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Research Article

Screening of Fungi Isolated from Fruit Waste Dumpsite Soil in Ipata Market Area of Ilorin for Proteolytic and Lipolytic Abilities

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ABSTRACT

The growing applications of enzymes in industries have necessitated the search for local protease and lipase-producing strains of microorganisms to discover indigenous microbes with such activity. Soil from waste dumpsites has been identified as repository for microbial diversity with potential industrial importance and biotechnological applications. This study focused on isolating, identifying, and screening fungi from soil samples collected at fruit waste dump sites in Ipata Market Area of Ilorin, Kwara State, for their ability to produce protease and lipase enzymes. Soil samples were collected from two distinct locations within the dump site and fungi were isolated using pour plate method on PDA plates. The fungi were characterized and identified, and screened for protease and lipase activities using skimmed milk and phenol red agar plates incubated at 27^0C for 48 hours respectively. Hyphal growth and clear zones of hydrolysis around colonies were measured to indicate protease and lipase activities respectively. Five fungal species were identified as *Aspergillus flavus*, *Aspergillus niger, Mucor* sp., and *Penicillium* sp from the two sites. *Aspergillus flavus* exhibited the highest lipase activity, with a significant clear zone of 83.69 mm, while *Penicillium sp*. showed the lowest lipase activity of 54.13 mm. For protease activity, *Aspergillus niger* recorded the highest hyphal growth diameter of 81.38 mm, whereas *Mucor* sp. displayed the least activity with hyphal growth diameter of 21.50 mm. The findings highlight the potential of local fungal strains isolated from fruit dumpsite in protease and lipase production, which could be leveraged on for industrial applications.

Keywords: Dump Site; Enzyme; Fungi; Lipase; Protease

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INTRODUCTION

The use of enzymes is pivotal in various industrial and biotechnological applications, particularly in medicine, agriculture, and food processing. Enzymes are unique secondary metabolites produced by all living organisms including microbes, especially bacteria and fungi, the use of enzymes have opened new avenues for industrial processes (Ozatay, 2020). Enzymes of fungal origin, such as proteases, amylases, cellulases, and lipases, are increasingly utilized in detergents to enhance the quality and properties of fabrics, drug production in pharmaceuticals, food processing and preservation. Enzymes generally have long history of use for baking, brewing, and cheese-making (Dhevagi *et al*., 2021). Today, the application of enzyme cut across every facet of life (Racheal *et al*., 2021).

Lipases are hydrolytic enzyme with catalytic ability to breakdown triglycerides bonds in fat and oil to release glycerol and free fatty acid. Proteases are involved in the hydrolysis of peptide bonds and conversion of protein to amino acids. Fungal produced lipases and proteases have both hydrolytic and oxidoreductive potentials and have wide application in several industrial processes spanning medical, pharmaceuticals, agricultural, bioremediation, textile, and food processing. Proteases and lipases from fungi are widely known for their specificity, stability over a wide range of temperature and pH, and uniformity in action (Riseh *et al*., 2024).

Species such as *Aspergillus* and *Penicillium* are prolific producers of lipases, proteases and other classes of enzymes with diverse applications (Kumar *et al.,* 2023).Microbial enzymes are preferred over those derived from plants and animals due to their cost-effectiveness, predictability, greater stability and reliability (Kranthi *et al*., 2021). The fungal microorganisms are ubiquitous in many environments and they produce enzyme extracellularly, making the production of fungal enzymes easier and more convenient. Moulds are particularly recognized for their uniqueness in producing attractive enzymes and as a major source of increase in national and global income generation though enzyme market.

Fungal enzymes including proteases and lipases offer significant advantages, such as easier handling, faster production in cost-effective media, higher yields, and enhanced catalytic activity. Therefore, discovery new organisms for its production will enhance commercial scale availability of local enzyme producer while reducing costs (Tafinta *et al*., 2024). Previous studies have sought for local enzyme producers from sources which include decomposing agricultural and industrial by-products as substrates, soil from locations ranging from forest soil to waste dumpsites (Ayinla *et al*., 2017). Though, new organisms were discovered, there is the need to search for more of novel enzyme producer to meet the enormous demand for enzyme in the industries. This will also mitigate the challenge of importing the enzymes at very expensive cost implications. Furthermore, fungi play a significant role in bioremediation and waste management, due to their ability to produce an array of enzymes with better affinity for organic pollutants and hence, contribute to nutrient cycling in ecosystems (Patel *et al*., 2021). This affinity highlights the importance of exploring fungal diversity in fruit waste environments and the potential benefits of harnessing their enzymatic capabilities for industrial applications.

