Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

Assessment of compost as a bio-fertilizer for the growth of paddy

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Publication Info

Paper received:
26 February 2011

Revised received:
20 May 2012

Re-revised received:
30 July 2012

Accepted:
22 October 2012

Abstract

The vegetable wastes were converted into compost by a stepwise degradation and its characteristics were studied and analysed at each stage. The temperature increased from 29^{0}C to 60^{0}C on 60th day and reached 33^{0}C on 90th day. Shift of pH from 7.6 to 7.3 on 60th day caused a shift of microflora from 12.01×10^7 to11.13×10^8 CFU ml^{-1} on 30th day and63.2×10^6 on 60th day and 36.75×10^6 on 90th day. Shift of microflora caused high decomposition of the waste into compost which were used for enriching the soil as manures. The other characteristics such as moisture, ash content and C:N ratio established the short period required for preparing a complete compost of good quality. The study showed the efficiency of these organisms as plant growth promoting rhizobacteria. Combinations of microorganisms with compost act as a good biofertilizer which improves the fertility of soil and increases plant growth. Better results were produced by organisms in combinations like *Azospirillum*, *Rhizobium* and *Azotobacter*. The least growth in shoot length (64 cm) total fresh weight (151g) and total dry weight (3.994 g) were observed in paddy grown in soil and *Bacillus* combination, but microbial mixture of compost and soil gave high paddy growth efficiency. The present study concludes that the rhizospheric organisms play well as plant growth promoting agents and gave a better yield and growth of plants in combination with the compost.

Key words

Biofertilizer, Compost analysis, Paddy growth

Introduction

The mineral nutrient status (including macro and micro) of the soil is considered as most essential for plant growth and yield. The mineral elements are known to be involved in metabolic functions of plants without which the plants cannot complete their life cycle (Choudhary *et al.*, 2005.). The nutrient status is known to vary depending upon the type, physico-chemical and biotic components of different soils. To enhance the nutrient level of the soil support plant growth, application of microbial fertilizers in agricultural lands has been a regular practice by the farmers (John.M.Whipps, 2000). Though the microbial fertilizers are known to enhance the NPK level of the soil to support the plant growth, continuous application of combined microbial fertilizer is reported to affect the soil fertility in the long run. Intensive use of only chemical fertilizers to achieve high production has created various problems. Some of the microorganisms like, *Azospirillum* and *Azotobacter* are known to increase the growth, yield and quality of crops by fixing atmospheric nitrogen, synthesizing plant growth hormones besides using organic manures and chemical fertilizers in combination. Plant growth promoting rhizobacteria (PGPR) are a heterogenous group of bacteria that can be found in rhizosphere at root surface and in association with roots which can improve the quality of plant growth (Joseph *et al.,* 2007) Plant growth promoting rhizobacteria use one or more direct mechanism of action to improve plant growth and health. This mechanism may be sequential or active simultaneously at different stages of

plant growth (Chaiharan *et al.,* 2008). The plant growth promoting rhizobacteria trigger an increase in root surface area which results in increased mineral uptake (Mantalin and Tournamine, 2003 and Kenjiiyama, 1994). So keeping in view the above constrains, the present study was conducted to screen and analyse the activity of plant growth promoting rhizobacteria (PGPR) belonging to genera *Rhizobium*, *Bacillus*, *Pseudomonas*, *Azotobacter* and *Azospirillum* in combination with compost to analyse their effect on the growth of paddy by pot culture technique.

Materials and Methods

Substrate and sample collection : The vegetable waste was collected from the canteen of Jaya College of Arts and Science, Thiruninravur, Chennai. The vegetable waste containted peels of onion, beans, carrot, cabbage, lady's finger, beetroot, tomato, greens and potatoes.

