Peertechz

LIFE SCIENCES GROUP

OPEN JOURNAL OF Plant Science OPEN ACCESS JOURNAL

ISSN: 2640-7906 DOI: https://dx.doi.org/10.17352/ojps

Research Article

Screening, Isolation and Characterisation of Fungal Species Causing Post-Harvest Spoilage of Mangoes in Uganda

Edgar Mugizi Ankwasa[1,2]*, Atuhaire Judith[1], Imade Francis[3] and Ssekandi Joseph[4]

[1]Department of Microbiology and Biotechnology Centre, Product Development Directory, Uganda Industrial Research Institute, P.O. Box 7086 Kampala, Uganda

[2]Department of Production, NEC Uzima Ltd, National Enterprise Corporation, Plot 2 Muwesi Road, P.O Box 3145, Bugolobi, Uganda

[3]Plant Science and Biotechnology Department, Faculty of Life Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria

[4]National Agricultural Research Laboratories - Kawanda, P.O. Box 7065 Kampala, Uganda

Received: 31 January, 2025
Accepted: 20 February, 2025
Published: 21 February, 2025

***Corresponding author:** Edgar Mugizi Ankwasa, Department of Microbiology and Biotechnology Centre, Product Development Directory, Uganda Industrial Research Institute, P.O. Box 7086 Kampala, Uganda, Email: eddieank@yahoo.com; generalmanager.uzima@nec.go.ug

ORCiD: https://orcid.org/0000-0002-1857-1747

Keywords: *Mangifera indica* L.; Post-harvest spoilage; Fungi; Pathogenicity

Abstract

Mangoes are grown in tropical and subtropical countries in the world. Post-harvest spoilage of mangoes greatly reduces their quality and market value. Pests of mangoes include fruit flies, stone weevils, and mealy bugs while the main pathogens are the fungi. Several diseases have been reported in mangoes such as anthracnose, stem end rot, *Aspergillus niger* rot, soft rot, and *Alternaria* rot. The aim of this study was to isolate, characterize, and identify fungal species causing post-harvest spoilage of mangoes in Uganda. Mangoes with signs of fungal infection were purchased from markets in Kampala, small pieces (2X2mm) were cut and extracted from the infected tissue of the mangoes, surface sterilized by dipping them in 1% (V/V) Sodium Hypochlorite (NaClO) solution for 60 seconds, rinsed 3 times with sterile distilled water and cultured on Potato Dextrose Agar (PDA) supplemented with chloramphenicol at 28 °C with 12-hour photoperiod for 7 days. Pure colonies were obtained from single spore isolation and identified as *Aspergillus fumigatus, Neofusicoccum parvum, Aspergillus krugeri,* and *Lasiodiplodia theobromae* after morphological and molecular characterization. Pathogenicity of the isolates was performed according to Koch's postulates and two control samples were included. The effect of fruit maturity and incubation temperature were also investigated by using ripe and unripe fruits and incubating at different 15 °C, 28 °C, and 35 °C. Ripe mangoes showed severe symptoms compared to the unripe mangoes when incubated at 28 °C and the results further showed that 28 °C was the favorable growth temperature for the fungi and that all the isolates were pathogenic.

Introduction

Mango *Mangifera indica L.* belongs to the Anacardiaceae family with so many cultivars grown in many different parts of the world. Mangoes are reported to have originated from the Indo-Burma region, and they have been cultivated for more than 4000 years [1]. Mangoes are popular commercial fruits grown in the tropical and subtropical areas of the world and they can be consumed fresh or in processed form [1-11]. Among all the tropical fruits grown globally, mango production represented more than half of their total production in 2012, and mangoes are grown in more than 100 countries in the world [12]. Mangoes provide vitamins and minerals which are very vital components of the human diet [2,6,8-10,13-16].

Global production and trade of mangoes

Asia ranks first in global mango production contributing about 77% of global mango production, in second place is the Americas which contributes about 13% and Africa comes last with a contribution of about 10% of the global mango production [1,12,17]. Global mango production data indicates that mango production has increased from 37.59 million metric tons in 2010 to 55.8 million metric tons in 2019. India tops the global ranking for mango-producing countries followed by Indonesia,

Citation: Ankwasa EM, Judith A, Francis I, Joseph S. Screening, Isolation and Characterisation of Fungal Species Causing Post-Harvest Spoilage of Mangoes in Uganda. Open J Plant Sci. 2025;10(1): 001-008. Available from: https://dx.doi.org/10.17352/ojps.000062

China, Mexico, and Pakistan. Table 1 shows the top five global mango producers in the world and these combined contribute 64.46% of the total global mango production [17]. Recently the global market size of mango has grown with a compound annual growth rate of 6.7% from 2023 to 2024 and it has been estimated to grow with 8.0% Compound Annual Growth Rate (CAGR) from 2025 to 2028 (Figure 1). [18] The global market in Malawi tops the ranking of mango-producing countries in Africa, followed by Egypt, Nigeria, Kenya, and Mali. Table 2 shows the top 5 mango-producing countries in Africa and these top five countries in Africa combined contribute 9.35% of the total global mango production [17]. There is a huge gap in production between the top five producers in the world, most of whom are from Asia, and the top five mango producers in Africa which clearly indicates that mango production is still low in Africa.

According to the data from the Tridge website [17], the world's top 2 mango producers (India and Indonesia) are not among the top 5 global exporters shown in Table 3 below and this could be due to high local demand and consumption for mangoes in these countries. Among the top 5 global mango producers, only Mexico appears in the top 5 mango exporters with an export value of 430.37 million dollars representing 26.67%.

The United States of America is the leading global importer of mangoes, importing mangoes worth 717 million dollars representing 35.97% the bulk of which comes from Mexico [17].

Among the top 5 global mango producers, only China appears in the top 5 importers (Table 4) with an import value worth 143.16 million dollars representing 7.16%.