Soil is a natural repository and home for millions of microorganisms including bacteria, fungi, protozoa and others where the survival and metabolic activities of these organisms is a direct function of their enzymatic potential (Kranthi *et al*., 2021; Mukunda *et al*., 2020). Current food scarcity, increase awareness in fruit consumption, methods of fruit processing, and urbanization have led to a surge in the generation of fruit waste leading to significant environmental challenges in the dumpsites. This leads to destruction in the aesthetic of the environment and contribute to the emission of greenhouse gas. Nevertheless, the soil in such dumpsites hosts a large population of bacteria and fungi with potentials in enzyme production, and can be sought for that purpose (Ayinla *et al*., 2017). This study therefore, aims to address isolating and screening fungi from soil samples at fruit waste dumpsites to identify strains with proteolytic and lipolytic activity.

MATERIALS AND METHODS

Study Area

Soil samples were collected from two locations within the fruit waste dump site at Ipata market area of Ilorin, Kwara State based on their high fruit waste accumulation.

Sample Collection

About 200 g each of soil samples were collected within the early hour of the day, from two locations identified as locations A and B, at a depth of about 5 cm from the surface in a sterile plastic container, the samples were carefully labeled with relevant information, including the sampling location and time, and were transported to the laboratory within 2 hours of collection.

Isolation of Fungi

Pour plate technique by Ogbuji *et al*. (2021) was adopted to isolate soil fungi. Each of the soil samples was used in ten folds serial dilution. Subsequently, 0.1 milliliters of the 1,000-fold dilution was aseptically spread onto the surface of freshly prepared Potato Dextrose Agar (included with 1ml of antibiotics streptomycin to inhibit bacterial contaminants) and allowed to solidify. The inoculated plates were incubated at room temperature (28°C ± 2°C) for 7 days to promote fungal growth and colony formation.

Identification of Fungal Isolates

Individual colonies was picked from the plates using sterile forcep and sub-cultured onto freshly prepared PDA plates to obtain pure cultures. The fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology, and pigmentation (Tafinta *et al*., 2013). The technique described by Oyeleke and Manga (2008) was adopted to identify the isolated fungi using cotton blue in lactophenol stain. The identification was achieved by placing a drop of the stain on clean slide and with the aid of a mounting needle, a small portion of the aerial mycelia from the representative fungi cultures was removed and placed in a drop of lactophenol. The mycelium was well spread on the slide with the needle and a cover slip was gently placed with little pressure to eliminate air bubbles. The slide was mounted and viewed under the light microscope with ×10 and ×40 objective lenses. The morphological characteristics and appearance of the fungal organisms seen were identified in accordance with Mailafia *et al*. (2017).

Screening for Lipase Production by Fungal Isolates

Lipase activity was evaluated according to the method demonstrated by Abdulmumini *et al*. (2022) using phenol red agar medium to detect pH changes arising due to the hydrolysis of oil into fatty acids; typically, the pH of the medium reduces upon hydrolysis and is characterized by a yellow halo (Singh *et al*., 2006). Phenol red medium was made up of the following ingredients: phenol red 0.01 percent (w/v), 1 percent (v/v) olive oil, 0.1 percent (w/v) $CaCl_2$, 2percent (w/v) agar, and this was dissolved in 100ml of distilled water in a conical flask, then 0.1ml of prepared streptomycin antibiotic was added to prevent the growth of bacteria and the pH was adjusted to 7.4. The medium was sterilized at 121^{o}c for 15 minutes before use. The phenol red agar was poured into Petri dishes, and left to solidify, after which the plates were inoculated with pure culture of the fungal isolates and incubated at 28^{0}C for 5 days. The activity of lipase produced by the organisms was indicated by a change in the color of the phenol red. The amount of lipase produced by each isolate was indicated by the zone of clearance diameter.

