# Inactivation of *Macrophomina phaseolina* propagules during composting and effect of composts on dry root rot severity and on seed yield of clusterbean

Satish Lodha, S.K. Sharma and R.K. Aggarwal Plant Pathology Laboratory, Central Arid Zone Research Institute, Jodhpur 342003, India (E-mail: sklodha@cazri.raj.nic.in)

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#### **Abstract**

Survival of a heat-tolerant pathogen *Macrophomina phaseolina*, causing dry root rot of clusterbean, was studied by incorporation and retrieval of infected residue samples at various stages of the composting process of pearl millet (*Pennisetum glaucum*) and clusterbean (*Cyamopsis tetragonoloba*) residues. During the heating phase, temperatures varied from 48–51 °C at 30 cm and 60–62 °C at 60 cm depth in compost pits. Reduction in survival of *M. phaseolina* propagules (13–23%) was significantly higher in the residues enriched with 4% urea-N and kept at 60 cm compared to 2% urea-N and at 30 cm. However, a heat phase (48–62 °C) was not enough to completely eradicate *M. phaseolina* propagules from infected residues. Further reductions (54–61%) in survived propagules were achieved by sub-lethal temperatures (48–53 °C) when moistened compost materials were exposed to heat during summer days. Beneficial effects of composts were ascertained on dry root rot intensity, seed yield of clusterbean and densities of *M. phaseolina*, *Nitrosomonas* and antagonists in soil. In a two-year field study, all the composts significantly reduced plant mortality due to dry root rot and increased the yield of clusterbean. The highest disease suppression and yield promotion were recorded in soil amended with pearl millet compost and cauliflower leaf residue compost, respectively. Soil amendment with compost also lead to a significant reduced density of *M. phaseolina* and an increased density of antagonistic actinomycetes, lytic bacteria and *Nitrosomonas*. Among composts, greater potential of cauliflower compost in enhancing population of antagonists in soil was discernible.

# Introduction

The incorporation of crop residues changes the soil characteristics in many ways including enrichment of nutrients, and improvement in microbial activity, water holding capacity, soil aeration and permeability. However, low moisture content, small microbial populations and high temperature prevailing in arid regions prolong the process of decomposition of crop residues when incorporated in a nutrient deficient sandy soil. Another disadvantage of direct incorporation is that crop residues may carry plant pathogens (Hoitink and Fahy, 1986). Composting is a method used to inactivate pathogens and decompose crop residues more rapidly. Most pathogens are inactivated during the heating

phase of composting (Bollen et al., 1989; Ylimaki et al., 1983), but no information on *Macrophomina phaseolina* is available.

In arid regions of India, many annual crops and weeds are susceptible to the dry root rot pathogen *M. phaseolina* (Tassi) Goid. (Lodha et al., 1986). It survives in the soil as sclerotia formed in infected plant tissues (Cook et al., 1973). Concerned with the risk of spreading *M. phaseolina* by incorporating compost in the soil, we analysed several samples from on-farm composts and detected 60–80 sclerotia per gram of compost. *Macrophomina phaseolina* is a heat-tolerant pathogen since sclerotia could withstand a temperature range of 60–65 °C (Bega and Smith, 1962; Mihail and Alcorn, 1984). Efforts, are therefore,

required to eliminate or bring down the sclerotial population of *M. phaseolina* from composts before their incorporation into soil.

During the crop-free summer period, polyethylene mulching (soil solarization) leads to a temperature increase in irrigated soil up to 58°C and consequently to a reduced density of viable propagules of M. phaseolina (Lodha and Solanki, 1992; Lodha et al., 1997). A dramatic increase in antagonistic bacteria and actinomycetes densities was also found in heated soil. However, scarcity of water and its high cost restricts use of this technique in low-input agriculture. Amendment of soil with composts prepared from organic wastes have been used with various levels of success for suppression of several soil-borne plant pathogens (Hoitink and Fahy, 1986; Ben-Yephet and Nelson, 1999), but information on M. phaseolina is not available. The present investigation deals with (i) the survival of M. phaseolina during composting and (ii) the disease suppressive characteristics of compost with respect to M. phaseolina.