Overview of mango production in Uganda

In Uganda, mangoes are the most common and popular fruits grown in almost all parts of the country and they have shown adaptability to all environmental and climatic zones of the country however the northern region, northeastern region, and west Nile regions have the highest mango production. The following exotic varieties of mangoes are grown in the West Nile region; Tommy Atkins, Kent, Haden, Keit, Zillate, Palvin, Palmer, Alphonso, and Irwin [4] Lake Victoria Crescent is another area or region which the government of Uganda has identified for commercial fruit production including commercial mango production. This region covers the districts of Luwero, Masaka, Iganga, Mayuge, and Kamuli [8]. Mango

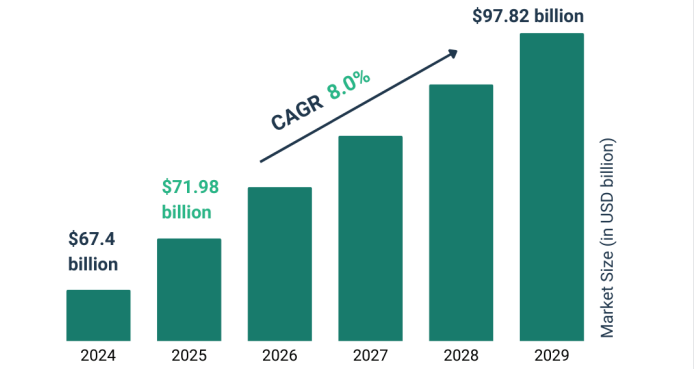

Figure 1: Mango global market report 2025 indicating Compound annual growth rate (CAGR) [32].

Table 1: Top 5 Global Mango Producing Countries in 2019.

Ranking	Country	Production volume (M MT)	Production (%)
-	Global	55.85	-
1	India	25.63	45.89
2	Indonesia	3.29	5.9
3	China	2.42	4.32
4	Mexico	2.40	4.29
5	Pakistan	2.27	4.06

Adapted from: (www.tridge.com/intelligences/mango/production accessed 2021-03-23)

Table 2: Top 5 Mango Producing Countries in Africa in 2019.

Ranking	Country	Production Volume	Production %
1	Malawi	2.1 M MT	3.73
2	Egypt	1.47 M MT	2.61
3	Nigeria	946.7 K MT	1.69
4	Kenya	868.0 K MT	1.55
5	Mali	814.9 K MT	1.46

Adapted from: (www.tridge.com/intelligences/mango/production accessed 2021-03-23)

Table 3: Top 5 Mango Exporting Countries in the World.

Ranking	Country	Export Percentage	Export Value USD
1	Mexico	26.67	430.37M
2	Brazil	13.2	262.19M
3	Peru	10.9	216.5M
4	Hong Kong	17.23	143.62M
5	Thailand	6.41	127.27M

Adapted from: (www.tridge.com/intelligences/mango/production accessed 2021-03-23)

Table 4: Top 5 Mango Importing Countries in the World.

Ranking	Country	Import Percentage	Import Value USD
1	USA	35.97	719M
2	German	10.93	218.5M
3	Hong Kong	8.6	173.33M
4	U.K	8.11	162.01M
5	China	7.16	143.16M

Adapted from: (www.tridge.com/intelligences/mango/production accessed 2021-03-23)

production in Uganda is still very low compared to other regional and global producers partly because mango growing has been majorly subsistence with many people having small mango plantations and others having several trees around their homes mainly for domestic consumption. The yield of mangoes in Uganda is 5.8 tons per hectare which is relatively low compared to other global producers for example India where the yield is 11.7 tons per hectare. [8] Most of the mango farmers still grow local varieties that are low-yielding, and this also partly explains the low yield. Several interventions have

Citation: Ankwasa EM, Judith A, Francis I, Joseph S. Screening, Isolation and Characterisation of Fungal Species Causing Post-Harvest Spoilage of Mangoes in Uganda. Open J Plant Sci. 2025;10(1): 001-008. Available from: https://dx.doi.org/10.17352/ojps.000062

been made to solve the problem of low yield, and these include introducing high-yielding varieties from other global mango-producing countries such as Kenya, South Africa, and Puerto Rico. Mango production has increased over time with the area under cultivation increasing from 6581 ha to 12,123 ha in the last 5 to 10 years [8]. Uganda has tropical climatic conditions with temperatures ranging between 21 °C and 25 °C, receives at least 600 mm/year of rainfall, and is on average 1,100 meters above sea level, these conditions are well suited for mango production and therefore can produce more mangoes [8,19]. Mangoes have been reported to flourish in altitudes of up to 1,500m, temperatures of 15 °C and 30 °C, and yearly rainfall of 850mm to 1,000 mm [17]. Globally there are over 1000 varieties of mangoes and these include Ataulfo, Haden, Keitt, Kent, Edward, Palmer, Manila, Kesar, Haden, Tommy Atkins, Alphonso, Dudhpeda, Kesar, Sindhu, Pairi, Desi, Chaunsa, Langra, and Katchamita to mention but a few [1]. In Uganda, additional varieties grown include Florigon, Glenn, Dancan, Early Gold, Erwin, Palvin, Zillate, Pinero, Alfonso, Apple, Boribo, Ssejjembe, Bire, Ssejjembe, Ssu and Kate [8].

