Chapter I

Introduction
INTRODUCTION

About the plant

*Brassica campestris* Var. Varuna belongs to the brassicaceae family and is one of the principal oil yielding crops of India and occupies a vast cultivated agricultural area in India.

The plant is grown as rabi crop during months of October and November at the onset of winter season in the planes of Northern India.

A number of phytopathogenic fungi and bacteria are known to infect Mustard causing severe damage to the crop. The most important ones in our country are: leaf spot of crucifers and white blisters disease caused by *Alternaria brassicae*, *A. brassicicola* and *Albugo candida*, respectively. During the course of present study leaf spot disease caused by *Alternaria* species was observed in the polluted as well as unpolluted control localities which have been taken into consideration for the present investigation.
SURVEY OF LITERATURE

Phylloplane microflora

The leaf surface serves as source of substrate for the growth of microorganisms as it provides essential organic and inorganic nutrients required for their physiological activities like germination, growth and sporulation. The leaf surface contains various substances that exude from underlying tissues (Tuckey, 1971; Godfrey, 1976). Climatic factors and microclimate such as precipitation and pH, relative humidity and dew formation govern the accumulation of exudates on the plant surface. The exudates contain organic substances such as carbohydrates (Chet et al., 1973), amino acids (Blakeman, 1972; Chet et al., 1973; Purkaystha and Mukhopadhyay, 1974), growth regulators (Good, 1974), growth inhibitors like phenolic compounds (Dix, 1974; Godfrey, 1974), antifungal substances (Egawa et al., 1973) and various other unidentified chemicals (Brillova, 1971; Schneider and Sinclair, 1975). Some important elements like K, Ca, Mg and Mn are among the inorganic nutrients which are exuded from the leaves.
In case of systematic studies of the leaf surface microbes, the term ‘phylosphere’ was proposed independently by last (1955a) Last and Deighton (1965), Last and Warren (1972) and Ruinen (1956) to describe the leaf surface and immediate adjacent area thereon. Kerling (1958) suggested that the term ‘phyloplane’ should be used when referring to the leaf surface habitat. Warren (1976) regarded these terms as conceptual rather than definitive. The term ‘phyloplane’ appears more meaningful and precise than the term ‘phylosphere’ (Brock, 1966; Gregory, 1973).

Development of foliar diseases is influenced by various phylloplane microorganisms. The work on phylloplane microflora has been reviewed by Preece and Dickinson (1971), Dickinson and Preece (1976), Blakeman (1981), Pugh (1984) and Fokkema and Heuvel (1986). In the recent past the phylloplane microflora of various plants have been studied by several workers (Mishra and Tewari, 1976a, 1978; Garg et al., 1978; Singh, 1978; Dixit and Gupta, 1982; Pandey, 1984; Rabbinge et al., 1984, Sharma et al., 1984; Tolstrup and Smedegaard-Petersen, 1984; Mathur et al., 1985; Srivastava, 1985; Vijay Kumar, 1981; Singh, 1988).
microbial population on the phylloplane is governed by a number of factors. In tropical countries where extreme meteorological fluctuations occur during different seasons, changes in the qualitative and quantitative nature of phylloplane microbial population are evident (Ruinen, 1961; Diem, 1967; Sinha, 1971; Mishra and Tewari, 1976a; Sharma and Mukerji, 1976; Bernstein and Carroll, 1977; Cox and Hall, 1987; Fell and Hunter, 1979). However, in temperate countries the same has been reported to be less influenced due to seasonal variations (Dickinson, 1976; McBride and Hayes, 1977).

The importance of saprobes microbes on competitive and antagonistic effects against foliar pathogens has already been realized by several workers (Preece and Dickinson, 1971; Dickinson and Preece, 1976; Brain et al., 1977; Fokkema, 1973 and 1976; Rai and Singh, 1980; Blakeman, 1981; Mukerji, 1983; Verma et al., 1983.

