Section E

CELLULOSE DECOMPOSITION BY LITTER FUNGI
INTRODUCTION

Cellulose is the most prominent carbonaceous constituent of the higher plants and is added continually in different forms to the soil along with the litter fall. Cellulose is associated with a number of other materials for example, lignin, cutin and other proteinaceous substances. The proportion of cellulose in higher plants changes with age and type of plant parts. The woody substances and leaves are specially rich in cellulose and the concentration increases as the plant matures. The decomposition of this carbohydrate has a special significance in the biological cycle of carbon and the processes are very much complicated. The biological degradation of cellulose has been reviewed in monographs or reviews by several workers like Siu (1951), Cascoigne et al. (1960), and Wood (1960).

Aerobic decomposition of fresh organic matter, such as leaf tissues follow a course which may be divided into two phases: the first is its conversion to humus and the second is the break-down of the humus to simple
gaseous and mineral constituents. Any substrate put into the soil will be exposed to an almost unlimited potential of microbial species and the changes in the physical and chemical characteristics of the habitat can alter the composition of the microflora or the cellulose degrading activity of individual organisms.

Many of the physiological studies on fungi have been carried out using noncellulose carbon source (Foster, 1939; Steinberg, 1939). Greathouse and Ames (1945) investigated the effects of different nitrogen sources for sixteen species of *Chaetomium* and Basu (1948) studied the same problem with *Aspergillus terreus*, *A. fumigatus*, *Stachybotrys atra* and *Chaetomium indicum*. Although a few scattered studies exist on the mineral nutrition of cellulose destroying fungi, there is very little work done on the effect of various conditions upon the mycoflora busy in decomposition of the forest litter.

The cellulose utilizing population includes aerobic and anaerobic mesophilic bacteria, filamentous fungi, actinomycetes, thermophilic bacteria and basidiomycetes. Uptil now a number of cellulolytic fungi have been studied in the pure culture, but the action in nature is clearly a result of a complex population and there is
definitely an intense microbiological competition for nutrients in the mixed population active in vivo (Pugh, 1958; Griffin, 1960; Hudson, 1962). In order to define precisely the population which is active in cellulose decomposition in the litter, it is necessary to use techniques which allow direct observation of the colonization of the cellulose and only by such methods a true information about the fungus flora busy in utilizing cellulose can be obtained. In the present study, colonization of cellophane paper, lens tissue, filter paper and wheat straw by litter fungi have been studied. An attempt is made here to elucidate the effects of nutrients on the occurrence and activity of selected cellulolytic fungi.

The present work is divided into several aspects of the cellulose decomposing fungi. At first isolation from litter are made with the help of such baits as filter paper, cellophane paper and wheat straw. The prominent fungi selected from these were then studied for their physiological characteristics such as media, carbohydrate nutrition, mineral nutrition, temperature and their capacity to decompose cellulose in the form of filter paper. These fungi were then also studied for the production of cellulolytic enzymes.
MATERIAL AND METHODS

Collection of the litter samples:

Two samples from each type of the three forest stands were collected for this study. The soil with litter was collected from surface to 9" depth. In all, six samples were brought to the laboratory. The samples were then processed by removing away big pebbles and root pieces, then ground with pestle and mortar and finally filtered through a 3 mm. sieve. The sieved litter was then stored in polythene bags at 10°C. in refrigerator for use when required. The pH of each sample was recorded and the moisture content was calculated by dry weight basis after drying it at 98°C. overnight.

Isolation of cellulolytic forms of fungi:

About 20 grams of soil litter sample was placed in sterile Petri dishes and the moisture adjusted to 100% moisture holding capacity (M.H.C.). To these dishes cellulotic baits in three different forms were added to capture the cellulolytic fungi. These baits were as follows:

(1) Cellophane paper pieces: The cellophane paper used was the brand of regenerated cellulose film made by
the British Cellophane Company. It is relatively a pure form of cellulose and its transparency provides perfect conditions for observation under the microscope.

Sheet cellophane was cut into pieces approximately 2 x 1 cm. and these pieces were boiled in distilled water to dissolve out plasticizers. After washing with distilled water, these pieces were used in two ways: (i) two cellophane pieces were placed directly on the sieved litter, in the prepared Petri dishes; (ii) the washed cellophane pieces were placed singly on glass slides, the excess water was drained off and the slides were buried vertically in small pots containing litter samples. These pots and Petri dishes were then kept at laboratory temperature for incubation.

(2) Filter paper (Whatman No. 1): Pieces of 2 x 1 cm. size were cut, moistened in water and sterilized. Two of these pieces were spread on the soil litter Petri dishes and incubated.

(3) Wheat straw: Pieces of wheat straw about 2 cm. long were moistened and sterilized. Four to six such pieces were then spread upon the soil litter dishes and incubated at room temperature.

These dishes and pots were allowed to incubate for
about 6-15 days. The paper pieces and straw pieces were lifted up with forceps at different intervals and placed on glass slides for examination under a binocular dissecting microscope. Help was also taken of a micromanipulator in picking up the spores and inoculum from the baits. The inoculum was then transferred to dishes containing suitable media for isolation, purification and identification.

In the Petri dishes the fungal species appeared within 6-15 days of incubation but in case of pots the decomposition of cellophane paper was more rapid so that after about two weeks it was not possible to recover the paper in good form. In late stages bacteria and mites predominated.

Results:

In all 24 species of fungi were isolated and purified. They were collected from different baits as shown in Table No. 14.

The relative occurrence of some of the important genera, in different localities, are given in Table No. 15.

A glance at these tables shows that most of the cellulolytic species grew in all the three types of
TABLE 14: Showing fungal species detected on different cellulosic baits.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Organism</th>
<th>Cellophane paper</th>
<th>Filter paper</th>
<th>Wheat straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Rhizopus nigricans</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td><em>Mucor luteus</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td><em>Choanephora cucurbitarum</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td><em>Chaetomium indicum</em></td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td><em>C. globosum</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td><em>Aspergillus fumigatus</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td><em>A. niger</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td><em>A. flavus</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td><em>A. ochraceus</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td><em>Penicillium variabile</em></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td><em>P. verruculosum</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td><em>Paecilomyces variot</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td><em>Mennoniella echinata</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td><em>Trichoderma viride</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td><em>Alternaria tenuis</em></td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td><em>Curvularia geniculata</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>17</td>
<td><em>Humincola brevis</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td><em>Pullularia pullulans</em></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td><em>Pestalotia lapagericola</em></td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td><em>Fusarium oxysporum</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>21</td>
<td><em>F. semitectum</em></td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>22</td>
<td><em>F. solani I</em></td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>23</td>
<td><em>F. culmorum</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>24</td>
<td><em>Rhizoctonia</em> sp.</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
**TABLE 15**: Showing distribution of important genera of fungi on cellophane paper buried in soil litter samples.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Sample No.</th>
<th>Number of cellophane pieces examined</th>
<th>Number of cellophane pieces colonized by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rhizopus</td>
</tr>
<tr>
<td>Rajababa hill</td>
<td>1</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Sajibhatar hill</td>
<td>1</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Gulla hill</td>
<td>1</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>12</td>
<td>3</td>
</tr>
</tbody>
</table>
cellulose baits with a few exceptions, for example, Choanephora cucurbitarum, Aspergillus ochraceus, Curvularia geniculata and Pestalotia lapagericola were collected only from wheat straw and not from the other baits, similarly the species Penicillium variabile and Pullularia pullulans were detected only on ordinary filter paper. There were eight species, viz., Rhizopus nigricans, Chaetomium globosum, Aspergillus niger, A. flavus, Penicillium verruculosum, Memnoniella echinata, Fusarium oxysporum and F. culmorum which occurred on all the three kinds of baits and were very widespread.

