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DEVELOPMENT AND TESTING OF SPECIFIED BIOPESTICIDE, BIOCOMPOST, BIOFERTILIZER ON MUSTARD PLANT

Biotechnology

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ABSTRACT

The selected microbial strains are produced in large quantity from the mother cultures by using specific Media, grown in conical flasks under room temperature on rotary orbital shaker, after incubation period the media has to be centrifuge and collect the pellet from it, then after count the number of cells per Milli liter with the help of Hemocytometer. The selected microbial strains have the properties of biopesticide, bio fertilizer that are essential for the healthy growth of Mustard plant. Bio compost was prepared separately on a tray with the help of different constituents, to that we added sterile soil, after 1 month of incubation the constituents are decomposed and it gives bio compost. To the biocompost selected microbial strains are added in different combination. During incubation period, the soil macro nutritional values are checked with the help of NICE soil testing kit. The activity of different biopesticide, biofertilizer, biocompost can be tested on mustard plant, at the same time, the growth characteristics are observed with the help of controller (normal soil+Biocompost). The specified biopesticide, biocompost, biofertilizer are very helpful to farmers. It increases the yield of plant, fertility of the soil, economically low cost and eco friendly.

KEYWORDS

Biofertilizer, Biopesticide, Biocompost, Mustard Plant, Nice Soil Testing Kit.

INTRODUCTION

Fertilizer is widely used to supply essential nutrient for plant to increase yield. In fact, yields of most crop plants increase linearly with the amount of fertilizer that they absorb. Due to this fact, agricultural sector are strongly depending on fertilization with mineral nutrients. When crop plants are grown under modern high-production conditions, substantial amounts of nutrients are removed from the soil. Then, to prevent deficiencies, nutrients can be added back to the soil in the form of fertilizers.

There are many types of fertilizer that existed such as inorganic fertilizer, organic fertilizer and biofertilizer. Fertilizers that provide nutrients in inorganic forms are called chemical fertilizers and those that derive from plant or animal residues are considered as organic fertilizers. Biofertilizers are the products containing living cells of different types of microorganisms which have an ability to mobilize nutritionally important elements from non-usable to usable form through biological process. Among of these fertilizers, chemical fertilizer is the most extensively used in plantation.

However, extensive use of chemical fertilizers in long term cause declining in productivity and also environmental quality .In the light of these problems, the use of organic fertilizers, biofertilizer and other microbial products is crucial in the current attempt to make the agriculture industry a viable component of a healthy and pleasant ecosystem.

Improvement should be done to these fertilizers to replace the usage of chemical fertilizer. Thus, this project is set up to improve the biofertilizer effectiveness to make it have superior potency over the chemical fertilizer.

Composting is a regulated, aerobic (requiring oxygen) process that uses natural decomposition to transform organic materials into a nutrient-rich, biologically-stable mulch or soil supplement. Compost is the final result.

Pesticides are substances that destroy, drive away, or manage species of plants and animals that are deemed harmful or bothersome in household and agricultural settings.

Scope of Study

The scope of this project is to determine the effect of strains in combination of specified Bio pesticide, Bio composting , Bio fertilizer and compare it effectiveness with normal soil.

Changes ofnitrogen, phosphorous, potassium content in biofertilizer will be determined by NICE soil testing kit. The effectiveness of enhanced biofertilizer, biopesticide, biocompost will be tested on mustard plant and proved by its physical changes. The techniques that will be employed include OD reading and physical changes observation. The equipment involved is hemocytometer, centrifugation, laminar air flow, autoclave.

Some of the Side Effects of Chemical Fertilizers and Pesticides:

1. They are leaching out in soil, water, especially in the drinking water.
2. They polluting the water basins.
3. Destroying the micro-organisms and friendly insects.
4. Making crop more susceptible to the attack of diseases.
5. Reducing the soil fertility.

To Reduce the Side Effects Produced by the Chemical Fertilizers and Pesticides :

a. A number of intellectuals throughout the world working on the alternatives.
b. Theyfound that Biofertilizers, Biopesticide, Biocompost can help in increasing the yield without causing damage associated with chemical fertilizers.
c. The price is lower and more competitive than organic fertilizer, which makes it more acceptable and often applied by users.

