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Comparative Analysis of the Phytochemical and Antifungal Properties of Lemon and Lime Peels in Preventing Rot Fungi in Irish and Sweet Potatoes

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ABSTRACT

Background and Objective: The use of plant extracts in treating plant diseases is gaining acceptance due to the adverse effect of chemical fungicides on plants, animals, humans, and the environment. The study was conducted to assess the phytochemical compounds and *in vitro* antimicrobial potential of the ethanol and methanol leaf extracts of lemon and lime peels. **Materials and Methods:** The study investigated the antifungal efficacy of plant extracts against the causative agent of dry rot in sweet and Irish potatoes. Diseased potato samples were collected, and the fungal pathogen was cultured using Potato Dextrose Agar (PDA) which was prepared according to the manufacturer's instructions and isolated. The organisms were identified using standard microbiological techniques. Plant extracts were obtained through Soxhlet extraction using aqueous and methanol solvents. The antifungal susceptibility assay was conducted using the agar well diffusion method, with inhibition zones measured to determine effectiveness. Data were analyzed using appropriate statistical tests to compare antifungal activity across treatments at the significance level 0.05. **Results:** Methanolic and ethanolic extracts of lemon and lime peels tested positive for tannins, alkaloids, and glycosides, with stronger reactivity in methanolic lemon peel extracts (24.16% yield). Methanol extract of lime peel had the highest concentration of steroids (++), while flavonoids and cardiac glycosides were the most abundant (+++). Fusarium oxysporum had the highest occurrence (50%) across isolates. Methanolic lemon peel extract showed inhibition zones of 77.90 mm (200 mg/mL) and 69.10 mm (150 mg/mL), while lime extract had 83.21 mm and 76.33 mm, respectively. Fluconazole exhibited the highest inhibition (100.0 mm) at all concentrations, with statistical significance varying ($p>0.05$ and $p<0.05$). **Conclusion:** The result showed that lime peel extract is more effective against the fungi causing dry rot of sweet and Irish potatoes compared to lemon peel extract.

KEYWORDS

Phytochemical, antifungal, lemon peel, lime peel, potatoes rot

INTRODUCTION

Due to the high yield per unit area and time, as well as their nutritional value, especially the protein-to-carbohydrate ratio, sweet potatoes (*Ipomoea batatas* (L.)) and Irish potatoes (*Solanum tuberosum*) have

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historically played an important role in food security. However, there are several obstacles that African farmers must overcome to produce crops, such as poor soil conditions, insufficient agricultural methods, dependence on local varieties, problems with land tenure, and damage from pests and diseases[1].

As with many other crops, potato tubers are susceptible to a variety of infections, including notably fungi, bacteria, nematodes, and viruses, which can result in large postharvest losses. Only a limited percentage of collected tubers are used, as a significant number are lost to rot diseases[2]. Dehydration, microbial invasion, tuber damage, and finally widespread postharvest spoiling can result from improper handling, inadequate packaging, and poor storage conditions. Field farming, harvesting, postharvest handling, and storage are just a few of the times that fungi can infect crops. Fungi are frequently the main cause of root crop spoiling, resulting in illnesses such as surface rot, root rot, soft rot, Java black rot, scurf, and black rot in sweet potatoes[3]. While certain illnesses, like scurf, are disseminated by soil or air, others, like Rhizopus soft rot, are found in many different parts of the environment. Postharvest losses can be considerably decreased by using good agricultural practices from crop development to harvest[4].

Pathological degeneration happens when fungi, typically enter through physical damage or the root attachment point, destroy the host tissue. One or a small number of pathogens frequently cause the first infections, which are then followed by secondary saprophytic microorganisms that worsen the harm. The growth capacity of the microbe, its enzymatic activity, and the physiological condition of the infected tissues all influence how the infection progresses[3].

