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Studies on Quantitative and Qualitative Analysis of Fungal Population of Decomposing Temple Wastes in Varanasi Region

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Abstract: To encounter the acute shortage of properly decomposed organic manure the present study was conducted for investigating the pattern of decomposition of the temple waste. Isolation of the fungi from decomposing temple waste and soil mixed with manure was done by direct observation method, damp chamber incubation method and dilution plate technique. Total 28 fungus were isolated and highest fungal population was recorded by dilution plate technique followed by damp chamber and then direct observation method and according to their occurrence they were divided into three categories (1) Dominant fungi which show 70% frequency, (2) Common fungi which shows less than 70% frequency and (3) Rare fungi were observed once or twice during isolation. The moisture content of the decomposing temple waste recorded maximum 29.65% at 15 days of intervals. Deuteromycotina fungi were recorded highest 64.28% in comparison to Zygomycotina (7.14%) and Ascomycotina (3.5%). In early stages of infection *Mucor racemosus*, *Rhizopus nigricans Alternaria alternata*, *Fusarium* spp. were found abundantly but in later stages of decomposition prevalence of *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigates*, *Aspergillus candidus*, *Penicillium rubrum*, *Penicillium citrinum* was recorded. During the process of decomposition the moisture content of the temple waste gradually decreases whereas changes in pH follow an erratic pattern due to the activity of above mentioned mycoflora throughout the process of biodegradation.

Key words: Temple wastes, decomposition, varanasi, fungal population

INTRODUCTION

In Post independence era Green Revolution in our nation ensured food security with huge surplus stocks of food-grains. By enhanced qualitative production damage due to famines and other natural calamities have been minimized. But yet even after 65 years of independence about 11.6 million hectares of agricultural land is affected by unbalanced and excessive use of synthetic fertilizers, absence of organic manures, depleting macro and micronutrients resulting in low-productivity (IFA, 2000). Due to intensive use of chemical pesticides, residues prevail in food chain and cause endangered the life sustaining system around the world (Miller, 2004). There is a ongoing global rat race to achieve short term high productivity of quality produce either to feed the hungry or for higher monetary benefit combined with the increasing intensity cultivation practices and injudicious use of chemical and synthetic fertilizers. This is the prime cause for the gradual propagation of these so called man made barren lands especially in developing and underdeveloped countries (Ho, 2010). TERI (The Energy and Resources Institute) estimated the economic loss of Rs. 1-3 Billion annually to these man-made barren lands (FAI's April 1997 issue). Places of historical, religious and touristic importance around the globe are often prone to large amount of solid waste leftovers by the pilgrims. This is responsible for several environmental issues pollution but also serves as source of inoculums for many diseases or some other health hazards/social problems (Blackman, 1995). In the one month long festivities organized on the banks of river ganga during the auspicious occasion of Ardh Kumbh Mela of 2004 at Allahabad the solid waste leftovers comprised of 62.20% biodegradable, 17.14% Non Biodegradable and 13.61% miscellaneous components (Gangwar and Joshi, 2008). It is hitherto proven fact that improper or injudicious disposal of solid waste not only impacts the soil quality by increasing the concentration of various hazardous elements in the soil but is the source of further ground water pollution that is primarily used for drinking and agricultural purposes (Ahel *et al*., 1998). Solid waste management needs thorough administrative supervision, financial flexibility and availability to the cause, legally

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binding measures, long term policy and planning going hand in hand with latest technological developments (Ramachandra and Bachamanda, 2007). The average per capita solid waste generation in our country has significantly gone up from 0.32 kg day^{-1} in 1973 to 0.48 kg day^{-1} in 1994. On an average the daily per capita MSW (Municipal Solid Waste) generation in India ranges between 100 g in small towns to 500 g in large towns but the figures in actuality may be much higher than estimated subject to the lack of sewage treatment plants and efficient government and public sector initiatives. Mineral fertilizers are used to improve crop yields as a major factor affecting food security (Dhere *et al.*, 2008). There is a need to identify locally available materials that can be extensively utilized for soil improvement. But the use of manures, dung, crop residue, mineral deposits and other organic matter will significantly improve soils health to sustain with higher crop yield. In many developing countries sector of recyclers, scavengers and collectors, whose business is to salvage 'waste' material and reclaim it for further use are exists. In addition, the mineral deposits can be use for several purposes including agriculture particularly soil fertility improvement. Use of organic matter such as agricultural waste, municipals waste, garbage, kitchen waste and temple waste with the objective of utilization of such waste in the form of compost and their application as organic manure. Therefore, the present investigation was undertaken to study the fungi involved in the process of decomposition of temple waste. Studied were also conducted to emphasize the effect of organic manure made from decomposition of temple waste.