Post-harvest spoilage and losses in mangoes

Food production in the world has continued to increase but unfortunately, one-third of the total global human food production is wasted. Sub-Saharan Africa alone loses 20 million metric tons of food annually according to reports by the United Nations and Food and Agriculture Organization. Many developing countries have limited capacity to handle and store food after harvesting and as a result, many farmers lose up to 40% of their harvest [20]. According to FAO [21]. Post-harvest losses (PHL) refer to any losses that occur after the product is separated from its growth point (harvest) to the time it is accessed by the final consumer. Post-harvest loss covers losses both in quantity (reduction in physical weight) and quality (nutritional value, acceptability, palatability) that occur between harvest time and final consumption or utilization. According to Codex standards, some of the attributes of quality mangoes should be whole, free of observable foreign matter, and free from pest and insect damage. Quality fruits should not show any signs of rotting, abrasion, or discoloration [21]. Mango fruits have high moisture content after harvest and this facilitates fungal growth and disease infestation and subsequently results in post-harvest losses [13,22]. As fruits ripen, they become very susceptible to post-harvest spoilage due to various physiological changes and senescence. Post-harvest spoilage of mangoes greatly reduces their quality and market value resulting in huge economic losses for the farmers, the traders, and the end consumers [23-25]. Post-harvest losses have been reported to be in the range of 20-50% in developing countries and they play a significant role in the overall quality of the fruits, the reported losses, and the final market value of the fruits [21]. Post-harvest losses in mangoes have been reported to be 25- 40% in India and this figure is even higher in Pakistan where it was reported to be 69% [19]. Baltazari, et al. [26], reported post-harvest losses in fresh mangoes in Tanzania ranging between 48–60% while Evans, et al. [12] reported post-harvest losses in mangoes in the range of 25% - 40%. Several factors are responsible for post-harvest losses in mangoes, and these include pests and diseases, mechanical injuries to the fruit as well as the handling, transportation, and storage conditions after harvesting [13,22,21]. Other factors such as delays during customs clearance and unexpected breakdown of trucks during transportation also contribute to post-harvest losses. Post-harvest spoilage of fruits can occur during or after harvesting, during transportation, during marketing, and during storage [27].

Social and economic impact of post-harvest spoilage of mangoes

Post-harvest losses globally cause food insecurity and contribute to high incidences of malnutrition and undernutrition, hunger and all these negative consequences directly affect the lives of poor small-holder farming communities mostly in developing countries [27]. Post-harvest losses have severe negative effects on the nutrition and health of both farmers and consumers as well as affecting their incomes and financial well-being. In many developing countries, post-harvest losses mostly affect rural women who are the main people handling most of the post-harvest activities [27]. Post-harvest losses not only cause food losses but also indirectly cause loss of resources that were used to produce all the wasted food such as land, water, fertilizers, time, and production costs [20]. The spoilage of mangoes during harvesting, handling, transport, storage, distribution, and marketing greatly increases losses incurred along the value chain. It is very important to employ good fruit-handling practices during the harvesting and post-harvest stages to reduce or minimize physical damage to the fruits and prevent spoilage [12]. Post-harvest spoilage and losses directly affect fruit quality and quantity and this reduces the market value and available quantities which results in a loss of revenue for the farmers and the country. By investing resources in post-harvest reduction interventions and strategies the problems of food security, and income loss will be addressed [28]. It is reported [22] that it is more important to focus on reducing post-harvest losses than focusing on increasing production to compensate for such losses, efforts should be made to reduce post-harvest spoilage and losses to save resources and maintain fruit quality and market value [23].

Common pests, pathogens and diseases of mangoes

Mangoes are greatly affected by different pests, pathogens, and diseases which lower their quality, acceptability, and market value. In Uganda pests, pathogens, and diseases are poorly managed and the farmers have limited knowledge of these pests and diseases which has negatively impacted the mango production sector [4]. According to a study [22], the major pests of mangoes include fruit flies, stone weevils, and mealy bugs, and fruit flies were reported in over 99 mango-producing countries [4], similarly, fruit flies and seed weevils as mango pests in addition to beetles, red-banded thrips, termites and mango tip borers were reported. In Uganda fruit flies are the main pests destroying mangos that were reported by most farmers. Pests cause huge losses to farmers and greatly reduce their incomes. In Uganda, fruit flies (*Bactrocera invadens*) were reported to be the most prevalent pests of mangoes followed by

Citation: Ankwasa EM, Judith A, Francis I, Joseph S. Screening, Isolation and Characterisation of Fungal Species Causing Post-Harvest Spoilage of Mangoes in Uganda. Open J Plant Sci. 2025;10(1): 001-008. Available from: https://dx.doi.org/10.17352/ojps.000062

mango seed weevils (*Deanolis albizonalis)*. The main pathogens responsible for postharvest losses in mangoes are fungi [15,24]. Mango fruits may be attacked by fungi at the farm during the growing stage, harvesting process, handling, and transportation as well as during storage and marketing both retail and wholesale [5,9,13,15,21,26]. Aggressive and rough handling of mangoes can create wounds that serve as entry points for pathogens. Several fungal species have been reported to cause fruit rotting during transportation, storage, marketing, and the major pathogens in mangoes include *Neofusicoccum parvum*, *Neofusicoccum mangiferae*, *Pestalotiopsis mangiferae*, *Cytosphaera mangiferae*, *Colletotrichum gloeosporioides*, *Alternaria alternata*, *Fusicoccum aesculi, Nattrassia mangiferae*, *Botryosphaeria* spp. and *Botrytis cinerea* [25]. According to Mairami, et al. [15], *Aspergillus niger, Rhizopus nigricans*, *Mucor mucedo*, and *Fusarium oxysporum* were identified in the spoilt fruits obtained from the Bwari market. Several diseases have been reported in mangoes and these include Anthracnose (caused by *Collectorichum gloeosporioides*), Stem end rot (caused by *Botryodiplodia theobromae* and *Dothiorell* spp), *Aspergillus niger* rot (caused by *Aspergillus niger*), soft rot, Alternaria rot (caused by *Alternaria alternata* and *Alternaria tenuissima* [4,13], reported additional mango diseases which included bacterial black spot caused by *Xanthomonas campestris*, algal leaf spot caused *Cephaleuros virescens* and powdery mildew caused by *Oidium mangiferae* [4]. The two most common and significant post-harvest diseases in mango fruits are Anthracnose disease and stem-end rots and this is due to poor disease management during production, pre-harvest and post-harvest [23].

The aim of this study was to isolate, characterize, and identify fungi causing post-harvest spoilage of mangoes in Uganda.

Materials and methods

Several chemicals were used during the research and these included culture media (PDA), Chloramphenicol supplement, Absolute Ethanol, Lactophenol cotton blue dye, Sodium Hypochlorite, Gotaq, Primers, and DNA markers. Several instruments and equipment were also used during the experiment and these included; DNA kits, Biological safety cabinets, an Incubator, a Vortex, and PCR instrument, a Microscope, and refrigerators.

