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ANTIFUNGAL ACTIVITY OF CITRUS LIMON LEAF EXTRACTS AGAINST FUNGAL PATHOGENS ISOLATED FROM DECAYING POTATO TUBERS IN SUPERMARKETS OF THE EASTERN CAPE PROVINCE, SOUTH AFRICA

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ABSTRACT

Globally, fungal pathogens pose severe threats to stored cereal and tuber crops, resulting in substantial losses in economic crops. This study investigated the inhibitory activities of aqueous, ethanol, and acetone leaf extracts of *Citrus limon* L. Osbeck against the mycelial growth of *Curvularia mebaldsii, Fusarium oxysporum*, and *Penicillium* species isolated from spoilt potato tubers retailed within supermarkets in Eastern Cape Province, South Africa. Various concentrations (100 g/L, 50 g/L, and 25 g/L) of the *C. limon* leaf extracts (aqueous, acetone and ethanol) were prepared and (5 mL) amended with potato dextrose agar (oxoid, UK) previously inoculated with the 7-day old culture of each fungal isolates before incubation at 28 ^{o}C. The percentage growth inhibition was determined to evaluate the antifungal efficacy of each extract. Aqueous, ethanol and acetone extracts at 50g/L and 100 g/L displayed 100 % inhibition against all three pathogens except *Penicillium* sp. with 91.0 - 90.97 % inhibition. The ethanol and acetone extracts had the most inhibitory effects against *F. oxysporum C. mebaldsii*. In contrast, the aqueous extracts displayed the least effects, though not significantly different ($p<0.05$) across the concentrations. The notable antifungal effects against *F. oxysporum*, a prominent pathogen of post-harvest spoilage of potatoes, suggest the potential use of *C. limon* extracts to enhance the shelf-life of potatoes in supermarkets, specifically, and for post-harvest storage in general.

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INTRODUCTION

Potato, scientifically known as *Solanum tuberosum,* is the fourth major food crop worldwide, behind wheat, rice, and maize (Liu et al., 2020). Belonging to the Solanaceae family, this plant originated in Peru, South America (Perez et al., 2022). Documented for its vital contributions to food, income, and employment in developing nations (Jenning et al., 2020), potato holds significant importance due to their rich energy content and ease of cultivation. This has positioned it as a major constituent of urban agriculture, supporting over 800 million individuals through job creation and food security (Jennning et al., 2020).

Over the years, potatoes have been identified as a staple food and serve as good sources of highly digestible carbohydrates, dietary fibre, essential vitamins and minerals (Zaheer and Akhtar, 2016). In developing countries, fresh potatoes are sourced as vegetables for healthy growth and development. Potatoes have been found to contain potential health-promoting

phytocompounds such as phenolic, carotenoids, dietary fibre, anthocyanin, and glycoalkaloids, which are effective biomolecules against cancer, cholesterol, inflammation, obesity diabetic cells (Burgos et al., 2020). Due to potato tubers' relatively high moisture content, they are readily prone to soft rots. Soft rot disease of potatoes is a serious fungal disease that reduces their shelf-life, economic and nutritive values (Pang et al., 2021; Nisa et al., 2022). These rots negatively impact their nutritional and organoleptic qualities and, ultimately, their economic importance. According to environmentalists, this challenge undermines the expansive exploitations of the benefits that potatoes offer (Changdrasekara and Kumar, 2016). Past studies have demonstrated that moulds such as *Fusarium* species, among others, are the predominant pathogens of potato rots globally (Ray and Hammerschmidt, 1998; Wharton et al., 2006; Heltoft et al., 2015; Tiwari et al., 2020; Xue et al., 2023).

Fungal pathogens contribute to one-third of global annual food losses (Ameida et al., 2019). In developing countries, fungal diseases are often categorized as neglected human diseases due to their threats to human health and crop yield. Plant protection against pathogens involves producing various secondary metabolites playing crucial roles in defense mechanisms against pathogenic organisms (Hiruma, 2019). These compounds can be classified into constitutive compounds in healthy plants and inducible compounds synthesized in response to pathogenic attacks (Huang et al., 2020). The first category comprises phenols, flavonoids, tannins, saponin glycosides, and alkaloids, while the second includes phytoalexins and phytoanticipins. These bioactive compounds have garnered attention for their potential as agents for biological pest and disease control.

A previous report recorded a high presence rate of rots in potato tubers sold in supermarkets within the Raymond Mhlaba local municipality of the Eastern Cape Province, South Africa (Ehiobu et al., 2020). Considering the global significance of potato tubers in food industries and human health, safeguarding them against pathogens' attacks cannot be overemphasized. While chemical fungicides have been applied to fight against fungal diseases, they come with ecological toxicity, high costs, and resistance development (Sabarwal et al., 2018; Iqbal and Mukhtar, 2020). Utilizing biofungicides derived from plant sources has emerged as an eco-friendly alternative to address this (Iqbal et al., 2014; Shahzaman et al., 2015; Shahbaz et al., 2023). This approach involves harnessing plant phytochemicals to create affordable and nontoxic fungicides (Balestrini et al., 2020). According to a study by Zanna et al. (2021), different plant extracts have demonstrated efficient *in vitro* antifungal activities against common spoilage fungi.

Moreover, using plant biomolecules as active bio-protective agents against rot fungal pathogens in potatoes has been recently recommended (Steglinska et al., 2023). Thus, this study was designed to investigate the *in vitro* potential antifungal activities of aqueous, acetone, and ethanol leaf extracts of *C. limon* on three fungi isolated from potato tubers sold in supermarkets within Raymond Mhlaba local municipality of Eastern Cape province, South Africa. Focusing on this particular geographic environment will provide insights that could directly impact provincial potato cultivation and contribute to ecologically sound agricultural practices. This unique investigation supports the increasing demand for innovative approaches that balance crop security and environmental conservation.

MATERIALS AND METHODS

Study area

The study was conducted during the summer at the Antimicrobial Laboratory, Botany Department, University of Fort Hare, Alice, South Africa. The geographical coordinates of the study area are 30 º 00 N to 34º 15' S and 22º 45'W to 30º 62´ E (Erasto et al., 2011).

Source of potato tubers

Rotten potato tubers were randomly gathered from supermarkets in Alice, King Williamstown, and Fort Beaufort in the Mhlabas local municipality of the Eastern Cape Province, South Africa. The infested tubers were placed in sterile polythene bags and transported to the laboratory to isolate and identify the pathogens associated with their spoilage.

Collection of plant samples and identification

Fresh leaves of *C. limon* (4 kg) were obtained from the staff quarters of the University of Fort Hare, Alice campus in the Eastern Cape Province, South Africa. The fresh leaves were identified as *C. limon* by a plant taxonomist at the University of Fort Hare Botany Department. The plant sample was deposited at the Botany Department Herbarium, Universities of Fort Hare, with voucher number UFH 2019-10-002.

Processing of plant sample and extraction procedure

The method of Onukwuorji et al. (2012) was adopted. According to this method, the collected *C. limon* leaves were washed in tap water to remove attached dirt and dust particles and dried at 40 °C for 72 hours. The dried leaves were pulverized into powder with an industrial electric blender (Hamilton Beach commercial HBF500S series China). The powdered leaves were stored at -20 °C. Ethanol, aqueous, and acetone solvents were used for extraction, which involved macerating 175 g of plant sample in 1 L of each solvent and subjected to 48 hours of shaking in a mechanical shaker (Gallenkamp orbital shaker). Buchner funnel with Whatman number 1 filter paper connected to the vacuum pump was used to filter the mixtures of ground plant samples with solvents. The aqueous filtrate from the aqueous solvent was chilled at -40 ^{o}C and later freeze-dried with a freeze dryer (Virti's bench top K). The organic solvent extracts (ethanol and acetone) were further concentrated at 45 ºC until dried with a rotary evaporator.

Reconstitution and Standardization extracts

The stored plant extracts were individually weighed and reconstituted using sterile distilled water to create concentrations of 25 g/L, 50 g/L, and 100 g/L, following previously established procedures (Tijiani et al., 2013; Giwa and Akombo, 2016).

