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SEED GERMINATION BIOASSAY USING MAIZE SEEDS FOR PHYTOXICITY EVALUATION OF DIFFERENT COMPOSTED MATERIALS

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Abstract

In this paper we evaluated the phytotoxicity of different composts obtained by two different composting methods using seed germination bioassay. Seeds of *Zea mays* were sown in 1:5 extract of composts and these were compared with the control (100% distilled water) for each type of material. Composting of herbal pharmaceutical solid waste (HPSW) was carried out using both conventional bin and pit method. HPSW was mixed separately with poultry manure, cow-manure and goat manure in three different ratios. Uncomposted and composted HPSW were tested to study the Phytotoxicity on *Zea mays* seed germination, after composting increase in percent germination as well as germination index (GI) values were observed in all combinations regardless, composted by pit or bin method. The results clearly showed that composting reduced Phytotoxicity. The results showed that use of completely composted organic waste reduces the phytotoxicity and is better than the use of uncomposted waste. It was found that pit method was more suitable than bin method. Herbal waste with goat manure in 1:1 ratio was found to be the most effective combination as compared to other combinations here. Germination was 100% and the germination index was 1.4 whereas uncomposted HPSW showed the lowest percent germination i.e., 77% and germination index 52.31 respectively.

Introduction

The organic waste materials, mainly of plant and animal origin, provide a good source of organic matter and nutrients to improve soil productivity (Sarwar *et al*., 2007; Sharma *et al*., 2005). Herbal pharmaceutical waste from herbal pharmaceutical industry contains high concentrations of organic matter. It contains several medicinal herbs like subistan, soanf (*Foeniculum vulgare*), Bad-e-musk, balchar (*Nardostachys jatamansi*), banafsha (*Viola odorata*) and tea (*Camellia sinensis*) .This waste material was collected and dumped into the municipal landfills, resulting loss of potentially valuable organic material that can be processed as organic fertilizer (Ferhan *et al*., 2010). Animal manures (poultry and cattle) have also been effectively used as fertilizers for centuries and recognized as the most considerable natural fertilizers because of its low cost and high content of macro- and micronutrients for crop growth (Sarwar *et al*., 2008; Sharpley & Smith, 1995; Sloan *et al*., 2003).

Composting is a useful strategy for the sustainable recycling of organic wastes (Tuomela *et al*., 2000). Composting has become a preferred method for municipalities and industries to recycle a variety of organic by-products into a safer and more stabilized material for application to soil (Butler *et al*., 2001; Carr *et al*., 1995). The organic waste material used for the composting varies in their nutrient and mineral composition as this affects the rate of decomposition of the compost and the composition of matured compost (Adegunloye *et al*., 2009).

Assessing the phytotoxicity of composts is one of the most important criteria being used to avoid environmental risk before the compost can be recycled back to agricultural land. Application of unstable compost onto the soil causes negative effects on seed germination, plant growth and development (Morel *et al*., 1985); it can induce anaerobic conditions (Mathur *et al*., 1993). Immature compost also introduce phytotoxic compounds such as heavy metals (Hue & Liu, 1995; Tam & Tiquia, 1994), phenloic compounds (Wong, 1985) ethylene and ammonia (Wong *et al*., 1983; Tam & Tiquia, 1994), excess accumulation of salts (Abdul *et al*., 2013; Salman *et al*., 2013) and organic acids (Manios *et al*., 1989) which could retard seed germination.

The present study was carried out with the objective to evaluate the suitable method for composting process i.e., bin and pit method. The most effective combinations and concentrations of herbal pharmaceutical waste with poultry; cow and goat manure on the basis of phytotoxicity assay.

Materials and Methods

Sample collection: Various composts were prepared using single and combination of substrates, these substrates included herbal waste (HW), cow manure (CM), poultry manure (PM) and goat manure (GM). Herbal waste sample were collected from herbal pharmaceutical industry and live stock waste (animal waste) collected from cattle farm and poultry farm located at Karachi region.

Sample preparation: Approximately 15-20 Kg of substrate mixtures were prepared separately by mixing herbal pharmaceutical solid waste, cow manure, goat manure and poultry manure in 1:1, 1:2, 1:3 ratios respectively (Table 1). Combinations of substrates were properly mixed on a concrete platform with occasional watering to bring the final moisture in the range of 40-60% (Buswell, 1984). All the test composts were run in triplicate and results were show the mean of three replicates. Composting mixtures were subjected to composting separately by two methods.

