

**PROJECT REPORT OF MINOR RESEARCH PROJECT**

**ON**

**“A Study of Lipase Enzyme Production and Its Industrial  
Application ”**

**Submitted to UGC, CRO, BHOPAL**



**ज्ञान-विज्ञान विमुक्तये**

**SUBMITTED BY**

**Dr. Bhawana Pandey  
Principal Investigator**

**&**

**Miss Chitra Bhattacharya  
Co-Principal Investigator**

**DEPARTMENT OF BIOTECHNOLOGY & MICROBIOLOGY  
BHILAI MAHILA MAHAVIDYALAYA, HOSPITAL SECTOR, BHILAI  
DISTT. DURG (C.G.)**

## **Objectives:**

Collection of the sample from specific sites.

1. Isolation and Screening of lipase producing fungal and bacterial species.
2. Qualitative Estimation of lipase producing Microbes by plate assay method.
3. Identification of Fungus and Bacteria.
4. Lipase Crude Enzyme Preparation.
5. Qualitative Estimation of Lipolytic Enzyme (Agar well Diffusion Assay of Crude Lipase).
6. Purification of Crude Lipase Enzyme.
7. Study of Lipase Activity.
8. Protein Estimation of Crude and Purified Lipase Extract by Lowry's Method.
9. Quantitative Estimation of Lipase Assay by Titrimetric Method.

Optimization of Lipase Enzyme Activity.

## A Study of Lipase Enzyme Production and Its Industrial Application

### SUMMARY:

Lipases are hydrolytic enzymes that *break* the ester bond of triacylglycerol, free fatty acids and glycerol. In its natural function, lipases can catalyze esterification, interesterification and transesterification reactions in non-aqueous media. Claude Bernard at 1856 was first discovered a lipase in animal pancreatic juice which was able to hydrolyze insoluble oil droplets and converted them into soluble products. Lipase has been traditionally obtained from animal pancreas and is used as a digestive aid for human consumption in crude mixture with other hydrolases. Collection of lipase enzyme is too difficult because of shortage of using animal pancreas to isolate lipase enzyme. So, initial interest was to isolate the lipase from microorganism. In 1901, Sir Eijkman first observed the presence of lipases in *Bacillus prodigiosus*, *B. pyocyaneus* and *B. fluorescens*. Cheetham (1995) reported best lipase producing bacteria were *Serratia marcescens*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. Microbial enzymes are useful because of the variety of catalytic activity, the high possible yields, regular supply due to absence of seasonal fluctuations and rapid growth of microorganisms on inexpensive medium. Microbial enzymes are stable than their corresponding plant and animal enzymes and their production is more convenient and safer. Most enzymes are produced by the fermentation of bio-based materials in industries. Certain microorganisms are the source of choice for lipase productions. Lipase enzymes are currently attracting an enormous attention for their biotechnological potential. Among the lipases sources, the microbial enzymes are preferred due their low cost, high stability in organic solvents (which are mostly used in synthesis reactions), no need for cofactors and large range of pH and temperature stabilities. Lipase-producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, oilseeds, and decaying food, compost heaps, coal tips, and hot springs. Lipase producing microorganisms include bacteria, fungi, yeasts, and actinomyces. Lipase-producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil processing factories, dairies, soil contaminated with oil, oil seeds, and decaying food, compost heaps, coal tips, and hot springs. Microbial lipases have gained special industrial attention due to their stability, selectivity, and broad substrate specificity. Microbial enzymes are also more stable than their corresponding plant and animal enzymes and their

production is more convenient and safer. Many microorganisms are known as potential producers of extracellular lipases, including bacteria, yeast, and fungi. In the present investigation we have isolated total 7 morphologically distinct bacterial strains from oil contaminated soils of different area of Bhilai-Durg region. The isolates were screened by tributyrin agar medium. Three of isolates produced clear zone than others, indicating higher lipase activity. In accordance with the Bergey's manual of systematic bacteriology, the isolates were likely to be belonging to genus *Staphylococcus* whereas we have also isolated same *Staphylococcus* sp., *Bacillus* sp. and *Clostridium* sp. as Bergey's manual system, and through biochemical characterization by Aneja (2003). The initial pH of the growth medium influences the rate of lipase production. It was inferred from the results that the bacteria is capable of producing lipase from the initial pH of medium from pH 4.0 to pH 10.0. In our results have obtained maximum lipase activity was observed at pH-7.0 on 37°C by *Bacillus* sp. (4.22), then *Staphylococcus* sp. (2.32) then after *Clostridium* sp. (1.89). Carbohydrates were screened for their efficiency to support lipase production like Glucose, Fructose, Sucrose, Xylose, Mannitol, Mannose, Arabinose and Dextrose were used as the basal carbon sources in basal medium and was assayed to check for the lipase activity. In the present study we had used only glucose as a carbon source it has been observed that in the presence of glucose medium, maximum growth was obtained in *Bacillus* sp. then *Staphylococcus* sp. then *Clostridium* sp. Optimization was carried out by using different organic nitrogen as nitrogen sources. Different nitrogen source used were Peptone, Beef extract and Yeast Extract, Urea, and Casein were added to the medium and incubated at 37°C for 24 hrs in a rotary shaker. We had used peptone as a nitrogen source. It observed that peptone is a good nitrogen source medium because of *Bacillus* sp. was giving maximum growth yielding as a comparison of *Staphylococcus* sp. and *Clostridium* sp. As well as the lipase production of ground shell medium and ground nut shell with peptone medium was carried out. *Aspergillus flavus* produces green colour, *Aspergillus niger* as black and *Aspergillus fumigatus* produces dark green colonies on the Potato Dextrose Agar plate. *A. flavus*, *A. fumigatus* and *A. niger* is mostly or entirely uniseriate aspergilla, strongly roughened conidia. The lipase enzyme production was carried out in ground nut shell medium in submerged fermentation. The lipase production was monitored at 28°C, 37°C, 50°C, pH 4, 6, 7, 8 and 9.0 is 0.90, 0.74 and 1.15 Unit/ml/min. The lipase production was increased at pH 7.0, 37°C. The maximum lipase production for ground nut shell medium was maintained at temperature 37°C. Lipase production by *A. niger* was in correlation with the growth temperature, whereby 37°C was the optimum temperature for incubation time. Maximum lipase production by pH range (pH

