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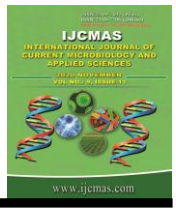

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Changes in Temperature, Microbial Population and Activity during the Process of Composting

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A B S T R A C T

The changes in temperature, microbial population dynamics and other factors were studied during the process of composting by layering residues of *Calotropis procera*, *Prosopis juliflora*, *Azadirachta indica*, *Acacia nilotica* and weeds in separate pits. Temperature began to rise soon after filling the residues and ranged between 55–59 °C for next 6 to 7 days with maximum being in *Calotropis* pit (59.2 °C). Estimation of microbial population dynamics and activity has shown that in the heat phase, fungal population remained low but reinfestation occurred after 30 days in all the composts except weed compost. There was a fluctuation in total actinomycetes and bacterial population in all the decomposing residues initially, however, at 150 days counts were maximum in *Calotropis* compost. *Trichoderma harzianum* disappeared during peak heating but reinfested occured at mature stage with maximum counts in *P. juliflora* compost, while *Bacillus* spp. was present throughout the composting period. At maturity, total fungi were maximum in neem followed by *P. juliflora* compost, but total actinomycetes and bacterial population was maximum in *Calotropis* compost. Maximum antagonistic actinomycetes (64.3 %) were estimated in *A. nilotica* compost. *P. juliflora* compost maintained its superiority in terms of microbial activity during the process of composting as well as at maturity. Among all the composts, maximum available micro-nutrients like Cu, Zn, Mn and Fe were estimated in compost prepared from *A. nilotica*.

Introduction

Incorporation of crop residues, composts or manures as soil amendment has been advocated for managing soil-borne plant pathogens (Hoitink and Fahy, 1986; Lazrovits *et al.*, 2000). In arid regions of India, direct incorporation of residues in soil has not been found successful due to low availability of soil moisture required for decomposition. Half digested crop residues in turn aggravate termite infestation in rainfed crops. More so, crop residues themselves are often infected with soil-borne plant pathogens; their release in soil during slow decomposition may augment inoculum density of pathogens (Lodha *et al.*, 1990).

Use of compost is an easy alternative for amending soil in arid soils where moisture retention is poor (Lodha and Burman 2000). Efforts were also made to inactivate released propagules of *M. phaseolina* from infected crop residues during heat phase of composting by increasing nitrogen concentration and exposing mature compost to dry summer heat (Lodha *et al.*, 2002).

In earlier studies, composts were prepared from residues of annual crops (Lodha *et al.*, 2002; Lodha and Burman, 2000). Most of these residues are also fed to livestock in resource deficient farming systems of the region. In recent years, it was thought worthwhile to utilize only above ground parts of those on-farm wastes in preparation of compost, which are not being utilized for any other purpose. In arid regions, such wastes which are amply available include *Calotropis procera, Prosopis juliflora, Azadirachta indica, Acacia nilotica* and weeds (*Aerva persica, Celosia argentea, Heliotropium subulatum, Euphorbia hirta*, etc.)

During the process of composting many physico-chemical and biological changes occur (Hoitink and Boehm, 1999). During first phase, temperature increases rapidly when mesophilic including beneficial micro organisms are killed due to a heat treatment (Hoitink and Fahy, 1986; Bollen, 1993). However, certain species of *Bacillus* and other thermophillic organisms survives this phase (Storm 1985). In curing phase, when temperature declines mesophilic organisms recolonize (Hoitink *et al.*, 1991). However, no such information on the recolonization of bio agents and other microbial communities is available for residues used for composting in a hot arid climate.

The present study, therefore, was undertaken to investigate survival of bio agents, microbial population dynamics and activity, availability of micronutrients during the process of composting and in mature composts prepared from certain on-farm wastes.

Materials and Methods

Experimental site

The experiments were conducted at the Central Arid Zone Research Institute, Jodhpur (Rajasthan, India). The Jodhpur city is situated at 26°N latitude and 73°E longitude at an elevation of 224 meters above sea level. It is located within south-eastern boundary of the Great Indian Thar desert. The soil had 85.0% sand, 8.9% clay, 5.5% silt, 0.031% total nitrogen, 0.25% organic carbon, 9 ppm available phosphorus, a pH value of 8.1, electrical conductivity 0.88%, bulk density 1.56 g cm^{-3} and moisture holding capacity (MHC) 10.4%.

