# Learning and training programs landt

Profession, Student

The learning and training program are providing a great opportunity to students learn and observe how to work? Mmanagement skills and deepbroad thinking on the basis of learning and training eventexperience, learning and training program are provide a surroundingenvironment to the student that they move toward experimental scientific understanding , using scientific words and development of scientific interpretation reasoning. In research how student think about science and use their idea concept and shape manipulate the biological/physical /chemical word for usefull purpose. their beneficial use. It is a great time for reassuring encouraging student by the surrounding environment which serve provide by learning and training programs. objective give a boost to mind for better resoning new thinking, hypothesis and linked conjunctive research to find new way alternative learning , training program provide a student to that they immerse engage in work , it also provide Research questioning , experiment designing and executing experiment, and analyzing data.

Argument and conclusion in programs their investigation are for better understanding scientific concept . It also develop deep scientific understanding and to promote their self work on their thinking. It also develop understanding the nature of science, working with Team, familiar with lab equipment and material, asking appropriate questions, also have a great reflection of other colleagues and guide, which also develop sophisticated practical skills using laboratory tools and procedure, analyzing data verify test and evaluate. Leraning and traning programs L&T programs provide a movment opportunity tofor student to interact with the people and

lab equipment utensils. which develops thinking ability. Leraning and traning L&T program work as a catalyst for a new practice researcher.

It was a great period of time, it was my first experience working with scientists and expertise in R&D department, to see how they work, how they react and think. My PI provide me a great chance to develop my mental and thinking strength by their motivational speech and set a opportunity in the form of training program.

The training program starts from 18/06/2018 to 29/06/2018 the main objective of training are based on project requirements.

1. :- sampling plan soil and rhizosphere, nodules, identify the angiosperm plant which forms the nodules by microbial interaction.
2. :-microbiology technique
3. :-quadrat formation and data analysis

The training program was assets by Dr. Arun jugran my PI in the supervision under co-pi Dr. Anita pandey. it was a good experience . on day first 18/06/2018 meet with Dr. Anit pandey a little disuses about myself and the project objective and goal, discus main key point of project how to evaluate.

## Plan suggest by dr. Anita pandey

Population status if actinorhizal plant in new Himalayan region through primary survey in Uttarakhand other states like hp and j&k you may option for secondary data base.

Status of nodules nodule morphology and related parameter like shape, size. colour etc.

Also try for isolation of Actinomycete frankia from the nodules followed by microscopy.

We start work as followed by in the direction of Dr. Anit pandey

Brief manuscripts present which work have done

- Sampling of root nodules in open field
- Sampling of root nodules of Alnus and myrica
- Sampling of soil rhizosphere /non rhizosphere soil
- sterilization
- Preparation of media for bacterial inoculation
- Microscopy of root nodules
- Quadrates design/observation

# SAMPLING

- Sampling of root nodules in open field.
- Sampling of soil rhizosphere /non rhizosphere soil.
- Rhizosphere soils collect from root.
- Non-rhizosphere soils collect from root free area.

# Material and method

Plouging tool, scatier, samplimg Polly bag’s, etc

Standardized protocol suggested for sampling bulk rhizoplan soil Angle et al., 1996

### SAMPLING PLAN OF ROOT NODULES

- Choose the site, were more availability of desirable material/specimen.

- In this case desirable material is root nodules of actinorhizal plant
- After finding the site, ploug slowly the base of tree(not harm the (tissue) plant part) were root is present
- If nodules are present in root , take a scatter and cut only the nodule part, do not harm root structure during sampling, hand shake the nodule to remove excess soil
- This excess rhizosphere soil help again in root nodulation due to abundance of frankia

**Sterilization technique and sterilizing material used in isolating and inoculation**

- Root nodules for inoculation.
- Petri
- Forceps
- Dissecting blade
- Sterile water
- Test tubes

**PROCEDURE OF SURFACE STERLILIZATION OF ROOT NODULES**

- The sterilization agent 0. 1% mercuric chloride and 3-5% of hydrogen per oxide.
- 0. 1% agcl and 3. 5%h2o2
- immerse nodule 4-5min in sterilizing agent.
- rinse with D. I water
- immerse nodules in 70% ethyl alcohol for 5 min
- rinse again with D. I water
- PREPARE SLIDE AND OBSERVE UNDER MICROSCOPE

**MEDIA PREPRATION**

- Aseptic condition provided to media.
- Sterilization is at 121 °C (15 lb in ¯²) for 15 minutes. pH values are 7. 0 unless stated otherwise.
- Allow 15 cm³ of agar for each Petri dish and 5-10 cm³ of broth for each McCartney bottle.
- All cotton wool plugs should be made of non-absorbent cotton wool