Biofertilizer

The term biofertilizer or called 'microbial inoculants' can be generally defined as a preparation containing live or latent cells of efficient strains of nitrogen fixing, Phosphate solubilizing or cellulytic microorganisms used for application of seed, soil or composting areas with the objective of increasing the numbers of such Microorganisms and accelerate certain microbial process to augment the extent of the availability of nutrients in a form which can assimilated by plant (NIIR Board, 2004).In large sense, the term may be used to include all organic resources (manure) for plant growth which are rendered in an available form for plant absorption through microorganisms or plant associations or interactions (NIIR Board, 2004).

Production of Bio fertilizer

There are several things need to be considered in biofertilizer making such as microbes' growth profile, types and optimum condition of organism, and formulation of inoculum. The formulation of inocula, method of application and storage of the product are all critical to the success of a biological product (Chen, 2008).

In general, there are 6 major steps in making biofertilizer. These includes choosing active organisms, isolation and selection of target microbes, selection of method and carrier material, selection of propagation method, prototype testing and large scale testing. First of all, active organisms must be decided. For example, we must decide to use whether organic acid bacteria or nitrogen fixer or the combination of some organisms. Then, isolation is made to separate target microbes from their habitation. Usually organism are isolate from plants root or by luring it using such as putting cool rice underground of bamboo plants.

Next, the isolated organisms will be grown on Petri plate, shake flask and then glasshouse to select the best candidates. It is also important to decide form of our biofertilizer product wisely so that the right carrier

material can be determined. If it is desired to produce biofertilizer in powder form, then tapioca flour or peat are the right carrier materials. Selection of propagation method is mainly to find out the optimum condition of organism. This can be achieved by obtaining growth profile at different parameter and conditions.

Common Organisms Used in Biofertilizer:
Organisms that are commonly used as biofertilizer components are Nitrogen fixers (N-fixer), Potassium solubilizer (K-solubilizer) and Phosphorus solubilizer (P solubilizer), or with the combination of molds or fungi. Most of the bacteria included in biofertilizer have close relationship with plant roots (FNCA Biofertilizer Project Group, 2006). Rhizobium has symbiotic interaction with legume roots, and Rhizobacteria Inhabit on root surface or in rhizosphere soil (FNCA Biofertilizer Project Group, 2006).

The phospho-microorganism mainly bacteria and fungi make insoluble phosphorus available to the plants (NIIR Board, 2004). Several soil bacteria and a few species of fungi posses the ability to bring insoluble phosphate in soil into soluble forms by secreting organic acids (NIIR Board, 2004). These acids lower the soil pH and bring about the dissolution of bound forms of phosphate.

While Rhizobium, Blue Green Algae (BGA) and Azolla are crop specific, bio inoculants like Azotobacter, Azospirillum, Phosphorus Solubilizing Bacteria (PSB), Vesicular Arbuscular Mycorrhiza (VAM) could be regarded as broad spectrum Biofertilizers (NIIR Board, 2004). VAM is fungi that are found associated with Majority of agriculture crops and enhanced accumulation of plant nutrients (NIIR rd, 2004). It has also been suggested that VAM stimulate plant growth by physiological effects or by reducing the severity of diseases caused by the soil pathogens (NIIR Board, 2004).

Biopesticides
Bio-pesticides are naturally occurring substances from living organisms (natural enemies) or their products (microbial products, phytochemicals) or their by-products (semiochemicals) that can control pest by nontoxic mechanisms (Salma and Jogen, 2011). According to Organization for Economic Cooperation and Development (2009), biopesticides are mass-produced, artificially created substances that are marketed for use in pest management and are derived from naturally occurring microorganisms.

Importance of Biopesticides
A chemical or biological substance, designed to kill or retard the growth of pests that damage or interfere with the growth of crops, shrubs, trees, timber and other vegetation desired by humans, is called pesticide. Practically all chemical pesticides, however, are poisons and pose long term danger to the environment and humans through their persistence in nature and body tissue.

Microbial Pesticides
Microbial Pesticides consist a microorganism (e.g., a bacterium, fungus, virus or protozoa) as the active ingredient. Microbial pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest. For example, there are fungi that control certain weeds, and other fungi that kill specific insects.

The most widely used microbial pesticides are subspecies and strains of Bacillus thuringiensis.

Each strain of this bacterium produces a different mixture of proteins and specifically kills one few related species of insect larvae. The target insect species are determined by whether the particular Bacillus thuringiensis produces a protein that can bind to a larval gut receptor, thereby causing the insect larvae to starve.

Bio Fungicides
Bio fungicides are fungal formulations used for the control of different diseases in crops.

