CHAPTER VI

CONTROL MEASURES

6.1 Introduction

A pathogen (Greek: *pathos*, “suffering passion” and *genes* “producer of”) or infectious agent – in conversational terms, a germ – is a microorganism in the widest sense that causes disease in its host. The host can be an animal including human, a plant or even another microorganism. The principal pathways wherein pathogens can invade a host can be air, water or soil and many more. The pathogens are highly abundant as they can manage to live just anywhere. Some thrive in heat, while others prefer the cold. Some type need oxygen while others do not. The different types of pathogen include virus, bacteria, fungi and protozoan parasites. All the above said pathogens differ in their host and differ in their site of infection also. Fungi are the most common cause of diseases in human, animals, crops and other plants.

The typical fungal spore size is 1-40 µm in length. The incidence of fungal infections has increased at an alarming rate in the past two decades (http://www.infoplease.com/dangerous-diseases-epidemics/fungal-infections.html). Most of this increase is due to opportunistic fungal infections related to the growing population of people with weakened immune systems due to HIV, cancer, and other diseases; and to modern medical practices such as the use of intensive chemotherapy and drugs that suppress the immune system. In most cases, fungi only cause disease if people have an underlying immune system problem.

Existing quantitative standards/guidelines for fungi in indoor air issued by governmental agencies and private professional organizations are based primarily on
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baseline data (rather than health effects data), and are either absolute (numerical) or relative (indoor/outdoor comparisons) or a combination of the two. Quantitative standards/guidelines range from less than 100 CFU/m$^3$ to greater than 1000 CFU/m$^3$ (total fungi) as the upper limit for non-contaminated indoor environments (Rao et al. 1996).

The air fungi are nature dwellers and depending upon season, meteorological parameters, microclimate and time of the day, their composition and concentrations changes. The microclimate, more precisely the habitat condition can be controlled to some extent. The other factors cannot be regulated and precautions are the only solution.

6.2 Review on control measures of Fungi

As air-control measures are crucial for reducing dissemination of airborne biological particles in hospitals, Gangneux et al. (2006) recommended air-control measures i.e, use of high-efficiency particulate air filtration, laminar air-flow systems, high rates of room-air exchange, positive pressure rooms and well-sealed rooms in hospital units housing high-risk patients.

Mould spores are ubiquitous in the environment. Partridge-Hinckley et al. (2009) cited the guidelines established by the Centers for Disease Control (CDC) and other authoritative organizations focusing to reduce exposure to mould spores. These recommendations include avoidance of areas and activities expected to result in high levels of mould spores (e.g., construction, gardening) and use of specially designed units (protected environments) where additional standards (e.g., HEPA-filtered rooms) are in place to minimize mould exposure.
The control measures are also developed by different workers at the genetic level for crop protection against fungal pathogen (Somers et al. 2002; Varshney et al. 2004; Santos et al. 2006 and Srivastava et al. 2011). Arun Arya (2010) reviewed large number plants tested for secondary metabolites against fruit pathogen. Integrated pest management using improved cultural practices, the use of solarisation, UV illumination, the application of growth hormone and application of minimum doses of fungicides are recommended to control various fruit diseases.