The hydrolytic enzymes produced by the fungi that cause sweet potato spoiling, including cellulases, pectinases, xylanases, and proteases, break down plant tissues and cause cell death. Consequently, fungi can obtain nutrients from the decomposing plant matter. Sweet potato flour's texture and consistency are changed by this spoiling, making it unfit for human eating or drastically lowering its market value[5]. Effective control strategies are required due to the economic impact of fungal infections. Postharvest fungal infections have been addressed using a variety of techniques, such as hydrowarming, gamma irradiation, and fungicide treatments. Although these methods have demonstrated some success in preventing spoiling and extending shelf life[6], they have disadvantages, including restricted availability for Nigerian farmers, possible environmental risks, phytotoxic effects on humans, and the possibility of fungal pathogens. The use of plant extracts as natural fungicides for crop protection has gained popularity as a result of these restrictions[7]. Many investigations have shown that plant extracts have antimicrobial properties, and botanicals like *Chromolaena odorata* (Siam weed), *Ocimum gratissimum* (wild basil), *Moringa oleifera* (moringa), and *Zingiber officinale* (ginger) have been extensively studied for their capacity to control plant diseases[7]

This study aims to evaluate and compare the antimicrobial properties of lemon (*Citrus limon*) and lime (*Citrus aurantifolia*) in controlling dry rot fungi affecting Irish potatoes (*Solanum tuberosum*) and sweet potatoes (*Ipomoea batatas*). The study was conducted to assess the phytochemical compounds and in vitro antimicrobial potential of the ethanol and methanol leaf extracts of lemon and lime peels.

MATERIALS AND METHODS

Study area: The study was conducted at the Department of Science Laboratory Technology, Faculty of Natural Sciences, University of Jos, Plateau State, Nigeria during the 2022-2023 academic session.

Collection of samples: Dry rot symptomatic Irish Fig. 1 and sweet potato Fig. 2 tubers (50 each) were collected from Faringada Market. The samples were collected in sterile polythene bags and were transported to the laboratory for analysis.

Expressing Fusarium dry rot, cut open longitudinally across the point of inoculation to expose the internal dry rot lesion.

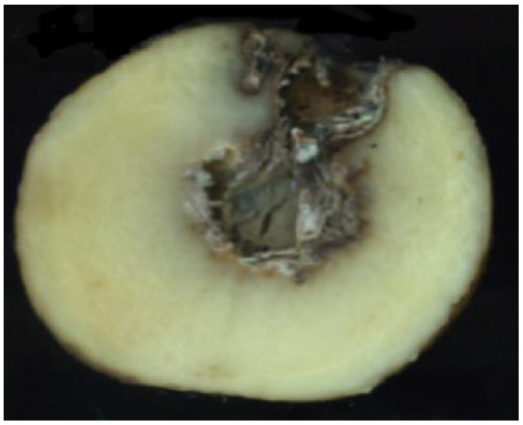

Fig. 1: Irish potato tubers

Fig. 2: Sweet potatoes tubers expressing rot

Fig. 3: Fungi isolated from the faringada market

Extraction of lime and lemon peel: Five lemon and lime fruits were peeled separately. The peels were air dried at room temperature and ground in powder form[8]. As 150 g of the powdered leaves were extracted with 600 mL of methanol. The extract was separately filtered using Whatman's No. 1 filter paper. Then the extract was concentrated in a vacuum using a rotatory evaporator at 40°C, the methanol remaining in the extract was removed by placing it at room temperature at 37°C overnight to give a residue weighing 8 g.

Fig. 4: Fungi isolated from the terminus market

Phytochemical analysis: The presence of glycosides, tannins, flavonoids, alkaloids, saponins, carbohydrates, proteins and water-soluble vitamins in the reconstituted fruit peel extract was examined by standard phytochemical methods[9].

Media preparation: The media used was (PDA agar) prepared according to the manufacturer's instructions[10].

Pathogen isolation, identification, and pathogenicity: Sections (0.5 cm in diameter, six pieces per tuber) were cut from the margins of necrotic or symptomatic tissue with a sterile scalpel, surface disinfested in 0.6% sodium hypochlorite for 10 sec, it was rinsed twice in sterile distilled water, and dried on sterile filter paper. The tissue sections were plated on half-strength potato dextrose agar (PDA; Difco Laboratories, Detroit, MI) amended with 0.5 g/L of streptomycin sulfate. Petri dishes were incubated at 23°C in the dark for 5 to 7 days. Putative isolates were transferred onto water agar (WA), and hyphal tips from the margin of actively growing cultures were removed with a sterile scalpel and plated to carnation leaf agar (CLA) and full-strength PDA to generate pure cultures, Fig. 3 and 4. The fungal strains were subjected to lactophenol cotton blue staining for morphological study. Preliminary identification of the isolates was based on conidial and colony morphology and pigmentation on PDA, respectively[11,12].