Screening for Protease Production by Fungal Isolates

The organisms were screened by using the prepared skimmed milk agar according to Adedayo *et al*. (2017). Skimmed milk agar was prepared by adding 1 g of casein, 0.5g of peptone, 0.3g of yeast extract, 1.5g of agar into 100 mL of distilled water in a conical flask, and 0.1ml of prepared streptomycin antibiotic was added to prevent the growth of bacteria, and sterilized at 121^{o}C for 15 minutes. The molten agar was poured into Petri dishes, and a pure culture of the isolated fungi was inoculated onto the plates and incubated at 28°C for 5 days. The zone of hydrolysis around fungal colonies were taken as the presence of proteolytic ability.

RESULTS

Fungi were isolated from the fruit waste dump site soil. A total of five fungal species were isolated from the soil samples, with three species from location A and two from location B. The fungal isolates from the two soil samples were identified with their morphological characteristics as *Aspergillus flavus*, *Aspergillus niger*, *Mucor* sp., *Aspergillus flavus* and *Penicillium* sp. The result is presented in Tables 1 and 2, and Plates 1 to 5.

Table 1: Morphological Characteristics and Identification of the Fungal Isolates

Isolates	Colonial Description of Isolated Fungi	Isolated Fungi
A	Growth on PDA was greenish-yellow colonies with whitish mycelium at the edge on the surface, but golden yellow inversely. It appeared under the microscope as a filamentous fungus with branched hyphae that form a dense network with stalk-like conidiophores.	*Aspergillus flavus*
B	The colony was initially whitish fast fast-growing colonies with pale-yellow reverse, and had black conidia. It appeared under the microscope with branched septate hyphae forming a mycelium network, with tall and slender conidiophores.	*Aspergillus niger*
C	White fluffy and cottony mass of colonies with pale yellow in reverse. It appeared under the microscope having a dense fluffy growth of hyphae and filamentous structure with round sporangiophores bearing sporangiospores.	*Mucor* sp.
D	Greenish-yellow colonies with whitish mycelium at the edge on the surface but golden yellow inversely. It appeared under the microscope as a filamentous fungus having with branched hyphae that form a dense network with a conidiophores resembling a stalk.	*Aspergillus flavus*
E	Colonies were initially gray-white, dense mycelia turning dirty green with age lined with whitish appearance at the edge. It appeared under the microscope as filamentous structure consisting of branched septate hyphae with finger like conidiophores extending from the hyphae and round blue conidia was also observed.	*Penicillium* sp.

Table 2: Frequency of Occurrence of Fungal Isolates

Isolated Fungi	Frequency of Occurrence	
	Location A	Location B
Aspergillus flavus	+	+
Aspergillus niger	+	-
Mucor sp.	+	-
Penicillium sp.	-	+

