

Low-Cost Agro-Industrial Waste–Based Production of Lipase by *Aspergillus flavus* and Its Application in *Brassica napus* Seed Germination

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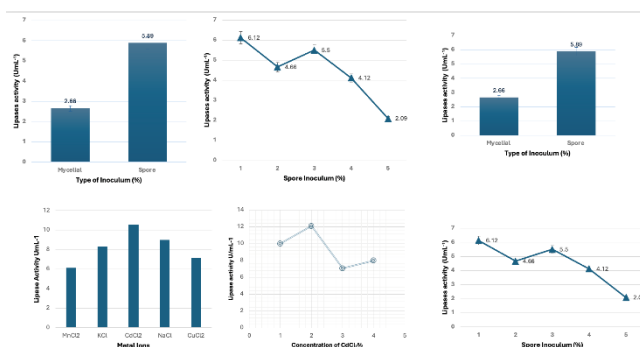
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Abstract: Lipases are biocatalysts of great industrial and agricultural importance. The production of lipase by microbial sources can be optimized by adjusting the physico-chemical and nutritional parameters. The aim of the present study was to determine the optimal production of extracellular lipase by *Aspergillus flavus* in solid-state fermentation and to evaluate its impact on lipid mobilization and seed germination in *Brassica napus*. *A. flavus*, a morphologically identified and molecularly confirmed strain, was initially isolated from oil-roasted bread and cultured on malt extract agar for enzyme production under solid-state fermentation. Optimization of fermentation parameters, including incubation time, temperature, pH, inoculum type and size, substrate type and concentration, and supplementation with metal ions, carbon, oil, and nitrogen sources, was performed using the one-variable-at-a-time (OVAT) approach as a preliminary optimization method. Lipase activity was determined by a titrimetric method using an olive oil emulsion. The optimized enzyme extract was applied to *B. napus* seeds to assess its effects on germination, seedling growth, and lipid mobilization. The optimal conditions were 48 h incubation, 40 °C, pH 8, spore inoculum (1 mL), 20 g Nigella meal, 2 % (w/v) CdCl₂, 3 % (w/v) fructose, 3 % (v/v) mustard oil, 2 % (w/v) ammonium sulfate, and 1 % (w/v) yeast extract, resulting in maximum extracellular lipase activity (14.99 ± 1.66 U mL⁻¹). Application of the optimized lipase significantly improved germination, radicle length, seedling fresh weight, and free fatty acid content by 12.8 %, 31.4 %, 16.4 %, and 50.4 %, respectively ($p \leq 0.05$) compared to the control, indicating enhanced lipid mobilization and seed vigor. These findings demonstrate the potential of fungal lipase in industrial production and agricultural applications, particularly in improving seed germination and early seedling development.



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Keywords: solid state fermentation, enzymes, lipases, *Aspergillus flavus*.

1 Introduction

Enzymes are basic proteins that play an important role in all organisms' metabolic systems and also in food spoilage, the breakdown of organic matter, and host infection. The enzymes are utilized in textile, food, detergents, paper, and in the leather industries on a huge scale for the welfare of human beings ¹⁾. Enzymes are protein catalysts that increase the rate of biochemical reactions ²⁾. Lipases belong to those enzyme groups that hydrolyze the various fatty esters ³⁾. Lipases are found in nature abundantly, but only microbial lipases are valuable commercially ⁴⁾. Due to their potential use in bioprocesses, lipases have drawn much consideration, predominantly because of their stability and accessibility ⁵⁾. "Microbial lipases, in particular, have garnered significant commercial interest due to their versatility, stability, and broad substrate specificity ⁶⁾.

The lipase produced in this study has potential applications across various industries. Oil seeds store triacylglycerols (TAG) in specialized storage organelles called oil bodies, which are important as reserve lipids for providing energy during germination and early seedling development. The activity of lipases (EC 3.1.1.3) during germination mobilizes lipids; TAGs are cleaved into free fatty acids and glycerol to facilitate the subsequent β -oxidation and gluconeogenesis processes in the growing seedling ⁷⁾. The potential of exogenous fungal lipases to stimulate the breakdown of reserve lipids during the germination of oilseeds is poorly investigated, despite the extensive study of microbial and industrial lipases. Optimization of lipase production using *Aspergillus niger*, therefore, has the potential to present a cost-efficient source of enzyme in commercial uses. Solid-state fermentation (SSF) is a process in which microorganisms grow on or within solid substrates without free water (Bellon-Maurel et al., 2003 ⁸⁾.

