



Effectiveness of biocontrol activity against two *Phaeoacremonium* species involved in grapevine pruning wound infection.

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ABSTRACT

Grapevine cuttings inoculated with *Phaeoacremonium* species (*Ph. aleophilum* and *Ph. hungaricum*) exhibited brownish discoloration and necrotic cankers. In both dual culture and food poisoning assays, *Trichoderma harzianum* and *Bacillus subtilis* showed the highest antagonistic activity against *P. aleophilum* and *P. hungaricum*, with *T. harzianum* demonstrating superior inhibition rates (up to 93.3%), followed by *B. subtilis* (up to 80.3%), while *Saccharomyces cerevisiae*. *cerevisiae* and *Clonostachys rosea* exhibited moderate to low efficacy against the tested pathogens. The potential dissemination of bacterial and fungal biocontrol agents via nursery propagation materials was investigated by assessing their effectiveness against *Phaeoacremonium aleophilum* and *P. hungaricum* in detached grapevine canes. Treatments included bacterial strains (*Pseudomonas fluorescens*, *Bacillus subtilis*) and fungal isolates (*Trichoderma harzianum*, *Clonostachys rosea*, *Saccharomyces cerevisiae*). Among these, *Clonostachys rosea* demonstrated the highest antagonistic activity, significantly reducing canker lengths to 10.6 mm and 13 mm for *P. aleophilum* and *P. hungaricum*, respectively. In contrast, *P. fluorescens* was the least effective in reducing canker lengths to 21.3 mm. These results confirm that both *T. harzianum* and *C. rosea* are effective biocontrol agents against *Phaeoacremonium* species. Their significant antagonistic activity was demonstrated in both in vitro and detached cane assays, where they significantly reduced pathogen growth and symptom development. This highlights their potential as sustainable, eco-friendly alternatives for managing grapevine trunk diseases, supporting their integration into effective disease management strategies.

Keywords: *Phaeoacremonium aleophilum*, *Ph. hungaricum*, Grapevine, *Trichoderma harizianum*, *Bacillus subtilis*.

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INTRODUCTION

Phaeoacremonium aleophilum is one of the most commonly identified species of *Phaeoacremonium* in diseased grapevines, playing a significant role in the development of grapevine trunk diseases (GTDs), including the Esca complex, Eutypa dieback, and Botryosphaeria dieback[1]. These diseases are considered major obstacles to the growth and sustainability of vineyards, particularly affecting the grapevine trunk, which is essential for long-term productivity. *Phaeoacremonium* species play a crucial role in this disease complex, with grapevine wood necrosis being a key symptom. This necrosis is characterized by brown internal streaking, leaf discoloration, and desiccation[2]. Grapevines are primarily infected by GTD fungal pathogens through annual pruning wounds made during dormancy[3]. The susceptibility of pruning wounds to GTD pathogens largely depends on the timing of pruning and the interval between pruning and potential infection. Studies utilizing artificial spore inoculations have demonstrated that grapevine pruning wounds are highly susceptible to fungal infections immediately after pruning. However, this susceptibility gradually decreases over several weeks or months [4];[5]. Seasonal variations in susceptibility have also been observed between grape-growing regions, primarily due to differences in climate[3]. The presence of *Ph. aleophilum* and *Ph. chlamydospora* as pioneers of young Esca underscores the importance of early detection and intervention to mitigate the spread of GTDs [6]. Studies have shown that infection by *Ph. aleophilum* can occur as early as the propagation stage, emphasizing the need for proactive management strategies [7]; [8]. Grapevine trunk diseases pose a significant economic challenge in all grape-growing regions, profoundly affecting grape production and long-term sustainability. black foot, and Esca diseases are primary culprits, causing substantial financial losses in the sector [9];[10];[11]. These issues are particularly prominent in older vineyards, typically over 10 years old, where diseases affecting the grapevine trunk are pervasive. However, even young grapevines in newly established vineyards exhibit signs of decline, a condition commonly referred to as Petri disease or young esca [12]. *Phaeoacremonium* species contribute significantly to this complex of diseases, with grape wood necrosis being a primary symptom. *Ph. aleophilum* has been reported for the first time by [13] as the cause of grapevine decline in Iraq. A study conducted by [2] demonstrated that *Ph. aleophilum* significantly decreased both the fresh and dry weight of grapevine green shoots compared to the non-inoculated treatment. *Ph. hungaricum* was recently identified by [14] as the cause of young vine decline in Iraq. To prevent fungal

