

Comparative Efficiency of Compost and Bioaugmented Compost on Growth of Mustard Plant

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ABSTRACT

Organic compost is made up of decomposing plant and food waste, recycling organic material and manure. Compost holds a huge microbial population with huge amount of plant available nutrients. Present study was conducted to assess organic carbon and other nutrients and different microbial population in the organic compost which has been made in Raja Narendra Lal khan women's college (Autonomous). The physical and chemical properties of compost were measured and amount of organic carbon, phosphorous nitrate- nitrogen also measured. To cheek the fertility of compost, mustard seeds were planted in compost and soil with the addition of isolated microorganism cultures. The results highlight that the health of compost is very good than the normal soil and the augmentation of isolated microorganism cultures makes it more fertile. The field experiment to study the "Comparative" efficiency of organics and biofertilizers on growth of mustard plants (Brassica juncea.)" was conducted during 15th April to 30th April-2023 in our college premises. The mustard plant seed was used in the study. Present result show that compost with bacterial consortia (free living nitrogen fixing bacteria, cellulose degrading bacteria and phosphate solubilizing bacteria) have high germination rate compare to other studies. Similarly, shoot length and number of leaves was highest for compost with bacterial consortia. The present work concluded the advances knowledge and importance of microorganism involved in the composting process and how compost promotes the maintenance and multiplication of beneficial microbial consortia and their ecosystem functions in agricultural soils, shifting towards a more sustainable and resilient agriculture.

Introduction

Compost is a decomposed organic material that can be used to help grow plants and keep soil healthy. Compost means to put together the correct amounts of compostable materials to make a great soil amendment. (R. Azcorn *et al.*, 1975). It is the biological reduction of organic material to humus; it is made from residues of plants and/or animals that are piled, moistened and allowed to decompose, and bacteria, insects and worms in the pile help this break down.

Adding finished compost to your soil (T. Khaing *et al.*,2019).

- Improves the structure and health of your soil by adding organic matter.
- Helps the soil retain moisture and nutrients.
- Attracts beneficial organisms to the soil and reduces the need for pesticides and fertilizers.
- Reduces the potential for soil erosion.
- Sequesters carbon in the soil.
- Builds resiliency to the impacts of climate change.

Microorganisms found in compost are mostly beneficial, bacteria provide the fastest and most efficient composting by excreting nutrients, nitrogen, phosphorus, and magnesium (A.Bakar et al., 2013). Bacteria can be especially helpful to plants by enriching the soil in which the plants are growing. This enrichment provides key molecules plants need to survive and thrive. The function of bacteria can effect both water and nutrient availability in the soil. Some specific examples include nitrogenfixing bacteria, the role of nitrogen-fixing bacteria is to supply plants with the vital nutrient that they cannot obtain from the air themselves, nitrogen-fixing microorganisms do what crops can't - get assimilative N for them. Bacteria take it from the air as a gas and release it to the soil, primarily as ammonia. (A. Das et al., 2007) It is the only

suitable option for plants because they can consume N only from the soil and only as nitrogenous inorganic compounds, (G. Valdés et al., 2023) phosphate-solubilizing bacteria (PSB) play an essential role in P cycling and promoting plant growth by increasing its P uptake in rhizosphere soils. Most PSB produces indole-3-acetic acid (IAA) which enables plant cells to grow, RNA/protein synthesis thus increasing plant growth. Moreover, the microbial metabolites and low molecular weight organic acids released along with the metabolic processes of PSB are essential for P solubilization in soils, (Z. Wang *et al.*, 2022) The plant growth promoting activity of the cellulose degrading bacteria (CDB) isolates not only reduc.es the composting period but also improves plant growth. These native cellulolytic microorganisms, either alone or in consortia, could be useful for effective bioconversion of plant biomasses into enriched compost. (L. Hagos et al., 2020) Consortium application improves efficiency, consistency and reliability of microbes under different soil conditions. Microbial consortium can establish beneficial interactions with plants, promoting plant growth and development, increasing the plant defence system against pathogens. (G. Padmaperuma et al., 2020) Taking into account the details as mentioned earlier, the experiment was conducted to determine the efficiency of microbes present in compost on plant growth (B. Glaser et

al., 2001). Material and methods 1.1. Study area

The compost sample was collected from compost forming pit, present in Raja Narendra Lal Khan Women's College (Autonomous), Paschim Medinipur, West Bengal. The sample was collected in a sterilized plastic container and transported to the laboratory for microbial and chemical profile analysis.

