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In Vitro Evaluation of the Fungicidal Activity of Garlic (*Allium sativum* L.) Extract Against Fungal Pathogens Associated with Potato (*Solanum tuberosum* L.) Tuber Rot in Bokkos, Plateau State, Nigeria

By

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Abstract

Nigerian potato (Solanum tuberosum L.) production is hindered by post-harvest fungal infections that lower tuber quality and market value. Therefore, this study examined the prevalence of fungal pathogens linked to potato tuber rot in Bokkos Local Government Area of Plateau State and assessed the aqueous garlic (Allium sativum L.) extract's in vitro fungicidal efficacy against the isolated fungi. Ninety-six rotten potato tubers were gathered from eight different places, and using conventional mycological methods on Potato Dextrose Agar, fungi were isolated and identified. Standard qualitative and quantitative techniques were used to assess the phytochemical components of garlic extract, and GC-MS analysis was used to identify the main bioactive chemicals. Agar disc diffusion was used to measure antifungal activity at various extract concentrations. Rhizopus stolonifer (23.5%) was the most common of the seven fungal species that were isolated, followed by Alternaria solani (19.6%) and Mucor hiemalis (17.6%). The incidence of fungal species did not change significantly between isolates, according to chi-square analysis ($\chi^2 = 9.2549$, $df = 6$, $P = 0.1597$). Garlic extract outperformed nystatin in some cases, displaying concentration-dependent antifungal activity with inhibitory zones ranging from 17 to 37 mm at 25-100% dosages. Phytochemical screening revealed flavonoids, alkaloids, saponins, terpenoids, and resins, whereas GC-MS identified bioactive compounds such as p-cymene, phenolic derivatives, and fatty acid esters. The study validates the use of garlic extract as a sustainable botanical fungicide for post-harvest disease management by demonstrating its excellent antifungal activity against potato rot pathogens.

Keywords: *Potato rot, Allium sativum, antifungal activity, GC–MS, phytochemicals, Bokkos, Nigeria*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a major food crop that helps to provide global food and nutritional security (Siamalube et al., 2025). The crop is an important component of human diets since it contains high-quality proteins, vitamins, minerals, and carbohydrates (Gikundi et al., 2021). Potatoes, after yam and cassava, are Nigeria's most important tuber crops, producing more than one million metric tonnes per year (Anayib et al., 2025). Plateau State, namely the Bokkos Local Government Area, is one of the country's most important potato production hubs. Diseases, poor storage conditions, and insufficient post-harvest processing processes continue to cause major losses in potato production, despite its economic and nutritional importance.

Fungal diseases pose a substantial challenge to potato production and storage around the world. Several fungi, including *Fusarium, Alternaria, Rhizoctonia, Penicillium, Aspergillus, Mucor,* and *Rhizopus*, infect potato tubers during cultivation, harvest, transportation, and storage, resulting in significant quality degradation and yield losses (Ahmed et al., 2024; Fayinminu et al., 2025; Tawose, 2025). These fungi have consistently been identified as substantial agents of potato rotting in research conducted throughout Nigeria (Rosemary et al., 2024; Iwuagwu et al., 2025; Nnebechukwu

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et al., 2025). Fungal genera like *Aspergillus, Cladosporium, Fusarium, Mucor, Penicillium, Rhizopus, Scopulariopsis,* and *Trichoderma* have been linked to potato tuber deterioration in Plateau State, underscoring the need for efficient management techniques to lower post-harvest losses (Danladi et al., 2023; Dawen et al., 2025; Tawose, 2025).

Ahmed et al. (2024) state that synthetic fungicides have a major role in controlling fungal diseases in potato production. Long-term usage of these compounds, notwithstanding their effectiveness, raises questions about potential health effects, fungicide resistance, environmental contamination, and chemical residues in food products (Fayinminu et al., 2025). Additionally, smallholder farmers frequently find it difficult to utilize commercial fungicides due to their high cost and restricted accessibility. These difficulties have raised interest in sustainable and environmentally friendly substitutes, especially antibacterial products made from plants.