Table 1. Quantity and C: N ratio of different substrate mixtures.

Ratio	Quantity (Kg)				C:N ratio
	HPSW	CM	GM	PM	
	HPSW+CM				
1:1	10	10	-	-	26
1:2	10	5	-	-	26
1:3	15	5	-	-	27
	HPSW+GM				
1:1	10	-	10	-	69
1:2	10	-	5	-	20
1:3	15	-	5	-	19
	HPSW+PM				
1:1	10	-	-	10	86
1:2	10	-	-	5	56
1:3	15	-	-	5	21
	Single Substrate				
HPSW	20	-	-	-	26

Pit composting method: Pits of 1.5 x 1.5 x 2 ft were constructed in the open sunny field, were piled with composting mixtures, and covered with layers of dried straws and observed for 60 days.

Bin composting method: Rectangular wooden boxes with the capacity about 30 K.g were used in this method. Wooden boxes were filled with the composting mixture then placed under sunlight for 60 days.

Phytotoxicity assay: The Phytotoxicity of the samples was determined in distilled water extracts (1:5, w/v) following the method of Zucconi & de Bertoldi (1987). According to method the extracts were agitated vigorously for 1 hour at room temperature in the dark and then centrifuged for 15 min at 3,000 rpm in order to separate the phases. The supernatant filtered through the Whatman No.1 filter paper filter. Ten maize (*Zea mays* L.*)* seeds were placed in a 90 mm diameter sterilized Petri dishes lined with filter paper containing 10 ml of each extract, petri dishes with filter paper and 10 ml of distilled water served as control, and test was run in triplicate. Petri dishes were left on laboratory bench and after 48 hours the total length of each maize root was measured. The dry weight was determined after drying seedlings at 60^{o}C for 24 hours (Ogbo *et al*., 2010).

Calculation

$$\text{Germination Index} = \%\ \text{Germination} \times \%\ \text{Relative root growth} \times 100$$

$$\%\ \text{Germination} = \frac{\text{No. of seeds germinated in extract}}{\text{No. of seeds germinated in control}} \times 100$$

$$\%\ \text{Relative root growth} = \frac{\text{Mean root length in extract}}{\text{Mean root length in control}} \times 100$$

$$\text{Mean Germination Time} = \sum Dn / \sum n$$

where n is the number of seed, which were germinated on day D, and D is number of days counted from the beginning of germination.

Results and Discussion

During turning of composting materials a pleasant earthy smell was noted in all combinations in both pits and bins during the whole process, this indicates proper aeration and absence of anaerobic decomposition. At the end of composting process great and consistent reduction in the size of the composting material was observed, also varying appearance (colour) of composting material was observed during the composting process. Materials composted by pit method contain cream to light brown colour while materials composted by bin method showed dark brown to chocolate brown colour.

The seed germination and root elongation technique has been devised to evaluate the damaging effects and toxicity of compost (Wong, 1985). In the present study mean germination time 02 days was observed in all the substrate ratios composted by bin and pit method. If we compare bin and pit method on the basis of GI value highest value was obtained when extracts of HPSW in combination with goat manure in 1:1 ratio composted by pit method was used, second highest value was observed when extracts of HPSW with cow manure in 1:2 ratio composted by pit method (Fig. 1). If we compare germination index values of different substrate rations, the highest and lowest GI were observed when extracts of HPSW with goat manure in 1:1 ratio and HPSW with poultry manure in 1:3 ratio were used, observed values were151.49 and 69.2 respectively (Fig. 1), on the other hand Fig. 2 show the comparison of seed germination in different composts prepared by bin and pit method. If we compare germination values of different substrate rations, composted herbal pharmaceutical waste in combination with poultry manure showed highest value which is 103.3 while CM 1:3 showed 92.2, similarly if we compare pit and bin method on the basis seed germination and GI values highest value was obtained by pit method while highest seed germination value was observed in both pit and bin method i.e., 103.3%.

After composting an increase in GI values was observed in all substrate mixtures regardless, composted by pit or bin method, this clearly showed that composting reduce phytotoxicity, similar results were also achieved by Delgado *et al*., 2010. If we observe GI values obtained by pit method, out of ten combinations nine combinations showed >100 value only PM1:3 showed a value of 69.2. Similarly substrate combinations treated with bin method, out of ten substrate combinations five showed >100 value while remaining five combinations showed values in the range of 67.39-99.24, here again GW1:1 combination composted by pit method showed highest GI value i.e. 151.49 while the lowest GI i.e., 67.39 was observed in PM1:3 combinations composted by bin method.

