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Enumeration of Microbes from Biofertilizer and Chemical Fertilizer Used Soil

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Abstract:
An experiment was conducted to know the bacterial population under organic and chemical fertilizer using agricultural fields. The organic fertilizer was made up with the mixture of several organic ingredients like rock phosphate, neem, and farmyard mixture. On the other hand, chemical fertilizer is made up with the mixture of muriate of potash, mixture of urea and single super phosphate. Microbial counts were observed and analyzer from organic and chemical fertilizer samples from the surface (0-10 cm) and sub-surface (10-30 cm) soil depth of the plots treated by plate and dilution plate method for bacteria. From this, obtained results showed that the organically treated field had the maximum microbial count. Organic field exhibit a significant variation in bacterial population (both the surface and sub-surface) with the chemically treated field and control. Based on the load of organic fertilizer usage, it increased the microbial biomass carbon, increased microbial count and also increase of organic carbon content in the soil. However, the inorganic fertilizer usage resulted in the decrease of microbial count, low organic carbon content in soil, microbial biomass carbon in soil, but it increased the level of other nutrients in soil like Nitrogen, Phosphorous and Potassium. It will also help to increase the level of micro nutrients in the soil.

Keywords: *biofertilizer, chemical fertilizer, microbial load, macro nutrients, micro nutrients, and soil condition.*

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I. Introduction:

Soil is the mother for every living organisms which include both the micro flora and fauna. Soil microbes plays a very important role in making the soil fertile because of their ability to carry out the biochemical transformation. Soil microorganisms, the major living part of soil organic matter, releasing of nutrients into the soil which will be taken by plants for its use (eg., Nitrogen, Potassium and Phosphorous) and as a transient nutrient sinker.

Changes in the microbial communities are used for the prediction of the efforts of ecosystem perturbations by organic and conventional management practices. Agricultural field activities like intercropping, tillage, rotation, use of fertilizers (organic and chemical), drainage and also use of pesticides have significant implications for the presence of microorganisms in the soil. The soil microbes have the capacity to change the soil surroundings and have shown the microbial load changes after the process of fertilization.

Fertilizer can directly and indirectly help for the growth of microbial populations as done by supplying the nutrients and may affect the particular microbial communities in the soil.

Because of the factors we collect from the two different fields

1) Biofertilizer soil field and
2) Chemical fertilizer soil field.

Soil samples are collected from the two different depths (surface and sub-surface). In both the biofertilizer and chemical fertilizers used fields, some amount of soil is taken from the surface (0-15cm) and some amount is taken from the sub-surface (15-30cm). Collected soil sample was weighed and serial diluted and chosen the correct dilution for bacteria and plated in nutrient agar and suitable agar plates. After the incubation of both the soil samples we go for the identification of organisms using staining process, using biochemical test and using selective media.

After analyzing the microorganisms in both the biofertilizer and chemical fertilizer used soil, we can know which type of beneficial microorganisms found in both the soil.

II. Materials And Methods:

STUDY SITE:

The investigation was carried out at agricultural fields of Annur and Sangothipalayam, Karumbathampatti, for a period of 15 days in the month of feb 2022. Biofertilizer used soil was collected from the Annur region and Chemical fertilizer used soil was collected from the regions of Sangothipalayam, Karumbathampatti.

- The geographical position of the study site in Annur is at 11.23* N latitude and 77.13* E longitude and is situated at an altitude of 338 m.s.l.
- The geographical position of the study site in Sangothipalayam, Karumbathampatti is at 11.1075* N Latitude and 77.3398* E Longitude and is situated at an altitude of 295 m. s. 1.

Bio fertilizer used agricultural field in Annur.

Chemical fertilizer used agricultural field in Sangothipalayam, karumbathampatti.

COLLECTION OF SOIL SAMPLE:

Soil sample was collected near plants in two different depths (surface and sub-surface areas).