Preparation of compost

Five pits of 3' x 2' size were dug each at a distance of 3 m under dense canopy of trees. Complete above ground plant part of *Calotropis procera* (Willd) Dryland, *Prosopis juliflora* (Swartz) DC, *Azadirachta indica* A. Juss., *Acacia nilotica* (L) Willd ex. Del. and on-farm weeds *Aerva persica* (Burm. F), *Celosia argentia* L) and *Euphorbia hirta* L) were crushed into small pieces (1-1.5 cm). The process of composting was initiated under partially anaerobic conditions in separate pits according to the principles of Indore type (Howard and Ward 1931). Four layers of residues with cow dung - urea mixture were piled in each pit and 60% moisture was maintained by regular addition of water. During the process of composting, two turnings were given at an interval of two months. The whole process of composting was studied carefully. Temperature of each pit was recorded by inserting thermometer to a depth of 30 cm during heat phase. Mature composts were ready as decomposed amendment in 5-6 months.

Antagonistic actinomycetes

Antagonistic actinomycetes to *M. phaseolina* were detected on Czapeck's Dox agar (pH 7.2) following the method of Ghaffar *et al.*, (1969). Isolated actinomycetes were transferred to liquid Ken-knight medium,

multiplied for 8 days at 28 ± 1° C and then placed at three equidistant points 1 cm from the edge of Petri dish (9 cm) containing Czapeck's Dox agar. After growth in the dark for 48 hours at 28 ± 2° C, a mycelial disc of *M. phaseolina* was placed in the centre of each Petri dish and incubated for three more days. Four replications were used for each strain of actinomycetes. Strains showing a minimum of 2 mm inhibition zone confirmed their antagonistic activity.

Determination of dehydrogenases activity

Dehydrogenases activity was measured at 120^{th}, 150^{th} day during process of composting and at maturity following the procedure of Tabatabai (1982).One-gram soil sample for each treatment was placed in clean dry screw capped borosilicate tubes and to this 0.2 ml of 3% (w/v) 2, 3, 5 - triphenyl tetrazolium chloride (TTC) solution and 0.5 ml of 1% (w/v) glucose solution was added. The contents of tubes mixed thoroughly, tightly capped and then were incubated at 30 ± 1^{o} C for a period of 24 hours. After incubation, 10 ml methanol was added to each tube and the contents were mixed thoroughly for exactly 1 minute. The tubes were kept in refrigerator (4^{0} C) for about 3 hours. Intensity of the color was measured at 485 nm using a Systronics Spectrophotometer. From the standard curve drawn by taking 1-10 ppm triphenyl formazan (TPF) in methanol, the amount of TPF produced was computed. Dehydrogenases activity was expressed in picokats as the amount of enzyme required to hydrolyze 1 p mole of TTC per second per gram compost. The compost samples were analyzed in triplicates for each treatment.

Micronutrient estimation

One gram sample from each compost was subjected to tri-acid digestion (Lindsay and Norvell 1978) for the estimation of micronutrients like copper, iron, zinc and manganese following the standard procedures. Available micronutrients were determined from digested samples with the help of an Atomic absorption Spectrophotometer (GBC – 932 AAS) at a wavelength of 325 nm, 248 nm, 279.2 nm and 214 nm, respectively. The instrument was calibrated with standard solutions made from pure metals. The results were expressed on oven dry basis.

Microbial population enumeration

During the preparation of compost, samples were collected from each pit at an interval of 30 days. The samples were air-dried and ground to pass through a 2 mm sieve for quantitative estimation of microbes. Total microbial populations were enumerated by serial dilutions on Martin's rose bengal agar (fungi), Thornton agar (bacteria), Ken knight agar (actinomycetes), Antagonistic actinomycetes (Czapeck's dox agar), *T. harzianum* and *Bacillus* spp. on their respective selective media. The means of six Petri-dishes were considered one estimation per replicate of each treatment.