infections in propagation material, various management approaches, including biological and physical treatments, have been proposed [15]; [16]. Additionally, chemical fungicides have been explored to control fungal infections in nurseries and enhance the quality of new grapevine material. Despite these efforts, effective control of GTDs remains a challenge, leading to increasing interest in biocontrol strategies. The use of endophytic microorganisms isolated from grapevine tissues has gained particular attention as a promising approach [15]. Several biocontrol agents, including *Bacillus subtilis* [16], *Clonostachys rosea* [15], [17], and *Trichoderma* species [18]; [19], have been extensively studied for their potential to combat GTDs. *Bacillus velezensis*, in particular, has emerged as a promising biocontrol agent, with recent studies highlighting its effectiveness in managing grapevine diseases [20]; [21]. The usage of biocontrol agents helps to increase the possibility of disease resistance, along with minimizing the usage of chemicals. Bacterial strains utilized as biocontrol agents mostly belong to the genera *Bacillus* and *Pseudomonas*. These bacterial biocontrol agents enhance plant growth by the suppression of either minor or major phytopathogens in addition to the production of plant growth-promoting metabolites such as gibberellins, auxins etc. [22];[23]. The objectives of this study were to conduct in vivo testing of the effectiveness of biocontrol agents (*Trichoderma harzianum*, *Clonostachys rosea*, *Bacillus* sp., *Pseudomonas* sp., *Saccharomyces* sp.) against *Ph. aleophilum* and *Ph. hungaricum* in grapevine pruning wounds, as well as in vitro testing using the Dual Culture Method and Food Poisoning Method.

Materials and Methods:

Pathogens, Source, and evaluated bioagents

Five bioagents were assessed for their microbial effectiveness against the two *Phaeoacremonium* species isolated from grapevine trunk wounds. Two *Phaeoacremonium* species included *Ph. aleophilum* and *Ph. hungaricum*, which were previously isolated and identified from grapevine trunk wounds by [13];[14] two bioagents included *Trichoderma harzianum* and *Clonostachys rosea* obtained from the Mycology Bank/Plant Protection Department at the University of Duhok. The bioagents *Bacillus subtilis* and *Pseudomonas fluorescens* were sourced from the Research Lab/Plant Protection Department at Salahaddin University and a species of yeast, *Saccharomyces cerevisiae*, was sourced from a trade powder formula available in local markets. Bacterial colonies were quantified using the serial dilution and plate count method.

Pathogenicity of *Phaeoacremonium* species

All grapevine pruning cuttings (canes) were surface-sterilized using 2% sodium hypochlorite, and surface-sterilized stems were wounded (approximately 1×1 mm, 4 mm deep) under the node to create fresh wounds before inoculation [24]. A hyphal specimen from each pathogen (*Phaeoacremonium aleophilum* and *Phaeoacremonium hungaricum*) was placed into each wound, then covered with sterilized moist cotton pads and incubated in a growth chamber at a regulated temperature of $25 \pm 2^\circ\text{C}$ and humidity of 70%. In the control treatment, the canes were wounded but not inoculated. At the end of the experiment, segments of tissue from the margins of the base of cuttings were transferred MEA plates to check for fungi presence and demonstrate necrosis symptoms in the injured area [25].

Evaluation of Microbial Activity Using Dual Culture Method.

