Chapter 3

Materials & Methods
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MATERIALS AND METHODS

3.1 SECTION I

This section includes experiments conducted to determine the impact of SO₂ and fly ash on growth and productivity of three cultivars of rapeseed and in turn the effect of SO₂ and fly ash on Alternaria blight of rapeseed caused by *Alternaria brassicicola* under different treatment conditions. Three cultivars of rapeseed were tested for their differential response to SO₂ and fly ash.

3.1.1 Experiment 1: Impact of SO₂ and Alternaria blight on growth and productivity of rapeseed

This experiment was conducted under artificial conditions.

3.1.1.1 Plant culture and treatment

Seeds of three cultivars of rapeseed, *Brassica campestris* L. (viz., T-7, TL-85 and TH-68) were surface sterilized for two minutes in 0.1% HgCl₂ solution followed by washing with sterilized double distilled water, at least twice. The surface sterilized seeds were sown in clay pots (30 cm in diam.) filled with autoclaved sandy loam field soil (66% sand, 24% silt, 8% clay, 2% OM, pH 7.7). After germination, seedlings were thinned to maintain three seedling per pot. Plants of different varieties (4-leaf stage) were exposed to SO₂ in the exposure chamber using three different concentrations. The plants designated to be inoculated with *Alternaria brassicicola* were inoculated with the pathogen by the method described later. The following were the treatments:
3.1.1.1.1 Control Set

1. B. campestris cv. T-7
   B. campestris cv. TL-85
   B. campestris cv. TH-68

2. B. campestris cv. T-7 + Alternaria brassicicola
   B. campestris cv. TL-85 + A. brassicicola
   B. campestris cv. TH-68 + A. brassicicola

3.1.1.1.2 Sets exposed with different levels of SO\textsubscript{2} and Alternaria brassicicola

(i) B. campestris cv. T-7 + SO\textsubscript{2} (142.85 µg m\textsuperscript{-3})
   B. campestris cv. TL-85 + SO\textsubscript{2} (142.85 µg m\textsuperscript{-3})
   B. campestris cv. TH-68 + SO\textsubscript{2} (142.85 µg m\textsuperscript{-3})

(ii) B. campestris cv. T-7 + SO\textsubscript{2} (142.85 µg m\textsuperscript{-3}) + A. brassicicola
    B. campestris cv. TL-85 + SO\textsubscript{2} (142.85 µg m\textsuperscript{-3}) + A. brassicicola
    B. campestris cv. TH-68 + SO\textsubscript{2} (142.85 µg m\textsuperscript{-3}) + A. brassicicola

(iii) B. campestris cv. T-7 + SO\textsubscript{2} (285.71 µg m\textsuperscript{-3})
     B. campestris cv. TL-85 + SO\textsubscript{2} (285.71 µg m\textsuperscript{-3})
     B. campestris cv. TH-68 + SO\textsubscript{2} (285.71 µg m\textsuperscript{-3})

(iv) B. campestris cv. T-7 + SO\textsubscript{2} (285.71 µg m\textsuperscript{-3}) + A. brassicicola
     B. campestris cv. TL-85 + SO\textsubscript{2} (285.71 µg m\textsuperscript{-3}) + A. brassicicola
     B. campestris cv. TH-68 + SO\textsubscript{2} (285.71 µg m\textsuperscript{-3}) + A. brassicicola

(v) B. campestris cv. T-7 + SO\textsubscript{2} (571.43 µg m\textsuperscript{-3})
    B. campestris cv. TL-85 + SO\textsubscript{2} (571.43 µg m\textsuperscript{-3})
    B. campestris cv. TH-68 + SO\textsubscript{2} (571.43 µg m\textsuperscript{-3})

(vi) B. campestris cv. T-7 + SO\textsubscript{2} (571.43 µg m\textsuperscript{-3}) + A. brassicicola
     B. campestris cv. TL-85 + SO\textsubscript{2} (571.43 µg m\textsuperscript{-3}) + A. brassicicola
     B. campestris cv. TH-68 + SO\textsubscript{2} (571.43 µg m\textsuperscript{-3}) + A. brassicicola
3.1.1.2 Exposure and doses

For exposure, pots were arranged inside the exposure chamber, according to factorial randomized block design. Three-weeks-old seedlings, grown in the pots and designated to receive SO$_2$ treatment were exposed to SO$_2$ on every alternate day for three hours. The exposure was continued till the termination of the experiments, after 120 days. The concentrations of SO$_2$ used were 142.85, 285.71 and 571.43 $\mu$g m$^{-3}$. Air flow rate 2.0 to 2.15 mS$^{-1}$. Ambient concentrations of SO$_2$ were 8.3±2.6 ppb.