Mustard Seed
Mustard seeds are the small round seeds of various mustard plants. The seeds are usually about 1 to 2 millimetres (0.039 to 0.079 in) in diameter and may be colored from yellowish white to black. They are an important spice in many regional foods and may come from one of three different plants: black mustard (Brassica nigra), brown Indian mustard (B. juncea), or white/yellow mustard (B. hirta/Sinapis alba).

Inflammatory Effects: The high source of magnesium in mustard seeds helps reducing the severity of asthma attacks and certain symptoms of rheumatoidarthritis and lowering blood pressure.

MATERIALS & METHODS
1. Microorganism are obtained from microbiology department Osmania University and wisdom life science company.
2. Mustard seeds are obtained from Tierra Seed Science Pvt. Ltd.
3. The equipment Laminar air flow, Auto clave, Microscope, Pots and Trays, soil(red), Hemocytometer, Centrifugation, Orbital shaker are from Osmania University campus.

Composition of Potato Dextrose Agar:
- Potato-200g, Dextrose -20g, Agar -20g, Distilled water -1000ml, PH -7.3

Composition of Nutrient Agar Media:
- Beef Extract – 3.0 g, Peptone - 5.0 g, Agar -15.0 g, Distilled water -1000ml, pH - 7.0

Microorganisms Used in the Production of Bio Fertilizer
- Rhizobium, Azospirillum, Azoto bacteria, Mixture of different bacillus specie's, Vam power, Mycorrhiza

Microorganisms Used in the Production of Bio Pesticide
- Trichoderma Viride, Pseudomonas fiuorescens, Beauveria bassiana.

Materials Used in the Production of Bio Composting
- Cow dung, Neem powder, Egg shell, Ash powder, Kitchen waste

Bio Compost Materials and their Volumes:
Table 1: Bio Compost Materials and Their Volumes.

S no	components	grams	%
1	Sterile soil	1000	50
2	Kitchen waste	2000	100
3	Neem powder	60	3
4	Ash powder	20	1
5	Egg shell	100	5
6	Cow dung	400	20

Hemocytomertry Values
Table 2: Microorganisms and Their Quantity.

S.no	Microorganisms	Quantity (cells/ml)
1	Rhizobium bacteria	1.16 X 10^5
2	Azospirillium bacteria	1.10 X 10^4
3	azotobacter	1.3 X 10^2
4	Bacillus Sediminis,	1.0 X 10^3
5	Bacilius Agricola,	1.1 X 10^2
6	Bacillus vedderi	1.8 X 10^4
7	Trichodemaviride	1.2 X 10^2
8	Pseudomonas flourosces	1.5 X 10^3
9	Baveriabassiana	1.1 X 10^3
10	Mycorrhiza	1.2 X 10^4

Methods of Testing
The cultures(as mentioned in Table 2)are used as Bio fertilizer, Bio pesticide, Bio composting,
- Testing process is in 2 stages.
- 1st stage: In vitro testing under aseptic conditions.
- 2nd stage: In vivo testing in soil.

Methodology
- Different microbial strains such as fungi species, bacterial species are grown in an agar media (potato dextrose agar, nutrient agar) on Petri plate.
- After growth of the cells, the cells are counted per milli liter with the help of hemocytometer.
- For large scale production of bio fertilizer, bio pesticide strains, we are prepared potato dextrose broth for fungi, nutrient broth for bacteria in a 2 litre conical flask incubated for 5 days in a room temperature under shaker during this period we are calculated cells growth by turbidity value with the help of colorimeter.
- After incubation period the media broth is centrifuged in centrifugal tubes.
- Pellet is collected and supernatant is removed, the collected pellet is added to the different trays with different microbial combination.

Experimental Setup

The Experimental set up consist of Control 1 and Control 2 along with Trays consisting of different combinations whuch are mentioned below:

Control 1- Kitchen waste + Neem powder Ash powder + Egg shell

Tray 1- Control 1+ azotobacter + azospirillum+ Rhizobium + sterile soil added in appropriate volume ratio.

Tray 2- Control 1 + Bacillus Species (sediminis,agri,vedderi) + sterile soil added in appropriate volume ratio.

Tray 3- Control 1 + vam power + mycorrhiza+ trichoderma + pseudomonas+ Beauveria + sterile soil added in appropriate volume ratio.

Tray 4- Control 1 + tray 1 + tray 2 + tray 3 + sterile soil added in appropriate volume ratio.

Control 2- Kitchen waste + cow dung + Ash powder + Egg Shell

Tray 5- Control2 + azotobacter + azospirillum + R + sterile soil added in appropriate volume ratio.