The technique offers an appropriate medium for microbial metabolism and growth, especially for fungi, as it has a lower moisture level than submerged systems. Different agro-industrial byproducts, such as wheat bran, rice bran, niggella meal, almond meal, coconut meal and sesame meal may be used as substrates in SSF. Rice bran is one of these, and it contains nutrients that are used extensively as a cheap source of obtaining enzymes. Process parameters and growth medium components are key factors that affect lipase biosynthesis in SSF. Optimization of these factors is typically performed using a one-factor-at-a-time (OFAT) approach, where each variable is modified individually while others are kept constant ²⁾. Despite the extensive literature available on microbial lipases, few studies have been conducted on low-cost production of agro-industrial waste under solid-state fermentation and its direct use in seed germination and lipid mobilization. Furthermore, the interdisciplinary analysis of optimizing the processes and their application in agriculture is not well investigated. The present study aimed to optimize extracellular lipase production by *Aspergillus flavus* using agro-industrial substrates under solid-state fermentation and to evaluate its effect on lipid mobilization and seed germination in *Brassica napus*.

2 Materials and Methods

2.1 Microorganism and Inoculum Preparation

The fungal isolate was initially identified morphologically on MEA medium and confirmed as *Aspergillus flavus* by ITS rDNA sequencing and comparison with the NCBI GenBank database ⁹⁾. For inoculum preparation, 10 mL of sterile distilled water was added to a sporulated slant, and spores were gently dislodged using an inoculating needle under aseptic conditions to create a spore suspension.

2.2 Solid-State Fermentation (SSF)

Fermentation was conducted in 250 mL Erlenmeyer flasks with 10 g of substrate. The substrate was moistened with 10 mL of distilled water; the substrate was sterilized in an autoclave at 121 °C for 15 minutes before inoculation. The flasks were cooled, and then 1.0 mL of the spore suspension was added and incubated at 30 °C, 48 hours. Each experiment was carried out in triplicate ¹⁰⁾.

2.3 Enzyme Extraction and Assay

After incubation, flasks were filled with 50 mL of water and shaken for 30 minutes. Whatman No. 1 filter paper was used to filter the contents. Titration was used to measure lipase activity. One milliliter of crude enzyme, ten milliliters of 10 % (w/v) olive oil-gum acacia emulsion, two milliliters of 0.6 % (w/v) CaCl₂ solution, and five milliliters of 0.1 M phosphate buffer (pH 7.0) were all added to the reaction mixture, and shaken at 200 rpm for an hour at 30 °C. The reaction was stopped after incubation with 20 mL of acetoneethanol mix of 1:1 % (v/v). A blank was prepared by adding all reagents except the enzyme to account for non-enzymatic hydrolysis. The lipase activity was then determined by

titrating the free fatty acids released with 0.1N NaOH, using phenolphthalein as an indicator. A unit of lipase activity (UmL^{-1}) is the quantity of enzyme that, under assay conditions, releases one micromole of fatty acid per minute ¹⁰. Activity was calculated using the following formula:

Lipase Activity (UmL^{-1}) = $[(V_2 - V_1) \times N \times 1000] / [V(\text{sample}) \times T]$ Where: V_1 = Volume of NaOH used for the control (mL), V_2 = Volume of NaOH used for the sample (mL), N = Normality of NaOH (0.1 N), $V(\text{sample})$ = Volume of enzyme extract used (1 mL), T = Incubation time (60 minutes).

2.4 Process Optimization using One-Factor-at-a-Time (OVAT) Approach

The classical one-variable-at-a-time (OVAT) approach was employed to systematically optimize the cultural and nutritional parameters for enhanced lipase production, incubation period (24 to 120 hours), type of inoculum (spore vs. mycelial), inoculum size (1 to 5 mL), and initial pH of the moistening agent (5 to 11). Furthermore, various substrates (mustard meal, rice bran, almond meal, sesame meal, wheat bran, coconut meal=10 to 50 g) and different metal ions (CdCl_2 , CuCl_2 , NaCl , MnCl_2 , KCl =1 %w/v) were evaluated. Additional carbon sources (glucose, dextrose, maltose, lactose, Fructose=1 %w/v), oil additives (mustard, almond, sesame, coconut, and nigella oil= %v/v), and inorganic (ammonium chloride, sulfate, carbonate, oxalate, nitrate) and organic (peptone, yeast extract, beef, tryptone, urea=1 %w/v). For each factor with the maximum effect, its optimal concentration was subsequently determined and selected for further studies. The impact of each variable was assessed based on the extracellular lipase activity (UmL^{-1}).

