Chapter IV

RESULTS AND DISCUSSION
Results and Discussion

Effects of air pollutants on phylloplane microflora of Mustard:

Results:

Total number of fungal species and fungi cm\(^{-2}\) increased from seedling stage to senescent stage of the plant in all the localities (Fig. 1. and Table 6).

An increased microbial population was recorded at site I in comparison to both unpolluted control and site II. (Figs. 1 and 2).

Statistical analysis of the data showed a significant variation (P = 0.01) in total number of species, number of fungi as well as bacterial and actinomycetes colonies cm\(^{-2}\) leaf area in relation to growth stages of the plants and the localities.

Twenty eight fungal species were recorded for one cropping seasons, at five growth stages of the host, from all the localities.

*Alternaria alternata*, *A. brassicae*, *A. tenuissima*,
*Aspergillus flavus*, *A. niger*, *A. luchuensis*,

- 90 -
Fig. 1. Total number of fungal species recorded at various growth stages in relation to the air pollutants.

Sdl- seedling, Prf- pre flowering, Flr- flowering

Pfl- Post flowering, Snt- senescent stage
*Cladosporium cladosporioides*, *C. herbarum*, *Curvularia lunata*, *Drechslera australiensis*, *Fusarium oxysporum*, *F. semitectum*, *Penicillium chrysogenum*, *P. citrinum*, *Phoma glomerata* and *Dark sterile mycelia* were generally recorded from all the polluted and unpolluted localities during the cropping season (November 06 to March 07).

An average of twenty five fungal species and $43.8 \times 10^2$ fungi cm$^{-2}$ leaf area were recorded from the unpolluted control locality. Twelve species and $18.19 \times 10^2$ fungi cm$^{-2}$ leaf area were recorded at the seedling stage which gradually increased with ageing of the plants up to 19 and $28.45 \times 10^2$ at prefloresing, 17 and $23.69 \times 10^2$ at flowering, 22 and $37.39 \times 10^2$ at post-flowering and 25 species and $43.88$ cm$^{-2}$ leaf area at senescent stage of the plants. Species of *Alternaria*, *Aspergillus*, *Cladosporium*, *Epicoccum* and *Fusarium* were the most dominant fungi in all the polluted and unpolluted localities (Tables 2-5).

A total of 28 species and $50.62 \times 10^2$ fungi cm$^{-2}$ leaf area were recorded from site I polluted locality. Fourteen, 24, 24, 27 and 28 species and $21.15$, $42.97$, $39.67$ and $50.62 \times 10^2$ fungi cm$^{-2}$ leaf area were recorded at seedling, prefloresing, flowering and senescent stages, respectively. *Alternaria brassicae*, *Aspergillus candidus*,
Fig. 2. Total number of fungi cm\(^{-2}\) (x10\(^2\)) on phylloplane recorded at various growth stages of the plant in relation to the air pollutants.

Sdl- seedling, Prf- pre flowering, Flr- flowering

Pfl- Post flowering, Snt- senescent stage
A. niger, A. terreus, Cladosporium cladosporioides, Curvularia lunata, Drechsslera australiensis, D. papendorfii, Epicoccum purpurascens, Fusarium oxysporum, Nigrospora sphaerica and Torula species were recorded with an increased per cent frequency and/or No. of fungi cm⁻² in case of site I (Table 2 and 3).

Twenty six species and 40.34 X 10² fungi cm⁻² leaf area were recorded from site II cement dust polluted locality. 13, 24.38 X 10⁻²; 22, 33.09 X 10²; 24, 40.34 X 10²; 26, 34.66 X 10² and 25, 34.28 X 10² species and fungi cm⁻² leaf area respectively were recorded at the seedling, preflowering, flowering, post-flowering and senescent stages respectively from site II cement dust polluted locality (Table 2-6).

Alternaria alternata, A. brassicae, A. tenuissima, Cladosporium cladosporioides, Curvularia lunata, Drechsslera australiensis, Fusarium oxysporum, Nigrospora sphaerica, Penicillium chrysogenum, Penicillium citrinum showed decreased population in case of site II as compared to site I which received comparatively low pollutant dose. (Table 2 and 3)
However some species e.g. *Aspergillus candidus*, *Epicoccum purpurascens*, *Myrothecium roridum*, *Populaspora* species, *Penicillium rugulosum*, and *Phoma* species were recorded as relatively tolerant fungal species (Table 2 and 3).

The highest number of *Alternaria brassicae* cm$^{-2}$ leaf area was recorded in site I polluted locality while least in the unpolluted control locality. The population of *Alternaria brassicae* in phylloplane at different growth stages of the host in site I polluted locality increased after pre-flowering stage (Fig. 5 and Table 5). However, its population decreased significantly in site II cement dust polluted locality in comparison to site I (Fig. 5).

The species predominantly recorded from various particular locality were: *Aspergillus candidus*, *Aureobasidium pullulans* and *Penicillium rugulosum* from site I and II of cement dust polluted locality where as *Penicillium citrinum*, *Torula herbarum* from unpolluted control locality (Table 2 and 3).

The number of colonies of actinomycetes cm$^{-2}$ leaf area increased gradually from seedling to senescent stage in all the localities (Fig. 3 and Table 6). An average
Fig. 3. Total number of actinomycetes cm$^{-2}$ leaf area on leaf surface recorded at various growth stages of the host plant in relation to the air pollutants.

Sdl- seedling, Prf- pre flowering, Flr- flowering

Pfl- Post flowering, Snt- senescent stage
Fig. 4. Total no. of bacterial population cm\(^{-2}\) leaf area on leaf surface of host plant in relation to air pollutants.

Sdl- seedling, Prf- pre flowering, Flr- flowering

Pfl- Post flowering, Snt- senescent stage
number of colonies recorded at different stages in each locality was 16.10 X 10^2 at seedling and 21.20 X 10^2 in senescent stage in unpolluted control; 15.70 X 10^2 at seedling and 35.4 X 10^2 at senescent stage in site I polluted; 20.0 X 10^2 at seedling and 42.3 X 10^2 at senescent stage in site II cement dust polluted locality. Overall, the maximum colonies of actinomycetes (42.3 X 10^2 cm^-2 leaf area) were recorded from site II polluted locality followed by site I (35.40 X 10^2) and control (21.2 X 10^2) (Fig. 3 and Table 6).