Numerous bioactive substances with established antifungal qualities can be found in botanical extracts (Iwuagwu et al., 2025). The efficacy of aromatic and therapeutic herbs against significant phytopathogenic fungi has been shown in numerous researches. Due to its high concentration of physiologically active sulfur compounds, such as allicin, ajoene, and diallyl sulphides, garlic (*Allium sativum* L.) has drawn a lot of interest (Akpan et al., 2024; Anjorin et al., 2024). By interfering with cellular membranes, enzyme systems, and metabolic processes, these substances prevent the growth of fungi (Jikah & Edo, 2023). Garlic extracts have demonstrated strong antifungal activity against a number of plant diseases in earlier research, indicating their promise as a natural and eco-friendly fungicide.

Garlic's antifungal qualities are becoming more well recognized, but nothing is known about how well it works against the fungi that cause potato tuber rot in Bokkos. Few researches have assessed locally accessible botanical alternatives for their control; the majority have concentrated on finding rotting microbes. The necessity to look into garlic extract's potential as a sustainable management strategy for potato rot pathogens is highlighted by this information gap. In order to assess the in vitro fungicidal activity of garlic (*Allium sativum* L.) extract against the recovered isolates, this study will isolate and identify the fungal species linked to potato tuber rot in Bokkos, Plateau State, Nigeria. The results will offer scientific support for the creation of locally available, reasonably priced, and ecologically friendly methods for controlling potato fungal infections and lowering post-harvest losses in Nigeria.

MATERIALS AND METHODS

Study Area

The study used rotten potato tubers gathered from key potato-producing towns in Plateau State's Bokkos Local Government Area. The sampling sites were Kuba (Kb), Tangur (Tg), Richa (Rc), Hottom (Ht), Horop (Hr), Mushere (Ms), Mundat (Md), and Amban (Ab). Laboratory analyses were performed at Plateau State University's Microbiology Laboratory in Bokkos, Nigeria, and the National Veterinary Research Institute's Microbiology Laboratory in Vom, Plateau State.

Sample Collection

A total of 96 potato tuber samples with evident indications of fungal rot were obtained from storage facilities and local marketplaces in the eight designated locations. Twelve (12) samples were collected from each area and brought to the laboratory in sterile polyethylene bags (Nnebechukwu et al., 2025) for mycological analysis.

Isolation of Fungi Associated with Potato Rot

Potato tubers were cleaned with flowing tap water to remove soil particles and debris. Small slices (about 5 mm × 5 mm) were aseptically cut from healthy and diseased tissue edges. The tissue segments were surface sterilized in 70% ethanol for 1 minute, then rinsed twice in sterile distilled water and blotted dry with sterile filter paper (Rosemary et al., 2024).

The sterilized tissues were infected into Potato Dextrose Agar (PDA) plates and incubated for 5-7 days at 28 ± 2 degrees Celsius. Emerging fungal colonies were regularly subcultured on new PDA plates until pure cultures were achieved. Pure isolates were stored on PDA slants at 4°C for further analysis (Ehiobu, 2023).

Identification of Fungal Isolates

The fungal isolates were identified using normal mycological protocols, which included colony morphology, pigmentation, growth characteristics, and microscopic features. Lactophenol cotton blue staining was used to investigate fungal structures under a light microscope. Established taxonomic keys and fungal identification manuals were used to identify the samples (Harborne, 1980; Khairan et al., 2019; Akpan et al., 2024).

Preparation of Garlic Extract

Fresh garlic bulbs (*Allium sativum* L.) were acquired at a Bokkos market and authenticated by a plant taxonomist. The bulbs were properly washed, peeled, sliced, and air-dried in the shade at room temperature. The dried samples were ground into a fine powder with a sterile laboratory blender.

Five grams (5 g) of powdered garlic were dissolved in 250 mL of sterile distilled water to make a stock concentration of 20 mg/mL (0.02 g/mL). The mixture was continually agitated for 24 hours and filtered through Whatman No. 1 filter paper. The filtrate was kept at 4°C until needed (Harborne, 1980; Khairan et al., 2019; Ahmed et al., 2024).

Qualitative Phytochemical Screening

Using standard phytochemical procedures described by Harborne (1973), Trease and Evans (2002), Edeoga et al. (2005), Kagbo and Ejebe (2009), and Prashant et al. (2011), the aqueous garlic extract was qualitatively screened for the presence of major phytochemical constituents such as alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, steroids, proteins, carbohydrates, reducing sugars, oxalates, resins, and phlobatannins.

