“OIL SEEDS AND BY PRODUCTS AS PER IOPEPC”

An Industrial Training Report submitted

For the partial fulfilment of the Degree of Master of Science by

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[M.Sc. (Biotechnology), Semester IV]

Under the supervision of

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2022-2023

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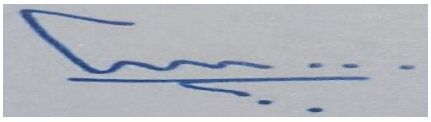
CERTIFICATE

This is to certify that this training report entitled **"OIL SEEDS AND BY PRODUCTS AS PER IOPEPC"** was successfully carried out by Mr. Radadiya Rahul Ashvinbhai towards the partial fulfillment of requirements for the degree of Master of Science in Biotechnology of Atmiya University, Rajkot. It is an authentic record of his/her own work, carried out by him/her under the guidance of Name of Supervisor for a period of three months during the academic year 2022–23. The content of this manuscript, in full or in parts, has not been submitted for the award of any other degree or certificate in this or any other university.



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Food, Water, Milk & it's Products Testing

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Date: 12/04/2023

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This is to certify that **Mr.Rahul Ashvinbhai Radadiya** (enrollment no - 210621035), 4th semester student of Atmiya university, has successfully completed his training in **THE EQUITY LABORATORIES**, Rajkot upto 11th Jan 2023 to 12th April 2023

During this period we found him sincere and hard working, Proved skills and Qualification successfully with regard to task assigned to him.

This certificate was issued to his upon him own request to be submitted To Whom It May Concern without any liabilities towards others.

We wish good luck for his future endeavours.

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DECLARATION

I hereby declare that the work incorporated in the present internship report entitled **"Oil seeds and By products as per IOPEPC**"ismyownworkandisoriginal. This work (in part or in full) has not been submitted to any University for the award of any Degree or aDiploma.

Date: 12/04/2023

Rahul A.Radadiya

ACKNOWLEDGEMENT

Thanks God, to the merciful and the passionate, for providing us the opportunity to step in the excellent world of science. To be able to step strong and smooth in this way, we have also been supported and supervised by many people to would like to express our deepest gratitude. The work was financially supported by **The Equity Laboratory-Rajkot**. The laboratory work was done in the microbiological testing laboratory. After thanking God, who gave us the power to finish this work, we take this opportunity to express our sincere gratitude to **Mr. Divyesh Marviya** and his myriad contributes for our work and for patience, motivation, enthusiasm, and immense knowledge. His focused guidance helps us during the writing of this project. I also like to thank to our university authorities and **Dr. Ragini Raghav** Head of Biotechnology department. I am also very grateful to all faculty members, Department of Biotechnology for their encouragement and support throughout the period of the study. Finally, I consider this as an opportunity to express my gratitude to all dignitaries who have been involved in successful completion of our project work.

Date:12/04/2023 Rahul A.Radadiya

LIST OF PRACTICES

LIST OF ABBREVIATION

IOPEPC	Indian Oilseed and Produce Export Promotion Council
TPC	Total Plate Count
PCA	Plate Count Agar
CYGA	Chloramphenicol Yeast Glucose Agar
PDA	Potato Dextrose Agar
IS	Indian Standard
D/W	Distilled Water
RS-1	Reference Stock-1
SCDM	Soyabean casein digest medium
N-Saline	Normal Saline
XLD	Xylose lysine decarboxylase
CFU	Colony forming unit
TNTC	Too numerous to count

LIST OF FIGURES

ABSTRACT

Food stuffs and Animal feeds are the great source of nutrition for microbes.It is important to check quality of food stuffs, animal feeds and water before consumption Because It has been provide opportunity to growth of pathogens and cause diseases in humans and animals. Total plates counts method and Yeast and mould detection and Enumeration method is used to determine Total number of microorganisms Which are present in the food stuffs and animal feeds By using IS:5887 (Part -3) Detection of *salmonella* spp. is done in to food stuffs and animals feeds. Because *salmonella* is the harmfull pathogen to cause sevier disease in the humans.As per IS 13428:2005 Detection of *pseudomonas* spp. into the water. *Pseudomonas* is the organism which are commonly found in water. It is also pathogen and harmful for humans and animals.By using IS 5403:1999 Detection of *A.niger* in the food stuffs and animal feeds . *A.niger* is one type of black mould and it is cause pathogenicity . It is found in penuts,onions and citrus fruit etc….Black mould is highly pathogenic for cause sevier disease in to plant ,humans and animals.