2.5 Application of Optimized Lipase in Seed Lipid Mobilization

Certified seeds of *B. napus* were obtained from the Punjab Seed Corporation, Lahore. Seeds were surface-sterilized with 1 % sodium hypochlorite for 2 minutes, rinsed with sterile distilled water, and air-dried before treatment. Surface-sterilized seeds were divided into two groups: (i) Control – soaked in phosphate buffer (0.1 M, pH 7.0), (ii) Treated – soaked in crude lipase solution (1.0 U mL^{-1} in 0.1 M phosphate buffer, pH 7.0). Seeds were soaked for 4 h at room temperature (100 rpm) and air-dried under sterile conditions. Germination tests were conducted on moistened filter paper in Petri dishes at $25 \pm 1^\circ\text{C}$ in the dark. In the germination assay, three replicas of the seed (20 seeds) were put in each Petri dish. Control seeds were soaked in 0.1 M phosphate buffer (pH 7.0), and treated seeds in 1.0 U mL^{-1} crude lipase solution (1.0 U mL^{-1} in 0.1 M phosphate buffer). The experiment was triplicated. Germination percentage was recorded up to 72 h. After 48 and 72 h of germination, 10 seedlings from each treatment were used for measurements. Radicle length was measured from the point of attachment to the tip using a digital Vernier caliper ($\pm 0.01 \text{ mm}$). Seedling fresh weight was recorded immediately after blotting on filter paper using a digital balance ($\pm 0.001 \text{ g}$). Free fatty acid (FFA) content in cotyledons was determined by titration ¹¹. Cotyledon extracts were prepared in isopropanol: hexane (2:1 v/v), and FFA (%) was calculated from NaOH titration values, representing lipid mobilization efficiency.

2.6 Data Analysis

Data from experiments performed in triplicate were expressed as mean $\pm$ SD and analyzed using one-way ANOVA at $p \leq 0.05$.

3 Results

The effect of incubation period on extracellular lipase production under solid-state fermentation (SSF) was evaluated. Maximum enzyme activity ($5.91 \pm 1.39 \text{ U mL}^{-1}$) was observed at 48 h, after which activity declined (Table 1).

Table 1 Impact of different incubation periods on lipase yield by *A. flavus* under SSF fermentation

Sr.no.	Incubation Period (h)	Lipase activity (U mL^{-1})
1	24h	$4.20 \pm 0.29 \text{ U mL}^{-1}$
2	48h	$5.91 \pm 1.39 \text{ U mL}^{-1}$
3	72h	$5.50 \pm 1.29 \text{ U mL}^{-1}$
4	96h	$4.77 \pm 1.19 \text{ U mL}^{-1}$
5	120h	$4.03 \pm 1.10 \text{ U mL}^{-1}$

Values are mean $\pm$ SD (n = 3).

Incubation temperature significantly influenced lipase yield, with 40 °C yielding the highest activity (5.81 ± 1.19 U mL⁻¹) (Table 2).

Among inoculum types, spore inoculum produced higher lipase activity than mycelial inoculum (5.89 ± 0.88 U mL⁻¹). Testing different spore concentrations showed that 1 mL was optimal (6.12 ± 1.37 U mL⁻¹) (Fig. 1).

The pH of the moistening agent significantly affected enzyme production, with pH 8 yielding the maximum activity (8.21 ± 1.01 U mL⁻¹) (Fig. 2).

Table 2 Effect of incubation temperature on the yield of lipases by *A. flavus* under SSF

Sr.no.	Incubation temperature(°C)	Lipase activity (U mL ⁻¹)
1	30 °C	5.20± 0.29 U mL ⁻¹
2	40 °C	5.81± 1.19 U mL ⁻¹
3	50 °C	5.50 ± 1.29 U mL ⁻¹
4	60 °C	4.17 ± 1.19 U mL ⁻¹
5	70 °C	4.07± 1.10 U mL ⁻¹

Values are mean ± SD (n = 3).

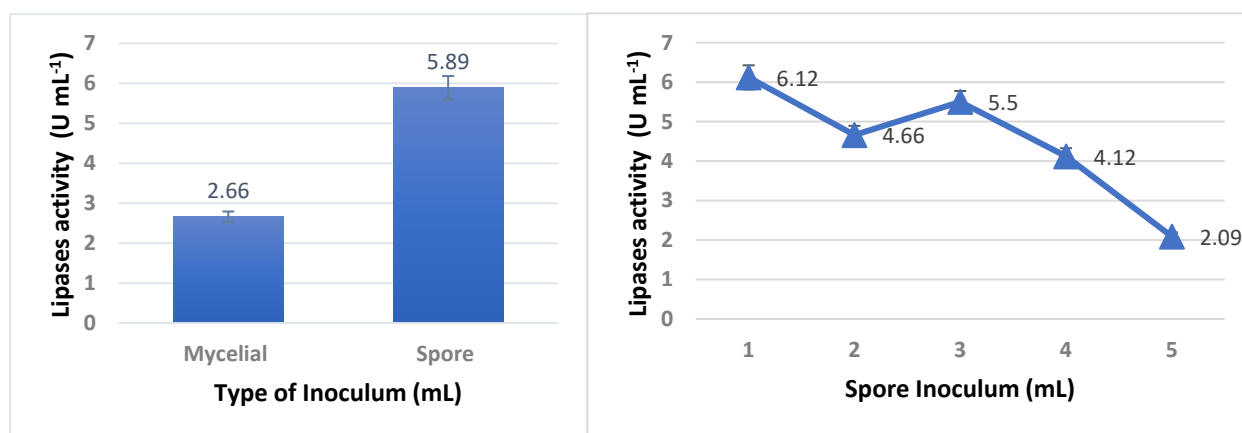


Fig.1 Effect of inoculum type and inoculum concentration on lipase yield in the presence of *A. flavus*. Values are mean ± SD (n = 3). Error bars represent standard deviation.