#### Materials and methods

### Soil and composts

The experimental soil was loamy sand (sand: 91%, clay: 5%, silt: 4%) having 0.031% N, 0.25% organic C, 9 ppm available P (Olsen and Dean, 1975), pH 8.1, EC  $0.088 \, dS \, m^{-1}$  (soil: water ratio 1: 2.5) and bulk density 1.56 g cm<sup>-3</sup>. Twelve samples of soil were collected at 0-30 cm depth randomly using a 2.5 cm diameter soil auger to estimate the density of M. phaseolina propagules in a field under repeated cultivation of legumes. Fully dried residues of pearl millet (Pennisetum glaucum), clusterbean (Cyamopsis tetragonoloba), cauliflower (Brassica oleracea var. capitata) and a mixture of off-season weeds (Boerhavia diffusa, Cenchrus biflorus, Cyanodon dactylon, Heliotropium subulatum and Tephrosia purpurea) were used for the preparation of composts. The process of composting was initiated under partially anaerobic conditions in separate pits (1.7 m<sup>3</sup>) according to the principles of the Indore type (Howard and Ward, 1931). A 5 cm cowdung layer completely saturated with water was spread over the base of all the pits. In each pit, a 30 cm layer was filled with 40 kg of residues enriched with 400 g gypsum (1%) and 800 g urea (2%). Each layer was provided with 60% moisture (w/w) and then covered with a 10 cm thick layer containing a mixture of 68 kg cowdung and 100 kg field soil (Figure 1). Four such layers of residues

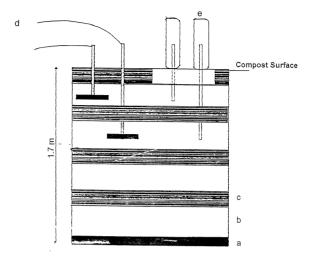


Figure 1. A cross-section of the compost pit. (a) Cowdung layer 5 cm, (b) crop residue (s) 30 cm, (c) cowdung + soil 10 cm, (d) residue in nylon bags 30/60 cm, (e) thermometers 30/60 cm.

with soil-dung mixture were placed in each pit. The soil having a native population of M. phaseolina (135 sclerotia per gram of soil), as estimated on selective medium (see biological assays section), was used in this mixture to enhance multiplication and activity of resident antagonists that can survive peak heating (Lodha and Solanki, 1992) and to absorb part of the liberated ammonia (Stevenson, 1986). Compost pits were finally covered with a 5 cm layer of weeds. Loss of moisture was replenished by adding 20 L of water every 10-15 days. C: N ratio of all the residues, cowdung, soil and each compost mixture was estimated separately. Carbon was estimated by oxidizing it with chromic acid in presence of H<sub>2</sub>SO<sub>4</sub> (Jackson, 1958). The excess chromic acid was back-titrated with ferrous ammonium sulphate. For N estimation, samples were digested with H2SO4 and distilled using Kjeltec Auto System II (Tecator). Once the composting process was over, matured composts were uniformly mixed separately and three sub-samples (500 g) were collected randomly from each compost to estimate C: N ratio. Macrophomina phaseolina and microbial populations including antagonists were estimated by the procedures described below.

Survival of M. phaseolina propagules during composting

In clusterbean and pearl millet residue-amended compost pits, several samples (20 g) each containing 2 g pieces (0.5–1.5 cm) of *M. phaseolina* infected roots and

18 g uninfected (apparently healthy) residues of clusterbean, enriched with 2% or 4% urea-N, were separately placed in small nylon pouches (120 µm pore). These were tied with long nylon strings and buried at 30 and 60 cm depth. Twenty-four samples, corresponding to 3 replications for each depths (2), each urea-N concentrations (2) and each date (2), were collected. Temperatures at both depths were recorded every day at 14 and 16 h for 6 weeks, because maximum temperature at 30 and 60 cm are attained at these two periods of the day. Pouches were retrieved after 2 and 4 months with a minimum disturbance from pits. These samples were air-dried and passed through a 2 mm sieve before estimating viable propagules of M. phaseolina. After 4 months, all the composting material of each pit was given 3 turnings at an interval of 15 days.

Effect of summer exposure of composts on survival of M. phaseolina

Composts retrieved from pits in May were spread separately over soil surface as a 10–12 cm thick layer in an open field during hot summer days of June and were moistened once at 10% (w/w) with water. Temperatures at 5 cm depth were recorded every day at 14 h. After seven days of exposure, three sub-samples (50 g) were collected randomly from each compost for estimating the density of *M. phaseolina* propagules.

Effect of composts on dry root rot severity and on clusterbean seed yield

Suppressive effect of composts on dry root rot was studied in a fixed layout using completely randomized block design with 5 replications during 1994 and 1995. The soil of the experimental field was naturally infested with sclerotia of M. phaseolina (640 per gram of soil) as determined on selective media as described in section biological assays. After summer exposure, 4-week-old composts (after retrieval from pits) of pearl millet, clusterbean, weeds and cauliflower residues (each 4 t ha<sup>-1</sup>) were incorporated to a depth of 0-30 cm by a spade in separate plots  $(4 \times 4 \text{ m})$ . Plots without any amendment served as control. Clusterbean seeds (cv. HG 75, 8 rows per plot) were sown on 25th (1994) and 22nd July (1995) and harvested on 5th November (1994) and 28th October (1995). Three soil samples from same depth were collected with a 2.5 cm diameter tubular probe before amendment and 15 days after harvest from each plot. For each date, these were bulked to form one sample for each of the 5 replications and processed for biological assays. Data on plant mortality due to dry root rot were recorded 12–15 days before harvest in the third and fourth row of each plot. Seed yield of clusterbean was also recorded.