6.0 to pH 7.0). The result showed that pH 7.0 supported both good growth and high lipase production. Minimum lipase production was observed in ground nut shell with peptone medium as a protein source. In this study we used different kinds of solvents like phenol, chloroform, glycerol and acetone for the production of lipase enzyme. The maximum lipase production was observed in phenol solvent of all *Aspergillus* sp. of fungal strains. We have optimized the effect of metal ions like  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ,  $\text{CaCl}_2$ ,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  for the maximum production of lipase it results obtained the maximum lipase production in  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  metal ion. For lipase production as a substrate ground nut shell powder was used which is low cost and best use of agro waste and easily available in local market. As per the above study this process appears useful as it allows the use of widely available agro industrial residues which naturally stimulate natural species of fungi and other microorganisms known to possess high performance and adaptability. It is important to mention that the use of agro-industrial residues as substrates in the production of lipase by solid-state fermentation can significantly reduce the final price of enzyme and also add value to low cost materials on the market. And hence the case study concluded that lipases are versatile enzymes that are used widely and distributed among yeast, fungi and bacteria. Lipases have gained importance to a certain extent especially in the area of organic synthesis, effect of metal ions, lipase inducers, non-specific reversible inhibitors and various industrial applications such as fat and oil processing, oleochemical industry, environment management, food industry, detergents, pulp and paper industry, tea processing, biosensors, diagnostic tools, cosmetic, perfumery and medical application.



For favour of ref. pl. 40/5 30/08/14

Chairman Sir

**UNIVERSITY GRANTS COMMISSION - CENTRAL REGIONAL OFFICE,**

Tawa Complex (Bittan Market), E-5, ARERA COLONY, BHOPAL-462 016  
Ph. : 0755 - 2467418, 2467892, Fax. : 0755 - 2467893, web site : www.ugc.ac.in

F.No. : MS-34/202002/XII/13-14/CRO

Date : \_\_\_\_\_

To  
The Principal,  
Bhilai Mahila Mahavidyalaya  
Bhilai Nagar, Bhilai (C.G.)

Return  
30/08/14

Code : 202002

Sub. : Financial Assistance for undertaking Minor Research Project by Dr. Bhawana Pandey, Asstt. Professor (Chemistry) Bhilai Mahila Mahavidyalaya, Bhilai Nagar, Bhilai (C.G.), in "A Study of Lipase Enzyme Production and Its Industrial Applications"

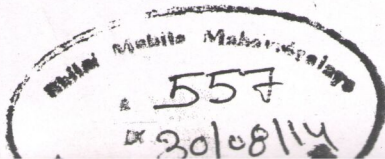
Sir,

The Commission on the recommendations of the Selection Committee has approved the research project entitled in "A Study of Lipase Enzyme Production and Its Industrial Applications." of by Dr. Bhawana Pandey, Asstt. Professor (Chemistry) Bhilai Mahila Mahavidyalaya, Bhilai Nagar, Bhilai (C.G.), and has agreed to provide a grant of Rs.330000/-.

Particular	Allocation	Grant being released
<b>NON RECURRING</b>		
1. Books & Journals	Rs. 50000.00	Rs. 50000.00
2. Equipments	Rs. 100000.00	Rs. 100000.00
<b>RECURRING</b>		
3. Travels, Field work	Rs. 30000.00	Rs. 15000.00
4. Contingency	Rs. 50000.00	Rs. 25000.00
5. Chemical & Glassware	Rs. 100000.00	Rs. 50000.00
6. Special Needs	Rs. 0.00	Rs. 0.00
<b>TOTAL</b>	<b>Rs. 330000.00</b>	<b>Rs. 240000.00</b>

I am directed to convey the sanction of the Commission for Payment of Rs. 240000/- as first installment to The Principal, Bhilai Mahila Mahavidyalaya, Bhilai Nagar, Bhilai (C.G.), under following terms and condition.