A pathogenicity test was carried out on healthy potato tubers to confirm that the isolated organisms caused the disease.

Determination of antifungal activity of the extracts: The crude extracts were screened for their antifungal activity, i.e. determination of the zone of inhibition against tested organisms by agar well diffusion method[13]. Sterile PDA gar plates were inoculated with prepared inoculum with a sterile cotton swab. Then with the help of a sterile cork borer no. 6, wells were made in the inoculated media plate. The 50 µL of the working solution/ suspension of different concentrations was transferred into the well with the help of a micropipette. The control was also placed in a separate well at the same time. After proper incubation, the plates were viewed for the zone of inhibition, which is suggested by clear areas without growth around the well.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): The crude extract, which showed antifungal activity, was subjected to a two-fold serial dilution method to determine minimum bactericidal concentration (MBC). A set of 12 screw-capped test tubes containing 1 mL nutrient broth was required. The test tubes were labeled as positive growth control, negative growth control, and numbers 1 to 10. Different dilutions of the crude extract were 7, 50, 25, and 5%. Then a fold serial dilution of the extract was prepared, each containing equal volume but decreasing concentration. The 50 µL of culture inoculum of test fungi was added to each tube with the help of a micropipette, except for the negative control.

All the tubes were incubated at 15 for 3 days and observed for turbidity by comparing with +ve and –ve control.

The results were interpreted based on the fact that growth occurs in the positive control and any other tube in which the concentration of the extract is not sufficient to inhibit growth and the lowest concentration of the agent inhibits the growth of the organism as detected by lack of visible turbidity as designated the minimum inhibitory concentration (MIC). However, in some cases, it was difficult to identify whether the turbidity was due to the growth of bacteria or due to the turbidity of the extract itself, the tubes were subcultured on a nutrient agar plate with a proper label followed by incubation at 15 for 2-3 days. Then they were examined for the growth of fungi. The antifungal activity of both plant extracts was observed and compared to determine the best plant extract that can be used to inhibit the fungi causing dry rot of Irish and sweet potato.

Analysis of data: The statistical analysis of the data and comparison of the means was done using the SAS software and Duncan's multiple range tests, respectively, at the significance level 0.05. Transformation of the data was done as needed.

RESULTS

From the phytochemicals assay carried out on all the extracts (Table 1), the methanolic and ethanolic extracts were positive for the presence of tannin, alkaloids and glycosides. although stronger reactivity was observed in the methanolic extracts in the lemon peels with *a total percentage yield* of 24.16%. Preliminary phytochemical investigation of methanol extract of lime peel (*Citrus aurantifolia.*) showed that Steroids were of greatest concentration (++) than the aqueous lemon peel extracts while flavonoids and cardiac glycoside were of high concentrations (+++) with the least concentration of steroids and terpenes, which appeared only in traces in lemon peels. Subsequently, the methanol extracts of each plant material were selected for the antimicrobial activity based on the percentage yield produced by the solvent.

Morphological characters of the isolated fungal pathogen were studied on culture plates as well as under a microscope. Colony morphology on culture plates was compared with the characteristics from the

Table 1: Phytochemical analysis of lemon and lime peels

Constituent	Lemon peels	Lime peels
Alkaloid	+++	+++
Saponin	-	-
Tannin	++	++
Flavonoids	+++	+++
Carbohydrates	++	++
Steroids	+	++
Teprenones	+	++
Anthraquinones	-	-
Cardiac glycoside	++	+++
% Yields	24.16%	18.88%

+: Present in trace amount, ++: Present in moderate amount/concentration, +++: Present in high amount/concentration and -: Absent

Table 2: Cultural and morphological characteristics of sweet potato samples isolated collected from the Faringada market

Cultural characteristics	Morphology characteristics	Isolate
Initially white and develops to a pin with age	Growth form is wooly. Conidiophores are septate and branched. Two types of conidia are found, the macroconidia and microconidia. The macroconidia are long, septate, and septas while the microconidia are small and ovoid. Non-septate hyphae and cotton mycelium. Produce clusters of root-like structure rhizoid and stolon.	*Fusarium oxysporum*

Table 3: Cultural and morphological characteristics of sweet potato samples isolate from the Terminus market