Collection of soil sample from different fields

PHYSIOCHEMICAL PROPERTIES:

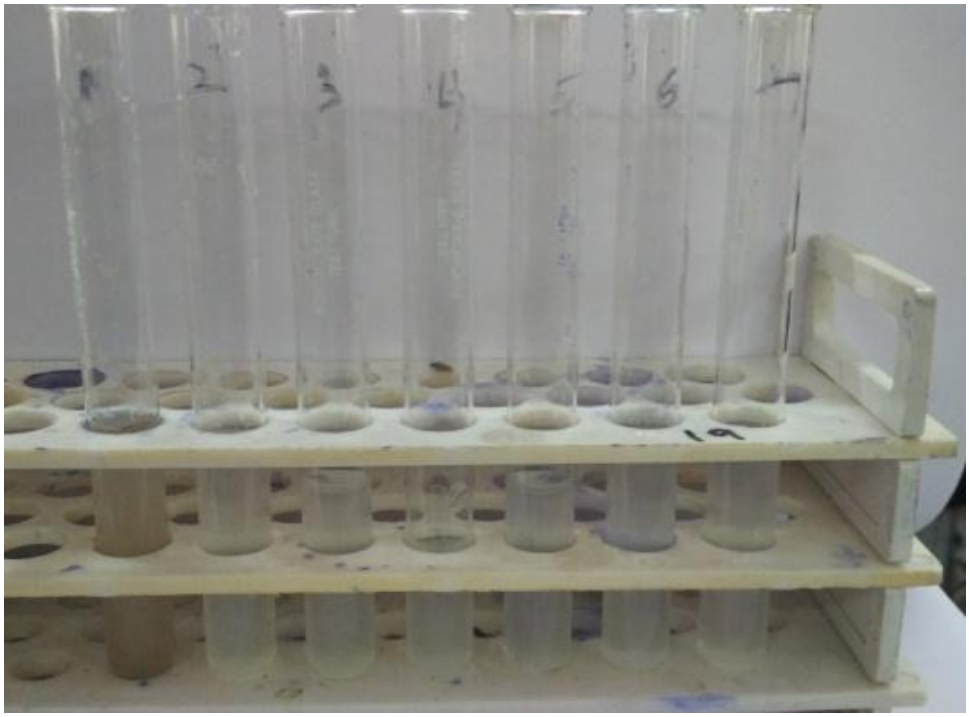
- Soil temperature was noted using soil thermometer during the time of soil sample collection.
- pH of the soil sample was measured using an electronic digital pH meter in 1:5 soil-water suspension.
- The moisture content of the soil samples were determined by weighing, drying in hot air oven at 100^{o}C for 24 hours and then reweighing.
- Organic carbon (C) was determined by the method formed by Anderson and Ingram.
- Total nitrogen (N), available phosphorous (P) and exchangeable potassium (K) were determined by distillation, molybdenum blue method and flame photometer method.

Soil salinity: soil salinity is usually assessed by either determining the Total Soluble Salts by evaporation of a soil water extracts (TSS), or by determining the electrical conductivity (TC) of either 1:5 distilled water: soil dilution, or a saturated paste extract.

Mineral test: Mineral test can be done by colorimeters and general laboratory equipments. SFA test is carried out to determine the minerals in the collected soil sample.

SERIAL DILUTION:

A serial dilution is a process of dilution of a substance in the solution. Usually the dilution factor at each step is constant, resulting in a geometric progression of the concentration in a logarithmic process. A ten-fold serial dilution could be 1M, 0.1M, 0.01M, 0.001M…Serial dilutions are used to create highly diluted solutions as well as solutions for experiments resulting in concentration curves with a logarithmic scale. In biology, it is often associated with the reducing the concentration of cells in a culture to simplify the operation. Serial dilution, because the call suggests, is a sequence of sequential dilutions which can be done to transform a dense answer right into a greater usable attention.



Serial dilution process is carried out after soil sample collection

OBJECTIVES OF SERIAL DILUTION:

The goal of the serial dilution technique is to estimate the attention (range of organisms, micro organism, viruses, or colonies) of an unknown pattern through enumeration of the range of colonies cultured from serial dilutions of the pattern.

In serial dilution, the density of cells is decreased in every step in order that it's miles less complicated to calculate the attention of the cells withinside the unique answer through calculating the overall dilution over the whole series.

Serial dilutions are typically achieved to keep away from having to pipette very small volumes (1-10 µl) to make a dilution of an answer.

By diluting a pattern in a managed way, it's miles viable to gain incubated lifestyle plates with an without difficulty countable range of colonies (round 30–100) and calculate the range of microbes gift withinside the pattern.

SERIAL DILUTION FORMULA/CALCULATIONS:

Serial dilution entails the system of taking a pattern and diluting it thru a sequence of popular volumes of sterile diluent, that could both be distilled water or 0.9 % saline.

