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Antifungal Potential of Cyanobacterium *Nostoc* sp. BCAC 1226 Suspension as a Biocontrol Agent Against Phytopathogenic Fungi and Oomycetes

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Abstract: Cyanobacteria are gaining significant importance as potential biocontrol agents against phytopathogenic fungi. We evaluated the inhibitory effects of a suspension of *Nostoc* sp. BCAC 1226 on *Penicillium* sp., *Phytophthora* sp., and *Stemphylium* sp. in vitro using potato dextrose agar medium. On the 7th day of incubation, *Phytophthora* sp. showed a reduction in colony area from 18.30 ± 1.68 to 8.55 ± 0.74 cm^2 (53.6% inhibition). Similarly, *Penicillium* sp. showed a reduction from 17.64 ± 1.46 to 8.90 ± 0.36 cm^2 (49.4% inhibition), and *Stemphylium* sp. showed a reduction from 17.76 ± 1.28 to 13.5 ± 0.73 cm^2 (23.7% inhibition). The inhibitory effects were more significant on the 14th day, with the growth of *Phytophthora* sp. further reduced to 4.9 ± 0.40 cm^2 (72.8% inhibition), *Penicillium* sp. to 5.54 ± 0.32 cm^2 (68.8% inhibition), and *Stemphylium* sp. to 8.71 ± 0.31 cm^2 (50.8% inhibition). These results indicate the potential antifungal activity of *Nostoc* sp. suspension, with the highest reduction observed in *Phytophthora* sp., followed by *Penicillium* sp. and *Stemphylium* sp. Future research should focus on the chemical characterization of the antifungal metabolites produced by *Nostoc* sp. BCAC 1226 and in vivo evaluations on economically important crops to evaluate their practical efficiency under field conditions.

Keywords: agriculture; antifungal activity; bio fungicide; cyanobacteria; fungal growth; ecological sustainability; plant protection; *Penicillium*; *Phytophthora*; *Stemphylium*

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1. Introduction

Despite the extensive application of various plant disease control agents, effective management of phytopathogens remains a major challenge in global agriculture. Furthermore, environmental pollution weakens plant defense mechanisms, making crops more vulnerable to infection. The increasing diversity of plant pathogens has also led to the adaptation of certain microorganisms and their transition from saprotrophic to facultative parasitic nutrition. In recent years, the use of biocontrol agents has gained significant attention as a sustainable alternative to chemical pesticides for plant disease management [1–3]. In this context, beneficial bacteria and fungi have been extensively studied for their biocontrol

potential in the management of plant diseases [4–9]. However, despite their diversity, distribution, and photosynthetic abilities, cyanobacteria have received comparatively less attention as potential biological control agents [10].

Cyanobacteria (blue-green algae) are a diverse group of Gram-negative photoautotrophic bacteria with two photosystems (PSI and PSII). These microorganisms use water as an electron donor during photosynthesis and release oxygen as a byproduct [11]. Cyanobacteria play a significant role in enhancing soil fertility through nitrogen and carbon fixation [12], thereby promoting plant growth and productivity [13]. Moreover, they produce a wide range of bioactive metabolites with antibacterial, antiviral, and antifungal properties [1,14,15].

Several bioactive compounds with antibacterial activity have been isolated from cyanobacteria, including ambiguine I isonitrile and hapalindole T, produced by *Fischerella* sp. [16,17], and lyngbyazothrins A-D from *Lyngbya* sp. [18]. Furthermore, fatty acids, carbohydrates, flavonoids, peptides, terpenes, and various secondary metabolites also contribute to antibacterial activity [14]. Cyanobacterial metabolites have also shown antiviral activities. For example, cyanovirin-N from *Nostoc ellipsosporum* and *Cyanothece* sp. [19], microvirin from *Microcystis aeruginosa* [14], and calcium spirulan from *Arthrospira platensis* [20] have demonstrated antiviral properties.

In relation to antifungal activity, cyanobacteria produce metabolites, such as butylated hydroxytoluene, hexadecanoic acid, and methyl ester from *M. aeruginosa*, which inhibit mycotoxigenic fungi [1]. Glycosylated lipopeptides, such as hassallidins from *Hassallia* sp. and *Anabaena* sp., inhibit the growth of *Candida albicans* and *Aspergillus flavus* [21–23]. Other valuable compounds include lyngbyabellins from *Lyngbya majuscula, Lyngbya bouillonii,* and *Moorea bouillonii*; microguanidines from *Microcystis* sp. (TAU IL-306) and *M. aeruginosa*; majusculamides from *Lyngbya polychroa* and *Lyngbya majuscule*; and 4,4′-dihydroxybiphenyl and norharmane from *Nostoc insulare* and *Nodularia harveyana* have also shown antifungal properties [1,14].