Cultural characteristics	Morphology characteristics	Isolate
Initially white and develops to a pin with age	Growth form is wooly. Conidiophores are septate and branched. Two types of conidia are found, the macroconidia and microconidia. The macroconidia are long, septate, and sickle-shaped with 3-4 septas while the microconidia are small and ovoid. Non-septate hyphae and cotton mycelium. Produce clusters of root-like structure rhizoid and stolon. The colour of the colony is light to dark violet purple with a cottony mycelium. The reverse colour is red. Macroconidia are produced on branched conidiospheres and are kidney shaped. Colony grows rapidly with white aerial mycelium often tinged with purple. Mycelium has a powdery appearance due to the presence of chains of micro-conidia. Abundant micro-conidia are formed and are hyaline and usually one-celled. They are oval and slightly flattened at each end.	*Fusarium oxysporum* *Fusarium moniliforme* *Rhizopus stolonifer*

Table 4: Cultural and morphological characteristics of Irish potato samples isolates collected from Faringada market

Cultural characteristics	Morphology characteristics	Isolate
Initially white and develops to a pin with age	Growth form is wooly. Conidiophores are septate and branched. Two types of conidia are found, the macroconidia and microconidia. The macroconidia are long, septate, and sickle-shaped with 3-4 septas while the microconidia are small and ovoid. Non-septate hyphae and cotton mycelium. Produce clusters of root-like structure rhizoid and stolon. The colour of the colony is light to dark violet purple with a cottony mycelium. The reverse colour is red. Macroconidia are produced on branched conidiospheres and are kidney shaped. Colony grows rapidly with white aerial mycelium often tinged with purple. Mycelium has a powdery appearance due to the presence of chains of micro-conidia. Abundant micro-conidia are formed and are hyaline and usually one-celled. They are oval and slightly flattened at each end.	*Fusarium oxysporum*

Table 5: Cultural and morphological characteristics of Irish potato samples isolates collected from the Terminus market

Cultural characteristics	Morphology characteristics	Isolate
Initially white and develops to a pin with age	Growth form is wooly. Conidiophores are septate and branched. Two types of conidia are found, the macroconidia and microconidia. The macroconidia are long, septate, and sickle-shaped with 3-4 septas while the microconidia are small and ovoid. Non-septate hyphae and cotton mycelium. Produce clusters of root-like structure rhizoid and stolon. The colour of the colony is light to dark violet purple with a cottony mycelium. The reverse colour is red. Macroconidia are produced on branched conidiospheres and are kidney shaped. Colony grows rapidly with white aerial mycelium often tinged with purple. Mycelium has a powdery appearance due to the presence of chains of micro-conidia. Abundant micro-conidia are formed and are hyaline and usually one-celled. They are oval and slightly flattened at each end.	*Fusarium oxysporum* *Penicillium* Spp.,

existing atlas. *Fusarium oxysporum* was identified as the fungus associated with the disease after Preliminary identification of the isolates based on conidial and colony morphology and pigmentation in all the culture plates, as shown Table 2-5 and Fig. 3 and 4.

Table 6: Mean zone of inhibition of methanol peel extract lemon on fungi associated with dry rot of Irish potato and sweet potato

		Concentration (mg/mL)					
Isolate	Extract	200	150	100	50	25	12.5
Fusarium oxysporum	Lemon peel extract	77.90^{b}	69.10^{b}	50.55^{b}	44.67^{b}	39.63^{b}	30.00^{b}
	Lime peel extract	88.11^{a}	80.12^{a}	77.55^{a}	63.01^{a}	59.04^{a}	50.11^{a}
	Positive control (fluconazole)	100.00^{c}	100.00^{c}	100.0^{c}	86.60^{c}	74.10^{c}	45.30^{c}

Means with different superscript along the column are statistically significant at $p<0.05$

Table 7: Test for the minimum inhibitory concentration as well as the minimum fungicidal concentration of methanol extracts against test organisms

Fungi	Minimum inhibitory concentration (MIC) (mg/mL)	Minimum fungicidal concentration (MFC) (mg/mL)
Fusarium oxysporum	10.25	11.65
Positive control	25.00	25.00

Means with different superscript along the column are statistically significant at $p<0.05$

