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BIOPESTICIDE EFFECT OF COMPOST GENERATED FROM MUNICIPAL SOLID WASTE

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ABSTRACT

Indiscriminate disposal of Municipal Solid Waste (MSW) creates several environmental and public health problems. Composting of MSW involves managing conditions to accelerate the biological decomposition of some of its organic components. In this process various bacteria and fungi, break down organic matter into simpler substances. Compost derived from MSW contains antagonistic microorganisms such as *Bacillus subtilis, Trichoderma sp.* And Pseudomonas sp., which can be used to control many soil borne plant pathogens.

Germination experiment was carried out to ascertain the influence of the stabilization process of compost derived from MSW on its biopesticide effect when *Fusarium oxysporum*, a plant pathogenic fungus was introduced into *Lycopersicon esculentum* (Tomato). Water extract of ten-year-old compost derived from MSW with heat-treated soil in different proportions were used in this experiment. The biopesticide effect of compost derived from ten and two-year-old MSW collected at different depths was evaluated from the number of germinated seeds and root elongated measurement. The compost samples were analysed for moisture content, pH, electrical conductivity, total solids, volatile solids, fixed solids, organic carbon, organic nitrogen, C/N ratio, total phosphate, potassium, and total microbial count to assess the characteristics of the compost.

Lycopersicon esculentum seeds inoculated with *Fusarium oxysporum* were placed in Petri plates. Seeds were watered with extracts of ten-year-old compost and heat-treated soil mixed in different proportions. Seeds without inoculation were maintained as control. After 8 days, the number of germinated seeds was observed and root elongated was measured. Then instead of ten-year-old compost, two-year-old compost was used and the experiment was conducted in the same way.

Addition of compost to soil resulted in the suppression of Fusarium wilt caused by *Fusarium oxysporum.* Microbial antagonism was responsible for this property. It was dependent on nutrient competition involving total micro flora of the soil and compost; and relied on the mechanism of general suppression.

KEYWORDS

Municipal Solid Waste; Compost; Biopesticide; *Fusarium oxysporum.*

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1 INTRODUCTION

Municipal Solid Waste (MSW) arising from human activities has become one of the major environmental problems causing excessive pollution and threat to human health. The generation of municipal solid waste in metro cities of India varies from 0.63 Kg/capita/day to 0.5 Kg/capita/day. 94% of this municipal solid waste is disposed by open dumping and about 5% is composted [1].

Methods for controlling pests and diseases using chemical pesticides are highly effective, but at the same time may be damaging to the environment [2]. Furthermore, the repeated use of such chemicals encourages the development of resistance in the target organisms and has a negative effect on beneficial organisms [3]. Pesticides may leach into groundwater supplies, and in addition some chemical pesticides may predispose plants to greater damage from root pathogens [4]. Compost derived from MSW can help control plant disease and reduce crop losses [5].

Compost technology is a valuable tool already being used to increase yields by farmers interested in sustainable agriculture. Professional growers discovered that the microorganisms in the compost are competitors of the pathogens such as *pythium* sp. and *rizoctonia* sp. [6]. These beneficial uses of compost can help growers save money, reduce their use of pesticides, and conserve natural resources. In the poultry industry, composting has also become a cost-effective method of mortality management. It destroys disease causing organisms and creates a nutrient-rich product that can be used or sold [7].

The application of municipal solid waste compost to agricultural soil can be a means to return the organic matter to agricultural soil and in some cases reduce the cost of municipal solid waste disposal. Municipal solid waste compost contains antagonistic microorganisms such as *Bacillus subtilis*, *Trichoderma* sp. and *Pseudomonas* sp., which can be used to control many soil borne plant pathogens [8].

A review of literature indicated that studies on the biopesticide effect of municipal solid waste compost in developing countries are very scarce. This study was designed in this context to investigate the bio pesticide effect of municipal solid waste composts on Fusarium wilt, caused by *Fusarium oxysporum*, a plant pathogenic fungus.

2 MATERIALS AND METHODS

2.1 Collection of compost derived from MSW

Compost derived from ten and two year old MSW was collected from Kodungaiyur dumpsite, using Auger sampler as and when required. Compost derived from ten-year-old MSW was collected from six different depths namely 10, 50, 75, 100, 125, 150 cm, and compost derived from two-year-old MSW was collected from two different depths namely10 and 100 cm. The compost samples were transported to the laboratory and stored at room temperature for further analyses.

2.2 Collection of soil

Soil containing 22% clay, 73% silt and 5% sand was collected from a local nursery and used for the study.