Statistical analysis

All the data were subjected to analysis of variance (ANOVA) and the treatment means compared by LSD (P = 0.05). Data on microbial populations were subjected to one-way analysis to identify the significance of main effects of microbial population and activity of their interaction with time intervals (Snedecor and Cochran, 1967).

Results and Discussion

Temperature

In all the pits, temperature began to increase soon after filling the residues (Fig. 1). After one week, temperature ranged between 55 –

59° C in all the pits and reached at a peak of 59.2 °C in *Calotropis* pit in next 6-7 days. Temperature declined in all the pits afterwards but *P. juliflora* and weeds pits recorded less decline compared to other pits. After 60 days also, the temperature in weed compost pit remained 5°C – 6°C higher than other pits until 90 days. After 120 days and till maturity no significant variations were recorded in all the compost pits.

Total microbial population

In general, fungal population remained low during heat phase in all the pits except in those having residues of *P. juliflora* and weeds but significant reinfestation occurred after 30 days. Population fluctuated at two subsequent sampling dates with a gradual decline except in neem and *A. nilotica* compost pits. Further at 150 days, a significant increase was estimated in all the composts, but significant difference could not be estimated except that prepared in compost by weed residues, which had significantly low fungal population (Table 1).

Population of actinomycetes remained significantly higher only in *Calotropis* and *P. juliflora* pits during heat phase compared to other pits (Table 1). At second sampling date (60 days), maximum counts were estimated in the pit having residues of weeds, but subsequently a gradual increase occurred in the population in all the pits with maximum being in *A. nilotica* pit. However, at 120 days and onwards a gradual decline in the counts of actinomycetes was estimated in all the pits except that having *Calotropis* compost, which had significantly higher counts than others at maturity also.

During heat phase, total counts of bacteria were not significantly different but a dramatic increase was estimated at second sampling date (60 days), with maximum being in *P. juliflora* and *Calotropis* compost (Table 1). However, the bacterial population decreased in next two sampling dates except in *A. nilotica* pit. Thereafter, reinfestation or multiplication again occurred, which was highest in *P. juliflora* and *Calotropis* composts. The population of bacteria, in general, remained low in mature composts than that recorded at 150 days interval.

Bacillus spp.

In general, *Bacillus* spp. were present in all the pits throughout the study (Table 2). The counts increased gradually at 60 days but declined in all the treatments afterwards. However, at subsequent sampling date a sudden upsurge was estimated in *Calotropis* compost with an exceptional decrease in rest of the composts. At final stage, the number decreased in *Calotropis*, *P. juliflora* and *A. nilotica*, but increased significantly in weeds and neem composts.

Trichoderma spp.

The population of *T. harzianum* was completely eliminated in all the treatments initially except in *A. nilotica* pit (Table 2). However, in *Calotropis* and *P. juliflora* compost pits reinfestation occurred only at second sampling date. After 90 days, *T. harzianum* propagules were estimated in all the composts, with maximum being in *P. juliflora* compost. This reinfestation remained visible till 120 days but again disappeared in the last phase.

Microbial activity

During the process of composting at 120 and 150 days, microbial activity was least in all the composts at the first sampling date (Table 3). Maximum microbial activity was estimated in neem compost followed by *Calotropis* and least in *P. juliflora* compost. A

tremendous increase (9 to 16- fold) was estimated in all the composts at second sampling date, with maximum being in *P. juliflora* compost. At this stage, activity was least in *A. nilotica* compared to other composts but still it was 10- fold higher than that estimated at first sampling date.

Composts at maturity

In mature composts, the population of total fungi was significantly higher in neem compared to all other composts (Table 4). However there were no significant ($P \leq 0.05$) differences were recorded in counts of fungi in *A. nilotica*, *Calotropis* and weed composts. Significantly higher numbers of actinomycetes were estimated in *Calotropis* compost as compared to others. Similarly, counts of bacteria though were highest in *Calotropis* compost but these were not significantly different than other composts. *P. juliflora* and *Calotropis* composts had significantly higher counts of *Bacillus spp.* compared to other composts. The population of *Trichoderma* spp. was dramatically higher in *P. juliflora* compared to other composts. Among different composts, maximum population of actinomycetes was estimated in *A. nilotica* compost, which was significantly equal to that of *Calotropis* compost. In rest other composts, populations of actinomycetes were significantly low. Antagonistic actinomycetes were also higher in *A. nilotica* and *Calotropis* compared to other three composts but proportionate population remained higher only in *A. nilotica* and neem composts (Fig. 2).

