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Sustainable Management of Pineapple Postharvest Decay: Antifungal Activity of Clove, Ginger, Garlic, and Turmeric against Key Pathogens

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ABSTRACT

Postharvest soft rot caused by fungal pathogens, including *Aspergillus niger*, *Penicillium digitatum*, *Aspergillus parasiticus*, *Fusarium chlamydosporum*, and yeast, severely compromises the shelf life and marketability of pineapple (*Ananas comosus* L.). Synthetic fungicides, though effective, raise environmental and health concerns, necessitating eco-friendly alternatives. This study evaluated the antifungal potential of four botanicals, *Syzygium aromaticum* (clove), *Zingiber officinale* (ginger), *Allium sativum* (garlic), and *Curcuma longa* (turmeric), against these pathogens using spice-amended potato dextrose agar (PDA) at concentrations of 1 g, 5 g, and 10 g per 250 mL. Pathogen susceptibility varied significantly ($p < 0.001$), with *A. niger* showing the highest resistance (mean growth: 57.81 mm), while *F. chlamydosporum* (26.94 mm) and yeast (29.94 mm) were the most inhibited. Clove exhibited the strongest antifungal activity (mean growth: 17.17 mm), achieving complete inhibition at all tested concentrations, followed by ginger (mean growth: 30.50 mm). Antifungal efficacy was concentration-dependent, with 10 g being most effective (mean growth: 28.25 mm). Garlic and turmeric showed moderate inhibition but were less consistent than clove and ginger. Clove and ginger emerged as potent, sustainable alternatives to synthetic fungicides for controlling postharvest pineapple spoilage. Their concentration-dependent efficacy underscores their practicality for commercial use. Further research should explore formulations for large-scale application and synergistic effects with other natural preservatives to enhance postharvest management strategies. Because *F. chlamydosporum* and Yeast inherently grow more slowly in control conditions, raw mycelial measurements may overestimate botanical efficacy; therefore, percentage inhibition offers a more accurate comparative metric for these organisms.

Keywords: *Ananas comosus*, postharvest decay, antifungal botanicals, *Syzygium aromaticum*, *Zingiber officinale*, fungal inhibition

Introduction

Pineapple (*Ananas comosus* L.), a tropical fruit of global economic significance, faces substantial challenges. Postharvest losses exceeding 40% in developing nations due to microbial spoilage (Begum *et al*., 2022; Kuruppu *et al*., 2025). Soft rot disease,

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characterized by rapid tissue maceration and enzymatic degradation of fruit parenchyma, poses particular challenges for producers and distributors (Maya *et al*., 2022; Nusaibah and Sapak, 2024). The condition arises from complex interactions between wound-invading fungal pathogens (*Aspergillus*, *Penicillium*, *Fusarium* spp.) and environmental factors, with losses escalating under suboptimal storage conditions (Thelma and Festus, 2018; Vignassa *et al*., 2021; Kuruppu *et al*., 2025).

Current management relies heavily on synthetic fungicides, such as thiabendazole and benomyl, to control diseases such as Black Rot and Soft Rot (Eckert and Ogawa, 1985; Pineapple; Nusaibah and Sapak, 2024). However, growing antimicrobial resistance (Atasoy *et al*., 2023; Mohd Anuar et al., 2024) and consumer demand for residue-free produce (Yao Fulgence *et al*., 2021; Chaudhary *et al*., 2024) necessitate alternative solutions. Plant-derived antimicrobials present a sustainable approach, with spices like clove (*Syzygium aromaticum*) and ginger (*Zingiber officinale*) containing bioactive compounds (eugenol, gingerol) demonstrating broad-spectrum antifungal activity in vitro (Ahmed *et al*. 2021; Khatri *et al*., 2023; Singh *et al*., 2025). These compounds interfere with fungal cell membranes and suppress mycelial growth via multiple mechanisms, which decreases the chances of resistance developing (Deresa and Diriba, 2023; Villavicencio-Vásquez *et al*., 2025).

This study investigates four underutilized botanicals although clove and turmeric possess well-known antimicrobial properties, they are considered underutilized specifically in applications targeting pineapple pathogens; clarifying this distinction highlights that their specific application to pineapple soft-rot pathogens is the novel contribution of this study - clove, ginger, garlic (*Allium sativum*), and turmeric (*Curcuma longa*) - for controlling pineapple soft rot pathogens. Our objectives were to identify predominant fungal pathogens in Benin City markets, evaluate the comparative efficacy of spice extracts at varying concentrations (1g, 5g, 10g/250mL PDA), and determine optimal botanical formulations for postharvest application. The research addresses critical gaps in developing locally available, low-cost antifungal treatments, establishing concentration thresholds for effective pathogen suppression, and providing scientific validation for traditional preservation practices. Findings will inform sustainable postharvest strategies for smallholder farmers and help reduce pineapple losses in tropical supply chains.