From Table No. 15, which shows the genera-wise breakup in different localities it is seen that the genera Aspergillus, Penicillium, Rhizopus and Fusarium are the most dominating ones in these isolations, while such genera as Chaetomium, Trichoderma, Alternaria and Rhizoctonia are comparatively less collected. Further it is seen that the maximum number of isolations in different sites of collection were in forest type I (Rajababa hill) followed by forest type II (Sajibhatar hill) and forest type III (Gulla hill).

**Nutritional studies of cellulolytic fungi:**

Certain of the fungi which appear to be most frequently associated with the materials rich in cellulose (as
cellophane paper, filter paper and wheat straw) were selected for detailed study. Since these fungi have a dominant role to play in litter decomposition it was thought proper to study their physiological characteristics in some detail. The organisms chosen were Rhizopus nigricans, Chaetomium globosum, Aspergillus fumigatus, A. niger, Penicillium verruculosum, Paecilomyces variotii, Memnoniella echinata, Trichoderma viride, Alternaria tenuis, Fusarium oxysporum, F. solani I, F. solani II, F. semitectum, F. culmorum and Rhizoctonia sp.

The fungi were first tried on a number of media at 28°C. A suitable medium for this study could be selected for each on which it grew best. A temperature study was then planned giving seven treatments so that a most suitable temperature could be selected for each fungal species. Czapek-Dox + Yeast agar on which most of the fungi grew well was selected for further experiments. In the carbohydrate study a number of them were tried mixed with a basal medium and put at the optimum temperature for each. Similar studies for cellulolytic activity of each of these fungi was planned and carried out.

**Effect of different media on the growth of fifteen selected cellulose decomposing fungi:**

**Method:**
All the 16 fungal species were grown on six different media namely, oatmeal agar, potato dextrose agar, malt extract agar, Richard's agar, czapek's agar and Czapek-Dox + Yeast agar. The dishes were inoculated in the centre by a 6 mm. disk of agar carrying the fungus. Inoculation was done under sterile conditions. Petri dishes were then incubated at 28°C. The colony size was measured after three and six days with the help of a divider and a millimeter scale. All the plates were run in duplicate, the average diameter after sixth day (144 hours) are recorded in Table No. 16.

The growth measurements of a few species as *Rhizopus nigricans*, *Trichoderma viride*, *Fusarium culmorum* and *Rhizoctonia* sp. were taken after 72 hours as their growth rate was quite rapid in comparison to others.

**Results:**

Growth of all the species in different media varied considerably. In general, there was very good growth and sporulation on Czapek-Dox + Yeast agar and potato dextrose agar. *Chaetomium globosum* grew well on malt extract agar. The species of *Fusarium* grew well on czapek's agar though the sporulation was less on this medium, while on oatmeal agar the sporulation was quite profuse. Most of the other fungi such as *Paecilomyces varioti*, *Trichoderma*
<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Organism</th>
<th>Oat meal agar</th>
<th>Potato dextrose agar</th>
<th>Malt extract agar</th>
<th>Richard's agar</th>
<th>Czapek's agar</th>
<th>Czapek, Dox + Yeast agar</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>*Rhizopus nigricans</td>
<td>62</td>
<td>68</td>
<td>72</td>
<td>66</td>
<td>64</td>
<td>78</td>
</tr>
<tr>
<td>2.</td>
<td>Chaetomium globosum</td>
<td>53</td>
<td>62</td>
<td>68</td>
<td>42</td>
<td>46</td>
<td>53</td>
</tr>
<tr>
<td>3.</td>
<td>Aspergillus fumigatus</td>
<td>64</td>
<td>72</td>
<td>68</td>
<td>58</td>
<td>62</td>
<td>76</td>
</tr>
<tr>
<td>4.</td>
<td>A. niger</td>
<td>62</td>
<td>70</td>
<td>64</td>
<td>52</td>
<td>66</td>
<td>72</td>
</tr>
<tr>
<td>5.</td>
<td>Penicillium verruculosum</td>
<td>60</td>
<td>72</td>
<td>62</td>
<td>48</td>
<td>58</td>
<td>74</td>
</tr>
<tr>
<td>6.</td>
<td>Paecilomyces variotii</td>
<td>54</td>
<td>68</td>
<td>52</td>
<td>44</td>
<td>52</td>
<td>64</td>
</tr>
<tr>
<td>7.</td>
<td>Memnoniella echinata</td>
<td>48</td>
<td>64</td>
<td>56</td>
<td>40</td>
<td>62</td>
<td>76</td>
</tr>
<tr>
<td>8.</td>
<td>*Trichoderma viride</td>
<td>42</td>
<td>52</td>
<td>40</td>
<td>38</td>
<td>46</td>
<td>48</td>
</tr>
<tr>
<td>9.</td>
<td>Alternaria tenenés</td>
<td>48</td>
<td>72</td>
<td>45</td>
<td>40</td>
<td>36</td>
<td>42</td>
</tr>
<tr>
<td>10.</td>
<td>Fusarium oxysporum</td>
<td>72</td>
<td>62</td>
<td>52</td>
<td>46</td>
<td>58</td>
<td>65</td>
</tr>
<tr>
<td>11.</td>
<td>*F. solani I</td>
<td>56</td>
<td>52</td>
<td>46</td>
<td>48</td>
<td>63</td>
<td>62</td>
</tr>
<tr>
<td>12.</td>
<td>*F. solani II</td>
<td>52</td>
<td>50</td>
<td>48</td>
<td>45</td>
<td>58</td>
<td>60</td>
</tr>
<tr>
<td>13.</td>
<td>*F. semitectum</td>
<td>68</td>
<td>64</td>
<td>58</td>
<td>62</td>
<td>54</td>
<td>70</td>
</tr>
<tr>
<td>14.</td>
<td>*F. culmorum</td>
<td>62</td>
<td>54</td>
<td>56</td>
<td>58</td>
<td>48</td>
<td>66</td>
</tr>
<tr>
<td>15.</td>
<td>*Rhizoctonia sp.</td>
<td>64</td>
<td>82</td>
<td>58</td>
<td>52</td>
<td>62</td>
<td>66</td>
</tr>
</tbody>
</table>

* Growth measurements after 72 hours of incubation.
viride, *Alternaria tenuis* and *Rhizoctonia* sp. grew luxuriantly on starchy media like oatmeal agar and potato dextrose agar. All the species of *Aspergillus*, *Penicillium*, *Rhizopus* and *Memnoniella* grew well on Czapek-Dox + Yeast agar.