To evaluate microbial activity, bacterial and fungal isolates were grown and examined against two *Phaeoacremonium* species responsible for grapevine stem canker. In dual culture assays, mycelium plugs (0.5 cm) from the actively growing edges of *Trichoderma harzianum* and *Clonostachys rosea* colonies were positioned 4 cm apart on 9-cm PDA plates. The antifungal potential of *Bacillus* sp., *Pseudomonas* sp., and the yeast *Saccharomyces* sp. was evaluated following the dual culture method described by [26]. Bacterial and yeast isolates were streaked on one side of a 9-cm Petri dish, 2 cm from the edge, while 5-mm disks from the margins of 7-day-old *Phaeoacremonium* cultures were placed on the opposite side, perpendicular to the bacterial streaks. Plates were incubated at $25 \pm 2^\circ\text{C}$ for 7 days, with three replicates for each. Control plates included solely *Phaeoacremonium* cultures. Fungal radial growth was measured once the pathogen in the control plates had fully colonized the medium. The experiment followed a factorial arrangement in a Complete Randomized Design (CRD) with three replications, each containing three plants. The formula proposed by [27] was used to calculate the inhibition rate of mycelial growth of test organisms compared to the control.

$I (\%) = (C - T) / C \times 100$; Where, I = rate inhibition, C= controlled Growth, T= Growth in treatment.

The data were first analyzed using analysis of variance (ANOVA), followed by the Duncan multiple range test ($p = 0.05$).

Microbial Activity Assessment Using the Food Poisoning Method

For each bioagent isolate, a loopful of inoculum from a 48-hour culture was added to a 250 mL Erlenmeyer flask containing 50 mL of potato dextrose broth (PDB). The flasks were incubated in the dark at 150 rpm for 72 hours. Following the modified protocol of [28], each bioagent culture was filtered through Whatman No. 4 filter paper and a $0.45 \mu\text{m}$ Millipore membrane to eliminate bacterial cells and fungal spores. A 10 mL aliquot of each filtrate, diluted to 50% concentration, was incorporated into PDA medium and dispensed into Petri dishes. Once solidified, a 7 mm mycelial disk of each *Phaeoacremonium* species was placed at the center of the plate. For the control, *Phaeoacremonium* species were grown on PDA containing sterile water instead of the bioagent filtrate. The mycelial diameter was measured in two perpendicular directions once the fungal growth in the control plates had completely covered the medium. Growth inhibition was calculated [27] by comparing test organisms to the control. The experiment was arranged in a factorial design using a Complete Randomized Design (CRD) with three replications, each consisting of three plants. Data were analyzed using analysis of variance (ANOVA), followed by Duncan's

multiple range test ($p = 0.05$).

Assessment of Microbial Antagonists Using Detached Cane Assay.

The microbial activity was conducted in grapevine cuttings under controlled conditions by modified detached cane assay according to [24]. All grapevine pruning cuttings (canes) were surface-sterilized using 2% sodium hypochlorite, and surface-sterilized stems were wounded (approximately 1×1 mm, 4 mm deep) under the node to create fresh wounds before inoculation. A hyphal sample of each pathogen was injected into each wound, then covered with sterilized wet cotton cushions and tied. For the control treatment, the shoots were wounded but not inoculated. The basal ~4 cm of inoculated cuttings were placed in a 500 ml beaker containing 250 ml of each bioagent suspension. Control cuttings were immersed in a beaker containing distilled water only. All treatments were incubated in a growth chamber at a controlled temperature of $25 \pm 2^\circ\text{C}$ and humidity of 70%. The canker length was measured after 60 days of incubation. The trial layout was factorial in a Complete Randomized Design (CRD) with three replications; each replicate had three plants. The data were first analyzed using analysis of variance (ANOVA), followed by the Duncan multiple range test ($p = 0.05$).

