SECTION - H

GENERAL SUMMARY
Paddy straw was selected as a material for studying composting process with nitrogenous and phosphorous amendments. The rate of decomposition was higher with nitrogenous amendments than without it. The rate of weight loss was greater during composting of ammonium biphosphate amended straw as compared to that in ammonium nitrate, urea, and superphosphate plus ammonium nitrate. The temperature at the centre of unamended straw compost pile was 47°C; in amended straw it varied between 47-53°C during the first 10-20 days but during the later period of composting it declined to the ambient. The temperature at the centre of the compost pile was higher than that at the periphery. The pH of the straw during early phase of composting increased to slight alkalinity possibly due to the evolution of ammonia; it later declined to slight acidity.

The length of the straw pieces used for the preparation of the compost and C:N ratio had marked influence on the rate of decomposition. As the length of the pieces was increased, the rate of decomposition declined. The rate of decomposition was considerably high with 25 mm long pieces and thus in the subsequent composting experiments, the straw was chopped into 20-25 long pieces; the moisture was maintained between 60-70%
level. As the nitrogen level of the straw was increased by the addition of ammonium biphosphate, the rate of decomposition was enhanced enormously; this was true up to C:N ratio of twenty and a further increase in nitrogen content did not result in further enhancement of the composting process.

During the study of microbial succession of composting paddy straw, five thermotolerant Aspergilli and thirteen species of thermophilic fungi belonging to 10 genera were recovered; these belonged to Phycomycetes, Ascomycetes and Deuteromycetes. The group-wise distribution is as detailed below:

**Phycomycetes:**

- Absidia corymbifera
- Rhizopus microsporus
- Rhizopus microsporus
- Mucor pusillus

**Ascomycetes:**

- Aspergillus nidulans
- Achaetomium macrosporum
- Chaetomium thermophile
- Thermoascus aurantiacus
- Thielavia minor
Deuteromycetes:

**Aspergillus fumigatus**

**A. oryzae**

**A. candidus**

**A. terreus**

**Humicola lanuginosa**

**H. grisea var. thermoidea**

**H. insolens**

**Sporotrichum sp**

**S. thermophile**

**Torula thermophile**

In the initial stages of composting, mesophilic microbes were present abundantly but their relative abundance declined gradually as temperature of the compost pile rose; when temperature once again dropped to the ambient, mesophilic microbes reappeared in great numbers. The relative abundance of the thermophilic microbes increased with the rise in temperature of the compost pile. The number of mesophilic as well as thermophilic bacteria was larger than the propagules of fungi and actinomycetes. Among the thermophilous fungi, *A. fumigatus*, *S. thermophile*, *C. thermophile* and *T. aurantiacus* were recorded in greater frequency.
The measurement of CO$_2$ evolved on the surface of the compost showed better correlation with the microbial activity than the number of propagules. The amount of carbon dioxide evolved gradually increased up to peak heating period with a gradual decline. One noteworthy point in this connection was that even though total microbial propagules were lower in number during peak heating period, CO$_2$ evolution was greater due to higher rate of respiration of abundant thermophilic microflora. The rate of decomposition was higher during the first 30 days corresponding to higher values of CO$_2$ evolution. After 20 days of composting the temperature dropped to the ambient with the result that the mesophilic microflora increased due to recolonization; the CO$_2$ evolution was, however, fairly low at this stage. This has suggested that the evolution of CO$_2$ is a better parameter to understand the trends in microbial activity than the determination of number of propagules.

The analysis of the samples collected at different intervals of composting showed that hemicelluloses and celluloses were highly susceptible to microbial attack whereas lignin was extremely resistant; the greater part of weight loss could be accounted for by the loss of these two substances. The loss of cellulose was not constant through the period of composting; it was at higher in the first 30 days. The lignin fraction showed negligible loss over a 100 days period of composting. The
ethanol soluble fraction declined constantly whereas the change in diastase soluble fraction was not significant due to conversion of starch of straw into the microbial reserve material, glycogen. The crude fat content declined gradually. The soluble nitrogen was utilized by microbes while loss in insoluble nitrogen was only negligible. The soluble nitrogen added as synthetic fertilizers was depleted rapidly whereas insoluble forms release utilizable forms by decomposition slowly and hence along with synthetic fertilizers the addition of organic manures has been recommended in agriculture. The ash content increased over a 100 day period of composting due to the depletion of organic matter and mineralization as indicated by an increase in the phosphorous content. The C:N ratio increased due to utilization of soluble nitrogen by microbes. The protein content showed an increase due to the incorporation of available nitrogen as microbial proteins. Both reducing and total sugars declined during the period of composting.

The weight loss in straw brought about by the mixed inoculum of twelve thermophilous fungi was less than that noted in a pure culture of Sporotrichum thermophile. The combination of S. thermophile, Sporotrichum sp., Thermoascus aurantiacus, Chaetomium thermophile and Torula thermophile decomposed more straw than the combination of all the cultures or any of the
single culture. The decomposition of straw by thermophiles was rapid at 45°C than that at 30°C. The rate of decomposition by thermophilic bacteria was more than that brought about by thermophilic actinomycetes.

