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Exploration of Microbial Flora Associated with the Spoilage of Fruits, Vegetables and Oilseeds

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ABSTRACT: The present study was carried out in Department of Agricultural Microbiology, University of Agricultural Sciences, Bengaluru to investigate the microbial populations responsible for spoilage in fruits, vegetables and oilseeds. The specific spoilage organisms in food samples were isolated and identified using 16s rRNA sequencing for bacterial isolates and ITS sequencing for fungal isolates. The enzymatic spoilage potentials of the isolates were studied qualitatively to understand the mechanisms of infection. The pathogenic abilities of the selected isolates were also assessed by inoculating them into fruits and subsequently re-isolating them from symptomatic tissues. The sequencing results revealed the dominant species present in spoiled food samples were *Klebsiella oxytoca, Enterobacter mori*, *Serratia marcescens, Bacillus subtilis*, *B. inaquosorum*, *Penicillium citrinum, Alternaria alstroemeriae, Aspergillus awamori, Aspergillus welwitschiae* and *Aspergillus aflatoxiformans*. The pathogenicity assay confirmed the isolated bacteria and fungi as potential phytopathogens that can induce rots in fruits including apple, papaya and tomato. The findings suggest that implementing improved management practices in food samples, such as protection from mechanical injuries or reducing microbial inoculum, may result in lowering post-harvest losses during storage and transportation.

Keywords: Spoilage microorganisms, 16s rRNA sequencing, ITS sequencing, Phylogenetic analyses, Pathogenicity test.

INTRODUCTION

Fruits, vegetables and oilseeds play a crucial role in human diet by providing essential factors for growth and development. However, a significant challenge that directly influence their economic value is their limited shelf life, influenced by the presence of intrinsic factors such as moisture content and chemical composition of food, as well as extrinsic factors such as pathogen attacks (Samuel *et al*., 2016). More than 25 % of fruits and vegetables produce are spoiled during post-harvest stages, particularly in transportation and storage (Synder *et al.,* 2024). Microbial growth changes the food's appearance, smell or flavor and texture, making it objectionable for consumption (Soomar and Ranani 2019).

Microbial invasion occurs at different developmental stages of fruits and vegetables. Many microorganisms, predominantly soil inhabitants are transmitted by soil particles, airborne spores and through irrigation water. The extent of contamination also depends upon handling practices, storage conditions and time duration (Pelczar *et al.,* 2005). Other factors that are responsible for food spoilage include external damages like cuts, bruises, cracks, *etc.*, paving the way for invasion and colonization (Sulieman *et al.,* 2023).

Fruits are more susceptible to fungal attack due to their acidic pH, compared to bacteria that prefer to thrive on foods with a neutral pH, such as vegetables, leading to rots with unpleasant odors. Various studies on spoilage-associated microflora confirmed the substantial abundance of both Gram-positive and Gram-negative bacteria in fruits and vegetables, indicating the roles of both bacterial and fungal populations in spoilage (Miedes and Lorences 2004; Watkins and Miller 2004). Some bacteria are capable of producing resistant structures such as endospores or biofilms, enabling them to survive for longer periods (Rudra *et al.,* 2022).

Oilseeds such as peanuts and maize, are more prone to fungal contamination as a result of the presence of low moisture content. The toxins produced by these fungi present a significant challenge to food security and post-harvest loss. Mycotoxins are secondary metabolites that contaminate raw agricultural commodities, foods and feeds, primarily due to inadequate post-harvest management practices (Bennett and Klich 2003). Exposure to mycotoxins can pose serious health problems for both humans and animals, ranging from long-term chronic diseases like immune

deficiency and cancer to acute poisoning leading to organ failure (Freire and da Rocha 2016; Davies *et al.,* 2021). Understanding the occurrence and characteristics of disease-causing microorganisms is critical for preventing spoilage and reducing post-harvest losses. Hence, the objective of the present study was to identify and characterize the microbial population responsible for food spoilage.