Results and Discussions:

Pathogenicity test

Detached grapevine cuttings inoculated with *Phaeoacremonium aleophilum* and *P. hungaricum* developed distinct brown to dark brown discoloration of the vascular tissue surrounding the inoculation site. This discoloration often extended longitudinally along the xylem vessels, indicating pathogen colonization and wood necrosis. Such brown streaking is a hallmark symptom associated with these pathogens and reflects the progressive degradation of vascular tissues in infected cuttings [29]. This discoloration may result from the oxidation and translocation of plant cell breakdown products caused by fungal enzymatic activity [30]. The vascular discoloration is commonly associated with fungal pathogens that cause grapevine trunk diseases, particularly esca and Petri disease. These pathogens disrupt water conduction by colonizing the xylem, leading to the accumulation of toxic metabolites that contribute to vascular dysfunction and necrosis [3]. Enzymes such as laccases, peroxidases, and cellulases secreted by *Phaeoacremonium* spp. degrade cell wall components, facilitating pathogen spread and tissue degradation [6]. Additionally, previous studies have linked vascular browning to the production of phenolic compounds in response to fungal invasion [31]. These compounds are part of the plant's defense mechanisms, which aim to limit pathogen movement; however, their oxidation often leads to tissue necrosis and further compromises vascular integrity [4]. The extent of discoloration may also depend on host susceptibility, environmental conditions, and the virulence of the fungal strain. These findings highlight the complex interactions between fungal pathogens and host plants.

In vitro evaluation of antagonistic fungi by Dual culture

The data presented in Figure 1 identified *Trichoderma harzianum* as the most effective bioagent in the dual culture method against *Phaeoacremonium aleophilum* and *Phaeoacremonium hungaricum*, by 93.3% and 75.8%, respectively. It covered over three-fourths of the plate, effectively dominating most pathogenic species. These results agree with [32] who tested the mycoparasitic potential of the *Trichoderma* strain IBWF 034-05 against the fungi *Phaeoacremonium aleophilum* in a dual culture test, after 5 days of inoculation at room temperature, radial growth of the pathogen was inhibited completely. *T. harzianum* also rapidly inhibited the mycelial growth of *P. hungaricum*, with its colonies extending into the inhibition zone and overlapping with *P. hungaricum* colonies. This rapid growth enabled *Trichoderma* to colonize a large area of the culture medium, giving it a significant advantage in the competition for space, nutrients, and dominance over its host [33]; [34]. *Trichoderma* sp. is among the most commonly recognized biological control agents, having been rigorously tested and studied globally. Enzymes like chitinase, glucanase, and other cell wall-degrading enzymes play vital roles in *Trichoderma*'s antagonistic effects on fungal pathogens [35]. To use environmentally friendly methods for protecting crops from diseases and pathogens worldwide, identifying and developing highly effective strains is the essential first step for successful biological control. *Saccharomyces cerevisiae* exhibited a growth decrease of 50.7% on *Ph. aleophilum*, while *Bacillus subtilis* thrived, effectively inhibiting *Ph. aleophilum* by 92.5% and moderately decreasing *Ph. hungaricum* growth by 68.1%. *Pseudomonas fluorescens* demonstrated different impacts on species, significantly hindering *Ph. aleophilum* by 92.3% and lowering *Ph. hungaricum* growth by 56.9%. *Clonostachys rosea* exhibited a moderate inhibitory impact on all species, with the most significant growth decline noted in *Ph. aleophilum* at 90.7% and *Ph. hungaricum* at 61.8%.

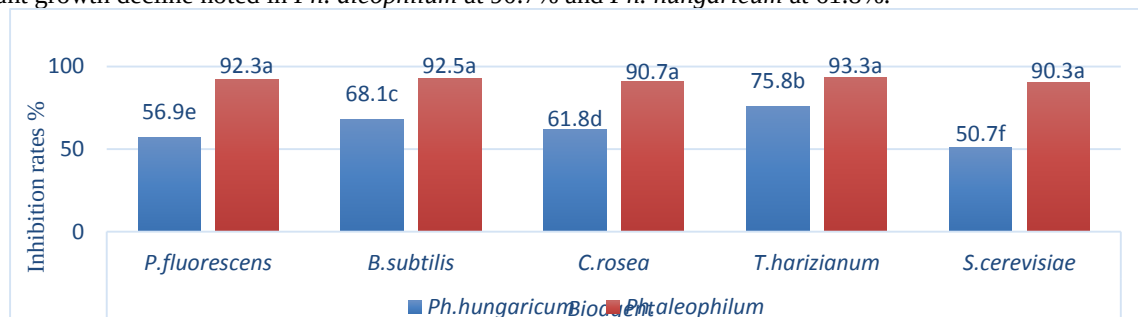


Figure 1: Inhibition percentage of mycelial growth of *Phaeoacrimonium* species treated with bioagents by Dual culture

method

The inhibition rates of the four bioagents, as illustrated in Figure 2, revealed that *T. harzianum* exhibited the highest inhibition rate at 84.5%. *B. subtilis* followed with an inhibition rate of 80.3%. *Saccharomyces cerevisiae* had the lowest inhibition rate at 70.5%, while *Clonostachys rosea* came in third place with a 76.2% growth inhibition rate.