1. The effective date of implementation of the Project will be the date of receipt of fund by the institution.
2. The tenure for the Minor Research Project will be 2 years.
3. On receipt of this letter the Principal Investigator must sign and return the Acceptance Certificate as enclosed duly countersigned by the Principal within 3 month of issue of this letter, failing which the approval should stand withdrawn.
4. In case, the grant is not settled within six months from the date of completion of the project, the same will lapse and no representation will be entertained on this behalf and Principal Investigator has to refund the whole grant.
5. Principal Investigator may undertake only one project at a time under UGC funding either by the UGC, H.O., New Delhi or by the C.R.O., Bhopal. The letter of undertaking enclosed may be sent to this office immediately after receiving this sanction. Failure to the submission of this and also in running two parallel projects funded by the UGC (Regional Office/Main Office at New Delhi), the Principal Investigator will be held solely responsible and have to refund the amount as and when it comes to the notice, of the authorities.
6. The College shall maintain proper accounts of the expenditure out of the Grants which shall be utilised only on approved item of expenditure as per detailed in XII Plan Guidelines.





# BHILAI MAHILA MAHAVIDYALAYA

HOSPITAL SECTOR, BHILAI NAGAR (C.G.) 490 009  
Affiliated to Pt. Ravishankar Shukla University, Raipur (C.G.)  
Recognized Under Section 2(f) and 12(B) of the UGC Act 1956

Ph : 0788-2242699  
0788-2210078  
Website : www.bmmbihilai.net

No. BMM / 2016/422

Date : 29/09/16

To

The Education officer,  
U.G.C. (C.R.O-2)  
Tawa Complex, Bittan Market,  
E-S, Arera Colony, Bhopal.

Subject:- Submission of Final Project REPORT.

Ref - F.NO. MRP F.NO. MS-35/202002/XII/13-14/CRO

Respected Sir,

Please find enclosed following documents in reference to F.No: MS-34/202002/XII/13-14/CRO dated 30/8/14 allotted to Dr. Bhawana Pandey as Principal Investigator and Ms. Chitra Bhattacharya, as Co Principal Investigator, on the topic "A Study of Lipase Enzyme Production and Its Industrial Application."

1. Annexure VI -Final Report.
2. Report about work-done till 31.08.2016

This is for your information and necessary action please.

Thanking you,

Yours Sincerely,

Dr. Zehra Hasan  
Principal

Dr. Bhawana Pandey  
Principal Investigator

Ms. Chitra Bhattacharya  
Co-Principal Investigator

College Code- 202002

To,  
The Educational Officer & Incharge  
U.G.C., C.R.O.,  
Tawa Complex (Bittan Market)  
E-5 Arera Colony, Bhopal.

File No. MS- F.No: MS-34/202002/XII/13-14/CRO dated 30/8/14.

Subject: Submission of Final Minor Research Project Report.

Respected Sir/Madam,

With regards, I am thankful to U.G.G., C.R.O., Bhopal for approving my project (MINOR RESEARCH PROJECT) F.No:MS-34/202002/XII/13-14/CRO dated 30/8/14. (letter attached) & releasing first installment of the grant sanctioned (letter attached).

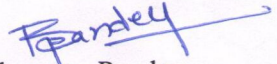
Sir, further it is to top mention that the concerned letter was received in the college and project work started from 1 September 2014.


This is to request you, please sanction second installment of the project as early as possible.

Please find final two copies of summary, hard binded project reports, CD and details of Expenditure statement. Bills and Audit Reports will be submitted within one month. This is for your information and necessary action please.

Thanking you

Yours sincerely

  
Dr. Bhawana Pandey  
Principal Investigator

  
Miss Chitra Bhattacharya  
Co- investigator



To,

The Educational Officer & Incharge  
U.G.C., C.R.O.,  
Tawa Complex (Bittan Market),  
E-5 Areara Colony,  
Bhopal.

File No. MS-34/202002/XII/13-14/CRO dated 30/8/14.

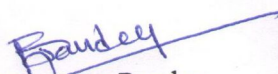
Subject: Submission of Final Minor Research Project Account.

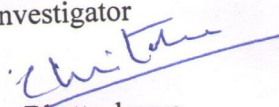
Respected Sir,

We are pleased to submit two copies of final report of the Minor Research Project sanctioned by C.R.O., Bhopal vide the above referred letter on **A Study of Lipase Enzyme Production and Its Industrial Applications**".

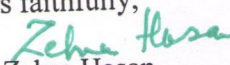
The UGC proforma vide Annexure II, III, V and VI (duly filled), Utilization Certificate, Statement of expenditure incurred on Field work, Certificate of Equipment, Chemicals and Books and Expenditure detail and original bills are also being submitted.

Thanking you

  
Dr. Bhawana Pandey  
Principal Investigator

  
Miss Chitra Bhattacharya  
Co- investigator

Yours faithfully,

  
Dr. Zehra Hasan  
Principal  
Bhilai Mahila Mahavidyalaya

- Enclosure: 1. Annexure II, III, V and VI (duly filled).  
2. Utilization Certificate.  
3. Statement of expenditure incurred on Field work.  
4. Certificate of Equipment, Chemicals and Books.  
5. Expenditure detail and original bills of expenditure.

UNIVERSITY GRANTS COMMISSION  
BAHADUR SHAH ZAFAR MARG  
NEW DELHI – 110 002.

