
Optimisation of Lipases from Fungi Associated with Palm Oil Mill Soils

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Abstract: Lipases are industrially significant enzymes applied in food processing, detergents, pharmaceuticals, cosmetics, and biodiesel manufacture. Palm oil mill effluent (POME), though nutrient-rich and supportive of microbial growth, is also a major pollutant of the environment. Exploring lipolytic fungi from such soils provides a dual benefit of waste management and enzyme generation. Soil samples from palm oil mills in Eyekanse via Owode-Yewa, Yewa South Local Government Area of Ogun State were analysed for fungal diversity and lipase enzyme optimisation. Isolates were obtained through serial dilution on mineral salts agar and screened for lipase activity on tributyrin agar. Promising strains including LFA1 *A. niger*, LFA2 *R. arrhizus*, LFA3 *A. flavus*, LFB7 *T. virens*, LFC9 *T. asperellum*, LFC10 *A. terreus* and LFC12 *A. tamarii* were cultured in submerged fermentation, using a lipase optimising medium enriched with mineral salts and yeast extract at 30°C for 96 hrs and crude lipase was recovered from the supernatant by centrifugation. Enzyme output was determined spectrophotometrically, while cultural and nutritional parameters were optimised using a one-variable-at-a-time approach. Out of sixteen isolates recovered, seven showed distinct lipase activity. Maximum enzyme levels were obtained at pH 8.0, 45°C, inoculum size 3.5 ml, 48 hrs incubation, and 160 rpm agitation. Palm oil served as the most suitable substrate, xylose supported highest yield as carbon source, while peptone and potassium nitrate proved effective nitrogen supplements. Sodium dodecyl sulphate enhanced enzyme activity. These findings offered valuable insights for optimising conditions to maximise lipase enzyme yield.

Key word: Bioremediation, Fungi, Lipase, Optimisation, Palm oil effluent

INTRODUCTION

Lipases (EC 3.1.1.3) are hydrolase enzymes that catalyse the breakdown of triglycerides into free fatty acids and glycerol (Yang, 2021). The versatility and robustness of lipases have made these enzymes highly valuable in diverse industries, including food processing, detergents, pharmaceuticals, cosmetics, and renewable energy. Microbial lipases are considered superior to plant and animal sources due to the ability to function under a wide range of conditions and act on multiple substrates. (Yang, 2021; Kurniawati *et al.*, 2023).

Palm oil processing generates a large volume of effluent known as palm oil mill effluent. This waste stream is rich in organic matter that favours microbial proliferation, yet also serves a major contributor to environmental pollution when released untreated into the environment (Yadav, *et al.*, 2022). As a result, POME-contaminated soils provide a unique reservoir of microorganisms with potential for industrial enzyme production.

Fungal lipases, in particular, are well recognised for their stability, high yields, and ease of production. However, the quantity and activity of the enzymes produced are strongly influenced by culture conditions such as pH, temperature, nutrient supply, and incubation period (Zhang *et al.*, 2023; Sharma, 2023; Safdar, 2023). For this reason, optimising fermentation parameters is a crucial step toward achieving economically viable enzyme production.

The present study was designed to optimise lipases from lipolytic fungi isolated from POME-impacted soils in Eyekanse via Owode-Yewa in the Yewa South Local Government Area of Ogun State, Nigeria, and to evaluate how environmental and nutritional factors influence lipase enzyme output. By identifying optimal growth conditions, this work seeks to contribute to the understanding of lipase production by indigenous fungal strains.

MATERIALS AND METHODS

Sample Collection: Soil samples were obtained from effluent discharge points at four palm oil mills located in Eyekanse-

Yewa (via Owode-Yewa), Yewa South, Ogun State, Nigeria. A clean, sterilised hand trowel was used for the collection process to minimise contamination and ensure that the samples were representative of the microbial communities associated with effluent-rich soils (Ezeji *et al.*, 2021). The samples were obtained from a depth of 4–6 cm beneath the soil surface, placed into clean, sterile polyethylene bags, clearly labelled. Samples were immediately transported to the laboratory under ambient conditions and processed within 24 hours of collection to preserve microbial viability. Upon arrival at the laboratory, the samples were stored at 4°C prior to microbiological analysis (Nema *et al.*, 2019).