The bacterial colonies cm^-2 leaf area increased gradually from seedling to senescent stage in all the localities (Fig. 4 and Table 6). An average number of colonies recorded at different stages in each locality was 102.00 X 10^3 at seedling and 173 X 10^3 at senescent in unpolluted control; 130 X 10^3 at seedling and 182 X 10^3 at senescent stage in site I polluted locality and 174 X 10^3 at seedling and 248 X 10^3 at senescent stage in site II cement dust polluted locality.

The maximum bacterial population (248 X 10^3 colonies cm^-2 leaf area) was observed in site II cement dust polluted locality followed by site I (182 X 10^2), and the unpolluted control (173 X 10^2) (Fig.4 and Table6).
Fig. 5. Population of *A. brassicae* on phylloplane in relation to air pollutants.

Sdl- seedling, Prf- pre flowering, Flr- flowering

Pfl- Post flowering, Snt- senescent stage
DISCUSSION

The total microbial population as well as the fungal species increased with ageing in all the polluted and unpolluted localities which might be attributed to a combination of factors like gradual accumulation of leachates on leaf surface and the longer duration of exposure of leaves to an environment. The increasing trend in microbial population and fungal species has been observed by other workers also. (Dickinson, 1967; Diem, 1967; 1974; Hudson, 1971; Mishra and Srivastava, 1971; Mishra and Tiwari, 1976; Sinha and Bahadur, 1974; Singh, 1978; Rai and Pathak, 1981; Srivastava, 1985; Singh et al., 1987; Singh, 1988).

Environmental factors influence the composition of microflora in each locality. One or more factors may also interact synergistically giving marked variations in species composition in a particular locality. This could be exemplified by the record of Aspergillus candidus, A. rugulosus, Epicoccum purpurascens, Myrothecium roridum, Populaspora species, Penicillium rugulosum, and Phoma species as predominant species in cement dust polluted localities and Penicillium citrinum and Torula herbarum from unpolluted control locality (Table 2 and 3). Similarly,
the maximum per cent frequency and number of colonies of *Alternaria brassicae* cm$^{-2}$ leaf area was recorded from site I polluted locality followed by site II and control locality.

Greatest fungal population was recorded from site I polluted locality. It decreased comparatively in case of site II polluted locality in comparison to site I polluted locality (Fig. 1 and 2, Table 6). The minimum fungal population dynamics was recorded from unpolluted control locality (Fig 1 and 2).

Pollutant-induced effects on nonpathogenic foliar microbes are less obvious than those observed for pathogens but likely involve similar mechanisms of action. Such changes may ultimately affect plant pathogens, since phylloplane saprophytes can interact with foliar pathogens, often suppressing but sometimes enhancing disease (Rist and Lorbeer, 1985). The population of *Alternaria brassicae* increased after the pre-flowering stage in the site I polluted locality but decreased in site II cement dust polluted locality. This shows favourable effect of low pollutant dose, adaptability and tolerance of the pathogen and adverse effect of increased cement dust dose(s) and increased microbial
population of antagonistic bacteria and actinomycetes on Alternaria brassicae caused a decrease in per cent frequency and no. of colonies cm$^{-2}$ in comparison to site I. Some of the other possible explanations could be:

(a) Modification of the pH of the leaf surface to favour colonization by the pathogen (Rai & Pathak, 1981).

(b) Pollutant-induced change in the host which could be favourable to the pathogen such as necrotic areas serving as infection courts and nutrient leakage providing nutrients for the pathogen.

(c) Pollutant induced effects on interactions between pathogenic and non-pathogenic foliar microbes in favour of the pathogen.

(d) Reduction of the resistance of leaf to infection and/or promotion of spore germination either by altering the pH or neutralizing the metabolic inhibitors on leaf surface (Rai and Pathak, 1981).

Smith (1976) has pointed out that under low doses of air pollutants, the microorganisms may function as "pollution sink" and the air pollutants might be useful as nutrient sources. Thus low doses of cement dust seems to be useful for Alternaria brassicae causing more
pathogenesis. Increased level of leaf spot disease in site I polluted locality seems to be due to adaptability and tolerance of the pathogen and some beneficial impacts of cement dust which favours the growth and sporulation of *Alternaria brassicae*.

The corrosive and adhesive properties of cement dust as well as Ca\(^++\) induced suppression of stomatal opening (Inoue and Katoh, 1987) and increased pH (7.8) of alkaline solutions formed on the leaf surface in presence of free moisture might be the cause of decreased pathogenesis at site II due to which the number of diseased plants per square meter in the field and the size of the lesions on the infected plants were found to be reduced in the cement polluted site II (cf, site I). Babich and Stotzky (1974) suggested that increase in pH might be responsible for decreased microbial growth. These finding support the view of Park et al.(1956); Evans (1968); Singh (1988; 1990 a & b)

The per cent frequency and colonies of *Alternaria brassicae* cm\(^{-2}\) leaf area of the host was recorded less in site II polluted locality than site I polluted locality. This seems to be due to toxicity of cement dust. Rai and Pathak (1981) and Rai (1987) made an extensive study.
on *Alternaria solani*, causing early blight of potato in relation to air pollutants and reported that incidence and severity of the disease was greater in NH$_3$ polluted locality than SO$_2$ and cement dust polluted localities. Singh *et al.* (1987) also studied the effect of air pollutants on *H. oryzae*, causing brown leaf spot disease of rice and mustered more severity and incidence of disease in NH$_3$ polluted locality in comparison to cement dust, SO$_2$ polluted and unpolluted localities. Thus it is evident that pollutant(s) affect Plant - Microbes - Pathogen interaction and, therefore, incidence and severity of the disease(s). The highest bacterial population was recorded from site II cement dust polluted locality followed by site I and control. Similarly in case of actinomycetes the maximum population was recorded from site II polluted locality followed by site I and least in case of control. The highest number of bacteria and actinomycetes but the least number of fungi was recorded in the phylloplane of mustard grown in site II cement dust polluted locality in comparison to site I and control locality. The increased bacterial population on leaf surface of host grown in site II cement dust polluted locality might be attributed to decreased fungal population, ability of bacteria to grow in
wide range of pH, withered leaf surface and their tolerance as well as ability to neutralize the toxic effects (Singh, 1988).