An increase in number of microorganisms in relation to ageing has been reported by several workers on many plants (Hudson, 1962; Dickinson, 1967, 76; Dickinson and Boarhman 1970; Diem, 1967, 69, 1974;

Two major techniques have been followed by the majority of workers for the study of phylloplane microorganisms: (a) Direct method through which the fungi are observed in situ and (b) Indirect method through which the leaf surface microbes are cultured on suitable growth media. Lindsey (1976) has pointed out that the techniques used in early studies of microfungal succession on the leaf surfaces were mainly indirect, such as plating (Pugh, 1958), spore fall (Last, 1955a) and humidity chamber incubation (Hudson and Webster, 1958; Pugh, 1958, Andrews, 1986). Dilution plate technique and moist chamber techniques are the most preferred ones for gross quantitative estimation of the number of species and for qualitative study at different stages of plant growth (Sharma et al., 1974).

**Air pollutants and plants**

India as well as other developing countries are at the threshold of a new industrial revolution due to which the level of air pollution is continuously increasing causing hazards to plants, animals and human health
and disturbing natural ecosystems. Air pollution involves a typical increase in atmospheric trace contaminants levels resulting from man’s activities. The ramifications of air pollutants may be global in nature but acute effects are generally local phenomena associated with commercial and industrial facilities in urban areas. Air pollutants of significance to the biota are varied in nature and include solid, liquid and gaseous materials. Particulates or aerosols are solid or liquid trace materials in the atmosphere and their composition is extremely varied. They may be viable or nonviable organic or inorganic substances. The inorganic fraction usually contains numerous elements, the most common being silicon, calcium, aluminum and iron. The organic fraction contains aliphatic and aromatic hydrocarbons, aldehydes, ketones and acids. Viable particles include pollen grains fungal spores and bacterial cells. The size of particulates may range from approximately 0.0005 to 500 μm in diameter. Due to their large mass, the particles are usually electrically charged and frequently attach to other particles. Particles larger than 10 μm frequently result from mechanical processes, such as wind erosion, grinding or spraying. Many fungal spores and pollen
grains are larger than 10 μm. Soil particles, processed dust; industrial combustion products and marine salt particles are typically between 1 and 10 μm in diameter. Particles in the 0.1 to 10 μm range frequently represent gases that have condensed to form non-volatile products (Smith, 1976)

Air pollutants may be released directly into the atmosphere (primary) or synthesized in the atmosphere (secondary). Because of their abundance and influence on biological system and on deterioration, the oxides of nitrogen, sulphur and carbon, hydrocarbons and ozeones are the most important.

Air pollutants influence biological systems directly or indirectly, singly or synergistically (Green halgh and Bevan 1978; Rai and Upadhyay, 1988, Singh, 1988 and Rai, Solanki and Upadhyay 1988). Interactive influence may be especially important in nature where mixtures of pollutants are common. Synergistic (Ricks and Williams, 1974; White et al., 1974), additive (Matsuschima and Brewer, 1972) and antagonistic (Majernik and Mansfield, 1972) interactions between air pollutants and plants have been described. Air contaminants influence both microorganisms (Heagle, 1973; Treshow, 1970) and host
AIR POLLUTANTS-PLANTS-MICROBES-INTERACTION (SINGH, 1988)

**DIRECT EFFECT**
- (INJURY, DISEASE)

**INDIRECT EFFECT**
- PHYSIOLOGICAL AND BIOCHEMICAL CHANGES
  - IMPAIR GERMINATION, GROWTH AND REPRODUCTION

**ENVIRONMENTAL FACTORS**

**HOST**

**MICROBES**

**HOST SPECIES, VEGETATION, MICROBIAL SPP. & THEIR POPULATION, TOPOGRAPHY, ETC.**

**INHIBITION/STIMULATION AND DISEASE CAUSATION ACCORDINGLY**

**SUPPRESSION/AGGRAVATION OF DISEASE**
plants (Naegele, 1973). Microorganisms and host plants also produce air pollutants. Air pollutants are rarely found alone and thus the environment consists of a complex mixture of phytotoxic gases (Rai and Upadhyay, 1988). Since in the present investigation only cement dust have been considered, the study carried out on the pollutants is summarized below:

**Cement Dust**

Cement dust pollution is a localized air pollution problem because it is emitted only in the vicinity of a cement factory. The dust emanating from cement kilns contains varying quantities of different components, particularly oxides of calcium, potassium and sodium, with lesser amounts of silica, aluminum, iron, manganese, magnesium and sulphur. The principal constituent, however, is calcium oxide which may comprise over 30 per cent of the total emission.