2.3 Collection of seeds

Certified seeds of *Lycopersicon esculentum* were collected from Tamil Nadu Agricultural University, Coimbatore. They were first washed with 0.1% $HgCl_2$ and then with distilled water. These seeds were chosen because they are commonly used vegetables. These plants are nitrogen demanding and grow quickly.

2.4 Collection of plant pathogen

Fusarium oxysporum, a fungal plant pathogen was collected from Department of Microbiology, Institute of Basic Medical Sciences, Taramani, Chennai. It was sub-cultured in Potato Dextrose Agar (PDA).

2.5 Analysis of compost

The compost was analyzed for the following as per standard methods [9]: Physico-chemical parameters such as moisture content, total solids, volatile solids, fixed solids, total carbon, Kjeldahl nitrogen, C-to-N ratio, available phosphorus and potassium, total bacterial and fungal populations before and after heat-treatment of the soil and the compost were determined using dilution technique [8].

2.6 Initial microbial count

Suspensions were plated on Acid Malt Agar (malt extract, 10 g/L ; citric acid added after autoclaving, 250 mg/L) for fungi, and on yeast peptone glucose agar (yeast extract, 5 g/L; peptone 5 g/L; glucose 10 g/L; pH adjusted to 7.5 with HCl or NaOH) for bacteria. Petri dishes were incubated at 25 and 50°C for the determination of mesophiles and thermophiles, respectively. Colonies were counted after 2 days of incubation for bacteria and 4 days for fungi. Total *Fusarium oxysporum* count was performed on Komada agar (K_2HPO_4, 1 g/L; KCl 0.5 g/L; $MgSO_4-7H_2O$, 0.5 g/L; Fe-Na-EDTA, 0.01 g/L; L-asparagine,2 g/L; D-galactose, 20 g/L; PCNB, 1 g/L; ox gall, 0.5 g/L; $Na_2B_4O_7$-10 H_2O, 1 g/L; streptomycin sulfate, 0.3 g/L) following Komada, [10]. Fluorescent *Pseudomonas* sp. was enumerated on King's B medium with cycloheximide (glycerol, 10 ml/L; F agar for *Pseudomonas* sp. (Merck), 35 g/L; cycloheximide, 0.1 g/L) following Larkin [11].

2.7 Preparation of compost tea

Compost tea (water extract) was prepared by mixing one part of compost with ten parts of distilled water.

2.8 Preparation of inoculum

A benomyl resistant strain of *Fusarium oxyspoum* [12] was grown on liquid Malt Extract medium (10 g/L) for 5 days. Conidia were then recovered by centrifugation (3000 g, 15 min), resuspended in sterile water and mixed with talc (1/3 conidia suspension, 2/3 talc, v/w). The talc inoculum mixture was dried at 20°C under forced air, sieved (200 µm) and stored at 4°C. Before utilization, the inoculum concentration in talc was determined by the suspension-dilution technique on Malt Agar (Malt extract, 10 g/L).

2.9 Soil -compost mixtures

Compost and soil were sieved (1 mm) and air-dried. Soil was used after heat-treatment by autoclaving for 1 h at 100°C, for three successive days. For phytotoxicity reasons, it was not possible to utilize the compost itself, therefore it was used in mixture with a conducive soil.

2.10 Experimental design

Water extract of compost and heat-treated soil mixture was used for this experiment (heat-treated soil + compost + distilled water in the following proportion – 5 g + 0 g + 20 mL; 4 g + 1 g + 20 mL; 3 g + 2 g + 20 mL; 2 g +3 g +20 mL; 1 g + 4 g +20 mL; 0 g + 5 g + 30 mL). Like wise, water extracts of compost collected at different depths were obtained.

Lycopersicon esculentum (Tomato) seeds were soaked in talc inoculum mixture infested with *Fusarium oxysporum.* Eight tomato seeds inoculated with *Fusarium oxysporum* and 8 non-inoculated seeds were placed in a clean sterilized Petri dish and 2 mL of water extract was added. In another Petri dish 8 tomato seeds inoculated with *Fusarium oxysporum* and 8 non-inoculated seeds were placed and 2 mL of distilled water (control) was added. The Petri dishes were maintained at room temperature. After 8 days, the number of germinated seedlings was recorded and root elongation measured. For all experiments triplicates were maintained.