Table.1 Changes in population of total fungi, actinomycetes and bacteria in different composts during process of composting

Compost		**Days**				
		30	**60**	**90**	**120**	**150**
Calotropis procera	**I**	3	16	10	12	**47**
	II	3	3	5	5	**11**
	III	1	19	11	5	**21**
Prosopis juliflora	I	25	124	28	21	**55**
	II	3	5	12	11	**6**
	III	2	27	23	14	**23**
Weeds	I	19	40	31	17	**20**
	II	2	14	17	10	**3**
	III	2	14	10	11	**17**
Neem	I	7	68	30	37	**46**
	II	1	7	14	4	**6**
	III	1	8	10	9	**17**
Acacia nilotica	I	6	13	18	38	**51**
	II	1	7	29	10	**3**
	III	1	9	10	13	**16**
LSD (*P*=0.05)	**I**	**3**	**15**	**5**	**6**	**10**
	II	**1**	**5**	**4**	**3**	**3**
	III	**0**	**5**	**4**	**8**	**7**

I Total fungi (x 10^3 cfu g^{-1}); II Total actinomycetes (x 10^5 cfu g^{-1}) and III Total bacteria (x 10^5 cfu g^{-1})
* Estimated on their respective media and average of six replicates

Table.2 Changes in population of *Bacillus* spp. and *Trichoderma harzianum* in different composts during process of composting

Compost		Days 30	60	90	120	150
Calotropis procera	I	21	18	15	43	**14**
	II	-	2.3	0.3	1.3	-
Prosopis juliflora	**I**	18	25	16	24	**17**
	II	-	0.3	13.3	1.3	-
Weeds	**I**	18	28	20	13	**24**
	II	-	-	2.3	1.3	-
Neem	I	11	27	13	10	**14**
	II	-	-	2.0	1.3	-
Acacia nilotica	I	10	34	33	30	**23**
	II	1	-	2.6	0.6	-
LSD(*P*=0.05)	**I**	**6**	**6**	**5**	**8**	**6**
	II	-	-	**2.2**	**1.0**	-

I *Bacillus* spp (x 10^{6}cfu g^{-1}).; II *Trichoderma* spp (x 10^{3}cfu g^{-1})

* Estimated on their respective specific medium and average of six replicates

Table.3 Microbial activity during the process of composting and of mature composts

Compost	Dehydrogenases assay (P Kat g^{-1})* Days 120	150	Mature
Calotropis	9.73	89.74	**97.93**
P. juliflora	6.60	119.02	**117.96**
Weeds	8.04	91.48	**61.15**
Neem	11.11	84.05	**97.44**
A. nilotica	8.07	80.61	**81.58**
LSD (*P*=0.05)	**Treatment = 3.5, Interval = 2.7 and Treatment x Interval = 6.1**		

* Estimated by Systronics Spectrophotometer

Table.4 Densities (cfu g^{-1}compost) of resident micro-flora including bio agents in mature composts

Compost	Fungi (x 10^{4})	Actinomycetes (x 10^{5})	Bacteria (x 10^{6})	*Bacillus* spp. (x 10^{6})	*T. harzianum* (x 10^{3})
Calotropis	3.9	6	8	10	**10**
P. juliflora	4.9	3	7	9	**19**
Weeds	3.6	1	6	4	**6**
Neem	5.9	4	6	7	**5**
A. nilotica	4.0	2	6	5	**9**
LSD(*P*=0.05)	**0.9**	**2**	**2**	**2**	**2**

*Estimated on their respective media and average of six replicates

Fig.1 Temperature at 30 cm depth of compost pits having decomposing residues of different plant materials

