SECTION - D

RESPONSE OF THE COPROPHILOUS ASCOMYCETES TO SOME VOLATILE COMPOUNDS
INTRODUCTION

Russian workers were the first to demonstrate the existence of volatile organic compounds acting upon fungi. As early as 1926 Doyarenko claimed that the soil air should be recognized as an organic constituent part of the soil, equally with soil solutions and the solid soil phase. Several workers (Mixon and Curl, 1967; Gilbert et al., 1969; Hora and Baker, 1970) showed that the growth of soil inhabiting fungi can be stimulated by gaseous products from higher plants. The inhibitory effects can also be obtained by volatile organic compounds.

The terpenes represent another group of volatile substances emanating from wood. Most of them are reported as inhibitory to fungi (Cobb et al., 1968; Hintikka, 1970). Not only woody plants but also herbs and grasses produce volatile compounds, which affect the growth and development of soil fungi. It is observed that the growth and respiration of soil microorganisms were stimulated by the presence of decaying residues of alfalfa hay. Spore germination in Trichoderma, Aspergillus and Penicillium species increased, as well as the germination and further growth of sclerotia of Sclerotium rolfsii buried in the soil (Menzies and Gilbert, 1967). On the other hand,
inhibiton in the growth of *S. rolfsii* by extracts from decaying oat and corn residue was reported by Mixon and Curl, (1967).

Volatile substances are also produced by the fungi themselves and by other microorganisms present in the substratum. Many of these substances are inhibitory rather than stimulatory. These may affect the growth or differentiation of producer mycelium itself or of other mycelia in the neighbourhood. A simple volatile compound, ethanol or ethyl alcohol was found to increase the efficiency of utilization of glucose in many fungi (Fries, 1973). It is now absolutely clear that fungi in practically all habitats live in an atmosphere containing various volatile organic compounds, which may be the exudates from various parts of higher plants or from the decomposing material like forest litter and dung.

Although dung is very suitable substrate for the growth of a large number of fungi as it contains a large quantity of carbohydrate, rich in vitamin, nitrogen and moisture content, also provide suitable pH for sporulation. Many gaseous emanations and other volatile compounds produced in the process of decomposition during digestion might be playing an important role in the survival or in the growth and sporulation of various coprophilic fungal species.

In the present study the effect of some volatile compounds on the mycelial growth and development of ascocarps by eight coprophilous ascomycetes have been evaluated.
MATERIAL AND METHOD

All the fungi used in this experiment were isolated from various types of dung (Table 1). The fungi were cultured on potato dextrose broth medium. The pH was adjusted to 6.0 before autoclaving.

Erlenmayer flasks of 150 ml capacity with side connecting tube were used as culture vessels. Each flask received 30 ml of medium. After autoclaving at fifteen pounds pressure for fifteen minutes, the flasks were inoculated with 6 mm inoculum disc grown on PDA. For a single treatment two flasks were inoculated with each test organism. The inoculated flasks were kept for incubation at 27°C for 24 hours and then transferred under treatment of different volatile compounds. A separate set of flasks for each test organism was kept for incubation without giving any treatment as control.

Eight different volatile compounds (Table 9) which were readily available in the laboratory and few of them are also well known fungicides were selected for treatments. Twenty ml of each volatile compound was taken in large glass tube having a side connecting tube. With the help of rubber tubing the glass tubes having volatile substances were connected with the inoculated flasks (Fig. 8B) and kept for incubation for 15 days at 27°C.

The dry weight of mycelium was determined at harvest. Before
drying the mycelium, the number of ascocarps (perithecia or cleistothecia) in each treatment were counted by floating the mycelial mat in a Petridish. These counts were made by use of a stereoscopic wide field microscope and different sporulation gradings were given.

RESULTS AND DISCUSSION

The results of these experiments are recorded in Table 9. These results showed that different volatile compounds have varied effects on different fungi.

Inhibition of fungal mycelium was shown in the presence of all the volatiles, but maximum inhibitory effect was shown by acetic acid and pyridine in Sordaria fimicola and S. humana respectively. A total inhibition in sporulation was shown by acetic acid, pyridine and acetone in both the test species of Sordaria. The atmosphere developed by ether and absolute alcohol seems not to be much toxic for vegetative as well as sexual reproductive structures in few of the test fungi.

Chaetomium species were found somewhat susceptible for ether, acetic acid, chloroform, formaldehyde and acetone. In the presence of all these volatiles the mycelial growth recorded in all the three species of Chaetomium was found less in comparison to control flasks. C. indicum showed less (25 mg) mycelial
growth in chloroform, sporulation was recorded only in the case of alcohol, pyridine and benzal. The same was the case with 
*C. bostrychodes*. In this fungus, the vapours emanating from pyridine were found very much toxic as only a little vegetative mycelium (7 mg) was produced here (Fig. 8). *C. robustum* grew only vegetatively in the presence of different volatiles showing cent per cent inhibition in the development of ascosporas, although the mycelium of this fungus was found somewhat resistant for emanating volatiles in comparison to the other species of *Chaetomium*.