It may be concluded, therefore, pollutant-induced effects on non-pathogenic foliar microbes are less obvious than those observed for the test pathogen i.e. *Alternaria brassicae* (Table 16 and 17).

**Change in amino acid and sugar contents of host in relation to cement dust pollution**

**RESULTS**

Overall, 18 amino acids and 12 sugars were recorded in leaves collected from all the localities out of which 9, 11 and 11 amino acids and 7, 06, and 06 sugars were recorded from unpolluted control, cement dust site I and site II polluted localities, respectively (Tables 7 and 8). The number of amino acids and sugars increased gradually with ageing of the plants in all the localities.

Alanine, arginine, cysteine, glycine, glutamine, asparagine, histidine, hydroxyproline, lysine, methionine, ornithine, proline, threonine, unidentified I and II were recorded from leaves collected from all the localities at
various growth stages of the host. Amino acid(s) recorded from particular locality(s) were:

Cement dust site I : Leucine

Cement dust site I and site II : Tyrosine

The amino acid(s) recorded at particular stage were:

**Seedling stage** :

Alanine, asparagine, histidine, methionine from all the localities; Tyrosine and unidentified II from site I and site II.

**Pre-flowering stage** :

Hydroxyproline and unidentified II from all the polluted localities; unidentified I in case of control and site I; Arginine, Asparagine in control and site II; Glutamine, glycine, histidine and threonine from site I and II.

**Flowering stage** :

Asparagine, threonine and unidentified II from all the localities; Glatamine, glycine from site I and II locality.
Post-flowering stage:

Asparagine, cysteine, hydroxyproline, lysine, threonine, and unidentified I from all the localities;
Glycine and glutamine in case of control;
Alanine and serine in site II;
Tyrosine and unidentified III from both the polluted localities. (Table 7)

Sugar(s) (Table 8):

The sugar(s) recorded from particular locality(s) were:

All the localities: Glucose, fructose, sucrose, sorbose, rhamnose and unidentified III.
Site I cement dust and control: Arabinose and unidentified I
Site I: Galactose
The sugar(s) recorded at particular growth stage(s) were:

Seedling stage:

Fructose and glucose from all the localities and sucrose from site I and site II.
Results and Discussion

Pre-flowering stage:

Fructose, glucose and sucrose from all the localities;

Rhamnose in case of cement dust site I and II;

Galactose and unidentified I in cement dust site I polluted locality;

Whereas sorbose in control locality.

Flowering stage:

Fructose, glucose and sucrose from all the localities;

Arabinose and unidentified III in case of site I cement dust;

Rhamnose in control;

Mannose and unidentified II in site II;

Unidentified IV in control locality only (Table 8)

Post-flowering stage:

Fructose, glucose, sucrose and mannose from all the localities;

Arabinose and unidentified II and III in control;

Sorbose in case of site I and site II cement dust locality;

Rhamnose in site II polluted locality (Table 8)
Changes in amino acids and sugar contents of host treated with cement dust and untreated control (Table 9 and 10)

AMINO ACIDS

A total of 18 amino acids and 12 sugar were detected in leaves of the host plants treated with cement dust and untreated control out which 9, 11 and 11 amino acids and 4, 6 and 5 sugars were recorded from untreated control, cement dust treated host plants, respectively (Table 9 and 10).

I Samplings :

Alanine, Arginine, Asparagine, Cysteine, Glutamine, Glycine, Histidine, Hydroxyproline, Lysine, Proline, Threonine, and Unidentified I were recorded from all the sampling (Table 9).

The amino acids detected from a particular locality:

Leucine and Serine in site I;

Methionine in unpolluted control and site I;

Unidentified II in site II;

Tyrosine in site I and site II of cement dust treated plants (Table 9)
II Sampling:

Alanine, Arginine, Asparagine, Cysteine, Glutamine, Glycine, Histidine, Hydroxyproline, Lysine, Ornithine, Proline, Threonine, and Unidentified I were detected from all the sampling.

The amino acids detected from particular samplings:

Methionine control and site I;

Leucine and Serine site I and unidentified II in case of site II only (Table 9).

III Sampling:

Asparagine, Glutamine, Proline in all the sampling cases;

Cysteine, Glycine, Hydroxyproline, Lysine, in site I and control;

Alanine site II;

Ornithine Unidentified I only in case of control (Table 9).

SUGARS (Table 10)

Fructose, glucose and sucrose were recorded in all the case throughout the samplings. The sugar(s) detected
in leaves of plants treated with particular doses of the pollutant were:

Control - Sorbose

Site I - Lactose

Site I and II - Raffinose, galactose

The sugar(s) recorded at particular sampling were:

I Sampling:

Fructose, sucrose and glucose in all cases of samplings.

Raffinose and galactose from site I and II,

Lactose from site I sampling (Table 10)

II Sampling:

Fructose, sucrose and glucose from all the samplings and galactose in case of site II of cement treated leaves.

III Sampling:

Glucose and sucrose were detected in all the sampling whereas other sugars could not be detected (Table 10).
DISCUSSION:

There was a marked qualitative variation in amino acid and sugar composition of the host plants collected from unpolluted control and polluted locality or treated with cement dust. The variation in amino acid and sugar composition of host seems to be due to direct or indirect effect of the pollutant(s) on physiology and biochemistry of the host.

Thus, on the basis of their presence or absence the amino acids present in leaves of treated and control plants can be briefly classified as follows:

I. Present in all the treated and controlled plants and relatively not affected by the pollutant: Alanine, Asparagine, Cysteine, Glutamine, Glycine, Histidine, Hydroxyproline, Methionine, Proline, Threonine, Unidentified I and II

II. Present in cement dust treated plants only and seems to be cement dust tolerant: Leucine, Serine (site I low dose treatment)

III. Unidentified II site II: Tyrosine in site I and II (higher level of the pollutant dose)
The qualitative variation in amino acid content of host might be due to altered metabolism of the test plants under influence of the particulate pollutant(s). Singh (1988) also studied the effects of NH₃, cement dust and SO₂ treatment on wheat plants and found a marked qualitative variation in amino acids and sugars content of the test plants in comparison to the control. The effect of cement dust on amino acid metabolism is still not very clear. However, Ahmed (1984) reported increased amino acid contents in several plants species growing around a cement factory. Zedler et al. (1986) observed increased amounts of Alanine, Threonine, isoleucine, Arginine, Tyrosine, *Fusarium oxysporum*, Histidine, Leucine, Methionine, Serine, valine and glycine whereas decreased amount of phenylalanine and cysteic acid in needles of *Picea abies* trees grown in SO₂ polluted locality. However, they suggested that increasing contents of amino acids were attributable to high amount of ammonia accumulated in the forest soils.