Biological assays

Population densities of M. phaseolina were determined by sprinkling 50 mg of native/amended soil and composts on chloroneb-mercury-rose bengalagar (CMRA) medium (Meyer et al., 1973) in 9 cm Petri dishes. Microbial populations were evaluated by serial dilution on Martin's rose bengal-agar for fungi (Martin, 1950), Thornton's agar for aerobic cultivable bacteria (Thornton, 1922) and Ken-Knight agar for actinomycetes (Allen, 1959). The populations of Nitrosomonas were estimated by the MPN method using ammonium-calcium carbonate medium (Alexander and Clark, 1965). The number of colony forming units (CFU) was measured after incubation of plates for 21 and 6 days at 30±1 °C for Nitrosomonas and different microbial groups, respectively. Six Petri dishes per medium were used for enumeration of the different microbial groups in each soil sample. The mean of six plates was considered as an estimation of microbial density in each of the 5 replicates of the different treatments.

Lytic bacterial density was estimated following the method of Greenberger et al. (1987). M. phaseolina was grown on potato dextrose broth for 7 days. The fungal mats obtained after filteration were thoroughly washed with sterile distilled water and cut into 1 cm diameter discs. Two discs and 500 mg air-dried soil from each of the 5 replications of all the treatments as carbon source were separately incorporated to flasks (3 replications) containing 30 ml Czapeck's mineral solution and incubated at 30  $\pm$  1  $^{\circ}$ C for 2 days for multiplication of lytic bacteria. One millilitre aliquot from each flask was withdrawn for serial dilution and 1 ml of the final suspension was poured on the Petri dishes containing nutrient agar medium. Colony forming units were counted after incubation for 3 days at  $30 \pm 1$  °C. Mean of three flasks was computed as one estimation for each of the 5 replications of all the treatments.

Actinomycetes antagonistic 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 dishes (9 cm) containing Czapeck's Dox agar. After growth

in the dark for 48 h at  $28 \pm 2$  °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 inducing a minimum of 2 mm inhibition zone confirmed their antagonistic activity.

## Statistical analyses

All the data were subjected to analysis of variance (ANOVA) and the treatment means were compared with LSD (P=0.05). Populations of total bacteria, actinomycetes, fungi, antagonistic actinomycetes and lytic bacteria were transformed to a log scale [ $\log(x+1)$ ] before statistical analysis. Data recorded on survival of M. phaseolina propagules in compost pits and soil were analysed using three factor factorial design (Snedecor and Cochran, 1967).

#### Results

#### Composts

The process of composting was completed in all the pits in a period of 6 months. Maximum temperatures observed were 48-51 °C at 30 cm and 60-62 °C at 60 cm depth after 9-13 days and 14-18 days, respectively, after filling the compost pits. The heat phase lasted 3-4 weeks. A reduction of 28%-35% of the initial volume was observed in compost pits with maximum being in the cauliflower pit. In general, considerable decrease in C:N ratio was estimated in all the composts (Table 1). Density of aerobic cultivable bacteria was maximal in cauliflower compost but that of actinomycetes, fungi, lytic bacterial and antagonistic actinomycetes were significantly higher in clusterbean compost (Table 2). In the final composts, viable sclerotia of M. phaseolina were significantly lower in cauliflower compost compared to other composts.

Table 1. C:N ratio of residues – soil-dung-urea mixture and their composted materials

Residue	C: N ratio					
	Residue	Mixture <sup>a</sup>	Compost			
Pearl millet	83.3	27.3	18.2			
Clusterbean	64.5	30.9	9.4			
Weeds	57.3	26.8	9.4			
Cauliflower leaves	19.6	15.1	11.6			

 $<sup>^{\</sup>mathrm{a}}\mathrm{C}:N$  ratio of soil, dung and urea were 8.1, 15.1 and 0.43, respectively.

Survival of M. phaseolina propagules during composting

Density of viable sclerotia of *M. phaseolina* was significantly lower in the partially decomposed clusterbean residue samples retrieved from 60 cm depth compared to those from 30 cm depth in both the pits (Table 3). The counts of *M. phaseolina* were significantly lower in the residues enriched with 4% compared to 2% urea-N. Viable propagules of *M. phaseolina* reduced drastically in all the samples retrieved after 4 months with similar trend for all the treatments except that differences were not significant in the samples enriched with urea-N at 30 cm depth from pearl millet pit (Table 3).