3 RESULTS AND DISCUSSION

3.1 Characteristics of compost derived from MSW

The Physic-Chemical characteristics of compost derived from MSW are furnished in *Table 1.* The moisture content increased with the depth and the values were comparable to the range (15 to 28%) reported by [13]. The pH of compost samples decreased gradually in deeper layers. Larkin [11] has stated that the mature composts have pH between 6.0 and 7.5. The present values fall in this range. The EC of ten-year-old composts varied from 3.7 to 2.0 and EC of two-year-old composts varied from 3.7 to 2.5. The electrical conductivity of compost was less than 4 dS/m which is within the reported range of 0 – 4.0 dS/m [1]. The volatile matter reduction was more in deeper layers because of microbial activity. Gotass (1996) has stated that living organisms feeding on organic matter for development of protoplasm account for the rapid reduction in volatile matter. Organic carbon started declining gradually with depth. It was 15.22% near the surface. The maximum reduction of organic carbon was at 100, 125, 150 cm below the surface. The reduction of organic carbon appeared to be influenced by the activity of microorganisms. The C/N ratio in ten-year-old composts varied from 15.53 to 14.15 and C/N ratio in two-year-old composts varied from 15.33 to 15.00. It is known that when the C/N ratio of the compost exceeds 20:1, the organisms become deficient in nitrogen and the process of decomposition is slowed down.

Table 1. Physico-chemical charecteristics of municipal solid waste compost.

S. No.	*Parameter*	*Ten-year-old compost*						*Two-year-old Compost*	
		Depth (cm)						Depth (cm)	
		10	50	75	100	125	150	10	100
1.	pH	7.4	7.2	6.8	6.7	6.4	6.2	7.8	7.4
2.	EC (dS/m)	3.7	3.5	2.7	2.5	2.3	2.0	3.7	2.5
3.	Moisture content (%)	2.78	15.2	20.97	22.00	24.5	25.42	2.78	20.97
4.	Total Solids (%)	97.22	84.8	79.03	78.00	71.5	74.58	97.22	79.03
5.	Volatile Solids (%TS)	25.42	23.24	20.20	18.88	15.55	12.23	25.42	20.20
6.	Fixed Solids (%TS)	75.24	76.76	79.8	81.12	84.45	87.77	75.24	79.8
7.	Organic carbon (%)	15.22	15.04	14.63	13.98	13.12	13.02	15.18	14.6
8.	Organic Nitrogen (%)	0.98	0.98	0.96	0.92	0.89	0.92	0.98	.93
9.	C/N Ratio	15.53	15.34	15.23	15.19	14.74	14.15	15.33	15.00
10.	Total phosphate (%)	0.602	0.584	0.581	0.563	0.543	0.532	0.632	0.558
11.	Potassium (%)	0.479	0.463	0.382	0.350	0.302	0.251	0.362	0.249

3.2 Initial microbial population of soil and compost

Compost contained about 10 times more fungi and 2.5 times more bacteria than soil (*Table 2*). Heat treatment of compost totally suppressed fungal populations, but thermophilic bacteria partially subsisted, developing at 50°C but not at 25°C. In the soil, a total *Fusarium* population of 1.4 x 10^3 cfu/g, including 7.7 x 10^4 cfu/g of *Fusarium oxysporum* population of 7.7 x 10^4 cfu/g was determined.

Table 2. Fungal and bacterial count in the soil and compost.

Sl.No.	Medium	Microbial population (cfu/g)				
		Fungi			Bacteria	
		Total	Total *Fusarium*	Fusarium oxysporum	Total	Pseudomonas sp.
1.	Compost	5.2 x 10^4	0	0	8.0 x 10^6	4.4 x 10^4
2.	Heat-treated compost	0	0	0	1.1 x 10^4	1.1 x 10^2
3.	Soil	2.5 x 10^4	1.4 x 10^3	7.7 x 10^4	2.2 x 10^7	7.7 x 10^4
4.	Heat-treated Soil (25°C)	0	N	N	6.1 x 10^4	N
5.	Heat-treated soil (50°C)	0	0	N	1.1 x 10^2	N

N: Negligible

3.3 Biopesticide effect of compost derived from MSW on *Lycopersicon esculentum* seeds

Biopesticide effect of compost derived from ten-year-old MSW (150 cm depth) on germination and root growth of control and pathogen-inoculated seeds of *Lycopersicon esculentum* seeds are presented in *Table 3*.

Table 3. Biopesticide effect of compost generated from ten-year-old MSW on germination and root growth of Lycopersicon esculentum (Tomato) seeds.