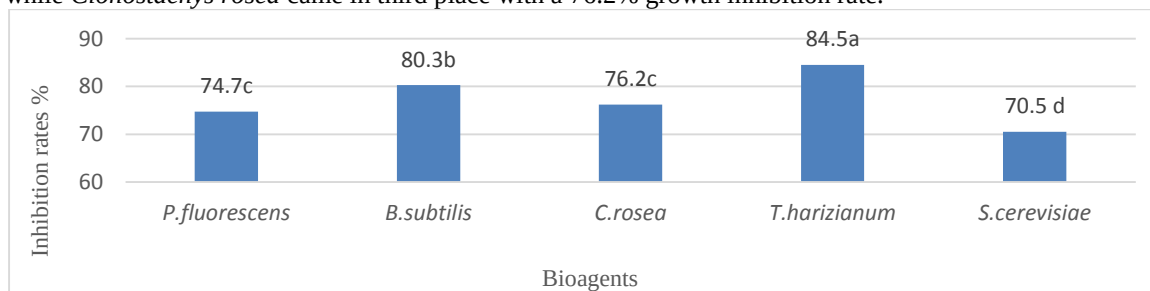


Figure 2: Inhibition percentage of Phaeoacrimonium species mycelial growth treated with bioagents

Its mycoparasitic nature, coupled with its ability to produce antifungal compounds and hydrolytic enzymes, makes it a strong contender for biological control applications [36]. However, environmental factors such as temperature, humidity, and substrate availability may influence its performance in real-world conditions, requiring further investigation to optimize its effectiveness in different agricultural settings [37].

Bacillus subtilis, the second most effective bioagent, also exhibited strong inhibitory potential, particularly against *Ph. aleophilum*. The ability of *B. subtilis* to produce lytic enzymes and secondary metabolites highlights its potential for broad-spectrum biocontrol applications [38]. However, its activity might depend on bacterial viability and environmental compatibility, which need to be considered for large-scale implementation [39].

Although *Clonostachys rosea* was effective, its slow growth could limit its field application, especially when rapid colonization is needed to suppress pathogens. This limitation suggests that its use might be more beneficial in integrated biocontrol strategies where it is introduced early or in combination with faster-growing antagonists [40]; [41]. Similarly, *Pseudomonas fluorescens* showed promise in inhibiting *Ph. minimum*, indicating that bacterial antagonists could play a significant role in preventing fungal infections at early infection stages by competing for nutrients and space [42].

The lower inhibition effect observed with *Saccharomyces* suggests that yeast-based biocontrol agents may require synergistic interactions with other bioagents to enhance their effectiveness. This aligns with previous studies that have shown yeast-antagonist combinations to be more successful than yeast alone in controlling fungal pathogens [43]; [44].

Food Poisoning assay

Figure 3 emphasizes the notable inhibition percentages attained by the antagonists *Trichoderma harzianum* demonstrated significant efficacy, inhibiting the mycelial growth of *Ph. hungaricum* by 70.5% and *Ph. aleophilum* by 86.8%. Compared to *S. cerevisiae*, *C. rosea*, and *B. subtilis*, *T. harzianum* demonstrated superior antagonistic activity, with inhibition rates of 70.5% and 86.8% for *Ph. hungaricum* and *Ph. aleophilum*, respectively. *T. harzianum* produces high concentrations of cell-wall-degrading enzymes, such as α -1,3-glucanases and various chitinolytic enzymes, which play a crucial role in mycoparasitism. These enzymes have been purified and characterized, demonstrating their ability to inhibit spore germination and hyphal elongation of pathogenic fungi in vitro [45].