**Effect of various temperatures on the growth of fifteen selected cellulolytic species:**

Selected fungal species were grown on Czapek-Dox + Yeast agar at seven different temperatures. The dishes were inoculated by a 6 mm. diameter agar disk taken from the edge of a vigorously growing colony. Incubation temperatures used were 15, 26, 28, 30, 35, 40 and 50°C. At the higher temperatures like 40 and 60°C, the Petri dishes were kept in a glass cabinet along with a beaker with distilled water to prevent the excessive drying of the cultures. The measurements were taken after 12, 36 and 60 hours intervals by averaging the two diameters at right angles across the colony. The observations are recorded in the Table No. 17 and temperature growth relationship has been shown in Figure No. 2-4.

**Results:**

From a perusal of the data the following conclusions
could be drawn:

1. *Rhizopus nigricans*: It sporulates very well within a range of temperatures between 26 to 35°C. The rate of growth was quite rapid at 30°C.; at 50°C. the rate of growth was recorded minimum whereas at 15°C. the fungus did not grow at all.

2. *Aspergillus fumigatus* and *A. niger*: Both the species grew well at 35°C. The initial growth was quite fast at 30°C., while at 40 and 50°C. the sporulation was much diminished and at the lower temperature of 15°C. there was no growth.

3. *Chaetomium globosum*: The growth of this fungus was rapid at 40°C. and 50°C. but formation of perithecia was delayed at the higher temperature. Mature perithecia were formed after 72 hours incubation at 40°C. and after 96 hours at 50°C.

4. *Penicillium verruculosum*, *Paecilomyces variotii* and *Memnoniella echinata*: These three species have almost the same growth rate at the range of 26 to 30°C. *Paecilomyces* grew fast at 26°C. whereas the maximum growth of the rest of the two species was at 30°C.

5. *Trichoderma viride*: Its sporulation was quite rapid
at 30°C. At 40 and 50°C. the fungus did not show any growth.

6. **Alternaria tenuis, Fusarium oxysporum, F. solani I, F. solani II, F. semitectum** and **F. culmorum**: All these species grew even at 15°C. but their rate of growth was very low. The best growth of all the species of Fusarium was at 26°C. They did not show any growth at 35 and 40°C. within 60 hours incubation.

7. **Rhizoctonia** sp.: This fungus grew in a very rapid way and even in 60 hours the whole dish was covered with it. Growth rate was very high at 26 and 30°C. Its mycelial growth was quite slow at higher temperature i.e., at 40 and 50°C. though it could grow slowly at even 50°C. At 15°C. there was no growth.

The litter fungi in general grew well between 26°C. and 35°C. with individual variations here and there for higher and lower temperatures. There are five species namely **Rhizopus nigricans, Aspergillus fumigatus, A. niger, Chaetomium globosum** and **Rhizoctonia** sp. which grew though sparingly upto 50°C. These could be termed as thermophilic forms in this context. Out of these five, none grew at the lower temperature of 15°C. The species of Fusarium in general grew best at 26°C. excepting **F. semitectum** for which the optimum temperature
TEMPERATURE GROWTH RELATIONSHIPS

**Rhizopus nigricans**

Penicillium verruculosum

**Aspergillus fumigatus**

Chaetomium globosum

**Aspergillus niger**

Paecilomyces variotii

**FIG. 2.**
TEMPERATURE GROWTH RELATIONSHIPS

TRICHODERMA VIRIDE

MEMNONIELLA ECHINATA

ALTERNARIA TENUISS

FUSARIUM OXYSPORUM

F. SOLANI I

F. SOLANI II

COLONY DIAM (M.M.)

COLONY DIAM (MM)

COLONY DIAM (M.M.)

COLONY DIAM (MM)

TIME (HOURS)

TIME (HOURS)

FIG. 3.
TEMPERATURE GROWTH RELATIONSHIPS

FUSARIUM SEMITECTUM

F. CULMORUM

RHIZOCOTONIA Sp.

FIG. 4.
was a little higher i.e., 28°C. For most other fungi which include such species as *Rhizopus nigricans*, *Penicillium verruculosum*, *Mmmnoniella echinata*, *Trichoderma viride* and *Alternaria tenuis* the optimum temperature recorded was 30°C. Very few fungi showed growth at lower temperatures of 15°C., these were *Alternaria tenuis*, *Fusarium oxysporum*, *F. solani* I, *F. solani* II, *F. semitectum* and *F. culmorum* though the growth was very much retarded. For the other experiments the optimum temperature for a particular species was selected.

Utilization of sugars by cellulolytic forms:

In this study the ability of all the fifteen selected fungal species, to use five different sugars, was determined by measuring the dry weight of mycelium produced in a mineral broth with different sugars as the main carbon source.

A 5% solution of the different sugars was made in a basal medium of the following composition:
Sodium nitrate (NaNO₃) . . . . . 5.0 gm.
Potassium dihydrogen phosphate (KH₂PO₄) 1.0 gm.
Magnesium sulphate (MgSO₄, 7H₂O) . . . 0.5 gm.
Calcium nitrate (Ca(NO₃)₂, 4H₂O) . . . 0.01 gm.
Ferric chloride (FeCl₃) . . . . . 0.001 gm.
Yeast extract . . . . . . 0.5 gm.
Distilled water . . . . . . 1000 ml.

The sugars chosen for this experiment were sucrose, glucose, xylose, mannose, and raffinose. Out of which some of the sugars such as glucose, xylose and mannose are known to be the hydrolytic products of cellulose and hemicelluloses.

15 ml. of the medium with different sugars was taken in 150 ml. flasks and autoclaved at 15 lbs. pressure for 15 minutes. Xylose was not autoclaved but its solution was added to the sterile basal medium by means of a hypodermic syringe fitted with a millipore filter. The solution of this sugar was thus sterilized by passage through the filter. This was done because xylose is known to decompose during autoclaving (Cochrane, 1958). A single flask for each fungus was taken as a control and no sugar was added in the basal medium. Each flask was inoculated with 6 mm. diameter disk of inoculum from the fungal colony growing on potato dextrose agar.
The flasks were inoculated at different temperatures according the optimum found for each fungal species as determined in the previous experiment. The ability to use sugars was determined by measuring the dry weight of mycelium produced by each fungal species after seven days incubation. The contents of the flasks were filtered through weighed (Whatman No. 1) filter papers and washed with distilled water. These filter papers with mycelial mat were dried at 85°C. for 24 hours. The net weight of mycelium was calculated by subtracting the weight of filter paper. The results are shown in Table No. 18 (Fig. no. 5-6).