A qualitative difference in sugar contents of the host of control and cement dust treated plants might be due to direct or indirect effect of the pollutant(s) on the test plants (Table 8 and 10). However, there is no relevant
information in support of the above explanation. It has been reported that treatment of wheat plants with low cumulative dose of SO₂ results in increased carbohydrate contents whereas it is reported to decrease at the higher cumulative dose (Prasad and Rao, 1981). Singh (1988) also reported qualitative variation in sugar contents of wheat plants treated with NH₃, cement dust and SO₂. Thus it may be inferred from the present study that the air pollutant(s) alter amino acid and sugar composition of plants.

**Responses of some dominant phylloplane fungi to particulate pollutant(s) in relation to population dynamics and growth behaviour**

**RESULTS**

Population dynamics (% frequency and colonies cm⁻²):

In case of the plant treated with low pollutant dose (5×10⁶ μg/m²); the per cent frequency of *Alternaria brassicae* was recorded highest and it was followed by *Epicoccum purpurascens*, *Alternaria alternata*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *Aspergillus candidus*, *Penicillium rugulosum*, *Aspergillus*
Fig. 6. Per cent frequency of some dominant phylloplane fungi recorded from untreated control and treated host plant.

- a. *A. alternata*
- b. *A. brassicae*
- c. *A. candidus*
- d. *A. niger*
- e. *Aureobasidium species*
- f. *C. cladosporioides*
- g. *E. purpurascens*
- h. *F. oxysporum*
- i. *P. citrinum*
- j. *P. rugulosum*
niger, *Aureobasidium* species and *Penicillium citrinum* (cf. control).

Likewise, the maximum number of colonies cm\(^{-2}\) was recorded in case of *Alternaria brassicae* and *Aureobasidium* species and minimum in case of *Fusarium oxysporum* (cf. control) (fig 6 and 7).

However, most of the fungi showed decreased percent frequency at increased dose(s) of the pollutant (1x10\(^7\) μg/m\(^2\)) except *Aspergillus niger*, *Fusarium oxysporum* *Penicillium citrinum* control and site I).

However, the no. of colonies cm\(^{-2}\) of most of the test fungi decreased at increased level of cement dust treatment except *Aspergillus niger*. (Table 13 and 14; Figs 6 and 7)

The most pronounced effect of cement dust treatment on population dynamics, in general, was recorded in case of *Penicillium citrinum* followed by *Alternaria alternata* and *Fusarium oxysporum*.

However, foliar application of cement dust caused an increased population dynamics of the test fungi at low level of the pollutant dose in comparison to the relatively higher dose (1x10\(^7\) μg/m\(^2\)) (Table 13 and 14; Figs 6 and 7)

:- 116 :-
Fig. 7. The number of fungi $cm^{-2} (x 10^2)$ on phylloplane of untreated control and cement dust treated host plant.
Sdl- seedling, Prf- pre flowering, Flr- flowering Pfl- Post flowering, Snt- senescent

f- C. cladosporioides g- E. purpurascens h- F. oxysporum i- P. citrinum j- P. rugulosum

- 117 -
It is evident from tables 13 and 14 that most of the test species showed reduction in per cent frequency and the number of colonies cm⁻² leaf area of the host on increasing doses of cement dust (1×10⁷ µg/m²) except *Aspergillus niger*. The maximum decrease was noted in case of *Alternaria brassicae* followed by *Alternaria alternata* and the minimum in case of *Aspergillus candidus*. However, other species showed variation in reduction with regard to dose of cement dust. (Table 13 and 14; Figs 6 and 7)

In general foliar application of cement dust caused statistically significant variation (p = 0.5) in per cent frequency and no. of colonies cm⁻² leaf area recorded in relation to test fungi and doses both during the treatment(s).

**Cement dust**: (Mean radial growth)

The radial growth of *Alternaria brassicae*, *A. niger*, *Aureobasidium* species, *Epicoccum purpurascens*, *Fusarium oxysporum*, and *Penicillium rugulosum* showed stimulation at both concentrations of cement dust (5 x $10^6$ µg/l and 1.0 x $10^7$ µg/litre) whereas *Cladosporium cladosporioides* and *Penicillium citrinum* showed inhibition
at low and high concentration of cement dust. The most pronounced stimulatory effect was seen on *Epicoccum purpurascens* followed by *Aspergillus niger*. The minimum stimulation was observed for *Aureobasidium* species. The maximum inhibition was recorded for *Cladosporium cladosporioides* and *Penicillium citrinum* followed by the minimum for *Alternaria alternata*. Reduction in stimulation of *Aureobasidium* or inhibition (*Alternaria alternata, Penicillium citrinum*) was recorded with prolonged incubation period. Other fungi showed variation in stimulation or inhibition at different periods of incubation (Table 15). Stimulation in case of *Alternaria alternata, A. brassicae, A. niger, Epicoccum purpurascens, Fusarium oxysporum*, and *Penicillium rugulosum* was recorded at low level of the pollutant (5 x 10^6 µg/l) and the said test fungi showed decreased mean radial growth at higher level of the pollutant dose (1 x 10^7 µg/l).

**DISCUSSION**

The results show that the population dynamics of the test species decreased at increased doses of the particulate pollutant(s) (Table 13, 14; Figs 6, 7). The ecology and survival of fungi can be considerably
influenced by atmospheric pollutants. The extent of any
damage will depend upon several factors of which the
principal ones are kinds of pollutants and their
concentrations, duration of exposure inherent resistance
of the organisms affected and nature of substrate
(Saunders, 1970).