## Summer exposure of moistened composts

During a 7-day period of natural heating, maximum air temperature ranged from 39 °C to 47 °C. Moistening the composts with water initially brought down the maximal soil temperature to 37 °C, but a gradual increase of 53 °C at 5 cm depth followed within 2–3 days. As a result, a 53–61% reduction in counts of *M. phaseolina* occurred in different composts (Table 2).

Effect of composts on disease severity and on clusterbean seed yield

The severity of dry root rot in 1994 was not severe on clusterbean crop due to high and evenly distributed rainfall (378 mm in 14 days) during the growing season.

Table 2. Densities of M. phaseolina propagules (cfu g $^{-1}$  plant) released from decomposing clusterbean residues enriched with urea-N embedded in clusterbean and pearl millet compost pits

Duration	Urea-N (%)	Compost pit*					
(month)		Pearl depth	millet (cm)		Clusterbean depth (cm)		
		30	60	30	60		
2	2	540	334	546	300		
	4	406	306	400	160		
4	2	103	119	131	112		
	4	109	95	95	57		
LSD ( $P < 0.05$ )							
Urea-N		1	19		23		
Month		28		17			
$\begin{array}{c} \text{Urea-N} \times \text{month} \times \\ \text{depth} \end{array}$		3	39		32		

<sup>\*</sup>Differences among pits were statistically non-significant.

Table 3. Densities (cfu  $g^{-1}$  compost) of M. phaseolina and other resident microflora including antagonists (before summer exposure) in different composts

Compost	M. phaseolina (g <sup>-1</sup> compost)		Bacteria (g <sup>-1</sup> compost)		Actinomycetes (g <sup>-1</sup> compost)		Fungi (g <sup>-1</sup> compost)	
	After withdrawal from pit	After summer exposure <sup>a</sup>	Aerobic cultivable log cfu <sup>c</sup>	Lytic <sup>b</sup> log cfu	Total log cfu	Antagonist <sup>d</sup> log cfu	Log cfu	
Pearl millet	67	31	15.08	13.93	14.09	13.52	13.94	
Clusterbean	45	18	14.80	14.34	15.54	14.22	15.23	
Weeds	55	23	14.93	14.16	14.07	13.37	14.09	
Cauliflower leaves	31	12	15.28	14.26	14.28	13.69	13.45	
LSD ( $P < 0.05$ )	10	4	0.15	0.14	0.13	0.18	0.16	

<sup>&</sup>lt;sup>a</sup>Summer exposure of moistened (10%) compost was done from 8 to 14 June, 1994.

However, a 27-day dry spell soon after sowing, leading to long duration of moisture stress, caused severe plant mortality due to dry root rot in 1995 (rainfall 260 mm in 9 days). Soil moisture in compost amended plots ranged from 2.4-8.8% and 2.1-5.7%, compared to 2.1-8.1% and 1.8-5.2% in non-amended plots during the crop growing seasons of 1994 and 1995, respectively. Thus, available soil moisture in amended plots remained 14-19% higher than in the non-amended control plots during different stages of the crop growth and the differences were more discernible during the rainless period in 1995. During both years of field experiments, disease severity due to dry root rot was significantly reduced and seed yield of clusterbean was significantly enhanced by soil amendment with the different composts (Table 4). Among composts, the lowest disease severity was recorded with pearl millet compost except in 1995 where disease suppression was not significantly different from that obtained in the presence of weed compost. However, seed yield was the highest in plots amended with cauliflower compost. The increase in seed yield in amended treatments over control was more conspicuous in a normal rainfall year 1994 (28.1–52.3%) than in a low rainfall year such as 1995 (28.9–38.4%).

Amendment of soil with compost, in general, increased the population of antagonistic actinomycetes, lytic bacteria and *Nitrosomonas* and decreased the population of *M. phaseolina* in soil (Table 5). Among composts, increase in antagonistic actinomycetes was significantly higher in cauliflower compost amended soil that also had maximum reduction of *M. phaseolina* density. In both years, the

Table 4. Effect of different compost amendment on dry root rot mortality induced by *M. phaseolina* and seed yield of clusterbean

Compost amended	Mortal	ity (%)	Seed yield (kg ha <sup>-1</sup> )		
soil*	1994	1995	1994	1995	
Pearl millet	2.0	4.4	508.3	296.8	
Clusterbean	3.0	6.3	572.7	299.5	
Weeds	4.2	6.8	585.7	311.4	
Cauliflower leaves	2.2	6.0	604.1	318.7	
Control	5.4	16.0	396.5	230.2	
LSD ( $P < 0.05$ )	1.2	5.5	54.5	45.5	

<sup>\*</sup>Soil was amended with 4 t compost per hectare.

population of *Nitrosomonas* spp. was also significantly higher in cauliflower compost followed by pearl millet compost amended soil compared to the other two composts.