3.1.1.3 Exposure chamber

Dynamic State Exposure Chamber (Standard Appliances, Varanasi, India) designed for continuous exposure of test materials for short or long durations of time to a mixture of air + gaseous pollutant(s), blowing through the chamber was used in the study (Fig. 1). The chamber was made up of transparent lucite sheets with a height of 120 cm and 8100 cm$^2$ cross sectional area. Lucite sheets were fixed in aluminium sections, which were coated with hard paint. The front of the chamber was provided with door in order to facilitate easy handling of test materials inside the chamber, while the bottom plate had several perforations with nozzles for smooth flow of thoroughly mixed air and pollutant gas. A meshed partition tray was placed 60 cm above the bottom plate in order to provide sufficient space for exposure of potted plants. Desired rate of air circulation through the chamber was set by electric regulator, the control pannel, which controlled the input voltage to the electric blower. The air or air +
Fig. 1. Exposure Chamber
gaseous pollutant mixture was injected through injection part, which entered the chamber from the perforated base and passed out through an exhaust duct located at the top of the chamber. The plants for treatment, in pots, were kept in the exposure chamber and exposed for a desired length of time.

3.1.1.4 SO₂ generation

SO₂ was generated in a generator which produced SO₂ gas by the action of sulphuric acid (H₂SO₄) on sodium sulphite (Na₂SO₃) under controlled reaction conditions. The amount of Na₂SO₃ and H₂SO₄ discharged from the reagent bottles mounted over the SO₂ generator was determined by collecting the solution dropping through capillary tube in a graduated cylinder for sometime and expressing the rate in ml/min. On the basis of flow rate, solution of sodium sulphite (Na₂SO₃) and sulphuric acid (10%) was prepared to produce required amount of SO₂ gas/min. On complete reaction 1M Na₂SO₃ produces 1M SO₂ or 126 mg Na₂SO₃ produces 64 mg SO₂.

\[
\text{Na}_2\text{SO}_3 + \text{H}_2\text{SO}_4 \rightarrow \text{SO}_2 + \text{Na}_2\text{SO}_4 + \text{H}_2\text{O}
\]

10% H₂SO₄ solution was used for all the working solutions of Na₂SO₃.

For determining the concentration of SO₂ during the exposure period, samplings were done by a handy air sampler (Kimoto Electricals, Japan) and analysed in the laboratory. The concentration of SO₂ in the sampled air was determined by West and Gaeke (1956) method as prescribed by National Environmental Engineering Research Institute, Nagpur, India in its Air Quality Monitoring Course Manual (Anon, 1986). Blower of the exposure chamber was run at constant
voltage (180 V) because at different voltages, the quantity of air blown into the chamber varied, which could change the desired concentrations of SO$_2$. Furthermore, as a precautionary measure, sampling was done after every 8 days and sample was again analysed in laboratory. Air flow rate ranged from 2.0 to 2.15 mS$^{-1}$, which was fast enough to overcome aerodynamic resistance. The air (with SO$_2$) inside the chamber was being replaced once every 5 seconds, approximately.

### 3.1.1.5 Collection of diseased material

Leaves of rapeseed showing characteristic symptoms of Alternaria blight (Fig. 2) were collected during the cropping season from the nearby fields in Aligarh. These naturally infected specimens were examined in the laboratory for the presence of the causal organism. The symptoms present on the leaves were thoroughly observed and characteristics were noted. The leaves were properly preserved, labelled and kept in wet and dry forms for further studies and future reference.

### 3.1.1.6 Isolation and purification of the pathogen

The leaves with fresh initial spots were selected for isolation of the pathogen. They were washed properly with sterilized water. Instruments used were also sterilized by 95% methylated spirit. Small bits of younger diseased leaf spots along with some healthy tissues were cut with the help of a scalpel, dipped in 0.1% HgCl$_2$ solution for about 30 seconds for external disinfection. After that they were washed with 3 to 4 changes of sterilized water. Excess moisture were removed by putting these pieces pressed in between the folds of sterilized
Fig. 2. Alternaria blight caused by *Alternaria brassicicola* on the leaf of *Brassica campesteris*
blotting paper in the inoculation chamber. The diseased fragments of leaves were then transferred to petriplates poured with potato dextrose agar (P.D.A.) medium. In each plate, three to four pieces were placed approximately at equal distance and then incubated at 25 ± 1°C. As soon as, the mycelial growth was seen around the diseased pieces, hyphal tips from the advancing mycelia of different petriplates were transferred to the culture tubes.

The culture was purified by single spore technique. A dilute spore suspension was poured on plain agar in petriplates and spores were allowed to settle down on the agar surfaces. Amount of the suspension was adjusted just enough to form a very thin layer over the surface of the agar. The spores settled quite apart from each other were selected under the microscope, marked and encircled with the help of inoculating needle. They were lifted along with agar and transferred to the petriplates containing P.D.A. After proper growth of transferred single spore, regular subculturing was done to check further contamination. After the purification accomplished, pure culture of the pathogen was multiplied and maintained on P.D.A. in culture tubes. The fungus in question causing the disease was identified as *Alternaria brassicicola*.

