International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 3 (2014) pp. 89-100**
http://www.ijcmas.com

Original Research Article

Bioremediation of vegetable wastes through biomanuring and enzyme production

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A B S T R A C T

Urbanization and industrialization accompanied by population flare-up has formed a serious problem of waste generation and its disposal, treatment and management. The solid wastes are generated more in some parts Salem city, Tamil Nadu. In the present study, the vegetable wastes were collected from various sources like vegetable market, reception halls, hospitals, schools and market areas which were mainly from Ammapet, Hasthampatti, Suramangalam and Kondalampatti region at Salem district. In this study, there are 40 different bacterial strains were isolated and identified. Among the strains, *Bacillus* sp. (B17), *Micrococcus* sp. (C3), and *Bacillus* sp. (P1) were identified as efficient starch hydrolyser and those were completely composted the market waste in very short duration when compared to the normal soil micro flora. The α-amylase enzyme assay also checked by Dinitrosalicylic Acid (DNS) method. In compost the NPK level was increased significantly and it could be helpful for the plant growth. In pot culture study, very lesser application of compost (2:1 - soil: compost) showed best results.

Keywords

Biomanureing; compost formation; amylase enzyme; waste management.

Introduction

Global increase in urbanization accompanied by population flare-up has formed a serious problem of waste generation and its disposal, treatment and management. Fruit and vegetable wastes (FVW) are produced in large quantities in markets and constitute a source of nuisance in municipal landfills because of their high biodegradability (Viturtia *et al*., 1989). In India, FVW constitute about more than 10 million tonnes annually and currently these wastes are disposed by dumping on the outskirts of cities. The main contributors of waste generation in Indian society are agriculture and municipal sectors. Industrial sectors have also not been able to handle and treat the wastes generated by them or control the emission of obnoxious gases into the atmosphere. In spite of the various environmental rules and regulations, very little has been achieved in terms of minimization of waste generations. In Indian cities total quantum of waste generation is increasing at rate of 1.33% (Srilatha *et al*., 1995). The solid waste

generated in the Salem city of Tamil Nadu from various sources like marriage receptions, hospitals, schools and market areas was collected from four zones viz Ammapet, Hasthampatti, Suramangalam and Kondalampatti. The MSW collected from all the zones was estimated at 335 MT per day including vegetable market waste of 24 MT. The total MSW generated is being dumped at Erumapalayam dumping yard for the past four decades without proper treatment. The total extent of the dumping area is about 22 acres and presently overflowing (Karthikeyan et al., 2007).

Composting is generally defined as the biological oxidative decomposition of organic constituents in wastes under controlled conditions which allow development of aerobic micro-organisms that convert biodegradable organic matter into a final product sufficiently stable for storage and application without adverse environmental effects (Nengwu, 2006; Lin, 2008). Because composting is an efficient method for recycling waste (Dumitrescu et al., 2009).

The enzyme α- Amylases are the first enzymes to be commercially produced and marketed. Dr. J. Takamine established the first industrial production of α-amylase from *A. oryzae* known as Taka diastase, which was used as a digestive aid. The global market for enzymes was about $2 billion in 2004. It is expected to have an average annual growth rate of 3.3 %. The share of carbohydrates comprising amylases, isomerises, pectinases and cellulases is about 40 % (Riegal and Bissinger, 2003). The food and beverage sectors utilize 90 % of the carbohydrases produced. The annual sale of α-amylases in global market is estimated to be $11 million (Kilara and Desai, 2002). The world production of a-amylases from *B. licheniformis* and *Aspergillus* sp. was about 300 tonnes of pure enzyme protein per year (Gupta et al., 2003). During the course of this investigation, isolate bacterial strains present in the market waste vegetables and screen that are capable to produce α-amylase by starch hydrolysis method and to form compost by using starch degrading bacterial strains in the presence of market waste and evaluate the soil: compost ratio to suitable for plant growth and compare composts produced by natural soil micro flora and specific bacterial strains by plantation study finally asses the production of α-amylase by market waste vegetables.