Tray 6- Control 2+ Bacillus Species (sediminis,agri,vedderi) + sterile soil added in appropriate volume ratio.

Tray 7- Control 2 +Vam power + mycorrhiza+ trichoderma + pseudomonas+ Beauveria+ sterile soil added in appropriate volume ratio.

Tray 8- Control 2 + Tray 5 + Tray 6 +Tray 7 + sterile soil added in appropriate volume ratio.

- Make sure that these trays are clean and free of dirt.
- After adding the microbial strains to tray, we kept it for incubation for 3 months. During this period the values of nitrogen, phosphorous, potassium during, PH, moisture content of soil using NICE Soil testing kit are calculated.
- For in vitro testing of different microbial strains activity on seed, we prepared nutrient agar media in test tubes, the seeds are inoculated with different microbial strains, before that mustard seeds are surface sterilized with ethanol, sodium hypo chloride and mercury chloride.
- After 3 months of incubation, different microbial cultured soil is collected
- 20 pots are taken, to that a fresh autoclaved 3 kg soil is added per every pot and 300 grams cultured soil is added in appropriate volume and maintains moisture for survival of microbial cells.
- Mustard seeds are taken from seed shop and inoculated into different pots
- After that, growth characteristics are observed with different microbial treatment pots of mustard plant.

Moisture Content of Soil (%)

Moisture content of cultured red soil is higher than normal red soil.

Moisture content (%) = I-F/I*100
= 150-80/150*100
=40% (cultured red soil)

Moisture content (%) = I-F/I*100
=150-90/150*100
=46% (normal red soil)

Where,
I= Initial weight of the soil sample (g)
F= final weight of the dried soil (g)

Seed Germination Rate

Mustard seeds were washed with tap water 3 to 5 times. After that, the seeds were rinsed with teepol solution to clean microbes and these seeds were cleaned with distilled water 2 to 3 times. Seeds were immersed with distilled water till overnight for softening the seeds coat. These seeds were mixed with bio fertilizer, bio pesticide in different combinations and dried under the shade place, after 15 days the germinated seeding were counted and calculate the rate of seed germination by the formula as follows.

Seed germination rate (sgr) % = (Total no of seeds germinated) / (Total no. of seeds sown) X 100

Water Holding Capacity (WHC)

Biofertilizers treated mustard plant red soil were powdered and dried on a hot air oven at 105 degree for 24 hrs. the weight of the empty beaker (diameter of 1.5cm and a height of 5.4 cm respectively) was noted initially (Wo) and a circular filter paper (whatman no.1) was placed inside the funnel, which was filled with the dried soil and again the second weight of the soil was noted (W1). After 15 minutes the funnel was taken out, beaker with water and the final weight (W2) was noted.

WHC % = (W2-Wo)-(W1-Wo) / (W1-Wo)*100

Where:
Wo= weight of the empty beaker (g)
W1=weight of the soil with filter paper (g)
W2=weight of the beaker after filtration or time duration (g)

Determination of Shoot, Root Length and Number of Leaves

The shoot, root length and number of leaves were calculated at different time intervals during growth of the plant (1st, 2nd, and 3rd week). The plants were uprooted carefully, the roots were washed with tap water to remove the soil particles and rinsed thrice with excess distilled water, and then blotted dry Shoot, root length and number of leaves were calculated.

RESULTS

Bacterial Growth Rate

In this method we are calculating the microbial strain growth rate, test sample is taken into a quartz cuvette, Broth solution is used as controller .at different intervals ranging from 0 - 120hrs after inoculation, growth was monitored by measuring turbidity at 540 nm.

Liquid Broth

Table 3: Liquid Broth Turbidity Values

DAYS	O.D VALUE at 540nm									
	A	B	C	D	E	F	G	H	I	J
1	0.40	0.32	0.42	0.45	0.52	0.32	0.44	0.48	0.46	0.51
2	0.46	0.35	0.48	0.48	0.61	0.36	0.51	0.52	0.55	0.55
3	0.57	0.42	0.53	0.52	0.71	0.42	0.57	0.53	0.60	0.57
4	0.69	0.49	0.56	0.61	0.73	0.53	0.63	0.61	0.65	0.64
5	0.84	0.54	0.61	0.69	0.75	0.61	0.71	0.65	0.71	0.71

Where;

A=Rhizobium E= Bacillus agricola
B= Azospirillium F= Bacillus veddena
C= Azotobacter G= Mycorrhiza
D= Bacillus sediminis H= Trichoderma viride
I= Pseudomonas flurosces J= Beauveria bassiana

Estimation of N, P, K, PH and Moisture Content Values Using Nice Soil Testing Kit- for 8 weeks

The values are presented according to the values of Nice soil testing kit.