In our country, there are many reports on the effect of pollutants like cement dust on the plants (Pierce, 1909; Rao and Rao, 1993; Rao, 1985; Singh, 1990; Singh and Rao, 1980 and 81; Shukla etal, 1990; Garg et al, 2000; Tripathi, et al, 2000). Dust from different sources
settles on leaves, flowers either as such or in combination with rain drops or mist to form thick crusts (Agrios, 1978). The thick crust of dust was demonstrated to interfere with light absorption and subsequent starch formation. Naturally deposited dust from cement factories may be responsible for chlorosis and death of tissues in both deciduous and coniferous species. Additional damage to plants is caused by the toxicity of some of the deposits on leaf tissue either directly or after formation of toxic solutions in the presence of moisture on the plant. Deposits also interfere with carbon dioxide exchange (Darley, 1966) and micro organisms.

**Impact of air pollutants on metabolic changes in plants**

The phototoxic effect of cement dust on plants have been assessed by many workers such as Horsman and Wellburn, 1976, 77; Swaminathan et al., 1989; Mishra et al., 1993; Shukla, et al., 1990; Garg, et al., 2000; Tripathi, et al., 2000 and many others.

Singh and Rao (1968, 1980a, b) studied the effect of cement dust on wheat and noted a decrease in nitrogen and phosphorus contents of dusted plants. Increased
amino acid contents were observed in several plant species growing in the vicinity of a cement factory (Ahmed, 1984). Zedler et al., 1986 studied the impact of atmospheric pollution on the protein and amino acid metabolism of spruce and *Picea abies* trees.

Garg et al., (2000) carried out work on effect of the dust on vegetation and crops. They reported the decreased chlorophyll content, increased percentage of closed stomata and decreased crop production in the case of wheat, gram and arhar.

**Influence of air pollutants on micro organisms and plant diseases**

Factors potentially important to the growth and development of both pathogenic and non-pathogenic foliar microbes, are the pollutants including SO$_2$, cement dust, ozone, hydrogen fluoride, heavy metals, acidic precipitation and particulates (in the form of dust from various industrial operations).

The interaction between host and pathogen is also governed by the environmental conditions. Air pollutants play an important role in pathogenesis. The incidence and severity of various plant diseases have been studied
in the laboratory as well as in the field by a number of workers (Daines et al., 1960; Couey and Uota, 1961; Couey, 1965; Gilbert, 1968; Skye, 1968; Saunders, 1966; Wellburn et al., 1972; Heagle, 1973; Saunders, 1973; Bevan and Greenhalgh, 1976; Laurence et al., 1979; Singh et al., 1987; Singh, 1988; Singh and Rai, 1990 a, b)

Despite increased awareness for air pollution and attempts to control it, emission of pollutants such as nitrogen and sulphur dioxides and the photogeneration of ozone are expected to increase in future (Laurence, and Aluisio 1981). It is reasonable to assume that the importance of these and other pollutant in regulating the growth and development of foliar microbes will increase simultaneously. Considerable information concerning air pollutant interactions with foliar microbes is presently available particularly with regard to pathogenic fungi (Heagle, 1973; Saunders, 1973; Babich and Stotzky, 1974, 1978, 1982; Treshow, 1975; Shriner, 1980; Laurence and Aluisio, 1981; Rist and Lorbeer, 1981, 1985; Rai and Upadhyay, 1988; Singh et al., 1987; Singh, 1988; Solanki, 1988; Singh and Rai, 1989a, 1989b, 1989c and 1990a).
Amongst various diseases of mustard leaf spot disease caused by Alternaria species accounts for the maximum crop damage all over the country. Researches on the influence of the individual air pollutant(s) on plant diseases are briefly reviewed below:

**Effect of cement dust**

Deposition of cement kiln dust on leaves of sugar beet increased the incidence of leaf spot disease caused by *Cercospora beticola* (Schöenbeck, 1960). It was postulated by Schöenbeck (1960) that lime dust altered the physiological balance and increased the plant susceptibility to infection. Manning (1971) also noted increased susceptibility of sassafrass and wild grape plants to infection by *Guignardia bidwelli* and *Gloeosporium sp.* on exposing plants to emissions of limestone dust. Rai and Pathak (1981) noted increased populations of bacteria and fungi but decreased population of actinomycetes on the phylloplane of potato in a cement dust polluted locality. *Penicillium javanicum* was constantly isolated from the polluted locality while *Mortierella subtilissima, Trichoderma koningii, T. viride, Aspergillus sulphureus, Helminthosporium sp.* were absent in polluted locality. However, *Aspergillus niger, A. flavus,*
A. luchuensis, Cladosporium cladosporioides, Curvularia lunata and Alternaria alternata were frequently isolated from the polluted locality.