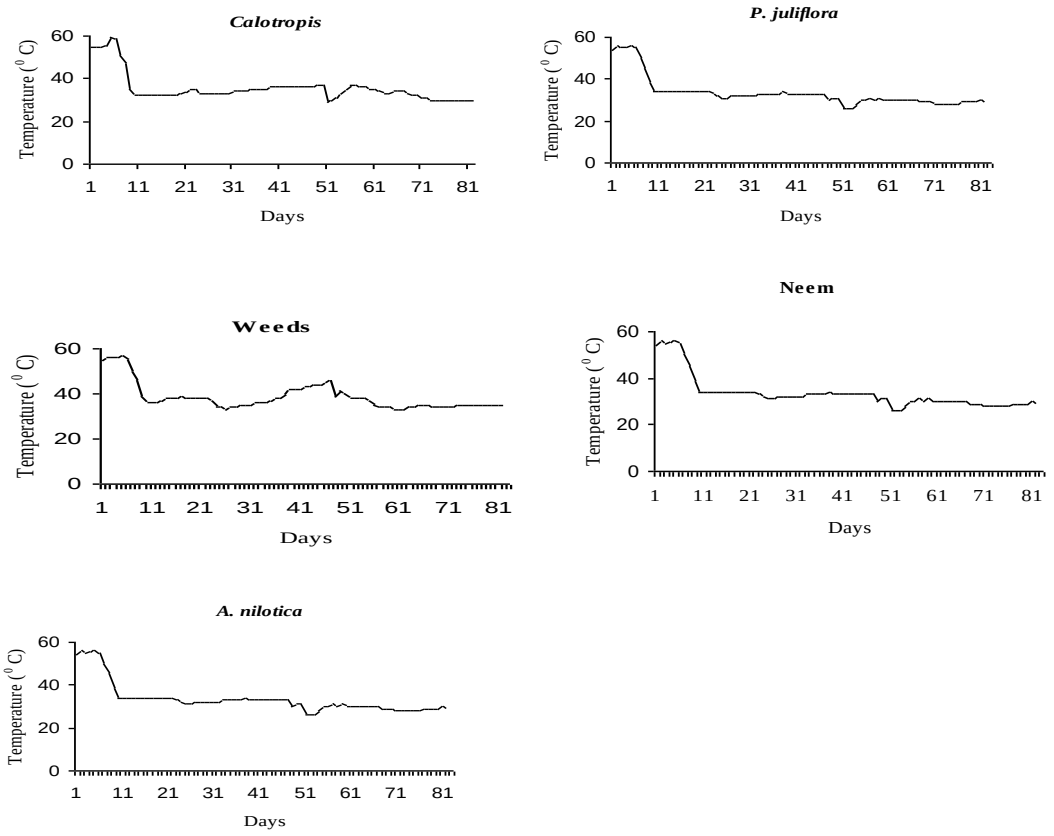


Fig.2 Population of actinomycetes antagonistic to *Macrophomina phaseolina* in different composts

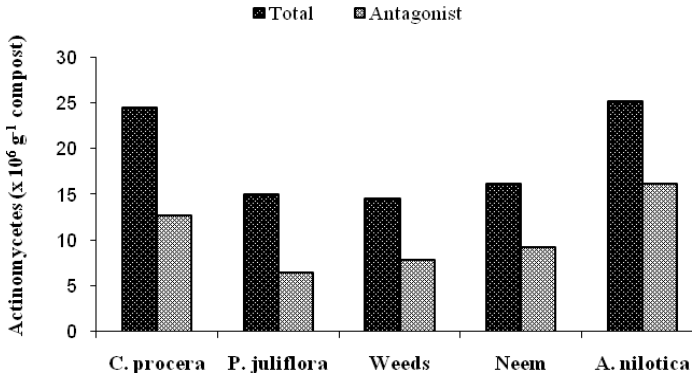
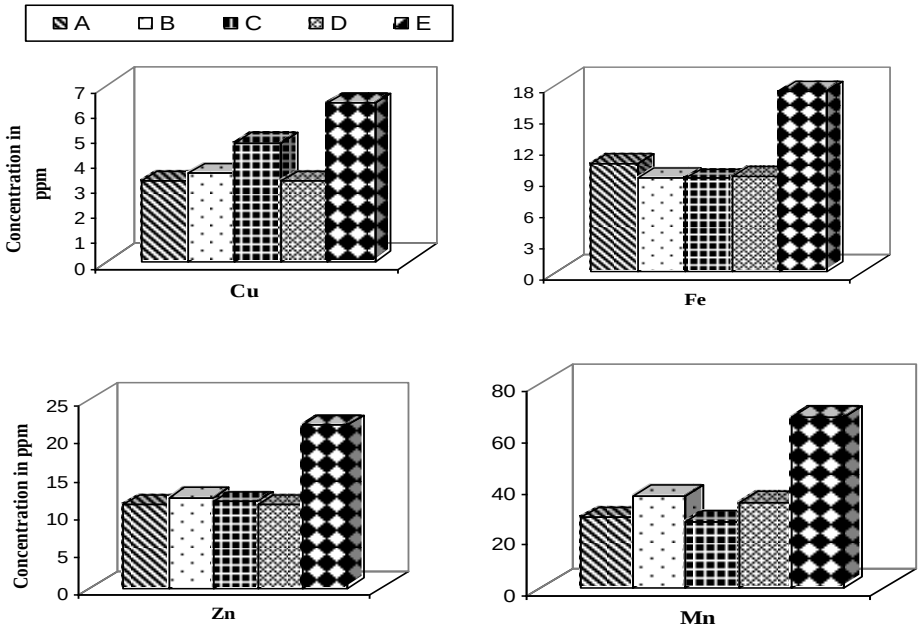