MATERIALS AND METHODS

Sample collection. The spoiled fruits, vegetables, and oilseeds were procured from local markets of Bengaluru, Karnataka, India, were individually placed in sterile polythene bags and brought to the Food Microbiology Laboratory in the Department of Agricultural Microbiology, University of Agricultural Sciences, Bengaluru for microbial analysis.

Examination of spoilage symptoms in food samples. In this study, the analyzed food samples include apples, papaya, pomegranate, tomatoes, brinjal, and groundnuts. Each specimen was comprehensively examined for the presence of various spoilage indicators like color, texture, rots, odor and other relevant characteristics.

Isolation and enumeration of microorganisms from spoiled samples. Food spoilage bacteria and fungi were isolated from the samples using nutrient agar and potato dextrose agar, respectively. The standard plate count method was employed to enumerate and isolate the spoilage microorganisms and incubated for 48 hours at 30°C for spoilage bacteria and 25°C for 96 hours for spoilage fungi. Colonies exhibiting distinct morphologies among bacteria and fungi were sub-cultured for subsequent analysis.

Morphological and biochemical characterization of spoilage microorganisms. All the bacterial isolates were studied for their colony and cell morphology; biochemical characteristics including Gram reaction, catalase test, IMViC test and carbohydrate fermentation profiles, as outlined in Bergey's Manual of Determinative Bacteriology (9^{th} edition). The fungal isolates were stained with lactophenol cotton blue and observed using a light microscope. The examination focused on assessing hyphal structure, fruiting bodies, and spore arrangement, with comparisons made to structures of known fungi referenced in the Illustrated Genera of Imperfect Fungi. Additionally, a qualitative screening of enzyme production for the hydrolysis of cellulose, starch and pectin was conducted.

DNA extraction and sequencing. DNA extraction from the isolated bacteria and fungi was conducted using the JETM DNA isolation kit following the manufacturer's protocol. Subsequently, the quality and concentration of DNA were assessed using a Nano Drop 1000 spectrophotometer. For the genetic analysis, five microliters of DNA samples were submitted to Barcode Biosciences for 16S rRNA gene (16S) and fungal internal transcribed spacer region (ITS) library preparation and sequencing. The samples were multiplexed using a dual-indexing approach and sequenced on an Illumina MiSeq with MiSeq Reagent Kit v3 (2 × 300 bp). Detailed information on PCR procedures, primers, and Illumina sequencing were followed as per Comeau *et al*. (2016); Yurgel *et al*. (2017). The sequence analysis and homology searches were obtained using online software from the National Center for Biotechnology Information (NCBI).

Phylogenetic analysis. Both bacterial and fungal cultures were subjected to phylogenetic analysis. The sequences of reference strains were obtained from Gen Bank and incorporated into the analysis for comparison. The sequence alignment was carried out using the Clustal W multiple sequence alignment program, and a phylogenetic tree was constructed with bootstrap support based on a neighbor-joining analysis in MEGA X. The branch strength was assessed through 1,000 bootstrap replications, and all missing data and gaps were deleted from the analysis.

Pathogenicity test. The healthy fruits were procured from the local markets of Bengaluru for experimentation in the year 2021-2022. The individual fruits (five) were used for each isolate. Each fruit was wounded using a sterile needle to create entry points and inoculated with 10 µl of a sterile water suspension containing 10^5 conidia/mL for each fungal isolate and 10^6-10^7cfu/mL for each bacterial isolate. The five fruits were kept as a control and were treated with an equivalent volume of sterile water. These were incubated at room temperature, with visual examinations conducted at various intervals to observe spoilage symptoms. The re-isolation and morphological identification of these isolates were carried out to fulfill Koch's postulates.

RESULTS AND DISCUSSION

The primary objective of this research was to screen and isolate microorganisms responsible for spoilage of fruits, vegetables and oilseeds. The identification and distribution of bacteria and fungi in spoiled food samples exposed potential pathogens responsible for post-harvest loss that might also cause risks to human health. The findings of the current research are discussed here.