In the recent years, the research works are now been focused on the higher plants for the search of antimicrobial substances (Wilson et al., 1997; Trakranrungsie et al., 2004; Udomkusonsri et al. 2007). The earlier notion was that, the antimicrobial substances can be obtained only from microorganisms. But with the progressive research works in antibiotics and antifungal substances, plants as a potential source of such substances are receiving considerable attention. Towards the end of twentieth century a lot of work has been done to study the biotoxic activity of plant extracts and several higher plant products have been standardized as potent biotoxicants against different plant pathogenic fungi. Research works in this field have also shown that plants do possess certain active principles or compounds the concentration of which varies with the varying seasons, age of the plant and the plant part used. The active principles, in the form of volatile oils are biochemical compounds that can be extracted from the plant parts by various methods (Guenther, 1972; Tewari, 1986). Volatile oils are sweet-smelling lipids synthesized and stored in various plant parts. These oils are essentially mixtures of two classes of terpenoids, i.e. the monoterpenes and sesquiterpenes, the former predominating in most cases. It has also been observed that many plants and their parts are used by man and animals alike, for the treatment of a variety of ailments.
Deshpande and Tipnis (1977), Singh (1993), Verma and Dubey (1999) suggested that a nearly 2% of the total higher plants have been screened for pesticidal properties and it has been found that extracts of the plants belonging to 157 families are frequently toxic against microorganisms. Out of them about 20% exhibit noticeable or prominent fungitoxic behaviour (Sehgal, 1961). Gilliver (1947) tested the extracts of 1915 flowering plants and reported that 23% of 113 families contained antifungal characteristics. Among the genera possessing essential oils, many of them belong to the families like – Myrtaceae, Rosaceae, Poaceae, Zingiberaceae, Asteraceae, Lamiaceae and Brassicaceae. Furthermore, the antifungal activity of some higher plant extracts has been established. The components of their extracts have been isolated, purified and tested by several workers (Kumar and Nene, 1963; Nene, 1971; Gupta et al., 1972; Sheikh and Agnihotri, 1972; Bambode and Shukla, 1973; Singh and Sharma, 1978; Kapoor et al., 1981; Kelasthane and Lakpale, 1994; Singh and Singh, 1997).

Fungitoxic activity of plant extracts can be tested by poisoned food technique of Grover and Moore (1962). Arya (1988) tested leaf extracts of Aegle mamelos, Ocimum sanctum, Azadirachta indica, Crataeva nurvala, Ephedra foliate (shoot), Eucalyptus occidentalis, Lawsonia inermis and Strichnos nux vomica in three different concentrations on two fruit rot pathogen, P. psidii and P. viticola. Most of the plants showed either moderate or maximum activity. The fungicidal nature of Azadirachta indica and Ocimum sanctum was reported earlier by Pandey et al. (1983) against Pestalotia psidii.

Tripathi (2005) tested 24 taxa belonging to 12 different families for their antifungal activity against Penicillium italicum. 100% activity against test fungus was shown by leaf extracts of Acacia nilotica (ethyl alcohol), Citrus aurantifolia (ethyl
Medicinal plants represent a rich source of antimicrobial agents (Arya and Perello, 2010). For centuries, indigenous plants have been used in herbal medicine for curing various diseases (Cowan, 1999). Recently, the acceptance of traditional medicine as an alternative form for health care and the development of microbial resistance to the available antibiotics (Srinivasan et al., 2001; Kumarasamy et al., 2002) have led researchers to investigate the antimicrobial activity of medicinal plants. Menghani (2012) reported the antimicrobial activity of the crude extracts of Tribulus terrestris and Piper cubeba showed good antimicrobial activity against selected test fungi. Bansod (2008) screened oils extracted from fifteen medicinal plants were for their activity against A. fumigatus and A. niger by disc diffusion method. The maximum antimycotic activity was demonstrated by oils of Cymbopogon martini, Eucalyptus globulus and Cinnamomum zylenicum as compared to control, followed by Cymbopogon citratus which showed activity similar to control (miconazole nitrate). The oils of Mentha spicata, Azadirachta indica, Eugenia caryophyllata, Withania somnifera and Zingiber officinale exhibited moderate activity. The oils of Cuminum cyminum, Allium sativum, Ocimum sanctum, Trachyspermum copticum, Foeniculum vulgare and Elettaria cardamomum demonstrated comparatively low activity against A. niger and A. fumigatus as compared to control. Mixed oils showed maximum activity as compared to standard.