2.0 Materials and Method

Study Site and Sample Collection

Field experiments were conducted at the University of Benin, Nigeria (6°24'N, 5°37'E; 239 m elevation), representing a humid tropical agroecology (annual rainfall: 1,208 mm; mean temperature: 26.7°C). Symptomatic pineapple fruits (n=30) exhibiting soft rot lesions (>2 cm diameter) were collected from Udo market using sterile sampling protocols, while healthy fruits (n=15) for pathogenicity assays were obtained from a commercial farm in Udo, Edo State, transported in sterilized crates (4°C), and processed within 2 h.

Pathogen Isolation and Characterization

Tissue samples (5 × 5 mm) from lesion margins were surface-sterilized (0.1% $HgCl_2$ for 2 min followed by triple rinsing in sterile distilled water) and plated on Potato Dextrose Agar (PDA; HiMedia, Mumbai) amended with chloramphenicol (100 mg/L) to suppress bacteria, then incubated (28 ± 2°C for 72 h). Pure cultures were obtained through hyphal tipping (Werres, 2015) and identified by examining their macroscopic colony features, such as color and texture, along with microscopic analysis of lactophenol cotton blue-stained structures (including conidiophores and spores) at a magnification of 400× using a Nikon Eclipse E200 microscope. A limitation of relying solely on morphological identification is the potential for misclassification among closely related fungal taxa; future studies should incorporate molecular confirmation (e.g., ITS sequencing) to validate isolate identity.

Pathogenicity Testing (Koch's Postulates)

Healthy fruits were surface-disinfected (1% NaOCl, 30 s), wounded (5 mm deep) with sterile cork borers, and inoculated with 5 mm mycelial plugs (7-day cultures). Controls received sterile

PDA plugs. Fruits were incubated in humidity chambers (RH 85%, 28°C) for 7 days. Re-isolation from symptomatic tissue confirmed pathogenicity.

Botanical Preparation

Botanical preparations included clove (*S. aromaticum* buds), ginger (*Z. officinale* rhizomes), garlic (*A. sativum* bulbs), and turmeric (*C. longa* rhizomes) purchased from Uselu market (Benin City) and authenticated at the University of Benin Herbarium. Samples were oven-dried (40°C for 48 h), ground to 80-µm powder (Willey mill), and stored in amber vials (4°C).

Antifungal Bioassay

An amended PDA was prepared by incorporating spice powders at low (1 g/250 mL, 0.4% w/v), medium (5 g/250 mL, 2% w/v), and high (10 g/250 mL, 4% w/v) concentrations. Sterilized media (121°C for 15 min) were poured into Petri dishes (20 mL/plate) and centrally inoculated with 5 mm pathogen plugs (n=3 replicates/treatment), then incubated (28°C). Radial growth was measured daily for 7 days using digital callipers (Mitutoyo), with percentage inhibition calculated versus controls. This concentration range (0.4–4% w/v) reflects values commonly reported in botanical antifungal studies and aligns with preliminary tests showing measurable inhibition within this interval. For clarity, all concentrations are expressed consistently as % (w/v) in the manuscript.

$$\text{The Percent Inhibition (PI)} = \frac{100(\text{Growth in control} - \text{Growth in treatment})}{\text{Growth in control}}$$

Storage Condition Trials

Storage condition trials used healthy fruits (n=9) stored under refrigeration (10°C, 85% RH), ambient conditions (27°C, 65% RH), and sunlight exposure (37°C peak, 6 h/day). Quality parameters were assessed every 3 days for 30 days using a chroma meter (Minolta CR-400) for color, a penetrometer (3 mm probe, 5 N load) for texture, and serial dilution plating on PDA for microbial load. Storage condition trials were conducted to assess how different postharvest environments (refrigeration, ambient conditions, and sunlight exposure) influence pineapple quality and pathogen proliferation. These trials provide an applied context for interpreting the antifungal findings by demonstrating how environmental conditions modulate decay progression and fruit shelf life, thereby illustrating the practical relevance of botanical treatments under real storage scenarios.

Statistical Analysis

Data were analyzed using GENSTAT 22.0 (VSN International), employing a three-way ANOVA to test the effects of fixed factors (botanical type, concentration, and pathogen species) and a random factor (replicate blocks). Tukey's HSD ($p<0.05$) separated means, with assumptions verified via Levene's and Shapiro-Wilk tests.

Results

Pathogen Identification and Prevalence

Six fungal species were consistently isolated from infected pineapples (Table 1). Yeast spp. dominated (77 colonies), followed by *Aspergillus niger* (22), *Rhizopus stolonifer* (19), and *Penicillium digitatum* (12). *Fusarium chlamydosporum* showed the lowest incidence (5 colonies). All isolates fulfilled Koch's postulates, reproducing soft rot symptoms in healthy fruits within 72 h.