The results (Table 9) showed that ether, pyridine and chloroform vapours have somewhat similar effects on the perithecia development in *Lophotrichus ampullus* and *L. bartelttii*. Minimum mycelium was developed in acetone and acetic acid by *L. ampullus* (31 mg) and *L. bartelttii* (5 mg) respectively. In other volatile compounds the growth varies but always found less than control flasks (Fig. 8). A poor ascospor development by *L. ampullus* was recorded in benzal whereas the same compound showed a total inhibition in ascospor formation by *L. bartelttii*.

None of the volatiles was found as effective as acetic acid in its toxic effect on sporulation and inhibition of mycelium of *Thielavia terricola*. However, the response of chloroform and acetone was very similar to acetic acid in the cent per cent inhibition of fruit bodies. The mycelial growth of *I. terricola* (Fig. 8) recorded in the emanating vapours of acetic acid,
### TABLE 9  
**Development of asccarps and mycelium (in mg) under various volatile compounds.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Organisms</th>
<th>Ether</th>
<th>Absolute alcohol</th>
<th>Acetic Acid</th>
<th>Lyridine</th>
<th>Benzal</th>
<th>Chloroform</th>
<th>Formaldehyde</th>
<th>Acetone</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Sordaria fimicola</em></td>
<td>118</td>
<td>203</td>
<td>31</td>
<td>54</td>
<td>79</td>
<td>102</td>
<td>107</td>
<td>100</td>
<td>227</td>
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<td>2.</td>
<td><em>S. humana</em></td>
<td>132</td>
<td>143</td>
<td>57</td>
<td>50</td>
<td>82</td>
<td>114</td>
<td>104</td>
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<td>151</td>
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<tr>
<td>3.</td>
<td><em>Chaetomium indicum</em></td>
<td>115</td>
<td>84</td>
<td>116</td>
<td>120</td>
<td>127</td>
<td>25</td>
<td>103</td>
<td>91</td>
<td>121</td>
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<tr>
<td>4.</td>
<td><em>C. bostrychodes</em></td>
<td>40</td>
<td>32</td>
<td>39</td>
<td>7</td>
<td>85</td>
<td>37</td>
<td>39</td>
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<tr>
<td>5.</td>
<td><em>C. robustum</em></td>
<td>120</td>
<td>123</td>
<td>74</td>
<td>104</td>
<td>75</td>
<td>52</td>
<td>100</td>
<td>117</td>
<td>146</td>
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<tr>
<td>6.</td>
<td><em>Lophotrichus ampullus</em></td>
<td>33</td>
<td>51</td>
<td>96</td>
<td>36</td>
<td>45</td>
<td>98</td>
<td>50</td>
<td>31</td>
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<tr>
<td>7.</td>
<td><em>L. barteltii</em></td>
<td>60</td>
<td>73</td>
<td>5</td>
<td>29</td>
<td>13</td>
<td>61</td>
<td>90</td>
<td>23</td>
<td>61</td>
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<td>+++</td>
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<tr>
<td>8.</td>
<td><em>Thielavia terricola</em></td>
<td>194</td>
<td>225</td>
<td>25</td>
<td>111</td>
<td>65</td>
<td>110</td>
<td>207</td>
<td>126</td>
<td>245</td>
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</tbody>
</table>

- : No sporulation  
+ : Sporulation poor (No. of asccarps less than 50)  
++ : Sporulation good (No. of asccarps 50-100)  
+++ : Sporulation best (No. of asccarps more than 100)
Fig. 8: Effect of volatile compounds on mycelial growth.

1. Ether
2. Absolute alcohol
3. Acetic acid
4. Pyridine
5. Benzal
6. Chloroform
7. Formaldehyde
8. Acetone

C. Control
FIG. 8 EFFECT OF VOLATILE COMPOUNDS ON MYCELIUM GROWTH
Fig. 8B ::

Apparatus used for providing the treatment of different volatile substances against the test fungi.
chloroform and acetone was 25, 110 and 126 mg respectively.

It is a common observation that sporulation is reduced in closed containers and that submerged mycelium fails to develop spores (Hampson, 1954). In the same way many ascomycetous forms fail to develop their sexual reproductive structures even in the presence of a good amount of organic matter like forest litter and dung. Although this is usually ascribed to an oxygen deficit, it seems more likely that the accumulation of various gaseous emanations from decomposing plant materials, produced by the fungi themselves and by other microorganisms present in their environment might be the critical factor. Weinhold (1963) and Weinhold & Garraway (1966) showed a dramatic effect of ethanol and other chemically related alcohol on the growth and rhizomorph formation in Armillaria mellea. A perusal of the data showed the complete inhibition in sporulation of all the test fungi in the presence of vapours emanated from acetic acid and acetone. Their response was also somewhat poor for the vegetative growth of coprophilous ascomycetes. The results presented here on eight coprophilous ascomycetes add evidence that the effect of one volatile compound can be different on different species of one genus as was found here in the species of Gordaria and Chaetomium.