# Discussion

Release of *M. phaseolina* inoculum from infected plant residues was significantly reduced by organic composting. Many factors may be involved in reducing the population of *M. phaseolina* during composting: (i) heat generated in the first phase (Bollen et al., 1989), (ii) toxicity of conversion products formed during or after the self-heating process (Berestetsky and Kravchenko, 1984) and (iii) microbial antagonism in presence of moisture (Dhingra and Sinclair, 1975). Amendment of soil with composts showing a reduced density of *M. phaseolina* leads to a significant decrease of dry root rot severity and a significant increase of seed yield of clusterbean both in normal and low rainfall conditions.

<sup>&</sup>lt;sup>b</sup>Erlenmeyer flasks containing Czapeck's mineral solution and mycelial disc of *M. phaseolina* as carbon source were inoculated with 500 mg of each compost.

<sup>&</sup>lt;sup>c</sup>Average of 3 replications for each sample.

<sup>&</sup>lt;sup>d</sup>Detected on Czapeck's Dox Agar against M. phaseolina.

Table 5. Densities of M. phaseolina, associated antagonists and Nitrosomonas in compost amended soil

Compost amendment	$C fu g^{-1} soil^a$								
	M. phaseolina <sup>b</sup>		Antagonistic actinomycetes <sup>c</sup> log cfu g <sup>-1</sup>		Lytic bacteria <sup>d</sup> log cfu g <sup>-1</sup>		Nitrosomonas <sup>e</sup> log cfu g <sup>-1</sup>		
	1994	1995	1994	1995	1994	1995	1994	1995	
Pearl millet	313	510	11.40	11.46	12.47	12.67	10.33	10.51	
Clusterbean	266	394	11.55	11.28	12.64	12.87	10.15	10.19	
Weeds	386	477	11.45	11.51	12.54	12.75	9.83	9.94	
Cauliflower leaves	253	381	11.90	12.20	12.69	12.85	10.42	11.04	
Control	426	768	11.28	11.16	12.36	12.38	9.84	9.78	
LSD (P<0.05)	76	43	0.19	0.13	0.12	0.07	0.23	0.18	

<sup>&</sup>lt;sup>a</sup>Populations estimated 15 days after the harvest of clusterbean crop.

Thermal death is considered to be the most important cause of eradication of plant pathogens from residues during composting (Hoitink and Fahy, 1986; Bollen et al., 1989). In the present study, low survival of M. phaseolina propagules, particularly at 60 cm could be attributed to elevated temperatures during the heat phase that reached values reported to be lethal (60 °C for 3 s) for sclerotia of M. phaseolina (Bega and Smith, 1962). This hypothesis is supported by the survival of a higher number of sclerotia in the samples kept at 30 cm depth where maximum temperatures (48–51 °C) were lower than at 60 cm depth. However, viable sclerotia could still be retrieved from the pits after 2 months. This observation could be ascribed to the heat-tolerant nature of M. phaseolina propagules, which withstand a temperature range of 58-63 °C under polyethylene mulching in hot arid conditions (Lodha, 1989; Lodha and Solanki, 1992; Mihail and Alcorn, 1984). Incomplete release of sclerotia from the organic matter due to partial decomposition of crop residues by the time of first sampling could be another possible explanation for this survival. Ylimaki et al. (1983) also found that a 3-week exposure to composting at 60-65 °C was insufficient to destroy Plasmodiophora brassicae in small-scale composting.

Further reduction in viable sclerotia of *M. phaseolina* after the heat phase could be a result of combined effects of fungitoxic compounds and microbial antagonism in the presence of moisture and nitrogen. Several workers have demonstrated production of fungitoxic volatile compound during decomposition

of crop residues (Berestetsky and Kravchenko, 1984; Spring et al., 1980). Release of such volatiles from decomposing pearl millet residues was shown to reduce density of viable propagules of *M. phaseolina* in a previous study (Sharma et al., 1995). The release of volatiles, particularly isothiocyanates from cruciferous residues was shown to be maximal, during the second and third week of decomposition (Lewis and Papavizas, 1970; 1971; Gamliel and Stapleton, 1993).