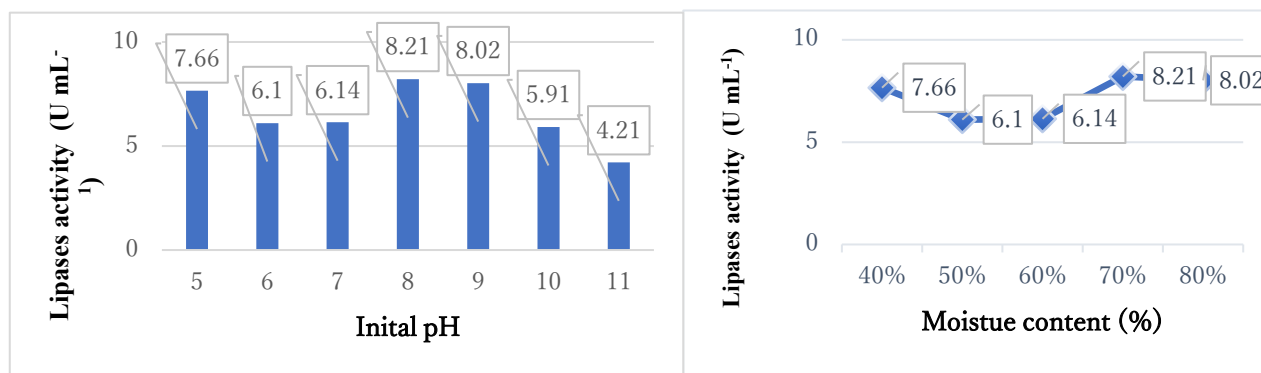


Fig.2. Effect of pH of the moistening agent on lipase yield by *A. flavus*. Values are mean ± SD (n = 3). Error bars represent standard deviation.

Among tested substrates, nigella meal produced the highest lipase activity ($9.02 \pm 1.16 \text{ U mL}^{-1}$), with an optimal substrate concentration of 20 g, resulting in $10.15 \pm 0.78 \text{ U mL}^{-1}$ (Fig. 3).

The addition of metal ions revealed that CdCl_2 enhanced lipase activity ($10.54 \pm 1.60 \text{ U mL}^{-1}$) at 2 % w/v, the optimized concentration for further experiments (Fig. 4).

After 48 h SSF, fructose (3 % w/v) gave the highest lipase activity ($12.32 \pm 1.66 \text{ U mL}^{-1}$), and nigella oil (3 % v/v) further enhanced it ($14.32 \pm 1.75 \text{ U mL}^{-1}$). These concentrations were selected as the optimized conditions for subsequent experiments (Fig. 5).

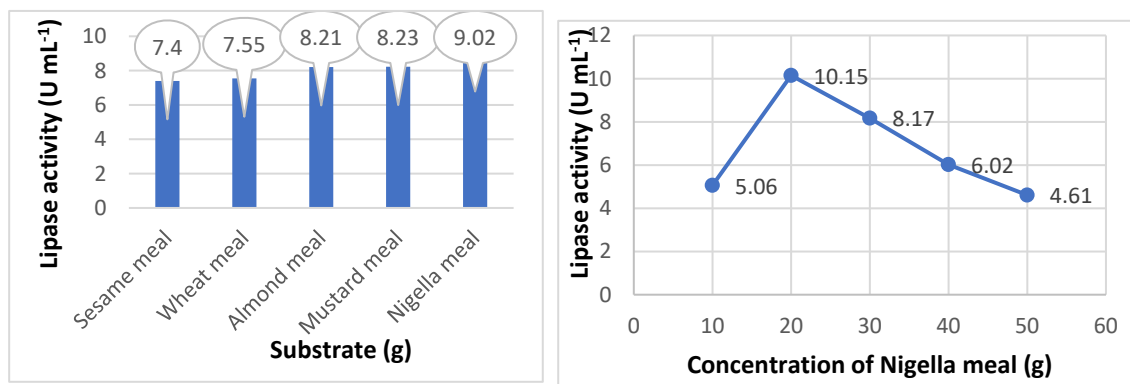


Fig.3 Effect of substrate type and substrate concentration on lipase yield
Values are mean $\pm$ SD (n = 3). Error bars represent standard deviation.