When four mesophilic fungi, viz., Cephalosporium humicola, Chaetomium globosum, Cladosporium herbarum and Irichoderma viride were mixed, the rate of decomposition was higher than that of any single culture. Among the four fungi, Chaetomium globosum decomposed more straw than the others. As in the case of thermophilic microbes, the rate of decomposition by mesophilic bacteria was greater than that of actinomycetes. The biochemical changes brought about by pure cultures of the thermophiles during the decomposition of paddy straw were closely similar to those observed with mixed inoculum of the microbes. Therefore, it is possible to prepare compost by pure cultures of thermophilic fungi by manipulating high temperature (45-50°C) and moisture (60-70%).

All the thermophilic fungi were able to grow in simple media indicating their autotrophic nature to vitamins. Glucose-asparagine, peptone-glucose and Sabouraud's-dextrose media supported good growth of the thermophiles. These moulds showed better growth in a medium containing organic nitrogen source. It is obvious from the measurement of mycelial growth of the thermophiles that NaNO₃, KNO₃ and (NH₄)₂HPO₄ were rapidly utilized.
On the basis of growth rates at different temperatures, thermophilic fungi can be categorized into three groups as listed below:

(i) **Strong thermophiles:**

This group includes *Chaetomium thermophile*, *Humincola lanuginosa*, *Rhizopus microsporus*, *Sporotrichum* sp., *S. thermophile*, *Thermoascus aurantiacus* and *Torula thermophile* with a high minimum temperature of 25°C or above and an optimum temperature at 45-50°C for growth. *Chaetomium thermophile*, *H. lanuginosa* and *T. aurantiacus* failed to grow at temperatures below 30°C suggesting their strong thermophilic nature; only *T. aurantiacus* and *H. lanuginosa* showed any growth at 60°C. Mature perithecia were produced by *T. aurantiacus* and *C. thermophile* in the temperature range 45-50°C and 40-45°C, respectively.

(ii) **Weak thermophiles:**

Moulds growing at 25°C or lower temperature but which failed to grow beyond 50-55°C were included in this category; these organisms showed an optimum of 40°C. This group includes *Absidia corymbifera*, *Mucor pusillus*, *Achaetomium macrosporum* and *Thielavia minor*. Matured perithecia were produced by *Ach. macrosporum* and *T. minor* at 35-40°C after 10 days.
(iii) **Thermotolerants:**

Moulds tolerating higher temperatures for growth like thermophiles but which are able to grow well at 20-28°C are classified in this group, e.g., *Aspergillus fumigatus*. This fungus failed to grow beyond 50°C.

The presence of glucose in the medium hastened the conidial germination of *Aspergillus fumigatus*, *Sporotrichum thermophile*, *Hymenoloma lanuginosa* and *Torula thermophila*. The temperature had an immense influence on spore germination and the germination percentage increased considerably as the temperature raised from 25 to 45°C; further increase in temperature resulted in decline in the rate of spore germination. Studies on thermal resistance of thermophilic fungi indicated that the majority survived temperatures of 60 and 64°C; only *H. lanuginosa* and *T. aurantiacus* survived 2 and 10 min exposure at 72°C, respectively.

The thermophiles were able to elaborate volatiles which inhibited the germination of conidia of *Aspergillus fumigatus*, *H. lanuginosa* and *Tor. thermophila* to a varied extent. The conidia of *Asp. fumigatus* and *Tor. thermophila* exposed to the volatiles of *Thermoascus aurantiacus* and *Mucor pusillus*, respectively failed to recover when they were displaced to a moist chamber. It would appear that the volatiles of these two thermophiles influence some essential metabolic process(es)
of the germinating spore whereby the recovery was hindered. The volatiles were also able to affect mycelial growth. The mycelial growth of *C. thermophila* was inhibited to a greater extent when it was grown in contact with the volatiles of *H. lanuginosa*. The ability to elaborate reasonably high concentration of fungistatic volatile factors appear to play an active role in establishing *Thermoascus aurantiacus*, *Sporotrichum thermophile* and *Chaetomium thermophile* as dominant inhabitants of composts.

The fast growing fungi, viz., *Asp. fumigatus* and *S. thermophile* exhibited higher competitive saprophytic ability (CSA) than the slow growing *H. lanuginosa* and *Tor. thermophila*. The colonization of unsterilized substrate units by *S. thermophile*, *H. lanuginosa* and *Tor. thermophila* was lesser than that noted for *Asp. fumigatus*. This would appear to be due to the appearance of fastgrowing *Thermoascus aurantiacus* and *Asp. fumigatus*, the inoculum of which was present on the unsterilized units. Because of their strong cellulolytic machinery, fast growth, and rapid production and germination of conidia, *Asp. fumigatus* and *S. thermophile* dominated over *Tor. thermophila* and non-cellulolytic *H. lanuginosa*. As the competitive saprophytic ability of a fungus depends not only on its innate ability but also on the composition and condition of the substrate and environment, the change in incubation temperature from 45°C to 50°C resulted change in CSA; *S. thermophile* thus
dominated over Asp. fumigatus at 50°C. The highest CSA of Asp. fumigatus and S. thermophile indicated by their lower RID50 values appeared to impart the advantage of occurring in higher number in the compost.