Sample collection

Mango fruits with clearly visible signs and symptoms of fungal infection were purchased from four different markets around Kampala city namely; Kireka market (0.34660N, 32.65010E), Banda market (0.34300N, 32.63700E), Nakawa market (0.32990N, 32.61230E), Kalerwe market (0.35060N, 3257170E), and were packed separately in polythene bags according to market source and were stored at 4 ºC until ready for use. A total of 40 mango samples were purchased and used in this experiment.

Isolation of fungi from infected tissues and culturing on PDA

Using a sterile and sharp surgical blade, small pieces (2X2mm) were cut and extracted from the infected tissue of the mangoes. The cut pieces were surface sterilized by dipping them in 1% (V/V) individually in Sodium Hypochlorite (NaClO) solution for 60 seconds, they were rinsed three times with sterile distilled water and were dried on sterile filter paper according to the method described by Ahmad, et al. (2019). Each of the pieces was carefully and individually transferred to sterile Petri dishes containing Potato Dextrose Agar (PDA) media (culture media). An anti-bacteria supplement: chloramphenicol (SR0078E) was added to the media to suppress bacterial growth according to the method of Hasan and Zanuddin, [14] The Petri dishes were incubated at 28 °C and 75% relative humidity with a 12-hr. photoperiod for 07 days.

Isolation and culturing of single colonies

After 7 days of incubation, the Petri dishes were observed for fungal growth, and mixed colonies were observed on different plates. Different colonies were marked out on the Petri dishes and isolated using the single spore technique as described by Hasan and Zanuddin, [14]. The single spores were cultured on Potato Dextrose Agar (PDA) media containing chloramphenicol supplement SR0078E and were incubated at 28 °C and 75% relative humidity with a 12 hr photoperiod for 7 days. This process was repeated 2 times to get pure colonies.

Microscopy and Morphological characterization of the fungi

Lacto phenol cotton blue dye was used to stain the chitin in the fungal cells and these were observed under the electron microscope according to the method described by Mailafia, et al. [22] and Hasan and Zanuddin, [14] The slides were prepared by adding a drop of cotton blue dye on clean slides using a dropper. Pure fungal isolates for examination were transferred onto the microscope slides by gently touching fungal culture using a sterile straight wire and then gently touching the slides containing cotton blue dye. The slides were covered gently with clean slide covers with little pressure to avoid air bubbles and the slides were mounted and observed under low and high-power magnification. Different morphological characteristics were observed such as the colour, hyphae, and general characteristics of the different mycelia.

Molecular Characterization of the fungal isolates

Deoxyribonucleic Acid (DNA) extraction: Deoxyribonucleic Acid (DNA) material was extracted from the pure cultures of the fungi according to the method described by [25] DNA extraction involved crushing 150 mg of fungus in 300 µl of TES extraction buffer (0.2M Tris-HCL [pH8], 10 mM EDTA [pH8], 0.5M NaCl, and 1% SDS), using acid sterilized sand, a motor, and pestle. Then 200 µl of TES buffer containing proteinase K (50µg/µl), was added and the mixture was transferred to a 1.5ml microfuge tube. The mixture in the tube was vortexed and incubated at 65 °C for 30 minutes. Thereafter one-half volume (250 µl) of 7.5M ammonium acetate was added (to precipitate proteins), and the sample was vortexed and incubated on ice for 10 min followed by centrifuging for 15 minutes at 13000 rpm. 500 µl of the supernatant was transferred to a new tube,

Citation: Ankwasa EM, Judith A, Francis I, Joseph S. Screening, Isolation and Characterisation of Fungal Species Causing Post-Harvest Spoilage of Mangoes in Uganda. Open J Plant Sci. 2025;10(1): 001-008. Available from: https://dx.doi.org/10.17352/ojps.000062

an equal volume of ice-cold isopropanol was added, and the mixture was incubated at minus 20 °C overnight (to precipitate the DNA). This was followed by centrifuging for 10 min at 13000 rpm to pellet the DNA. The supernatant was discarded, and the pellet was washed by adding 800 µl of 70% ethanol and centrifuging at 13000 rpm for 5 min. The supernatant was discarded, and the pellet was then left to dry air on a sterile paper towel for 6 hours. The dry DNA pellet was re-suspended in nuclease-free water, and the amount of DNA was quantified using a Nanodrop. DNA concentration was then diluted to 100 ng/µl and stored at minus 20 °C to be used in Polymerase Chain Reactions (PCR).

Polymerase chain reaction (PCR) with universal primers ITS1/4: The 5.8S rRNA gene was amplified using the primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-CCTCCGCTTATTGATATGC-3') (Masoud, et al. 2004; Hasan and Zanuddin, [14]. The PCR reaction mix comprised 12.5 µl of 2X Gotaq premix (Bioneer Corporation, 2016), 0.625 µl of 10 µM forward and reverse primer each, 2 µl of 50 ng/µl DNA sample and this was topped up to 25 µl using nuclease-free water. The PCR cycle conditions for amplifying the 5.8S rRNA gene were four minutes of initial denaturation at 95 °C, 35 cycles of 40 s of denaturation at 95 °C, 30s annealing at 55 °C, 40s extension at 72 °C, and 5 min of final extension at 72 °C. The PCR products (amplicons) and a 1kb plus DNA ladder (Thermo Scientific) were separated by agarose gel electrophoresis using a 1.5% agarose gel in 1X TAE buffer (40 mM Tris, 20 mM acetate, and 1mM EDTA) at 80 volts for 60 min. The gel was then stained with ethidium bromide at 0.5µg/ml final concentration and the bands were visualized using a Gel documentation machine (UVP 97-0664-02, Fisher Scientific, and Norway). The bands were then excised using a surgical blade, after which they were purified using a Gene Elute TM Gel Extraction kit (NA1111-1KT, Sigma-Aldrich, German) according to the manufacturer's instructions elaborated below. Briefly, the DNA fragment of interest was excised from an agarose gel using a clean, sharp scalpel, placed in a 1.5ml Eppendorf tube, and weighed. Three gel volumes of the gel solubilisation solution were added to the slice (for example 100mg of gel was added to 300 ml of gel solubilisation solution). The gel mixture was then incubated at 60 °C until the gel was completely dissolved. Binding columns were prepared by placing them in 2ml collection tubes, adding 500 µl of column preparation solution to each of them, and centrifuging at 12000 rpm for one minute. One gel volume of isopropanol was added into the solubilized gel mixture and this mixture was pipetted into a prepared binding column and centrifuged at 12000 rpm for one minute. The binding column was removed from the 2ml collection tube and the flow-through liquid was discarded. Then it was returned to the collection tube and 700 µl of wash solution was added, centrifuged at 12000 rpm for one minute and the flow-through liquid was discarded too. Lastly, the binding column was transferred to a fresh collection tube and 50 µl of elution solution was added at the center of the membrane, incubated for one minute at room temperature followed by centrifuging at 12000 rpm for one minute. The DNA contained in the flow-through was then quantified using a Nano-drop and then stored at -20 °C to be sent for sequencing.