Isolation and Identification of Fungi from Palm Oil Mill Soils: To recover fungal isolates, one gram of each soil sample was suspended in sterile distilled water and serially diluted. Aliquots of appropriate dilutions were spread on mineral salts agar plates containing palm oil as a lipid source (emulsified in Tween 80 at 10 ml oil:1 ml Tween); (NH₄)₂SO₄, 5 g; Na₂HPO₄, 6 g; KH₂PO₄, 2 g; MgSO₄, 3 g; CaCl₂, 3g and Agar (Yadav *et al.*, 2022). The plates were incubated at 28°C for 5-7 days. Distinct colonies were purified through repeated subculturing on potato dextrose agar (PDA). Identification was carried out based on cultural features and microscopic characteristics observed with lactophenol cotton blue preparations, using standard mycological keys (Raja, 2022).

SCREENING OF FUNGAL ISOLATES FOR LIPASE PRODUCTION

Isolates were initially screened for lipase production by spotting on tributyrin agar plates. Formation of clear zones around colonies indicated lipase activity. Positive isolates were selected for further evaluation in mineral salts medium without agar using palm oil as the principal carbon source ((NH₄)₂SO₄, 5 g; Na₂HPO₄, 6 g; KH₂PO₄, 2 g; MgSO₄, 3 g; CaCl₂, 3 g). Following incubation for 72 hrs at 28°C, the broth cultures were centrifuged at 100 rpm using a bench-top centrifuge (Nuve, Turkey; Model

NF 400; Serial No. NF400-21-4587), and the cell-free supernatants were collected as crude enzyme extracts (Safdar, 2023).

Optimisation of Variables for Lipase Production by the Fungal Isolates: A one-variable-at-a-time (OVAT) strategy was applied to assess the effect of environmental and nutritional parameters on lipase output. The conditions investigated included: pH values ranging from 2.0 to 11.0; temperature range between 25°C and 85°C; inoculum sizes from 1.0 to 4.0 ml; agitation speeds between 100 and 180 rpm; incubation periods from 12 to 96 hrs; various lipid substrates; supplementary carbon and nitrogen sources; and different surfactants (Chen, 2021).

Enzyme Assay: Lipase activity was determined spectrophotometrically at 410-420 nm using a UV-Visible spectrophotometer (Model: UV1100, Yoke Instrument Co., Ltd., China; Serial No. UV1100-2019-4587). The reaction mixture consisted of an olive oil emulsion as the substrate and phenolphthalein as the indicator. Activity was expressed as the change in absorbance per minute. All assays were performed in triplicate, and average values were reported (Gupta, 2022).

RESULTS

A total of sixteen fungal isolates were recovered, of which seven demonstrated significant lipase activity (Table 1). Palm oil was the most effective substrate for enzyme production, followed by olive oil, while soybean oil and tributyrin were less effective (Figure 1). Peptone was found to be the best organic nitrogen source for the optimum production of lipase, while potassium nitrate was the most effective inorganic nitrogen source (Figure 2). Among additional carbon sources, xylose recorded the highest lipase enzyme yields by the fungal isolates, followed by lactose, while glucose and starch were less favourable (Figure 3). Surfactant supplementation revealed that SDS was the most effective inducer of enzyme activity, followed by gum arabic and Tween 80 (Figure 4). Physical

(environmental) parameters also influenced lipase yields. Optimum production of lipase enzyme by the fungal isolates was achieved at 45°C (Figure 5) and pH 8.0 (Figure 6). Enzyme activity of the fungal isolates was maximal at an inoculum concentration of 3.5

ml (Figure 7) and agitation speed of 160 rpm (Figure 8). Maximum enzyme activity occurred between 36-48 h of incubation, followed by decline with longer incubation (Figure 9).