Examination of spoilage symptoms in food samples. The food samples were collected and segregated based on the presence of spoilage symptoms including soft rot, mold growth on fruits' surface, spots, slime, discoloration, foul odor, *etc*. The visual examination of spoilage symptoms in food samples was: soft, rotten tissue with black rot or greyish mold growth in tomatoes; brown spots or mold growth in apples; brownish-red discoloration and rotten tissue in pomegranates; white mold growth on the surface of papaya; presence of molds in groundnut seeds; and brinjal developed water lesions with a foul odor. The manifestation of spoilage symptoms varies depending on the causative agents coupled with other factors such as the chemical composition of food, pH, temperature of storage, season, moisture content present in food and physical injuries during transportation and storage (Ramprasad *et al*., 2014).

Isolation and enumeration of bacteria and fungi from spoiled samples. The enumeration of the microbial population within the spoiled food samples is

presented in Table 1. The total viable count of bacterial isolates ranged from 2.1×10^6 cfu. g^{-1} to 11.7×10^6 cfu g^{-1}, while fungal isolates ranged from 0.5×10^6 cfu g^{-1} to 1.1×10^6 cfu g^{-1}. This highlights the considerable abundance of microorganisms in the spoiled food samples. The chemical composition of foods provides an ideal environment for the survival and growth of these microorganisms. (Hasan and Zulkahar 2020).

Table 1: Microbial population in spoiled foods.

Source	Viable count ($\times10^6$cfu. g^{-1})	
	Bacteria	Fungi
Apple	11.7 ± 0.65^{a}	0.7 ± 0.25^{ab}
Pomegranate	2.1 ± 0.32^{d}	1.1 ± 0.15^{a}
Papaya	4.6 ± 0.15^{c}	1.0 ± 0.15^{ab}
Tomato	7.6 ± 0.21^{b}	0.8 ± 0.21^{ab}
Groundnut	0.0 ± 0.00^{e}	0.5 ± 0.15^{bc}
Brinjal	7.2 ± 0.17^{b}	0.0 ± 0.00^{c}

Note: The values in the table are mean of three replications and standard errors. [a] and [b] within the rows refer to significant differences ($p < 0.05$) as per DMRT analysis.

Morphological and biochemical of spoilage microorganisms. A total of nine bacterial strains and six fungal strains were isolated from spoiled food samples based on differences in their appearance and color of colonies, cell morphology and biochemical characteristics for bacteria, and the hyphal structure, conidiophore and conidia for fungi. These isolates were selected, pure-cultured and preserved for further investigations. Also, a qualitative analysis was carried out to determine the degradation of starch, pectin and cellulose by these bacterial and fungal isolates. The results, as elucidated in Table 2, show the ability of spoilage bacteria and fungi to produce the extracellular enzymes for the degradation of starch, pectin and cellulose. The combined morphological and biochemical characterization of isolates are reported below.

***Klebsiella* spp.** An isolate APB1 obtained from spoiled apples was identified as *Klebsiella* spp. The colonies appeared to be tiny, circular and translucent. The microscopic observations showed Gram-negative, rod-shaped cells with sizes ranging from 0.8×2.0 µm, and non-endospore formers when observed for endospore formation. Biochemical characterization indicated positive results for catalase, citrate utilization, and the Voges-Proskauer test, while showed negative results for Methyl red test and indole production. The carbohydrate fermentation test confirmed its ability to utilize various sugars such as glucose, sucrose, fructose and mannitol. It also exhibited positive results for pectin hydrolysis during enzymatic screening.

***Bacillus* spp.** The four isolates of spoiled tomato, brinjal and pomegranate were identified as *Bacillus* spp. The isolate POB1 from spoiled pomegranate developed large, round, off-white colonies. Microscopic observations revealed Gram-positive, rod-shaped cells with sizes ranging from 1.5×4.0 µm, and was endospore former. The spoiled tomatoes and brinjal isolates collectively designated as TOB1 and TOB2 exhibited large, circular, off-white and white colonies. Both were Gram-positive rods with cell size 1.0×2.0 µm, and were endospore formers. Biochemical characterization of all the isolates were positive results for catalase and negative for Methyl red test, citrate utilization, Voges-Proskauer test and indole production. The carbohydrate fermentation test indicated that POB1 was able to ferment fructose and mannitol while TOB1 and TOB2 were able to utilize only fructose. The hydrolysis of starch, cellulose and pectin was studied by the capacity to produce respective enzymes and only isolate TOB1 was able to hydrolyze starch.

