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International Journal of Current Research
Vol. 6, Issue, 08, pp.8026-8031, August, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ENRICHMENT OF COMPOST THROUGH MICROBIAL INOCULATION – EFFECT ON QUALITY

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ARTICLE INFO

Article History:
Received 24th May, 2014
Received in revised form
10th June, 2014
Accepted 20th July, 2014
Published online 31st August, 2014

Key words:
Compost enrichment,
Vermicompost,
Farm yard manure,
Biofertilizer.

ABSTRACT

An incubation study was carried out to assess the quality of the vermicompost and farmyard manure in terms of total nutrient content and microbial population count following inoculation of various microbial cultures. *Azotobacter, Azospirillum* and phosphate solubilizing bacteria (PSB) culture @ 0.2 % each was inoculated in different consortia to farmyard manure (FYM) or vermicompost and incubated for thirty days in a completely randomized design laboratory experiment maintaining moisture content at about 25±1 % (w/w). The population of *Azotobacter*, *Azospirillum* and PSB significantly increased in the composts by about 35 to 133% during the 30 days incubation period with different consortia. The C:N ratio reduced significantly in FYM and vermicompost after 30 days incubation due to significant decrease in total carbon content. The content of total N, P and K was not affected in vermicompost at 30 days after inoculation of microbial culture, but the same in FYM decreased significantly barring few occasions.

INTRODUCTION

Use of properly prepared compost facilitates low-input agriculture system into profitable and sustainable one and inoculation of microbial culture into it improves its quality and productivity. Intensive cropping resulted in more reliance on chemical fertilizer corresponding to a disproportionate use of organic manure for sustaining crop and soil productivity. The focus had been on to recycle the bio-waste through improved methods as the traditional methods of composting result in losses of about 55% of organic matter and 30 to 50% of nitrogen (Kumaraswamy, 2001). Among various composting methods, vermicompost has gained wide acceptance and popularity because of its faster decomposition (Venter and Reinecke, 1988; Kaushik and Garg, 2003), superior quality (Gupta, 2003; Alam *et al.,* 2007; Hand *et al.,* 1988; Garg *et al.,* 2006; Suthar, 2006; Singh *et al.,* 2008) and positive effect on yields of crops in general (Reddy and Ohkura, 2004; Sinha and Heart, 2009), of legumes (Benik and Bejbaruah, 2004; Suthar, 2006), ornamental and flowering plants (Kale *et al,* 1987; Nethra *et al,* 1999), vegetables (Edwards and Burrows, 1988; Atiyeh *et al.,* 2000). Enrichment of compost in terms of increasing the nutrient content of final compost product had been studied (Shinde *et al,* 1990a; Bhanawase *et al.,* 1994; Hajra *et al.,* 1994; Zayed and Abdel-Motaal, 2005; Gaind *et al.,* 2006). Microbial enrichment technique with bio-inoculants to composting material had been shown to improve the quality of compost (Gaur, 1982, Rasal *et al.,* 1990; Shinde *et al.,* 1990b; Arora and Garg, 1992; Murkute *et al.,* 1992; Arora *et al,* 1994; Manna *et al,* 1997; Dey *et al.,* 2002), and even in low-grade city compost (Talashilkar, 1985). In spite of considerable attention both from researchers and farming community, the trend in consumption of biofertilizer is not encouraging (Ghosh 2004). This is mainly due to unavailability of the commercial product in time and in proper places. Further, the most of the work relating to compost enrichment are done during composting period. Thus information on quality of the compost due to microbial inoculation into finished compost product would provide possible avenue of using biofertilizer in a big yet simple way for the farming community. Accordingly, the present study was carried out to assess the quality of vermicompost and farm yard manure (FYM) in terms of nutrient content and microbial population following inoculation with *Azotobacter*, *Azospirillum* and PSB.