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Quantitative Phytochemical Analysis

The total phenolics, flavonoids, tannins, alkaloids, saponins, and steroids were quantified using spectrophotometric methods described by Harborne (1973), Singh et al. (2004), and Edeoga et al. (2005). A UV-visible spectrophotometer was used to detect absorbance values at relevant wavelengths, and concentrations were computed using standard calibration curves. The results were presented as percentage composition or equivalent concentrations of the different standards.

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The bioactive elements of the garlic extract were detected by Gas Chromatography-Mass Spectrometry (GC-MS) analysis with an Agilent 7890A Gas Chromatograph connected to a Mass Spectrometry detector (Abu et al., 2023). The chromatographic profiles were compared to those in the National Institute of Standards and Technology (NIST) spectrum collection to identify the compounds. Compounds were identified based on retention duration, peak area percentage, molecular weight, and mass spectral properties.

Evaluation of Antifungal Activity of Garlic Extract

The antifungal activity of the garlic extract against the isolated fungus species was assessed using the agar disc diffusion technique (Anjorin et al., 2024). Sterile filter paper discs impregnated with garlic extract were placed on PDA plates pre-inoculated with fungal cultures (Daradka et al., 2021). A commercial nystatin disc served as the positive control, and sterile distilled water as the negative control.

Plates were incubated at 28 ± 2°C for 72-120 h. The diameter of the inhibition zones around each disc was measured with a digital Vernier caliper to assess antifungal activity. All experiments were run in triplicate, and mean inhibitory zone diameters were recorded.

DATA ANALYSIS

The frequency and percentage occurrence of fungal isolates linked to potato tuber rot were calculated using descriptive statistics. Percentage occurrence was estimated as the proportion of each fungal species to the total number of isolates collected. A chi-square (χ^2) goodness-of-fit test was employed to identify significant variations in the occurrence of fungal species. The antifungal activity of garlic extract was assessed using inhibitory zone widths (mm) produced against each fungal strain at various extract doses and compared to a positive control (nystatin). Qualitative phytochemical elements were classified as present or missing, whilst quantitative phytochemical levels were expressed as percentages. Compound identification was performed on GC-MS data using the National Institute of Standards and Technology (NIST) spectrum library. P-value < 0.05 indicated statistical significance.

RESULTS

Incidence and Frequency of Fungal Species Associated with Potato Tuber Rot

A total of 96 potato tuber samples were analysed from eight locations in Bokkos Local Government Area, Plateau State, Nigeria. Fungal organisms were isolated from the rotted potato tissues, yielding a total of 51 fungal isolates. The distribution of fungal species varied across the samples. *Rhizopus stolonifer* recorded the highest frequency of occurrence (23.5%), followed by *Alternaria solani* (19.6%) and *Mucor hiemalis* (17.6%) however, *Rhizoctonia solani* (3.9%) recorded the lowest occurrence among the isolates (Table 1). The incidence and frequency of fungal species associated with potato tuber rot in Bokkos LGA showed no significant difference (χ^2 = 9.2549, df = 6, P = 0.1597).

Table 1: Incidence and Frequency of Fungal Species Associated with Potato Tuber Rot

Isolate	Frequency of occurrence	Percentage (%) of occurrence
Aspergillus niger	6	11.8
Aspergillus flavus	5	9.8
Alternaria solani	10	19.6
Mucor hiemalis	9	17.6
Rhizopus stolonifer	12	23.5
Rhizoctonia solani	2	3.9
Penicillium spp.	7	13.7
Total (%)	**51**	