Then, a small measured extent of every dilution is used to make a sequence of pour or unfold plates. Depending at the envisioned attention of cells/organisms in a pattern, the volume of dilution is decided. For e.g., if a water pattern is taken from an incredibly polluted environment, the dilution thing is increased. In contrast, for a much less infected pattern, a low dilution thing is probably sufficient.

Serial two-fold and ten-fold dilutions are typically used to titer antibodies or put together diluted analytes withinside the laboratory.

The dilution thing in a serial dilution may be decided both for an character check tube or may be calculated as a complete dilution thing withinside the whole series.

The dilution thing of every tube in a set:

$$\frac{volume\ of\ sample}{volume\ of\ sample + volume\ of\ diluent}$$

For a ten-fold dilution, 1 ml of pattern is introduced to nine ml of diluent. In this case, the dilution thing for that check tube might be:

$$\text{Dilution factor} = \frac{1\ ml}{1\ ml + 9\ ml} = \frac{1}{10} = 10^{-1}$$

After the primary tube, every tube is the dilution of the preceding dilution tube.
Now, for general dilution factors,
Total dilution thing for the second one tube = dilution of first tube × dilution of the second one tube. Example:
For the primary tube, dilution thing = 10^{-1} (1 ml introduced to 9 ml)
For the second one tube, dilution thing = 10^{-1} (1ml introduced to 9 ml)
Total dilution thing = preceding dilution × dilution of subsequent tube
= general dilution of $10^{-1} \times 10^{-1} = 10^{-2}$

PROCEDURE FOR SERIAL DILUTION:
The following is the process for a ten-fold dilution of a pattern to a dilution thing of 10^{-6}:
1) The pattern/lifestyle is taken in a check tube and 6 check tubes, every with nine ml of sterile diluents, that could both be distilled water or 0.9% saline, are taken.
2) A sterile pipette is taken.
3) 1 ml of well combined pattern/lifestyle is drawn into the pipette.
4) The pattern is then introduced to the primary tube to make the overall extent of 10 ml. This presents an preliminary dilution of 10^{-1}.
5) The dilution is very well combined through emptying and filling the pipette numerous times.
6) The pipette tip is discarded, and a brand new pipette tip is connected to the pipette.
7) Now, 1 ml of combination is taken from the 10^{-1} dilution and is emptied into the second one tube. The second tube now has a complete dilution thing of 10^{-2}.
8) The equal system is then repeated for the final tube, taking 1 ml from the preceding tube and including it to the following 9 ml diluents.
9) As six tubes are used, the very last dilution for the micro organism/cells might be 10-6 (1 in 1,000,000).

SPREAD PLATE METHOD: The unfold plate method entails the usage of a sterilized spreader with a clean floor made from steel or glass to use a small quantity of micro organism suspended in an answer over a plate. The plate desires to be dry and at room temperature in order that the agar can soak up the micro organism extra readily. (commonly 0.1 ml)

POUR PLATE METHOD: Pour plate technique is commonly the technique of desire for counting the range of colony-forming micro organism found in a liquid specimen. Because the pattern is blended with the molten agar medium, a bigger extent may be used than with the unfold plate. In this technique, a hard and fast quantity of inoculum (commonly 1 ml) from a broth/pattern is positioned withinside the middle of a sterile Petri dish the usage of a sterile pipette. Molten cooled agar (approx. 15mL) is then poured into the Petri dish containing the inoculum and combined well. After the solidification of the agar, the plate is inverted and incubated at 37°C for 24-48 hours.

INCUBATION & OBSERVATION OF GROWTH:
After the plating system incubate the plates at 37* C for 48 hours and have a look at the results. Observe the boom (growth) and do staining system, biochemical exams, 16 RNA sequence exams and different essential exams to verify the organism.

COLONY COUNTING:
The reason of plate counting is to estimate the range of cells gift primarily based totally on their potential to present upward push to colonies below particular situations of nutrient medium, temperature and time. Theoretically, one possible mobileular can supply upward push to a colony thru replication. However, solitary cells are the exception in nature, and maximum probable the progenitor of the colony become a mass of cells deposited together. In addition, many bacteria develop in chains (e.g. Streptococcus) or clumps (e.g., Staphylococcus). Estimation of microbial numbers through CFU will, in maximum cases, undercount the range of dwelling cells found in a pattern for those reasons. This is due to the fact the counting of CFU assumes that each colony is separate and based through a unmarried possible microbial mobileular.