The higher reduction in viable propagules of M. phaseolina in the decomposing tissues enriched with 4% compared to 2% urea-N recorded in the present study could result from a higher concentration of nitrogen. Filho and Dhingra (1980) have also reported that higher nitrogen levels hasten the decomposition of plant residues accompanied by an increase in microbial populations that in turn caused reduction in M. phaseolina counts. Germination of sclerotia of M. phaseolina has been found to be stimulated by amendments (Smith, 1969). The resulting germ-tube and hyphae are known to be sensitive to bacteria and actinomycetes leading to lysis of fungal cell walls (Kovoor, 1954). In our experiment, high populations of antagonists estimated in final compost samples might have accelerated antagonism, particularly the activity of lytic bacteria in the presence of moisture and nitrogen.

Survival of *M. phaseolina* propagules in matured composts could be due to static state of residues in pits where lethal temperatures was not reached at all the sites. However, efforts to expose moistened composts

<sup>&</sup>lt;sup>b</sup>Initial population of *M. phaseolina* was 640 propagules per gram of soil.

<sup>&</sup>lt;sup>c</sup>Detected on Czapeck's Dox Agar against M. phaseolina.

<sup>&</sup>lt;sup>d</sup>Erlenmeyer flasks containing Czapeck's mineral solution and mycelial discs of *M. phaseolina* as carbon source were inoculated with 500 mg of compost amended soil.

<sup>&</sup>lt;sup>e</sup>Estimated by MPN method.

to prevailing high temperatures was found highly effective in further reducing the viable propagules of *M. phaseolina*. Sub-lethal temperatures (48–53 °C) exerted a weakening effect on remaining sclerotia of *M. phaseolina* which might have accelerated microbial antagonism. In hot arid regions, merely exposing moistened cruciferous residue amended soil (46–53 °C) during summer days could reduce the population of *M. phaseolina* by 90–96% at 0-15 cm depth (Lodha et al., 1997).

Organic amendments may increase, decrease or not affect diseases caused by soil-borne pathogens (Hoitink and Fahy, 1986; Huber and Watson, 1970). In the present study, disease suppression due to addition of composts into the soil could possibly be ascribed to the following factors: (i) composts support high levels of total microbial population including antagonists and (ii) composts improves the moisture holding of the soil which in turn could reduce the pathogenic propagules and disease.

Increased densities of bacteria and actinomycetes along with antagonists by amendment of compost in the field study could be ascribed to its blending with field soil in each layer of residues during composting. Their population multiplied rapidly from initial  $1.6 \times 10^5 \,\mathrm{g}^{-1}$  soil to  $1.2 \times 10^6 \,\mathrm{g}^{-1}$  compost at high temperature attained during heat phase, more so, in the presence of nutrients like urea-N. Bacillus spp. that can withstand peak heating may induce biological control in composts. One Bacillus strain was found to inhibit growth of M. phaseolina in separate in vitro tests (data not presented). Phae et al. (1990) also isolated a strain of Bacillus subtilis from compost that induced suppressive effect on phytopathogenic microorganisms. Amended soil held more water than the non-amended sandy soil, which in turn reduced M. phaseolina population or its infection on the host plant due to enhanced antagonism or competition for site (Filho and Dhingra, 1980; Sheikh and Ghaffar, 1979). Soil moisture is the most critical factor in determining M. phaseolina infection besides increasing antagonism particularly by bacteria (Dhingra and Sinclair, 1975; Lodha, 1996). Low disease levels (2.2–6.8%) in spite of substantial inoculum density (253–510 sclerotia per gram of soil) may be due to suppressive effects of the compost. In contrast, pathogen density as low as 64 sclerotia per gram of soil could cause 27% mortality due to dry root rot in non-amended soils (Lodha, 1996). Lewis et al. (1992) also observed induction of suppression without a reduction in population of Pythium ultimum in compost amended soils. Mechanisms involved in the suppressiveness of amended soils described here could result from enhanced lytic bacterial activity and antagonism by actinomycetes, microbial competition and increased availability of soil moisture. These hypotheses, however, remain to be further evaluated. Availability of soil moisture might have also helped in maintaining high water potential of plants at vulnerable stage, which did not express symptoms of *Macrophomina* infection (Burman and Lodha, 1996).