The biological properties of the extracts of several species of Zingiberaceae have been investigated by many workers. Some of the reported biological and
pharmacological effects were antifungal (Apisariyakul et al., 1995), antioxidant (Selvam et al., 1995), antiinflammation (Claeson et al., 1996), anticancer (Limtrakul et al., 1997), hypolipidemic (Babu & Srinivasan, 1997) and antibacterial (Pattnaik et al., 1997). The effectiveness of the extracts have been shown in many studies to be attributed to the chemical constituents of the essential oils of the plants (Pattnaik et al., 1997).

According to Sbragia (1975), the green plants especially those growing in the tropics are reservoirs of various biologically active substances. But very little study has been made to ascertain the activity of rich plant products against human pathogenic fungi.

The rhizome oils of nine Zingiberaceae species showed selective toxicity against were investigated for their antifungal activities against dermatophytes, three filamentous fungi (Aspergillus niger, A. fumigatus and Mucor sp.) and strains of yeast. (Apisariyakul et al. 1995; Jantan et al., 2003; Jirovetz et al., 2003).

6.3 Application of Zingiberaceae Oil

The Zingiberaceae is one of the largest families from the order Zingiberales, with approximately 50 genera and over 1,000 species. The family is an important natural resources that provide many useful products for food, spices, medicines, dyes, perfumes and aesthetics to man. Zingiberaceae is distributed mainly in the tropical and subtropical areas. The centre of distribution is South East Asia (Burkill, 1966). The most studied genera are Curcuma sp. Alpinia sp. Zingiber sp. and Kaempferia sp.

Zingiberaceae species grow naturally in damp, shaded parts of the low-land or on hill slopes, as scattered plants or thickets. Most members of the family are easily recognized by the characteristic aromatic leaves and fleshy rhizome when both of
them are crushed and also by the elliptic to elliptic-oblong leaves arranged in two ranks on the leaf-shoot. Various ginger rhizome provide health-promoting effects and have been utilized to treat certain illnesses such as nausea, motion sickness, stomach ache, asthma, diarrhea, digestive disorder, vomiting, rheumatism, swelling, common cold, cough and other disorders. Several studies have revealed that the members of the Zingiberaceae family consist of a wide variety of active phytochemicals and possess antioxidative, anti-inflammatory, anticancer and anti-tumour promoting activity (Ling et al., 2005).

In traditional medicines, the powdered forms or aqueous extracts of the rhizomes of several species have been used to treat various symptoms and diseases such as abdominal distensions, coughing, vomiting, diarrhoea, fever, stomachache, toothaches, skin infections, asthma, rheumatism, yellow fever, urinary tract infections, malaria and gonorrhea (Burkill, 1966; Grosvenor et al., 1995).

In recent years, several reports have been published concerning the composition and/or the biological properties (antimicrobial, antioxidant, anticancer and a stimulated effect on the immune system) of Zingiberaceae extracts (Claeson, 1996; Tylor, 1996; Negi et al., 1999; Scartezzini and Speroni, 2000; Sudibyo, 2000; Youko et al., 2000; Bendjeddou et al., 2003; Jirovetz et al., 2003; Patricia et al., 2003; Nguefack et al., 2004; Kummee, 2008; Natta et al., 2008). These studies have emphasized the existence of marked chemical differences among oils extracted from different species or varieties. These variations are likely to influence the antimicrobial activity of the oil and are generally a function of three factors: genetically determined properties, the age of the plant and the environment.

In North Eastern region of India, where the floral diversity is in abundance, it provides a valuable base for the search and collection of such plant and their further
analysis to explore their hidden potentials. The present study is concentrated on the screening of certain plant rhizome of the Zingiberaceae family for their anti-microbial activity. The aim is to test the efficacy of essential oils extracted from some species of Zingiberaceae family over selected fungal strain. The pathogenic organism Aspergillus niger was selected for the study on the basis of clinical, pharmaceutical importance as well as for potential to cause contamination of food and drugs. Aspergillus niger is the well known causative organism of respiratory diseases in human and post harvest diseases in plants.