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MATERIALS AND METHODS

Laboratory experiments were conducted during summer season of 2009 and 2010 at Assam Agricultural University, Jorhat. Ten kilogram (on oven dry weight basis at 65^0 C for 24 hours) of ready to use compost, taken in gunny bag, was inoculated @ 0.2 % (w/w) each of *Azotobacter, Azospirillum* and PSB culture in different consortia and incubated for thirty days maintaining moisture content at about 25±1 % (w/w). The consortia used were *Azotobacter* + PSB, *Azospirillum* + PSB and *Azotobacter* + *Azospirillum* + PSB in both the seasons. In the first season, only vermicompost collected from DWSR Centre, Department of Agronomy of the University was evaluated using two different brands of commercial biofertilizer. The biofertilizer brands included one produced by All India Network Project on Biofertilizer, Department of Soil Science of the University (AAU culture), and the other was Mukta, produced and marketed by Brahmaputra Valley Fertilizer Corporation, Namrup, Assam (Commercial culture). Both the samples were collected from the source one day before inoculation and stored under refrigeration (4^0 C). In the second season, summer 2010, both vermicompost and FYM were inoculated with same consortia with AAU culture.

Each treatment was replicated thrice in a completely randomized design and the bags were closed, tied with rope, and incubated keeping on a concrete floor. The bags were opened time to time to check for the moisture status and accordingly watered to maintain 25±1 % moisture content (w/w), mixed properly and closed. Samples were drawn periodically after mixing the compost of a bag properly, analysed for total nutrient content (C, N, P and K) and total count for population of *Azotobacter*, *Azospirillum* and PSB as colony forming unit (cfu) using appropriate media and serial dilution technique.

Viable bacterial counts

The bacterial count was done by taking 1 gram of the compost sample and was serially diluted. Hundred microlitres of it was plated separately for *Azotobacter* (Burk's medium), *Azospirillum* (NFb medium) and PSB (Pikovskaya's medium) and the plates were then incubated at 28±2 ^{0}C for 48–72 hours and the colony-forming units were counted.

RESULTS AND DISCUSSION

Microbial population

The total population of inoculated microorganisms in the compost, expressed as colony forming unit (cfu) per gram of soil, are presented in Table 1 and 2 for the seasons 2009 and 2010, respectively. Irrespective of the organisms, the population in the compost increased due to incubation. The highest population was observed at 30 days after incubation in case of *Azotobacter* in 2009 and PSB in 2010, both in vermicompost with *Azotobacter* + PSB inoculation. On the other hand, the lowest population was observed at 15 days after incubation for *Azospirillum* in vermicompost in 2009 and for PSB in FYM in 2010, both without inoculation of biofertilizer.

Effect of consortia

Irrespective of the compost, the *Azospirillum* population significantly increased due to inoculation as consortia with PSB. In case of *Azotobacter*, both dual inoculation with PSB and triple inoculation with PSB + *Azospirillum* had significant effect on the population. Inoculation of PSB, irrespective of the consortia, increased the PSB population compared to that in the compost without inoculation. There was no statistical difference in PSB population in the first season among the consortia, however significant increase was noticed in FYM with *Azospirillum* + *Azotobacter* + PSB, and in vermicompost with *Azotobacter* + PSB in the second season. More *Azotobacter* and "phosphobacteria" were found in the rhizospheres when plants were inoculated with both groups of organisms together than when they were inoculated singly (Ocampo *et al.,* 1975). Synergistic interactions on plant growth have been observed by co-inoculation of PSB with nitrogen fixers such as *Azospirillum* (Belimov *et al.,* 1995) and *Azotobacter* (Kundu and Gaur, 1984). The higher population of PSB may be due to its ability to use the both the substrates efficiently thereby favouring its growth (Gutierrez-Rojas, 2011), while *Azospirillum* possesses a versatile metabolic system where carbon and nitrogen are metabolized readily (Parmar and Dufresne, 2011).

Source of biofertilizer

The population of bacteria in the compost differed between the two sources of biofertilizer, studied in the first season. But the difference was statistically significant only for *Azotobacter* population in the vermicompost. Inoculation of *Azotobacter* with AAU culture resulted in its higher population in the compost compared to the commercial culture inoculation (Table 1). This might be due to difference in the effective population of *Azotobacter* in respective cultures.