The plate rely is linear for E. coli over the variety of 30 to three hundred CFU on a fashionable sized Petri dish. Therefore, to make certain that a pattern will yield CFU on this variety calls for dilution of the pattern

and plating of numerous dilutions. Typically, ten-fold dilutions are used, and the dilution collection is plated in replicates of two or three over the selected variety of dilutions. Often 100µl are plated however additionally large quantities as much as 1ml are used. Higher plating volumes growth drying instances however frequently do not bring about better accuracy, given that extra dilution steps can be needed. The CFU/plate is study from a plate withinside the linear variety, after which the CFU/g (or CFU/mL) of the unique is deduced mathematically, factoring in the quantity plated and its dilution thing (e.g. CLSI VET01S).
A answer of bacteria at an unknown attention is frequently serially diluted to be able to acquire at the least one plate with a countable range of bacteria. In this figure, the "x10" plate is appropriate for counting.
A gain to this technique is that distinct microbial species might also additionally provide upward push to colonies which might be truly distinct from every other, each microscopically and macroscopically. The colony morphology may be of good use in the identity of the microorganism present.
A previous expertise of the microscopic anatomy of the organism can provide a higher expertise of the way the found CFU/mL pertains to the range of feasible cells in line with milliliter. Alternatively, it's miles feasible to lower the common range of cells in line with CFU in a few instances with the aid of using vortexing the pattern earlier than accomplishing the dilution. However, many microorganisms are sensitive and might go through a lower in the share of cells which might be feasible whilst positioned in a vortex.

CFU/ml = (no. of colonies x dilution factor) / extent of culture plate
Now which you have a few data, you could do the CFU calculation withinside the unique pattern.
1. Take the quantity you plated (0.five mL) and multiply with the aid of using the dilution thing (0.01) to yield 0.005. You have to try this to discover the dilution thing which yielded your CFU rely. Here, 1/2 of a milliliter of the 1:100 dilution allowed you to rely CFU.
2. Divide the CFU from the dilution (179) with the aid of using the end result from Step 1 (0.005) to yield 35,800 CFU.
This approach that the unique 1 mL of pattern that become diluted includes 35,800 CFU. Another manner to position that is to mention that the unique pattern has 35,800 CFU/ml.
CFU is a superb device to discover what number of micro organism, fungi or any microorganisms there are in a given pattern. The plating technique defined right here is especially useful for extremely focused samples or organisms that do not develop nicely in liquid lifestyle.

PROCEDURE:
Collected the soil pattern from distinct agricultural fields the usage of bio fertilizer and chemical fertilizers for the technique of agriculture.
Collected the soil from floor area (0-15cm) and Sub floor area (15-30cm) to discover the distinct bacterial populace of each the biofertilizer and chemical fertilizer the usage of fields.
Taken the soil samples to the laboratory and completed physiological exams for the soil pattern.
The physiological exams have been,
1) **Soil temperature** take a look at the usage of soil the thermometer
2) **pH** of the samples becomes taken the usage of an digital virtual pH meter.
3) The **moisture content material of the soil** samples have been decided gravimetrically with the aid of using weighing, drying in warm air oven at 105°C for twenty-four h after which reweighing.
4) **Soil nitrogen** decided with the aid of using kjeldahl distillation.
5) **Soil phosphorous** decided with the aid of using Molybdenum blue technique.
6) **Soil Potassium** decided with the aid of using flame photometer take a look at.
7) **Soil salinity** decided with the aid of using salinity.
8) **Soil minerals** decided with the aid of using SFA take a look at.
After finishing physiological take a look at finished serial dilution method for reducing the microbial load.
10 gm of soil and blended it withinside the one hundred ml of distilled water, this is 10-1 dilution.
Filled 10 take a look at tubes with nine ml of distilled water.
Taken 1 ml of water from the 100ml soil mixer the usage of pipette and poured it withinside the take a look at tube and marked it as 10^{-2}
Done this technique for the final nine tubes and mark the dilution of the tubes properly.
The dilutions of 10^{-4}, 10^{-5} and 10^{-6} for micro organism become taken.
Nutrient agar become organized and poured withinside the petri plates.
Four petri plates of sets (one set is for bio fertilizer used soil pattern & every other set is for chemical fertilizer used soil pattern) become taken.
Three plates have been used for the inoculation of diluted pattern and one plate is used for control.
The serial diluted samples have been inoculated with the aid of using the usage of unfold plate method, and the samples have been incubated on the temperature of 37* C for forty eight hours.
After the incubation found the plates to discover distinct sorts of bacterial colonies.

