Pak. J. Bot., 5 (1) : 57-63, 1973.

SOME STUDIES ON THE FUNGI OF KALLAR GRASS (*DIPLACHNE FUSCA* (L.) P. BEAUV.) COMPOST

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Abstract

Both thermophilic and mesophilic fungi have been isolated from an experimental kallar grass (*Diplachne fusca*) straw compost. The occurrence of these fungi has been correlated to the variation in temperature and pH of the compost. The decomposition of kallar grass has been studied by the sequential estimation of dry matter. Humus has also been estimated during the composting of kallar grass cuttings.

Introduction

Much work has been done on the isolation of fungi from mushroom composts (Waksman *et al*, 1939; Waksman & Cordon, 1939; Webley, 1947; Eastwood, 1952; Fergus, 1964), but only a few detailed investigations have been reported on the succession and development of fungi during the various phases in the preparation of a compost. Eastwood (1952) studied different phases in cut barley straw, but she could not find any thermophilic fungi in her compost. Chang & Hudson (1967) studied the wheat straw compost and they reported a detailed succession of fungi and also observed a marked thermophilic phase favouring thermophilic fungi. Studies on the town waste refuse composts are also beginning to receive attention (Stutzenberger *et al*, 1970). The idea underlying such researches is the conversion of town wastes or any other waste material into a compost which is a rich source of humus, a substance vital for the maintenance of soil fertility and the improvement of soil structure.

The purpose of the present investigation was to study the composting of kallar grass (*Diplachne fusca*) cuttings. *D. fusca* is a halophytic grass which has shown its ability to get established under highly saline and sodic conditions. It is a common belief among the cultivators that it 'eats up' salts and thus helps in amelioration of saline and saline-sodic soils (Hussain & Hussain, 1970). As recommended, this process is very time consuming since it requires cultivation of this grass for a long time (Khan, 1966).

The approach being worked out at this Institute is to plough under *D. fusca* grass when 2-3 cuttings have been obtained after its establishment and allow it to decompose in the same salt affected soil. It will not only help in aggregation of soil particles but also provide a good source of organic matter to the saline soil and thus help in its improvement. In order to hasten the decomposition of this plant material, it is essential to study the mycoflora naturally occurring on it before coming to the soil. The activity and roleof such mycoflora can be best studied during composting of such a material.

The object of the present study was to carry out composting of kallar grass cuttings on a small scale and to follow the succession of different fungi and to observe the environmental changes that take place during composting.

Materials and Methods

Chang & Hudson (1967) used bottomless one meter cube wooden bins for study-in wheat straw compost. In these investigations similar wooden compost bins were c nstructed but were smaller in size (2ft cube) and had a wooden bottom. The interior of he bin was lined with a thick polythene sheet so as to avoid any damage to the wood. the bins had five holes in the lid for sampling and three holes on each side for ventilation Holes for inserting thermometers were also provided.

Soft branches of kallar grass were cut into pieces of about 1-2 inches by a cutter, spread on a plastic sheet, moistened by sprinkling water and left overnight. Thirty kg of these cuttings were loosely packed into the composting bin. Before packing, 1 kg ammonium nitrate was thoroughly mixed with the grass cuttings. The bin was then placed in a controlled temperature room (32-34°C).

Samples for fungal isolations were taken more frequently in the beginning; the time between sampling was increased with the compost age. At each sampling time, 3 samples were taken out from different depths and from different sampling holes. All the four samples were mixed into a composite sample and then used for fungal isolations. Three methods were used for fungal isolations. Firstly, direct observation method was used. Some pieces of kallar grass compost were put in Petri dishes lined with wet filter pepers and incubated at 30°C and 45°C. After 2 days of incubation, fungi appearing on the kallar grass pieces were observed under sterioscopic microscope and recorded for estimating frequency of occurrence.