Singh and Garg (1985) reviewed the work on microbial life under hypersaline environment and showed Alternaria tenuis, Cladosporium herbarum and Stemophyllum lanuginosum etc. occurred widely in high saline conditions. Blaker and Mac Donald (1986) reported that incidence root rot of citrus caused by Phytophthora parasitica, increased with increasing soil salinity.

Kao and Ko (1986a, b) observed suppression in growth of Pythium splendens causing damping off of cucumber by calcium which is an important constituent of cement. Bayles and Aist (1987) observed that calcium increased the resistance of a barley mutant to powdery mildew. Manning (1971) observed reduced numbers of bacteria and fungi on phylloplane of grape heavily incrusted with dust. Singh et al. (1987) observed the least incidence and severity of leaf spot disease of rice caused by Helminthosporium oryzae in polluted locality in comparison to control. They also observed qualitative and quantitative variations in distribution of phylloplane microorganisms.
Some mechanisms which are potentially involved in changing the physiology and biochemistry of leaves and modifying host-parasite relationship.

a. Direct chemical effects of polluted rain on the pathogen.
b. Direct chemical effects of polluted rain on leaf epidermal tissue.
c. Combined physical/chemical degradation of protective waxes on leaf surface by polluted rain.
d. Indirect effect on leaf surface microflora resulting from shifts in pH or nutrient leaching and causing shifts in competitive microflora.
e. Pollutant induced changes in response of the plant to other pollutants resulting in alteration of plant tissue susceptibility to infection.
f. Pathogen induced changes in resistance or sensitivity to pollutant injury.

Rai and Pathak (1981) frequently isolated *Cladosporium cladosporioides*, *C. herbarum*, *Mortierella subtilissima*, *Alternaria solani*, *Curvularia lunata* and *Rhizopus nigricans* on phylloplane of potato from SO$_2$ polluted locality whereas *Cephalosporium roseo-griseum*,
Aspergillus sydowi, Bipolaris tetramera, Torula herbarum
and T. graminis were absent in this locality.

**Air pollution and plant surface microorganisms**

Babich and Stotzky (1974) and Rai and Upadhyay
(1988) have provided comprehensive reviews of the
relationship between air pollution and microorganisms.
Saunders (1971, 1973) has examined the influence of air
pollutants, particularly SO₂, on the leaf surface
microflora. Boughton et. al., 1978; Craker and Manning
1974 Heagle (1973), Treshow (1975), Rist and Lorbeer
(1985) have described the inter-relations between air
contaminants and fungal and bacterial pathogens of
plants.

Smith (1976) postulated a relationship between air
contaminants and plant surface microbes and divided
into three classes:

**Class I: Low air pollution dose**

The micro organisms may function as an important
source or sinks for air contaminants. Soil
microorganisms are extremely important contributors of
trace contaminants to the atmosphere. Microbes are
primary agents for the transformation and release of
inorganic sulphur compounds in soils and aquatic sediments from which substantial amounts reach to the atmosphere. Soil micro organisms also produce large quantities of nitrous oxide, ammonia, methyl mercaptan, carbon monoxide and various hydrocarbons (Babich and Stotzky, 1974). Plant surface microorganisms also release the trace contaminants to the atmosphere though the amounts are very much less than those released by soil microbes e.g., *Puccinia graminis tritici* uredospores release n-nonanal (French and Weintraub, 1957) and *Saccharomyces cerevisae* releases methyl or ethyl mercaptan when metabolizing S methyl or S ethyl cysteine (Cherest et al., 1970). Carbon monoxide is released by several fungi including *Aspergillus niger*, *Fusarium spp.* and *Cephalosporium spp.* (Westlake et al., 1961). The effect on ecosystem of microbially produced air pollutants might range from no effect to some manner of allelopathic influence (Smith, 1976).