Annual/Final Report of the work done on the Major/Minor Research Project. (Report to be submitted within 6 weeks after completion of each year)

1. Project report No. 1<sup>st</sup>/2<sup>nd</sup>/3<sup>rd</sup>/Final \_\_\_\_\_ 2<sup>nd</sup> \_\_\_\_\_

2. UGC Reference No. \_\_\_ F.No: MS-34/202002/XII/13-14/CRO \_\_\_\_\_

Period of report: from 31/03/2015 to 31/03/2016

3. Title of research project “A Study of Lipase Enzyme Production and Its Industrial Application”

4. (a) Name of the Principal Investigator **Dr. (Mrs.) Bhawana Pandey**

Deptt. and University/College where work has progressed **Bhilai Mahila Mahavidyalaya, Hospital Sector, Bhilai**

(b) Effective date of starting of the project - **01/09/2014**

5. Grant approved and expenditure incurred during the period of the report:

a. Total amount approved Rs. 3,30,000/-

b. Total expenditure Rs. 2,40,000/-

c. Report of the work done: (Please attach a separate sheet)

i. Brief objective of the project **ENCLOSURE 1**

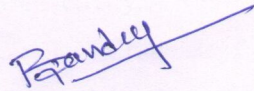
ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication - **ENCLOSURE 2**

iii. Has the progress been according to original plan of work and towards achieving the objective? if not, state reasons: **Yes**

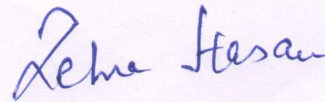
iv. Please indicate the difficulties, if any, experienced in implementing the project **Nil**

- v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet.
- vi. If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of work done may also be sent to the Commission – N.A.
- vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any

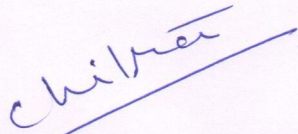
N. A.



SIGNATURE OF THE PRINCIPAL  
INVESTIGATOR



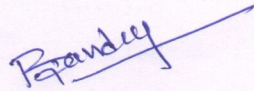
REGISTRAR/PRINCIPAL



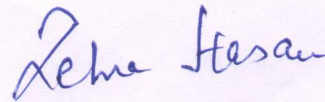
SIGNATURE OF THE CO-INVESTIGATOR

- v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet.
- vi. If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of work done may also be sent to the Commission – N.A.
- vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any

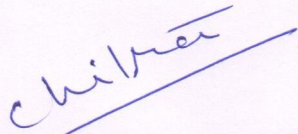
N. A.



SIGNATURE OF THE PRINCIPAL  
INVESTIGATOR



REGISTRAR/PRINCIPAL



SIGNATURE OF THE CO-INVESTIGATOR



# BHILAI MAHILA MAHAVIDYALAYA

HOSPITAL SECTOR, BHILAI NAGAR (C.G.) 490 009

(Managed by Bhilai Education Trust)

(Affiliated to Hemchand Yadav Vishwavidyalaya, Durg)

Recognized Under Section 2(f) and 12(B) of the UGC Act 1956

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0788-2242078

Website : www.bmmbilai.com

No. BMM / 2016/422

Date : 29/09/16

## MINOR RESEARCH PROJECT

MS-34/202002/XII/13-14/CRO Bhopal

### TO WHOM SO EVER IT MAY CONCERN

This is to certify that all the equipments and books which were purchased by Dr. Bhawana Pandey for her Minor Research Project MS-34/202002/XII/13-14/CRO Bhopal, dated 30/08/2014. Titled "A study of Lipase Enzyme production and its industrial application" have been submitted in the college.

Dated- 29.9.16

*Zeina Hasan*

Principal

Bhilai Mahila Mahavidyalaya

**Annexure III**  
**University Grants Commission**  
**Central Regional Office**  
**Bhopal 462016**

**STATEMENT OF EXPENDITURE IN RESPECT OF MINOR RESEARCH  
PROJECT**

1. Name of Principal Investigator: Dr. Bhawana Pandey
2. Department Of PI: Biotechnology  
Name of College: Bhilai Mahila Mahavidyalaya
3. UGC approval Letter No. and Date: **MS-34/202002/XII/13-14/CRO Bhopal**
4. Title of Research Project: A study of Lipase Enzyme production and its industrial application.
5. Effective date of starting the project: 29/11/2014
6. A. Period of Expenditure: from Dec 2014 to August 2016  
B. Details of Expenditure

S.N	Item	Amount Approved	Expenditure
1	Books and Journals	50000.00	50,582/-
2	Equipments	100000.00	101990/-
3	Contingency	25000.00	25026/-
4	Field Work/Travel/(Give detail in performa)	15000.00	20050/-
5	Chemicals and Glasswares	50000.00	49243/-
	Total	2,40,000/-	2,46,891/-

7. If as a results of check or audit objection some irregularity is noticed at later date, action will be taken to refund, adjust or regularize the objected amounts.
8. It is certified that the grant of Rs. 2,40,000/- (Rupees Two Lakh Forty Thousands only) received from the University Grants Commission under the scheme of support of Minor Research Project entitled "A study of Lipase Enzyme production and its industrial application". Vide UGC letter no. **MS-34/202002/XII/13-14/CRO, dated 30/08/2014** has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

*B. Pandey*  
*29.9.16*  
Signature of Principal Investigator

*Reha Hexan*  
Principal

## ISOLATION AND SCREENING OF FUNGAL STRAINS FORM PADDY FIELD SOIL FOR PRODUCTION OF LIPASE

\*CHITRA BHATTACHARYA<sup>1</sup>, ASHIS KUMAR SARKAR<sup>2</sup> AND BHAWANA PANDEY<sup>3</sup>

<sup>1,2</sup> School of Biological & Chemical Sciences,  
MATS University, RAIPUR (C.G.) INDIA

<sup>3</sup> Department of Biotechnology & Microbiology,  
Bhilai Mahila Mahavidyalaya,  
BHILAI (C.G.) INDIA

\*Corresponding Author:  
E-mail. : [chitra16b@gmail.com](mailto:chitra16b@gmail.com)

### ABSTRACT

Three different fungal strains of *Aspergillus* sp. were isolated and screened. These isolates were identified on the basis of morphological and microscopic studies. Among these fungal strains, *Aspergillus fumigatus* given best results for these studies. Olive oil substrates were optimized and maximum lipase activity of 85.51 U/g was observed at pH 7.0. Maximum lipase activity was observed for an incubation period of 72 hrs at 30°C.