Second method employed was a washing technique. Some kallar grass cuttings from the sample were vigorously shaken in universal containers containing 10 ml distilled sterile water. These pieces were further given four washings with sterile water. After washing, kallar grass pieces were inoculated on to agar Petri dishes (four pieces on each Petri dish). Two media were used, namely Eggins & Pugh's cellulose agar (Eggins & Pugh, 1962) and Eggins & Pugh's glucose agar (Eggins & Malik, 1969). Three replicates of each media were used. Such isolations were always made at both 30°C and 45°C. All the Petri dishes were observed after 7 days of incubation and fungi appearing were identified and recorded.

Thirdly, dilution technique was employed to ascertain the number of fungi. One gramme of the composite sample was shaken on a reciprocating shaker for one hour in a medical bottle containing 200 ml sterile water. Eggins & Pugh's glucose agar medium containing rosebengal, penicillin and streptomycin was used.

In addition to fungal isolations, pH, weight loss and humus content of the compost were also estimated. For this purpose the method used by Chang (1967) was adopted. Five nylon bags filled with 100 g of kallar grass cuttings were buried in the kallar grass cuttings in the compost bin. Each bag was taken out at required intervals and determinations on weight loss, pH and humus content were made. For humus estimation the method described by Malik & Sandhu (1972) was employed.

Results and Discussion

The variations in temperature, pH and dry matter ot the kallar grass compost are represented in Figs. 1-3. The temperature curve gave a peak on 6th day of composting (Fig. 1). The maximum temperature r ache was 53°C and it started dropping after

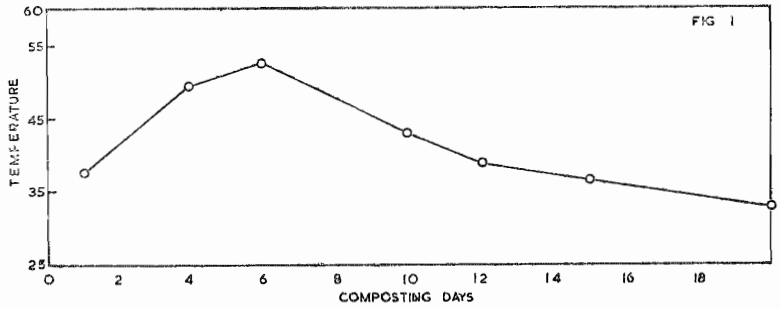

Fig. 1. Temperature curve of kallar grass during composting period.

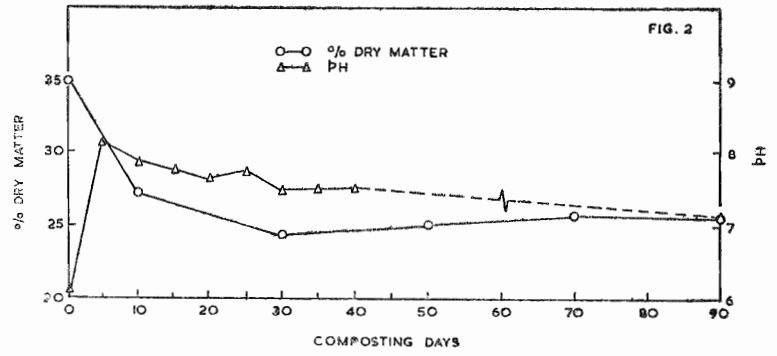

Fig. 2. Graph showing changes in drymatter percentage and pH during composting of kallar grass cuttings.

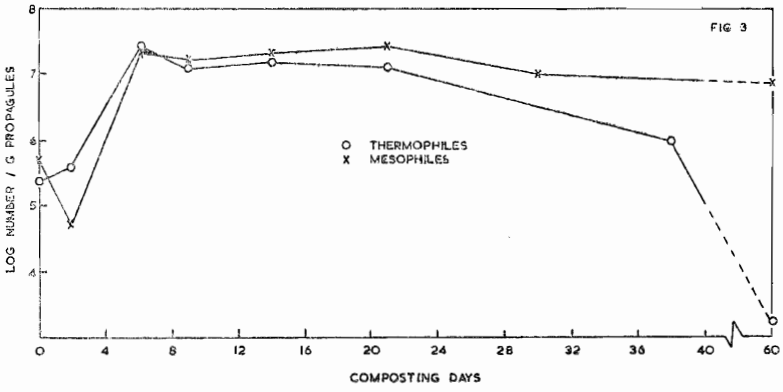

Fig. 3. Fungal population during composting of kallar grass cuttings.