The result shows the susceptibility patterns of the test isolate to the varying concentrations. At higher concentrations, higher antifungal activity was exerted on the test organisms by all the extracts. The methanolic extract of lemon peels was 77.90 mm followed by 69.10 mm) at a highest concentration of 200 and 150 mg/mL respectively on *Fusarium oxysporum* Meanwhile, susceptibility rates of the methanol leaf extract of the lime for the isolates showed the highest zones of inhibitions of 83.21 and 76.33 mm all on *Fusarium oxysporum* at a concentration of 200 and 150 mg/mL. However, susceptibility rates of the control drug (Fluconazole) of the fungi isolates showed the highest percentage zones of inhibitions of 100.0mm each at a concentration of 200, 150, and 100 mg/mL, respectively. Differences in the susceptibility rates of all the bacterial and fungal isolates across concentrations were statistically significant and non-significance ($p>0.05$ and $p<0.05$) (Table 6).

Table 7 represents the minimum inhibitory concentration (MIC) of the extracts against the organisms. A constant MIC value of 10.25 mg/mL was obtained for the methanolic extracts against the test isolate; the methanolic extracts had MIC values varying between 10.25-20 mg/mL on the isolate. The methanolic extracts of both lemon and lime showed fungicidal effects on the test organisms with values as low as 11.65 mg/mL for all the isolates.

The result of the minimum inhibitory concentration as well as the minimum fungicidal concentration of methanolic extracts and the fungal control drug (fluconazole) showed that all the test organisms had their MIC and MFC at 10.25 and 11.65 mg/mL, respectively.

DISCUSSION

Plants have served as a source of therapy for infectious diseases ranging from mild to fatal. Various research has concentrated on the antimicrobial properties of different plants as well as the presence of secondary metabolites with pharmacologic and therapeutic values. These reports have to a large extent provided insights into the predisposing factors contributing to the efficacy of natural plants in the management of infections. Generally, the potency of plants as antimicrobial agents is determined by the nature of the extractant/solvent, the concentration of the extracts and the presence of phytochemicals[14].

Methanolic extracts of the plant revealed that Tannins, saponins, flavonoids, and anthraquinones are highly present while steroids and cardiac glycosides are moderately present. Tannins, saponins, anthraquinones, and flavonoids have also been reported in similar research[14]. Carbohydrate is present in both ethanolic and aqueous extracts. On the other hand, the aqueous extract of the plant shows that saponin is highly present while tannins, flavonoids, and anthraquinones are moderately present. Cardiac glycoside is also present. This is in tandem with the research conducted by Banso[15].

Phytochemical screening also carried out indicated the presence of biologically active constituents including saponin, tannin, glycosides, alkaloids and steroids. In this study, the methanolic extract had the highest concentration of the assayed phytochemicals, followed by the water extract.

The present research showed that methanolic extracts of lemon and lime peels have the greatest inhibitory range against all tested *Fusarium* spp., indicating high antifungal activity. The richness of phytochemicals in the extracts, which is boosted by methanol's exceptional solubility qualities as an extraction solvent, is responsible for this great potency. Studies by Raubilu *et al.*[16] had revealed similar results. The chemical composition, molecular structure, and complexity of methanol make it an organic solvent that is more capable of dissolving plant phytoconstituents than ethanol and water[17].

The most commonly recovered species among the fungal isolates from Irish and sweet potato tubers affected with dry rot was *Fusarium oxysporum*. According to earlier research, *F. oxysporum* was common in Michigan's potato seed stocks in 2009 and 2010[18]. *Fusarium oxysporum* was responsible for 38 and 50% of the total isolates from sweet potatoes and sick Irish potatoes in this investigation, respectively. According to Aktaruzzaman *et al.*[19], *F. oxysporum* is a common cause of potato dry rot in the Northern United States. These results are in line with their findings.