3.1.1.7 Pathogenicity test

The pathogenicity of the fungus was established following Koch's postulates on potted plants of rapeseed cv. T-7, TL-85 and TH-68.
3.1.1.8 Exposure of *Alternaria* spores to sulphur dioxide

The spores of *Alternaria brassicicola* were exposed to sterilized sulphur dioxide in micro-gas exposure cabinets which were placed in exposure chambers of 90 x 90 x 120 cm dimensions. The micro-gas exposure system consisted of a small air sterilization unit and an exposure cabinet (Fig. 3). The sterilization unit had a compact suction pump and a filtering device, fitted to the inlet of the pump. The filtering material prevented entry of any fungal and bacterial spores in the exposure cabinet. The exposure cabinet (33x26x3 cm) was made of transparent glass-fibre. The cabinet had a removable upper cover and one inlet and one outlet. The outlet of the sterilization unit was connected to the inlet of the cabinet. The sulphur dioxide mixed with air in exposure chamber was sterilized by micro-exposure system and hence the sulphur dioxide was used to fumigate the spores of *A. brassicicola*.

Spore suspension of *A. brassicicola* was prepared by blending mycelial mats (from a pure culture) in 1000 ml of sterilized water in an electric blender. Sterilized petriplates of 3 cm diameter containing slides were placed in micro-gas cabinet to expose spore suspension to sulphur dioxide at 142.85, 285.71 and 571.43 µg m\(^{-3}\) for 3 and 6 h. The control (unexposed) set, petriplates with spore suspension was exposed to sterilized ambient air (2 m/sec\(^2\)) for 3 and 6 h. After completion of the desired exposures, the plates containing spore suspension was covered and incubated for 48 h, in a B.O.D. incubator at 25±2°C. All the slides were placed on a glass triangle and kept in petriplates
containing sterilized water, at the bottom. Five plates were maintained for each treatment. After incubation, the plates were examined under compound microscope and 100 conidia were considered to determine their germination percentage of various treatments.

3.1.1.9 Parameters

The following parameters were determined for each treatment of the experiment:

1. Length of shoot and root, at harvest
2. Fresh and dry weight of shoot and root, at harvest
3. Number of flowers per plant, at 80 days after sowing
4. Number of pods per plant, at harvest
5. Pigment content of the leaves (chl. a, chl. b, total chlorophyll and carotenoids) at 60 days after sowing
6. Nitrogen, phosphorus, potassium and sulphur content in leaves at 60 days, after sowing
7. Oil content of the seeds (%)
8. Seed yield per plant

The methods for determining the above mentioned parameters are described in detail below.

3.1.1.10 Plant growth and yield

A few hours before termination of the experiment, an excess amount of water was added to the pots to soften the soil for easy uprooting of the plants without excessive root loss. Uprooted plants, from different treatments, were kept in labelled polythene bags, and
brought to the laboratory. Thereafter, the length of the shoot and root was measured. Dry weight of the plants was determined by wrapping plants in labelled blotting sheets and dried in hot air oven at 60°C for 24 h and weighed. The number of flowers was counted at 80 days, after seed sowing. Number of pods/plant and number of seeds/plant were counted, before dry weight was taken to determine the yield in each treatment.

3.1.1.11 Plant analysis

Leaf samples of the three cultivars of rapeseed, used in the experiment, were collected at 60 days after sowing for estimating chlorophyll, carotenoid, nitrogen, phosphorus, potassium and sulphur contents and at harvest, oil contents of seeds.

3.1.1.11.1 Estimation of leaf pigments

Leaf pigments (chlorophyll a, b, total chlorophyll and carotenoids) were determined by grinding 1 g of fresh leaves from interveinal areas in 40 ml of 80% acetone with the help of mortar and pestle. The suspension was decanted in Buchner funnel having two Whatman filter paper No. 1. Then filtration was done with the help of suction pump. The residue was ground thrice by adding acetone. The suspension was decanted in Buchner funnel and filtered in vacuum. At last, mortar and pestle were rinsed with acetone, transferred in Buchner funnel and filtered. The filtrate was transferred to 100 ml volumetric flask and the volume was made upto the capacity by adding acetone. Optical density (O.D.), by spectrophotometer, was read at 480 nm and 510 nm for carotenoids and 645 and 663 nm for chlorophyll.
Carotenoids and chlorophyll contents were calculated by using the following formulae:

\[
\text{Carotenoids} = \frac{7.6 \times (\text{OD. } 480) - 1.49 \times (\text{OD. } 510)}{d \times 1000 \times W}
\]

\[
\text{Chlorophyll 'a'} = \frac{12.7 \times (\text{OD. } 663) - 2.69 \times (\text{OD. } 645)}{1000 \times W} \times V
\]

\[
\text{Chlorophyll 'b'} = \frac{22.9 \times (\text{OD. } 645) - 4.68 \times (\text{OD. } 663)}{1000 \times W} \times V
\]

\[
\text{Total Chlorophyll} = \frac{20.2 \times (\text{OD. } 645) + 8.02 \times (\text{OD. } 663)}{1000 \times W} \times V
\]