6th day and came down to ambient (32-34°C) after 18 days. As the temperature was recorded by the thermometers fixed on the sides of compost bins, the relatively low rise in temperature could be due to lack of insulation. Chang & Hudson (1967) recorded a maximum of 40°C from their side thermometers whereas their central dial thermometer recorded a temperature as high as 67°C. Fergus (1964) reported a maximum

temperature of 62°C as maximum temperature but it was reached after 8-9 days of composting whereas Miehe (1907) had earlier recorded 68.5°C after 5 days. This variation in temperature found by various workers is probably due to the varying size of compost heaps and the degree of insulation achieved.

The average pH values of kallar grass compost were determined by homogenizing 5 g. of sample in 50 ml. boiled distilled water (Fig. 2). The initial pH of kallar grass homogenate was found to be 6.2. After mixing with ammonium nitrate and 2 days of composting, it rose to 8.2. After 5 days, the pH decreased to 7.2 and then increased to 7.4 after 40days. Later on the compost reached neutrality. Chang & Hudson (1967) also observed an initial rise in pH and then it dropped to neutrality. Stutzenberger *et al*(1970) reported a gradual rise in pH starting from 5.7 to about neutrality.

The loss in dry weight during composting is also represented in Fig. 2. The rate of decomposition was not uniform throughout this period. Maximum decomposition took place during first ten days and dry matter decreased from 35.7 to 27.9%. It further decreased to 24.5% after 30 days of composting. Later on the dry matter slightly increased to 25.5% presumably due to the accumulation of fungal mycelium after 90 days of composting.

The results of humus estimation showed a maximum humus content of 14% after 60 days of composting. The initial humus content of kallar grass cuttings was negligible and a sharp rise was observed after 14 days of composting when 6.5% humus was recorded.

Total number of fungi was estimated by dilution plate technique. The logarithms of the numbers of fungi present after different periods of composting are presented in Fig. 3. Mesophiles and thermophiles are plotted separately in the same figure. Initially kallar grass cuttings had a higher number of mesophilic mycoflora as compared to thermophilic one. Number of mesophilic fungi sharply decreased after 2 days of composting. Both mesophilic and thermophilic mycoflora rose to a maximum as the temperature increased. Number of thermophiles was higher at this stage. Later on both mesophiles and thermophiles remained fairly constant; the thermophiles started decreasing after 22 days of composting and were quite low at the end of the composting period. After ini ial decrease in number, mesophiles maintained their high number throughout the composting period.

During composting period neither mesophiles nor thermophile were killed off during peak heating period as reported by Chang & Hudson (1967). This was probably due to the development of relatively low heating phase as the maximum temperature reached was only 53°C. The initial ecrease in mesophilic number after 2 days of composting can be explained on the basis of change in micro-environmental conditions in the compost which greatly inhibited the already active mycoflora on the kallar grass pieces.

Inspite of the relatively low temperature development, kallar grass compost exhibited a conspicuous succession of fungi during the different stages of decomposition. This pattern has been elucidated by the other two methods used to isolate fungi from the compost. The percentage frequency of different fungi has been summarized in Table I.

TABLE I. Percentage frequency of fungi isolated on cellulose agar (CA) and glucose agar (G) during composting of kallar grass (*Diplachne fusca*).