Results:

A perusal of the graphs shows that all the fungi were able to grow on all the five sugar sources used in the present investigation. The fungi in general showed almost no growth in the carbon free control medium except a few like Paecilomyces variotii and Fusarium solani I which showed some growth in these flasks. Here too the records were more or less negligible. Amongst the hexoses, glucose supported an all round good growth. It was specially suited to Rhizopus nigricans, Chaetomium globosum, Paecilomyces variotii, Fusarium oxysporum and F. culmorum which had the maximum growth
TABLE 18: Oven dry weight of mycelium in different carbohydrates after 7 days incubation. (Weight in mg)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Organism</th>
<th>Sucrose 5%</th>
<th>Glucose 5%</th>
<th>Xylose 5%</th>
<th>Mannose 5%</th>
<th>Raffinose 5%</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Rhizopus nigricans</em></td>
<td>135</td>
<td>210</td>
<td>59</td>
<td>152</td>
<td>182</td>
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<tr>
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<td><em>Chaetomium globosum</em></td>
<td>268</td>
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<td><em>A. niger</em></td>
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<td><em>Penicillium verruculosum</em></td>
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<td>6.</td>
<td><em>Paecilomyces variotii</em></td>
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<td>276</td>
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<td>280</td>
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<td>11.</td>
<td><em>F. solani</em> I</td>
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<td>192</td>
<td>79</td>
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<td><em>F. solani</em> II</td>
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<td>244</td>
<td>166</td>
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<tr>
<td>13.</td>
<td><em>F. semitectum</em></td>
<td>145</td>
<td>165</td>
<td>382</td>
<td>110</td>
<td>88</td>
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<tr>
<td>14.</td>
<td><em>F. culmorum</em></td>
<td>268</td>
<td>462</td>
<td>319</td>
<td>203</td>
<td>289</td>
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</tr>
<tr>
<td>15.</td>
<td><em>Rhizoctonia</em> sp.</td>
<td>272</td>
<td>170</td>
<td>150</td>
<td>260</td>
<td>270</td>
<td>7</td>
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</tbody>
</table>
Fig. 5  Utilization of carbohydrates in terms of mycelial growth.

1  *Rhizopus nigricans*
2  *Aspergillus fumigatus*
3  *A. niger*
4  *Penicillium verruculosum*
5  *Chaetomium globosum*
6  *Paecilomyces variotii*
7  *Aemmoniella echinata*
8  *Trichoderma viride*
9  *Alternaria tenuis*
10  *Fusarium oxysporum*
Fig. 6 Utilization of carbohydrates in terms of mycelial growth.

11  *Fusarium solani* I

12  *F. solani* II

13  *F. semitectum*

14  *F. culmorum*

15  *Rhizoctonia* sp.
UTILIZATION OF CARBOHYDRATES IN TERMS OF MYCELIAL GROWTH

FIG. 6
on it. Mannose was again a good source for the general growth of fungi tested. It was however best suited to Aspergillus fumigatus, A. niger, Memnoniella echinata, Trichoderma viride and Fusarium solani I which recorded their maximum growth on it. The disaccharide viz., sucrose supported a good all round growth excepting that Paecilomyces variotii, did not grow well on it. Maximum growth of Penicillium verruculosum and Rhizoctonia sp. was obtained on it. The result on raffinose was varied, some of the fungi like Alternaria tenuis and Fusarium solani II had their maximum growth on this sugar, while Paecilomyces variotii and Fusarium semitectum grew very poorly. The other fungi grew in a moderate way. The results on xylose (pentose sugar) were rather poor when compared to other sugars. Only a few fungi like Penicillium verruculosum, F. semitectum and F. culmorum grew exceptionally well on it. On the other hand, Rhizopus nigricans and Fusarium solani I grew very poorly in this sugar.

Coming to the individual species we find that there are some species which are discriminative for sugars. For example, Paecilomyces variotii grows very poorly on sucrose and raffinose and Fusarium semitectum grows exceptionally well on xylose while poorly on others. Rhizopus nigricans was peculiar in having a very poor
growth on xylose and similar observation was for *Fusarium solani* I. The maximum growth in the entire experiment was recorded on glucose for *Fusarium culmorum* which is a fast growing species.

Another point worthy of note was that the species of the same genus may differ in utilizing the same source of carbon e.g. *Fusarium solani* I and *F. solani* II grew poorly on xylose but *F. semitectum* utilized it favourably and showed best growth on it. In the same way *F. oxysporum* and *F. culmorum* grew well on glucose while rest of the Fusarium species did not show the same response. Even the two strains of *Fusarium solani* differ in their choice of sugar utilization, one grew well on mannose while the other on raffinose.

**Utilization of cellulose:**

The capacity to utilize cellulose by fifteen selected organisms was tested by the method as described by Garrett (1962). Whatman No. 1 filter paper of 6 cm. diameter was moistened in 150 ml. conical flask. A 15 ml. aliquot of mineral solution of the composition described for sugar utilization was taken. The pH of the basal medium was 5.3 before sterilization. The flasks were autoclaved for 30 minutes at 15 lbs. pressure. Each
flask was inoculated with a 6 mm. agar disk cut with a cork borer from the growing margin of the colony of a test fungus. Control flasks were set up using disks of blank agar as inoculum. All the flasks were incubated for 24 days at their appropriate (optimum) temperatures. After 8-10 days the filter papers were covered with a fairly dense growth of fungal mycelium.

The filter paper pads were then removed and all the pads were dried (without washing) to constant weight at 80°C. and loss in dry weight was noted. The ability of the individual fungi to use cellulose was calculated as net loss in weight of cellulose after substracting the value of control series. In control flasks it was noted that in each flask the weight of filter paper pad increases after 24 days incubation, this increase in weight was due to the weight of minerals sedimented from the basal medium and from the agar disk. It will be common to both, the treatments as well as control. The results are shown with the help of the bargraphs (Fig. no. 7), which show the net value of cellulose utilized by different species.

Results:

All the five thermophilic fungi tested, viz.:

*Rhizopus nigricans*, *Aspergillus fumicatus*, *A. niger*,
<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Rhizopus nigricans</em></td>
</tr>
<tr>
<td>2</td>
<td><em>Chaetomium globosum</em></td>
</tr>
<tr>
<td>3</td>
<td><em>Aspergillus fumigatus</em></td>
</tr>
<tr>
<td>4</td>
<td><em>A. niger</em></td>
</tr>
<tr>
<td>5</td>
<td><em>Penicillium verruculosum</em></td>
</tr>
<tr>
<td>6</td>
<td><em>Paecilomyces variotii</em></td>
</tr>
<tr>
<td>7</td>
<td><em>Mennioniella echinata</em></td>
</tr>
<tr>
<td>8</td>
<td><em>Trichoderma viride</em></td>
</tr>
<tr>
<td>9</td>
<td><em>Alternaria tenuis</em></td>
</tr>
<tr>
<td>10</td>
<td><em>Fusarium oxysporum</em></td>
</tr>
<tr>
<td>11</td>
<td><em>F. solani</em> I</td>
</tr>
<tr>
<td>12</td>
<td><em>F. solani</em> II</td>
</tr>
<tr>
<td>13</td>
<td><em>F. semitectum</em></td>
</tr>
<tr>
<td>14</td>
<td><em>F. culmorum</em></td>
</tr>
<tr>
<td>15</td>
<td><em>Rhizoctonia sp.</em></td>
</tr>
</tbody>
</table>
UTILIZATION OF CELLULOSE

TEMPERATURE OF INCUBATION

- 26°C
- 28°C
- 30°C
- 35°C
- 40°C

FIG. 7.
Chaetomium globosum and Rhizoctonia sp. were found strongly cellulolytic in their action. Out of these five, Aspergillus niger, Chaetomium globosum and Penicillium verruculosum were found to be most active and could be designated as strongly cellulolytic. Among the other organisms Trichoderma viride, Paecilomyces variotii, Rhizoctonia sp. and Rhizopus nigricans were found to be next in order in their capacity to utilize cellulose. The rest of the fungal species were poor in this respect and they could use very little of cellulose even after 24 days of incubation. On seeing the data for the utilization of cellulose by species of Fusarium it was found that F. semitectum and F. solani I have some capacity to utilize cellulose while the rest of the Fusarium species were found to be very low in this respect.