Materials and Methods

Sample Collection and Processing

Market waste vegetables (Beetroot, Carrot and Potato) were collected from Uzhavar Sandhai, Dadagapatti gate, Periyar Colony, Salem, Tamil Nadu. The samples were transport to the laboratory using sterile plastic bags for further analysis. The spoiled vegetables were crushed by Mortar and pestle and from that the bacterial colonies were enumerated by serial dilution method. The colonies were counted and enumerated and morphologically different strains were selected and stored at 4^{o}C in agar slants.

Screening and identification of amylase producing bacteria

Starch agar medium was prepared and sterilized. The medium was poured into sterile plates and allowed for solidification. All the test organisms were inoculated by spot inoculation and they were incubated at 37^{o}C for 24-72 hours. After incubation the medium was flooded

with iodine solution. The iodine reacts with starch to form a dark blue-coloured complex. Clear area around the growth of the culture after the addition of the iodine indicates the breakdown of starch by the organism due to its production of amylase. The clear zone around the colonies were measured and noted. The starch hydrolysing bacterial strains were identified by biochemical analysis according to the bergey's manual of determinative bacteriology.

Compost formation

The lab scale compost was done by tray method. Here two types of compost setup created. First one was compost-I, using sterile soil with inoculums and the second one is compost-II, non-sterile soil without inoculums. The spoiled root crops vegetables were collected and weighed. The vegetables were cut at small pieces. The plastic trays were sterilized by surface sterilization. Totally six layers formed by soil and vegetables. In compost-I had sterile soil mixed with specific bacterial strains. The non-sterile soil had natural microbial population. The tray setups were keeping it in sunlight for 1 month duration.

Plantation for analysing compost and soil ratio

Effects of microbial compost on the growth of plants were studied under laboratory condition. The green gram and black gram seeds were selected for plantation. The different soil and compost ratio were prepared. Compost-I and soil ratio was 1:1, 2:1, 3:1, 4:1, and compost as a control. And the same the compost-II and soil ratio were prepared. Totally 4 sets of pots were created (compost-I: soil, soil: compost-I and compost-II: soil, soil: compost-II). After plantation each and every day the growth of the plant were measured and tabulated. This process maintained up to 10 days. After duration the plants were plugged from pots and the roots were rinsed with water, and root length was measured, the nodules were counted and tabulated.

Inoculum preparation and inoculation

About 100 ml sample of nutrient broth solution with 2% Nutrient broth (Hi-Media, India) was sterilised at 121°C for 20 min and cooled, and was inoculated under aseptic conditions with Specific bacterial strain, from a nutrient agar slant. The broth culture was incubated for 24 h on a rotary shaker (150 rpm) at 30°C (Chundakkadu, 1999).

Enzyme production

The vegetables (Carrot and Potato) were weighed (each 50 gm) and crushed by mortar and pestle. The crushed vegetables were sterilized by autoclaving. The 2000 ml aspirator bottle were taken and sterilized. The crushed and sterilized vegetable mixes were transfer to aspirator bottle in aseptically and make up the volume at 1000 ml. And add 10 ml of 24 hrs bacterial inoculums. This set up was keeping in agitation by magnetic stirrer. The 10 ml of sample was collected every day for enzyme assay.

Amylase enzyme assay by DNS (3, 5-dinitrosalicylic acid) method

The crude enzyme sample (500 µl) and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) were incubated at 25^{o}C for 10 min. Then, 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M

NaCl) was added to each tube. The reaction mixtures was incubated at 25oC for 10 min and stopped with 1.0 ml of dinitrosalicylic acid colour reagent. Thereafter, the mixture was incubated in a boiling water bath for 15 min, and cooled to room temperature. The reaction mixture was then diluted by adding 10 ml of distilled water, and absorbance measured at 540 nm (Ganiyu et al., 2012).

Results and Discussion

The total of 6 market waste samples processed by serially dilution plate count method and bacterial population were enumerated (Table 1). Morphologically different kinds of bacterial strains (40 Strains) were found in spoiled vegetables and the results were noted (Table-2). Based on the screening (starch hydrolysis test) three bacterial strains (B17, C3, P1) were able to degrade starch. In this the B17 were formed 2.2 cm zone in starch agar plate. According to the identification these three strains are *Aerobic bacillus* B17, *Micrococcus SPP* C3, *and Aerobic Bacillus* P1 (Table-3). In the same revealed Vihinen and Mantasala, 1989 that among bacteria, *Bacillus* sp. is widely used for thermostable α-amylase production to meet industrial needs. *B. subtilis, B. stearothermophilus, B. licheniformis* and *B. amyloliquefaciens* are known to be good producers of α-amylase and these have been widely used for commercial production of the enzyme for various applications. Pandey et al., 2000 said that *Bacillus* species are considered to be the most important sources of α-amylase and have been used for enzyme production using SSF.