1.2. Compost Sample Collection

The compost sample was collected from compost forming pit The sample was collected in a sterilized plastic container and transported to the laboratory for microbial and chemical profile analysis.



Fig-1 Compost forming pit

1.3. Compost Dilution Preparation

For compost sample, 10g was suspended in a 500 ml conical flask containing 100 ml of sterile water .The flask was shaken thoroughly by mechanical shaker for 5 min .The suspension was allowed to stand for 15 min to settle down the heavy particles and then the stock solution was prepared. sample from each stock solution was then serially diluted .During dilution 1.0ml suspension was taken and added to 9.0ml of sterilized distilled water in test tubes and thus 8 times diluted compost sample was made that is up to 10^{-8} dilution.

1.4. Enumeration of total bacteria number

Media Preparation

Enumeration of total bacteria was made by direct plate count method on Muller Hinton Agar media (Kaper *et al.*, 1977). The composition of the medium was(g/l): Beef extract 2.0; Acid Hydrolysate of Casein 17.50; starch 1.5; agar 20.0. The pH was adjusted to 7.0 before sterilization. The medium was sterilized for 15 min at 121ÚC.

Then 0.1 ml of the diluted compost sample $(10^{-6}, 10^{-7}, 10^{-8})$ was pipette out and poured aseptically in the respectively labelled sterilized petri plate (Muller Hinton agar medium) separately and spreaded All the plates were incubated for 24 hours at 37 úC in invert position.

Number of bacteria/gram of soil (CFU/g of soil) =CFU/ml sample X dilution factor (X. Luo *et al.*, 2017).

1.5. Enumeration of total nitrogen fixing bacteria

Media preparation

Enumeration of total nitrogen fixing bacteria was made by direct plate count method on Ashby media. The composition of the medium was(g/l) : Glucose 10.0 ; Di potassium phosphate 1.0 ; magnesium sulphate 0.5 ; sodium chloride 0.5 ; ferrous sulphate 0.1 ; sodium molybdate 0.005 ; calcium carbonate 2.0 ; agar 20.0 .The pH was adjusted to 7.0 before sterilization .The medium was sterilized for 15 minutes at 121ÚC.

Then 0.1 ml of the diluted compost sample $(10^{-6}, 10^{-7}, 10^{-8})$ was pipette out and poured aseptically in the respectively labelled sterilized petri plate (Ashby medium) separately and spreaded. All the plates were incubated for 5 to 6 days at 28ÚC in invert position (X. Luo *et al.*, 2017).

1.6. Enumeration of total phosphate solubilizing bacteria

Media prepatration

Enumeration of total phosphate solubilizing bacteria was made by direct plate count method on Pikovskaya's agar media. The composition of the medium was(g/l) : dextrose(D-glucose) 10.0 ; calcium phosphate 5.0 ; magnesium sulphate 0.1 ; potassium chloride 0.2 ; ammonium sulphate 0.1 ; yeast extract 0.5 ; manganese sulphate 0.0001 ; ferrous sulphate 0.0001 ; agar 20.0. The pH was adjusted to 7.0 before sterilization. The medium was sterilized for 15 min at 121ÚC.

Then 0.1 ml of the diluted compost sample $(10^{-6}, 10^{-7}, 10^{-8})$ was pipette out and poured aseptically in the respectively labelled sterilized petri plate (Pikovskaya's agar medium) separately and spreaded. All the plates were incubated for 4 to 5 days at

28ÚC in invert position (X. Luo et al., 2017).

1.7. Enumeration of total cellulose degrading bacteria

Media Preparation

Enumeration of total phosphate solubilizing bacteria was made by direct plate count method on carboxy methyl cellulose (CMC) agar media. The composition of the medium was (g/l): CMC 10.0; tryptone 2.0; magnesium sulphate 0.1; potassium di hydrogen phosphate 4.0; di sodium hydrogen phosphate 4.0; calcium chloride 0.001; magnesium sulphate 0.2; ferrous sulphate 0.004; agar 20.0. The pH was adjusted to 7.0 before sterilization. The medium was sterilized for 15 min at 121ÚC.