INTRODUCTION

- **NAME OF ORGANIZATION –** THE EQUITY LABORATORY
- **NATURE OF BUSINESS:**

THE EQUITY

1. Analytical Testing ,
2. Research & Development ,
3. Training ,
4. Services Provider.

- **Year of Established –** August 2021
- **Total number of Personnel –** 08
- **Type of Testing Services :**
    1. Microbiological Testing Of Foods ,
    2. Food & Agriculture Commodities Testing ,
    3. Water And Soil Testing For Agricultural Work.
    4. Snacks & Namkeen Testing

Introduction

- Oilseeds are generally processed (90% of soybeans and 87% of other oilseeds) into protein meal, almost entirely used for feed, and into vegetable oil for food, and biodiesel uses.
- Soybean production and exports are dominated by two countries: Brazil and the United States.

Introduction

- Indian Oilseed and Produce Export Promotion Council (IOPEPC) is concerned with the promotion of various Oilseeds and Oils.
- The formation of IOPEA was, in fact, the first organized effort to promote and protect the interests of India's export trade in commodities like Oilseeds, Vegetable Oils and Oilcakes in a collective and concerted manner through a representative body.

Method For Yeast & Mould Count From Peanut Sample.

Aim: Enumeration of Yeast and Mould .

Introduction:

Foods are generally sources of different types of nutrients, generally foods contain carbohydrates, fats, proteins, water, minerals and vitamins etc. Because of both yeast and mould cause various degrees of deterioration and decomposition of foods, that's why We need to detection of yeast and mould from the food sample is important. If foods are infected withpathogenic fungi like *Candida* Spp and*Aspergillus* Spp.and. It may cause serious diseases in consumerbody.

Principle:

Preparationofpouredplatesusingaspecifiedselectiveculturemediumandaspecified quantityofthetestsampleiftheinitialproductisliquid,orofaninitialsuspensioninthecaseofother products. Preparation of other plates, under the same conditions, using decimal dilutions of the test sample or -of the initial suspension. Aerobic incubation of the plates at 25°C for 3, 4 or 5 days. Calculation of the number of yeasts and moulds per gram or per milliliter of sample from the number of colonies obtained on plates chosen at dilution levels so as to give a significantresult.

Materials:

1. Peanut sample
2. Instruments: Autoclave, Laminar air flow,Incubator.
3. Mediaandreagents:PDA(Potatodextroseagar),Agar–agarpowder,Isopropylalcohol 70%, Distilledwater.
4. Glassware: Test tubes, Flask, Spreader, Petridish

Media preparation:

- Weigh 3.9gm **PDA media** and 2 gm agar powder
- Autoclaved the media at 121˚C and 15 lbs pressure for 20minutes
- Then pour the media in Petri dish under laminar air flow

Sample preparation:

- Make 10 fold dilution[10^{-1} to 10^{-5}]

- First start the dilution with 9 ml water + 1mlsample
- If sample is solid that take 1 gmsample
- Performed serial dilution till 10^{-5}dilutio

Procedure:

- Take 0.1ml sample from 10^{-1} tube and spread over a 10^{-1} PDAplate
- Same procedure will perform from 10^{-1} to 10^{-5} dilution, and spread over the respective PDAplates
- Incubation at **25˚C for 3, 4, 5 days**
- Observe the result and go forcalculation.

Calculation: Count those plate only in which colonies less than 150 number.

SET-1	SET-2
D1-TNTC (Too numerous to count)	D1-TNTC (Too numerous to count)
D2-58	D2-43
D3-7	D3-0
D4-0	D4-0
D5-0	D5-0

- ThenumberofYeastandMouldpergmorpermlisequalto =$\Sigma c/[n_1+0.1(n_2)]d$

  Σc= the sum of the colonies counted on all theplates.

  N_1= the number of plate counted in the firstdilution.

  N_2= the number of plates counted in the seconddilution

  d= the dilution from which the first counts wereobtain.

  $= \underline{\Sigma C}[N1+0.1(N_2)]\ d$

  $= 108 / 2+0.1\ (2) \times 10^{-2}$

  $= 108 / 0.022$

= 4,909.09090

= 4.909×10^3 CFU / ml

Observation:

Figure 1 Yeast And Mould 10x-1 dilution. 2. 10x-2 dilution 3. 10x-3 dilution 4. 10x-4 dilution

Result:

By performing this experiment the value of yeast and mould enumeration to be found is 4.909×10^3 CFU/ ml.

Conclusion:

Given food sample not consumable.

NOTE- Consumable range of yeast and mould in milk and milk products is 10 CFU / ml.

Method for Enumeration of Total Bacterial Count

Aim: Enumeration of Total Plate Count Method.