FUNGI	DAYS OF COMPOSTING																	
	0		2		6		9		14		21		30		38		56	
	CA	G	CA	G	CA	G	CA	G	CA	G	CA	G	CA	G	CA	G	CA	G
Mesophiles:																		
Alternaria humicola	75	67																
Aspergillus fumigatus				8	16					8	33							
A. niger										16								
A. tamarii									33	16		58						
Cephalosporium sp.							100		92	58	100	66						8
Chaetomium globosum					8		100		83		66		100		83		83	
Curvularia sp.	8																	
Fusarium solani	42		100	100	100	66												
Helminthosporium graminium ..	58		33															
Helminthosporium sp.	8		33															
Mucor sp.	8	100					92	100										
Paecilomyces sp.										8	8		100	100				
Penicillium variable									16	8								
Pullularia pullulans											8							
Thermophiles																		
Aspergillus fumigatus					50		8		16		42		8		8			
Cephalosporium thermophile ..		8									100		8		66			
Chaetomium thermophile	25						83		100									
Humicola grisea			42		25													
H. insolens							83		100		100							
Malbranchea pulchella					8								42					
Mucor pusillus	75	100	100		33	33							33		25		16	
Myriococcum albomyces ..					66										8			
Streptomyces sp.																		
Talaromyces dupontii																		

The initial mycoflora of kallar grass pieces consisted of *Alternaria humicola*, *Helminthosporium graminium*, *Helminthosporium sp*, *Fusarium solani*, *Curvularia sp.* and *Mucor sp.* Out of these *A. humicola*, *H. graminium* and *F. solani* were observed by direct observation method. Their frequency of occurrence was quite high on cellulose agar. Glucose agar supported the growth of sugar fungi like *Mucor sp.* Other fungal species also made their appearance on this agar but their frequency was quite low.

After 2 days of composting, *A. humicola* disappeared and the frequency of both species of *Helminthosporium* decreased whereas that of *Fusarium solani* increased and was most dominant. It covered all the pieces in the Petri dish lined with wet filter paper. It remained dominant for 6 days whereafter it disappeared. *Apsergillus fumigatus* and *Chaetomium globosum* also made their appearance on 6th day of composting.

Among thermophilic fungi *Chaetomium thermophile*, *Cephalosporium sp.* and *Mucor pusillus* were present on the kallar grass pieces before composting, but their frequency of occurrence was quite low. None of these species appeared on the kallar grass pieces in the Petri dishes lined with wet filter paper. After 2 days of composting *Humicola grisea* appeared on cellulose agar whereas *M. pusillus* was 100% on both cellulose and glucose agar. After 6 days of composting *H. insolens*, *H. grisea*, *A. fumigatus* and *Malbranchea pulchella* were isolated from the cellulose agar. *Streptomyces sp.* was observed growing on glucose agar.

After 9 days of composting most of the thermophilic fungi appeared and were quite high in their frequency of occurrence. In addition to the thermophilic fungi, *C. globosum* and *Cephalosporium sp.* (both mesophiles), were also dominant. Between 6th and 14th day *H. insolens* was seen growing profusely on the compost surface. All these thermophilic species also appeared when compost pieces were incubated in Petri dishes lined with wet filter papers.

After 14 days of composting percentage frequency of nearly all the thermophilic fungi considerably decreased, only *Malbranchea pulchella* reached 42% after 30 days and *Myriococ cum albomyces* was recorded after 30, 38 and 50 days of composiing. It appeared on cellulose agar only.

Among mesophilic fungi, *C. globosum*, *Cephalosporium sp*, *Paecilomyces sp*, *Penicillium variable*, *A. niger* and *A. tamarii* appeared between 14th and 30th day of composting whereas *C. globosum* continued upto 56th day. *Pullularia pullulans* was also isolated once after 21 days of composting. *Mucor sp.* also appeared occasionaly on glucose agar and had quite high percentage frequency.

The results presented in this paper have elucidated the role played by various fungi in bringing about decompostion of kallar grass pieces and the successional patterns in which they actively colonize the substrate during composting. Most of the studies regarding the colonization and succession of fungi on plant remains above the soil have been reviewed by Hudson (1968). Such studies have shown definite mycofloristic patterns which vary with different plants. In order to hasten the rate of decomposition of kallar grass after ploughing under, reinoculation of saline soil with those fungi which have been found active during composting, may afford a good help in the biological amelioration ot saline soils. This would need further studies on the active cellulolytic mycoflora naturally inhabiting saline soils and their interaction with the reinoculated fungi.

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