Then 0.1 ml of the diluted compost sample $(10^{-6}, 10^{-7}, 10^{-8})$ was pipette out and poured aseptically in the respectively labelled sterilized petri plate (CMC agar medium) separately and spreaded. All the plates were incubated for 2 to 3 days at 28ÚC in invert position (M. Benito *et al.*,2003)

1.8. Gram staining

The smear was prepared in a grease free glass slide it was firstly stained with crystal violet for 1 minutes gently the stain was washed off with tap water Gram's iodine was then applied to the smear for 1 minutes the Gram's iodine was washed off with tap water 95% alcohol was added to the smear drop by drop until the alcohol runs almost clear the 95% alcohol was washed off with tap water the smear was then counterstained with safranin for 45 seconds the safranin was washed off with tap water the slide was air dried and then it was observed under microscope (M. Hemalatha et al., 2002).

1.9. Estimation of percentage of organic carbon by walklay method of compost and back rapid titration methods present in compost sample.

Principle

The organic matter in the compost gets oxidized by chromic acid (in the potassium dichromate and sulphuric acid) utilizing the heat of dilution of sulphuric acid. The uncreated dichromate is determined by back titration with ferrous ammonium sulphate (Mohr salt) solution (redox titration) using barium diphenyl amine sulphate indicator.

Reagent preparation

Potassium dichromate(K₂Cr₂O₇) solution: 4.903 gm in 100 ml volumetric flask. It will be 1N.

Mohr Salt: 20 gm ammonium ferrous sulphate + 1 ml H_2SO_4 + 100 ml water.BDS: 15 mg BDS in 10 ml water.

Procedure

1 gm compost in a 500 ml conical flask 10 ml 1N potassium dichromate solution was added and 20 ml conc. H_2SO_4 was added the conical flask containing the sample and the above solution along with the conc. H_2SO_4 was left undisturbed for 30 minutes 200ml water was added and was left to cool 10 ml phosphoric acid was added 1 ml BDS indicator solution was added the solution was titrated against 0.5N Mohr salt solutions (M. Benito *et al.*,2003).

Calculation

% Of organic carbon= $3 \times (B - T/T)$ [B=

Required Mohr solution for blank

titration

T=Required Mohr solution for test titration]

1.10. Determination of phosphate phosphorous level present in compost sample

Reagent preparation

Blank: chloro molybdate + $H_{2}O$

Olsen reagent: 4.2 gm NaHCO₃ +H₂O Chloro molybdate solution: 1.5 gm ammonium molybdate + 30 cc HCL +70 cc H₂O

Stannous chloride: 1 ml S.C + 2.5 HCL(c). we take 0.153 ml in 10 ml distilled water.

Procedure

5 gm soil in 150 ml conical flask In 25 ml Olsen reagent was added, shaken for 30 minutes and filtered 10 ml of filtrate was taken in 50 ml volumetric flask 10 ml of chloro molybdate solution was added water was added to make up the volume up to 50 ml 1ml stannous chloride was added Reading in absorbance at 720 nm/660nm was taken (M. Hemalatha et al., 2002).

1.11. Estimation of percentage of total nitrate – nitrogen present by kjeldahl method in compost sample

Principle

Organic and nitrate nitrogen is converted to

ammonium sulphate and the ammonium is distilled into boric acid and titrated with HCL

or H₂SO₄ using appropriate indicator. Reagent

- 1. Digestion mixture:
- a. Potassium sulphate
- b. Copper sulphate
- c. Selenium

A,b,c are mixed with proportion of 10:1:0.5 respectively

- 2. H₂SO₄ Conc.
- 3. NaOH solution (40%)
- 4. H₃BO₃ solution (4%)
- 5. 0.001 N HCL

6. Tashiro's indicator (0.248 g methylene blue and 0.375 g methyl red dissolved in 300 ml ethyl alcohol, Allen, 1953)

Procedure

Digestion

1 Weigh 5 g compost into digestion flask.

2 Add 5 g digestion mixture and 20 ml H₂SO₄ Conc.

3 Put the flask on digestion board with electric heaters. Heat gradually; low at 10-30 minutes, then raise heating degree.

4 After the end of fuming, the digestion is continued for 1 hour after the solution had cleared with white colour of digestion mixture.

5 Transfer the sample to 250 ml volumetric flask, complete the volume with distilled water.

Distillation

1. Put 20 ml H_3BO_3 in Erlenmeyer flask and 4 drops of the indicator. Put the flask so that the lower tip of the glass receiver tube is below the boric acid surface.

2. Start running the cooling water in condenser.

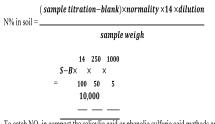
3. Start boiling the water in the boilers.