A pit of 1m X 1m X 1m was dug and piled with the vegetable waste mixture. A 10cm of vegetable waste was over laid with 10 cm of soil and the process was repeated to fill the pit. The pit contained 5 piles of vegetable waste over laid with 5 piles of soil. It was moistened with water and covered with straw to maintain the moisture. The compost was turned once in 15 days

Collection of sample : The sample of vegetable waste compost was collected at a depth of 25 cms for determining of temperature, moisture, ash content, analysis of nitrogen and carbon, and assay for bacterial and fungal contents as well as cellulose activity. The temperature was determined by inserting the thermometer 25 cm deep into the pile or composting wastes. The samples were collected from different 6 parts of composting wastes with long forceps and 100 mg of each sample was added to double amount of distilled water and mixed with magnetic stirrer for 15 min. The mixture was filtered 4 times and centrifuged at 1500 rpm for 5 min. The supernatant was collected and pH was analysed using pH meter by using distilled water as control. 10 mg of sample was weighed and heated in a heating mantle at 550ºC for 3 hrs and the remains were weighed for ash content analysis. 10 mg of sample was weighed and kept in hot air oven at 100ºC for 72 hrs and the remains were weighed and moisture content was calculated.

Analysis of compost : The total nitrogen content was estimated by Micro Kjeldhal method. The moisture and carbon was estimated following the method suggested by Jackson (1975). Microbial load was enumerated by standard plate count method from 10^{-1} to 10^{-12} dilutions using plate count agar, nutrient agar Sabraouds dextrose agar and PBYG medium. After incubation, the Petriplates were examined carefully for morpholocial similarity and dominant bacterial colonies were selected and poured on to the PBYG medium (Alsina and Blanch, 1994)

One gm of soil sample was weighed and dissolved in 99 ml of distilled water and plated on Wilson Blair bismuth sulphite medium, deoxy cholate agar, *Salmonella* and *Shigella* agar, eosin methylene blue agar, Mac conkey agar, nutrient agar. Colonies were confirmed by standard biochemical assessments. Degrading efficiency of cellulose was estimated by the method of Kiyohika (1985).

Plant growth promoting rhizobacterial analysis : The organisms isolated were tested for the production of ammonia in peptone water incubated for 48 hrs at 30ºC. Addition of Nesslers reagent, a filtrate of mercuric iodide (100µg), potassium iodide (90µg), distilled water (400µl) mixed with (100µg) sodium hydroxide in 500µl of distilled water, induced brown to yellow color indicating production of amonia. The isolated rhizospheric organisms were inoculated in sterile nitrite broth and incubated at 37ºC for 24 hrs. Nitrate reagent A and B were added to observe a red colour change. The isolated organisms were tested for carbohydrate metabolism in a medium containing 2g peptone, 5g NaCl, 0.3g K_2HPO_4,3 ml phenol red, 3g agar and 10ml distilled water. The oxidation and fermentation of carbohydrate were determined by change in colour.

Pot culture : The seeds were obtained from Agricultural Office, Tiruvallur, Chennai The rice variety IR-8 was used for the present investigation. A pot experiment was performed with different combinations *viz.,* T_1 = garden soil (control); T_2 = Soil + *Azotobacter* (10:1); T_3 = Soil + *Bacillus;* T_4 = Soil + *Pseudomonas;* T_5 = Soil + Rhizobium ; T_6 = Soil + Mixed organisms+compost (4:1:4); T_7 = Soil + Compost (5:5) and T_8 = Soil + Mixing of organisms (4:1:4)

The treatments were replicated six times and laid out in a randomized block design(RBD) manner. Three replicates were harvested at 30 days of plant growth and checked every 5 day interval. Plants were grown in a pot culture at day and night temperature of 15 to 25^{0}C at natural tropical environment. After growth for 4 weeks, fresh weight and dry weight were estimated (Chanway *et al.,* 1988).