d = Length of the light path

V = Total volume of the chlorophyll solution

W = Fresh weight of the leaf

3.1.1.11.2 Estimation of nitrogen, phosphorus, potassium and sulphur contents

3.1.1.11.2.1 Digestion of powder

Fifty mg of the leaf dry powder was taken in a Kjeldhal flask (100 ml) and 1 ml of concentrated sulphuric acid was pipetted into it, followed by heating on a digestion assembly for 2 h. 0.5 ml of chemically pure \( \text{H}_2\text{O}_2 \) (30%) was added after the flask cooled down. The solution was heated again for 30 min. till the colour changed from black to light yellow. The flask was cooled for 15 min. and an additional amount of 3-4 drops of \( \text{H}_2\text{O}_2 \) (30%) was added, followed by gentle heating for another 15 min. to get a clear and colourless solution. At this stage, excess of \( \text{H}_2\text{O}_2 \) was avoided as it would oxidise ammonia in
the absence of organic matter. The peroxide digested material was transferred to a volumetric flask (50 ml) with three washings with distilled water. The final volume was made upto the mark with distilled water.

3.1.1.11.2.2 Nitrogen content

The nitrogen content in the leaf samples was estimated according to the method described by Lindner (1944). Five ml of the peroxide digested material was transferred to a 25 ml volumetric flask. Two ml of 2.5N NaOH and 1 ml of 10% sodium silicate were added to the flask to neutralize the excess of acid and to prevent turbidity. Volume was made upto the mark with distilled water. Five ml of this sample was pipetted into a 20 ml graduated test tube to which 0.5 ml of Nessler's reagent was added dropwise, with repeated shakings. The final volume was made upto 10 ml with distilled water. After waiting for 5 min., to get optimum colour development, per cent transmittance of the solution was read at 525 nm on a spectrophotometer. A blank consisting of Nessler's reagent and distilled water was run simultaneously with each set of samples. Standard curve was plotted by using known graded dilutions of ammonium sulphate solution. The optical density of each sample was compared with that on the calibrated curve and per cent nitrogen in each sample was noted on dry weight basis.

3.1.1.11.2.3 Phosphorus content

Phosphorus content was estimated by the method of Fiske and Subba Row (1925). Five ml of the digested peroxide solution was taken in a 20 ml graduated test tube and 1 ml of molybdic acid was added
carefully followed by addition of 0.4 ml of 1 amino-2-naphthol-4-sulphonic acid. The colour of the solution turned blue. Distilled water was added to make the final volume upto 10 ml. The per cent transmittance was read at 620 nm on spectrophotometer. A blank (control) was also run simultaneously with each set of samples. The standard curve was plotted by using known gradual concentrations of monobasic potassium phosphate solution. The optical density of the unknown samples was compared with that of the standard curve to calculate the per cent phosphorus contents.

3.1.1.11.2.4 Potassium content

Potassium content in the digested material of leaf was estimated directly by flame photometer using potassium filter. A blank of distilled water was run side by side. The reading was compared with a calibration curve plotted by using known graded dilutions of a standard potassium chloride solution.

3.1.1.11.2.5 Sulphur content

Sulphur was estimated by adopting the methodology of Patterson (1958). One hundred mg oven dried leaf powder was digested with 0.1 ml of 8.2% selenium dioxide solution, 10 ml nitric acid, 1 ml hydrochloric acid and 5.8 mg NaCl for 20-30 min., followed by evaporation to dryness. To the hot residue, 2 ml of 2.9 N HCl was added and the mixture was re-evaporated to dryness. The last treatment was repeated twice to ensure reduction of nitrates. Finally, 25 ml distilled water and 1 ml of 2.9 N HCl were added. The contents were allowed to boil followed by filtration. The filtrate was collected in a 100 ml
volumetric flask to which 10 ml of 3% glycerol and 5 ml of 2% barium chloride in 2% HCl were added. The volume of the flask was made up to the mark with D.D.W. The reading was recorded, after maximum colour development, at 420 nm, using a blank, with the help of spectrophotometer. The reading of each sample was compared with the standard curve.

3.1.1.11.3 Oil analysis

The seed samples was crushed to get a final meal for extracting the oil, after separating them from extraneous material.

3.1.1.11.3.1 Determination of oil content

25 gm of grind seeds, meal were transferred to a soxhlet apparatus and sufficient quantity of petroleum ether was added. The apparatus was kept on a hot water bath running at 60°C for about 6 h, for complete extraction of the oil. The petroleum ether from the extracted oil was evaporated. The extracted oil was expressed as a percentage by mass of the seeds and calculated by the following formula:

$$ \frac{100 \times m}{m^0} $$

where, $m = \text{Sum of the mass in grams of oil}$

$m^0 = \text{Seed samples in grams}$

3.1.1.12 Statistical analysis

The experimental data was analysed statistically by following the standard procedures laid down by Gomez and Gomez (1984). The 'F' test was applied to assess the significance of the data at 5% level of
probability. Critical difference (C.D.) was calculated to compare the effect of various treatments, varieties and their interactions, using the following formula:

\[
\text{C.D.} = \frac{\text{Standard Error} \times 2}{t \text{ (value) } 5\%} \times \text{Replicates}
\]

3.1.2 Experiment 2: Impact of fly ash as soil amendment and Alternaria blight on growth and productivity of rapeseed

This experiment was conducted to determine the effect of fly ash amendment to the soil and Alternaria blight on growth and productivity of rapeseed.