Several studies have used standard microbiological assays, such as the agar plate diffusion test, to evaluate the antifungal properties of cyanobacteria [24]. For example, cell extracts and culture media from ten cyanobacterial strains, including *Oscillatoria*, *Hapalosiphon*, *Fischerella*, and *Stigonema* species, have shown antifungal activities against *Candida kefyr* (Beij.) Uden et Buckley ex Mey. et Ahearn ATCC 38296. Among these, only the petroleum ether and methanol extracts of *Hapalosiphon hibernicus* West et West FS33 showed antifungal properties against *C. albicans* ATCC 14053 [13]. Similarly, 15% extracts from *Phormidium fragile* Gomont have shown antifungal effects against *A. flavus*, *C. albicans*, and *Trichoderma viride* Schumach [25]. In South Korea, a screening of 14 cyanobacterial genera identified antifungal activity in nine strains against phytopathogenic fungi affecting *Capsicum annuum* L. [26]. In addition, species such as *Anabaena subcylindrica* Borge, *Nostoc muscorum* C. Agardh ex Bornet & Flahault, and *Oscillatoria angusta* Koppe exhibited antifungal effects against fungal pathogens isolated from *Vicia faba* [27]. A previous study reported that an increase in the concentration of cyanobacterial filtrates decreased fungal growth [27].

Among cyanobacteria, the genus *Nostoc* Vaucher ex Bornet & Flahault is characterized by its cosmopolitan distribution [28–30] and ability to synthesize diverse biologically active compounds [31,32]. These properties highlight the valuable applications of *Nostoc* spp. in ecology, biotechnology, and medicine [33]. Moreover, antifungal peptides, such as nostocyclamide, a cyclic hexapeptide with allelochemical properties, have been identified in *Nostoc* spp. [34,35]. Nostocyclamide has shown significant antifungal activity, particularly against *Saccharomyces cerevisiae* (Desm.) Meyen [34,36]. Another antifungal peptide, nostofungicidine, a cyclic lipopeptide from *Nostoc commune*, also inhibited the growth of

Aspergillus candidus [37]. Cyclopeptides from the Laxaphycin family, such as heinamide, exhibit antagonistic activity against *A. flavus* [38,39].

Cyanobacterial oligopeptides also exhibit antifungal properties, including cryptophycin, a cyclic depsipeptide isolated from *Nostoc* spp. [40,41]. Initially, it was studied for its antifungal activity against *Cryptococcus neoformans* and later gained significant attention for its anticancer potential [42]. Other bioactive compounds include phycobiliproteins (phycoerythrin) [43], polyphenolic metabolites (4,4′-dihydroxybiphenyl) [44,45], and alkaloids (norharmane and nostocarboline), which have shown diverse antimicrobial activities [46,47].

Several commercial biofungicides have been developed based on the use of beneficial microorganisms. *Trichoderma* spp. are widely used as an active component in products such as Promot WP® (JH Biotech Inc., Ventura, CA, USA), Trichosoil® (Lage y CIA, Montevideo, Uruguay), Trianum -P® (Koppert, Haverhill, UK), TUSAL® (Certis, Columbia, MD, USA), ASPERELLO T34® (Biocontrol, Barcelona, Belgium), EsquiveWP® (Agrauxine, Marq-en-Baroeul, France), Vintec ® (Bi-PA NV/SA, Destelbergen, Belgium), and Remedier® (Isagro, Milan, Italy) [3,48]. Other fungal biocontrol agents include *Clonostachys* spp., which are utilized in products such as Lalstop G46® WG (Lallemand Plant Care, Milwaukee, WI, USA) [48]. Similarly, bacterial genera such as *Bacillus subtilis* have been incorporated into formulations such as Serenada®, Kodiak® (St. Louis, MO, USA), Rhizo-plus® (Andermatt Group AG, Grossdietwil, Germany), Baktofit® (Sibbiopharm, Sibiryak, Novosibirsk Region, Russia), and Phytosporin® (BashInkom, Ufa, Bashkortostan, Russia); *Streptomyces griseus* is the active component in Phytolavin® (Pharmbiomed, Kursk, Russia); and *Pseudomonas fluorescens* is used in the development of Planriz® (KompaBelarus, Minsk, Russia) [2]. However, biofungicides formulated with cyanobacteria, particularly *Nostoc* spp., as active components, remain largely unexplored. Although numerous studies have reported the antifungal properties of cyanobacteria, including *Nostoc* spp., there is currently a lack of commercially approved biofungicide formulations based on these organisms [1,14,49–52].

Despite the growing evidence of the antifungal properties of *Nostoc* spp., their fungicidal activity against phytopathogens remains largely unexplored. Fungal pathogens from the genera *Phytophthora* de Bary [53], *Stemphylium* Wallroth [54], and *Penicillium* [55] are among the most harmful and destructive microorganisms, affecting food and ornamental plants and causing significant economic losses. The genus *Phytophthora* comprises 203 species that are phylogenetically placed within the phylum Oomycota, although they exhibit morphological resemblance to true fungi [56]. Several species within this genus pose a serious threat to global agriculture, with certain strains causing yield losses of more than 50% in crops, such as potatoes [2,57]. *Stemphylium* is a genus of filamentous ascomycetes that includes both plant pathogenic and saprophytic representatives that are widely distributed across diverse agroecological zones [54,58,59]. The genus *Penicillium*, which belongs to the phylum Ascomycota, comprises 354 species that are widely distributed across diverse habitats [55]. While certain *Penicillium* species have a significant impact on plant health and soil ecosystems, several others act as phytopathogens [60,61], leading to plant diseases and extensive tissue damage, including leaf spotting, seed necrosis, fruit rot, and post-harvest spoilage [62–64].