Introduction:

Oil seeds is highly susceptible to contaminated with various type of microorganisms along withthisOther seedsalsocontaindifferenttypesofnormalfloralike*E.Coli.*It is important to determine the numberofmicroorganismwhichispresentintheSeeds,forthispurposeweareuseatotalplatecount method.

Principle:

Two poured plates are prepared using a specified culture medium and a specified quantity of the test sample, if the initial product is liquid, or using a specified quantity of an initial suspension in the case of other products. Other pairs of poured plates are prepared, under the same conditions, using decimal dilutions of the test sample or of the initial suspension. The plates are aerobically incubated at 30 °C for 72 hrs. The number of microorganisms per milliliter or per gram of sample is calculated from the number of colonies obtained on selected plates.

Materials:

1. Sample(Peanut) ,
2. Plate count agar[PCA],
3. Spreader,
4. Laminar air flow,
5. Glasswares,
6. Isopropyl alcohol –Disinfectant,
7. Test Tubes forDilution,
8. DistilledWater.

Media Preparation:

- Weigh 3gm PCA (plate count agar) media for100ml
- Add 2gm agarpowder in 100 ml D/W water. Add 100 ml water and mixit
- Autoclaved the media at 121˚C and 15 lbs pressure for 20minutes
- Then pour the media in Petri dish under laminar air flow

Sample Preparation:

- Take 10 gm Sample Add in 90 ml Sterile Distilled Water, [10^{-1}]
- Mixed the sample

Procedure:

- After serial dilution take 0.1ml sample from 1:10 tube and spread into 10^{-1} PCA mediaplate.
- Again take 0.1ml sample from 10^{-2} tube and spread into 10^{-2} PCA agarplate
- Same procedure is done till 10^{-5}dilution.
- Incubate plate at 30˚C for 72 Hrs.
- Observed the result and then go for calculation.

Serial Dilution image:

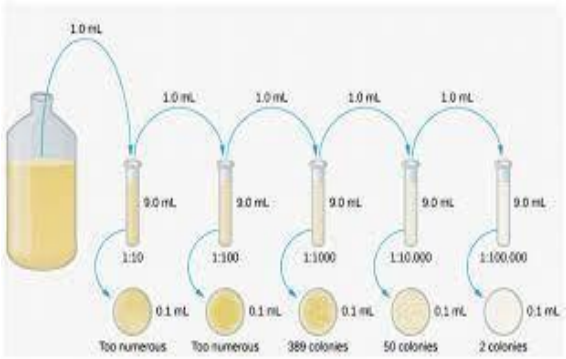

Figure 2Ten fold serial dilution

Calculation:

Count those plate only in which colonies between 15 to 300 numbers.

SET-1	SET-2
D1-TNTC (Too numerous to count)	D1-TNTC (Too numerous to count)
D2-219	D2-228
D3-17	D3-26
D4-4	D4-0
D5-0	D5-0

ThenumberofYeastandMouldpergmorpermlisequalto=Σc/[N_1+0.1(N_2)]d

Σc= the sum of the colonies counted on all theplates.

n_1= the number of plate counted in the firstdilution.

n_2= the number of plates counted in the seconddilution

d= the dilution from which the first counts wereobtain.

= Σc / [n1+0.1(n2)] d

= 490/ 2+0.1 (2) ×10 -2

= 490 /0.022

=22,272.727

= 2.2272 × 10^4 CFU / ml.

Observation :

Figure 32 Yeast And Mould 10x-1 dilution. 2. 10x-2 dilution 3. 10x-3 dilution 4. 10x-4 dilution

Result:

By performing this experiment the value of Bacterial enumeration to be found is 2.2272 × 10^4CFU / ml.

Conclusion:

As per Total bacterial counts sample should not consumable .

Isolation of *Salmonella* Spp.from the Peanut Seeds

Aim: Detection of *Salmonella* Spp.from the Peanut Seeds.

Introduction:

- Salmonella infection (salmonellosis) is a common bacterial disease that affects the intestinal tract. Salmonella bacteria typically live in animal and human intestines and are shed through feces. Humans become infected most frequently through contaminated water or food.

Classification:

Domain: Bacteria

Phylum: Pseudomonadota

Class: Gammaproteobacteria

Order: Enterobacterales

Family: Enterobacteriaceae

Genus: *Salmonella*

Species: *Salmonella Typhimurium*

Principle:

- This method is used to detect *Salmonella* spp. In sample.
- Salmonella belong to Enterobacteriaceae family.
- Their principal habitat is the intestinal tract of humans and other animals.