Figures: 08

References:08

Table:05

KEY WORDS: *Aspergillus* spp., Lipase enzyme, Olive oil.

### Introduction

Lipases are hydrolytic enzymes that *break* the ester bond of triacylglycerol, free fatty acids and glycerol. In its natural function, lipases can catalyze esterification, interesterification and transesterification reactions in non-aqueous media<sup>2</sup>. Fungi are widely recognized as the best lipase sources and are used preferably for industrial applications. Uses of waste biomaterials for biotechnological products, especially enzymes, have been noticed in the recent years. Solid substrate fermentation (SSF) has built up credibility in recent years for the production of different microbial products including enzymes through inexpensive media. Lipases can be found in animal and vegetable cells. Certain microorganisms are the source of choice for lipase productions. Lipase enzymes are currently attracting an enormous attention for their biotechnological potential. Among the lipases sources, the microbial enzymes are preferred due their low cost, high stability in organic solvents (which are mostly used in synthesis reactions), no need for cofactors and large range of pH and temperature stabilities<sup>4</sup>.

To obtain the microbial enzymes, two processes can be employed: submerged fermentation (SmF) and solid-state fermentation (SSF)<sup>6</sup>. The SSF is the process where substrate given to the microorganism is solid, being moistened by a nutritious solution or a buffer solution. This process appears useful as it allows the use of widely available agro industrial residues which naturally stimulate natural species of fungi and other microorganisms known to possess high performance and adaptability<sup>1</sup>. It is important to mention that the use of agro-industrial residues as substrates in the production of lipase by solid-state fermentation can significantly reduce the final price of enzyme and also add value to low cost materials on the market.

### Material and Methods

**Sample Collection & Isolation of lipase producers:** The soil sample was collected from paddy field located at Dist.-Durg, Chhattisgarh enriched by periodic sub-culturing of sample in Potato Dextrose Agar (PDA) media.

They were aseptically subjected to serial dilutions and plated on Potato Dextrose Agar (PDA) and incubated at 28°C for 72 hrs. The pH of medium was adjusted by 1N NaOH/1N HCl. After incubation predominant fungal colonies were isolated and screened for lipase activity and then subjected to morphological examinations.

#### **Qualitative Estimation of Lipase Production**

The fungus is producing lipase or not, a tributyrin agar medium plate was prepared and point inoculated with respective fungi. A clear zone was observed around the fungal colony due to the hydrolysis of fat, which showed extracellular lipase producing potential by the fungal colony.

#### **Identification of lipase producing fungi**

Lipase producing fungi was further point inoculated on PDA slants and incubated in incubator for 72 hours at 30°C. An isolated fungus was identified in the basis of morphological and Physiological characters (Lacto-phenol cotton blue stain).

#### **Substrate Selection**

The Agriculture supplement (groundnut shell) was collected from local market. The groundnut shell was dried for 10 days and grind well to make powder. The groundnut samples were used as powder for the production of lipase<sup>7</sup>.

#### **Screening for extracellular lipase production**

Modified basal salt medium (BSM) containing (g/l): NH<sub>4</sub>NO<sub>3</sub>, 1; K<sub>2</sub>HPO<sub>4</sub>, 1.5; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.025; CaCl<sub>2</sub>, 0.025; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.015; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.005, distilled water; 1000 ml and pH adjust to 7.0. After sterilization of medium streptomycin, 250 mg and 1% (v/v) sterilized olive oil was added<sup>5,7</sup>. One ml of the spore suspension was transferred to 20 ml of pervious medium. Inoculated flasks were incubated at 30°C under shaking (150 rpm) for 6 days, and then filtrate was centrifuged under cooling at 10000 x g for 30 min at 4°C, and the supernatants was subsequently collected for lipase activity determination.

#### **Optimization of Lipase Production at Different Sources:**

**Effect of Different Temperature on Lipase Activity:** To ascertain the optimum temperature for the enzyme activity, the assay mixture was incubated in the temperature range of 10°C, 15°C, 20°C, 25°C, 30°C, 35°C and 40°C.

**Effect of Different pH on Lipase Activity:** For determining the effect of different pH on lipase activity, the enzyme was incubated with different pH (4.0, 5.0, 6.0, 7.0, 8.0 and 9.0) for 30 min at 35°C under standard assay conditions.

**Effect of Metal Ions on Lipase Activity:** For determining the effect of different metal ions on lipase activity, the enzyme was incubated with different metal ions (concentration 50µl/mg each). CU<sup>+2</sup> (CUSO<sub>4</sub>), Ca<sup>+2</sup> (CaCl<sub>2</sub>·6H<sub>2</sub>O), (MgCl<sub>2</sub>·6H<sub>2</sub>O) for 30 min at 35°C under standard assay conditions.