Deoxyribonucleic Acid (DNA) sequencing and species identification: The PCR amplicons were sequenced at the Joint Clinical Research Council (JCRC, Uganda). Sanger sequencing was done, and ab1 DNA sequence files obtained were edited in Bio-edit version 7.2, and converted to FASTA file format. The obtained FASTA files were deposited in the National Centre for Biotechnology Information (NCBI) nucleotide database to be assigned accession numbers by which they can be internationally accessed. Each of the sequences was subjected to the Basic Local Alignment Search Tool (BLAST) of NCBI's nucleotide database to identify which reference species sequences it aligned to with the highest similarity.

Pathogenicity test

Pathogenicity of the isolates was performed according to Koch's postulates. Six fresh (04) and healthy mangoes were surface sterilized with 90% ethanol, pricked using sterile needles, and inoculated with 10 µL of the pathogens. All this work was done under sterile conditions inside the laminar flow chamber. Two controls were run without any inoculation. The experimental fruits and the control fruits were incubated at 28 °C with 90% relative humidity and were observed for 5 to 7 days according to the method of [29] with slight modification. To investigate the effect of maturity of the mango fruit on the rate of spoilage both ripe and unripe mangoes were inoculated with fungal pathogens and the samples were incubated at 28 °C and were observed for 7 days for symptoms of fungal infection. To investigate the effect of different temperatures on the rate of spoilage 04 fresh fruits were inoculated with pathogens and cultured at different temperatures 15 °C, 28 °C, and 35 °C and observed for 5 to 7 days. Fungal isolates were re-isolated from the infected fruits, re-examined, and compared with the original fungal isolates.

Experimental results

Isolation of fungi from infected tissues and culturing on PDA

Small pieces (2X2 mm) were cut and extracted from the infected tissue of the mangoes and Figure 2 below shows mangoes with signs of fungal infection from which we extracted different fungal isolates.

Isolation of pure colonies and culturing on PDA

Mixed colonies were observed to have grown on the Petri dishes after incubation at 28°C for 7 days on PDA media and were purified into single colonies (pure colonies) using the single spore technique (Figure 3). A total of four (04) fungal isolates were isolated namely; Mango Ban 5(2)1, Mango Nak 7(1)1, Mango Nak 6(3)1, and Mango Nak 11(4)1.

Microscopy and morphological characterization

All the mycelia of the 04 fungal isolates were examined and identified under the microscope. Table 5 describes the colors of the colonies, hyphae, and mycelia growth characteristics.

Citation: Ankwasa EM, Judith A, Francis I, Joseph S. Screening, Isolation and Characterisation of Fungal Species Causing Post-Harvest Spoilage of Mangoes in Uganda. Open J Plant Sci. 2025;10(1): 001-008. Available from: https://dx.doi.org/10.17352/ojps.000062

The morphological characteristics of the fungi such as colony color and mycelia growth were observed after 5 days of incubation at 28°C on PDA Media. All the colonies were fast-growing with different colors ranging from green, grey, and whitish changing to grey and dark grey, and the colors became darker as the fungi grew changing from whitish or grey to dark grey.

Molecular characterization of the fungal isolates

Four fungal isolates were obtained from the spoilt mangoes bought from different markets in Kampala and were identified as *Aspergillus fumigatus*, *Neofusicoccum parvum*, *Aspergillus krugeri*, and *Lasiodiplodia theobromae* based on their molecular characterization (Table 6).

DNA extracted from the fungal isolates was amplified and run on Gel electrophoresis and the results showed that there were regions specific for fungi (Figure 4).

Pathogenicity test

The points of inoculation with the pathogens developed symptoms of fungal infection which in due course spread to the fruit exterior in 4 to 7 days after inoculation and the symptoms that were seen on the fruits were very comparable to those seen on the original fruits (Figure 5). Fungal pathogens were re-isolated from the diseased parts and these were found to be similar to the original pathogens based on molecular characterisation. This gave a clear confirmation that these pathogens were responsible for post-harvest losses earlier observed on the fruit samples bought from different markets around Kampala city. It was observed that ripe fruits were more susceptible to fungal attack and spoilage than unripe ones. *Lasiodiplodia theobromae* was the most aggressive fungal species causing severe rotting in ripe fruits in 4 days.

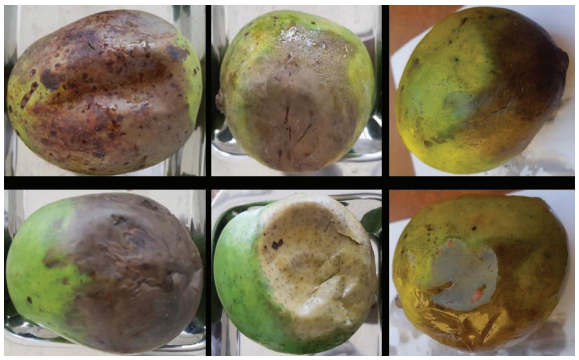

Figure 2: Mangoes with signs and symptoms of fungal infestation.