Table 1: Lipase Activity of Fungal Isolates Recovered from Palm Oil Mill Effluent Soils

Isolate code	Fungal species	Lipase activity (IU/ml)
LFA1	<i>Aspergillus niger</i>	27.1
LFA2	<i>Rhizopus arrhizus</i>	24.2
LFA3	<i>Aspergillus flavus</i>	31.3
LFB7	<i>Trichoderma virens</i>	20.0
LFC9	<i>Trichoderma asperellum</i>	30.1
LFC10	<i>Aspergillus terreus</i>	24.7
LFC12	<i>Aspergillus tamarii</i>	30.0

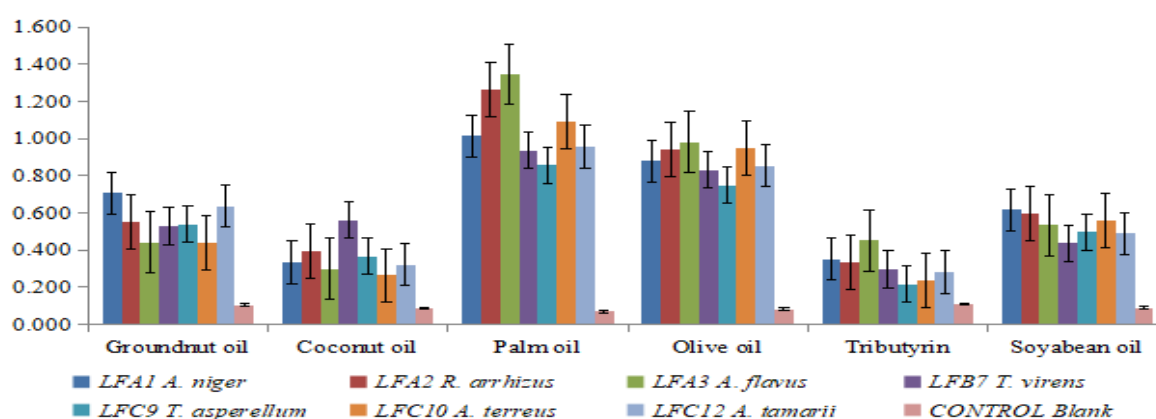


Figure 1: Influence of Different Lipid Substrates on the Lipase Yield of Fungal Isolates
Keys: LFA, B, C, D – Isolates code for lipolytic fungi from soil samples from palm oil mills A, B, C and D respectively; Y-axis (vertical) – Performance mean values per ml of isolates; X-axis (horizontal) – Various substrates sources

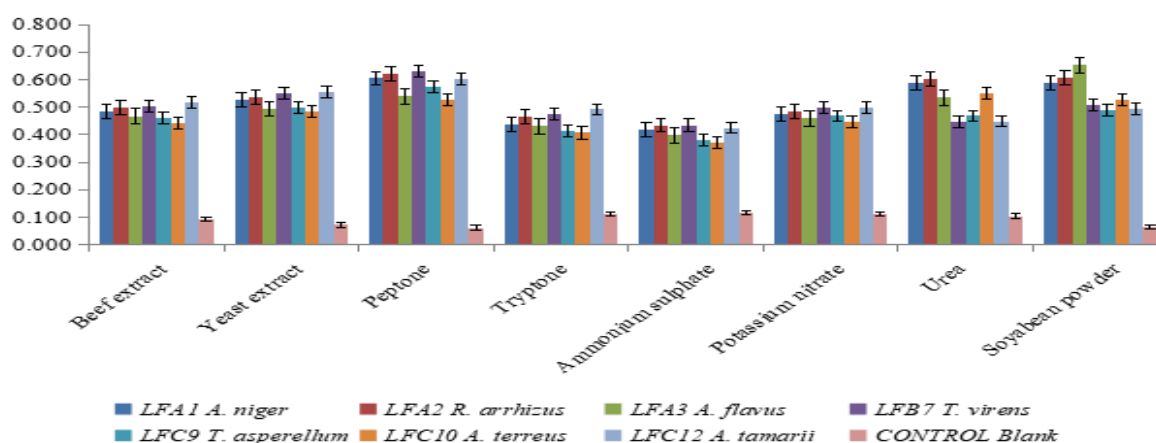


Figure 2: Comparative Performance of Organic and Inorganic Nitrogen Sources in Supporting Lipase Production

Keys: LFA, B, C, D – Isolates code for Lipolytic Fungi from soil samples from palm oil mills A, B, C and D respectively; Y-axis (vertical) – Performance mean values per ml of isolates; X-axis (horizontal) – Various nitrogen sources