Isolation and identification of fungal pathogens of potato rot disease

The sampled rotted potato tubers were washed under running tap water and sliced into tiny bits. With a sterilised inoculating needle, the tiny bits of rotted potato tuber samples were inoculated onto freshly prepared PDA and incubated at an ambient temperature of (35^{o}C) for ten days. Pure fungal cultures were obtained through successive culturing. The pure cultures were characterized by examining their colonial morphologies and Methyl blue-stained microscopic features, such as the shape and colour of the conidiophore, conidia, mycelium, sporangiophore and vesicles. The observed characters were compared with already identified species using the identification key, as reported by Barnett and Hunter (1972).

Scanning electron microscope (SEM) preparation for fungal isolate identity

A sterile dissection knife was used to carefully extract one cm^2 section of pipe deposition or encrustation from the surface matrix of each of the three test samples. Each fragment was then immersed in 2.5 % glutaraldehyde in cacodylate buffer for 2 hours and rinsed with distilled water. Post-fixation was conducted with 2 % osmium tetroxide for 1 hour. Subsequently, the post-fixed samples were rinsed in distilled water for 15 minutes. The rinsed samples were sequentially placed in 50 %, 60 %, 70 %, 80 %, and 90 % ethanol for 10 minutes each and then transferred to 100 % ethanol for 20 minutes. The fungal samples were air-dried in a desiccator, mounted on an aluminium stub using double-sided carbon tape, and sputter-coated with gold/palladium (Au/Pd) before being observed under a scanning electron microscope (SEM).

Molecular characterization of fungal isolates

For DNA extraction, 50 mg of fungal wet weight was re-suspended in 200µl of an isotonic buffer. To this, 750µl of lysis solution was carefully added. The tubes containing the wet samples were processed in a bead beater with a 2mL tube holder assembly for 3 minutes. Cell disruption was also performed for 20 minutes using standard bench-top vortexes. After centrifugation at 10,000 ×g, 400 µl of supernatant was transferred to a Zymo-spin filter (Orange top) in a collection tube, followed by centrifugation for 1 minute at 7000 ×g. Subsequently, 1200 µl of fungal DNA binding buffer was added to the filtrate, and 800µl of the resulting mixture was transferred to a Zymo-spin column and centrifuged at 10,000 ×g for 1 minute. The elution process was repeated, and the purified DNA was further subjected to genomic DNA extraction using the quick DNA fungal mini-prep kit (Zymo Research Catalogue number D6005). The Information technology service (ITS) target region was amplified using NEB's quick-load 2× Master Mix (Catalogue number M0486) with primers listed in Table 1. PCR products were analyzed using a gel and purified using the Zymoclean Gel DNA recovery kit (Zymo Research Catalogue number D4050). The resulting purified fragments were analyzed on the ABI 3500×1 genetic analyzer (Applied Biosystems, Thermo Fisher Scientific), as delineated in Table 1.

Pathogenicity assay

To confirm pathogenicity, the assay was done following the procedure reported by Gwa and Richard (2018) with slight modifications. Healthy potato tubers were washed and sterilized with a 5 % sodium hypochlorite solution for 30 seconds. Sterilized healthy potato tubers were punctured with a 5 mm diameter cork borer to create holes for inoculation. A 5-7 day-old culture isolate disc was inoculated into one hole, sealed with petroleum

jelly. Control tubers received a disc of uninoculated potato dextrose agar (PDA). After 14 days of incubation at 35°C, tubers were examined for disease symptoms. The symptoms observed in the diseased tubers matched those of the treated fresh tuber, confirming Koch's postulates.

Table 1: ITS Primers Sequences.

Name of Primer	Target	Sequences (5′to3′)	Reference
ITS1	Small Sub-Unit	TCCGTAGGTGAACCTGCGG	(White et al., 1990)
ITS4	Large Sub-Unit	TCCTCCGCTTATTGATATGC	(White et al., 1990)

BLASTN 2231+.

***In vitro* inhibition assay of plant leaf extract**

The *in vitro* inhibitory effects of aqueous, acetone, and ethanol plant extracts were evaluated on mycelial growth using the method of Gwar and Richard (2018). This method was initiated by creating four equal sections on each plate through the drawing of two perpendicular lines at the bottom. The point of intersection was indicated as the centre of the plate. The plates were amended with 5 mL of plant extracts at various concentrations (25 g/L, 50 g/L, 100 g/L), except for the control. After that, a 5mm diameter disc cut from the periphery of the colony of the pure culture of each isolated pathogen pure culture was placed in the centre of each dish containing the amended potato dextrose agar (PDA) and incubated at 35 °C. The Petri dishes were laid out in a completely randomized design and, readings were obtained, and the experiment terminated on the seventh day. After seven days, the percentage growth inhibition (PGI) was calculated using the formula:

$$PGI(\%) = \frac{DC - DT}{DC} \times 100$$

Where DC is the average diameter of the fungi pathogen colony in the control plate, and DT is the average diameter of the fungi pathogen colony in the treated plate.

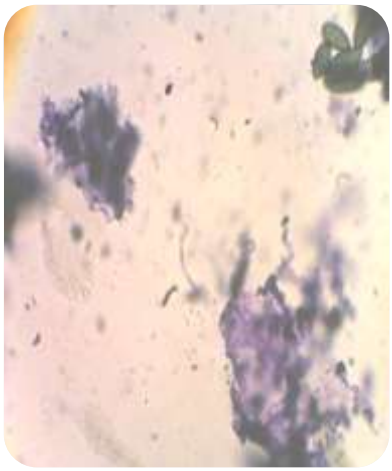
Data analysis

The data from the mycelial growth inhibition assay was expressed as a percentage (%). The means of the various treatments were compared through a one-way Analysis of Variance (ANOVA) with a significance level of 0.05 between treatments.

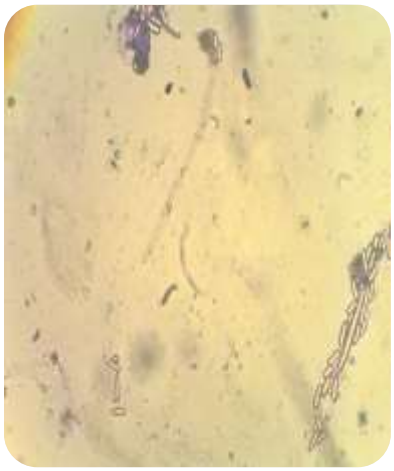
RESULTS

Colonial morphology and light microscope micrograph

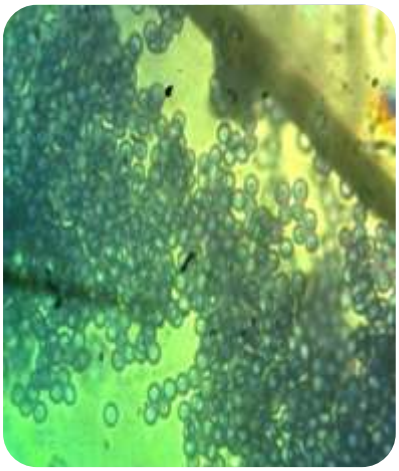
The result of the colonial morphology of the pure colonies on PDA and subsequent microscopic examination of each stained colony revealed a preliminary identity of hyphal structures and spores, which are typical of *Curvularia mebaldsii*, *Fusarium oxysporum*, and bunches of vesicles of *Penicillium* species as shown in Figure 1.



Curvularia mebaldsii × 40



Fusarium oxysporum × 40



Penicillium species × 40

Figure 1: Light microscopic micrographs of the fungal isolates from potato rot disease.

Molecular characterization of fungal isolates

The phylogenetic tree of the three fungal isolates using their characterized ITS gene is shown in Figure 2.

Pathogenicity test

The results of the pathogenicity test to establish the isolates as the causative agent of the observed potato rot disease are displayed in Figure 3. The result revealed that the inoculated fresh tuber manifested the disease symptom of macerated soft tissue with a foul odour. The control showed slight tissue maceration due to the wounds.