The compost formation study, the two type of tray maintained (Table-4; Fig-4). In this compost-I was changed black in colour and the market waste was ruined quickly compare to the compost-II. Here, the vegetable wastes act as a carbon and nitrogen source for bacterial strain. The starch degrading bacterial strains utilizes the starch in the vegetable waste. Djekrif et al., 2005; Haq et al., 2005; Swamy and Seenayya, 1996 exposed that are Agricultural wastes (orange waste, pearl millet starch, potato, corn, tapioca, wheat and rice as flours) are being used for composting.

After one month composting period the plantation study were proceeded. In this different ratio of soil and compost were prepared in plastic cups. The leguminous plant seed such as green gram and black gram was planted. This also revealed the same, the compost-I quick germination and the growth were serially increased. But the compost-II germination was started at second day and the growth also in very slow. The plant growth was measured every day and results were tabulated (Table 6 and 7). After one month composting period the plant were plugged out and the root length was measured and the nodule formation also counted and tabulated (Table 6, Table 7). Based on this result both green gram and black gram seeds the 2:1 ratio is very effective to plant growth.

This ratio was equal efficiency to normal fertilizer soil but the root length and nodule formation is very high in compost I (2:1) (Fig-1, Fig-2), because the nodule formation and root length also affect plant growth and yield. And also depend on macro nutrients like Nitrogen (N), Phosphorus (P) and Potassium (K) which are essential for the growth of plants and crops. In here the N, P, K level of the compost I and II were increased ultimately with in short period (Fig-3). Compare to compost II the compost I had

Table.1 Processing of Vegetable Waste Sample by Pour Plate Method

S. No.	Sample	10^{-3} CFU/g	10^{-4} CFU/g	10^{-5} CFU/g
1.	Market waste 1	40	18	13
2.	Market waste 2	1280	650	345
3.	Market waste 3	45	10	2
4.	Market waste 4	1280	1240	684
5.	Market waste 5	550	179	88
6.	Market waste 6	1680	720	427

Table.2 Microbial Population Present In Vegetable Waste

S. No.	Sample	Morphology	No. of Colonies	Dilution
1.	B1	White colour rough colony	1	10^{-3}
2.	B2	White colour mucoid colonies	15	10^{-3}
3.	B3	Yellow colour spreaded colony	1	10^{-4}
4.	B4	Deep yellow colour mucoid colonies	28	10^{-4}
5.	B5	Light red colour colonies	3	10^{-5}
6.	B6	White spreaded colonies	2	10^{-5}
7.	B7	White colour smooth colonies	26	10^{-5}
8.	B8	White colour smooth colonies	3	10^{-5}
9.	B9	White colour smooth small colonies	20	10^{-5}
10.	B10	White colour spreaded colonies	2	10^{-5}
11.	B11	White colour small colonies	2	10^{-5}
12.	B12	White colour rough colonies	2	10^{-5}
13.	B13	White colour spreaded colony	1	10^{-5}
14.	B14	White colour spreaded colonies	5	10^{-4}
15.	B15	Yellow colour colonies	42	10^{-5}
16.	B16	White colour smooth colonies	4	10^{-5}
17.	B17	Yellow colour smooth colonies	5	10^{-5}
18.	B18	White colour smooth mucoid colonies	8	10^{-5}
19.	B19	Red colour smooth colonies	4	10^{-5}
20.	C1	White colour mucoid colonies	8	10^{-3}
21.	C2	White colour smooth mucoid colonies	1	10^{-3}
22.	C3	Rough White colour colonies	1	10^{-3}
23.	C4	White colour colonies	2	10^{-4}
24.	C5	Light yellow colour spreaded colony	1	10^{-5}
25.	C6	White colour mucoid colonies	20	10^{-5}
26.	C7	White colour mucoid colonies	48	10^{-5}
27.	C8	White colour smooth colonies	8	10^{-5}
28.	C9	Dirty white colour colonies	12	10^{-5}
29.	P1	Rough white colour colonies	1	10^{-4}
30.	P2	White colour smooth big colonies	3	10^{-4}
31.	P3	White colour mixture colonies	3	10^{-4}