Fig.3 Available micronutrients (ppm) in different composts at maturity (A-*Calotropis*, B -*P. juliflora*, C- weeds, D -neem and E-*A. nilotica*)



In mature composts, microbial activity significantly increased only in *Calotropis* and neem composts compared to that estimated at 150 days (Table 3). At this stage also, *P. juliflora* compost maintained its superiority in terms of time and interval over other composts. However, microbial activity was not significantly ($P \leq 0.05$) different in *Calotropis* and neem composts, while minimum microbial activity was estimated in weeds compost. Among composts, maximum available micronutrients like Cu, Zn, Fe and Mn were estimated in compost prepared from *A. nilotica* residues (Fig. 3). After that, maximum contents of Zn and Mn were estimated in *P. juliflora* compost, while Cu and Fe contents were highest in weeds and *Calotropis* composts, respectively. Among five composts, neem had least content of Cu, Zn and Fe.

Our studies demonstrate that considerable variations in all the physio-chemical properties during process of composting of different agro-waste residues during the initial phase of composting, increases in temperature recorded in different pits are in close agreement with those observed by other workers in case of small scale composting (Surez-Estrella *et al.*, 2003). Bollen *et al.*, (1989) recorded a temperature range of 50-70^{o} C within 6 days, and in their study also this phase lasted for 3-4 weeks. Lodha *et al.*, (2002) recorded a temperature range of 48-51^{o} C at 30 cm and 60-62^{o}C at 60 cm after 9-13 and 14-18 days, respectively. Increase in temperature during initial phase is attributed to exothermic reactions. As the composting proceeds, indigenous microorganisms start to utilize the organic materials for available carbon, nitrogen and other nutrients. As the activity continues, the temperature begins to increase from heat that is generated through microbial oxidations and respiratory functions known as bio oxidative phase. Increased temperature observed in weed residues pit compared to others may be due to slow rate of decomposition of stem and other portions.

More so, temperature varies with the type of residues used (Bollen *et al.,* 1989). One of the basic advantages of heat phase is sanitation of residues as temperature is considered as most reliable parameter to relate inactivation of pathogens in infected plant residues (Hoitink *et al.,* 1987). In general, low level of fungal population at first sampling date is yet another indication that only thermophilic fungi could survive in compost pits. Similarly, low fungal population in weed residues even at 150 days could be assigned to relatively high temperature in this pit and slow rate of decomposition of these residues (*Aerva, Celosia*), which are also known to have antifungal properties against soil-borne pathogens (Mawar and Lodha, 2006).