Root and shoot lengths of 48 hr old seedlings in aqueous extracts of different substrates are presented in Tables 2 & 3. the longest roots were observed in GM 1:1 and CM 1:2 composted by pit method i.e. 31.3 mm and 30.5 mm respectively, extracts of uncomposted HPSW had an adverse effect on root length, here shortest root length i.e., 9.8 mm was observed.

Similarly, if we observe mean shoot lengths again highest shoot length 11.5 mm and 10.5 mm were observed in GM 1:1 & CM1:2 respectively composted by pit method.

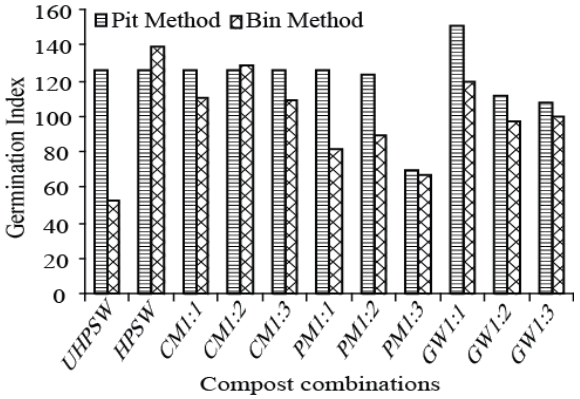

Fig. 1. Comparison of germination index of different composts produced by pit and bin method.

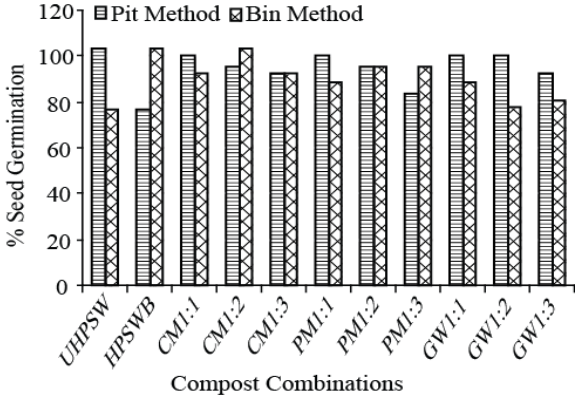

Fig. 2. Comparison of different composts produced by pit and bin method.

Table 2. Phytotoxicity assay results of composts produced by pit method.

Sample code	MGT (day)	Mean shoot length (mm)	Mean root length (mm)	Shoot / Root ratio	Seedling dry wt. (g)
Control	02	7.3 ± 1.50	20.7 ± 0.77	0.35 ± 0.11	1.92 ± 0.03
CHPSWP	02	8.0 ± 0.65	25.3 ± 3.67	0.31 ± 0.07	2.12 ± 0.33
UNCHPW	02	3.8 ± 2.96	9.874 ± 3.74	0.38.14	0.96 ± 1.84
CM1:1 P	02	8.0 ± 3.09	25.0 ± 8.13	0.32 ± 0.05	1.96 ± 0.51
CM1:2P	02	10.5 ± 2.64	30.5 ± 8.34	0.34 ± 0.00	1.78 ± 0.20
CM1:3P	02	8.2 ± 3.38	26.1 ± 6.85	0.31 ± 0.05	1.58 ± 0.34
PM1:1P	02	6.9 ± 1.78	22.6 ± 1.48	0.30 ± 0.05	2.13 ± 0.25
PM1:2P	02	8.9 ± 2.20	26.6 ± 5.44	0.33 ± 0.03	1.76 ± 0.20
PM1:3P	02	7.6 ± 3.31	17.0 ± 6.71	0.44 ± 0.09	1.63 ± 0.46
GW1:1P	02	11.5 ± 0.30	31.3 ± 2.61	0.36 ± 0.06	2.07 ± 0.12
GW1:2P	02	9.0 ± 1.07	22.9 ± 0.35	0.39 ± 0.05	1.99 ± 0.09
GW1:3P	02	8.7 ± 0.95	24.2 ± 5.72	0.35 ± 0.02	2.26 ± 0.41