Results and Discussion

The physico-chemical analaysis of compost showed increase in ash content and decrease in moisture level with increse in incubation time. Most important feature of compost is that during processing of various organic wastes, many of the nutrients are changed to available forms that are more readily taken up by plants (Chaudhuri, 2005). During composting there was change in the odour of compost with time. The change in odour, colour and texture of the compost indicates the degradation process is in progress. The most effective compost managing plan was

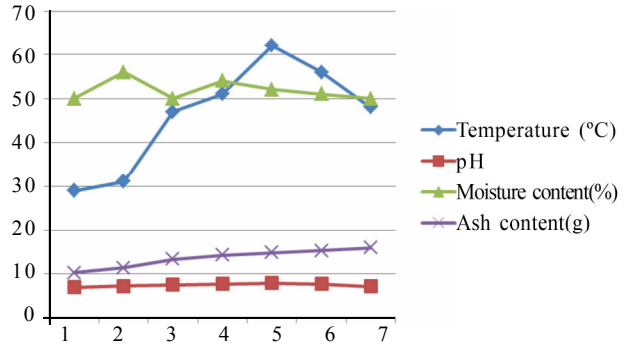

Fig. 1 : Physical parameters of vegetable waste compost at the depth of 25cms

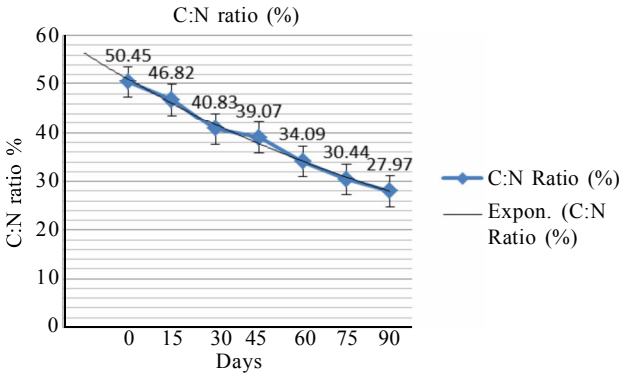

Fig. 2 : Chemical parameters of vegetable waste compost

to maintain temperature at the highest level possible without inhibiting the rate of microbial decomposition (Wu *et al.*, 2000). If temperature was less than 20ºC, micro-organisms were inhibited or killed and the reduced diversity of organisms resulted in lower rate of decomposition (Wu *et al.,* 2000). In the present study, the moisture content was nearing 50% in control and it was maintained till 90th day using sprinkler. Microorganisms require moisture to assimilate nutrient, metabolise new cells and reproduce. They also produced water as a part of decomposition process. If water accumulation is faster than evaporation, the oxygen flow is impeded and results in anaerobic condition. (Kiyohiko, 1985). This usually occured at moisture level above 65% when microbial activity decreases and the composting process slows down. Below 20% moisture level very little microbial activity occured in the media.

As compared to control soil, there was reduction in the odour of compost. The rotten smell changed to slightly tolerable smell on 60th day and became odour less 90th day. The odour usually occured from following three sources: raw material, ammonium released from high nitrogen materials and anaerobic conditions within the windrow and piles.