3.1.2.1 Source of fly ash

Fly ash used in the experiment was obtained from the thermal power plant, Kasimpur (Fig. 4). The thermal power plant at Kasimpur (530 MW capacity) consumes daily 3192 MT bituminus type coal. The field soil and fly ash were mixed in requisite quantities to obtain different levels of fly ash i.e. 20, 40, 60, 80 and 100%. The field soil (without addition of fly ash) served as control. The mixture of soil and fly ash were filled in clay pots (30 cm diam.) and the pots were autoclaved. Surface sterilized seeds of four varieties of sunflower were sown in the pots placed in glass house. The following treatments were used in the experiment.

3.1.2.2 Amendment of soil with fly ash

The sandy loam soil used in the experiment, contained 66, 24 and 8% sand, silt and clay particles and 2% OM, pH 7.7, respectively.
Fig. 4. Source of Fly Ash, Thermal Power Plant, Kasimpur, India
The field soil and fly ash were mixed together (wt/wt) in 6 proportions i.e. 0, 20, 40, 60, 80 and 100%.

1. Control (%) = 1000 g soil + No fly ash
2. 20% = 800 g soil + 200 g fly ash
3. 40% = 600 g soil + 400 g fly ash
4. 60% = 400 g soil + 600 g fly ash
5. 80% = 200 g soil + 800 g fly ash
6. 100% = No soil + 1000 g fly ash

After properly mixing the fly ash with soil, pots (30 cm in diam.) were filled with one kg of each type of mixture (soil or/and fly ash). The pots were autoclaved at 20 lb for 20 min. Each treatment was replicated three times.

3.1.2.3 Fly ash analysis

Fly ash obtained from the thermal power plant, Kasimpur was analysed to determine the following:

1. pH was measured by pH meter and electrical conductivity (EC) by electrical conductivity meter (Elico Co. Ltd; Hyderabad, India) in the extract from 1:1 fly ash/water suspension (w/v).

2. The texture of fly ash in relation to particle size was determined by hydrometer method (Allen et al. 1974).

3. Total organic carbon was estimated by Degtjareff method (Walkley and Black, 1934).

4. Total nitrogen was determined by micro-Kjeldahl method (Nelson and Sommers, 1972).
5. Total phosphorus was estimated by molybdenum blue method (Allen et al., 1974).

6. Heavy metal contents in soil and fly ash were analysed by mixed acid digestion using conc. HNO₃, conc. H₂SO₄ and HClO₄ followed by atomic absorption spectrophotometry (Allen et al., 1974).

3.1.2.4 Plant culture and treatment

Surface sterilized seeds of three cultivars of rapeseed Brassica campestris L. (viz., T-7, TL-85 and TH-68) were used in the experiment. Plants designated to be inoculated with Alternaria brassicicola were inoculated according to the treatment. Inoculation method was same as given in the experiment 1. All the other agricultural practices were same as in experiment 1. The following were the treatments.

3.1.2.4.1 Control set

(i) Brassica campestris cv. T-7 + fly ash (0%)
   B. campestris cv. TL-85 + fly ash (0%)
   B. campestris cv. TH-68 + fly ash (0%)

(ii) B. campestris cv. T-7 + fly ash (0%) + A. brassicicola
    B. campestris cv. TL-85 + fly ash (0%) + A. brassicicola
    B. campestris cv. TH-68 + fly ash (0%) + A. brassicicola

3.1.2.4.2 Sets treated with different levels of fly ash and Alternaria brassicicola

(i) B. campestris cv. T-7 + fly ash (20%)
    B. campestris cv. TL-85 + fly ash (20%)
    B. campestris cv. TH-68 + fly ash (20%)
(ii)  
B. *campestris* cv. T-7+ fly ash (20%) + *A. brassicicola*

B. *campestris* cv. TL-85+ fly ash (20%) + *A. brassicicola*

B. *campestris* cv. TH-68 + fly ash (20%) + *A. brassicicola*

(iii)  
B. *campestris* cv. T-7+ fly ash (40%)

B. *campestris* cv. TL-85 + fly ash (40%)

B. *campestris* cv. TH-68 + fly ash (40%)

(iv)  
B. *campestris* cv. T-7+ fly ash (40%) + *A. brassicicola*

B. *campestris* cv. TL-85+ fly ash (40%) + *A. brassicicola*

B. *campestris* cv. TH-68 + fly ash (40%) + *A. brassicicola*

(v)  
B. *campestris* cv. T-7+ fly ash (60%)

B. *campestris* cv. TL-85 + fly ash (60%)

B. *campestris* cv. TH-68 + fly ash (60%)

(vi)  
B. *campestris* cv. T-7+ fly ash (60%) + *A. brassicicola*

B. *campestris* cv. TL-85+ fly ash (60%) + *A. brassicicola*

B. *campestris* cv. TH-68 + fly ash (60%) + *A. brassicicola*

(vii)  
B. *campestris* cv. T-7+ fly ash (80%)