**Effect of macro-nutrients on colonization of cellulose by litter decomposers:**

In the previous experiment the cellulolytic activity of fifteen selected fungi was determined. In the present test an attempt has been made to find out how this activity is affected by some of the macro-nutrients. The macro-nutrients used in this case were Potassium dihydrogen phosphate (\( \text{KH}_2\text{PO}_4 \)) and Potassium nitrate (\( \text{KNO}_3 \)) to provide mainly the phosphate and nitrate ions.
Experiment - I

Effect of nutrients added in solution:

Method:

Collection of litter: Litter samples from the teak forest of Sagar were collected in new polythene bags. From the litter samples the root materials and undecomposed plant parts were removed. The samples were passed through a sieve of 3 mm. mesh. The pH of the sample was determined. The sieved litter was placed in large sterilized Petri dishes (6" diam.) and sets of five dishes were treated as follows:

(i)  1% solution of KH₂PO₄ was added in such a way that water content rose to 100% moisture holding capacity (M.H.C.).

(ii) 1% solution of KNO₃ was added in the same way.

(iii) 2% solution of KN₂PO₄ was similarly added.

(iv) 2% solution of KNO₃ was similarly added.

(v) A mixture of 1% KH₂PO₄ and 1% KNO₃ was added.

(vi) A mixture of 2% KH₂PO₄ and 2% KNO₃ was added.

(vii) Instead of mineral solution only sterile distilled water was added to act as control.
In this way a set 5 x 7 = 35 Petri dishes were prepared. To these dishes a set of ten sterilized lens tissue (a pure form of cellulose made from cotton fibres) disks (5 mm. diam.) were placed equidistantly to note the colonization on them from the soil-litter. The dishes were incubated at 26°C. for ten days.

Observation and records:

The disks were examined and the fungi growing on these disks were isolated in pure culture and identified. The percentage of disks colonized by each fungal species have been recorded in Table No. 19 (Fig. no. 3).

Only six fungal species colonized the disks in this experiment showing that the treatments given were highly selective for the fungal flora. The fungi isolated also occur among the fifteen cellulolytic forms already discussed. Considering the performance of different species we find that Cheatommium globosum which was totally absent from the control, occurred on these disks in which phosphate and nitrate was supplied in various proportions. The maximum colonization was found to occur in 2% nitrate. Then there were three species, viz., Aspergillus niger, Penicillum verruculosum and Fusarium oxysporum which occurred almost throughout including the control disks. They responded both to phosphate and nitrate, though in
TABLE 19: Showing colonization of litter fungi on lens tissue disks under different doses of macro-nutrients.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Organism</th>
<th>Percentage of lens tissue disks colonized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P₁</td>
</tr>
<tr>
<td>1.</td>
<td>Chaetomium globosum</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Aspergillus niger</td>
<td>26</td>
</tr>
<tr>
<td>3.</td>
<td>Penicillium verruculosum</td>
<td>22</td>
</tr>
<tr>
<td>4.</td>
<td>Trichoderma viride</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Fusarium oxysporum</td>
<td>46</td>
</tr>
<tr>
<td>6.</td>
<td>Rhizoctonia sp.</td>
<td>32</td>
</tr>
</tbody>
</table>

P₁ - 1%KH₂PO₄; P₂ - 2%KH₂PO₄; N₁ - 1%KNO₃; N₂ - 2%KNO₃; C - Control.
Fig. 3 Occurrence of cellulolytic fungi on lens tissue disks placed on litter samples amended with phosphate and nitrate.

1 *Chaetomium globosum*
2 *Aspergillus niger*
3 *Penicillium verruculosum*
4 *Fusarium oxysporum*
5 *Trichoderma viride*
6 *Rhizoctonia* sp.
OCCURRENCE OF CELLULOLYTIC FUNGI ON LEN S TISSUE DISKS PLACED ON LITTER SAMPLES AMENDED WITH PHOSPHATE AND NITRATE.

$P_1, 1\% \text{ KH}_2\text{PO}_4$; $P_2, 2\% \text{ KH}_2\text{PO}_4$; $N_1, 1\% \text{ KNO}_3$; $N_2, 2\% \text{ KNO}_3$; $C$, CONTROL.

FIG. 8.
varying degrees in different doses. The behaviour of *Trichoderma viride* was rather peculiar. It did not occur on control disks but occurred sparingly in some of the treatments. Phosphate alone appears to have no effect on the activity of this fungus while nitrate alone or the two mixed nutrients stimulate its development. *Rhizoctonia* sp. though not found in control disks appeared to be stimulated by both nitrate and phosphate in various doses.

**Experiment - II**

**Effect of nutrients placed in solid form on lens tissue:**

**Method:**

In this experiment instead of keeping the lens tissue disks, the surface of the litter in dishes was completely covered with a sheet of lens tissue. The mineral nutrients were put at three points in solid form on the lens tissue the points of application being marked. The amount of minerals applied at each point was of the order of about 20 mg. In this case a control dish was kept in which no mineral was kept on lens tissue. The plates were incubated for three weeks. The fungi could
grow through the tissue and appeared above around the treated spots and these portions were examined and picked up with the help of forceps for isolation and identification of the species.

**Observations:**

After 21 days incubation the dishes containing the mineral nutrients at three points were observed. The following observations were recorded:

1. Around the points of application of phosphate a cottony growth of *Fusarium* was noted. The other organism isolated was *Aspergillus niger* growing in patches around the *Fusarium*.

2. In case of nitrate *Penicillium verruculosum* was predominant along with some greyish colonies of sterile mycelium.

3. At the point of application of lime (pH of sample was 5.2) the lens tissue was utilized by bacteria and only a round circle formed by the utilization of cellulose was visible, none of the fungal species was isolated.

4. In the control dishes no microbial activity was recorded except a species of *Fusarium* which was seen at one point only.
STUDY OF CELLULOYTIC ENZYMES PRODUCED BY
SELECTED LITTER FUNGI

INTRODUCTION

The botanical significance of the microbiological decomposition of cellulose has attracted the attention of botanists by the hundreds. Chemically cellulose is a high polymer of β-1,4 linked D-glucose residues. It is mainly broken down through the agency of bacteria and fungi, representing different taxonomic groups specializing in different cellulosic materials in nature. As has been described in a previous experiment the ascomycetes and fungi imperfecti play an important role in the decomposition of plant residues in the forest litter. So far, the major basic research has been on the problem of prevention of deterioration of cellulose in case of cotton fibres. Economically the other most important form of cellulose is the wood. In the present studies the organisms studied were isolated from the decomposing forest litter.

The biochemical processes involved in the decomposition of the various substrates differ considerably. Readily soluble substances such as the simple sugars and amino acids appear to be absorbed directly and then
metabolized within the microbial cells. The cellulases are generally considered to be formed only in the presence of cellulose. According to Reese and Mandels (1962) not cellulose itself but the soluble cellobiose, a hydrolytic product, is the true inducer of cellulase in cellulose cultures. Decomposition of cellulose is brought about by a hydrolytic enzyme system. As yet no one has demonstrated any enzyme system capable of decomposing the other constituents of the plants like phenolic polymers and lignin. The enzymatic breakdown of cellulose involves two or more components which act differently on the polymer chain. Although the enzyme systems are extracellular, they seldom act at any great distance from the microorganisms which produce them. Any organism which produces enzymes in culture and hydrolyse soluble cellulose derivatives will not be called cellulolytic, because that may or may not be able to attack or utilize native cellulose.