Pure culture isolates

Figure 4 shows the pure culture of the disease isolate obtained from culturing and subsequent sub-culturing of the rot pathogen tuber isolates randomly picked from supermarkets across the major studied towns of Alice, King Williams and Fort Beaufort in the study area.

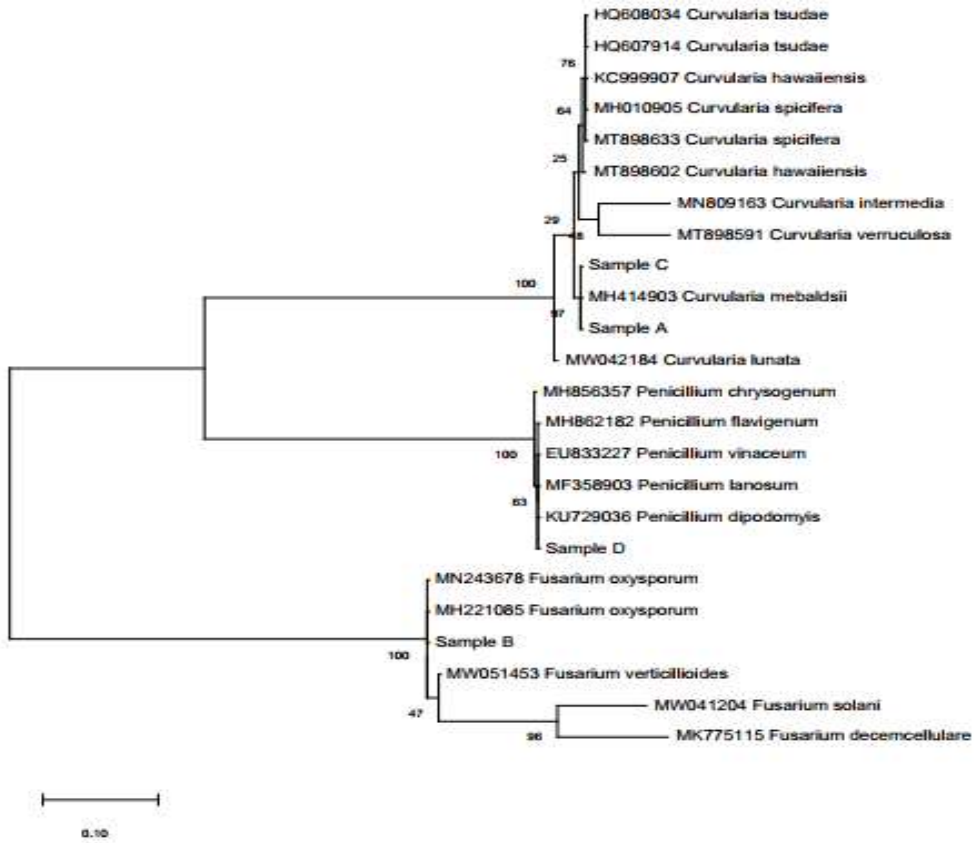


Figure 2: Phylogenetic tree of three fungal isolates from potato rot disease.

Figure 3: The pathogenicity test results of isolate of potato rot disease incubated at 35ºC for 14 days.

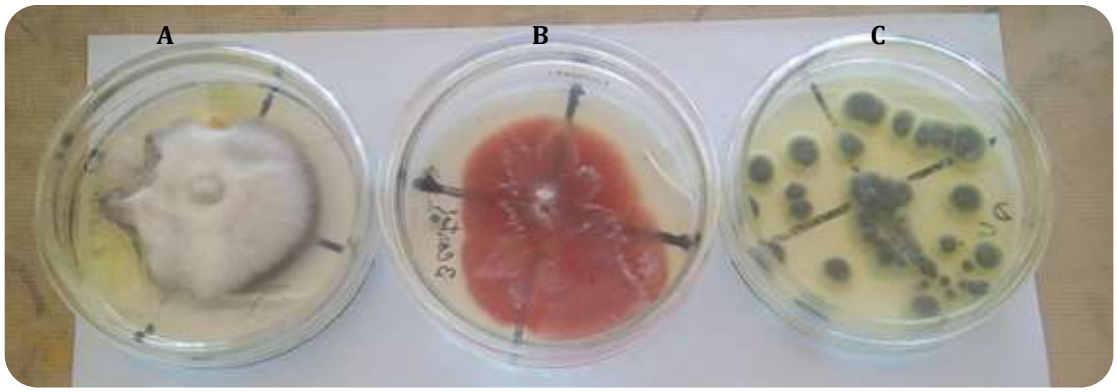


Figure 4: Purified cultures of fungal pathogens isolated from potato rot: A= *Curvularia mebaldsii*; B = *Fusarium oxysporum*; C = *Penicillium* spp.

***In vitro* antifungal activity of the extracts**

Figure 5 shows the growth inhibition patterns of three fungal isolates from rotten potato tubers using aqueous leaf extract of *C. limon.* At a plant extract concentration of 100 g/L, the examined pathogens exhibited 100 % inhibition in mycelial growth rate. At 50 g/L, the plant extract displayed 100 % inhibition in mycelial growth rate for both *F. oxysporum* and *C. malbaldsii* and 91 % for *Penicillium* sp. The lowest concentration (25 g/L) resulted in 99 %, 89.7 % and 86 % inhibition of mycelial growth rate for *F. oxysporum, C. malbaldsii* and *Penicillium* sp.

Similarly, the growth inhibition patterns of the three fungal pathogens when exposed to acetone extracts of *C. limon* are presented in Figure 6. The highest acetone extract concentration (100 g/L) led to 100 % inhibition in the mycelial growth rate of the three examined pathogens. At a concentration of 50 g/L, a 100 % inhibition rate was recorded against the mycelial growth of *F. oxysporum* and *C. malbaldsii*, while that of *Penicillium* sp. was 91 %. At 2 5g/L acetone extract concentration, the antifungal effect percentage against *F. oxysporum* was 91 %, followed by *C. malbaldsii* (97 %) and *Penicillium* sp. (86%).

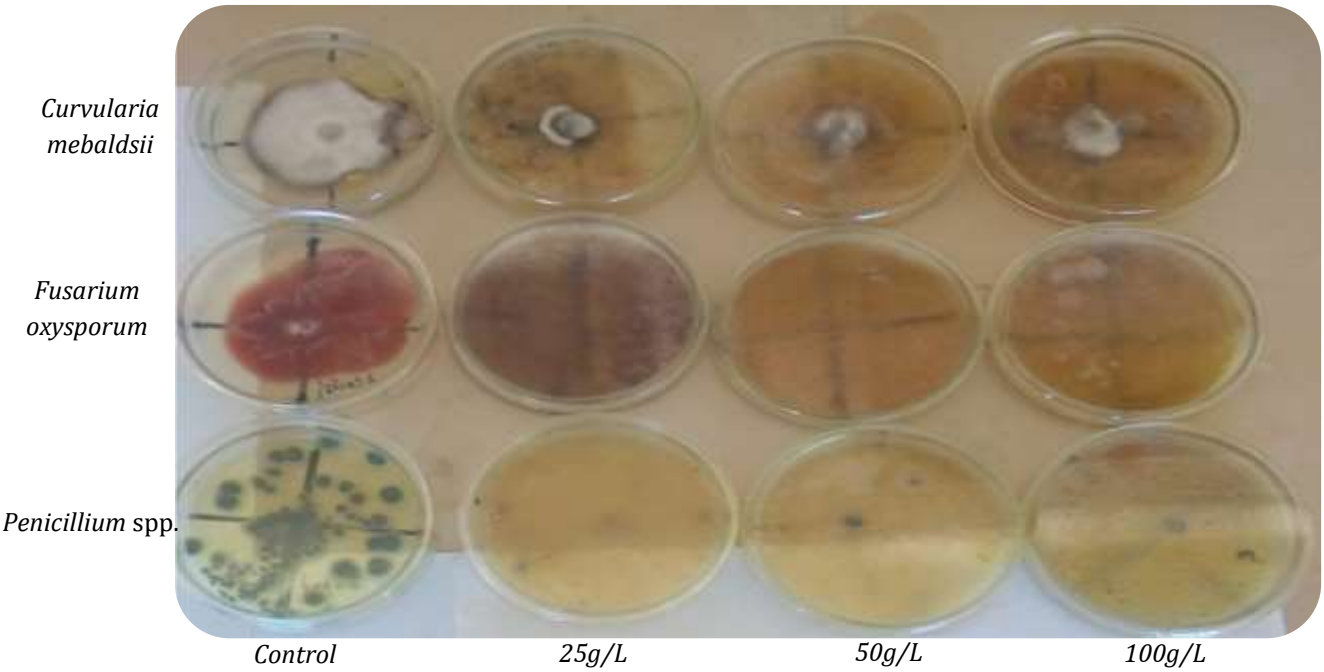


Figure 5: Exposure of 7-day-old cultures of fungal rot disease isolates to aqueous leaf extracts of *C. limon* at concentrations of 25 g/L, 50 g/L, and 100 g/L, with a negative control (no plant extract).