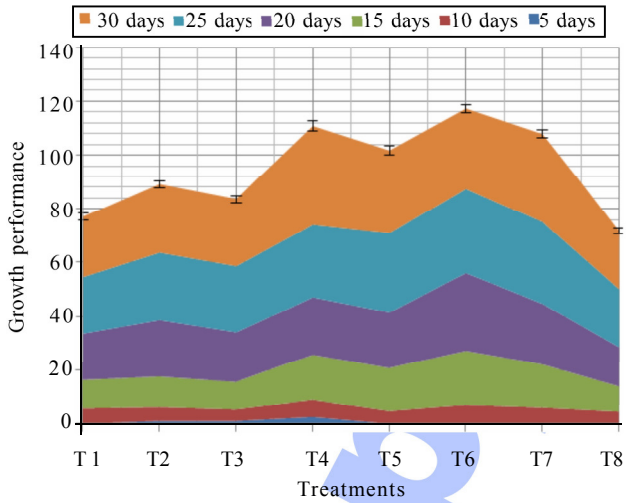

Fig. 3 : Growth performance of paddy with different treatments

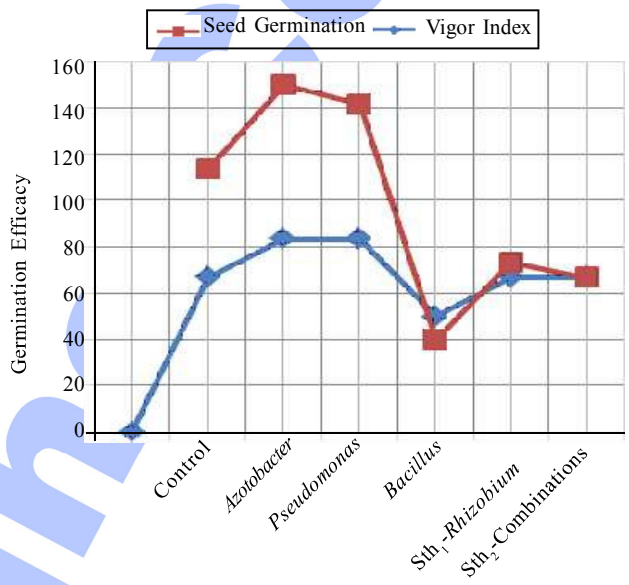

Fig. 4 : Seed germination and vigor index of paddy plant

The ash content of the compost showed significant increase with time (Fig. 1). The changes in ash content reflect trends in mineralization of organic matter. The similar result was reported by Wu *et al.* (2000). The nitrogen (mg g^{-1}) concentration was found to be 0.89 in control, 0.94 on 15th day , 0.98 on 30th day 1.02 on 45th day, 1.13 on 60th day and 1.18 on 90th day. The organic carbon concentration (mg g^{-1}) was found to be 48.4 in control, 44.0 on 15th day, 40.0 on 30th day, 39.86 on 45th day, 38.52 on 60th day and 35.01 on 90th day, respectively. The compost showed decrease in their organic carbon content with incubation time.

The C : N ratio was 50.45 in control, 46.52 on 15th day, 40.83 on 30th day, 39.07 on 45th day, 34.09 on 60th day and 30.08 on 90th day, respectively (Fig. 2). The decrease in C:N ratio with incubation time is indicative of compost maturation progress. The compost showed increase in the bacterial load up to 60th day and slight reduction on 90th day. Fungal load increased up to 60th day and slight reduction on 90th day. The isolated bacteria were *Staphylococcus aures, Bacillus species, Vibrio cholera, Vibrio paraheamolyticus, Serratia species, Klebsiella species, Escherichia coli, Salmonella paratyphi* and *Pseudomonas* species while the fungal species isolated were *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Fusarium species, Penicillium species, Rhizopus species, Microsporum gypseum and Trichophyton,* respectively.

The biological decomposition of organic matter is mediated by a variety of biochemical processes in which enzymes play a key role (Garcia *et al.,* 1992; Vuorinen, 1999). The major constituents like cellulose, hemicellulose, lignin, starch and different protein compounds present in waste are degraded by specific enzymes. Therefore, the quantification of enzyme activity during composting can reflect the dynamics of composting process in terms of decomposition of organic matter and nitrogen transformation. In correlation with enzyme activity, the changes in microbial number and types is also helpful in providing information about the maturity of the composted product (Tiquia, 2002, 2005). The cellulose (µg ml^{-1}) concentration of compost was found to be decrease from 34.42 in control and reduced to16.54 on 45th day and 4.12 on 90th day (Table 1).This explains that cellulose degradation occurs at high rate in the mesophilic phase than in thermophilic phase . Abundant enzyme activities in compost leads to the decomposition process which decreases the cellulose concentration in compost. This is also enhanced by the activity of aerobic and heterotrophic microbial population and its degradation activity in compost. In correlation with the introduced enzyme activity the microbial numbers reached maximum of $11.1x10^8$ and $11.3x10^{10}$ CFU of bacteria and fungi ml^{-1} respectively (Table1). Availability of partially digested nutrient rich organic wastes contributes to the proliferation of microbes and causes an increase in pH which causes the shift of microbial population that decreased to $36.75x10^6$ and $94.61x10^4$ CFU of bacteria and fungi ml^{-1} of compost. In correlation with the results of Haritha *et al.* (2009) introduced enzyme activity the microbial numbers reached the maximum of $126x10^6$, $28x10^4$ and $93x10^5$ CFU of bacteria, fungi and actinomycetes ml^{-1} samples respectively.