32.	P4	White colour mucoid colonies	30	10^{-5}
33.	P5	Yellow colour colonies	20	10^{-5}
34.	P6	Yellow colour spreaded colonies	2	10^{-5}
35.	P7	Small in white colour colonies	12	10^{-5}
36.	P8	White colour single colony	22	10^{-5}
37.	P9	White colour spreaded colon ies	9	10^{-5}
38.	P10	White colour rough colonies	3	10^{-5}
39.	P11	White colour spreaded colonies	15	10^{-5}
40.	P12	Dirty whitecolour spreaded coonies	12	10^{-5}

Table.3 Starch hydrolysis test

S. No.	Strain. No	Zone of Inhibition(Cm)
1.	B17	2.2
2.	C3	1.6
3.	P1	1.7

Table.4 Formation of Soil and Market Waste layers for Compost preparation for screening amylase producing organisms

S. No.	Layer	Compost-I		Compost-II	
		Sterilized Soil with *Bacillus* Sp B17	Kg	Non-Sterilized Soil	Kg
1.	I	Vegetables waste	600g	Vegetables waste	600g
2.	II	Soil	1.5g	Soil	1.5g
3.	III	Vegetables waste	600g	Vegetables waste	600g
4.	IV	Soil	1.5kg	Soil	1.5kg
5.	V	Vegetables waste	600kg	Vegetables waste	600kg
6.	VI	Clay Soil	1kg	Clay Soil	1kg

Plate.1 Graphical modal for compost formation

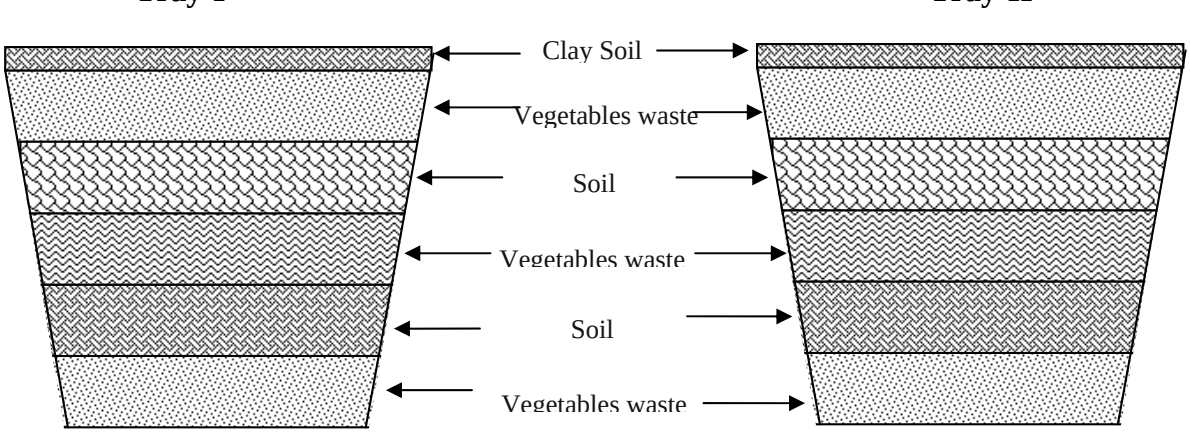


Table.6 Growth rate and Nodule formation in Different Ratio of Sterile soil and compost

