CHAPTER VI

VOLATILES AND FUNGAL GROWTH
INTRODUCTION

Organic volatile compounds are produced either during decomposition of organic waste or by micro-organisms themselves and play a significant role in soil fungistasis (Lockwood, 1977). These volatiles belong to alkenes, aldehyde, ketones and inorganic compounds (Robinson et al., 1968; Robinson and Garrett, 1969; Smith and Cook, 1974). Pavlica et al. (1978) has reported that at certain concentrations ammonia, ethylene, and acetone stimulated mycelial growth or spore germination in fungi. Soil organic matter is predominantly anionic, with the principal functional groups being carboxyl, phenolic and alcoholic hydroxyl, methyl, amino and carbonyl and the sign and density of charge will vary according to the pH and the isoelectric point (pI) constituents moieties. Soil organic colloids are described in detail by Schnitzer and Khan (1972); Flaig et al. (1975) and Hayes and Swift (1978). Norman (1969) had evaluated the production of volatiles by Dipodescus aggregatus and identified 11 esters, 9 alcohols, 5 acids and 3 carbonils by gas chromatography and mass spectrometry.

According to Balis (1976) Allyl alcohol is found as one of the volatile fungistatic compound in soil. It is reported that such volatile metabolites may some times be stimulatory rather than inhibitory to
fungi (Hutchinson, 1971; Fries, 1973). Luckwood (1964) had originally suggested that fungistasis is a localized result of microbial activation in the sporosphere; antibiotic production appeared to be mainly responsible for this localized reaction. Industrial and domestic activities have accelerated the rates of transfer of numerous elements through the hydrosphere, lithosphere and atmosphere. This extraneous impact on the natural cycling of these elements has resulted in their increased mobilisation, transport, deposition and subsequent accumulation in the various microbial ecosystems. Atmospheric pollutants also include their effects on the activities, ecology and population dynamics of microbes in terrestrial and aquatic environments (Babich and Stotzky, 1974, 1978).

Microorganisms are directly involved in atmospheric pollution in that they are the source of substantial quantities of various gaseous pollutants, both inorganic and organic, and they serve as sinks for the removal of numerous atmospheric constituents. Microbes and viruses are recipients of, and responders to, atmospheric pollutants. Studies performed in vitro and in situ have demonstrated that atmospheric pollutants may adversely affect the generation time of bacteria; spore germination, mycelial proliferation, fruiting body formation and spore production by fungi; microbial respiratory activity;
photosynthesis of cyanobacteria, algae and lichens; nitrogenase activity of microbes involved in dinitrogen fixation and viral infectivity (Babich & Stotzky, 1974, 1978). Keratinophilic fungi can survive saprophytically in nature particularly in the habitats rich in an atmosphere of various volatile organic compounds produced by the microorganisms or during the process of decomposition of organic waste and they play a significant role in soil fungistasis (Mixon and Curl, 1965; Hora and Baker, 1970). The volatile substances may be inhibitory or stimulatory to fungal growth (Stotzky and Schenk, 1976; Deshmukh and Agrawal, 1982). In most of the Industrial waste ammonia and ethylene are considered to be a prime factor for inhibiting the growth of microorganisms. Literature showed that these factors differ in their action from the organism to other and organism also differ in their sensitivity to different volatile factors found in their habitats.

Many of the gaseous constituents in the troposphere arise from biological and abiological processes occurring on or near the surface of the Earth. These atmospheric gases are derived from both natural (biotic and abiotic) and anthropogenic (industrial and domestic) activities. Natural biotic sources include emissions from volcanoes, forest fires ignited by lighting, chemical reactions in soil and aquatic systems. Volatiles compounds are
also detected in natural soils. Formaldehyde was detected in some soils at a concentration high enough to inhibit the fungal spore germination. A simple volatile compound ethanol was found to increase the efficiency of utilization of glucose in some fungi (Fries, 1973). Terrestrial green plants, fresh water algae, soil fungi and germinated seeds also produce carbon monoxide (Babich and Stotzky, 1972, 1974). Natural emissions of nitrogen dioxide hydrogen sulphide and ammonia also exceed those from anthropogenic sources (Rasmussen et al., 1975).