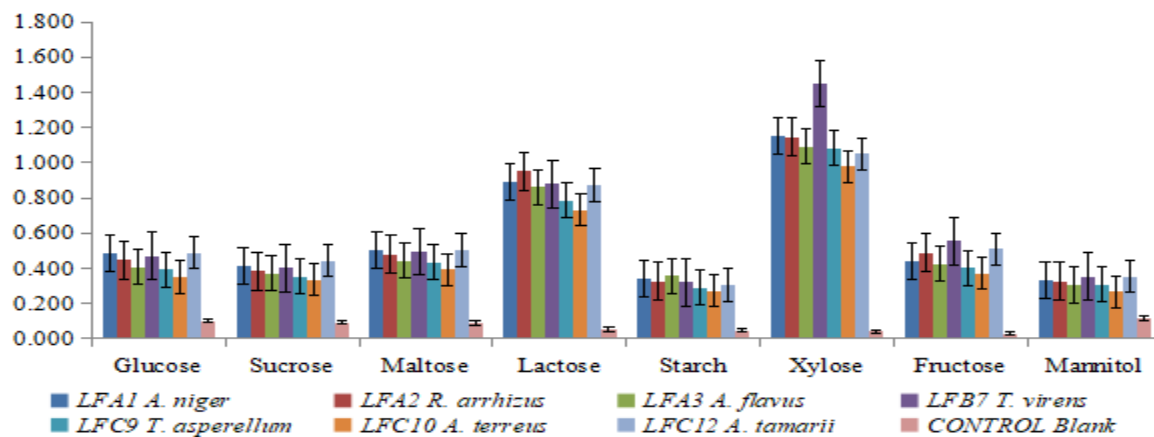


Figure 3: Changes in Lipase Activity when Isolates were Supplemented with Alternative Carbon Sources

Keys: LFA, B, C, D – Isolates code for Lipolytic Fungi from soil samples from palm oil mills A, B, C and D respectively; Y-axis (vertical) – Performance mean values per ml of isolates; X-axis (horizontal) – Various additional carbon sources

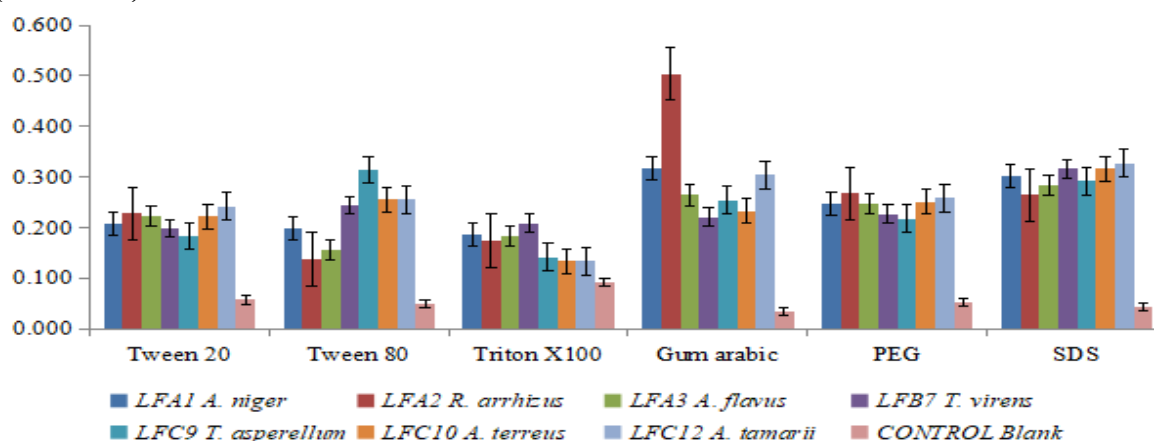


Figure 4: Impact of Surfactant Supplementation on Lipase Secretion by Fungal Isolates

Keys: LFA, B, C, D – Isolates code for lipolytic fungi from soil samples from palm oil mills A, B, C and D respectively; Y-axis (vertical) – Performance mean values per ml of isolates; X-axis (horizontal) – Various surfactants