***Enterobacter* spp.** Three isolates from spoiled apple, papaya and brinjal, were collectively labeled as PAB1, based on similar morphological and biochemical observations. The colonies appeared small circular translucent with a glistening surface. Microscopic observations showed Gram-negative rods with cell dimensions of 0.8×2.0 µm, and no evidence of endospore formation. Biochemical characterization yielded positive results for catalase, citrate utilization, Voges-Proskauer test and indole production while expressing negative results for Methyl red test. An isolate PAB1 was able to utilize glucose, sucrose, lactose, fructose and mannitol.

***Serratia* spp.** An isolate PAB2, obtained from spoiled papaya, developed small, circular, deep red-pigmented colonies. Microscopic observations revealed Gram-negative, rod-shaped cells with sizes ranging from 0.8×2.0 µm and a non-endospore formation. The results were positive for catalase, citrate utilization, Voges-Proskauer test and indole production whereas negative for Methyl red test. The carbohydrate fermentation test demonstrated its ability to utilize glucose, sucrose, fructose and mannitol.

***Penicillium* spp.** The colony morphology of the isolate APF1 obtained from spoiled apples, characterized by a bluish-green color with a velvety appearance and vigorous growth on PDA medium. Microscopic examination revealed septate hyphae, branched conidiophores, 2-3 metula, and phialides swollen at the base. The sterigmeta were branched. Conidia were produced in chains from phialides and appeared to be smooth and round. They could hydrolyze starch.

***Alternaria* spp.** The spoiled tomatoes and apples isolates were collectively designated as APF2, based on similar morphological characteristics. The colony exhibited a whitish-grey coloration with velvety margins and slow growth on PDA medium. Microscopic analysis revealed septate hyphae, and the presence of a wide, septate, and curved conidiophore. The conidia were solitary, having a short conical beak with germ tubes. Conidia also showed two to seven transverse septa along with one or two longitudinal septa. In enzyme production studies for the hydrolysis of starch, cellulose and pectin, it showed positive results for the starch hydrolysis and cellulose hydrolysis.

***Aspergillus* spp.** The spoiled papayas and pomegranates (two) isolates collectively designated as POF1 based on similar observations and morphological characteristics. An isolate PAF1 from spoiled papayas and an isolate GRF1 from spoiled groundnuts, were identified as *Aspergillus* spp. The colonies of isolates POF1 and PAF1 exhibited a brownish-black color and had rapid growth on Potato Dextrose Agar (PDA)

medium. The conidiophores were sub-hyaline, erect, smooth, unbranched, and aseptate. The conidial heads were biseriate, featuring globular, aseptate, unbranched chains of conidia. The conidia appeared globose and had a rough texture. The colony of isolate GRF1 obtained from spoiled groundnuts, exhibited a greenish-yellow color with a granular appearance on PDA medium. The conidiophores were rough-walled, bearing vesicles that were globose to subglobose. The conidial heads were radiating, featuring both biseriate and uniseriate arrangements. Phialides were present, bearing conidia. It was positive for starch hydrolysis and couldn't hydrolyze cellulose and pectin.

Table 2: Enzymatic activities of spoilage bacterial and fungal isolates.

Source	Bacterial isolates						Fungal isolates				
	APB1	POB1	PAB1	PAB2	TOB1	TOB2	APF1	APF2	POF1	PAF1	GRF1
Starch	-	-	-	-	+	+	+	+	-	-	+
Pectin	+	-	-	-	-	-	-	-	-	-	-
Cellulose	-	-	-	-	-	-	-	+	-	-	-

Note: '+'- Positive, '-'- Negative.