χ^2 = 9.2549, df = 6, P = 0.1597

Antifungal Activity of Aqueous Garlic Extract Against Fungal Isolates

The antifungal activity of aqueous garlic (*Allium sativum* L.) extract was concentration-dependent and inhibited all tested fungus species. At the lowest dose (10%), no significant inhibition was seen, while higher concentrations (25-100%) demonstrated moderate to strong antifungal action. At a 25% concentration, inhibition zones ranged from 17 mm for *Rhizoctonia solani* to 22 mm for *Aspergillus niger* and *Mucor* were more susceptible to garlic extract than *Penicillium* spp. and *Rhizoctonia solani. hiemalis*. At 50%, inhibition increased, ranging from 20 mm to 27 mm among isolates. The 75% concentration demonstrated much stronger antifungal effects, with inhibition zones ranging from 24 mm to 28 mm. The maximum concentration (100%) had the strongest antifungal efficacy, with inhibition zones ranging from 24 mm (*Penicillium* spp.) to 37 mm (*Aspergillus niger*). Across all treatments, *Aspergillus niger* and *Mucor hiemalis*

*Corresponding Author: **Musa Filibus Gugu.**

Table 2: Antifungal Activity of Aqueous Garlic Extract Against Fungal Isolates

Concentration (%)	Diameters of inhibition zone (mm)						
	Isolated fungi						
	Aspergillus niger	*Aspergillus flavus*	*Alternaria sp.*	*Mucor hiemalis*	*Rhizopus stolonifer*	*Rhizoctonia solani*	*Penicillium spp.*
C+	23.0	24.0	24.0	24.0	24.0	23.0	14.0
10	0.1	0.1	0.1	0.1	0.1	0.1	0.0
25	22	21	18	22	18	17	20
50	27	24	20	25	22	20	21
75	28	26	25	26	26	24	22
100	37	32	28	30	28	28	24

(C+) positive control

Phytochemical Composition of Aqueous Garlic Extract

Qualitative Phytochemical Screening

Qualitative phytochemical screening revealed the presence of several bioactive compounds in garlic extract (Table 3). Carbohydrates, proteins, alkaloids, flavonoids, saponins, resins, steroids, reducing sugars, and terpenoids were detected in varying intensities.

Resins were highly abundant (+++), while alkaloids, flavonoids, proteins, and saponins were moderately present (++). Steroids and terpenoids were detected in low abundance (+). Oxalates, phenols, phlobatannins, and tannins were not detected in the extract.

Table 3: Qualitative Phytochemical Screening

Phytochemical Test	Status in Plant Material
Carbohydrate	++
Oxalate	ND
Phenols	ND
Phlobatanins	ND
Protein	++
Reducing Sugar	+
Resins	+++
Saponin	++
Steroids	+
Tannins	ND
Terpenes	+
Alkaloid	++
Flavonoid	++

Keys: + = present, ++ = moderately present, +++ = highly present, ND = Not Detected

Quantitative Phytochemical Composition

Quantitative analysis confirmed the presence of major phytochemical constituents in varying proportions (Table 4). Alkaloids recorded the highest concentration (7.28%), followed by flavonoids (5.56%) and saponins (4.68%). Steroids were present at 3.23%, while tannins (0.20%) and phenolic compounds (0.07%) were present in relatively low concentrations. The overall phytochemical profile indicates that garlic extract contains a mixture of bioactive compounds with potential antimicrobial properties.

Table 4: Quantitative Phytochemical Composition

Phytochemical Component	Status in Plant Material (%)
Alkaloid	7.28
Flavonoid	5.56
Phenols	0.07
Saponins	4.68
Steroid	3.23
Tannin	0.20

GC–MS Analysis of Garlic Extract

The GC-MS analysis of aqueous garlic extract revealed a complex variety of volatile and semi-volatile ingredients, such as phenolic compounds, fatty acid methyl esters, alcohols, hydrocarbons, and terpenoid derivatives (Table 5; Figure 1). Mass spectrum library matching identified 35 chemicals; however, only ten of these compounds with probable biological significance and adequate spectral matching quality were retained for interpretation.

Major compounds identified included Phenol, 2,4-bis(1,1-dimethylethyl)-, hexadecanoic acid methyl ester, tridecanoic acid methyl ester, 1-decanol, and 1-undecanol. The chromatogram displayed several prominent peaks between retention times of approximately 8 and 15 minutes, indicating the presence of biologically active metabolites within the extract.