MATERIALS AND METHODS

Formation of pits for decomposition of temples wastes: Temples wastes were collected from different temples of Varanasi region (i.e., Vishwanath temple of B.H.U. campus and Godwalia) in 2010. Three pits were made (1M×1M×0.5M) at the field of the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University. Waste of 35 kg was kept in each pit and covered by a thin layer of soil for 3 months for decomposition. After three months temple wastes were converted into organic manure.

Studies on decomposing fungi of temple wastes: Ten samples were randomly collected from each pits of decomposed temple waste at 15 days of interval. The entire sample were brought into the laboratory and mixed together for determining the temple wastes inhabiting mycoflora, pH and moisture content during decomposition. Quantitative and qualitative analysis of fungal population of decomposed temple wastes were observed by the use of three different methods.

Direct observation: The waste decomposing fungi were isolated by the help of binocular microscope from the decomposing temple wastes by a method of Garrett (1981).

Damp chamber incubation method: In the initial stage of decomposition (15 days) of temple wastes, fungi were observed by damp chamber incubation method. The temple wastes were cut into 3-4 mm pieces and placed on sterilized blotting paper in Petri plates having moist chamber and the plates were incubated at 25±20°C for 7 days.

Dilution plate technique: The fungi were isolated by dilution plate technique. The ten gram sample of decomposed waste were taken from the pit and suspended into 100 mL of sterilized water in a 200 mL of conical flask. Further dilution series (1:102, 1:103, 1:104 and 1:105) were prepared. 1 mL of dilution was poured in sterile Petri plate and 15-20 mL of pre-sterilized PDA media was added. Five replication of each treatment were prepared and all the plate incubated at 25±20°C for a week.

Moisture content: The ten gram samples of decomposed temple waste were taken from the pits and oven dried at 105°C for 24 hours and the moisture content of the samples were determined on the basis of loss in the dry weight.

pH: The decomposed temple wastes were mixed in distilled water in ratio of 1:5 and the pH was determined with the help of Elico-electric pH meter.

Species of fungi observed during present investigation have been isolated by the method already described and cultivated on a variety of prepared solidified media. The portion of mycelium was transferred on (3%) solidified water agar medium plate. When the development of colony was seen, single hyphal tip was cut-out and placed on another fresh plate to get pure culture and transferred on nutrient agar slant and maintain as stock culture at an interval of one month. Fungi were identified with the help of permanent slid.

RESULTS

The experiment was set up in the month of September 2011, at the end of the monsoon. Initially, the temple waste was mixed with different colours viz. green, yellow, red and blue, turn into brownish black colour at the end of

Table 1: pH and Moisture content (%) in oven dried temple waste

Day's	pH	Moisture content (%)
At initial	6.7	27.32
At 15 days	6.9	29.65
At 30 days	6.5	29.24
At 45 days	7.1	29.07
At 60 days	7.0	29.00
At 75 days	7.2	28.75
At 90 days	7.1	28.55

Table 2: Fungi associated with decomposing temple waste based on direct observation

Fungal species	At initial days	At 30 days	At 45 days	At 60 days	At 90 days
Rhizopus stolonifer	+	+	+	+	+
Aspergillus niger	+	+	+	+	+
Aspergillus flavus	-	+	+	+	+
Penicillium rubrum	-	+	+	+	+
Penicillium citrinum	-	-	-	+	+
Cladosporium claddosporioids	-	-	+	+	+
Chaetomiuum globosum	-	-	-	+	+
Fussarium semitectum	-	+	+	+	+
Alternaria alternata	+	+	+	+	-
Alternaria solani	+	+	-	-	-
Dark sterile mycelium	+	+	-	+	+
White sterile mycelium	-	-	-	+	+