Identification of spoilage bacteria and fungi. The identification of these selected isolates was carried out through 16S rRNA gene sequencing for bacteria and ITS gene sequencing for fungi. The results, along with the NCBI accession numbers for the submitted sequences, are mentioned in Table 3. According to 16s rRNA sequencing results, the bacterial isolates were identified as *Klebsiella oxytoca*, *Bacillus spizizenii, Enterobacter mori*, *Serratia marcescens, Bacillus subtilis,* and *Bacillus inaquosorum.* While the ITS sequencing results identified fungal organisms as *Penicillium citrinum, Alternaria alstroemeriae, Aspergillus awamori, Aspergillus welwitschiae,* and *Aspergillus aflatoxiformans.*

Table 3: Identification of microorganisms isolated from spoiled fruits, vegetables and oilseed.

Isolate Code	Organism identified	Accession no.	Closest type strain in NCBI database	Sequence lengthbp	Sequence similarity
APB1	*Klebsiella oxytoca* strain UASBMIC_OO6	ON413770	*Klebsiella oxytoca* strain BLPS7	1440	99.58
POB1	*Bacillus spizizenii* strain UASBMIC_010	ON413913	*Bacillus spizizenii* strain NBRC 101239	1489	97.08
PAB1	*Enterobacter mori* strain UASBMIC_007	ON413771	*Enterobacter mori* strain YIM Hb-3	1523	99.18
PAB2	*Serratia marcescens* strain UASBMIC_011	ON413914	*Serratia marcescens* strain NBRC 102204	1401	99.43
TOB1	*Bacillus subtilis* strain UASBMIC_008	ON413772	*Bacillus subtilis* strain IAM 12118	1534	100.0
TOB2	*Bacillus inaquosorum* strain UASBMIC_009	ON413773	*Bacillus inaquosorum* strain BGSC 3A28	1532	99.88
APF1	*Penicillium citrinum* isolate APF1	ON454381	*Penicillium citrinum* NRRL 1841	506	100.0
APF2	*Alternaria alstroemeriae* isolate APF2	ON470193	*Alternaria alstroemeriae* CBS 118809	492	99.00
POF1	*Aspergillus awamori* isolate POF1	ON470195	*Aspergillus awamori*	519	99.81
PAF1	*Aspergillus welwitschiae* isolate PAF1	ON479653	*Aspergillus welwitschiae* CBS 139.54	502	99.81
GRF1	*Aspergillus aflatoxiformans* isolate GRF1	ON470194	*Aspergillus aflatoxiformans* DTO 228-G2	512	99.28

Phylogenetic analyses. The most similar sequences were aligned using Clustal W software, and a phylogenetic tree was constructed to analyze the evolutionary relationships among the sequences of isolated microorganisms and their nearest neighbors. The phylogenetic trees for bacterial isolates and fungal isolates are depicted in Fig. 1 and 2, respectively. In the phylogenetic analysis, isolate APB1 clustered with a strong bootstrap support value (BS=100) with the reference strain *Klebsiella oxytoca* strain JCM 1665; isolate POB1 grouped with the reference strain *Bacillus spizizenii* strain NRRL B- 23049, supported by a bootstrap support value of 85; isolate PAB1 clustered with the reference strain *Enterobacter mori* strain YIM Hb-3; isolate PAB2 formed a cluster with a bootstrap support value of 73 with reference strain *Serratia marcescens* strain NBRC 102204; isolate TOB1 grouped with the reference strain *Bacillus subtilis* strain IAM 12118, supported by a high bootstrap support value of 95; isolate TOB2 clustered with the reference strain *Bacillus inaquosorum* strain BGSC 3A28 (BS= 96).

In neighbor-joining analyses using ITS gene, the results were on par with morphological characterization, as the isolate APF1 clustered with a strong bootstrap support value (BS=99) with the reference strain *Penicillium citrinum* NRRL1841; isolate APF2 grouped with the reference strain *Alternaria alstroemeriae* CBS 118809, supported by a high bootstrap support value of 95; isolatePOF1 formed a cluster with a bootstrap support

value of 89 with reference strain *Aspergillus awamori*; isolate PAF1 grouped with the reference strain *Aspergillus welwitschiae* CBS 139.54, supported by a high bootstrap support value of 92; isolate GRF1 clustered with the reference strain *Aspergillus aflatoxiformans* DTO 228-G2 (BS= 99).