Bacillus subtilis emerged as the second most potent antagonist, suppressing the mycelial development of *Ph. hungaricum* (61.6%) and *Ph. aleophilum* (80.3%). *Bacillus* species use various methods, such as producing extracellular enzymes, antibiotic lipopeptides, surfactants, and hormones, to inhibit pathogenic fungi and consequently lower disease occurrence. [46]; [47]; [48]. Key genes involved in lipopeptide biosynthesis have been identified in the tested endophytic bacterial strains. The efficacy of bacterial antagonists in controlling fungal diseases is often enhanced when used alone and, in some cases, in combination with fungicides [49].

Saccharomyces showed lower effectiveness, diminishing the growth of *Ph. hungaricum* by 17%, but it exhibited significant inhibition against *Ph. aleophilum*, resulting in an 80.4% decrease. This indicates that the antagonist's efficacy might change based on the specific pathogen and control strategy. Earlier research has indicated that metabolites derived from various yeast species frequently demonstrate restricted inhibitory activities against fungal pathogens. [50].

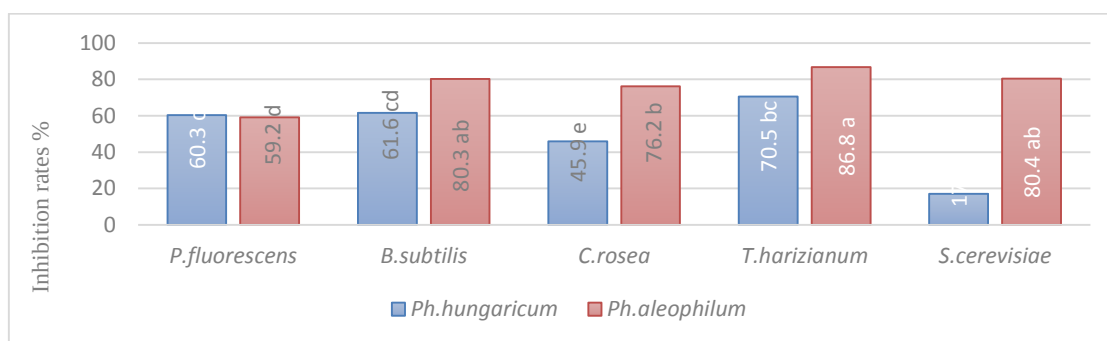


Figure 3: inhibition percentage of *Phaeoacremonium* species mycelial growth by bioagent concentration

Figure 4 shows the efficacy of bioagent filtrates, where *T. harzianum* demonstrates the greatest inhibition rate, decreasing the growth of all examined species by 78.6%. *B. subtilis* came in second with a 70.9% inhibition rate, while *C. rosea* followed with a 61% inhibition.