Characteristics:

- Rod shaped
- Gram negative, Facultative anaerobic bacteria

Materials:

- Buffered Peptone Water

- RV medium(Rappaport-Vassiliadis)
- Selenite / Cystine broth
- Xylse lysine decarboxylase agar
- Bismuth Sulphate AgarN-Agar

Procedure:

- Take 25 gm Peanut Seedssample
- Add in 225 ml 0.1% Buffered peptonewater
- Keep it 37 ºC for 24 hrs inincubator
- Prepare 100 ml Selenite/Cystine broth & 10ml Rappaport-Vassiliadis Medium and inoculated the sample 10 ml & 0.1ml in respective broth
- Incubate the broth at 37 ºC for 24hrs & 42 ºC for 24 hrs Respectively ,
- Prepared the XLD agar ,
- Streak out of plates from inoculated broth ,
- Incubate at 37 ºC for 24-48hrs,
- Observed the plates if obtained Black color colony than performed further confirmation test,
- Prepared Nutrient agar,
- Streak out of plates from inoculated media
- Incubate 37 ºC for 18-24hrs

Confirmation test: Bio-chemical .

- L-Lysine decarboxylase medium: Inoculate a colony just below the surface of the liquid L-!ysine decarboxylase medium . Overlay the medium afterwards with sterile liquid paraffih or oil. Place in an incubator at 36º C ± 2º C for 24 h
- Lactose/glucose fermentation and hydrogen sulfide formation: Streak a colony and stab the butt into iron/two-sugar agar . Place in an incubator at 36 ºC ± 2 ºC for 24 h.

Observation:

- L-Lysine decarboxylase test: Typical Salmonella cultures show a purple colour.Lactose/glucose fermentation and hydrogen sulfide formation: Typical Salmonella cultures show red slants with gas formation and yellows butts with blackening of the agar

Figure 4 Whole Salmonella spp. results

Result: Salmonella is Absent in Peanut Seeds .

Conclusion: So here the Peanut is safe for human consumption.

Method fordetection of *Escherichia Coli* & *Coliforms* bacteria

Aim: Detection ofbacteriafrom CashewaccordingtoIndian Standard.

Introduction:

To enumerate *E.coli* and *coliforms* present in sample. *Coliform* are found in the soil, surface water & human and animal waste whereas *E.coli* are found in human intestine.

Classification:

Domain: Bacteria

Phylum:Pseudomonadota

Class: Gammaproteobacteria

Order: Enterobacterales

Family: Enterobacteriaceae

Genus: *Escherichia*

Species*: Escherichia Coli*

Principle:

On EMB if *E. coli* is grown it will give a distinctive metallic green sheen (due to the metachromatic properties of the dyes, *E. coli* movement using flagella, and strong acid end-products of fermentation). Some species of Citrobacter and Enterobacter will also react this way to EMB.The *coliform* group consists of several genera of bacteria belonging to the family Enterobacteriaceae. The historical definition of this group has been based on the method used for detection i.e. lactose fermentation. This group is defined as all aerobic and facultative anaerobic, gram-negative, non-spore-forming rod shaped bacteria that ferment lactose with gas and acid formation within 48 hour at 35°C . Examination of foods, ingredients and raw materials, for the presence of marker groups such as *coliforms* is the one of the common tests.

Materials:

- Cashew Sample
- Eosin methylene blue & MacConkey's agar

- Petri-plates
- Test Tube

MediaPreparation:

- MacConkey's broth
- Eosin methylene blue agar
- Tergitol -7 agar
- MacConkey agar

Procedure

- Take 200 ml peptone water and add 25 gram sample
- Take 10 ml of Mac-Conkey broth add 1 ml of sample Incubate at 37°C for 24 hrs.
- Streak on EMB or Tergitol-7 agar or Mac-Conkey agar Incubate at 37°C for 24 hrs.

Observation:

EMB: dark colonies with green metallic sheen

MaCconkey's: Light Pink colonies

Figure 5Bio-chemical results

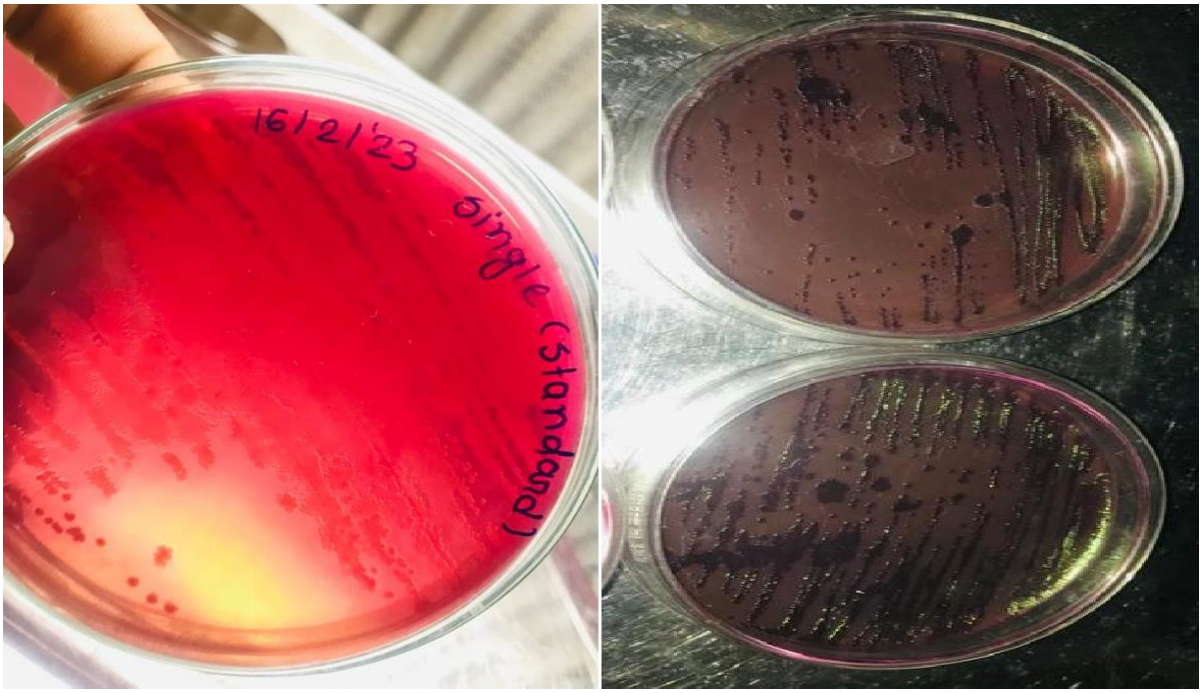

Figure 6E.Coli on MacConkey's & EMB Plates Respectively

Results: *E.Coli*&*Coliforms* are Presents in Peanut sample**.**

Conclusion: The Peanut is not safe for human or animal consumption

Preparation of Glycerol Stock

Aim: To Maintain the Organism via glycerol solutions.

Principle:

A Glycerol stock is type of suspension used to store bacterial cultures over long periods of time in laboratory settings.When liquid bacterial culture is added to 50% glycerol solution,the glycerol enters the bacterial cells,rendering them structurally stable and allowing them to be stored safely.

Requirements:

- 0.86% Normal Saline,
- Glycerol solution,
- Double distilled water,
- Screw cap bottle,
- Cryophills vials,
- Petri plates,
- Safety Items,

Procedure:

- **Preparation of Master Stock**
  - Take 5 ml Normal saline,
  - 1gm bacterial powder added into 10ml flask and mix it,
  - Transfer 1ml suspension to 4 cryophills vials and add 1ml 80% glycerol solution and mix it,
  - Master stock preserve in Deep Freezer for 1-2 year.
- **Preparation of Reference Stock**
  - Take 1ml solution from N-saline + bacterial powder suspension and add into soya bean casein digest medium,
  - Incubate at 37˚c for 24 hrs,
  - After incubation observe growth in SCDM,
  - Make 12 cryophils vials in this add 1ml solution from SCDM broth + 1ml 80% glycerol to each tube,
  - Reference stock RS-1 vilas time duration is 1 month,

- **Preparation of Working Stock**
    - Take loop full culture from reference stock (RS-1) vials,Streak into respective agar according to selected organism,If take broth (biochemical) then transfer 0.1ml culture from RS-1 vials and also do Gram staining,
    - Incubate at 37°c and observe result,
    - Same procedure repeat with RS 2 to 12 vials,
    - Make 4 nutrient agar slant,
    - Streak into 4 nutrient slant from RS 1 vials,
    - Incubate at 37° c,
    - Observe growth and store culture in refrigerator and use as a standard when pathogen are run,
    - Working stock time duration is 1 week,

REFERENCES

1. IS 5403:1999 -- Yeast and mould count of foodstuffs ,
2. IS 5402: 2012 -- Total plate count(TPC) ,
3. IS 5887: (Part – III) – 1999 –Detection of *Salmonella* Spp,
4. IS 5887Part–I) – 1976 –Method for Detection and Enumeration of bacteria Responsible for food Poisoning,
5. https://images.app.goo.gl/48KRFiuY