the decomposition process. The temple waste maintained its identity up to 5-6 days while some parts up to 10-12 days due to presence of slow degrading material and cellulolytic materials, takes longer time in decomposition. After decomposition temple waste was converted into black-brown powdery mass which was finally incorporated as humus in soil. The data of moisture content in different day's interval are given in the Table 1. Maximum moisture was recorded after 15 days of decomposition is 29.65%, moisture content were gradually increased with period of decomposition. Increasing moisture content helps in increasing the microbial activity. It has been observed that the high humidity, the atmospheric condition like light intensity, temperature and humidity had effect on the fungal colonization. The increase in pH during decomposition is probably due to higher calcium, which belongs to the structural component that is release at a slower rate in comparison to other compounds. According to percent occurrences of fungi which colonized the Temple waste mostly belong to class deuteromycotina are strong colonizer and their occurrence was observed 64.28%, occurrences of class Zygomycotina was 7.14% and 3.5% weak colonizer was found in Ascomycotina occurrence.

Qualitative analysis of fungal population of decomposed temple wastes

Direct observation method: A total number of 12 fungal species were isolated by this method. Fungal species observed under the stereoscopic binocular microscope are listed in Table 2. The dominant fungi were found *Aspergillus niger*, *Rhizopus stolonifer*, *Penicillium rubrum*, *Alternaria alternata*, *Fusarium semitectum* and Dark sterile mycelium.

Table 3: Fungi recorded by damp chamber incubation method from decomposing temple wastes

Fungal species	At initial	At 10 days	At 20 days	At 30 days
Mucor racemonus	+	+	+	-
Rhizopus stolonifer	+	+	+	-
Aspergillus niger	+	+	+	+
Aspergillus flavus	-	+	+	+
Aspergillus fumigates	-	-	+	+
Chaetomiuum globosum	-	-	+	+
Penicillium rubrum	+	+	+	+
Trichoderma viride	-	-	+	+
Trichoderma harzarinum	+	-	+	+
Alternaria alternate	+	-	-	-
Cladosporium cladosporiodes	-	-	+	+
Fusararium semitectum	-	-	+	-
Curvularia lunata	+	-	-	+
Dark sterile mycelium	-	-	+	+

+: Present, -: Absent

Table 4: Fungi recorded by Dilution plate Technique from decomposing temple waste

Fungal species	At initial stage	At 15 days	At 30 days	At 45 days	At 60 days	At 75 days	At 90 days
Aspergillus niger	-	+	+	+	+	+	+
Aspergillus terrus	+	-	+	+	+	+	+
Aspergillus flavus	-	+	+	+	+	+	+
Aspergillus sulfureus	-	-	-	-	+	+	+
Aspergillus candidus	-	+	+	-	-	+	-
Aspergillus luchuensis	-	-	-	+	+	-	+
Rhizopus stolonifer	+	+	+	+	+	+	-
Mucor racemonus	+	+	+	+	+	+	-
Penicillium expensum	-	+	+	+	+	+	+
Penicillium citrinum	-	+	+	+	+	+	+
Penicillium rubrum	-	+	+	+	+	+	+
Fusarium semitectum	-	-	-	+	-	+	+
Fusarium roseum	-	-	-	-	-	+	-
Alternaria alternaria	+	+	-	-	-	+	+
Cladosporium cladosporioides	-	+	+	+	-	-	-
Trichoderma viride	-	+	+	+	+	+	+
Trichoderma harzianum	-	-	-	+	-	+	+
Dark sterile mycelium	+	-	-	+	-	+	+
Pink sterile mycelium	-	-	-	-	-	+	+
Unknown -1	-	-	-	-	+	+	+
Unknown-2	-	-	-	+	+	+	+

Damp chamber incubation method: This method was used for isolation of fungi by different days interval, when the temple waste can be handled properly. After decomposition, the waste was broken down into small pieces. A total number of 14 fungal species were isolated by this method (Table 3). The prominent fungal species are *Aspergillus niger*, A. flavus, *Penicillium rubrum*, *Rhizopus stolonifer* and *Mucor racemonus*.