The colonies have been counted the usage of the colony counter and calculated with the aid of using CFU formula.
Finally distinct sorts of micro organism in bio fertilizer used soil and chemical fertilizer used soil become diagnosed and effects have been plotted.

IDENTIFICATION OF BACTERIA IN NUTRIENT AGAR PLATE USING APPERANCE, COLONY MORPHOLOGY, STAINING & BIOCHEMICAL TESTS:
In bio fertilizer used soil pattern:
1) Gram negative, Rod shaped & Catalase and oxidase positive
Appearance in plate - greenish colour colonies (manufacturing of pyoverdin pigment).
Bacteria Name: ***Pseudomonas aeroginosa.***
2) Gram negative, Rod shaped.
Appearance in plate – yellow in colour
Bacteria name: ***Nitrobacter***
3) Gram negative, Rod shaped, ordinarily found in root nodules.
Appearance in plate: white transluent, glistening and small colonies.
Bacteria name: ***Rhizobium***
4) Gram negative, Rod shaped.
Appearance in plate - round colonies, hard opaque, fuzzy white or barely yellow colonies.
Bacteria name: ***Bacillus subtilis***
5) Gram negative, barely twisted rectangular rod shaped.
Appearance in plate – Colonies with flat morphology, glossy, large, scarlet pink colour.
Bacteria name: ***Azospirillum***
6) Gram negative, Blue inexperienced algae.
Appearance in plate - colonies are inexperienced in colour.
Bacteria name: ***Cyanobacteria***
7) Gram positive, Filamentous micro organism.
Appearance in plate - golden yellow and whitish colony, bubble like look in Centre of the colony.
Bacteria name: ***Streptomyces***
In chemical fertilizer used soil sample:
1) Gram-poor, flourishes optimally at 30°C.
Appearance in plate - Gold rusty colour.
Bacteria name: ***Acidithiobacillus ferrooxidans***.
2) Gram-positive, Gram- poor or Gram-variable, spore-forming and
facultatively anaerobic or strictly aerobic. catalase positive. It does now no longer have a capsule. Positive for starch hydrolysis, produces amylase.
Appearance in plate – translucent (whitish yellow colour).
Bacteria name: ***Paenibacillus spp.***
3) Gram poor, Rod shaped, frequently found in root nodules.
Appearance in plate: white transluent, glistening and small colonies.
Bacteria name: ***Rhizobium***
four) Gram poor, Rod shaped & Catalase and oxidase positive
Appearance in plate - greenish colour colonies (manufacturing of pyoverdin pigment).
Bacteria Name: ***Pseudomonas aeroginosa***.

USES OF THOSE MICRO ORGANISM FOR SOIL & PLANT:
Pseudomonas aeruginosa: Play a crucial position withinside the biodegradation and bioremediation of those poisonous compounds located in soil and water via way of means of using the insecticides as its carbon supply and energy.
Nitrobacter: Play a crucial position withinside the nitrogen cycle via way of means of oxidizing nitrite into nitrate in soil and marine systems.
Azotobacter: These micro organism make use of atmospheric nitrogen fueloline for his or her mobileular protein synthesis. This mobileular protein is then mineralized in soil after the loss of life of Azotobacter cells thereby contributing closer to the nitrogen availability of the crop plants.
Bacillus subtilis: solubilize soil Phosphorous, beautify nitrogen fixation, and bring siderophores that sell its boom and suppresses the boom of pathogens.
Cyanobacteria: They play a couple of roles withinside the soil via way of means of solving C and N and synthesizing exopolysaccharides, which boom soil fertility and water retention and enhance soil shape and stability.

Rhizobium: enables in solving nitrogen in leguminous plants. It attaches to the roots of the leguminous plant and produces nodules. These nodules repair atmospheric nitrogen and convert it into ammonia that may be utilized by the plant for its boom and improvement.

Streptomyces: produce PGP substances, secondary metabolites (along with antibiotics), and enzymes.
Acidithiobacillus ferrooxidans: affecting the plant improvement at once thru the atmospheric nitrogen fixation.
Paenibacillus spp.: act as a plant boom-selling rhizobacteria, affecting the plant improvement at once thru the atmospheric nitrogen fixation, mineral solubilization, siderophores or phytohormone manufacturing.