Of the twelve thermophilic fungi tested for cellulose utilization, seven brought about significant weight loss of filter paper suggesting their ability to produce C1-cellulase component. There was not much difference in cellulase production in the two media containing filter paper and paddy straw as cellulose sources. The culture filtrates obtained from five thermophiles, viz., C. thermophile, Sporotrichum sp, S. thermophile, T. aurantiacus and Tor. thermophila released reducing sugars by acting on CMC and filter paper suggesting the presence of C1-, Cx- and β-glucosidase components in their cellulase systems. The cellulase production increased up to 15-20 days with a decline at later period due to either repression by hydrolysis products like cellobiose and glucose or increasingly resistant crystalline cellulose left after degradation of the most susceptible portions of cellulose. The combination of the above mentioned five organisms or any of the other combinations tested did not give better production of cellulase than the individual organisms. The optimum temperature for cellulase production by T. aurantiacus was 50°C while for the others it was 45°C. The optimum temperature for the activity of cellulase was 55°C for T. aurantiacus and 50°C
for others. The hydrolysis of CMC and maximum accumulation of reducing sugars occurred at pH 5.5. The cellulase production by these organisms even in a medium lacking cellulose suggested their constitutive nature. But the enzyme production was higher in a medium containing cellulose than that lacking it. The cellulase preparations of these organisms can be used for the production of hydrolysate from cellulosic wastes to grow yeasts in order to produce single cell protein.

All the thermophilous fungi tested in this investigation liquefied gelatin and showed caseinolysis suggesting their ability to elaborate extracellular proteases. The presence of glucose in the medium promoted enzyme production in case of *H. lanuginosa*, *Tor. thermophila*, *S. thermophile* and *Ach. macrosorum*; it was repressed in *C. thermophile*. Excepting *C. thermophile*, protease was constitutive for all other thermophiles. The medium containing maltose and gelatin as carbon and nitrogen sources enhanced enzyme production. Since all the fungi were able to produce in a paddy straw containing medium also, it would appear to play a decisive role in the composting process.

All the organisms produced amylase in starch as well as paddy straw broth; but *H. lanuginosa*, *M. pusillus*, *Tor. thermophila*, *Ach. macrosorum* and *Asp. fumigatus* produced more
enzyme as compared to others. The amylase of these organisms was constitutive as the enzyme was secreted in glucose-asparagine medium lacking starch. But the enzyme production was promoted by the presence of starch in the medium. There was a direct correlation between dry mycelial weight and enzyme production in Asp. fumigatus and Rhizopus microsporus while it remained constant in S. thermophile and C. thermophile irrespective of the decline in mycelial weight. The increase in concentration of starch and yeast extract in the medium enhanced enzyme production in Ach. macrosorum, H. lanuginosa, Asp. fumigatus and Tor. thermophile while there was a decline in M. pusillus. The production of amylase increased in the temperature range 35-45°C for all thermophiles; only H. lanuginosa and Tor. thermophile were able to produce enzyme at 55°C. In the case of Ach. macrosorum, Asp. fumigatus and M. pusillus the enzyme activity increased up to 45°C and up to 50°C in H. lanuginosa and Tor. thermophile; any further increase resulted in decreased amylase activity. It is very interesting to mention that H. lanuginosa was a very good producer of amylase and protease which could be exploited for medical and industrial purposes.

Among all the thermophiles, H. lanuginosa, H. grisea, Absidia corymbifera, C. thermophile, Sporotrichum sp and S. thermophile showed zones of precipitation of calcium laurate suggesting their ability to produce extracellular lipases.
Sporotrichum sp, Mucor pusillus, H. lanuginosa and A. corymbifera produced more lipase indicated by lipolysis of butter and coconut oil.

When the twelve thermophilous fungi were mixed, the rate of decomposition was less as compared to that of S. thermophile. It appears to be due to the competition existing among the organisms for the substrate and interaction of volatiles. Though the production of cellulase was not enhanced by the combination of C. thermophile, Sporotrichum sp, S. thermophile, I. aurantiacus and Tor. thermophila in a flask due to limitation of surface area, this combination decomposed straw at a higher rate in a big bottle where greater surface area of the substrate was available. As indicated earlier, the majority of weight loss could be accounted by the loss in cellulose and hemicellulose. In this investigation, a definite correlation was noted only between the rate of decomposition and the cellulolytic ability of the organism and thus the rate of decomposition of highly cellulolytic I. aurantiacus, C. thermophile, Sporotrichum sp, S. thermophile and Tor. thermophila was justified. As the protein, starch and lipid contents of straw are very little, other enzymes might just be supplementing the decomposition of cellulose and hemicellulose during the composting process.