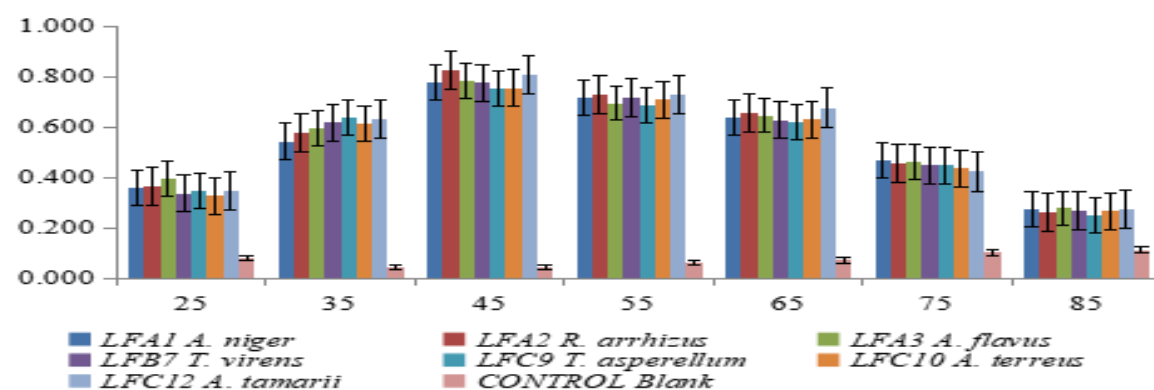


Figure 5: Variation in Enzyme Output across Different Incubation Temperatures

Keys: LFA, B, C, D – Isolates code for lipolytic fungi from soil samples from palm oil mills A, B, C and D respectively; Y-axis (vertical) – Performance mean values per ml of isolates; X-axis (horizontal) – Temperature range 25°C – 85°C

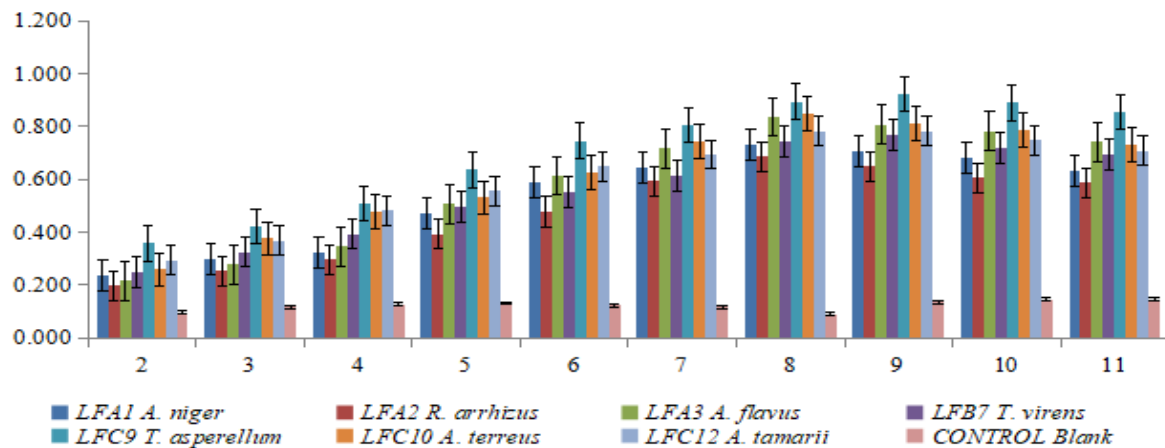


Figure 6: Lipase Activity Profiles of Fungal Isolates at Different pH Levels

Keys: LFA, B, C, D – Isolates code for lipolytic fungi from soil samples from palm oil mills A, B, C and D respectively; Y-axis (vertical) – Performance mean values per ml of isolates; X-axis (horizontal) – pH range 2.0 – 11.0