The present study aimed to evaluate the anti-phytopathogenic potential of *Nostoc* sp. suspension against representatives of the genera *Phytophthora*, *Stemphylium*, and *Penicillium*, using a modified methodology for quantifying colony dimensions. This study aims to contribute to the development of sustainable, biologically derived alternatives for the effective management of plant diseases.

2. Materials and Methods

2.1. Cyanobacterial Cultivation

The cyanobacterial strain *Nostoc* sp. BCAC 1226 (Figure 1), obtained from the Bashkortostan Collection of Algae and Cyanobacteria (BCAC), was used in this study. This strain was originally isolated from the LB2 sample collected from the Kabeku River area near the Shiveluch volcano, Kamchatka Peninsula, within a larch–birch forest with poplar vegetation (Figure 1).

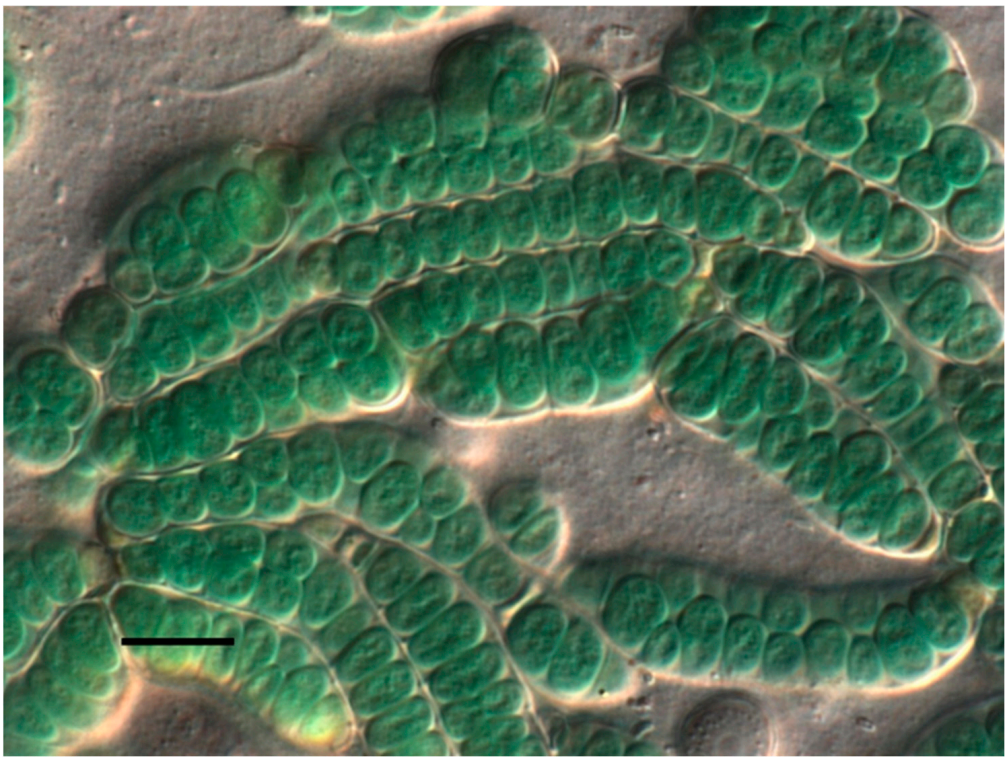

Figure 1. Microscopic visualization of *Nostoc* sp. BCAC 1226. Scale bar 10 µm.

Nostoc sp. BCAC 1226 was cultivated in Z8 medium, as described by Carmichael et al. [65]. The composition and concentrations of nutrients in the Z8 medium are detailed in the Supplementary Materials (Table S1). Briefly, *Nostoc* sp. BCAC 1226 cultures were maintained in sterile glass tubes and 250 mL Erlenmeyer flasks under continuous aeration and illumination using 18W cool fluorescent tubes (Philips TLD18W/33, Philips Lighting Poland S.A., Pila, Poland) at an intensity of 40 µmol m^{-2} s^{-1}. The photoperiod was maintained at a 12:12 h light–dark cycle, and the incubation temperature was maintained at 25 °C. Prior to the experimental assay, the *Nostoc* sp. BCAC 1226 suspensions were scaled up in 1 L Erlenmeyer flasks and incubated for two weeks. The biomass was harvested during the exponential growth phase, typically achieved within 10 days under the specified growth conditions, to ensure maximum metabolic activity and cell viability. The resulting suspension had a cell density of approximately 10^7 cells mL^{-1}, which was standardized for all the experimental procedures. The biomass concentration was determined by measuring the optical density (OD) at 680 nm using a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan). The suspension was adjusted to an OD of ~0.8, which corresponded to the target cell concentration (10^7 cells mL^{-1}), based on the calibration curves established during preliminary experiments.