The foliar spray of cement dust significantly reduced
the per cent frequency as well as number of colonies of
generally all the test species at increased dose of the
pollutant except *Aureobasidium* (Tables 13 and 14; Figs.
6 and 7). The adverse effect of cement dust on test
species seems to be due to toxicity of alkaline solutions
(formed when dusts are deposited in presence of free
moisture), corrosive and adhesive properties of cement
dust and Ca⁺⁺ induced suppression of stomatal opening
(Inoue and Katoh, 1987) and it might be responsible for
decreased population of the test species at increased level
of the pollutant.

Babich and Stotzky (1974) suggested that increase
in pH might be responsible for decreased population of
microbes. Suppressed growth of *Pythium splendens*, a
causal organism of damping off of cucumber, was
reported by Kao and Ko (1986a, b). Increased population
of most of the test fungi (Tables 13 and 14) might be due to their ability to neutralize toxic effects and Ca\(^{++}\) induced biological activities or synergistic effects of several factors like withering of leaf surface, increased amount of leaf exudates and resistance and adaptability of these species to cement dust. Similarly, cement kiln dust deposits have been reported to increase incidence of *Cercospora* leaf spot infections on sugar beet (Schoenbeck, 1960). Limestone dust deposits also appear to stimulate leaf spot infections on wild grape and sassafras leaves (Manning, 1970). Increased number of bacteria and fungi were found on leaves with light to moderate dust deposits (Manning, 1975).

It is evident from the cultural studies that the radial growth of *Alternaria brassicae*, *A. niger*, *Aureobasidium* species, *Epicoccum purpurascens*, *Fusarium oxysporum*, and *Penicillium rugulosum*, increased while it decreased in the case of *Aspergillus candidus*, *Cladosporium cladosporioides*, and *Penicillium citrinum* at high concentration of cement dust (cf. site I). The basic mechanism of such stimulation is still not clear and it needs further investigation. However, the growth inhibition of *Alternaria alternata*, *Aspergillus candidus*,
Cladosporium cladosporioides, in case of high dose treatment and Penicillium citrinum in both the treatments might be due to their high sensitivity and inability to neutralize the toxic effects. Thus the present study shows that the particulate pollutant(s) considerably affect the microorganisms on leaf surface as well as under cultural conditions.

Influence of air pollutants on incidence and severity of leaf spot disease of host

RESULTS

The survey of the leaf spot disease was conducted thrice in the polluted and unpolluted control localities from pre-flowering to post flowering stages at 15 days intervals in the year 2006 and 2007.

It is evident from Tables 16 and 17 that the per cent incidence of the disease and the total infected leaf area increased in each locality from I to III sampling, during both the cropping seasons. The least per cent incidence of the disease and total leaf area infected was recorded in case of site II polluted locality (10, 18, 27% and 18, 24, 30% respectively) whereas the maximum per cent incidence and leaf area infected was noticed in case of
site I (15, 24, 35% and 20, 32 and 38%, respectively). The infection level recorded were bcc and bdd in case of site II and I, respectively during cropping season 2006. It is further verified that the disease severity increased in case of site I in comparison to unpolluted control locality (Table 16).

During cropping season, 2007; The least incidence of the disease and total area infected were recorded in unpolluted control (10, 15, 24% and 15, 23, 34%, respectively) It was followed by site II (11, 18, 30% and 19, 27 and 31% respectively). However, maximum incidence of the disease and total leaf area infected was recorded in case of crop grown in site I. It showed a similarity with the observation(s) recorded during cropping season, 2006.

The disease was observed in all the localities which increased with ageing. The level of the disease incidence in the field was influenced by the particulate pollutant(s) (cf. control). It was aggravated in site I polluted locality (Tables 16 and 17). However, in case of site II cement dust polluted locality the disease severity was slightly suppressed as compared to the control (Table 17). A careful study of the Tables 16 and 17 indicated that the
least incidence and severity of the disease was recorded in site II cement dust polluted locality while more in case of site I during the respective cropping seasons.

The severity of disease in all the polluted and unpolluted control localities during both the cropping seasons was found to be in rating group (11 – 20%) in the first and d – c (31-40%; 21-30%) in the last sampling during cropping season 2006. Whereas it was found to be in rating group b – c (11-20%; 21-30%) in the first and d (31-40%) in the last sampling during cropping season, 2007 (Table 16 and 17). However, the severity of the disease was higher in the site I locality than control and site II (Table 16). The maximum severity of the disease was recorded in case of site I followed by site II and the least in case of control locality (Table 17).

DISCUSSION

The least incidence and severity of leaf spot disease in site II cement dust polluted locality might be attributed to the corrosive and adhesive properties of cement dust. Increased pH (7.8) (Table 11 & 12) could also be the cause of decreased pathogenesis as reported by Singh et al. (1987). In a similar study the least occurrence of A.
solani, the causal organism of early blight of potato, was reported in cement dust polluted locality in comparison to unpolluted control (Rai and Pathak, 1981; Rai, 1987). Calcium is one of the most important constituents of cement dust which plays as important role in suppression of various diseases. Kao and Ko (1986a) noted that calcium suppresses the growth of Pythium splendens, a causal agent of cucumber damping-off. The application of various calcium salts to soil reduces crop losses caused by Sclerotium rolfssii (Sitterly, 1962), Rhizoctonia solani (Bateman, 1964), Fusarium oxysporum f. species. Lycopersici (Jones and Woltz, 1967, 1970), P. myriotylum and P. aphanidermatum (Gill, 1972), Aphanomyces euteiches Phytophthora cinnamomi ( Boughton et al., 1978; Lee and Zentmyer, 1982).

The lower incidence and severity of the disease in site II polluted locality in comparison to unpolluted control (Table 16) might be attributed to toxicity of the increased cement dust doses (Singh et al., 1987).

The highest incidence and severity of the disease was recorded in site I polluted locality (Table 16 & 17). In another study the highest per cent occurrence of the pathogen on leaf surfaces was recorded in site I (Fig 5) in
comparison to other localities (Table 5-6). Smith (1976) has stated that under influence of low doses of air pollutants micro organisms may act as “pollution sink” and cement dust might be directly/indirectly useful as nutrient source(s) or facilitates pathogenesis and fungal colonization.