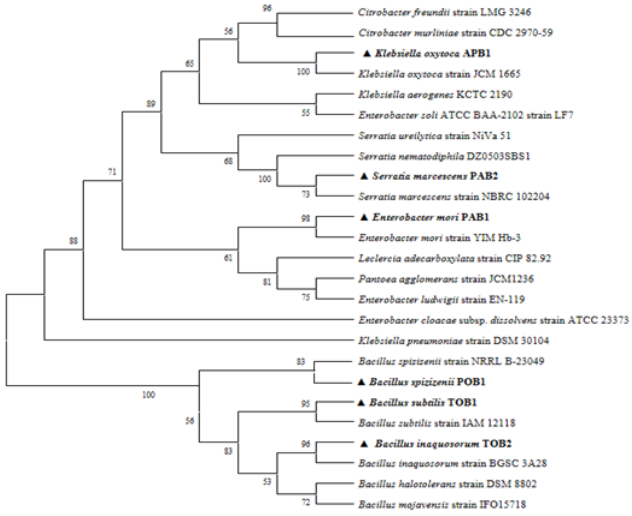

Fig. 1. A Phylogenetic tree constructed using Neighbor-Joining (NJ) analysis, based on genetic data from 16s rRNA datasets of bacterial isolates. The NJ bootstrap support (BS) ≥70 values are displayed at the nodes. The bacterial isolates from this study are highlighted and indicated with a triangle.

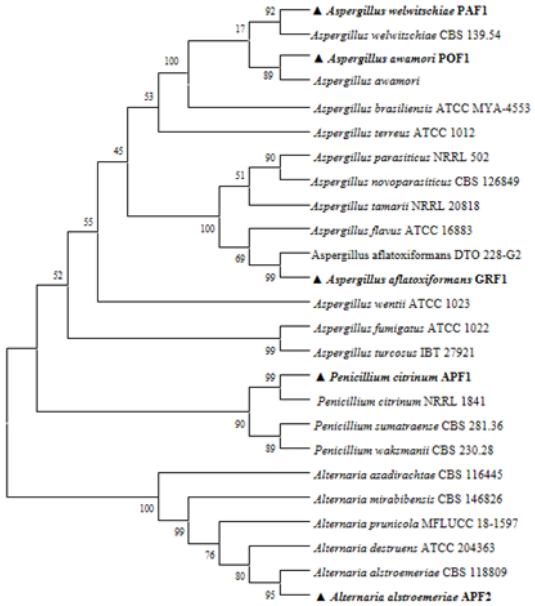

Fig. 2. A Phylogenetic tree constructed using Neighbor-Joining (NJ) analysis, based on genetic data from ITS datasets of fungal isolates. The NJ bootstrap support (BS) ≥70 values are displayed at the nodes. The fungal isolates from this study are highlighted and indicated with a triangle.

Pathogenicity tests. All the isolates used in this experiment, were able to infect the tested fruits *viz.*, apples, papayas and tomatoes, exhibiting rot symptoms (Fig. 3). Subsequently, these microorganisms were re-isolated from the symptomatic tissues proving Koch's postulates. Brown to black lesions developed on each fruit from the inoculation point. These lesions enlarged to cover the entire fruit with the occurrence of sporulation on the fruit's surface after inoculation with bacterial and fungal isolates within a duration of three to six days. The time duration required for the development of symptoms varied in all fruits inoculated. The spoilage incidence was significantly higher in fruits inoculated with isolates compared to control.