Table 5: Major Bioactive Compounds Identified in Aqueous Garlic Extract by GC–MS Analysis

S/No	Compound	Molecular Formula	Molecular Weight (g/mol)
1.	1-Decanol	$C_{10}H_{22}O$	158
2.	1-Undecanol	$C_{11}H_{24}O$	172

*Corresponding Author: **Musa Filibus Gugu.**

3.	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206
4.	Pentanoic acid	$C_5H_{10}O_2$	102
5.	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	228
6.	Hexadecanoic acid, methyl ester (Methyl palmitate)	$C_{17}H_{34}O_2$	270
7.	Oxalic acid, allyl hexadecyl ester	$C_{21}H_{38}O_4$	354
8.	5,10-Pentadecadien-1-ol (Z,Z)	$C_{15}H_{28}O$	224
9.	1,3,8-p-Menthatriene	$C_{10}H_{14}$	134
10.	p-Cymene	$C_{10}H_{14}$	134

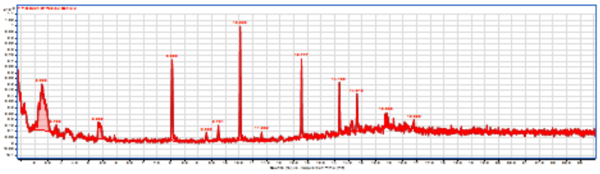

Figure 1: Chromatogram of phytoconstituents present in garlic extract

DISCUSSION

The current study looked at fungal pathogens linked with potato tuber rot in Bokkos, Plateau State, Nigeria, and assessed the in vitro fungicidal activity of aqueous garlic (*Allium sativum* L.) extract against the recovered isolates. The study also evaluated the extract's phytochemical ingredients and bioactive chemicals in order to get insight into its antifungal properties. The results show that garlic extract has significant antifungal efficacy against fungal species linked with potato tuber degeneration and could be a promising botanical alternative for disease treatment.

The discovery of *Rhizopus stolonifer, Alternaria solani, Mucor hiemalis, Penicillium* spp., *Aspergillus niger, Aspergillus flavus,* and *Rhizoctonia solani* shows the complex fungal population linked to potato tuber rot in Bokkos. The prevalence of R. stolonifer (23.5%) is consistent with other publications (Ahmed et al., 2024; Nnebechukwu et al., 2025; Tawose, 2025; Tula et al., 2026), which identified this fungus as one of the leading causes of soft rot and post-harvest degradation in potato tubers. The abundance of *Alternaria solani* and *Mucor hiemalis* shows that both field-acquired and storage-associated fungal infections play a substantial role in potato losses in the studied area. Similar fungal taxa have been identified from potato-producing locations in Plateau, Kano, Benue, Borno, and Sokoto States (Danladi et al., 2023; Tawose, 2025; Tula et al., 2026), demonstrating that these organisms continue to pose significant challenges to potato production and storage in Nigeria. The lack of a statistically significant difference in the incidence of fungal species associated with potato tuber rot across the sampled isolates indicates that, while there were variations in frequency among the fungal species, their distribution did not differ significantly from expected patterns across the study area. The findings show a very homogeneous presence of the major fungal pathogens in Bokkos, showing that potato tuber rot in the area is caused by a diverse fungal population rather than a single statistically significant pathogen.

The antifungal testing demonstrated that garlic extract has a clear concentration-dependent inhibitory impact on all fungal isolates tested. The steady increase in inhibition zones from 25% to 100% concentration shows that the extract's potency is directly proportional to the concentration of bioactive chemicals available to prevent fungal growth. At full dosage, the extract inhibited *Aspergillus niger* (37 mm), *Aspergillus flavus* (32 mm), and *Mucor hiemalis* (30 mm) most effectively. These results show that garlic extract has broad-spectrum antifungal action against both storage fungi and phytopathogenic species associated with potato rot. Danladi et al. (2023), Atsen et al. (2024), and Ndifon (2025) found similar concentration-dependent antifungal activities in garlic extracts, with considerable suppression of fungal pathogens such as *Aspergillus, Fusarium, Rhizoctonia,* and *Alternaria*. The observed increase in antifungal activity with increasing extract concentration shows that higher amounts of bioactive phytochemicals promote fungal growth inhibition. The current findings give additional evidence that garlic has significant potential as a natural alternative to synthetic fungicides for the treatment of post-harvest fungal infections.