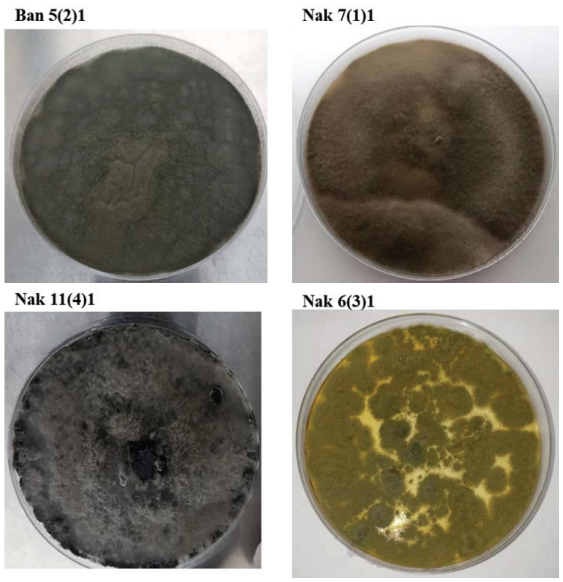

Figure 3: Isolated fungal species after incubation at 28 °C on PDA.

Table 5: Morphological characteristics of the different mycelia.

Isolate	Species	Color	Hyphae
Ban 5(2)1	*Aspergillus fumigatus*	Fast-growing colonies with grey spores	Septate
Nak 7(1)1	*Neofusicoccum parvum*	Fast-growing colonies with white to grey fluffy aerial mycelia	Non-septate
Nak 6(3)1	*Aspergillus krugeri*	Fast-growing colonies with green spores	Septate
Nak 11(4)1	*Lasiodiplodia theobromae*	Fast-growing colonies with grey to dark grey fluffy aerial mycelia	Septate

Table 6: Identified species after DNA sequencing of the 04 isolates.

Sample Name	Identified Species	Reported sequence	Similarity
Mango Ban 5(2)1	*Aspergillus fumigatus*	MT597433.1	100%
Mango Nak 7(1)1	*Neofusicoccum parvum*	MW393581.1	100%
Mango Nak 6(3)1	*Aspergillus krugeri*	MK450654.1	100%
Mango Nak 11(4)1	*Lasiodiplodia theobromae*	MW590685.1	100%

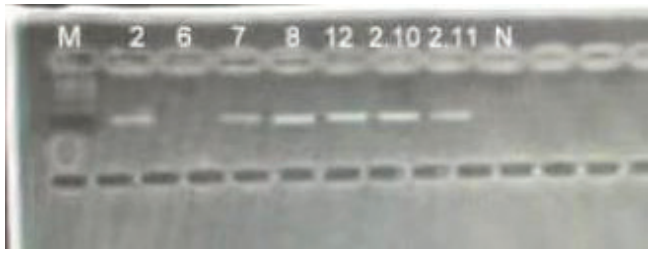

Figure 4: 1.5% TAE agarose gel stained with Ethidium Bromide M1kb ladder. 2 - 2.11 5.8s rRNA amplicons, and N negative control.

Figure 5: Mango post inoculation with Pathogens and incubation at 28 °C for 5 days.

Citation: Ankwasa EM, Judith A, Francis I, Joseph S. Screening, Isolation and Characterisation of Fungal Species Causing Post-Harvest Spoilage of Mangoes in Uganda. Open J Plant Sci. 2025;10(1): 001-008. Available from: https://dx.doi.org/10.17352/ojps.000062

Discussion of results

The four fungal species isolated from the spoilt mangoes namely; *Aspergillus fumigatus, Neofusicoccum parvum*, *Aspergillus krugeri*, and *Lasiodiplodia theobromae* have previously been reported to cause post-harvest spoilage and diseases in mangoes in many other parts of the world.

Lasiodiplodia theobromae was reported to cause dieback disease in Brazil, Korea, India, Oman, Pakistan, USA [26]. In Pakistan, *Lasiodiplodia theobromae* were reported to be the causative agents for stem-end rot and quick decline [23]. *Lasiodiplodia theobromae* was reported to cause many other diseases like tree dieback, root rot, fruit rots, and leaf spots in several other fruits like citrus, avocados, mangos, papayas, bananas, and guavas [30]. Gummosis of mango trees caused by *Neofusicoccum parvum* was reported in Sichuan, southwest China [21]. In 2012, post-harvest rot of mango fruits was reported in Okinawa Prefecture, Japan, and the fungus responsible for this rot disease was identified as *N. parvum* [31]. Experimental results showed that both ripe and unripe mangoes showed symptoms of fungal infection when they were inoculated with different fungal pathogens, however, it was also observed that the ripe mangoes showed more severe symptoms in a shorter growth period compared to the unripe mangoes when incubated at 28 °C and this could be possible because unripe mangoes do not meet the nutritional requirements of the fungi. Some of the fruits developed visible symptoms of fungal attack by the third day of incubation and the symptoms became very severe on the sixth and seventh day of incubation. It is suggested [13] that green or unripe fruits have high amounts of antifungal agents or even enzymes that could resist fungal spoilage compared to ripe fruits and that also there was a possibility of green or unripe fruits producing certain toxins that could disappear as the fruit ripened. These findings are similar to earlier reports by Jerin, et al. [7], who reported that ripened mangoes were more vulnerable to microbial attack and spoilage and that the microbes causing spoilage flourished in temperatures ranging between 25 °C to 37 °C. The higher rate of fungal spoilage in ripe fruits could be attributed to the physiological changes that occur during ripening which result in a reduction in acidity and an increase in sugar conditions that favor fungal growth. To investigate the effect of temperature on the rate of spoilage 4 ripe mangoes were inoculated with the pathogens and cultured under three different temperatures; 15 °C, 28 °C, and 35 °C and the results showed that 28 °C was the most favorable growth temperature for the fungal isolates causing fruit rot in 4 to 6 days. The rate of spoilage was observed to be slowest at 15 °C compared with growth at the other temperatures 28 °C, and 35 °C, was highest at 28 °C and averaged at 35 °C. In this study, it was observed that all 04 fungal isolates grew well at 28 °C and 35 °C and these findings were supported by earlier studies which reported that the optimum temperature for fungal growth was between 25 °C to 37 °C [7]. A study conducted by Rahi, et al. [24] reported that the optimum temperature for fungal growth was 25°C and that there was bad growth at 35 °C. Ullah, et al. [23], reported that the majority of the *Botryodiplodia* sp. that cause die-back diseases in mangoes were favored by temperatures ranging between 25 - 30 °C and that *L. theobromae* isolates showed maximum growth at 30 °C. Zhang, [31] reported that high relative humidity and high temperatures favor the growth of *L. theobromae* and that the fungus grows well in temperatures between 15 to 35 °C with an optimum growth temperature of 30 °C. It is reported [27] that when fresh fruits were inoculated with fungal spores and incubated at 25±2 °C for 7 days with a 12 hr photoperiod, all the fruits developed symptoms of *Stemphylium* rot except for the control fruit. In another experiment done by Lopes, et al. (2014), it was reported that all the isolates showed maximum growth between 25 °C and 30 °C. According to the experiment conducted by [13], it was reported that the maximum rotting of mangos occurred at 30 °C which is in line with previous findings in pomegranate rot, guava fruit rot, papaya rot, apple rot where it was reported that maximum fungal rot occurred between 30 °C - 35 °C. The results of the study further revealed that fruits incubated at 10 °C did not develop any symptoms of mango fruit rot. The results of the pathogenicity test indicated that the 4 fungal isolates were pathogenic and symptoms of spoilage and rotting similar to those previously observed were indeed observed and further confirmatory tests were done through morphological and molecular characterization which confirmed their pathogenicity.