Bacillus subtilis and *B. inaquosorum* caused the softening of tissues in tomatoes. It further progressed the formation of brown to black spots with the emission of a foul odor one-week post-inoculation. Tomatoes inoculated with bacterial isolates had a mean spoilage rate of 32% after three days, reaching complete spoilage by six days post-inoculation compared to control which had 65 % spoilage incidence after 6 days of inoculation and complete spoilage 12 days post-

inoculation. Furthermore, *Enterobacter mori, Serratia marcescens* and *Klebsiella oxytoca* induced similar symptoms in papayas and apples within three days post-inoculation. The spoilage incidence in papayas was recorded 100 % three days post-inoculation, whereas, in apples, complete spoilage incidence was observed after nine days of post-inoculation (Data not shown).

Fungal growth was evident on the surface of the apples inoculated with *Penicillium citrinum*, tomatoes inoculated with *Alternaria alstroemeriae*, and papayas inoculated with *Aspergillus awamori* and *Aspergillus welwitschiae*, after three to five days of inoculation. The spoilageincidence of 100 % was recorded in tomatoes six days of post-inoculation and apples inoculated with fungal cultures after nine days post-treatment, compared to control which had spoilage incidence of 66 % in tomatoes and apples six days and nine days post-inoculation, respectively. Meanwhile, a rapid spoilage incidence in papayas was observed, reaching 100 % within three days post-inoculation (Data not shown). The fungal growth was localized only at the point of inoculation in the case of apples resulting in softening of tissues and brown spots. These spots had covered a significant portion of the fruit by the sixth day of post-inoculation.

These results provide a clear understanding of the progression of bacterial and fungal growth and their contribution to spoilage of inoculated fruits. Fruits are considered perishable due to their high moisture content and nutritional composition, making them favorable for microbial activity. *Klebsiella* species are recognized for causing contamination in various foods, contributing to both disease and spoilage. It also acts as an opportunistic pathogen and is commonly associated with nosocomial infections of urinary and respiratory tracts. It can survive by forming biofilms despite disinfection and pasteurization treatments. Notably, this species was isolated and identified as a soft rot-causing bacterium in spoiled potatoes and as a dominant species in *Botrytis*-infected grapes (Nisiotou *et al*., 2011; Ponvizhi Ramya *et al*., 2014). *K. oxytoca* along with *Serratia marcescens* and other bacteria was identified as a causative agent for soymilk spoilage (Xilin Xu *et al.,* 2011). *S. marcescens* was identified as a spoilage bacterium in several foods including apples, coconuts and carrots (Samuel *et al*., 2016; Yaqoob *et al*., 2019; Rajamani Mohanram *et al*., 2020). It is also reported for causing cucurbit yellow vine disease in watermelon and zucchini (Rascoe *et al*., 2003). *Enterobacter mori* has recently been evidenced as an emerging pathogen in research conducted by Zhang *et al*. (2021), causing canker in kiwifruit and mulberry. Furthermore, it was identified as one of the causal agents responsible for spoilage in carrots, decline and offshoot rot of date palm and pre-harvest soft rot of peach fruit (Mahamud *et al*., 2013; Ahmad *et al*., 2021; Abedinzadeh *et al*., 2023). Various findings on *Bacillus subtilis* and *B. inaquosorum* have emphasized their role in the spoilage of several food items and fresh produce. *Bacillus subtilis* was identified as one of the spoilage bacteria isolated from rotten tomatoes, as a causative agent of rope spoilage in bread and spoilage in soybeans and soybean curd (Sorokulova *et al*., 2003; Wang *et al.,* 2019; Erena, 2020). It was also isolated from spoiled canned bamboo shoots and is responsible for the softening of bamboo shoots (Kawabata *et al*., 1999). Similarly, *B. inaquosorum* was identified as a causal agent for soft rot in potatoes, and as a spoilage pathogen in onions (Ponvizhi *et al*., 2014). Gyorgy *et al*. (2020) highlighted *B. inaquosorum* in their research as the bacterial population present with the highest occurrence on the surface of fresh vegetables.