6.4 Materials and Method

(a) Plant collection: The plant specimens were collected from different parts of Assam. The oil samples from the following species were extracted and tested during the work:

I. Zingiber officinale Rosc.var. moran

II. Z. officinale Rosc.

III. Z. zerumbet (L.) J. E. Smith.

IV. Curcuma amada Roxb.

(b) Preparation of crude oil extract: The crude oil was extracted from the dried rhizomes of the plant specimen using Clevenger apparatus. The oil was extracted using water as solvents.

(c) Test micro-organism: The fungal strains were recovered from air and hard synthetic fungal media plates maintained at $^{40}$C.

Fungal Strains-

I. Aspergillus niger van Tiegham
(d) Revival and maintenance of micro-organisms:

(i) **Systematic fungal media with the composition:** Glucose (50g/L), Potassium dihydrogen phosphate (2.0g/L), Potassium hydrogen phosphate (2.0g/L), Magnesium sulphate heptahydrate (1.0g/L), peptone (8.0g/L), yeast extract (2.0g/L), agar for hard agar plate (15g/L), agar for soft agar plate (8g/L) and no agar for liquid media were used for the growth of fungal strain. The fungal strains from hard agar plates were revived by taking a loopful of it and inoculating it into 2ml liquid media in a test tube. These were then incubated at 28°C, 180 rpm. After 48hrs. each of these was sub cultured up to second subculture and incubated. Then 200µl from this was plated on Petri plates with hard agar media and incubated at 28°C for 48 hrs. From here a single spore was picked and inoculated into 2 ml liquid media and incubated it at 28°C, 180 rpm for 48 hrs. This was then used for antifungal assay of oil samples.

(ii) **Antifungal assay of oil samples:** Antifungal activity of the oil samples were tested by the disc diffusion technique for each oil extracts separately. Here the positive control was taken as a Petri plate with octal disc placed over the media. Here negative control was taken as DMSO. Incubation of the plates was done at 28°C.

100µl of 10 hrs. old culture of a selected fungal strain, mixed with 9 ml soft agar media was poured into Petri plates marked into five parts with marker containing 10 ml of hard agar media. Sterile filter pare disc (9mm in diameter) were placed on the surface of the medium. DMSO served as negative control. Using sterilized dropping pipettes, 10µl of extract with different dilutions (25%, 50%, 75% and crude extract) was carefully added into the filter paper discs and incubation was done 12 hrs at 37°C. The assessment of antifungal efficacy was based on the measurement of diameter of inhibition zone formed around the disc.

Five independent sets of similar trials were carried out adding different extracts of a particular oil samples with the most effective dilution on a plate. These set of experiments were conducted individually and the efficacy of the oil samples were noted.
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Figure 1: Plate seeded with *Aspergillus niger* showing zone of inhibition by different concentration of *Z. officinale* (water extract).

Figure 2: Plate seeded with *Aspergillus niger* showing zone of inhibition by different conc. of *Z. zerumbet* (water extracts).

Figure 3: Plate seeded with *Aspergillus niger* showing zone of inhibition by different conc. of *Z. moran* (water extract).

Figure 5: Plate seeded with *Aspergillus niger* showing zone of inhibition by different conc. of *C. amada* (water extract).

Plate 13: Photo plate showing zone of inhibition by different concentrations of Zingiberaceae oil on *Aspergillus niger*. 
6.5 Results

(a) For antifungal assay, zone of inhibition was observed up to 24hrs. from the period of incubation.

Table 6.1: Zone of inhibition in antibacterial assay

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Plant species</th>
<th>Fungal species</th>
<th>Zone of inhibition in hours</th>
<th>Measurement of Zone of inhibition in cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Zingiber officinale</em> var. <em>moran</em></td>
<td><em>Aspergillus niger</em></td>
<td>24</td>
<td>1.7-2.5</td>
</tr>
<tr>
<td>2</td>
<td><em>Z. officinale</em></td>
<td><em>A. niger</em></td>
<td>14</td>
<td>1-1.7</td>
</tr>
<tr>
<td>3</td>
<td><em>Z. zerumbet</em></td>
<td><em>A. niger</em></td>
<td>16</td>
<td>0.9-1.5</td>
</tr>
<tr>
<td>4</td>
<td><em>Curcuma amada</em></td>
<td><em>A. niger</em></td>
<td>20</td>
<td>1.2-1.8</td>
</tr>
</tbody>
</table>

(b) DMSO taken as negative control do not show any zone of inhibition, providing that the solvent does not affect the antibacterial property of the oil samples.