Conclusion

The greatest threat to the mango industry development in Uganda is post-harvest spoilage and losses due to fungal infection and diseases. It is very crucial to minimize injury to the fruits during post-harvest handling to extend their shelf life, minimize losses, and maintain their market value. The line government ministries, agencies, development partners, and other stakeholders should allocate more resources towards research on mango pests and diseases as well as towards educating the different actors along the mango value chain on appropriate post-harvest handling and Good Agricultural Practices (GAP).

Acknowledgment

I thankfully acknowledge the support from the Department of Microbiology and Biotechnology Centre, Product Development Directory, Uganda Industrial Research Institute, P.O. Box 7086 Kampala, Uganda, and the Department of Production, NEC Uzima Ltd, National Enterprise Corporation, Plot 2 Muwesi Road, P.O Box 3145, Bugolobi, Uganda.

References

1. Matheyambath AC, Subramanian J, Paliyath G. Mangoes. In: Reference Module in Food Science. Encyclopedia of Food and Health. 2016; 641–5. Available from: https://doi.org/10.1016/B978-0-12-384947-2.00442-6

2. Onyeani C, Osunlaja SO, Owuru OO, Sosanya O. Mango fruit anthracnose and the effects on mango yield and market values in Southwestern Nigeria. Asian J Agric Res. 2012;6:171–9. Available from: http://dx.doi.org/10.3923/ajar.2012.171.179

3. Khanzada M, Lodhi A, And L, Shahzad S. Pathogenicity of Lasiodiplodia theobromae and Fusarium solani on mango. Pak J Bot. 2004;36. Available from: https://www.researchgate.net/publication/266069789_Pathogenicity_of_Lasiodiplodia_theobromae_and_Fusarium_solani_on_mango

Citation: Ankwasa EM, Judith A, Francis I, Joseph S. Screening, Isolation and Characterisation of Fungal Species Causing Post-Harvest Spoilage of Mangoes in Uganda. Open J Plant Sci. 2025;10(1): 001-008. Available from: https://dx.doi.org/10.17352/ojps.000062

4. Acema DE, Asiku B, Odama E. Assessment of mango pests, diseases and orchard management practices in West Nile Zone of Uganda. Agric For Fish. 2016;5(3):57–63. Available from: https://www.sciencepublishinggroup.com/article/10.11648/10015029

5. Islam F, Shamsi S, Bashar MD. Fungi associated with anthracnose of mango (Mangifera indica L.) fruits and their pathogenic potentiality. Dhaka Univ J Biol Sci. 2018;27:93–100. Available from: http://dx.doi.org/10.3329/dujbs.v27i1.46415

6. Bui R, Sinha B, Devi PS, Dinesh K, Dilip RK. First report of Lasiodiplodia theobromae associated with stem-end rot of mango in Manipur, India. Indian Phytopathol. 2018;71(4):631–2. Available from: http://dx.doi.org/10.1007/s42360-018-0096-x

7. Jerin I, Sajib S, Rahi M, Islam M, Waliullah, Chadni Z, et al. Characterization and control of two unknown fungal strains isolated from postharvest mango spoilage. J Adv Biol Biotechnol. 2018;18:1–10. Available from: https://doi.org/10.9734/JABB/2018/41591

8. Ddamulira MMG, Ramathani I, Sebikejje T, Naluyimba R, Otim A, Pariyo A. Mango yield performance in Lake Victoria Crescent region of Uganda. Am J Plant Sci. 2019;10:1142–53. Available from: https://doi.org/10.4236/ajps.2019.107082

9. Ali SMY, Hossain MM, Zakaria M, Hoque MA, Ahiduzzaman M. Postharvest losses of mangoes at different stages from harvesting to consumption. Int J Bus Soc Sci Res. 2019;7(4):21–6. Available from: https://www.researchgate.net/profile/Md-Azizul-Hoque/publication/337149963_POSTHARVEST_LOSSES_OF_MANGOES_AT_DIFFERENT_STAGES_FROM_HARVESTING_TO_CONSUMPTION/links/5e7e271e299bf1a91b82653b/POSTHARVEST-LOSSES-OF-MANGOES-AT-DIFFERENT-STAGES-FROM-HARVESTING-TO-CONSUMPTION.pdf