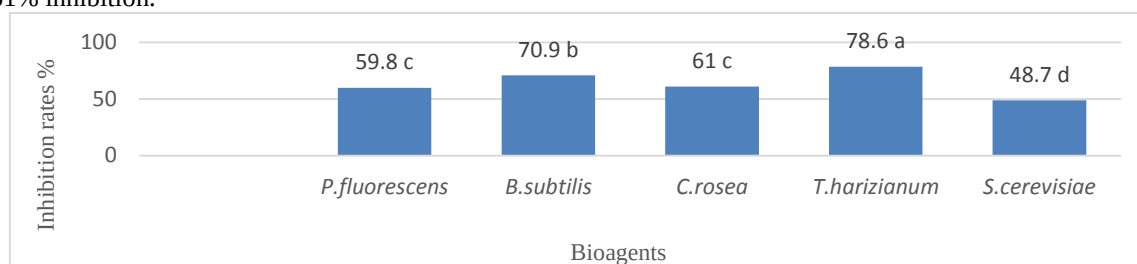


Figure 4: Inhibition percentages by different bioagents

These findings emphasize the differential effectiveness of biocontrol agents against various fungal pathogens. The variation in inhibition rates may be attributed to the unique mechanisms of action employed by each antagonist. *T. harzianum*'s superior inhibitory capacity can be linked to its aggressive enzymatic degradation of fungal cell walls through the production of chitinases, glucanases, and proteases [51];[52]. *B. subtilis*'s effectiveness is associated with its multifaceted antimicrobial strategies, including the production of lipopeptides, antibiotics, and biofilm formation that inhibit pathogen growth and colonization [53];[54]. The relatively lower efficacy of *Saccharomyces cerevisiae* may be due to its indirect antagonistic mechanisms, such as competition for nutrients and space, rather than direct enzymatic or antibiotic actions [55];[56]. Antagonistic microorganisms, including bacteria, yeast, and filamentous fungi, are part of various taxonomic groups and possess various antagonistic properties. These microbes produce pathogen-specific antifungal metabolites that can suppress or even eradicate pathogens, inhibiting their growth and disrupting their normal development. In doing so, they reduce the impact of nearby phytopathogens. The intensity and methods of fungal growth inhibition differ across various phytopathogens. Microscopic examination of inhibition zones has revealed significant alterations in the fungal growth structure. Despite these differences, the general pattern of mycelial changes caused by biocontrol agents tends to be consistent for the same fungal species. Even if a particular bioagent is not fully effective against *Phaeoacremonium* species, it can still demonstrate strong antagonistic activity against one or more species. Effectively controlling one pathogenic species with fewer side effects can be particularly advantageous, as the combined presence of multiple pathogens can lead to more severe disease than each pathogen acting alone. This makes the targeted use of antagonistic microbes a valuable strategy in integrated pest and disease management [57];[58].

Evaluation of Microbial Antagonists Using Detached Cane Assay.

The potential spread of bacterial strains, such as *Pseudomonas* and *Bacillus*, along with fungal strains like *Trichoderma*, *Clonostachys*, and *Saccharomyces*, through nursery propagation materials was assessed by inoculating the grapevine canes with *Phaeoacremonium aleophilum* and *P. hungaricum*. As shown in Figure 5, *Clonostachys rosea* exhibited the most significant effect in suppressing both pathogens, reducing canker length to 10.6 mm and 13 mm, respectively. In contrast, the weakest impact was observed when grapevine canes were immersed in a *Pseudomonas fluorescens* cell suspension, resulting in canker lengths of 21.3 mm. Treatment with a *Trichoderma harzianum* conidial suspension limited the canker lengths caused by *P. aleophilum* and *P. hungaricum* to 16.3 mm and 18.3 mm, respectively. Similarly, a *Bacillus subtilis* cell suspension effectively inhibited *P. aleophilum* (16.3 mm) and *P. hungaricum* (14 mm). Additionally, *Saccharomyces cerevisiae* restricted the development of *Ph. aleophilum* (16.6 mm) and *P. hungaricum* (13.3 mm).

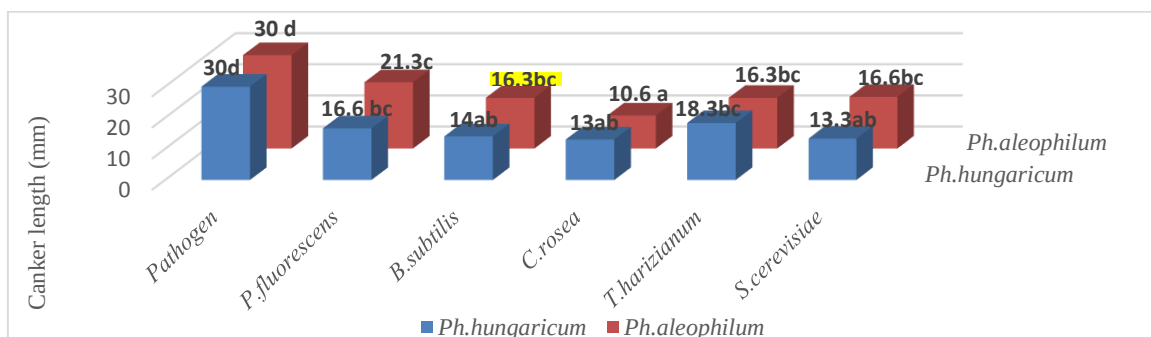


Figure 5: Detached Grapevine Cane Assay of Microbial Biocontrol Agents Against *Ph. aleophilum* and *Ph. hungaricum*