Keys: + = Isolated**;** - = Not Isolated

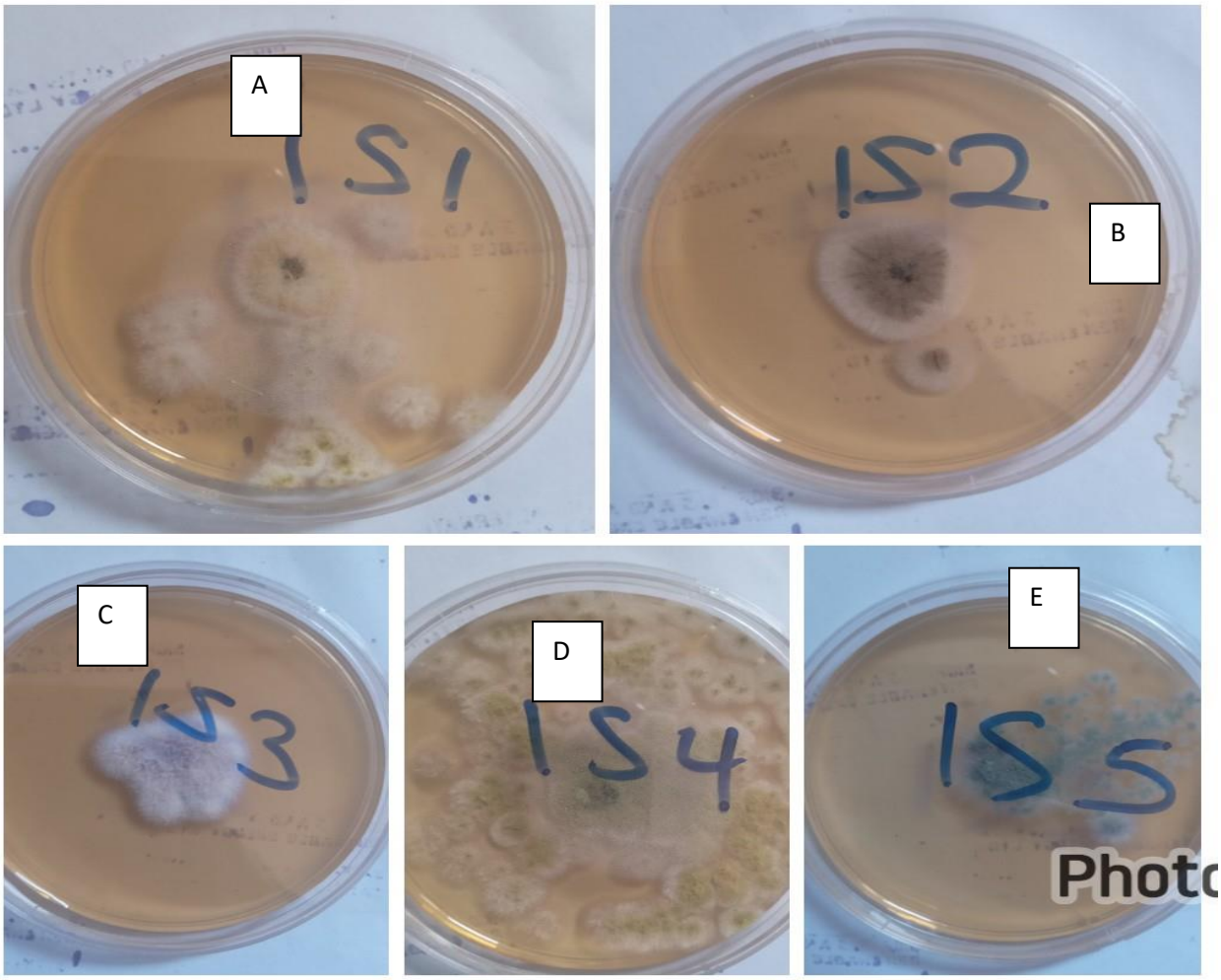

Plate 1 (A-E): Pure Culture of the Fungal Isolates

Lipolyic and Proteolytic Potentials of the Isolated Fungi

On the phenol red agar plate for lipase detection, *Aspergillus flavus* exhibited the highest zone of clearance of 83.69 mm while *Penicillium* sp. had the lowest zone of clearance of 54.13 mm, *Aspergillus niger* had the highest zone of clearance of 81.38 mm for protease production whereas *Mucor* sp. exhibited the lowest of 21.50 mm, as indicated in Tables 3 and 4, and Plate 2 (A-E) and Plate 3 (A-E). Notably, the lipase activity of *Aspergillus flavus* isolated from both location A and location B showed identical zones of clearance. In contrast, the protease activity had different zones of clearance for the same species isolated from the two locations.