For the antifungal bioassays, 10 mL of whole-cell suspensions of *Nostoc* sp. BCAC 1226 was then applied to the fungal plates. All cultivation and experimental procedures

were conducted under sterile conditions using a laminar airflow hood, following standard microbiological protocols to prevent contamination [66].

2.2. Cultivation of Phytopathogens

Phytopathogenic representatives from the genera *Phytophthora*, *Stemphylium*, and *Penicillium* were isolated from infected plant tissues, including potato leaves, tomato fruits, and tulip bulbs, respectively. The isolates were cultured on potato dextrose agar (PDA) plates according to standard methods [67]. Briefly, PDA medium was prepared by boiling 200 g of washed and sliced potatoes in 1000 mL of distilled water for 40 min. The extract was filtered through sterile gauze and 20 g of agar was added. The final volume was adjusted to 1000 mL with distilled water and autoclaved at 121 °C for 20 min. The sterilized medium was poured into Petri dishes (7–10 mL per dish) under aseptic conditions.

The repeatability of the experiments was set to ten independent trials. Fungal cultures were inoculated onto PDA plates using a sterile loop and evenly spread across the surface with a glass spatula. The plates were incubated at 25 °C for seven days prior to the antifungal assays.

2.3. Experimental Design

Antifungal and anti-oomycete activities of *Nostoc* sp. BCAC 1226 suspension was evaluated using three sets of Petri dishes containing pre-grown phytopathogens treated with 30-day-old *Nostoc* sp. BCAC 1226 suspension. Control plates were treated with an equal volume of Z8 medium instead of *Nostoc* sp. BCAC 1226 suspension. All plates were incubated under the same photoperiod (12:12 h light–dark cycle) and temperature (25 °C), as described previously. Pathogen growth was monitored at 7 days and 14 days post-treatment.

The anti-phytopathogenic activity was evaluated using the agar diffusion method [68] with modifications, wherein the inhibitory effect of *Nostoc* sp. BCAC 1226 was determined by measuring the reduction in fungi and oomycetes compared to that in the control. The experiment was conducted in triplicate, with ten independent repetitions per treatment.

2.4. Data and Statistical Analysis

The growth area of phytopathogens in Petri dishes was calculated using a modified version of the established methodologies [69,70]. Photographs of all Petri dishes from each experimental treatment were captured to enhance the accuracy of the analysis. The fungal growth regions were marked directly on the Petri dish surface using a permanent marker and segmented into geometric shapes, such as circles, rectangles, triangles, and rhombuses, to facilitate area calculation. The total colony area was determined using standard geometric formulas, as described by Bhuyar et al. [71]:

$$\text{Circle: } S = \frac{\pi d^2}{4} \tag{1}$$

where d is the diameter

$$\text{Rectangle: } S = a \times b \tag{2}$$

where a and b are the sides

$$\text{Isosceles Triangle: } S = \frac{\left(a \times \sqrt{b^2 - \frac{a^2}{4}}\right)}{2} \tag{3}$$

where a is the isosceles side and b is the base

$$\text{Scalene Triangle (Heron's formula): } S = \sqrt{p(p-a)(p-b)(p-c)} \quad (4)$$

where a, b, c are the sides and $p = \frac{(a+b+c)}{2}$

$$\text{Rhombus: } S = \frac{d_1 d_2}{2} \quad (5)$$

where d_1 and d_2 are the diagonals.

The total fungal growth area per Petri dish was calculated by adding the individual geometric areas of the colonies.

In the statistical analysis, descriptive statistics such as arithmetic mean, standard error, median, standard deviation, and coefficient of variation were determined [72]. The statistical significance was determined using Student's *t*-test [73] to compare pathogen growth between treated and control samples. All statistical analyses were performed using Statistica for Windows, version 10.0 (StatSoft Inc., Tulsa, OK, USA) [46]. Differences were considered statistically significant at $p < 0.05$.

3. Results

The antifungal and anti-oomycete activities of *Nostoc* sp. BCAC 1226 were evaluated by quantifying the colony growth areas of the phytopathogenic fungi *Stemphylium* sp. and *Penicillium* sp. and the oomycete *Phytophthora* sp. on the 7th and 14th days after inoculation. The application of *Nostoc* sp. BCAC 1226 suspension resulted in a significant reduction in fungal growth compared to the untreated control (Table 1).

Table 1. Effect of *Nostoc* sp. BCAC 1226 suspension on colony area measurements of phytopathogens on the 7th and 14th days post-inoculation.