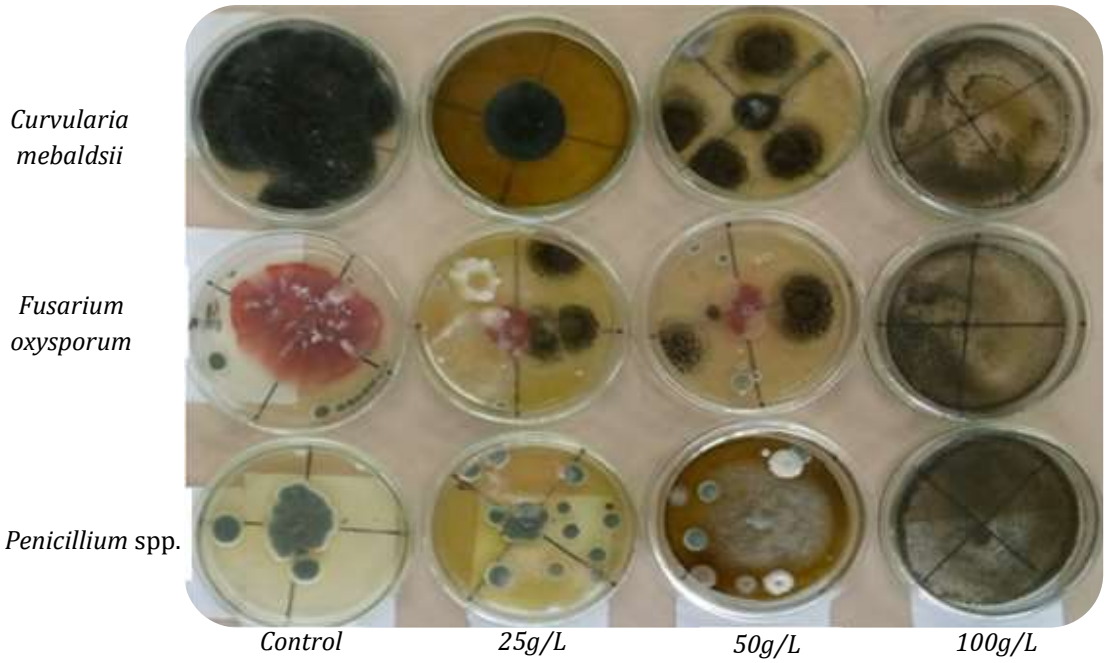


Figure 6: Exposure of 7-day-old cultures of fungal rot disease isolates to acetone leaf extracts of *C. limon* at concentrations of 25 g/L, 50 g/L, and 100 g/L, with a negative control (no plant extract).

Regarding the antifungal activity of the ethanol extracts of *C. limon,* a 100 % mycelial growth inhibition rate was observed against each of the three fungal isolates at an extract concentration of 100 g/L (Figure 7). Also, at a concentration of 50g/L, *F. oxysporum* and *C. malbaldsii* exhibited a 100 % inhibition growth rate, while *Penicillium* sp. mycelial growth was reduced by 90.97 %. Finally, at an ethanol concentration of 25 g/L, *F. oxysporum* and *C. malbaldsii* mycelial growth inhibition rates were 96.52%, while *Penicillium* sp exhibited 85.57 % inhibition in mycelial growth rate.

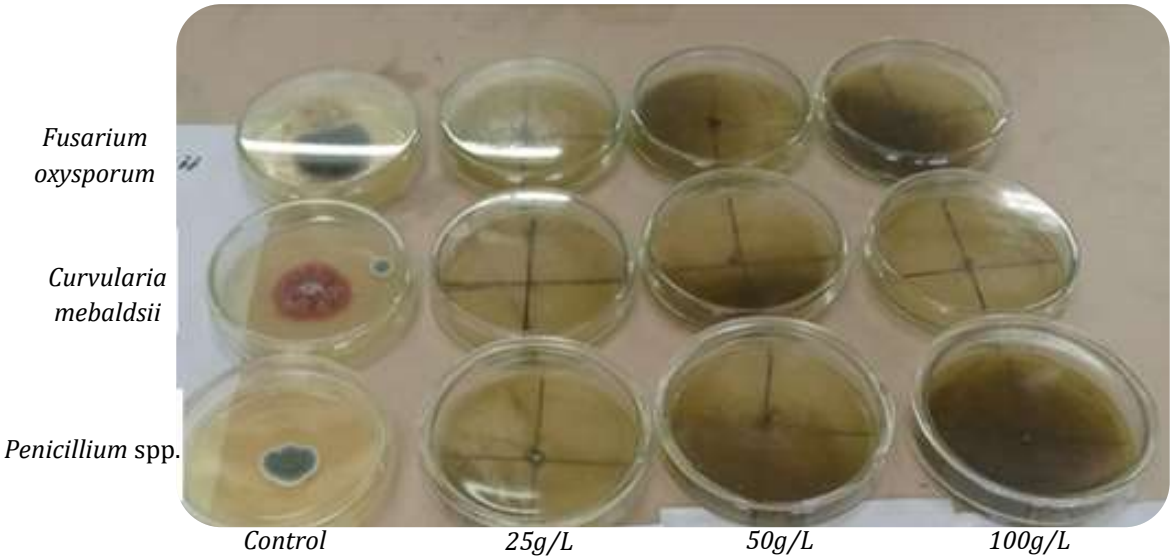


Figure 7: Inhibitory pattern of fungal isolates from diseased potato tubers with concentrations of 25 g/L, 50 g/L, and 100 g/L of ethanol leaf extracts of *C. limon* with a negative control (no plant extract) after 7 days of incubation at 28ºC.

Figure 8 shows growth inhibition patterns of the three fungal isolates from potato rot disease prevalence in

supermarkets using aqueous leaf extracts of *C. limon.* At 100g/L plant extract concentration, the examined pathogens exhibited 100% mycelial growth rate inhibition. At 50g/L, plant extract *F. oxysporum* and *C.malbaldsii* displayed 100% mycelial growth rate inhibition, while the growth rate of *Penicillium species* was reduced by 91%. Lastly, at 21g/l concentration of the same plant extracts, the mycelial growth of *F. oxysporum was* 99%, followed by *C.malbaldsii* (897%) and *Penicillium species* (86%).

Figure 9 shows the growth inhibition patterns of the three fungal pathogens isolated from potato rot disease prevalence in supermarkets using acetone extracts of *C. limon.* At 100g/L acetone, plant leaf extract shows 100% inhibition on the mycelial growth rate of the three examined pathogens. At 50g/L concentration, *F. oxysporum* and *C. malbaldsii* exhibited 100% mycelial growth inhibition, while that of *Penicillium species* was reduced to 91%. Lastly, at 25g/L concentration, *F. oxysporum* displayed a mycelial growth rate of 91%, followed by *C. malbaldsii* (97%) and *Penicillium species* (86%).