Natural environments generally are heterogenous open systems in which populations of microorganisms rarely exist in complete isolation from each other. In fact it is now axiomatic that different kinds of organism coexist in nature in the same habitat because each type of organism has its own functional status in the ecosystem. Gause (1934) recognised many years ago that, not only are the constraints imposed upon a particular population of one or more species by the physical and chemical environment important, but so too are the activities of neighbouring populations. Various types of interactions between pairs of microorganisms have been encountered, both negative and beneficial (Slater & Bull 1978). Of these interactions competition is probably the most important as it is the basis, as Gause (1934) had also observed that struggle
for existence and occurs when populations of different organisms are limited in terms of growth rate or final population size by the common dependence on an external factor required for growth (Burns and Slater, 1982).

It is now generally accepted that in nature competition most often occurs at sub maximal growth rates because substrates for microbial growth become available in low concentration (Veldkamp and Jannasch, 1972). However, the maximum specific growth rate of a microorganism which is expressed when all nutrients are in excess may also have a selective advantage in those natural environments in which fluctuations in nutrient concentrations occur. The faster growing organism will be able to build up a larger population during periods of excess substrate and thereby gradually increase their proportion of the total microbial biomass.

Pathak and Agrawal (1977) evaluated the inhibitory effect of organic volatile compounds on the vegetative growth and sporulation in coprophilous fungi. The effect of several organic volatiles on the growth and sporulation of soil inhabiting keratinophilic fungi have been also shown by Jain and Agrawal, 1978b; Singh and Agrawal 1981b, Rathore et al., 1982.
PART - A

I. MYCELIAL GROWTH AND SPORULATION IN DIFFERENT ORGANIC VOLATILES

MATERIALS AND METHODS

The effect of volatile vapours emanating from ten different compounds was studied against the growth and sporulation of *A. benhamiae*, *C. indicum*, *M. aurantiaca* and *M. gypseum*. For this 50 ml of Sabouraud's dextrose broth (Peptone 10.0 gm, Dextrose 40.0 gm and Distilled water 1000.00 ml) was taken in 250 ml Erlenmeyer flasks. The flasks were sterilized at 15 lbs pressure for 15 minutes and inoculated with 1 ml spore suspension having $2 \times 10^6$ spore ml$^{-1}$ in 0.1% Tween-80 of the test fungus. After 24 hours of incubation at $28^\circ C \pm 1^\circ C$ the flasks of 150 ml size having a side connecting tube, containing 10 ml volatile compound was connected with the help of a rubber tube with stopper. A separate set of flasks for each test organism was also kept without any treatment as control. The mouth of all the flasks were twined with polythene and kept for incubation at $28^\circ C \pm 1^\circ C$ for 7 days. The mycelium was harvested on a preweighted filter paper and dried at $80^\circ C \pm 2^\circ C$ till constant weight. Dry weight of the mycelium was calculated by subtracting the initial weight of the filter paper. Before harvesting the mycelium, tip of a needle was touched with the
spores and dipped in a cavity slide containing 0.05 ml sterilized distilled water and observed under microscope. The sporulation was determined and different spore gradings was recorded on arbitrary scale (Table-18). The percentage mycelial growth, stimulation or inhibition was determined in comparison to control.

RESULTS

The results of the present investigation are given in Table-18 Fig. 20. A perusal of the data showed that emanation of chloroform, formaldehyde, formic acid, pyridine and n-butyric acid caused mycelial growth inhibition in most of the test organisms. Maximum growth inhibition i.e., 76.74% in _A.benhamiae_ was recorded in the vapours emanated from chloroform. In _A.benhamiae_ growth stimulation of 6.97, 11.62, 16.27, 39.53 and 44.13% in the presence of vapours emanating from benzene, ethyl alcohol, isopropanol, ethyl acetate and acetaldehyde, respectively was noted. Growth stimulation of 4.85, 10.85 and 14.28% in _C.indicum_ was observed in the atmosphere saturated with the vapours of acetaldehyde, benzene, ethyl alcohol and ethyl acetate, respectively. Acetaldehyde, n-butyric acid, ethyl acetate and benzene could cause 3.54, 7.35, 8.99 and 28.06% growth stimulation in _M.aurantiaca_, respectively. However, vapours emanating from formaldehyde, formic acid,
Table-18: Mycelial growth and sporulation of keratinophilic fungi induced by volatile substances