4. Put 25 ml of the sample in the funnel with distilled water. Released ammonia is trapped in boric acid (R. Raabe.,2004).

Titration

1. Ammonia is titrated with HCL or H₂SO₄ At end point the green colour just disappears.

Calculation



To catch NO3 in compost the salicylic acid or phenolic sulfuric acid methods are necessary.

To catch NO_3 in compost the salicylic acid or phenolic sulfuric acid methods are necessary.

Result

• Table-1: Isolation and enumeration of bacteria from compost

Sample	Dilution	Total bacteria count (CFU)/gm	N ₂ fixing bacteria count (CFU)/gm	Phosphate solubilizing bacteria (CFU)/gm	Cellulose degrading bacteria (CFU)/gm
compost	10-6	405×10 ⁷	30×10 ⁷	21×10 ⁷	50×10
	10-7	85×10 ⁸	20×10 ⁸	10×10 ⁸	10×10 ⁷
	10-8	63×10 ⁹	0	0	0



Fig: -2 Bacterial growth on Muller Hinton agar media

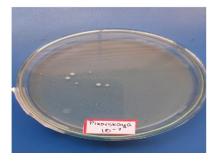


Fig: -4 Phosphate solubilizing bacteria Pikovskaya's agar media



Fig:3 N₂ fixing bacteria growth on Ashby **agar media**

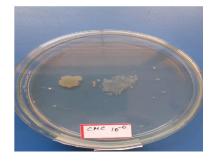


Fig:-5 Cellulosedegrading bacteria on growth on CMC agar growth media

Isolated bacteria	colour	Gram nature	shape
Nitrogen fixing bacteria	Pink	Negative	Rod
Phosphate solubilizing bacteria	Pink	Negative	Rod
Cellulose degrading bacteria	Violet	Positive	Rod

Table-2: Gram staining of isolated bacteria

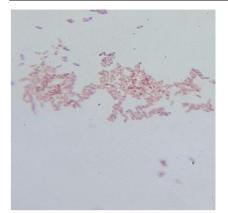


Fig: -6 Microscopic view of N₂ fixing bacteria

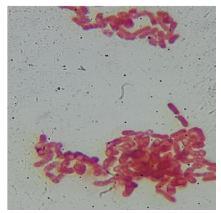


Fig: -7 Microscopic view of phosphate solubilizing bacteria



Fig: -8 Microscopic view of cellulose degrading bacteria

sample	Average percentage of organic carbon (%)	Average percentage of nitrate nitrogen (%)	Average percentage of phosphate phosphorus (%)
Soil	6.21% (observed)	0.72% (observed)	3.7% (observed)
	(12-18%, standard) (1.5-4%, standard)		(0.003-0.005%, standard)
compost	29.7% (observed)	1.07%(observed)	13.22%(observed)

Table-3: Chemical properties of soil and compost

Table-4: Rate of germination of mustard seed [Soil -200gm(T1), total seed-5]

		C1	C2	C3	C4
Date	Control	(Soil + Nitrogen	(Soil + Cellulose	(Soil +PSB)	(Soil + Mixture
		fixing bacteria)	degrading bacteria)		of cultures)
Day1	-	-	-	-	-
Day4	-	-	-	-	-
Day8	-	1(20%)	-	-	-
Day12	-	1(20%)	-	-	-
Day16	-	1(20%)	-	-	-

Table-5: Rate of germination of mustard seed [Soil 175 gm +Compost 25gm (T2), total seed- 5]

Date	Cont rol	C1 (Compost + Nitrogen bacteria) + Soil fixing	C2(Compost Cellulose bacteria) + Soil + degrading	C3(Soil +Compost +PSB)	C4(Compost +Soil +Mixture cultures) of
Day1	-	-	-	-	-
Day4	-	-	-	-	-
Day8	-	-	-	1(20%)	-
Day12	-	-	-	1(20%)	-
Day16	-	-	-	1(20%)	-

Table-6: Rate of germination of mustard seed [Compost 200gm (T3), total seed-5]

Date	Contro l	C1 (Compofixing Nitrogen bacteria)	C2(Compost +Cellulose degrading bacteria)	C3(Compost +PSB)	C4(Compost of +Mixture cultures)
Day1	-	-	-	-	-
Day4	1(20%)	2(40%)	-	4(80%)	5(100%)
Day8	1(20%)	5(100%)	2(40%)	5(100%)	5(100%)
Day 12	1(20%)	5(100%)	2(40%)	5(100%)	5(100%)
Day 16	1(20%)	5(100%)	3(60%)	5(100%)	5(100%)