B. *campestris* cv. TL-85 + fly ash (80%)

B. *campestris* cv. TH-68 + fly ash (80%)

(viii)  
B. *campestris* cv. T-7+ fly ash (80%) + *A. brassicicola*

B. *campestris* cv. TL-85+ fly ash (80%) + *A. brassicicola*

B. *campestris* cv. TH-68 + fly ash (80%) + *A. brassicicola*

(ix)  
B. *campestris* cv. T-7+ fly ash (100%)

B. *campestris* cv. TL-85 + fly ash (100%)

B. *campestris* cv. TH-68 + fly ash (100%)

(x)  
B. *campestris* cv. T-7+ fly ash (100%) + *A. brassicicola*

B. *campestris* cv. TL-85+ fly ash (100%) + *A. brassicicola*

B. *campestris* cv. TH-68 + fly ash (100%) + *A. brassicicola*
3.1.2.5 Parameters

The following parameters were considered.

1. Length of shoot and root, at harvest
2. Fresh and dry weight of shoot and root, at harvest
3. Number of flowers per plant at 80 days, after sowing
4. Number of pods per plant, at harvest
5. Pigment content of the leaves (chl. a, chl. b, total chlorophyll and carotenoids) at 60 days, after sowing
6. Nitrogen, phosphorus, potassium and sulphur contents in leaves at 60 days, after sowing
7. Oil contents of the seeds (%)
8. Seed yield per plant.

The same methods were used for determining the above mentioned parameters as in experiment 1.

3.1.3 Experiment 3 : Impact of fly ash dusting and Alternaria blight on growth and productivity of rape seed

This experiment was conducted to study the foliar application of fly ash and Alternaria blight on growth and productivity of rape seed.

3.1.3.1 Fly ash application

The fly ash used in the present study was obtained from the thermal power plant, Kasimpur. Dusting of fly ash was done with ASPEE Plastic duster (American Spring and Pressing Works, Pvt. Ltd., Bombay) delivering fly ash particles uniformly. For application, potted plants of the treatments were placed in a metersquare area and 2, 5 and
8 g of fly ash was dusted over the pots every day according to the dosage of the treatment. The application was continued upto 60 days. To prevent fly ash from being deposited on the soil surface in pots, before dusting of fly ash, the pots were kept in polythene bags and the upper part of each bag was tagged with the plants with the help of rubber band. Plants were watered by removing the rubber band, and exposing the soil surface as and when required. Plants receiving no fly ash served as control.

### 3.1.3.2 Plant culture and treatment

Surface sterilized seeds of rapeseed cultivars (*Brassica campestris* L. viz. T-7, TL-85 and TH-68) were used in the experiment. Plants designated to be inoculated with *Alternaria brassicicola* were inoculated according to the treatment. Inoculation and other agricultural practices were same as in experiment 1. The following treatments were used in the study.

#### 3.1.3.2.1 Control set

(i)  $B. \text{campestris} \ \text{cv. T-7} + \ 0 \ \text{g fly ash day}^{-1}$

$B. \text{campestris} \ \text{cv. TL-85} + \ 0 \ \text{g fly ash day}^{-1}$

$B. \text{campestris} \ \text{cv. TH-68} + \ 0 \ \text{g fly ash day}^{-1}$

(ii) $B. \text{campestris} \ \text{cv. T-7} + \ 0 \ \text{g fly ash day}^{-1} + A. \text{brassicicola}$

$B. \text{campestris} \ \text{cv. TL-85} + \ 0 \ \text{g fly ash day}^{-1} + A. \text{brassicicola}$

$B. \text{campestris} \ \text{cv. TH-68} + \ 0 \ \text{g fly ash day}^{-1} + A. \text{brassicicola}$

#### 3.1.3.2.2 Sets with foliar application of fly ash and *A. brassicicola*

(i) $B. \text{campestris} \ \text{cv. T-7} + \ 2 \ \text{g fly ash day}^{-1}$

$B. \text{campestris} \ \text{cv. TL-85} + \ 2 \ \text{g fly ash day}^{-1}$
B. campestris cv. TH-68 + 2 g fly ash day$^{-1}$

(ii) B. campestris cv. T-7 + 2 g fly ash day$^{-1}$ + A. brassicicola
    B. campestris cv. TL-85 + 2 g fly ash day$^{-1}$ + A. brassicicola
    B. campestris cv. TH-68 + 2 g fly ash day$^{-1}$ + A. brassicicola

(iii) B. campestris cv. T-7 + 5 g fly ash day$^{-1}$
      B. campestris cv. TL-85 + 5 g fly ash day$^{-1}$
      B. campestris cv. TH-68 + 5 g fly ash day$^{-1}$

(iv) B. campestris cv. T-7 + 5 g fly ash day$^{-1}$ + A. brassicicola
      B. campestris cv. TL-85 + 5 g fly ash day$^{-1}$ + A. brassicicola
      B. campestris cv. TH-68 + 5 g fly ash day$^{-1}$ + A. brassicicola

(v)  B. campestris cv. T-7 + 8 g fly ash day$^{-1}$
      B. campestris cv. TL-85 + 8 g fly ash day$^{-1}$
      B. campestris cv. TH-68 + 8 g fly ash day$^{-1}$

(vi) B. campestris cv. T-7 + 8 g fly ash day$^{-1}$ + A. brassicicola
      B. campestris cv. TL-85 + 8 g fly ash day$^{-1}$ + A. brassicicola
      B. campestris cv. TH-68 + 8 g fly ash day$^{-1}$ + A. brassicicola

3.1.3.3 Parameters

The following parameters were considered.