Table 3. Phytotoxicity assay results of composts produced by bin method

Sample code	MGT (day)	Mean shoot length (mm)	Mean root length (mm)	Shoot / Root ratio	Seedling dry wt. (g)
Control	02	7.3 ± 1.50	20.7 ± 0.77	0.35 ± 0.11	1.92 ± 0.03
UNCHPW	02	3.8 ± 2.96	9.874 ± 3.74	0.38.14	0.96 ± 1.84
CHPSWB	02	9.8 ± 3.96	27.8 ± 12.7	0.35 ± 0.06	1.81 ± 1.72
CM1:1B	02	8.7 ± 2.54	24.9 ± 2.19	0.34 ± 0.04	1.64 ± 1.76
CM1:2B	02	6.2 ± 1.57	25.7 ± 4.61	0.24 ± 0.06	2.17 ± 1.02
CM1:3B	02	9.2 ± 1.13	24.6 ± 1.76	0.37 ± 0.01	1.61 ± 1.14
PM1:1B	02	5.8 ± 0.87	18.8 ± 0.56	0.30 ± 0.05	1.82 ± 2.16
PM1:2B	02	5.5 ± 0.36	19.2 ± 0.28	0.28 ± 0.01	2.01 ± 0.05
PM1:3B	02	4.8 ± 2.96	14.6 ± 3.74	0.32 ± 0.14	2.06 ± 1.84
GW1:1B	02	7.8 ± 3.25	27.7 ± 0.07	0.28 ± 0.10	3.66 ± 0.28
GW1:2B	02	7.5 ± 2.44	25.9 ± 11.59	0.29 ± 0.02	3.33 ± 0.75
GW1:3B	02	7.4 ± 0.88	25.3 ± 3.46	0.29 ± 0.04	3.70 ± 0.69

MGT= Mean germination time. Mean and standard deviation are shown

The shoot: root ratios (S/R ratio) of seedlings in aqueous extracts of different substrates composted by bin and pit method were calculated (Tables 2 & 3). S/R ratio of seedlings in aqueous extracts of all the substrates either composted by bin or pit method were decreased in comparison with the S/R ratio of uncomposted HPSW, thus a decrease in the S/R ratio indicated that root growth dominated shoot growth even though both increased or root growth either increased or remained unchanged, and shoot growth was either unaffected or even decreased (Bashan & Dubrovsky, 1996).

GI and seed germination of *Zea mays* was significantly retarded by the uncomposted HPSW water extracts and had GI and seed germination 52.31% and 77% respectively, the relative root growth of *Zea mays* was also inhibited by the uncomposted HPSW, it was only about 52.40%, indicate an insufficient stabilized product or the presence of any other toxic substances for these seeds (Said-Pullicino *et al*., 2007), but these disappeared after composting process. Substrates that show a GI >100 could be considered as carrier of stimulating growth properties (Warman, 1999) whereas a GI > 80 to 85% indicated the disappearance of

Phytotoxicity in the compost, therefore the disappearance of the Phytotoxicity from the compost. Figure 3 shows the results obtained for the relative root growth (RRG) of different compost prepared by bin and pit method. There was a higher RRG 151.4% was observed in extracts of HPSW with goat manure in 1:1 ratio. Mixture of HPSW and cow manure in 1:2 ratios had got also a higher RRG 147.3% which is again composted by pit method.

On the basis of results it can be concluded that composting reduces phytotoxicity (Delgado *et al*., 2010), it was also found out that pit method is more suitable than bin method and substrate mixture of HPSW and GM in 1:1 ratio found to be the most effective as compared to other substrate mixtures.

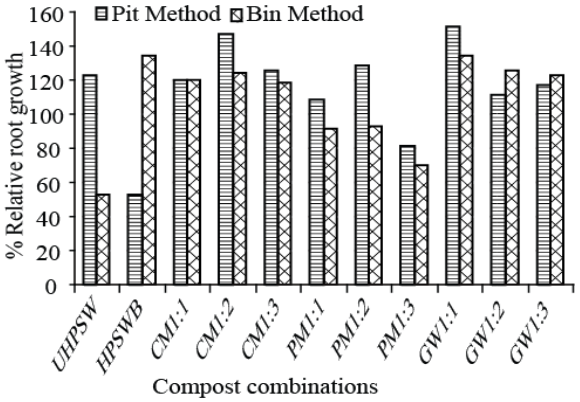

Fig. 3. Comparison of % relative root growth of different composts produced by pit and bin method.

Conclusion

The study concluded that co-composting of animal and herbal pharmaceutical solid wastes by pit method enhances maize seed germination by reducing phytotoxicity.

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