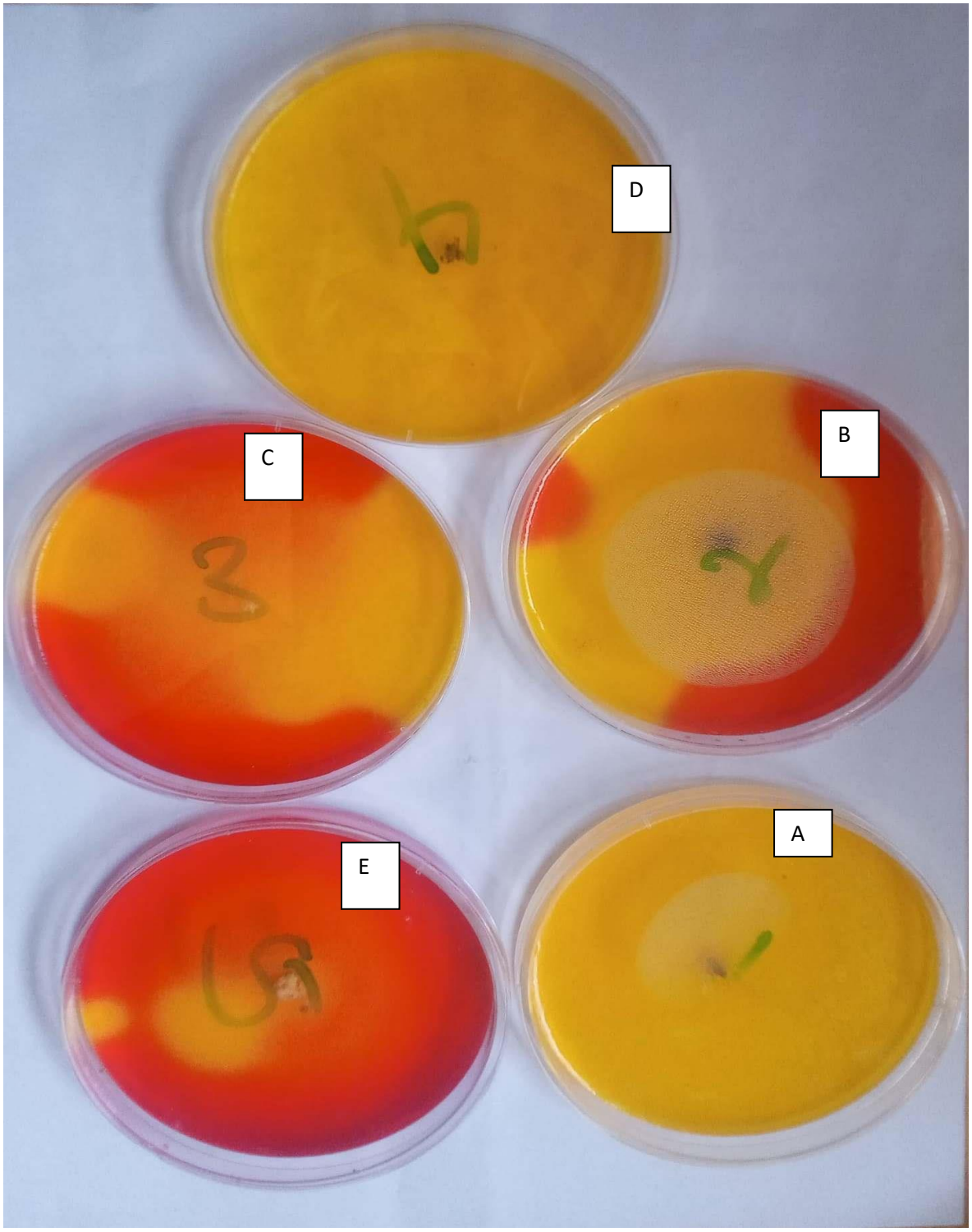

Plate 2 (A-E): Lipolytic Ability of the Fungal Isolates as Evidenced by the Zones of Clearance