Variant of Experiment		X_{min}, cm²	X_{max}, cm²	X ± S, cm²	σ	Me	CV (%)	*t*
7th Day Post-Inoculation								
Phytophthora sp.	Control	11.35	29.29	18.30 ± 1.68	5.31	16.78	29.03	-
	Experiment	5.32	13.28	8.55 ± 0.74	2.35	8.14	27.44	5.31 *
Stemphylium sp.	Control	11.94	24.17	17.76 ± 1.28	4.04	17.54	22.72	-
	Experiment	10.43	16.74	13.50 ± 0.73	2.31	13.24	17.08	2.89 *
Penicillium sp.	Control	11.36	25.38	17.64 ± 1.46	4.63	17.47	26.24	-
	Experiment	6.62	10.93	8.90 ± 0.36	1.13	8.95	12.72	5.80 *
14th Day Post-Inoculation								
Phytophthora sp.	Control	11.13	29.30	18.03 ± 1.73	5.46	16.04	30.26	-
	Experiment	3.17	7.16	4.96 ± 0.41	1.29	4.70	26.03	7.37 *
Stemphylium sp.	Control	11.78	24.03	17.60 ± 1.28	4.04	17.20	22.95	-
	Experiment	6.97	9.97	8.71 ± 0.31	0.97	8.83	11.09	6.77 *
Penicillium sp.	Control	11.20	24.37	17.50 ± 1.43	4.53	18.06	25.88	-
	Experiment	3.91	7.33	5.54 ± 0.32	1.03	5.31	18.52	8.15 *

Note: X_{min}—minimum value of the attribute; X_{max}—maximum value of the attribute; X ± S—arithmetic mean and its standard error; Me—median; σ—standard deviation; and CV—coefficient of variation. *t* represents the Student's coefficient. Values marked with an asterisk (*) indicate statistical significance at $p < 0.05$, based on Student's criterion.

On the 7th day post-inoculation, a significant reduction in the colony area of fungal and oomycete growth was observed in the *Nostoc*-treated plates compared to the control (Table 1). *Phytophthora* sp. showed a significant reduction in mean colony area from 18.30 ± 1.68 cm^2 in the control to 8.55 ± 0.74 cm^2 in the treated samples ($t = 5.31$, $p < 0.05$) (Table 1). Similarly, *Stemphylium* sp. showed a significant decrease in colony size from 17.76 ± 1.28 cm^2 in the control to 13.50 ± 0.73 cm^2 in the treated samples ($t = 2.89$, $p < 0.05$). The highest inhibition was recorded in *Penicillium* sp., where the colony area was reduced from 17.64 ± 1.46 cm^2 in the control group to 8.90 ± 0.36 cm^2 in the treated group ($t = 5.80$, $p < 0.05$). The coefficient of variation (CV) values indicated consistent inhibition of fungal growth, with *Stemphylium* sp. showing the lowest variability (CV = 17.08%) and *Phytophthora* sp. showing the highest degree of variation (CV = 27.44%) among the treated samples (Table 1).

Macroscopic examination further confirmed these results, with changes in morphological characteristics, including pigment loss and reduced colony expansion (Figure 2(B1–B3)). The uneven surface of the culture medium resulted from the interaction between *Nostoc* sp. BCAC 1226 suspension and phytopathogenic fungi. The production of extracellular metabolites by *Nostoc* sp., combined with the enzymatic activities of phytopathogenic fungi, likely contributed to the partial degradation of the agar medium. Such interactions have been previously reported, wherein microbial activity, through the secretion of lytic enzymes and organic acids, changes the physical characteristics of culture media. In contrast, untreated control plates showed typical morphological characteristics, such as gray coloration in *Stemphylium* sp., whereas treated samples showed lighter coloration. In addition, *Phytophthora* sp. and *Penicillium* sp. showed distinct zones of colorless inhibition, indicating significant inhibition of fungal growth. Quantitative assessments further confirmed these findings, showing a significant decrease in the fungal colony area in response to *Nostoc* sp. BCAC 1226 treatment (Table 1).

A further reduction in the fungal colony area was observed on the 14th day post-inoculation (Table 1, Figure 2C), confirming the sustained antifungal and anti-oomycete potential of *Nostoc* sp. BCAC 1226. The most significant inhibition was recorded for *Phytophthora* sp., where the colony area decreased to 4.96 ± 0.41 cm^2 in the treated samples compared to 18.03 ± 1.73 cm^2 in the control ($t = 7.37$, $p < 0.05$) (Table 1). Similarly, the mean colony area of *Stemphylium* sp. decreased to 8.71 ± 0.31 cm^2 in treated samples, from 17.60 ± 1.28 cm^2 in the control group ($t = 6.77$, $p < 0.05$). The highest reduction in growth was recorded for *Penicillium* sp., with the treated colonies showing a mean area of 5.54 ± 0.32 cm^2, which was significantly lower than that of the control (17.50 ± 1.43 cm^2; $t = 8.15$, $p < 0.05$). Analysis of CV values between the 7th and 14th days further confirmed the sustainability of the fungal growth inhibition (Table 1). Among the treated groups, *Stemphylium* sp. showed the least variability (CV = 11.09%), whereas *Phytophthora* sp. and *Penicillium* sp. showed moderate levels of variation (CV = 26.03% and 18.52%, respectively) (Table 1).