1. Length of shoot and root, at harvest
2. Fresh and dry weight of shoot and root, at harvest
3. Number of flowers per plant at 80 days after sowing
4. Number of pods per plant, at harvest
5. Pigment content of the leaves (chl. a, chl. b, total chlorophyll and carotenoids) at 60 days after sowing
6. Nitrogen, phosphorus, potassium and sulphur contents in leaves at 60 days after sowing
7. Oil content of the seeds (%)
8. Seed yield per plant.

The same procedures were adopted as given in the experiment 1 for the determination of the above parameters.

3.2 SECTION II

This section includes experiments conducted to determine the impact of SO$_2$ and fly ash on growth and productivity of sunflower. Four cultivars of sunflower were tested for their differential response to SO$_2$.

3.2.1 Experiment 1 : Impact of SO$_2$ on growth and productivity of sunflower

3.2.1.2 Exposure and doses

Same procedure was adopted for exposing the plants to SO$_2$ as described for rapeseed in Section I (experiment 1). Three doses of SO$_2$ (142.85, 285.71 and 571.43 $\mu$g m$^{-3}$) were used, as in the case of rapeseed.

3.2.1.2 Plant culture and treatment

Seeds of four cultivars of sunflower, *Helianthus annuus* L. (viz., Morden, PSF-5, CSFH-778 and NSFH-110) were surface sterilized, for two minutes, in 0.1% HgCl$_2$ solution followed by washing with distilled, sterilized water, at least twice. The surface sterilized seeds were sown in clay pots (30 cm in diam.) filled with
autoclaved sandy loam field soil (66% sand, 24% silt, 8% clay, 2% OM, pH 7.7). After germination, seedlings were thinned to maintain three seedling per pot. Plants of different varieties (4-leaf stage) were exposed to SO₂ in the exposure chamber. The following were the treatments.

3.2.1.2.1 Control set

*H. annuus* cv. Morden

*H. annuus* cv. PSF-5

*H. annuus* cv. CSFH-778

*H. annuus* cv. NSFH-110

3.2.1.2.2 Exposed set

(i) *H. annuus* cv. Morden + SO₂ (142.85 μgm⁻³)

*H. annuus* cv. PSF-5 + SO₂ (142.85 μgm⁻³)

*H. annuus* cv. CSFH-778 + SO₂ (142.85 μgm⁻³)

*H. annuus* cv. NSFH-110 + SO₂ (142.85 μgm⁻³)

(ii) *H. annuus* cv. Morden + SO₂ (285.71 μgm⁻³)

*H. annuus* cv. PSF-5 + SO₂ (285.71 μgm⁻³)

*H. annuus* cv. CSFH-778 + SO₂ (285.71 μgm⁻³)

*H. annuus* cv. NSFH-110 + SO₂ (285.71 μgm⁻³)

(iii) *H. annuus* cv. Morden + SO₂ (571.43 μgm⁻³)

*H. annuus* cv. PSF-5 + SO₂ (571.43 μgm⁻³)

*H. annuus* cv. CSFH-778 + SO₂ (571.43 μgm⁻³)

*H. annuus* cv. NSFH-110 + SO₂ (571.43 μgm⁻³)

Each treatment was replicated three times and pots were arranged in factorial randomised block design on the glasshouse benches.
3.2.1.3 Parameters

The following parameters were determined for each treatment of the experiment:

1. Length of shoot and root, at harvest
2. Fresh and dry weight of shoot and root, at harvest
3. Fresh and dry weight of floral head, at harvest
4. Floral head diameter and 100 seed weight, at harvest
5. Pigment content of the leaves (chl. a, chl. b, total chlorophyll and carotenoids) at 60 days, after sowing
6. Nitrogen, phosphorus, potassium and sulphur content in leaves at 60 days, after sowing
7. Oil content of seeds (%)
8. Seed yield per plant.

The same methods were used for determining the above mentioned parameters, as described for rapeseed in the Section I.

3.2.2 Experiment 2: Impact of fly ash amended soil on growth and productivity of sunflower

The experiment was conducted in glass house, to assess the effect of fly ash artificially added to the soil in various proportions on the plant growth and productivity of sunflower. Four cultivars of sunflower were used in this experiment.