Many research findings have highlighted the versatility of fungal species in contaminating and potentially causing spoilage in a range of fruits, as well as their ability to produce mycotoxins with implications for food safety. *Penicillium citrinum* was isolated from spoiled tomatoes, peppers and onions, and also confirmed for infecting healthy cucumbers (Yaradua *et al*., 2018). Rodrigues *et al*. (2022) mentioned it as one of the dominant fungi causing spoilage in chestnut fruits, contributing to the development of green rots. *P. citrinum* is also studied for its capability to produce mycotoxins such as citrinin and patulin and is associated with the contamination of different fruits including grapes, apples and pears (Bragulat *et al.,* 2008; Sadashivam *et al*., 2021). *Alternaria alstroemeriae* was reported to cause spoilage in apple and tomato fruits, associated with heart rot in pomegranate and a phytopathogen infecting an ornamental palm, *Caryota mitis* L., commonly (Clustering fishtail palm) by causing leaf spots (Ulhaq *et al*., 2020; Aloi *et al*., 2021; Gomomo *et al*., 2022; Dandago *et al.,* 2023). *Aspergillus awamori* was isolated from different fruits and vegetables, contributing to fruit rot and post-harvest spoilage including pomegranate, papaya, orange, tomato, grapes, lemons, sweet potato, *etc.* (Al-Hindi *et al*., 2011;Saif*et al*., 2020; Alum *et al*., 2019). Additionally, it is also identified as a phytopathogen responsible for causing black mold rot in onion bulbs (Yeon *et al*., 2022). *Aspergillus welwitschiae* is a toxigenic fungus posing potential health risks due to the production of toxins such as Ochratoxin A, fumonisins and patulin. Habib *et al.* (2021) investigated pre- and post-harvest loss in table grapes due to fungal infection caused by *A. welwitschiae*. It is also identified as a spoilage fungus in onions and studied for its implications in post-harvest losses in onion bulbs (Bharath Kumar *et al.,* 2022). *Aspergillus aflatoxiformans* belongs to *Aspergillus* section *flavi* and is an aflatoxigenic strain capable of producing aflatoxin B and G. It was first isolated and identified in peanuts by Frisvad *et al*. (2005). It has also been isolated from infected maize, cassava flour and rice, along with other fungal species including *A. welwitschiae* and *Penicillium citrinum* (Ekpakpale *et al.,* 2021). *A. aflatoxiformans* is also reported to cause infection in nutmeg kernels and rubber wood (Salman *et al*., 2020; Arifah *et al*., 2023).

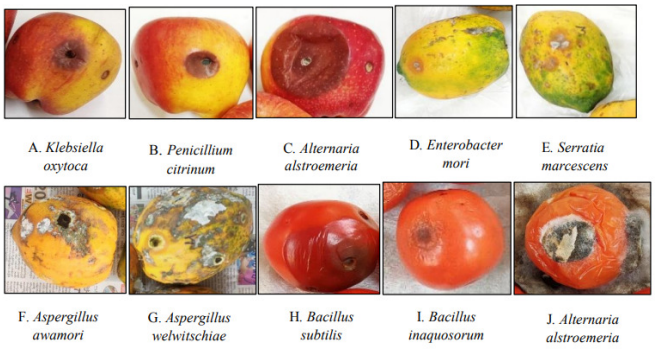

Fig. 3. Fruit rot symptoms observed in apples, papayas and tomatoes infected with bacterial and fungal isolates.

CONCLUSIONS

This study identified and characterized the major microflora associated with spoilage and post-harvest losses in fruits, vegetables and oilseeds, which are also pathogenic to humans. All bacterial and fungal isolates, except for *Bacillus spizizenii,* were confirmed as pathogens causing fruit rots in the respective fruits. These microbes were able to produce extracellular enzymes to hydrolyze starch, cellulose and pectin revealing the possible mechanisms of infection. This represents an initial step in comprehending the disease incidence in food samples. The severity of the disease was found to be related to higher inoculum concentrations, which resulted in rapid spoilage. To mitigate such issues, natural practices were suggested such as the removal of infected plant material or application of chemical or biological antimicrobials, *etc*. These measures can decrease the inoculum concentration, thereby reducing the chances of infection. Further research is being conducted on bio-preservation as a potential strategy that could be employed to reduce the spoilage occurrence in fruits, vegetables and oilseeds during storage and post-harvest stages.

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Conflict of Interest. None.

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