The variation in incidence and severity of disease in the polluted and unpolluted localities recorded for two cropping season might be due to differences in predisposing factors (Table 1) including the intensity of ambient air pollutant(s).

Effects of air pollutants on microbial interactions between *Alternaria brassicae* and the test fungi

RESULTS AND DISCUSSION

It is evident from Tables 18; Figure 8 that several species inhibited the lesion development under the influence of cement dust pollution. The maximum inhibition in lesion development was noted in the case of *Aspergillus candidus* and the minimum in the case of *Alternaria alternata* in case of low dose of the pollutant. The application of composite microflora on foliar surface
caused significant \((p = 0.05)\) inhibition of lesion development (Fig 8 and Table 18).

Inhibition in lesion development by interaction with several test species under influence of cement dust might be due to corrosive and adhesive properties of cement dust and tolerance of the test fungi. Increased pH (7.8) (Table 11 & 12) might be also a reason which causes inhibition in lesion development. Rai and Pathak (1981), Rai (1987), Singh et al. (1987) and Rai et al. (1988) also reported the least incidence of disease under influence of cement dust.

Similarly, the maximum inhibition in lesion development was recorded in case of Aspergillus candidus followed by Aspergillus niger, Penicillium rugulosum, Epicoccum purpurascens, Penicillium citrinum, Aureobasidium species, Fusarium oxysporum, Cladosporium cladosporioides and the least in case of Alternaria alternata (Fig. 8 and Table 18).

Stimulation in lesion development at low dose of the cement dust treatment seems to be due to alteration of environment of leaves either by disturbing the equilibrium of leaf surface microflora of host or by
Fig. 8. Influence of cement dust on microbial interaction of some selected dominant phylloplane fungi and *Alternaria brassicae* on leaf surface of mustard in relation to leaf spot disease

- A. alternata  b. A. candidus  c. A. niger  d. *Aureobasidium* species  e. C. cladosporoides
f. E. purpurascens  g. F. oxysporum  h. P. citrinum  i. P. rugulosum
j. Composite microflora
altering the pH of the host leaves or by both (Wellburn et al., 1972; Rai and Pathak, 1981). Schoenbeck (1960) reported that cement dust deposition on sugar beet increased the incidence of leaf spot disease caused by *Cercospora beticola* and concluded that the cement dust altered the physiological balance and increased the plant susceptibility to infection. Manning (1971) also reported that sassafras and wild grape plant continuously exposed to emission of limestone dust were more susceptible to infection by *Guignardia bidwellii* and *Gloeosporium sp.*, the causal organisms of leaf spot disease of grape. This stimulation of disease development might be due to favourable effect of cement dust on the pathogen but not on the test fungi. However, the test viz., *Aspergillus candidus*, *A. niger*, *Penicillium rugulosum*, *Epicoccum purpurascens*, were recorded as cement dust tolerant species and caused increased inhibition of lesion development in comparison to other test fungi (Fig. 8 and Table 18).

It is evident from the present study that the particulate pollutants, in the natural atmosphere, greatly influence the microorganisms, their interactions and their harmful and beneficial activities. The antagonism of the
saprophytes against the pathogen on leaf surface is markedly altered under the influence of air pollutants (Heagle, 1973). The behavior of Aspergillus candidus, A. niger and Penicillium rugulosum which are saprophyte on leaves is interesting. The antagonistic role of the test fungi in the phylloplane had been recognized by several workers (Newhook, 1957; Bhatt and Vaughan, 1963; Diem 1967; Warren, 1972; Rai and Singh, 1980 and Rai et al., 1988 and Solanki, 1988). In the present study it was observed that Aspergillus candidus was more effective in reducing the disease under the influence of the both does of the pollutant(s). This behaviour of the test fungal species may be attributed to its natural affinity with phylloplane of Brassicae species which has been recorded by Singh and Rai (1980); Singh et al. (1987) Dickinson (1976) reported that Cladosporium cladosporioides has an ability to grow and sporulate in fluctuating environmental and nutritional conditions. It has also caused an inhibition in lesion development caused due to Alternaria infection and may correlated with its nature of dominant phylloplane mycoflora.
The following conclusions can be drawn from the study:

(i) the incidence and severity of the disease is changed by the particulate pollutant(s);

(ii) the pollutants(s) may exert unfavourable effect on the pathogen but not on antagonists; thereby reducing the disease;

(iii) the pollutants may have unfavourable effect on the antagonists but not on the pathogen; thereby stimulating the disease;

(iv) the pollutants may have favourable or unfavourable effect(s) on both, the pathogen and antagonist(s), resulting inhibition at \(1 \times 10^7 \, \mu g/m^2\) high doses.

**Effect of cement dust on colony interaction between**

**Alternaria brassicae and test fungi**

Less per cent inhibition in colony growth of *Alternaria brassicae* (cf. control) was recorded at all the concentrations of cement dust in case of interactions with *Alternaria alternata, Aureobasidium, Fusarium oxysporum*. However, *A. candidus, Epicoccum purpurascens* and *Penicillium rugulosum* caused more per cent inhibition of growth of the pathogen at \(5.0 \times 10^6 \, \mu g/l\) concentration.
More per cent growth inhibition of the pathogen (cf. control) was recorded due to interaction with *A. candidus*, *Epicoccum purpurascens*, *Penicillium citrinum*, *P. rugulosum* at all the concentrations of cement dust used whereas relatively less per cent inhibition was recorded due to *A. niger*, *Fusarium oxysporum* at (5.0 x 10^6 µg/l) and *A. niger*, *Penicillium citrinum* at 1.0 x 10^7 µg/l. Less per cent growth inhibition of the test species was recorded by the pathogen at each concentration in the cases of interactions with *Alternaria alternata*, *Aureobasidium*, *Epicoccum purpurascens*, and *Penicillium citrinum*. Relatively more per cent inhibition (cf. control) was noted for *Aspergillus candidus* *A. niger*, and *Fusarium oxysporum*, (Table 19)

The grading of colony interaction was altered from A to B₂ (*A. alternata*); C to D (*E. purpurascens*); B₂ to D (*A. niger*); D to B₁ (*Aspergillus candidus*); A to C (*Aureobasidium* and *Fusarium oxysporum*) and B₁ to C (*Penicillium citrinum*). However, the grading of colony interaction in case of *Cladosporium cladosporioides* remain unchanged (D to D) (Table 19).
DISCUSSION