Type of compost

The population of bacteria before and after incubation of biofertilizer was smaller in farm yard manure (Table 2). Except for the population of *Azospirillum*, the percent increase in the population of *Azotobacter* and PSB was higher in FYM than that in vermicompost. A relatively faster growth in the bacterial population in FYM may be attributed to the sufficient availability of decomposable organic substances for their multiplication (Tripathy and Ayappan, 2005). In case of *Azotobacter*, which grows well in low-N medium, adding nitrogen to culture medium had positive influence on biomass production regarding both the quantity produced and on growth rate (Oppenheim and Marcus, 1970; Vela and Rosenthal, 1972; Revillas *et al.,* 2000). Similar to this, the increased growth of bacteria observed in vermicompost may be explained on the basis of its higher nitrogen content relative to farm yard manure. The differences in the parameters in vermicompost between the two seasons are ascribed to the natural variation in organic manures. Application of vermicompost prepared from raw materials with varying type and composition had been reported to have differential effect on soil microbial population (Sabu *et al.,* 2000).

Incubation time

The incubation time had a pronounced effect on the microbial population, irrespective of composts and consortia used (Table 1 and 2). Except for PSB population at 15 days after incubation in 2009 season, inoculation significantly increased the population of bacteria due to incubation up to 30 days. Kavitha and Subramanian (2007) also reported about two-fold increase in the population of *Azotobacter* and phosphobacteria, but comparatively lower increase in the latter, after 20 days of

Table 1. Total microbial population (cfu x 10^5) in vermicompost at days after incubation with different biofertilizer sources in summer 2009

Treatment	*Azospirillum*		*Azotobacter*		PSB	
	15	30	15	30	15	30
Initial (before inoculation)	3.8		5.2		8.2	
Without inoculation	5.2	7.8	6.8	11.2	14.3	17.5
Azospirillum + PSB (Commercial culture)	7.9	12.7	8.9	15.8	16.8	29.2
Azotobacter + PSB (Commercial culture)	6.3	8.3	18.7	26.1	18.5	30.3
Azospirillum +*Azotobacter* + PSB (Commercial culture)	9.3	14.2	18.6	25.5	18.8	28.5
Azospirillum + PSB (AAU culture)	8.6	14.4	9.3	16.4	21.5	36.9
Azotobacter + PSB (AAU culture)	6.6	9.6	22.3	38.9	19.9	38.3
Azospirillum +*Azotobacter* + PSB (AAU culture)	9.1	16.0	22.9	36.8	20.3	35.9
$CD_{P=0.05}$	1.4	1.9	2.8	5.4	NS	8.3
CV (%)	13.1	8.9	8.7	10.5	12.8	17.3

Table 2. Total microbial population (cfu 10^5 g^{-1}) in different composts at days after incubation with AAU culture in summer 2010

Treatment	*Azospirillum*		*Azotobacter*		PSB	
	15	30	15	30	15	30
Farm yard manure (FYM)						
Initial (before inoculation)	3.2		4.4		2.8	
Without inoculation	4.4	6.3	5.5	6.6	3.6	5.4
Azospirillum + PSB	5.9	8.2	6.0	7.0	6.4	10.6
Azotobacter + PSB	4.7	6.4	8.5	14.8	8.0	16.4
Azospirillum +*Azotobacter* + PSB	5.4	7.1	8.2	16.2	10.4	22.1
$CD_{P=0.05}$	1.0	1.3	1.2	1.6	1.4	1.7
CV (%)	10.8	8.6	11.2	13.5	7.6	9.2
Vermicompost						
Initial (before inoculation)	4.6		8.5		7.4	
Without inoculation	5.8	9.6	11.5	14.5	8.1	15.5
Azospirillum + PSB	9.3	15.3	13.1	16.0	10.5	18.8
Azotobacter + PSB	7.1	10.9	14.4	25.6	12.5	26.3
Azospirillum +*Azotobacter* + PSB	6.8	11.1	13.5	20.2	10.2	20.6
$CD_{P=0.05}$	1.4	1.5	1.7	2.2	2.0	2.5
CV (%)	13.7	14.7	12.1	11.7	20.1	11.4

Table 3. Total nutrient content (%) in vermicompost 15 days after incubation with different cultures in summer 2009