Seed	Ratio	Growth Rate (cm)										Root Length (cm)	Nodules Formation (No's)
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day		
Green Gram	C-I ; S:C ; 1:1	G	G	G	G	G	3	3.5	4	4.3	4.5	3	-
	C-I ; S:C ; 2:1	G	1	6	9	9.5	10.5	12.5	13.5	14	14.5	7	28
	C-I ; S:C ; 3:1	G	G	5	7.5	9	9.5	10	10.5	11	11.5	-	-
	C-I ; S:C ; 4:1	G	G	G	G	G	-	-	-	-	-	5.5	10
	C-I ; Soil Control	G	6	9	10	11.8	12	12.5	13.5	14	14.5	3	17
	C-I ; C:S ; 1:1	G	G	1	5	6.5	6.5	8	8.4	8.5	9	5.5	-
	C-I ; C:S ; 2:1	G	G	G	G	1	4.5	5	5.4	5.7	6	5	-
	C-I ; C:S ; 3:1	-	-	-	-	-	-	-	-	-	-	-	-
	C-I ; C:S ; 4:1	G	G	G	1	3	4	4.5	5	5.8	6	1.5	-
	C-I ; Compost Control	-	-	-	-	-	-	-	-	-	-	-	-
Black Gram	C-I ; C:S ; 1:1	G	G	6.5	8.5	10.8	11.5	11.8	12.5	14.8	15.5	7	8
	C-I ; C:S ; 2:1	G	G	4	6.3	7.5	8.5	9.3	9.5	10	10.5	8	4
	C-I ; C:S ; 3:1	G	G	1	4.5	6	7	8.5	9	9.5	10.5	4.2	3
	C-I ; C:S ; 4:1	G	G	G	1	4.5	5.5	6.5	7.5	9	9.5	6	-
	C-I ; Compost Control	-	-	-	-	-	-	-	-	-	-	-	-
	C-I ; S:C ; 1:1	G	G	G	G	G	G	G	G	G	G	-	-
	C-I ; S:C ; 2:1	G	2.5	7	8	9	9.5	10	10.5	11	11.5	7	30
	C-I ; S:C ; 3:1	G	1	7	6.5	8.2	9	9.5	10	10.7	11	5	21
	C-I ; S:C ; 4:1	G	G	4	6.5	8	9.8	10	11	12	12.5	6	6
	C-I ; Soil Control	G	6	10	12	13	13.5	14.5	15	16	16	8	28

C-I: Sterile Soil for compost, C-II: Non Sterile Soil for compost, C: Compost, S: Soil, G: Germination

Table.7 Growth rate and Nodule formation in Different Ratio of Non Sterile soil and compost

Seed	Ratio	Growth Rate (cm)										Root Length (cm)	Nodules Formation (No's)
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day		
Green Gram	C-II ; C:S ; 1:1	G	G	G	G	3	3	5	5.5	6	6.5	2	-
	C-II ; C:S ; 2:1	-	G	G	2	4.5	6	7	8	9	9.5	4	-
	C-II ; C:S ; 3:1	-	G	G	G	3	5	6	6	6	6	4	-
	C-II ; C:S ; 4:1	-	-	-	-	-	-	-	-	-	-	-	-
	C-II ; Compost Control	-	-	-	-	-	-	-	-	-	-	-	-
	C-II ; S:C ; 1:1	G	G	G	1	2	3	4.5	5	5.8	6	2.5	-
	C-II ; S:C ; 2:1	G	G	G	3.5	5.5	7	8.5	9	9.5	9.8	6	-
	C-II ; S:C ; 3:1	-	-	-	-	-	-	-	-	-	-	-	-
	C-II ; S:C ; 4:1	-	-	-	-	-	-	-	-	-	-	-	-
	C-II ; Soil Control	G	1	7	9	11	12.5	14	15	16	16	6	6
Black Gram	C-II ; C:S ; 1:1	G	G	2	3	5	4.5	7.5	8.5	10.3	11.5	5.5	5
	C-II ; C:S ; 2:1	-	-	-	-	-	-	-	-	-	-	-	-
	C-II ; C:S ; 3:1	-	-	-	-	-	-	-	-	-	-	-	-
	C-II ; C:S ; 4:1	G	G	G	1	3	4	6	8	9	9.5	4.5	-
	C-II ; Compost Control	_	G	G	1	2	1	2.5	2.5	2.5	2.5	-	-
	C-II ; S:C ; 1:1	G	G	G	1	3	4	6	8.5	9	9.5	5.2	6
	C-II ; S:C ; 2:1	G	G	1	6.5	7	8.5	9.5	10	11.5	12	6	6.6
	C-II ; S:C ; 3:1	G	G	5.5	6.5	7.5	8	8.6	9.5	10.8	11.5	5.5	6
	C-II ; S:C ; 4:1	_	G	G	5	5	7	7.5	8.5	9.2	9.5	7	18
	C-II ; Soil Control	G	G	8.8	11.5	13	13.5	14.8	16	16.5	17.5	10.2	24