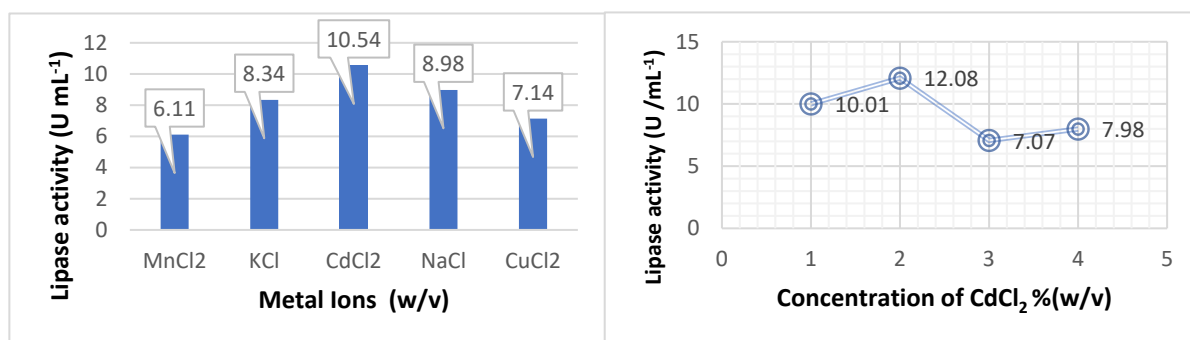


Fig.4 Study of metal ion type and concentration on lipase yield in presence of *A. flavus*
Values are mean $\pm$ SD (n = 3). Error bars represent standard deviation.

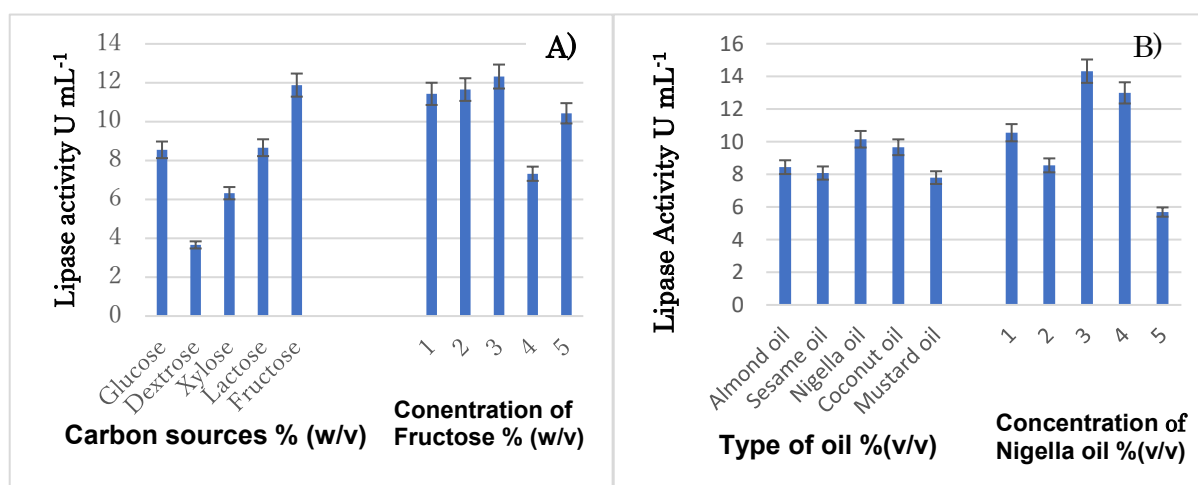


Fig. 5 Effect of A) additional carbon source type and concentration B) Effect of oil additive type and concentration on lipase yield under SFF
Values are mean $\pm$ SD (n = 3). Error bars represent standard deviation.