Our results showed greater potential of cauliflower compost amendment to reduce M. phaseolina density compared to other composts, in which increased antagonist density brought proportionate reduction in dry root rot incidence. Beneficial effects of incorporating cruciferous residues on growth and yield of watermelon and wheat due to disease suppression and increase in microbes beneficial to plant growth has been well documented (Keinath, 1996; Kirkegaard et al., 1994). Increased seed yield in all the compost amended treatments may be a cumulative effect of reduced disease incidence, more retention of soil moisture, availability of nutrients and qualitative and quantitative improvements in microbiological properties. Improvement in the population of *Nitrosomonas* in amended soil in our study is an indication of mineralization of N from compost (Hadas et al., 1996). Nitrosomonas oxidises ammonium to nitrite, which in turn is converted into nitrate by Nitrobacter. The Nitrosomonas population is more stable in soil throughout the year than of Nitrobacter (Skujins and Fulgham, 1978), even in dry soil (Allison and Prosser, 1991). Increased microbial populations particularly those of antagonistic actinomycetes and lytic bacteria against M. phaseolina in compost amended soil probably lead to long-term beneficial effects. The high microbial activity and biomass caused by the 'general soil microflora' in compost-amended soil prevents germination of pathogenic propagules and infection of the host, presumably through microbiostasis (Hoitink and Boehm, 1999).

Our study demonstrated that after sanitation of crop residues from *M. phaseolina*, amendment of compost in nutrient deficient sandy soil reduced dry root severity, improved microbial properties of soil and seed yield. In resource-deficient farming of arid regions, the beneficial effect of compost as an integral part of low-input sustainable agriculture can also be a practical way of managing soil-borne pathogens.

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#### References

- Alexander M and Clark FE (1965) Nitrifying bacteria. In: Black CA, Evans DO, White JL, Esminger LE and Clark FE (eds) Method of Soil Analysis Chemical and Microbiological Properties (pp 1377–1483) American Society of Agronomy, Madison, WI. 1572 pp
- Allen ON (1959) Experiments in Soil Bacteriology, 3rd edn (117 pp) Burgess, MN
- Allison SM and Prosser JI (1991) Survival of ammonia oxidising bacteria in air-dried soil. FEMS- Microbiology Letters 79: 65–68
- Bega RV and Smith RS (1962) Time–temperature relationships in thermal inactivation of sclerotia of *Macrophomina phaseolina*. Phytopathology 52: 632–635
- Ben-Yephet Y and Nelson EB (1999) Differential suppression of damping-off caused by *Pythium aphanidermatum*, *P. irregulare* and *P. myriotylum* in composts at different temperatures. Plant Disease 83: 356–360
- Berestetsky OA and Kravchenko LV (1984) Volatile products of plant residue decomposition and their effect on soil microflora. In: Jszegi (ed.) Soil Biology and Conservation of the Biosphere, Vol I (pp 419–425) Academiai Kaido, Budapest
- Bollen GJ, Volker D and Wijnen AP (1989) Inactivation of soilborne plant pathogens during small scale composting of crop residues. Netherlands Journal of Plant Pathology 95(suppl 1): 19–30
- Burman U and Lodha S (1996) *Macrophomina phaseolina* induced changes in plant water relations of resistant and susceptible cowpea genotypes. Indian Phytopathology 49: 254–259
- Cook GE, Boosalis MG, Dunkle LD and Odvodny GN (1973) Survival of *Macrophomina phaseolina* in corn and sorghum stalk residue. Plant Disease Reporter 57: 873–875
- Dhingra OD and Sinclair JB (1975) Survival of *Macrophomina* phaseolina sclerotia in soil: effects of soil moisture, carbon: nitrogen ratios, carbon sources and nitrogen concentration. Phytopathology 65: 236–240
- Filho ES and Dhingra OD (1980) Population changes of *Macrophomina phaseolina* in amended soils. Transactions of British Mycological Society 74: 471–481
- Gamliel A and Stapleton JJ (1993) Characterization of antifungal compounds evolved from solarized soil amended with cabbage residue. Phytopathology 83: 899–905
- Ghaffar A, Zentmeyer GA and Erwin DC (1969) Effect of organic amendments on severity of *Macrophomina phaseolina* root rot of cotton. Phytopathology 59: 1267–1269.
- Greenberger A, Yogev A and Katan J (1987) Induced suppressiveness in solarized soils. Phytopathology 77: 1663–1667.
- Hadas A, Kautsky L and Portnoy R (1996) Mineralization of composted manure and microbial dynamics in soil as affected by long-term nitrogen management. Soil biology and Biochemistry 28: 733–738