C-I: Sterile Soil for compost, C-II: Non Sterile Soil for compost, C: Compost, S: Soil, G: Germination

Fig.1 Growth rate of sterile soil and compost

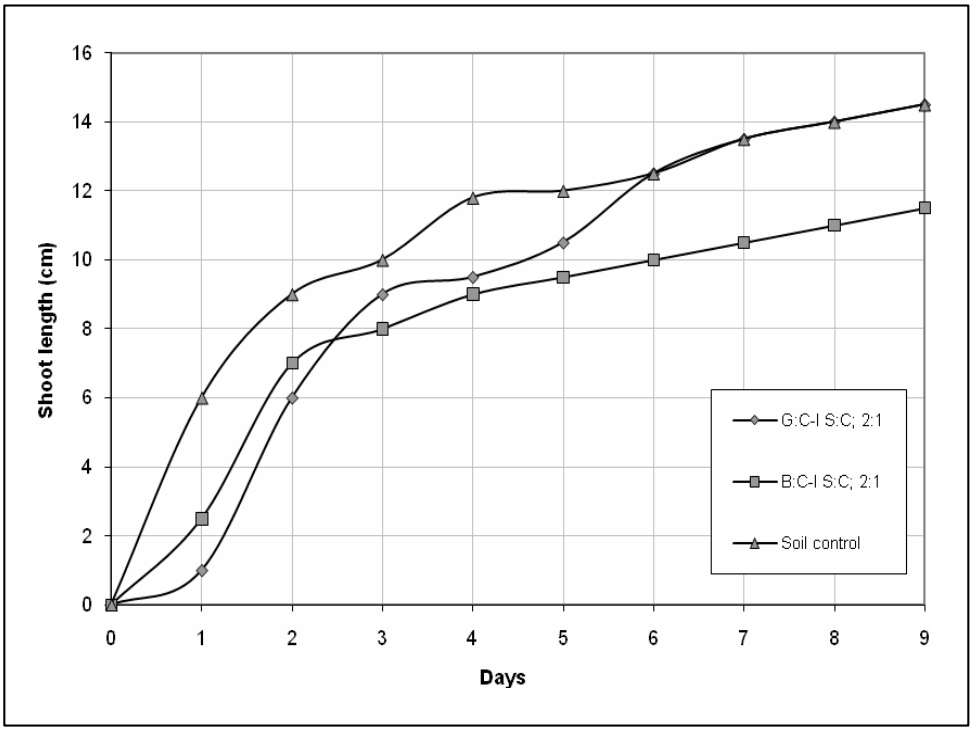


Fig.2 Root length and Nodule formation in sterile soil and compost