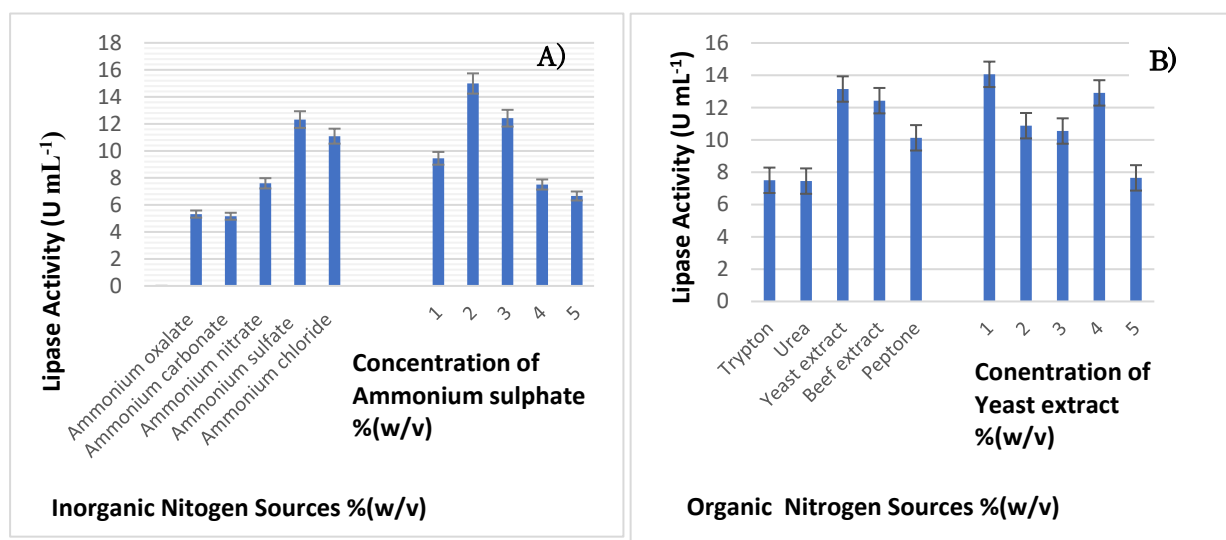


Fig. 6 Impact of (A) inorganic nitrogen source type and concentration, and (B) organic nitrogen source type and concentration on lipase yield under SSF
Values are mean $\pm$ SD (n = 3). Error bars represent standard deviation

Table 3 Effect of optimized *A. flavus* lipase on germination and growth parameters of *B. napus*

Parameters	Control	Lipase-treated	Increase (%)
Germination (%)	78 $\pm$ 2.3 %	88 $\pm$ 2.0 %	12.8 %
Radicle length (cm)	1.21 $\pm$ 0.09 cm	1.59 $\pm$ 0.12 cm	31.4 %
Seedling fresh weight (mg)	45.6 $\pm$ 2.1 mg	53.1 $\pm$ 1.7 mg	16.4 %
Free fatty acids (% of seed DW)	12.1 $\pm$ 0.6 %	18.2 $\pm$ 0.8 %	50.4 %

Values represent mean $\pm$ SD (n = 3). DW = dry weight. Percentage increase calculated relative to the control.

Inorganic nitrogen sources showed ammonium sulfate (2 % w/v) to maximize lipase production (14.99 ± 1.66 U mL⁻¹), while among organic sources, yeast extract (1 % w/v) was optimal (14.06 ± 0.88 U mL⁻¹) (Fig. 6).

Seeds treated with optimized *A. flavus* lipase exhibited higher germination and growth parameters than the control after 72 hours of germination. Seed germination rate increased from 78 ± 2.3 % in the control to 88 ± 2.0 % in the treated seeds. The treated seedlings had a mean radicle length of 1.59 ± 0.12 cm, compared to the control at 1.21 ± 0.09 cm. The enzyme-treated group showed an increase in seedling fresh weight from 45.6 ± 2.1 mg to 53.1 ± 1.7 mg. Treated seedlings had significantly higher free fatty acid content in their cotyledons (18.2 ± 0.8 % of seed dry weight) compared to the control (12.1 ± 0.6 %), indicating improved lipid mobilization (Table 3).

4 Discussion

Microbial sources, especially fungi, are preferred for industrial enzyme production due to their stability in organic solvents and cost-effectiveness. In the present study, *Aspergillus flavus* was screened for extracellular lipase production under solid-state fermentation, and several physicochemical parameters were optimized to enhance enzyme production. The maximum activity was observed after 48 h of incubation, while shorter or longer periods resulted in reduced enzyme production, likely due to nutrient depletion or toxic metabolite accumulation. This observation is consistent with previous studies reporting optimal lipase production within 48–72 h in filamentous fungi under SSF conditions¹². Spore inoculum produced higher lipase activity (6.66 ± 0.88 U mL⁻¹) than mycelial inoculum (3.66 ± 1.19 U mL⁻¹), and an inoculum size of 1 mL gave the best production (8.62 ± 1.38 U mL⁻¹)¹³.

Optimal enzyme activity was obtained at pH 8 (13.43 ± 1.67 U mL⁻¹), consistent with reports of alkaline stability in fungal lipases⁵. Similar alkaline optima (pH 7–9) have been widely reported for *Aspergillus*-derived lipases, supporting their suitability for industrial applications. *A. flavus* exhibited the highest production on Nigella meal, likely due to its

rich nutrient composition; this substrate was consistently used throughout the study to avoid ambiguity. The use of 20 g substrate was found optimal for enzyme secretion, while higher substrate levels decreased activity due to oxygen limitation ¹⁴). Among carbon sources, fructose supported maximum lipase production, whereas dextrose showed the lowest activity, confirming that non-glucose sugars alleviate catabolite repression ¹⁵). Nigella oil was the most effective lipid inducer among the tested oils, possibly owing to its favorable fatty acid profile. Comparable findings have been reported, showing that plant-derived oils enhanced lipase induction due to their triglyceride composition ¹⁶).