Table 1 : Microbial load of compost and cellulose concentration in definite time interval

Days	Microbial load (cfu ml^{-1})		
	Bacterial	Fungal	Cellulose (µg ml^{-1})
0	$12.01x10^7$	$51.3x10^8$	46.42
15	$11.1x10^8$	$11.3x10^{10}$	34.42
30	$11.3x10^7$	$95.93x10^7$	20.81
45	$24.6x10^5$	$10.12x10^7$	16.54
60	$63.2x10^6$	$93.15x10^5$	13.21
75	$24.29x10^7$	$13.21x10^5$	10.02
90	$36.75x10^6$	$94.61x10^4$	4.12

The seed germination percentage indicates the speed of germination of plants and the efficiency of the organism to initiate the seed germination. The germination index of paddy seeds was less in *Bacillus* (66.7%) on 4th day but varying results was obtained with *Azotobacter*, *Pseudomonas*, *Bacillus*, Sth_1 (*Rhizobium*) and Sth_2 (combinations) of organisms as 83.3%, 83.3%, 50%, 66.7% and 66.7% respectively. (Table 2, Fig. 4).

The pot culture study gives a clear view of practical applications and explain the synergistic or antagonistic effect of microorganisms with the seeds.The results of the present study suggests that bacterial treatments have a significant effect on seedling growth of paddy. The pot culture analysis revealed that seed inoculation with combination of bacteria resulted in increased plant height and weight. The single organism that gaves maximum height and weight for paddy was *Pseudomonas* (Fig 3). These results are in confirmation with the previous work of Chanway *et.al.* (1988). According to the study, rice seed bacterization with *C. agropyri, E. gergoviae* and *B. amyloliquefaciens* significantly enhanced seed germination and seedling growth under aerobic cultivation. The three studied rhizobacteria, especially *E. gergoviae*, are highly efficient IAA producer and were found to produce higher IAA than the values reported by Yasmin *et al.* (2007). The variations among different rhizobacteria species and strains, culture conditions, growth stages and substrates availability were reported to affect the production of IAA (Mirza *et al.,* 2001). The significant improvement of seed germination, radical length and vigor index might be due to the production of IAA by the rhizobacteria. It is well known that IAA is an important regulator in shoot and root growth (Kaufman *et al.,* 1995), and seed bacterization with IAA-producing rhizobacteria significantly enhanced early seedling establishment (Noel *et al.,* 1996). Future, more study can be done on the role of enzyme activity in enhancing the seedling growth.

Thus a consortium was designed to act as a biofertilizer to increase the seed germination, height and weight of paddy, the consortium contains plant growth promoting rhizobacteria such as *Bacillus* sp., *Pseudomonas*, *Azotobacter* and *Rhizobium*. According to Singh *et al.* (2006)

Table 2 : Effects of PGPR on germination and seedling growth of paddy

Isolates	Days of germination			Seed germination (4^{th} Day)%	Germination	Root length index	Shoot length
	2	4	6				
Tl	-	3	5	50	1.75	0.8	0.9
T2	-	5	6	83.33	2.25	0.9	0.9
T3	-	5	6	83.33	2.25	0.9	0.8
T4	-	3	6	50	1.75	0.7	0.5
T5	-	4	6	66.7	2	0.5	0.6
T6	-	4	6	66.7	2	0.3	0.5
T7	-	5	6	83.33	2.25	1.11	1.26
T8	-	6	6	100	2.5	1.23	1.31

the inoculation of seeds or seedlings with microbial innocula has been adopted as a method of modifying microbial populations around crop plants to promote both development and yield. The stimulation of seedling development by bacteria has also been attributed to the production of biologically active compounds. A significant increase in grain yield was also recorded in rice plants inoculated with plant growth promoting bacteria (Singh *et al.,* 2006). Similarly inoculation of *Rhizobia* to rice produced significantly higher root and shoot biomass; increased their photosynthetic rate and accumulated higher levels of growth regulating phytohaqrmones (IAA and gibberlline) (Feng *et al.,* 2005).

Future study can be done on the increase of seed number and yield. The designed consortium can be further formulated based on concentrations and designed for large scale production and commercialization. This when applied would increase the quality and fertility of the land.

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