Nitrogen Test Values

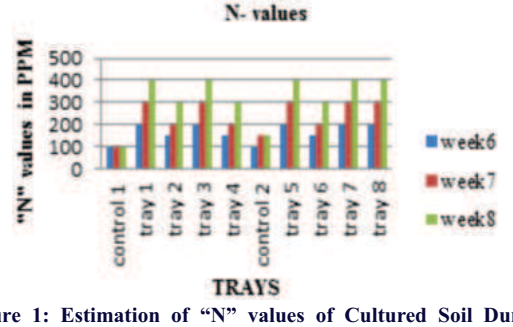

Figure 1: Estimation of "N" values of Cultured Soil During Incubation Period.

Phosphorous Test Values

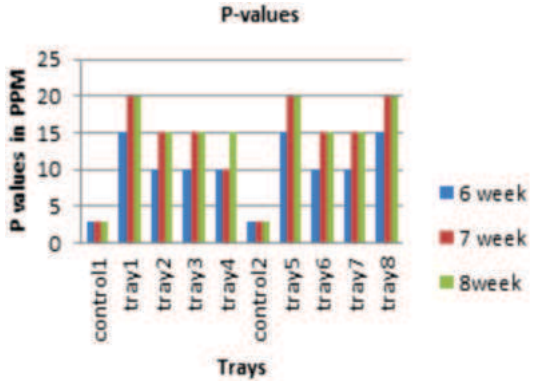

Figure 2: Estimation of "P" values of Cultured Soil During Incubation Period.

Potassium Test Values

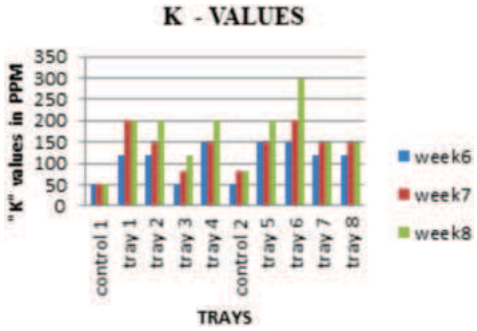

Figure 3: Estimation of "K" values of Cultured Soil During Incubation Period.

pH values of Cultured Soil:

Table 4: pHvalues of Cultured Soil During Incubation Period.

Trays	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control 1	8.0	7.5	7.5	7.5	7	7	7	6.5
Tray1	7.5	7.0	6.9	6.5	6.5	6.5	6.5	6.5
Tray2	8.0	8.0	7.5	6.5	6.5	6.5	6.5	6.5
Tray3	7.5	7.0	7.0	6.5	6.5	6.5	6.5	6.5
Tray4	7.5	7.5	7.0	6.5	6.5	6.5	6.5	6.5
Control 2	8.0	7.5	7.0	6.5	6.5	6.5	6.5	6.5
Tray5	7.5	7.5	7.0	6.5	6.5	6.5	6.5	6.5
Tray6	8.0	8.0	7.5	6.5	6.5	6.5	6.5	6.5
Tray7	8.0	8.0	7.5	6.5	6.5	6.5	6.5	6.5
Tray8	7.5	7.5	7.0	6.5	6.5	6.5	6.5	6.5

Moisture % of Cultured Soil:

Table 5: Moisture % Values for Cultured Soil

Trays	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Control 1	40%	45%	65%	65%	70%	75%	75%	80%
Tray1	70%	75%	78%	79%	85%	90%	91%	91%
Tray2	52%	55%	62%	70%	70%	80%	85%	93%
Tray3	50%	64%	64%	70%	81%	81%	82%	85%
Tray4	40%	66%	73%	82%	82%	90%	92%	92%
Control 2	40%	45%	65%	65%	70%	75%	75%	80%
Tray5	70%	75%	78%	79%	85%	90%	91%	91%
Tray6	52%	55%	62%	70%	70%	80%	85%	93%
Tray8	50%	64%	64%	70%	81%	81%	82%	85%
Tray9	40%	66%	73%	82%	82%	90%	92%	92%

Seed Germination Rate:

Table 6: Seed Germination % Values for Cultured Soil

Pots	Week 1	Week 2
Control 1	33.3%	50%
Pot 1	66%	83.3%
Pot 2	50%	66%
Pot 3	58.3%	75%
Pot 4	58.3%	66%
Control 2	33.3%	41.6%
Pot 5	75%	83.3%
Pot 6	66%	83..3%
Pot 7	58.3%	75%
Pot 8	58.3%	66%

Water Holding Capacity:

Table 7: Water Holding Capacity

Pots	Week 1	Week 2	Week 3
Control 1	40%	45%	48%
Pot 1	61%	73%	88%
Pot 2	48%	57%	69%
Pot 3	56%	59%	66%
Pot 4	61%	67%	73%
Control 2	40%	45%	48%
Pot 5	61%	72%	83%
Pot 6	47%	56%	63%
Pot 7	57%	63%	71%
Pot 8	61%	78%	82%

Shoot Length in Centimetre (cm):

Table 8: Shoot Length Values

Pots	Week 1	Week 2	Week 3
Control 1	1.1	1.9	2.8
Pot 1	1.4	2.2	3.3
Pot 2	1.3	2.8	3.0
Pot 3	1.3	2.8	3.3
Pot 4	1.3	2.7	3.2
Control 2	1.2	2.6	3.2
Pot 5	1.5	2.4	3.5
Pot 6	1.5	2.0	3.2
Pot 7	1.4	2.9	3.0
Pot 8	1.4	2.0	3.1

Root Length in Centimetre (cm):

Table 9: Root Length Values

Pots	Week 1	Week 2	Week 3
Control 1	0.1	0.4	0.9
Pots 1	0.4	0.8	1.2
Pots 2	0.3	0.6	1.1
Pots 3	0.3	0.7	1.1
Pots 4	0.4	0.7	1.1
Control 2	0.1	0.5	0.8
Pots 5	0.4	0.9	1.3
Pots 6	0.3	0.6	1.2
Pots 7	0.3	0.8	1.0
Pots 8	0.4	0.9	1.1

Number of Leaves Per Plant:

Table 10: Number of Leaves Per Plant

Pots	Week 1	Week 2	Week 3
Control 1	2	4	5
Pots 1	4	6	8
Pots 2	3	5	6
Pots 3	3	6	7
Pots 4	4	6	7
Control 2	2	3	4
Pots 5	5	7	8
Pots 6	4	6	7
Pots 7	3	4	5
Pots 8	3	5	6

DISCUSSION

Nutrient values (N, P, K) of different cultured trays, varies from tray to tray

The increasing order of N, P, K, Ph, moisture % values of trays are:
TRAY 1 > TRAY 5 > TRAY 2 > TRAY 6 > TRAY 4 > TRAY 8 > TRAY 3 > TRAY 7 > CONTROL 1 > CONTROL 2.

Water holding capacity, Seed germination rate, shoot length, root length, number of leaves per plant varies from pot to pot, the increasing order is:
POT 1 > POT 5 > POT 2 > POT 6 > POT 4 > POT 8 > POT 3 > POT 7 > CONTROL 1 > CONTROL 2.

The N-fixers bacteria showing good results on mustard plant in nutrient up taking such as N, P, K.

Bacillus species also showing good results on soil in case of water holding capacity.

Trichoderma viride showing good seed germination rate.

Mycorrhiza, vam power showing impact on root growth.

Among all the trays and pots showing good results in N-fixer bacteria combination later, bacillusspecies, then trichoderma, beauveria, pseudomonas, vam power.

These all characteristic are studied and compared with control 1 and control 2.

CONCLUSION

The bio fertilizers, bio pesticide, bio compost increase physio-chemical properties of soils such as soil structure, texture, water holding capacity and PH by providing several nutrients and sufficient organic matter. These are also well recognized as an important

component of integrated plant nutrient management for sustainable agriculture and are good tools to improve the crop yield, productivity and quantity, reduce the rate of chemical fertilizers and the cost of crop production and allow for better environment.

Bio fertilizer includes mainly the nitrogen fixing, Bacillus species, mycorrhiza and plant growth promoting microorganisms.

Bio pesticides are biological or biologically derived agents that are usually applied in a manner similar to chemical pesticides but achieve pest management in an environmentally friendly way. With all pest management products, but especially microbial agents, effective control requires appropriate formulation and application.

A major growth area for bio pesticides is in the area of seed treatments and soil amendments.

The specified biopesticide, biocompost, biofertilizer are very helpful to farmers as it increases the yield of plant and fertility of the soil, due to its economically low cost and is also eco-friendly.

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