- Hointink HAJ and Boehm MJ (1999) Biocontrol within the context of soil microbial communities: a substratedependent phenomenon. Annual Review of Phytopathology 37: 427–446
- Hoitink HAJ and Fahy PC (1986) Basis for the control of soil-borne plant pathogens with composts. Annual Review of Phytopathology 24: 93–114
- Howard A and Wad YD (1931) The Waste Products of Agriculture and their Utilisation as Humus (p 167) Oxford University Press, London
- Huber DM and Watson RD (1970) Effect of organic amendments on soil-borne plant pathogens. Phytopathology 60: 22–26
- Jackson ML (1958) Soil Chemical Analysis (498 pp) Prentice Hall, New Delhi, India
- Keinath AP (1996) Soil amendment with cabbage residue and crop rotation to reduce gummy stem blight and increase growth and yield of watermelon. Plant Disease 80: 564–570
- Kirkegaard JA, Gardner PA, Angus JF and Koetz E (1994) Effect of brassica crops on the growth and yield of wheat. Australian Journal of Agricultural Research 45: 529–545
- Kovoor ATA (1954) Some factors affecting the growth of Rhizoctonia bataticola in soil. Journal of Madras University 24: 47–52
- Lewis JA and Papavizas GC (1970) Evolution of volatile sulfur containing compounds from decomposition of crucifers in soil. Soil Biology and Biochemistry 2: 239–246
- Lewis JA and Papavizas GC (1971) Effect of sulfur containing volatile compounds and vapors from cabbage decomposition on *Aphanomyces euteiches*. Phytopathology 61: 208–214
- Lewis JA, Lumsden RD, Millner PD and Keinath AP (1992) Suppression of damping off peas and cotton in the field with composted sewage sludge. Crop Protection 11: 260–266
- Lodha S (1989) Soil solarization for control of *Macrophomina phaseolina* (Tassi) Goid in arid soils. Indian Phytopathology 42: 170–171
- Lodha S (1996) Influence of moisture conservation techniques on *Macrophomina phaseolina* population, dry root rot and yield of clusterbean. Indian Phytopathology 49: 342–349
- Lodha S, Gupta GK and Singh S (1986) Crop disease situation and some new records in Indian arid zone. Annals of Arid Zone 25: 311–320
- Lodha S and Solanki KR (1992) Influence of solar heating on control of dry root rot (*Macrophomina phaseolina*) and weeds in arid environment. Indian Journal of Agricultural Science 62: 838–843
- Lodha S, Sharma SK and Aggarwal RK (1997) Solarization and natural heating of irrigated soil amended with cruciferous residues for improved control of *Macrophomina phaseolina*. Plant Pathology 46: 186–190
- Martin JP (1950) Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Science 69: 215–232
- Meyer WA, Sinclair JB and Khare MN (1973) Biology of *Macrophomina phaseolina* in soil studied with selective media. Phytopathology 63: 613–620
- Mihail JD and Alcorn M (1984) Effects of soil solarization on *Macrophomina phaseolina* and *Sclerotium rolfsii*. Plant Disease 68: 156–159

- Olsen SR and Dean LA (1965) Phosphorus. In: Black CA (ed.) Methods of Soil Analysis. Part 2: Agronomy Monographs No. 9 (pp 1035–1049) American Society of Agronomy, Madison, Wisconsin
- Phae CG, Saski M, Shoda M, Kubota H (1990) Characteristics of *Bacillus subtilis* isolated from composts suppressing phytopathogenic microorganisms. Soil Science Plant Nutrition 36: 575–586
- Sharma SK, Aggarwal RK and Lodha S (1995) Population changes of *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *cumini* in oil cake and crop residue amended sandy soils. Applied Soil Ecology 2: 281–284
- Sheikh AH and Ghaffar A (1979) Relation of sclerotial inoculum density and soil moisture to infection of field crops by *Macrophomina phaseolina*. Pakistan Journal of Botany 11: 185–189
- Skujins J and Fulgham PT (1978) Nitrification in great basin desert soils. In: West NE and Skujins J (eds) Nitrogen in Desert Ecosystems (pp 60–76) Dowden, Hutchinson & Ross Inc. Stroudsburg, Pennsylvania

- Smith WH (1969) Germination of *Macrophomina* phaseolina sclerotia as affected by *Pinus lambertiana* root exudate. Canadian Journal of Microbiology 15: 1387–1391
- Snedecor GE and Cochran WJ (1967) Statistical Methods (pp 258–298) Oxford & IBH Publishing Co., Calcutta
- Spring DE, Ellis MA, Spotts RA, Hoitink HAJ and Schmitthenner AF (1980) Suppression of the apple collar root pathogen in composted hardwood bark. Phytopathology 70: 1209–1212
- Stevenson FJ (1986) Cycles of Soil (380 pp) John Wiley & Sons, Inc. Canada
- Thornton HG (1922) On the development of a standardized agar medium for counting soil bacteria with special regard to the expression of spreading colonies. Annals of Biology 9: 241–274
- Ylimaki A, Toiviainen A, Kallio H and Tikanmaki E (1983) Survival of some plant pathogens during industrial-scale composting of wastes from a food processing plant. Annals Agriculturae Fenniae 22: 77–85