Table 1: Fungal isolates from pineapple soft rot lesions

Pathogen	Colony Count	Incidence (%)	Lesion Diameter (mm)*
Yeast spp.	77	41.2	18.3 ± 2.1
Aspergillus niger	22	11.8	15.7 ± 1.8
Rhizopus stolonifer	19	10.2	22.4 ± 3.2
Penicillium digitatum	12	6.4	12.9 ± 1.5
Aspergillus parasiticus	5	2.7	9.8 ± 1.2
Fusarium chlamydosporum	2	1.1	9.8 ± 1.2

**Mean ± SD after 7 days post-inoculation*

Antifungal Efficacy of Botanicals

Two-way ANOVA revealed significant effects of botanical type ($F_{3,\ 48} = 87.6$, $p < 0.001$) and concentration ($F_{2,\ 48} = 64.3$, $p < 0.001$) on mycelial inhibition. Clove showed 100% inhibition at all concentrations (0 mm growth)

(Figure 1a-b, while ginger exhibited dose-dependent suppression (84.2% inhibition at 10 g/250 mL, 16.3 ± 1.1 mm vs. control: 88.0 ± 0.0 mm). Garlic and turmeric showed limited efficacy (<50% inhibition at 10 g). Effect sizes (η^2) indicated strong contributions of botanical type (η^2 = 0.54) and concentration (η^2 = 0.47), complementing the reported p- values.



Figure 1: (a) Inhibition of test organisms with Clove at 10 g. (b) Growth of test organisms in the control plates.

Pathogen-Specific Responses

Pathogen susceptibility varied significantly ($p < 0.05$), with *Aspergillus niger* being the least susceptible (57.8 ± 2.3 mm mean growth), and *F. chlamydosporum* (26.9 ± 1.8 mm) and yeast (29.9 ± 2.0 mm) being most inhibited. Clove suppressed all pathogens completely, including resistant *A. niger* (Table 2).

Table 2: Mean mycelial growth (mm) of fungal pathogens under 10 g of botanical treatment

Pathogen	Clove	Ginger	Garlic	Turmeric	Control
A. niger	0.0[a]	17.7[b]	69.0[c]	75.7[c]	88.0[d]
R. stolonifer	0.0[a]	24.3[b]	52.1[c]	68.5[d]	86.2[e]
P. digitatum	0.0[a]	15.2[b]	48.6[c]	62.4[d]	84.9[e]
A. parasiticus	0.0[a]	18.5[b]	58.3[c]	70.8[d]	87.1[e]
F. chlamydosporum	0.0[a]	18.0[b]	16.3[b]	35.7[c]	44.3[d]
Yeast spp.	0.0[a]	20.0[b]	17.3[b]	47.3[c]	41.0[e]

Values with different superscripts within a row differ significantly (Tukey's HSD, $p < 0.05$).

Storage Condition Effects

Refrigeration (10°C) extended shelf life to 27 days versus 9 days (ambient) and 6 days (sunlight) (Log-rank test, $\chi^2 = 38.4$, $p < 0.001$). Refrigerated fruits retained green hue (Chroma meter a = -12.3) versus browning in sunlight (a = +4.7) by Day 12, maintained firmness (3.2 ± 0.4 N vs. ambient: 1.1 ± 0.2 N), and showed lower microbial loads (yeast counts $<10^6$ CFU/g versus sunlight-stored fruits exceeding this threshold by Day 9).

Discussion

The findings of this study demonstrate that *S. aromaticum* (clove) exhibits exceptional antifungal efficacy against pineapple soft rot pathogens, achieving complete mycelial inhibition (100%) across all tested concentrations (1–10 g/250 mL). This strong inhibitory effect aligns with previous reports on eugenol's fungicidal properties (Braga *et al*., 2007; Park *et al*., 2009; Guo *et al*., 2024), which disrupt fungal membrane integrity and mitochondrial function at concentrations ≥0.4%. Notably, clove's ability to completely suppress *Aspergillus niger,* a pathogen known for resistance to many synthetic fungicides (Jahani *et al*., 2020; Muñoz Castellanos *et al*., 2020; Zheng *et al*., 2022),

positions it as a promising broad-spectrum alternative for postharvest disease management.

Zingiber officinale (ginger) displayed dose-dependent inhibition, with 10 g treatments reducing *Fusarium chlamydosporum* growth by 84%. This effect correlates with gingerol's documented capacity to interfere with fungal ergosterol biosynthesis (Xi *et al*., 2022). However, ginger's limited efficacy against *A. niger* (17.7 mm residual growth) suggests pathogen-specific limitations that warrant further investigation.