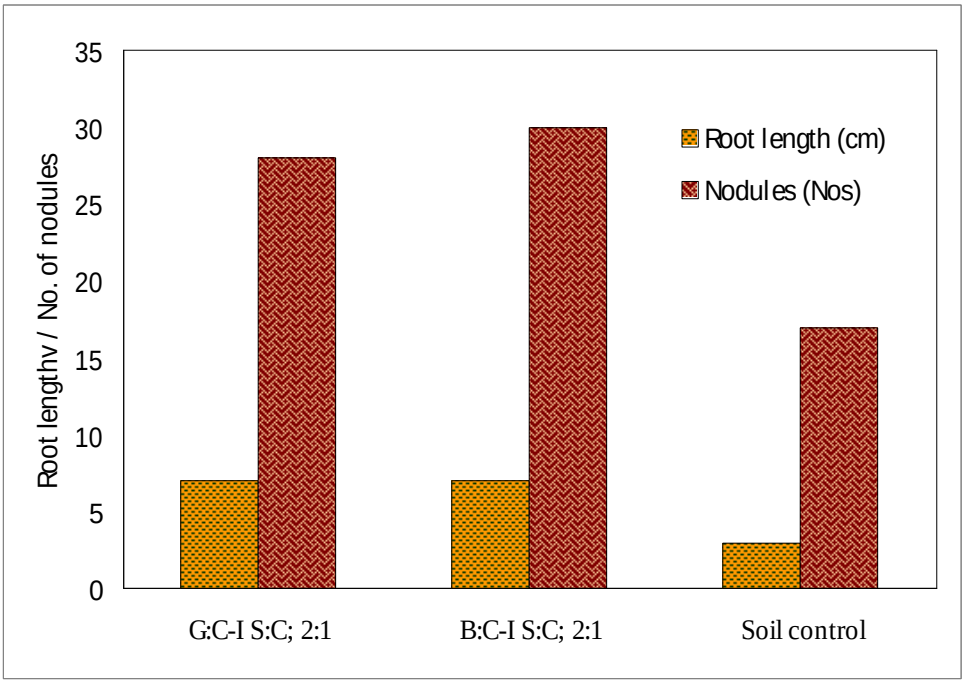


Fig.3 pH, Electrical Conductivity (EC), Nitrogen (N), Phosphorus (P), and Potassium (K) Levels in Natural Soil, Compost-I and Compost-II

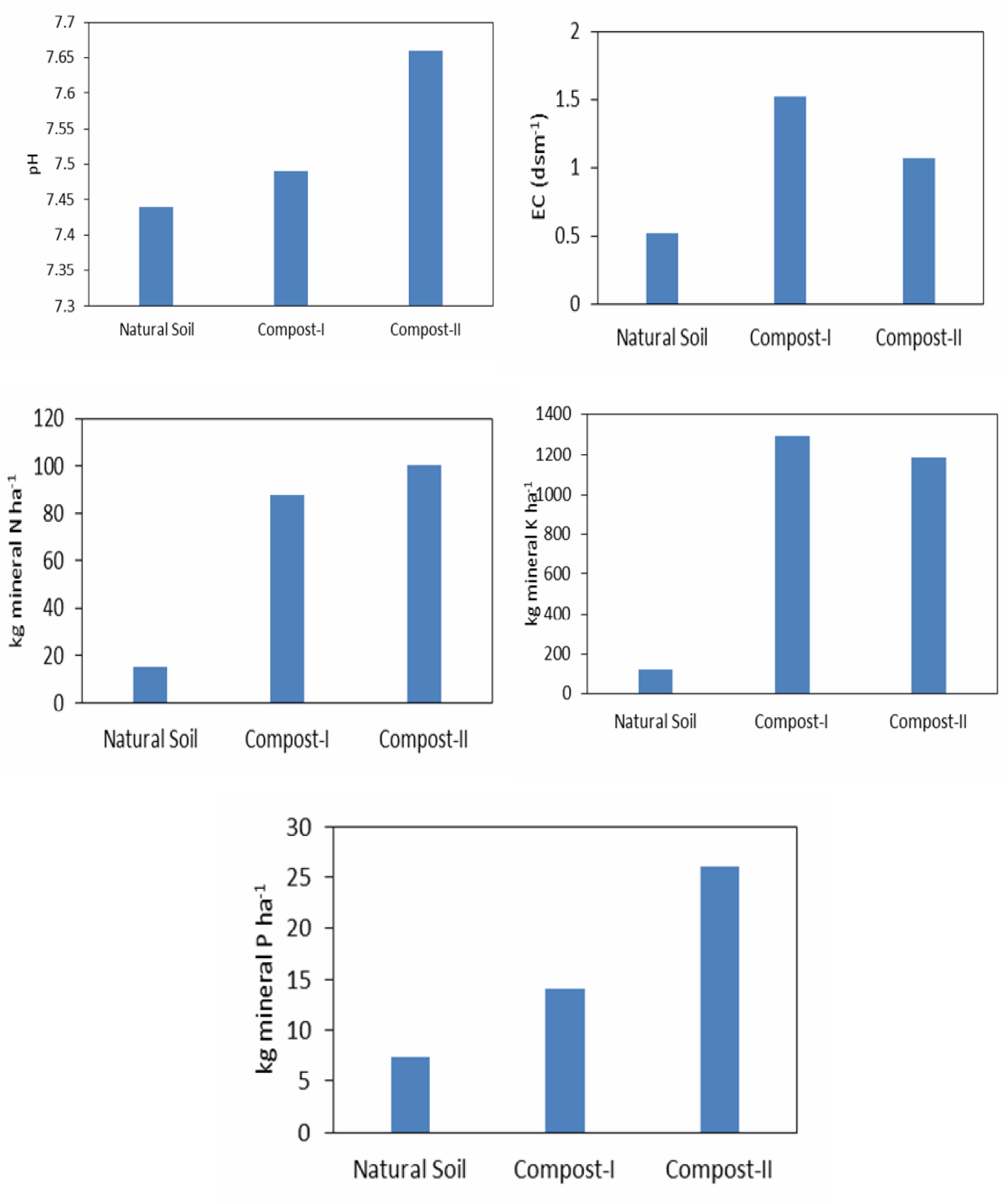
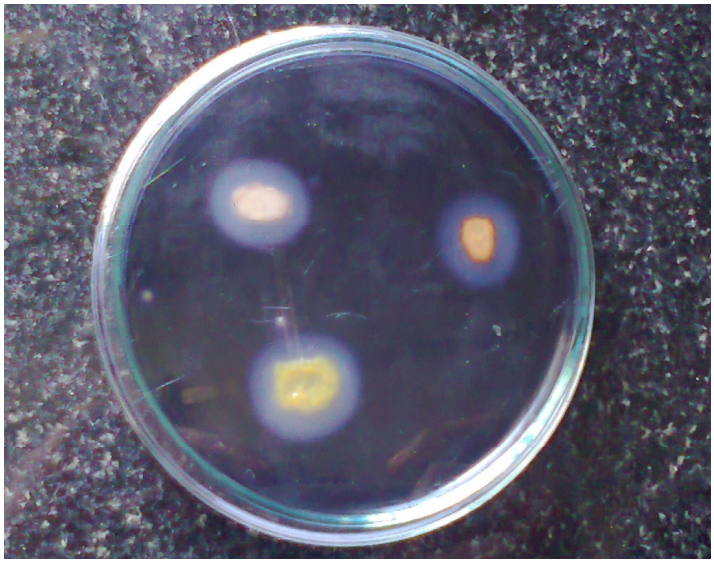