Relatively higher population of actinomycetes observed in *Calotropis, P. juliflora* and neem composts and those of bacteria in *P. juliflora* and *Calotropis* composts can be assigned to different composition of residues used. However, better growth of these organisms in *P. juliflora* composts can be due to availability of nutrients, sugars and growth hormones, etc. (Kolarkar and Sharma 1990 ; Santos and Pereria 1990; Khan *et al.,* 1992). Major fungal species detected in different composts include *Aspergillus versicolor, A. niger, Emericella rugulosa, Penicillium rubrum*, etc. Most of these are thermophilic and are known to contribute towards antagonism against soil-borne pathogens. Mesophilic microorganisms have also been implicated in the suppressive action of composts on *Pythium aphanidermatum* (Hadar and Mandelbaum 1986). In the present study, presence of good number of actinomycetes having antagonistic property against *M. phaseolina* in matured compost is of greater beneficial consequences in arid regions. This group of microbes can perform antagonistic activity even in fairly dry soils (Lodha *et al.,* 1990). Significant contribution of actinomycetes to biological control provided by composts has also been reported by You and Sivasithamparam (1995).

Bacillus spp., which could withstand peak heating, may induce biological control potential of composts. One *Bacillus* strain, *B firmus* was found to inhibit growth of *M. phaseolina* in separate *in vitro* tests (Lodha *et al.,* 2013). Phae *et al.,* (1990) also isolated a strain of *B. subtilis* from compost that induced suppressive effect on phytopathogenic microorganisms. Survival of *Bacillus* spp. alongwith other facultative thermopiles during heat phase has also been reported by Strom (1985).

In the present study, *Trichoderma* spp. disappeared during heat phase started to reinfest in curing phase onwards. The third or curing phase of the composting process begins as the concentration of readily biodegradable materials become limiting to the microflora in the compost. As a result, microbial activity decreased in the samples collected at 120 days of composting. After the temperature declined below 40°C, mesophilic microorganisms, some with bio-control activity colonized the now semi pasteurized composts from the outer low-temperature layers into the center of the pile, as also reported by Hoitink and Fahy (1986). During the entire process of composting, frequent turning and adequate maintenance of optimum moisture conditions enhanced recolonization by mesophiles and development of natural disease suppression (Hoitink *et al.,* 1991). As a result, microbial activity again increased in the samples collected on 150 days and in mature composts. However, reduction and eradication of *Trichoderma* spp. at 120 and 150 days was not related to microbial activity. Increased population of total bacteria, fungi and *Bacillus* spp. might have contributed in enhanced microbial activity at 150 days. Low population of actinomycetes at this sampling

date indicates that this group of microbe might not be playing important role in enhanced microbial activity.

Increased availability of micronutrients in mature composts can be attributed to fairly good decomposition of bio-degradable materials. Dried plants of *P. juliflora, A. nilotica*, neem and few of the weeds used in the present study contains high amount of Fe, Zn, Cu and Mn (Kolarkar and Sharma, 1990). Compost prepared from *A. nilotica* and weeds had higher concentration of these nutrients, which might have promoted growth of different types of microbes. *Trichoderma* strains effective as biological control agents in mature compost grow as saprophytes in fresh organic matter because the high concentrations of free nutrients repress hyperparasitism (Hoitink and Boehm, 1999). In our study, mature composts harbor good population of all these types of microbes, which can compete or hyper parasitize soil-borne plant pathogens including *M. phaseolina*. Greater population of *T. harzianum*, *Bacillus* spp and antagonistic actinomycetes are reducing incidence of *M. phaseolina* in arid soils (Mawar and Lodha 2008). Interactions among all of these strains seem to be required to achieve maximum efficacy against a broad array of soil-borne fungal or fungal like pathogens (Hardy and Sivasithamparam, 1995). Utilization of weeds in the preparation of compost has a potential advantage in nutrient deficient sandy soil. Many weeds are host of *M.phaseolina* (Singh *et al.*, 1990). However, in process of composting heat generated through exothermic reactions inactivate pathogenic peopagules to a greater extent (Lodha *et al.*, 2002). More so, weeds used to prepare compost are known to reduce *M.phasolina* population besides enhancing antagonistic actinomycetes (Mawar and Lodha, 2006). Earlier, use of *P.juliflora* compost as soil amendment has been found to reduce incidence of dry root rot of guar and cowpea in field studies with increase in micronutrients and microbial activity (Bareja *et al.,* 2013). The objective envisaged in the present study to utilize some of the available on farm wastes in the preparation of compost has been achieved to a considerable extent. Returning a part of residues what has been extracted from soil during course of agriculture can be an appropriate alternative for restoring nutrients and improving organic base of nutrient deficient arid soils.

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