The susceptibility patterns of the test isolate to the varying concentrations. At concentration, higher antibacterial activity was e xerted on the test organisms by all the extracts. The methanolic extract of lemon peels was 77.90 mm followed by 69.10 mm at the highest concentration of 200 and 150 mg/mL, respectively on *Fusarium oxysporum* while *F. solani* has the lowest susceptibility rate on the lemon extract with 60.10 and 58.88 at a high concentration of 200 and 150 mg/mL, respectively. Meanwhile, susceptibility rates of the methanol leaf extract of the lime for the isolates showed the highest zones of inhibitions of 83.21 and 76.33 mm all on *Fusarium oxysporum* at a concentration of 200 and 150 mg/mL, respectively while *F. sambucinum* has the least zone of inhibition of 75.75 and 69.67 at a highest concentration of 200 and 150 mg/mL. respectively. The least zones of inhibition of the methanol extract of lemon peel was 30.00 mm at 12.5 mg/mL while the least zone of inhibition of the methanol extract of lime was 31.00. However, susceptibility rates of the control drug (Fluconazole) of the fungi isolates showed the highest percentage zones of inhibitions of 100.0 mm each at a concentration of 200, 150, and 100 mg/mL, respectively. The findings of this study support those of Oluwalana *et al.*[20], who found that certain plants contain active compounds that can inhibit specific microorganisms. The researcher clarified that these compounds vary in their chemical structures, which in turn affects their inhibitory activity in fungus growth and survival[21]. Additionally, the sensitivity of *Fusarium oxysporum*, *F. sambucinum*, and *F. solani* to different plant extracts varies[22].

Low MIC values were obtained for the extracts, with a constant value of 12.5 mg/mL for the methanolic extract. This result also emphasizes the high antimicrobial potency of extracts of lemon peel and lime peels because a low MIC indicates a high efficacy of the extract[23]. At a low concentration of 12.5 mg/mL, the methanolic extract exerted fungicidal effects on *Fusarium oxysporum*. The methanolic extracts also had MFC values of 11.25-25 mg/mL. Lime and lemon peel extracts may therefore efficiently lyse fungal cells at low concentrations, so validating the plants' previously indicated excellent antibacterial activity. Nevertheless, mycelia growth was suppressed by all test plant extracts at different doses. As the extract concentration rose, so did the test plants' inhibitory impact. The test organism was significantly ($p<0.05$) controlled to varying degrees by the various plant extracts.

The antibacterial effect of the methanol lime peel extract on *Fusarium oxysporum* and the highest resistance *F. solani* was similar to the work by Okeke *et al.*[24]. Again, the work of Neela *et al.*[25] stated that "lime oil had a more consistent antimicrobial activity when used along with organic solvent (methanol) than the neat extract (orange essential oil)[14].

The two plant extracts' significant growth inhibitions of the test organisms suggest their possible use in controlling these organisms in disease-causing situations and opportunistic human infections. The lime peel extract's methanol extract was the most effective, revealing its potential efficiency for the treatment of diseases arising from this organism. The antifungal profile of these plant extracts underlines their recognition and application as medicinal plants.

CONCLUSION

The study revealed that methanolic and ethanolic extracts of lemon and lime peels contain significant phytochemicals, with methanol extracts showing higher yields. Fusarium oxysporum was identified as the primary fungal pathogen associated with dry rot in Irish and sweet potatoes. Methanolic lime peel extract exhibited the highest antifungal activity, with an 88.11 mm zone of inhibition at 200 mg/mL, outperforming lemon peel extract (77.90 mm) but remaining less effective than fluconazole (100.00 mm). The MIC and MFC values of methanolic extracts were 10.25 and 11.65 mg/mL, respectively, indicating strong fungicidal properties. The research revealed that the antifungal effect of the methanol extract of lemon and lime peel on fungi isolated from Irish and sweet potatoes was statistically significant. The results of this study therefore imply that both plant extracts have great antifungal properties. These findings suggest that citrus peel extracts have potential as natural antifungal agents for dry rot management. Future research should explore formulation methods to enhance their stability and field application as biofungicides.

SIGNIFICANCE STATEMENT

To avoid rot fungi in Irish (*Solanum tuberosum)* and sweet potato (*Ipomoea batatas*), this study compares the phytochemical content and antifungal qualities of lemon (*Citrus limon*) and lime (*Citrus aurantifolia*) peels. Food security and economic sustainability are greatly impacted by postharvest losses brought on by fungal diseases, especially in areas that depend on root and tuber crops. This study provides information on natural, environmentally acceptable antifungal substitutes by assessing the bioactive substances found in lemon and lime peels and their effectiveness against rot-causing fungi. The results may encourage the creation of bio preservatives to prolong shelf life, lessen reliance on chemical fungicides, and reduce postharvest losses, supporting sustainable farming methods and food preservation techniques.

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