Comparative macroscopic analysis between the treated and control groups (Figure 2) revealed a time-dependent inhibition of fungal and oomycete growth. Untreated colonies showed extensive radial expansion, whereas *Nostoc* sp.-treated colonies showed decreased growth, irregular margins, and reduced hyphal density.

Statistical analyses confirmed the inhibitory effects of *Nostoc* sp. BCAC 1226 suspensions. Student's *t*-test revealed significant differences between the treated and control groups for all phytopathogens ($p < 0.05$), confirming the effectiveness of all the tested antifungal agents. The inhibitory responses varied among species, with *Penicillium* sp. and *Phytophthora* sp. showing rapid inhibition within the first week, whereas *Stemphylium* sp. showed a delayed but significant reduction in growth between days 7 and 14. This

variability suggests that the antifungal and anti-oomycete activities of *Nostoc* sp. BCAC 1226 may be influenced by species-specific factors, such as differences in cell wall structure, metabolic activity, and sensitivity to cyanobacterial bioactive compounds. These findings highlight the potential application of *Nostoc* sp. BCAC 1226 as a natural antifungal agent for plant protection.

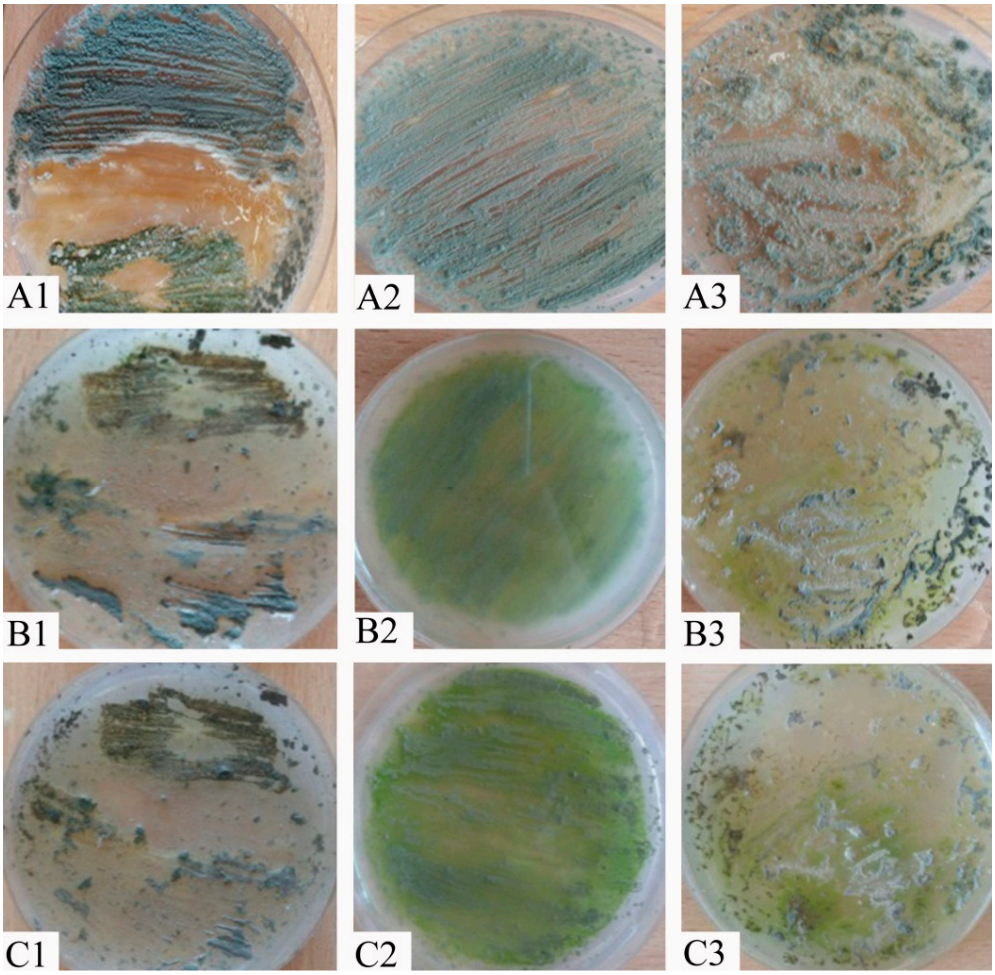

Figure 2. Morphological changes in *Phytophthora* sp. (1st column), *Stemphylium* sp. (2nd column), and *Penicillium* sp. (3rd column) in response to *Nostoc* sp. BCAC 1226 suspension: (**A**) pre-treatment (control); (**B**) 7th day and (**C**) 14th day post-treatment.

4. Discussion

The findings of this study demonstrate the potential antifungal and anti-oomycete activities of the *Nostoc* sp. BCAC 1226 against three significant phytopathogenic genera: *Phytophthora*, *Penicillium*, and *Stemphylium*. These results are consistent with previous research findings, which highlight the antifungal potential of *Nostoc* spp. and other cyanobacteria against fungi.