<table>
<thead>
<tr>
<th>Valatile compounds</th>
<th>A. benhamiae</th>
<th></th>
<th>C. indicum</th>
<th></th>
<th>M. aurantiaca</th>
<th></th>
<th>M. gypseum</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry wt. (mg)</td>
<td>% Growth</td>
<td>Sp.</td>
<td>Dry wt. (mg)</td>
<td>% Growth</td>
<td>Sp.</td>
<td>Dry wt. (mg)</td>
<td>% Growth</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>240</td>
<td>11.62</td>
<td>-</td>
<td>390</td>
<td>11.42</td>
<td>-</td>
<td>231</td>
<td>37.05</td>
</tr>
<tr>
<td>Benzene</td>
<td>230</td>
<td>6.97</td>
<td>-</td>
<td>388</td>
<td>10.85</td>
<td>-</td>
<td>470</td>
<td>28.06</td>
</tr>
<tr>
<td>Chloroform</td>
<td>50</td>
<td>-</td>
<td>76.74</td>
<td>190</td>
<td>-</td>
<td>45.71</td>
<td>205</td>
<td>-</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>250</td>
<td>16.27</td>
<td>-</td>
<td>319</td>
<td>-</td>
<td>8.85</td>
<td>339</td>
<td>-</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>195</td>
<td>-</td>
<td>9.30</td>
<td>345</td>
<td>-</td>
<td>1.42</td>
<td>355</td>
<td>-</td>
</tr>
<tr>
<td>Formic acid</td>
<td>180</td>
<td>-</td>
<td>16.27</td>
<td>277</td>
<td>-</td>
<td>20.85</td>
<td>287</td>
<td>-</td>
</tr>
<tr>
<td>Pyridine</td>
<td>200</td>
<td>-</td>
<td>6.97</td>
<td>310</td>
<td>-</td>
<td>11.42</td>
<td>330</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>300</td>
<td>39.53</td>
<td>-</td>
<td>400</td>
<td>14.28</td>
<td>-</td>
<td>400</td>
<td>8.99</td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>310</td>
<td>44.13</td>
<td>-</td>
<td>367</td>
<td>4.85</td>
<td>-</td>
<td>380</td>
<td>3.54</td>
</tr>
<tr>
<td>n-butyric acid</td>
<td>200</td>
<td>-</td>
<td>6.97</td>
<td>330</td>
<td>-</td>
<td>5.71</td>
<td>340</td>
<td>7.35</td>
</tr>
<tr>
<td>Control</td>
<td>215</td>
<td>***</td>
<td>350</td>
<td>***</td>
<td>367</td>
<td>++</td>
<td>583</td>
<td>***</td>
</tr>
</tbody>
</table>

Each datum given in the table is an average of two replicates.
Sporulation grades: *** - good; ++ - Fair; + - Poor; - Nil.
S. - Stimulation; I. - Inhibition; Sp. - Sporulation
FIG. 20: MYCELIAL GROWTH OF KERATINOPHILIC FUNGI INDUCED BY VOLATILE SUBSTANCES.
isopropyd alcohol, pyridine, ethyl alcohol and chloroform were also found somewhat toxic for this fungus. An increase in the vegetative growth of *M. gypseum* was also noted in the presence of emanating vapours of ethyl acetate (0.85%), benzene (1.20%) and isopropanol (5.48%). In most of the cases decrease in sporulation was recorded when the volatile vapours inhibited the growth of these fungi. However, in case of *M. gypseum* good sporulation was recorded when cultured in atmosphere of vapours emanating from benzene and acetaldehyde.
II. FUNGISTATIC EFFECT OF SOME SOIL VOLATILES