Dilution plate technique: This technique is widely used in isolation of fungi. Total 21 fungal species were isolated by this method (Table 4). The prominent fungal species *Aspergillus niger*, *A. flavus*, *A. terrus*, *Mucor racemonus*, *Rhizopus stolonifer*, *Trichoderma viride*, *Penicillium*

Table 5: Percent occurrence of various classes of fungi colonizing on the decomposing temple waste

Classes of fungi	No. of spp. isolated	Occurrence (%)
Zygomycotina	2	7.14
Zygomycetes	2	
Ascomycotina	1	3.5
Pyrenomycetes	1	
Chaetomiales	1	
Deuteromycotina	18	64.28
Hyphomycetes	18	
Moniliales	18	
Moniliaceae	12	42.85
Dematiaceae	4	14.28
Tuberculariaceae	2	7.14
Mycelia sterilia	3	10.71
Un-known	2	7.14
Total No. of fungi	28	

citrinum, *Penicillium rubrum* and *Penicillium expensum* were found in this technique. Different species of fungi belonging to various genera observed at different stage of decomposition. For the study of qualitative nature of fungi associated with decomposition of temple waste classified into three different groups on the basis of their appearance in different time of intervals:

- **Group (1) Dominant:** This group of fungi appeared more than 70% incidence and more in number. The fungi were *Aspergillus niger*, *Aspergillus flavus*, *Mucor racemosus*, *Rhizopus stolonifer*, *Penicillium rubrum* and *Penicillium citrinum*
- **Group (2) Common:** This group includes those fungi which appeared less than 70% incidence but more than 50%. These are *Trichoderma viride*, *Aspergillus terreus*, *Cladosporium cladosporioides*, *Alternaria alternata*, *Penicillium citrinum* and white sterile mycelium
- **Group (3) Rare:** This group include those fungi which appeared once or twice during isolation. These are *Aspergillus sulfureus*, *Aspergillus luchuensis* and pink sterile mycelium

DISCUSSION

Maximum moisture content (29.65%) was recorded at 15 days (Table 5). Moisture content plays an important role in microbial activity in the soil. Increasing moisture contents helps in increasing microbial activities. Madge (1965) has shown marked effect of moisture on the number of soil fungi. Moisture content is chiefly responsible for the colonization of microorganisms (Dunn *et al*., 1985; Christensen, 1989; Sinha *et al*., 1998; Vijay and Naidu, 1995; Pal and Broadbent, 1975; Fioretto *et al*., 1998 and Vibha and Sinha, 2008). The pH of the soil mixed Temple waste varied from 6.5 to 7.2. Increase in pH due to higher biomass and bioactivity occurs when wood ash is incorporated in the soil as organic amendments (Zimmermann and Frey, 2002). Isolation of the fungi from decomposing temple waste and soil mixed with manure was done by direct observation method, damp chamber incubation method and dilution plate technique. Among these methods the distribution of higher percentage of Deuteromycetous fungi are strong colonizer whereas Ascomycotina were weak colonizer was reported by several worker (Rai *et al*., 2001; De Santo *et al*., 2002). Dominant fungi in this group which appeared more in number recorded from the decomposition of temple waste were Aspergillus niger, Aspergillus flavus, *Rhizopus stolonifer*, *Penicillium rubrum*, *Mucor racemosus* and *Rhizopus nigricans*. Fungal species such as *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer*, *Penicillium rubrum* and *Penicillium citrinum* have been studied by some various workers (Gupta and Kumar, 1979; Van Wyk *et al*., 1986; Bridge and Spooner, 2001; Gurav and Pathade, 2011; Klich, 2002; Manzoor *et al*., 2004; Elmholt and Labouriau, 2005 and Sinha *et al*., 2009). Fungal population such as *Alternaria alternata*, *Fusarium* spp. and *Rhizopus nigricans* increased at initial stage but at later stages *Aspergillus niger*, *Aspergillus flavus*, *Penicillium rubrum* and *Penicillium citrinum* were recorded predominantly at increasing days. Similar result were reported by Elmholt and Labouriau (2005), Guerrero *et al*. (2007) and Mandal *et al*. (2007).

CONCLUSION

During the process of decomposition the moisture content of the temple waste gradually decreases whereas changes in pH follow an erratic pattern due to the activity of several fungal species out of which *Aspergillus niger* and *Penicillium rubrum are* most abundant throughout the process of biodegradation.

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