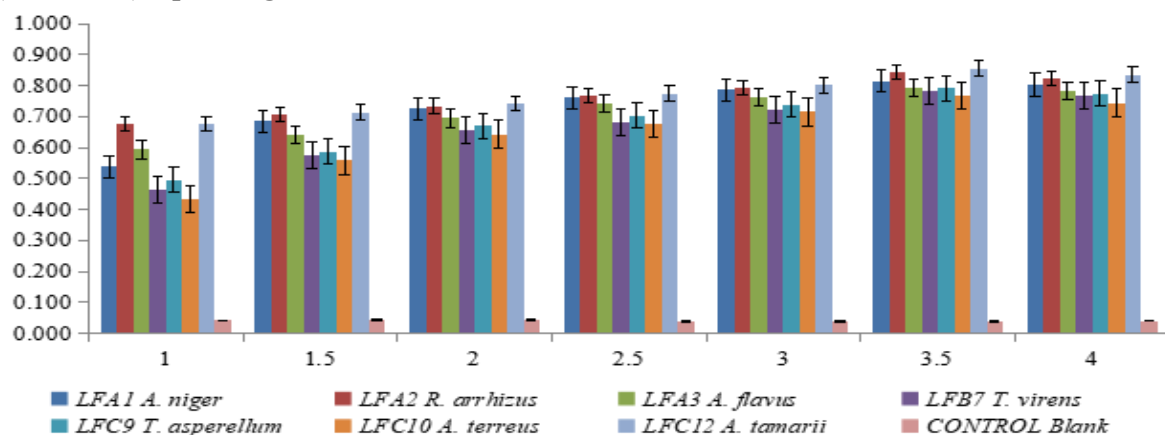


Figure 7: Relationship between Inoculum Concentration and Lipase Production Efficiency

Keys: LFA, B, C, D – Isolates code for lipolytic fungi from soil samples from palm oil mills A, B, C and D respectively; Y-axis (vertical) – Performance mean values per ml of isolates; X-axis (horizontal) – Inoculum concentration range 1.0 ml – 4.0 ml

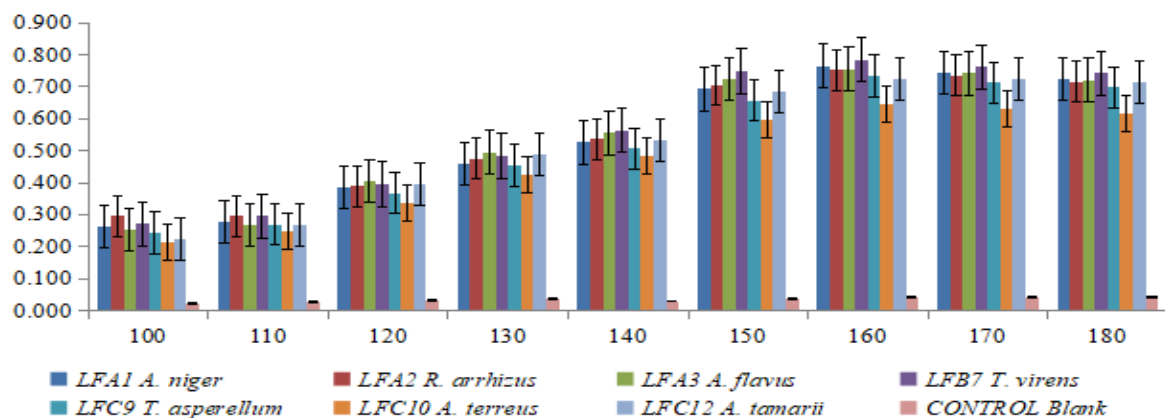


Figure 8: Response of Fungal Isolates to Varying Agitation Speeds during Fermentation

Keys: LFA, B, C, D – Isolates code for lipolytic fungi from soil samples from palm oil mills A, B, C and D respectively; Y-axis (vertical) – Performance mean values per ml of isolates; X-axis (horizontal) – Agitation range 100 rpm – 180 rpm