Sl. No.	Experimental condition	Germination (%)		Root length (cm)	
		Noninoculated seeds	Seeds inoculated with *Fusarium. oxysporum*	Noninoculated seeds	Seeds inoculated with *Fusarium oxysporum*
1.	Control Dist. water (C_1)	0	0	0	0
2.	5 g Heat-treated soil + 20 ml Dist. water – compost (T_1)	95.0	0	12.0	0
3.	4 g Heat-treated soil + 1 g compost + 20 ml Dist. water (T_2)	94.0	0	11.5	0
4.	3 g Heat-treated soil + 2 g compost + 20 ml Dist. water (T_3)	93.5	80.0	10.5	10.2
5.	2 g Heat-treated soil + 3 g compost + 20 ml Dist. water (T_4)	92.0	0	10.2	0
6.	1 g Heat-treated soil + 4 g compost + 20 ml Dist. water (T_5)	0	0	0	0
7.	0 g Heat-treated soil + 5 g compost + 20 ml Dist. water (T_6)	0	0	0	0

For phyto-toxicity reasons, it was not possible to use the compost itself. Therefore, compost was mixed with heat-treated soil and used as the medium. Seeds watered with distilled water (control) did not germinate. This may be due to the lack of nutrients present in the distilled water. Non-inoculated seeds were germinated. The inoculated seeds watered with extracts of ten-year-old compost collected at 150 cm depth showed positive results on germination and root elongation. There were clear differences in the germination of tomato seeds and emergence among the different organic extracts. Water extract of 2 g compost derived from ten-year-old MSW with 3 g heat-treated soil (T_3) produced the most rapid emergence, averaging over 80%, and the root length was 10.2 cm after one week. Growth of the emerged seeds was rapid initially but declined towards the end of the experiment, probably because of the influence of restricted space in the Petri plates.

Biopesticide effect of compost derived from two-year-old MSW (100 cm depth) on the germination and root growth of control and pathogen-inoculated seeds of *Lycopersicon esculentum* (Tomato) seeds are presented in *Table 4*.

Table 4. Biopesticide effect of compost generated from two-year-old MSW on germination and root growth of Lycopersicon esculentum (Tomato) seeds.

Sl. No.	Experimental condition	Germination (%)		Root length (cm)	
		Noninoculated seeds	Seeds inoculated with *Fusarium. oxysporum*	Noninoculated seeds	Seeds inoculated with *Fusarium oxysporum*
1.	Control Dist. water (C_1)	0	0	0	0
2.	5 g Heat-treated soil + 20 ml Dist. water –compost (T_1)	96.5	0	13.0	0
3.	4 g Heat-treated soil + 1 g compost + 20 ml Dist. water (T_2)	95.5	0	12.5	0
4.	3 g Heat-treated soil + 2 g compost + 20 ml Dist. water (T_3)	93	85	11.5	12.2
5.	2 g Heat-treated soil + 3 g compost + 20 ml Dist. water (T_4)	93.0	0	10.5	0
6.	1 g Heat-treated soil + 4 g compost + 20 ml Dist. water (T_5)	0	0	0	0
7.	0 g Heat-treated soil + 5 g compost + 20 ml Dist. water (T_6)	0	0	0	0

Seeds watered with distilled water (control) did not germinate. This may be due to the lack of nutrients in distilled water. Non-inoculated seeds were germinated. The inoculated seeds watered with extracts of compost collected at 100 cm depth showed positive results on germination and root elongation. Water extract of 1 g of compost derived from two-year-old MSW with 4 g heat-treated soil (T_8) produced the most rapid emergence, averaging over 85%, and the root length was 12.2 cm after one week. The bio pesticide effect was also strong when compost was used as organic amendment in the presence of *Fusarium oxysporum*, as could be seen from stem and shoot length.

Control compost was as effective as heat-treated compost in making the soil suppressive, although the *Fusarium oxysporum* population decreased to a lesser extent in the soil with control compost than in the soil with treated compost. These results differed from those of Trillas *et al* [14] who observed that supressiveness to *Fusarium* wilt disappeared with control compost. Therefore, mesophilic microorganisms or compounds could still be responsible of the compost. Composts are naturally colonized by mesophilic microorganisms during the mesophilic phase of composting.

The low disease incidence in heat-treated soil and control compost could be related to the compost microflora which actively colonized the space provided by the nutrient-rich heat-treated soil resulting in higher population density. Similar observations on the controlling ability of compost have been observed by Filippi and Pera [15] also.

The suppression of *Fusarium oxysporum* could be attributed to competition for carbon nutrients between the compost micro flora and the pathogen. This competition was non-specific, involving the compost microflora (general suppression mechanism). The soil micro flora was less effective in suppressing *Fusarium oxysporum* than the compost microflora, as the compost-treated soil mixture, essentially colonized by compost microflora.

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