The continuous natural production of air pollutants without subsequent accumulation of these materials implies the existence of natural scavenging processes, both biotic and abiotic, termed as "sinks" (Babich and Stotzky, 1974; Warren, 1973). The phyllosphere
microbiota also serves as major sinks for removal of gaseous hydrocarbons from the atmosphere. For example, approximately $7 \times 10^6$ tons yr$^{-1}$ ethylene is removed through aerobic microbial decomposition by soil microbiota. The nitrogen fixing organisms are a potential sinks for acetylene which is reduced to C$_2$H$_4$. Several bacteria (e.g., *Mycobacterium methanicum*, *Methanomonas methanica*), some fungi (e.g., *Acremonium* sp, *Graphium* sp.) and a few algae like *Chlorella* are able to oxidize and utilize methane (cited by Babich and Stotzky, 1978).

**Class II: Intermediate air pollution dose**

Intermediate doses may adversely and subtly affect individual microbial species or individual member of a given species reducing their biological activity. As the dosage of air pollution increased direct alterations of microbial metabolism is induced and abnormalities may be manifested in morphology, pigmentation or enzyme activity. Numerous fungi, including *Trichoderma viride*, *Penicillium egyptiacum*, *Botrytis allii*, *Sclerotinia fructicola*, *Penicillium expansum* and *Phytophthora cactorum* grow in *vitro* by appressed mycelium when exposed to ozone which appears to suppress the production of aerial hyphae (Babich and Stotzky, 1974). Alterations in
reproductive potential may be an important response to air pollution. Two primary mechanisms of influence may occur; altered spore production or dispersal and altered ability to germinate or rate of germination (Smith, 1976).

At the cellular level air contaminants may alter metabolism. The inhibition of hydrogenase by NO was observed in *Penicillium vulgaris* by Krasna and Rittenberg (1954). *Helminthosporium sativum* exhibited suppressed lipid synthesis when exposed to high ozone dose (Price, 1968). The ecosystem response could include altered species composition and succession, reduced microbial biomass and altered structure and function (energy flow, nutrient cycling, competition and succession).

**Class III: High air pollution dose**

Higher doses of air pollutants might cause stimulation of individual species or induce acute morbidity or mortality. If stimulated, the ecosystem changes could include increased microbial biomass and altered structure and function. With morbidity or mortality ecosystem consequences might eventually lead to reduced microbial biomass and simplification. The
effect on host plant of these various relationships would depend on the nature of microbe-air pollution interactions (Smith, 1976).

Babich and Stotzty (1978) also stated that volatile, gaseous and particulate air pollutants, derived both from natural and anthropogenic sources, affect not only the growth, reproduction, morphology and other characteristics of microbes but also their activity, ecology and population dynamics in natural habitats. Microorganisms occupy a unique ecological position in their interactions with an impact on air pollution. They pointed out that microbes are recipients of air borne contaminants similarly to plant and animals. However, microorganisms are distinct in following ways:

(a) They are sources of substantial quantities of various particulates and gaseous atmospheric pollutants.
(b) They can utilize, either as an energy source or a nutrient source, numerous air pollutants and thereby serve as 'sink' for many pollutants.
(c) When in air borne state, they are themselves air contaminants.
Effect of air pollutants on microbial interactions in the phylloplane:

A lot of work has been done on microbial interactions between pathogenic and saprophytic fungi which has been reviewed by Bier and Rowat (1962a, b) Bier (1966), Grosclaude (1970), Mc Bride (1970, 1971), Swinburne (1973), Skidmore and Dickinson (1976), Rai and Singh (1980), Singh (1988), and Solanki (1988).


Interactions of air pollutants with foliar pathogens have received more attention than interactions with non pathogens because of highly visible symptoms associated with pathogens and of the economic importance of plant diseases. Increasing awareness of the possible role of air pollutants in microbial interactions affecting disease...
development has received considerable attention of pathologists in recent years. Air pollutants, together with other environmental factors influence the biotic agents causing disease or they alter the morphology, anatomy and physiology of the plants and may render them more susceptible to infection (Treshow, 1975).