**Effect of Solvents on Lipase Activity:** The effect of different solvents on lipase activity was determine by incubating the assay mixture with acetone, chloroform, glycerol, phenol (concentration 50µl/ml each) individually for 30 min at 35°C under standard assay conditions.

**Estimation of Lipase activity:** For lipase production, *Aspergillus* spp. was grown in modified PDA medium containing 1.5% olive oil (broth). The cultivation of fungi was carried out at 35°C for 72 h in shake flasks each containing 50 ml of the above mentioned medium. The fungal culture was filtered through muslin cloth. Centrifuged the filtrate at 10,000 rpm at 4 °C for 10 min to obtain supernatant. The extracellular lipase activity was measured by Spectrophotometric procedure using p-nitrophenylacetate. Freshly prepared 1.2 ml of p-nitrophenylacetate solution was incubated in a water bath at 37°C for 10 min. After 10 min, 0.5ml of crude enzyme sample was added and the reaction mixture was further keep alive at 37°C in a water bath for 30 min. The formation of yellow colour indicate the lipase activity. To terminate the reaction, 0.1ml of 100 mM CaCl<sub>2</sub>·2H<sub>2</sub>O was added to the solution. The absorbance of yellow colour was calculated at 410 nm against a control. (Mahmoud *et al.*, 2015)



### Result and Discussion

In the present study *Aspergillus* spp. are the isolated from agricultural (Paddy) soil. The lipase production in submerged fermentation of ground shell medium and ground nut shell with peptone medium was carried out. The *Aspergillus* spp. produced significant quantities of enzyme when grown in synthetic oil based medium under fermentation. The lipase enzyme production was carried out in

ground nut shell medium in submerged fermentation. The lipase production was monitored at 35°C and pH 7.0 in between range of 70-85 U/g. The result showed that substrates such as Ground nut shell medium supported the good growth and high lipase production. The total protein content was monitored on ground nut shell medium was 85.47 and ground nut shell with peptone medium was 60.0<sup>3</sup>.