The classical cellulase enzyme of Pringsheim (1912) is made up of at least two entities designated as $C_1$ and $C_x$ respectively. The same steps were also referred by other workers. The cellulases produced in culture have been referred to as $C_x$ cellulases; those organisms which can hydrolyze or alter the native cellulose have been considered to produce an enzyme referred to as $C_1$ enzyme.
Convenient soluble cellulose derivatives that have become popular for these tests is carboxymethylcellulose (CMC). Use of these derivatives has made possible application of viscometric techniques.

In this series of experiments it has been the endeavour to determine the nature of the fifteen selected cellulolytic fungi and to categorize them into two main types on the basis of their enzymatic potentialities: (i) those which are truly cellulolytic capable of attacking native cellulose of the forest debris, (ii) those which are utilizers and can use only the later formed products of the first type of decomposition.

MATERIAL AND METHODS

Enzyme assay was done in culture filtrates of the selected organisms to test their ability to decompose native cellulosic material.

The fungi were grown on a basal modified Czapek's-Mox medium where the carbon source was partly substituted with different cellulose sources. The native cellulose sources tried were filter paper pulp and cotton fibres. The composition of the medium was as follows:
Sodium nitrate (NaNO₃) . . . . . . . . . 3.0 gm.
Potassium dihydrogen phosphate (KH₂PO₄) . . 1.0 gm.
Magnesium sulphate (MgSO₄.7H₂O) . . . 0.5 gm.
Potassium chloride (KCl) . . . . . 0.5 gm.
Sucrose . . . . . . . . . 10.0 gm.
Ferrous sulphate (FeSO₄.7H₂O) . . . In a trace.
Cellulose source . . . . . . . . 10.0 gm.
Distilled water . . . . . . . . 1000 ml.

35 ml. of the sterilised broth medium was taken in 150 ml. conical flask. The initial pH of the medium was recorded. Each flask was then inoculated with a test fungus by adding single disk of 6 mm. diam. obtained from the margins of a freshly grown colony on potato dextrose agar plates and incubated at 26°C. for seven days. A parallel series of control flasks was run with basal medium in which no cellulose source was added in order to determine if enzymes were produced even without the induction by cellulose source (C₉ activity). In another series of flasks with basal medium only CMC was added which is not regarded as a native source of cellulose but a special type to detect cellulase activity. In this way four types of flasks were run in series of each of the fifteen fungal species, viz.

(i) Basal medium + filter paper pulp (native source)
(ii) " " + cotton fibres ( " " )
(iii) " " + CMC
(iv) Basal medium only as control.
Culture filtrates were obtained by filtering under suction in a Buchner funnel. After taking the final pH, the filtrates were centrifuged at 3000 r.p.m. for 15 minutes to clear any suspended hyphal fragments or fibres. The supernatant liquid was taken as enzyme preparation and used for test. If required the enzyme preparations were frozen for the purpose of storage and later use.

The enzyme assay was done viscometrically in Fenske-Ostwald viscometers. The reaction mixture consisted of:

1.0% CMC solution . . . . . . . 3.5 ml.
Water . . . . . . . 1.5 ml.
McIlvaines buffer (pH 6.5) . . . . . 1.5 ml.
Enzyme preparation . . . . . 1.5 ml.

The viscosity measurements of this reaction mixture were recorded at 30°C in a water bath, and at intervals of 10 minutes over a total period of 60 minutes. The observations were recorded for percentage loss in the viscosity of the reaction mixture at different periods of reaction times, as per the formula:

\[ \% \text{ loss in viscosity} = \frac{E_{T_0} - E_{T_t}}{E_{T_0} - E_{T_w}} \times 100 \]
where \( ET_o \) = Efflux time of reaction mixture at 0'hour
\( ET_t \) = Efflux time of reaction mixture at time 't'
\( ET_w \) = Efflux time of water.

Relative enzyme activity was calculated as

\[
REA = \frac{1000}{t \text{ at } V_{25}}
\]

where \( t \) \( V_{25} \) represented the time in minutes required to reduce the viscosity of the reaction mixture by 25% of the initial.

Results:

Determinations of the capacity to produce cellulase for colonization of the dead organic matter by the few selected fungi of the litter mycoflora, were made as to the activity and the type of enzyme involved. The fungi taken were *Rhizopus nigricans*, *Aspergillus fumigatus*, *A. niger*, *Penicillium verruculosum*, *Chaetomium globosum*, *Paecilomyces variotii*, *Mennoniella echinata*, *Trichoderma viride*, *Alternaria tenuis*, *Fusarium oxysporum*, *F. solani* I, *F. solani* II, *F. semitectum*, *F. culmorum* and *Rhizoctonia* sp. The data have been presented in Table no. 20 and Figure no. 9-10.

The results obtained showed that almost all the
organisms produced cellulase invitro, but not all were capable of utilising native cellulosic material provided. This showed that the cellulase that was produced differed in activity in certain cases, depending upon the inherent ($C_x$) or the adaptive ($C_1$) types of cellulase.

Fungi having $C_1$ and $C_x$ activity (in native cellulosic sources and in CMC):

It was found that cellulase activity was quite high in culture filtrates of *Aspergillus niger*, *A. fumigatus*, *Penicillium verruculosum*, *Chaetomium globosum*, *Memnoniella echinata*, *Trichoderma viride*, *Fusarium solani I*, *F. semitectum* and *Rhizoctonia* sp. on CMC, cotton and filter pulp. Maximum REA values of 59, 34, 14, 18 and 25 were obtained for *Aspergillus niger*, *Memnoniella echinata*, *Fusarium solani I*, *F. semitectum* and *Rhizoctonia* sp. respectively. REA of 21, 14 and 142 were obtained in cotton for *Aspergillus fumigatus*, *Penicillium verruculosum* and *Chaetomium globosum*. REA of 14 on filter paper for *Trichoderma viride* was recorded. These fungal species were showing much less or negligible activity in control.

Fungi having $C_1$ and $C_x$ activity (in native cellulosic sources, CMC and in control):

The cellulase activity of *Paecilomyces variotii* was
found to be present in control as well as in native sources. However, more cellulase activity was found in filter paper pulp for this fungus.

Fungi having $C_x$ activity (in CMC):

Fusarium oxysporum, F. solani II and F. culmorum produced more cellulase in CMC in control and other cellulosic sources. Maximum REA values 33, 10 and 9 were obtained for F. oxysporum, F. solani II and F. culmorum respectively. In certain cases there was very little difference in the cellulase activity in culture filtrates of CMC and control.