3.2.2.1 Amendment of soil with fly ash

Same procedure was adopted as in Section 1 (experiment 2).
3.2.2.2 Treatments

The following were the treatments:

3.2.2.2.1 Control set

 Helianthus annuus cv. Morden + fly ash (0%)
 Hel. annuus cv. NSFH-110 + fly ash (0%)
 Hel. annuus cv. CSFH-778 + fly ash (0%)
 Hel. annuus cv. PSF-5 + fly ash (0%)

3.2.2.2.2 Sets treated with different levels of fly ash

(i) Hel. annuus cv. Morden + fly ash (20%)
 Hel. annuus cv. NSFH-110 + fly ash (20%)
 Hel. annuus cv. CSFH-778 + fly ash (20%)
 Hel. annuus cv. PSF-5 + fly ash (20%)

(ii) Hel. annuus cv. Morden + fly ash (40%)
 Hel. annuus cv. NSFH-110 + fly ash (40%)
 Hel. annuus cv. CSFH-778 + fly ash (40%)
 Hel. annuus cv. PSF-5 + fly ash (40%)

(iii) Hel. annuus cv. Morden + fly ash (60%)
 Hel. annuus cv. NSFH-110 + fly ash (60%)
 Hel. annuus cv. CSFH-778 + fly ash (60%)
 Hel. annuus cv. PSF-5 + fly ash (60%)

(iv) Hel. annuus cv. Morden + fly ash (80%)
 Hel. annuus cv. NSFH-110 + fly ash (80%)
 Hel. annuus cv. CSFH-778 + fly ash (80%)
 Hel. annuus cv. PSF-5 + fly ash (80%)

(v) Hel. annuus cv. Morden + fly ash (100%)
 Hel. annuus cv. NSFH-110 + fly ash (100%)
H. annuus cv. CSFG-778 + fly ash (100%)

H. annuus cv. PSF-5 + fly ash (100%)

Each treatment was replicated three times and pots were arranged on the glass house benches in a factorial randomised block design. The following parameters were considered:

1. Length of shoot and root, at harvest
2. Fresh and dry weight of shoot and root, at harvest
3. Fresh and dry weight of floral head, at harvest
4. Floral head diameter and 100 seed weight, at harvest
5. Pigment content of leaves (chl. a, chl. b, total chlorophyll and carotenoids) at 60 days after sowing
6. Nitrogen, phosphorus, potassium and sulphur contents of leaves at 60 days after sowing
7. Oil contents of seeds (%)
8. Seed yield per plant.

The same methods were used for determining the above parameters as described in the Section I.

3.2.3 Experiment 3: Impact of fly ash dusting on growth and productivity of sunflower

The present experiment was designed to assess the impact of foliage-deposited fly ash on plant growth and productivity of sunflower.

3.2.3.1 Fly ash application

The procedure described in section 1 (experiment 3) were adopted for fly ash application.
3.2.3.2 Plant culture

Seedling of sunflower were raised in autoclaved sandy loam field soil (66% sand, 24% silt, 8% clay, 2% OM, pH 7.7) filled in 30 cm diam. clay pots. After the establishment of seedlings, they were thinned to three seedling/pot. The fly ash was applied on three weeks old seedlings. Each treatment consisted of three replicates and pots were placed on glass house benches in factorial randomised block design.

3.2.3.3 Treatments

Plants of sunflower were grown in clay pots filled with sandy loam soil as in the previous experiment, with fly ash spray. The following were the treatments.

3.2.3.3.1 Control set

- H. annuus cv. Morden + 0 g fly ash day$^{-1}$
- H. annuus cv. NSFH-110 + 0 g fly ash day$^{-1}$
- H. annuus cv. CSFH-778 + 0 g fly ash day$^{-1}$
- H. annuus cv. PSF-5 + 0 g fly ash day$^{-1}$

3.2.3.3.2 Sets with foliar application of fly ash

(i) H. annuus cv. Morden + 2 g fly ash day$^{-1}$
- H. annuus cv. NSFH-110 + 2 g fly ash day$^{-1}$
- H. annuus cv. CSFH-778 + 2 g fly ash day$^{-1}$
- H. annuus cv. PSF-5 + 2 g fly ash day$^{-1}$

(ii) H. annuus cv. Morden + 5 g fly ash day$^{-1}$
- H. annuus cv. NSFH-110 + 5 g fly ash day$^{-1}$
H. annuus cv. CSFH-778 + 5 g fly ash day$^{-1}$
H. annuus cv. PSF-5 + 5 g fly ash day$^{-1}$

(iii) H. annuus cv. Morden + 8 g fly ash day$^{-1}$
H. annuus cv. NSFH-110 + 8 g fly ash day$^{-1}$
H. annuus cv. CSFH-778 + 8 g fly ash day$^{-1}$
H. annuus cv. PSF-5 + 8 g fly ash day$^{-1}$

3.2.3.4 Parameters

The following parameters were considered.

1. Length of shoot and root, at harvest
2. Fresh and dry weight of shoot and root, at harvest
3. Fresh and dry weight of floral head, at harvest
4. Floral head diameter and 100 seed weight, at harvest
5. Pigment content of the leaves (chl. a, chl. b, total chlorophyll and carotenoids) at 60 days, after sowing
6. Nitrogen, phosphorus, potassium and sulphur contents in leaves at 60 days, after sowing
7. Oil content of the seeds (%)
8. Seed yield per plant.

The same methods were applied for determining the above mentioned parameters, as described earlier.