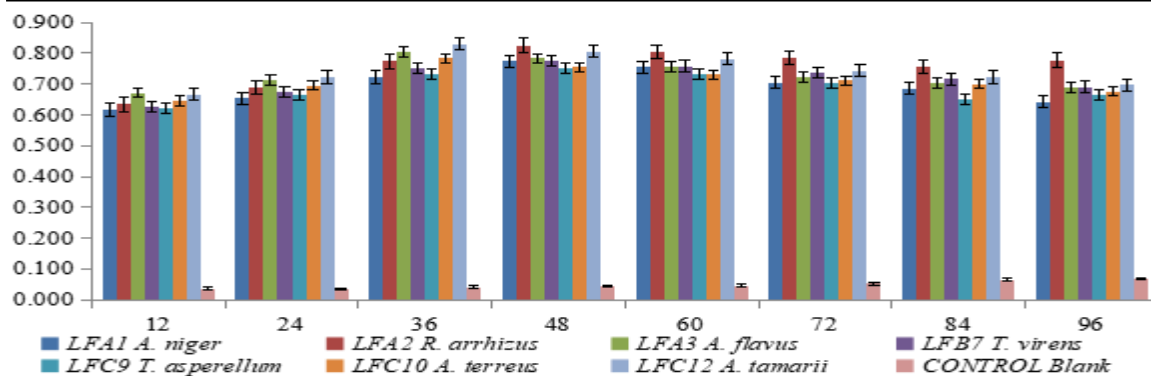


Figure 9: Time-Course of Lipase Production by Fungal Isolates at 45°C

Keys: LFA, B, C, D – Isolates code for Lipolytic Fungi from soil samples from palm oil mills A, B, C and D respectively; Y-axis (vertical) – Performance mean values per ml of isolates; X-axis (horizontal) – Incubation period range 12 – 96 h at 45°C

DISCUSSION

The present study demonstrates that fungi isolated from soils impacted by palm oil mill effluent are efficient lipase producers when cultivated under optimised conditions. Among the substrates tested, palm oil promoted the highest enzyme yields, which agrees with previous reports that natural oils act as strong inducers of lipase biosynthesis (Ezeji *et al.*, 2021). This suggests that the native lipid profile of palm oil may serve as both a nutrient source and an enzyme production trigger.

Of all the isolates examined, *Aspergillus flavus* exhibited the highest lipase enzyme activity, closely followed by *Rhizopus arrhizus* and *Trichoderma virens*. These findings are consistent with earlier work that identified members of the *Aspergillus* and *Rhizopus* genera as prolific lipase producers (Nema *et al.*, 2019; Ren, 2023). The ability of these fungi to secrete large quantities of extracellular enzymes makes them attractive for industrial exploitation.

Nutritional factors were found to play a significant role in modulating enzyme yield. Supplementation with peptone and potassium nitrate supported enhanced lipase activity, which aligns with previous studies indicating that a balanced supply of organic and inorganic nitrogen promotes enzyme biosynthesis (Wu, 2021). Xylose was identified as the most effective additional

carbon source, corroborating earlier reports on its capacity to stimulate fungal lipase production (Singh, 2022).

The optimal pH (8.0) and temperature (45°C) obtained in this work fall within the range reported for industrially relevant fungal lipases, further reinforcing the suitability of these isolates for practical applications (Chen, 2021; Raja, 2022). The variations in aeration and inoculum sizes also influenced enzyme output, underscoring the importance of optimising physical culture conditions to achieve maximum productivity (Gupta, 2022).

Overall, this study strengthens the evidence that indigenous fungi from POME-contaminated soils can be harnessed for sustainable lipase production. Beyond offering an avenue for waste utilisation, fungi hold promise for use in biodiesel processing, bioremediation, and other biotechnological industries.

CONCLUSION

This work demonstrates that soils contaminated with palm oil mill effluent harbour fungal species with strong lipase-producing capacity. The study established that enzyme output is greatly shaped by culture conditions, with peak activity recorded at pH 8.0, 45°C, 3.5 ml inoculum size, 48 hrs incubation, and 160 rpm agitation. Palm oil was identified as the most

effective inducer, while xylose, peptone, and potassium nitrate provided additional nutritional support. Surfactant supplementation with SDS further improved enzyme yield. Among the isolates, *Aspergillus flavus*, *Rhizopus arrhizus*, and *Trichoderma virens* consistently produced the highest lipase levels, highlighting their promise as candidates for industrial exploitation. The findings not only validate

the potential of palm oil mill soils as reservoirs of useful microbial enzymes, but also provide a basis for large-scale optimisation and application. These indigenously sourced fungi could be harnessed for eco-friendly bioremediation strategies and for advancing Nigeria's contribution to biodiesel and other biotechnological industries.

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