CHAPTER - VII
DISCUSSION

All over the world aeroallergens pose a major problem for people suffering from allergic disorders. It has been reported that about 15-20% of the world population suffers from major allergic diseases; while in India almost 10% of the population suffer from allergic rhinitis (Singh and Malik, 1992). In Delhi the scenario is even more alarming, with an estimated 60% of the population suffering from respiratory disorders (Singh, 1979) the scenario. It is caused by Aspergillus species like *A. flavus*, *A. fumigatus*, *A. nidulans* and *A. niger*. An attempt has been made in the present study to find out suitable means for rooting out such allergic disorders.

The work has been divided into three phases, namely –

(i) Selection of plant species.

(ii) Aerobiological studies and selection of test fungal studies.

(iii) Testing the antifungal efficacy of the selected plant oils against the test fungal species.

In the first phase, the test plant species was selected on the basis of plant parts used for daily diets and also their medicinal properties. Spices form an
integral part of our daily food preparations, where they are used as flavoring agents. This property is due to the presence of essential oils, which impart a characteristic flavor to the food preparations. The essential oils of many spice-yielding plants have antifungal properties (Singh et al., 1997; Purohit et al., 2002).

The genus *Zingiber* includes a number of species, which are used regularly as spices and also have tremendous medicinal properties. Among the species found in Assam, four have been reported to have medicinal properties. These species are *Zingiber capitatum*, *Z. officinale*, *Z. purpureum* and *Z. zerumbet*.

The above-mentioned *Zingiber* species were collected and the different parts subjected to hydro distillation. The rhizomes of all the four species yielded noticeable amounts of essential oil. This oil was collected and stored for testing their antifungal efficacy.

In the second phase, a survey of the atmospheric mycoflora i.e., aerobiological studies of Greater Guwahati was carried out. The study was conducted with the help of an Andersen’s Sampler. Seven sites were selected, which covered the entire length of the city. The study revealed the presence of a large number of fungal genera of which the two most dominant genera are –
Cladosporium and Aspergillus. This is supported by the findings of Bora et al., 1996.

The genus Aspergillus dominated in terms of the number of species occurring. Altogether fourteen different *Aspergillus* species were collected from the air of Guwahati. They were observed all round the year and in all the sites. Their numbers peaked during the hot, humid months of June-July and August-September. The species *A. niger* and *A. nidulans* dominated in all the seven sites.

As discussed earlier, a number of *Aspergillus* species are responsible for a number of allergic disorders in man. It also affects a large number of plants and animals. So an attempt has been made to control the growth of these harmful *Aspergillus* species. As the species *A. flavus, A. fumigatus, A. nidulans* and *A. niger* were found at all the sites all round the year they were selected as the test fungal species.

The third phase, which is the most important phase, includes the efficacy tests. The efficacy of Zingiber oils against the four test *Aspergillus* species was tested.

Four sets of experiments were conducted, viz. –

(a) Antifungal efficacy of the essential oil and its vapors.
(b) Minimum inhibitory concentration (MIC) of the oils and their nature of toxicity i.e. fungicidal (minimum lethal concentration, MLC) or fungistatic.

(c) Antifungal efficacy of the essential oils stored for different periods.

(d) Antifungal efficacy of the essential oils, exposed to different temperatures.

Antifungal efficacy of the essential oil and its vapors:

The oil (at 1000 ppm) – The oil of *Zingiber zerumbet* completely inhibited the mycelial growth of all the four *Aspergillus* species, i.e. the percent mycelial inhibition was 100%.

- The oil of *Z. officinale* completely inhibited the growth of *A. nidulans* at 1000 ppm, i.e. the percent mycelial inhibition was 100%. In the other *Aspergillus* species, the inhibition percent was not significant.

- The oil of *Z. purpureum* also completely (100%) inhibited the growth of all the four *Aspergillus* species, at 1000 ppm.
- The oil of *Z. capitatum* inhibits the mycelial growth of *only A. niger* and *A. nidulans* by 100%.