The detached cane assay further confirmed the efficacy of these microbial antagonists in a plant-based model. *Clonostachys rosea* exhibited the strongest suppression of *P. aleophilum* and *P. hungaricum*, significantly reducing canker lengths. These findings align with previous reports suggesting that *C. rosea* effectively controls fungal pathogens through competition, mycoparasitism, and the production of antifungal metabolites [59]. In contrast, the least effective treatment was *Pseudomonas fluorescens*, which resulted in longer canker lengths, suggesting a weaker biocontrol effect in this assay. *Trichoderma harzianum* is widely recognized for its strong biocontrol properties, particularly against fungal pathogens, due to its ability to produce a variety of bioactive compounds. These include cell-wall-degrading enzymes such as chitinases, glucanases, and proteases, which contribute to its mycoparasitic behavior. Mycoparasitism involves the parasitization of pathogenic fungal hyphae, leading to their suppression and eventual death. Furthermore, *T. harzianum* produces secondary metabolites, such as gliotoxins and viridins, which exhibit antifungal activity by disrupting cellular functions in pathogens [60]; [61].

Conclusion

This study evaluated the biocontrol potential of various microbial agents against *Phaeoacremonium* species that cause grapevine trunk diseases. *Trichoderma harzianum* showed the highest antagonistic activity in both dual culture and food poisoning assays, significantly inhibiting *P. aleophilum* and *P. hungaricum*. In Detached Cane Assay, *Clonostachys rosea* proved to be the most effective in reducing canker length, while *Bacillus subtilis* and *Saccharomyces cerevisiae* exhibited moderate and variable effects. These findings support the integration of microbial antagonists as environmentally friendly alternatives in strategies for managing diseases in grapevines.

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فعالية المكافحة الحيوية ضد نوعين من فطريات *Phaeoacremonium* المرتبط باصابات جروح التقليم في كرمات العنب.

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الخلاصة

تم تلقيح عقلات الكرمة بأنواع *Phaeoacremonium* (*Ph. hungaricum* و *Ph. aleophilum*)، وظهرت على العقل تغيراً في اللون إلى البني وتقرحات نخرية. في كل من اختباري التثبيط المزدوج واختبار التسمم الغذائي، أظهر كل من *Trichoderma harzianum* و *Bacillus subtilis* أعلى نشاطاً تضادياً ضد *Phaeoacremonium aleophilum* و *P. hungaricum*، حيث سجل *T. harzianum* معدلات تثبيط تصل إلى 93.3%، يليه *B. subtilis* بنسبة تصل إلى 80.3%، في حين أظهر كل من *Saccharomyces cerevisiae* و *Clonostachys rosea* فعالية متوسطة إلى ضعيفة ضد العزلات الفطرية المختبرة. وظهر *Clonostachys rosea* قدرة على تثبيط *P. aleophilum* بنسبة 67.5% و *P. hungaricum* بنسبة 42.3%. وقد تم تقييم كفاءة العوامل الحيوية في الحد من الممرضات باستخدام اختبار القصب المنفصل (*Detached Cane Assay*) على مواد الإكثار في المشاتل، وأظهرت النتائج أن *C. rosea* كان الأكثر فعالية في تقليل طول التقرحات (10.6–13 مم)، في حين أن *Pseudomonas fluorescens* كان الأقل تأثيراً (21.3 مم). وأظهرت المعاملات الأخرى، بما في ذلك *T. harzianum* و *B. subtilis* و *S. cerevisiae*، درجات متفاوتة من تقليل التقرحات.

الكلمات المفتاحية: *Phaeoacremonium aleophilum*، *Ph. hungaricum*، العنب، *Trichoderma harzianum*، *Bacillus subtilis*.