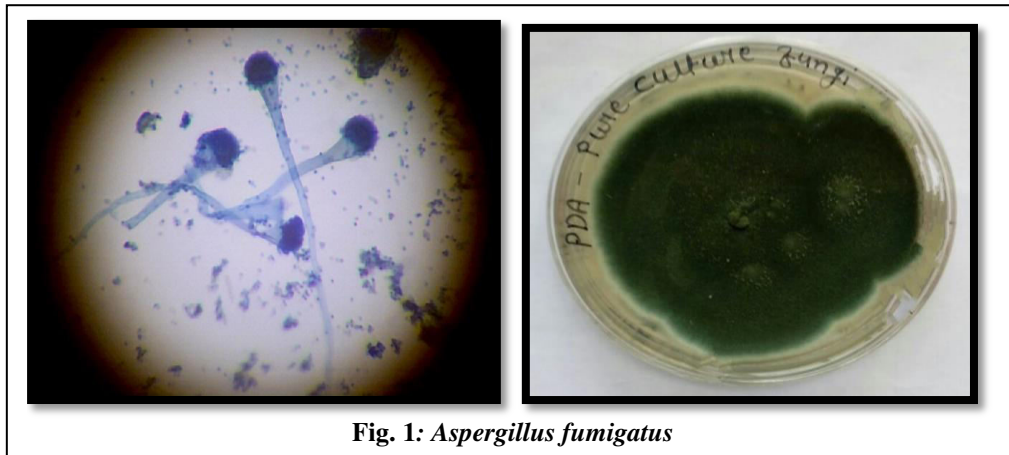


Fig. 1: *Aspergillus fumigatus*

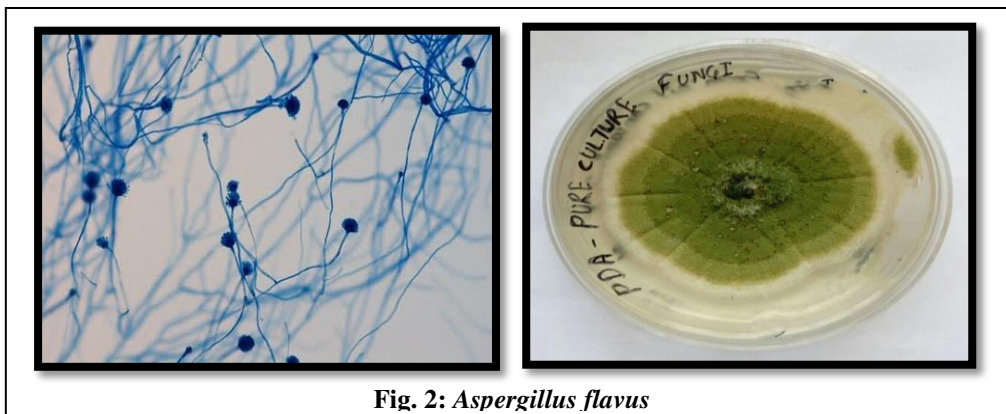


Fig. 2: *Aspergillus flavus*

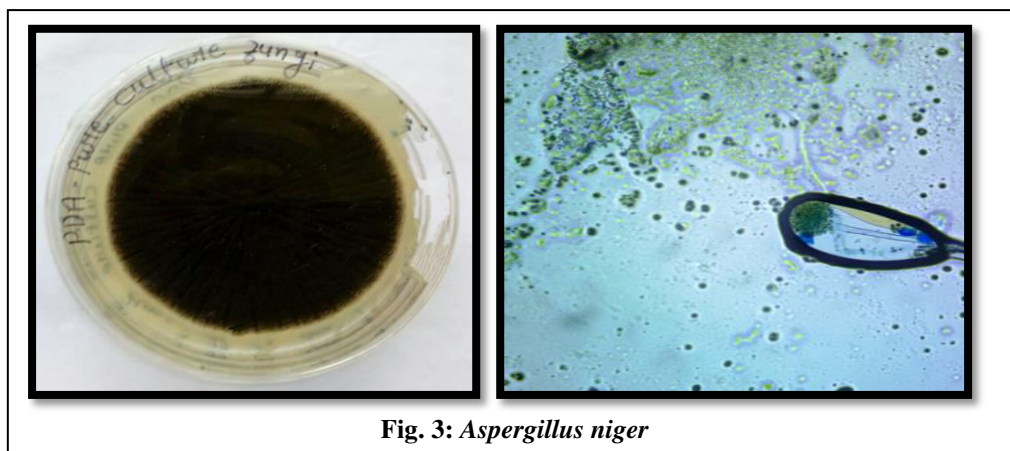
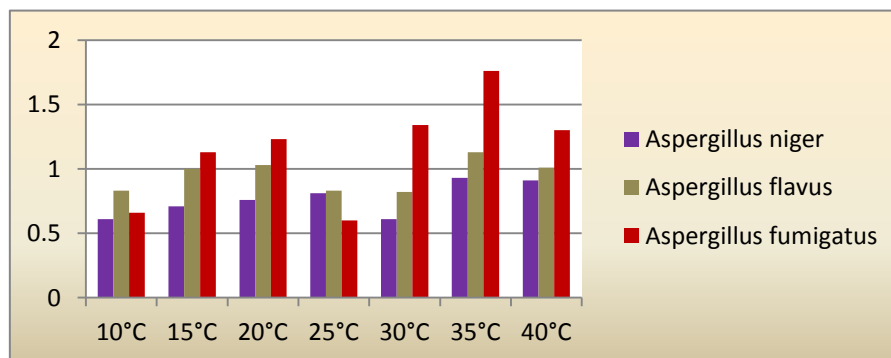


Fig. 3: *Aspergillus niger*

**TABLE-1: Effect of different temp. on lipase activity determine by colorimetric method**

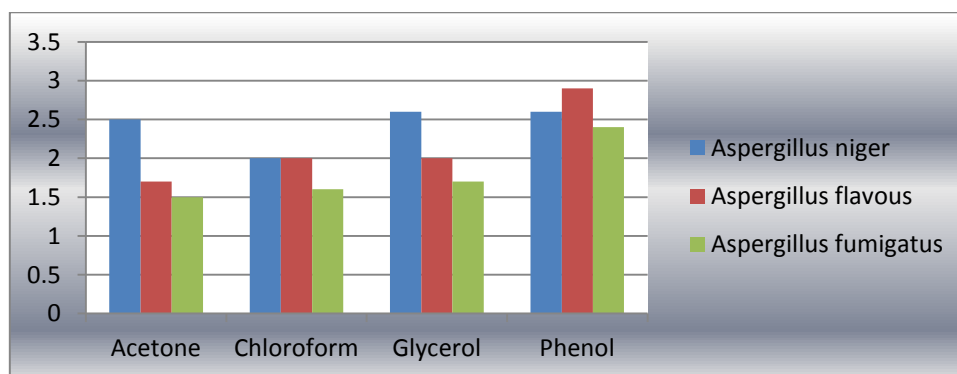
Name of Lipase Producing Fungi	10° C	15° C	20° C	25° C	30° C	35° C	40° C
<i>Aspergillus niger</i>	0.61	0.71	0.76	0.81	0.61	0.93	0.91
<i>Aspergillus flavus</i>	0.83	1.00	1.03	0.83	0.82	1.13	1.01
<i>Aspergillus fumigatus</i>	0.66	1.13	1.23	0.60	1.34	1.76	1.30



**FIG. 4: Effect of different temp. on lipase activity determine by Colorimetric method**

**TABLE-2: Effect of different solvent on lipase activity by colorimetric method**

Name of Lipase Producing fungi	Acetone	Chloroform	Glycerol	Phenol
<i>Aspergillus niger</i>	2.5	2.0	2.6	2.6
<i>Aspergillus flavous</i>	1.7	2.0	2.0	2.9
<i>Aspergillus fumigatus</i>	1.5	1.6	1.7	2.4



**Fig. 5: Effect of different solvent on lipase activity by colorimetric method.**

**TABLE-3: Effect of metal ions on lipase activity by colorimetric method.**

Name of Lipase Producing fungi	CaCl <sub>2</sub> .6H <sub>2</sub> O	CuSO <sub>4</sub> .5H <sub>2</sub> O	MgCl <sub>2</sub> .6H <sub>2</sub> O
<i>Aspergillus niger</i>	1.05	1.01	1.10
<i>Aspergillus flavous</i>	1.11	0.09	1.05
<i>Aspergillus fumigatus</i>	1.51	1.11	1.03