Treatment	C	N	P	K	C:N ratio
Initial (before inoculation)	22.8	1.65	1.24	1.86	13.82
Without inoculation	18.3	1.62	1.19	1.79	11.3
Azospirillum + PSB (Commercial culture)	11.4	1.51	1.11	1.80	7.4
Azotobacter + PSB (Commercial culture)	11.5	1.56	1.10	1.71	7.4
Azospirillum +*Azotobacter* + PSB (Commercial culture)	10.7	1.53	1.11	1.69	7.0
Azospirillum + PSB (AAU culture)	11.0	1.58	1.09	1.68	7.0
Azotobacter + PSB (AAU culture)	11.4	1.58	1.14	1.64	7.2
Azospirillum +*Azotobacter* + PSB (AAU culture)	11.3	1.57	1.10	1.61	7.2
$CD_{P=0.05}$	1.7	NS	NS	NS	1.1
CV (%)	8.1	6.2	7.46	6.1	7.7

Table 4. Total nutrient content (%) in vermicompost 30 days after incubation with different cultures in summer 2009

Treatment	C	N	P	K	C:N ratio
Initial (before inoculation)	22.8	1.65	1.24	1.86	13.8
Without inoculation	13.7	1.58	0.95	1.73	8.7
Azospirillum + PSB (Commercial culture)	8.7	1.43	0.79	1.73	6.0
Azotobacter + PSB (Commercial culture)	9.1	1.49	0.80	1.42	6.1
Azospirillum +*Azotobacter* + PSB (Commercial culture)	8.5	1.42	0.79	1.52	6.0
Azospirillum + PSB (AAU culture)	8.7	1.42	1.07	1.67	6.1
Azotobacter + PSB (AAU culture)	8.9	1.47	1.00	1.55	6.1
Azospirillum +*Azotobacter* + PSB (AAU culture)	9.0	1.47	0.97	1.59	6.2
$CD_{P=0.05}$	0.8	NS	0.20	NS	0.7
CV (%)	4.8	5.8	12.3	9.1	6.2

incubation of city compost. Contrary to this, the better growth in PSB due to incubation in the present study may be due to higher P content of the composts.

Total nutrient content in the compost

The total nutrient content in the vermicompost at various days after incubation with commercial culture or AAU culture are presented in Table 3 and 4, and that of FYM and vermicompost in 2010 season presented in Table 5 and 6.

Total carbon

Irrespective of the compost or cultures used, the total carbon content of the compost decreased significantly due to incubation with biofertilizer. Besides, there was no significant difference among the treatments, except for FYM where Azospirillum+*Azotobacter* + PSB inoculation yielded statistically lower total carbon content both at 15 and 30 days after incubation (Table 5 and 6).

The decrease in carbon content of the incubated compost may be attributed to the increasing population of microorganisms which utilize decomposable organic waste both as a source of food and energy (Chefetez *et al.,* 1998; Becking, 2006). Similarly, the low content of total carbon in FYM with Azospirillum +*Azotobacter* + PSB inoculation may be correlated to relatively higher population of microorganisms both at 15 and 30 days after incubation, compared to other consortia inoculation (Table 2).

Total nitrogen, phosphorous and potassium

Except for total potassium content in vermicompost in summer 2009, the values decreased significantly in compost inoculated with various consortia of microorganisms, compared to those without inoculation of biofertilizer. Barring few occasions, the total content of the nutrients in composts was not affected statistically by different consortia. The phosphorous content of vermicompost inoculated with *Azospirillum* + PSB significantly differed 30 days after incubation due to source of biofertilizer, while the content of total N, P and K was at par among the treatments.