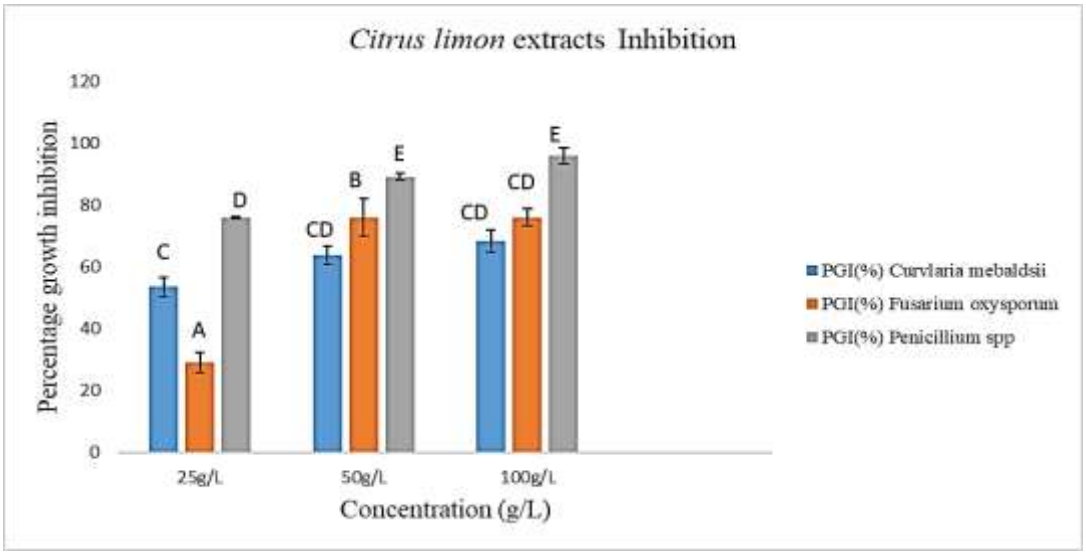


Figure 8: Growth inhibition of the three fungal pathogens isolated from rotten potatos using aqueous leaf extracts of *C. limon* (values with different alphabetical letters indicate significant differences at P < 0.05).

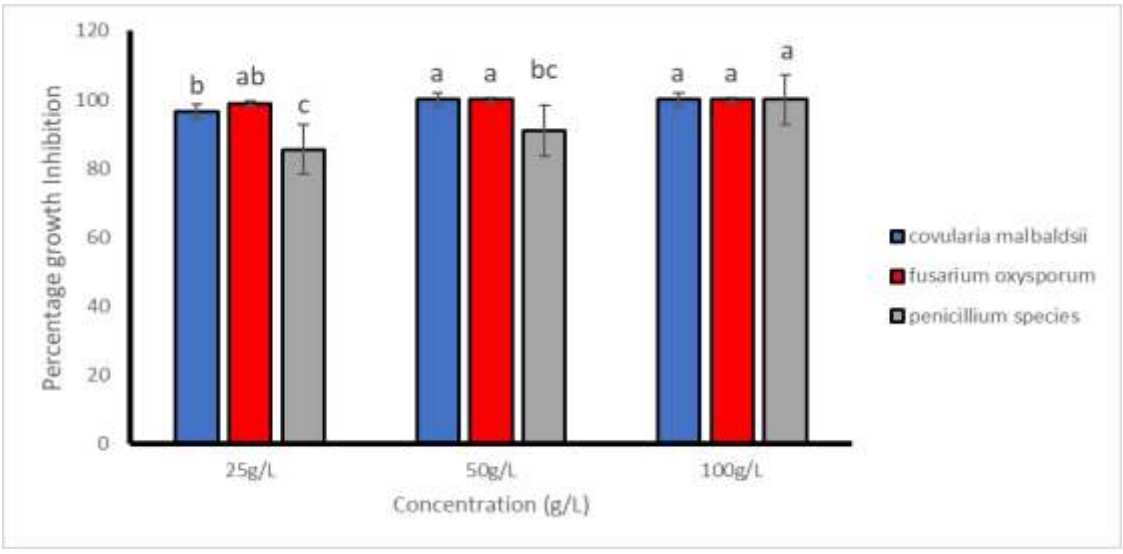


Figure 9: Growth inhibition of the three fungal pathogens isolated from rotten potatos using acetone leaf extracts of *C. limon* (values with different alphabetical letters indicate significant differences at P < 0.05).

Figure 10 shows the growth inhibition patterns of the three fungal pathogens isolated from potato rot disease prevalence in supermarkets using ethanol extracts of *C. limon.* The three examined potato rot disease pathogens displayed 100% mycelial growth inhibition at 100g/L plant leaf extract concentration. At 50g/L concentration, *F. oxysporum* and *C. malbaldsii* exhibited a 100% inhibition growth rate, while *Penicillium* species mycelial growth was reduced by 90.97%. Finally, at 25g/L concentration, *F. oxysporum* and *C.malbaldsii* mycelial growth inhibition was 96.52%, while *Penicillium species* exhibited 85.57% mycelial growth rate inhibition.

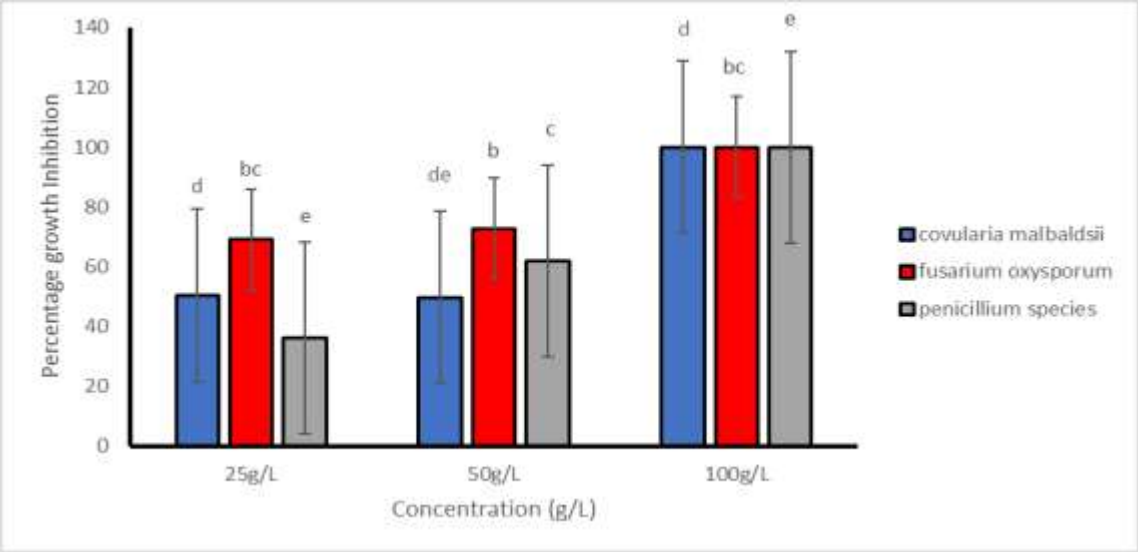


Figure 10: Growth inhibition of the three fungal pathogens isolated from rotten potatos using ethanol leaf extracts of *C. limon* (values with different alphabetical letters indicate significant differences at P < 0.05).

DISCUSSION

All food crops are subject to post-harvest microbial spoilage, depending on the intrinsic and extrinsic prevailing conditions. Among the readily attacked tuber crops is sweet potato, predominantly cultivated in most African countries (Singh et al., 2021). The biological management of these plant pathogens has continued to gain the attention of scientists in recent times (Zaria, 2014). This study evaluated the antifungal efficacy of acetone, aqueous, and ethanol leaf extracts of *C. limon* on three significant pathogens of potato rot diseases.