Air pollutants influence biological systems both directly and indirectly and singly and interactively (fig. I) (Rai and Upadhyay, 1988; Singh 1988). This influence may be especially important in nature where mixtures of pollutants are common. Synergistic (Ricks and Williams, 1974; White et al., 1974), additive (Matsuschima and Brewer, 1972) and antagonistic (Majernik and Mansfield, 1972) interactions between air pollutants and plants have been described. Significant alteration of the structure and function of the plant surface microflora by air pollution may influence the metabolism of the host plant. The response of host to change, induced in the saprophytic component of the surface microflora, would be related to the nature of the relationship between host and saprobes. In the case of pathogenic microorganisms host response to pathogen air pollution interaction might result in increased or decreased disease incidence.
**Effect of leaf extract on phylloplane microfungi:**

A number of workers have studied the effect of leaf extracts of various plants on pathogenic and non-pathogenic fungi (Kumar and Nene, 1968; Shekhawat and Prasada, 1971; Ahmad et al., 1973; Tiwari, 1975). However, no attempt has been made to study the effect of leaf extract of mustard leaves exposed to different air pollutants on growth of fungi, Singh (1978) studied the effect of leaf extracts of mustard and barley plants on some phylloplane fungi including two pathogenic fungi i.e. *Alternaria brassicae* and *Drechslera graminea* and reported that the leaf extracts considerably stimulated the growth of all the test fungi including pathogenic ones. However, the autoclaved extract was found to be most effective. Mishra and Tiwari (1978) studied the effect of leaf extracts and exudates of wheat and barley on some phylloplane fungi and noted a stimulatory effect of the exudates and extracts on most of the fungi. Nevell and Wainwright (1986) studied microbial growth on leaves from polluted sites and litter leachates and its effect on litter pH and sulphate concentration and found profuse growth of bacteria and fungi in all leachates. Although many workers have studied several aspects of phylloplane
microflora of many plants, there is paucity of information on the influence of leaf exudates collected from air polluted localities on leaf surface microfungi.

**Effect of air pollutants on fungal colonization and succession on decaying plant parts:**

Succession can be defined as a change in the composition of the biological community with time. Fungal colonization begins soon after the senescence of a leaf. Different kinds of fungi appear at various stages of decay and the fungus flora changes as the leaf tissues decompose and disorganize. On the basis of sequence of colonization of fungi these fungi are characterized as primary, secondary and tertiary colonizers by some workers (Rai, 1973; Sharma, 1973). Work on the colonization and succession of mycoflora on various plant parts have been reviewed by several workers (Webster, 1956, 1957; Khanna, 1964, 1970; Hudson, 1962, 1971; Sharma, 1967; Rai, 1974; Singh, 1978; Frankland, 1981; Garrett, 1981; Harper and Lunch, 1981, 1985). However, no attempt has been made so far to study colonization and succession of plant parts exposed to different polluted environments. Since the air pollutants greatly influence the aeromycoflora (Rai and Singh, 1988) and
phyloplane microflora (Singh, 1988) they may influence colonization of senescent leaves by the microorganisms and their subsequent decomposition.

**Plan of work**

The following aspects have been studied during the course of the present investigation:

1. Isolation and study of phylloplane microflora of mustard from unpolluted control and cement dust air polluted locality of at monthly intervals from seeding up to senescent stage during the cropping season.

2. Physico-chemical analysis of leaf extracts from the polluted and unpolluted control localities.

3. Responses of dominant phylloplane fungi to air pollutant in relation to growth behavior and population dynamics.


5. Isolation and study of the pathogen *Alternaria brassicae*, *A. brassicicola* causing leaf spot disease of mustard.
6. Influence of air pollutants on microbial interactions between *Alternaria* species and phylloplane fungi in relation to leaf spot disease.

7. Studies on effects of leaf extracts of mustard collected at different growth stages from the localities on growth behavior of dominant/tolerant test phylloplane fungi and the test pathogen *Alternaria brassicae* and *A. brassicicola*.

8. Studies on colonization and succession of fungi on decaying leaves of host plants from senescent stage up to the final stage of their decay in the polluted and unpolluted control localities.