Fungi having $C_x$ activity (in control):

In Alternaria tenuis much cellulase activity was obtained in control where no cellulosic substrate was provided. Maximum REA value of 12 was found for the above fungus. It was showing negligible cellulase activity in CMC and much less on other cellulosic sources. Rhizopus nigricans was exceptional; it did not show recordable enzyme activity in cellulosic sources as well as in control even after 21 days of incubation.
<table>
<thead>
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<th>Sl. No.</th>
<th>Organism</th>
<th>CMC</th>
<th></th>
<th>Filter paper</th>
<th></th>
<th>Cotton</th>
<th></th>
<th>Control</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>% loss at</td>
<td>REA</td>
<td>% loss at</td>
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<td>REA</td>
<td>% loss at</td>
<td>REA</td>
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<tr>
<td></td>
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<td>20 min.</td>
<td>40 min.</td>
<td>60 min.</td>
<td>20 min.</td>
<td>40 min.</td>
<td>60 min.</td>
<td>20 min.</td>
<td>40 min.</td>
</tr>
<tr>
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<td><em>Rhizopus nigricans</em></td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
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<td><em>Chaetomium globosum</em></td>
<td>26</td>
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<td>31</td>
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<td>12</td>
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<td>11</td>
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<td><em>A. niger</em></td>
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</tr>
<tr>
<td>5.</td>
<td><em>Penicillium verruculosum</em></td>
<td>5</td>
<td>8</td>
<td>15</td>
<td>12</td>
<td>3</td>
<td>5</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>6.</td>
<td><em>Paecilomyces varioti</em></td>
<td>5</td>
<td>10</td>
<td>11</td>
<td>0</td>
<td>9</td>
<td>14</td>
<td>21</td>
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<tr>
<td>7.</td>
<td><em>Memnoniella echinata</em></td>
<td>20</td>
<td>31</td>
<td>37</td>
<td>34</td>
<td>18</td>
<td>28</td>
<td>33</td>
<td>29</td>
</tr>
<tr>
<td>8.</td>
<td><em>Trichoderma viride</em></td>
<td>6</td>
<td>12</td>
<td>15</td>
<td>9</td>
<td>11</td>
<td>16</td>
<td>22</td>
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</tr>
<tr>
<td>9.</td>
<td><em>Alternaria tenuis</em></td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>9</td>
<td>17</td>
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<td>10.</td>
<td><em>Fusarium oxysporum</em></td>
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<td>33</td>
<td>14</td>
<td>20</td>
<td>23</td>
<td>14</td>
</tr>
<tr>
<td>11.</td>
<td><em>F. solani I</em></td>
<td>6</td>
<td>13</td>
<td>20</td>
<td>14</td>
<td>10</td>
<td>14</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
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<td>12</td>
<td>18</td>
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<td>10</td>
<td>9</td>
<td>12</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>13.</td>
<td><em>F. semitectum</em></td>
<td>15</td>
<td>21</td>
<td>26</td>
<td>18</td>
<td>12</td>
<td>19</td>
<td>23</td>
<td>14</td>
</tr>
<tr>
<td>14.</td>
<td><em>F. culmorum</em></td>
<td>8</td>
<td>13</td>
<td>16</td>
<td>9</td>
<td>5</td>
<td>7</td>
<td>10</td>
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TABLE 20: Table showing activity of cellulase (i) as % loss in viscosity of reaction mixture at time 't'. (ii) relative enzyme units.
Fig. 9  Activity of cellulase as % loss in viscosity of reaction mixture.

1  Rhizopus nigricans
2  Aspergillus fumigatus
3  A. niger
4  Penicillium verruculosum
5  Chaetomium globosum
6  Paecilomyces variotii
7  Hemioniscia echinata
8  Trichoderma viride
9  Alternaria tenuis
10  Fusarium oxysporum
11  F. solani I
12  F. solani II
13  F. semitectum
14  F. culmorum
15  Rhizoctonia sp.
ACTIVITY OF CELLULASE AS % LOSS IN VISCOSITY OF REACTION MIXTURE

FIG. 9.
Fig. 10  Activity of cellulase as relative enzyme units.

1  Rhizopus nigricans
2  Aspergillus fumigatus
3  A. niger
4  Penicillium verruculosum
5  Chaetomium globosum
6  Paecilomyces variotii
7  Mmmoniella echinata
8  Trichoderma viride
9  Alternaria tenuis
10  Fusarium oxysporum
11  F. solani I
12  F. solani II
13  F. semitectum
14  F. culmorum
15  Rhizoctonia sp.
ACTIVITY OF CELLULASE AS RELATIVE ENZYME UNITS

Figure 10.

RELATIVE ENZYME ACTIVITY

CMC
FILTER PAPER
COTTON
CONTROL

ORGANISM
DISCUSSION

The fungi studied here can be classified into four groups depending upon their enzymatic activities against cellulosic sources. The first category can be called as having $C_1$ and $C_x$ activity which are further divisible into two types: first, those fungi like Aspergillus niger, A. fumigatus, Penicillium verruculosum, Chaetomium globosum, Memnoniella echinata, Trichoderma viride, Fusarium solani I, F. semitectum and Rhizoctonia sp. which are able to assimilate the native cellulose as well as CMC, and most of the fungal species tested will come under this category. These do not show much enzymatic activity in control. Under the second type those fungi will be included which attack native cellulose ($C_1$), CMC and can also produce $C_x$ enzyme in the control flasks in which no cellulose source was present. This type of activity was shown by Paecilomyces variotii only.

The third category comprises of those fungi which attacked CMC but showed less activity in other cellulosic sources as well as in control. Some Fusarium species were of this nature. Lastly, there was a category of fungi which produced almost no $C_1$ activity and showed plenty of $C_x$ activity. These produced enzymes predominantly in control flasks. These are of the nature of
utilizers which mainly depend upon the initial degradation by other fungi. The enzyme $C_x$ is unable to act on native cellulose but with the help of these enzymes the cleavage products brought about by $C_1$ enzyme such as cellotriose and cellotetraose are utilized.

It should, however, be mentioned that these enzymes are very often overlapping and not sharp cut. Basic data on these lines of work are still being gathered before any finality is reached.

The ability to attack native cellulose is naturally of greater significance in the decomposition of forest litter. By considering the enzyme activity of Aspergillus niger, A. fumigatus, Chaetomium globosum, Penicillium verruculosum, Memnoniella echinata, Trichoderma viride, Fusarium solani I, F. semitectum and Rhizoctonia sp. it seems that these possess the necessary enzymatic mechanism needed to degrade native cellulose. This enzyme system thus plays an important role with respect to the saprophytic activities of these fungi in the decomposition of the plant parts.

Cellulolytic enzymes are also produced by many plant pathogenic organisms (Husain, 1953; Bateman, 1964) and has been demonstrated in the host tissues. Thus cellulase enzymes would seem to be of paramount importance in saprogenesis as well as in pathogenesis.
GENERAL DISCUSSION, SUMMARY AND CONCLUSION ON CELLULOSE DEGRADATION

From a biological point of view the decomposition of cellulose is one of the most important processes in nature. It is estimated that approximately one-third of the organic material produced in the world is cellulose, most of which occurs in higher plants as the skeletal substance of cell wall. In fully mature wood tissues the proportion of cellulose amounts to about 40 to 60% of the oven dry material. The decomposition of this product is therefore of utmost importance in maintaining the balance between the opposite synthetic and degradative reactions in the carbon cycle. Besides this role in the general economy of nature, cellulose decomposition is also of importance in plant pathological context as well as industrial and commercial spheres.