(c) The dilute samples of oil were noted to be more effective then the crude samples, with some exceptions.

(d) Of all the oil samples, the oil extracted from *Z. officinale* var. *moran* was found to be most effective against the test organisms.

(e) For *Z. officinale* var. *moran* all the concentration was found to be effective against microbial strain tested.
(f) In antifungal assay carried out with the said oil samples, the degree of efficacy in descending order was *Z. officinale* var. *moran*, *C. amada*, *Z. zerumbet* and *Z. officinale*.

(g) *Aspergillus niger* showed different zone of inhibition for different oil samples tested ranging from 0.9 to 2.5 cm.

### 6.6 Discussion

Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated (Uniyal et al. 2006).

This study is a preliminary evaluation of antimicrobial activity of the selective species of Zingiberaceae of Northeast India. As it is an indigenous family found in the region, unrevealing its hidden potential would be a great achievement in cure of many harmful diseases. All the species tested here showed marked antimicrobial efficacy against the test organism. Through this work, it has been concluded that *Zingiber officinale* var *moran* has the maximum antimicrobial effect, among those studied here. The species can be utilized for further study to discover new class of antibiotics of plant origin that can be affective against some pathogenic strain of bacteria and fungi for maintenance of plant, animal and human health and provide biochemical tools for the study of infectious diseases.

Further study of the major components of these species is to be done so that the active principles can be isolated and purified and undergo further testing in the way of drug designing.
6.7 Conclusion

In addition to the above mentioned strategies, the safe disposal of organic waste and reduction of the pollution load of air with the help of biological sink is highly recommended to restrict the fungal growth from health ground of peoples. Hot and humid conditions pamper fungal growth. So, means can be taken to reduce the indoor humidity, especially of educational institutes, offices, hospitals and industries using organic substrates, where air and sun rays is very restricted. The maintenance of cleanliness in the indoor environment including domestic houses can be an effective control measure to reduce fungal infection. The fungi having heterogenous life cycle can be controlled by the destruction of alternate host.

In case the cause cannot be regulated e.g. the outdoor environments, the harmful effect can be controlled by spreading mass awareness. In this respect, database management is very much essential. The basic research to obtain the data on which these policies will depend should be encouraged.

From an infection control viewpoint, the nearby building works, nearby parks and gardens are the most common urban source of fungi and their spores, which possess daily risks to immune compromised patients. Nearby parks and gardens may also act as potential sources of fungal infections in such patients. Thus, infection control of fungi and their spores in healthcare premises should probably focus more on either physical barrier means to reduce their intrusion, such as the installation of permanently sealed windows in the rooms of immune-compromised patients, or their physical removal by circulating hospital indoor air through HEPA filters in the
vicinity of such patients. Such prioritization will be required when specific environmental recommendations are made for healthcare premises.

Individual-level interventions are also prescribed to protect staff and patients against airborne pathogens. These include specific vaccinations (e.g. for influenza), as well as the wearing of masks and other personal protective equipment, mainly by healthcare workers. It is likely that a combination of these methods, adapted to specific situations as required, will be used to control the nosocomial transmission of airborne infectious agents.

Finally the time of exposure and susceptibility of individual are important criteria in fungal diseases incidence. So immune-compromised individuals are suggested to avoid long term outing during the optimised days. Also practicing yoga specially Pranayama is useful to strengthen the immunity system and the cleansing process of respiratory track. So regular practice of yoga can be an essential tool of control measures against aeromycoflora.