MATERIALS AND METHODS

In the present study the fungistatic effect in different type of soils was evaluated. Four type of soils i.e., garden, forest, wheat-field and roadside soil samples were collected from different sites and brought to laboratory. Thirty gm of unsterilized soil was kept in each petridishes and moist with sterile water to provide sufficient humidity. The effect of soil volatiles was tested using the method described by Hora and Baker (1972) with modifications:

Soil Emanation agar method (SEA) : In this method a sterile glass slide was attached in the centre of the upper lid of the petridish with the help of a tape. The agar discs placed were on its free surface. Agar discs are prepared by pouring 9 ml molten 2% (w/v) water agar into a petridish standing on a level surface, giving a layer of agar just under 1.5 mm in thickness. The discs are cut from the agar with a flamed 8 mm diameter cork-borer and removed on the tip of a flamed scalpel. The cover of the petridish having slide and agar discs was replaced on the bottom plate containing different type of soils. The agar discs were exposed to the volatile substances emanating
from the different type of soils. These discs were preactivated in manner for 24 hours at 28±1°C, and then transferred to sterile glass slide kept on a glass U-tube in a moistened petridish. Discs were inoculated with a drop of spore suspension of the test fungus. After 24 hours of incubation at 28±1°C, the agar discs were examined for the germination of spores. Approximately 100 spores were observed in each count. A set of petridishes without any soil was also kept as control. The percentage inhibition in spore germination was also calculated with the help of percentage spore germination in control.

RESULTS

The data of in vitro production of volatile metabolites by different type of soil samples are presented in Table 19 Fig. 21. The data indicate that volatile metabolites from none of the soil samples could cause 100% inhibition in spore germination of A. benhamiae. Volatile emanations from garden soil cause 75% and 60% inhibition in M. aurantiaca and M. gypseum, respectively. A. benhamiae and C. indicum showed only 30.58% and 50% inhibition, respectively. The spores of these fungi seem to be most sensitive to the volatiles of the test soil samples. The volatiles of the forest soil showed the cent percent inhibition in the spore
<table>
<thead>
<tr>
<th>Soil samples</th>
<th>A. benhamiae</th>
<th>C. indicum</th>
<th>M. aurantiaca</th>
<th>M. gypseum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SG %</td>
<td>Inh %</td>
<td>SG %</td>
<td>Inh %</td>
</tr>
<tr>
<td>Garden soil</td>
<td>59</td>
<td>30.58</td>
<td>35</td>
<td>50</td>
</tr>
<tr>
<td>Forest soil</td>
<td>12</td>
<td>85.88</td>
<td>10</td>
<td>85.71</td>
</tr>
<tr>
<td>Wheat field soil</td>
<td>51</td>
<td>40</td>
<td>40</td>
<td>42.85</td>
</tr>
<tr>
<td>Road side soil</td>
<td>6</td>
<td>92.94</td>
<td>15</td>
<td>78.58</td>
</tr>
<tr>
<td>Control</td>
<td>85</td>
<td>70</td>
<td>80</td>
<td>75</td>
</tr>
</tbody>
</table>

Each datum given in the table is an average of five readings.

SG % = Percentage of spore germination
Inh % = Percentage of Inhibition
FIG. 21: FUNGISTATIC ACTIVITY IN DIFFERENT TYPE OF SOILS.
germination of *M. aurantiaca* and *M. gypseum*, while only 85.88% and 85.71% inhibition in *A. benhamiae* and *C. indicum*, respectively was recorded.

The volatiles emanated from wheat field soil also showed cent percent inhibition in *M. aurantiaca*, spores of the other test fungi also found to be quite susceptible to the volatile emanations of this soil. A total of 73.33%, 42.85% and 40% inhibition in spore germination of *M. gypseum*, *C. indicum* and *A. benhamiae* was recorded, respectively. The volatile emanations of road-side soil showed 92.42%, 84%, 78.57 and 75% inhibition in *A. benhamiae*, *M. gypseum*, *C. indicum* and *M. aurantiaca*, respectively was noted. These volatiles seem to be quite toxic showing maximum inhibition in most of the test keratinophilic fungi and related dermatophytes.