The results obtained show that different types of interactions occur between paired fungi on agar media which have been observed earlier by several workers (Van den Heuvel, 1970; Dickinson and Boardman, 1970; Fokkema, 1973; Skidmore 1976; Skidmore and Dickinson, 1976). These interaction are altered by the kind and concentration of the air pollutants. The alteration in interactions particularly growth inhibition of the pathogen as well as test saprophytic fungi occur due to direct favourable or adverse effect of the air pollutants on growth of the pathogen and other test fungi. Radial growth inhibition on agar plates is commonly ascribed to production of antibiotics by the antagonists, competition for nutrients, mechanical obstruction and hyperparasite reaction (Diem, 1969; Ikediugwu and Webster, 1970; Fokkema, 1973, 1976; Skidmore and Dickinson, 1976). These activities seem to be altered under influence of air pollutants and might be responsible for the changes in per cent growth inhibition of pathogenic and saprophytic species. Change in pH of the nutrient medium due to antagonistic fungi and its inhibitory effect on the growth
of *Septoria nodorum* has been recorded by Skidmore (1976).

Intermingled growth (grade A) of interacting fungi is possible only when both the fungal colonies show equal growth rate and equal competitive and resistance capacity. Overgrowth (grade B₁, B₂) is possible when one of the interacting species has a higher growth rate and/or produces antibiotic substances and has tolerance against any such substance produced by the other. Diffusion of active staling growth substances in nutrient agar by the antibiotic producing fungal species may be inhibitory/lethal to the test fungi (grade C or D).

The alteration of grade A to C in the case of *Aureobasidium*, and *Fusarium oxysporum* under influence of both doses of cement dust: B₁ to C (*Penicillium citrinum*) and B₂ to D in case of *A. niger* under influence of cement dust; A to B₂ (*A. alternata*) and C to D in case of *Epicoccum purpurascens*, under cement dust treatment(s) seems to be due to some effect of the respective doses of the cement dust on growth of interacting fungal species. If D grade is altered to any one of the remaining grades eg. *A. candidus* it may be attributed to favourable effect of the pollutants on the interacting species. Thus it is
evident from the present study that the air pollutants greatly influence the fungal colony interactions.

**Effect of leaf extract of Brassica species on radial growth of A. brassicae and some phylloplane fungi in relation to particulate pollutants**

**RESULTS**

It is apparent from Table 20 that amendment of culture medium with leaf extracts of Mustard collected from different polluted and unpolluted control localities and sterilized through autoclaving and seitz filtering showed varied effect (Stimulatory and/or inhibitory) on growth behavior of all the test fungi. Effects also varied with regard to vegetative growth stage of the host. (Table 20)

The growth of all the test fungi including the pathogenic one, *A. brassicae*, was stimulated in the case of both autoclaved and seitz filtered leaf extracts of cement dust polluted locality. However, an inhibition was noticed in the case of *A. brassicae*, *Aureobasidium species*, *Fusarium oxysporum* and *P. citrinum* on addition of leaf extracts of plants collected from site II (Table 20).
The leaf extracts of site I polluted locality exhibited stimulatory effect against *A. alternata*, *A. brassicae*, *A. candidus*, *A. niger*, *Aureobasidium* species, *Cladosporium cladosporioides*, *Epicoccum purpurascens*, *Fusarium oxysporum*, and *P. rugulosum*. However, *A. alternata*, *A. brassicae*, *A. candidus*, *A. niger*, *Aureobasidium* species, *Epicoccum purpurascens*, *Fusarium oxysporum*, and *P. rugulosum* showed decreased radial growth in case of extracts collected from site II (Table 20). A significant (P=0.01) increase in radial growth of *A. candidus*, *Aureobasidium*, *E. purpurascens*, *A. niger*, *Cladosporium cladosporioides*, *F. oxysporum* and *P. rugulosum* was noticed at certain growth stages on addition of extracts obtained form site I polluted locality. However, the effect was insignificant in case of other test fungi. The maximum increase was noticed in case of *F. oxysporum* while the minimum for *Penicillium citrinum*.

The Seitz filtered leaf extracts obtained from plants grown in cement dust polluted locality was stimulatory to all the test fungi. The maximum stimulation was recorded in case of *A. niger* and *Fusarium oxysporum* followed by *Alternaria brassicae*, *Aureobasidium*, *Cladosporium cladosporioides*, *Epicoccum purpurascens*, *Penicillium*
*rugulosum*. It was also noted that the stimulatory effect was higher at the flowering stages in case of all the test fungi except *Penicillium citrinum*. The autoclaved leaf extract of site I was found stimulatory to *A. alternata*, *A. candidus*, *A. niger* and *Cladosporium cladosporioides* at all the growth stages (Table 20).

**DISCUSSION**

Stimulatory responses by the leaf extract of site I polluted locality against the test species might be due to increased nutritional value of host or due to induction of stimulatory factor (Singh et al., 1987).

The extract from site I and site II cement dust polluted localities showed either stimulation or inhibition depending upon the situation. The stimulatory effect might be attributed to sensitivity of species to some products of altered metabolism like amino acids, sugars, enzymes etc. probably induced by air pollutants (Zedler et al., 1986; Breman et al., 1962; Grill and Esterbauer, 1973; Miyake, 1984; Horsman and Wellburn, 1976; Jager and Pahlich, 1972; Prasad and Rao, 1981; Singh and Rao, 1979, 1980; Stratigakos et al., 1985) while inhibitory effect of autoclaved extract might be due to
inactivation of growth promoting substances present in the leaf extract during autoclaving. Saunders (1970) stated that degradation of products of thioglycocides (formed as a result of SO₂ uptake) have considerable bactericidal and fungicidal property.

Alteration in pH of the leaf medium due to influence of air pollutants is also inevitable which also contribute considerable effect on growth of microorganisms in amended culture medium.

Thus it is evident from the present study that particulate pollutant(s) play a vital role in distribution and growth of microorganisms on phylloplane either directly or indirectly by influencing the metabolism of the host plants.