# MEDIA COMPOSITON

**For actinobacteria isolation here Ty, and Actinomycete isolation agar is used**

- Composition Ty media
- Composition
- Ingredients Gms / Litre
- Casein enzymic hydrolysate 5. 000
- Yeast extract 3. 000
- Final pH ( at 25°C) 7. 0±0. 2
- Actinomycete Isolation Agar composition
- Composition
- Ingredients Gms / Litre
- Sodium caseinate 2. 000
- L-Asparagine 0. 100
- Sodium propionate 4. 000
- Dipotassium phosphate 0. 500
- Magnesium sulphate 0. 100
- Ferrous sulphate 0. 001

- Agar 15. 000
- Final pH ( at 25°C) 8. 1±0. 2
- Inoculation of microbe by different method

# Pour plate method

In pour plate method we directly pour diluted solution of sample in culture plate and then pour media on it.

In pour plate bacterial mycelia growth start from inner part in the media.

Pour plate method is usually the method of choice for counting the number of colony-forming bacteria present in a liquid specimen

### Streak plate method

In streak plate method, a loop (metallic wire) is used for inoculating the bacterial specimen in media plate for pure culture.

Streaking is a technique used to isolate a pure strain from a single species of microorganism

### Spread plate method

Spread plate technique is a method employed to plate a liquid sample for the purpose of isolating or counting the bacteria present in that sample.

spread plate technique will results visible and isolated colonies of m/o that are evenly distributed in the plate and are countable.

## ISOLATION OF MICROBES

The term isolation refers to the separation of a strain from a natural, mixed population of living microbes, as present in the environment, for example in water or soil flora.

Here we use Serial dilution technique.

### INOCULATION OF MICROB IN MEDIA

Inoculation of the sample onto certain solid or liquid media.

It depend upon the which type of isolate is needed.

For example; for isolation of frankia actinobacteria, there is a need of Actinomycete Isolation Agar or selective media that promotes growth of desired microbe.

### Microscopy

Root colonization method is used for microscopy.

Her e we show step wise root colonization method

- Wash the root nodules.
- Immersed root nodules in to15%w/v koh for 15 min done until melanin content remove.
- Wash D. I water.
- Bleach with H2O2 for 5 min.
- Acidify 1% Hcl.
- wash D. I water.
- staining root 0. 05 typen blue in lacto phenol for 30 min at 90ºc.

## Microscopic view and preparation of slide

Prepare slide of root nodules, myrica and Alnus

### QUADRATS DESGINE/OBSERVATION

Quadrat is frame, square shape, used in ecology and geography to isolate a standards unit of area for study of the flora distribution in over a large area.

Soil sampling plan in open field by using z scheme

Quadrat theory and mathematical calculation

Use 50×50m² size of plot, it covers 2500m² area was observed.

In 50×50m² plot , draw 10 quadrat that size 10×10m² , that cover 100m² area it is used for total tree vegetation.

In 10×10 m² quadrat drawn 2 quadrat size 5×5m² in one 10×10m² quadrat. It is used for shrubs.

In 10×10 m² quadrat drawn 10 quadrat size 1×1m² in one 10×10m² quadrat. It is used for herbs.

## Mathematical formulas for observation

- Density = no of indv. of a species/quadrat in which studied frequency
- Frequency= no of quadrat in which species occurred/quadrat studied×100
- Abundance= no of indv. Of a species/no of quadrat in which species occurred
- Relative density(R. D)= density of a species/total density×100
- Relative frequency= frequency of a species/total frequency(sum of frequency of all species) ×100
- Basal area=$(CBH)^2/4\pi$
- Relative basal area= (BA)/sum of total BA×100
- IVI (IMPORTANT VALUE INDEX)

IVI= RA+RF+RBA

- RIVI (RELATIVE IVI)
- RIVI= IVI/TOTAL IVI×100
- H'(Shannon-weinger index)

Here, $P^{I}$= Ni/N, (no. of individual of a particular species)/total no. of individual (sum).

Log pi= log Ni/N

[-pi×log pi]

12. H‘=(Ni/N) ×Ni/N

E. g.=-0. 5 ×-0. 0002=+0. 00001

# TYPE OF DIVERSITY

1. alpha diversity (α-diversity)

2. beta diversity (β-diversity)

3. gamma diversity (γ-diversity)

1. Alpha diversity (α-diversity) is the mean species diversity in sites or habitats at a local scale.

2. Beta diversity (β-diversity or true beta diversity) is the ratio between regional and local species diversity.

3. Gamma diversity (γ-diversity) is the total species diversity in a landscape.

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