**DISCUSSION**

The data showed that the poor survival of keratinophilic fungi in forest soil may be due to high soil fungistatic factor against all the test fungi. Volatile emanations from forest soil caused cent percent inhibition in the spore germination of *M. aurantiaca* and *M. gypseum*, while in *A. benhamiae* and *C. indicum* 85.88 and 85.71% inhibition was recorded. The volatile substances from wheat-field soil showed
percent inhibition in *M. aurantiaca* only. Spores of other fungi were also found somewhat susceptible to the volatile metabolites to this soil. Road side and garden soil samples were also found to possess good fungistatic properties against keratinophilic fungi. Mankau (1962) was able to demonstrate that a water diffusible compound was present in all California soils tested which inhibited the germination of the conidia of the predatory fungi studied, namely, *Arthrobotrys arthrobotryoides*, *A. dactyloides* and *Dactylella ellipsospora*. Jackson (1958) used an agar-disc technique to demonstrate that fungi showed a range of sensitivity to soil fungistasis. Dobbs and Hinson (1953) coined the term 'fungistasis' to explain this phenomenon under conditions which would appear to the favourable to the spores of most fungi, fail to germinate when placed in contact with natural soil. Mitchell and Dix (1975) attempted to quantify the differences in the potential of several *Trichoderma* spp. to colonize substrate in fungistatic soil by the calculation of a theoretical colonization index.

Ethanol and other chemically related compounds can induce the mycelial growth and rhizomorph production in *Armillaria mellea* (Weinhold, 1963; Weinhold and Garraway, 1966; Pennlant, 1967). Robinson et al. (1966) studied the volatile metabolites and showed the phenomenon
of sporostasis in several fungal culture. Fungistatic property in the volatile emanations may be due to their effect on different metabolic properties of the germinating spores which might interfere with them on entering in the spores through their membranes.

According to Fries (1973) any one or more specific substances can affect cellular metabolism but without causing inhibition of the metabolic processes may be due to:

(i) Activation (or blocking) of an enzyme reaction;
(ii) removal or neutralization of an inhibitor;
(iii) influencing nutrient uptake from the medium;
(iv) action as a derepressor or otherwise affecting enzyme synthesis at nuclear or ribosomal level;
(v) changing membrane structures by

substitution of a limiting factor in intermediary metabolism.

The physiological activity of many of these volatile products is analogous to the growth hormone of higher plants or as inhibitor of various metabolic process. Thus, the volatile organic compounds can act on fungi in three different ways:

(a) as nutrients (indispensable as carbon and energy sources): large quantities necessary;
(b) as metabolic regulators: small quantities sufficient;
(c) as vitamins (indispensable as co-enzyme components): very small quantities sufficient.

Garraway and Weinhold (1968a,b) and Fries (1973) have reported that the change in glucose metabolism takes place by the presence of ethanol. The enzyme mechanism through which the ethanol cause their metabolic shift remain obscure. It is reported that these volatiles also act as regulators or intermediary metabolism influencing nutrient uptake from the medium acting as depressor or otherwise affecting enzyme synthesis at nuclear or ribosomal level; changing membrane structures by substituting a limiting factor in intermediary metabolism (Fries, 1973). In nature, we find the accumulation of various gaseous emanations during the decomposition of organic matter by a large number of saprophytic fungi and other microorganisms, it must first penetrate the aqueous film surrounding the cell or spore and hence the effect of such type of gaseous substance is mediated by its rate of entry in to an aqueous external and, subsequently, aqueous internal environment. Thus, the effect of the toxic gases is not the result of their gaseous form, but of their solubility product (Babich and Stotzky, 1974; Stotzky and Schenk, 1976). Some workers have also
reported the inhibitory/stimulatory effect of organic volatile compounds against keratinophilic fungi and related dermatophytes (Jain and Agrawal, 1978b, Singh and Agrawal, 1981; Deshmukh and Agrawal, 1982; Rathore and Agrawal, 1986; Geetha Singh and Agrawal, 1987; Jain, 1991; Suhane and Agrawal, 1993). Most workers are concerned only with the nature of the phenomenon (Ko and Lockwood, 1967; Hora and Baker, 1972). The data obtained in the present study showed that different volatile compounds differ in their action against different keratinophilic fungi which may play an important role in the survival of these pathogens in different type of environment and soil.
PART - B