Fig.4 Starch Hydrolysis Test for *Bacillus* sp *B17, Micrococcus* sp *C3, Bacillus* sp *P1* isolated from Market waste vegetables



low NPK level, but in the plantation study the compost I gave good results compare to compost II, it may be the high amount of macro nutrients may affect the plant growth. In compost I the bacterial strain *Bacillus* sp optimize the macro nutrients and compost the market waste vegetables and also the compost I were give good plant growth in very low ratio of soil and compost (2:1). The market waste vegetables were taken under the starch rich vegetables. In this amylase activity also give suitable result. One unit of amylase activity was defined as the amount of enzyme that releases 1 mol of reducing sugar as glucose per min, under assay conditions and expressed as U/g of dry substrate. Enzyme activity in units can be measured by using the formula below:

$$\text{Enzyme activity} = \frac{\text{mg /ml maltose released x 0.36}}{\text{Volume of enzyme taken x incubation time}}$$

The α- amylase production was observed by DNS method (Table-8). On the basis of amylase productivity level in aspirator bottle cultures after 72 h of growing, growth medium containing vegetable waste was selected as the best medium. Moreover, α-amylase biosynthesis appeared to be independent of starch availability. In vegetable waste the 0.001466 U/mL of amylase to be secreted after 12 h of cultivation. But the production was decreased at the time of increasing the incubation period.

In this study the market waste completely composted in very short duration compare to the normal soil micro flora. Based on the pot culture result the *Bacillus* Sp B17 was able to form efficient compost by using market waste compare to the normal soil micro flora. In this the soil and compost (2:1) ratio is identified as an efficient one for both plants. This ratio give good plant growth, high amount of nodule formation and root length. The NPK levels also increased very high in short period in compost-I. In starch hydrolysis test the *Bacillus* Sp B17 was given a good zone were comparing to others.

So this strain was selected for α- amylase production. The aspirator bottle study was used in this enzyme production. The enzyme content was assessed by Dinitrosalicylic Acid (DNS) every day of the duration (10days).

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