Fig:9 - Day 1 application result



Fig:10 - Day 4 application result

Table-7: Measurement	of shoot	length	of mustard	plant	[Soil200gm (T1) total
seed-5]					

Date	Control	C1 (Soil + Nitrogen fixing bacteria)	C2(Soil + Cellulose degrading bacteria)	C3(Soil +PSB)	C4(Soil Mixture of culturs)
Dayl	-	-	-	-	-
Day4	-	-	-	-	-
Day8	-	1.5cm	-	-	-
Day12	-	2.5cm	-	-	-
Day16	-	2.5cm	-	-	-

Table-8: Measurement of shoot length of mustard plant Soil 175gm + compost25gm (T2) total seed -5]

Date	Contr ol	C1 (Soil+ Compost + Nitrogen fixing bacteria)	C2(Soil + Compost+ cellulose degrading bacteria)	C3(Soil +Compost +PSB)	C4(Compost +Mixture cultures) of
Day1	-	-	-	-	-
Day4	-	-	-	-	-
Day8	-	-	-	2cm	-
Day	-	-	-	2.5cm	-
12					
Day	-	-	-	3cm	-
16					

Table-9: Measurement of shoot length of mustard plant(T3) total seed-5]

[Compost 200gm

Date	Contro I	C1 (Compost+ Nitrogen fixing bacteria)	C2(Compost +Cellulose degrading bacteria)	C3(Compost +PSB)	C4(Compostof Mixture cultures)
Dayl	-	-	-	-	-
Day4	2.8cm	2cm	-	-	5cm
Day8	3cm	2.9cm	1.25cm	5cm	3.2cm
Day 12	4cm	3.5cm	2.25cm	4.4cm	4.1cm
Day 16	4.5cm	4cm	2.25cm	4.5cm	4.5cm

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Fig:11 - Shoot length of mustard plant

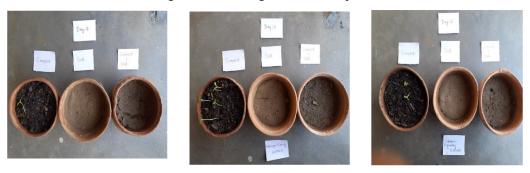




Fig: 12-Day 8 application result

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Date	Control	C1 (Soil + Nitrogen fixing bacteria)	C2(Soil + Cellulose degrading bacteria)	C3(Soil +PSB)	C4(Soil +Mixture of cultures)
Day1	-	-	-	-	-
Day4	-	-	-	-	-
Day8	-	2	-	-	-
Day 12	-	2	-	-	-
Day 16	-	3	-	-	-

Table-10: Number of Leaves of mustard plant[Soil 200gm (T1) total seed-5]

Table-11: Number of Leaves of mustard plant [Soil 175gm +compost 52gm (T2) total seed -5]

Date	Contro l	C1 (Soil + C + mpost Nitrogen fixing bacteria)		C3(Soil +Compost +PSB)	C4(Soil +Compost +Mixture of cultures)
Day1	-	-	-	-	-
Day4	-	-	-	-	-
Day8	-	-	-	2	-
Day	-	-	-	2	-
12					
Day	-	-	-	2	-
16					

Date	Contro l	C1 (Com + Nitrogen ^{fixing} bacteria)	C2(Compost +Cellulose degrading bacteria)	C3(Compost +PSB)	C4(Compost +Mixture of cultures)
Day1	-	-	-	-	-
Day4	2	2	-	2	2
Day8	2	2	2	2	2
Day 12	2	2	4&3	3Plant×3leaves 2Plant×2leaves	2Plant×4leaves 3Plant×3leaves
Day 16	3	3Plant×3leaves 2Plant×4leaves	4	3Plant×4leaves 1Plant×3leaves, 1plant×2leaves	4Plant×4leaves 1Plant×4leaves

Table-12: Number of Leaves of mustard plant [Compost 200gm (T3) total -5]





Fig:13-Day 12 application result



Fig:14 - Day 16 application result

Discussion

Application of isolated bacterial Consortia on Mustard plants:

Agricultural soil bacterial communities are essential for maintaining soil health and plant productivity. Soil bacteria perform multiple metabolic activities that allow, among others, the degradation of organic compounds, mineralization of nutrients, nitrification, and dissimilatory reduction of nitrate to ammonium and transform organic residues into plant nutrients such as amino acids, ammonium, phosphate, and potassium, among others (S. Wongkiew *et al.*, 2022). Many research studies suggest that an optimal ecology of agricultural techniques as manure application, organic farming, biochar modification, tillage management and

improvement of the soil carbon-nitrogen ratio promotes the bacteria and activity, increasing the efficiency of nutrient use by plants (T. Huang *el al.*,2014). Keeping the importance of compost and bacterial consortia, present project done on the application of isolated bacteria with compost on mustard plants. The diversity of bacteria in agricultural soils is diverse and depends largely on soil nutrition as shown for Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes which are fastgrowing copiotrophs when nutrients are abundant, while Acidobacteria is a slowgrowing oligotrophic bacterium but adaptable to low nutrients (S. Wongkiew et amendment, al.. 2022). Organic biostimulation, and biodegradation using nutrient-rich organic substrates are

recommended techniques to stimulate the establishment and activity of bacteria that degrade organic matter and release inorganic nutrients to plants through mineralization, which in turn improves the quality of agricultural soils. In general, it is suggested that healthy bacterial communities in soils could be maintained by adding organic matter at near-neutral pH levels (S. Wongkiew et al., 2022). It has been shown that the soil after compost application is dominated by bacteria. Compost treatment has been reported to impact the composition and activity of the soil microbial community, especially increasing the presence of Grampositive bacteria and rhizobacterial populations (C. Viti et al., 2010). Compost fertilizes the soil with nutrients that enhance the growth of organotrophic bacteria, oligotrophic bacteria, and actinobacteria, finding up to 383 operational taxonomic units (OTUs) (Z. Zhen et al., 2014) of the benefits of bacterial communities in agricultural soils has been proposed, the application of bacterial fertiliser to provide a variety of nitrogen-fixing bacteria and phosphorussolubilizing bacteria that could improve the soil microbial community structure (C.Viti et al., 2010).

Composting is an important technique in sustainable agriculture for the transformation of organic matter in fertiliser or organics amendments favouring soil fertility and pathogen suppression, while promoting microbial biodiversity in the soil. This will impact both the quantity and quality of crops and foods. It is currently widely used in organic and conventional agriculture throughout the world which indicates how the composting process is regulated by the succession of microorganisms and their environmental conditions while promoting sustainable agriculture (X. Liu el al., 2022). This wide microbial diversity gives it the properties that benefit soil fertility and health. Present studies in general show that the most relevant microorganisms in the composting process are free living nitrogen fixing bacteria and phosphate solubilising bacteria. The effect of compost and its components promotes sustainable agriculture. The composting process is determined by a set of successions of bacteria and fungi from diverse taxonomic groups. This wide microbial diversity gives it the properties that benefit soil fertility and health. Due to the environmental conditions, the different composting processes that exist, the different raw materials, and the initial microbial composition, it is difficult to generalize the microbial composition of the composting process (A. Anastasi el al., 2005)

Project work done the isolation of nitrogen fixing bacteria, phosphate solubilizing bacteria, cellulose degrading bacteria from compost.

Nutrient analysis report of organic carbon (29.7 %), nitrate nitrogen (1.07%) and

phosphate phosphorus (13.22%) which is higher than the normal soil (Table :3).

The population of isolated studied bacteria was comparatively higher which is more suitable for crops (Table:1). Isolated bacteria some are gram positive and some are gram negative (Table: 2).

After 16 days of observation, it was concluded that rate of germination of mustard seed was higher for compost with bacterial consortia than the other studies. (Table:4,5,6). So single bacteria are not enough for agriculture crops as a bio fertilizer but mixture of bacteria (consortia) is more suitable for the crops development because bacterial consortia provide different types of nutrients for the plants growth.

After 16 days' observation, it was also indicated that the compost with bacterial consortia show the higher growth of mustard plants (Table: 7,8,9,10,11,12).

Conclusion

According to project report, chemical properties and microbial population of compost showed that the compost is suitable for plant growth and health. In addition, microbial augmentation with compost was observed to be suitable for sustainable crops. The compost should be used widely in agriculture because of their low cost, good fertility of the soil and supplying more trace elements. It provides many essential nutrients for plant growth and therefore is often used as fertilizer. Therefore, prepared compost with bacterial consortia has greater potential for the development of crops.

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