III. Result And Discussion:

Effect of natural fertilizer soil and inorganic fertilizer soil on bacterial populace at surface (0-15 cm) and sub-surface (15-30 cm) soil depths in agriculture discipline. Result of evaluation of variance confirmed that there have been enormous distinction among the software bio-fertilizer and chemical fertilizer used soil for yield and yield thing traits. The effects indicated that the impact of bio fertilizer and chemical fertilizer on characters such as, pH, temperature, salinity, moisture and different elements like range of styles of micro organism's gift, yielding of the crop and fitness blessings vary.

Result among biofertilizer become gift micro organism like ***Pseudomonas aeruginosa*** become facilitates in agriculture because of this that Solubilization of vitamins, synthesis of antibiotics etc., The subsequent microbes which facilitates in agriculture become *Nitrobacter* it become oxidize nitrite to nitrate.

The ultimate microbes become additionally facilitates in agriculture discipline it become found in soil, it become our test, we takes a distinct soil discipline become biofertilizer soil and chemical fertilizer soil. The soil samples have been taken and take a serial dilution and pour with inside the particular media and incubate it for forty eight hours at 37°C.

Based at the boom of the colony we decide the microbes gift with inside the soil pattern. It become identity check for the microbes gift with inside the soil, the 2 soil pattern become differentiated with the aid of using the microbes gift with inside the soil pattern.

The biofertilizer offers extra microbes then a chemical fertilizer .Some microbes are found in biofertilizer which facilitates in agriculture discipline it way it facilitates in plant boom.

The micro organism become facilitates in plant boom it become extra in biofertilizer than a chemical fertilizer. The particular media used with inside the test become facilitates with inside the boom with inside the microbes to get colonies for the identity of the boom. The particular media for the Pseudomonas become blood agar, chocolate agar, macconkey agar it offers a big and particular boom for the pseudomonas sp.

The particular media for the Nitrobacter become Nitrobacter agar medium its facilitates withinside the particular boom of the nitrobacter. The particular media for the Rhizobium become Yeast extract mannitol agar it become a particular boom of rhizobium and its species. The particular media for the bacillus subtilis become Macconkey agar and chapman agar medium that is a particular boom for the Bacillus subtilis. The particular media for the Azospirillum become Azospirillum medium it become a particular boom of the Azospirillum. The particular media for the Cyanobacteria become BG11 medium it become excellent medium for the cyanobacteria. The particular media for the Streptomyces become Oatmeal agar, MYM agar, ISP4 agar media it become a particular and excellent medium for the boom of Streptomyces. The particular media for the ***acidiothibacillus ferrooxidans*** become thiosulfate containing DSMZ medium. The above media is a instance for the boom for the particular bacterial cultivation wherein utilized in our test. The micro organism are useful for the boom of the flora withinside the agricultural discipline, the microorganism in biofertilizer is extra beneficial than a chemical fertilizer.

The biofertilizer is extra beneficial to develop a beneficial micro organism to develop a flora and its continue to exist. Chemical fertilizer is stops or kills the best micro organism with inside the soil it become have an effect on the boom of the flora however it additionally facilitates with inside the boom of flora much less than biofertilizer. Chemical fertilizer it have an effect on the vitamins and the strength supply with inside the soil which become used with inside the agricultural discipline.

So we use biofertilizer to product the vitamins with inside the soil so it become extra useful than a chemical fertilizer. Usage of bio fertilizer facilitates to continue to exist maximum range of exact micro organism in soil and that micro organism's facilitates to develop the plant healthy. In now a days extra chemical fertilizer are used with inside the discipline of agriculture it have an effect on the vitamins gift with inside the soil, it lower the nutrient cost in destiny. So we use biofertilizer to keep away from the destiny trouble it become our test to show that biofertilizer is extra useful than we use chemical fertilizer.

IV. Conclusion:

The final conclusion about this article is using of biofertilizer is better than using chemical fertilizer to improve the quality of the soil. This will also increase the microbial population in the soil where it will also help for the better plant's growth. Using of chemical fertilizer will the decrease the growth rate of the microbes in the soil which will also affect the quality of the soil. By continue use of chemical fertilizer will increase the unwanted content in the soil which will affect the soil.

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