ANTIMYTIC ACTIVITY OF SOME ESSENTIAL OILS

INTRODUCTION

The essential oils and chemically related compounds are chief products of the above ground parts of seeded plants. The antifungal properties of the essential oils may be due to the presence of certain antifungal active ingredients in them. A survey of Indian medicinal plants was made in 1956 by Chopra et al. They showed the presence of antifungal components in some of the medicinal plants. Inhibitory properties of essential oils and their components against pathogenic and non-pathogenic fungi have been also reported by several workers viz., Goutam and Purohit, 1973; Thind and Dahiya, 1976; Kher and Chaurasia, 1977; Jain and Agrawal, 1978. However, reports on the antifungal pathogenic fungi are meagre and fragmentary. The vapours emanated from the freshly cut onion possess bacteriocidal properties and crushed onion exhibits both antifungal and antibacterial properties (Klopping and Vanderkerk, 1951; Wei et al., 1967). The antifungal activity of garlic has been earlier reported by Tansey and Appleton (1975) and Moore and Atkins (1977) against some plant pathogenic fungi.
Present investigation was undertaken to evaluate the efficacy of some essential oils obtained from medicinal plants against keratinophilic fungi and related dermatophytes.

**MATERIALS AND METHODS**

In the present study ten essential oils i.e., Young-Young oil, Gamma terpene, Alpha terpene, Linayl acetate, essential oils obtained from *Mentha arvensis*, *Ocimun basilicum*, Ginger oil and three essential oils of *Ocimum americanum* Linn., i.e., eugenol pure, eugenol acetate and geranyl acetate were determined. These different oils were obtained from the dried seeds of the medicinal plants by water and steam distillation method and dried over anhydrous calcium chloride in desiccator. These oil samples were obtained from the university, Department of Chemistry. Four keratinophilic fungi i.e., *A.benhamiae*, *C.indicum*, *M.aurantiacus* and *M.gypseum* were tested in the present study. Antifungal activity was assayed, following the method described by Loo et al., (1945). Twenty five ml of sterile Sabouraud's Dextrose Agar medium (SDA) was taken in each petridish and allow to solidify. Spore suspension (2 ml) was placed and distributed thoroughly by rotatory motion with the help of a glass 'U' tube over the surface of the media. Sterilized filter paper discs (Whatman
No. 1) of 6 mm diameter were dipped in the oil samples and placed on the centre of the seeded plates. A set of control dishes with filter paper disc dipped in 1000 ppm solution of griseofulvin was also run for each organism. Dishes were incubated at 28±1°C for three days. The diameter of the zone showing no fungal growth was measured, an average of maximum diamensions of the zone around the filter paper disc was determined and recorded every time.

RESULTS AND DISCUSSION

The data (Table-20, Fig. 22) obtained in the oil of Ocimum basilicum seems to be toxic against most of the test organisms. It causes a maximum inhibition of 40 mm against A. benhamiae and M. gypseum. Eugenol acetate was also found to be highly toxic against A. benhamiae showing 34 mm inhibition zone. Geranyl acetate showed 31 mm inhibition zone was tested against M. aurantiaca. A prusal of the data showed that most of the essential oils tested here have antifungal activity. However, Young-Young oil showed inhibition zone of 9 mm against A. benhamiae. In this oil inhibition zone of 9, 10 and 18 mm was recorded in M. aurantiaca, C. indicum and M. gypseum respectively. Among the constituents of essential oil of O. americanum, acetates of eugenol and geranyl showed toxic effect against almost all the keratinophilic fungi tested here. In eugenol acetate
Table-20: Antifungal activity of some essential oils against keratinophilic fungi