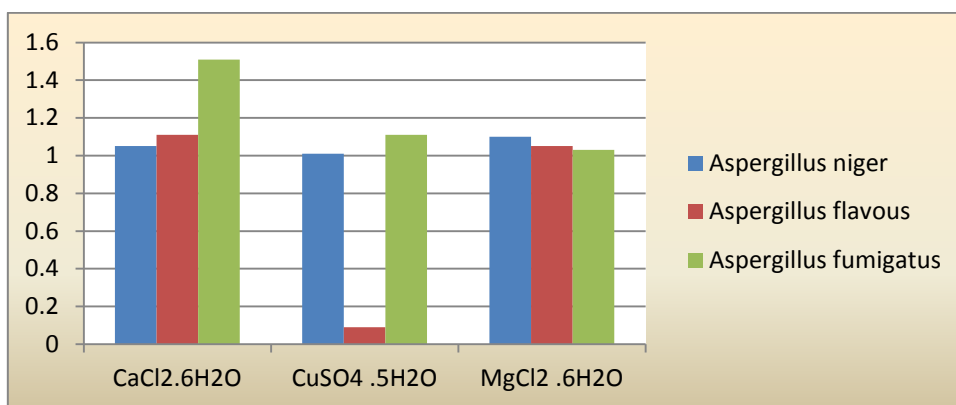


Fig. 6: Effect of metal ions on lipase activity by colorimetric method

TABLE-4: Effect of different pH on lipase activity by Colorimetric method.

Name of Lipase Producing fungi	4.0	5.0	6.0	7.0	8.0	9.0
<i>Aspergillus niger</i>	0.69	1.21	1.49	1.91	1.44	1.11
<i>Aspergillus flavous</i>	0.82	1.01	1.01	2.12	1.72	0.12
<i>Aspergillus fumigatus</i>	0.12	1.10	1.61	1.87	1.94	1.21

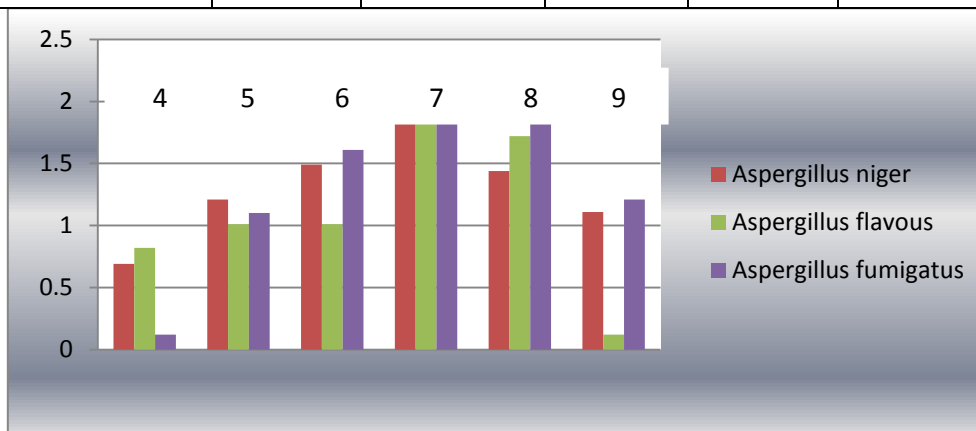


Fig.7: Effect of different pH on lipase activity by Colorimetric method.

TABLE-5: Lipase Assay

Name of Lipase Producing fungi	Lipase Activity (U/g)
<i>Aspergillus niger</i>	72.05
<i>Aspergillus flavous</i>	71.11
<i>Aspergillus fumigatus</i>	85.51

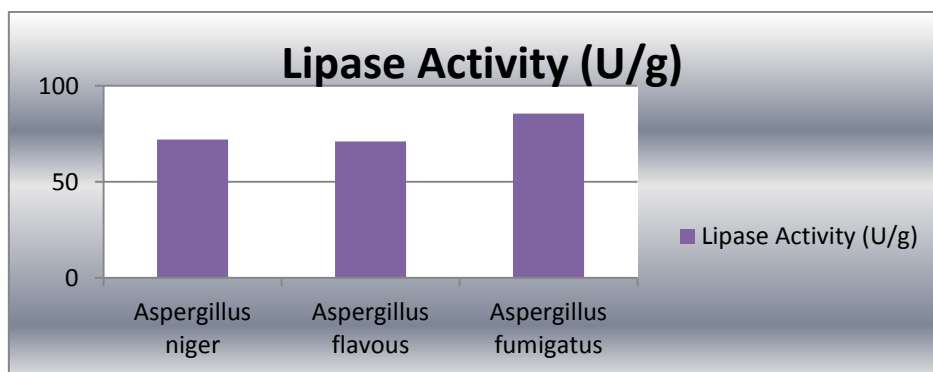


Fig. 8: Lipase Assay

In this study we used different kinds of solvents like phenol, chloroform, glycerol and acetone for the production of lipase enzyme. The maximum lipase production was observed in phenol solvent of all *Aspergillus* spp. of fungal strains. We have optimized the effect of metal ions like  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ,  $\text{CaCl}_2$ ,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  for the maximum production of lipase it results obtained the maximum lipase production in  $\text{CaCl}_2$ ,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  metal ion.

### Conclusion

In present study the results presents that *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus fumigatus* isolates from Agricultural (Paddy) soils can be potentially used in lipase production and optimum lipase activity are shown in the substrate of olive oil.

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