**Effects of the air pollutants on colonization and succession of fungi on decaying leaves of host (Brassica species)**

**RESULTS AND DISCUSSION**

It is evident from Tables 21 and 22 and Figs. 9 and 10 that the pattern of fungal colonization on senescent leaves of host is markedly influenced by the particulate pollutant(s). However, no definite trend of succession of
Fig. 9. Total No. of fungal species on leaves of decaying host in relation to cement dust pollution.
Fig. 10. The number of fungi cm$^2$ ($\times 10^3$) on leaves of decaying host in relation to cement dust polluted localities.
fungi was observed on the decaying leaves placed in the same field in each polluted and unpolluted locality. Statistically significant variation (P = 0.05) in the total number of fungal species and fungi cm\(^{-2}\) on senescent leaves in relation to monthly samplings and localities were recorded (Tables 23 and 24).

The total number of fungal species and their colonies cm\(^{-2}\) leaf area increased on senescent leaves up to June and thereafter it decreased in control and site II polluted localities (Figs. 9 and 10; Tables 23 and 24). *Alternaria brassicae, Aspergillus flavus, A. niger, Cladosporium cladosporioides, C. herbarum, Chaetomium, Epicoccum purpurascens, Fusarium oxysporum, Fusarium semitectum, Phoma species, P. rugulosum and P. javanicum* were dominant species in all the localities. These species were followed by *Alternaria alternata, Aspergillus candidus, A. clavatus, Curvularia lunata, C. pallescens, Rhizopus nigricans*. However, the average maximum population (18 species and 116.83 X 10\(^3\) fungi cm\(^{-2}\) leaf area) was recorded from the unpolluted locality; site I (16 and 132.77 X 10\(^3\)) and site II (17 and 147.62 X 10\(^3\)), respectively. *Nigrospora sphaerica, Penicillium citrinum, Torula herbarum*, and Dark sterile mycelia were
not recorded from the control locality. *Aspergillus candidus*, *A. clavatus*, *Cephalosporium*, *Chaetomium species*, *Drechslera australiensis*, and *Epicoccum species* were recorded as dominant species in the polluted localities. However, rare occurrence of *A. alternata*, *A. tenuissima*, *A. clavatus*, *A. flavus*, *A. luchuensis*, *A. sydowi*, *Aureobasidium*, *Cephalosporium*, *C. pallenscens*, *F. moniliforme* and *Mucor species* were observed in all the localities. The no. of fungi/cm² leaf area of decaying host plant in control was recorded as 102.03 X 10³ during April and it increased up to may only (116.83 X 10³) and the least in case of August (94.30 fungi/cm² leaf area). However, the no. of fungi/cm² leaf area increased in case of site I polluted locality from initial to final stage of observation (90.91 X 10³ – 132.31 X 10³). The maximum no. of fungi/cm² leaf area was recorded during July in case of site II polluted locality (147.62 fungi/cm²) (Fig. 10).

*A. sydowi*, *Choanephora species*, *Pestalotia species*, *Pithomyces species* were recorded only from unpolluted control locality (Table 21-22). *Aspergillus candidus*, *A. clavatus*, *A. luchuensis*, *Torula*, *Cephalosporium*, *Chaetomium species*, *D. australiensis*, *P. citrinum*,
Sclerotium species and dark Sterile mycelia showed predominance in the polluted localities of site I/site II (Table 21 and 22)

The fungal population increased owing to the fact that the age of senescent leaves provided rich amount of moribund tissues for their growth and reproduction. On the other hand the surface area of decaying leaves also increased due to activity of initial colonists. This allowed the colonization of such fungi which were unable to grow on fresh decaying leaves. A decrease in population after July seems to be due to increased microbial population of antagonists on decaying plants, depletion of organic nutrients from the substrates and competition among the fungal species and other microbes, which started growing on substrates later during the process of decomposition.

High temperature affects the colonization of fungi in various ways like (a) directly by affecting the germination of fungal spores and their mycelial growth and (b) indirectly by lowering the moisture content of substrates and relative humidity of the atmosphere. Several workers reported that the low moisture content of decaying plant parts limits the germination, growth and sporulation of fungi (Webster, 1956, 1957; Hudson and Webster, 1958;
Webster and Dix, 1980; Hudson, 1962; Meredith, 1962a; Rai, 1973, 1974; Singh, 1978; Singh, 1988). Competition among the microorganisms also play a vital role in disappearance of some fungi during the colonization of litter which has already been emphasized by earlier workers (Webster, 1956; Hudson and Webster, 1958; Meredith, 1962a; Harper and Webster, 1964; Khanna, 1964; Macauley and Thrower, 1966; Yadava, 1966a, b; Sharma, 1967; Rai, 1973, 1974; Singh, 1978; Singh, 1988).

The microbial population decreased considerably in the unpolluted and site II polluted localities (cf. site I) (Fig. 10; Table 24). This may be attributed to their toxic effects on microorganisms (Singh, 1988). A comparative decrease in total number of fungal species at site I and site II in comparison to control may be attributed to limited number of species being capable of tolerating cement dust toxicity, competitive saprophytic ability and antagonism (Singh 1988).

However, the increase in number of fungi/cm² leaf area at site I and site II in comparison to the control may be attributed to the fact that some microbes may serve as pollutant sink (Smith, 1976, Babaich and stotzky, 1978;
The species isolated only from the unpolluted control locality eg. *A. sydowi*, *Choanephora*, *Pestalotia* and *Pithomyces* species seems to be sensitive to the cement dust pollution and may serve as bio-indicator of the pollutant(s). The selective presence of some species in the polluted locality eg. *A. candidus*, *A. clavatus*, *Cephalosporium*, *Chaetomium*, *P. rugulosum*, *Sclerotium* species seems due to their ability to neutralize the toxic effect(s) of cement dust and serve as pollutant sink. These species may be utilized as biocontrol agent of plant pathogen(s). The principal step in litter decomposition and humus formation are principally controlled by extracellular enzymes of microbial origin which accumulate in the environment (Burns, 1978). Pollutants can influence the rates and extent of organic matter decomposition either through the inhibition of enzymes activity or decreased the production of enzymes.

The reason for an increase or decrease of microflora due to effects of various air pollutants have been discussed elsewhere in the preceding sections (Smith 1976, Babaich and Stotzky 1978, 82; Heagle, 1973; Singh, 1988)