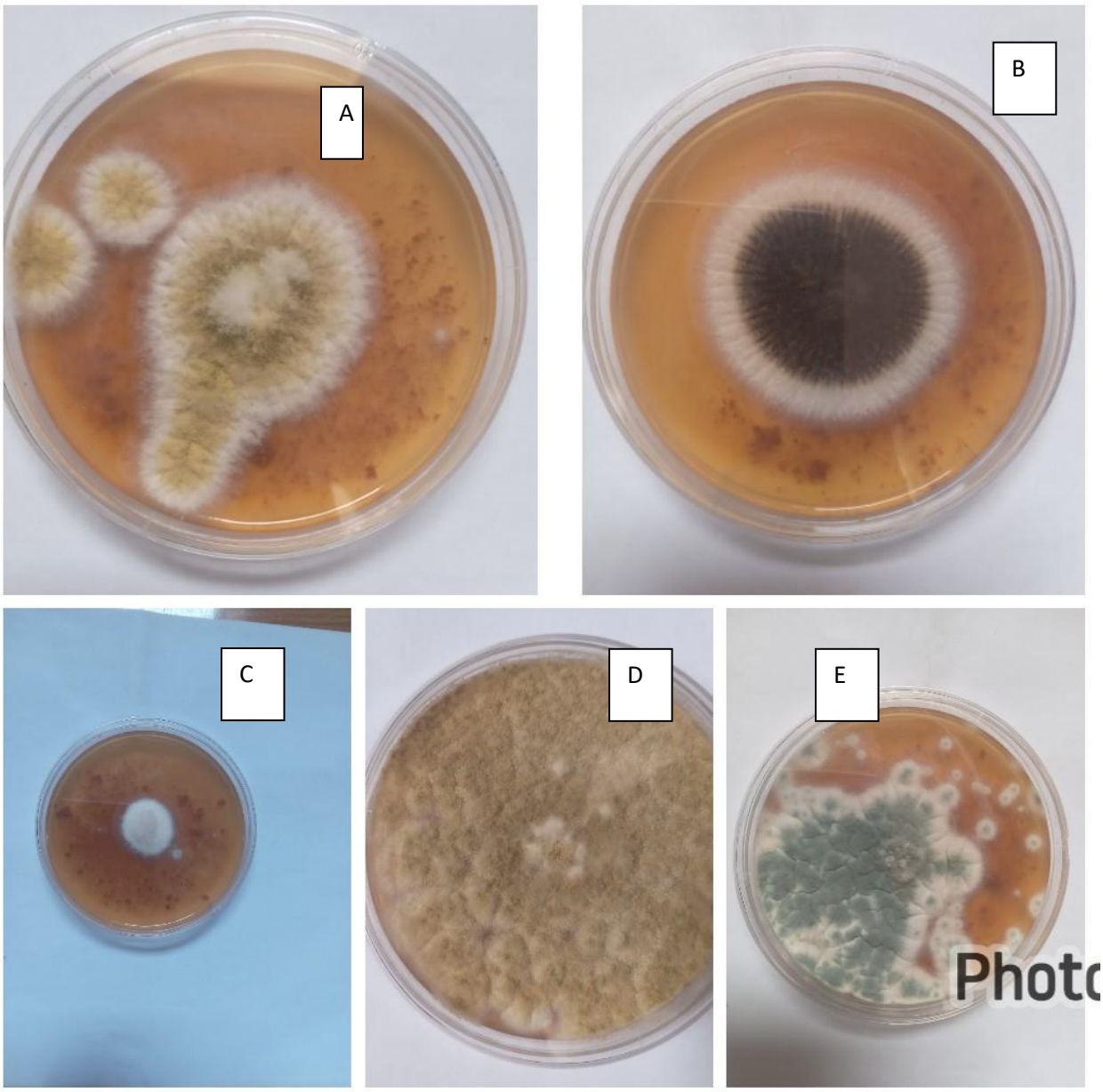

Plate 3 (A-E): Proteolytic Ability of the Fungal Isolates as evidenced by the Zones of Clearance

Table 3: Lipolytic Ability of the Fungal Isolates

Isolates	Names of the Isolates	Zones of Clearance (mm)
A	*Aspergillus flavus*	83.69
B	*Aspergillus niger*	83.52
C	*Mucor* sp.	83.22
D	*Aspergillus flavus*	83.69
E	*Penicillium* sp.	54.13

Table 4: Proteolytic Ability of the Fungal Isolates

Isolates	Names of the Isolates	Zones of Clearance (mm)
A	*Aspergillus flavus*	63.84
B	*Aspergillus niger*	81.38
C	*Mucor* sp.	21.50
D	*Aspergillus flavus*	77.49
E	*Penicillium* sp.	79.66