The isolates from the potato rot diseases were confirmed phenotypically and molecularly as *Curvularia mebaldsii*, *Fusarium oxysporum*, and *Penicillium* sp. The finding from the pathogenicity test established the isolates as the causative agents of the observed potato rot disease. The presence of *F. oxysporum*, *Penicillium* species and *C. mebaldsii* in this study, therefore, suggests that they are responsible for the spoilage of potatoes retailed within the supermarket, especially during the summer season in Mhlaba local municipality of Eastern Cape province, South Africa. The isolation of *F. oxysporum* from potato dry rot disease has also been previously studied (Shuping and Eloff, 2017). Also, Ibrahim et al. (2014) reported the isolation of *Rhizopus stolonifer, Aspergillus niger, A. flavus, Penicillium* species, *Mucor racemosus, F. oxysporum* and *Alternaria alternata* from rotted Irish potatoes in Sokoto Metropolis, Nigeria. Similarly, *A. flavus*, *A. niger, R. stolonifer, Trichoderma viride, F. oxysporum, P. digitatum, Cladosporium herbarium,* and *A. ochraceus* were associated with the deterioration of white variety sweet potato (*Ipomoea batatas*) under different storage structures (Tortoe et al., 2019). Other studies reported *A.s flavus, A. niger, F. oxysporum, Geotrichum candidum* and *R. oryzae* were obtained from the rotten potato samples from Odisha, India (Khatoon et al., 2017). In Uruguay, *F. oxysporum* was reported to occur in sweet potato rot *(Ipomoea batatas* L & Lamb*)* (Scattolini et al., 2020). It appears from previous reports that *Fusarium* spp. seems to be one of the major isolates from rotted potato tubers, suggesting that they are majorly responsible for their spoilage. Therefore, the presence of *F. oxysporum*, and *Penicillium* species, agrees with earlier reports that these

fungi are majorly associated with the spoilage of potato tubers (Lui et al., 2021; Paul et al., 2021; Paul et al., 2020; Gyasi et al., 2022; Lai et al., 2022). Several species of *Curvularia* have recently been reported from clinical samples (Madrid et al., 2014; Marin-Feli et al., 2020), but some are well-known for the global spoilage of cereals and tuber crops. Previous studies have linked the fungal isolates with spoilage of several post-harvested crops, leading to global colossal economic losses (Ameida et al., 2019). Apart from the potential financial losses, the phytopathogens isolated from this study have been reported to pose serious health risks to consumers of infested potatoes and other food crops (Egbuta et al., 2017). The ailments range from superficial to systemic and opportunistic infections in exposed individuals, especially the immunocompromised, very young and the elderly (Madrid et al., 2014; Egbuta et al., 2017). The presence of these isolates is of public health relevance, hence the need for proper management of commercial potato tubers to avoid public health hazards. Environmentalists and researchers have recommended using botanicals and other natural plant products for pest and disease management to achieve sustainable crop production (Lengal et al., 2020; Seepe et al., 2021).

The findings on the *in vitro* antifungal effects of *C. limon* leaf extracts probably offer potential choices for managing these phytopathogens of potato tubers. This study revealed that *C. limon* leaf extracts significantly inhibited the mycelial growth of *F. oxysporum*, *Penicillium* species and *C. mebaldsii.* This finding is concurrent with previous studies on the *in vitro* effects of plant extracts against bacterial and fungal isolates of crops (Ezeonu et al., 2018; Gwa and Richard, 2018; Giwa and Akombo, 2016; Ehiobu and Ogu, 2016; Musto et al., 2014; Raji and Raveendran, 2013). The 100% inhibition in mycelial growth rates of *F. oxysporum* by the aqueous, acetone and ethanol extract concentrations at 50 and 100 g/L is worth noting. This suggests the crude extract's complete suppression of the fungal growth at a relatively lower concentration. *Fusarium* one of the major pathogens of post-harvest spoilage of potatoes and other crops in different regions of Africa and Asian countries (Nsofor et al., 2020; Tiwari et al., 2020; Lui et al., 2021; Paul et al., 2021; Paul et al., 2020; Gyasi et al., 2022; Lai et al., 2022; Ikechi-Nwogu and Nworuka, 2023). This could have important implications for the potential use of aqueous, acetone and ethanol extracts as natural alternatives to synthetic fungicides in pre-and post-agricultural practices.

Earlier studies have shown that the fungal isolates were susceptible to different plant extracts. According to the report by Musto et al. (2014), the aqueous leaf extract of *Solanum nigrum* displayed significant inhibitory activity against the mycelial growth of *Penicillium digitatum*, the causal organism of *Citrus* blue mould. Similarly, aqueous *Allium sativum* extract (Gallic) exhibited high inhibitory activity against the mycelial growth of *P. digitatum* and *P. italicum.* In addition, Zhao et al. (2020) reported that tea saponins derived from *Camellia sinensis* expressed potent inhibitory activities against the mycelial growth of *P. digitatum and P. italicum.* Also, aqueous extracts of *Arenora rubra*, a Moroccan plant, demonstrated high inhibitory activity against *P. italicum,* the pathogen of *Citrus* blue mould (Askane et al., 2012). Furthermore, Zaria (2014) reported that the aqueous and ethanol extracts of five medicinal plant extracts significantly inhibited the mycelial growth of *Phytophthora infestan,* the fungal pathogen of late blight diseases of potatoes and tomatoes. Our findings agree with earlier reports regarding the potential inhibitory effects of aqueous and ethanol extracts of *C. limon* against the isolated pathogens of potato rot disease.

The findings from this study revealed extract concentration-dependent mycelial growth inhibition patterns. This is in concordance with the reports of several studies on the use of medicinal plants for the control of fungal diseases in crop plants (Sohail and Bani-Hassan, 2018; Ezeonu et al., 2018; Raji and Raveendran, 2013; Askane et al., 2012). This indicates that the leaf extracts' active ingredients are likely present in various fractions of the extracts in varying amounts. The observed variations in the inhibitory effects of the aqueous and organic solvents could be attributed to the differences in the extracting powers of the solvents and probably the extraction techniques. Studies have shown that the efficacy of extracting solvent from crude plant extracts is directly linked to the polarity of the metabolites and the solvent used for the extraction, which in turn determines the quality, quantity, extracting velocity, inhibitory compounds and biosafety (Zhang et al., 2019). The observed antifungal activities in this study could be linked to the bioactive compounds: alkaloids, saponins, flavonoids and phenolic compounds, reported in our previous study (Ehiobu et al., 2021). Volatile oil, sabinene, carene, limonene and ß-ocimene identified in *C. limon* leaf extract compounds

were reported to possess antimicrobial activities against pathogenic bacteria (Asker et al., 2020). Other antimicrobial phytcompounds reported in C*. limon* extracts were caffeoyl N-Tryptophan, Hydroxycinnmoyl-O-glucoside acid, Vicenin 2, Eriocitrin, Kaempferol-3-O-rutinoside, and Quercetin-3-rutinoside (Makni et al., 2018).

CONCLUSIONS

The fungal pathogens involved in the potato rot disease were isolated, the pure culture recovered, and the pathogenicity assay and molecular characterizations established the isolates as *Fusarium oxysporum*, *Penicillium* species and *Curvularia mebaldsii.* The study revealed that the aqueous, acetone and ethanol leaf extracts of *Citrus limon* demonstrated significant *in vitro* inhibitory activity against the investigated pathogens in a concentration-related pattern. Therefore, the plant leaf could be considered a biofungicide to enhance the shelf-life of potatoes in supermarkets, specifically, and for post-harvest storage in general. Further research on the purification of the specific bioactive ingredients and investigation of their *in vivo* activities is recommended.

AUTHOR'S CONTRIBUTIONS

JME designed the research, carried out all the experiments and wrote the manuscript as part of his PhD thesis.

CONFLICT Of INTEREST

The authors declare no conflict of interest.

REFERENCES

Alara, O.R., Abdurahman, N.H., Ukaegbu, C.I., Kabbashi, N.A., 2019. Extraction and characterization of bioactive compounds in *Vernonia amygdalina* leaf ethanolic extract comparing Soxhlet and microwave-assisted extraction techniques. Journal of Taibah University for Science 13, 414-422.

Ameida, F., Rodrigues, M.L., Coelho, C., 2019. The still understanding problem of fungal diseases worldwide. Frontiers in Microbiology 10, 1-5.