In the soil-ecology of forests the role of cellulose decomposition is obviously of very great importance. The annual litter fall is formidable and its availability is achieved through the mediation of microorganisms. The biological utilization of cellulose can proceed from temperatures near freezing to a point up to 65°C, and the various cellulolytic organisms are affected differently by temperature. Mesophiles dominate at moderate temperatures while a thermophilic microflora adapted to hotter
localities can bring about a rapid cellulose dissimilation at higher temperature which may be due to direct effect of temperature upon enzymatic action. It is with this background that these studies on cellulose degradation and utilization were undertaken.

The initial isolation of cellulolytic fungi was done with the help of baits added to the soil-litter plates prepared from the 0-9" depth of the forest floor; only those which were frequently gathered were selected out. Fifteen such fungi, namely *Rhizopus nigricans*, *Chaetomium globosum*, *Aspergillus fumigatus*, *A. niger*, *Penicillium verruculosum*, *Paecilomyces variotii*, *Meponicella echinata*, *Trichoderma viride*, *Alternaria tenuis*, *Fusarium oxysporum*, F. solani I, F. solani II, F. semitectum, F. culmorum and *Rhizoctonia* sp. were finally selected for further studies. Next step was to know something about the physiology of the individual species. For this the fungi were grown in several media (oatmeal agar, potato dextrose agar, malt extract agar, Richard's agar, czapek's agar and czapek's-Dox + yeast agar), several temperatures (15, 26, 29, 30, 35, 40 and 50°C.) and in different carbohydrate sources (sucrose, glucose, xylose, mannose and raffinose). The effect of some macro-nutrients (nitrate and phosphate) on colonization of cellulose and finally their capacity to decompose cellulose in the form of filter paper was determined.
From the results it is apparent that out of fifteen tested fungal species only a few fungi like *Rhizopus nigricans*, *Aspergillus fumigatus*, *Aspergillus niger*, *Chaetomium globosum* and *Rhizoctonia* sp. can tolerate the higher temperature which often prevails in Indian conditions. Out of these fifteen, some species were mesophilic and were very soon killed off by the high temperatures.

The fungi which possess high growth rates can rapidly utilize cellulose as shown by *Aspergillus niger*, *A. fumigatus*, *Chaetomium globosum* and *Rhizoctonia* sp. It may be noted that the optimum temperature for *Chaetomium globosum* which is a well-known cellulose fungus was 40-50°C, which was the highest. By seeing the Figure no. 7, it will be found that the rate of utilization of cellulose in the form of filter paper was found to be considerable in the case of *Aspergillus fumigatus*, *A. niger*, *Penicillium verruculosum*, *Chaetomium globosum* and *Trichoderma viride*. *Rhizoctonia* sp. does not utilize much cellulose in these experiments. The same observations were recorded by Tribe (1960) while examining the possibility that *Rhizoctonia solani* is an active cellulose decomposer in the soil. Using the buried cellulose film in natural soil he observed that in several litter samples *R. solani* was the dominant fungal colonizer. However, it should be emphasised that by this method the
capacity to decompose native cellulose is not measured. 
*R. solani* has a high growth rate and a rapid utilizer of 
the decomposition products of its own activity and also 
the products formed by other soil organisms. *Hemmosiella 
echinata* is also an active and destructive fungus as seen 
on cellophane. Its ecology has been thoroughly discussed 
by White et al. (1949) who also observed its preference 
for the purer form of cellulose. In these experiments 
the same species was often found to colonize all the cellu-
losic sources added to the litter. *Trichoderma viride* 
which often appeared on normal soil plates did not show 
any preference to cellulosic baits. Certain fungi recorded 
on straw have not been seen on cellulose film and on 
filter paper, which shows their preference to particular 
type of cellulose. Species of *Fusarium* specially *F. 
culmorum*, *Penicillium* and *Trichoderma* colonized straws 
placed on soil-litter samples. These results are in 
accord with those of Butler (1953). Sadasivan (1939) and 
Walker (1941) obtained species of *Penicillium* and 
*Trichoderma* only after surface sterilization of the straws. 
Species of all these fungi possess cellulose destroying 
power which confirms the results of Norman (1930) and 
Siu (1951).

Another factor contributing towards specificity in 
colonizing flora is the difference in chemical composition
of the substrates. While the filter paper and the cellophane paper are pure cellulose forms, wheat straws vary greatly qualitatively as well as quantitatively in their constituents. Wheat straws also differ in the nature of the noncellulosic components and in the degree of hardness. As a result, each cellulosic substrate lying in the litter is likely to support its own microflora.

Siu (1951) by a number of techniques isolated organisms responsible for the deterioration of cotton fabric. Most of his isolates were quite common to all the samples studied by him and consisted of *Penicillium citrinum*, *P. funiculosum*, *Aspergillus niger*, *A. fumigatus* Chaetomium globosum, *Mennonicilla echinata* and *Trichoderma viride*. In the present studies also many of these fungi figured prominently and can be considered to be a good evidence for these fungi playing a great role in the deterioration processes.

Generally a variety of carbohydrates are preferred by fungi as the source of carbon, but no single sugar was found to be the best source for all the organisms tested. However, most of the fungi could use a large variety of carbon sources for their growth and sporulation. It was also found that species of the same genus differed in utilizing the sources of carbon. Maximum utilization of carbon source in terms of mycelial growth of *Fusarium*
culmorum and F. oxysporum was found on glucose whereas
F. solani and F. semitectum could utilize maximum carbon
from mannose and xylose respectively. Lal and Tandon
(1968) while studying the utilization of monosaccharides
by three isolates of Colletotrichum capsici marked the
variation in the rate of utilization of various sugars
by different isolates of the same species. Siu and
Sinden (1951) while studying the effects of temperature
and mineral nutrition of cellulolytic fungi concluded
that the presence of soluble carbon sources such as
glucose, xylose, cellobiose and sucrose inhibited the
simultaneous utilization of cellulose by fungi. It seems
that an organism does not degrade the cellulose till such
time as the availability of other soluble sources of
carbon has been exhausted.

The cellulolytic fungi are special types which are
capable of acting upon the cellulosic sources when other
soluble carbon sources are not present. This they do by
virtue of special enzymatic equipment. In this work
fifteen fungi were tested for this capacity by culturing
them in different cellulosic sources, like cotton fibres
and filter paper. These tests revealed that these fungi
could be classified into four categories: (i) those
which have C1 and Cx activity and are able to act on
native cellulosic sources and CMC. These were Aspergillus
niger, A. fumigatus, Penicillium verruculosum, Chaetomium globosum, Demoniaella echinata, Irichoderma viride, Fusarium solani I, F. semitectum and Rhizoctonia sp.; (ii) those which have similar activity i.e., C₁ and Cₓ but in addition they produced enzyme in control without any inducing substrate. There was only one species of this type namely Paecilomyces variotii; (iii) those which produced Cₓ activity only and that too only in the presence of CMC which acted as inducer. The species noted here were F. oxysporum, F. solani and F. culmorum; and (iv) those which produced enzyme (Cₓ) activity in control flasks i.e., it needed no inducer at all. There was only one species of this type viz., Alternaria tenuis. Rhizopus nigricans was exceptional in showing rather poor activity (C₁ and Cₓ, Fig. 10). It survives in the soil in the form of a utilizer which lives on the cellulosic decomposing products released by other fungi.

It can be said in conclusion that, cellulose, which is so abundantly available, is not an obligate carbon source for fungi. They have a broad nutritional base and utilize many carbonaceous materials.