C:N ratio

The C:N ratio significantly decreased due to incubation with or without inoculation of biofertilizer. The value for vermicompost declined to about 6.1 from an initial ratio of 13.8:1 in 2009 and 14.1:1 in 2010, the same in FYM decreased from 55.8:1 to as low as 25.9:1 at 30 days after incubation. However, there was no statistical difference in the values among the consortia. The reduction in C:N ratio due to incubation with biofertilizer is desirable as a C:N ratio 5 to 20 is indicative of acceptable maturity (Golueke, 1981), although a ratio of 15 or less is preferable (Morel *et al.*,1985; Bernal *et al.,* 2009). The reduction in pH, organic carbon and C:N ratio was also noticed during incubation of farmyard manure and phosphobacteria compost (Ravindrana *et al.,* 2007). Non-composted manure may have adverse effects on plant growth and seed germination (Hoekstra *et al.,* 2002) induce anaerobic conditions as microorganisms utilize oxygen in the soil pores to break down the material (Mathur *et al.,* 1993), phytotoxicity

Table 5. Total nutrient content (%) in different compost at 15 days after inoculation with AAU culture in summer 2010

Treatment	C	N	P	K	C:N ratio
Farm yard manure (FYM)					
Initial (before inoculation)	36.3	0.65	0.32	0.84	55.8
Without inoculation	20.1	0.55	0.31	0.82	36.9
Azospirillum + PSB	16.3	0.47	0.24	0.66	34.7
Azotobacter + PSB	16.4	0.48	0.26	0.68	34.2
Azospirillum +*Azotobacter* + PSB	15.1	0.43	0.22	0.72	35.1
$CD_{P=0.05}$	0.7	0.06	0.06	0.08	1.9
CV (%)					
Vermicompost					
Initial (before inoculation)	24.5	1.74	1.28	2.14	14.1
Without inoculation	19.5	1.69	1.21	2.11	11.6
Azospirillum + PSB	17.6	1.54	1.17	1.97	11.5
Azotobacter + PSB	17.2	1.61	1.05	1.89	10.7
Azospirillum +*Azotobacter* + PSB	17.4	1.56	1.01	1.96	11.1
$CD_{P=0.05}$	0.9	0.09	0.08	0.12	1.4
CV (%)	4.4	7.11	10.01	6.97	9.4

Table 6. Total nutrient content (%) in different composts at 30 days after inoculation with AAU culture in summer 2010

Treatment	C	N	P	K	C:N ratio
Farm yard manure (FYM)					
Initial (before inoculation)	36.3	0.65	0.32	0.84	55.8
Without inoculation	16.8	0.48	0.26	0.73	35.0
Azospirillum + PSB	11.9	0.42	0.21	0.65	28.3
Azotobacter + PSB	11.4	0.44	0.19	0.62	25.9
Azospirillum +*Azotobacter* + PSB	10.5	0.40	0.22	0.60	26.3
$CD_{P=0.05}$	0.7	0.06	0.06	0.08	2.5
CV (%)	6.8	10.14	8.32	12.33	10.2
Vermicompost					
Initial (before inoculation)	24.5	1.74	1.28	2.14	14.1
Without inoculation	11.6	1.48	1.13	2.05	7.9
Azospirillum + PSB	8.9	1.46	1.06	1.97	6.1
Azotobacter + PSB	8.8	1.40	1.02	1.89	6.3
Azospirillum +*Azotobacter* + PSB	8.6	1.36	0.97	1.96	6.3
$CD_{P=0.05}$	1.0	0.08	0.12	NS	1.1
CV (%)	7.5	7.9	11.4	7.3	16.2

due to the presence of organic acids (Fuchs, 2002; Cambardella *et al.,* 2003). Mesophilic and thermophilic microorganisms are involved in composting and their succession is important in effective management of the composting process (Ishii *et al.,* 2000). Many parameters have been considered as maturity indices for compost, and most focus on the chemical and physical parameters of compost (Wu *et al.,* 2000; Ko *et al.,* 2008; Bernal *et al.,* 2009; Chang and Chen, 2010) but due to variation in raw materials and composting technology parameters and methods of their estimation may not be universally accepted (Itavaara *et al.,* 2002). The maturity of the compost may be assessed by the biological activity of the product, including total microorganisms count, monitoring biochemical parameters of microbial activity and analysis of biodegradable constituents (Morel *et al.,* 1985). In view of the results obtained in this study and the discussions above, it may be concluded that inoculation of vermicompost and FYM with biofertilizer improved the quality of the composts. The effect of consortia, though showed some difference, could not be conclusively explained. Further studies may be carried out to characterize the process and to evaluate the composts on growth and yield of crops.

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