.	9.00a	θ	9.00a	$^{(1)}$
$S\ddot{\mathrm{O}}$ 1-4	Bacillus spp.	6.6 _b	26.6	9.00a	
$S\ddot{\mathrm{O}}$ 1-5	Bacillus spp.	6.2 _b	31.1	9.00a	
$S\ddot{\mathrm{O}}$ 1-7	Bacillus cereus	4.7c	47.7	1.5c	83.3
$S\ddot{\mathrm{O}}$ 1-14	<i>Bacillus</i> spp.	6.b	23.3	9.00a	$^{(1)}$
$S\ddot{\mathrm{O}}$ 1-16	Bacillus megaterium	3.3d	63.3	9.00a	
Control	B. cinerea/S. sclerotiorum	9.00a	θ	9.00a	

^a The bacterial isolates were inoculated onto PDA and marked with a circular zone immediately after pathogen inoculation. The mycelial growth (cm) of the fungus towards the bacterial zone was measured in the petri dishes containing bacteria. The growth was compared with that in the control petri dishes, and the inhibition rates (%) were calculated.; ^b The experimental data were measured in 2 different petri dishes, and the averages were taken. The experiment was repeated twice at different times. The similar lowercase letters next to the mean values within the same column indicate that there is no statistically significant difference between the isolates. (Tukey Test, P≤0.05)

Figure 1. The potential of different antagonist bacterial isolates to inhibit mycelial growth of *B. cinerea* (A) and *S. sclerotiorum* (B) in *in vitro* dual culture tests.

The continuous use of synthetic pesticides for controlling air and soil-borne pathogens leads to resistance development and environmental pollution (Savary et al., 2019; Wang et al., 2021; Imran et al., 2023). This situation highlights the need for sustainable alternatives to manage plant diseases, including methods compatible with organic farming. Therefore, using compost as a biological control approach is a potential method to mitigate the damage caused by these chemicals and can be economically advantageous when employed. Biological control methods may involve a single organism or a group of beneficial organisms working together to suppress a pathogen and control disease (Collinge et al., 2022). This study has demonstrated that bacterial isolates derived from the compost produced are highly suppressive. Our results indicate the high microbial activity of the compost. Among the isolates with antagonistic properties in dual culture experiments, three exhibited 47.7%-63.3% inhibition rates, effectively suppressing the mycelial growth of *B. cinerea*. These results are consistent with studies on the *in vitro* suppressive effects of compost against various plant pathogens (Pane et al., 2010; Pane et al., 2012; Kara et al., 2016; Barghouth et al., 2023; Jiménez et al., 2023). In contrast, the effects of the isolates on the development of *S. sclerotiorum* showed significant variability, with two isolates proving to be the most effective against the soil-borne fungus, exhibiting 83.3% and 80% inhibition rates in mycelial growth. Previous studies have reported the role of such bacterial isolates in suppressing the development of significant soilborne plant pathogens (Soylu et al., 2005; Gupta et al., 2006; Kumar et al., 2012; Sönmez, 2018). Overall, our data support the conclusion that microorganisms thriving in the examined compost play a crucial role in pathogen suppression. It has been reported that solid compost and organic amendments can suppress soil-borne pathogens and play a significant role in stimulating the beneficial microbial components responsible for disease suppression (Giotis et al., 2009; Zaccardelli et al., 2011). Additionally, composts have been used as a source for isolating potential biological control agents. Bacterial isolates such as *B. subtilis*, *B. licheniformis*, and *P. chrysogenum* from compost have shown significant antagonistic effects against plant pathogens (Kone et al., 2010; Suarez-Estrella et al., 2012). The results obtained in this study suggest that compost harbors beneficial antagonists that could serve as a resource against plant pathogens.

The Bacillus species, known for its various antagonistic properties, are among the most studied in biological research due to their ability to synthesize antibiotic substances.These bacteria are noted for their production of antimicrobial compounds with different molecular structures, their resistance to pathogen resistance to these compounds, and their ability to form endospores that allow survival under harsh environmental conditions. As a result, they are considered the most suitable microbial species for biopesticide production (Stein, 2005; Abbas et al., 2019; Alvindia & Natsuaki, 2009; Kara & Soylu, 2022). In our study, three bacterial isolates identified as *Bacillus* were isolated, and all three isolates were found to be effective in vitro against *B. cinerea* and *S. sclerotiorum* diseases.

Recently, various approaches have been explored to enhance biocontrol activity, leading to significant advancements in the formulation and production of beneficial microorganisms, such as antagonistic bacteria, and the commercialization of biopesticides. However, to create a genuine alternative to synthetic chemicals by increasing biocontrol potential, further research is needed to discover new microbiomes, clarify their effects on pathogens, and improve our understanding of the interactions between pathogens, antagonists, and hosts.

Declarations

This paper was presented at the 7th International Anatolian Agriculture, Food, Environment and Biology Congress.

The authors declare no conflict of interest.

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