Several studies have reported the antifungal activity of various *Nostoc* species. For instance, *N. commune* FK-103 has demonstrated potential anti-phytopathogenic activity against *Phytophthora capsici* [26]. Similarly, ethanolic extracts of *Nostoc calcicola*, isolated from Central India, have shown inhibitory effects against diverse fungal pathogens, including *Penicillium chrysogenum* [74]. Moreover, *Nostoc* strain ATCC 53789 produces cryptophycin, an antifungal compound that effectively inhibits the growth of *Penicillium expansum* [42]. In addition, *Nostoc enthophytum* and *N. muscorum* have shown antifungal activity against *Rizoctonia solani* under both in vitro and greenhouse conditions [75]. Notably, most previous

studies evaluating the antifungal properties of *Nostoc* relied on methanolic extracts, whereas the present study used whole-cell suspensions of live cyanobacteria. While methanolic extracts may contain concentrated secondary metabolites, the extraction process may lead to the degradation of thermolabile or volatile bioactive compounds, such as vitamins, hormones, and certain signaling molecules. Therefore, the use of intact suspensions provides a wide range of biologically active compounds that potentially enhance antifungal efficiency by preserving their natural biochemical diversity.

This study expands on previous findings by demonstrating that whole-cell suspensions of *Nostoc* sp. BCAC 1226 exhibited significant antifungal effects compared to those of the extracted metabolites. This suggests that the inhibitory activity is likely due to a complex mixture of secondary metabolites, potentially through synergistic interactions between the compounds.

The antifungal effects observed in *Nostoc* sp. BCAC 1226 are mediated through diverse mechanisms, including antibiosis, competitive exclusion, and stimulation of plant defense response. Antibiosis, which involves the production of antifungal metabolites, is a well-established strategy used by *Nostoc* spp. to inhibit pathogenic fungi [76]. *Nostoc* sp. are known to synthesize a diverse range of antifungal compounds, including cryptophycin, nostodione A, nostocyclamide, cyanopeptolins, nostocyclopeptides, muscotoxins A and B, and other bioactive peptides [26,77–82]. For instance, strains of *Nostoc* spp. 6sf Calc and CENA 219 have been reported to produce antifungal glycolipopeptides against *C. albicans* and *A. flavus* [22]. *Nostoc linckia,* under Zn and Cu stress conditions, produces flavonoids, phycobiliproteins, phenolic compounds, and tannins [83]. Moreover, *Aliinostoc alkaliphilum* sp. nov. strains isolated from a shallow lake in Brazil excreted Nocuolin A, which exhibits antibacterial and antifungal properties [49]. In addition, *Desmonostoc alborizicum* produces hydrolytic enzymes (chitosanase, protease, FPase, carboxymethyl cellulose, xylanase, cellobiohydrolases, and cellobiase), which are effective against several phytopathogens, such as *Alternaria alternata*, *Fusarium solani*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Verticillium dahlia,* and *Phytophthora* spp. [50]. These antifungal and anti-oomycete metabolites disrupt fungal cell membranes, inhibit spore germination, and interfere with critical metabolic pathways, thereby preventing the establishment and proliferation of pathogens.

The antifungal properties observed in this study are consistent with those reported for *Nostoc* spp. For example, Domracheva et al. [84] demonstrated that biofilms of *Nostoc paludosum* N18 and *Nostoc linckia* N273 have significantly inhibited the growth of *F. oxysporum*, *Fusarium nivale*, and *Fusarium culmorum*. Similarly, a methanolic extract of *N. linckia* moderately reduced the radial growth of *F. oxysporum* f. sp. lycopersici [85]. The methanolic extract of *Desmonostoc alborizicum* significantly inhibited the development of *F. oxysporum* [86]. *N. muscorum* has also been shown to reduce the linear growth of *Alternaria porri* (20.37%) [46]. Similarly, *N. linckia* 612 has been shown to be active against phytopathogens of the genera *Alternaria* and *Fusarium* [82]. In another study, *Nostoc piscinale* SCAU04 and *Anabaena variabilis* SCAU26 effectively inhibited *R. solani*, the causal agent of sheath blight in rice [87]. These findings highlight the wide range of anti-phytopathogenic potentials of cyanobacteria.

The anti-phytopathogenic activity of *Nostoc* sp. BCAC 1226 strain is, in some instances, comparable to that of widely used commercial biofungicides. The genus *Trichoderma* is one of the most commonly used microorganisms in various biofungicides [3,88], and its representatives have been shown to inhibit the growth of numerous fungal species [3,88]. However, against the studied pathogens (*Phytophthora*, *Penicillium*, and *Stemphylium* spp.), Nostoc sp. BCAC 1226 exhibited potentially more effective inhibitory action than that of *Trichoderma*.