The above findings are supported by the work of Dubey and Mishra, 1990; Singh *et al.*, 1995; Singh *et al.*, 1997 and Dubey *et al.*, 1998.

The oil vapour (at various doses) - The vapors of *Zingiber zerumbet* completely inhibited the mycelial growth of all the four Aspergillus species between 800 ppm and 1000 ppm, but below these two doses the inhibition is partial.

- The vapors of *Zingiber officinale* are cent percent effective only on *A. nidulans* at 1000 ppm.

- The vapors of *Zingiber purpureum* is very strong and inhibits all the four species by 100% at 600 ppm doses.

- The vapors of *Zingiber capitatum* is completely effective only on *A. nidulans* and *A. niger* at 1000 ppm doses.

The above findings are also supported by the findings of Dubey and Mishra, 1990.
Minimum inhibitory concentration (MIC) of the oils and their nature of toxicity i.e. fungicidal (MLC) or fungi static:

*Zingiber zerumbet* – For *A. niger*, the MIC was found to be 600 ppm and the MLC is 800 ppm. In *A. nidulans* the MIC has been found to be 500 ppm and the MLC, 800 ppm. The MIC for *A. flavus* and *A. fumigatus* was 700 ppm and the MLC, 900 ppm.

*Zingiber officinale* - The oil of this species has not been found to be very effective against the test Aspergillus species. It has been observed that at 1000 ppm only *A. nidulans* is inhibited (MIC). There is no MLC in this case.

*Zingiber purpureum* - This species has been found to be very effective against all the four Aspergillus species. The MIC for *A. nidulans* and *A. niger* has been found to be 500 ppm and for *A. flavus* and *A. fumigatus* 600 ppm. The MLC for *A. nidulans* was found to be 700 ppm, for *A. niger*, 800 ppm, for *A. flavus* and *A. fumigatus*, 900 ppm.
*Zingiber capitatum* - This Zingiber species is effective only against two Aspergillus species, namely *A. niger* and *A. nidulans*. The MIC for *A. niger* was found to be 800 ppm and for *A. nidulans*, 900 ppm. The MLC was 1000 ppm for both the species.

Antifungal efficacy of the essential oils stored for different periods:

The oils of all the four *Zingiber* species were stored for different periods of time and tested for their antifungal efficacy. It has been observed that they retain 100% efficacy even after 36 months i.e., 3 years.

Antifungal efficacy of the essential oils, exposed to different temperatures:

The oils of the four *Zingiber* species were heated to different temperatures, prior to testing their efficacy. It has been observed that there is no change in their efficacy even on heating them to 100°C.

All the above observations suggest that the essential oils of all the four selected *Zingiber* species are effective against all the four Aspergillus species up to a certain extent. The two species *Zingiber zerumbet* and *Zingiber purpureum* were found to be more effective, as they completely inhibited (fungicidal) the growth of all the four Aspergillus species between 800 ppm to
1000 ppm dose of their oil or its vapors. *Zingiber officinale* was effective only against *A. nidulans*, (fungi static) at 1000 ppm; while *Zingiber capitatum* was effective against *A. niger* and *A. nidulans*, completely inhibiting (fungicidal) them at 1000 ppm.

Thus, we can say that the above four *Zingiber* species can be safely considered for further studies as antifungal agents, to check or control the growth of human pathogenic fungal species, like *Aspergillus flavus*, *A. fumigatus*, *A. nidulans* and *A. niger*. The above four *Aspergillus* species are known to cause Allergic Broncho Pulmonary Aspergillosis in man (Ellis *et al.*, 1992; Singh *et al.*, 1997) and it has been found that the selected *Zingiber* species oils are effective against the growth of these fungal species, to varying degrees. So, the four *Zingiber* species can be used for the control of Allergic Broncho Pulmonary Aspergillosis.