10. Khaskheli M. Mango diseases: impact of fungicides. 2020. Available from: https://www.intechopen.com/chapters/69065

11. Konsue W, Dethoup T, Limtong S. Biological control of fruit rot and anthracnose of postharvest mango by antagonistic yeasts from economic crops leaves. Microorganisms. 2020;8:317. Available from: https://doi.org/10.3390/microorganisms8030317

12. Evans E, Ballen F, Siddiq M. Mango production, global trade, consumption trends, and postharvest processing and nutrition. In: Handbook of Mango Fruit: Production, Postharvest Science, Processing Technology and Nutrition. 2017. p. 1–16. Available from: https://doi.org/10.1002/9781119014362.ch1

13. Rajmane, Korekar. Isolation and identification of fungi associated with spoilage of mango fruit (Mangifera indica), India. Int J Sci Res. 2016;5(8).

14. Hasan NA, Zanuddin NAM. Molecular identification of isolated fungi from banana, mango, and pineapple spoiled fruits. AIP Conf Proc. 2018;2020. Available from: https://doi.org/10.1063/1.5062700

15. Mairami FM, Ndana R, Umar I. Isolation and identification of fungal species associated with fruits spoilage in Bwari Market, Abuja, Nigeria. J Adv Microbiol. 2018;12:1–6. Available from: https://doi.org/10.9734/JAMB/2018/42620

16. Uddin M, Afroz M, Moon N, Shefat S. Management of Anthracnose Disease of Mango Caused by Colletotrichum gloeosporioides: A Review. 2018 Dec. Available from: https://www.researchgate.net/publication/329519803_Management_of_Anthracnose_Disease_of_Mango_Caused_by_Colletotrichum_gloeosporioides_A_Review

17. Tridge. Top Producing Countries of Mango [Internet]. 2021 Mar 23 [cited 2021 Mar 23]. Available from: https://www.tridge.com/intelligences/mango/production

18. The Business Research Company. Mango Growth Factors Market Report 2025, In-Depth Analysis to 2034 [Internet]. 2025 Feb 13 [cited 2025 Feb 13]. Available from: https://www.thebusinessresearchcompany.com/report/mango-global-market-report

19. Ahmed R, Mohammed S. Isolation and classification of fungi associated with spoilage of post-harvest mango (Mangifera indica L.) in Saudi Arabia. Afr J Microbiol Res. 2014;8:685–8. Available from: http://dx.doi.org/10.5897/AJMR12.1898

20. World Food Programme. Taking it to scale: Post-Harvest Loss Eradication in Uganda 2014–2015. 2015.

21. Li Q, Deng TJ, Huang SP, Guo TX, Mo JY, Hsiang T. First report of gummosis of mango trees caused by Neofusicoccum parvum in Sichuan, Southwest China. 2014;96.

22. Boateng CN. Analysis of post-harvest losses in the mango marketing channel in Southern Ghana [dissertation]. University of Ghana; 2016.

23. Food and Agriculture Organization (FAO). Post-harvest management of mango for quality and safety assurance: Guidance for horticultural supply chain stakeholders [Internet]. 2018. Available from: https://www.readkong.com/page/post-harvest-management-of-mango-for-quality-and-safety-9944777

24. Mascarenhas P, Behere A, Sharma A, Padwal-Desai SR. Post-harvest spoilage of mango (Mangifera indica) by Botryodiplodia theobromae. Mycol Res. 1996;100(1):27–30. Available from: https://doi.org/10.1016/S0953-7562(96)80096-7

25. Mahuku G. A simple extraction method suitable for PCR-based analysis of plant, fungal, and bacterial DNA. Plant Mol Biol Rep. 2012;22:71–81. Available from: https://link.springer.com/article/10.1007/bf02773351

26. Kamil F, Saeed E, El-Tarabily K, AbuQamar S. Biological Control of Mango Dieback Disease Caused by Lasiodiplodia theobromae Using Streptomycete and Non-streptomycete Actinobacteria in the United Arab Emirates. Front Microbiol. 2018;9. Available from: https://doi.org/10.3389/fmicb.2018.00829

27. Baltazari A, Mtui HD, Chove LM, Msogoya T, Kudra A, Muhamba T, et al. Evaluation of post-harvest losses and shelf life of fresh mango (Mangifera indica L.) in Eastern Zone of Tanzania. Int J Fruit Sci. 2020;20(4):855–70. Available from: http://dx.doi.org/10.1080/15538362.2019.1697411

28. Food and Agriculture Organization (FAO). Government of Uganda, FAO, WFP, IFAD for the development of a comprehensive strategy to reduce PHL in grains. 2019.

29. Ahmad Z, Hussain R, Riaz M, Khan MA, Nadeem M, Akram K, et al. 23. 2019 - Ahmad et al 2019 (PakJas). Pak J Agric Sci. 2019;56:435–42. Available from: https://www.researchgate.net/publication/332912852_23_2019_-_Ahmad_et_al_2019_PakJas

30. Zhang J. Chapter 10 - Lasiodiplodia theobromae in Citrus Fruit (Diplodia Stem-End Rot). In: Bautista-Baños SB, editor. San Diego: Academic Press; 2014;309–35. Available from: https://doi.org/10.1016/B978-0-12-411552-1.00010-7

31. Takushi T, Ajitomi A, Arasaki C, Ooshiro A, Sato T. Stem-end rot of mango (Mangifera indica) caused by Neofusicoccum parvum in Japan. Jpn J Phytopathol. 2017;83:102–6. Available from: https://www.gene.affrc.go.jp/databases-micro_search_detail_en.php?maff=245650

Citation: Ankwasa EM, Judith A, Francis I, Joseph S. Screening, Isolation and Characterisation of Fungal Species Causing Post-Harvest Spoilage of Mangoes in Uganda. Open J Plant Sci. 2025;10(1): 001-008. Available from: https://dx.doi.org/10.17352/ojps.000062