Notably, the inhibitory zones created by the 100% garlic extract outperformed the nystatin control for several fungal isolates. Although direct comparisons between crude plant extracts and refined antifungal medications should be viewed with caution, this finding shows garlic's significant antifungal activity and supports its prospective use as a botanical fungicide. Previous studies (Steglińska et al., 2022; Teixeira et al., 2023; Anjorin et al., 2024) found that garlic extract inhibited phytopathogenic fungi such as *Fusarium, Alternaria, Rhizoctonia*, and *Aspergillus* in a concentration-dependent manner. The current findings support emerging evidence that garlic-derived compounds can be effective and ecologically friendly alternatives to synthetic fungicides.

The phytochemical examination identified alkaloids, flavonoids, saponins, steroids, terpenoids, resins, proteins, carbohydrates, and reducing sugars. Among the measured metabolites, alkaloids (7.28%), flavonoids (5.56%), and saponins (4.68%) were found in comparatively high amounts. These compounds have been widely reported to have antimicrobial properties through a variety of mechanisms, including disruption of cell membranes, inhibition of enzyme systems, interference with nutrient uptake, and induction of oxidative stress in microbial cells (Akpan et al., 2024; Fayinminu et al., 2025). The presence of these metabolites in garlic extract suggests a potential metabolic foundation for the antifungal effect observed in this investigation.

Flavonoids have been shown to prevent fungal development by modifying membrane permeability and blocking key metabolic pathways (Rodríguez et al., 2023). Similarly, saponins have antifungal effects via interacting with

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membrane sterols, resulting in enhanced membrane permeability and, eventually, cell lysis (Dervishi et al., 2026). Alkaloids have also been demonstrated to suppress nucleic acid production and disrupt fungal cellular metabolism. The existence of these chemicals shows that garlic extract's antifungal action is the consequence of many mechanisms acting simultaneously against susceptible fungal infections (Dantas et al. 2025).

The GC-MS analysis revealed that the aqueous garlic extract included a diverse range of bioactive ingredients, including phenolic compounds, alcohols, fatty acid esters, and terpenoid derivatives. Among the key chemicals discovered were phenol, 2,4-bis(1,1-dimethylethyl)-, p-cymene, 1-decanol, 1-undecanol, tridecanoic acid methyl ester, and hexadecanoic acid methyl ester. Previous research has linked these chemicals with antibacterial, antioxidant, and antifungal properties (Keke et al., 2023; Sharmin et al., 2025). Phenolic chemicals can denature proteins and alter microbial membranes, whereas fatty acid esters can impair membrane integrity and cellular respiration. Similarly, terpenoid compounds like p-cymene exhibit antibacterial action against a wide range of fungal infections.

Although the typical sulfur-containing compounds associated with garlic, such as allicin and diallyl sulfides, were not detected in the current study, this could be attributed to the aqueous extraction procedure and the inherent instability of organosulfur compounds during extraction and GC-MS analysis. Allicin is known to deteriorate quickly after tissue disruption and may undergo additional transformation during sample preparation and chromatographic analysis (Hu et al., 2024; Zhou et al., 2025). As a result, the antifungal activity found in this investigation was most likely caused by the synergistic action of numerous phytochemical ingredients rather than a single dominant molecule.

The study's findings have significant implications for sustainable potato production and post-harvest disease management. The growing worries about synthetic fungicide use, including as environmental contamination, disease resistance, and chemical residues in food products, have fueled the hunt for safer alternatives. Garlic is affordable, widely available, biodegradable, and generally considered safe for agricultural usage. The high antifungal activity of garlic extracts against the primary fungal infections associated with potato tuber rot implies that it could be developed into a locally derived botanical fungicide to reduce post-harvest losses among smallholder potato producers.

CONCLUSION

In conclusion, the study found that garlic extract had considerable fungicidal action against fungal infections linked to potato tuber rot in Bokkos, Plateau State. The antifungal activity appears to be mediated by a varied set of phytochemicals and bioactive metabolites that work together to limit fungal growth. These findings lend credence to garlic's potential use as an environmentally acceptable and sustainable alternative for managing potato storage diseases, as well as laying the groundwork for future research into garlic-based biofungicidal formulations for agricultural uses.

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