Askane, L., Talib, I., Boubaer, H., Boubaker, H., Boudyach, E.H., 2012. *In vitro* and *in vivo* antifungal activity of several Moroccan plants against *Penicillium italicum*, the causal agent of Citrus blue mold. Crop Protection 40, 53-58.

Asker, M., Elgengaihi, S.E., Hassan, E.M., Mohammed, M.A., Arafa, M.M., 2020. Phytochemical constituents and antibacterial activity of *Citrus lemon* leaves. Bulletin of the National Research Centre 44, 194.

Balestrini, R., Ghiagnone, S., Quiroga, G., Fiorilli, V., Romano, I., Gambino, G., 2020. Long term impact of chemical and alternative fungicides applied to grape vine CV Nebbiolo on berry transcriptome. International Journal of Molecular Sciences 21, 1-18.

Barnett, H.L., Hunter, B.B., 1972. Illustrated genera of imperfect fungi. Burgess publishing company. Minneapolis, Minneapolis, Minnessota, USA 90-241.

Burgos, G., Zum, Felde T., Andre, C., Kubow, S., 2020. The potato and its contribution to the human diet and health. The potato crop: Its agricultural, nutritional and social contribution to humankind, pp. 37-74.

Chandrasekara ,A., Kumar, T.J., 2016. Roots and tuber crops as functional foods: a review on phytochemical constituents and their potential health benefits. International Journal of Food Science 2016, 3631647.

Egbuta, M.A., Mwanza,_M., Babalola, O.O., 2017. Health risks associated with exposure to filamentous fungi. International Journal of Environmental Research and Public Health 14, 719.

Ehiobu, J., Idamokoro, E.M., Afolayan, A.J., 2020. Potato rot disease incidence among supermarket owners within the Raymond Mhlaba local municipality of South Africa. AIMS Agriculture and Food 52, 181-189.

Ehiobu, J., Idamokoro, E.M., Afolayan, A.J., 2021. Phytochemical content and antioxidant potential of leaf extracts of *Citrus limon* L. Osbeck collected in the Eastern Cape Province South Africa. South African Journal of Botany 1411, 480-486.

Ehiobu, J.M., Ogu, G.I., 2016. *In vitro* effect of *Colocasia esculenta* l. leaf extracts on mycelial growth and spore germination of *Fusarium* species. International Journal of Health Sciencs and Research 1, 47-52.

Ehiobu, J.M., Ogu, G.I., 2018. Phytochemical content and in vitro anti-mycelial efficacy of *Colocasia esculenta* L, *Manihot esculenta* Crantz and *Dioscorea rotundata* Poir leaf extracts on *Aspergillus niger* and *Botryodiplodia*

theobromae. Journal of Horticulture and Plant Research 1, 9-18.

Erasto, P., Mbwambu, H., Nondo, R.S.O., Lall, N., Lubschagne, A., 2011. Actimycobacterial antioxidant activity and toxicity of extracts from the root of *Rauvolfia vomitoria* R. *caffra*. ScopeMed 1, 73-80.

Ezeonu, C.S., Imo, C., Agwaranze, D., Iruka, A., Joseph, A., 2018. Antifungal effect of aqueous and ethanolic extracts of neem leaves, stem, bark and seeds on fungal rot diseases of yam and cocoyam. Chemistry and Biology 5, 1-9.

Giwa, V.I., Akombo, A., 2016. Studies on the antimicrobial potency of five crude plants and chemical fungicides in *in vitro* control of *Aspergillus flavus* causal agents of White yam *Dioscorea alata* tuber rot. Journal of Plant Science and Research 1, 1-8.

Gwa, V.I., Richard, I.B., 2018. Susceptibility of white yam *Dioscorea rotundata* Poir tuber to rot fungi and control with extracts of *Zingiber officinale* Rosc. *Azadirachta Indica* A. Juss. and *Piper guineense* Schumach. Journal of Plant Pathology and Microbiology 9, 452.

Gyasi, E., Akrofi, S., Adongo, B.A., Osafo, E.A., Kotey, D.A., Mohammed, A., 2022. Fungi associated with sweet potato tuber rot at CSIR - PGRRI Bunso Eastern Region, Ghana. Ghana Journal of Agricultural Science 57(1), 106-112.

Heltoft, P., Molteberg, E.L., Nastad, R., Hermansen, A., 2015. Effect of maturity level and potato cultivar on development of *Fusarium* dry rot in Norway. Potato Research 58, 205-219.

Hiruma, K., 2019. Roles of plant-derived secondary metabolites during interaction with pathogenic and beneficial microbes under conditions of environmental stress. Microorganisms 7, 1-9.

Huang, J., Rucker, A., Schmidt, A., Gleixner, G., Gershenzon, J., Trumbore, S., Hartmann, H., 2020. Production of constitutive and induced secondary metabolites in a coordination with growth and storage in Norway spruce saplings. Tree Physiology 40, 928-942.

Ibrahim, M., Shehu, K., Sambo, S., Tukur, I.A., Tafinta, I.Y., 2014. Identification of fungi associated with storage rots of Irish Potato *Solanum tuberosum* L. tubers in Sokoto Metropolis. Annals of Biological Sciences 2, 1-4.

Ikechi-Nwogu, C.G., Nworuka, N., 2023. Isolation and identification of common fungal pathogens invading sweet potatoes *Ipomoea batatas* sold in Choba market port Harcourt Nigeria. Journal of Applied Sciences and Environmental Management 27, e2717.

Iqbal, U., Mukhtar, T., 2020. Inhibitory effects of some fungicides against *Macrophomina phaseolina* causing charcoal rot. Pakistan Journal of Zoology 52(2), 709-715.

Iqbal, U., Mukhtar, T., Iqbal, S.M., 2014. *In vitro* and *in vivo* evaluation of antifungal activities of some antagonistic plants against charcoal rot causing fungus, *Macrophomina phaseolina*. Pakistan Journal of Agricultural Sciences 51 (3), 689-694.

Jennings, S.A., Kochler, A., Nicklin, K.J., Deva, C., Salt, S.M., Challinor, A.J., 2020. Global potato yields increase under climate change with adaptation and CO_2 fertilization. Frontiers in Food Sustainable Systems 4, 1-17.

Khatoon, A., Mohapatra, A., Satapathy, K.B., 2017. Studies on fungi associated with storage rot of sweet potato [*Ipomoea batatas* L. Lam.] root tubers in Odisha, India. International Journal of Microbiology and Mycology 5, 1-7.

Lai, J., Liu, T., Liu, B., Kuang, W., Song, S., 2022. First Report of *Curvularia plantarum* causing leaf spot on sweet potato *Ipomoea batatas* in China. Plant Disease 106, e1753

Lengai, G.M.W., Muthomi, J.W., Mbega, E.R., 2020. Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. Scientific African 7 e00239.

Liu, J., Sun, Z., Zou, Y., Li, W., He, F., Huang, X., Lin, C., Cai, Q., Wisniewski, M., Wu, X., 2021. Pre and post-harvest measures used to control decay and mycotoxigenic fungi in potato *Solanum tuberosum* L. during storage. Critical Review in Food Science and Nutrition 62, 415-428.

Liu, N., Zao, R., Qlao, L., Zhang, Y., Li, M., Sun, H., Zing, Z., Wang, X., 2020. Growth stages, classification of potato crop based on analysis of optimization. Sensors 10, 20.

Madrid, H., Cunha, KC, Da., Gené, J., Cano, J., Sutton, D.A., Guarro, J., Crous, P.W., 2014. Novel *Curvularia* species from clinical specimens. Persoonia 33, 48-60.

Makni, M., Jemai, R., Kriaa, W., Chtourou, Y., Fetoui, H.,

2018. *Citrus limon* from Tunisia, phytochemical and physicochemical properties and biological activities. BioMed Research International 2018, e6251546

Marin-Felix, Y., Hernández-Restrepo, M., Crous, P.W., 2020. Multi-locus phylogeny of the Genus *Curvularia* and description of ten new species. Mycological Progress 19, 559-588.