In addition to antibiosis, cyanobacteria have been shown to produce phytohormones and other bioactive compounds that enhance plant resistance to infections. The dual role of *Nostoc* spp. as biocontrol agents and plant growth promoters makes them particularly promising for sustainable agriculture [81,89]. *Nostoc* sp. BCAC 1226 inhibits various fungal and oomycete genera, suggesting that it produces a complex mixture of bioactive metabolites that act through synergistic or complementary mechanisms to enhance pathogen inhibition.

While numerous studies have focused on the antifungal properties of isolated cyanobacterial metabolites, this study demonstrates that whole-cell suspensions provide additional advantages. Cyanobacterial suspensions contain a complex mixture of bioactive compounds, enzymes, and secondary metabolites that interact to enhance their anti-phytopathogenic activity [90,91]. In contrast, purified compounds may have limited efficacy because of degradation or the absence of synergistic factors present in the natural cyanobacterial matrix.

Previous studies have utilized cyanobacterial suspensions to assess their anti-phytopathogenic activity and plant growth-promoting effects on plants. For instance, many researchers have utilized microalgal suspensions with cell densities ranging from 8×10^5 to 3×10^7 cells mL^{-1} to evaluate their effects on phytopathogens and plant health [84,92–94]. Similarly, our study suggests that whole-cell suspensions of *Nostoc* sp. BCAC 1226 represent an ecologically relevant and sustainable approach for evaluating biocontrol potential in natural and agricultural ecosystems. Furthermore, cyanobacterial suspensions are environmentally sustainable alternatives to synthetic fungicides. Unlike chemical fungicides, which pose risks such as environmental contamination, toxicity to non-target organisms, and the emergence of fungicide-resistant strains, cyanobacterial suspensions provide a biologically derived, biodegradable, and potentially more resilient strategy for pathogen control in agriculture.

The findings of this study contribute to the growing body of evidence supporting the use of cyanobacteria as biocontrol agents in agricultural systems. *Nostoc* sp. BCAC 1226 inhibited *Phytophthora*, *Penicillium*, and *Stemphylium* spp., suggesting its potential application in integrated disease management programs to reduce reliance on synthetic fungicides. Future research should focus on optimizing application strategies, evaluating the persistence of cyanobacterial suspensions under field conditions, and assessing their compatibility with conventional agricultural practices to enhance efficacy.

In addition, detailed chemical characterization of the antifungal metabolites produced by *Nostoc* sp. BCAC 1226 provides valuable insights into its mode of action. Genome sequencing and metabolomic analyses could further elucidate the biosynthetic pathways responsible for antifungal activity, enabling targeted enhancement of antifungal properties through biotechnological approaches.

The sustainability of *Nostoc* suspensions in inhibiting fungal pathogens can be further discussed by comparing their efficiency with that of existing biological control agents, such as plant extracts and beneficial bacteria. Several studies have reported that *Nostoc* sp., particularly the Nostoc ATCC strain 53789, exhibits significant antifungal activity against *Armillaria* sp., *Penicillium expansum*, and *Sclerotinia sclerotiorum*, even at relatively low concentrations (0.25 g L^{-1}) [42]. Moreover, *N. muscorum* has been reported to enhance disease resistance against *Alternaria porri* in onion plants by up to 66.1%, indicating its promising role in biocontrol [46]. Plant-derived extracts have been extensively studied for their antifungal properties and are considered viable alternatives for sustainable pathogen control in agriculture. Phytochemical screening of various plant species has revealed potent antifungal activity against common pathogens, such as *Aspergillus niger* and *Rhizopus stolonifer*, with minimum inhibitory concentrations below 100 µg mL^{-1} [95]. Similarly,

beneficial bacterial strains, such as *Bacillus velezensis*, produce secondary metabolites, such as iturins, fengycins, and surfactins, which exhibit antagonistic effects against fungal pathogens, such as *F. oxysporum* and *Colletotrichum*, and have the potential to be valuable biocontrol agents in agriculture [96].

In this context, *Nostoc* suspensions present a promising and eco-friendly alternative to conventional biocontrol agents, with significant potential for integration into sustainable plant disease management strategies. Future research should focus on the chemical characterization of antifungal metabolites produced by *Nostoc* sp. BCAC 1226, as well as in vivo evaluations on economically important crops, to evaluate their practical efficacy under field conditions.

5. Conclusions

This study demonstrated the antifungal potential of *Nostoc* sp. BCAC 1226 against phytopathogenic fungi, including *Phytophthora*, *Stemphylium*, and *Penicillium*, highlighting its potential as a biocontrol agent for sustainable agricultural practices. The observed antifungal effects were associated with the whole suspension of intact, live cyanobacterial cells, which exhibited promising natural biocontrol activity. Previous research has indicated that a combination of antibiosis and bioactive metabolites likely contributes to the observed effects. Further studies should focus on chemical characterization of the active antifungal compounds produced by Nostoc sp. BCAC 1226 to deepen the understanding of its bioactivity and enhance practical applications. Moreover, future research should focus on the in vivo evaluation of economically important crops to evaluate their practical efficiency under field conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/applmicrobiol5020046/s1; Table S1. Composition of modified Z-8 medium (Carmichael 1986, with modifications) [65].

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