DISCUSSION

The study detected four fungal species from three prominent genera; *Aspergillus, Penicillium and Mucor*. The isolated genera; especially *Aspergillus* and *Penicillium* were among microorganisms frequently encountered in the search for enzyme producing strains of fungi (Adedayo *et al*., 2017).Moulds are commonly found in decomposing organic matter where they derive organic carbon for survival as heterotroph. Soil and decomposing waste/litter most suitably host a large fungal population hence the continuous search of these environments for microbes of commerce with significant potential for enzyme production. The presence and isolation of these fungi in fruit waste dumpsite environment highlights their ecological role in breaking down of organic components, biogeochemical cycling and enhancement of waste management. The recovery of these fungi also suggests that fruit waste dumpsite supports a rich microbial ecosystem that is essential for the complete breakdown of the inherent organic carbon in the fruit waste. The rich diversity of fungi is an indication that these fungi have adapted to utilize the organic substrates available in the fruit waste, such as sugars, fats, and proteins for growth and metabolism. The identification of multiple *Aspergillu*s species aligns with previous studies by Abdullahi *et al*. (2024) and Abdulmumini *et al*. (2022), which reported the prevalence of this genus in similar ecological niches, underscoring its importance in enzyme production and other biotechnological applications. The growth pattern of the isolated fungi and enzyme activity follow the normal growth pattern expected of moulds. Enzyme activity corresponds to growth since growth of an organism on a substrate is directly related to its ability to metabolize the substrate which is directly related to its enzymatic complexes (Rosa *et al*., 2024).

The large clear zones around the colonies of *Aspergillus* on agar plates as qualitative indicators of lipase and protease activity, confirms the ability of the fungus to produce extracellular enzymes. All fungi produce and release diverse types of enzyme upon the substrate on which they are growing. The extracellular enzyme is secreted for outward conversion of complex organic molecules into simple form for absorption through the hyphae network. *Aspergilli* are noted and well-documented for its complex enzyme system. Species of *Aspergillus* have been reported to produce many enzymes for during laboratory work and for industrial purposes. *Aspergillus* has been implicated in enzyme production more than any other genera with ability to produce cellulases, pectinases, lipases, xylanase and proteases among others used in food processing, detergent formulation, and bioremediation (Bellaouchi *et al*., 2021).

The implications of this study are significant for both environmental sustainability and biotechnological innovation. Utilizing fruit waste as a substrate for fungal growth aids in waste management by reducing organic waste volume while promoting the recovery of valuable enzymes for industrial applications. The enzymes produced by the isolated fungi could be employed in various sectors, including food and beverage, pharmaceuticals, and biofuels, contributing to a circular economy. This is particularly relevant in sustainable waste management and resource recovery, as emphasized by Bharathiraja *et al*. (2016), who highlighted the importance of using agricultural waste for enzyme production. *Penicillium* species are another group of fungi with great potential for enzyme production. Species of this fungus have been isolated from soil and other environments including leaf litters, waste dumpsites, landfills and domestic wastes (Adedayo and Ayilara, 2021), and used in production of industrially applicable enzymes with good qualities and quantities. *Mucor* species is another fungus that is reportedly associated with decomposing organic matter and has been variously isolated from spoilt food; it is very notorious as a food spoilage fungus especially on fruits. The ability to produce enzyme on commercial scale by this fungus is not commonly reported though it is not impossible. The ability to produce enzyme has been documented by Adedayo and Sani (2019).

The need for identifying local fungal strains with high enzyme production capabilities underscores the importance of exploring indigenous microbial resources. As noted by Andrio and Demain (2014), this approach can lead to discovering novel enzymes with unique properties suitable for specific industrial applications not met by commercially available enzymes.

CONCLUSION

In conclusion, the isolation and identification of fungi from the fruit waste dump site soil in the Ipata Market Area of Ilorin yielded positive outcomes as two species with high lipolytic and proteolytic capacities (*Aspergillus flavus* and *niger*) were discovered. The discovery of these fungal strains can be further researched upon to expose their optimum culture requirements for lipase and protease production towards sustainability and resource recovery in the local and global enzyme industry. It is therefore recommended that future research should be focused on optimization of culture parameters for production of these enzymes for the local industries while also exploring the potential commercial applications of these indigenous fungal strains to maximize their benefits across various industries.

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