Musto, M., Potenza, G., Cellini, F., 2014. Inhibition of *Penicillium digitatum* by a crude extract from *Solanum nigrum* leaves. Biotechnology Agronomy Society and Environment 18, 174-180.

Nisa, T., Haq, M.I., Mukhtar, T., Khan, M.A., Irshad, G., 2022. Incidence and severity of common scab of potato caused by *Streptomyces scabies* in Punjab, Pakistan. Pakistan Journal of Botany 54(2), 723-729.

Nsofor, G.C., 2020. Fungal and bacterial pathogens associated with soft rot disease of sweet potato *Ipomoea batatas* L Lam. Nigeria Agricultural Journal 51, 213-218.

Onukwuorji, C.A., Ramesh, R.P., Okigbo, R.N., 2012. Isolation of fungi causing rot disease of Cocoyam *Colocasia esculenta* L Schott and control with plants extracts. Global Advanced Research Journal of Agricultural Science C1, 33-47.

Pang, L-J., Adeel, M., Shakoor, N., Guo, K-R., Ma, D-F., Ahmad, M.A., Lu, G-Q., Zhao, M-H., Li, S-E., Rui, Y-K., 2021. Engineered nanomaterials suppress the soft rot disease (*Rhizopus stolonifer*) and slow down the loss of nutrient in sweet potato. Nanomat 11, 2572.

Paul, N.C, Park, S., Liu, H., Lee, J.G., Han, G.H., Kim, H., Sang, H., 2021. Fungi associated with post-harvest diseases of sweet potato storage roots and *in vitro* antagonistic assay of *Trichoderma harzianum* against the diseases. Journal of Fungi Basel 711, 927

Paul, N.C., Park, W., Lee, S., Chung, M.N., Lee, H.U., Yang, J.W., 2020. Occurrence of sweet potato *Ipomoea batatas* wilt and surface rot disease and determining resistance of selected varieties to the pathogen in Korea. Plants 94, 497.

Perez, W., Forbes, G.A., Arias, R., Pradel, W., Kawarazuka, N., Andrade-Piedra, J., 2022. Farmer perceptions related to potato production and late blight management in two communities in the *Peruvian Andes*. Frontiers in Sustainable Food Systems 6, 7-14.

Raji, R., Raveendran, K., 2013. Antifungal activity of selected plant extracts against phytopathogenic fungi *Aspergillus niger.* Asian Journal of Plant Science and Research 3, 13-15.

Ray, H., Hammerschmidt, R., 1998. Responses of potato tuber to infection by *Fusarium sambucinum*. Phytopathological and Molecular Plant Pathology 53, 82-91.

Sabarwal, A., Kumer, K., Sing, A.P., 2018. Hazardous effects of chemical pesticides on human health cancer and other associated disorders. Environmental Toxicology and Pharmacology Journal 63, 103-114.

Scattolini, A., Hernández, R.L., González, I.H., 2020. Identification of *Fusarium oxysporum* causing sweet potato rot *Ipomoea batatas* L and Lamb in Uruguay. Agrociencia 24, 124.

Seepe, H.A., Nxumalo, W., Amoo, S.O., 2021. Natural products from medicinal plants against phytopathogenic *Fusarium* species: current research endeavours, challenges and prospects. Molecules 26, 6539.

Shahbaz, M., Akram, A., Raja, N.I., Mukhtar, T., Mehak, A., Fatima, N., Ajmal, M., Ali, K., Mustafa, N., Abasi, F. 2023. Antifungal activity of green synthesized selenium nanoparticles and their effect on physiological, biochemical, and antioxidant defense system of mango under mango malformation disease. PLoS ONE 18(2), e0274679.

Shahzaman, S., Inam-ul-Haq, M., Mukhtar, T., Naeem M., 2015. Isolation, identification of antagonistic rhizobacterial strains obtained from chickpea (*Cicer arietinum* L.) field and their *in-vitro* evaluation against fungal root pathogens. Pakistan Journal of Botany 47(4), 1553-1558.

Shuping, D.S.S., Eloff, J.N., 2017. The use of plants to protect plants and food against fungal pathogens. African Journal of Traditional, Complementary and Alternative Medicines 14, 120-127.

Singh, R., Gupta, M., Singal, P., Goyal, S., Updhyay, S.K., 2021. *In vitro* antimicrobial activities of vegetables potato cucumber, sweet potato and ginger peel wastes for eco-friendly microbial management. International Journal of Botany Sciences 6, 134-137.

Sohail, A.A., Bani-Hassan, B.M., 2018. Morphological and molecular identification of fungi isolated from

different environmental sources in the Northern Eastern desert of Jordan. Journal of Biological Sciences 11, 329-337.

Stefanczyk, E., Sabkowiak, S., Brylinska, M., Jadwiga, S.J., 2016. Diversity of *Fusarium* species associated with dry rot disease of potato tubers in Poland. European Journal of Plant Pathology 145, 871-844.

Steglińska, A., Sulyok, M., Janas, R., Grzesik, M., Liszkowska, W., Kręgiel, D., Gutarowska, B., 2023. Metabolite Formation by fungal pathogens of potatoes (*Solanum tuberosum* L.) in the presence of bioprotective agents. International Journal of Environmental Research and Public Health 20, 5221.

Tijiani, A., Adebitan, S.A., Gurama, A., Aliyu, M., Dawaji, A.Y., Haruna, S.G., Muhammmed, N.A., 2013. Efficacy of some Botanicals for the control of wet rot diseases on mechanically injured sweet potato caused by *Rhizopus stolonifer* in Bauchi state. International Journal of Scientific and Research Publications 3, 1-10

Tiwari, R.K., Kumar, R., Sharma, S., Sagar, V., Aggarwal, R., Naga, K.C., Lal, M.K., Chourasia, K.N., Kumar, D., Kumar, M., 2020. Potato dry rot disease, current status pathogenomics and management. BioTech 1011, 503.

Tortoe, C., Obodai, M., Amoa-Awua, W., 2019. Microbial deterioration of white variety sweet potato *Ipomoea batatas* under different storage structures. International Journal of Plant Biology 1, 52-55.

Trabelsi, B.M., Abdallah, R.A.B., Kthiri, H.W., Remadi, M.D., 2016. Assessment of the antifungal activity of non-pathogenic potato-associated fungi towards *Fusarium* species causing tuber dry rot disease. Journal of Plant Pathology and Microbiology 7, 1-7.

Wharton, P.S., Tumbalam, P., Kirk, W.W., 2006. First report of potato tuber sprout rot caused by *Fusarium sambucinum* in Michigan. Plant Disease 90, 1460.

Xue, H., Liu, Q., Yang, Z., 2023. Pathogenicity, mycotoxin production, and control of potato dry rot caused by *Fusarium* spp.: a review. Journal of Fungi 98, 843.

Zaheer, K., Akhtar, M.H., 2016. Potato production, usage, and nutrition-a review. Critical Reviews in Food Science and Nutrition 56, 711-21.

Zanna, H., Tijani, Y., Abubakar, S., Modu, B., Damasak, A.A., Uzairu, S.M., 2021. Fungicidal potential of selected plant extracts against human pathogenic fungi. Scientific African 13, 1-6.

Zaria, A.M.B., 2014. Antifungal activity of extracts from five Egyptian wild medicinal plants against late blight disease of tomato and potato. Pflanzenschutz 47, 1988-200

Zhang, H., Birch, J., Pei, J., Ahmed, I.A.M., Yang, H., Dias, G., Abd El-Aty, A.M., Bekhit, A.E-D., 2019. Identification of six phytochemical compounds from *Asparagus officinalis* L. root cultivars from New Zealand and China using UAE-SPE-UPLC-MS/MS: effects of extracts on H_2O_2-induced oxidative stress. Nutrients 11, 1-17.

Zhao, J., Zhang, J., Li, P., Xu, Y., He, Y., Fan, L., 2020. Triterpenoid saponins in tea *Camellia senensis* plants: biosynthetic gene–expression, content variations chemical identification and cytotoxicity. International Journal of Food Science and Nutrition 72, 308-323.