The relatively poor performance of *A. sativum* (garlic) and *C. longa* (turmeric) contrasts with their reported antibacterial activity (Elkhtam *et al*., 2014; Wada *et al*., 2021). This discrepancy may be attributed to two key factors: the thermal instability of allicin (garlic's primary bioactive compound), which degrades rapidly at temperatures >28°C (Fujisawa *et al*., 2008), and *Penicillium digitatum*'s apparent ability to detoxify curcuminoids (Song *et al*., 2020; Li *et al*., 2022), resulting in only 47% inhibition by turmeric treatments.

Refrigeration at 10°C demonstrated significant synergy with botanical treatments, extending pineapple shelf life threefold compared to ambient storage. These results support the "hurdle technology" approach (Gomez, 2023; Saha *et al*., 2023), where combined physical and biochemical preservation methods enhance food safety and quality.

Several limitations should be acknowledged when interpreting these findings. First, *in vitro* results may overestimate field efficacy due to uncontrolled environmental variables (e.g., humidity fluctuations). Second, the sensory impact of high clove concentrations (10 g) on fruit aroma remains unverified; consumer acceptance trials are needed. Third, while effective, clove's current market price ($5/kg in Nigeria at $1 to N1,600) may limit scalability compared to synthetic fungicides ($1.2/kg). A logical next step would involve an *in vivo* validation trial, such as applying clove- or ginger-based treatments as pre-storage dips on freshly harvested pineapples under controlled supply-chain simulations, allowing comparison of decay incidence, sensory acceptance, and microbial dynamics across realistic temperature and handling conditions; this would directly extend and strengthen the current findings while retaining the noted sensory and cost limitations.

These findings suggest several practical applications. Farmers could implement clove-ginger emulsions (5 g/L) as pre-storage dip treatments, while policymakers might consider subsidizing spice-based formulations to replace thiabendazole in export supply chains. Researchers should prioritize investigations into nanoencapsulation technologies (Osman *et al*., 2024; Prieto and Lagaron, 2024; Fakhariha *et al*., 2025) to enhance the stability and efficacy of botanical active compounds.

The study's implications extend beyond immediate applications, offering a model for developing sustainable postharvest solutions that combine traditional knowledge with scientific validation. Future research should address the identified gaps while exploring opportunities to optimize treatment protocols for different production scales and market requirements.

Conclusion

This study provides compelling evidence that botanical extracts, particularly from *S. aromaticum*, offer effective control against pineapple soft rot pathogens. Clove demonstrated complete inhibition of all tested fungal isolates at concentrations as low as 1 g/250 mL PDA, including resistant strains such as *Aspergillus niger*. The superior antifungal performance of clove is attributed to its high eugenol content, which disrupts fungal cell membranes and metabolic processes. *Zingiber officinale* (ginger) showed significant but concentration-dependent efficacy, with 10 g treatments achieving 84% inhibition of *Fusarium chlamydosporum*.

The research confirmed that refrigeration at 10°C substantially extends pineapple shelf life by slowing pathogen proliferation and maintaining fruit quality. However, even under optimal storage conditions, yeast species emerged as persistent spoilage organisms, suggesting the need for targeted control measures. The differential susceptibility of pathogens to botanical treatments highlights the importance of pathogen-specific management strategies. These findings validate the potential of plant-derived

antimicrobials as sustainable alternatives to synthetic fungicides in postharvest systems.

Recommendations

Based on the study outcomes, we propose the following actionable measures: Farmers and postharvest handlers should implement clove extract treatments (5-10 g/L) as a dip or spray application for pineapples intended for storage or transport. This approach is particularly recommended for organic production systems and export markets where chemical residues are restricted. Extension services should develop training programs to educate stakeholders on proper preparation and application methods for botanical treatments. This recommended 5–10 g/L range is intended only as a preliminary dip or spray dosage and should be further validated under field and commercial storage conditions before operational adoption.

For commercial scalability, public and private sector partnerships should invest in the development of standardized, stabilized formulations of clove and ginger extracts. Research institutions should prioritize field trials to evaluate treatment efficacy across different agroecological zones and storage infrastructures. Future studies should investigate nanoencapsulation technologies to enhance the stability and controlled release of bioactive compounds during storage.

Policymakers are encouraged to establish regulatory frameworks for botanical postharvest treatments and consider subsidy programs to improve smallholder access to these solutions. Consumer acceptance studies should be conducted to assess market readiness for botanically treated produce. Lastly, further research should explore synergistic combinations of botanical extracts with other non-chemical preservation methods to develop integrated postharvest management systems.

These recommendations provide a pathway for translating research findings into practical solutions that can reduce postharvest losses while promoting sustainable agricultural practices. These strategies have the potential to greatly improve pineapple value chains, especially in tropical developing nations where postharvest losses are particularly high.

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Conflict of Interest

The authors declare that they have no competing interests

Declaration of AI generative tools

During the preparation of this work, the author(s) used Grammarly to reduce grammatical errors. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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