Regarding nitrogen sources, ammonium sulphate (2 %) was the most suitable inorganic source, enhancing enzyme activity as observed in *Penicillium* ¹⁷). This is consistent with recent research, which also affirms that ammonium salts have been effective inorganic sources of nitrogen, promoting the growth of microbes and enzyme production through regulations of metabolic pathways involved in the production of lipase ¹⁵). Whereas, yeast extract (1 %) was the best organic source, supplying amino acids, vitamins, and growth factors that promote enzyme secretion ¹⁸). Nonetheless, there is variability across species, with some reporting reduced activity with inorganic sources or varying activity levels across different strains of fungi or under varying culture conditions ^{19, 20}).

Moreover, seed treatment with the optimized alkaline lipase significantly improved germination percentage, radicle length, seedling fresh weight, and free fatty acid content in *Brassica napus*, indicating enhanced lipid mobilization during germination ²¹). The increase in germination (+12.8 %) and radicle length (+31.4 %) suggests improved energy availability and metabolic activity. Mechanistically, lipase catalyzes the hydrolysis of stored triacylglycerols into free fatty acids and glycerol, which are further utilized via β -oxidation and the glyoxylate cycle to generate energy required for early seedling growth. This aligns with findings that efficient lipase activity accelerates reserve mobilization and supports seed vigor ^{22,23}). The enzyme's optimal stability at pH 8 and 40 °C further highlights its potential industrial application in seed treatment and other biotechnological processes.

However, certain limitations must be acknowledged. Aflatoxin production by *A. flavus* and residual cadmium from CdCl₂ were not assessed, which may raise biosafety concerns. Additionally, OVAT approach does not account for interactions among variables, and the germination assay was conducted with a single enzyme concentration and lacked appropriate controls. Future studies should focus on non-aflatoxigenic strains, toxin and metal residue analysis, safer inducers, multivariate optimization approaches such as response surface methodology, and dose–response validation with proper controls.

5 Conclusion

Aspergillus flavus produced high yields of extracellular lipase under optimized solid-state fermentation using low-cost agro-industrial substrates. Application of the enzyme significantly enhanced *Brassica napus* seed germination, seedling growth, and lipid mobilization, demonstrating its potential both for industrial applications and as a biostimulant to improve crop establishment. This study highlights a sustainable approach for valorizing agricultural waste into value-added products for agriculture. Future work should focus on the use of non-aflatoxigenic strains, assessment of potential toxin or heavy metal residues, dose–response evaluation, and multivariate optimization to further ensure biosafety and maximize enzyme efficiency.

Competing Interests

The authors declare that there are no conflicts of interest.

Availability of Data and Material

All the data generated in this research work has been included in this manuscript.