<table>
<thead>
<tr>
<th>Essential oil used</th>
<th>A. benhamiae</th>
<th>C. indicum</th>
<th>M. aurantiaca</th>
<th>M. gypseum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young-Young oil</td>
<td>09</td>
<td>10</td>
<td>09</td>
<td>18</td>
</tr>
<tr>
<td>Linayl acetate</td>
<td>17</td>
<td>15</td>
<td>17</td>
<td>09</td>
</tr>
<tr>
<td>Gamma terpene</td>
<td>11</td>
<td>14</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Alfa terpene</td>
<td>20</td>
<td>18</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>Mentha ariensis</td>
<td>17</td>
<td>20</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>Ocimum basilicum</td>
<td>40</td>
<td>32</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Ginger oil</td>
<td>18</td>
<td>19</td>
<td>15</td>
<td>29</td>
</tr>
<tr>
<td>Ocimum americanum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>i) Eugenol acetate</td>
<td>34</td>
<td>15</td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>ii) Eugenol pure</td>
<td>10</td>
<td>12</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>iii) Geranyl acetate</td>
<td>16</td>
<td>20</td>
<td>31</td>
<td>15</td>
</tr>
<tr>
<td>Control</td>
<td>22</td>
<td>26</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>

Each datum given in the table is an average of three independent determination.
FIG. 22: ANTIFUNGAL ACTIVITY OF SOME ESSENTIAL OILS AGAINST KERATINOPHILIC FUNGI.
34, 33, 30 and 15 mm zone of inhibition was recorded against *A. benhamiae*, *M. aurantiaca*, *M. gypseum* and *C. indicum*, respectively. Geranyl acetate showed 31 mm inhibitory zone against *M. aurantiaca*.

A perusal of the data (Table-20, Fig. 22) showed a minimum inhibition in the growth of *A. benhamiae* (9 mm) and *M. aurantiaca* (9 mm) when the filter paper discs dipped in Young-Young oil were kept on seeded plates. It is to be noted that these organisms also showed poor growth in the presence of eugenol pure. Among all the essential oils under test, only two i.e., *Ocimum basilicum* and eugenol acetate were found to be toxic to all the fungi. It can be revealed that activity depends the nature of their effective components or their capacity of diffusion in the agar medium.

According to Maruzzella *et al.* (1960) the antimicrobial activity in the essential oils of various plants is due to the presence of some compounds like aliphatic acids and aldehydes. Antifungal property of eugenol acetate, geranyl acetate and methyl heptonone have also been evaluated by Grovr and Rao (1977). They have reported the toxic effects of eugenol acetate on the growth of *Candida albicans*. It has been observed that the fungitoxicity in plants in neither a generic character nor a family one as members of different
families have been found active against different micro-
organisms. Strong fungitoxicity of an essential oil
may be on account of the presence of certain antifungal
active principles which after isolation may prove of
great therapeutic value. Such isolated substances
from higher plants have been demonstrated to possess
systemic activity and less phytotoxicity as compared
with synthetic fungicides (Fawcett and Spencer, 1970).

The exact mechanism of the action of these
active principles of plants is still not known. The
active ingredients of plant products having strong
fungitoxicity, after chemical investigations and
purification may prove to be of great therapeutic value
to cure. The large number of fungal diseases. The
crude extract obtained from various plants is reported
to be fungitoxic and can also cure some fungal disease
(Dagis, 1958; Tokin, 1960; Gera et al., 1963; Kavacs,
1964; Thapliyal and Nene, 1967). Fries (1973) described
that the growth inhibitory activity of essential oils
may be due to their direct effect and suppression of
enzymatic activity, acting as depressor or affecting
enzyme synthesis at nuclear or ribosomal level, changing
membrane structures. The essential oils showing strong
antifungal activity can be employed as surface applicants
as preventive measures against the skin diseases caused by
various keratinophilic fungi and related dermatophytes.