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References

- 1) Alves, M.H., Campos-Takaki, G.M., Porto, A.L.F., Milanez, A.I. Screening of *Mucor* spp. for the production of amylase, lipase, polygalacturonase and protease. *Braz. J. Microbiol.*, **33**, 325-330 (2002). doi: 10.1590/S1517-83822002000400009
- 2) Hosseinpour, M.N., Najafpour, G.D., Younesi, H., Khorrami, M., Vaseghi, Z. Lipase production in solid state fermentation using *Aspergillus niger*: response surface methodology. *IJE Trans. B Appl.*, **25**, 151-159 (2012). doi: 10.5829/idosi.ije.2012.25.03b.01
- 3) Gaoa, X.G., Cao, S.G., Zhang, K.C. Production, properties and application to nonaqueous enzymatic catalysis of lipase from a newly isolated *Pseudomonas* strain. *Enzyme Microb. Technol.*, **27**, 74-82 (2000). doi: 10.1016/S0141-0229(00)00191-5
- 4) Sharma, N., Rathore, M., Sharma, M. Microbial pectinase: sources, characterization and applications. *Rev. Environ. Sci. Bio/Technol.*, **12**, 45-60 (2013). doi: 10.1007/s11157-012-9276-9
- 5) Aulakh, S.S., Prakash, R. Optimization of medium and process parameters for the production of lipase from an oil-tolerant *Aspergillus* sp. (RBD-01). *J. Basic Microbiol.*, **50**, 37-42 (2010). doi: 10.1002/jobm.200900361
- 6) Chandra, K., Patel, A.K., Kumar, P., Chen, C.W., Dong, C.D., et al. Marine thraustochytrids a potential source for lipase: challenges in industrial applications: marine thraustochytrids: challenges in industrial applications. *Indian J. Exp. Biol.*, **62**, 463-474 (2024). doi: 10.56042/ijeb.v62i07.12065
- 7) Choi, Y.J., Zaikova, K., Yeom, S.J., Kim, Y.S., Lee, D.W. Biogenesis and lipase-mediated mobilization of lipid droplets in plants. *Plants*, **11**, 1243 (2022). doi: 10.3390/plants11091243
- 8) Bellon-Maurel, V., Orliac, O., Christen, P. Sensors and measurements in solid state fermentation: a review. *Process Biochem.*, **38**, 881-896 (2003). doi: 10.1016/S0032-9592(02)00093-6
- 9) Bashir, T., Ullah, N., Sarwar, A., Naeem, M., Shami, A., et al. Enhanced optimisation techniques for solid state fermentation: enhancing extracellular lipase production by *Candida albicans*, *Mucor* sp., and *Pseudomonas stutzeri*. *Acta Aliment.*, **2025**, 066-2025 (2025). doi: 10.1556/066.2025.00204
- 10) Bashir, T., Majeed, H., Iftikhar, T. Isolation and screening of thermophilic bacteria and its subsequent evaluation for lipases production. *Pak. J. Bot.*, **56**, 759-764 (2024). doi: 10.30848/PJB2024-2(3)
- 11) Zhang, J., Liu, H., Sun, J., Li, B., Zhu, Q., et al. *Arabidopsis* fatty acid desaturase FAD2 is required for salt tolerance during seed germination and early seedling growth. *PLoS One*, **7**, e30355 (2012). doi: 10.1371/journal.pone.0030355
- 12) Sanchez, S., Demain, A.L. Metabolic regulation and overproduction of primary metabolites. *Microb. Biotechnol.*, **1**, 283-319 (2008). doi: 10.1111/j.1751-7915.2007.00015.x
- 13) Suhail, K.C. Comparative study on the production of lipase by using solid state fermentation and submerged fermentation from fungal sources (Master's thesis, Rajiv Gandhi University of Health Sciences (India)). (2009).
- 14) Mahadik, N.D., Puntambekar, U.S., Bastawde, K.B., Khire, J.M., Gokhale, D.V. Production of acidic lipase by *Aspergillus niger* in solid state fermentation. *Process Biochem.*, **38**, 715-721 (2002). doi: 10.1016/S0032-9592(02)00194-2
- 15) Kumar, A., Verma, V., Dubey, V.K., Srivastava, A., Garg, S.K., et al. Industrial applications of fungal lipases: a review. *Front. Microbiol.*, **14**, 1142536 (2023). doi: 10.3389/fmicb.2023.1142536
- 16) Costa, A.R., Salgado, J.M., Lopes, M., Belo, I. Valorization of by-products from vegetable oil industries: enzymes production by *Yarrowia lipolytica* through solid state fermentation. *Front. Sustain. Food Syst.*, **6**, 1006467 (2022). doi: 10.3389/fsufs.2022.1006467
- 17) Lima, V.M., Krieger, N., Sarquis, M.I., Mitchell, D.A., Ramos, L.P., et al. Effect of nitrogen and carbon sources on lipase production by *Penicillium aurantiogriseum*. *Food Technol. Biotechnol.*, **41**, 105-110 (2003).
- 18) Alabdallal, A.H., Alanazi, N.A., Aldakeel, S.A., AbdulAzeez, S., Borgio, J.F. Molecular, physiological, and biochemical characterization of extracellular lipase production by *Aspergillus niger* using submerged fermentation. *PeerJ*, **8**, e9425 (2020). doi: 10.7717/peerj.9425
- 19) de Almeida, A.F., Taulk-Tornisiello, S.M., Carmona, E.C. Influence of carbon and nitrogen sources on lipase production by a newly isolated *Candida viswanathii* strain. *Ann. Microbiol.*, **63**, 1225-1234 (2013).
- 20) Mendes, D.B., Silva, F.D., Guarda, P.M., Almeida, A.F., Oliveira, D.D., et al. Lipolytic enzymes with hydrolytic and esterification activities produced by filamentous fungi isolated from decomposition leaves in an aquatic environment. *Enzyme Res.*, **2019**, 8182425 (2019).
- 21) Dolui, A.K., Vijayaraj, P. Functional omics identifies serine hydrolases that mobilize storage lipids during rice seed germination. *Plant Physiol.*, **184**, 693-708 (2020). doi: 10.1104/pp.20.00268
- 22) Barros, M., Fleuri, L.F., Macedo, G.A. Seed lipases: sources, applications and properties-a review. *Braz. J. Chem. Eng.*, **27**, 15-29 (2010). doi: 10.1590/S0104-66322010000100002

- 23) Kelly, A.A., Quettier, A.L., Shaw, E., Eastmond, P.J. Seed storage oil mobilization is important but not essential for germination or seedling establishment in *Arabidopsis*. *Plant Physiol.*, **157**, 866-875 (2011). doi: 10.1104/pp.111.181784

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